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Protective effect of *Juglans regia* L., against ultraviolet-B induced photoaging in human epidermal keratinocytes



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Keywords: Juglans regia L. Ultraviolet-B MAPKs MMPs Photoaging markers

ABSTRACT

Solar ultraviolet-B radiation (UVB) has severe adverse effects on the structure and functions of the skin. Although, UVB (290–320 nm) represents only 5–10% of UV light reaching earth's surface, its contribution towards photoaging is tremendous. In this present study was investigate the photoprotective effect of methanolic extract of the male flower of *J. regia* L. (MEJR) against UVB induced photoaging in human epidermal keratinocytes (HaCaT). Cells were exposed to UVB-irradiation at a dose of 20 mJ/cm², induces the activation of several signaling pathways which are associated with oxidative stress and photoaging. A single dose of UVB irradiation increased the protein and mRNA expression of MAPKs, AP-1, MMPs, Smad7 and decreased expression of TIMP-1/2, TGF- β 1, Smad3, procollagen type-1 in HaCaT cells. In contrast, pretreatment of MEJR (80 µg/ml) prior to UVB-irradiation significantly prevented the overexpression of MAPKs, AP-1, MMPs, Smad7 and decreased expression of TIMP-1/2, TGF- β 1, Smad3 and procollagen type-1 in HaCaT cells. Moreover, pretreatment of MEJR (80 µg/ml) prior to UVB-irradiation significantly prevents apoptosis in sub G_o-phase. Thus, MEJR protects UVB-mediated photoaging in human skin cells, by modulating the expression of photoaging markers. The protection might be because of the presence of the good amount of bioactive compounds in MEJR.

1. Introduction

Skin is the largest major organ of our system; it protects the body from several environmental insults [1]. It has been directly exposed to the ambient environment and thereby it is the primary target organ for all sorts of environmental stresses including harmful radiations [2]. Ultraviolet (UV) radiation is a major environmental factor that directly affects the skin according with their wavelengths [3]. Ultraviolet-B (UVB) irradiation induces several deleterious effects on human ssskin, which includes sunburn, tanning, immune suppression, photoaging and skin carcinogenesis [4,5]. Acute and chronic exposure of UVB-radiation alters the structural integrity of skin leads to photoaging [6]. UVB-irradiation generates highly destructive molecules called reactive oxygen species (ROS), which are extremely caused oxidative damage to proteins, lipids, and DNA in the skin leading to ageing [7].

Over production of ROS during UVB irradiation leads to significant disturbances in signaling pathways resulting in changes in gene

expression [8]. UVB radiation induced ROS generation activates the serine therione kinases called as mitogen-activated protein kinases (MAPKs), which has a vital role in UVB induced skin photodamage [9]. The MAPK family includes c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinases (ERK) and p38 kinase, plays an important role in the regulation of cellular functions such as differentiation, apoptosis, proliferation, inflammation, and photoaging [10].

Activation of MAPKs family proteins by the exposure of UVB radiation stimulates the overexpression of transcriptional factor activator protein-1 (AP-1) resulted in the matrix metaallo proteinase (MMPs) [11]. MMPs are zinc dependent endopeptatides which are involved in the degradation of extracellular matrix and collagen synthesis and also play key roles in the activation of inflammation and skin aging [9,12,13]. MMPs are secreted by keratinocytes and dermal fibroblasts in response to UV radiation mediated oxidative stress and photoaging of the skin [11]. Degradation of collagen is generally regulated by MMPs by the activity of their natural inhibitors known as tissue inhibitor of

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Abbreviations: UVB, ultraviolet-B radiation; MEJR, methanolic extract of male flower of *J. regia* L; HaCaT, human epidermal keratinocytes; MAPKs, mitogenactivated protein kinases; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinases; NF-κB, nuclear factor-kappa; AP-1, transcription factor activator protein-1; MMPs, matrix metalloproteinases; MTT, 3-(4, 5 -dimethyl- 2-thiaozolyl)-2,5-diphenyl -2H-tetrazolium bromide; TBA, 2-thiobarbituric acid; TCA, trichloroacetic acid; RIPA, Radio immuno precipitation assay; BSA, buffer and bovine serum albumins

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Research Article

IN VIVO AMELIORATIVE POTENTIAL OF CAFFEIC ACID AGAINST HEPATOTOXICITY AND NEPHROTOXICITY INDUCED BY MERCURIC CHLORIDE IN ALBINO WISTAR RATS

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ABSTRACT

Objective: The current experimental study is designed to scrutinize the possible defensive effect of caffeic acid (CA) against mercury-induced hepatotoxicity in male rats.

Methods: During the treatment periods, a sub-lethal dose of HgCl₂ (1.29 mg/kg body weight) was given to rats for 15 days through oral administration and the CA was administrated for 15 days, on mercuric intoxicated rats were observed. After completing the programmed period of experimental work, the blood was drawn through a micro glass tube in the ocular region and separated the serum by centrifuge.

Results: The subsequent serum enzymes were analyzed as the alkaline phosphatase (ALP), alanine transferase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH). Further, the bilirubin, albumin, total cholesterol, urea, and creatinine levels were also noticed in the blood serum of both normal and treated rats. CA is one of the well-known phenolic compounds, and a strong antioxidant helps to prevent oxidative damage and reducing oxidative stress. The biological action of liver biomarker enzymes such as ALT, AST, ALP, and LDH was significantly enhanced and at the same time the drastic increased in the level of albumin was all together decreased. The level of bilirubin, total cholesterol, urea, and creatinine was significantly increased in HgCl₂ intoxicated rats. When the treatment of CA on mercury intoxicated rats for 15 days (5 mg/kg body weight), in the serum enzymes (ALT, AST, ALP, and LDH) also declined to near normal level. The level of bilirubin, total cholesterol, urea, and creatinine activities was also significantly declined to near normal level when compared to mercury-treated group. The level of albumin was significantly enhanced. The CA alone treatment showed the enhanced antioxidant levels and not any alteration in the levels of biochemical parameters when compared to control.

Conclusion: These observations of the present experimental work demonstrated the detoxify effects and defensive effect of CA against HgCl₂ toxicity in liver tissue.

Keywords: Alanine transferase, Aspartate transaminase, Alkaline phosphatase, Lactate dehydrogenase, Bilirubin, Albumin, Total cholesterol, Urea and creatinine, Mercuric chloride, Caffeic acid.

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INTRODUCTION

Heavy metals are known to be toxic to all type of animals including human beings [1,2]. Even at low concentration, mercury and its compounds cause deleterious effects to the animals [3]. Mercury and its compounds have been recognized as one of widespread environmental and industrial pollution [4,5]. Exposure of this heavy metal is causing numerous types of adverse health effects in animals [6]. In most manufacturing industries such as thermometers, barometers, and instruments for measuring blood pressure are responsible for releasing these heavy metals in the abiotic system [7,8]. Liver is the central organ to carry out all type of metabolic activities and detoxification in the animal and also carried out a different type of functions which is associated with metabolic activities and detoxification process of toxic substances [9]. The measurement of mitochondrial enzymes in the liver is considered to be a very good tool to understand the occurrence of hepatic necrosis which is associated with liver diseases [10,11]. In serum, the assessment and determination of liver enzymes such as alanine transferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and serum lipid profile, cholesterol, triacylglycerides, and lipoproteins, are providing clear picture of the liver function in an animal. It also used to evaluate the exact functional status of the liver and to detect liver cellular injury occurred in animal. If any type of alteration occurred in the enzymatic activity (biomarkers) caused drastic physiological and biochemical changes occurred in animals, and it is also considered as a marker index of hepatotoxicity [12], and it can serve as an index of liver biosynthetic capacity [13-16]. India is well-known for a plethora of medicinal plants.

The medicinal uses of many plants and its products have been reported in literature [17]. The present experimental work evaluated both the hepatoprotective and nephroprotective effect of Caffeic acid (CA) on mercuric chloride induced hepatotoxicity and nephrotoxicity in rats. In the present experimental study, hepatotoxicity and nephrotoxicity have been mediated by reactive oxygen species which is generated by administration of mercuric chloride in the rat. This type of toxicity could be managed completely by way of administration of remedial agents which is possess antioxidants [18], free radical scavengers [19], and anti-lipid peroxidation [20] activities. Administration of CA on mercury intoxicated rats was able to get complete recovery from mercuric chloride induced hepatocellular damage as evidenced in the present experimental work by prevention of any increase in serum biomarker enzymes and biochemical levels subsequent to toxin exposure and the known antioxidant, free radical scavenging, and antilipid peroxidation properties of CA might be the contributing factor for these manifestations.

METHODS

Chemicals

In the present experimental work, Mercuric chloride $(HgCl_2)$, CA, and all other necessary reagents of analytical grade were used and it was purchased from HiMedia Laboratories Ltd, Mumbai, India.

Animals

Healthy male albino rats, *Rattus norvegicus* (180–200 g), were obtained from the Central Animal House, Department of Experimental Medicine,

ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH



IN VIVO HEPATOPROTECTIVE EFFECT OF CAFFEIC ACID ON MERCURIC CHLORIDE-INDUCED BIOCHEMICAL CHANGES IN ALBINO WISTAR RATS

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ABSTRACT

Objective: Mercury (Hg) is a highly dangerous and also one of the harmful heavy metals which induces oxidative stress in the animal body. The present study is planned to examine the possible defensive result of caffeic acid (CA) against mercury chloride (HgCl₂)-induced hepatotoxicity in male albino Wistar rats, *Rattus norvegicus*.

Methods: Sublethal dose of HgCl₂ (1.29 mg/kg body weight) was administrated in rats for 15 days through oral dose and the CA was administrated for another 15 days on mercuric-intoxicated rats. After completing the scheduled exposure time, the rats were sacrificed and the whole liver organ was removed immediately from the animal, and it was used to carry out for biochemical and bioenzymological studies to observe.

Results: The level of lipid peroxidation (LPO) content reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the liver tissue. CA is an energetic component in the phenolic propolis extract and also in a wide variety of plants, and a strong antioxidant helps to prevent oxidative damage and to reduce oxidative stress. The antioxidants such as GPx, CAT, and SOD and non-antioxidant GSH were significantly decreased, and also, the LPO level was increased in mercury toxicity rats. The treatment of CA (5 mg/kg body weight) in the liver tissue shows considerable declining in the level of oxidant content and along with an increase in the level of antioxidant properties by the way of improvement in liver tissues. Antioxidant and non-antioxidant enzyme (LOP, GSH, GPx, SOD, and CAT) activities were also significantly decreased to near untreated control level when compared to Hg-treated group. The CA acid alone treatment showed the enhanced antioxidant levels and not any alteration in the levels of biochemical parameters when compared with control.

Conclusion: These observations of the present experimental study clearly explained the detoxify effects and protective effect of CA against HgCl₂ toxicity in liver tissue.

Keywords: Mercuric chloride, Hepatotoxicity, Caffeic acid, Lipid peroxidation, Superoxide dismutase, Catalase, Glutathione and glutathione peroxidase.

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INTRODUCTION

Mercury (Hg) and its compounds are called as worldwide pollutant because it easily distributed in air, water, and soil and possess high risk to global health. Nowadays, most of the researcher's attention has been refocused on the toxicity of the heavy metal which is present in various forms of mercury compounds. Mostly, inorganic forms of mercury compounds have used in the production of various products and agriculture medicines, etc. [1]. Normally, heavy metals are having high affinity with thiol groups (-SH). If the sulfhydryl group is available at the enzyme active site, the heavy metals immediately react with it and decrease the availability of total sulfhydryl groups in cell to get oxidative stress [2,3]. Mercury (Hg) and its compounds will bind to any free thiol available in the system, and the heavy metals are most frequently bound with the thiols due to its high concentrations [4]. Once the reaction rate was initiated, the stable mercury-sulfhydryl bond was formed because it is liable in the presence of other free total sulfhydryl groups [5]. Chen [6] have also suggested that mercury and its compounds are biochemically toxic as it binds to sulfhydryl groups (-SH) and leads to disruption of protein structures and functions. In the present experimental study, the decreasing level of total protein content presents in the liver tissues of rats when treated with mercury toxicity also supporting the above findings. Proteins are common fundamental biomolecules to all living organisms, and they are responsible for most of the biocomplex functions that make life in a possible way. and proteins also act as major structural ingredients of living beings. Proteins play a vital role to carry out various types of physiological functions of an organism with the help of the enzymes or subunits of enzymes [7]. Naturally, formation of free radical process

causes oxidative decoration of lipid leading to promote the production of lipid peroxidation (LPO) content and it also used as an indicator of oxidative stress. Promoting the imbalance between the production of reactive oxygen species (ROS) and free radicals (FR) in the organs due to the accumulation of LPO content caused the pathogenesis of different types of diseases, damages, and defects [8-10]. During the accumulation of LPO content such as aldehydes, inter alia, and malondialdehyde (MDA) leads to cellular membrane damages by oxidative destruction of polyunsaturated fatty acids [11,12]. Total amino acids are the building blocks of the protein in animal tissues. Most of the amino acids can easily interact with one another and with other molecules to form different complex molecules and also useful in the synthesis of cellular, subcellular, and other organic substances. The formations of different types of proteins mainly depend on the arrangement of amino acid sequences and in varying numbers. Different types of amino acid pool and their arrangement in the tissues may get vary in their chemical composition [13]. During the stressful condition, total free amino acids would also serve as energy precursor and also utilized for the synthesis of required proteins to overcome the stress. A number of experimental works have been conducted and explained the biological toxicity of HgCl₂ in an organism by carrying out the biochemical fate of mercury toxicity [14]. Nowadays, an emerging interest has been focused on the use of phytochelating plant products with ameliorative potentials and its antioxidant activity in heavy metal intoxication therapy. Caffeic acid (CA) is one of the non-flavonoid catecholic compounds (the most common phenolic acids). It is easily available in several species of plants, and it also occurs in parts of fruits, vegetables, tea, coffee, and wine [15] and is regularly consumed in human diet [16,17]. In the past



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Neuroprotective and Ameliorative Effect of Caffeic Acid On Antioxidant and Acetylcholine Status of Mercury Intoxicated Rats

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Abstract

Mercury (Hg), a well-known environmental and industrial hazard, exerts a number of toxic effects in humans and animals. And also it acts as a neurotoxin. In the present experimental study, the antioxidant and acetylcholine property of mercury intoxicated brain tissue of rat was investigated. And withdrawal effects of mercury toxicity in the brain tissue with the help of caffeic acid treatments were also investigated. Caffeic acid at a concentration of 5 mg/kg body weight, accelerate the oxidation of neurotoxicity induced by HgCl2 (1.29mg/kg Body weight of the animal). The aim of this study was to investigate the protective potential of Caffeic acid against Hgcl2 induced brain damage. The antioxidant activities of caffeic acid concentration were mainly due to the scavenging of lipid peroxide in this system. The oxidant mechanism for caffeic acid is most likely due to the strong reducing power and weak metal chelating ability. During the Hgcl2 treatment, the mercury toxicants mainly induced the toxicity in the brain tissues or rats to exhibit elevated level of lipid peroxidation (LOP). At the same time the level of enzymic antioxidants Superoxide dismutase (SOD), Catalase (CAT), and glutathione peroxidase (GPx), AchE activity and non-enzymic antioxidants reduced glutathion (GSH) were significantly decreased in brian tissues. But during the recovery period (Caffeic acid treatment on mercury intoxicated animal), are enhances the not only the GSH levels, and also promote the AchE activities. It leads to protects cell damage against neurotoxicity induced by mercuric chloride. Histological and histopathogical observation are also supporting these findings by the way of restoration of brain level or nearl normal level. Thus, result of the present study caffeic acid exhibited potent antioxidant, neuroprotective activities on HgCl2 induced neurotoxicity in rats.

Keywords

Mercuric chloride, Caffeic acid, Antioxidant, Neurotoxicity.

85

RESEARCH ARTICLE



Sulforaphane potentially attenuates arsenic-induced nephrotoxicity via the PI3K/Akt/Nrf2 pathway in albino Wistar rats

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Abstract

Oxidative stress plays a significant role in the pathophysiology of numerous kidney diseases, generally mediated by reactive oxygen species (ROS). Arsenic (Ar) is known to exert its toxicity through the generation of ROS and inflammation. The current study investigates the protective effects of sulforaphane (SFN) against arsenic-induced renal damage via PI3K/Akt-mediated Nrf2 pathway signaling. Thirty-two male albino Wistar rats were randomly divided into four groups of eight animals each, designated as control, arsenic (Ar), sulforaphane plus Ar (SFN+Ar), and sulforaphane alone (SFN), with oral administration of Ar (5 mg/kg BW) and SFN (80 mg/kg BW) daily for 28 days. Ar administration significantly (P < 0.05) increased the levels of ROS, OHdG, Ar accumulation, and lipid peroxidation, and decreased levels of enzymatic and nonenzymatic antioxidants. Notably, a significant (P < 0.05) increase was observed in markers of apoptosis, DNA damage, TUNEL-positive cells, and dark staining of ICAM-1 in renal tissue with decreased PI3K/Akt/Nrf2 gene expression. The biochemical findings were supported by histopathological and electron microscopy evaluation, which showed severe renal damage in rats treated with Ar. Pretreatment with SFN significantly (P < 0.05) attenuated renal ROS, OHdG, lipid peroxidation, and DNA damage, and increased phase II antioxidants via PI3K/Akt-mediated Nrf2 activation in renal tissue. These results show that dietary supplementation with SFN protects against Ar-induced nephrotoxicity via the PI3K/Akt-mediated Nrf2 signaling pathway in the rat kidney.

Keywords Sulforaphane · Arsenic · Rat · Nephrotoxicity · PI3K/Akt · Nrf2 pathway

Introduction

Arsenic (Ar) is a major toxicant that is ubiquitous in the environment, and a high level of exposure carries an increased risk of cancers of the skin, liver, lung, and kidney, and cardiovascular and reproductive abnormalities (Yu et al. 2013). Sources of human exposure to heavy metals and metalloids such as

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arsenic, lead, cadmium, and uranium include both industrial and environmental surroundings. Among many toxicants, arsenic ranks as an important antineoplastic agent that has been used medicinally to treat a variety of ailments for more than 2400 years (Smeester et al. 2011). More than 200 million people worldwide are at risk of arsenic poisoning, among which 100 million live in West Bengal, India (Council 2001), where groundwater arsenic absorption exceeds the maximum permissible level of 50 μ g/L, according to the World Health Organization (WHO). Ar generally enters the body via the ingestion of contaminated drinking water, and long-term consumption of Ar-contaminated water results in Ar toxicity in nearly all organ systems of the body (Mandal and Suzuki 2002).

Consumption of arsenic, either through the supply of drinking water or medicinal administration, may cause renal damage. The kidney is a target organ for Ar toxicity, as it has been reported that around 60% of the daily dose of arsenic is excreted through urine. Chronic arsenic exposure has been found to

Research Article



The molecular and biochemical insight view of grape seed proanthocyanidins in ameliorating cadmium-induced testes-toxicity in rat model: implication of PI3K/Akt/Nrf-2 signaling

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The present study aims to evaluate the protective effect of grape seed proanthocyanidins (GSP) on cadmium (Cd)-induced testicular apoptosis, inflammation, and oxidative stress in rats. A total of 24 male Wistar rats were divided into four groups, namely control, GSP (100 mg/kg BW), Cd (5 mg/kg BW), and Cd+GSP. Cd-treated rat testes exhibited a significant increment in oxidative stress mediated inflammation and apoptosis. Pre-administration of GSP exhibit significant protection against the apoptotic and inflammatory damages elicited by Cd and uphold the intercellular antioxidant status in testes. Histological changes were studied and the immunohistochemical staining for caspase 3, HSP70, and eNOS protein expressions were also analyzed to justify the protective action of GSP. Furthermore, GSP prevented DNA damage, and enhanced the expression of antioxidant responsive elements Nrf2/HO-1 by PI3K/Akt-dependent pathway. Therefore, our results suggest that GSP acts as a multipotent antioxidant entity against Cd-induced oxidative testicular toxicity in rats.

Introduction

Cadmium (Cd) is considered as a potent reproductive toxicant, and has severe toxic effects in testis of rats and humans associated with the male infertility and deprived semen quality [1]. Cd brings testicular changes at various stages of growth and maturity which comprised severe edema, hemorrhage, necrosis and atrophy, as well as reduction in counts and motility of sperm and decreased concentrations of testos-terone in plasma and testes [2]. Cd has been found to induce the non-reversible tissue necrosis at relatively low concentrations [3] and alters gene expression in testis even at non-toxic doses of Cd. Cd has potent estrogen and androgen like activities *in vivo* and *in vitro*, by directly binding to estrogen and androgen receptors [4].

The pathogenesis of testicular damage following Cd exposure are generally attributed to oxidative damage which cause damage to cells, stimulates free radical production, resulting in oxidative deterioration of lipids, proteins, and DNA. Cd exposure is one of the main causes of human prostate and testicular cancers due to the elevated levels of Cd in prostatic tissues and Leydig cell adenomas in the testis and epithelial cell adenomas in the ventral prostate of rats [5]. High levels of Cd in the modulation of male reproductive system are in seminal fluid which is associated with asthenozoospermia in infertile males. Based on variability of testicular damage, several mechanisms of Cd-induced testicular toxicity have been proposed. The physical and chemical properties of the Cd⁺² ions, specifically its similarities to calcium and zinc put forth the oxidative stress of Cd. Cd substitutes the calcium or zinc in crucial physiological processes that

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Outline

Highlights

Abstract

Graphical abstract

Keywords

1. Introduction

2. Materials and methods

3. Results

4. Discussion

5. Conclusions

Conflict of interest

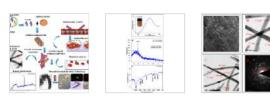
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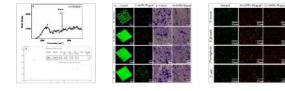
Appendix A. Supplementary data

References

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Tables (3)

Table 1 田 Table 2
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- Table 3
- Extras (1)

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Journal of Trace Elements in Medicine and Biology Volume 51, January 2019, Pages 191-203

Toxicology

Crustin-capped selenium nanowires against microbial pathogens and Japanese encephalitis mosquito vectors – Insights on their toxicity and internalization

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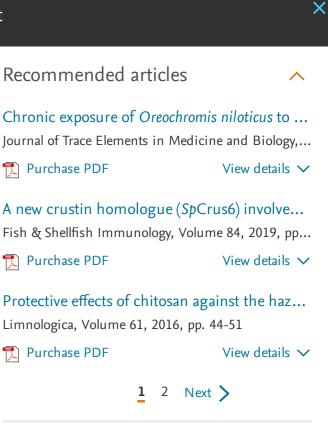
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Highlights

- Selenium nanowires (Cr-SeNWs) were synthesized using the • antimicrobial peptide crustin.
- They showed effective antibacterial and antibiofilm properties.
- Hemolytic assays showed good biocompatibility and antioxidant activity.
- Cr-SeNWs are highly toxic to Japanese encephalitis mosquito • vectors.
- Histological and green-red channel analysis showed mosquito larval tissue damages.

Abstract

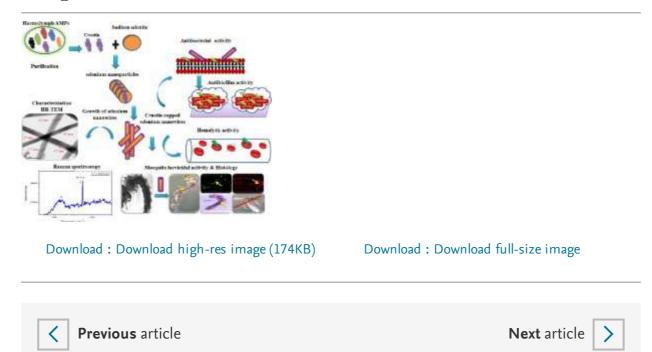
Herein, we reported a method to synthesize selenium nanowires (Cr-SeNWs) relying to purified cysteine-rich antimicrobial peptide crustin in presence of



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ascorbic acid. Cr-SeNWs were characterized by UV-vis, XRD, FTIR and Raman spectroscopy, as well as SEM, HR-TEM and EDAX. The UV-vis spectroscopy peak was noted at 350 nm. XRD showed the crystalline nature of Cr-SeNWs through diffraction peaks observed 2θ at 12° and 28° corresponding to (020), and (241) lattice planes, respectively. HR-TEM results shed light on the size of Cr-SeNWs, ranging from 17 to 47 nm. Raman spectroscopy and EDAX analysis of Cr-SeNWs showed presence of 57% selenium element. Furthermore, Cr-SeNWs showed higher antimicrobial activity on Gram-positive bacteria (Staphylococcus aureus, Enterococcus faecalis) over Gram-negative ones (Pseudomonas aeruginosa, Escherichia coli). The zone of inhibition was larger on S. aureus (50 μ g/ml = 4.0 mm, 75 μ g/ml = 7.2 mm) and E. faecalis (50 μ g/ml = 3.1 mm, 75 μ g/ml = 5.1 mm), over *P. aeruginosa* (50 μ g/ml = 2.1 mm, 75 μ g/ml = 4.8 mm), E. coli (50 μ g/ml = 1.3 mm, 75 μ g/ml = 4.3 mm) bacteria. The antibiofilm activity of Cr-SeNWs was also investigated and biofilm reduction was observed at 75 μ g/ml. In addition, Cr-SeNWs were highly effective as larvicides against Zika virus and Japanese encephalitis mosquito vectors, i.e., Culex quinquefasciatus and Culex tritaeniorhynchus, with LC_{50} values of 4.15 and 4.85 mg/l, respectively. The nanowire toxicity and internalization was investigated through confocal laser scanning microscopy and histological studies. To investigate the potential of Cr-SeNWs for real-world applications, we also evaluated Cr-SeNWs in hemolytic assays, showing no cytotoxicity till 5 mg/ml. Besides, higher antioxidant activity at the concentration at 100 μ g/ml was noted, if compared with purified crustin. The strong antioxidant potential of this nanomaterial can be helpful to boost the shelf-life potential of Cr-SeNWsbased pesticides and antimicrobials.

Graphical abstract



Keywords

Biocompatibility; Culex quinquefasciatus; Culex tritaeniorhynchus; Integrated vector management; Mosquito control; Culicidae; Nanomaterial; Pesticide

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Table 3

Extras (1)

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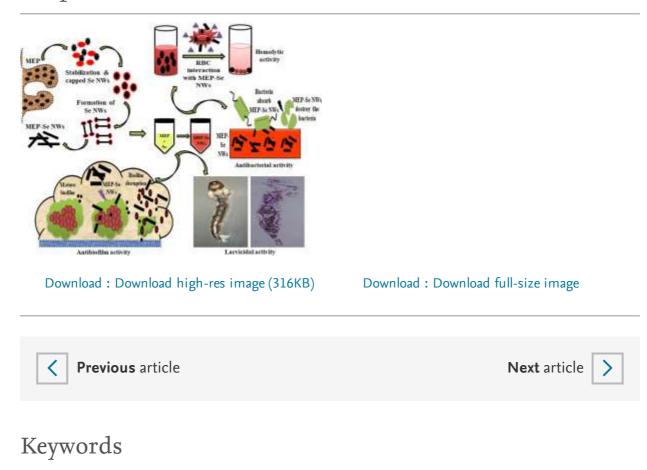
Q Search ScienceDirect Get Access Share Export Advanced X COVID-19 campus closures: see options for Remote Access to subscribed content Outline **Recommended** articles Journal of Photochemistry and Photobiology B: \wedge Biology Highlights Chronic exposure of Oreochromis niloticus to ... Volume 192, March 2019, Pages 55-67 Journal of Trace Elements in Medicine and Biology,... ELSEVIER Abstract Purchase PDF View details 🗸 **Graphical Abstract** Microbial exopolymer-capped selenium Keywords Synthesis of ZnO nanoparticles using insulin... nanowires - Towards new antibacterial, Journal of Photochemistry and Photobiology B: Bi... 1. Introduction 📆 Purchase PDF View details 🗸 2. Materials and Methods antibiofilm and arbovirus vector larvicides? 3. Results Protective activity of beta-1, 3-glucan bindin... International Journal of Biological Macromolecules,... Muthukumar Abinaya ^a, Baskaralingam Vaseeharan ^a \approx \boxtimes , Ravichandran Rekha ^a, Sivakumar Shanthini ^b, 4. Discussion Marimuthu Govindarajan ^{c, d}, Naiyf S. Alharbi ^e ightarrow [©], Shine Kadaikunnan ^e, Jamal M. Khaled ^e, Mohammed Purchase PDF View details 🗸 5. Conclusions N. Al-Anbr ^e Acknowledgments 1 2 Next > **⊞** Show more Conflict of Interest https://doi.org/10.1016/j.jphotobiol.2019.01.009 Get rights and content Appendix A. Supplementary data Citing articles (1) References Show full outline 🗸 Article Metrics Highlights Citations Se nanowires (Se NWs) were synthesized using microbial Figures (9) exopolymers (MEP) from Bacillus licheniformis Dahb1. Citation Indexes: 1 MEP-Se NWs were characterized by UV–Vis, XRD, FTIR, FE-SEM, • Captures HR-TEM and EDX. Exports-Saves: 1 • MEP-Se NWs revealed great antioxidant and antibiofilm activity. Readers: 47 Low hemolytic activity of MEP-Se NWs pointed out their • Social Media biocompatible nature. 3 Tweets: Show all figures 🗸 MEP-Se NWs were highly toxic to Aedes aegypti and Culex • quinquefasicatus larvae. ÖPLUMX View details > Tables (3) 田 Table 1 Table 2

Abstract

Arboviral diseases and microbial pathogens resistant to commercially available drugs are on the rise. Herein, a facile microbial-based approach was developed to synthesize selenium nanowires (Se NWs) using microbial exopolymer (MEP) extracted from the *Bacillus licheniformis* (probiotic bacteria). MEP-Se NWs were

characterized using UV-Visible, XRD, FTIR, HR-TEM, FE-SEM and EDX. An UV–Visible peak was detected at 330 nm while XRD spectrum data pointed out the crystalline nature of MEP-Se NWs. FTIR spectrum revealed functional groups with strong absorption peaks in the range 3898.52–477.97 cm⁻¹. FE-SEM and HR-TEM revealed that the obtained structures were nanowires of 10-30 nm diameter. Se presence was confirmed by EDX analysis. MEP-Se NWs at 100 µg/ml highly suppressed the growth of both Gram ⁽⁻⁾ and Gram ⁽⁺⁾ bacteria. Further, microscopic analysis evidenced that 75 µg/ml MEP-Se NWs suppressed biofilm formation. Hemolytic assays showed that MEP-Se NWs were moderately cytotoxic. In addition, LC_{50} values lower than $10\,\mu\text{g/ml}$ were estimated testing MEP-Se NWs on both Aedes aegypti and Culex quinquefasciatus 3rd instar larvae. Morphological and histological techniques were used to elucidate on the damages triggered in mosquito tissues, with special reference to midgut, post-exposure to MEP-Se NWs. Therefore, based on our findings, MEP-Se NWs can be considered for entomological and biomedical applications, with special reference to the management of biofilm forming microbial pathogens and arbovirus mosquito vectors.

Graphical Abstract



Dengue; Lymphatic filariasis; Microbial pathogens; Nanobiotechnology; Nanoparticles, Zika virus

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1. Introduction

2. Materials and methods

3. Results and discussion

4. Conclusions

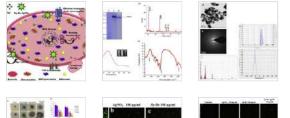
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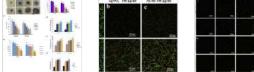
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Journal of Trace Elements in Medicine and Biology Volume 54, July 2019, Pages 8-20

Bioinorganic chemistry

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Enhanced antibacterial activity of hemocyanin purified from *Portunus pelagicus* hemolymph combined with silver nanoparticles – Intracellular uptake and mode of action

Ramachandran Ishwarya ^a, Baskaralingam Vaseeharan ^a 은 쩓, Sivakumar Shanthini ^b, Marimuthu Govindarajan ^{c, d}, Naiyf S. Alharbi ^e 은 쩓, Shine Kadaikunnan ^e, Jamal M. Khaled ^e, Mohammed N. Al-anbr ^e

https://doi.org/10.1016/j.jtemb.2019.03.005

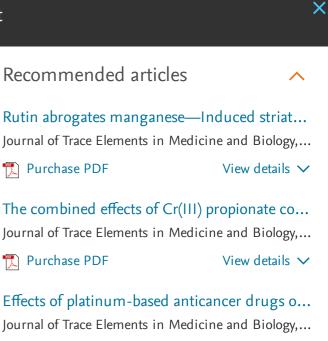
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Highlights

- Nanosilver (AgNPs) was fabricated using hemocyanin (Hc, 78 kDa) purified from of *Portunus pelagigus* hemolymph.
- Hc-AgNPs were more efficient than purified Hc against bacteria.
- Hc-fabricated AgNPs led to higher membrane permeability and ROS formation in bacterial pathogens.
- Hc-fabricated AgNPs affected bacterial replication, leading to protein leakage and cell death.

Abstract

Recently, biogenic nanoparticles have been considered promising candidates for manufacturing antibacterial nanodrugs. Here, we synthesized AgNPs using the crab-borne antibacterial agent hemocyanin and assessed the antibacterial action against several pathogenic bacteria. In this study, the crustacean immune protein hemocyanin (Pp-Hc, 78 kDa) purified from Portunus pelagicus hemolymph was used to fabricate silver nanoparticles. Characterization of hemocyanin-fabricated AgNPs (*Pp*-Hc AgNPs) were achieved using ultravioletvisible spectrophotometer, X-ray powder diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), high-resolution-transmission electron microscopy (HR-TEM), and energy-dispersive X-ray spectroscopy. The antibacterial efficacy of AgNO₃, *Pp*-Hc and *Pp*-Hc AgNPs was compared by growth inhibition, antibiofilm and live and dead analyses. Based on the results, Pp-Hc AgNPs was more efficient than Pp-Hc and AgNO₃ against pathogenic bacteria. Mechanistic analysis revealed membrane damage and reactive oxygen species (ROS) generation, suggesting that Pp-Hc and Pp-Hc AgNPs rely to similar modes of action. Intracellular protein molecules and nucleic acid leakage confirmed that *Pp*-Hc AgNPs increase membrane permeability, leading to cell death. Based on our results, capping of the exterior surface of nanoparticles with antimicrobial crab-borne peptides, such as *Pp*-Hc, improves their functions as potential agents against bacterial diseases, which may be useful in clinical applications.



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Keywords

Bacterial biofilm; DNA fragmentation; Reactive oxygen species; Malondialdehyde; Membrane damage; Nucleic acid leakage; Peroxidase; Superoxide dismutase

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Original Paper | Published: 30 April 2019

Facile and Cost-Effective Ag Nanoparticles Fabricated by *Lilium lancifolium* Leaf Extract: Antibacterial and Antibiofilm Potential

<u>Mysoon Al-Ansari</u> , <u>Noorah Alkubaisi</u>, <u>Kasi Gopinath</u>, <u>Viswanathan Karthika</u>, <u>Ayyakannu Arumugam</u> & <u>Marimuthu Govindarajan</u>

<u>Journal of Cluster Science</u> **30**, 1081–1089(2019) | <u>Cite this article</u> **124** Accesses | **1** Citations | <u>Metrics</u>

Abstract

In the present study one-pot green fabrication of silver nanoparticles (Ag NPs) with aqueous leaf extract of *Lilium lancifolium*. Synthesized Ag NPs were illustrated by UV–Vis, FT-IR, XRD, AFM and TEM analysis. A surface plasmon resonance peak was noticed at 411 nm in UV–Vis analysis. XRD patterns of Ag NPs showed high crystalline nature with face centered cubic structure. AFM and TEM analysis of Ag NPs exhibit special and anisotropic morphology with accurate particle size about 10–70 nm and average mean value of 45 nm. Ag NPs were tested for antibacterial and anti-biofilm activity against Gram positive and Gram-negative bacterial strains. The exposure to 25 μ L exhibited better antibacterial activity and 50 μ L significantly control the bacterial biofilm growth. Overall, the proposed green synthesis method of Ag NPs is facile, convenient, less time consuming and environmentally safe.

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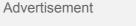
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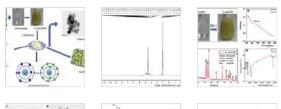
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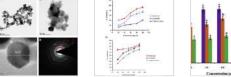
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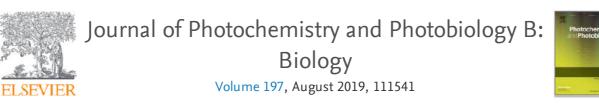
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Table 4

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Supplementary material



Synthesis of ZnO nanoparticles using insulinrich leaf extract: Anti-diabetic, antibiofilm and anti-oxidant properties

Viswanathan Vinotha ^a, Arokiadhas Iswarya ^a, Rajagopalan Thaya ^b, Marimuthu Govindarajan ^{c, d}, Naiyf S. Alharbi ^e, Shine Kadaikunnan ^e, Jamal M. Khaled ^e, Mohammed N. Al-Anbr ^e, Baskaralingam Vaseeharan ^a $\stackrel{\circ}{\sim}$ \boxtimes

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Highlights

- ZnO NPs was synthesized using leaf extract of *Costus igneus*.
- The synthesized Ci-ZnO NPs was characterized by UV, XRD, FTIR, and TEM.
- Ci-ZnO NPs shows potent anti diabetic activity and antioxidant activity.
- Ci-ZnO NPs shows antibacterial and antibiofilm activity. •

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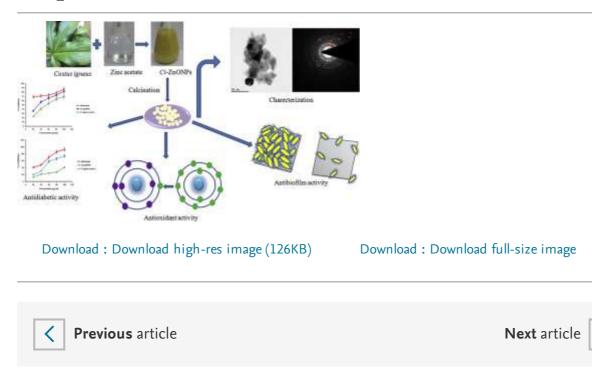
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Abstract

Here, we report the novel fabrication of ZnO nanoparticles using the Costus igneus leaf extract. Gas chromatography-mass spectrometry (GC-MS) and proton nuclear magnetic resonance (¹H NMR) spectroscopy to determine the bioactive components present in the plant extract. The synthesis of Ci-ZnO NPs (C. igneus- coated zinc oxide nanoparticles) was accomplished using a costeffective and simple technique. Ci-ZnO NPs were specified using UV-visible spectroscopy, FTIR, XRD, and TEM. Ci-ZnO NPs was authenticated by UV-Vis and exhibited a peak at 365 nm. The XRD spectra proved the crystalline character of the Ci-ZnO NPs synthesized as hexagonal wurtzite. The FTIR spectrum illustrated the presence of possible functional groups present in Ci-ZnO NPs. The TEM micrograph showed evidence of the presence of a hexagonal organization with a size of 26.55 nm typical of Ci-ZnO NPs. The $\alpha\text{-}$ amylase and α -glucosidase inhibition assays demonstrated antidiabetic activity of Ci-ZnO NPs (74 % and 82 %, respectively), and the DPPH [2,2-diphenyl-1picrylhydrazyl hydrate] assay demonstrated the antioxidant activity of the nanoparticles (75%) at a concentration of 100 μ g/ml. The Ci-ZnO NPs exhibited promising antibacterial and biofilm inhibition activity against the pathogenic bacteria Streptococcus mutans, Lysinibacillus fusiformis, Proteus vulgaris, and Vibrio parahaemolyticus. Additionally, the Ci-ZnO NPs showed biocompatibility with mammalian RBCs with minimum hemolytic activity ($0.633 \% \pm 0.005 \%$) at a concentration of $200 \,\mu g/ml$.

Graphical Abstract



Keywords

Costus igneus; Biosynthesis; XRD; TEM; ZnO nanoparticles; Biological properties

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Cu exposure induces oxidative stress effects and down regulates the • non-specific immune parameters in O. niloticus.

Background

Trace elements of copper (Cu) are one of the main forms of ecological noxious waste in freshwater systems that affect the survival and development of organisms. The objective of the current study was to investigate the effects of chronic exposure to Cu on the growth, oxidative stress, immune and biochemical response in the Nile tilapia, Oreochromis niloticus.

Methods

Three groups of *O. niloticus* were tested as follows; the first group was used as the control (not treated with Cu in water), while the 2nd and 3rd groups were exposed to (low) $40 \,\mu g \, L^{-1}$ and (high) $400 \,\mu g \, L^{-1}$ concentrations of Cu added to water, respectively. The duration of the experiment, which was conducted in triplicate, was 60 d. End points were evaluated on days 30 and 60. Following 30 d and 60 d of exposure to Cu, the fish were removed from experimental tanks to determine growth. Consequently, blood samples were collected from caudal veins at the end of the trial period (30 d and 60 d) and serum was separated to evaluate different immunological parameters, such as lysozymes (LYZ), respiratory burst activity (RBA) and myeloperoxidase (MPO). Gill and liver tissues were collected for evaluation of Cu and certain biochemical parameters as follows: antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST); nonenzymatic antioxidants such as glutathione (GSH) and metallothionein (MT), and oxidative stress indicators such as malondialdehyde (MDA) and protein carbonyl (PCO). The results pertaining to treatments and the control were compared using two-way ANOVA and Tukey's HSD test. The level of significance was set at $P \le 0.05$. Data were expressed as mean \pm SD.

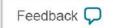
Results

Chronic exposure to Cu did not induce any mortality in fish during the test period. However, following exposure to Cu, growth of fish in the exposed groups was affected more than that in the control group (unexposed to Cu). In addition, accumulation of Cu in the liver tissue was higher than that in the gill tissues of fish exposed to Cu, compared to that in the control. Gill and liver tissues of Cu-exposed fish showed a significant ($P \le 0.05$) reduction in the activities of the antioxidant enzymes, SOD, CAT, GPx, and GST, compared to those of unexposed fish. Non-enzymatic antioxidants, GSH and MT, in gill and liver tissues were significantly increased ($P \le 0.05$) in fish exposed to both concentrations of Cu, compared to those in unexposed fish. Oxidative stress indicators, MDA and PCO in gills and liver of Cu-exposed fish was significantly ($P \le 0.05$) at both tested concentrations, when compared to control group. Non-specific immune response of LYZ, RBA, and MPO activity in serum decreased significantly ($P \le 0.05$) in Cu-exposed fish, compared with that of unexposed fish.

Conclusion

Overall, the present results highlighted that chronic exposure to Cu ions may exert a strong effect on the antioxidant and immune responses of O. niloticus. Changes in antioxidant enzymes, oxidative stress effects and immune parameters during post-chronic metal exposure may indicate the potential of these parameters as biomarkers of metal toxicity in aquatic ecosystems.

Graphical abstract





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Phytochemical analysis, in vitro antioxidant and antimicrobial activities of male flower of Juglans regia L.

Umar Muzaffer and V. I. Paul

Department of Zoology, Faculty of Science, Annamalai University, Tamilnadu, India

ABSTRACT

The male flower of Juglans regia L., were investigated for its in vitro antioxidant activity, antimicrobial activity, and chemical constituents. The antioxidant activity showed that the methanol extract of J. regia male flower (MEJR) had highest scavenging potential than the other solvents (ethanolic = EEJR and aqueous = AEJR). The antimicrobial activity showed that Staphylococcus aureus and Escherichia coli were the most sensitive organisms and significant activity was also recorded against both the fungal strains tested, with highest activity against Candida albicans. Totally, 26 constituents were identified by high-resolution-liquid chromatographymass spectrometry analyses from which seven compounds were identified first time from the extract.

ARTICLE HISTORY

Received 22 November 2016 Accepted 22 November 2017

KEYWORDS

Antimicrobial; Antioxidant; FTIR; Juglans regia L; LCMS

Introduction

The Juglandaceae family includes several genera among which Juglans genus is the important representative, with 7-45 species. Among these Juglans regia L. is one of the premium tree traditionally cultivated for its valuable wood and fruits. The seed is a nut of high economic interest to the food industry and is globally popular and valued for its nutritional, health, and sensory attributes.^[1] The vast biodiversity of Himalaya provides this royal species mostly in the Kashmir region, growing up to 25-35 m.

Juglans regia is considered to treat a variety of health complaints traditionally, including Cancer, Inflammation, Diabetes, Antiradicalar, Hyperhidrosis, Antidiarriec, Prostate, Antiradicalar, and Cardiovascular disorders.^[2-5] However, researchers investigated that almost all parts of the plant are important against different health disorders as well as for preservation of food grains.^[6] The extracts from *J. regia* nut inhibited oxidative damages,^[7-10] inflammation,^[11,12] tumor growth,^[8,13] antiwrinkle, and photoageing.^[14] Kernels as a dietary food, against diabetes, hypoxia, some skin diseases, and inflammation^[15,16]; leaves as antidiarrheals, anthelmintic, depurative^[17] and also mixed with stored-grains as an insecticide and fungicide.^[17,18] Stem bark as an astringent, anthelmintic, depurative, bactericide, diuretic, digestive, laxative, stimulant, detergent, and insecticidal.^[19] Juglans regia L. shell is reported for polishing gun-casings, jewelry, and metal material and is used as media to separate water and crude oil.^[20]

Juglans regia L. is a good source of flavonoids, Polyphenols, flavonols, carbohydrates, fatty acids, cardiac glycosides, steroids, minerals, tannins, protein, dietary fiber, melatonin, plant sterols, α -tocopherol, folate, tannins, vitamin A and C, and vitamin E family compound.^[1,21–23] Several studies demonstrated the antimicrobial activity of phenolic extracts,^[20–25] making them as best substitute to antibiotics and food preservatives. Juglans regia L. is a natural product of high economic interest to the food industry and is very popular and largely consumed as royal food globally and valued for its

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RESEARCH ARTICLE



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Impact of ethanol extract of Anamirta cocculus (Linn.) seeds on tissue damage biomarkers of the predatory catfish Heteropneustes fossilis (Bloch.)

Umer Qadir Dar and V. I. Paul 回

Department of Zoology, Annamalai University, Annamalainagar, India

ABSTRACT

Seeds of the phytopiscicide Anamirta cocculus are used recently to eradicate unwanted fishes from aquaculture ponds during pond preparation. However, the mode of action of A. cocculus and the biochemical responses caused to exposed fishes largely remain unexplained. The present study attempts to assess the impact of 24 h LC₅₀ (18.79 ppm) of the ethanolic extract of A. Cocculus seeds on the tissue damage biomarkers viz., alkaline phosphatase (ALP), acid phosphatase (ACP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of the predatory wild fish Heteropneustes fossilis. The tissues assayed were brain, gills, liver, kidney, muscle, accessory respiratory organs (ARO) and blood. The exposure has caused varying levels of significant reductions in the activities of all these enzymes in various tissues except blood. The activities of serum phosphatases and aminotransferases became significantly higher in the later stages of exposure. The observations clearly indicate the tissue-damaging effects of A. cocculus seed extract.

ARTICLE HISTORY Received 17 March 2015 Accepted 23 March 2018

KEYWORDS

Anamirta cocculus; piscicidal plant; Heteropneustes fossilis; ethanolic extract: phosphatases; aminotransferases

1. Introduction

Many civilizations throughout the world have exploited piscicidal plants (ichtyotoxicants) as barbascos for capturing wild fish for human consumption [1,2]. Anamirta cocculus (Linn.) is one such piscicidal plant extensively used by the native tribes and local people in the Indian subcontinent and neighbouring geographical regions for traditional fishing from the wild [3,4]. In addition to their primary use as a fishing agent, reports also indicate the probabilities of utilizing the piscicidal plants in the management of nuisance fishes in aguaculture ponds [5,6]. Eradication of weed fishes and other nuisance organisms such as predatory fishes and insects from culture ponds before stocking the desired species is an important step in aquaculture management. This is significant in view of the high rate of fecundity of weed fishes and voracious carnivorous nature of the predatory ones. While the high fecundity rate of weed fishes leads to severe competition for food, space and dissolved oxygen with the cultured species, predatory species may compete as well as prey upon the desired ones. The difficulty in eliminating air-breathing predatory fishes from even the dewatered culture ponds is mainly attributed to their capacity to survive inside moist cracks, crevices, burrows and even inside the bottom mud [7].

In the absence of safe and effective means to eradicate the unwanted fish fauna in stocking ponds, aqua

farmers find it easy to use synthetic chemicals such as malachite green, sodium cyanide and antimycin, and even chemical pesticides to get rid of them and thereby leading to ecological as well as public health concerns [6,8–10]. Use of pesticides and chemicals for the eradication of unwanted fish fauna and insects in fish culture ponds during pond preparation could have long-lasting impacts and may affect non-target organisms as well as human health through food chains and bioaccumulation. Non-target animals including the cultivated fishes are greatly affected by the indiscriminate use of the synthetic piscicidal agents [11]. Therefore, plant origin piscicides are preferred to synthetic ones because of the biodegradation potential and nonbioaccumulative nature of the former [7]. Studies by Jothivel and Paul [7,12] have established the significance and effectiveness of the seeds of A. cocculus as an organic management tool for the eradication of weed and predatory fishes including air-breathing ones.

Since the fish caught by applying A. cocculus seeds are traditionally consumed by humans, it is important to examine the toxic effects of the seed extract on the physiology and biochemistry of fishes as the seeds are reported to contain picrotoxin derivatives [3,13]. The activities of some enzymes like alkaline phosphatase (ALP; E.C., 3.1.3.1), acid phosphatase (ACP; E.C., 3.1.3.2), aspartate aminotransferase (AST; E.C., 2.6.1.1)

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Juglans regia L. protects against UVB induced apoptosis in human epidermal keratinocytes



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Keywords: Juglans regia L. Ultraviolet-B DNA damage Apoptotic markers

ABSTRACT

The present study was aimed to investigate the photoprotective effect of the male flower of *J. regia* L. (MEJR) against ultraviolet-B induced apoptosis in human skin cells. Human skin epidermal keratinocytes were pretreated with the MEJR (80 μ g/ml, has been selected after MTT assay), prior to 30 min UVB-irradiation at a dose of 20 mJ/cm². Mitochondrial membrane potential was evaluated using Rhodamine-123 staining; the % apoptosis by Hoechst staining and acridine orange staining; DNA damage was measured by comet assay. The levels of p53, Bax, Bcl-xL, Bcl-2, Cytochrome c, Caspase-9 and Caspase-3 expression in HaCaT cells were analyzed by western blotting and RT-PCR. Pretreatment with MEJR 80 μ g/ml prior to UVB-irradiation significantly prevents apoptotic characteristics, DNA damage and loss of mitochondrial membrane potential. Thus, MEJR protects UVB-mediated human skin cells, by modulating the expression of apoptotic markers and UVB-induced DNA damage in HaCaT cells.

1. Introduction

Sunlight is the main source of ultraviolet radiation, having three major components such as UVA (400-320 nm), UVB (320-280 nm) and UVC (280-100 nm). Among the three, UVB radiation is the most damaging component, reaching the surface of the earth. It mainly affects the epidermal layer of the skin [1] and is considered as a major risk factor responsible for the formation of free radicals, acute inflammation and increased risk of non-melanoma skin cancer. More than 90% of skin cancer caused to fair-skinned populations is due to their exposure to intensive UVB radiation [2]. Generally, people are exposed to 15 MED (Minimal Erythema Dose) of UVB in a day time [3]. In case of fairskinned population, epidemiological studies have reported that individuals get erythema only after 20 min of exposure to sun light in mid-summer. This corresponds to $15-70 \text{ mJ/cm}^2$ of UVB and equals to 1 MED especially in case of fair-skinned population. Skin cancer is one of the most common cancers in the United States [4]. Current estimates show that one out of five, suffers from skin cancer in their lifetime and more than 8500 people in the United States, are diagnosed with skin cancer every day [5].

Apoptosis is an active suicidal machinery or programmed cell death, which participates in eliminating the unwanted or potentially harmful cells, under pathophysiological conditions. UVB radiation is a strong genotoxic agent, which may lead to apoptosis, characterized by membrane blebbing and nuclear fragmentation [6]. Exposure of skin cells to UVB irradiation may respond either by activating protective mechanisms or ultimately undergoing apoptosis. However, damaged cells when escapes apoptotic process, may lead to the tumorigenesis also. Therefore, apoptosis is an important approach for protecting normal cells from the UVB radiation [7]. UV radiation and other environmental toxicants induced apoptosis have two pathways; death receptor mediated apoptosis and mitochondria mediated apoptosis. The mitochondrial membrane permeability is mainly regulated by anti- and pro-apoptotic proteins belonging to the Bcl-2 family [8]. The antiapoptotic proteins, including Bcl-2, Bcl-XL, neutralize apoptosis by interacting with some pro-apoptotic members on the mitochondrial membrane. Moreover, tumor suppressor protein p53, induced by DNA damage, can also lead to the onset of apoptosis [9]. UVB-induced damage to the keratinocytes, frequently promotes hot spot mutations in the p53 gene that ultimately may lead to a faulty trigger to apoptosis and can promote the non-melanoma skin cancers.

J. regia L. (Juglandaceae) is found primarily in temperate areas and commercially cultivated in the United States, Asia Minor and also in Central and Southern Europe [10]. It is one of the oldest cultivated species, for nuts, in history and is considered to be a source of sesquiterpene, alcohols, tocopherols, phospholipids, sphingolipids, sterols, hydrocarbons, unsaturated fatty acids, flavonoids, steroids, and terpenoids. It has also been used in traditional medicine for treatment of

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RESEARCH





A mechanism underlying the neurotoxicity induced by sodium fluoride and its reversal by epigallocatechin gallate in the rat hippocampus: involvement of NrF2/Keap-1 signaling pathway

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Abstract

Background: Fluoride (FI) exposure engenders neurodegeneration and induces oxidative stress in the brain. Therefore, the mechanism of FI-induced neurotoxic effects needs to be determined. The aim of this study was to investigate the neuroprotective effects of EGCG (40 mg/kg) on FI (25 mg/kg/bw)-induced oxidative stress mediated neurotoxicity with special emphasis on the hippocampus (4 weeks).

Results: Fl-intoxicated rat shows an increased Fl concentration along with the decreased neurotransmitter (AChE, NP, DA and 5-HT) activity in the brain. The oxidative stress markers (ROS, TBARS, NO, and PC) was significantly increased with decreased enzymatic (SOD, CAT, GPx, GR, GST, and G6PD) and nonenzymatic antioxidants (GSH, TSH, and Vit.C) in the rat hippocampus. Moreover, results showed that increases in intrinsic and extrinsic apoptotic pathway leading to DNA damage and cell death were also proved by the immunohistochemical, histological, and ultra-structural studies in the Fl-treated rat hippocampus. In this context, pre-administration of EGCG significantly improved the oxidative stress, biochemical changes, cellular apoptotic and histological alternations by Fl in the hippocampus of rats.

Conclusions: These results confirmed the EGCG supplementation might attenuate the FI-induced neurotoxicity via Nrf2/Keap1 signaling pathway in the rat hippocampus.

Keywords: Fluoride, Epigallocatechin gallate, ROS, Nrf2/Keap1, Rat, Hippocampus

Background

Fluoride (Fl) is a potent central nervous system toxin and affects the brain function even at low doses (Mullenix, Denbensten, Sehunior, & Kernan, 1995). The research on Fl and the brain that have been studied in humans in India, China, Iran, and Mexico showed that high levels of Fl exposure were associated with IQ deficits in children (NRC, 2006). The animal studies have also documented

the considerable evidence on the direct toxic effects of Fl on the brain, even at the low level of 1 ppm Fl in water (Varner, Jensen, Horvath, & Isaacson, 1998). These effects include a reduction in nicotinic acetylcholine receptors, reduction in lipid content, impaired anti-oxidant defense, damage to the hippocampus, Purkinje cells and accumulation of Fl in the pineal gland and the formation of betaamyloid plaques (NRC, 2006). Earlier epidemiological studies and animal experiments have also demonstrated that the intelligence of children and animals exposed to high levels of fluoride were severely impaired, especially with the symptoms of decreased learning and memory capacity (Lu et al., 2000).



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Outline

Background

Purpose

Methods

Results

Conclusion

Q

Q

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287 - Protective effect of Sulforaphane against Arsenic-induced hepatotoxicity in rats: Role of PI3K/Akt-mediated Nrf2 signaling pathway

Thangapandiyan Shanmugam^{*1}, Ramesh Mathan ¹, Miltonprabu Selvaraj ²

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Background

Industrial and agricultural growths in recent years has resulted in the extreme discharge of arsenic into the environment, making arsenic toxicity a major worldwide concern. Oxidative stress is considered the primary mechanism for arsenic toxicity.

Purpose

The main objective of this study was to evaluate sulforaphane protective ability against arsenic-induced hepatotoxicity in rats via PI3K/Akt mediated Nrf2 activation.

Methods

For this purpose, male Wistar rats were divided into six groups of 8 rats each: control, Arsenic (As) (5mg/kg BW), As + SFN (5mg/kg; 20, 40, 80 mg/kg BW) and Vitamin C (As (5mg/kg) +100mg/kg). The animals were gavaged for 28 consecutive days. Liver tissue samples were extracted 24 hours after the last treatment and later analyzed for biochemical, molecular and histological alterations.

Results

Pretreatment with SFN led to decreased levels of ALAD, Ar accumulation, and brought antioxidant enzymes into normal levels without affecting Arsenic metabolism. This was accompanied by stabilizes the apoptotic markers via

PI3K/Akt mediated Nrf2 activation as evidenced by western blotting and PCR techniques. Furthermore, SFN pretreatment shield the histoarchitecture of liver tissue in As treated rats.

Conclusion

The present study has provided mechanistic insights to the phytotherapeutic potential of SFN against As-induced liver injury by up-regulating Nrf2 gene via PI3K/Akt signalling pathway.

<	Previous article	Next article	>	

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Effect of isolated bioactive flavonoid apigenin-7-o-βd-glucuronide methyl ester on cyclooxygenase-2 gene expression in the breast cancer MCF-7 cell lines

K. Kamalakararao¹, D. Govinda Rao², M. Muthulingam³, V. K. Gopalakrishnan⁴, Zenebe Hagos⁴, P. John Dogulas⁵, K. Krishna Chaithanya⁴*

ABSTRACT

Introduction: Breast cancer is one of the most commonly diagnosed cancers worldwide. Apigenin-7-O- β -D-glucuronide methyl ester is a bioactive flavonoid and has been isolated from the ethyl acetate leaf extract of *Manilkara zapota*, shown *in vitro* anticancer activities. **Objective:** The present study was aimed to investigate the possible inhibitory activity of apigenin-7-O- β -D-glucuronide methyl ester on the cyclooxygenase (COX)-2 gene expression in the breast cancer MCF-7 cell lines. **Materials and Methods:** MCF-7 cell lines were cultured in monolayers in RPMI 1640 and antiproliferative activity of apigenin-7-O- β -D-glucuronide methyl ester was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), a yellow tetrazolium (MTT) assay with different concentrations, the levels of COX-2 gene expression were measured by reverse transcription real-time polymerase chain reaction (RT-PCR). **Results:** The results showed that apigenin-7-O- β -D-glucuronide methyl ester (1, 5, 10, 50, and 100 µg/mL) shown significantly exhibited a concentration-dependent cytotoxic effect on MCF-7 breast cancer cell with half maximal inhibitory concentration (IC₅₀) value of 40.17 µg/ml comparing with standard tamoxifen with IC₅₀ values of 7.72 µg/ml. From the RT-PCR analysis showed apigenin-7-O- β -D-glucuronide methyl ester significantly inhibited the COX-2 gene expression fold -2.5 and -10.31 in MCF-7 cell lines. **Conclusion:** From this study, it can be concluded that the apigenin-7-O- β -D-glucuronide methyl ester, significantly inhibits the growth and attenuates the COX-2 gene expression in MCF-7 breast cancer cells.

KEY WORDS: Anticancer activity, Apigenin-7-O-β-D-glucuronide methyl ester, Cytotoxicity, *Manilkara zapota*, MCF-7, Real-time polymerase chain reaction

INTRODUCTION

Breast cancer is one of the usually diagnosed cancers and the second leading cause of cancer death in women worldwide. The incidence of breast cancer is approximately 10.4%, and it is the most common cancer in women, showing annual 1–2% increases, and causes high morbidity and mortality.^[1,2] About 30% of the patients with early-stage breast cancer have recurrent disease. The prognosis of breast cancer depends on various biological and molecular

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factors.^[3,4] Cyclooxygenase (COX) group of enzymes is important for the conversion of arachidonic acid to prostaglandins and thromboxane, COX-1 is constitutively expressed at a constant level throughout the cell cycle in most of the tissues for maintain normal functioning of gastrointestinal tract and COX-2, an inducible enzyme, plays a key role in pathophysiological processes of inflammation as well as several cancers including breast, prostate, lung, liver, cervical, intestinal, and skin.^[5]

From the recent studies suggest that COX-2-derived metabolites such as PGE₂ and TXA₂ contribute to the maintenance of tumor viability, premalignant hyperproliferation, tumor growth, transformation, invasion, and metastatic spread,^[6,7] and COX-2 has been

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Antiproliferative activity of isolated bioactive flavonoid apigenin-7-O-β-D-glucuronide methyl ester from ethyl acetate leaf extract of *Manilkara zapota*

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ABSTRACT

Introduction: Cancer is one of the major causes of death among the world, demonstrating a substantial public health problem. Medicinal plants have been a great source of secondary metabolites with antiproliferative activities. Apigenin-7-O-β-D-glucuronide methyl ester is a bioactive flavonoid and has been isolated from the ethyl acetate leaf extract of Manilkara zapota, shown in vitro antiproliferative activities. Objective: The present study was aimed to evaluate the antiproliferative activity of bioactive flavonoid apigenin-7-O-β-D-glucuronide methyl ester against cancer cell lines such as human breast cancer (MCF-7), human hepatocellular carcinoma HEP G2, and human colon cancer cell lines (HT-29). Materials and Methods: Antiproliferative activity of apigenin-7-O-β-D-glucuronide methyl ester was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, a yellow tetrazolium assay. Results: The results showed that the apigenin-7-O-β-D-glucuronide methyl ester (1, 5, 10, 50, and 100 μg/mL) has exhibited significant shown a concentrationdependent moderate anticancer activities against HT-29 (colon cancer cell line) and MCF-7 breast cancer cell with half maximal inhibitory concentration (IC50) values are of 42.09 µg/ml and 40.17 µg/ml comparing with standards 5-fluorouracil and tamoxifen with IC50 values of 8.17 μg/ml and 7.72 μg/ml. Apigenin-7-O-β-D-glucuronide methyl ester shown strongest antiproliferative activity against HEP G2 (liver cancer cell) with IC50 value of 38.92 µg/ml compared with standard paclitaxel with IC50 values of 7.72 μ g/ml. Conclusion: From this study, it can be concluded that the apigenin-7-O- β -D-glucuronide methyl ester exhibited significant anticancer activity on HEP G2 (liver cancer cell) and could serve as a potential source of plant derived, anticancer agents for the development of therapeutic anticancer drugs.

