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Coleus aromaticus leaf extract fractions: A source of novel ovicides, larvicides and repellents against *Anopheles*, *Aedes* and *Culex* mosquito vectors?

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ABSTRACT

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

1. Introduction

Mosquitoes are important vectors of deadly pathogens and parasites (Benelli, 2015; Benelli and Mehlhorn, 2016; Murugan et al., 2015a,b; Benelli et al., 2016). About 40% of the world's population is at risk from mosquito-borne diseases. In 2015, 2.35 million cases of dengue were reported in the Americas, of which 10 200 cases were identified as severe dengue causing 1181 deaths. The year 2015 was characterized by abundant global dengue outbreak, then Philippines reporting more than 169 000 cases and Malaysia exceeding 111 000 doubtful

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cases of dengue, defining a 59.5% and 16% increase in case numbers to the previous year, respectively. Brazil separately documented over 1.5 million cases in 2015, approximately 3 times higher than in 2014. Also in 2015, Delhi in India, recorded its worst outbreak by reason of 2006 with over 15 000 cases (WHO, 2014a,b,c). In the last few years, dengue has re-appeared in the United States of America and has made encroachment into Europe (Alves et al., 2013). In India, dengue is widespread and endemic in most major cities (NVBDCP, 2013; Govindarajan and Sivakumar, 2014a,b). *Ae. aegypti* and *Ae. albopictus* are two primary mosquito vectors for dengue and yellow fever in Taiwan, where the quantity of dengue fever cases has expanded significantly recently (WHO, 2008, 2010; Benelli, 2015).

Anopheles stephensi is the most important vector of malaria fever in the urban districts of India and other West Asian countries. Malaria remains one of the most widespread disease in the tropical world (Geng et al., 2009; Sharma et al., 2009; Jayaprasad et al., 2015). Malaria afflicts 36% of the world people i.e. 2020 million in 107 countries and territories situated in the tropical and subtropical regions (Panneerselvam et al., 2013). However, malaria death rates among children in Africa have been decreased by an expected 58% since 2000 (WHO, 2014a,b,c). Malaria slaughtered an expected 306 000 under-fives comprehensively, incorporating 292 000 kids in the African Region (WHO, 2015). *Culex quinquefasciatus* is an important vector of filariasis, West Nile virus, avian malaria and St. Louis encephalitis (Samba Shiva et al., 2015). Worldwide, 25 million people showed sex organ sickness and over 15 million are afflicted with lymphedema (Ghosh et al., 2013; WHO, 2014a,b,c).

The use of synthetic chemicals for management of mosquitoes raises many issues connected to the environment and human health (Benelli, 2015). Eco-friendly tools to manage mosquito young instar populations in an IPM framework are urgently required. Natural products are usually most well-liked because of their less harmful nature and fast biodegradability (Bilal et al., 2012; Shaalan, 2012). Plant products have been utilized traditionally by human communities in different rural areas worldwide against insect vectors and parasites (Kovendan et al., 2014; Govindaraju et al., 2015; Pavela and Benelli, 2016).

Coleus aromaticus, a species native to Middle East and Indian areas (Rashmi Sahay et al., 2011), is a plant of multipurpose effectiveness against a wide range of diseases in Asian enthnomedicine (Wong, 2007) It also shows antimicrobial (Murthy et al., 2009), antileishmanial and antioxidant activities (Gurgel et al., 2009; Buznego and Perez-Saad, 1999). The leaves are used for treatment of throat infection, cough and nasal congestion. From a phytochemical point of view, the chemical composition of *C. aromaticus* has been investigated by several studies (Shivaji and Kumudini, 2014; Rout et al., 2012; Himesh and Akhlesh Kumar, 2012). Later on, Govindarajan et al. (2013) showed that the essential oil from this plant and its pure isolated constituent thymol was toxic to larvae of *Culex tritaeniorhynchus*, *Aedes albopictus*, and *Anopheles subpictus*.

In this research, we analyzed six ethyl acetate and methanol fractions of *C. aromaticus* leaf extract using thin layer chromatography and GC–MS in order to shed light on the main chemical constituents with mosquitocidal activity. The fractions were tested as ovicides, larvicides and repellents against the mosquito vectors *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*.

2. Materials and methods

2.1. Plant material

Fully developed leaves of *C. aromaticus* were collected from Pagasalai village (11°10′32.0″N and 79°43′16.6″E), Nagapattinam District, Tamil Nadu, India. The samples were authenticated at the Department of Botany of Annamalai University. Voucher specimens were stored at the herbarium of Plant Phytochemistry division, Department of Zoology, Annamalai University, Tamil Nadu, India.

2.2. Extraction

The leaves were washed carefully with water to remove impurities. Then, the leaves were shade-dried under room temperature and kept in a hot air oven at 50 °C for half an hour. After that, the material was ground by using electric blender. 500 g of powdered plant material was packed inside a Soxhlet apparatus, and successive extraction was carried out using as solvents methanol, ethyl acetate, acetone and benzene for 72 h. The solvents were evaporated under vacuum in a rotary evaporator (Heidolph, Germany), and the dried extracts were stored at 4 °C until further bioassay.

2.3. Phytochemical screening

Following the methods by Kokate 1994 and Sathish Kumar et al. (2013), we screened the bioactive chemical constituents detecting the presence of secondary metabolites such as alkaloids, flavonoids, saponins, steroids, tannins, terpenoids, tri-terpenoids, anthraquinones, amino acid, phenol, glycosides, carbohydrate, protein and phytosteroids in the different extract fractions of *C. aromaticus*.

2.4. Gas chromatography–mass spectrometry analysis

Gas chromatography–mass spectroscopy (GC–MS) was performed using a mass detector Turbo mass gold-Perkin Elmer particular identifier and a Elite-5MS (5% diphenyl/95% dimethy poly siloxane) slender segment. The stove temperature was customized from 50 to 280 °C at the rate of 5 °C min^{-1} and stopped at this temperature for 36 min. The delta and interface temperatures were 250 and 280 °C, respectively. The transporter gas was helium at a stream rate of 1.0 ml min^{-1} (consistent stream). The sample (2 μl) was injected at a split of 10:1. Electron sway mass spectrometry was conveyed at 70 eV. Particle source and fourfold temperature were kept up at 250 and 200 °C separately (Kumaravel et al., 2010).

2.5. Larvicidal activity

The larvicidal activity of *C. aromaticus* extract fractions were assessed according to the method by WHO (2005). All doses ranged from 10 to 50 ppm and were tested on early third instars of the targeted mosquitoes. The plants products were dissolved in 1 ml dimethyl sulfoxide (DMSO) and diluted in 249 ml of dechlorinated water. Control was 1 ml of DMSO in 249 ml of dechlorinated water. Per each tested species, 25 individuals per replicate were stored in 249 ml of dechlorinated water and 1 ml of DMSO plus the required dose of mosquitocidal. Larval mortality was recorded after 24 h. For each dose, 5 replicates were carried out. Percent mortality was rectified for control mortality utilizing the formula by Abbott (1925).

2.6. Ovicidal activity

To evaluate the ovicidal potential of the leaf extract and AgNPs, the method of Su and Mulla (1998) was followed. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a photomicroscope (Leica, Germany). Each experiment was replicated 5 times (n = 100 per replicate), along with controls. The hatch rates were assessed 48 h post-treatment.