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Target and non-target toxicity of fern extracts against mosquito vectors and beneficial aquatic organisms

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ABSTRACT

Dengue and malaria are significant mosquito-borne diseases that are rapidly spread worldwide, mainly in temperate countries. Pteridophytes were identified to be a significant source of novel mosquitocidal agents. The present research was to explore the eco-friendly larvicides from methanol extracts of ferns, *viz., Actiniopteris radiata*, *Adiantum caudatum*, *Cheilanthes swartzii*, *Hemionitis arifolia* and *Lycopodium clavatum*. The larvicidal potential of the extracts screened using larvae of dengue vector *Aedes aegypti* (III and IV instar) and malarial vector *Anopheles stephensi* (III and IV instar), showed 10–100% mortality rates. Biosafety assessment was made on embryos of *Danio rerio* and *Artemia nauplii*. The phyto-constituents of the methanol extract of *A. radiata* leaves were identified through gas chromatography-mass spectrometry (GC-MS). Methanolic leaf extracts of *A. radiata*, *A. caudatum* and *C. swartzii* exhibited larvicidal activity against III and IV instar larvae of *Ae. aegypti* (LC_{50}: 37.47, 74.51 and 152.38 and 67.58, 95.89 and 271.46 ppm) and *An. stephensi* (LC_{50}: 70.35, 112.12 and 301.05 and 113.83, 175.30 and 315.19 ppm), respectively. The GC-MS of the methanol extract of *A. radiata* leaves revealed the presence of 7 phyto-components among which, Carbamic acid, phenyl-, (2-Nitrophenyl) methyl ester (**1**), Benzoic acid, 3- methylbenzoate (**2**) and 4-(benzylimino) − 1,4-dihydro-1-(p-toluoylmethyl) pyridine (**3**) were dominant. Biosafety assessment of methanol extract of *A. radiata* leaves on embryos of *Danio rerio* (Zebra fish) and *Artemia nauplii* (micro crustacean) revealed that there were no destructive or teratogenic effects. To conclude, the larvicidal activity and insignificant toxicity to non-target aquatic organisms of *A. radiata* leaves makes it a potential and environment safe biocontrol agent against dengue and malarial vectors.

1. Introduction

Mosquitoes constitute a major group of vectors that concerns community health issues, transmitting numerous life-threatening diseases, such as dengue, malaria, yellow fever, chikungunya, filariasis, encephalitis, Zika virus and West Nile virus infections (Benelli and Romano, 2017b). The female *Aedes aegypti*, is a major devastating vector inhabiting the waste dump sites that feeds exclusively on humans (Kraemer et al., 2015), thereby spreading chickungunya, yellow fever, dengue and Zika virus (Benelli and Mehlhorn, 2016; Ribeiro and Kitron, 2016; Benelli and Mehlhorn, 2016; Ribeiro and Kitron, 2016). Malaria, caused by the parasitic protozoan, *Plasmodium,* transmitted by the bites of female *Anopheles* mosquitoes that is present throughout India and other tropical countries (Chatterjee and Chandra, 2000), thereby resulting in epidemics (WHO, 2017). Deaths caused by malaria stretched to four hundred thousand in 2016 with an estimated 216 million cases, with the African Region accounting for an inconsistent prevalence of the disease with 91% deaths as reported by WHO (2017).

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Of the 30% of global population affected by malaria in the South East Asian region, 70% malarial cases are reported from India alone (Das and Sarma, 2017; White, 2015). Malaria, as well other mosquito-borne diseases, has a major impact on the economy of developing countries, through direct medical costs and indirect costs such as loss of productivity and tourism (WHO, 2016; Benelli and Beier, 2017a).

Vector control is the main strategy to prevent the transmission of mosquito. This can be achieved by creating public awareness regarding vector control measures which is being practiced worldwide, especially in temperate countries. During the past decades, the chemical pesticides are in practice for their rapid response in reducing the insect pest population. However, the continuous usage of man-made chemicals has produced various harmful effects *viz.*, environmental pollution, decreasing the natural biodiversity, adversative effects on non-target organisms, induced pest resistance as well as causing several health complications to humans (Chengala, 2017). To overcome these difficulties, scaling up of alternative pesticides that do not produce harmful effects to the non-targeted species that are cheaper and easily degradable is required (Ansari et al., 2000). Therefore, bio-pesticides that are either microbial (entomopathogenic viruses, bacteria, fungi and nematodes) or plant based (plant secondary metabolites, essential oils and phytochemicals) are acquiring cumulative reputation as they are replacements to synthetic pesticides as they are environment safe, cost effective in addition to their easily biodegradable nature (Kamaraj et al., 2008a, 2008b; Rawani et al., 2012; Bhattacharya and Chandra, 2014; Senthil-Nathan, 2015). Besides, biopesticides are key components of many pest control programs (Senthil-Nathan, 2015). Phytochemicals such as flavonoids, alkaloid compounds like anabasine and lupinine, rotenone and pyrethrums (Zubairi et al., 2004; Benelli, 2016), steroids, terpenoids and tannins were stated by Shaalan et al. (2005) and Chellappandian et al. (2017) to exhibit insecticidal properties.