KEYWORDS: Antiproliferative activity, Apigenin-7-O-β-D-glucuronide methyl ester, HEP G2, HT-29, *Manilkara zapota*, MCF-7

INTRODUCTION

Cancer in one of the second major causes of mortality in the worldwide associated with 8.2 million of death in the world in 2012 after cardiovascular diseases.^[1] Cancer is the uncontrolled autonomous growth of abnormal cells that can arise in any organ or tissue of the body. The cancer cell is a once normal cell caused by genetic mutations in DNA,

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continues to develop, and invasion causes changes in surrounded tissues.^[2] According to GLOBOCAN 2012, an estimated 14.1 million new cancer cases and 8.2 million cancer-related deaths reported in 2012, prevalence estimates for 2012 show that there were 32.6 million people (over the age of 15 years) alive who had a cancer diagnosed in the previous 5 years. The most commonly diagnosed cancers worldwide were those of the lung (1.8 million, 13.0% of the total), breast (1.7 million, 11.9%), and colorectal (1.4 million, 9.7%). The most common causes of cancer death were cancers of the lung (1.6 million, 19.4% of the total), liver (0.8 million, 9.1%), and stomach (0.7 million,

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Total antioxidant capacity, ferric reducing power, and iron-chelating activities of methanolic leaf extract of *Rhizophora apiculata* Blume

M. Muthulingam^{1*}, K. Krishna Chaithanya²

ABSTRACT

Introduction: Reactive oxygen species and reactive nitrogen species (RNS) are a class of highly reactive molecules derived from the metabolism of oxygen in both pathological and nonpathological conditions. Accumulation of these reactive oxygen and nitrogen species has been involved in the development of degenerative diseases. *Rhizophora apiculata* Blume is an Indian traditional medicinal plant used in the curing of oxidative stress-related diseases. *Objective:* The present was an attempt to evaluate the total antioxidant, reducing power, and metal chelating activity of methanolic leaf extract of *R. apiculata* Blume. **Methods:** The total antioxidant activity, reducing power, and iron-chelating activity of methanolic leaf extract of *R. apiculata* Blume Blume were performed using standard procedures. **Results:** The results have indicated that the total antioxidant activity of methanolic leaf extract was 126.18 μ g/500 μ g, showed maximum reducing power of 0.264 O.D at 700 nm and significant Fe²⁺ chelating of 76.21% with the half-maximal inhibitory concentration value of 205 μ g/ml. **Conclusion:** The present study revealed that the methanolic leaf extract of *R. apiculata* Blume leaves is potential source of natural antioxidants.

KEY WORDS: Antioxidant assay, Iron chelating, Reducing power activity, Rhizophora apiculata Blume

INTRODUCTION

Oxidative stress is generated due to the existence of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) which are formed under normal physiological conditions but become harmful when not being eliminated by the endogenous antioxidant systems. In fact, oxidative stress results from an imbalance between the generation of ROS and endogenous antioxidant systems.^[1,2] The upregulated ROS and RNS both in pathological and oxidative stress result in numerous diseases and disorders such as cancer,^[3] cardiovascular disease, neural disorders,^[4] ulcerative colitis,^[5] and aging.^[6] Antioxidants can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions.^[7] It has been suggested that natural antioxidants like L-ascorbic acid are more safe and healthy than synthetic antioxidants such as butylated hydroxyl toluene (BHT), the commercial synthetic antioxidants with phenolic structure such as butylated

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hydroxyanisole, BHT, and tert-butylhydroquinone are used to cure different types of oxidative stress-related diseases, but long-term use causing cancer due to their mutagenic nature.^[8]

Medicinal plants have played a key role in Indian traditional medicinal systems, they are rich sources of therapeutic bioactive compounds and thus serve as an important raw material for the preparation of drugs and have become a target for development plant-derived drugs,^[9] natural bioactive of compounds such as phenols and flavonoids are the important secondary metabolites in plants having intrinsic biological properties such as antioxidant, anticarcinogen, anti-aging. and autoimmune diseases.[10] Particularly plant-derived low-molecularweight antioxidants such as glutathione and ascorbate are produced in the presence NADPH acting as the final electron source these low-molecular-weight antioxidants balance antioxidant system. Rhizophora apiculata is the species of mangrove tree. It is widely distributed in the tropical countries such as India, Malaysia, and Ethiopia. R. apiculata tree root, leaf, and/or stem extracts shown a significant inhibitory effect on the growth of human bacterial, viral, and

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Qualitative and quantitative phytochemical analysis and *in vitro* antioxidant activities of methanolic leaf extract of *Rhizophora apiculata* blume

M. Muthulingam^{1*}, K. Krishna Chaithanya²

ABSTRACT

Introduction: An oxidative stress results from the imbalance between the free radicals and the antioxidant system, and up regulated reactive oxygen and nitrogen species have been associated with oxidative stress diseases. Rhizophora apiculata Blume is an important medicinal plant long time used by many people in Asia and Africa continents for curing both the infectious and non-infectious diseases. Objective: The objective of the present study was to find the qualitative and quantitative phytochemical and evaluate the in vitro antioxidant activity of the methanolic leaf extract of R. apiculata Blume. Materials and Methods: The qualitative phytochemical analysis, quantitative estimations of total phenolic, alkaloid, flavonoid, tannin, and saponin content, and in vitro antioxidant activities (1,1-diphenyl-2-picrylhydrazyl [DPPH], hydrogen peroxide, superoxide, and hydroxy radical) of the methanolic leaf extract of R. apiculata Blume were performed using standard procedures. Results: The quantitative phytochemical analysis of the methanolic leaf extract of R. apiculata Blume revealed the presence of considerable amounts of tannins (95.14 µg/ml tannic acid equivalents/g), flavonoids (87.18 µg/ ml quercetin equivalents/g), phenolic compounds (54.56 µg/ml, gallic acid equivalents/g), saponins (4.17 µg/ml), and alkaloids (3.78 µg/ml), and further, the *in vitro* antioxidant activity of the ethanolic leaf extract of *R. apiculata* Blume showed significant scavenging activity on increasing order of $H_2O_2 > DPPH > OH > O_2$ compared to that of standard ascorbic acid. Conclusion: Based on the present results, the methanolic leaf extract of R. apiculata Blume contained high amount of flavonoid, tannin, and phenolic compounds, thereby existing significant in vitro antioxidant activity, and the ethanolic leaf extract of R. apiculata Blume can be used as a potential source of desired bioactive natural antioxidants for the development of therapeutic antioxidant drugs.

KEY WORDS: Antioxidants, Free radicals, Medicinal plant, Phytochemicals, Rhizophora apiculata Blume

INTRODUCTION

The high amounts of free radicals and other reactive species produced during aerobic respiration in the body can cause oxidative injury of essential macromolecules including amino acids, lipids, proteins, and DNA. It has been established that oxidative stress induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS) is the major causative factors in the development of many chronic and degenerative diseases including atherosclerosis, heart disease, aging, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases, and others.^[1,2] The most effective way to eliminate free radicals which cause

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the oxidative stress is with the help of antioxidants. Antioxidants are the substances capable of stabilizing or deactivating free radicals before they are damaging to the cell.^[3] Both exogenous and endogenous antioxidants act as an effective free radical scavenger by preventing and repairing damages caused by ROS and RNS and promoting their free radical decomposition and suppressing oxidative stress disorders.^[4] Several synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, and tertiary butyl hydroquinone are commercially available for the treatment of oxidative stress-related diseases, but the long-term use concerns due to their side effects such as kidney damage and mutagenesis.^[5]

Therefore, there is a great tendency toward natural antioxidant sources such as plant derivatives. Several Indian medicinal plants were used for curing oxidative

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Evaluation of qualitative, quantitative phytochemicals and *in vitro* antioxidant activities of methanolic leaf extract of *Boerhaavia diffusa* (Linn.)

M. Muthulingam¹*, K. Krishna Chaithanya²

ABSTRACT

Introduction: An oxidative stress result from the imbalance between the free radicals and enzymatic and non-enzymatic antioxidant systems. Boerhaavia diffusa (BD) (Linn.) is a well-known medicinal plant used in Indian traditional medicinal system. **Objective:** The objective of the present study was to find the qualitative, quantitative phytochemical and evaluate the *in vitro* antioxidant activity of methanolic leaf extract of BD (Linn.). **Materials and Methods:** The qualitative, quantitative phytochemical, and reducing power of methanolic leaf extract of BD (Linn.) were performed using established procedures. **Results:** The quantitative phytochemical analysis of the methanolic leaf extract of BD (Linn.) revealed that the presence of considerable amount of flavonoids, tannins, phenolic compounds, saponins, and alkaloids, and further, the *in vitro* antioxidant activity of methanolic leaf extract of BD (Linn.) showed significant scavenging activity on increasing order of H₂O₂ (inhibitory concentration [IC_{50]} value 178 µg/ml) DPPH (IC₅₀ value 205 µg/ml) OH (197 µg/ml) and O₂ (313 µg/ml) compared to that of standard ascorbic acid. **Conclusion:** It can be concluded that the methanolic leaf extract of BD (Linn.) has shown the significant *in vitro* antioxidant activity and it may be used as a potential source used as antioxidants to prevent oxidative stress-related diseases.

KEY WORDS: Antioxidants, Boerhaavia diffusa (Linn.), Inhibitory concentration, Qualitative phytochemical analysis, analysis

INTRODUCTION

A free radical is a molecule or molecular fragment containing one or more unpaired electrons in its outermost molecular orbital. These formed free radicals are highly reactive and immediately involved in the start a chain reaction.^[1] The sources of free radicals can be exogenous and endogenous in nature. The exogenous free radicals are generated by tobacco smoke, pollutants, organic solvents and endogenous sources of free radicals are generated during infections and aerobic respiration in the body.^[2] Both exogenous and endogenous generated free radicals can cause oxidative damages of biomolecules such as amino acids, lipids, proteins, and DNA leads to induction of many chronic and degenerative diseases

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including atherosclerosis, ischemic heart disease, ageing, cancer, and neurodegenerative diseases.^[3] Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage. Antioxidants exist both enzymatic (endogenous) and non-enzymatic (exogenous) forms in the intracellular and extracellular environment, can be effectively used in the preventing free radical formation by scavenging them or promoting their decomposition.^[4,5]

Antioxidant-based drugs such as butylated hydroxytoluene, butylated hydroxyanisole, and tertiary butyl hydroquinone are used for the treatment of oxidative stress-related diseases, but long-term use of these synthetic antioxidants has been causing liver and kidney damage and mutagenesis. Recent studies have shown that the derivatives such as polyphenols, terpenes, and various plant extracts exert an antioxidant action and alleviate the chronic diseases caused by oxidative stress.^[6] In India, several medicinal plants have been extensively used for the treatment of oxidative

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In vitro anticancer activity of methanolic leaf extract of *Boerhaavia diffusa* Linn. against MCF-7 cell line

M. Muthulingam^{1*}, K. Krishna Chaithanya²

ABSTRACT

Introduction: Breast cancer is the leading cause of cancer-related deaths among the females, the medicinal plants are potential source for the treatment of cancer. Breast cancer cells have increasingly shown resistance to chemotherapeutic drugs. Plant-derived secondary metabolites are proved to be an important source of anticancer drugs. *Boerhaavia diffusa* is an important medicinal plant used in many people to curing both infectious and non-infectious diseases. **Objective:** The present study was aimed to evaluate the cytotoxic effect (anticancer activity) and observation of cell morphology changes of breast cancer (MCF-7) cell lines when treated with the methanolic leaf extract of *B. diffusa*. **Materials and Methods:** The cytotoxic effect of the methanolic leave extract of *B. diffusa* against MCF-7 cell lines was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), a yellow tetrazolium (MTT) assay and observable changes of cell morphology of MCF-7 on exposure to the extract using phase contrast microscope. **Results:** The results demonstrated that the methanolic leave extract of *B. diffusa* (2550, 100, and 200 µg/mL) had shown a dose-dependent significant cytotoxic activity of 13.9%, 27.96%, 43.65%, and 52.86, respectively, with half maximal inhibitory concentration of 69.18 µg/ml and 47.14% of cell viability was observed at 200 µg/ml concentration of *B. diffusa* shown significant anticancer activity against MCF-7 cell lines and the methanolic leave extract of *B. diffusa* shown significant anticancer activity against MCF-7 cell lines and the methanolic leave extract of *B. diffusa* shown a dose-dependent significant anticancer activity against MCF-7 cell lines and the methanolic leave extract of *B. diffusa* shown significant anticancer activity against MCF-7 cell lines and the methanolic leave extract of *B. diffusa* shown significant anticancer activity against MCF-7 cell lines and the methanolic leave extract of *B. diffusa* shown significant anticancer activity against M

KEY WORDS: Anticancer activity, *Boerhaavia diffusa*, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, Cytotoxicity, MCF-7 cell lines

INTRODUCTION

Cancer is a multistep disease caused by physical, environmental, metabolic, chemical, and genetic factors.^[1] Breast cancer has been increased universally^[2,3] and is considered as the second most mortality cause factor in women. Breast cancer is the most common female cancer worldwide representing nearly a quarter (23%) of all among top five cancers in Americans, it is the third fatal cancer^[4] while it is also the most widespread cancer in Asian and African continents.^[5] Since breast cancer is a miscellaneous illness,^[6] it has several indicators^[7] associated to typical features of tissues, therapeutic evaluation, and reaction to therapy.^[8] Improvements in the diagnosis and management of breast cancer have yielded drop in mortality frequency, but it varies widely between

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diverse geographic areas.^[9,10] For early and locally advanced breast carcinoma, the intention of treatment is cure while for metastasis, it is improvement in clinical presentation of disease and quality of life. Various clinical approaches are employed for breast cancer management such as radiation, chemotherapy, immunomodulation, and surgery in treating cancer are commonly used strategies in the treatment of breast cancer, which can lead to several side effects such as bone marrow depression, leukopenia, anemia, alopecia, and hyperuricemia, teratogenicity, carcinogenicity, and also its reduced spermatogenesis in men, amenorrhea in women due to this indication, there is an imperative need of new cancer management.^[11]

Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with current chemotherapeutic agents. The plant-derived agents are being used for the treatment of cancer. Several anticancer agents

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Phytochemical screening and cytotoxic effect of methanolic leaf extract of *Rhizophora apiculata* blume against Michigan Cancer Foundation-7 breast cancer cell lines

M. Muthulingam¹*, K. Krishna Chaithanya²

ABSTRACT

Introduction: *Rhizophora apiculata* is an important traditional medicinal plant used in many people in Asia and Africa for curing of both infectious and non-infectious diseases. **Objective:** The present study was aimed to evaluate the cytotoxic effect and observation of cell morphology changes of breast cancer (Michigan Cancer Foundation-7 [MCF-7]) cell lines of after the treatment with the methanolic leaf extract of *R. apiculata*. **Materials and Methods:** Cytotoxicity of the methanolic leaf extract of *R. apiculata* was tested against MCF-7 cell lines by using MTT assay and to find the cell morphology of MCF-7 cells upon exposure to the methanolic leaf extract of *R. apiculata* by using phase contrast microscope **Results:** The results showed that the methanolic leaf extract of *R. apiculata* with different concentrations (25,50, 100, and 200 µg/mL) had shown significant concentration-dependent anticancer (cytotoxic) activity against MCF-7 breast cancer cell by showing 48.27% of cell viability with half maximal inhibitory concentration (IC₅₀) values are of 140.97 µg/ml, comparing with standard tamoxifen by showing 80.80% of cell viability with IC₅₀ values of 61.88 µg/ml. **Conclusion:** From this study, it can be concluded that the methanolic leave extract of *R. apiculata* could be serve as a potential source of plant-derived, anticancer agents for the development of therapeutic anticancer drugs and further study has been focused on the isolation of bioactive anticancer compounds present in the methanolic leaf extract of *R. apiculata* by bioactivity-guided fractionation

KEY WORDS: *Rhizophora apiculata*, Michigan cancer foundation-7, Cytotoxicity, Cell morphology, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, Anticancer agents

INTRODUCTION

Cancer is one of the foremost deadly diseases and public health burdens in both developed and developing countries. Cancer is the continual and unregulated proliferation and differentiation of individual cells in a multicellular organism results from the defects in the fundamental regulatory mechanism at molecular and cellular levels.^[1,2] The International Agency for Research on Cancer announced that the incidence of mortality and prevalence of all major types of cancer, among the world shown that there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer in 2012 worldwide and by year 2030, it is estimated that there will be 26 million new cancer cases and 17 million cancer deaths

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per year.^[3,4] Breast cancer is the second leading cause of cancer deaths among women in the United States, chemotherapy commonly used strategies in treatment of breast cancer, due to adverse side effects, ranging from nausea to bone marrow failure and development of multidrug resistance;^[5,6] hence, discovery of natural compounds from plants may be an alternative source for cancer treatment, The plant based drugs have less toxic and more potent anticancer agents that are comparable to the drugs available in the market.

Medicinal plants occupy a premier position in human history as an ultimate source of phytocompounds having high therapeutic value. It is found that 40% of all medicines derived from natural sources and out of the 25% are from plant sources. Medicinal plants have various advantages over chemical products because plant-derived compounds are more tolerant and non-toxic to the normal human cells.^[7] Several studies have been reported that plant-

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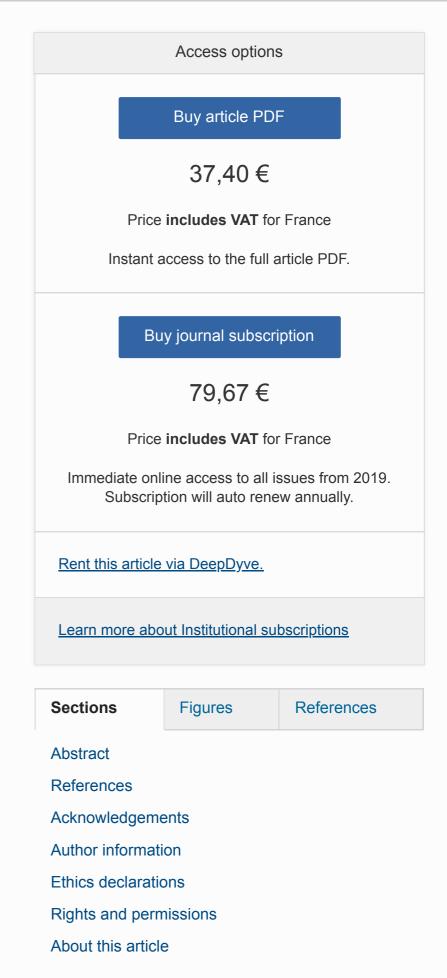
Orchids as Sources of Novel Nanoinsecticides? Efficacy of Bacillus sphaericus and Zeuxine gracilis-Fabricated Silver Nanoparticles Against Dengue, Malaria and Filariasis Mosquito Vectors

Kalimuthu Kovendan 🖂, Balamurugan Chandramohan, Marimuthu Govindarajan, Arulsamy Jebanesan, Siva Kamalakannan, Savariar Vincent & Giovanni Benelli 🖂

Journal of Cluster Science 29, 345–357(2018) Cite this article 246 Accesses | 6 Citations | Metrics

Abstract

Mosquitoes are the most critical group of insects in the context of public health, since they transmit key parasites and pathogens, causing millions of deaths annually. Insecticides from natural products may boost the effectiveness of vector control programs. In this study, we tested silver nanoparticles (AgNPs) fabricated using the leaf extract of the orchid Zeuxine gracilis as reducing agent, and the microbial pesticide Bacillus sphaericus, against the mosquitoes Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. The synthesis of AgNP was confirmed analyzing the excitation of surface Plasmon resonance using ultraviolet-visible (UV-Vis) spectrophotometry. SEM and TEM showed the irregular shapes of AgNPs. EDX spectroscopy, FTIR spectroscopy, X-ray diffraction and dynamic light scattering analysis were carried out. AgNPs were highly effective against the larvae of An. stephensi $(LC_{50} = 8.48 \ \mu g/mL)$, Ae. aegypti $(LC_{50} = 10.39 \ \mu g/mL)$ and Cx. quinquefasciatus $(LC_{50} = 13.21 \mu g/mL)$, respectively. Combined treatments testing *B. sphaericus* with AgNPs were also effective against An. stephensi (LC₅₀ = 12.32 μ g/mL), Ae. aegypti (LC₅₀ = 14.78 μ g/mL) and Cx. quinquefasciatus (LC₅₀ = 19.19 μ g/mL). Overall, this study suggests that the orchid-synthesized AgNPs can be a rapid, environmentally safer bio-pesticide to be used in synergy with B. sphaericus to control mosquito vectors.



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Protective Role of Sulforaphane against Multiorgan Toxicity in Rats: An *In-vivo* and *In-vitro* Review Study

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Abstract

Sulforaphane [1-isothiocyanate-(4R) -(methylsulfinyl) butane] is a natural dietary isothiocyanate produced by the enzymatic accomplishment of the myrosinase on glucopharanin, a 4-methylsulfinylbutyl glucosinolate contained in cruciferous vegetables such as broccoli, brussel sprouts, and cabbage. Exploring of this compound against various oxidative degenerative diseases such as hepatic, nephro and cardiac diseases is growing because of its anticarcinogenic and cytoprotective properties. In several invivo and invitro experimental paradigms proved SFN and its compounds are having capacity to protects against various xenobiotics induced oxidative stress mediated diseases such as focal cerebral ischemia, lung inflammation, intracerebral hemorrhage, ischemia and reperfusion induced acute cardiac, hepatic, renal failure, and cancer etc. Sulforaphane is an indirect antioxidant compound is able to induce/activate numerous cytoprotective proteins, including antioxidant enzymes, through the Nrf2/ARE pathway. Because, it poses the powerful electrophiles, attributable to the central carbon atom of the -N = C = S group, which reacts readily with sulfur- nitrogen- and oxygen-based respective antioxidant genes such as heme oxygenase-1, NAD(P)H: quinone oxidoreductase, glutathione-S-transferase, gamma-glutamyl cysteine ligase, and glutathione reductase and induced Nrf2 gene for Phase II enzymes. In conclusion, sulforaphane is a promising antioxidant agent that is effective to attenuate oxidative stress and tissue/cell damage in different in-vivo and in-vitro experimental animals.

Keywords: Sulforaphane, Oxidative stress, rats, liver, kidney, heart, ROS

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INTRODUCTION

Nutrition plays an imperative role in our health and well-being. According to the great quotes by Hippocrates (431 BC) "Let food be thy medicine; and medicine be thy food". Apart from the already known dietary constituent; in the recent years, there has been a growing attention in certain compounds having massive health property which comprises antioxidants and phytochemicals. The term phytochemical refers to a diversity of non-nutritional biologically dynamic compounds occurring in plant foods that give a mixture of health benefits beyond basic nutrition. Phytochemical accurately mean "Plant chemicals". Scientists have recognized thousands of diverse phytochemicals, found in vegetables, fruits, beans, whole grains, nuts and seeds (Table 1). Consumption of plant foods loaded in phytochemical may assist to prevent at least one in every five cases of cancer, as well as other serious ailments such as liver, kidney, heart, lungs, brain and testicular diseases. Since, researchers exploring the way of phytochemicals prevent disease, but not clear. However, they identified the following ancestry. i) It stimulates the immune system, the body's defence against viruses, bacteria and other disease-causing agents. ii) Reduces oxidation that is caused by molecules called "free radicals" can cause abnormalities in cells that may eventually lead to cancer. iii) Triggers death of damaged cells that may be precursors to cancer. iv) Prevents DNA damage and helps with DNA repair mechanisms. v) Helps to regulate hormones, reduces inflammation and slows the growth rate of cancer cells. Table 1 shows the types of phytochemicals derived from different plant sources and its possible benefits. Intake of

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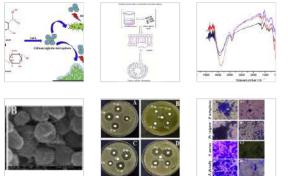
4. Discussion

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pathogens

M. Khaled ^d, Giovanni Benelli ^{e, f}

Microbial Pathogenesis Volume 114, January 2018, Pages 17-24

Synthesis of chitosan-alginate microspheres

activity against multi-drug resistant microbial

Rajagopalan Thaya ^a, Baskaralingam Vaseeharan ^a 은 쩓, Jeyachandran Sivakamavalli ^a, Arokiadhas Iswarya ^a,

Marimuthu Govindarajan ^{b, c}, Naiyf S. Alharbi ^d, Shine Kadaikunnan ^d \otimes \boxtimes , Mohammed N. Al-anbr ^d, Jamal

with high antimicrobial and antibiofilm



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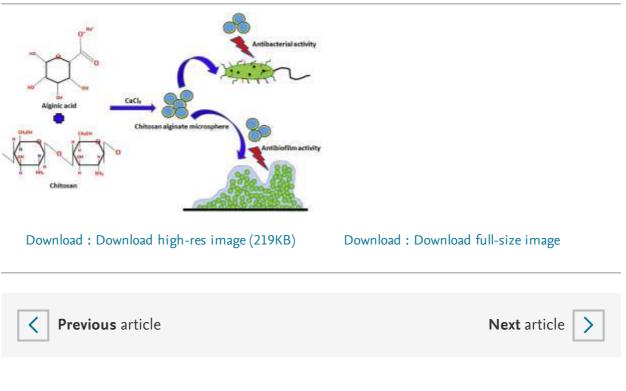
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• CS/ALG microspheres showed high antibac activity on key bacterial pathogens.	• CS/ALG microspheres showed high antibacterial	and antibiofilm	Social Media	
	activity on key bacterial pathogens.		Tweets:	3
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Abstract

The successful treatment of multi-drug resistant microbial pathogens represents a major challenge for public health management. Here, chitosanalginate (CS/ALG) microspheres with narrow size distribution were fabricated by ionically cross linking method using Ca²⁺ ions as agents for polymer solidification. The physicochemical properties of CS/ALG microspheres, such as surface morphology and size, were studied by SEM. The functional group interactions were confirmed by Fourier transform infrared (FTIR) spectroscopy. SEM revealed that the CS/ALG microspheres were spherical in shape with smooth surfaces, size was 50–100 μ m. The synthesized CS/ALG microspheres showed antibacterial and antibiofilm activity on bacteria of public health relevance. CS/ALG microspheres exhibited antibacterial activity at the concentration of 5–20 µg, with significant inhibitory zones on multiple antibiotic resistant pathogens, including Gram positive *Staphylococcus aureus*, Enterococcus faecalis, and Gram negative Pseudomonas aeruginosa and Proteus vulgaris. Furthermore, in situ light microscopy and confocal laser scanning microscopy (CLSM) showed that CS/ALG microspheres inhibited the bacterial biofilm formation in S. aureus, E. faecalis P. aeruginosa and P. vulgaris after a single treatment with 40 µg. Overall, our findings underlined that chemically synthesized CS/ALG biomaterial has high antibacterial and antibiofilm activity against a number of microbial pathogens of interest for human health, thus this synthesis route can be further exploited for drug development in current biomedical science.

Graphical abstract



Keywords

Chitosan/alginate microsphere; Enterococcus faecalis; Proteus vulgaris; Pseudomonas aeruginosa; Staphylococcus aureus

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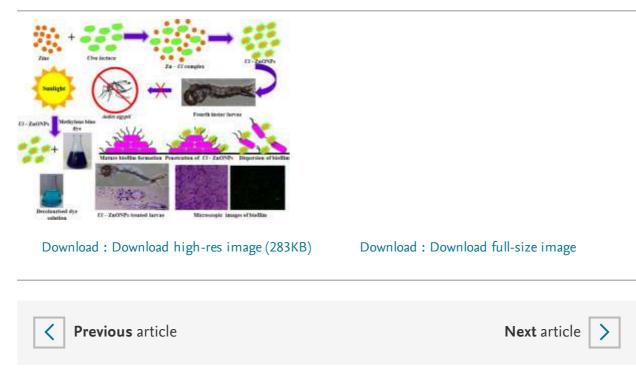
Q Search ScienceDirect Get Access Share Export Advanced X COVID-19 campus closures: see options for Remote Access to subscribed content Outline **Recommended** articles Journal of Photochemistry and Photobiology B: \wedge Biology Highlights Synthesis and characterization of phytochem... Volume 178, January 2018, Pages 249-258 Journal of Photochemistry and Photobiology B: Bi... ELSEVIER Abstract Purchase PDF View details \checkmark **Graphical Abstract** Facile green synthesis of zinc oxide Keywords Greener synthesis of zinc oxide nanoparticles... Journal of Photochemistry and Photobiology B: Bi... nanoparticles using Ulva lactuca seaweed 1. Introduction 📆 Purchase PDF View details 🗸 2. Materials and Methods extract and evaluation of their photocatalytic, 3. Results and Discussion Facile green synthesis of zinc oxide nanopart... antibiofilm and insecticidal activity Advanced Powder Technology, Volume 28, Issue 3,... 4. Conclusions Purchase PDF View details 🗸 Acknowledgements Ramachandran Ishwarya ^a, Baskaralingam Vaseeharan ^a $\stackrel{ heta}{\sim}$ 🖾, Subramanian Kalyani ^a, Balan Banumathi ^a, Marimuthu Govindarajan ^{b, c}, Naiyf S. Alharbi ^d, Shine Kadaikunnan ^d 은 쩓, Mohammed N. Al-anbr ^d, Jamal References 1 2 Next > M. Khaled ^d, Giovanni Benelli ^{e, f} Show full outline 🗸 **∃** Show more Citing articles (58) https://doi.org/10.1016/j.jphotobiol.2017.11.006 Get rights and content Figures (7) Article Metrics Citations Highlights Citation Indexes: 58 Eco-friendly green synthesis of ZnO nanoparticles was done using the Ulva lactuca extract (Ul). Captures The Ul-ZnO nanoparticles showed efficient photocatalytic activity 522 Readers: on methylene blue. Show all figures 🗸 Social Media Ul-ZnO nanoparticles showed high antibiofilm activity on 4 species • of Gram positive and negative bacteria. Tweets: 1 Tables (2) High toxicity of ZnO nanoparticles was observed against Aedes • 🗄 Table 1 ÖPLUMX View details > agypti larvae. Table 2 Histopathological effects caused by ZnO nanoparticles penetration in the insect's body were studied.

Abstract

The bioactivity of semiconductor nanocomplexes has been poorly studied in

the field of pesticide science. In this research, the synthesis of zinc nanoparticles was accomplished through new effortless green chemistry process, using the *Ulva lactuca* seaweed extract as a reducing and capping agent. The production of *U. lactuca*-fabricated ZnO nanoparticles (*Ul*-ZnO Nps) was characterized by powder X-ray diffraction (XRD), UV-visible, Fourier transform infrared (FTIR) spectroscopy, selected area electron diffraction (SAED) analysis and transmission electron microscopy (TEM). The U. lactucafabricated ZnO NPs were tested for their photodegradative action against organic dyes, as well as for antibiofilm and larvicidal activities. The UV visible absorbance spectrum of Ul-ZnO NPs exhibited the absorbance band at 325 nm and TEM highlighted average crystallite sizes of nanoparticles of 10–50 nm. Methylene blue (MB) dye was efficiently corrupted under sunlight in presence of Ul-ZnO NPs. Excellent bactericidal activity was shown by the Ul-ZnO Nps on Gram positive (Bacillus licheniformis and Bacillus pumilis) and Gram negative (*Escherichia* coliand *Proteus vulgaris*) bacteria. High antibiofilm potential was noted under both dark and sunlight conditions. The impact of a single treatment with *Ul*-ZnO NPs on biofilm architecture was also analyzed by confocal laser scanning microscopy (CLSM) on both Gram positive and Gram negative bacteria. Moreover, Ul-ZnO NPs led to 100% mortality of Aedes aegypti fourth instar larvae at the concentration of 50 μ g/ml within 24 h. The effects of ZnO nanoparticle-based treatment on mosquito larval morphology and histology were monitored. Overall, based on our results, we believe that the synthesis of multifunctional Ul-ZnO Nps using widely available seaweed products can be promoted as a potential eco-friendly option to chemical methods currently used for nanosynthesis of antimicrobials and insecticides.

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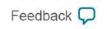
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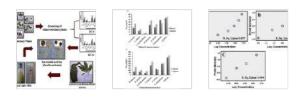
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■ Table 1 Table 2

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Green larvicides against blowflies, Lucilia sericata (Diptera, Calliphoridae): Screening of seven plants used in Indian ethno-veterinary medicine and production of green-coated zinc oxide nanoparticles \star

Balan Banumathi ^a, Baskaralingam Vaseeharan ^a $\stackrel{ heta}{\sim}$ 🖾, Balasubramanian Malaikozhundan ^a, Palaniappan Ramasamy ^b, Marimuthu Govindarajan ^c, Naiyf S. Alharbi ^d, Shine Kadaikunnan ^d, Angelo Canale ^e, Giovanni Benelli[®]

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Highlights

- Seven plants were screened for larvicidal activity against Lucilia • sericata.
- Lobelia leschenaultiana-coated ZnO nanoparticles were prepared.
- High larvicidal efficacy of ZnO nanoparticles was observed against • L. sericata.

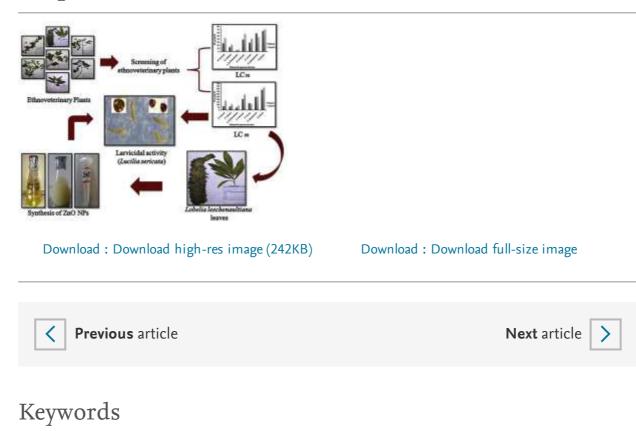
Abstract

Dipteran flies are responsible of myiasis, a common parasitic infestation leading to the invasion of living, necrotic or dead tissues. They also transmit mycobacterial infections to livestock. In the present investigation, seven plant species employed in Southern India for medical and ethno-veterinary purposes were extracted using ethanol or water and tested against second instar larvae of Lucilia sericata. 100% larval mortality was observed testing Lobelia leschenaultiana ethanol extract at 60 mg/L ($LC_{50} = 3.4$ mg/L). Based on the highest percentage of larval mortality, we selected L. leschenaultiana for fabrication of ZnO nanoparticles to control L. sericata. Zinc acetate showed 82.2% mortality at 60 mg/L ($LC_{50} = 38.2 \text{ mg/L}$) whereas L. leschenaultiana-coated ZnO nanoparticles (Ll-ZnO NPs) showed 100% mortality at 6 mg/L, the LC_{50} was 0.78 mg/L. Overall, our research represents an attempt to integrate current ethno-veterinary knowledge from native tribes of Southern India, in order to identify effective botanicals for the development of nano-biopesticides against livestock pests and parasites.





Graphical abstract



Biosafety; Diptera; Ethnopharmacology; Green synthesis; Myiasis; Nilgiri

This article is part of a Special Issue entitled 'Natural Pesticide Research' published at the journal Physiological and Molecular Plant Pathology 101C, 2018.

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Get Access Share Export COVID-19 campus closures: see options for Remote Access to subscribed content Journal of Trace Elements in Medicine and Biology Volume 45, January 2018, Pages 93-103 Purchase PDF Technical note Bacterial exopolysaccharide (EPS)-coated ZnO nanoparticles showed high antibiofilm activity Purchase PDF and larvicidal toxicity against malaria and Zika virus vectors Purchase PDF Muthukumar Abinaya ^a, Baskaralingam Vaseeharan ^a $\approx \boxtimes$, Mani Divya ^a, Aruna Sharmili ^b, Marimuthu Govindarajan ^c, Naiyf S. Alharbi ^d, Shine Kadaikunnan ^d, Jamal M. Khaled ^d, Giovanni Benelli ^{e, f} **⊞** Show more

Citing articles (34) https://doi.org/10.1016/j.jtemb.2017.10.002 Get rights and content Article Metrics Citations Highlights Citation Indexes: 34 • ZnO nanoparticles were synthesized using the exopolysaccharide (EPS) from probiotic Bacillus licheniformis. Captures • EPS-ZnO NPs were characterized by UV-vis, XRD, FTIR, SEM, 7 Exports-Saves: TEM, EDAX and Zeta potential analyses. Readers: 170 EPS-ZnO NPs showed high antioxidant, antibiofilm and antifungal • Social Media activity. 2 Tweets: Cytotoxicity of EPS-ZnO NPs by hemolysis potential test pointed ٠ out their non-toxic and biocompatible nature. ÖPLUMX View details > • The larvicidal potential of EPS-ZnO NPs was showed on key mosquito vectors Anopheles stephensi and Aedes aegypti.

Abstract

In this study, a novel and effective approach was performed to synthesize ZnO nanoparticles (ZnO NPs) using the exopolysaccharides (EPS) from the

Outline Highlights

Abstract

Graphical abstract

Keywords

1. Introduction

2. Materials and methods

3. Results

4. Discussion

5. Conclusions

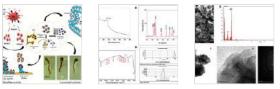
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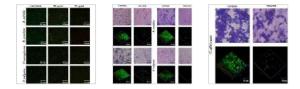
Appendix A. Supplementary data

References

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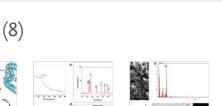
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1 2 Next >

probiotic strain Bacillus licheniformis Dahb1. EPS acted as reducing and stabilizing agent for the formation of EPS-ZnO NPs by co-precipitation method. Structural characterization was investigated by a surface plasma resonance centered at 375 nm in UV-vis spectrum. FTIR spectrum exhibited functional groups with strong absorption peak at 3814.7–420 cm⁻¹. XRD showed the crystalline nature of EPS-ZnO NPs. TEM showed that the EPS-ZnO NPs were hexagonal in shape, with size within the range of 10–100 nm. The presence of Zn was confirmed by EDX analysis. Antibacterial activity of EPS-ZnO NPs was demonstrated as 100 µg/ml significantly inhibited the effective growth control of Gram-negative (Pseudomonas aeruginosa and Proteus vulgaris) and Gram-positive (Bacillus subtilis and Bacillus pumilus) bacteria. Light microscopy and confocal laser scanning microscopy evidenced that the antibiofilm activity of EPS-ZnO NPs was higher against Gram-negative bacteria over Gram positive bacteria. EPS-ZnO NPs also inhibited the biofilm growth of *Candida albicans* at the concentration of 75 µg/ml. The hemolytic test showed low cytotoxicity of EPS-ZnO NPs at 5 mg/ml. In addition, EPS-ZnO NPs achieved 100% mortality against third instars mosquito larvae of Anopheles stephensi and Aedes aegypti at very low doses. Moreover, histology studies revealed the presence of damaged cells and tissues in the mid-gut of treated mosquito larvae. The multipurpose properties of EPS-ZnO NPs revealed in the present study can be further considered for pharmaceutical, parasitological and entomological applications.

Graphical abstract



Keywords

Exopolysaccharides; Zinc oxide nanoparticles; Antibiofilm; Malaria; Dengue

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> spectroscopy (FTIR) revealed the possible functional groups of Ge-ZnO NPs, with strong bands at 3851, 3447, and 2923 cm⁻¹. Moreover, transmission electron microscopy (TEM) highlighted the presence of spherically shaped Ge-ZnO NPs that were 20 nm in size. Energy dispersive analysis X-ray (EDX) analysis showed that the zinc elemental content of Ge-ZnO NPs was 59.10%. The results of antibacterial activity assays revealed higher inhibition of Ge-ZnO NPs against Gram-negative *Pseudomonas aeruginosa* at 100 µg/ml over that against Gram-positive Enterococcus faecalis. Greater inhibition of biofilm formation was observed for Gram-negative bacteria compared to Grampositive bacteria. In addition, Ge-ZnO NPs effectively inhibited the biofilm growth of the fungus *Candida albicans* at 50 µg/ml. Ge-ZnO NPs reduced the viability of hepatocarcinoma cancer cell lines at 100 µg/ml. Moreover, in chick embryos, notable anti-angiogenesis effects were observed for Ge-ZnO NPs and zinc acetate at 50 µg/ml compared to that observed testing gelatin. Overall, based on the results, Ge-ZnO NPs may be used as a novel agent for the control of biofilm-forming microbial pathogens.

biopolymers have been shown to be useful for many purposes. This study

particles were characterized by UV-Vis spectrum, showing a main peak at

Zeta potential and X-ray diffraction analysis. Fourier transform infrared

375 nm. The stability and crystalline nature of the particles was evaluated by

focused on gelatin-coated zinc oxide NPs synthesized by co-precipitation. The

Graphical Abstract



ZnO nanoparticles; Gelatin; Characterization; Biofilm; Chorioallantoic membrane assay

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Outline

Highlights

Graphical abstract

1. Introduction

2. Materials and methods

Abstract

Keywords

3. Results

4. Discussion

5. Conclusions

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Tables (4)

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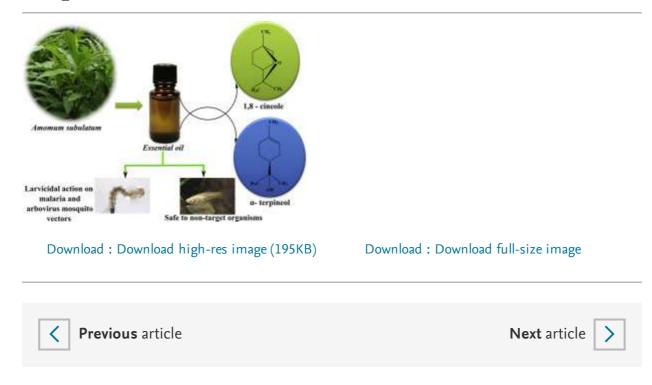
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Mosquitoes (Diptera: Culicidae) are the primary vectors of several serious diseases that affect both animals and humans. Most of these diseases, especially the ones that are caused by arboviruses such as Zika, dengue and Japanese encephalitis, cannot be effectively prevented or treated, thereby rendering the discovery of alternative measures for the control of mosquito-borne diseases an absolute necessity. The current study evaluates the acute toxicity of Amonum subulatum-derived essential oil against Anopheles subpictus, Aedes albopictus, and Culex tritaeniorhynchus. We used gas chromatography-mass spectroscopy to analyze the chemical composition of A. subulatum EO. Analysis revealed thirtythree components. Primary constituents were 1,8- cineole (39.8%) and α terpineol (11.5%). The essential oil from A. subulatum demonstrated a significant toxic effect against *An. subpictus*, *Ae. albopictus* and *Cx.* tritaeniorhynchus third instar larvae, with LC₅₀ values of 41.25, 44.11 and 48.12 µg/ml and LC₉₀ values of 80.29, 85.6 and 89.30 µg/ml, respectively. Concerning non-target effects in the aquatic environment, we evaluated the acute toxicity of A. subulatum oil on 4 mosquito biocontrol agents, Anisops bouvieri, Diplonychus indicus, Poecilia reticulata and Gambusia affinis. We observed that the toxicity of this oil was extremely low, with LC₅₀ ranging between 3123 and 9104 μ g/ml. In conclusion, the A. subulatum essential oil is an interesting bioresource to develop environmental friendly mosquito larvicides. Indeed, it is effective against different Culicidae species with LC₅₀ always lower than $50 \,\mu\text{g/ml}$, while LC₅₀ calculated for non-target organisms are about 100-fold higher.

Graphical abstract



Keywords

Biosafety; Gambusia affinis; GC-MS; Malaria; Japanese encephalitis; West Nile virus; Zika virus

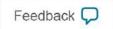
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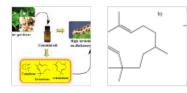
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Table 2

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Ecotoxicology and Environmental Safety Volume 148, February 2018, Pages 781-786

Insecticidal activity of camphene, zerumbone

and α -humulene from Cheilocostus speciosus

rhizome essential oil against the Old-World

^e, Sami A. Alyahya ^f, Naiyf S. Alharbi ^g, Shine Kadaikunnan ^g, Jamal M. Khaled ^g, Filippo Maggi ^h

Giovanni Benelli ^{a, b} ^A [⊠], Marimuthu Govindarajan ^{c, d} ^A [⊠], Mohan Rajeswary ^c, Baskaralingam Vaseeharan

bollworm, Helicoverpa armigera



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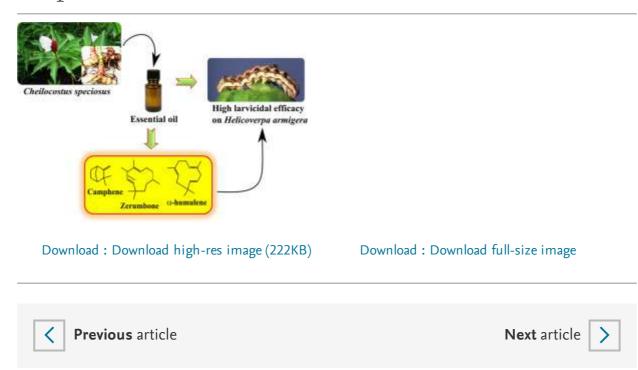
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Highlights		Citations	
• <i>Helicoverpa armigera</i> is a polyphagous pest showing several synthetic insecticides.	resistance to	Citation Indexes:	15
• We focused on the essential oil of <i>Cheilocostus specie</i>	osus as a source of	Captures	
insecticidal compounds.		Exports-Saves:	4
• GC and GC-MS were done, then the oil and its ma	jor constituents	Readers:	53
were evaluated in ovicidal and larvicidal toxicity test	sts.	Social Media	
 Camphene, zerumbone and α-humulene showed la 10.64, 17.16 and 20.86 µg/ml, respectively. 	rvicidal LC ₅₀ of	Tweets:	2
 Camphene, zerumbone and α-humulene have a propotential as eco-friendly botanical insecticides. 	omising	őplumx	View details >

Abstract

The fast-growing resistance development to several synthetic and microbial insecticides currently marketed highlighted the pressing need to develop novel

and eco-friendly pesticides. Among the latter, botanical ones are attracting high research interest due to their multiple mechanisms of action and reduced toxicity on non-target vertebrates. Helicoverpa armigera (Lepidoptera: Noctuidae) is a key polyphagous insect pest showing insecticide resistance to several synthetic molecules used for its control. Therefore, here we focused on the rhizome essential oil extracted from an overlooked Asian plant species, Cheilocostus speciosus (J. Konig) C. Specht (Costaceae), as a source of compounds showing ingestion toxicity against *H. armigera* third instar larvae, as well as ovicidal toxicity. In acute larvicidal assays conducted after 24 h, the C. speciosus essential oil achieved a LC₅₀ value of 207.45 μ g/ml. GC and GC-MS analyses highlighted the presence of zerumbone (38.6%), α -humulene (14.5%) and camphene (9.3%) as the major compounds of the oil. Ingestion toxicity tests carried out testing these pure molecules showed LC₅₀ values of 10.64, 17.16 and 20.86 μ g/ml, for camphene, zerumbone and α -humulene, respectively. Moreover, EC_{50} values calculated on *H. armigera* eggs were 35.39, 59.51 and 77.10 μ g/ml for camphene, zerumbone and α -humulene, respectively. Overall, this study represents the first report on the toxicity of *C. speciosus* essential oil against insect pests of agricultural and medical veterinary importance, highlighting that camphene, zerumbone and α -humulene have a promising potential as eco-friendly botanical insecticides.

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Keywords

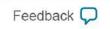
Acute toxicity; *Cheilocostus speciosus*; Essential oil; Green pesticides; Insecticide resistance; Larvicide

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ORIGINAL PAPER



Single Step Fabrication of Chitosan Nanocrystals Using *Penaeus semisulcatus*: Potential as New Insecticides, Antimicrobials and Plant Growth Promoters

Vijayan Thamilarasan^{1,2} · Venkatesan Sethuraman³ · Kasi Gopinath⁴ · Chinnasamy Balalakshmi⁵ · Marimuthu Govindarajan^{6,7} · Ramzi A. Mothana⁸ · Nasir A. Siddiqui⁸ · Jamal M. Khaled⁹ · Giovanni Benelli^{10,11}

Received: 15 December 2017 / Published online: 23 January 2018 © Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

In this study, chitosan nanoparticles (CH-NPs) were synthesized using *Penaeus semisulcatus* shrimp shells and characterized using UV–Vis and FT-IR spectroscopy, as well as XRD and HR-TEM analyses. CH-NPs were investigated for growth inhibition properties against selected species of bacterial and fungal pathogens, showing performances higher or comparable over positive controls, respectively. Furthermore, CH-NPs were tested on three important mosquito vectors, achieving LC_{50} from 12.27 to 14.62 µg/ml. In addition, CH-NPs were evaluated using in vitro plant tissue culture by rooting gel method, to enhance the vegetative growth of the medicinal plant species *Sphaeranthus indicus*. With the simple technique presented here, large-scale industrial production of CH-NPs is possible. They can be used to develop pesticides highly effective against mosquito vectors of high medical and veterinary importance, as well as for plant tissue culture and food packaging applications.

Keywords Chitosan · Nanomaterials · XRD, TEM · Microbial pathogens · Dengue, malaria

Introduction

Advancements in science and technology has resulted in a modern world with changes in our lifestyle. For the past two decades, nanotechnology has been an intensive and a

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vibrant field of research in science and technology [1]. This technology is involved in multiple applications, such that a single device could now replace a rather wide number of individual applications, for example, a smartphone [2]. Notably, the size and shape of nanomaterial play a vital

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Bio-mining drugs from the sea: High antibiofilm properties of haemocyanin purified from the haemolymph of flower crab Portunus pelagicus (L.) (Decapoda: Portunidae)

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ARTICLE INFO

Keywords: Arthropod Candida albicans Cell surface hydrophobicity Crustacean Growth inhibition

ABSTRACT

Marine organisms are an outstanding source of molecules with wide biological activities. In this research, we investigated the antimicrobial protein haemocyanin isolated from the haemolymph of flower crab (Portunus pelagicus) using gel filtration chromatography. Purified P. pelagicus haemocyanin (Pp-Hc) was subjected to SDS-PAGE analysis and the fraction containing Pp-Hc ran as a distinct band, its molecular weight was 78 kDa. The Pp-Hc was characterized through HPLC, XRD, CD and FTIR analyses. The antibacterial potential of Pp-Hc was evaluated against five Gram-positive and ten Gram-negative bacteria through the agar well diffusion method, which clearly revealed the high bactericidal activity of Pp-Hc. Furthermore, Pp-Hc was studied for its antibiofilm activity against five biofilm-forming Gram-negative bacteria, i.e., Vibrio alginolyticus, Vibrio harvevi, Vibrio parahaemolyticus, Pseudomonas aeruginosa, and Proteus vulgaris. Confocal laser scanning microscopy (CLSM) and light microscopy evidenced significant antibiofilm properties of Pp-Hc at 100 µg/ml concentration. Based on exopolysaccharide (EPS) quantification and cell surface hydrophobicity (CSH) index, the antibiofilm action of Pp-Hc was linked to its ability to reduce EPS production and increase cell surface hydrophobicity. The pathogen recognition mechanism of purified Pp-Hc showed a wide spectrum of binding activities, leading to high toxicity against both Gram-positive and Gram-negative bacterial species. Furthermore, the purified Pp-Hc showed high antifungal activity against the pathogen Candida albicans. Haemocyanin is not only involved in transporting oxygen, it has a relevant function in the flower crab immune system. This work adds useful knowledge for aquaculture concerns dealing with microbial pathogen control. Overall, our results outlined the high potential of purified Pp-Hc as a starting material to develop new and effective antimicrobial drugs for aquaculture purposes.

1. Introduction

The flower crab, Portunus pelagicus (L.) (Decapoda: Portunidae) is an edible crab, which inhabits the intertidal zones all over the Indo-Pacific region, therefore playing a chief role in the marine ecosystems (Lestang et al., 2003; Kunsook and Dumrongrojwatthana, 2017). It is a vital species in aquaculture industries. However, in the last few years, crab farming has been threatened by the increase of infectious diseases due to bacterial, fungal and viral pathogens (Destoumieux-Garzon et al., 2001). Current microbiology and parasitology are facing the growing lack of effective antibiotic drugs. Indeed, antibiotics must be used prudently to treat humans and animals, since their prolonged overuse led to the fast development of resistance in targeted organisms (Davies and Davies, 2010). Antibiotic resistance now represents a serious

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PLANT-BORNE COMPOUNDS AND NANOPARTICLES: CHALLENGES FOR MEDICINE, PARASITOLOGY AND ENTOMOLOGY

Mosquito control with green nanopesticides: towards the One Health approach? A review of non-target effects

Giovanni Benelli¹ • Filippo Maggi² • Roman Pavela³ • Kadarkarai Murugan⁴ • Marimuthu Govindarajan⁵ • Baskaralingam Vaseeharan⁶ • Riccardo Petrelli² • Loredana Cappellacci² • Suresh Kumar⁷ • Anders Hofer⁸ • Mohammad Reza Youssefi⁹ • Abdullah A. Alarfaj¹⁰ • Jiang-Shiou Hwang¹¹ • Akon Higuchi¹²

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Abstract The rapid spread of highly aggressive arboviruses, parasites, and bacteria along with the development of resistance in the pathogens and parasites, as well as in their arthropod vectors, represents a huge challenge in modern parasitology and tropical medicine. Eco-friendly vector control programs are crucial to fight, besides malaria, the spread of dengue, West Nile, chikungunya, and Zika virus, as well as other arboviruses such as St. Louis encephalitis and Japanese encephalitis. However, research efforts on the control of mosquito vectors are experiencing a serious lack of ecofriendly and highly effective pesticides, as well as the limited success of most biocontrol tools currently applied. Most importantly, a cooperative interface between the two disciplines is still lacking. To face this challenge, we have reviewed a wide number of promising results in the field of green-fabricated pesticides tested against mosquito vectors, outlining several examples of synergy with classic biological control tools. The nontarget effects of green-fabricated nanopesticides, including acute toxicity, genotoxicity, and impact on behavioral traits of mosquito predators, have been critically discussed. In the final section, we have identified several key challenges at the interface between "green" nanotechnology and classic biological control, which deserve further research attention.

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PLANT-BORNE COMPOUNDS AND NANOPARTICLES: CHALLENGES FOR MEDICINE, PARASITOLOGY AND ENTOMOLOGY

Brevibacillus laterosporus isolated from the digestive tract of honeybees has high antimicrobial activity and promotes growth and productivity of honeybee's colonies

Jamal M. Khaled^{1,2} • Fahd A. Al-Mekhlafi^{3,4} • Ramzi A. Mothana⁵ • Naiyf S. Alharbi¹ • Khalid E. Alzaharni^{6,7} • Anwar H. Sharafaddin⁸ • Shine Kadaikunnan¹ • Ahmed S. Alobaidi¹ • Noofal I. Bayaqoob⁸ • Marimuthu Govindarajan⁹ • Giovanni Benelli^{10,11}

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Abstract The development of novel antimicrobial drugs, as well as the discovery of novel compounds able to promote honeybee's growth, represents major challenges for modern entomology. The main aim of this study was to investigate whether *Brevibacillus laterosporus* isolated from the digestive tract of Saudi honeybees, *Apis mellifera*, was able to stimulate colony strength parameters of honeybees and to evaluate its ability to produce antimicrobial agents. Honeybees were collected in Dirab, Riyadh Region, Saudi Arabia, and microorganisms were isolated and identified by 16S ribosomal RNA analysis. Microscopic identification of the microorganism in its native state was facilitated by atomic force microscopy at high-resolution imaging. Active biological compounds were produced by submerged fermentation with *B. laterosporus*.

The fermented broth was subjected to extraction and purification, and then semi-pure compounds were analyzed by gas chromatography–mass spectrometry. The effectiveness of the crude extract and semi-pure compounds as antimicrobial agents was evaluated by susceptibility assays. More than 22% of the microorganisms isolated from the digestive tract of healthy honeybees have been identified as *B. laterosporus*, this kind of species has a unique shape and morphological structure. The cyclic dipeptide cyclo(Leu-Pro) produced by *B. laterosporus* showed biological activity against several pathogenic microorganisms. Furthermore, the total counts of workers, closed brood, and open brood, as well as the production of bee pollen and honey, were better in honeybees treated with a *B. laterosporus* suspension. The data indicated that the

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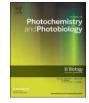
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Swift fabrication of Ag nanostructures using a colloidal solution of *Holostemma ada-kodien* (Apocynaceae) – Antibiofilm potential, insecticidal activity against mosquitoes and non-target impact on water bugs



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ABSTRACT

Recent research in entomology and parasitology focused on the efficacy of green fabricated nanomaterials as novel insecticides. In this study, we synthesized poly-dispersed and stable silver nanoparticles (AgNPs) using the leaf extract of Holostemma ada-kodien. The nanostructures were characterized by ultraviolet-visible spectroscopy, Fourier-transform infrared spectroscopy, scanning electron microscopy, transmission electron microscopy, energy dispersive X-ray, and X-ray diffraction analysis. The efficacy of H. ada-kodien leaf extract and AgNPs in vector control was evaluated against the mosquitoes Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus, which act as major vectors of important parasitic and arboviral diseases. AgNPs showed higher toxicity if compared to the H. ada-kodien leaf aqueous extract, LC50 towards larvae of A. stephensi, A. aegypti, and C. quinquefasciatus were 12.18, 13.30, and 14.70 µg/mL, respectively. When the AgNPs were tested on non-target water bugs, Diplonychus indicus, the LC_{50} value was 623.48 µg/mL. Furthermore, 100 µl/mL of AgNPs achieved significant antimicrobial activity against Bacillus pumilus, Enterococcus faecalis, Pseudomonas aeruginosa, Proteus vulgaris, and Candida albicans. Light and confocal laser scanning microscopy highlighted a major impact of the H. ada-kodien-synthesized AgNPs on the external topography and architecture of microbial biofilms, both on Grampositive and Gram-negative bacteria. Overall, this study sheds light on the insecticidal and antibiofilm potential of H. ada-kodien-synthesized AgNPs, a potential green resource for the rapid synthesis of polydispersed and highly stable AgNPs.

1. Introduction

Pesticide science is currently facing a challenge due to the development of resistance to chemical insecticides, resulting in arthropod pest and vector outbreaks [1] as well as toxicity to non-target organisms and human health concerns [2–4]. In this framework, plant secondary metabolites may be a possible resource to produce mosquito control agents [5-8].

Recently, green synthetic routes to fabricate mosquitocidal nanomaterials using plant, bacterial, and fungal species have been proposed [9,10]. Plant and microbial nano-products are particularly cost-effective and eco-friendly [11–13]. Recent studies have pointed to the importance of testing different botanicals as reducing and capping agents for nanosynthesis, since they lead to the production of nanoparticles

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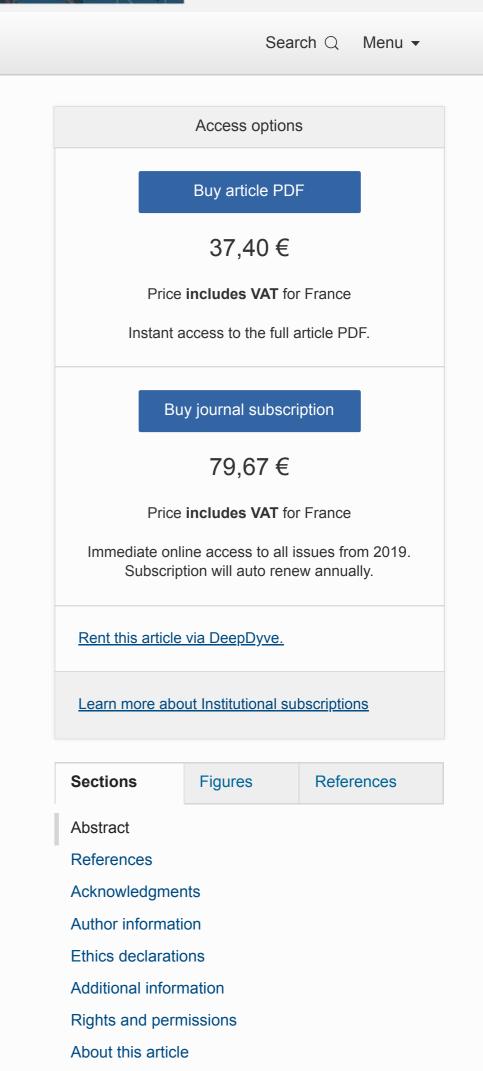
Eco-friendly and cost-effective Ag nanocrystals fabricated using the leaf extract of *Habenaria plantaginea*: toxicity on six mosquito vectors and four non-target species

<u>Chinnadurai Aarthi</u>, <u>Marimuthu Govindarajan</u> ⊡, <u>Pichaimuthu Rajaraman</u>, <u>Naiyf S. Alharbi</u>, <u>Shine</u> <u>Kadaikunnan</u>, <u>Jamal M. Khaled</u>, <u>Ramzi A. Mothana</u>, <u>Nasir A. Siddiqui</u> & <u>Giovanni Benelli</u> ⊡

<u>Environmental Science and Pollution Research</u> 25, 10317–10327(2018) | <u>Cite this article</u>
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Abstract

Recently, the biofabrication of metal nanoparticles has gained wide interest owing to its inherent features such as swift, simplicity, eco-friendliness, and cheaper costs. Different green-reducing agents led to the production of nanoparticles with varying toxicity on insects. In the current study, silver nanoparticles (AgNPs) were successfully synthesized using Habenaria plantaginea leaf extract. Ag nanoparticles were studied by UV–Vis spectroscopy (UV-Vis), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), atomic force microscopy (AFM), scanning electron microscopy (SEM) coupled with energy-dispersive spectroscopy (EDS), and transmission electron microscopy (TEM). H. plantaginea extract and AgNPs were tested for mosquito larvicidal activity on *Anopheles stephensi*, *Aedes aegypti*, *Culex quinquefasciatus, An. subpictus, Ae. albopictus, and Cx. tritaeniorhynchus.* LC₅₀ values were 102.51, 111.99, 123.47, 123.96, 136.56, 149.42 µg/ml and 12.23, 13.38, 14.78, 14.37, 15.39, 16.89 µg/ml, respectively. Moreover, *H. plantaginea* aqueous extract and AgNPs were tested against the non-target species Anisops bouvieri, Diplonychus indicus, Poecilia reticulata, and Gambusia affinis obtaining LC₅₀ values ranging from 831.82 to 36,212.67 μ g/ml. Overall, this study showed the effectiveness of H. plantaginea-fabricated nanoparticles on a wide range of important mosquito vectors, highlighting their scarce toxicity on four natural enemies predating mosquito larvae and pupae.



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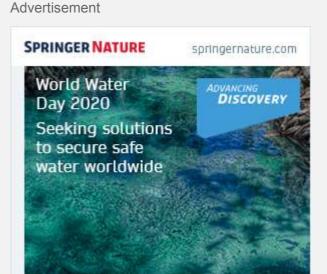
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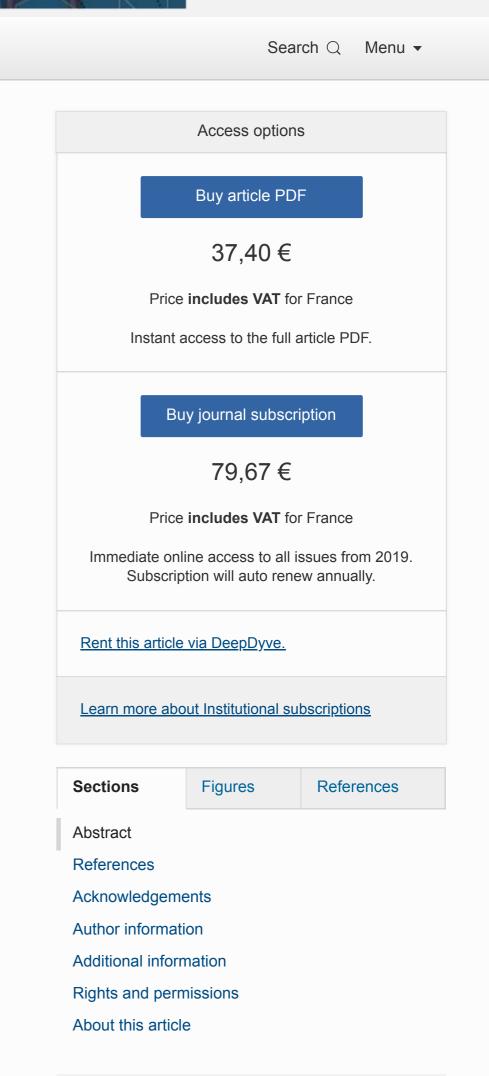
High efficacy of (Z)- γ -bisabolene from the essential oil of Galinsoga parviflora (Asteraceae) as larvicide and oviposition deterrent against six mosquito vectors

Marimuthu Govindarajan , Baskaralingam Vaseeharan, Naiyf S. Alharbi, Shine Kadaikunnan, Jamal M. Khaled, Mohammed N. Al-anbr, Sami A. Alyahya, Filippo Maggi & Giovanni Benelli 🖂 Environmental Science and Pollution Research 25, 10555–10566(2018) | Cite this article

265 Accesses | 8 Citations | 1 Altmetric | Metrics

Abstract

The eco-friendly management of mosquitoes with novel and effective larvicides and oviposition deterrents is a crucial challenge to prevent outbreaks of mosquito-borne diseases. However, most of the herbal formulations tested in these years showed LC₅₀ values higher of 40 ppm, and significant oviposition deterrent activity only when tested at relatively higher doses (> 50 μ g/ml). Herein, we studied the chemical composition of the Galinsoga parviflora essential oil (EO). This plant is an annual herb native to South America naturalized all over the world. We tested the EO larvicidal and oviposition deterrent action on 6 mosquito species. Totally 37 compounds were identified in the EO of *G. parviflora* by GC and GC-MS analyses. The major constituent was (Z)- γ -bisabolene (38.9%). The G. parviflora EO and (Z)- γ bisabolene showed acute toxicity on An. stephensi ($LC_{50} = 31.04$ and 2.04 µg/ml, respectively), Ae. aegypti (LC₅₀ = 34.22 and 2.26 μ g/ml, respectively), Cx. quinquefasciatus (LC₅₀ = 37.10 and 2.47 μ g/ml, respectively), *An. subpictus* (LC₅₀ = 40.97 and 4.09 μ g/ml, respectively), *Ae.* albopictus (LC₅₀ = 45.55 and 4.50 μ g/ml, respectively) and *Cx. tritaeniorhynchus* (LC₅₀ = 49.56 and 4.87 µg/ml, respectively) larvae. Furthermore, the oviposition deterrent potential of the G. parviflora EO and (Z)- γ -bisabolene was studied on six mosquito vectors, showing that $25 \,\mu\text{g/ml}$ of (*Z*)- γ -bisabolene led to an Oviposition Activity Index lower of -0.79 in all tested mosquito vectors. Overall, all larvicidal LC_{50} values estimated for (Z)- γ -bisabolene were lower than 5 μ g/ml. This result far encompasses current evidences of toxicity reported for the large



majority of botanical products currently tested against mosquito young instars, allowing us to propose this compound as an highly effective mosquito larvicide and oviposition deterrent.

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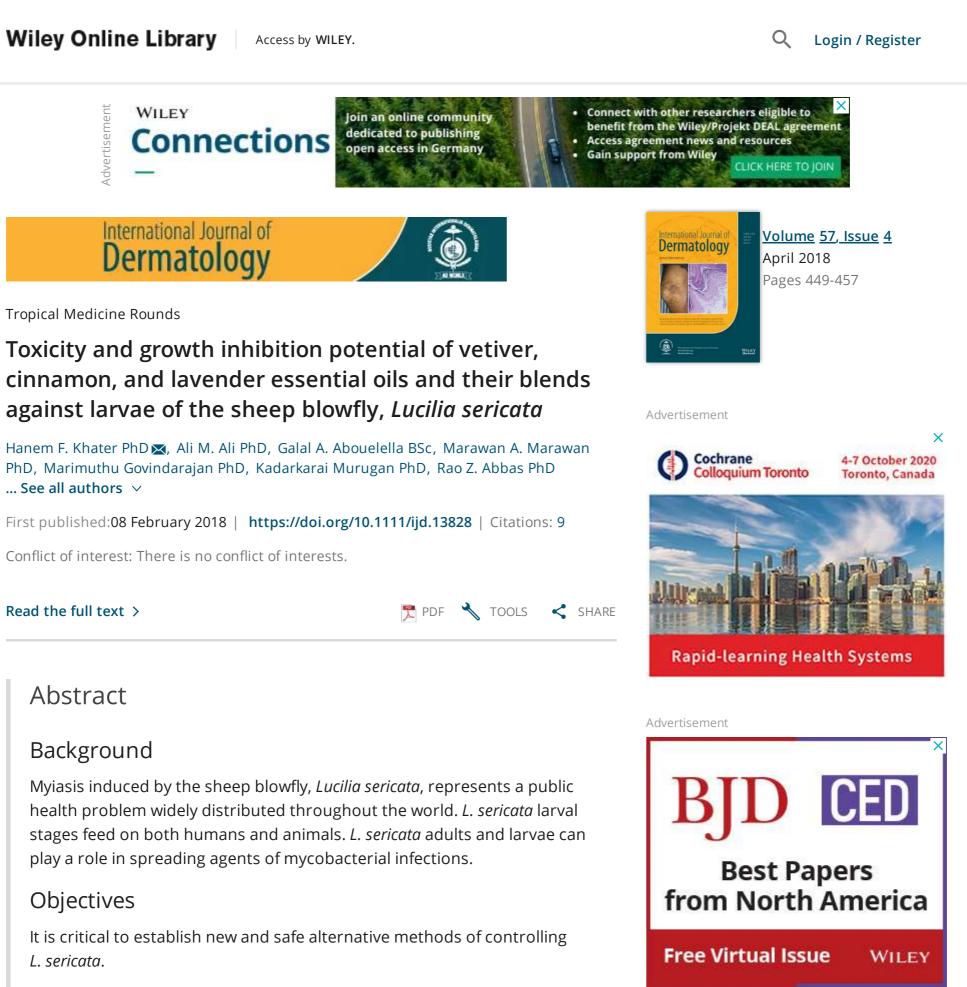
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Benelli G (2015a) Research in mosquito control: current challenges for a brighter future.

Parasitol Res 114(8):2801-2805. https://doi.org/10.1007/s00436-015-4586-9





Methods

The insecticidal effectiveness and growth inhibition potential of three commercially available essential oils (EOs), vetiver (Chrysopogon zizanioides), cinnamon (Cinnamomum zeylanicum), and lavender (Lavandula angustifolia), as well as their blends, were tested against the second (L2) and third (L3) larval stages of *L. sericata*. Sunflower (*Helianthus annuus*) oil was used as a carrier and tested on L2 and L3 larvae. To the best of our knowledge, all applied essential oils, except lavender, and oil blends were tested against L. sericata for the first time.

Results

All applied oils did not repel L2 from the treated liver but adversely affected their development. Contact treatments on *L. sericata* L3 indicated that vetiver and cinnamon oils significantly affected treated larvae. Total mortality rates were 93.33 and 95.56%, respectively. Furthermore, oil blends tested through contact assays killed larvae when used at higher concentrations; adult emergence was eliminated post-treatment with doses >30% for oil blend 1 and >10% for oil blend 2.

Conclusion

Overall, cinnamon and vetiver oils (5%) were selected as reliable and cheap biopesticides for controlling larvae of L. sericata. The tested oils are inexpensive and represent new promising botanical insecticides in the fight against blowflies causing myiasis.

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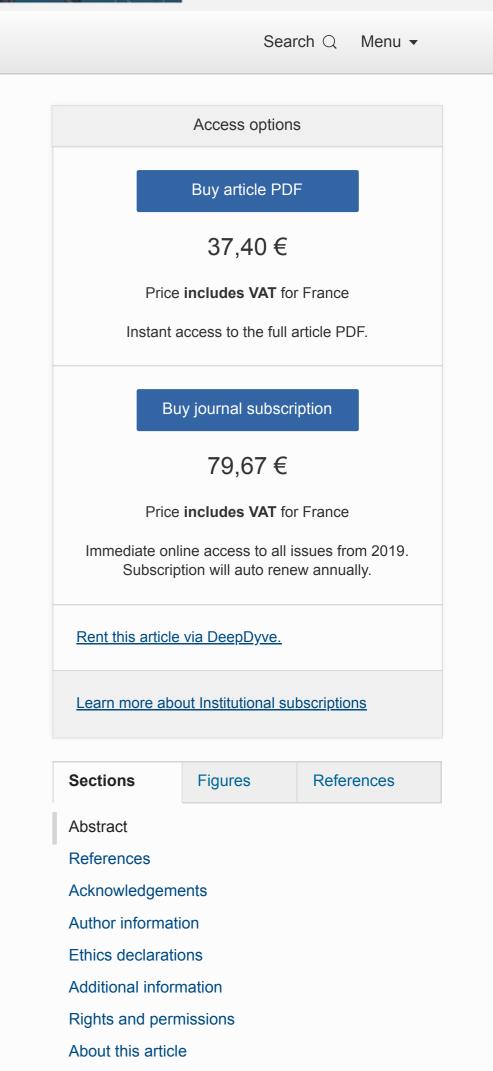
Curzerene, trans- β -elemenone, and γ -elemene as effective larvicides against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*: toxicity on non-target aquatic predators

Marimuthu Govindarajan 🖂, Mohan Rajeswary, Sengamalai Senthilmurugan, Periasamy Vijayan, Naiyf S. Alharbi, Shine Kadaikunnan, Jamal M. Khaled & Giovanni Benelli 🖂

Environmental Science and Pollution Research 25, 10272–10282(2018) | Cite this article
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Abstract

A wide number of studies dealing with mosquito control focus on toxicity screenings of whole plant essential oils, while limited efforts shed light on main molecules responsible of toxicity, as well as their mechanisms of action on non-target organisms. In this study, GC-MS shed light on main essential oil components extracted from leaves of the Suriname cherry Eugenia *uniflora*, i.e., curzerene (35.7%), trans- β -elemenone (11.5%), and γ -elemene (13.6%), testing them on Anopheles subpictus, Aedes albopictus, and Culex tritaeniorhynchus larvae. Nontarget toxicity experiments were carried out on four species of aquatic larvivorous organisms, including fishes, backswimmers, and waterbugs. The essential oil from *E. uniflora* leaves tested on An. subpictus, Ae. Albopictus, and Cx. tritaeniorhynchus showed LC₅₀ of 31.08, 33.50, and 36.35 μ g/ml, respectively. Curzerene, trans- β -elemenone, and γ -elemene were extremely toxic to An. subpictus (LC₅₀ = 4.14, 6.13, and 10.53 μ g/ml), Ae. albopictus $(LC_{50} = 4.57, 6.74, and 11.29 \mu g/ml)$, and *Cx. tritaeniorhynchus* $(LC_{50} = 5.01, 7.32, and$ 12.18 μ g/ml). The essential oil from *E. uniflora* leaves, curzerene, trans- β -elemenone, and γ elemene showed low toxicity to larvivorous fishes, backswimmers, and waterbugs, with LC₅₀ ranging from 303.77 to 6765.56 µg/ml. Predator safety factor (PSF) ranged from 55.72 to 273.45. Overall, we believe that curzerene isolated from the essential oil from E. uniflora leaves can represent an ideal molecule to formulate novel mosquito larvicides, due to its



extremely low LC_{50} on all tested mosquito vectors (4.14–5.01 µg/ml), which far encompasses most of the botanical pesticides tested till now. Notably, the above-mentioned LC_{50} did not damage the four aquatic predators tested in this study.

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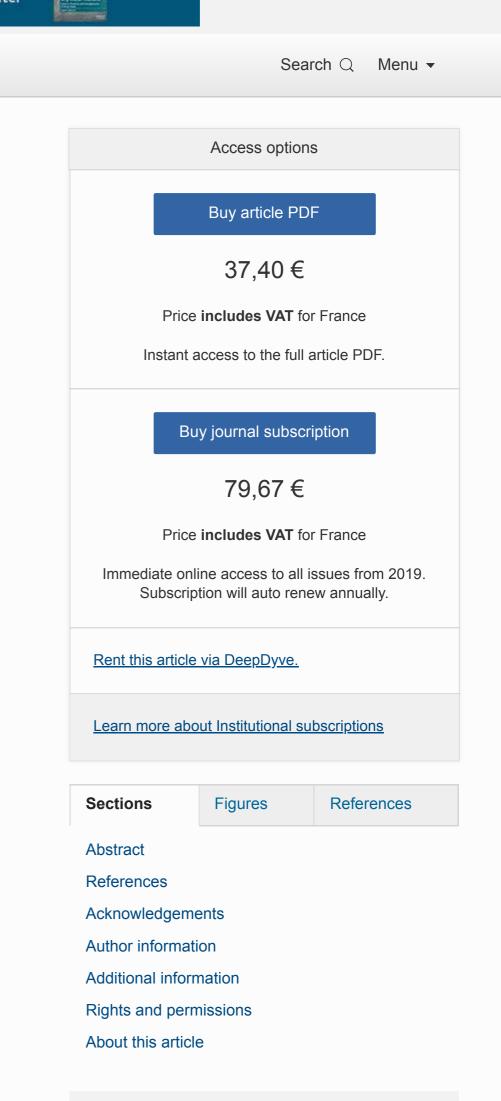
Fabrication of highly effective mosquito nanolarvicides using an Asian plant of ethno-pharmacological interest, Priyangu (*Aglaia elaeagnoidea*): toxicity on non-target mosquito natural enemies

<u>Giovanni Benelli</u> ⊡, <u>Marimuthu Govindarajan</u> ⊡, <u>Sengamalai Senthilmurugan</u>, <u>Periasamy Vijayan</u>, <u>Shine</u> <u>Kadaikunnan</u>, <u>Naiyf S. Alharbi</u> & <u>Jamal M. Khaled</u>

<u>Environmental Science and Pollution Research</u> 25, 10283–10293(2018) | <u>Cite this article</u>
1820 Accesses | 6 Citations | 1 Altmetric | <u>Metrics</u>

Abstract

Mosquitoes threaten the lives of humans, livestock, pets and wildlife around the globe, due to their ability to vector devastating diseases. Aglaia elaeagnoidea, commonly known as Priyangu, is widely employed in Asian traditional medicine and pest control. Medicinal activities include antiinflammatory, analgesic, anticancer, and anesthetic actions. Flavaglines, six cyclopenta[b]benzofurans, a cyclopenta[bc]benzopyran, a benzo[b]oxepine, and an aromatic butyrolactone showed antifungal properties, and aglaroxin A and rocaglamide were effective to control moth pests. Here, we determined the larvicidal action of A. elaeagnoidea leaf aqueous extract. Furthermore, we focused on Priyangumediated synthesis of Ag nanoparticles toxic to Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi. The plant extract and the nanolarvicide were tested on three mosquito vectors, following the WHO protocol, as well as on three non-target mosquito predators. Priyangu-synthesized Ag nanoparticles were characterized by spectroscopic (UV, FTIR, XRD, and EDX) and microscopic (AFM, SEM, and TEM) analyses. Priyangu extract toxicity was moderate on Cx. quinquefasciatus (LC₅₀) 246.43; LC₉₀ 462.09 µg/mL), Ae. aegypti (LC₅₀ 229.79; LC₉₀ 442.71 µg/mL), and An. stephensi (LC₅₀ 207.06; LC₉₀ 408.46 µg/mL), respectively, while Priyangu-synthesized Ag nanoparticles were highly toxic to *Cx. quinquefasciatus* (LC₅₀ 24.91; LC₉₀ 45.96 µg/mL), *Ae. aegypti* (LC₅₀ 22.80; LC₉₀ 43.23 µg/mL), and An. stephensi (LC₅₀ 20.66; LC₉₀ 39.94 µg/mL), respectively. Priyangu extract and Ag nanoparticles were found safer to non-target larvivorous fishes, backswimmers, and waterbugs, with



 LC_{50} ranging from 1247 to 37,254.45 µg/mL, if compared to target pests. Overall, the current research represents a modern approach integrating traditional botanical pesticides and nanotechnology to the control of larval populations of mosquito vectors, with negligible toxicity against non-target including larvivorous fishes, backswimmers, and waterbugs.

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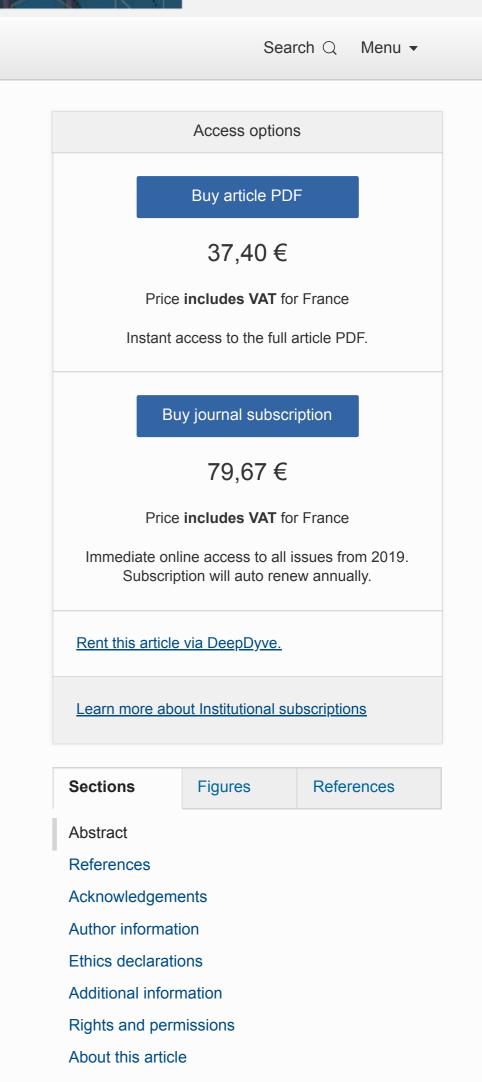
High toxicity of camphene and y-elemene from *Wedelia* prostrata essential oil against larvae of Spodoptera litura (Lepidoptera: Noctuidae)

Giovanni Benelli 🖂, Marimuthu Govindarajan 🖂, Mohamad S. AlSalhi, Sandhanasamy Devanesan & Filippo Maggi

Environmental Science and Pollution Research 25, 10383–10391(2018) | Cite this article 440 Accesses | 11 Citations | 1 Altmetric | Metrics

Abstract

The development of eco-friendly biopesticides in the fight against agricultural pests is an important challenge nowadays. The essential oil of *Wedelia prostrata* Hemsl. is known for its multipurpose biological activities in Chinese folk medicine. However, limited efforts attempted to understand the potential insecticidal activity of its main individual constituents and related mechanism of action. In this research, we investigated the insecticidal activity of W. prostrata against the crop pest Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae). Toxicity on 4th instar larvae was assessed after 24 h of exposure. The LC_{50} and LC_{90} of the W. prostrata essential oil were 167.46 and 322.12 µg/ml, respectively. GC-MS analyses were carried out to shed light on the oil chemical composition. Main constituents were the monoterpene camphene (9.6%) and the sesquiterpenes γ -elemene (7.6%), α -humulene (6.9%), and (E,E)- α -farnesene (7.3%). The pure constituents were evaluated for their insecticidal activity on S. litura 4th instar larvae. The most toxic molecule was camphene $(LC_{50} = 6.28 \mu g/ml)$, followed by γ -elemene, $(LC_{50} = 10.64 \mu g/ml)$, α -humulene $(LC_{50} = 12.89 \ \mu g/ml)$, and (E,E)- α -farnesene $(LC_{50} = 16.77 \ \mu g/ml)$. Overall, our experiments highlighted the promising potential of camphene and γ-elemene from *W. prostrata* essential oil against larvae of *S. litura*, allowing us to propose these two compounds for the development of newer pesticides in the fight against crop pests. Further studies aimed at evaluating the potential synergy between these two molecules, as well as their stability in field conditions are ongoing.



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Plant-borne compounds and nanoparticles: challenges for medicine, parasitology and entomology Published: 11 May 2017

Zingiber cernuum (Zingiberaceae) essential oil as effective larvicide and oviposition deterrent on six mosquito vectors, with little non-target toxicity on four aquatic mosquito predators

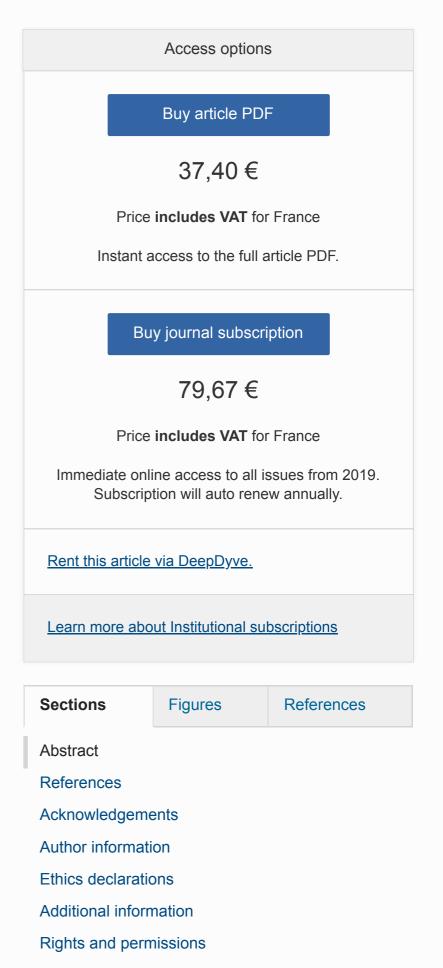
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Environmental Science and Pollution Research 25, 10307–10316(2018) Cite this article 1810 Accesses | 7 Citations | 1 Altmetric | Metrics

Abstract

Mosquitoes are responsible for the transmission of many pathogens and parasites, which cause serious diseases in humans and animals. Currently, botanical products have been suggested as alternative tools in the fight against arthropod vectors. In this study, the essential oil (EO) extracted from Zingiber cernuum was tested as larvicide and oviposition deterrent on six mosquito species of public health relevance, including malaria and Zika virus vectors. The EO showed high toxicity on third instar larvae of Anopheles stephensi ($LC_{50} = 41.34 \mu g/ml$), Aedes aegypti (LC₅₀ = 44.88 μ g/ml), Culex quinquefasciatus (LC₅₀ = 48.44 μ g/ml), Anopheles subpictus ($LC_{50} = 51.42 \ \mu g/ml$), Aedes albopictus ($LC_{50} = 55.84 \ \mu g/ml$), and Culex *tritaeniorhynchus* (LC₅₀ = 60.20 μ g/ml). In addition, low doses of *Z. cernuum* EO reduced oviposition rates in six mosquito species. The acute toxicity of Z. cernuum EO on four mosquito predators was scarce; LC_{50} ranged from 3119 to 11,233 µg/ml. Overall, our results revealed that the Z. cernuum EO can be considered for the development of effective and environmental-friendly mosquito larvicides and oviposition deterrents.

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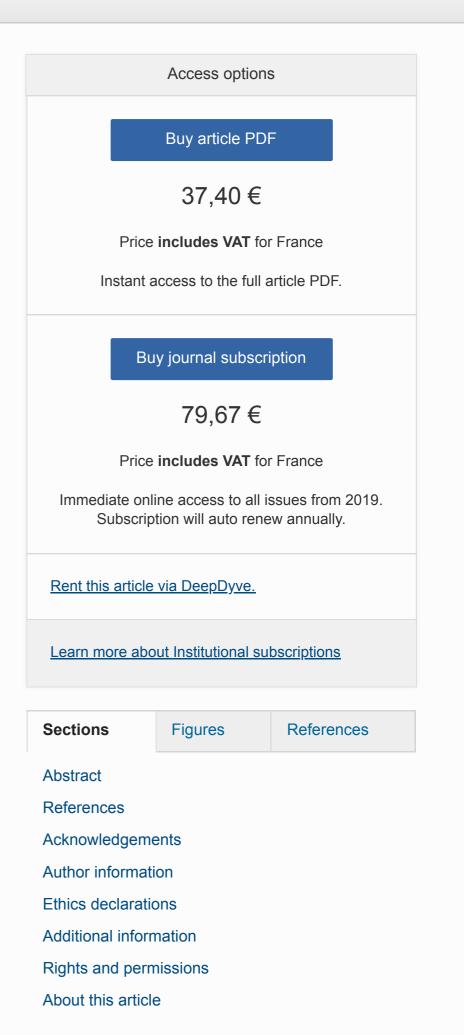
Plant-borne compounds and nanoparticles: challenges for medicine, parasitology and entomology Published: 05 February 2017

Biophysical characterization of *Acacia caesia*-fabricated silver nanoparticles: effectiveness on mosquito vectors of public health relevance and impact on non-target aquatic biocontrol agents

<u>Giovanni Benelli</u> ⊡, <u>Shine Kadaikunnan</u>, <u>Naiyf S. Alharbi</u> & <u>Marimuthu Govindarajan</u> ⊡ <u>Environmental Science and Pollution Research</u> **25**, 10228–10242(2018) | <u>Cite this article</u> **1925** Accesses | **11** Citations | <u>Metrics</u>

Abstract

Mosquito-borne diseases lead to serious public health concerns in tropical and sub-tropical countries worldwide, due to development of mosquito resistance to synthetic pesticides, non-target effects of pesticides, and socioeconomic reasons. Currently, green nanotechnology is a promising research field, showing a wide range of potential applications in vector control programs. The employ of natural products as reducing agents to fabricate insecticidal nanocomposites is gaining research attention worldwide, due to low costs and high effectiveness. Interestingly, biophysical features of greensynthesized nanoparticles strongly differ when different botanicals are employed for nanosynthesis. In this study, a cheap Acacia caesia leaf extract was employed to fabricate silver nanoparticles (Ag NPs) with ovicidal, larvicidal, and adulticidal toxicity against three mosquito vectors, Anopheles subpictus, Aedes albopictus, and Culex tritaeniorhynchus. Ag NPs were analyzed by various biophysical methods, including spectroscopy (UV-visible spectrophotometry, XRD, FTIR, EDX) and microscopy (SEM, TEM, AFM) techniques. High acute larvicidal potential was observed against larvae of An. subpictus $(LC_{50} = 10.33 \mu g/ml)$, Ae. albopictus $(LC_{50} = 11.32 \mu g/ml)$, and Cx. tritaeniorhynchus $(LC_{50} = 12.35 \mu g/ml)$. Ag NPs completely inhibited egg hatchability on three vectors at 60, 75, and 90 µg/ml, respectively. In adulticidal assays, LD₅₀ values were 18.66, 20.94, and 22.63 µg/ml. If compared to mosquito larvae, Ag NPs were safer to three non-target aquatic biocontrol agents, with LC₅₀ ranging from 684 to 2245 µg/ml. Overall, our study highlights the potential of A. caesia as an abundant and cheap bioresource to fabricate biogenic Ag NPs effective against mosquito young instars



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and adults, with moderate impact on non-target aquatic biocontrol agents.

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Plant-borne compounds and nanoparticles: challenges for medicine, parasitology and entomology Published: 05 December 2016

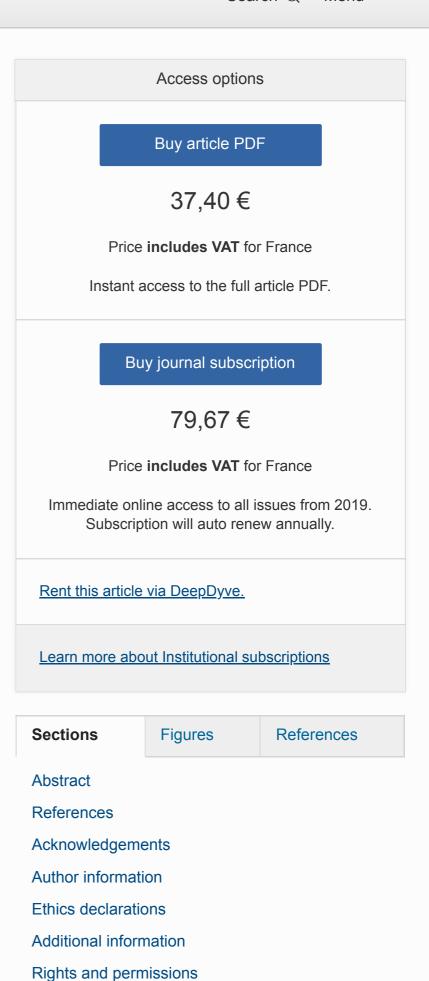
Towards green oviposition deterrents? Effectiveness of *Syzygium lanceolatum* (Myrtaceae) essential oil against six mosquito vectors and impact on four aquatic biological control agents

<u>Giovanni Benelli</u> 🖂, <u>Mohan Rajeswary</u> & <u>Marimuthu Govindarajan</u> 🖂

<u>Environmental Science and Pollution Research</u> 25, 10218–10227(2018) | <u>Cite this article</u>
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Abstract

Essential oils (EOs) from plants may be alternative sources of molecules toxic against mosquito vectors of public health relevance. Most of researches in this field focused on EOs as larvicides or ovicides, while limited efforts focused on the exploitation of EOs as oviposition deterrents. In the present study, the larvicidal and oviposition deterrent activity of Syzygium lanceolatum leaf EO was evaluated against six mosquito species, Anopheles stephensi, An. subpictus, Aedes aegypti, Ae. albopictus, Culex quinquefasciatus, and Cx. tritaeniorhynchus. The chemical composition of the S. lanceolatum EO was analyzed by GC-MS analysis, showing the presence of phenyl propanal, β -caryophyllene, α -humulene, and caryophyllene oxide as major constituents. S. lanceolatum EO showed high acute toxicity on An. stephensi (LC₅₀ = 51.20 μ g/ml), Ae. aegypti (LC₅₀ = 55.11 μ g/ml), Cx. quinquefasciatus $(LC_{50} = 60.01 \ \mu g/ml)$, An. subpictus $(LC_{50} = 61.34 \ \mu g/ml)$, Ae. albopictus $(LC_{50} = 66.71 \ \mu g/ml)$, and *Cx. tritaeniorhynchus* (LC₅₀ = 72.24 μ g/ml) larvae. Furthermore, the EO was effective as oviposition deterrent against the six tested mosquito species, with OAI on An. stephensi, An. subpictus, Ae. aegypti, Ae. albopictus, Cx. quinquefasciatus, and Cx. tritaeniorhynchus reaching -0.83, -0.81, -0.84, -0.83, -0.84, and -0.86, respectively. The toxicity of S. lanceolatum EO against several biological control agents of mosquitoes, including water bugs (Anisops bouvieri and Diplonychus indicus) and fishes (Gambusia affinis and Poecilia reticulata), was extremely low, with LC₅₀ ranging between 4148 and 15,762 µg/ml. Overall, our results pointed out the promising potential of the S. lanceolatum leaf EO as a source of environmental-friendly oviposition deterrents and larvicides effective against a wide number



of mosquito species of importance for parasitology.

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ZnO NPs nanoparticles showed high antibiofilm activity on Gram-

• They were toxic to larvae of *Aedes aegypti* mosquitoes, which vector

■ Table 1

Abstract

Penaeus semisulcatus.

Zika virus.

positive and negative bacteria.

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The green synthesis of metal nanoparticles using phytochemical from marine seaweeds is a fast-growing research field in nanotechnology. Here, the biosynthesis of zinc oxide nanoparticles was achieved using the hot water extract of Sargassum wightii. The hot water extract prepared from S. wightii (H Sw) and ZnO NPs were studied by UV-visible and FTIR spectroscopy, SEM and XRD. Then, both products were evaluated for antibiofilm activity towards aquatic pathogens. The nanoparticles' immunostimulating potential on green tiger prawns, *Penaeus* semisulcatus was studied through immersion and dietary administration. Shrimp immune parameters (i.e., total hemocytes count (THC), respiratory bursts (RBs), phenoloxidase (PO) and superoxide dismutase (SOD) activity) were significantly affected by exposure or ingestion of ZnO nanoparticles. In addition, the hot water extract and ZnO nanoparticles had high antibiofilm activity against Gram-positive (B. subtilis, S. aureus) and Gramnegative (S. sonnei, P. aeruginosa) microbial pathogens. It was accomplished that the ZnO nanoparticles can be used as the bacteriostatic and immunostimulant agents through immersion and dietary administration enhancing immunity of green tiger shrimp. Furthermore, the toxicity effects of ZnO nanoparticles were 100% at 24 h on Aedes aegypti 3 rd instar larvae at the concentration of 100 µg/mL and the greatest efficacy was accomplished by H Sw ZnO NPs against the Ae. aegypti after 24 h (LC₅₀ 49.22; LC₉₀ 86.96 mg/mL), if compared to the seaweed extract alone. Morphological and histological damages triggered by nanoexposure were investigated.

Graphical Abstract



Keywords

Crustaceans; Antibiofilm activity; Dengue; Larvicides; Microbial pathogens; Zika virus

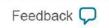
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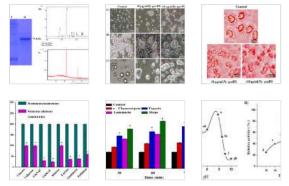
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Identification, characterization and immune

response of prophenoloxidase from the blue

Sangily Jayanthi ^a, Baskaralingam Vaseeharan ^a $\stackrel{ ineq}{\sim}$ $\stackrel{ ineq}{\sim}$, Ramachandran Ishwarya ^a, Sivashanmugam Karthikeyan ^b, Marimuthu Govindarajan ^{c, d}, Naiyf S. Alharbi ^e, Shine Kadaikunnan ^e, Jamal M. Khaled ^e, Csaba Vágvölgyi

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antibiofilm activity

Prophenoloxidase is a conserved Cu-containing enzyme acting as a major defense molecule in the immune response of crustaceans. In the present research, we purified prophenoloxidase from the haemolymph of Portunus pelagicus (Pp-proPO) by Blue Sepharose CL-6B chromatography. Pp-proPO exhibited only one band with molecular weight of 75 kDa on SDS-PAGE. The purified Pp-proPO was characterized through X-ray diffraction (XRD) and high-performance liquid chromatography (HPLC). Pp-proPO showed phagocytic activity on the yeast Saccharomyces cerevisiae as well as encapsulation on sepharose CL-6B beads associated with CM sepharose and beads of sodium alginate. Pp-proPO also led to strong agglutination on human erythrocytes. Furthermore, *Pp*-proPO showed magnified PO activity when altered with activated particles acting as pathogen combined molecular patterns (PAMPs), metal ions or other chemicals. Pp-proPO showed relevant antibiofilm activity on Gram negative bacteria Pseudomonas aeruginosa and Escherichia coli. Overall, the above results allowed us to claim that *Pp*-proPO play a key role in immune defense mechanisms of P. pelagicus crabs, in particular towards microbial pathogens; notably we added basic information to the functional characterization of *Pp*-proPO, as well as to understand its immunological role in crustaceans defense systems.



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Keywords

Antibiofilm activity; Functional analysis; Portunus pelagicus

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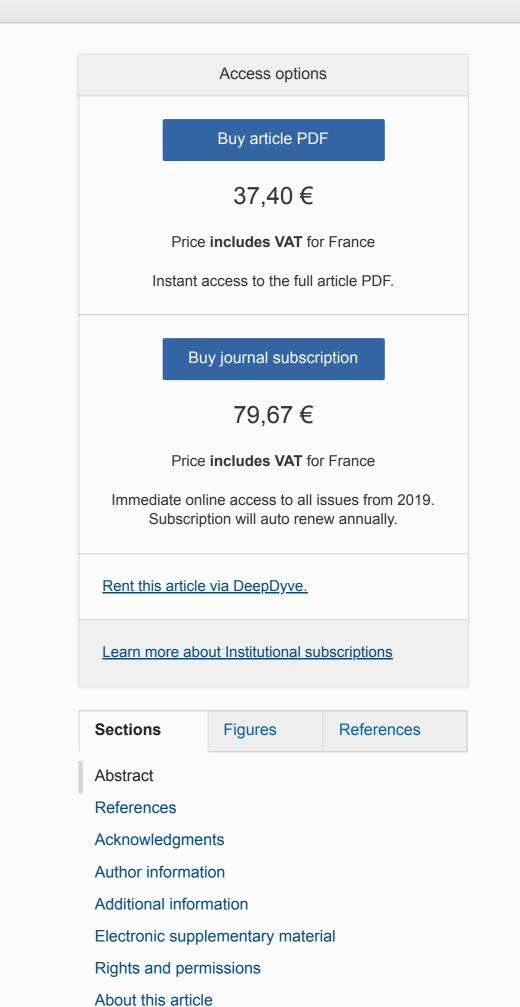
Structural characterization of *Bacillus licheniformis* Dahb1 exopolysaccharide—antimicrobial potential and larvicidal activity on malaria and Zika virus mosquito vectors

Muthukumar Abinaya, Baskaralingam Vaseeharan ⊡, Mani Divya, Sekar Vijayakumar, Marimuthu Govindarajan, Naiyf S. Alharbi, Jamal M. Khaled, Mohammed N. Al-anbr & Giovanni Benelli
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Abstract

Microbial polysaccharides produced by marine species play a key role in food and cosmetic industry, as they are nontoxic and biodegradable polymers. This investigation reports the isolation of exopolysaccharide from *Bacillus licheniformis* Dahb1 and its biomedical applications. *Bacillus licheniformis* Dahb1 exopolysaccharide (*Bl*-EPS) was extracted using the ethanol precipitation method and structurally characterized. FTIR and ¹H-NMR pointed out the presence of various functional groups and primary aromatic compounds, respectively. *Bl*-EPS exhibited strong antioxidant potential confirmed via DPPH radical, reducing power and superoxide anion scavenging assays. Microscopic analysis revealed that the antibiofilm activity of *Bl*-EPS (75 µg/ml) was higher against Gram-negative (*Pseudomonas aeruginosa* and *Proteus vulgaris*) bacteria over Gram-positive species (*Bacillus subtilis* and *Bacillus pumilus*). *Bl*-EPS led to biofilm inhibition against *Candida albicans* when tested at 75 µg/ml. The hemolytic assay showed low cytotoxicity of *Bl*-EPS at 5 mg/ml. Besides, *Bl*-EPS achieved LC₅₀ values < 80 µg/ml against larvae of mosquito vectors *Anopheles stephensi* and *Aedes aegypti*. Overall, our findings pointed out the multipurpose bioactivity of *Bl*-EPS, which deserves further consideration for pharmaceutical, environmental and entomological applications.

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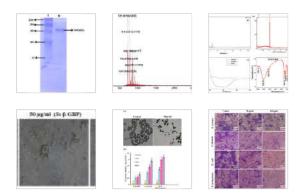
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International Journal of Biological Macromolecules Volume 114, 15 July 2018, Pages 864-873

Phenoloxidase activation, antimicrobial, and antibiofilm properties of β -glucan binding protein from *Scylla serrata* crab hemolymph

Mani Divya ^a, Baskaralingam Vaseeharan ^a ^A [⊠], Mahalingam Anjugam ^a, Arokiadhas Iswarya ^a, Sivashanmugam Karthikeyan ^b, Palaniyandi Velusamy ^c, Marimuthu Govindarajan ^{d, e}, Naiyf S. Alharbi ^f, Shine Kadaikunnan ^f, Jamal M. Khaled ^f, Csaba Vágvölgyi ^{f, g}

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Highlights		Article Metrics
• β-Glucan binding protein (β-GBP) from Scylla serve	ata was purified	Citations
and characterized.		

- Functional analysis included both PO activity and encapsulation assays.
- MIC was estimated on microbial pathogens, along with live and dead assay.
- High antibiofilm activity of β -GBP was found against microbial pathogens.
- β -GBP could be used to develop antibiofilm drugs in current biomedical science.

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Abstract

In this study, we purified β -GBP from hemolymph of *Scylla serrata* crabs using affinity chromatography. The purified *S. serrata* β -GBP (*Ss*- β -GBP) had 100 kDa molecular mass in the SDS-PAGE. MALDI-TOF/TOF analysis was conducted,

revealing that the purified 100 kDa protein had 96% similarity with β -GBP of Astacus leptodactylus. Ss- β -GBP was characterized using high-performance liquid chromatography (HPLC), X-ray diffraction (XRD) analysis, circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopy, which confirmed the structure of the Ss- β -GBP. The purified Ss- β -GBP was functionally analyzed by yeast agglutination and phagocytic reaction assays. Moreover, the PO enhancing ability of $Ss-\beta$ -GBP was evidenced through PO activity. Specifically, the antibacterial activity of the $Ss-\beta$ -GBP against Grampositive (Enterococcus faecalis and Staphylococcus aureus) and Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacteria was evaluated by determining its minimum inhibitory concentration (MIC) < 60 µg/ml for all tested species. Furthermore, the antibiofilm efficacy of Ss- β -GBP at 50 and 100 µg/ml was outlined using light microscopy and confocal laser scanning microscopy (CLSM). Bacterial viability assays also outlined the dose-dependent activity of Ss- β -GBP based on the ratio of live/dead bacterial cells. The results of this study revealed that crab-borne Ss- β -GBP might be widely used to suppress the growth of pathogenic bacteria.



Next article >

Keywords

Arthropods; β -Glucan binding protein; Crabs; Encapsulation; Live and dead assay; SDS-PAGE

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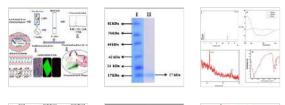
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Searching for crab-borne antimicrobial

peptides: Crustin from Portunus pelagicus

triggers biofilm inhibition and immune

Vibrio parahaemolyticus Dahv2

through in vivo and in vitro analysis.

treatment with *Pp*-Cru.

crustacean.

observed against key bacterial pathogens.

https://doi.org/10.1016/j.molimm.2018.07.024

Highlights

responses of Artemia salina against GFP tagged

Ravichandran Rekha ^a, Baskaralingam Vaseeharan ^a \approx \boxtimes , Ramachandran Ishwarya ^a, Mahalingam Anjugam ^a,

Naiyf S. Alharbi^b, Shine Kadaikunnan^b, Jamal M. Khaled^b, Mohammed N. Al-anbr^b, Marimuthu

Purification of crustin (Pp-Cru) from Portunus pelagicus was

We reported an enhancement of the immune system by *Pp*-Cru

Synergic antibacterial and antibiofilm properties of *Pp*-Cru were

Live and dead assay showed ultra-low bacterial cell viability post-

In vivo study of crustin was assessed with Artemia salina as a model

characterized by HPLC, CD, XRD, and FTIR analyses.

Molecular Immunology Volume 101, September 2018, Pages 396-408



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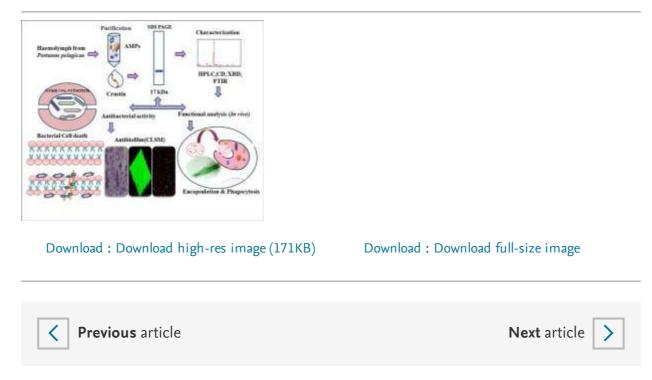
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Abstract

Marine organisms represent a huge source of novel compounds for the development of effective antimicrobial drugs. The present study focus on the purification of the antimicrobial peptide crustin from the haemolymph of the blue swimmer crab, Portunus pelagicus, by blue Sepharose CL-6B matrix assisted affinity column chromatography. Crustin showed a single band with a molecular mass of 17 kDa in SDS-PAGE analysis. The XRD analysis exhibited peaks at 32° and 45° while a distinct peak with a retention time of 1.8 min resulted in high performance liquid chromatography (HPLC) pointing out the crystalline nature and purity of crustin, respectively. Crustin purified from P. pelagicus (Pp-Cru) showed immunological activities, triggering encapsulation, phagocytosis on Sepharose beads and yeast (Saccharomyces cerevisiae) respectively. Furthermore, encapsulation of GFP tagged V. parahaemolyticus in Artemia salina and challenging study were assessed under CLSM and the potential of *Pp*-Cru was examined *in vivo*. In addition, the growth reduction and biofilm inhibition potential of Pp-Cru on Staphylococcus aureus, Enterococcus faecalis (Gram- positive bacteria) and Pseudomonas aeruginosa, Escherichia coli (Gram-negative bacteria) was evidenced by inverted and confocal laser scanning microscopic analysis, revealing that 100 µg/ml of Pp-Cru can disrupt the biofilm matrix thereby the thickness of biofilm was significantly reduced. Overall, the present investigation might provide a sensitive platform to realize the significant function of *Pp*-Cru in crustacean immune mechanism as well as its potential to bacterial growth inhibitor. The functional properties of purified *Pp*-Cru antimicrobial peptide may lead to a superior understanding of innate immune response in *P. pelagicus* species, which suggest the promising application for drug development in aquaculture.

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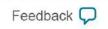
In vivo; Antibacterial; Antibiofilm; Crustin; Haemolymph; Portunus pelagicus

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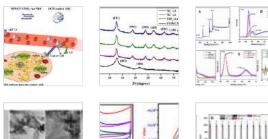
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nanotubes for targeted drug delivery

Viswanathan Karthika ^a, Periyannan Kaleeswarran ^a, Kasi Gopinath ^b, Ayyakannu Arumugam ^b 으 쯔, Marimuthu Govindarajan ^{c, d}, Naiyf S. Alharbi ^e, Jamal M. Khaled ^e, Mohammed N. Al-anbr ^e, Giovanni

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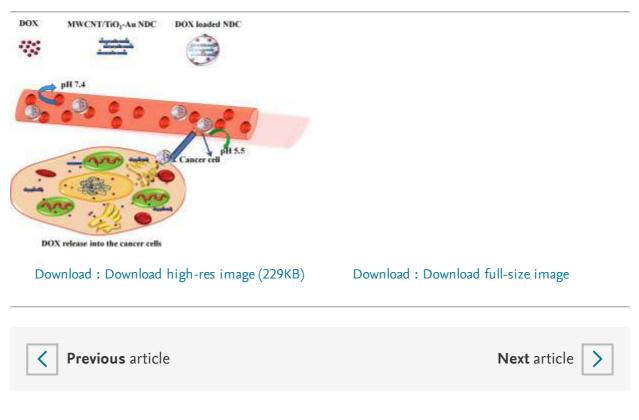
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 Highlights We fabricated novel biocompatible carbon nanwith TiO₂-Au nanocomposite (NC). Biocompatibility studies were done on human zebrafish embryos. High antimicrobial and antibiofilm activities volume 0.45 mg/mL. 	blood cells and were observed.	Article Metrics Citations Citation Indexes: Captures Readers: Social Media Tweets:	10 59 1 View details

ADSTRACT

Nanomaterial-based drug carriers have become a hot spot of research at the interface of nanotechnology and biomedicine because they allow efficient loading, targeted delivery, controlled release of drugs, and therefore are promising for biomedical applications. The current study made an attempt to decorate the multiwalled carbon nanotubes (MWCNT) with titanium dioxide-gold nanoparticles in order to enhance the biocompatibility for doxorubicin (DOX) delivery. The successful synthesis of nano drug carrier (NDC) was confirmed by XRD, XPS and UV–Visible spectroscopy. FESEM and TEM revealed that the morphology of NDC can be controlled by manipulating the reaction duration, MWCNT concentration and TiO₂-Au source concentration. Results showed that TiO₂ and Au nanoparticles were well coated on MWCNT. NDC had finely tuned biocompatible properties, as elucidated by hemolytic and antimicrobial assays. NDC also showed a high antioxidant potential, 80.7% expressed as ascorbic acid equivalents. Commercial DOX drug was utilized to treat A549 and MCF7 cancer cell lines showing improved efficiency by formulating it with NDC, which selectively delivered at the pH 5.5 with drug loading capacity of 0.45 mg/mL. The drug releasing capacity achieved by NDC was 90.66% for 10 h, a performance that far encompasses a wide number of current literature reports.

Graphical abstract



Keywords

Anticancer drugs; Antimicrobial activity; Doxorubicin; Green synthesis; Nanoparticle characterization

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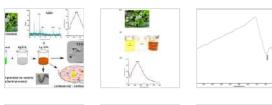
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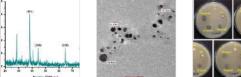
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Nanosilver crystals capped with Bauhinia acuminata phytochemicals as new antimicrobials and mosquito larvicides

Naiyf S. Alharbi ^a 은 쯔, Marimuthu Govindarajan ^{b, c} 은 쯔, Shine Kadaikunnan ^a, Jamal M. Khaled ^a, Taghreed N. Almanaa ^a, Sami A. Alyahya ^d, Mohammed N. Al-anbr ^a, Kasi Gopinath ^e, Arumugam Sudha ^f

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https://doi.org/10.1016/j.jtemb.2018.06.016

Highlights

- AgNPs fabricated with Bauhinia acuminata extract was studied for • entomological and biomedical purposes.
- AgNPs were characterized by UV–vis, FTIR, XRD and TEM.
- AgNPs exhibited high antimicrobial activity. •
- Larvicidal toxicity against malaria, Zika virus and filariasis vectors • was reported.

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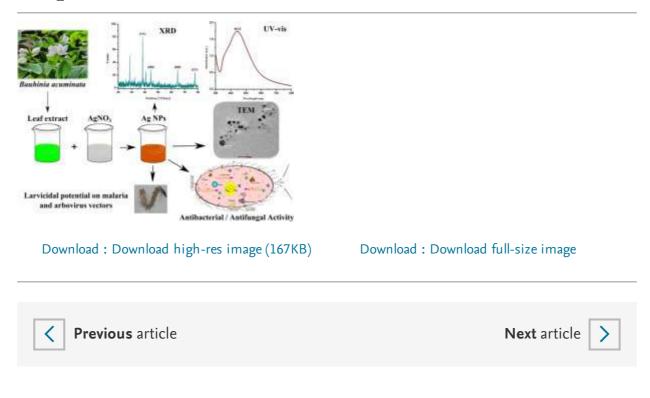
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Abstract

To develop novel nanoformulated insecticides and antimicrobials, herein we produced Ag nanoparticles (AgNPs) using the Bauhinia acuminata leaf extract. This unexpensive aqueous extract acted as a capping and reducing agent for the formation of AgNPs. We characterized *B. acuminata*-synthesized AgNPs by UV-vis and FTIR spectroscopy, XRD and TEM analyses. UV-vis spectroscopy analysis of B. acuminata-synthesized AgNPs showed a peak at 441.5 nm. FTIR shed light on functional groups from the phytoconstituents involved in nanosynthesis. XRD of B. acuminata-synthesized AgNPs suggested a facecentered cubic structure, with a highly crystalline nature. TEM of B. acuminatasynthesized AgNPs revealed mean size of 25 nm, with round shape. AgNPs tested at $60 \,\mu\text{g/mL}$ inhibited the growth of 5 bacteria and 3 fungal pathogens. In the insecticidal assays on important mosquito species, LC₅₀ of the aqueous extract of B. acuminata leaves on the larvae of Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus were 204.07, 226.02, and 249.24 µg/mL, respectively. The B. acuminata-synthesized AgNPs exhibited higher larvicidal efficacy, with LC_{50} values of 24.59, 27.19, and 30.19 $\mu g/mL$, respectively. Therefore, herein we developed a single-step, reliable, inexpensive, and environmentally non-toxic synthesis process to obtain AgNPs with high bioactivity against pathogens and vectors. Given the effective antimicrobial and larvicidal activity, nanoparticles fabricated using plant extracts and extremely low concentrations of trace elements, such as silver, can be exploited for multipurpose activities. Our results pointed out that B. acuminata-synthesized AgNPs have a promising potential in antimicrobial food packaging, as well as a foliar spray to control plant pathogens in the field, and to synergize the efficacy of fungicidal and larvicidal formulations.

Graphical abstract



Keywords

Antibacterial activity; Antifungal activity; Insecticides; Mosquito control; Pesticides

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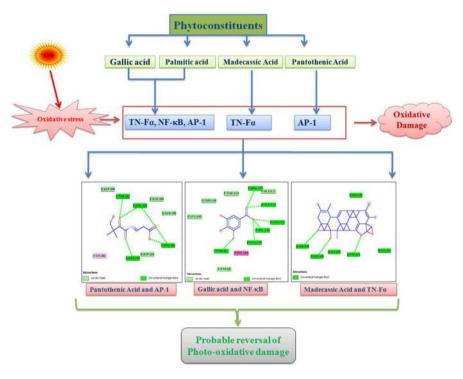
Molecular docking of selected phytoconstituents with signaling molecules of Ultraviolet-B induced oxidative damage

Umar Muzaffer¹ · V. I. Paul¹ · N. Rajendra Prasad²

Received: 13 May 2017 / Accepted: 6 November 2017 © Springer-Verlag GmbH Germany, part of Springer Nature 2017

Abstract

The signaling molecules TNF- α , AP-1, and NF- κ B act to integrate multiple stress signals into a series of diverse antiproliferative responses. Disruption of these processes can promote tumor progression and chemoresistance. Naturally occurring plant derived compounds are considered as attractive candidates for cancer treatment and prevention. Phytoconstituents can control and modify various biological activities by interacting with molecules involved in concerned signaling pathways. The aim of this study was to find binding conformations between phytoconstituents and these signaling molecules responsible for multiple stress signals of UVB induced photodamage. Induced fit docking was carried out for understanding the binding interactions of pantothenic acid (vitamin B5); 3,4,5-trihydroxy benzoic acid (gallic acid); madecassic acid and hexadecanoic acid, ethyl ester (palmitic acid) with TNF- α , AP-1, and NF- κ B. Favorable binding conformations between these signaling molecules and the four phytoconstituents were observed. A number of poses were generated to evaluate the binding conformations and common interacting residues between the ligands and proteins. Among them, the best ligands against TNF- α , AP-1, and NF- κ B are reported. The present investigation strongly suggests the probable use of these flavonoids for the amelioration of UVB induced photodamage. **Graphical abstract**



Keywords Oxidative damage \cdot Docking \cdot Flavonoids \cdot TNF- α \cdot AP-1 \cdot NF- κ B

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Process Safety and Environmental Protection

Volume 106, February 2017, Pages 23-33

Coleus aromaticus leaf extract fractions: A source

of novel ovicides, larvicides and repellents

against Anopheles, Aedes and Culex mosquito

Mathalaimuthu, Baranitharan ^a, Shanmugam, Dhanasekaran ^{a, b}, Kalimuthu Kovendan ^c $\stackrel{ heta}{\to}$ Kadarkarai,



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 We studied the toxicity of Coleus aromaticus extract of 	on 3 mosquito	Citation Indexes:	7
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• Methanol fraction 4 showed high toxicity on A. ace	gypti, A. stephensi	Exports-Saves:	10
and C. quinquefasciatus.		Readers:	22
• 100% mortality was achieved by 40 ppm of the methon mosquito eggs.	hanol fraction 4	ÖPLUMX	View details
Mothernal fraction 4 tested at 2.5 mg/cm^2 repairing	auita adulta far		

- Methanol fraction 4 tested at 2.5 mg/cm² repel mosquito adults for • at least 320 min.
- GC–MS showed 9 compounds in the methanol extract, the main • one was 11-octadecenoic acid, methyl ester.

Abstract

Eco-friendly tools to manage mosquito young instar populations in an IPM framework are urgently required. Here, we analyzed six ethyl acetate and

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Highlights

Abstract

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Keywords

1. Introduction

2. Materials and methods

3. Results

4. Discussion

5. Conclusions

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References

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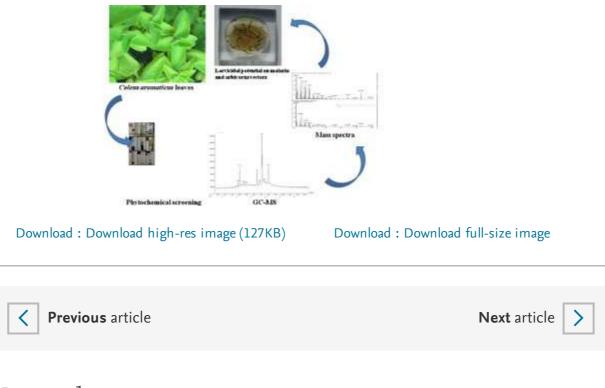
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methanol fractions of Coleus aromaticus leaf extract using thin layer chromatography and GC–MS, in order to shed light on the main chemical constituents with toxicity on mosquitoes. The fractions were tested as ovicides, larvicides and repellents against the mosquito vectors Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. In GC–MS analysis, a total of nine compounds were identified in the methanolic extract composition, the main component was 11-octadecenoic acid, methyl ester. The highest larvicidal activity was observed for the methanol fraction 4 against Ae. aegypti, An. stephensi and Cx. quinquefasciatus with LC_{50} values of 23.90, 22.32 and 20.51 ppm. In the ovicidal experiments, 100% mortality was exterted by methanol fraction 4 tested at 40 ppm against Ae. aegypti, An. stephensi and Cx. quinquefasciatus. Furthermore, high repellence of methanol fraction 4 tested at 2.5 mg/cm^2 was observed in "arm in cage" tests for at least 320 min. We hypothesized that 11-octadecenoic acid, methyl ester was the main constituent responsible for the mosquitocidal and repellent activity of C. aromaticus fractions.

Graphical abstract



Keywords

Acute toxicity; Aedes aegypti; Anopheles stephensi; Culex quinquefasciatus; GC–MS; Thin layer chromatography

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ORIGINAL PAPER



One-Pot Synthesis of Dysprosium Oxide Nano-Sheets: Antimicrobial Potential and Cyotoxicity on A549 Lung Cancer Cells

Kasi Gopinath¹ · Mari Chinnadurai² · Natarajan Parimala Devi¹ · Kasi Bhakyaraj³ · Shanmugasundaram Kumaraguru³ · Tamilvanan Baranisri² · Arumugam Sudha⁴ · Mohammed Zeeshan⁵ · Ayyakannu Arumugam⁶ · Marimuthu Govindarajan⁷ · Naiyf S. Alharbi⁸ · Shine Kadaikunnan⁸ · Giovanni Benelli⁹ Received: 9 December 2016 © Springer Science+Business Media New York 2016

Abstract The present work deals with green synthesis of dysprosium oxide nanosheets (Dy_2O_3 NS) using *Syzygium travancoricum* leaf extract. Synthesized NS were characterized by UV–VIS–DRS, ATR-FT-IR, PL, Raman spectroscopy, XRD, TEM with EDX and XPS analysis. UV–VIS–DRS analysis showed the absorption peak at 355 nm and band gap value of 4.8 eV. ATR-FT-IR analysis was performed to analyze the biomolecules responsible for formation of Dy_2O_3 NS. Micro-Raman analysis showed a peak at 373 cm⁻¹. XRD confirmed the presence of Dy_2O_3 NS with body-centered cubic structure. TEM images revealed the presence of nano sheets with size ranging from 100 to 200 nm and EDX analysis showed Dy and O elements and no other elements were detected, which indicates the purity of the

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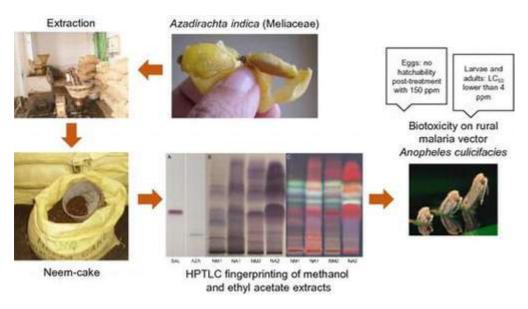
Neem cake as a promising larvicide and adulticide against the rural malaria vector *Anopheles culicifacies* (Diptera: Culicidae): a HPTLC fingerprinting approach

Giovanni Benelli S, Balamurugan Chandramohan, Kadarkarai Murugan, Pari Madhiyazhagan, Kalimuthu Kovendan, Chellasamy Panneerselvam, ...show all Pages 1185-1190 | Received 07 Jun 2016, Accepted 11 Jul 2016, Published online: 24 Aug 2016

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Abstract

Mosquitoes are insects of huge public health importance, since they act as vectors for important pathogens and parasites. Here, we focused on the possibility of using the neem cake in the fight against mosquito vectors. The neem cake chemical composition significantly changes among producers, as evidenced by our HPTLC (High performance thin layer chromatography) analyses of different marketed products. Neem cake extracts were tested to evaluate the ovicidal, larvicidal and adulticidal activity against the rural malaria vector *Anopheles culicifacies*. Ovicidal activity of both types of extracts was statistically significant, and 150 ppm completely inhibited egg hatching. LC₅₀ values were extremely low against fourth instar larvae, ranging from 1.321 (NM1) to 1.818 ppm (NA2). Adulticidal activity was also high, with LC₅₀ ranging from 3.015 (NM1) to 3.637 ppm (NM2). This study pointed out the utility of neem cake as a source of eco-friendly mosquitocides in Anopheline vector control programmes.



Keywords: Anopheline, arbovirus, eco-friendly insecticides, mosquito vectors, ovicidal activity

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ORIGINAL PAPER



Swift Fabrication of Silver Nanoparticles Using Bougainvillea glabra: Potential Against the Japanese Encephalitis Vector, Culex tritaeniorhynchus Giles (Diptera: Culicidae)

Savariar Vincent¹ · Kalimuthu Kovendan² · Balamurugan Chandramohan² · Siva Kamalakannan² · Palanisamy Mahesh Kumar² · Chellamuthu Vasugi³ · Cheruparambath Praseeja⁴ · Jayapal Subramaniam² · Marimuthu Govindarajan⁵ · Kadarkarai Murugan² · Giovanni Benelli⁶

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Abstract Mosquitoes are the most critical group of insects in the context of public health, since they transmit key parasites and pathogens, causing millions of deaths annually. *Culex tritaeniorhynchus* is an important vector of Japanese encephalitis (JE) across urban and semi-urban areas of Asia. In this study, we bio-fabricated silver nanoparticles (Ag NP) using the leaf extract of *Bougainvillea glabra* as reducing and stabilizing agent. The synthesis of Ag NP was confirmed analyzing the excitation of surface Plasmon resonance using ultraviolet–visible (UV–vis) spectrophotometry. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) showed the clustered and irregular shapes of Ag NP. The presence of silver was determined by energy dispersive X-ray (EDX) spectroscopy.

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Original Paper | Published: 28 November 2016

Gum-Mediated Fabrication of Eco-Friendly Gold Nanoparticles Promoting Cell Division and Pollen Germination in Plant Cells

Naiyf S. Alharbi, Kasi Bhakyaraj, Kasi Gopinath 🖂, Marimuthu Govindarajan, Shanmugasundaram Kumuraguru, Subramanian Mohan, Periyannan Kaleeswarran, Shine Kadaikunnan, Jamal M. Khaled & Giovanni Benelli

Journal of Cluster Science 28, 507–517(2017) | Cite this article 214 Accesses 9 Citations Metrics

Abstract

In this study we reported a simple approach for the green synthesis of gold nanoparticles (AuNPs) using the *Terminalia arjuna* gum extract as a source of reducing and stabilizing compounds. The fabrication of AuNPs was confirmed by UV-Vis spectroscopy, XRD and TEM. UV–Vis spectrum clearly showed the absorption peak at 520 nm, which is due to the surface Plasmon resonance on AuNPs. XRD analysis highlighted the crystalline nature of AuNPs, with face-centered cubic structure. TEM showed that spherical shape of nanoparticles, with average particles size of 10-20 nm. AuNPs did not show any inhibitory activity both on Gram-positive and Gram-negative bacteria. Furthermore, different doses of AuNPs (0, 100, 500 and 1000 μ M) were tested to assess their potential impact on mitotic division and pollen germination. A treatment with 100 µM of AuNPs significantly induced both mitotic division and pollen germination. In the plant cells, the passive uptake of AuNPs through cytosol allowed them to move towards the nuclear pore complex. Subsequently, they interacted with histone protein leading to mitotic cell division and pollen germination. Overall, our study highlighted the potential of gum-fabricated AuNPs in cytogenetic, gene expression and plant tissue culture applications.

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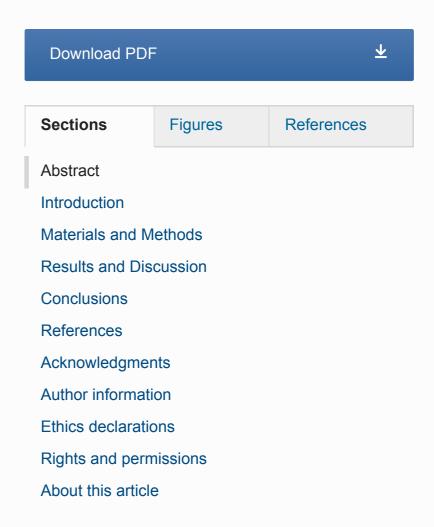
One Pot Green Synthesis of Colloidal Silver Nanocrystals Using the Ventilago maderaspatana Leaf Extract: Acute Toxicity on Malaria, Zika Virus and Filariasis Mosquito Vectors

Raja Mohamed Sait Thameem Azarudeen, Marimuthu Govindarajan , Mashael Marzouq AlShebly, Fatma Saeed AlQahtani, Abubucker Amsath & Giovanni Benelli 🖂

Journal of Cluster Science 28, 369–392(2017) Cite this article 1163 Accesses 6 Citations Metrics

Abstract

Mosquitoes act as a vector for most of the life-threatening diseases. The green synthesis of ecofriendly metal nanoparticles is a fast-growing branch of current nanoscience with a number of biomedical and entomological implications. Here, we have synthesized silver nanoparticles (AgNPs) by using a poorly studied Indian plant, Ventilago maderaspatana. The results recorded from UV–Visible spectrophotometry, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), atomic force microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) confirmed the quick synthesis of AgNPs. The toxic activity of V. maderaspatana leaf extract and biosynthesized AgNPs was evaluated against the malaria vector *Anopheles stephensi*, the Zika virus vector Aedes aegypti and the filariasis vector *Culex quinquefasciatus*. Compared to the leaf aqueous extract, AgNPs showed higher toxicity against A. stephensi, A. aegypti, and C. $\mathit{quinquefasciatus}$ with LC_{50} values of 24.89, 26.92 and 29.24 $\mu g/mL$, respectively. A single treatment with AgNPs tested at 120, 150 and 180 μg/mL led to no egg hatchability. In adulticidal experiments, the maximum efficacy was observed on A. stephensi (LD₅₀ = 41.19 μ g/mL), followed by A. *aegypti* (LD₅₀ = 44.85 μ g/mL) and C. *quinquefasciatus* $(LD_{50} = 48.94 \mu g/mL)$, respectively. V. maderaspatana extract and green fabricated AgNPs were found safer to non-target aquatic biocontrol organisms Anisops bouvieri, Diplonychus indicus and Gambusia affinis, with LC_{50} ranging from 1673 to 41,854 µg/ml.



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Introduction

Mosquitoes transmit more diseases than any other group of arthropods affecting millions of people throughout the world. Mosquito vectors are solely responsible for transmitting diseases such as malaria, dengue, chikungunya, Japanese encephalitis, lymphatic filariasis and, very recently, Zika virus [1]. Vector control is an essential requirement in the management of epidemic diseases that are transmitted by mosquitoes. Notably, the excessive use of synthetic pesticides causes emergence of resistance in treated pests and also lead to harmful effect on non-target organisms [2]. This has required an urgent search for development of new and improved mosquito control methods that are cheap, effective and safe for human health and the environment [3-7].

Noble metal nanoparticles have received attention over the last few years, as they can be used in medicine, biology, material science, physics, and chemistry [8]. Among the several noble metal nanoparticles, silver nanoparticles (AgNPs) have attracted special attention due to their properties, which include favorable electrical conductivity, chemical stability, catalytic and antibacterial activity [9, 10]. The green synthesis of AgNPs has been accomplished using plants, microorganisms, and biopolymers [9]. The use of plant-borne products for nanoparticle synthesis offers a wide range of benefits over other biological synthesis methods because it does not require the maintenance of cell cultures and incorporates support for the large-scale synthesis of nanoparticles [11]. The extracellular nanoparticle synthesis, which utilizes extracts different plant parts, is often inexpensive due to easier downstream processing [<u>12</u>–<u>16</u>].

Nowadays, a growing number of plants have been screened successfully for nanosynthesis of silver-based mosquitocides, including Sida acuta [17], Barleria cristata [18], Chomelia asiatica [19], Gmelina asiatica [20], Feronia elephantum [21], and Heliotropium indicum [22]. Phyto-synthesized AgNPs have been recently proposed as effective mosquito larvicides, and are gaining importance over synthetic chemical pesticides because of their reduced harmful effects to non-targeted species and novelty in the mechanism(s) of action $[\underline{12}-\underline{14},\underline{23},\underline{23},\underline{14},\underline{23},\underline{14},\underline{23},\underline{14},\underline{23},\underline{14},\underline{23},\underline{14},\underline{14},\underline{23},\underline{14}$ <u>24</u>].

Ventilago maderaspatana is a medicinal herb belonging to family Rhamnaceae. It is distributed in Forests of low elevations-South Greece, India, Indonesia, Myanmar, Sri-Lanka. It is commonly known as red creeper. This plant is a woody climber. Traditionally, the root bark of V. maderaspatana is used as a carminative, stomachic, vitiated conditions of kapha, dyspepsia, colic flatulence, erysipelas, leprosy, scabies, and pruritus. The powdered stem bark

mixed with gingerly oil is applied externally to treat skin diseases and itch [25]. Apart from

these, antifeedant activity of V. maderaspatana on insects was also reported [26]. The

phytochemical reports on the root bark of V. maderaspatana show the presence of various anthraquinones, including ventinone-A.B, chrysophanol, physcion, emodin, islandicin, xanthorin and xanthorin-5-methyl ether [27]. The plant extracts act as an antibacterial agent [28], and also shows anti-denaturation and antioxidant properties [29]. The stem bark of V. maderaspatana was found to possess anti inflammatory and anticancer activities [30].

In this research, we proposed a cheap and rapid method of biosynthesis of poly-dispersed AgNPs using the aqueous leaf extract of V. maderaspatana. Bio-reduced AgNPs were characterized by UV-Vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), atomic force microscopy (AFM), scanning electron

Original Paper | Published: 25 August 2016

Single-Step Biofabrication of Silver Nanocrystals Using *Naregamia alata*: A Cost Effective and Eco-Friendly Control Tool in the Fight Against Malaria, Zika Virus and St. Louis Encephalitis Mosquito Vectors

Raja Mohamed Sait Thameem Azarudeen, Marimuthu Govindarajan , Abubucker Amsath, Udaiyan Muthukumaran & Giovanni Benelli

Journal of Cluster Science **28**, 179–203(2017) | <u>Cite this article</u> **1275** Accesses | **12** Citations | <u>Metrics</u>

Abstract

Botanical-based nanosynthesis has been recently reported as a cheap alternative for mosquito management. Screening different botanicals as reducing and capping agents led to the production of metal nanoparticles with different biophysical and mosquitocidal features. Here, *Naregamia alata*-mediated biosynthesis of silver nanoparticles (AgNPs) was conducted. AgNPs were tested on egg, larval and adult populations of three important mosquito vectors, Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. AgNPs were characterized using UV–Vis spectrophotometry, FTIR, AFM, SEM, TEM, EDX, and XRD analyses. Maximum larvicidal toxicity was detected against A. stephensi ($LC_{50} = 12.40 \mu g/ml$), followed by *A. aegypti* (LC₅₀ = 13.57 μ g/ml) and *C. quinquefasciatus* (LC₅₀ = 14.84 μ g/ml). A single treatment with AgNPs tested at 60, 75 and 90 μ g/ml led to no egg hatchability. In adulticidal experiments, the maximum efficacy was observed on A. stephensi ($LD_{50} = 31.60 \mu g/ml$), followed by A. aegypti (LD₅₀ = 34.31 μ g/ml) and C. quinquefasciatus (LD₅₀ = 37.52 μ g/ml), respectively. AgNPs were safer for three non-target mosquito natural enemies, Anisops *bouvieri, Diplonychus indicus* and *Gambusia affinis*, with LC₅₀ ranging from 629 to 2111 µg/ml. Overall, N. alata-fabricated AgNPs are a promising and eco-friendly tool against Anopheles, Aedes and Culex mosquito vectors, with negligible toxicity against non-target aquatic organisms.



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Introduction

Mosquitoes (Diptera: Culicidae) pose an important threat to public health since of their ability to act as vectors of pathogens and parasites causing malaria, lymphatic filariasis, dengue, chikungunya, yellow fever, Zika virus and Japanese encephalitis. These diseases are responsible for mortality, morbidity, economic loss and social disruption [1–3]. *Anopheles stephensi* is the primary vector of malaria in India and other west Asian countries. Every year, an estimated 300–500 million new infections and 600,000 cases based on world malaria report 2013 [4] deaths result from malaria worldwide. Malaria is a protozoan infection of erythrocytes caused in human beings by five species of the genus *Plasmodium (P. falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi*). In most cases, malaria is transmitted via the bite of an infected female anopheline mosquito, but congenital malaria and acquisition through infected blood transfusion are well described [5]. More than 40 per cent of the world's population-approximately 3 billion people are exposed to malaria in 108 endemic countries [6]. About one million cases of malaria are reported in India every year.

Aedes mosquito borne diseases have become one of the major threats to human population. Zika virus infection is a mosquito-borne illness like dengue (DEN) and chikungunya (CHIK) viruses. A lot about the biology of vectors and its role in disease transmission is known but *Aedes* mosquito-borne infections continue to be the major cause of mortality in many subtropical and tropical countries. The *Aedes*-borne infections like Zika are a potential threat, especially in urban settings where *A. aegypti* is abundant. The growing population in urban settings also increased the need for potable water, which necessitated storage practices in households, making ideal breeding habitats for *A. aegypti* mosquitoes that also increase dengue and CHIKV infections. Due to this mosquito-borne viral diseases are going to be a major threat in the 21st century [7].

Culex quinquefasciatus is a vector of many pathogens and parasites of humans, and both domestic and wild animals. Viruses transmitted by this species include West Nile virus, St. Louis encephalitis virus and Western equine encephalitis virus. *C. quinquefasciatus* is the principal vector of St. Louis encephalitis virus in the southern U.S.A. The virus increases its numbers in birds and then infects mosquitoes feeding on birds during the bird-nesting season in the spring. The mosquito may then transmit the virus to humans. St. Louis encephalitis is age-dependent, affecting older humans more than the young. Symptoms of the disease are flulike and can range from fever and headaches to stiffness and confusion. After a period of several days the brain may begin to swell, accompanied by depression, extreme excitement, sleepiness, or sleeplessness. Humans do not develop high levels of the virus in the blood and therefore are considered dead-end hosts unable to infect mosquitoes [8].

The use of synthetic insecticides to control mosquito vectors lead to resistance, adverse environmental effects and high operational costs [1]. Therefore, eco-friendly control tools are urgently needed [9]. In recent years, the green processes for the synthesis of silver nanoparticles (AgNPs) are evolving into an important branch of nanotechnology, due to low cost, simple procedures and absence of toxic chemicals or energy inputs [9]. In particular, AgNPs can be used for purification of drinking water, degradation of pesticides, to kill human pathogenic bacteria, as well as novel mosquitocides [10].

Recently, a growing number of plants have been successfully used for efficient and rapid extracellular synthesis of silver, copper and gold nanoparticles [<u>11</u>]. Good examples include cheap extracts of neem, *Azadirachta indica* [<u>12</u>], *Pongamia pinnata* [<u>13</u>], *Feronia elephantum*



Original Paper | Published: 05 January 2017

What Kind of Reducing Botanical? High Mosquitocidal Efficacy of a Silver Nanocomposite Synthesized Using a Leaf Aqueous Extract of *Fumaria indica*

<u>Giovanni Benelli</u> 🖂, <u>Marimuthu Govindarajan</u> 🖂, <u>Shine Kadaikunnan</u> & <u>Naiyf S. Alharbi</u>

Journal of Cluster Science **28**, 637–643(2017) | <u>Cite this article</u> **162** Accesses | **4** Citations | <u>Metrics</u>

Abstract

Mosquito vector control is a crucial challenge nowadays. Recently, the relevance of screening poorly studied plant products for green synthesis of nanopesticides was highlighted. Different plant products for nanosynthesis led to nano-composites with distinct biophysical features and toxicity. Here we fabricated silver nanoparticles capped with metabolites from *Fumaria indica* aqueous extract. Several biophysical methods were used to validate nanosynthesis *F. indica*-synthesized nanomaterial showed high efficacy as pesticide on *Anopheles subpictus* ($LC_{50} = 10.34 \mu g/ml$), *Aedes albopictus* ($LC_{50} = 11.42 \mu g/ml$) and *Culex tritaeniorhynchus* ($LC_{50} = 12.50 \mu g/ml$) larvae, allowing us to propose this easy to obtain nano-material as an effective source of novel mosquito pesticides.

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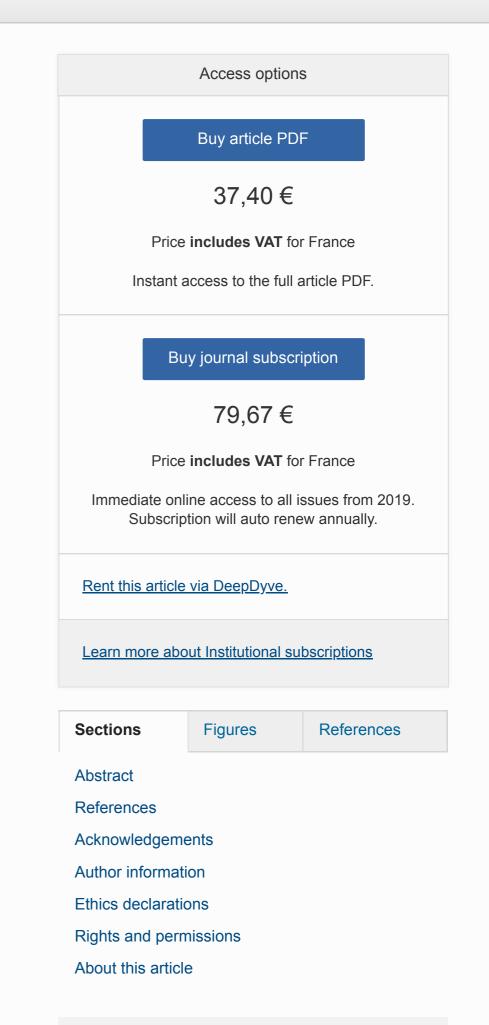
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Original Paper | Published: 22 June 2016

A Facile One-Pot Synthesis of Eco-Friendly Nanoparticles Using *Carissa carandas*: Ovicidal and Larvicidal Potential on Malaria, Dengue and Filariasis Mosquito Vectors

Marimuthu Govindarajan 🖂 & Giovanni Benelli

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Abstract

Mosquito-borne diseases represent a major human and animal health problem in all tropical and subtropical countries worldwide. In this study, we investigated the one-pot synthesis of silver nanoparticles (AgNPs) using a cheap leaf extract of *Carissa carandas* (Apocynaceae). Bio-reduced AgNPs were characterized by UV–visible spectrophotometry, Fourier transform infrared spectroscopy, X-ray diffraction analysis, atomic force microscopy, scanning electron microscopy and transmission electron microscopy. The acute toxicity of *C. carandas* extract and green-synthesized AgNPs was evaluated on eggs and larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. AgNPs showed high toxicity against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* larvae with LC₅₀ values of 14.33, 15.69 and 16.95 µg/mL, respectively. A single treatment with AgNPs tested at 60 µg/mL led to no egg hatchability. The egg rafts of *C. quinquefasciatus* were more resistant to the toxic action of AgNPs if compared to *A. aegypti* and *A. stephensi*. *C. carandas*-fabricated AgNPs were found safer to non-target organisms *Anisops bouvieri*, *Diplonychus indicus* and *Gambusia affinis*, with LC₅₀ ranging from 1097.87 to 17249.89 µg/ml. Overall, this research shed light on the mosquitocidal potential of *C. carandas*, a potential bio-resource for rapid, cheap and effective synthesis of poly-disperse and stable silver nanocrystals.

⊻ Download PDF Sections Figures References Abstract Introduction Materials and Methods **Results and Discussion** Conclusions References Acknowledgments Author information Ethics declarations **Rights and permissions** About this article

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Introduction

Mosquitoes (Diptera: Culicidae) are vectors of many diseases, including malaria, filariasis, dengue, West Nile virus, yellow fever, chikungunya, Zika virus and Japanese encephalitis. Among them, Search Q Menu -

malaria, spread by *Anopheles* females, dengue, spread by *Aedes* mosquito and filariasis, spread by *Culex* mosquito, are the three vector borne diseases characterizing tropical and subtropical regions worldwide, considered as major public health concerns [1]. The better strategy to lower the incidence of mosquito-transmitted diseases is to avoid mosquito biting using skin repellents, insecticide treated bed nets, and targeting mosquito young instars with pesticides [2]. Mosquito young instars have less mobility in breeding habitat so devising control measures at this stage are comparatively easy [3]. Current practice aimed to the control of mosquito larvae mainly rely to the use of synthetic pesticides such as carbamates, organophosphates and pyrethroids, as well as microbial control agents. In the early days of their use, insecticides showed success in reducing vector populations but the frequent and blind use of them increased the selection pressure on mosquitoes creating resistance and also had negative impact on human health and non-target species [4, 5]. In view of these facts, plant-borne insect control tools can be considered a safer and effective alternative [<u>6–9</u>].

Nanotechnology is a growing field making an outstanding impact in all spheres of human life [10]. Nanomaterials can be synthesized by different methods including chemical, physical, irradiation, and biological methods. The development of new chemical or physical methods has resulted in environmental contaminations, since the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts [11]. Thus, there is a need for "green nanotechnology" that includes a clean, safe, eco-friendly, and environmentally nontoxic method of nanoparticle synthesis, and in this method there is no need to use high pressure, energy, temperature, and toxic chemicals [12, 13]. The biological methods include synthesis of nanomaterials from the extracts of plant, bacterial and fungal species [14].

Currently, a growing number of plants have been recently screened for nanosynthesis of silver nanomosquitocides, including *Sida acuta* [15], *Barleria cristata* [16], *Gmelina asiatica* [10], *Chomelia asiatica* [17], *Feronia elephantum* [18], *Heliotropium indicum* [19], *Clerodendrum chinense* [20], *Bauhinia variegata* [21] and *Anisomeles indica* [22]. Phyto-synthesized silver nanoparticles (AgNPs) have been recently proposed as effective mosquito larvicides, and are gaining importance over synthetic chemical pesticides because of their reduced harmful effects to non-targeted species and novelty in the mechanism(s) of action [23, 24].

Carissa carandas Linn. is a large dichotomously branched evergreen shrub with short stem and strong thorns in pairs. It belongs to the family of Apocynaceae, and is commonly known as Christ's thorn or Bengal Currant, 'Kalakke' in Tamil [25]. The plant is native and common throughout India, Sri Lanka, Java, Malaysia, Myanmar and Pakistan. In traditional and folk medicine, this species is widely used as an anthelmintic, astringent, appetizer, antipyretic, in biliary, stomach disorders, rheumatism and disease of the brain [26]. Earlier studies have shown that the extract of the plant possesses cardiotonic, antipyretic and antiviral activity [27, 28]. Various cardiac glycosides, a triterpenoidal constituent

carissone and β -sitosterol were reported from the root extract of the plant [29]. However, to the best of

our knowledge, its mosquitocidal activity is currently unknown.

In this research, we proposed a cheap and rapid method of one-pot bio-fabrication of poly-dispersed AgNPs using the aqueous leaf extract of *C. carandas*. Bio-reduced AgNPs were characterized by UV– vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), atomic force microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The acute toxicity of *C. carandas* leaf extract and green-synthesized AgNPs was evaluated against the eggs and larvae of malaria vector *A. stephensi*, the dengue vector *A. aegypti* and the filariasis vector *C. quinquefasciatus*. Furthermore, we evaluated the biotoxicity of *C.*

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Green-Synthesized Mosquito Oviposition Attractants and Ovicides: Towards a Nanoparticle-Based "Lure and Kill" Approach?

Giovanni Benelli 🖂 & Marimuthu Govindarajan

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Abstract

Mosquitoes are key vectors of malaria, dengue, yellow fever, chikungunya, West Nile, Japanese encephalitis, lymphatic filariasis, Zika virus and St. Louis encephalitis virus. Ecofriendly control tools of Culicidae vectors are a priority. Green nanotechnologies may help to boost the effectiveness of mosquito vector control. We proposed a facile fabrication of polydisperse and stable silver nanoparticles (AgNPs) using the Aganosma cymosa leaf extract. Nanoparticles were characterized by UV-visible spectroscopy, Fourier transform infrared spectroscopy, X-ray diffraction analysis, atomic force microscopy, scanning electron microscopy and transmission electron microscopy. Nanoparticles showed high toxicity on eggs and larvae of Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. LC₅₀ values were 12.45, 13.58 and 14.79 μ g/mL, respectively. No egg hatchability was noted post-treatment with 40, 50 and 60 µg/mL, respectively. Nanoparticles were found safer to non-target mosquito predators Anisops bouvieri, Diplonychus indicus and Gambusia affinis, LC₅₀ values ranged from 673.36 to 2247.43 µg/mL. Notably, AgNPs showed high oviposition attractiveness towards the three mosquito species. Overall, the oviposition attractiveness of the A. cymosa extract coupled with the ovicidal action of AgNPs can help to develop "lure and kill" tools to be used at mosquito breeding sites.

Introduction

Mosquitoes (Diptera: Culicidae) represent a key threat for millions of humans and animals

Download PDF		<u>*</u>			
Sections	Figures	References			
Abstract					
Introduction					
Materials and Methods					
Results and Discussion					
Conclusions					
References					
Acknowledgments					
Author informat	ion				
Ethics declaration	ons				
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About this article	е				

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worldwide, since they act as vectors for important parasites and pathogens, including malaria, filariasis and important arboviruses, such as dengue, West Nile and Zika virus [1, 2]. Current control strategies mainly rely to synthetic pesticides, insect growth regulators and microbial control agents. However, synthetic chemicals lead to a number of negative implications, including high operational costs, development of resistance and toxic effects on non-target organisms and human health [3].

Nanotechnology is a growing field making an outstanding impact in all spheres of human life [4]. Nanomaterials can be synthesized by different methods including chemical, physical, and biological methods. The development of new chemical or physical methods has resulted in environmental contaminations, since the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts [5]. Thus, there is a need for "green nanotechnology" that includes a clean, safe, eco-friendly, and environmentally nontoxic method of nanoparticle synthesis, and in this method there is no need to use high pressure, energy, temperature, and toxic chemicals [6, 7]. The biological methods include synthesis of nanomaterials from the extracts of plant, bacterial and fungal species [8].

Currently, a growing number of plants have been screened for nanosynthesis of silver nanomosquitocides, including *Sida acuta* [9], *Barleria cristata* [10], *Gmelina asiatica* [4], *Chomelia asiatica* [11], *Feronia elephantum* [12], *Heliotropium indicum* [13], *Clerodendrum chinense* [14], *Bauhinia variegata* [15], and *Anisomeles indica* [16]. Phyto-synthesized silver nanoparticles (AgNPs) have been recently proposed as effective mosquito larvicides, and are gaining importance over synthetic chemical pesticides because of their reduced harmful effects to non-target species and novelty in the mechanism(s) of action [17]. Notably, the employ of different botanicals as reducing and capping agents lead to the production of nanoparticles with different biophysical features and toxicity against vectors, parasites and pathogens, pointing out the key importance of screening local botanical resources for nanosynthesis [17].

Aganosma cymosa (Apocynaceae), also known as *Echites cymosa*, is a climbing shrub found commonly growing in Indian hills. The distribution is restricted to peninsular region in India and Bengal [<u>18</u>]. Plant samples of *A. cymosa* yielded 10.3 % oil, while strictly limited information is available on the composition of the polar fraction of the plant extract [<u>19</u>]. However, despite the lack of information on *A. cymosa* chemical composition, the plant is traditionally used as an emetic and anthelmintic. It is also used in the treatment of bronchitis, leprosy and skin diseases. Flower-borne constituents are effective against diseases of eyes [<u>20</u>].

In this research, we proposed a cheap and rapid method of synthesis of poly-dispersed AgNPs

using the aqueous leaf extract of *A. cymosa*. Bio-reduced AgNPs were characterized by UV–vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), atomic force microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The acute toxicity of *A. cymosa* leaf extract and biosynthesized AgNPs was evaluated against the eggs and larvae of malaria vector *Anopheles stephensi*, the dengue and Zika virus vector *Aedes aegypti* and the filariasis and St. Louis encephalitis vector *Culex quinquefasciatus*. Furthermore, we evaluated the biotoxicity of *A. cymosa* aqueous extract and green-synthesized AgNPs on three non-target aquatic predators of *Anopheles, Aedes* and *Culex* mosquitoes, *Anisops bouvieri, Diplonychus indicus* and *Gambusia affinis*. We also focused on the impact of a single treatment with the plant extract and the green synthesized AgNPs on mosquito oviposition activity. Notably, these AgNPs showed high oviposition attractiveness towards the three mosquito species. Thus, the

oviposition attractiveness of the *A. cymosa* extract coupled with the ovicidal action of AgNPs can help to develop "lure and kill" tools to be used at mosquito breeding sites.

Materials and Methods

Materials

Silver nitrate was procured from Merck, India. The glassware was acid-washed thoroughly and then rinsed with Millipore Milli-Q water. Healthy and fresh leaves of *A. cymosa* were collected from Nilgiris, Western Ghats (11°10′N–11°45′N latitude and 76°14′E–77°2′E longitude), Tamil Nadu State, India. The identity was confirmed at the Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu. Voucher specimens were numbered and kept in our research laboratory for further reference.

Preparation of Plant Extracts

Leaves of *A. cymosa* were dried in the shade and ground to fine powder in an electric grinder. Aqueous extract was prepared by mixing 50 g of dried leaf powder with 500 mL of water (boiled and cooled distilled water) with constant mized using a magnetic stirrer. The suspension of dried leaf powder in water was left for 3 h and filtered through Whatman n. 1 filter paper and the aqueous filtrate were stored in an amber-colored airtight bottle at 10 °C temperature until testing.

Green synthesis of Silver Nanoparticles

Ten grams of thoroughly washed and finely cut leaves were added in a 300-mL Erlenmeyer flask along with 100 mL of sterilized double-distilled water, the mixture was boiled for 5 min before finally decanting it. The colloidal extract was filtered with Whatman filter paper n. 1, stored at -15 °C and tested within a week. The filtrate was treated with aqueous 1 mM AgNO₃ (21.2 mg of AgNO₃ powder in 125 mL of Milli-Q water) solution in an Erlenmeyer flask and incubated at room temperature. Eighty-eight milliliters of an aqueous solution of 1 mM silver nitrate was reduced using 12 mL of *A. cymosa* leaf extract at room temperature for 10 min, resulting in a brown–yellow solution indicating the formation of AgNPs.

Characterization of Silver Nanoparticles

The bioreduction of Ag⁺ ions was monitored using a UV–vis spectrophotometer (UV-160v, Shimadzu, Japan). Analysis on size, morphology, agglomeration pattern and dispersed nature of AgNPs were performed by atomic force microscopy (Agilent Technologies AFM-5500), scanning electron microscopy (Hitachi S3000 H SEM) and transmission electron microscopy (TEM Technite 10 Philips). The purified AgNPs were examined for the presence of biomolecules using FTIR spectroscopy (Thermo Scientific Nicolet 380 FT-IR Spectrometer) KBr pellets and crystalline AgNPs were determined by XRD analysis.

Mosquito Rearing

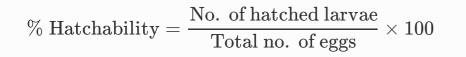
Following the method by Govindarajan et al. [21] laboratory-bred pathogen-free strains of mosquitoes were reared in the vector control laboratory, Department of Zoology, Annamalai University. At the time of adult feeding, these mosquitoes were 3-4 days old after emergences (maintained on raisins and water) and were starved for 12 h before feeding. Each time, 500 mosquitoes per cage were fed on blood using a feeding unit fitted with Parafilm as membrane for 4 h. *A. aegypti* feeding was done from 12 noon to 4.00 p.m. and *A. stephensi*, and *C. quinquefasciatus* were fed during 6.00-10.00 p.m. A membrane feeder with the bottom end fitted with Parafilm was placed with 2.0 mL of the blood sample (obtained from a slaughterhouse by collecting in a heparinized vial and stored at 4 °C) and kept over a netted cage of mosquitoes. The blood was stirred continuously using an automated stirring device, and a constant temperature of 37 °C were maintained using a water jacket circulating system. After feeding, the fully engorged females were separated and maintained on raisins. Mosquitoes were held at 28 ± 2 °C, 70-85 % relative humidity, with a photoperiod of 12-h light and 12-h dark.

Larvicidal Activity

Larvicidal activity of the aqueous crude extract and AgNPs from *A. cymosa* was evaluated according to WHO [22]. The aqueous extract was tested at 90, 180, 270, 360 and 450 µg/mL concentrations and Ag NP was tested at 6, 12, 18, 24 and 30 µg/mL concentrations. Twenty numbers of late III instar larvae were introduced into a 500-mL glass beaker containing 250 mL of dechlorinated water, plus the desired concentrations of leaf extract or AgNPs. For each concentration, five replicates were performed. Larval mortality was recorded at 24 h after exposure, during which no food was given to the larvae. Each test included a set control groups (silver nitrate and distilled water) with five replicates for each individual concentration.

Ovicidal Activity

For ovicidal activity, the slightly modified method of Su and Mulla [23] was followed. *A. stephensi, A. aegypti* and *C. quinquefasciatus* eggs were collected from vector control laboratory, Department of Zoology, Annamalai University. The leaf aqueous extract and AgNPs were to achieve various concentrations ranging from 10 to 600 µg/mL. Eggs of these mosquito species (100 per replicate) were exposed to each concentration of aqueous extract and AgNPs. After 24 h treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated six times along with appropriate control. The hatch rates were assessed 48 h post treatment by following formula:



Oviposition Activity

Oviposition behaviour assays were carried out following the method by Xue et al. [24] Fifteen gravid female mosquitoes (10 days old, 5 days after blood feeding) were transferred to each mosquito cage ($45 \times 38 \times 38$ cm). Concentrations from 50 to 250 µg/mL and from 10 to 50 µg/mL were made from aqueous crude extract and AgNPs, respectively, in 100 ml of water. Two enamel bowls holding 100 ml of water were placed in opposite corners of each cage, one treated with the test material and the other with water and AgNO₃ as control. The positions of the bowls were alternated between the different replicates to nullify any effect of position on oviposition. Five replicates for each concentration were run, with cages placed side by side for each bioassay. After 24 h, the number of eggs laid in treated and control bowls were counted under a stereomicroscope (Olympus, Japan). The effective attractiveness (EA %) for each oviposition concentration was calculated [24] and the oviposition active index (OAI) was assessed [25].

Percentage of
$$EA = \frac{NT - NC}{NT} \times 100$$

where: EA, effective attractiveness, NT, number of eggs in treated, NC number of eggs in control.

$$\mathrm{OAI} = rac{\mathrm{NT} - \mathrm{NS}}{\mathrm{NT} + \mathrm{NS}} imes 100$$

where: NT total number of eggs in the treated water and NS total number of eggs lay in the control water.

Biotoxicity on Non-Target Organisms

The effect of non-target organisms was assessed following the method by Sivagnaname and Kalyanasundaram [26]. The effect of aqueous extract and AgNPs of the potential plant was tested against adults of the non-target organisms *A. bouvieri, D. indicus,* and *G. affinis.* The species were field collected and separately maintained in cement tanks (85 cm diameter and 30 cm depth) containing water at 27 ± 3 °C and external relative humidity 85 %. The aqueous extract and AgNPs of *A. cymosa* were evaluated at concentration of even 50 times higher the LC₅₀ dose for mosquito larvae. Ten replicates will be performed for each concentration along with four replicates of untreated controls. The non-target organisms were observed for mortality and other abnormalities such as sluggishness and reduced swimming activity after 48 h exposure. The exposed non-target organisms were also observed continuously for 10 days to understand the post treatment effect of this extract on survival and swimming activity.

Data Analysis

Larval mortality data were subjected to probit analysis. LC₅₀ and LC₉₀ were calculated using the method by Finney [<u>27</u>]. Ovicidal mortality data were analyzed by two-way ANOVA followed by Tukey's HSD test. In experiments evaluating biotoxicity on non-target organisms, the Suitability Index (SI) was calculated for each non-target species using the following formula [<u>28</u>].

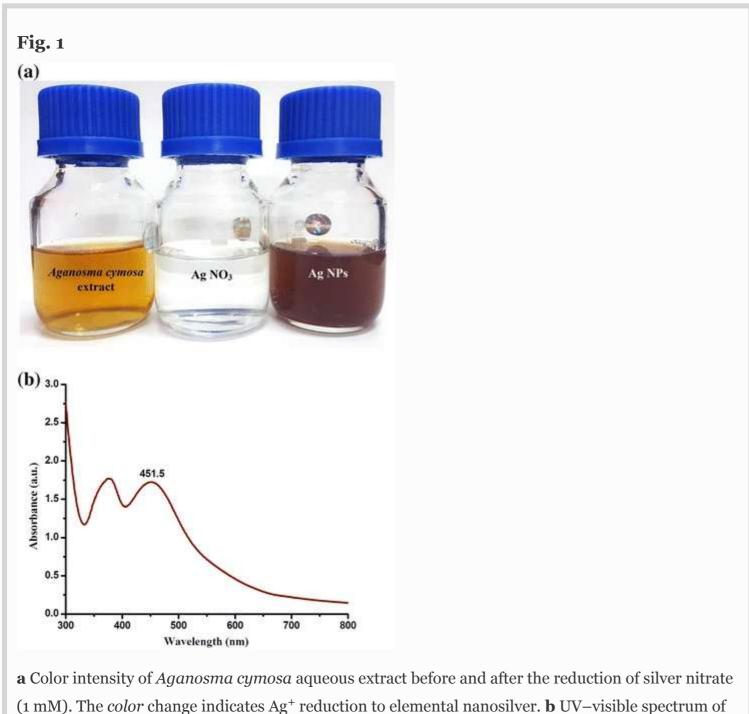
 $\mathrm{SI} = rac{\mathrm{LC}_{50} ext{ of non-target organisms}}{\mathrm{LC}_{50} ext{ of target vector species}}$

All data were analyzed using the SPSS Statistical Software Package version 16.0. A probability level of P < 0.05 was used for the significance of differences between values.

Results and Discussion

Fabrication and Characterization of Silver Nanoparticles

Silver ions were rapidly reduced to metallic nanosilver when the *A. cymosa* plant extract was added to the solution. It was observed that the color of the solution turned from yellowish to bright yellow and to dark brown after the reaction (Fig. <u>1</u>a), which indicated the formation of AgNPs, in agreement with recent research on other plant species [<u>10</u>, <u>12</u>]. The formation and stability of AgNPs in aqueous solution was confirmed using UV–vis spectroscopy. Figure <u>1</u>b shows that the absorption spectrum of AgNPs formed in the reaction media, with a peak at 451.5 nm. This peak was probably due to the surface plasmon resonance of AgNPs. It has been reported earlier that absorbance at around 450 nm is a characteristic of noble metal nanoparticles [<u>11</u>].

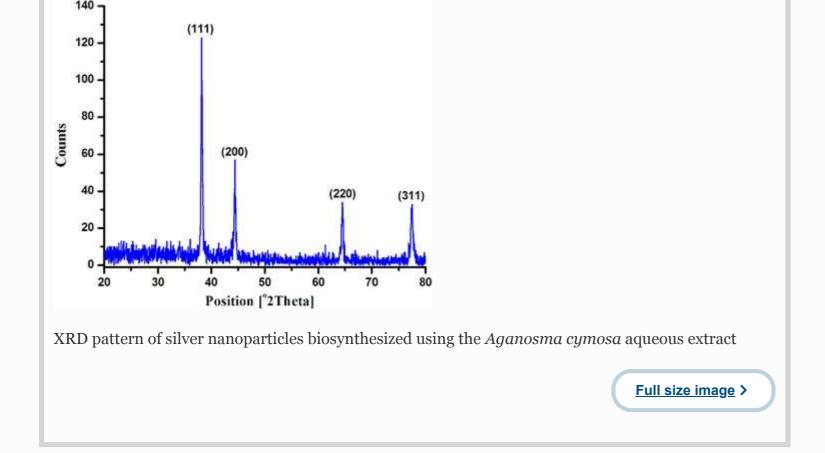


silver nanoparticles after 180 min from the reaction



The XRD pattern showed diffraction peaks at (111), (200), (220) and (311), which correspond to 37.28°, 44.24°, 65.28° and 77.42° and depict crystalline nature of the biofabricated AgNPs. The obtained XRD pattern illustrated that green-synthesized AgNPs showed both cubic and hexagonal structures (Fig. <u>2</u>). Similar phenomena were reported for AgNPs biofabricated using *Trianthema decandra*, which showed interplanar distance corresponds to mixed phase of cubic and hexagonal structures [<u>29</u>].

Fig. 2



FTIR spectroscopy shed light on the identity of the biomolecules responsible for reduction and efficient stabilization of the AgNPs [21]. The band at 3349 cm⁻¹ (Fig. 3) corresponds to O–H, as also the H-bonded alcohols and phenols [30]. The peak at 2205 cm⁻¹ may indicate the presence of nitrile group, C=N [31]. Similarly, bands at 1404 cm⁻¹ could be due to the C,C stretching of an aromatic ring, which may suggest the presence of benzene ring. Shoulder peaks at 1114 cm⁻¹ probably indicate that the amine stretch, C–N bond. The bands at 867 cm⁻¹ might indicate the presence of aromatic C–H bending. The peak at 617 cm⁻¹ probably corresponds to C–H stretching strong vinyl di-substituted alkenes [32]. Overall, the rapid reduction of silver ions might be linked with the presence of water-soluble phytochemicals such as flavones, quinones, and organic acids present in the leaf extract of *A. cymosa*.

AFM is a primary tool for analyzing size, shape, and agglomeration pattern [33]. It offers visualizations of three-dimensional views of the nanoparticles unlike the electron microscopes. It has an advantage over combination of high resolution, samples does not have to be conductive and does not require the high-pressure vacuum conditions. 2.5 µm resolution studies of biologically fabricated AgNPs with AFM reveal the particles are poly-dispersed, spherical in shape, having the size range from 1 to 16.5 nm and there is no agglomeration observed between the particles (Fig. 4a). Raw data obtained from AFM were treated with a specially designed image processing software (NOVA-TX) to further exploit the 3D image of AgNPs (Fig. <u>4</u>b). The average particle size obtained from the corresponding diameter distribution ranged from 8 to 14 nm (Fig. <u>4</u>c). SEM of the biofabricated AgNPs was performed in order to investigate the morphology and size distribution of AgNPs (Fig. 5). SEM showed that the morphology of AgNPs is mostly spherical, in agreement with the shape of SPR band in the UV-vis spectrum. The average particle size measured from all the SEM images was in agreement with AFM data. Figure 6b showed the EDX profile with a strong Ag signal along with other peaks, which may be originated from biomolecules bounding to the surface of AgNPs. Metallic AgNPs generally show typical absorption peak approximately at 3 keV due to surface Plasmon resonance [34]. In addition, TEM micrograph shows fine configuration of crystalline, spherical AgNPs, with size slightly higher of that recorded in AFM and SEM assays, since it ranged between 27 and 49 nm (Fig. 7). It was also noted that AgNPs bound with thin layer of biomolecule coating on their surface which act as stabilizing agent, thus AgNPs were poly-dispersed without direct contact and stable for long period of time [35].

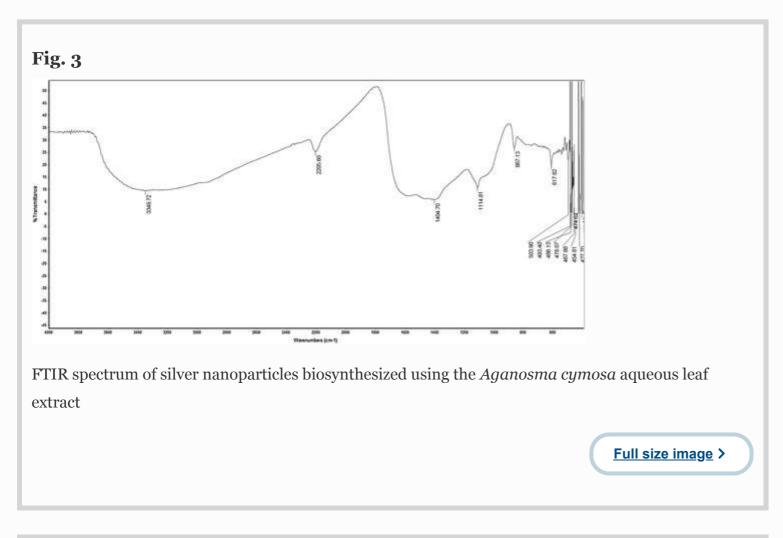
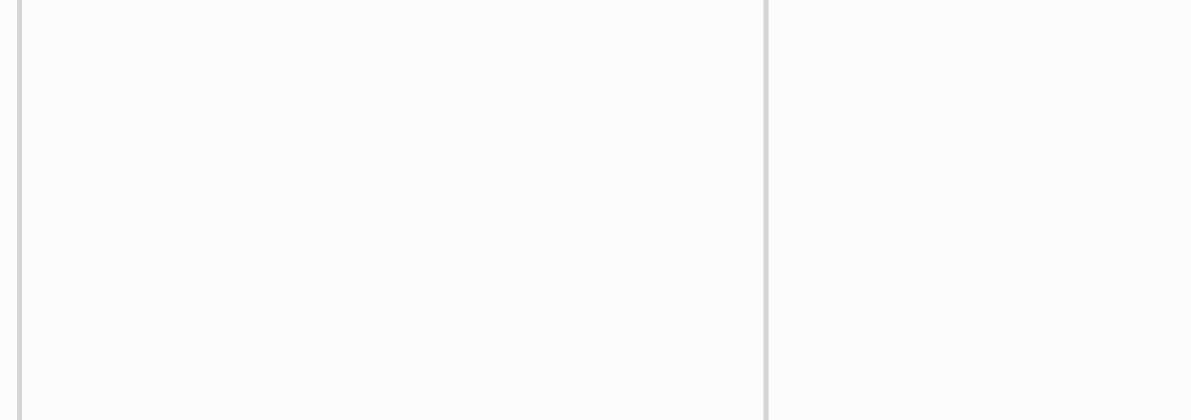
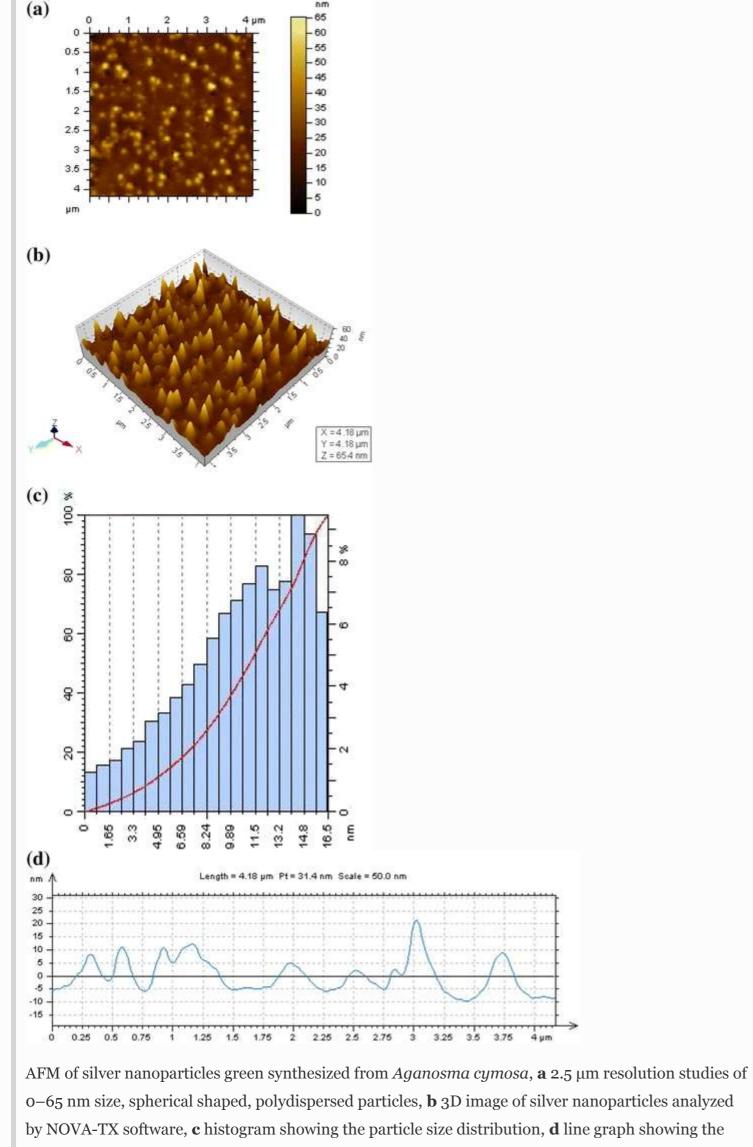


Fig. 4

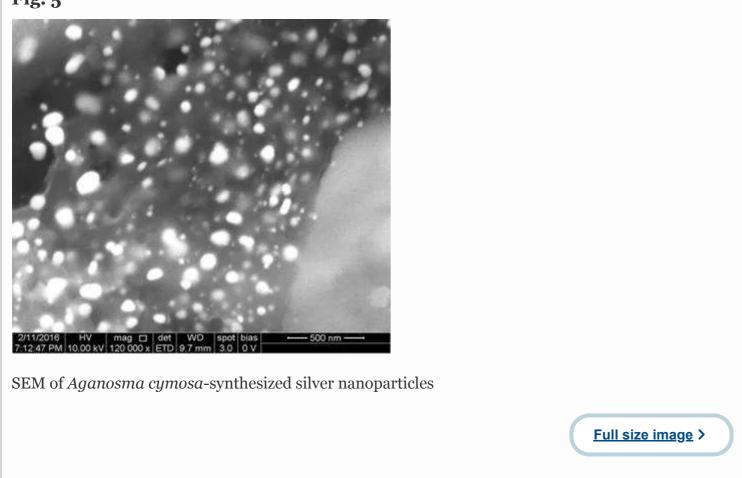




size distribution of green-synthesized silver nanoparticles

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Fig. 5



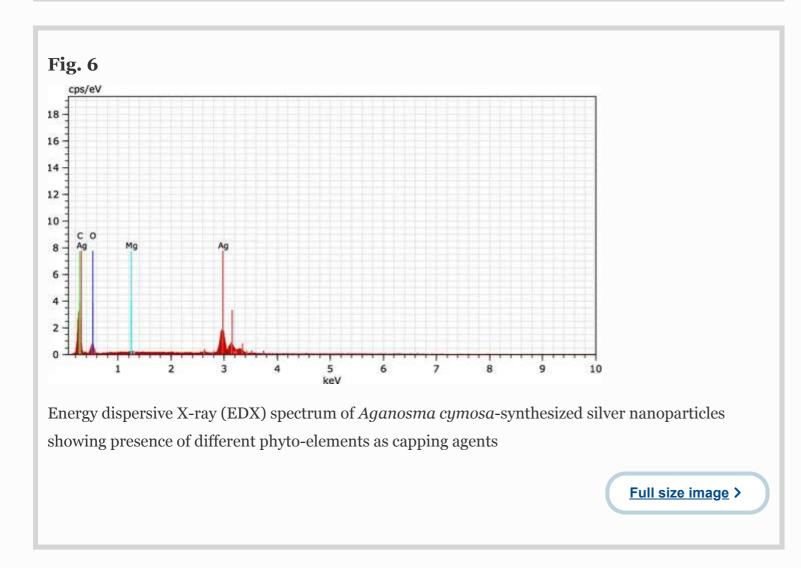
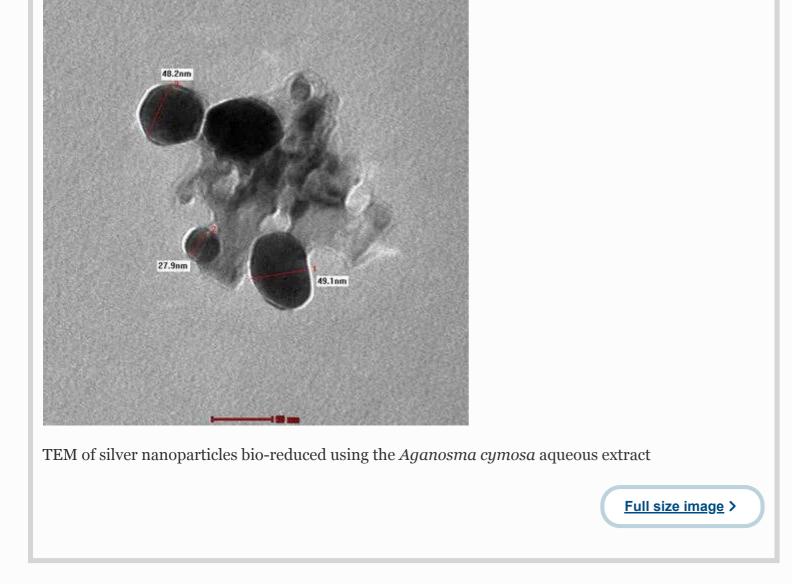


Fig. 7



Toxicity and Oviposition Attractiveness on Mosquito Vectors

The larvicidal action of both the A. cymosa leaf extract and AgNPs showed dose-dependent larvicidal effect against all tested mosquito species (Tables <u>1</u>, <u>2</u>). Compared to the leaf aqueous extract, biofabricated AgNPs showed higher toxicity against A. stephensi, A. aegypti, and C. *quinquefasciatus* with LC₅₀ values of 12.45, 13.58 and 14.79 μ g/mL, respectively (Table <u>2</u>). The egg hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs (Table 3, 4). AgNPs exerted zero hatchability at 40, 50 and 60 µg/mL for A. stephensi, A. aegypti, and C. quinquefasciatus, respectively. Notably, AgNPs showed high oviposition attractiveness towards the three mosquito species (Tables 5, <u>6</u>, <u>7</u>).

Table 1 Larvicidal activity of Aganosma cymosa aqueous leaf extract against the mosquito vectors Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus

Full size table >



Table 2 Larvicidal activity of silver nanoparticles

biosynthesized using the Aganosma cymosa leaf extract against the mosquito vectors Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus	
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Table 3 Ovicidal activity of Aganosma cymosa aqueous leafextract against Anopheles stephensi, Aedes aegypti andCulex quinquefasciatus	
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Table 4 Ovicidal activity of silver nanoparticles fabricated using the Aganosma cymosa leaf extract against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus

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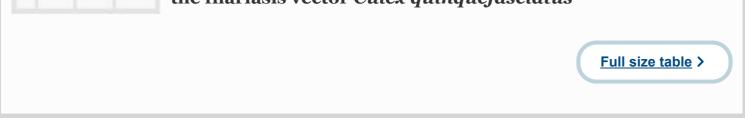
Table 5 Oviposition attractant activity of Aganosma cymosa leaf extract and green synthesized silver nanoparticles against the malaria vector Anopheles stephensi

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Table 6 Oviposition attractancy of Aganosma cymosa leaf extract and green synthesized silver nanoparticles against the dengue vector Aedes aegypti

Full size table >

Table 7 Oviposition attractancy of Aganosma cymosa leaf extract and green synthesized silver nanoparticles against the filariasis vector Culex quinquefasciatus



In latest years, a growing number of evidences have been provided about the larvicidal efficacy of plant-fabricated nanolarvicides, while only moderate efforts focused on their ovicidal potential and oviposition manipulation activity [17, 36]. Each plant material containing unique bioactivity including repellent, larvicidal, ovicidal, and adulticidal activity, thus their efficacy of nanosynthetical routes varies from species to species and also according to the tested plant parts [1, 2, 37, 38]. Combination of nanoparticles with bioactive principles bestows improved efficiency. The present study showed that the percentage of mosquito larvicidal mortality increased by many folds with the addition of bio-stabilized AgNPs. Similarly, Muthukumaran

et al. [11] studied the potential of *G. asiatica*-mediated synthesis of AgNPs against *An. stephensi, Ae. aegypti*, and *Cx. quinquefasciatus*, obtaining high LC_{50} values (i.e., 22.44, 25.77, and 27.83 µg/mL, respectively). In addition, Murugan et al. [39] reported that low doses (i.e., 1 ppm) of *Cymbopogon citratus*-synthesized gold nanoparticles control malaria and dengue vectors boosting early instar mosquito larvae predation by copepods in a gold nanoparticle-contaminated environment. Thus, it has been supposed that ultra-low doses of metal nanoparticles reduced the motility of mosquito larvae, enhancing predation of odonate nymphs and other mosquito natural enemies [17].

Biotoxicity on Non-Target Aquatic Organisms

Our toxicity treatments achieved negligible toxicity against *A. bouvieri, D. indicus* and *G. affinis* adults, with LC_{50} values ranging from 673 to 32494 µg/mL (Tables <u>8</u>, <u>9</u>). Longevity and swimming activity of the study species were not altered for a week after testing. SI indicated that *A. cymosa* -fabricated AgNPs were less toxic to the non-target organism tested if compared to the targeted mosquito larval populations (Table <u>10</u>).

Table 8 Toxicity of Aganosma cymosa aqueous leaf extractagainst three non-target natural enemies of Anopheles,Aedes and Culex mosquito vectors

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Table 9 Toxicity of green-synthesized silver nanoparticles using the *Aganosma cymosa* leaf extract against three nontarget natural enemies sharing the same ecological niche of *Anopheles, Aedes* and *Culex* mosquito vectors

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Table 10 Suitability index of three non-target mosquito natural enemies over young instars of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* exposed to *Aganosma cymosa* aqueous leaf extract and greensynthesized silver nanoparticles

Nowadays, moderate knowledge is available about the acute toxicity of mosquitocidal nanoparticles towards non-target aquatic species [17]. Recently, Govindarajan et al. [38] reported that the biotoxicity of *M. sylvestris* aqueous extract and green-synthesized AgNPs was negligible also on non-target organisms D. indicus and G. affinis, since the toxicity treatments achieved LC₅₀ ranging from 813.16 to 10,459.13 μ g/mL. In addition, the biotoxicity of *B. cristata* aqueous extract and green-synthesized AgNPs was evaluated on non-target organisms D. indicus, A. bouvieri, and G. affinis with LC₅₀ ranging from 633.26 to 8595.89 μg/mL, respectively [<u>10</u>]. The *Berberis tinctoria* extract was tested against two other non-target mosquito predators *T. splendens* and *M. thermocyclopoids*, with LC₅₀ values of 552.28 and 480.92 ppm, respectively [40]. Govindarajan et al. [21] investigated the biotoxicity of C. spinarum aqueous extract and green-synthesized AgNPs on non-target organisms D. *indicus*, *A. bouvieri* and *G. affinis*. Toxicity treatments achieved negligible toxicity with LC₅₀ values ranging from 424.09 to 6402.68 µg/mL. Concerning sub-lethal effects, G. affinis exposed to nanoparticle-contaminated aquatic environments showed higher predation rates against An. stephensi and Ae. albopictus larvae. In standard laboratory conditions, the predation efficiency of G. affinis on An. stephensi and Ae. albopictus III instar larvae was 86.2 and 81.7 %, respectively. In AgNPs-contaminated environments, predation was 93.7 and 88.6 %, respectively [<u>41</u>].

Conclusions

Overall, the plant-mediated synthesis of silver nanoparticles is one-pot, eco-friendly and costeffective [42]. In the present study, AgNPs were rapidly biosynthesized at room temperature using a cheap *A. cymosa* leaf extract. Bio-reduced AgNPs were polydispersed and stable in solution for at least four weeks. *A. cymosa*-synthesized AgNPs are easy to produce, stable over time, and can be employed at low dosages to strongly reduce populations of vectors mosquitoes with little detrimental effects on predation rates of mosquito predators, such as *D. indicus*, *A. bouvieri* and *G. affinis*. Notably, AgNPs showed high oviposition attractiveness towards the three mosquito species. Thus, the oviposition attractiveness of the *A. cymosa* extract coupled with the ovicidal action of AgNPs can help to develop "lure and kill" tools to be used at mosquito breeding sites [1].

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Ethics declarations

Ethical approval

All applicable international and national guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Conflicts of interest

The authors declare no conflicts of interest.

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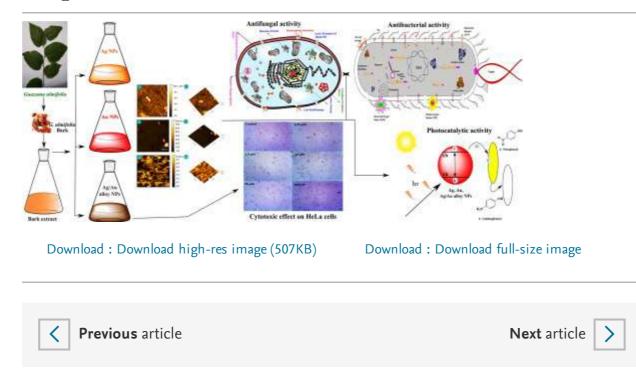
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Abstract

In the present study, we focused on a quick and green method to fabricate Ag,

Au and Ag/Au alloy nanoparticles (NPs) using the bark extract of Guazuma ulmifolia L. Green synthesized metal NPs were characterized using different techniques, including UV-Vis spectroscopy, FT-IR, XRD, AFM and HR-TEM analyses. The production of Ag, Au and Ag/Au alloy NPs was observed monitoring color change from colorless to brown, followed by pink and dark brown, as confirmed by UV–Vis spectroscopy characteristic peaks at 436, 522 and 510 nm, respectively. TEM shed light on the spherical shapes of NPs with size ranges of 10–15, 20–25 and 10–20 nm. Biosynthesized NPs showed good catalytic activity reducing two organic dyes, 4-nitrophenol (4-NP) and Congo red (CR). UV–vis spectroscopy, fluorescence, circular dichroism spectroscopy and viscosity analyses were used to investigate the NP binding with calf thymus DNA. The binding constant of NPs with DNA calculated in UV–Vis absorption studies were 1.18×10^4 , 1.83×10^4 and 2.91×10^4 M⁻¹, respectively, indicating that NPs were able to bind DNA with variable binding affinity: Ag/Au alloy NPs > Ag NPs > Au NPs. Ag/Au alloy NPs also showed binding activity to bovine serum albumin (BSA) over the other NPs. Ag and Ag/Au alloy NPs exhibited good antimicrobial activity on 14 species of microbial pathogens. In addition, the cytotoxic effects of Ag/Au alloy NPs were studied on human cervical cancer cells (HeLa) using MTT assay. Overall, our work showed the promising potential of bark-synthesized Ag and Ag/Au alloy NPs as cheap sources to develop novel and safer photocatalytic, antimicrobial and anticancer agents.

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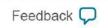
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The essential oil from *Zanthoxylum monophyllum* a potential mosquito larvicide with low toxicity to the non-target fish *Gambusia affinis*

Roman Pavela 🖂 & Marimuthu Govindarajan

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Abstract

The mosquito larvicidal activity of *Zanthoxylum monophyllum* leaf essential oil (EO) and its major chemical constituents was tested against the three mosquito vectors *Anopheles subpictus*, *Aedes albopictus* and *Culex tritaeniorhynchus*. In the EO of *Z. monophyllum*, it contains 36 compounds with the two major compounds being Germacrene D-4-ol (19.40 %) and α -Cadinol (12.30). The larvicidal activity of the essential oil against *An. subpictus*, *Ae. albopictus* and *Cx. tritaeniorhynchus* was determined and LC₅₀ values were estimated at 41.50, 45.35 and 49.01 µg/mL, respectively. The two major compounds Germacrene D-4-ol showed a significantly higher efficacy compared to α -Cadinol. While LC₅₀ for Germacrene D-4-ol ranged from 6.12 to 7.26 µg/mL, LC₅₀ values for α -Cadinol were estimated in the range from 10.27 to 12.28 µg/mL. The EO, Germacrene D-4-ol and α -Cadinol were found safer to the non-target organism *Gambusia affinis* (LC₅₀ = 4234.07, 414.05 and 635.12 µg/mL, respectively), which was manifested in the high suitability of the index/predator safety factor value, ranging from 86.36 for the least sensitive larvae of *Cx. tritaeniorhynchus* to 102.02 for the most sensitive larvae of *An. subpictus*.

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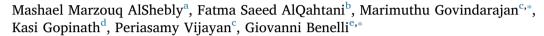


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Toxicity of ar-curcumene and epi-β-bisabolol from *Hedychium larsenii* (Zingiberaceae) essential oil on malaria, chikungunya and St. Louis encephalitis mosquito vectors



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ABSTRACT

Mosquitoes act as vectors of key pathogens and parasites. Plant essential oils have been recognized as important sources of biopesticides, which do not induce resistance and have limited toxic effects on human health and non-target organisms. In this research, we evaluated the larvicidal and oviposition deterrence activity of *Hedychium larsenii* essential oil (EO) and its major compounds ar-curcumene and epi- β -bisabolol. Both molecules showed high toxicity against early third instars of *Anopheles stephensi* (LC₅₀=10.45 and 14.68 µg/ml), *Aedes aegypti* (LC₅₀=11.24 and 15.83 µg/ml) and *Culex quinquefasciatus* (LC₅₀=12.24 and 17.27 µg/ml). In addition, low doses of ar-curcumene and epi- β -bisabolol were effective as oviposition deterrents against the three tested mosquito species. Notably, the acute toxicity of *H. larsenii* oil and its major compounds against the mosquito biocontrol agent *Poecilia reticulata* was low, with LC₅₀ higher than 1500 ppm. Overall, the results from this study revealed that ar-curcumene and epi- β -bisabolol from the *H. larsenii* oil can be considered for the development of novel and effective mosquito larvicides.

1. Introduction

Mosquito-borne diseases represent a major public health problem in tropical and subtropical countries worldwide (Benelli et al., 2016a). Malaria is a life threatening disease that caused an estimated 627000 deaths in 2012. Malaria parasites (Plasmodium spp.) are vectored through the bites of Anopheles mosquitoes (WHO, 2014; Benelli and Mehlhorn, 2016). Anopheles stephensi Liston (Diptera: Culicidae) is a major vector of malaria worldwide, and has been shown to be directly responsible for about 40-50% of the annual malarial incidence (Benelli, 2015a). Aedes aegypti (L.), the yellow fever mosquito is a main vector of dengue and Zika virus. This species is widely distributed in the tropical and subtropical zones, where it is closely associated with the human habitat. The geographical range of A. aegypti is increasing in part due to rapid urbanization and increased global movement of people and cargo (Benelli, 2015a; Benelli and Mehlhorn, 2016). Furthermore, Culex quinquefasciatus (Say), is an important vector of lymphatic filariasis, West Nile and St. Louis encephalitis virus. In particular, lymphatic

filariasis is one of the most widely distributed neglected tropical diseases, with approximately 120 million people infected worldwide and 44 million people having common chronic manifestation (Bernhard et al., 2003).

Currently, synthetic insecticides and insect growth regulators are widely used to control larval instars of mosquitoes, while knowledge on botanical-based oviposition deterrents remains scarce (Benelli, 2015b, 2015c). However, the adverse effects linked with their overuse, i.e. insecticide resistance, environmental pollution, and risks to human and other organisms, represent major limits to their successful employment (Naqqash et al., 2016). In recent years, it has been pointed out that some products derived from plants, including essential oils, can provide lower risk alternatives to synthetic pesticides and repellents (Benelli, 2015b; Govindarajan and Benelli, 2016a, 2016b, 2016c; Pavela and Benelli, 2016; Govindarajan et al., 2016a, 2016b, 2016c).

The genus *Hedychium*, commonly known as butterfly lilies, belongs to Zingiberaceae family (Wu and Larsen, 2000). The genus comprises of about 80 species mostly distributed in the Indo-Malayan region

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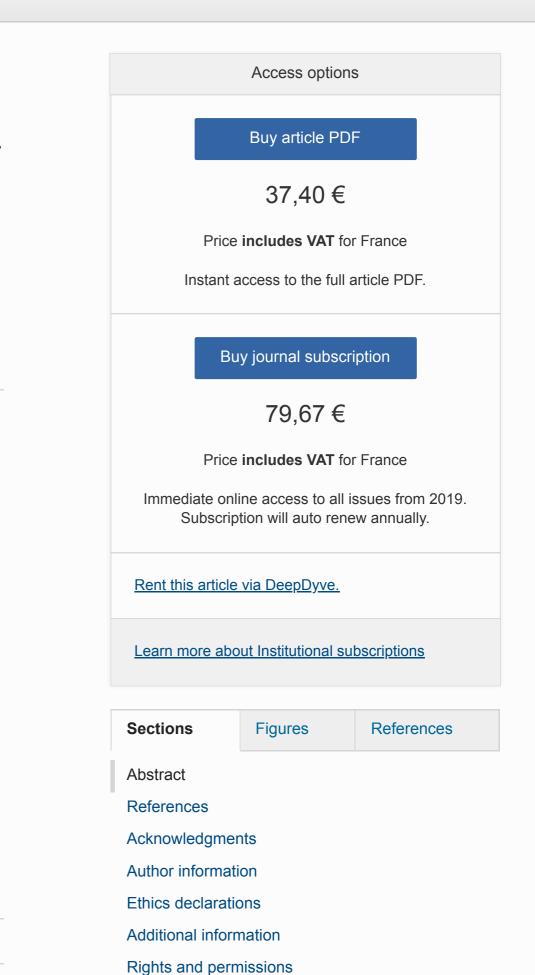
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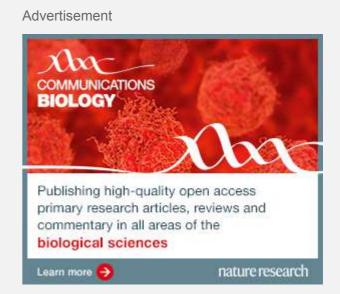
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One-Pot Green Synthesis of Silver Nanoparticles Using the Orchid Leaf Extracts of Anoectochilus elatus: Growth Inhibition Activity on Seven Microbial Pathogens

Kasi Gopinath 🖂, Natarajan Parimala Devi, Marimuthu Govindarajan, Kasi Bhakyaraj, Shanmugasundaram Kumaraguru, Ayyakannu Arumugam, Naiyf S. Alharbi, Shine Kadaikunnan & Giovanni Benelli

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Abstract

The development of reliable antibiotics in the fight against multidrug resistant bacteria is a crucial challenge nowadays. Here, we describe a green chemistry approach for the synthesis of antimicrobial silver nanoparticles (AgNPs) employing the leaf extract of the orchid Anoectochilus elatus. Synthesized Ag NPs were studied by UV–visible and ATR-FT-IR spectroscopy, as well as by XRD, SEM and TEM. The UV–Visible spectra of AgNPs exhibited a surface plasmon resonance peak at 420 nm. XRD analysis showed that peaks at 38.09°, 44.59°, 64.67° and 77.54° confirmed the crystalline nature of AgNPs. TEM revealed spherical shapes with an average particle size of 20 nm. Synthesized AgNPs were screened to evaluate their antimicrobial activity against five Gram-negative and two Gram-positive human bacterial pathogens. AgNPs showed effective antibacterial activity against all tested bacterial strains at the concentration of 30–50 μL. Furthermore, the bactericidal efficacy of AgNPs can be useful to develop new eco-friendly antibacterial products to be used for photocatalytic, textile fabric applications, as well as to co-formulate detergents.

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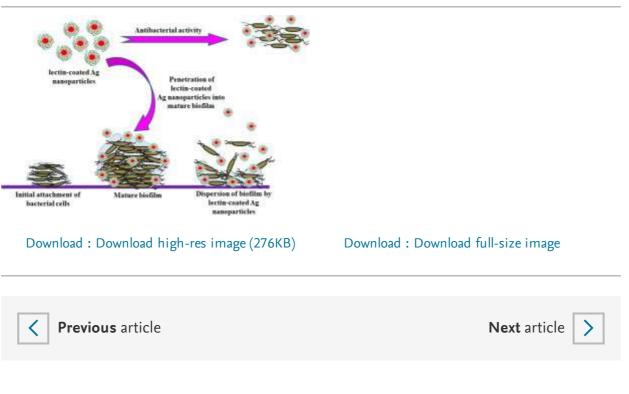
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Abstract

Lectins from the haemolymph of arthropods, including crustaceans, are molecules potentially involved in the immune recognition and phagocytosis. Here, lectin was purified from the haemolymph of blue swimmer crab Portunus pelagicus, using mannose-coupled sepharose CL-4B affinity column chromatography. In SDS-PAGE analysis, lectin showed a molecular mass of approximately 155 kDa. The synthesis of lectin-coated silver nanoparticles (lectin-coated AgNPs) was confirmed by UV–Vis spectroscopy, XRD, FTIR, SEM, TEM, SAED, and EDX analysis. TEM analysis revealed that lectin-coated AgNPs were spherical in shape with size of 30–57 nm. Their antibacterial activity against human pathogenic Gram negative and Gram-positive bacteria was determined by agar well diffusion method. Lectin-coated AgNPs showed significant antimicrobial activity when compared to lectin and silver nitrate tested alone. The antibiofilm properties of lectin-coated AgNPs were also investigated on human pathogenic Gram-negative Proteus vulgaris, Pseudomonas aeruginosa and Gram-positive Enterococcus faecalis and Bacillus pumilus. Lectincoated AgNPs showed antibiofilm activity on the bacteria as well as on *Candida* albicans. Lectin-coated AgNPs reduced the biofilm architecture interfering with cell adhesion and polysaccharide matrix. This was additionally confirmed by exopolysaccharide (EPS) quantification index revealing the trouble in the structural reliability of biofilm by decrease in EPS and bacterial adhesion to hydrocarbons.

Graphical abstract



Keywords

Antibiofilm; Exopolysaccharide; Blue swimmer crab; Candida albicans

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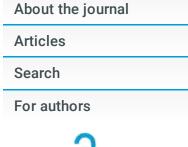
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One-pot and eco-friendly synthesis of silver nanocrystals using *Adiantum raddianum*: Toxicity against mosquito vectors of medical and veterinary importance

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Mosquitoes (Diptera: Culicidae) represent a key threat for millions of humans and animals worldwide, since they act as vectors for devastating parasites and pathogens. Eco-friendly control tools are a priority. Plant-mediated biosynthesis of nanoparticles is rapid and cost-effective. Here we biosynthesized poly-dispersed silver nanocrystals (AgNPs) using a cheap aqueous leaf extract of *Adiantum raddianum*. AgNPs were characterized by UV-vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive spectroscopy (EDX) and X-ray diffraction analysis (XRD). The acute toxicity of *A. raddianum* extract and biosynthesized AgNPs was evaluated against larvae of the malaria vector *Anopheles stephensi*, the dengue vector *Aedes aegypti* and the filariasis vector *Culex quinquefasciatus*. Compared to the leaf aqueous extract, AgNPs showed higher toxicity against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* with LC₅₀ values of 10.33, 11.23 and 12.19 µg/ml, respectively. Biosynthesized AgNPs were found safer to non-target organisms *Diplonychus indicus*, *Anisops bouvieri* and *Gambusia affinis*, with respective LC₅₀ values ranging from 517.86 to 635.98 µg/ml. Overall, this study firstly shed light on the potential of *A. raddianum* as a potential bio-resource for rapid, cheap and effective nanosynthesis of novel mosquitocides.

Keywords: Arbovirus; Filariasis; Malaria; Mosquito-borne diseases; Nanotechnology

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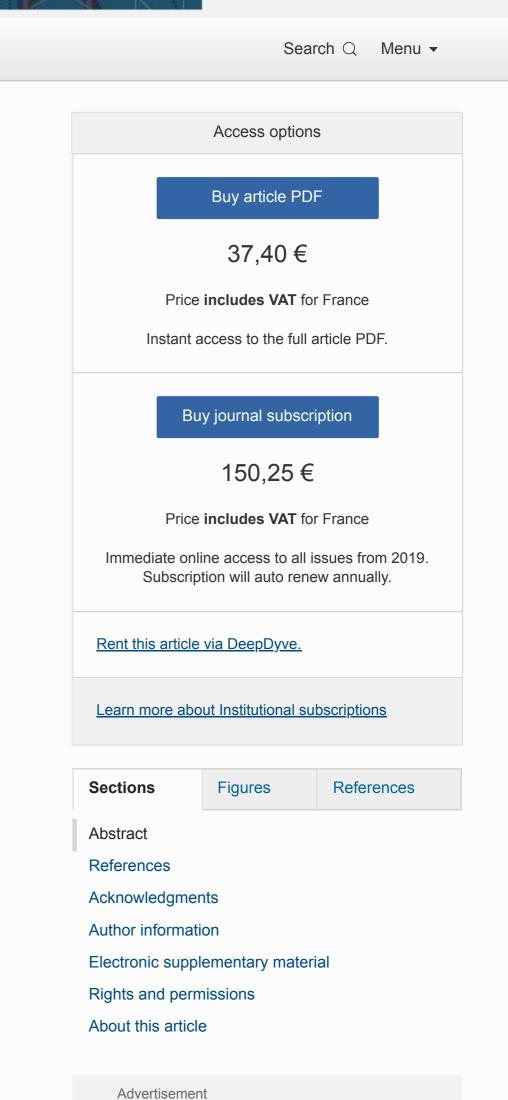
Toxicity of herbal extracts used in ethno-veterinary medicine and green-encapsulated ZnO nanoparticles against *Aedes aegypti* and microbial pathogens

Balan Banumathi, Baskaralingam Vaseeharan ⊡, Ramachandran Ishwarya, Marimuthu Govindarajan, Naiyf S. Alharbi, Shine Kadaikunnan, Jamal M. Khaled & Giovanni Benelli

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Abstract

Dengue and chikungunya are arboviral diseases mainly vectored by the mosquito *Aedes aegypti*. Presently, there is no treatment for these viral diseases and their prevention is still based on vector control measures. Nanopesticides fabricated using herbal extracts as reducing and capping agents currently represent an excellent platform for pest control. In this scenario, the present study assessed the acute toxicity of seven plants employed in ethno-veterinary medicine of southern India, as well as the green synthesis of zinc oxide nanoparticles, on third-instar larvae of A. aegypti. Larvae were exposed to extracts of the seven plants obtained with solvents of different polarity (acetone, ethanol, petroleum ether, and water) for 24 h. Maximum efficacy was observed for Lobelia leschenaultiana leaf extracts prepared using all the four solvent extracts (LC $_{50}$ = 22.83, 28.12, 32.61, and 36.85 mg/L, respectively). Therefore, this plant species was used for the synthesis and stabilization of ZnO nanoparticles based on its maximum efficacy against third-instar larvae of A. aegypti. L. leschenaultianaencapsulated ZnO nanoparticles showed 100% mortality when tested at 10 mg/L, the LC_{50} was extremely low, 1.57 mg/L. Zinc acetate achieved only 65.33% when tested at 60 mg/L, with a LC₅₀ of 51.62 mg/L. Additionally, ZnO nanoparticles inhibited growth of Pseudomonas aeruginosa, Proteus vulgaris, Shigella sonnei, and Vibrio parahaemolyticus and also inhibited biofilm formation on selected microbila pathogens, showing impact on EPS production and hydrophobicity. Overall, our results suggest that L. leschenaultiana-fabricated ZnO nanoparticles have a significant potential to control A. aegypti mosquitoes and Gram-



negative bacterial pathogens.

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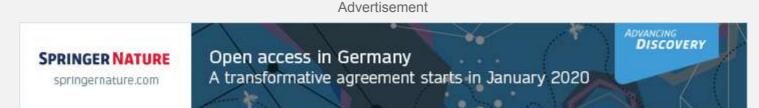
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Toxicity of *Camellia sinensis*-Fabricated Silver Nanoparticles on Invertebrate and Vertebrate Organisms: Morphological Abnormalities and DNA Damages

Balan Banumathi, Baskaralingam Vaseeharan ▷, Periyakaruppan Suganya, Thavasimuthu Citarasu, Marimuthu Govindarajan, Naiyf S. Alharbi, Shine Kadaikunnan, Jamal M. Khaled & Giovanni Benelli
 Journal of Cluster Science 28, 2027–2040(2017) | Cite this article
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Abstract

Our understanding of nanoparticle toxicity and fate in the aquatic environment is still patchy. In the present study, the toxicity of silver nanoparticles coated by *Camellia sinensis* (*Cs*) leaf extract metabolites (*Cs*-AgNPs) was investigated in comparison with *C. sinensis* leaf extract and AgNO₃ on a micro-crustacean, *Ceriodaphnia cornuta*, and a fish *Poecilia reticulata*. 100% mortality of *C. cornuta* was observed post-exposure to AgNO₃ (40 µg/ml) if compared to the *Cs* leaf extract and *Cs*-AgNPs, showing 30 and 56% mortality at the same concentration, respectively. In *P. reticulata* 100% mortality was observed testing AgNO₃ and *Cs*-AgNPs post-exposure to 1 and 30 µg/ml, respectively. Light microscopy and CLSM images showed the accumulation of nanoparticles in the intestine of *C. cornuta* treated with *Cs*-AgNPs at 40 µg/ml. In addition, histological observations confirmed the abnormal tissue texture in nanoparticle-exposed *P. reticulata*, if compared to control fishes. Furthermore, *C. cornuta* and *P. reticulata* treated with *Cs*-AgNPs showed DNA damages compared to the control. Overall, these findings indicated relevant limits about the employ of silver-based pesticides in the environment, and also pointed out the *Cs*-AgNPs were less toxic to *C. cornuta* and *P. reticulata* if compared to silver ions.

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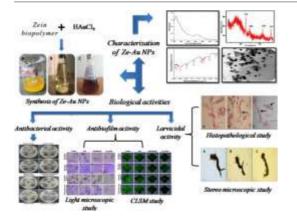
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Abstract

The control of multidrug-resistant bacteria as well as insect pests and vectors is timely and important now a days. The present study was designed to evaluate the in vitro antibacterial, antibiofilm and mosquito larvicidal effects of gold nanoparticles synthesized using the zein biopolymer (Ze-AuNPs) against Gram positive (Bacillus pumilus and Bacillus subtilis), Gram negative (Shigella sonnei and *Pseudomonas aeruginosa*) bacteria and third instar larvae of the dengue and Zika virus vector Aedes aegypti. The synthesized Ze-AuNPs were characterized by UV-vis spectroscopy, X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The antibacterial assays testing Ze-AuNPs at 100 µg/ml showed that the zones of inhibition against Gram positive species B. pumilus and B. subtilis were 13.9 and 14.2 mm, respectively, while for Gram negative S. sonnei and P. aeruginosa they were 18.1 and 18.4 mm, respectively. Light and confocal laser scanning microscopy (CLSM) confirmed the interruption and disintegration of bacterial biofilm post-treatment with Ze-AuNPs at 100 µg/ml. In larvicidal assays on A. aegypti, HAuCl₄ and Ze-AuNPs treated third instar larvae of *A. aegypti* showed LC₅₀ of 26.6 and 6.81 mg/L, respectively, and LC₉₀ of 81.1 and 13.6 mg/L respectively. The histopathological analysis of A. aegypti treated with Ze-AuNPs showed complete disintegration of abdominal region, particularly the midgut and caeca, with loss of lateral and caudal hairs. The stereomicroscopic visualization of A. aegypti treated with Ze-AuNPs showed the loss of upper head hair, lower head hair, antenna hair, lateral hair and caudal hair. Overall, the study concludes that Ze-AuNPs have excellent antibacterial, antibiofilm effects and has ability to control the larval populations of A. aegypti mosquitoes.

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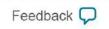
Antimicrobial; Dengue; Mosquito control; Bionanotechnology; Polymermediated nanosynthesis

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Abstract

The impact of green-fabricated gold nanoparticles on plant cells and nontarget aquatic species is scarcely studied. In this research, we reported an environment friendly technique for the synthesis of gold nanoparticles (Au NPs) using the Sphaeranthus indicus leaf extract. The formation of the metal NPs was characterized by UV-Visible and FT-IR spectroscopy, XRD, SEM and TEM analyses. The UV–Visible spectra of Au NPs showed a surface plasmon resonance peak at 531 nm. FT-IR analysis indicated functional bio-molecules associated with Au NPs formation. The crystalline nature of Au nanoparticles was confirmed by their XRD diffraction pattern. TEM revealed the spherical shape with a mean particle size of 25 nm. Au NPs was tested at 0, 1, 3, 5, 7 and 10% doses in mitotic cell division assays, pollen germination experiments, and in vivo toxicity trials against the aquatic crustacean Artemia nauplii. Au NPs did not show any toxic effects on plant cells and aquatic invertebrates. Notably, Au NPs promoted mitotic cell division in Allium cepa root tip cells and germination of Gloriosa superba pollen grains. Au NPs showed no mortality on A. nauplii, all the tested animals showed 100% survivability. Therefore, these Au NPs have potential applications in the development of pollen germination media and plant tissue culture.

Graphical Abstract



Keywords

Biosafety; Green synthesis; Microcrustacean; Biocompatibility; Pollen germination; Mitotic index

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Original Paper | Published: 11 July 2017

Euphorbia rothiana-Fabricated Ag Nanoparticles Showed High Toxicity on *Aedes aegypti* Larvae and Growth Inhibition on Microbial Pathogens: A Focus on Morphological Changes in Mosquitoes and Antibiofilm Potential Against Bacteria

Balan Banumathi, Baskaralingam Vaseeharan ⊡, Thenmozhi Chinnasamy, Sekar Vijayakumar, Marimuthu Govindarajan, Naiyf S. Alharbi, Shine Kadaikunnan, Jamal M. Khaled & Giovanni Benelli
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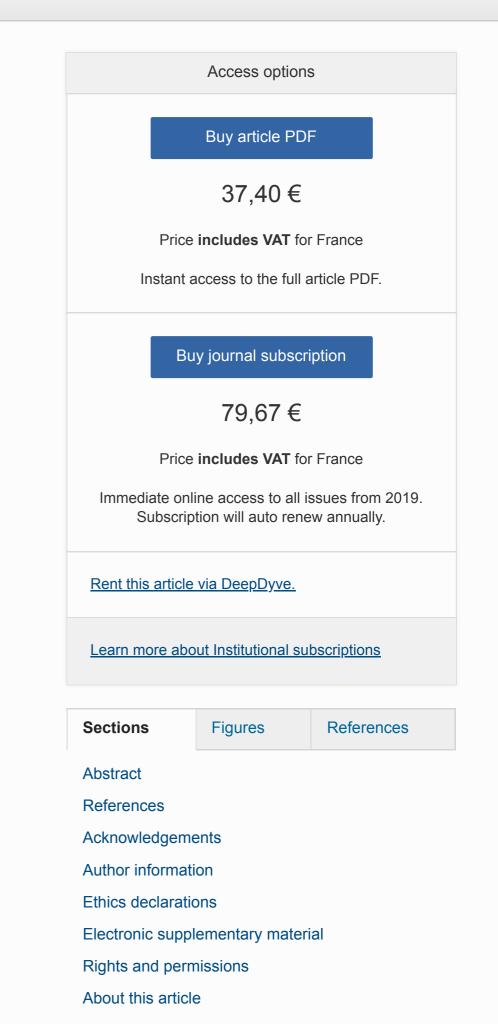
Abstract

The green synthesis of pesticides and antibiotics is gaining increasing importance nowadays. We fabricated *Euphorbia rothiana*-capped Ag nanoparticles (Er-AgNPs), testing their antibiofilm and growth inhibition potential on various microbial pathogens. We evaluated the toxicity of *E. rothiana*-capped Ag nanoparticles on larvae of the Zika virus vector *Aedes aegypti*, shedding light on histological changes post-exposure to the plant leaf extract and Er-AgNPs. Nanoparticles were characterized by UV–Vis, and FTIR spectroscopy, XRD and TEM. Light microscopy showed the greatest inhibition of biofilm formation in Gram positive and Gram-negative bacteria post-treatment with *E. rothiana*-capped Ag nanoparticles at 75 µg/ml. Confocal microscopy confirmed the biofilm interruption and disintegration post-treatment with nanoparticles at 75 µg/ml. *E. rothiana* extract and Er-AgNPs showed 100% larvicidal activity on *A.aegypti* at 50 and 8 mg/L, respectively. AgNO₃ led to 100% mortality of *A. aegypti* larvae at 40 mg/L after 48 h. Stereomicroscopic and histopathological analysis reported tissue damages and loss of cuticular parts in mosquito larvae.

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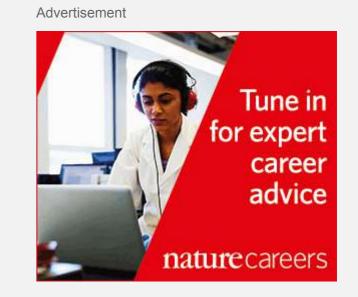
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Original Paper | Published: 21 July 2017

Green Synthesis of Ag Nanoparticles with Anti-bacterial Activity Using the Leaf Extract of an African Medicinal Plant, *Ipomoea asarifolia* (Convolvulaceae)

Jamal M. Khaled, <u>Naiyf S. Alharbi</u>, <u>Shine Kadaikunnan</u> ⊡, <u>Ahmed S. Alobaidi</u>, <u>Mohammed N. Al-Anbr</u>, <u>Kasi Gopinath</u> ⊡, <u>Ayyakannu Aurmugam</u>, <u>Marimuthu Govindarajan</u> & <u>Giovanni Benelli</u>

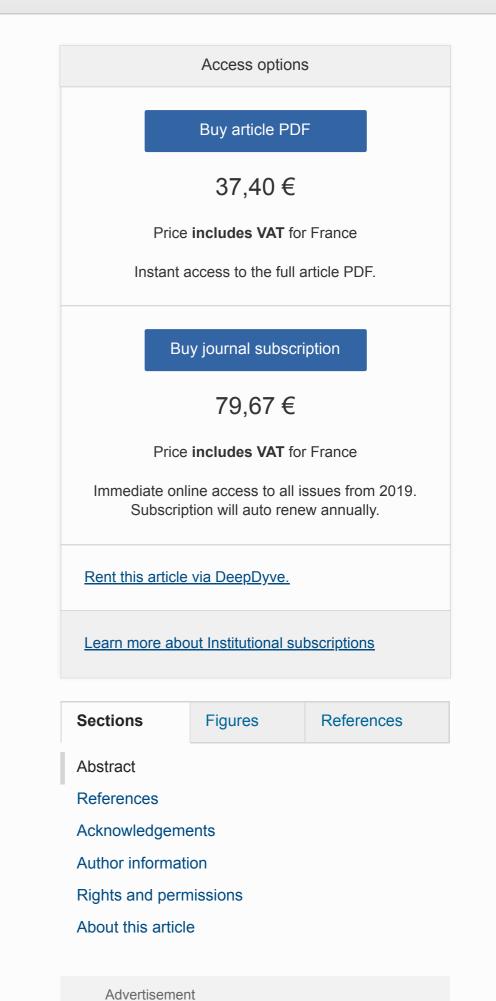
Journal of Cluster Science **28**, 3009–3019(2017) | <u>Cite this article</u> **311** Accesses | **8** Citations | <u>Metrics</u>

Abstract

Herbal drugs prepared with the plant *Ipomoea asarifolia* has many medicinal uses throughout West Africa, including its employ as diuretic, emmenagogue and purgative. This species is a good source of acylated anthocyanins, and the methanol and ethyl acetate extracts of *I. asarifolia* are known for their strong acetylcholinesterase inhibitory effects. Here, green synthesis of Ag nanoparticles (Ag NPs) was performed at room temperature using the *I. asarifolia* leaf extract. The Ag NPs were characterized by UV–visible spectroscopy, FT-IR, XRD, AFM, SEM with EDX and TEM. AFM and SEM analyses showed that spherical morphology of *I. asarifolia*-synthesized Ag NPs with a size range of 20 to 60 nm. TEM revealed that *I. asarifolia*-synthesized Ag NPs had a mean particle size of 40 nm. The *I. asarifolia*-synthesized Ag NPs was tested against four bacterial pathogens using the disk diffusion method. The zone of inhibition varied significantly in a dose-dependent manner. Overall, our results underlined the potential of cheap aqueous extracts from this African medicinal plant species for the synthesis of silver nanocomplexes with wide efficacy against human pathogenic bacteria.

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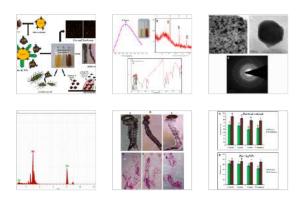
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Tables (1)

Table 1





Antibacterial activity and antibiotic modulati... Journal of Photochemistry and Photobiology B: Bi...

Eco-friendly fabrication of Ag nanostructures using the seed extract of Pedalium murex, an ancient Indian medicinal plant: Histopathological effects on the Zika virus vector Aedes aegypti and inhibition of biofilmforming pathogenic bacteria

Ramachandran Ishwarya ^a, Baskaralingam Vaseeharan ^a pprox oxtimes, Ramasamy Anuradha ^a, Ravichandran Rekha ^a, Marimuthu Govindarajan ^b, Naiyf S. Alharbi ^c, Shine Kadaikunnan ^c, Jamal M. Khaled ^c, Giovanni Benelli ^{d, e} **∃** Show more

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- Facile green synthesis of silver nanoparticles was performed using the Pedalium murex seed extract.
- Nanoparticles were characterized using an extensive array of • biophysical methods.
- Nanoparticles' larvicidal activity on Zika virus vectors was substantiated by histopathological analyses.
- Nanoparticles also showed high antibacterial and antibiofilm • activity.

Abstract

The control of Zika virus mosquito vectors and well as the development of drugs in the fight against biofilm-forming microbial pathogens, are timely and important challenges in current bionanoscience. Here we focused on the ecofriendly fabrication of Ag nanostructures using the seed extract of *Pedalium murex*, an ancient Indian medicinal plant. Initial confirmation of Ag nanoparticles (AgNPs) production was showed by a color change from transparent to dark brown. The UV–Visible spectrum (476 nm), X-ray diffraction peaks (101, 200, 220 and 311) and Fourier transform infrared spectroscopy shed light on the production of green-capped AgNPs. Morphological structure analysis using HR-TEM showed that the AgNPs were mostly hexagonal in shape with rough edges, and a size of 20–30 nm. The larvicidal potential of P. murex seed extract and AgNPs fabricated using the P. murex seed extract (Pm-AgNPs) was tested on fourth instar mosquito larvae of the Zika virus vector Aedes aegypti. Maximum efficacy was achieved by Pm– AgNPs against Ae. *aegypti* after 24 h (LC_{50} 34.88; LC_{90} 64.56 mg/ml), if compared to the *P. murex* seed extract. Histopathological analyses showed severe damages to the hindgut and larval muscles in NPs-treated Ae. aegypti larvae. The sub-MIC concentrations of *Pm*-AgNPs exhibited significant anti-biofilm activity against Gram positive (Enterococcus faecalis, Staphylococcus aureus) and Gram negative (Shigella sonnei, Pseudomonas aeruginosa) bacterial pathogens, as showed by EPS and MTP assays. Light and CLSM microscopic studies highlighted a significant impact of *P. murex* seed extract and *Pm*–synthesized AgNPs on the surface topography and architecture of bacterial biofilm, both in Gram positive and Gram negative species. Overall, results reported here contribute to the development of reliable large-scale protocols for the green fabrication of effective mosquito larvicides and biofilm inhibitors.



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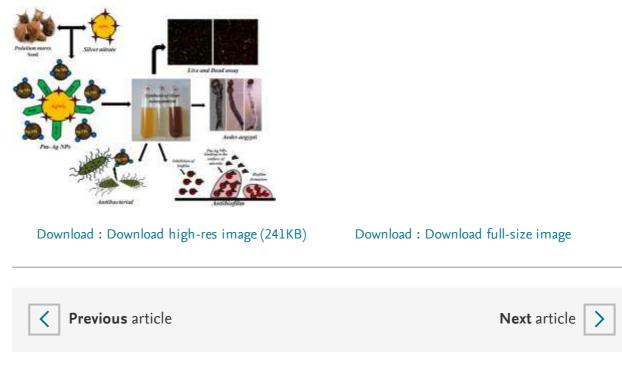
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Ag nanoparticles; CLSM; Dengue; EPS assay; Microbial pathogens; TEM

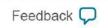
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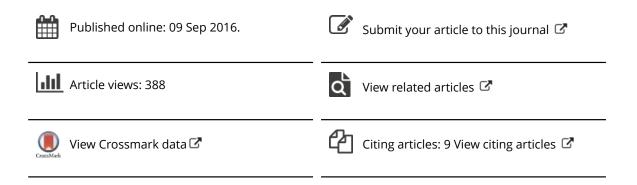
ISSN: 2169-1401 (Print) 2169-141X (Online) Journal homepage: https://www.tandfonline.com/loi/ianb20

Single-step biological fabrication of colloidal silver nanoparticles using *Hugonia mystax:* larvicidal potential against Zika virus, dengue, and malaria vector mosquitoes

Marimuthu Govindarajan, Shine Kadaikunnan, Naiyf S. Alharbi & Giovanni Benelli

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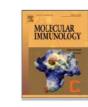
A study on β -glucan binding protein (β -GBP)

and its involvement in phenoloxidase cascade

in Indian white shrimp Fenneropenaeus indicus

Govindarajan ^b, Naiyf S. Alharbi ^c, Shine Kadaikunnan ^c, Jamal M. Khaled ^c, Giovanni Benelli ^{d, e}

Mahalingam Anjugam ^a, Baskaralingam Vaseeharan ^a $\stackrel{ heta}{\sim}$ $\stackrel{ heta}{\sim}$, Arokiadhas Iswarya ^a, Muthu Amala ^a, Marimuthu



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Abstract

The present study reports the purification of novel immune molecule β -1, 3 glucan binding protein from the heamolymph of the Indian white shrimp, Fenneropenaeus indicus (Fiβ-GBP). The purified Fiβ-GBP had 95 kDa molecular weight in SDS-PAGE analysis. MALDI-TOF/TOF analysis revealed that the purified $Fi\beta$ -GBP showed similarity to various crustacean proteins; 48 and 46% similarity was observed for β -1, 3 glucan binding protein of Chinese white shrimp Fenneropenaeus chinensis and banana shrimp Fenneropenaeus merguiensis, with MOWSE score of 3.11e + 12 and 2.05e + 8, respectively. The phenoloxidase activity (PO) of $Fi\beta$ -GBP was evaluated and, in the presence of laminarin, PO activity increased significantly. Substrate specificity assay demonstrated that *Fi* β -GBP had the specific binding site for soluble or insoluble β -glucan (laminarin), since the PO activity increased in the presence of laminarin when compared to other sugars. Enzymatic activities revealed that the optimum temperature and pH for Fiβ-GBP activating PO were 40 °C and pH 7–8. Moreover, even at 100 °C Fiβ-GBP enhanced PO activity highlighting that Fiβ-GBP was thermostable and thermophilic in nature. Among various divalent metallic ions, $Fi\beta$ -GBP significantly promoted the PO activity in presence of Mg^{2+} and Ca^{2+} . The breakdown of para nitroanilide from N_{α} -Benzoyl-L-Arginine 4-Nitroanilide hydrochloride showed that serine protease activity was induced by $Fi\beta$ -GBP and also increased concentration of $Fi\beta$ -GBP evoked the activity. Furthermore, hemolytic activity tests revealed that PO reaction product induced RBC membrane damage and cell shrinkage. Lastly, Baclight bacterial viability assays showed maximum killing effect of PO reaction product on both Gram positive and Gram negative bacteria.

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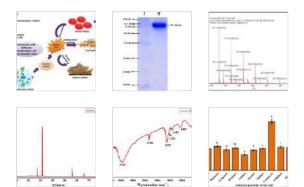
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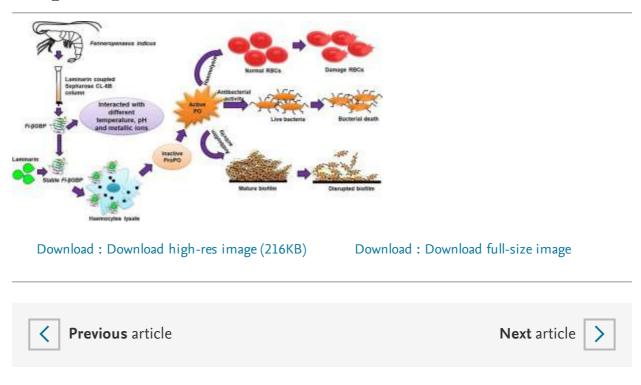


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Innate immunity; β -1, 3 glucan binding protein; Phenoloxidase; Serine protease



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Journal of Nutritional Biochemistry

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Grape seed proanthocyanidins protects against cadmium induced oxidative pancreatitis in rats by attenuating oxidative stress, inflammation and apoptosis via Nrf-2/HO-1 signaling

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Received 20 August 2015; received in revised form 9 February 2016; accepted 3 March 2016

Abstract

The present study has been designed and carried out to explore the role of grape seed proanthocyanidins (GSP) in the pancreas of cadmium (Cd)-induced cellular oxidative stress-mediated toxicity in rats. Four groups of healthy rats were given oral doses of Cd (5-mg/kg BW) and to identify the possible mechanism of action of GSP 100-mg/kg BW was selected and was given 90 min before Cd intoxication. The causative molecular and cellular mechanism of Cd was determined using various biochemical assays, histology, western blotting and ELISA. Cd intoxication revealed increased levels of proinflammatory cytokines (TNF- α , IL1 β and IFN- γ), reduced levels of cellular defense proteins (Nrf-2 and HO-1) and glucose transporter (GLUT-2 and GLUT-4) along with the enhanced levels of signaling molecules of apoptosis (cleaved Caspase-12/9/8/3) in the pancreas of Cd-intoxicated rats. Results suggested that the treatment with GSP reduced blood glucose level, increased plasma insulin and mitigated oxidative stress-related markers. GSP protects pancreatic tissue by attenuated inflammatory responses and inhibited apoptosis. This uniqueness and absence of any detectable adverse effect of GSP proposes the possibility of using it as an effective protector in the oxidative stress-mediated pancreatic dysfunction in rats. (2016 Elsevier Inc. All rights reserved.

Keywords: Cadmium; Inflammation; Oxidative stress; Apoptosis; Antioxidant; GSP

1. Introduction

Cadmium (Cd) is a superfluous trace element, which is toxic to many plants and animals. It is an industrial-used substance with negative long-time effects on human health. Cd content in human can gradually increase by exposure to Cd contaminated foods which may cause a higher incidence of many Cd allied diseases, including renal

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dysfunction, hepatotoxicity, osteoporosis and cancers [1]. Although Cd accumulates in the liver, kidney and bone, pancreas is also considered as an important target organ of Cd [1]. The pancreas is a pinkish white glandular organ and is considered as the second largest gland after the liver. The pancreas is serene of four different cell types, including alpha, beta, delta and pancreatic polypeptide producing (PP) cells, and located within the endocrine part of the tissues [2]. Cd in pancreas of rats is mostly bound to MT [3]. However, MT in the pancreas is highly inclined to oxidative reactions compared to MT in the liver, kidney and spleen [3]. Pancreatic cells are susceptible to oxidative damage because of the two reasons: one is their lower antioxidant defense machinery, and other is the overproduction of ROS within the cell due to the exposition of the general population to the toxic substances in the daily life [4]. Cd is known to cause the pancreatic beta (β) -cell damage and affect carbohydrate metabolism by injuring the Langerhans islet β -cells and reducing the insulin secretion [5]. Long-term exposure to Cd leads to necrosis, degeneration and degranulation of β cells, causing an increase in the serum glucose level and decrease in plasma insulin secretion, that are associated with the altered blood and urine levels of Cd [6]. Cd toxicity has also been observed in the diabetic conditions, which are accompanied with significant Cd accumulation in the blood and/or pancreas [5,6].

Flavonoids are widely being studied due to their medicinal properties, especially their antioxidant activities and are the most common group of polyphenolic compounds in the human diet having

Abbreviations: Cd, Cadmium; GSP, Grape seed proanthocyanidins; β-cell, Beta- cells; MT, Metallothionein; EDTA, Ethylene diamine tetra acetic acid; TC, Total cholesterol; TG, Tri glycerides; LDL, Low- density lipoprotein; HDL, Highdensity lipoprotein; GK, Glucokinase; PK, pyruvate kinase; DCFH-DA, Dichloro dehydro fluorescein diacetate; NADPH, Nicotinamide adenine dinucleotide phosphate; TrxR, Thioredoxin reductases; TNF-α, Tumor necrosis factor; IL-1β, Interleukin 1 beta; IFN-γ, interferon gamma; ELISA, Enzyme-linked immunosorbent assay; GLUT-2, Glucose transporter 2; GLUT-4, Glucose transporter 4; Nrf-2, Nuclear factor-erythroid 2-related factor 2; HO-1, Heme oxygenase-1; iNOS, Inducible nitric oxide synthetase; Bax, Bcl-2 associated X protein; Bcl-2, B- cell lymphoma 2; NFκB, Nuclear factor kappa B; PARP, Poly (ADP ribose) polymerase

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4. Hepatoprotective effects of quercetin

5. Conclusion

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Figures (1)



Tables (4)

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■ Table 3

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Food and Chemical Toxicology Volume 108, Part B, October 2017, Pages 365-374

Hepatoprotective effect of quercetin: From

Selvaraj Miltonprabu ^a lpha \boxtimes , Michał Tomczyk ^b, Krystyna Skalicka-Woźniak ^c, Luca Rastrelli ^d, Maria Daglia ^e,

Liver diseases caused by viral hepatitis infections have a negative impact on global health. Approximately 30 million people in the USA and 29 million

people in the European Union suffer from chronic liver disease. There are

excessive alcohol consumption. Many herbal medicines are used in traditional medicine for their protective and therapeutic properties against liver diseases.

Among their bioactive components, flavonoids have been found to be active

against liver dysfunction and damage caused by liver diseases. Extensive

including anti-oxidative, anti-inflammatory, anti-apoptotic and

evidences report that quercetin (QE), a major flavonol commonly found in

apple, berries, onion, citrus fruits, cruciferous vegetables, tea, pepper, tomato,

whole gain, cocoa and red wine, displays a wide range of healthy properties,

hepatoprotective activities against various hepatic ailments. This review aims

effects of QE with special emphasis on its mechanisms of actions. To provide a

bioavailability are reported. Overall, data in literature shows that QE appears to

to critically analyze the available literature regarding the hepatoprotective

complete picture of QE, its distribution, chemistry, biosynthesis and

many kinds of diseases of the liver, caused by viruses, such as hepatitis A,

hepatitis B and hepatitis C, or by certain drugs and poisons including



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be a promising hepatoprotective compound.

Keywords

Flavonoid; Hepatoprotection; Liver diseases; Oxidative stress; Quercetin

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Hepatoprotective effect of grape seed proanthocyanidins on Cadmium-induced hepatic injury in rats: Possible involvement of mitochondrial dysfunction, inflammation and apoptosis



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Keywords: Cd GSP Mitochondria Oxidative stress Liver Rats

ABSTRACT

The present study was undertaken to evaluate the possible ameliorative role of grape seed proanthocyanidins (GSP) against Cadmium (Cd) induced hepatic inflammation, apoptosis and hepatic mitochondrial toxicity in rats. Male Wistar rats were distributed in four experimental groups: control, GSP, Cd and Cd + GSP. Exposure to a hepatotoxic dose of Cd (5 mg/kg BW) caused liver damage, coupled with enhanced reactive oxygen species (ROS) generation, increased inflammation and apoptosis in liver with increased DNA damage in hepatocytes of rats. Mitochondria were isolated from the hepatic tissues of rats from each group. Our results showed significant decrease in the tri-carboxylic acid cycle enzymes, increased mitochondrial swelling, inhibition of cytochrome c oxidase activity and complex I–III, II–III and IV mediated electron transfer, decreased mitochondrial ATPases, a reduction in calcium content and mitochondrial oxygen consumption in Cd treated rats. All these molecular changes caused by Cd were alleviated by the pre-supplementation with GSP (100 mg/kg BW). The ultra structural changes in the liver also support our findings. From our results, it is clearly indicated that the free radical scavenging, metal chelating and antioxidant potentials of GSP might be the possible reason, responsible for the rescue action against Cd induced mitochondrial damage in the liver of rats.

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1. Introduction

Cadmium (Cd) is one of the most toxic metals released into the environment and is known to be a hepatotoxic facet that can be transferred between various levels of the food chain. Cd belongs to the group of transition elements and adopts almost only one oxidation state +2 [1]. The toxic action of the Cd is recognized to be multifactorial. Cd acts as a catalyst in the oxidative reactions of biological micro molecules and therefore toxicities associated with this metal might be due to the oxidative tissue damage. Cd increases the production of reactive oxygen species (ROS) not through the Fenton like reaction [1], but the mechanism involves the interference with the activities of antioxidant, pro-oxidant and some other enzymes, alteration in thiol proteins, inhibition of energy metabolism and alteration in DNA structure and inhibits the activity of several crucial enzymes of the antioxidative defense system. Binding of Cd to sulphydryl group results in the primary injury

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of cells in mitochondria and secondary injury initiated by the activation of Kupffer cells have been mentioned as a possible mechanism of Cd induced toxic effects [2]. Activated Kupffer cells release proinflammatory cytokines and chemokines, which stimulate the migration and accumulation of neutrophils and monocytes in the liver, which amplificate the Cd, induced primary injury in hepatocytes [3]. The liver is one of the major target organs of both chronic and acute Cd exposure. While hepatocytes and endothelial cells of the liver sinusoids are supposed to be the primary cellular targets in liver [4].

Mitochondria are the key intracellular targets for different stresses, including Cd [5], but the mechanism of metal-induced mitochondrial damage is not fully understood. Mitochondria are the major source of ROS and thus a prime target of Cd induced hepatoxicity. About 1–4% of total consumed mitochondrial oxygen is incompletely reduced to the production of ROS [5]. Cd causes mitochondrial swelling, decreases the expression of genes that control mitochondrial activity, and finally, decreases mitochondrial oxidative capacity and ATP synthesis. Cd insult to mitochondria pertains to a structural damage as well as impairment in the activity of certain enzymes [6] modifies mitochondrial function and inhibits oxidative phosphorylation in the liver of rats.

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Defensive role of silibinin against arsenic induced oxidative stress mediated dyslipidemia and neurotoxicity in rats

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Abstract

Arsenic (As) is an environmental toxic metalloid that is present in everywhere such as air, water and soil. Generally, inorganic arsenic has a tendency to be more toxic than organic arsenic. The present study was designed to determine whether oral administration of silibinin (SB), which has been shown to have substantial antioxidant properties, when pre-administered (75 mg/kg body weight) once daily for 4 weeks along with arsenic (5 mg/kg) would prevent arsenic-induced changes in antioxidant defense system, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX),glutathione-S-transferase (GST),glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PD), reduced glutathione (GSH), total sulfhydryl groups (TSH) and vitamin C in rat brain regions such as cortex, striatum, cerebellum, hippocampus and brain stem. Our study also examined the effect of SB over arsenic-induced reactive oxygen species (ROS) production and lipid peroxidation level (LPO) and protein carbonyl content (PC) in distinct brain regions of rats. Moreover, As also alters the lipid profiles such as total lipids, phospholipids, cholesterol, cerebrosides and gangliosides in various regions of the brain. Pre-administration of SB restores the altered enzymatic and non-enzymatic antioxidants, lipid profiles and also markedly reduced the ROS, LPO, PC and accumulation of As in various regions of the brain. These results suggested that arsenic-induced deficits in antioxidant enzyme activities and increase in ROS production and lipid peroxidation levels in brain regions can be remarkably prevented by pre-administration of SB.

Keywords: Arsenic; Oxidative Stress; Brain; Lipid Profile; Silibinin; Rat.

1. Introduction

Arsenic (As), a Group I carcinogen (International Agency for Research on Cancer (IARC), 1989) and an environmental pollutant, has been implicated in the occurrence of various cancers and numerous health problems (Yoshida et al., 2004). High levels of inorganic arsenic are found in ground water in many regions of the world as a result of geochemical processes posing serious chronic health risks to humans (Yadav et al., 2009; Brinkel et al., 2009). Arsenic exposure has been associated with health problems, including hypertension (Yadav et al., 2009), cardiovascular diseases, developmental abnormalities, diabetes, hearing loss, fibrosis of the liver and lung, hematological disorders, neurological and reproductiveproblems, blackfoot disease and cancer (Kapaj et al., 2006; Khan et al., 2006). Moreover, peripheral neuropathy following arsenic toxicity has been reported (Kapaj et al., 2006; Vahidnia et al., 2007).

In view of the adverse effects of arsenic on brain, extensive studies have been undertaken to understand the mechanisms of arsenic-induced neurotoxicity. Enhanced generation of reactive oxygen species (ROS) and nitrogen species (RNS) associated with a deficient antioxidant system leading to increased oxidative stress is largely accepted as one of the potential mechanisms of arsenic neurotoxicity (Flora and Gupta, 2007; Shila et al., 2005a; Sinha et al., 2008a; Das et al., 2010) results in damage to mitochondrial membrane and subsequently to cell death. Involvement of neuronal nitric oxide synthase (nNOS) and nitric oxide (NO) levels has also been shown in arsenic neurotoxicity (Chattopadhyay et al., 2002; Zarazua et al., 2006; Flora et al., 2009; Rios et al., 2009).

Further, brain is a soft target of arsenic toxicity since it easily crosses the blood-brain barrier (Rosado et al., 2007; Brinkel et al., 2009). A number of studies have shown vulnerability of corpus striatum, Cerebellum, Brain stem, cortex and hippocampus to arsenic toxicity that are associated with behavioral and neurochemical abnormalities (ShilaEt al., 2005b; Yadav et al., 2009). Furthermore, arsenic also alters the contents of lipid classes, proteins, glutathione and the ascorbic acid in various regions of brain (Haider and Najar, 2008) Due to increasing exposure to environmental neurotoxicants like arsenic, there is an increasing interest to investigate the prophylactic and protective efficacy of natural products found in plant extracts. Silibinin (SB) is the major compound of the silymarin isolated from seeds of mediterranean milk thistle, Silybum marianum (L.) Gaertn. (Asteraceae), and clinically used as a hepatoprotectant (Pradhan and Girish, 2006). Silibinin (SB) has antilipoperoxidative activity with anti-inflammatory, anti-allergic and anti-oxidant activities by scavenging various free radicals (Basiglio et al., 2009). SB is reported to have a broad spectrum of biological activities such as hepatoprotective (Ferenci, 1989), antioxidant (Saller et al., 2001), metal chelation (Pietrangelo et al., 1995) free radical-scavenging (Winterbourn, 2008) and etc.,

The present study was carried out to investigate the protective effect of SB against arsenic induced oxidative stress, contents of lipids, proteins, antioxidant defense systems in various regions of rats.



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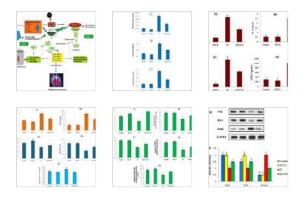
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International Immunopharmacology Volume 39, October 2016, Pages 128-139

Epigallocatechin gallate potentially abrogates

pathway in rats: An in-vivo and in-silico study

fluoride induced lung oxidative stress,

inflammation via Nrf2/Keap1 signaling

Thangapandiyan Shanmugam ^a pprox $minesize{M}$, Miltonprabu Selvaraj ^a, Senthilraja Poomalai ^b



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Abstract

Background

Since this Nrf2-dependent cellular defense response is able to protect multiorgans, including cancer, neurodegenerative diseases, cardiovascular diseases, inflammation and chronic lung injury. The antioxidant and antiinflammatory potential of Epigallocatechin gallate (EGCG) and Nrf2/Keap1 signaling mechanisms in pulmonary toxicity have not been clarified. In the present study, we demonstrated that protective efficacy of EGCG against fluoride (Fl) induced oxidative stress mediated lung injury in rats.

Methods

The animals were divided in to four groups. Group 1: Control rats received normal saline; Group 2 rats received EGCG (40 mg/kg/bw) alone for four weeks; Group 3 rats received Fl (25 mg/kg/bw) alone for four weeks, Group 4 rats received EGCG (90 min before administration) along with Fl for four weeks.

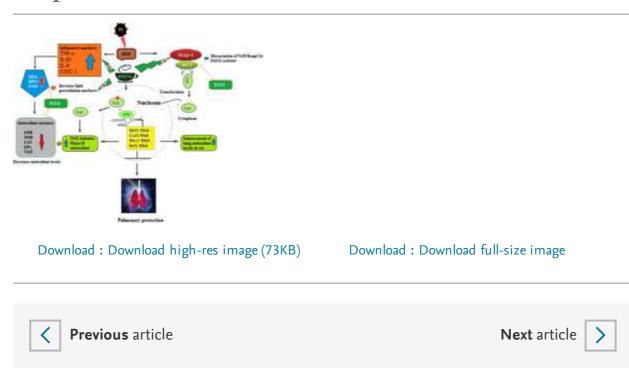
Results

Oral administration of Fl (25 mg/kg/bw) significantly (p < 0.05) increased the ROS, inflammatory cytokines, lung edema, melonaldehyde (MDA) and myeloperoxidase (MPO) in rats. In addition, upon administration of Fl significantly (p < 0.05) decreased the antioxidant status, Nrf2, and HO-1 with increased Keap1 protein. Histological and immunohistochemical (iNOS) study also revealed the Fl induced significant (p < 0.05) changes in the lung tissue of rats. Pre-administration of EGCG significantly (p < 0.05) improved the antioxidant status, and inhibited the oxidative stress, inflammatory cytokines, and Keap1 protein via the activation of Nrf2 translocation in to the nucleus. Moreover, the molecular docking studies also support the antioxidant potential of EGCG and Nrf2 activation.

Conclusion

Taken together, our data indicate that EGCG potentially abrogates Fl induced oxidative lung injury by activation of the Nrf2/Keap1 pathway in rats.

Graphical abstract



Keywords EGCG; NaF; Lung; ROS; Nrf2; Rat

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Entomofaunal diversity of tree hole

ranges of Tamilnadu, India

P. Senthamarai Selvan ^a $\stackrel{\circ}{\sim}$ $\stackrel{\boxtimes}{\sim}$, A. Jebanesan ^a, D. Reetha ^b

https://doi.org/10.1016/j.actatropica.2016.03.029

Acta Tropica Volume 159, July 2016, Pages 69-82

mosquitoes in Western and Eastern Ghats hill



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Highlights

- To understand the distribution patterns of tree hole mosquitoes may provide basic information about the disease incident.
- Information about mosquito's species diversity, distribution in tree • holes are fundamental to take proper control strategies in hills fringe villages.
- Reports on diversity and distribution of tree hole mosquito species • in the selected study areas are sparse.
- Hence, this research contribution has more significance for basic • biological research and developing control strategies for vector borne diseases.
- Results of the present study highlights diversity and distribution • patterns of tree hole mosquito species in Western and Eastern Ghats hill ranges of Tamilnadu, India.

Abstract

The distribution and abundance of various mosquito vectors is important in the determination of disease prevalence in disease endemic areas. The aim of the present study was to conduct regular entomological surveillance and to determine the relative abundance of tree hole mosquito species in Tamilnadu, India. In addition to this, the impact of weather-conditions on tree hole mosquito population were evaluated between June, 2014 and May, 2015. Six hills ranges viz., Anaimalai hills, Kodaikanal hills, Sitheri hills, Kolli hills, Yercaud hills, and Megamalai were selected, the immatures collected from tree holes by the help of suction tube. Collections were made at dusk and dawn at randomly selected 15 different tree species. The collected samples were stored and morphologically identified to species level in the laboratory. Mosquito diversity was calculated by Simpson's and Shannon–Weiner diversity indicies with spatial and temporal aspects. Over 2642 mosquitoes comprising the primary vectors of dengue, chickungunya, malaria, filariasis were identified. Other species collected from the fifteen sites in each hill during the study included Christophersiomyia annularis, Christophersiomyia thomsoni, Downsiomyia albolateralis, Downsiomyia nivea and Toxorhynchites splendens, etc. Study revealed high species diversity and relative density associated with different study sites. Based on the Shannon diversity index high number of species was recorded with Aedes pseudoalbopicta (0.0829) followed by Ae. aegypti (0.0805) and least species was recorded as Anopheles elegans (0.0059). The distribution of the primary vectors of DF along the high occurrence was evident with most study sites representing proportions of this vector population. This showed the high risk level associated with the livestock movement in amplification and circulation of the virus during the outbreaks. The findings of this study, therefore, demonstrated the potential vulnerability of nomadic communities to infection by arboviral diseases transmitted by mosquito vectors.

Extras (1)

Highlights

Outline

Abstract

Graphical abstract

Keywords

1. Introduction

2. Materials and methods

3. Results

4. Discussion

5. Conclusion

Conflict of interest

Acknowled gements

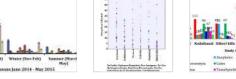
Appendix A. Supplementary data

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Figures (10)





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Tables (5)

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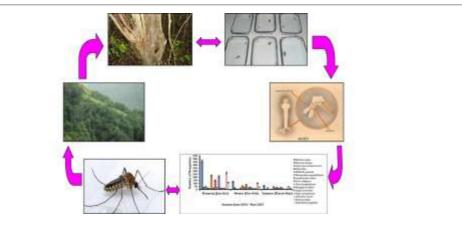
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Keywords

Tree hole mosquitoes; Diversity; Western and Eastern Ghats; Tamilnadu; India

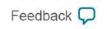
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ORIGINAL PAPER



Do Chenopodium ambrosioides-Synthesized Silver Nanoparticles Impact Oryzias melastigma Predation Against Aedes albopictus Larvae?

Jayapal Subramaniam^{1,2} · Kadarkarai Murugan^{2,3} · Arulsamy Jebanesan¹ · Philips Pontheckan² · Devakumar Dinesh² · Marcello Nicoletti⁴ · Hui Wei⁵ · Akon Higuchi⁶ · Suresh Kumar⁷ · Angelo Canale⁸ · Giovanni Benelli⁸

Received: 23 October 2016/Published online: 7 November 2016 © Springer Science+Business Media New York 2016

Abstract The green synthesis of nanopesticides has been recently proposed to improve the efficacy of mosquito control programs. However, limited efforts shed light on the impact of sub-lethal doses of nanopesticides on behavioral traits of mosquito biocontrol agents. We described the synthesis of silver nanoparticles (AgNP) at room temperature using the aqueous extract of *Chenopodium ambrosioides*, and their high toxicity against the invasive mosquito *Aedes albopictus*. LC₅₀ calculated on young instars ranged from 13 ppm (first instar larvae) to 19 ppm (pupae). LC₅₀ calculated on adults was 14 ppm. The chemical composition of the *C. ambrosioides* extract was characterized by GC–MS analysis. The production of

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Asian Pacific Journal of Tropical Disease Volume 5, Supplement 1, 2015, Pages S59-S66



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Original article

Diversity of mosquitoes and larval breeding preference based on physico-chemical parameters in Western Ghats, Tamilnadu, India

Periyasamy Senthamarai Selvan ^a $\stackrel{ heta}{\sim}$ 🖾, Arulsamy Jebanesan ^a, Govindaraj Divya ^b, Velu Ramesh ^c

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https://doi.org/10.1016/S2222-1808(15)60858-1

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Abstract

Objective

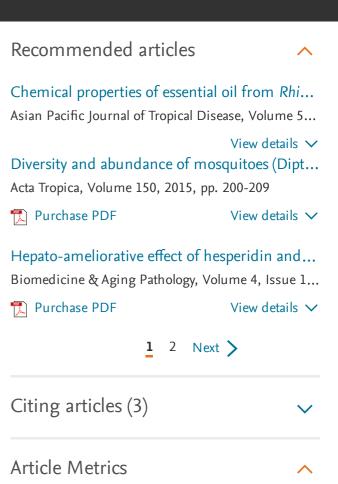
To study the diversity and distribution of mosquitoes in Western Ghats of Coimbatore and Nilgiris District, Tamilnadu, India.

Methods

Random collections were carried out during August-2013 to July-2014 in cesspits, animal footprints, rock holes, tree holes, drainages at study areas of Marudhamalai, Valparai, Mettupalayam in Coimbatore District and Dhottapeta, Coonoor, Gudalur in Nilgiris District of Tamilnadu, India by using suction tube and kerosene pump. Mosquitoes were identified by standard entomological procedures.

Results

A total of 1 018 mosquitoes (larvae and pupae) were collected from all over the study areas comprising 6 genera and 23 species. They are, *Culex mimulus, Culex* pseudovishnui, Culex quinquefasciatus, Culex vishnui, Culex khazani, Culex uniformis, Heizmannia chandi, Heizmannia grenii, Heizmannia indica, Oclerotatus anureostriatus, Oclerotatus albotaeniatis, Oclerotatus deccanus, Oclerotatus gubernatoris, Aedes aegypti, Aedes albopictus, Aedes edwardsi, Aedes krombeini, Toxorhynchites minimus, Toxorhynchites splendens, Anopheles aitkenii, Anopheles barbirostris, Anopheles culiciformis and Anopheles maculatus. Shannon-Weaver diversity index, Margalef's index of richness and Simpsons dominance index was also studied. From 6 sites, the highest mosquitoes were collected from Marudhamalai (309) and the least mosquitoes were collected in Mettupalayam (68). The study determined whether physicochemical characteristics differ between habitats with high and low presence of mosquito larvae. Based on Margalef's index of richness (D_{mg}) , the highest values were present in Mettupalayam (5.214) study area and the lowest in Marudhamalai (3.837). It can be concluded from Shanon-Weaver index of diversity that, the highest values were present in Mettupalayam (2.947) and the least value were in Gudalur (2.410) during the study period.



Conclusions

In areas with reservoirs of disease, mosquito abundance information can help to identify the areas at higher risk of disease transmission. The study identifies mosquito species density and diversity of culicine and anopheline larvae. Hence, this research contribution has more significance for basic biological research and developing control strategies for vector borne diseases.



Next article

Keywords

Mosquitoes; Diversity; Aedes; Anopheles; Culex; Physico-Chemical characters; Western Ghats; Coimbatore; Nilgiris

Available online 4 Jun 2015

Foundation Project: Supported by the University Grants Commission for financial assistance under UGC-Major Research Project Scheme, UGC Ref. Letter No: 42-558/2013 (SR) dated 22.03.2013.

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Cadmium and Apoptosis: A Molecular Approach

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Abstract

Apoptosis, also known as programmed cell death is a highly regulated and fundamental process found in all multicellular organisms. It is not only implicated in regulatory mechanisms of cells, but has been attributed to a number of diseases, like inflammation, malignancy, autoimmunity and neuro degeneration. A variability of pollutants can persuade apoptosis. Oncogenic transition metals like cadmium, promote apoptosis alongside DNA base modifications, strand breaks and rearrangements. Generation of reactive oxygen species, accumulation of Ca^{2+} , upregulation of caspase-3, downregulation of bcl-2, and deficiency of p-53 lead to the Cadmium (Cd) induced apoptosis. Metallothionein expression determines the choice between apoptosis and necrosis in Cd induced toxicity. Thus, disorders of apoptosis may play a critical role in some of the most debilitating metal-induced afflictions including hepato toxicity, renal toxicity, neuro toxicity, autoimmunity and carcinogenesis. An understanding of Cd-induced apoptosis will be obliging in the development of precautionary molecular approach.

Keywords: Metals, cadmium, apoptosis, cell-proliferation, carcinogenicity

*Author of correspondence E-mail: smprabu73@gmail.com

INTRODUCTION

Metals have been exploited by man since antiquity. Anthropogenic uses have led to a global dispersion of metals in the environment. Man, animals and plants are exposed to a variety of metals through environment, food, water and soil. Metals being nonbiodegradable persist in the environment for a period and long causes serious ecotoxicological problems. Additionally, many metallic compounds like zinc, copper, calcium, trivalent chromium, and iron being essential to life have formed complex biogeochemical cycles. Essential elements are involved in a variety of critical functions including the control of gene transcription, nerve conductance and oxygen transport and as active centers in enzymes. Therefore, the critical molecular events within the cell such as gene expression, cell proliferation and cell death are affected by trace elements. Some toxic metals may mimic the essential metals thereby gain access to important and molecular targets. It is flawless that toxic metals can together activate and inactivate the cellular procedures regimented by the indispensable metals. Even crucial metals can be toxic too. An uncommon of the

environmental metals, i.e., arsenic, chromium is carcinogenic.

Apoptosis is considered as an ongoing normal event in the control of cell populations. However, apoptosis can also be induced by a variety of xenobiotics including many of the toxic metals resulting in the loss of affected cell populations. Apoptosis essentially occurs when cellular damage, including damage to genetic material, has exceeded the capacity for repair (Figure 1).

Environmental metals can damage apoptosis and that suppression of the apoptotic response could expedite aberrant cell accumulation, which may be a life-threatening step in the pathogenesis of malignancy or autoimmunity. One of the primary genes shown to regulate apoptosis was Bcl-2. Consequently, a number of Bcl-2 related proteins were also recognized. Although, the overwhelming evidence that Bcl-2 proteins are evolutionarily preserved their regulators of apoptosis, specific biochemical function remains scandalous. There have been a multitude of reports showing that enforced over expression of Bcl-2 or of Bcl- XL acts to delay the onset of

Studies on potential breeding habitats of dengue and chickungunya vector mosquitoes in Ramanathapuram district, Tamil Nadu, India

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In recent past, entomological survey has not been carried out in the rural villages of Ramanathapuram district, Tamil Nadu, India. Keeping this in view, larvae and pupae of *Aedes* mosquitoes from different artificial containers were collected from higher altitudes of the Ramanathapuram, viz. Paramakudi, Rameshwaram, Tiruvadanai, Kadaladi, and Ramanathapuram during pre (May-June) and post (November-December) monsoon seasons in 2015 to understand the seasonal distribution, so as to forecast the risk of dengue transmission. Collected immature were transported to laboratory and allowed to emerge as adult. The adults were identified up to the species level. *Aedes aegypti* mosquitoes were highest in water tanks followed by in discarded tyres and the maximum number of collection was made from Ramanathapuram followed by Rameshwaram. The Shannon-Weiner diversity index value of *Aedes* mosquitoes in the study areas recorded for *Ae. aegypti*, *Ae. albopictus*, and *Ae. vittatus* were 1.1571, 0.1105, and 0.0674, respectively. Presence of *Ae. aegypti* and *Ae. albopictus* in both seasons reveals re-emergence of vector borne diseases at higher altitudes.

Keywords: *Aedes*, Diversity, Ramanathapuram, Tamil Nadu, India. IPC code; Int. cl. (2015.01)– A01M 1/00

Introduction

Dengue fever (DF) and its severe forms, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are mosquito transmitted arboviral diseases belonging to genus *Flavivirus*, family Flaviviridae. It affects the tropical and the subtropical regions of the world¹. The incidences of the disease have increased over the last 50 years² with 2.5 billion people living in areas where dengue is endemic and it affects up to 100 million people each year, with 500,000 cases of DHF and DSS, and around 30,000 deaths, mostly children³. In recent years, dengue fever and its more serious forms, DHF and DSS, have emerged as a major public health problem with expanded geographic distribution and increased epidemic activity⁴.

Mosquitoes breed in various habitats such as ponds, marshes, ditches, pools, drains, water containers, tree holes, etc. Different genera of mosquitoes may have a specific breeding preference⁵. The abundance of *Aedes* mosquitoes is strongly influenced by availability of water sources and

changes in climate. Human ecology is responsible for the creation of mosquitogenic environment; humans directly or indirectly create such a situation. Containers are probably the most important factor for the breeding of *Aedes* mosquitoes⁶.

In India, DF and DHF have spread to many different parts of the country⁷ including Southern India^{8,9}. Among the 32 districts of Tamil Nadu, 29 districts were found to be affected with dengue infections, which include DHF outbreaks in Chennai⁸, Dharmapuri¹⁰, Tiruchirappalli¹¹, and Virudhunagar district¹².

Materials and Methods

Study area

Investigations on *Aedes* mosquitoes were carried out in Ramanathapuram district, Tamil Nadu, India at Paramakudi, Rameshwaram, Tiruvadanai, Kadaladi, and Ramanathapuram in two different seasons, premonsoon (May-June, 2015) and post-monsoon (November-December, 2015). The study areas were situated in 9° 23' 0" N latitude and 78° 50' 0" E longitude with the elevation of 7 feet. During the survey, *Aedes* mosquitoes breeding was observed in a variety of domestic and rain water containers. The most important breeding habitats observed were water

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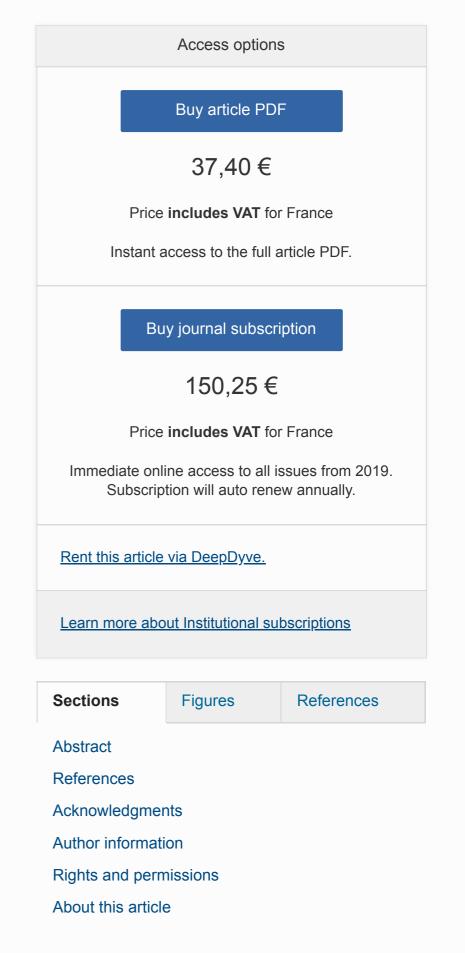
Green synthesis of silver nanoparticles from Cassia roxburghii-a most potent power for mosquito control

Udaiyan Muthukumaran, Marimuthu Govindarajan 🖂 & Mohan Rajeswary

Parasitology Research 114, 4385–4395(2015) Cite this article 451 Accesses | 13 Citations | Metrics

Abstract

Mosquitoes transmit serious human diseases, causing millions of deaths every year. The use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. Insecticides of synthesized natural products for vector control have been a priority in this area. In the present study, silver nanoparticles (AgNPs) synthesized using Cassia roxburghii plant leaf extract against Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus were determined. Larvae were exposed to varying concentrations of synthesized AgNPs (12, 24, 36, 48, and 60 µg/mL) and aqueous leaf extracts (60, 120, 180, 240, and 300 µg/mL) for 24 h. The synthesized AgNPs were characterized by UV-Vis spectrum, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), with energy-dispersive X-ray spectroscopy analysis (EDX), transmission electron microscopy, and X-ray diffraction analysis (XRD). Compare to aqueous extracted synthesized AgNPs showed extensive mortality rate against An. stephensi, Ae. aegypti, and C. quinquefasciatus with the LC₅₀ and LC₉₀ values that were 26.35, 28.67, 31.27 and 48.81, 53.24, and 58.11 µg/mL, respectively. No mortality was observed in the control. This is the first report on mosquito larvicidal activity of plant-synthesized nanoparticles. Thus, the use of C. roxburghii to synthesize silver nanoparticles is a rapid, eco-friendly, and a single-step approach, and the AgNPs formed can be potential mosquito larvicidal agents. Therefore, this study proves that C. roxburghii is a potential bioresource for stable, reproducible nanoparticle synthesis (AgNPs) and also can be used as an efficient mosquito control agent. This is the first report on the larvicidal activity of the plant extract and AgNPs.





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δ -Cadinene,Calarene and δ -4-Carene from Kadsura heteroclita Essential Oil as Novel Larvicides Against Malaria, Dengue and Filariasis Mosquitoes

Author(s): Marimuthu Govindarajan, Mohan Rajeswary, Giovanni Benelli.

Journal Name: Combinatorial Chemistry & High Throughput Screening Accelerated Technologies for Biotechnology, Bioassays, Medicinal Chemistry and Natural Products Research

Volume 19 , Issue 7 , 2016

DOI: 10.2174/1386207319666160506123 520

Abstract:

Mosquitoes (Diptera: Culicidae) are major vectors of important pathogens and parasites. Malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis cause millions of deaths every year. Mosquito control is being challenging due to the development of pesticide resistance and negative environmental concerns. In this scenario, plants employed in traditional Asian medicine may be alternative sources of newer and effective mosquitocides. In this research, we evaluated the larvicidal activity of Kadsura heteroclita leaf essential oil (EO) and its major chemical constituents (δ -Cadinene, Calarene and δ -4-Carene) against the malaria vector Anopheles stephensi, the dengue vector Aedes aegypti and the filariasis vector Culex quinquefasciatus. The chemical composition of the EO was analyzed by gas chromatography–mass spectroscopy. GC-MS revealed that the essential oil of K. heteroclita contained 33 compounds. The major chemical components were δ -Cadinene (18.3%), Calarene (14.8%) and δ -4-Carene (12.5%). The EO had a significant toxic effect against early third-stage larvae of An. stephensi, Ae. aegypti and Cx. quinquefasciatus, with LC₅₀ values of 102.86, 111.79 and 121.97 µg/mL. The three major constituents extracted from the K. heteroclita EO were tested individually for acute toxicity against larvae of the three mosquito vectors. δ -Cadinene, Calarene and δ -4-Carene appeared most effective against An. stephensi (LC₅₀ = 8.23, 12.34 and 16.37 µg/mL, respectively) followed by Ae. aegypti (LC₅₀ = 9.03, 13.33 and 17.91 µg/mL), and Cx. quinquefasciatus (LC₅₀ = 9.86, 14.49 and 19.50 µg/mL). Overall, this study adds knowledge to develop newer and safer natural larvicides against malaria, dengue and filariasis mosquito.

Keywords: Arbovirus, biosafety, Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus, mosquito vectors.





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Novel synthesis of silver nanoparticles using *Bauhinia* variegata: a recent eco-friendly approach for mosquito control

Marimuthu Govindarajan , Mohan Rajeswary, Kaliyan Veerakumar, Udaiyan Muthukumaran, S. L. Hoti, Heinz Mehlhorn, Donald R. Barnard & Giovanni Benelli 🖂

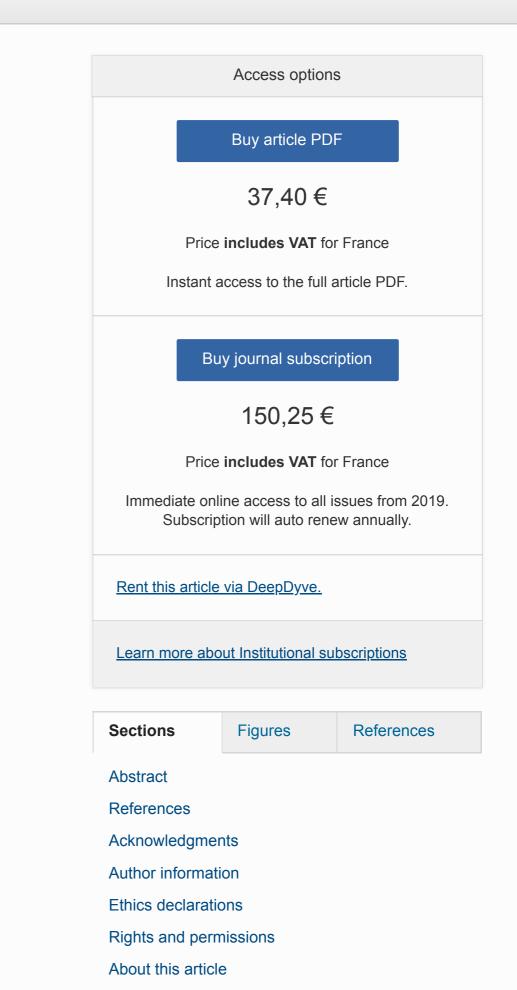
Parasitology Research 115, 723–733(2016) Cite this article 1119 Accesses | 48 Citations | 2 Altmetric | Metrics

Abstract

Mosquito vectors are responsible for transmitting diseases such as malaria, dengue, chikungunya, Japanese encephalitis, dengue, and lymphatic filariasis. The use of synthetic insecticides to control mosquito vectors has caused physiological resistance and adverse environmental effects, in addition to high operational cost. Biosynthesis of silver nanoparticles has been proposed as an alternative to traditional control tools. In the present study, green synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract of *Bauhinia variegata* by reduction of Ag⁺ ions from silver nitrate solution has been investigated. The bioreduced silver nanoparticles were characterized by UV-visible spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive X-ray analysis (EDX), and X-ray diffraction analysis (XRD). Leaf extract and synthesized AgNPs were evaluated against the larvae of Anopheles subpictus, Aedes albopictus, and Culex tritaeniorhynchus. Compared to aqueous extract, synthesized AgNPs showed higher toxicity against An. subpictus, Ae. albopictus, and Cx. *tritaeniorhynchus* with LC₅₀ and LC₉₀ values of 41.96, 46.16, and 51.92 µg/mL and 82.93, 89.42, and 97.12 µg/mL, respectively. Overall, this study proves that *B. variegata* is a potential bioresource for stable, reproducible nanoparticle synthesis and may be proposed as an efficient mosquito control agent.

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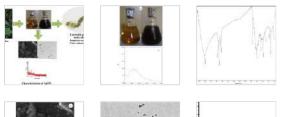
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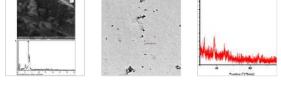
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■ Table 1

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Experimental Parasitology Volume 161, February 2016, Pages 40-47

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Green synthesis and characterization of silver nanoparticles fabricated using Anisomeles indica: Mosquitocidal potential against malaria, dengue and Japanese encephalitis vectors

Marimuthu Govindarajan ^a $\stackrel{>}{\sim}$ ⊠, Mohan Rajeswary ^a, Kaliyan Veerakumar ^a, Udaiyan Muthukumaran ^a, S.L. Hoti^b, Giovanni Benelli^c

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Highlights

- Eco-friendly green synthesis of silver nanoparticles (AgNP) was performed using Anisomeles indica leaf extract.
- Green synthesized AgNP were characterized by UV-vis, FTIR, SEM, • EDX, TEM and XRD.
- Potent larvicidal activity was noticed against filariasis, malaria and ٠ dengue mosquito vectors.
- LC_{50} value ranged from 31.56 to 38.08 μ g/mL.

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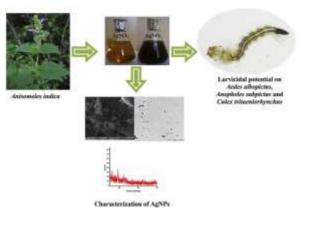
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Abstract

Mosquitoes (Diptera: Culicidae) represent a key threat for millions of people worldwide, since they act as vectors for devastating parasites and pathogens. In this scenario, eco-friendly control tools against mosquito vectors are a priority. Green synthesis of silver nanoparticles (AgNP) using a cheap, aqueous leaf extract of Anisomeles indica by reduction of Ag⁺ ions from silver nitrate solution has been investigated. Bio-reduced AgNP were characterized by UV-visible spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energydispersive spectroscopy (EDX) and X-ray diffraction analysis (XRD). The acute toxicity of A. indica leaf extract and biosynthesized AgNP was evaluated against larvae of the malaria vector Anopheles subpictus, the dengue vector Aedes albopictus and the Japanese encephalitis vector Culex tritaeniorhynchus. Both the A. indica leaf extract and AgNP showed dose dependent larvicidal effect against all tested mosquito species. Compared to the leaf aqueous extract, biosynthesized AgNP showed higher toxicity against An. subpictus, Ae. albopictus, and Cx. tritaeniorhynchus with LC_{50} values of 31.56, 35.21 and 38.08 µg/mL, respectively. Overall, this study firstly shed light on the mosquitocidal potential of A. indica, a potential bioresource for rapid, cheap and effective AgNP synthesis.

Graphical abstract



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Keywords

Aedes albopictus; Anopheles subpictus; Culex tritaeniorhynchus; Nanomosquitocidals; Nanotechnology; Plant-borne larvicides

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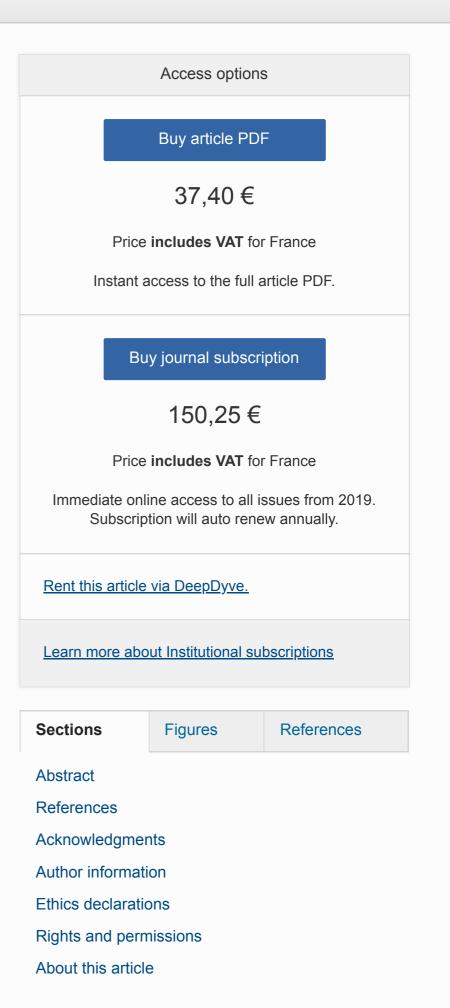
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Eugenol, α-pinene and β-caryophyllene from *Plectranthus barbatus* essential oil as eco-friendly larvicides against malaria, dengue and Japanese encephalitis mosquito vectors <u>Marimuthu Govindarajan</u> , <u>Mohan Rajeswary</u>, <u>S. L. Hoti</u>, <u>Atanu Bhattacharyya</u> & <u>Giovanni Benelli</u>

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Abstract

Mosquito-borne diseases represent a deadly threat for millions of people worldwide. Eco-friendly mosquitocides are a priority. In Ayurvedic medicine, Plectranthus species have been used to treat heart disease, convulsions, spasmodic pain and painful urination. In this research, we evaluated the acute toxicity of essential oil from *Plectranthus barbatus* and its major constituents, against larvae of the malaria vector Anopheles subpictus, the dengue vector Aedes albopictus and the Japanese encephalitis vector *Culex tritaeniorhynchus*. The chemical composition of *P. barbatus* essential oil was analyzed by gas chromatography-mass spectroscopy. Nineteen components were identified. Major constituents were eugenol (31.12 %), α-pinene (19.38 %) and β-caryophyllene (18.42 %). Acute toxicity against early third-instar larvae of An. subpictus, Ae. albopictus and Cx. tritaeniorhynchus was investigated. The essential oil had a significant toxic effect against larvae of An. subpictus, Ae. albopictus and Cx. tritaeniorhynchus, with 50 % lethal concentration (LC₅₀) values of 84.20, 87.25 and 94.34 µg/ml and 90 % lethal concentration (LC₉₀) values of 165.25, 170.56 and 179.58 µg/ml, respectively. Concerning major constituents, eugenol, α -pinene and β -caryophyllene appeared to be most effective against An. subpictus (LC₅₀ = 25.45, 32.09 and 41.66 μ g/ml, respectively), followed by Ae. albopictus (LC₅₀ = 28.14, 34.09 and 44.77 μ g/ml, respectively) and Cx. tritaeniorhynchus (LC₅₀ = 30.80, 36.75 and 48.17 µg/ml, respectively). Overall, the chance to use metabolites from *P. barbatus* essential oil against mosquito vectors seems promising, since they are effective at low doses and could be an advantageous alternative to build newer and safer mosquito control tools.



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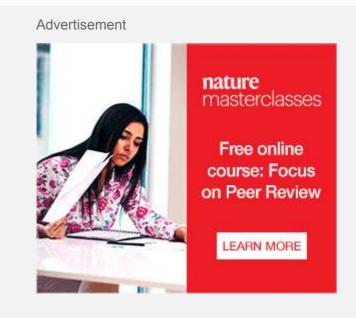
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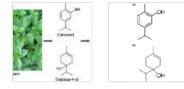
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Tables (4)

- 田 Table 1
- Table 2
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Research in Veterinary Science Volume 104, February 2016, Pages 77-82

Larvicidal potential of carvacrol and terpinen-

4-ol from the essential oil of Origanum vulgare

Anopheles subpictus, Culex quinquefasciatus and

Culex tritaeniorhynchus (Diptera: Culicidae)

(Lamiaceae) against Anopheles stephensi,

Marimuthu Govindarajan ^a 은 쯔, Mohan Rajeswary ^a, S.L. Hoti ^b, Giovanni Benelli ^c 은 쯔



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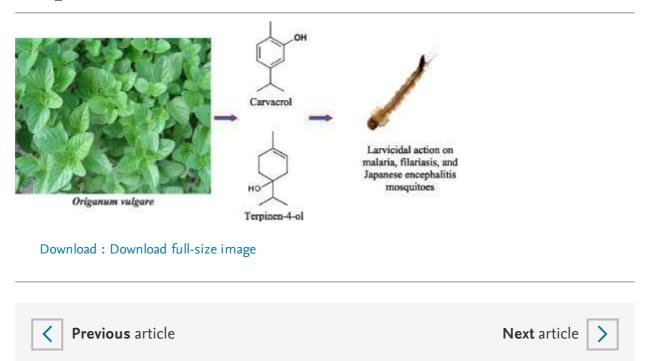
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• Carvacrol and terpinen-4-ol were the major con	nstituents of the EO.	Social Media	
- LC_{50} values against the four mosquito vectors ranged from 21.15 to		Shares, Likes & Comments:	1
27.95 μg/ml.		Tweets:	2
• Carvacrol and terpinen-4-ol may be considered larvicides.	as eco-friendly	آن ال	View details

Abstract

Mosquito-borne diseases represent a deadly threat for millions of people worldwide. However, the use of synthetic insecticides to control Culicidae may

lead to resistance, high operational costs and adverse non-target effects. Nowadays, plant-borne mosquitocides may serve as suitable alternative in the fight against mosquito vectors. In this study, the mosquito larvicidal activity of Origanum vulgare (Lamiaceae) leaf essential oil (EO) and its major chemical constituents was evaluated against the malaria vectors Anopheles stephensi and An. subpictus, the filariasis vector Culex quinquefasciatus and the Japanese encephalitis vector Cx. tritaeniorhynchus. The chemical composition of the EO was analyzed by gas chromatography–mass spectroscopy. GC–MS revealed that the essential oil of O. vulgare contained 17 compounds. The major chemical components were carvacrol (38.30%) and terpinen-4-ol (28.70%). EO had a significant toxic effect against early third-stage larvae of An. stephensi, An. subpictus, Cx. quinquefasciatus and Cx. tritaeniorhynchus, with LC50 values of 67.00, 74.14, 80.35 and 84.93 μ g/ml. The two major constituents extracted from the O. vulgare EO were tested individually for acute toxicity against larvae of the four mosquito vectors. Carvacrol and terpinen-4-ol appeared to be most effective against An. stephensi (LC₅₀ = 21.15 and 43.27 μ g/ml, respectively) followed by An. subpictus (LC₅₀ = 24.06 and 47.73 μ g/ml), Cx. quinquefasciatus (LC₅₀ = 26.08 and 52.19 μ g/ml) and Cx. tritaeniorhynchus (LC₅₀ = 27.95 and 54.87 μ g/ml). Overall, this research adds knowledge to develop newer and safer natural larvicides against malaria, filariasis and Japanese encephalitis mosquito vectors.

Graphical abstract



Keywords

Arbovirus; Filariasis; Malaria; Eco-friendly larvicides; Mosquito vectors; Plantborne mosquitocides

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Abstract

Mosquito-borne diseases represent a deadly threat for millions of people worldwide. However, the use of synthetic insecticides to control Culicidae may lead to high operational costs and adverse non-target effects. Plant-borne

compounds have been proposed for rapid extracellular synthesis of mosquitocidal nanoparticles. Their impact against biological control agents of mosquito larval populations has been poorly studied. In this study, we synthesized silver nanoparticles (Ag NPs) using the *Clerodendrum* chinense leaf extract as reducing and stabilizing agent. The biosynthesis of AgNP was confirmed analyzing the excitation of surface Plasmon resonance using ultraviolet–visible (UV–vis) spectrophotometry. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) showed the clustered and irregular shapes of Ag NP. The presence of silver was determined by energy dispersive X-ray (EDX) spectroscopy. Fourier transform infrared (FTIR) spectroscopy analysis investigated the identity of secondary metabolites, which may act as Ag NP capping agents. The acute toxicity of *C. chinense* leaf extract and biosynthesized Ag NP was evaluated against larvae of Anopheles subpictus, Aedes albopictus and Culex tritaeniorhynchus. Compared to the leaf aqueous extract, biosynthesized Ag NP showed higher toxicity against A. subpictus, A. albopictus, and C. tritaeniorhynchus with LC_{50} values of 10.23, 11.10 and 12.38 µg/mL, respectively. Biosynthesized Ag NPs were found safer to nontarget organisms Diplonychus indicus, Anisops bouvieri and Gambusia affinis, with respective LC₅₀ values ranging from 647.05 to 6877.28 μ g/ml. Overall, our results highlight that C. chinense-fabricated Ag NP are a promising and ecofriendly tool against larval populations of mosquito vectors of medical and veterinary importance, with negligible toxicity against non-target aquatic organisms.

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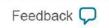
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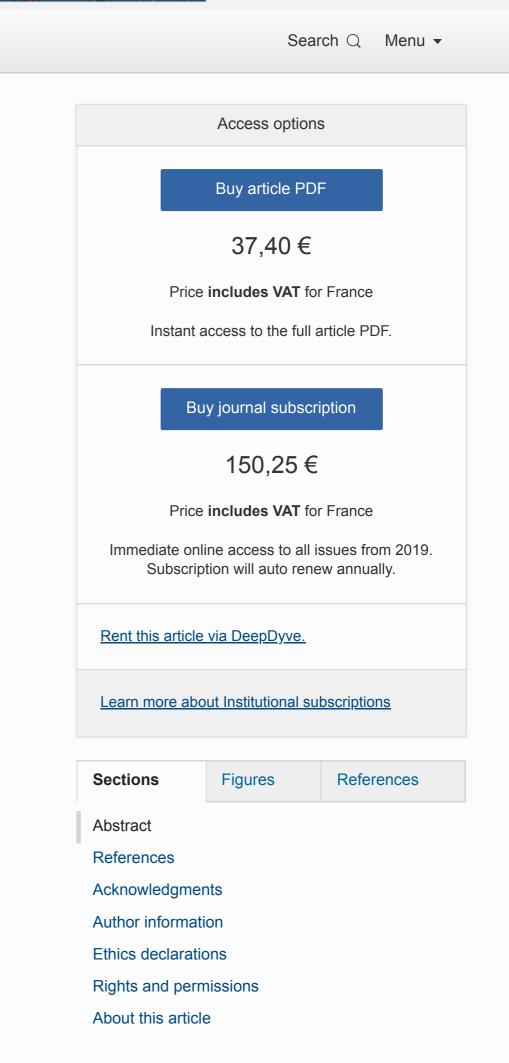
Facile biosynthesis of silver nanoparticles using *Barleria cristata*: mosquitocidal potential and biotoxicity on three non-target aquatic organisms

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<u>Parasitology Research</u> 115, 925–935(2016) | <u>Cite this article</u>
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Abstract

Mosquitoes (Diptera: Culicidae) act as vectors of important pathogens and parasites, such as malaria, dengue, chikungunya, Japanese encephalitis and lymphatic filariasis. The use of synthetic mosquitocides often leads to high operational costs and adverse non-target effects. Recently, plant-borne compounds have been proposed for rapid extracellular biosynthesis of mosquitocidal nanoparticles. However, the impact of these nanomosquitocides against biological control agents of mosquito larval populations has been poorly studied. In this research, we biosynthesized silver nanoparticles (Ag NP) using the Barleria cristata leaf extract as a reducing and stabilizing agent. The biosynthesis of Ag NP was confirmed analyzing the excitation of surface plasmon resonance using ultraviolet-visible (UV-vis) spectrophotometry. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) showed the clustered and irregular shapes of Ag NP. The presence of silver was confirmed by energy-dispersive X-ray (EDX) spectroscopy. Fourier transform infrared (FTIR) spectroscopy investigated the identity of secondary metabolites, which may also act as Ag NP capping agents. The acute toxicity of *B. cristata* leaf extract and biosynthesized Ag NP was evaluated against larvae of Anopheles subpictus, Aedes albopictus, and Culex tritaeniorhynchus. Compared to the leaf aqueous extract, biosynthesized Ag NP showed higher toxicity against An. subpictus, Ae. albopictus, and Cx. tritaeniorhynchus with lethal concentration (LC)₅₀ values of 12.46, 13.49, and 15.01 μ g/mL, respectively. Notably, biosynthesized Ag NP were found safer to non-target organisms Diplonychus indicus, Anisops *bouvieri*, and *Gambusia affinis*, with respective LC_{50} values ranging from 633.26 to 866.92 µg/mL. Overall, our results highlight that *B. cristata*-fabricated Ag NP are a promising and eco-friendly tool against young instar populations of mosquito vectors of medical and veterinary importance.



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Bio-physical Characterization of Poly-dispersed Silver Nanocrystals Fabricated Using *Carissa spinarum*: A Potent Tool Against Mosquito Vectors

Marimuthu Govindarajan , Marcello Nicoletti & Giovanni Benelli

Journal of Cluster Science **27**, 745–761(2016) | <u>Cite this article</u> **199** Accesses | **45** Citations | <u>Metrics</u>

Abstract

In this research, green synthesis of silver nanoparticles (Ag NP) using a cheap, aqueous leaf extract of *Carissa spinarum* has been investigated. Bio-reduced Ag NP were characterized by UV–visible spectroscopy, Fourier transform infrared spectroscopy, scanning electron microscopy, transmission electron microscopy and X-ray diffraction analysis. The acute toxicity of *C. spinarum* leaf extract and biosynthesized Ag NP was evaluated against larvae of the malaria vector *Anopheles subpictus*, the dengue vector *Aedes albopictus* and the Japanese encephalitis vector *Culex tritaeniorhynchus*. Both the *C. spinarum* leaf extract and Ag NP showed dose dependent larvicidal effect against all tested mosquito species. Compared to the leaf aqueous extract, biosynthesized Ag NP showed higher toxicity against *A. subpictus*, *A. albopictus*, and *C. tritaeniorhynchus* with LC₅₀ values of 8.37, 9.01 and 10.04 µg/mL, respectively. Biosynthesized Ag NP were found safer to non-target organisms *Diplonychus indicus*, *Anisops bouvieri* and *Gambusia affinis*, with respective LC₅₀ values ranging from 424.09 to 647.45 µg/mL. Overall, this study highlights the concrete potential of *C. spinarum* as a potential bio-resource for rapid, cheap and effective synthesis of mosquitocides.

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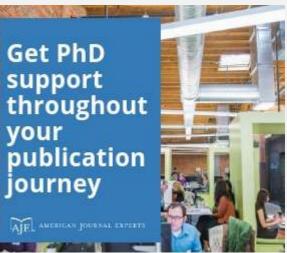


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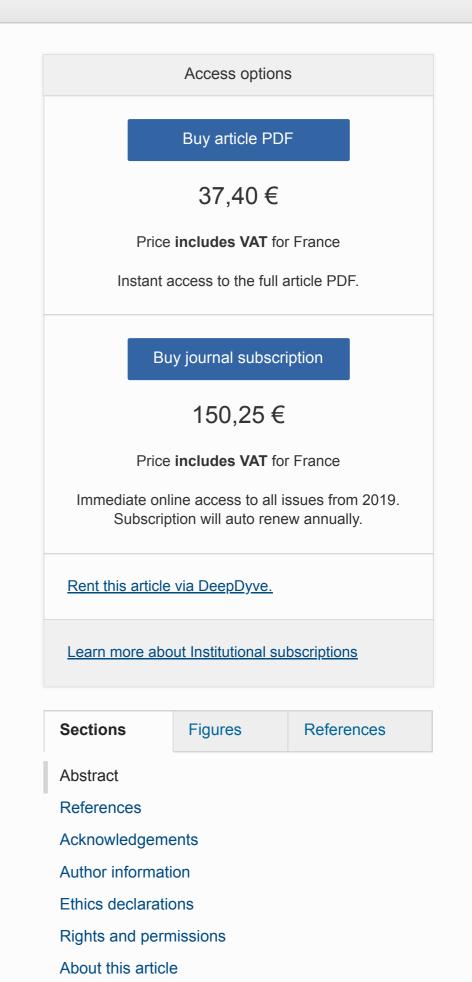
Larvicidal and repellent potential of *Zingiber nimmonii* (J. Graham) Dalzell (Zingiberaceae) essential oil: an ecofriendly tool against malaria, dengue, and lymphatic filariasis mosquito vectors?

<u>Marimuthu Govindarajan</u> ⊠, <u>Mohan Rajeswary</u>, <u>Subramanian Arivoli</u>, <u>Samuel Tennyson</u> & <u>Giovanni</u> <u>Benelli</u> ⊠

<u>Parasitology Research</u> 115, 1807–1816(2016) | <u>Cite this article</u>
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Abstract

Mosquitoes (Diptera: Culicidae) are important vectors of terms of public health relevance, especially in tropical and sub-tropical regions. The continuous and indiscriminate use of conventional pesticides for the control of mosquito vectors has resulted in the development of resistance and negative impacts on non-target organisms and the environment. Therefore, there is a need for development of effective mosquito control tools. In this study, the larvicidal and repellent activity of *Zingiber nimmonii* rhizome essential oil (EO) was evaluated against the malaria vector *Anopheles stephensi*, the dengue vector *Aedes aegypti*, and the lymphatic filariasis vector *Culex quinquefasciatus*. The chemical composition of the EO was analyzed by gas chromatography-mass spectroscopy (GC-MS). GC-MS revealed that the Z. nimmonii EO contained at least 33 compounds. Major constituents were myrcene, β -caryophyllene, α humulene, and α -cadinol. In acute toxicity assays, the EO showed significant toxicity against early third-stage larvae of An. stephensi, Ae. aegypti, and Cx. quinquefasciatus, with LC₅₀ values of 41.19, 44.46, and 48.26 µg/ml, respectively. Repellency bioassays at 1.0, 2.0, and 5.0 mg/cm² of Z. nimmonii EO gave 100 % protection up to 120, 150, and 180 min. against An. stephensi, followed by Ae. aegypti (90, 120, and 150 min) and Cx. quinquefasciatus (60, 90, and 120 min). Furthermore, the EO was safer towards two non-target aquatic organisms, Diplonychus indicus and Gambusia affinis, with LC_{50} values of 3241.53 and 9250.12 µg/ml, respectively. Overall, this research adds basic knowledge to develop newer and safer natural larvicides and repellent from Zingiberaceae plants against malaria, dengue, and filariasis mosquito vectors.



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Graphical abstract

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Materials and methods

Results and discussion

Conclusions

Compliance with ethical standards

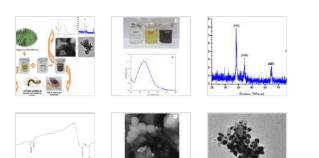
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Tables (5)

- Table 1
- Table 2
- 田 Table 4
- Table 5

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One-pot fabrication of silver nanocrystals using Ormocarpum cochinchinense: Biophysical characterization of a potent mosquitocidal and toxicity on non-target mosquito predators

Marimuthu Govindarajan ^a ightarrow ⊠, Giovanni Benelli ^b ightarrow ⊠

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https://doi.org/10.1016/j.aspen.2016.04.003

Highlights

- Facile synthesis of silver nanoparticles (Ag NPs) was performed • using Ormocarpum cochinchinense extract.
- Ag NPs were characterized by UV–vis, XRD, FTIR, SEM, TEM and • EDX.
- Ag NPs were potent larvicides against filariasis, malaria and dengue • mosquitoes, and LC₅₀ ranged from 10.43 to 12.35 μ g/mL.
- Toxicity against two non-target aquatic organisms was moderate. •

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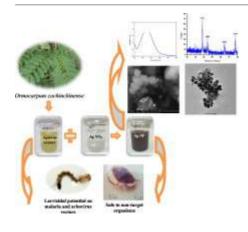
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Exports-Saves: Readers:	1 29
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Abstract

Mosquito vectors (Diptera: Culicidae) are vectors of pathogens and parasites of public health importance, including malaria, dengue, chikungunya, Japanese encephalitis, lymphatic filariasis and Zika virus. Novel eco-friendly control tools against Culicidae are a priority. In this research, silver nanoparticles (Ag NPs) were rapidly synthesized using the aqueous leaf extract of Ormocarpum cochinchinense as a cheap, non-toxic material. The bio-reduced Ag NPs were characterized by UV-visible spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive spectroscopy (EDX) and X-ray diffraction analysis (XRD). The acute toxicity of O. cochinchinense leaf extract and synthesized Ag NP was evaluated against larvae of the malaria vector Anopheles stephensi, the dengue vector Aedes aegypti and the filariasis vector Culex quinquefasciatus. Compared to the leaf aqueous extract, synthesized Ag NPs showed high toxicity against A. stephensi, A. aegypti and C. quinquefasciatus, with LC_{50} values of 10.43, 11.26 and 12.35 μ g/mL, respectively. Synthesized Ag NPs were found safer to non-target mosquito predators Diplonychus indicus and Gambusia affinis, with LC₅₀ values ranging from 522.13 to 637.70 μ g/mL. Overall, this study firstly shed light to O. cochinchinense's potential as a bioresource for rapid, cheap and effective nanosynthesis of mosquitocides.

Graphical abstract



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Keywords

Biological control agent; Biosafety; Culicidae; Fabaceae; Plant-borne larvicide; Nanobiotechnology

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One-step synthesis of polydispersed silver nanocrystals using Malva sylvestris: an eco-friendly mosquito larvicide with negligible impact on non-target aquatic organisms

Marimuthu Govindarajan , S. L. Hoti, Mohan Rajeswary & Giovanni Benelli

Parasitology Research 115, 2685–2695(2016) | Cite this article 1842 Accesses | 39 Citations | Metrics

Abstract

The synthesis of eco-friendly nanoparticles is evergreen branch of nanoscience with a growing number of biomedical implications. In this study, we investigated the synthesis of polydisperse and stable silver nanoparticles (AgNP) using a cheap leaf extract of Malva sylvestris (Malvaceae). Bio-reduced AgNP were characterized by UV-visible spectrophotometry, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), atomic force microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The acute toxicity of M. sylvestris leaf extract and green-synthesized AgNP was evaluated against larvae of the malaria vector Anopheles stephensi, the dengue vector Aedes aegypti and the filariasis vector Culex quinquefasciatus. Compared to the leaf aqueous extract, AgNP showed higher toxicity against A. stephensi, A. aegypti, and C. quinquefasciatus with LC₅₀ values of 10.33, 11.23, and 12.19 µg/mL, respectively. Green-synthesized AgNP were found safer to non-target organisms *Diplonychus indicus* and *Gambusia affinis*, with respective LC₅₀ values ranging from 813.16 to 1044.52 µg/mL. Overall, this research firstly shed light on the mosquitocidal potential of *M. sylvestris*, a potential bio-resource for rapid, cheap and effective synthesis of polydisperse and highly stable silver nanocrystals.

Introduction

Nanotechnology is a developing interdisciplinary field of research interspersing material science, bionanoscience, and technology. Remarkable advances are made in the field of biotechnology and nanotechnology to harness the benefit of life sciences, health care, and industrial biotechnology

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Abstract		
Introduction		
Materials and m	ethods	
Results and disc	cussion	
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References		
Acknowledgmer	nts	
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Ethics declaration	ons	
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About this article	9	

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(Gardea-Torresdey et al. 2002; Lee et al. 2003; Benelli 2016; Murugan et al. 2016a, b, c). A reliable and eco-friendly process for synthesis of metallic nanoparticles is an important step in the field of nanotechnology. Many materials are synthesized in nano-size for various applications including medicine, engineering, biomedical electronics, and pest management (Kalimuthu et al. 2008; Smitha et al. <u>2008</u>). Metals are commonly used for the synthesis of nanoparticles relying to chemical and biological methods. However, chemical methods usually involve use of highly toxic chemicals for synthesis of nanoparticles, which makes them certainly unsuitable for a number of purposes of public health importance. An alternative, eco-friendly, and advantageous approach to chemical methods is the biological nanosynthesis (Benelli 2016).

Recently, biological synthesis of silver nanoparticles has received a special attention due to its cheap and eco-friendly features. Current research shows that rapid green synthesis of metal nanoparticles can be easily performed using metabolites from bacteria, fungi, and plants. In particular, plant-mediated synthesis has been successfully applied for the production of silver nanoparticles, due to its faster rate of synthesis and also the possibility to reduce the steps in downstream processing (Huang et al. 2007; Salam et al. <u>2012</u>).

Mosquitoes (Diptera: Culicidae) are vectors of high public health importance. Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus are vectors of many pathogens and parasites accounting for huge mortality and morbidity worldwide, including malaria, dengue, Zika virus, Japanese encephalitis and filariasis (Benelli and Mehlhorn 2016; Benelli et al. 2016). The better strategy to lower the incidence of mosquito-transmitted diseases is to avoid mosquito biting using skin repellents, insecticide-treated bed nets, and targeting mosquito young instars with pesticides (Benelli <u>2015a</u>). In larval stage, mosquitoes have less mobility in breeding habitat, so devising control measures at this stage is comparatively easy (Howard et al. <u>2007</u>). Current practice aimed to the control of mosquito larvae mainly relies to the use of synthetic pesticides carbamates, organophosphates, and pyrethroids. In the early days of their use, insecticides showed success in reducing vector population, but the frequent and blind use of insecticides increases selection pressure on mosquitoes creating resistance and also had negative impact on human health and non-target species (Raghvendra 2002; Sarwar et al. 2009). In view of these facts, plant-borne control tools can be considered a safer and effective alternative (Benelli <u>2015b;</u> Pavela <u>2015a, b</u>).

Nowadays, a growing number of plants are screened successfully for nanosynthesis of silver nanoparticles (AgNP), including Sida acuta (Veerekumar et al. 2013), Barleria cristata (Govindarajan and Benelli 2015), Chomelia asiatica (Muthukumaran et al. 2015a), Gmelina asiatica (Muthukumaran <u>2015b</u>), *Feronia elephantum* (Veerakumar et al. <u>2014a</u>), and *Heliotropium indicum* (Veerakumar et et al. al. <u>2014b</u>). Phyto-synthesized silver nanoparticles (AgNP) have been recently proposed as effective mosquito larvicides and are gaining importance over synthetic chemical pesticides because of their

reduced harmful effects to non-target species and novelty in the mechanism(s) of action (Patil et al.

2012a; Benelli 2016).

Malva sylvestris (Malvaceae), also known as common mallow, is an herb widely present in India, growing to a height of 4 ft. In traditional medicine, the most commonly used parts of the plant are flowers and leaves without petiole. The active ingredients include mucilage, tannins, malvyn, and malvidin (Tomoda et al. 1989). The plant leaves are also rich in nutraceuticals such as antioxidants (phenols, flavonoids, carotenoids, and tocopherols), unsaturated fatty acids (e.g., α -linolenic acid), and minerals (Barros et al. 2010). The anti-inflammatory, antimicrobial, and antioxidant activities of this plant have been documented by many studies (Billeter et al. 1991; Pirbalouti et al. 2010). However, its

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Keywords

1. Introduction

2. Materials and methods

3. Results

4. Discussion

5. Conclusions

Conflicts of interest

Compliance with ethical standards

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References

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Figures (2)



Tables (4)

田 Table 1

- Table 2
- 田 Table 3
- 田 Table 4



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Ecotoxicology and Environmental Safety Volume 129, July 2016, Pages 85-90

Chemical composition, toxicity and non-target

effects of Pinus kesiya essential oil: An eco-

dengue and lymphatic filariasis mosquito

Marimuthu Govindarajan ^a $\stackrel{>}{\sim}$ $\stackrel{\boxtimes}{\sim}$, Mohan Rajeswary ^a, Giovanni Benelli ^b $\stackrel{>}{\sim}$ $\stackrel{\boxtimes}{\sim}$

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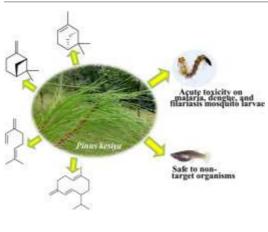
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 LC₅₀ ranged from 52 to 62 μg/ml. Biotoxicity against three non-target organisms was li ranged from 4135 to 8390 μg/ml. 	ttle, LC ₅₀	Exports-Saves: Readers: Social Media	9 85
		Tweets:	3
Abstract		ÖPLUMX	View details >

Mosquitoes (Diptera: Culicidae) are vectors of important parasites and pathogens causing death, poverty and social disability worldwide, with special reference to tropical and subtropical countries. The overuse of synthetic insecticides to control mosquito vectors lead to resistance, adverse environmental effects and high operational costs. Therefore, the development of eco-friendly control tools is an important public health challenge. In this study, the mosquito larvicidal activity of Pinus kesiya leaf essential oil (EO) was evaluated against the malaria vector Anopheles stephensi, the dengue vector Aedes aegypti and the lymphatic filariasis vector Culex quinquefasciatus. The chemical composition of the EO was analyzed by gas chromatography-mass spectroscopy. GC-MS revealed that the P. kesiya EO contained 18 compounds. Major constituents were α -pinene, β -pinene, myrcene and germacrene D. In acute toxicity assays, the EO showed significant toxicity against early thirdstage larvae of An. stephensi, Ae. aegypti and Cx. quinquefasciatus, with LC₅₀ values of 52, 57, and 62 μ g/ml, respectively. Notably, the EO was safer towards several aquatic non-target organisms Anisops bouvieri, Diplonychus indicus and Gambusia affinis, with LC_{50} values ranging from 4135 to 8390 µg/ml. Overall, this research adds basic knowledge to develop newer and safer natural larvicides from Pinaceae plants against malaria, dengue and filariasis mosquito vectors.

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Keywords

Arbovirus; Biosafety; GC–MS; Medical entomology; Mosquito borne diseases; Pinaceae

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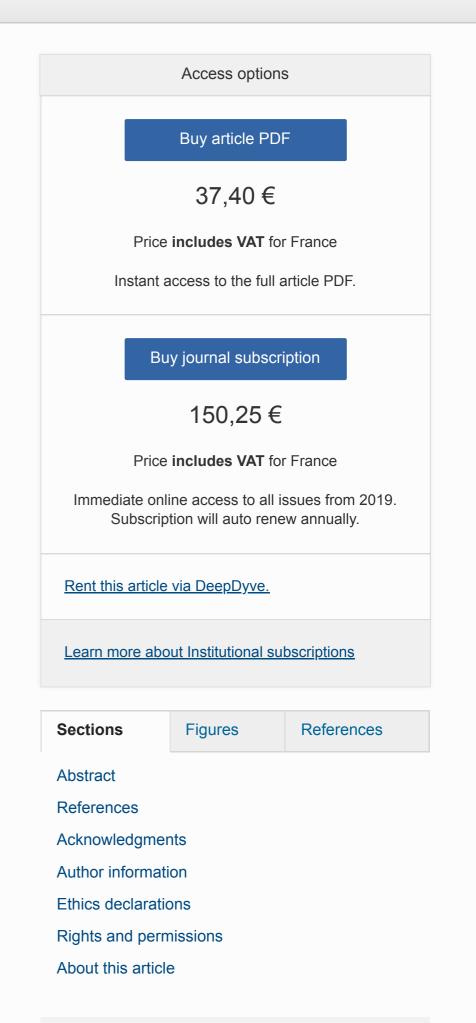
α-Humulene and β-elemene from *Syzygium zeylanicum* (Myrtaceae) essential oil: highly effective and eco-friendly larvicides against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus* (Diptera: Culicidae)

Marimuthu Govindarajan 🖂 & Giovanni Benelli

<u>Parasitology Research</u> 115, 2771–2778(2016) | <u>Cite this article</u>
527 Accesses | 43 Citations | 2 Altmetric | <u>Metrics</u>

Abstract

Mosquitoes transmit serious pathogens and parasites to humans and animals, including malaria, dengue, Japanese encephalitis and filariasis. The extensive use of chemical pesticides leads to the development of resistance in mosquito vector populations and serious non-target effects on human health and the environment. Myrtaceae plants can be a useful reservoir of natural products effective against Culicidae young instars. In this research, we evaluated the mosquitocidal potential of the essential oil (EO) from *Syzygium zeylanicum* leaves against larvae of three mosquitoes of medical and veterinary importance, the malaria vector Anopheles subpictus, the dengue vector Aedes albopictus, and the Japanese encephalitis vector *Culex tritaeniorhynchus*. The chemical composition of the EO was analyzed by gas chromatography–mass spectroscopy. GC-MS revealed that the S. zeylanicum EO contained at least 18 compounds. The major chemical components were α -humulene (37.8.5 %) and β -elemene (10.7 %). The EO had a significant toxic effect against early third-stage larvae of An. subpictus, Ae. albopictus, and Cx. tritaeniorhynchus, with LC₅₀ values of 83.11, 90.45, and 97.96 µg/ml, respectively. The two major constituents extracted from the S. zeylanicum EO were tested individually for acute toxicity against larvae of the three mosquito vectors. α -Humulene and β -elemene appeared highly effective against An. subpictus (LC₅₀ = 6.19 and 10.26 μ g/ml, respectively), followed by Ae. albopictus (LC₅₀ = 6.86 and 11.15 μ g/ml) and *Cx. tritaeniorhynchus* (LC₅₀ = 7.39 and 12.05 μ g/ml). Furthermore, the EO and its major components was safe towards the non-target fish Gambusia affinis; LC₅₀ values were 20374.26, 1024.95, and 2073.18 μ g/ml, respectively for EO, α -humulene and β -elemene. Overall, this



study highlighted that the acute toxicity of *S. zeylanicum* EO towards mosquito larvae was mainly due to the presence of α -humulene and β -elemene. Furthermore, we pointed out the concrete possibility to exploit these two compounds from *S. zeylanicum* EO as highly effective larvicides against young instars of *An. subpictus*, *Ae. albopictus*, and *Cx. tritaeniorhynchus*, with little effect on non-target organisms.

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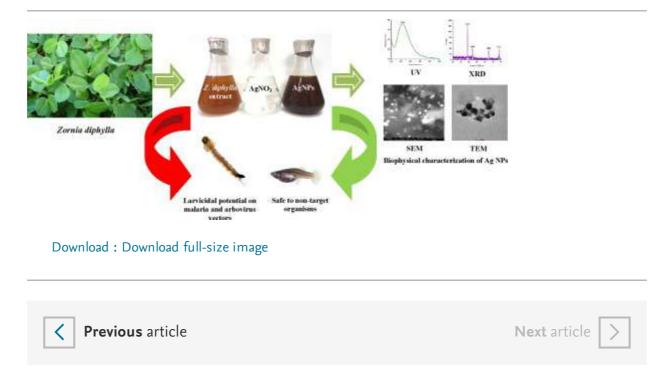
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Abstract

Mosquitoes (Diptera: Culicidae) are vectors of important pathogens and parasites, including malaria, dengue, chikungunya, Japanese encephalitis, lymphatic filariasis and Zika virus. The application of synthetic insecticides causes development of resistance, biological magnification of toxic substances through the food chain, and adverse effects on the environment and human health. In this scenario, eco-friendly control tools of mosquito vectors are a priority. Here single-step fabrication of silver nanoparticles (AgNP) using a cheap aqueous leaf extract of Zornia diphylla as reducing and capping agent pf Ag⁺ ions has been carried out. Biosynthesized AgNP were characterized by UVvisible spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive spectroscopy (EDX) and X-ray diffraction analysis (XRD). The acute toxicity of Z. diphylla leaf extract and biosynthesized AgNP was evaluated against larvae of the malaria vector Anopheles subpictus, the dengue vector Aedes albopictus and the Japanese encephalitis vector Culex tritaeniorhynchus. Both the Z. diphylla leaf extract and Ag NP showed dose dependent larvicidal effect against all tested mosquito species. Compared to the leaf aqueous extract, biosynthesized Ag NP showed higher toxicity against An. subpictus, Ae. albopictus, and Cx. tritaeniorhynchus with LC₅₀ values of 12.53, 13.42 and 14.61 µg/ml, respectively. Biosynthesized Ag NP were found safer to nontarget organisms Chironomus circumdatus, Anisops bouvieri and Gambusia affinis, with the respective LC₅₀ values ranging from 613.11 to 6903.93 μ g/ml, if compared to target mosquitoes. Overall, our results highlight that Z. diphyllafabricated Ag NP are a promising and eco-friendly tool against larval populations of mosquito vectors of medical and veterinary importance, with negligible toxicity against other non-target organisms.

Graphical Abstract



Keywords

Aedes albopictus; Anopheles subpictus; Culex tritaeniorhynchus; Nanomosquitocidals; Plant-borne larvicides; Non-target effects

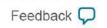
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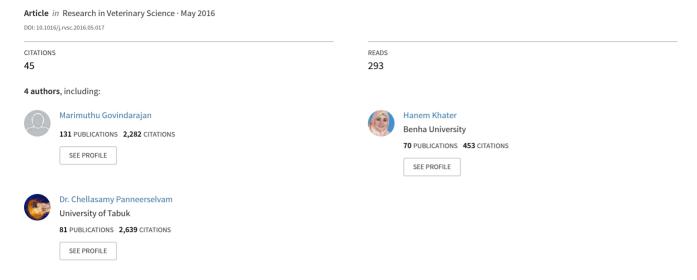
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One-pot fabrication of silver nanocrystals using Nicandra physalodes: A novel route for mosquito vector control with moderate toxicity on non-target water bugs



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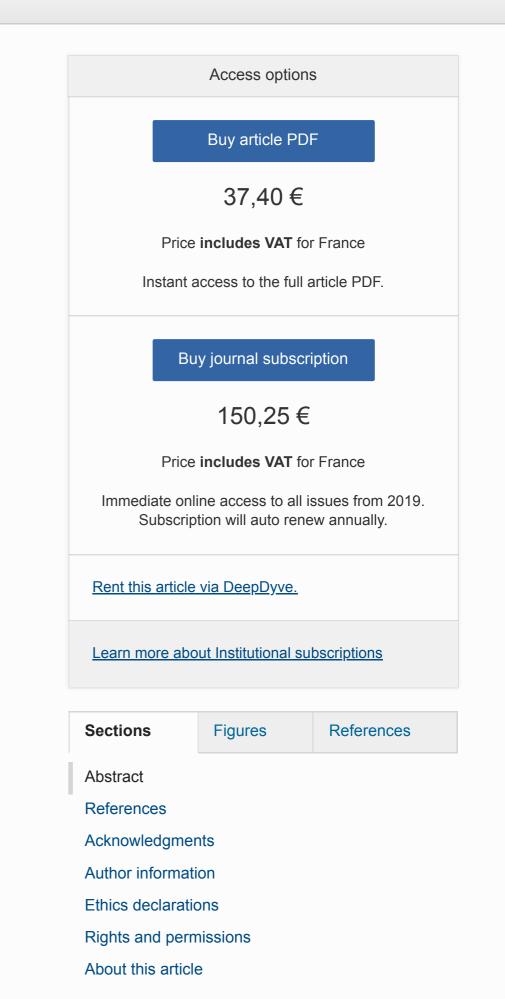
Photosensitizers in the fight against ticks: safranin as a novel photodynamic fluorescent acaricide to control the camel tick *Hyalomma dromedarii* (Ixodidae)

Hanem Khater, Nabil Hendawy, Marimuthu Govindarajan, Kadarkarai Murugan & Giovanni Benelli 🖂

<u>Parasitology Research</u> 115, 3747–3758(2016) | <u>Cite this article</u>
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Abstract

Ticks transmit more pathogen species than any other group of blood-feeding arthropods worldwide, affecting humans, livestock, and companion animals. Hyalomma dromedarii is the predominant tick species infesting camels, and its effective control is of pivotal importance. In this research, we compared the phytoefficacy of safranin (SF), a fluorescent dye applied as an acaricide for the first time, to that of tetramethrin (TM) against engorged females of H. dromedarii through in vitro immersion bioassays. Furthermore, the effect of SF exposure was evaluated on the reproductive potential of surviving tick females. Different concentrations of SF (0.03, 0.06, 0.3, 1, and 4 % *w*:*v*) and TM (0.03, 0.13, 0.5, 2, and 4 %) were prepared in distilled water and administered to engorged females of H. dromedarii. SFtreated ticks were illuminated with a light source for 30 min post-treatment (PT). Photophysical properties of SF were studied, and the relative efficacy of the used light source and sunlight was calculated. Results showed that the minimum least concentration that causes 100 % acaricidal effect was 4 % PT with SF and TM, for 8 and 48 h, respectively. LC₅₀ values 8 and 24 h PT were 0.08, 0.03 and 0.78, 0.20 %, respectively. Comparing LC₅₀ and LC₉₀ 2 h PT, SF was 33 and 22 times more potent than TM. LT₅₀ of 4 % SF and TM were 0.80 and 2.17 h, respectively. Treatment with the lowest concentrations of SF and TM induced reduction of the number of ovipositing females, eggs per female, ticks laying viable eggs, and hatched eggs. Overall, our results highlighted that SF is highly effective if compared to TM, allowing use to candidate it for the development of novel and safer acaricides.



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of all parasitic stages of H. (Euhyalomma) dromedarii and H. (E.) schulzei (Acari: Ixodidae).

J Med Entomol 45:817-831

Abstract

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Abstract

ranged from 12.52 to 14.63 μ g/mL.

ranged from 653.05 to 2183.16 µg/mL.

Little toxicity on three non-target organisms was found, LC₅₀

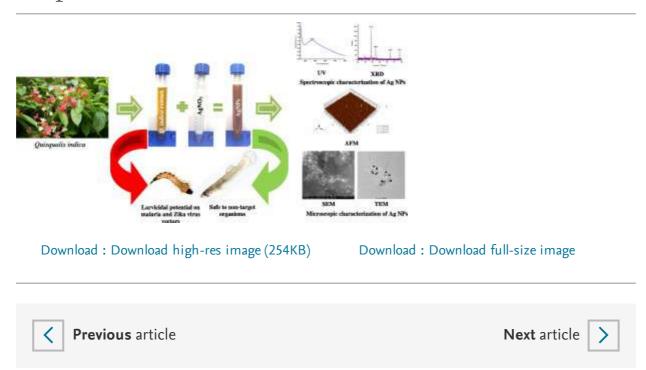
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Currently, mosquito vector control is facing a number of key challenges, including the rapid development of resistance to synthetic pesticides and the recent spread of aggressive arbovirus outbreaks. The biosynthesis of silver nanoparticles (AgNPs) is currently considered an environmental friendly alternative to the employ of pyrethroids, carbamates and microbial agents (e.g. Bacillus thuringiensis var. israelensis), since AgNPs are easy to produce, effective and stable in the aquatic environment. However, their biophysical features showed wide variations according to the botanical agent using for the green synthesis, outlining the importance of screening local floral resources used as reducing and stabilizing agents. In this study, we focused on the biophysical properties and the mosquitocidal action of Quisqualis indica-fabricated AgNPs. AgNPs were characterized using spectroscopic (UV, FTIR, XRD) and microscopic (AFM, SEM, TEM and EDX) techniques. AFM, SEM and TEM confirmed the synthesis of poly-dispersed AgNPs with spherical shape and size ranging from 1 to 30 nm. XRD shed light on the crystalline structure of these AgNPs. The acute toxicity of Quisqualis indica extract and AgNPs was evaluated against malaria, arbovirus, and filariasis vectors, Anopheles stephensi, Aedes aegypti and *Culex* quinquefasciatus, as well as on three important non-target aquatic organisms. The Q. indica leaf extract showed moderate larvicidal effectiveness on Cx. quinquefasciatus (LC₅₀ = 220.42), Ae. aegypti (LC₅₀ = 203.63) and An. stephensi (LC₅₀ = 185.98). Q. indica-fabricated AgNPs showed high toxicity against Cx. quinquefasciatus (LC₅₀ = 14.63), Ae. aegypti (LC₅₀ = 13.55) and An. stephensi (LC₅₀ = 12.52), respectively. Notably, Q. indica-synthesized AgNPs were moderately toxic to non-target aquatic mosquito predators Anisops bouvieri (LC₅₀ = 653.05 μ g/mL), Diplonychus indicus (LC₅₀ = 860.94 μ g/mL) and Gambusia affinis (LC₅₀ = 2183.16 μ g/mL), if compared to the targeted mosquitoes. Overall, the proposed one-pot biogenic fabrication of AgNPs using Q. indica is a lowcost and eco-friendly tool in the fight against Zika virus, malaria and filariasis vectors, with little impact against non-target aquatic mosquito predators.

Graphical Abstract



Keywords

Arbovirus; Mosquito-borne diseases; Green synthesis; Nanobiotechnology; AFM

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Acute toxicity and repellent activity of the *Origanum scabrum* Boiss. & Heldr. (Lamiaceae) essential oil against four mosquito vectors of public health importance and its biosafety on non-target aquatic organisms

<u>Marimuthu Govindarajan</u> ⊡, <u>Shine Kadaikunnan</u>, <u>Naiyf S. Alharbi</u> & <u>Giovanni Benelli</u> ⊡
 <u>Environmental Science and Pollution Research</u> 23, 23228–23238(2016) | <u>Cite this article</u>
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Abstract

The recent outbreaks of dengue, chikungunya, and Zika virus highlighted the pivotal importance of mosquito vector control in tropical and subtropical areas worldwide. However, mosquito control is facing hot challenges, mainly due to the rapid development of pesticide resistance in Culicidae and the limited success of biocontrol programs on Aedes mosquitoes. In this framework, screening botanicals for their mosquitocidal potential may offer effective and eco-friendly tools in the fight against mosquitoes. In the present study, the essential oil (EO) obtained from the medicinal plant Origanum scabrum was analyzed by GC-MS and evaluated for its mosquitocidal and repellent activities towards Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus, and Culex tritaeniorhynchus. GC-MS analysis showed a total of 28 compounds, representing 97.1 % of the EO. The major constituents were carvacrol (48.2 %) and thymol (16.6 %). The EO was toxic effect to the A. stephensi, A. aegypti, C. quinquefasciatus, and C. tritaeniorhynchus larvae, with LC₅₀ of 61.65, 67.13, 72.45, and 78.87 µg/ml, respectively. Complete ovicidal activity was observed at 160, 200, 240, and 280 µg/ml, respectively. Against adult mosquitoes, LD₅₀ were 122.38, 134.39, 144.53, and 158.87 µg/ml, respectively. In repellency assays, the EOs tested at 1.0, 2.5, and 5.0 mg/cm² concentration of O. scabrum gave 100 % protection from mosquito bites up to 210, 180, 150, and 120 min, respectively. From an ecotoxicological point of view, the EO was tested on three non-target mosquito predators, Gambusia affinis, Diplonychus indicus, and Anisops bouvieri, with LC₅₀ ranging from 4162 to 12,425 µg/ml. Overall, the EO from O. scabrum may be considered as a low-cost and eco-friendly source of

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phytochemicals to develop novel repellents against Culicidae.

Introduction

Mosquito-borne diseases include malaria, dengue, West Nile virus, chikungunya, yellow fever, Japanese encephalitis, filariasis, and Zika virus, which are major public health problems (Mehlhorn 2008; Mehlhorn et al. 2012; Benelli et al. 2016a, b, c). The incidence of malaria is, however, gradually receding, with a consistent decline in case over the past few years, even if the spread of chloroquine-and artemisinin-resistant *Plasmodium* strains is a real threat nowadays (Dev et al. 2004; Jensen and Mehlhorn 2009; Benelli and Mehlhorn 2016). On the other hand, the recent outbreaks of dengue, chikungunya, and Zika virus highlighted the pivotal importance of mosquito vector control (Benelli 2015a). Furthermore, Japanese encephalitis (JE) is a serious illness with lifelong neuropsychiatric sequelae. The risk of infection is high for geographical locations of human habitation near paddy fields/water bodies (the breeding habitat of JE vectors), with the presence of pigs (amplification host) in close proximity. The menace of JE is growing and spreading in areas hitherto free from the disease, with increased morbidity and mortality (Sharma et al. 2014). Unfortunately, there is no specific treatment for the arboviruses mentioned above; thus, the constructive and environment-friendly restraint of mosquito vectors is of great importance for public health (Benelli 2016a, b; Benelli and Mehlhorn 2016; Benelli et al. 2015a, b).

The employment of synthetic pesticides, including organophosphates such as fenthion, temephos, and insect growth regulators, such as methoprene and diflubenzuron, currently represent an effective control method to control young instar populations of mosquito vectors (Liu et al. 2012). However, the extensive and indiscriminate use of these synthetic insecticides has led to heavy concerns for human health and the environment (Isman 2006; Benelli 2015a; Naqqash et al. 2016). Hence, there is a request of novel tools for Culicidae control. Plant-borne insecticides, as well as essential oils (EOs), are encouraging since they are effective at reduced doses, environmental-friendly, biodegradable, and often economical (Benelli 2015b; Pavela 2015a; Govindarajan and Benelli 2016a, b, c; Govindarajan et al. 2011, 2013a, b). Thus, recent research focused on plant EOs and extracts as possible sources of arthropod ovicidal, larvicidal, adulticidal, and repellent compounds (e.g., Cheng et al. 2003, 2004; Govindarajan 2010a; Govindarajan et al. 2011; Dinesh et al. 2015; Madhiyazhagan et al. 2015; Murugan et al. 2015a, b, c; Pavela 2015b; Suresh et al. 2015; Benelli et al. 2016b; Jaganathan et al. 2016; Panneerselvam et al. 2015).

Notably, the EOs extracted from plants belonging to the Lamiaceae family have been widely studied for their toxic action against several mosquito species (Benelli <u>2015b</u>; Pavela <u>2015a</u>). Good examples are the larvicidal EOs obtained from *Clausena anisata* (Govindarajan <u>2010b</u>), *Coleus aromaticus* (Govindarajan et al. <u>2013b</u>), *Dalbergia sisoo* (Ansari et al. <u>2000a</u>), *Hyptis martiusii* (Araujo et al. <u>2003</u>), *Hyptis suaveolens* (Sakthivadivel et al. <u>2015</u>), *Lavandula gibsoni* (Kulkarni et al. <u>2013</u>), *Lippia sidoides* (Lima et al. <u>2013</u>), *Moschosma polystachyum* (Rajkumar and Jebanesan <u>2004</u>), *Mentha spicata* (Govindarajan et al. <u>2012</u>), *Mentha piperita* (Ansari et al. <u>2000b</u>), *Mentha longifolia* (Pavela et al. <u>2014</u>), *Ocimum basilicum* (Govindarajan et al. <u>2013a</u>), *Ocimum selloi* (Padilha de Paula et al. <u>2003</u>), *Ocimum americanum* (Tawatsin et al. <u>2001</u>), *Ocimum gratissimum* (Cavalcanti et al. <u>2004</u>), *Ocimum sanctum* (Gbolade and Lockwood <u>2008</u>), *Plectranthus barbatus* (Govindarajan et al. <u>2016a</u>), *Plectranthus ambonicus* (Lima et al. <u>2011</u>), *Plectranthus mollis* (Kulkarni et al. <u>2013</u>), *Pogostemon cablin* (Trongtokit et al. <u>2005</u>), *Pulegium vulgare* (Pavela et al. <u>2014</u>), *Rosmarinus officinalis* (Prajapati et al. <u>2005</u>), *Satureja hortensis* (Pavela <u>2009</u>), *Tagetus minuta* (Perich et al. <u>1995</u>), *Thymus vulgaris* (El-Akhal et al. <u>2016</u>), *Thymus leucospermus*, *Thymus teucrioides* (Pitarokili et al. <u>2011</u>), *Vitex agnus*

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4. Conclusions

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Acknowledgments

Appendix A. Supplementary material

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Tables (7)

🗄 Table 1 Ħ Table 2 Ħ Table 3 Table 4

Table 5 Table 6

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Marimuthu Govindarajan ^a ペ ⊠, Giovanni Benelli ^b ペ ⊠

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Ecotoxicology and Environmental Safety Volume 133, November 2016, Pages 395-402

Eco-friendly larvicides from Indian plants:

bicyclogermacrene on malaria, dengue and

Effectiveness of lavandulyl acetate and

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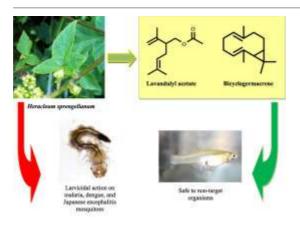
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HighlightsHeracleum sprengelianum essential oil was mainly contact	omposed of	Article Metrics Citations	^
lavandulyl acetate and bicyclogermacrene.		Citation Indexes:	31
• Larvicidal activity was investigated against mosqui	itoes.	Captures	
 Lavandulyl acetate showed the highest toxicity, LC 4.17 to 5.11 μg/ml. 	50 ranged from	Exports-Saves: Readers:	6 89
• Lavandulyl acetate is proposed as a newer and safe larvicide.	r mosquito	Social Media	
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Abstract			

Mosquitoes (Diptera: Culicidae) are a key threat for millions of people and animals worldwide, since they act as vectors for devastating pathogens and parasites, including malaria, dengue, Japanese encephalitis, filiariasis and Zika virus. Mosquito young instars are usually targeted using organophosphates, insect growth regulators and microbial agents. Indoor residual spraying and insecticide-treated bed nets are also employed. However, these chemicals have negative effects on human health and the environment and induce resistance in a number of vectors. In this scenario, newer and safer tools have been recently implemented to enhance mosquito control. The concrete potential of screening plant species as sources of metabolites for entomological and parasitological purposes is worthy of attention, as recently elucidated by the Y. Tu's example. Here we investigated the toxicity of Heracleum sprengelianum (Apiaceae) leaf essential oil and its major compounds toward third instar larvae of the malaria vector Anopheles subpictus, the arbovirus vector Aedes albopictus and the Japanese encephalitis vector *Culex tritaeniorhynchus*. GC–MS analysis showed that EO major components were lavandulyl acetate (17.8%) and bicyclogermacrene (12.9%). The EO was toxic to A. subpictus, A. albopictus, and C. tritaeniorhynchus, with LC₅₀ of 33.4, 37.5 and 40.9 μ g/ml, respectively. Lavandulyl acetate was more toxic to mosquito larvae if compared to bicyclogermacrene. Their LC₅₀ were 4.17 and 10.3 µg/ml for A. subpictus, 4.60 and 11.1 µg/ml for A. albopictus, 5.11 and 12.5 µg/ml for C. tritaeniorhynchus. Notably, the EO and its major compounds were safer to three non-target mosquito predators, Anisops bouvieri, Diplonychus indicus and Gambusia affinis, with LC_{50} ranging from 206 to 4219 $\mu g/ml.$ Overall, this study highlights that H. sprengelianum EO is a promising source of eco-friendly larvicides against three important mosquito vectors with moderate toxicity against non-target aquatic organisms.

Graphical abstract



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Keywords

Apiaceae; Heracleum sprengelianum; Mosquitoes; Biosafety; Non-target effects; Gambusia affinis

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Green synthesis of silver, gold and silver/gold bimetallic nanoparticles using the *Gloriosa superba* leaf extract and their antibacterial and antibiofilm activities

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ARTICLE INFO

ABSTRACT

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Keywords: Biofabrication Biosafety Gloriosa superba Nanotechnology Surface plasmon resonance

1. Introduction

The green synthesis, also known as phytosynthesis, of noble metal nanoparticles (NPs) is one of the emerging fields in nanoscience and nanotechnology. The nanoparticle size and shapes strongly impact the physical, chemical, electrical and optical properties of nanomaterials. Nowadays, silver (Ag) and gold (Au) NPs are used in a wide range of biomedical [1,2], drug delivery [3,4], catalytic [5,6], agriculture [7,8] antioxidant [9,10], anticancer [11,12] antibacterial [13,14] antifungal [15], antibiofilm [16,17], entomological and parasitological applications [18,19]. Recently, botanical-based synthesis of Ag and Au NPs have gained a growing attention due to its low cost, simplicity and environmentally benign synthesis protocols [20–26]. For example, Ag, Au and Ag/Au bimetallic NPs have been synthesized by *Anacardium occidentale* [27], *Piper pedicellatum* [28] *Punica granatum* [29] and *Brassica oleracea* [30].

The biofilm is a microbial population enclosed in a matrix. It grows in three steps, namely (i) initial adhesion, (ii) proliferation and (iii) detachment. Bacterial cells bind together by extracellular polymeric substances and connected to a surface (substrate), involving in

* Corresponding author.

The green fabrication of metal nanoparticles using botanical extracts is gaining increasing research attention in nanotechnology, since it does not require high energy inputs or the production of highly toxic chemical byproducts. Here, silver (Ag), gold (Au) and their bimetallic (Ag/Au) nanoparticles (NPs) were green synthesized using the *Gloriosa superba* aqueous leaf extract. Metal NPs were studied by spectroscopic (UV–visible spectroscopy, fluorescence spectroscopy, FT-IR spectroscopy, XRD and EDX) and microscopic (AFM and TEM) analysis. AFM and TEM showed that Ag and Au NPs had triangular and spherical morphologies, with an average size of 20 nm. Bimetallic Ag/Au NPs showed spherical shapes with an average size of 10 nm. Ag and Ag/Au bimetallic NPs showed high antibacterial and antibiofilm activities towards Gram-positive and Gram-negative bacteria. Overall, the proposed synthesis route of Ag, Au and Ag/Au bimetallic NPs can be exploited by the pharmaceutical industry to develop drugs effective in the fight against microbic infections. © 2016 Published by Elsevier Ltd.

> each cell-surface and cell-cell interactions as a part of the developmental process [31,32]. Biofilms show thousand times of resistance towards toxicants if compared to planktonic cells [33]. They can cause serious problems in the environment and leads to infections both humans and animals [34-37]. Recently, metal and metal oxide NPs have been used to inhibit the microbial growth and forestall the biofilm formation [17,38,39]. The green synthesis of protein-stabilized Ag NPs, coated on the surface of the polycaprolactam (polymer) evidences the high antibiofilm activity against bacterial and fungal pathogens [40]. Biosynthesized Ag NPs showed high activity against the primary biofilm formation of Pseudomonas aeruginosa and Staphylococcus aureus [41]. Also, sixteen different marine biofilm bacterial isolates have been investigated for the antibiofilm activity, treating them different concentration of biosynthesized Ag NPs. It has been noted that 50 µg/mL of Ag NPs significantly exhibited the prevention of biofilm with some variations among the tested bacteria [16].

> *Gloriosa superba* L., (Colchicaceae) is a climbing herb native of South Africa. It is a national flower of Zimbabwe and state flower of Tamil Nadu [42]. *G. superba* is a tuberous plant with plow shaped cylindrical tubers. It is commercially cultivated in Tamil Nadu, and particularly in Karur and Tirupur districts. It has been used in ailments of arthritis, gout, rheumatism, inflammation, ulcers, bleeding piles, skin diseases, leprosy and snakebites [43,44]. The leaf extract contains superbine, colchicine, gloriosine, gloriosol, phytosterils and

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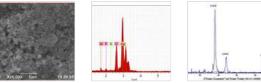
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- Table 5

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Journal of Asia-Pacific Entomology Volume 19, Issue 4, December 2016, Pages 1001-1007

Green-synthesized silver nanoparticles using

dengue vector Aedes aegypti (Diptera: Culicidae)

Kalimuthu Kovendan ^a $\stackrel{>}{\sim}$ $\stackrel{\boxtimes}{\sim}$, Balamurugan Chandramohan ^a, Devakumar Dinesh ^a, Dhandapani Abirami ^b,

and impact on the predatory efficiency of the

Psychotria nilgiriensis: toxicity against the

non-target organism Poecilia sphenops

Periasamy Vijayan ^c, Marimuthu Govindarajan ^c, Savariar Vincent ^d, Giovanni Benelli ^e $\stackrel{ imes}{\sim}$ 🖾

(Cyprinodontiformes: Poeciliidae)



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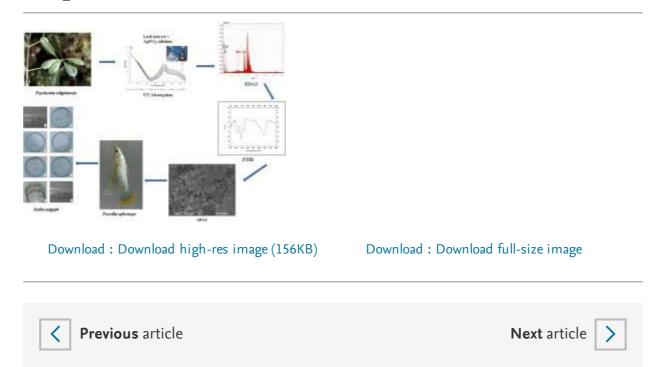
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- Silver nanoparticles (AgNPs) were biosynthesized using the aqueous • leaf extract of *P. nilgiriensis*.
- AgNPs were characterized by UV–vis spectrophotometry, SEM, EDX analysis, XRD and FTIR spectroscopy.
- AgNPs combined with the plant extract were highly toxic against • young instars of dengue mosquitoes.
- Ultra-low doses of AgNPs boost predation of Poecilia sphenops on • mosquito larvae.

Abstract

Mosquitoes transmit serious diseases to humans and animals, causing millions of deaths every year. Effective insecticides of natural origin for eco-friendly vector control are a priority. In this study, silver nanoparticles (AgNPs) biosynthesized using as cheap *Psychotria* nilgiriensis leaf extract were tested on larvae and pupae of *Aedes aegypti* (Diptera: Culicidae). Biophysical characterization was carried out with UV–vis spectrophotometry, Fourier transform infrared spectroscopy, scanning electron microscopy, and energydispersive X-ray spectroscopy. Larvae were exposed to varying concentrations of aqueous extract of synthesized AgNPs for 24 h. The maximum mortality was observed for green-synthesized AgNPs. Moreover, the combined treatment of leaf extract of *P. nilgiriensis* and AgNPs lowered the plant extract LC₅₀ to 92.87, 115.27, 140.37, 169.30 and 212.55 μ g/ml. The effectiveness of green synthesized AgNPs was confirmed against eggs and adults of *A. aegypti*. Furthermore, we showed that the predatory efficiency of Poecilia sphenops on A. aegypti was not reduced after the exposure at sublethal doses of AgNPs. Predation in the control was 65% (larva I) and 49.62% (larva II). Predation against late-instar larvae was minimal. In AgNPs-treated environment (3 ppm), predation was boosted to 90.25% (larva I) and 76.50% (larva II), respectively. Overall, P. nilgiriensis-synthesized AgNPs could be proposed at ultra-low doses to reduce larval population of dengue vectors enhancing predation rates of *P. sphenops*.

Graphical abstract



Keywords

Biosafety; Ovicidal activity; Adulticidal activity; UV-vis spectrophotometry, XRD, SEM, non-target aquatic organisms

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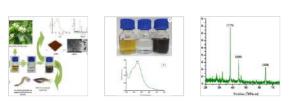
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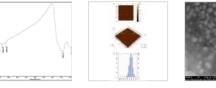
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Enzyme and Microbial Technology Volume 95, December 2016, Pages 155-163



Part of special issue:

Biosynthetic nanoparticles for biotechnological and biomedical applications

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mosquito vectors

Highlights

- Eco-friendly control tools against malaria and Japanese encephalitis • vectors are a priority.
- Silver nanoparticles (AgNP) were fabricated using *Ichnocarpus* • *frutescens* aqueous extract as a reducing and capping agent.
- AgNP were characterized by UV-vis spectrophotometry, FTIR, SEM, • TEM, AFM, EDX and XRD.
- AgNP showed potent larvicidal activity against malaria and • arbovirus vectors, LC₅₀ ranged from 14 to 17 μ g/mL.
- Biotoxicity against non-target aquatic organisms was neglible, LC₅₀ ranged from 636 to 2098 μ g/mL.

Abstract

Mosquito (Diptera: Culicidae) vectors are solely responsible for transmitting important diseases such as malaria, dengue, chikungunya, Japanese encephalitis, lymphatic filariasis and Zika virus. Eco-friendly control tools of

Marimuthu Govindarajan ^a ightarrow ⊠, S.L. Hoti ^b, Giovanni Benelli ^c ightarrow ⊠ **∃** Show more https://doi.org/10.1016/j.enzmictec.2016.05.005

Culicidae vectors are a priority. In this study, we proposed a facile fabrication process of poly-disperse and stable silver nanoparticles (Ag NPs) using a cheap leaf extract of Ichnocarpus frutescens (Apocyanaceae). Bio-reduced Ag NPs were characterized by UV-vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), atomic force microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The acute toxicity of *I. frutescens* leaf extract and greensynthesized Ag NPs was evaluated against larvae of the malaria vector Anopheles subpictus, the dengue vector Aedes albopictus and the Japanese encephalitis vector Culex tritaeniorhynchus. Compared to the leaf aqueous extract, Ag NPs showed higher toxicity against A. subpictus, A. albopictus, and C. tritaeniorhynchus with LC_{50} values of 14.22, 15.84 and 17.26 μ g/mL, respectively. Ag NPs were found safer to non-target mosquito predators Anisops bouvieri, Diplonychus indicus and Gambusia affinis, with LC_{50} values ranging from 636.61 to 2098.61 µg/mL. Overall, this research firstly shed light on the mosquitocidal potential of *I*. frutescens, a potential bio-resource for rapid, cheap and effective synthesis of poly-disperse and highly stable silver nanocrystals.

Graphical abstract



Keywords

Aedes albopictus; Anopheles subpictus; Culex tritaeniorhynchus; Green synthesis; Nanotechnology; Plant-borne larvicides



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Artemisia absinthium-borne compounds as novel larvicides: effectiveness against six mosquito vectors and acute toxicity on non-target aquatic organisms

Marimuthu Govindarajan 🖂 & Giovanni Benelli 🖂

Parasitology Research 115, 4649–4661(2016) | Cite this article 1688 Accesses | 29 Citations | Metrics

Abstract

The eco-friendly control of mosquito vectors is a crucial challenge of public health importance. Here we evaluated the larvicidal potential of Artemisia absinthium essential oil (EO) and its three major chemical constituents against six mosquito vectors: Anopheles stephensi, Anopheles subpictus, Aedes aegypti, Aedes albopictus, Culex quinquefasciatus, and Culex tritaeniorhynchus. The EO was obtained by leaf hydro-distillation. Its chemical composition was analyzed using gas chromatography-mass spectrometry. Major components were (E)- β -farnesene (31.6 %), (Z)-en-yn-dicycloether (11.12 %), and (Z)- β -ocimene (27.8 %). The EO was toxic effect against larval populations of An. stephensi, An. subpictus, Ae. aegypti, Ae. albopictus, Cx. quinquefasciatus, and Cx. tritaeniorhynchus, with LC₅₀ values of 41.85, 52.02, 46.33, 57.57, 50.57, and 62.16 μg/ml. (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)- β -ocimene were highly effective on *An. stephensi* (LC₅₀ = 8.13, 16.24 and 25.84 µg/ml) followed by An. subpictus (LC₅₀ = 10.18, 20.99, and 30.86 μ g/ml), Ae. aegypti (LC₅₀ = 8.83, 17.66, and 28.35 μ g/ml), Ae. albopictus (LC₅₀ = 11.38,23.47, and 33.72 μ g/ml), Cx. quinquefasciatus (LC₅₀ = 9.66, 19.76, and 31.52 μ g/ml), and *Cx. tritaeniorhynchus* (LC₅₀ = 12.51,25.88, and 37.13 μ g/ml). Notably, the EO and its major compounds were safer to the non-target organisms Chironomous circumdatus, Anisops *bouvieri* and *Gambusia affinis*, with LC₅₀ values ranging from 207.22 to 4385 µg/ml. Overall, our results highlight that (*E*)- β -farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)- β -ocimene from the *A*. absinthium EO represent promising eco-friendly larvicides against six key mosquito vectors with moderate toxicity against non-target organisms.

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Introduction

Arthropods are important vectors of a great number of pathogens and parasites, which may hit as epidemics or pandemics in the increasing world populations of humans and animals (Mehlhorn 2015; Benelli and Mehlhorn 2016; Benelli et al. 2016a). Mosquitoes (Diptera: Culicidae) represent a key threat for millions of organisms worldwide, since they act as vectors of the agents of malaria, dengue, yellow fever, West Nile virus fever, Japanese encephalitis, filariasis and, more recently, Zika virus (Mehlhorn et al. <u>2012</u>; Benelli <u>2015a</u>; Benelli et al. <u>2016b</u>, <u>c</u>).

According to the latest estimates, there were at least 198 million cases of malaria in 2013 and an estimated 584,000 deaths. Malaria mortality rates have fallen by 47 % globally since 2000 and by 54 % in the African region, but are still high. Most deaths occur among children living in Africa, where a child dies every minute from malaria (Jensen and Mehlhorn 2009; WHO 2014). Dengue is ranked among the most important mosquito-borne viral diseases in the world. In the last 50 years, the incidence has increased 30-fold. An estimated 2.5 billion people live in over 100 endemic countries and areas where dengue viruses can be transmitted. Up to 50 million infections occur annually with 500,000 cases of dengue hemorrhagic fever and 22,000 deaths, mainly among children (WHO 2012a). In the past decade, West Nile virus has emerged in the Americas, becoming endemic throughout the region. Chikungunya, a formerly obscure arbovirus endemic to East Africa, has also emerged, causing millions of cases in the Indian Ocean basin and mainland South and Southeast Asia. The Japanese encephalitis virus has expanded its range in the Indian subcontinent and Australasia, where it chiefly affects children (Tolle 2009; Benelli and Mehlhorn 2016).

Currently, the use of mosquito larvicides faces several serious problems. Besides the negative effects of synthetic insecticides on the environment and non-target organisms, including man (Hodgson and Levi <u>1996</u>; WHO <u>2012b</u>), the development of resistant mosquito populations in particular is one of the most serious problems (Hemingway and Ranson 2000; Naqqash et al. 2016). Insecticide resistance is viewed as an extremely serious threat to crop protection and vector control, and is considered by many parties, including industry, the WHO, regulatory bodies, and the public, to be an issue that needs a proactive approach (Hemingway and Ranson 2000; McCaffery and Nauen 2006; Nauen 2007; WHO 2012b).

These problems highlighted the needing of new pest control alternatives, acceptable for the environment and human health (Benelli 2016a, b; Pavela and Benelli 2016). Among the existing alternative tools aimed at decreasing pest populations, the use of pesticides based on plant extracts is currently one of the most promising (Amer and Mehlhorn 2006a, b, c, d; Dubey 2011; Benelli 2016c; Govindarajan et al. <u>2016a</u>, <u>b</u>, <u>c</u>, <u>d</u>). Essential oils and related main compounds are also an environmentally interesting tool because they are biodegradable and have minimal side effects on nontarget organisms, as well as on the environment (Govindarajan 2010; Govindarajan et al. 2012, 2013; Pavela 2014, 2015; Benelli 2015b, c Govindarajan and Benelli 2016a, b).

Essential oils can be used as an alternative to synthetic larvicides for vector control programs (Pavela

2015). It is well known that plant-derived natural products are extensively used as biologically active compounds (Zebitz <u>1984</u>). Among them, essential oils were the first preservatives used by the man (Bakkali et al. 2008). Essential oils are mainly composed by isoprenoid compounds, mainly mono- and sesquiterpenes, which are mainly responsible of the smell of many aromatic plants (Franzios et al. 1997). Commercially, essential oils are used in four primary ways: as pharmaceuticals, as flavor enhancers in many food products, as odorants in fragrances, and as insecticides (Zhu et al. 2001; Pavela <u>2015;</u> Benelli <u>2015a</u>).

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RESEARCH ARTICLE

Diallyl Trisulfide Ameliorates Arsenic Induced Dyslipidemia in Rats

Selvaraj Miltonprabu and Naorem Chanu Sumedha

Received August 12, 2013; revised June 1, 2014; accepted July 29, 2014; published online April 30, 2015 © KoSFoST and Springer 2015

Abstract The efficacy of diallyl trisulfide (DATS) against arsenic (As) induced dyslipidemia in the plasma and livers of rats was studied. Oral administration of As [5 mg/kg of body weight (BW)/day] for 4 weeks showed significant (p<0.05) increases in TC, LDL-C, VLDL-C, FFA, PL, and TG levels and the activity of HMG-CoA reductase in the plasma and liver, with significant (p < 0.05) reductions in levels of HDL-C hepatic ubiquinones and activities of LCAT and LPL in the plasma and liver. Oral administration of DATS (80 mg/kg of BW) for 4 weeks in As intoxicated rats significantly (p < 0.05) reduced plasma and liver TC, FFA, TGs, VLDL-C, and LDL-C levels, and the activity of HMG-CoA reductase, and significantly (p < 0.05) increased the activities of LCAT and LPL and the levels of HDL-C and ubiquinone in the livers of rats. DATS protects against As-induced dyslipidemia in rats.

Keywords: diallyl trisulfide, arsenic, lipid, lipoprotein, ubiquinone

Introduction

Arsenic (As) is a metalloid found in water, soil, and air from natural and anthropogenic sources and exists in inorganic and organic forms. Many recent studies have provided experimental evidence that arsenic induced generation of free radicals can cause cell damage and death through activation of oxidative signaling pathways (1). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are capable of damaging a wide variety of cellular macromolecules, including DNA, lipids, and proteins. Occurrence of carotid atherosclerosis was accounted in people exposed

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Tel: +91-04144-238282; Fax: +91-04144-238080 E-mail: smprabu73@gmail.com to As for a long period of time (2). Peoples from Chile, Mexico, Poland, United States and Germany winery workers have been publicized to have As linked atherosclerotic vascular ailments.

Arsenic posses a self-directed threat to individuals with atherosclerotic vascular syndrome in integration with the common threat features like cigarette smoking, diabetes, hypertension, and hyperlipidemia. These are the means through which As stimulated the development of atherogenesis. In particular, a disproportion between atheroprotective and atherogenic lipoproteins in plasma is one of the most important contributors to atherosclerosis (3). It has been reported that the disclosure of arsenic, there is an atherosclerosis succession in the denizens of the Lanyang Basin in Taiwan (4). A possibility of induced alterations in lipid profiles has been reported (5).

It has been reported that As, even at very low levels, selectively inhibits the abilities of glucocorticoid and the glucocorticoid receptor to switch-on genes normally under glucocorticoid control (6). In addition, As has been reported to increase plasma lipid, plasma total lipid (TL), total cholesterol (TC), triglyceride (TG), bilurubin, and LDL-C levels, and decrease high density lipoprotein (HDL) levels, contributing to the effect of arsenic on the permeability of the liver cell membrane, and/or liver dysfunction (6). Specifically, an As induced elevated LDL level and a reduced HDL level in plasma were found to be useful for independent prediction of a risk for developing cardiovascular disease (7).

Induction of ROS and depletion of antioxidant defenses due to As are important factors governing the toxic effects of arsenic. In the cell's DNA, proteins, and lipids are found to be injured by ROS as it can react with the biological molecules leading to necrobiosis. The action of some enzymes which are concerned with gluconeogenesis, uptake of glucose in cells, and engenderment of glutathione are found to be restrained by arsenic as it can binds to the sulfhydryl groups of these proteins (6). A therapeutic

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Introduction

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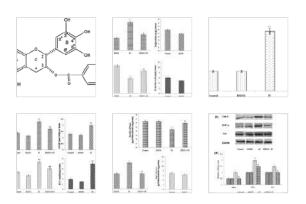
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Journal of Trace Elements in Medicine and Biology Volume 29, January 2015, Pages 321-335

TOXICOLOGY

Epigallocatechin gallate potentially attenuates Fluoride induced oxidative stress mediated cardiotoxicity and dyslipidemia in rats

S. Miltonprabu ペ ⊠, S. Thangapandiyan

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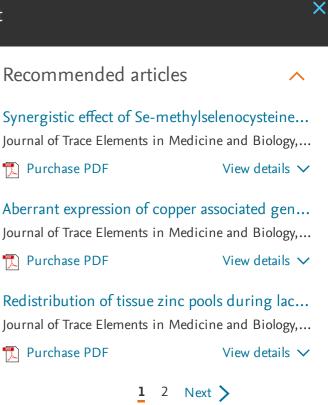
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Highlights

- Fl induced cardiotoxicity is mediated via ROS generation.
- Fl also provoked oxidative stress, inflammation and apoptosis in cardiomyocytes.
- EGCG ameliorates Fl induced cardiotoxicity via its enhanced antioxidant activity.
- EGCG improves enzymatic and non-enzymatic status in Fl intoxicated cardiac tissue.
- EGCG ameliorates Fl induced dyslipidemia via its ROS scavenging activity.

Abstract

The present study was undertaken to evaluate the cardioprotective role of (–)epigallocatechin-gallate (EGCG) against Fluoride (F) induced oxidative stress mediated cardiotoxicity in rats. The animals exposed to F as sodium Fluoride (NaF) (25 mg/kg BW) for 4 weeks exhibited a significant increase in the levels of cardiac troponins T and I (cTnT & I), cardiac serum markers, lipid peroxidative markers and plasma total cholesterol (TC), triglycerides (TG), phospholipids (PL), free fatty acids (FFA), low density lipoprotein cholesterol, very low density lipoprotein cholesterol as well as cardiac lipids profile (TC, TG and FFA) with the significant decrease of high density lipoprotein cholesterol and cardiac phospholipids. F intoxication also decreased the levels of mitochondrial enzymes such as ICDH, SDH, MDH, α -KGDH and NADH in the cardiac tissue of rats. The mitochondrial Ca²⁺ ion level was also significantly reduced along with the significant decrease in the levels of enzymatic and non enzymatic antioxidants. Furthermore, F treatment significantly increased the DNA fragmentation, up regulate cardiac proapoptotic markers, inflammatory markers and down-regulate the antiapoptotic markers in the cardiac tissue. Pre administration of EGCG (40 mg/kg/bw) in F intoxicated rats remarkably recovered all these altered parameters to near normalcy through its antioxidant nature. Thus, results of the present study clearly demonstrated that treatment with EGCG prior to F intoxication has a significant role in protecting F-induced cardiotoxicity and dyslipidemia in rats.



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Fluoride; EGCG; Heart; Lipids; Oxidative stress

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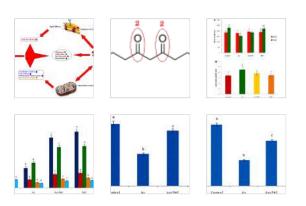
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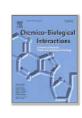
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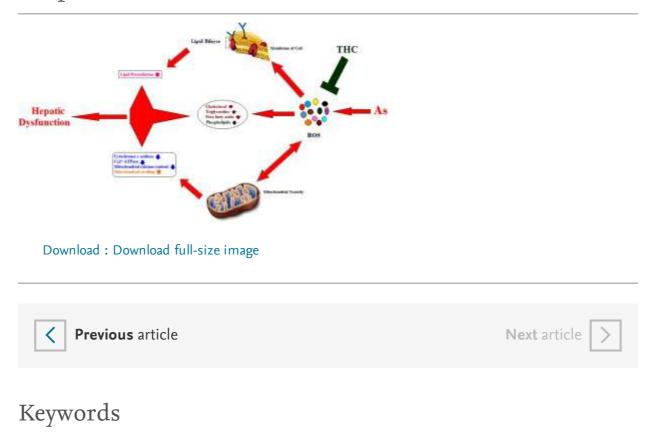
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Abstract

Arsenic (As) is a well-known human carcinogen and a potent hepatotoxin. Environmental exposure to arsenic imposes a serious health hazard to humans and other animals worldwide. Tetrahydrocurcumin (THC), one of the major metabolites of curcumin, exhibits many of the same physiological and pharmacological activities as curcumin and in some systems may exert greater antioxidant activity than the curcumin. It has been reported that THC has antioxidant efficacy attributable to the presence of identical β -diketone of 3rd and 5th substitution in heptane moiety. In the present study, rats were orally treated with arsenic alone (5 mg kg⁻¹ bw/day) with THC (80 mg kg⁻¹ bw/day) for 28 days. Hepatotoxicity was measured by the increased activities of serum hepatospecific enzymes, namely aspartate transaminase, alanine transaminase, alkaline phosphatase and bilirubin along with increased elevation of lipid peroxidative markers, thiobarbituric acid reactive substances. And also elevated levels of serum cholesterol, triglycerides, free fatty acids and phospholipids were observed in arsenic intoxicated rats. These effects of arsenic were coupled with enhanced mitochondrial swelling, inhibition of cytochrome c oxidase, Ca²⁺ATPase and a decrease in mitochondrial calcium content. The toxic effect of arsenic was also indicated by significantly decreased activities of enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase along with non-enzymatic antioxidant such as reduced glutathione. Administration of THC exhibited significant reversal of arsenic induced toxicity in hepatic tissue. All these changes were supported by the reduction of arsenic concentration and histopathological observations of the liver. These results suggest that THC has a protective effect over arsenic induced toxicity in rat.

Graphical abstract



Arsenic; Tetrahydrocurcumin; Liver; Mitochondria; Rat

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Chemico-Biological Interactions Volume 242, 5 December 2015, Pages 179-193

rats and its attenuation by GSP through the

activation of Nrf2 signaling pathway



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Abstract

Cadmium (Cd) is one of the toxic heavy metals in the environment, which induces oxidative stress, dyslipidemia and membrane disturbances in heart. The present study was designed to evaluate the role of grape seed proanthocyanidins (GSP) against Cd induced oxidative stress mediated cardiotoxicity in rats. In this study, male Wistar rats were treated with Cd as cadmium chloride (CdCl₂, 5 mg/ kg bw, PO) and pre-administered with GSP (100 mg/kg bw, PO) 90 min before the Cd intoxication for 4 weeks. Our results demonstrate a significant increase in the levels of cardiac troponins T and I (cTnT & I), cardiac serum markers, lipid peroxidative markers and plasma total cholesterol (TC), triglycerides (TG), phospholipids (PL) and free fatty acids (FFA). Cd induced oxidative stress decreased the levels of mitochondrial Krebs cycle enzymes as well as the respiratory chain enzyme activities and altered the levels of cardiac enzymatic and non-enzymatic antioxidants. The inflammatory (NF-kB, NO, TNF- α , IL-6), apoptotic markers (caspase 3, cytochrome C, Bax, Bcl-2), membrane bound ATPases and antioxidant Nrf2 (HO-1, keap1) markers were also measured in the control and experimental rats. All these alterations caused by Cd could be lessened by the pre-supplementation of GSP. The preadministration of GSP significantly increased the activities of mitochondrial and respiratory chain enzymes, reduced the levels of cardio-oxidative stress markers in Cd-treated rats, which examines the stress stabilizing action of GSP. GSP also prevented the cytochrome C release, inhibited the caspase activation and maintained the ratio of Bcl-2/Bax by its free radical scavenging ability. Nrf2 expression was transiently increased while the impaired cardiac markers were restored near to their basal levels by the pre-treatment with GSP in Cd intoxicated rats. The cardioprotective nature of the GSP was further fortified by our light microscopic and ultra structural findings. Overall, our results suggest that GSP has an ability to inhibit the membrane disturbances in cardiomyocytes, apoptotic pathway, inflammation and activate the Nrf2 expression through which it recuperated the Cd induced oxidative stress mediated cardiac dysfunction.

Outline

Highlights

Abstract

Graphical abstract

Keywords

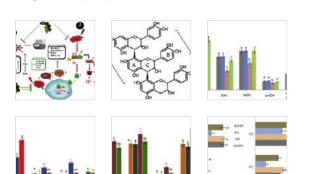
- 1. Introduction
- 2. Materials and methods
- 3. Results
- 4. Discussion
- Acknowledgments

Appendix A. Supplementary data

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Figures (13)



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Tables (7)

- ${f H}$ Table 1 Table 2 Ħ Table 3 \blacksquare Ħ Table 4
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https://doi.org/10.1016/j.cbi.2015.10.005

Highlights

- Effect of GSP on Cadmium-induced cardiac injury in rats investigated.
- GSP abrogated the state of oxidative stress and mitigated a •
- GSP inhibited NFkB, TNFα, IL2 and IL6 over expressions. •
- Both biochemical and histological data correlate the prote • effect of GSP.
- GSP enhanced the activation of Nrf2 and its related antiox enzymes.

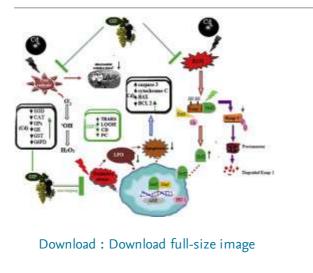
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Keywords

Cadmium; GSP; ROS; Oxidative stress; Lipid peroxidation; Rats

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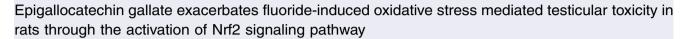
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ARTICLE INFO

ABSTRACT

Article history: Received 10 Feb 2015 Received in revised form 16 Jun 2015 Accepted 12 Jul 2015 Available online 4 Aug 2015

Keywords:

Fluoride EGCG Testes Oxidative stress Inflammation Apoptosis Nrf2 **Objective:** To explore the ameliorative potential of Epigallocatechin gallate (EGCG) by evaluating markers of oxidative stress, apoptosis, and inflammation and antioxidant competence in Fl intoxicated rats.

Methods: The animals were divided in to four groups that is control, EGCG alone, NaF, and EGCG with NaF. Group III animal were exposed to Fl as sodium Fluoride (NaF) (25 mg/kg BW) for 4 weeks. After the completion of the treatment, the testis tissues has been removed and used for the experimental observations.

Results: Pre-administration of EGCG to Fl intoxicated rats showed a significant normalization in the levels of steroidogenic enzymes, testosterone, sperm functions, oxidative stress markers and antioxidant status. The altered levels of proinflammatory cytokines and apoptotic markers were also relapsed in close proximity to control. In addition, EGCG significantly improved antioxidant status and reduced the oxidative stress and pathological changes in testes. The mRNA and protein analysis also substantiated that EGCG pre-treatment markedly enhanced the expression of Nrf2 and its target genes HO-1, NQO1 and γ GCS and suppressed the expression of Keap1 in testis. **Conclusion:** Altogether, our findings supports that EGCG attenuates Fl toxicity in testis through Nrf2 activation.

1. Introduction

Fluoride (Fl) is an essential trace element to the human body, for decades it has been employed for the prevention of dental caries [1]. Meanwhile, as a pervasive natural pollutant, fluoride can normally enter into the human body through drinking water, food, industrial pollution, drugs, cosmetics, etc. However, drinking water is the major source of daily intake of fluoride [2]. Accumulating evidences illustrated that in addition to hard tissues like teeth and skeleton, excessive fluoride exposure can also cause injury to the soft tissues such as, brain, heart, liver, kidney and testis [3,4]. These findings heightened the concerns regarding the multi-exposure routes as well as the multi-organ toxicity elicited by fluoride. The reproductive toxicity is of great concern to the public, as clearly demonstrated by epidemiological studies that environmental

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Tel: +91 04144 238282, +91 9842325222 Fax: +91 04144 238080 E-mail: smprabu73@gmail.com exposure to fluoride was associated with male infertility and low birth rates of people living in the area of endemic fluorosis [5.6]. However, increased evidences demonstrated that fluoride exposure also affected the soft tissues including testes [7]. Epidemiological data have also indicated that fluoride may adversely affect the reproductive systems of men living in endemic areas of fluorosis [8].

A number of mechanisms have been proposed to explain fluoride induced testicular toxicity. Among them, oxidative stress has been observed as the major cause for its toxicity in tissues, liver, kidney, brain, and testes in animals and in people living in areas of endemic fluorosis [9-13]. Fluoride has also been reported to hamper the activity of many antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase. Moreover, fluoride can deplete glutathione levels, often resulting in too much production of reactive oxygen species (ROS) at the mitochondrial level, and directing to the damage of cellular components [14,15]. Although the fertility of spermatozoa mainly on the maintenance of mitochondrial transmembrane potential ($\Delta \Psi m$) which could be altered by Fl, proposing a possible mechanism of Fl induced toxicity in testes [16]. However, the possible mechanism(s) by which Fl damages the testicular function remains ambiguous.

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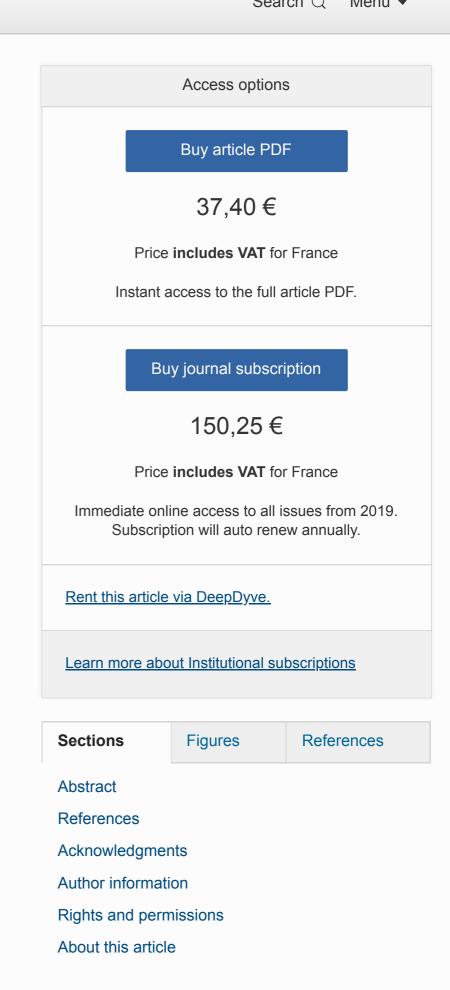
Laboratory evaluation of Indian medicinal plants as repellents against malaria, dengue, and filariasis vector mosquitoes

Marimuthu Govindarajan 🖂 & Rajamohan Sivakumar

Parasitology Research 114, 601–612(2015) | Cite this article 541 Accesses 8 Citations 1 Altmetric Metrics

Abstract

Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases. Mosquitoes are the carriers of severe and well-known illnesses such as malaria, arboviral encephalitis, dengue fever, chikungunya fever, West Nile virus, and yellow fever. These diseases produce significant morbidity and mortality in humans and livestock around the world. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticides, in the present study, the repellent activity of crude hexane, ethyl acetate, benzene, chloroform, and methanol extracts of leaf of Erythrina indica and root of Asparagus racemosus were assayed for their repellency against three important vector mosquitoes, viz., Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus. The crude extract was applied on a membrane used for membrane feeding of unfed mosquitoes in a 1-ft cage. About 50 unfed 3-4-day-old laboratory-reared pathogenfree strains of A. stephensi, A. aegypti, and C. quinquefasciatus were introduced in a 1-ft cage fitted with a membrane with blood for feeding with temperature maintained at 37 °C through circulating water bath maintained at 40–45 °C. Three concentrations (1.0, 2.0, and 5.0 mg/cm²) of the crude extracts were evaluated. Repellents in E. indica afforded longer protection time against A. stephensi, A. *aegypti*, and *C. quinquefasciatus* than those in *A. racemosus* at 5.0 mg/cm² concentration, and the mean complete protection time ranged from 120 to 210 min with the different extracts tested. In this observation, these two plant crude extracts gave protection against mosquito bites; also, the repellent activity is dependent on the strength of the plant extracts. These results suggest that the leaf extract of E. indica and root extract of A. racemosus have the potential to be used as an ideal eco-friendly approach for the control of mosquitoes. This is the first report on the mosquito repellent activity of the



reported A. racemosus and E. indica plants.

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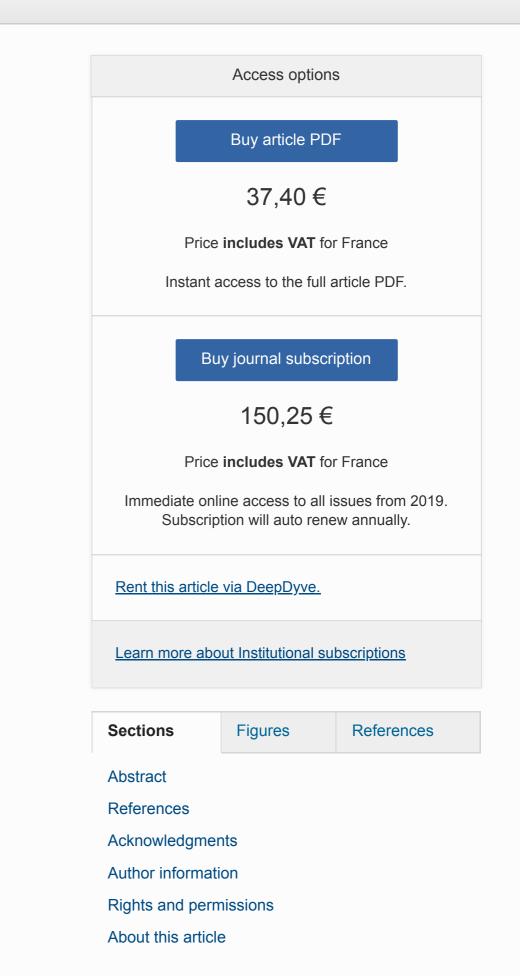
Mosquito larvicidal potential of silver nanoparticles synthesized using Chomelia asiatica (Rubiaceae) against Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus (Diptera: Culicidae)

<u>Udaiyan Muthukumaran, Marimuthu Govindarajan 🖂 & Mohan Rajeswary</u>

Parasitology Research 114, 989–999(2015) Cite this article 948 Accesses | 48 Citations | Metrics

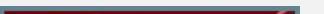
Abstract

Mosquitoes transmit serious human diseases, causing millions of deaths every year. Mosquito control is to enhance the health and quality of life of county residents and visitors through the reduction of mosquito populations. Mosquito control is a serious concern in developing countries like India due to the lack of general awareness, development of resistance, and socioeconomic reasons. Today, nanotechnology is a promising research domain which has a wide ranging application in vector control programs. These are nontoxic, easily available at affordable prices, biodegradable, and show broadspectrum target-specific activities against different species of vector mosquitoes. In the present study, larvicidal activity of aqueous leaf extract and silver nanoparticles (AgNPs) synthesized using C. asiatica plant leaves against late third instar larvae of Anopheles stephensi, Aedes aegypti, and Cx. quinquefasciatus. The range of varying concentrations of synthesized AgNPs (8, 16, 24, 32, and 40 µg/mL) and aqueous leaf extract (40, 80, 120, 160, and 200 µg/mL) were tested against the larvae of An. stephensi, Ae. aegypti, and Cx. quinquefasciatus. The synthesized AgNPs from C. asiatica were highly toxic than crude leaf aqueous extract in three important vector mosquito species. The results were recorded from UV-Vis spectrum, Fourier transform infrared spectroscopy, scanning electron microscopy, and energy-dispersive X-ray spectroscopy analysis (EDX). Considerable mortality was evident after the treatment of C. asiatica for all three important vector mosquitoes. The LC₅₀ and LC₉₀ values of C. asiatica aqueous leaf extract appeared to be effective against An. stephensi (LC₅₀, 90.17 μg/mL; LC₉₀, 165.18 μg/mL) followed by Ae. aegypti (LC₅₀, 96.59 μg/mL; LC₉₀, 173.83 μg/mL) and Cx. quinquefasciatus (LC₅₀, 103.08 µg/mL; LC₉₀, 183.16 µg/mL). Synthesized AgNPs against the vector mosquitoes of An. stephensi, Ae. aegypti, and Cx. quinquefasciatus had the following LC₅₀ and LC₉₀ values: An. stephensi had LC₅₀ and LC₉₀ values of 17.95 and 33.03 µg/mL; Ae. aegypti had LC₅₀ and LC₉₀ values of 19.32 and 34.87 µg/mL; and Cx. quinquefasciatus had LC₅₀ and LC₉₀ values of 20.92 and 37.41 µg/mL. No mortality was observed in the control. These results suggest that the leaf aqueous extracts of C. asiatica and green synthesis of silver nanoparticles have the potential to be used as an ideal eco-friendly approach for the control of An. stephensi, Ae. aegypti, and Cx. quinquefasciatus. This is the first report on the mosquito larvicidal activity of the plant extracts and synthesized AgNPs.



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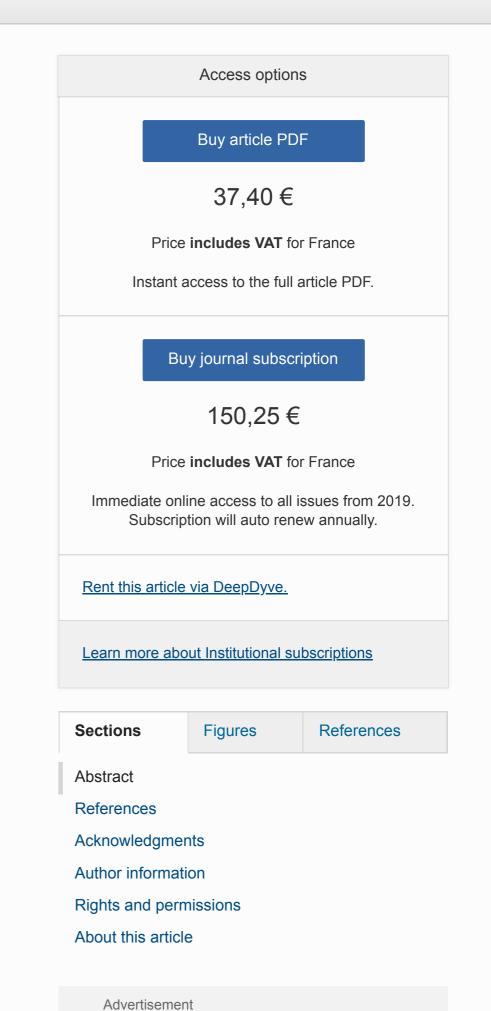
Synthesis and characterization of silver nanoparticles using *Gmelina asiatica* leaf extract against filariasis, dengue, and malaria vector mosquitoes

Udaiyan Muthukumaran, Marimuthu Govindarajan 🖂, Mohan Rajeswary & S L Hoti

Parasitology Research 114, 1817–1827(2015) | Cite this article
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Abstract

Mosquitoes are blood-feeding insects and serve as the most important vectors for spreading human diseases such as malaria, yellow fever, dengue fever, and filariasis. The continued use of synthetic insecticides has resulted in resistance in mosquitoes. Synthetic insecticides are toxic and affect the environment by contaminating soil, water, and air, and then natural products may be an alternative to synthetic insecticides because they are effective, biodegradable, eco-friendly, and safe to environment. Botanical origin may serve as suitable alternative biocontrol techniques in the future. The present study was carried out to establish the larvicidal potential of leaf extracts of Gmelina asiatica and synthesized silver nanoparticles using aqueous leaf extract against late third instar larvae of Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus. Larvae were exposed to varying concentrations of plant extracts and synthesized AgNPs for 24 h. The results were recorded from UV-visible spectroscopy, Fourier transform infrared spectroscopy, scanning electron microscopy, transmission electron microscopy, and energy-dispersive X-ray spectroscopy analysis support the biosynthesis and characterization of AgNPs. The maximum efficacy was observed in synthesized AgNPs against the larvae of An. stephensi (lethal dose (LC_{50}) = 22.44 μ g/mL; LC₉₀ 40.65 μ g/mL), Ae. aegypti (LC₅₀ = 25.77 μ g/mL; LC₉₀ 45.98 μ g/mL), and C. quinquefasciatus (LC₅₀ = 27.83 μ g/mL; LC₉₀ 48.92 μ g/mL), respectively. No mortality was observed in the control. This is the first report on mosquito larvicidal activity of plantsynthesized nanoparticles. Thus, the use of G. asiatica to synthesize silver nanoparticles is a rapid, eco-friendly, and a single-step approach and the AgNps formed can be potential mosquito larvicidal agents.



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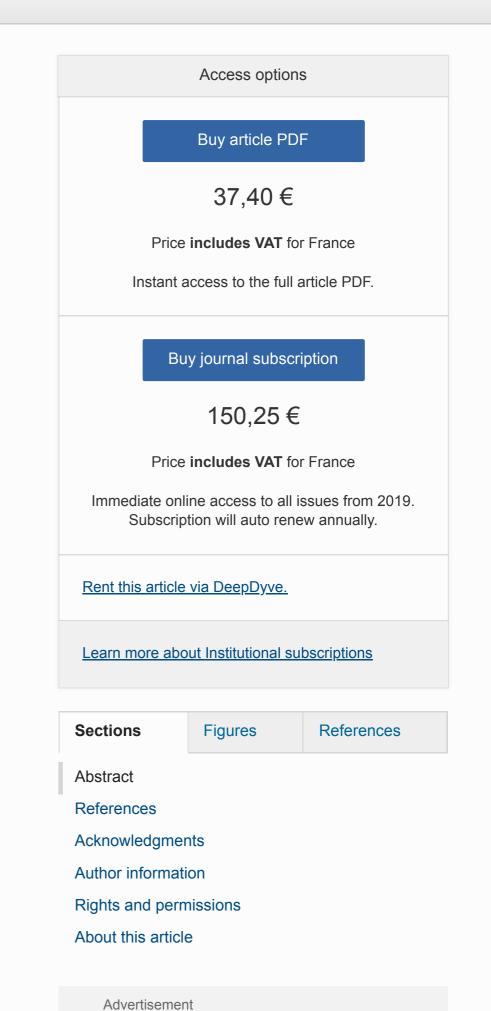
Synthesis and characterization of silver nanoparticles using *Gmelina asiatica* leaf extract against filariasis, dengue, and malaria vector mosquitoes

Udaiyan Muthukumaran, Marimuthu Govindarajan 🖂, Mohan Rajeswary & S L Hoti

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Abstract

Mosquitoes are blood-feeding insects and serve as the most important vectors for spreading human diseases such as malaria, yellow fever, dengue fever, and filariasis. The continued use of synthetic insecticides has resulted in resistance in mosquitoes. Synthetic insecticides are toxic and affect the environment by contaminating soil, water, and air, and then natural products may be an alternative to synthetic insecticides because they are effective, biodegradable, eco-friendly, and safe to environment. Botanical origin may serve as suitable alternative biocontrol techniques in the future. The present study was carried out to establish the larvicidal potential of leaf extracts of Gmelina asiatica and synthesized silver nanoparticles using aqueous leaf extract against late third instar larvae of Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus. Larvae were exposed to varying concentrations of plant extracts and synthesized AgNPs for 24 h. The results were recorded from UV-visible spectroscopy, Fourier transform infrared spectroscopy, scanning electron microscopy, transmission electron microscopy, and energy-dispersive X-ray spectroscopy analysis support the biosynthesis and characterization of AgNPs. The maximum efficacy was observed in synthesized AgNPs against the larvae of An. stephensi (lethal dose (LC_{50}) = 22.44 μ g/mL; LC₉₀ 40.65 μ g/mL), Ae. aegypti (LC₅₀ = 25.77 μ g/mL; LC₉₀ 45.98 μ g/mL), and C. quinquefasciatus (LC₅₀ = 27.83 μ g/mL; LC₉₀ 48.92 μ g/mL), respectively. No mortality was observed in the control. This is the first report on mosquito larvicidal activity of plantsynthesized nanoparticles. Thus, the use of G. asiatica to synthesize silver nanoparticles is a rapid, eco-friendly, and a single-step approach and the AgNps formed can be potential mosquito larvicidal agents.



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FULL LENGTH ARTICLE

Repellent properties of *Delonix elata* (L.) Gamble (Family: Fabaceae) against malaria vector *Anopheles stephensi* (Liston) (Diptera: Culicidae)

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Received 30 November 2012; accepted 23 August 2013

KEYWORDS

Repellent activity; Delonix elata; Leaf and seed; Malarial vector; Anopheles stephensi **Abstract** Mosquito control is facing a threat because of the emergence of resistance to synthetic insecticides. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. The purpose of the present study was to assess the effects of leaf and seed hexane, ethyl acetate, benzene, chloroform and methanol extract of *Delonix elata* on repellent activity against the malaria vector mosquito *Anopheles stephensi* (Diptera: Culicidae). Evaluation was carried out in a net cage ($45 \times 30 \times 45$ cm) containing 100 blood starved female mosquitoes of *An. stephensi*. Repellent activity was carried out in laboratory conditions. Plant crude extracts of *D. elata* were applied at 1.0, 2.5, and 5.0 mg/cm² separately in the exposed forearm of volunteers. Ethanol was used as the sole control. Applied leaf and seed crude extracts were observed to protect against mosquito bites. There were no allergic reactions experienced by the volunteer subjects. The repellent activity of the extract was dependent on the strength of the extract. Among the tested solvents, both the leaf and seed methanol extracts showed maximum efficacy. The highest concentration of 5.0 mg/cm² provided over 210 and 180 min protection for the leaf and seed extracts, respectively. Crude extracts of *D. elata* (leaf and seed) exhibit the potential for controlling *An. stephensi*.

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1. Introduction

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Malaria remains one of the most prevalent diseases in the tropical world. With 200–450 million infections annually worldwide, it causes up to 2.7 million deaths (WHO, 2010). In India, malaria is transmitted by six vector species, in which *Anopheles stephensi* is responsible in urban areas. It is endemic in all parts of India, and periodic epidemics of malaria occur every 5–7 years. Malaria continues to be a major public health problem in the tropical world. Of the total world population of about 5.4 billion people, 2200 million are exposed to malarial

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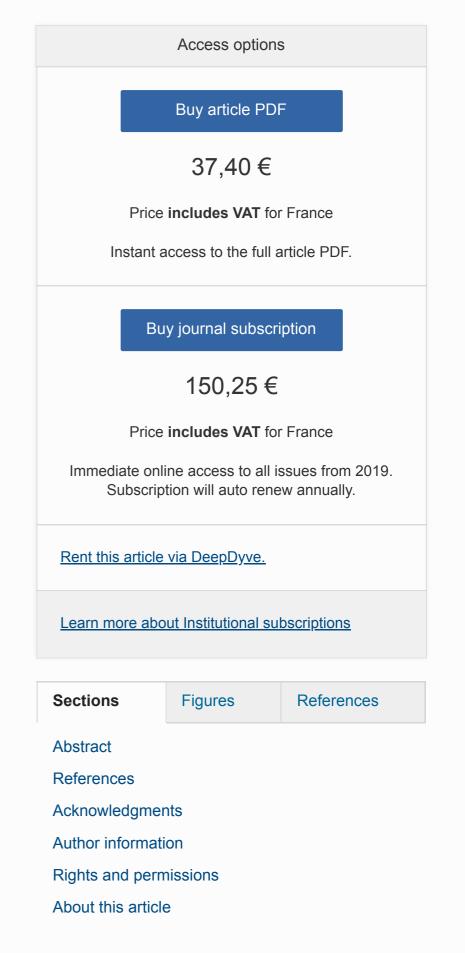
Green synthesis of silver nanoparticles from Cassia roxburghii-a most potent power for mosquito control

Udaiyan Muthukumaran, Marimuthu Govindarajan 🖂 & Mohan Rajeswary

Parasitology Research 114, 4385–4395(2015) Cite this article 451 Accesses | 13 Citations | Metrics

Abstract

Mosquitoes transmit serious human diseases, causing millions of deaths every year. The use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. Insecticides of synthesized natural products for vector control have been a priority in this area. In the present study, silver nanoparticles (AgNPs) synthesized using Cassia roxburghii plant leaf extract against Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus were determined. Larvae were exposed to varying concentrations of synthesized AgNPs (12, 24, 36, 48, and 60 µg/mL) and aqueous leaf extracts (60, 120, 180, 240, and 300 µg/mL) for 24 h. The synthesized AgNPs were characterized by UV-Vis spectrum, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), with energy-dispersive X-ray spectroscopy analysis (EDX), transmission electron microscopy, and X-ray diffraction analysis (XRD). Compare to aqueous extracted synthesized AgNPs showed extensive mortality rate against An. stephensi, Ae. aegypti, and C. quinquefasciatus with the LC₅₀ and LC₉₀ values that were 26.35, 28.67, 31.27 and 48.81, 53.24, and 58.11 µg/mL, respectively. No mortality was observed in the control. This is the first report on mosquito larvicidal activity of plant-synthesized nanoparticles. Thus, the use of C. roxburghii to synthesize silver nanoparticles is a rapid, eco-friendly, and a single-step approach, and the AgNPs formed can be potential mosquito larvicidal agents. Therefore, this study proves that C. roxburghii is a potential bioresource for stable, reproducible nanoparticle synthesis (AgNPs) and also can be used as an efficient mosquito control agent. This is the first report on the larvicidal activity of the plant extract and AgNPs.





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Outline

Abstract

Keywords

1. Introduction

2. Materials and methods

3. Results

4. Discussion

Disclosure of interest

Acknowled gements

References

Show full outline \checkmark

Figures (1)



Tables (2)

☐ Table 1☐ Table 2



Biomedicine & Preventive Nutrition Volume 4, Issue 2, April–June 2014, Pages 131-136

Original article

Antioxidant potential of hesperidin and ellagic acid on renal toxicity induced by mercuric chloride in rats

Erusan Bharathi, Ganesan Jagadeesan 🖰 🖾

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https://doi.org/10.1016/j.bionut.2013.12.007

Abstract

The health effects of mercury are highly dependent on the different chemical forms of mercury. Inorganic mercury has a non-uniform distribution after absorption being accumulated mainly in kidney tissue causing acute renal failure. The purpose of this work was to study the influence of hesperidin and ellagic acid followed by mercuric chloride induced kidney damage. At sublethal dose of mercuric chloride (1.23 mg/kg B.W) was administrated in rats for 7 days. The results revealed that treatment of mercuric chloride caused marked enhanced level of lipid peroxidation (LPO) content and significantly decreased in the level of reduced glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activities in the kidney tissue. Hesperidin is a natural flavonoid and a strong antioxidant helps to prevent oxidative damage. Ellagic acid has a chemo protective effect in cellular models by reducing oxidative stress. The treatment of hesperidin and ellagic acid (5 mg/kg B. W) in the kidney tissue shows a significantly decreasing in the level of oxidant content and simultaneously an enhanced level of antioxidant properties by the way of recovery in kidney tissues. Antioxidant and nonantioxidant enzymes (LPO, GSH, GPx, SOD, CAT) activities were also an enhanced to near normal level when compared to mercury treated group. These observations of the present experimental study demonstrated a preliminary protective effect of hesperidin and ellagic acid against mercuric chloride intoxicated rat kidney tissue.



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Keywords

LPO; GSH; GPx; CAT; SOD; Mercuric chloride; Hesperidin; Ellagic acid

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Abstract

Keywords

1. Introduction

2. Materials and methods

3. Results

4. Discussion

5. Conclusion

Disclosure of interest

Acknowled gements

References

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Figures (1)



Tables (3)

田 Table 1田 Table 2



Biomedicine & Preventive Nutrition Volume 4, Issue 2, April–June 2014, Pages 239-243

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Ameliorative potential of ferulic acid on cardiotoxicity induced by mercuric chloride

Cardiovascular disease affects more people and causes more death commonly.

associated with an antioxidant deficit as well as increased myocardial oxidative

infarction induced by mercuric chloride and the productive role of ferulic acid

in rats. At sub-lethal dose of mercury chloride (1.30 mg/kg body weight 45 days

stress. The aim of the present study was designed to evaluate the myocardial

Heart failure mainly occurs due to myocardial infarction and it may be

daily) administered in rat, heart tissue shows an elevated level of lipid

marker enzymes. Occurrence of cardiotoxicity is mainly due to the

alanine transferase (ALT), aspartate transaminasas (AST), creative

cardiac tissues against mercury chloride-induced oxidative stress.

peroxidation (LPO) content and simultaneously decreased level of cardiac

accumulation of heavy metal in cardiac tissues and increase in the level of

blood serum specific markers. The following serum enzymes were drastically

increased. Due to the mercury toxicity, the level of alkaline phosphatase (ALP),

phosphokinase (CPK), total cholesterol (TC) and lactate dehydrogenase (LDH) were increased. The administration of sub-lethal dose of ferulic acid (5 mg/kg

body weight 45 days daily) restores all the serum marker enzymes to near-

normal level. This result suggests that the administration of ferulic acid not

only promotes the marker enzymes but it also acts as a protective effect of

Murugan Vijayakumar, Ganesan Jagadeesan ^A⊠, Erusan Bharathi

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Keywords

Mercuric chloride; Cardiac toxicity; Ferulic acid; ALT; AST; ALP; LDH; CPK; TC; LPO

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Abstract

Keywords

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2. Materials and methods

3. Result

4. Discussion

Disclosure of interest

Acknowledgment

References

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Figures (1)



Tables (2)

☐ Table 1☐ Table 2



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Biomedicine & Aging Pathology Volume 4, Issue 1, January–March 2014, Pages 17-21

Original article

Hepato-ameliorative effect of hesperidin and ellagic acid on mercuric chloride intoxicated rats

Erusan Bharathi 🖾, Ganesan Jagadeesan 🙁 🖾, Murugan Vijayakumar

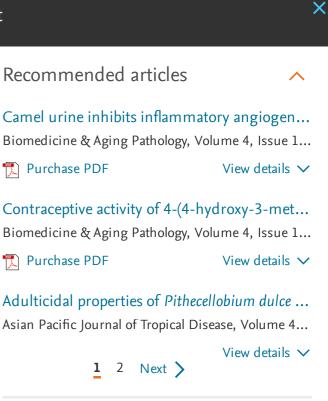
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Abstract

Protective efficacy of Hesperidin and Ellagic acid on hepatotoxicity induced by mercuric chloride for 7 days in rats, Rattus norvegicus, were carried out in the present study. At sub-lethal dose of mercuric chloride (1.23 mg/kg body weight) was administrated in rats for 7 days through oral dose. After completing the scheduled exposure time, the intoxicated rats were sacrificed and then whole liver organ was isolated immediately and they used for bio-enzymological analyses. In the present experimental study, the following biochemical and bio-enzymological studies were carried out to find the levels of lipid peroxidation (LPO) and reduced glutathione (GSH) and super oxidedismutase (SOD), catalase (CAT), glutathione peroxidase (GP_X) activities in whole liver tissue. The results revealed that treatment with mercuric chloride caused marked elevation in the level of free radicals (LPO) and simultaneously decreased in the level of SOD, CAT, GP_X, activities and GSH content in rat liver tissue. The hesperidin and ellagic acid (5 mg/kg body weight) treatment on mercury-intoxicated rats were restoring these oxidant and antioxidant activities near to normal level when compared to mercuric chloride intoxicated groups. These results suggested that the protective efficacy of hesperidin and ellagic acid on mercuric chloride induced hepatotoxicity in rats have been proven.



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Keywords

Mercuric chloride; Hesperidin; Ellagic acid; LPO; SOD; CAT; GPx; GSH

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Keywords

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2. Materials and methods

3. Results

4. Discussion

Disclosure of interest

Acknowledgement

References

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■ Table 1 Table 2



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Biomedicine & Aging Pathology Volume 4, Issue 3, July-September 2014, Pages 219-222

Original article

In vivo restoration of hepatic and nephro protective potential of hesperidin and ellagic acid against mercuric chloride intoxicated rats

G. Jagadeesan ペ ⊠, E. Bharathi

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https://doi.org/10.1016/j.biomag.2014.01.008

Abstract

The present experimental study is to investigate the efficacy of some phytochemicals on heavy metal intoxicated animals. Commonly phytochemicals have played a vital role for protective effect of oxidative stress, which is induced by heavymetals in animals. At sub-lethal dose of mercuric chloride (1.23 mg/kg body weight) treated rat liver tissue shows hepatic cell damage and alteration of its metabolic activities by the way of liver marker enzymes. The hepato-protective effect of Hesperidin and ellagic acid was tested against mercuric chloride induced hepato-toxicity in rats. In the present study, drastically altered in the level of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Lactic dehyrogenase (LDH), bilirubin, albumin, cholesterol, urea and creatinine levels were observed in the blood serum of mercury intoxicated rats. The activity of liver marker enzymes such as ALT AST, ALP and LDH were significantly increased and albumin was simultaneously decreased in mercuric chloride intoxicated rats. Administration of Hesperidin and Ellagic acid (5 mg/kg body weight) on mercuric chloride intoxicated rats not only reduced the liver markers enzymes and bilirubin and cholesterol levels and also maintain their level to near normal condition. Hesperidin and ellagic acid alone treated animals did not alter the ALT, AST, ALP, LDH, bilirubin, albumin, cholesterol urea and creatinine levels in serum. Our results indicate that treatment for hesperidin and ellagic acid exhibited the strong hepatoprotective activity against mercuric chloride induced hepatotoxicity.



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ALT; AST; ALP; LDH; Bilirubin; Albumin; Cholesterol; Urea; Creatinine; Mercuric chloride hesperidin; Ellagic acid

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Selvaraj Milton Prabu* and Naorem Chanu Sumedha

Ameliorative effect of diallyl trisulphide on arsenic-induced oxidative stress in rat erythrocytes and DNA damage in lymphocytes

Abstract

Background: Arsenic (As) is a naturally occurring semimetallic element that is classified as a toxicant and a human carcinogen. Diallyl trisulphide (DATS), an organosulphur compound, is an antioxidative substance that is extracted from garlic (*Allium sativum*). Erythrocytes are very expedient models to understand the susceptibility of membrane to oxidative damage induced by different xenobiotic compounds. Arsenic has been reported to induce oxidative stress to erythrocytes due to lipid peroxidation and alteration in defence mechanism as erythrocytes are the first target that arsenic compounds attack in the body after systemic absorption. In the light of this fact, the purpose of this study is to characterise the ameliorative effect of DATS on arsenic-induced oxidative stress in rat erythrocytes.

Methods: Experimental rats were randomly divided into four groups and treated orally for 28 days: control, As [5 mg/kg body weight (BW)] treated, As+DATS (80 mg/kg BW) treated, DATS (80 mg/kg BW) treated and As+vitamin C (100 mg/kg BW) treated. Oxidative stress in erythrocytes was recorded by estimating plasma marker enzymes, plasma and erythrocyte membrane oxidative stress markers, erythrocyte membrane antioxidant enzymes and non-antioxidant enzymes, etc.

Results: Oral administration of arsenic at 5 mg/kg BW per day elevated the levels of plasma marker enzymes, namely, aspartate transaminase (AST), alanine transaminase (ALT), acid phosphatase (ACP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and γ -glutamyl transferase (γ GT) (U/L) with significantly increased lipid peroxidation markers such as thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), lipid hydroperoxides (LH), conjugated dienes (CD), and protein carbonyl (PC) contents were also elevated in As-treated rat plasma and erythrocytes. The levels of non-enzymatic antioxidants (reduced glutathione, vitamins C and E) and enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G6PD) were also decreased in As-treated rats. The toxic effect of As significantly decreased the activities of membrane-bound ATPases (Na⁺/K⁺-ATPase, Mg²⁺-ATPase, and Ca²⁺-ATPase), with a significant increase in% tail DNA of rat lymphocytes measured by means of a single-cell gel electrophoresis assay. Administration of DATS for 28 days significantly reduced the levels of plasma markers. The levels of TBARS, MDA, LH, CD, and PC were significantly decreased and there was a significant increase in ATPase activities and non-enzymatic and enzymatic antioxidants on treatment with DATS in a dose-related manner.

Conclusions: All these changes were supported by reduction of DNA damage in lymphocytes with DATS treatment. DATS at a dose of 80 mg/kg BW was found to be most effective and the results revealed the same. The results of the study showed that DATS shows a protective effect against As-induced oxidative stress in rat erythrocytes and lymphocytes.

Keywords: antioxidant enzymes; arsenic; comet assay; diallyl trisulphide; erythrocyte membrane; erythrocytes; oxidative stress.

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Introduction

Increasing human activities have modified the global cycle of heavy metals, nonmetals and metalloids. In today's world, environmental and occupational surroundings can generate a variety of modes for exposure to various forms of metals. Arsenic, the 33rd element in the periodic table, is one of the most widely studied elements in the field of metal intoxication [1, 2]. Arsenic is one of the most common environmental contaminants and carcinogens; it is a popular murder weapon and is known as the "king of



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Ameliorative effects of grape seed proanthocyanidins on cadmium induced metabolic alterations in rats

NazimaBashir, Vaihundam Manoharan, Selvaraj Milton Prabu *

Faculty of science, Department of zoology, Annamalai University, Annamalai Nagar *Corresponding author E-mail: smprabu73@gmail.com

Abstract

Objective: To study the ameliorative effects of grape seed proanthocyanidins (GSP) against cadmium (Cd) induced hyperglycemia, hypercholesterolemia and hyperlipidemia in rats.

Methods: Wistar rats, body weight 180-200g, were randomly divided into four groups, six rats in each group. First group (control) represented the control animal, the second group (Cd) was fed orally with Cd in the form of CdCl₂ (5mg/kg bw), the third group (GSP) was fed with GSP (200 mg/kg bw) and to the fourth group (Cd+GSP), CdCl₂ was administered orally at a dose of (5 mg/kg bw) and pretreated with GSP (200 mg/kg bw) 90 min before Cd intoxication for 4 weeks. At the end of experiment, rats were sacrificed; blood and liver samples were collected and used for analysis of various metabolic parameters.

Results: The result of the present study revealed that Cd exposure caused significant reduction in food intake and body weight gain, but increased the liver weight. GSP administration significantly revert all these changes to normal level. Cd intoxication induced hyperglycemia, elevate plasma glucose and lipid profiles and decline in high density lipoprotein (HDL) -cholesterol respectively. The GSP pretreatment regimen was beneficial in the restoration of the increased serum levels of TC, TG and lipid profiles and of the suppressed insulin and total antioxidants on Cd exposure.

Conclusion: From the present investigation, it may be concluded that Cd intoxication caused deleterious effects on the metabolism of rats which were successfully restored by GSP. Therefore, the present work suggests that GSP is a feasible therapeutic agent to improve and treat patients with hyperlipidemia, obesity, hyperglycemia in addition to its anti-oxidant properties and can be used as a component in food to promote the health of people.

Keywords: Cd, GSP, Carbohydrate Metabolism, Lipid Metabolism, Liver.

1. Introduction

Cadmium (Cd) is one of the most important environmental and occupational metallic toxicant arising primarily from battery, electroplating, pigment, fertilizer industries and cigarette smoke. Since Cd does not degrade in the environment, the risk of human exposure is constantly increased due to Cd as it also enters the foodchain (ATSDR, 2005). Exposure to Cd produce both acute and chronic tissue injury and can damage various organs and tissues, including liver, kidney, lung, bone, testis and blood, depending on the dose, route and duration of exposure (Sarkar et al., 1995) One of the basis of Cd toxicity is its negative influence on Cd enzymatic systems of cells, owing to the substitution of other metal ions (mainly Zn^{2+} and Cu^{2+}) in metalloenzymes and its very strong affinity to biological structures containing SH, carboxyl and phosphate group (Timbrell.,2000). Exposure to Cd metal is known to induce the formation of reactive oxygen species (ROS) like superoxide radicals, hydroxyl ion and hydrogen peroxide (Stohs et al., 2000) They inhibit many enzymes and disturb many processes, including lipid metabolism and alters in the serum and tissue concentration of some lipid compounds, including free fatty acids (FFA), triglycerides (TG), phospholipids as well as total cholesterol and high and low density lipoprotein cholesterol (HDL and LDL) causing hypercholesterolemia and hyperlipidemia (Larregle et al., 2008)

Grape seed proanthocyanidins (GSP) are polyphenolic compounds that can be found in the plant physiology of various plant species, are mainly concentrated in tree barks and outer skin of the seeds. GSP is of a diphenylpropane structure of C₆-C₃-C₆. Most often found as a glycoside derivative, this compound class is composed of three monomer units of catechin, epicatechin, and epigallocatechin (Harborne and Mabry., 1982) GSP can clear off free radicals, protect the over-oxidative damage and prevent a range of diseases caused by free radicals, such as myocardial infarction, atherosclerosis, drug-induced liver and kidney injury more, it has functions of anti-thrombotic, anti-tumor, antimutagenic, anti-radiation, and anti-fatigue (Engelbrecht et al., 2007). GSP has been shown to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility and to affect enzyme systems, including phospholipase A2, cyclooxygenase and lipooxygenase (Robert et al., 1990). Oral administration of GSP significantly reduces the postprandial levels of TG-rich and apolipoprotein-B-containing lipoproteins and improves several atherosclerotic risk indexes in normolipidemic rats (Delbas et al., 2005). We have studied the protective role of GSP on cadmium induced hepatic dysfunction in rats (Nazimabashir et al., 2014) now the present study was devoted to investigate the influence of (GSP) on some metabolic and biochemical changes associated with hyperglycemia, antihyperlipidemic and antiperoxidative effect on cadmium intoxicated rats.



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Silibinin potentially attenuates arsenic-induced oxidative stress mediated cardiotoxicity and dyslipidemia in rats

M. Muthumani · S. Milton Prabu

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Abstract Cardiac dysfunction is one of the major causes of mortality and morbidity throughout the world. Chronic exposure of arsenic (As) mainly leads to cardiotoxic effect. Cardiotoxicity was induced by the sodium arsenite as the source of As (5 mg/kg BW, PO) for 4 weeks. As intoxication significantly (p < 0.05) increased the serum cardiac markers, viz. creatine kinase-MB, lactate dehydrogenase, aspartate transaminase, alanine transaminase and alkaline phosphatase, oxidative stress markers in heart, plasma total cholesterol (TC), triglycerides (TG), phospholipids (PL), free fatty acids (FFA), low density lipoprotein cholesterol, very low density lipoprotein cholesterol as well as cardiac lipid profile (TC, TG and FFA) and significantly (p < 0.05) decreased the level of serum high density lipoprotein cholesterol, cardiac PL, mitochondrial enzymes such as ICDH, SDH, MDH, α -KDH and NADH dehydrogenase, levels of enzymatic antioxidant, nonenzymatic antioxidants and membrane-bound ATPases in heart. In addition, Asintoxicated rats showed a significant (p < 0.05) up-regulation of myocardial NADPH (NOX) oxidase sub units such as NOX2 and NOX4 as well as Keap-1 and downregulation of Nrf2 and HO-1 protein expressions. Preadministration of silibinin (SB) (75 mg/kg BW) remarkably recovered all these altered parameters to near normalcy in As-induced cardiotoxic rat. Moreover, the light microscopic and transmission electron microscopic study further supports the protective efficacy of SB on the heart mitochondria. In conclusion, our data demonstrate that SB has a potential to extenuate the arsenic-induced cardiotoxicity and dyslipidemia in rat.

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Keywords Arsenic · Silibinin · Heart · Oxidative stress · Antioxidant · Rat

Introduction

Arsenic (As) and many of its compounds are potent cardiotoxins. Contaminated drinking water is the main source of intake of arsenic in worldwide as well as contaminated food [1, 2]. In general, inorganic trivalent arsenic (As (III)) is more toxic, which increases reactive oxygen species (ROS) by binding vicinal thiols or sulfur-containing groups [3, 4]. Arsenic is the first metalloid to be recognized as a human carcinogen. Once absorbed, As redistributes itself to nearly entire organ systems of the body including heart [5, 6]. As-induced ROS causes lipid peroxidation, protein oxidation, enzymes inactivation and DNA damage in the myocardial tissue [7]. Arsenic exposure plays a key role in the pathogenesis of myocardial tissue, which is associated with myocardial injury, cardiac arrhythmias and cardiomyopathy [6]. Furthermore, several earlier literatures have publicized that chronic exposure to arsenic has been linked with myriad of human diseases, such as diabetes, atherosclerosis, cardiovascular diseases and hyperkeratosis [8].

Mitochondria are the main patrons of molecular oxygen in the cardiac cell in addition to act as a major source of ROS [9]. Although recent studies support the role of free radical-mediated oxidative damage in the As-mediated organ injury and cell death, few evidences are known about the mechanism of As-induced cardiac abnormalities. The proposed pathway of arsenic-induced cardiotoxicity chiefly mediated through the generation of ROS which impose mitochondrial dysfunction, oxidation of lipids, proteins and DNA and changes in cardiac ion channels and apoptosis

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Grape seed proanthocyanidins (GSP) are polyphenolic compounds which exert a novel spectrum of biological, pharmacological and therapeutic properties against oxidative stress. Recent findings have shown that GSP exhibit numerous antioxidant and free radical scavenging properties. So, it was of special concern to investigate the protective efficacy of GSP against cadmium induced hepatic dysfunction in rats. Male Wistar rats were treated with cadmium (Cd) as cadmium chloride (CdCl₂, 5 mg kg⁻¹ bw, orally) and orally pre-administered with GSP (100 mg kg⁻¹ bw) 90 minutes before Cd intoxication for 4 weeks to evaluate hepatic damage of Cd and antioxidant potential of GSP. Our results demonstrate a significant (p < 0.05) increase in the levels of serum hepatic markers and hepatic oxidative stress markers and a notable (p < 0.05) decrease in hepatic enzymatic and non-enzymatic antioxidant contents along with histopathological alterations on Cd exposure. The GSP pre-treatment regimen was beneficial in the recovery of altered serum and hepatic biochemical and histological variables by Cd. Our results clearly indicate that the free radical scavenging and antioxidant potential of GSP benefits the recovery of rats exposed to Cd. We thus recommend that diets rich in GSP help in attenuating Cd-induced oxidative hepatic dysfunction in rats.

Protective role of grape seed proanthocyanidins

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Introduction

Cadmium (Cd) is a heavy metal that is linked to carcinogens and many other harmful disorders in the human body. The major industrial sources of Cd include oil refining, smelting of metals such as copper and zinc, combustion of fossil fuel and nickel-cadmium battery manufacture and disposal.¹ Cd usage has increased dramatically during the last 40–50 years because of its high resistance to corrosion and valuable electrochemical properties. Less than 5% of the metal is recycled. So, environmental pollution due to Cd is evident.² The basis of cadmium toxicity is its negative influence on enzymatic systems of cells, owing to the substitution of other metal ions (mainly Zn^{2+} and Cu^{2+}) in metallic enzymes and its strong affinity to biological structures containing SH groups.³

The mechanism pertaining to Cd toxicity may be multifactorial. It has been suggested that Cd acts as a catalyst in the oxidative reactions of biological macromolecules, and therefore, the toxicity associated with Cd is due to the oxidative tissue damage.⁴ Heavy metals like Cd inhibit the activity of vital antioxidant enzymes by substituting the metal cofactor in its active site⁵ elevating the free radicals and the associated health problems under intoxicated conditions. Cd depletes glutathione and protein bound sulfhydryl groups resulting in enhanced production of reactive oxygen species (ROS) which consist mainly of the superoxide anion radicals (O_2^{--}), hydrogen peroxide (H_2O_2) and hydroxyl radicals ('OH).⁶ Reactions of these reactive oxygen species with cellular biomolecules have been shown to lead to lipid peroxidation, membrane protein damage, an altered antioxidant system, DNA damage, altered gene expression and apoptosis.^{7,8} Due to growing evidence that even relatively low exposure to Cd creates a risk to health^{9,10} and prognosis indicating that exposure to this metal will rise,¹⁰ in recent years increasing interest has been focused on the prevention of health damage induced by Cd and on therapeutic interventions.

Grape seed proanthocyanidins (GSP) are high-molecularweight oligomers and polymers of polyhydroxyflavan-3-ol units, monomer units such as ((+) catechin and (-) epicatechin) linked most commonly by acid-labile $4 \rightarrow 8$ and in some cases by $4 \rightarrow 6$ bonds. $4 \rightarrow 8$ bonds (*e.g.*, B-type dimer and C-type dimer) are more common than $4 \rightarrow 6$ bonds and some branching may occur in the chain of higher oligomers and polymers. GSP are present essentially in polymeric forms (60–80%), followed by oligomeric forms (15–30%), while the monomer flavan-3-ols (catechins) represent only a small



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Diallyl trisulfide (DATS) abrogates arsenic induced testicular oxidative stress in rats

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Abstract

Background: Arsenic is an emblematic heavy metal and enters the animals through intake of arsenic contaminated water and gets stored in primitive organs.

Aim: The present study was designed to investigate the protective effect of diallyl trisulfide (DATS) on arsenic (As) induced testicular oxidative stress in male rats.

Methods: Experimental rats were randomly divided into four groups and treated orally for 28 days: control, as (5mg/kg/bw) treated, DATS (80mg/kg/bw) + As treated and DATS (80mg/kg/bw) alone treated rats. Testicular toxicities were recorded by plasma hormonal analysis, sperm function test, estimation of testicular oxidative stress markers, biochemical analysis, antioxidant enzymes and non-antioxidant enzymes and histopathological changes.

Results: Short term exposure to as resulted in decreased testicular - weight; sperm count, plasma hormonal concentration and significantly increased levels of oxidative stress markers, testis ALP, ACP and cholesterol with a significant decreased in the enzymatic antioxidant, non-antioxidant enzymes and ATPases enzymes when compared to the control group. Pre-administration of DATS to as treated rats were found to protect against adverse changes in the reproductive organ weight, sperm count, enhance plasma hormone level along with restored antioxidant enzymes, non-antioxidant enzymes and ATPases enzymes supporting with revived histological structure of testes. **Conclusion:** Our results show that DATS exerts a protective effect against as induced testicular toxicity associated with reduced testicular oxidative stress as evidenced by the clear restoration of plasma hormone levels and antioxidant activities.

Keywords: Arsenic, Diallyl Trisulfide, Oxidative Stress, Rat, Testis.

1. Introduction

Arsenical compounds are environmental contaminant with manifold effects in animal and human populations. Human are exposed to arsenic mainly through water, food and drugs. The repeated use of arsenic is as herbicides, insecticides, rodenticides and food preservatives (Mehranjani and Hemadi 2007). Though adverse, on the other hand, reality relics that a large number of populations in some areas of the world are drinking arsenic-contaminated ground water and the number of cases torment from As-induced organ dysfunctions are increasing terrifically (Das et al. 2009). Arsenic is a major environmental chemical and a known reproductive toxicant via the depression of spermatogenesis and androgenesis in males (Morakinyo et al. 2010). Arsenic is also extremely sensitive in the process of reproduction, leading to increased rates of abortion in women and infertility in man (Skakkeback et al. 1991).

Arsenic affects the mitochondrial enzymes, impairs the cellular respiration and causes cellular toxicity. It can also substitute phosphate intermediates, which could theoretically slow down the rate of metabolism and interrupt the production of energy. Male infertility is reflected by low sperm count, low sperm motility and bad quality of sperms (Sarkar et al. 2003). Arsenic has been found to have an inhibitory effect on the activity of testicular steroidogenic enzyme D5-3b- hydroxysteroid dehydrogenase (D5-3b-HSD) and 17ß-hydroxysteroid dehydrogenase (17ß-HSD) and to reduce the weight of the testes and accessory sex glands in rats (Ali et al. 2013). High As level may suppress the sensitivity of gonadotroph

cells to GnRH (Gonodotropin releasing hormone) as well as gonadotropin secretion by elevating plasma levels of glucocorticoids (Ali et al. 2013). These ultimately lead to the development of gonadal toxicity.

Arsenic exposure has also been associated with severe metabolic disorders, including reproductive toxicity. Male germ cells may be susceptible to oxidative stress because of high concentration of polyunsaturated fatty acids and low antioxidant capacity. Arsenic exerts its toxicity by generating reactive oxygen species (ROS) during redox cycling and metabolic activation processes that causes tissue damages. Free radicals damage biomembrane, reflected by increased lipid peroxidation oxidation of nucleic acid and protein, thereby compromising cellular integrity and function. In the testes enhanced production of ROS causes significant alteration in tissue physiology, spermatogenic process or induces oxidative damage to DNA, which is potential risk to offspring (Sharma and Kumar 2011).

At the same time, renewed attention has been created due to the therapeutic application of arsenic in the treatment of lymphoid and hemopoietic neoplasms such as acute promyelocytic leukemia (Das et al. 2009). Arsenic has been found to impair male reproductive function like spermatogenesis and testosterone release, inhibited testicular enzyme function, and reduced the weight of the male sex organs (Sarkar et al. 1991) probably by inducing oxidative stress (Chang et al. 2007). However, the mechanism(s) by which arsenic impairs male reproductive function remains unclear. Emerging evidence from the literature supports the role of free



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Epigallocatechin gallate supplementation protects against renal injury induced by fluoride intoxication in rats: Role of Nrf2/HO-1 signaling

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ABSTRACT

Fluoride intoxication generates free radicals, causing oxidative stress that plays a critical role in the progression of nephropathy. In the present study, we hypothesized that epigallocatechin gallate (EGCG), found in green tea, protects the kidneys of rats treated with fluoride by preventing oxidative stress, inflammation, and apoptosis. Pretreatment of fluoridetreated rats with EGCG resulted in a significant normalization of creatinine clearance and levels of urea, uric acid, and creatinine. Fluoride intoxication significantly increased renal oxidative stress markers and decreased the levels of renal enzymatic and non-enzymatic antioxidants. In addition, renal NO, TNF- α , IL-6 and NF- κ B were also increased in the renal tissue of fluoride-treated rats. Further, EGCG pretreatment produced a significant improvement in renal antioxidant status and reduced lipid peroxidation, protein carbonylation and the levels of inflammatory markers in fluoride-treated kidney. Similarly, mRNA and protein analyses showed that EGCG pretreatment normalized the renal expression of Nrf2/Keap1 and its downstream regulatory proteins in fluoride-treated rat kidney. EGCG also effectively attenuated fluoride-induced renal apoptosis by the up-regulation of anti-apoptotic proteins such as Bcl-2 and down-regulation of Bax, caspase-3, caspase-9 and cytochrome c. Histology and immunohistochemical observations of Kim-1 provided further evidence that EGCG effectively protects the kidney from fluoride-mediated oxidative damage. These results suggest that EGCG ameliorates fluoride-induced oxidative renal injury by activation of the Nrf2/HO-1 pathway.

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Abbreviations: ATPase, adenosine triphosphatase; Bcl-2, B-cell lymphoma 2; Bax, B-cell associated X protein; CAT, catalase; EDTA, ethylenediaminetetraacetic acid; EGCG, epigallocatechin gallate; GAPDH, glyceraldehyde 3 phosphate dehydrogenase; GCSH, γ -glutamylcysteine synthetase heavy subunit; G6PD, glucose 6-phosphate dehydrogenase; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione S-transferease; GSTM, glutathione S-transferase Mu; HO-1, heme oxygenase-1; IL-6, interleukin-6; Keap-1, Kelch-like ECH-associated protein 1; Kim-1, kidney injury molecule-1; LOOH, lipid hydroperoxide; NaF, sodium fluoride; NF-kB, Nuclear factor kappa B; Nrf2, nuclear factor erythroid-2 related factor-2; PC, protein carbonyl; ROS/RNS, reactive oxygen species/reactive nitrogen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TNF- α , tumor necrosis factor- α ; TSH, total sulfhydryl groups.

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Original article

Dimethoxycurcumin potentially protects arsenic induced oxidative hepatic injury, inflammation and apoptosis via Nrf2-Keap1 signaling in rats

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ABSTRACT

NADPH oxidase mediated ROS generation plays a decisive role in the pathogenesis of arsenic (As) hepatotoxicity. Antioxidant phytochemicals, like dimethoxycurcumin (DiMC) has a tremendous scope in attenuating the ROS mediated hepatic injury. Hence, the present study has been designed to investigate the hepatoprotective action of DiMC by analysing the markers of hepatic oxidative stress, pro-inflammatory cytokines, apoptotic markers and antioxidant competence in As (5 mg/kg BW) induced hepatotoxic rats. Oral administration of DiMC (80 mg/kg BW) to As intoxicated rats showed a significant amelioration in the levels of serum hepatic markers, pro-inflammatory cytokines and the expression of NADPH oxidase subunits (Nox2, Nox4, and p47phox) in liver. The elevated levels of hepatic oxidative stress markers lipid peroxides, hydroperoxides, protein carbonyls and conjugated dienes and decreased levels of enzymatic and non-enzymatic antioxidants status were also reverted back to near normalcy by DiMC when compared with As treated rats. In addition, mRNA and protein expression analysis also confirms that DiMC pre-treatment significantly downregulates the NOX subunits and upregulates the Nrf2 and its related enzymes in the liver. Studies on the mechanism of apoptosis showed that As accelerated the markers of mitochondrial dependent apoptotic pathway (enhanced cytochrome c release in cytosol from mitochondria, altered the expression of Bax, Bcl-2, Bad, caspase-9, caspase-3). However, DiMC pretreatment effectively restored the As-induced alterations in liver. Histological and immunohistochemical results were also evidenced that DiMC potentially protects the liver from As-induced oxidative stress, inflammation and apoptosis. These findings encourage the use of DiMC as a prospective salutary entity for As hepatotoxicty through the suppression of NADPH oxidase and Nrf2 activation.

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1. Introduction

Arsenic (As) is a pervasive environmental toxin. It enters the organisms by dermal contact, inhalation, or ingestion of contaminated drinking water and has an effect on the entire organ systems of the body [1]. Among numerous mechanisms, oxidative stress due to accelerated generation of free radicals has also been associated for As-induced hepatic injury [2]. Investigations at the cellular and molecular levels disclose that As intoxication augments the production of reactive oxygen species (i.e. superoxide and

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hydrogen peroxide), causes lipid peroxidation, enhances oxidation of proteins, enzymes as well as DNA disrupt mitosis and promotes apoptosis [3]. Recent studies suggest that As exerts its toxic effects through a variety of mechanisms in which the most noticeable one is the excess generation of reactive oxygen species (ROS) via NADPH oxidases and by the diminution of antioxidant defense system [4–6]. At present, As is considered to be the most potent hepatotoxic agent because liver is the primary target organ for arsenic methylation [7]. Lin et al. [8] have reported that the oxidative stress may produce acquired tolerance to apoptosis, enhanced cell proliferation, altered DNA methylation, genomic instability, and aberrant estrogen signalling involved in the liver toxicity caused by As [9]. In addition, As intoxication has been reported to depress the antioxidant defense system, leading to the oxidative deterioration of cellular macromolecules including DNA, proteins and lipids which results in membrane damage and ultimately cause chaos to the biological system. Oxidative stress in the liver may lead to

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Cadmium and Apoptosis: A Molecular Approach

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Abstract

Apoptosis, also known as programmed cell death is a highly regulated and fundamental process found in all multicellular organisms. It is not only implicated in regulatory mechanisms of cells, but has been attributed to a number of diseases, like inflammation, malignancy, autoimmunity and neuro degeneration. A variability of pollutants can persuade apoptosis. Oncogenic transition metals like cadmium, promote apoptosis alongside DNA base modifications, strand breaks and rearrangements. Generation of reactive oxygen species, accumulation of Ca^{2+} , upregulation of caspase-3, downregulation of bcl-2, and deficiency of p-53 lead to the Cadmium (Cd) induced apoptosis. Metallothionein expression determines the choice between apoptosis and necrosis in Cd induced toxicity. Thus, disorders of apoptosis may play a critical role in some of the most debilitating metal-induced afflictions including hepato toxicity, renal toxicity, neuro toxicity, autoimmunity and carcinogenesis. An understanding of Cd-induced apoptosis will be obliging in the development of precautionary molecular approach.

Keywords: Metals, cadmium, apoptosis, cell-proliferation, carcinogenicity

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INTRODUCTION

Metals have been exploited by man since antiquity. Anthropogenic uses have led to a global dispersion of metals in the environment. Man, animals and plants are exposed to a variety of metals through environment, food, water and soil. Metals being nonbiodegradable persist in the environment for a period and long causes serious ecotoxicological problems. Additionally, many metallic compounds like zinc, copper, calcium, trivalent chromium, and iron being essential to life have formed complex biogeochemical cycles. Essential elements are involved in a variety of critical functions including the control of gene transcription, nerve conductance and oxygen transport and as active centers in enzymes. Therefore, the critical molecular events within the cell such as gene expression, cell proliferation and cell death are affected by trace elements. Some toxic metals may mimic the essential metals thereby gain access to important and molecular targets. It is flawless that toxic metals can together activate and inactivate the cellular procedures regimented by the indispensable metals. Even crucial metals can be toxic too. An uncommon of the

environmental metals, i.e., arsenic, chromium is carcinogenic.

Apoptosis is considered as an ongoing normal event in the control of cell populations. However, apoptosis can also be induced by a variety of xenobiotics including many of the toxic metals resulting in the loss of affected cell populations. Apoptosis essentially occurs when cellular damage, including damage to genetic material, has exceeded the capacity for repair (Figure 1).

Environmental metals can damage apoptosis and that suppression of the apoptotic response could expedite aberrant cell accumulation, which may be a life-threatening step in the pathogenesis of malignancy or autoimmunity. One of the primary genes shown to regulate apoptosis was Bcl-2. Consequently, a number of Bcl-2 related proteins were also recognized. Although, the overwhelming evidence that Bcl-2 proteins are evolutionarily preserved their regulators of apoptosis, specific biochemical function remains scandalous. There have been a multitude of reports showing that enforced over expression of Bcl-2 or of Bcl- XL acts to delay the onset of

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Low-cost and eco-friendly green synthesis of silver nanoparticles using Feronia elephantum (Rutaceae) against Culex quinquefasciatus, Anopheles stephensi, and Aedes aegypti (Diptera: Culicidae)

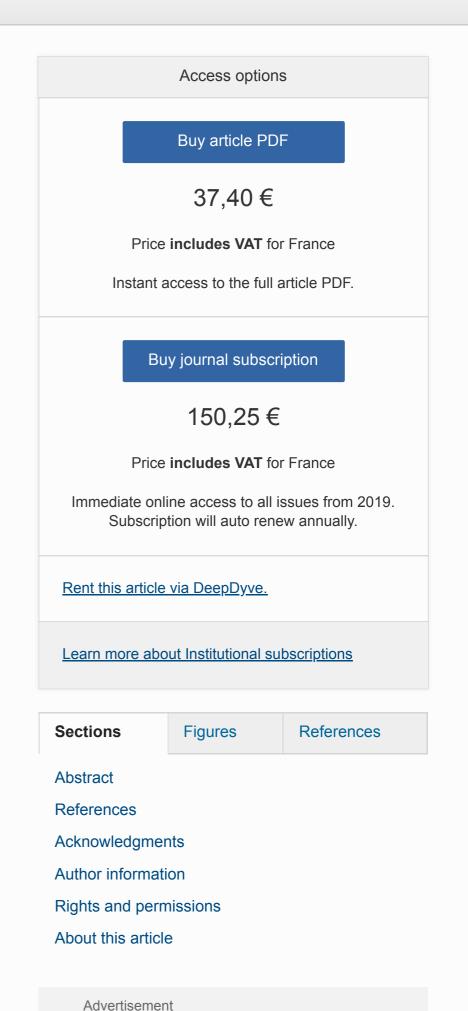
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Abstract

Mosquitoes transmit serious human diseases, causing millions of deaths every year. The use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. Insecticides of synthesized natural products for vector control have been a priority in this area. In the present study, the larvicidal activity of silver nanoparticles (AgNPs) synthesized using Feronia elephantum plant leaf extract against late third-instar larvae of Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus was determined. The range of concentrations of synthesized AgNPs (5, 10, 15, 20, and 25 μ g mL⁻¹) and aqueous leaf extract (25, 50, 75, 100, and 125 μ g mL⁻¹) were tested against the larvae of A. stephensi, A. aegypti, and C. quinquefasciatus. Larvae were exposed to varying concentrations of aqueous crude extract and synthesized AgNPs for 24 h. Considerable mortality was evident after the treatment of F. elephantum for all three important vector mosquitoes. The synthesized AgNPs from F. elephantum were highly toxic than crude leaf aqueous extract to three important vector mosquito species. The results were recorded from UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy analysis (EDX). Synthesized AgNPs against the vector mosquitoes A. stephensi, A. aegypti, and C. quinquefasciatus had the following LC₅₀ and LC₉₀ values: A. stephensi had LC₅₀ and LC₉₀ values of 11.56 and 20.56 μ g mL⁻¹; A. aegypti had LC₅₀ and LC₉₀ values of 13.13 and 23.12 μ g mL⁻¹; and C. quinquefasciatus had LC₅₀ and LC_{90} values of 14.19 and 24.30 µg mL⁻¹. No mortality was observed in the control. These results suggest that the green synthesis of silver nanoparticles using F. elephantum has the potential to be used as an ideal eco-friendly approach for the control of A. stephensi, A. aegypti, and C. quinquefasciatus. This is the first report on the mosquito larvicidal activity of the plant extracts and synthesized nanoparticles.



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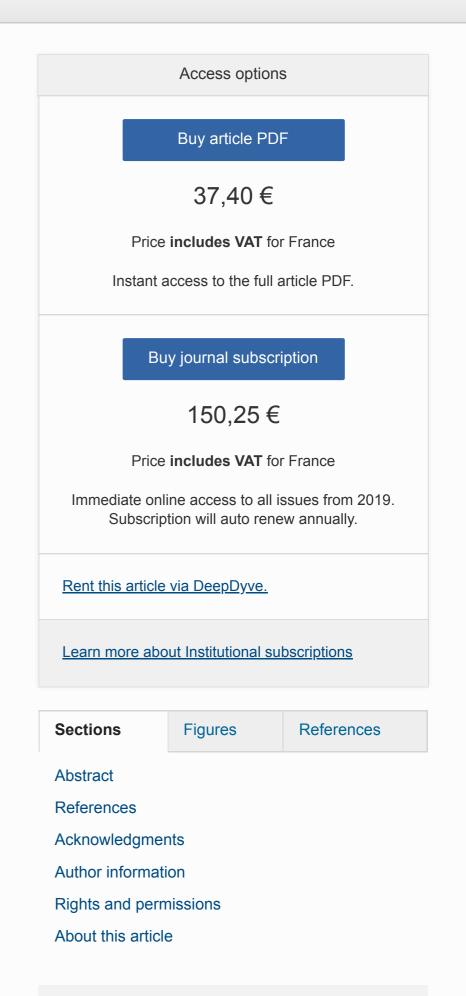
Mosquito larvicidal properties of silver nanoparticles synthesized using *Heliotropium indicum* (Boraginaceae) against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract

Mosquitoes transmit dreadful diseases to human beings wherein biological control of these vectors using plant-derived molecules would be an alternative to reduce mosquito population. In the present study activity of aqueous leaf extract and silver nanoparticles (AgNPs) synthesized using Helitropium indicum plant leaves against late third instar larvae of Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus. The range of varying concentrations of synthesized AgNPs (8, 16, 24, 32, and 40 µg/mL) and aqueous leaf extract (30, 60, 90, 120, and 150 µg/mL) were tested against the larvae of Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus. The synthesized AgNPs from H. *indicum* were highly toxic than crude leaf aqueous extract in three important vector mosquito species. The results were recorded from UV–Vis spectrum, Fourier transform infrared spectroscopy, scanning electron microscopy, energy-dispersive X-ray spectroscopy analysis, transmission electron microscopy, and histogram. The synthesized AgNPs showed larvicidal effects after 24 h of exposure. Considerable mortality was evident after the treatment of *H. indicum* for all three important vector mosquitoes. The LC₅₀ and LC₉₀ values of *H. indicum* aqueous leaf extract appeared to be effective against *A. stephensi* (LC₅₀, 68.73 µg/mL; LC₉₀, 121.07 µg/mL) followed by *A. aegypti* (LC₅₀, 72.72 µg/mL; LC₉₀, 126.86 µg/mL) and C. quinquefasciatus (LC₅₀, 78.74 µg/mL; LC₉₀, 134.39 µg/mL). Synthesized AgNPs against the vector mosquitoes of A. stephensi, A. aegypti, and C. quinquefasciatus had the following LC₅₀ and LC₉₀ values: A. stephensi had LC₅₀ and LC₉₀ values of 18.40 and 32.45 µg/mL, A. aegypti had LC₅₀ and LC₉₀ values of 20.10 and 35.97 µg/mL, and C. quinquefasciatus had LC₅₀ and LC_{90} values of 21.84 and 38.10 μ g/mL. No mortality was observed in the control. These results suggest that the leaf aqueous extracts of *H. indicum* and green synthesis of silver nanoparticles have the potential to be used as an ideal ecofriendly approach for the control of A. stephensi, A. aegypti, and C. quinquefasciatus. This is the first report on the mosquito larvicidal activity of the plant extracts and synthesized nanoparticles.



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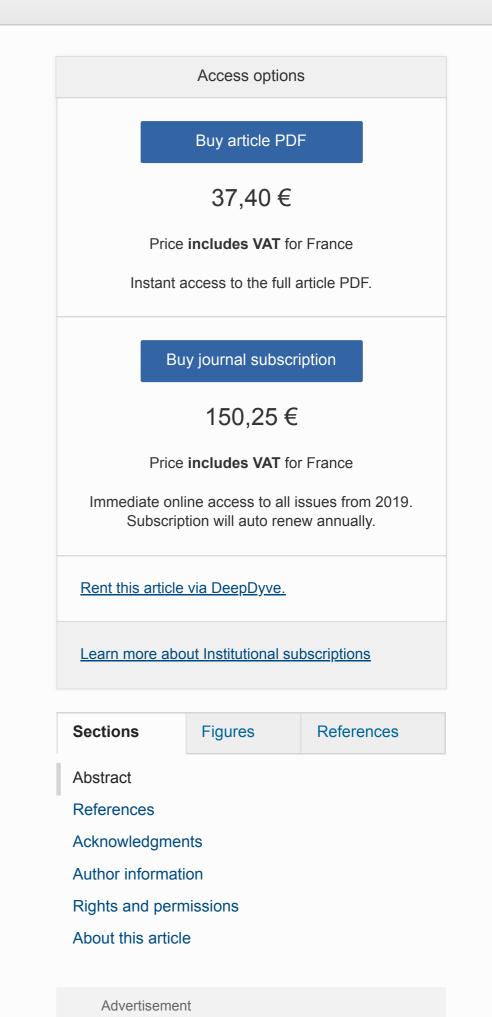
Ovicidal, larvicidal and adulticidal properties of Asparagus racemosus (Willd.) (Family: Asparagaceae) root extracts against filariasis (*Culex quinquefasciatus*), dengue (Aedes aegypti) and malaria (Anopheles stephensi) vector mosquitoes (Diptera: Culicidae)

Marimuthu Govindarajan 🖂 & Rajamohan Sivakumar

Parasitology Research **113**, 1435–1449(2014) | Cite this article 787 Accesses | 11 Citations | 1 Altmetric | Metrics

Abstract

Several diseases are associated to the mosquito-human interaction. Mosquitoes are the carriers of severe and well-known illnesses such as malaria, arboviral encephalitis, dengue fever, chikungunya fever, West Nile virus and yellow fever. These diseases produce significant morbidity and mortality in humans and livestock around the world. The present investigation was undertaken to study the ovicidal, larvicidal and adulticidal activities of crude hexane, ethyl acetate, benzene, chloroform and methanol extracts of root of Asparagus racemosus were assayed for their toxicity against three important vector mosquitoes, viz., *Culex* quinquefasciatus, Aedes aegypti and Anopheles stephensi (Diptera: Culicidae). The mean percent hatchability of the eggs was observed after 48 h post-treatment. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. All the five solvent extracts showed moderate ovicidal activity; however, the methanol extract showed the highest ovicidal activity. The methanol extract of Asparagus racemosus against Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi exerted 100 % mortality (zero hatchability) at 375, 300 and 225 ppm, respectively. Control eggs showed 99–100 % hatchability. The larval mortality was observed after 24 h of exposure. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in methanol extract of root of Asparagus racemosus against the larvae of Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi with the LC50 and LC90 values were 115.13, 97.71 and 90.97 ppm and 210.96, 179.92, and 168.82 ppm, respectively. The adult mortality was observed after 24 h recovery period. The plant crude extracts showed dosedependent mortality. At higher concentrations, the adult showed restless movement for some times with abnormal wagging and then died. Among the extracts tested, the highest adulticidal activity was observed in methanol extract against Anopheles stephensi followed by Aedes *aegypti* and *Culex quinquefasciatus* with the LD_{50} and LD_{90} values were 120.44, 135.60, and 157.71 ppm and 214.65, 248.35, and 290.95 ppm, respectively. No mortality was recorded in the control. The finding of the present investigation revealed that the root extract of Asparagus racemosus possess remarkable ovicidal, larvicidal and adulticidal activity against medically important vector mosquitoes and this is the low cost and ideal eco-friendly approach for the control of mosquitoes. This is the first report on the mosquito ovicidal, larvicidal and adulticidal activities of the reported Asparagus racemosus root.



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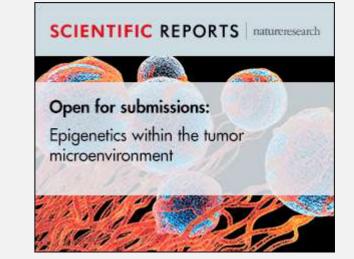
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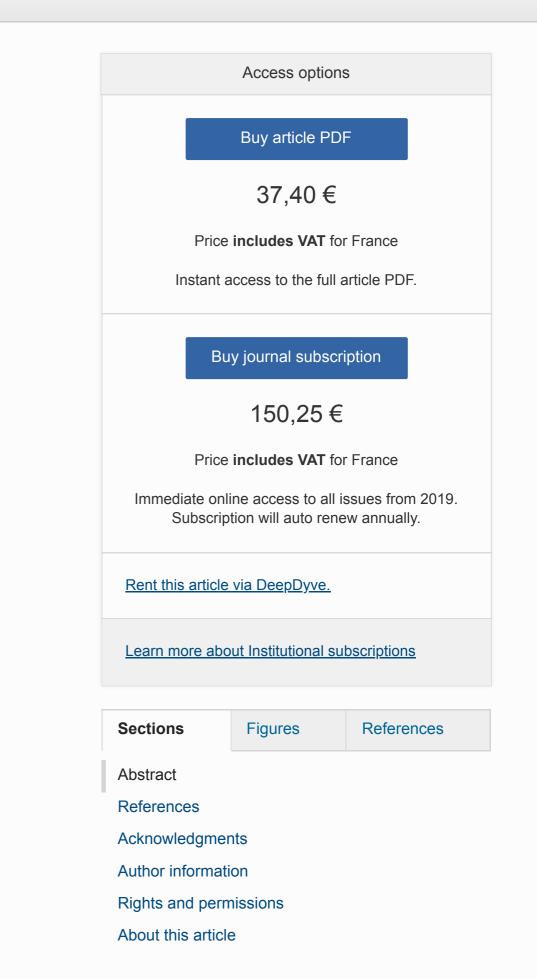
Larvicidal, ovicidal, and adulticidal efficacy of *Erythrina* indica (Lam.) (Family: Fabaceae) against Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus (Diptera: Culicidae)

Marimuthu Govindarajan 🖂 & Rajamohan Sivakumar

Parasitology Research 113, 777–791(2014) | Cite this article 576 Accesses | 24 Citations | 6 Altmetric | Metrics

Abstract

Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis, and Japanese encephalitis. Mosquito control is facing a threat because of the emergence of resistance to synthetic insecticides. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal, ovicidal, and adulticidal potential of the crude hexane, benzene, chloroform, ethyl acetate, and methanol solvent extracts from the medicinal plant *Erythrina indica* against the medically important mosquito vectors, Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus (Diptera: Culicidae). The larval mortality was observed after 24 h of exposure. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in methanol extract of leaf of E. indica against the larvae of A. stephensi, A. aegypti, and C. quinquefasciatus with the LC_{50} and LC₉₀ values of 69.43, 75.13, and 91.41 ppm and 125.49, 134.31, and 167.14 ppm, respectively. The mean percent hatchability of the eggs was observed after 48 h post treatment. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. All the five solvent extracts showed moderate ovicidal activity; however, the methanol extract showed the highest ovicidal activity. The methanol extract of *E. indica* against *A. stephensi*, *A. aegypti*, and *C. guinguefasciatus* exerted 100 % mortality (zero hatchability) at 150, 200, and 250 ppm, respectively. Control eggs showed above 99.3–100 % hatchability. The adult mortality was observed after 24 h recovery period. The plant crude extracts showed dose-dependent mortality. At higher concentrations, the adult showed restless movement for some times with abnormal wagging and then died. Among the extracts tested, the highest adulticidal activity was observed in methanol extract against A. stephensi followed by A. aegypti and C. quinquefasciatus with the LD₅₀ and LD₉₀ values of 88.76, 94.09, and 119.64 ppm and 160.83, 169.01, and 219.77 ppm, respectively. No mortality was recorded in the control. Our data suggest that the crude hexane, benzene, chloroform, ethyl acetate, and methanol solvent extracts of *E. indica* have the potential to be used as an eco-friendly approach for the control of the *A. stephensi*, *A. aegypti*, and *C.* quinquefasciatus.



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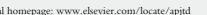
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Adulticidal properties of *Pithecellobium dulce* (Roxb.) Benth. (Family: Fabaceae) against dengue vector, Aedes aegypti (Linn.) (Diptera: Culicidae)

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PEER REVIEW

Peer reviewer

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Comments

The research work is very much important to the society to control morbidity and other defects caused by mosquitoes and also other insect vectors. This work reports a novel approach for the control of vector mosquitoes. The study is interesting and important in its field. It has scientific value and will be of help for the mankind.

Details on Page S452

ABSTRACT

Objective: To examine the toxicity of mosquito adulticidal activity of different solvent leaf and seed extracts of Pithecellobium dulce (P. dulce) against dengue vector, Aedes aegypti (Ae. aegypti).

Methods: Adulticidal efficacy of the crude leaf and seed extracts of P. dulce with five different solvents like benzene, hexane, ethyl acetate, methanol and chloroform was tested against the five to six day old adult female mosquitoes of Ae. aegypti. The adult mortality was observed after 24 h under the laboratory conditions.

Results: Among the tested solvents the maximum efficacy was observed in the leaf and seed methanol extract. The LC_{s_0} and LC_{s_0} values of *P. dulce* leaf and seed extract against adults of *Ae*. aegypti were 218.64, 257.99 mg/L and 426.05, 507.73 mg/L, respectively. No mortality was observed in controls. The *Chi*-square values were significant at *P*<0.05 level.

Conclusions: From the results it can be concluded the crude extract of *P. dulce* leaf and seed was an excellent potential for controlling dengue vector mosquito, Ae. aegypti.

KEYWORDS Adulticidal activity, Pithecellobium dulce, Leaf, Seed, Aedes aegypti, Dengue vector

1. Introduction

The occurrence of dengue has grown dramatically around the world in recent decades. Some 2.5 billion people-two fifths of the world's population-are now at risk from dengue. WHO currently estimates there may be 50 million dengue infections worldwide every year[1]. In the Indian scenario, almost the entire country is endemic to the mosquito-borne diseases due to favorable ecological conditions. Aedes aegypti (Ae. aegypti), a vector of dengue that carries the arbovirus responsible for this

disease, is widely distributed in the tropical and subtropical zones. The only way to prevent dengue virus transmission is to combat the disease-carrying mosquitoes. Mosquito control is being strengthened in many areas, but there are significant challenges, including an increasing mosquito resistance to insecticides and a lack of alternative, cost-effective, and safe insecticides. The use of phytochemicals is one such strategy that may be suitable for mosquito control. Thus, attempts to develop novel materials as mosquitocide are still necessary. Biologically active plant materials have attracted considerable

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S449

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Mosquito repellent properties of *Delonix elata* (L.) gamble (Family: Fabaceae) against filariasis vector, *Culex quinquefasciatus* Say. (Diptera: Culicidae)

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PEER REVIEW

Peer reviewer

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Comments

This is a good study in which the authors evaluated the mosquito repellent activity of *D. elata* against medically important filariasis vector, *Cx. quinquefasciatus*. The results are interesting and suggested that plant derived *D. elata* are present especially in repellent activity. This study provides the first report on the mosquito repellent activity of the different solvent extracts of *D. elata* plant. Details on Page S197

ABSTRACT

Objective: To determine the repellent activity of hexane, ethyl acetate, benzene, chloroform and methanol extract of *Delonix elata (D. elata)* leaf and seed against *Culex quinquefasciatus (Cx. quinquefasciatus)*.

Methods: Evaluation was carried out in a net cage (45 cm×30 cm×25 cm) containing 100 blood starved female mosquitoes of *Cx. quinquefasciatus*. Repellent activity was carried out in the laboratory conditions according to the WHO 2009 protocol. Plant crude extracts of *D. elata* were applied at 1.0, 2.5, and 5.0 mg/cm² separately in the exposed fore arm of study subjects. Ethanol was used as the sole control.

Results: In this study, the applied plant crude extracts were observed to protect against mosquito bites. There were no allergic reactions experienced by the study subjects. The repellent activity of the extract was dependent on the strength of the extract. Among the tested solvents, the leaf and seed methanol extract showed the maximum efficacy. The highest concentration of 5.0 mg/cm² provided over 150 min and 120 min protection, respectively.

Conclusions: Crude extracts of *D. elata* exhibit the potential for controlling *Cx. quinquefasciatus*, the mosquito vector of filariasis.

KEYWORDS Repellency, *Delonix elata*, Leaf and seed, *Culex quinquefasciatus*, Filariasis

1. Introduction

Mosquitoes are primary vectors for many dreadful and fatal diseases such as dengue, malaria, yellow fever and filariasis. They transmit diseases to more than 700 million people each year. Insect-transmitted disease remains a

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major source of illness and death worldwide. Diseases that are health care associated transmission of viruses to humans by mosquitoes are an expanding problem in tropical and subtropical regions. Some of them such as filariasis, dengue, malaria and West Nile virus are amongst the most prevalent diseases in the world^[1]. Lymphatic filariasis is a

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Adulticidal properties of synthesized silver nanoparticles using leaf extracts of *Feronia elephantum* (Rutaceae) against filariasis, malaria, and dengue vector mosquitoes

Kaliyan Veerakumar & Marimuthu Govindarajan 🖂

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Abstract

Mosquito-borne diseases with an economic impact create loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates. Mosquito control is facing a threat because of the emergence of resistance to synthetic insecticides. Extracts from plants may be alternative sources of mosquito control agents because they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use to control mosquitoes. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, in the present study, the adulticidal activity of silver nanoparticles (AgNPs) synthesized using Feronia elephantum plant leaf extract against adults of Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus was determined. The range of concentrations of synthesized AgNPs (8, 16, 24, 32, and 40 μ g mL⁻¹) and aqueous leaf extract (40, 80, 120, 160, and 200 μ g mL⁻¹) were tested against the adults of A. stephensi, A. aegypti, and C. quinquefasciatus. Adults were exposed to varying concentrations of aqueous crude extract and synthesized AgNPs for 24 h. Considerable mortality was evident after the treatment of *F. elephantum* for all three important vector mosquitoes. The synthesized AgNPs from F. elephantum were highly toxic than crude leaf aqueous extract to three important vector mosquito species. The results were recorded from UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy analysis (EDX), and transmission electron microscopy (TEM). Synthesized AgNPs against the vector mosquitoes A. stephensi, A. aegypti, and C. quinquefasciatus had the following lethal dose (LD)₅₀ and LD₉₀ values: A. stephensi had LD₅₀ and LD₉₀ values of 18.041 and 32.575 μ g mL⁻¹; A. aegypti had LD₅₀ and LD₉₀ values of 20.399 and 37.534 μ g mL⁻¹; and C. quinquefasciatus had LD₅₀ and LD_{90} values of 21.798 and 39.596 µg mL⁻¹. No mortality was observed in the control. These results suggest that the leaf aqueous extracts of F. elephantum and green synthesis of AgNPs have the potential to be used as an ideal eco-friendly approach for the control of the A. stephensi, A. aegypti, and C. quinquefasciatus. This is the first report on the adulticidal activity of the plant extracts and AgNPs.

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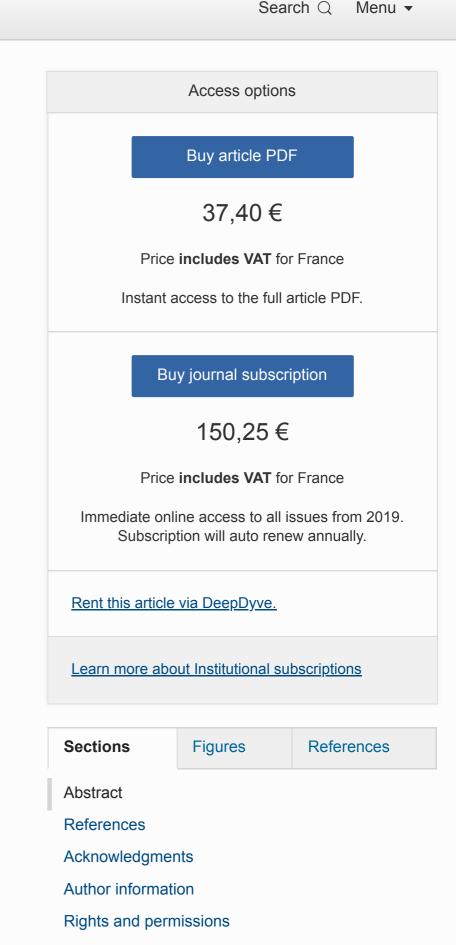
Evaluation of plant-mediated synthesized silver nanoparticles against vector mosquitoes

Kaliyan Veerakumar, Marimuthu Govindarajan 🖂 & S. L. Hoti

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Abstract

Diseases transmitted by blood-feeding mosquitoes, such as dengue fever, dengue hemorrhagic fever, Japanese encephalitis, malaria, and filariasis, are increasing in prevalence, particularly in tropical and subtropical zones. To control mosquitoes and mosquito-borne diseases, which have worldwide health and economic impacts, synthetic insecticide-based interventions are still necessary, particularly in situations of epidemic outbreak and sudden increases of adult mosquitoes. Green nanoparticle synthesis has been achieved using environmentally acceptable plant extract and eco-friendly reducing and capping agents. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, in the present study, the adulticidal activity of silver nanoparticles (AgNPs) synthesized using Heliotropium indicum plant leaf extract against adults of Anopheles stephensi, Aedes aegypti, and *Culex quinquefasciatus* was determined. Adult mosquitoes were exposed to varying concentrations of aqueous extract of *H. indicum* and synthesized AgNPs for 24 h. AgNPs were rapidly synthesized using the leaf extract of *H. indicum*, and the formation of nanoparticles was observed within 6 h. The results recorded from UV-vis spectrum, Fourier transform infrared, X-ray diffraction, scanning electron microscopy, and transmission electron microscopy support the biosynthesis and characterization of AgNPs. The maximum efficacy was observed in synthesized AgNPs against the adult of A. stephensi (lethal dose $(LD)_{50}$ = $26.712 \ \mu g/mL; LD_{90} = 49.061 \ \mu g/mL), A. aegypti (LD_{50} = 29.626 \ \mu g/mL; LD_{90} =$ 54.269 μ g/mL), and *C. quinquefasciatus* (LD₅₀ = 32.077 μ g/mL; LD₉₀ = 58.426 μ g/mL), respectively. No mortality was observed in the control. These results suggest that the leaf aqueous extracts of *H.indicum* and green synthesis of AgNPs have the potential to be used as



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an ideal eco-friendly approach for the control of the A. stephensi, A. aegypti, and C. quinquefasciatus. This is the first report on the adulticidal activity of the plant extracts and AgNPs.

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