Secondary metabolites from plants were also proved as effective insecticidal agents by affecting their physiology and biochemistry (Senthil-Nathan, 2013; Kamaraj et al., 2017a). Indigenous plants such as *Eucalyptus tereticornis* Sm. (Senthil-Nathan, 2007) and Neem were reported with larvicidal potential against *An. stephensi* Liston and Lepidopteran insects (Senthil-Nathan, 2013). Development of insecticidal resistance in pests can be minimised by use of natural pesticides. Azadirachtin, from neem has been found to hinder acetylcholinesterase enzyme, thereby preventing insect resistance towards pesticides (Senthil-Nathan et al., 2008a). As a vital part of integrated pest management, combined action of botanical as well as microbial pesticide was proved to be very effective against larvae of the rice leaffolder, *Cnaphalocrocis medinalis* (Senthil-Nathan et al., 2004).

Kamaraj et al. (2010a, 2010b, 2011a, 2011b) have also examined the underlying principle behind mosquito control using several therapeutically important plants and their active derivatives. Also, their positive impacts with their minimal toxicity towards non-targeted organisms as well as environment was also tested.

Of late, environmentally benign and innovative approaches from plant-based source have been recognized to reduce the mosquito populations (Pavela and Benelli, 2016). The plant extracts encompass different phytocompounds whereas certain compound demonstrated numerous mechanisms leading to mosquito death. Limonoids of neem (Senthil-Nathan et al., 2005), triterpenes of *Dysoxylum malabaricum* and *D. beddomei* (Senthil-Nathan et al. 2006a, 2008b) as well as extracts of *Melia azedarach* L. (Senthil-Nathan et al., 2006b) showed larvicidal as well as growth inhibitory activities against mosquito vector *An. stephensi*.

The pteridophytes (Fern) were thought to be rather unusable members of the plant kingdom and their beneficial parts have gone mostly unnoticed. Conversely, some pteridophytes are used for traditional medicine, food, fibre, crafts and ornamentation *etc.,* (Shrivastava, 2007; Kamaraj et al., 2017b). A small terrestrial herb, *Adiantum caudatum* L. (Adiantaceae) grows in shaded environment in soil cuttings, slopes, and splits of rocks in forest. Shrivastava (2007) have reported that some significant medicinal uses of *Adiantum* species for the treatment of various communicable diseases. The *A. caudatum* possess effective antimicrobial action against *Micrococcus luteus*, *Streptococcus pneumoniae*, *Aspergillus terreus* and *Candida albicans* (Singh et al., 2008). *Actiniopteris radiata* (Sw.) Link. (Actiniopteridaceae), are of pronounced therapeutic importance. *A. radiata* possess numerous activities such as an astringent, anti-inflammatory, antifertility, styptic, anthelmintic, antitubercular, antistress, antiallergic, anticholinergic, antibacterial and antioxidant (Vadnere et al., 2013). *Cheilanthes swartzii* Webb. et Benth. (Cheilanthaceae) is a tiny, green attractive garden fern (Abraham et al., 2012). *C. farinosa* has been reported to have anti-inflammatory and anti-nociceptive activities (Yonathan et al., 2006).

Lycopodium clavatum L. (club mosses) belongs to Lycopodiaceae family, found on flora and fauna (Ollgaard, 1992). The aerial parts of *L. clavatum* are commonly used as carminatives, laxatives and diuretics. *Hemionitis arifolia* (Burm.) belonging to the family Hemionitidaceae, exist very rarely in South India (Moore, 1859). Nair et al. (2006) studied the antidiabetic effect of *H. arifolia* extract in mice system. The crude and alcoholic extracts of *H. arifolia* leaves exhibited antimicrobial activities against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (Karmakar et al., 2011).

The present investigation is focused on toxic effect of fern extracts against dengue and malaria mosquito vectors and thus exploring the potential of lower plants as candidates with anti-mosquitocidal properties. The pteridophytes were selected based on the therapeutic and biological activities, which were stated earlier in the literature. By way of our literature review, there was no evidence on the mosquito larvicidal activities of the five selected fern species from South India. This is the new report on the mosquitocidal activity of selected fern extracts against the III and IV instar larvae of *Ae. aegypti* and *An. stephensi*. In addition, eco-safety assessment of the extracts was done on zebrafish (*Danio rerio*) embryo and *Artemia nauplii*. Furthermore, the major phyto-constituents and identification was done by gas chromatography-mass spectrometry (GC-MS) of effective fern *A. radiata*.

2. Materials and methods

2.1. Collection and extraction of selected pteridophytes

The leaves of *Actiniopteris radiata*, *Adiantum caudatum*, *Cheilanthes swartzii*, *Hemionitis arifolia* and *Lycopodium clavatum* were obtained from Malaiyur Hills, Dharmapuri district (11°53′28″ N, 078°30′ 26″ E, altitude 959 m), Tamil Nadu, South India, in May 2017. The collected pteridophytes taxonomic identifications were made by Dr. A. Akbar, Head of the Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore, India. The dried fern leaf samples of each species (100 g) were individually extracted by cold percolation in methanol (250 mL) (successively three times) at room temperature for 48 h then subsequently the extracts were filtered and concentrated to dryness under reduced pressure below 40 °C in rotary evaporator. The achieved crude extracts were lyophilized for further study. The (%) yields of 10.72%, 6.80%, 9.42%, 8.36% and 9.84% were attained from methanol extract of *A. radiata*, *A. caudatum*, *C. swartzii*, *H. arifolia* and L. *clavatum*, respectively.

2.2. Mosquito larvae culture

The larvae of dengue and malaria vectors *Ae. aegypti* and *An. stephensi* were obtained from the Centre for Research in Medical Entomology (CRME), Madurai, Tamil Nadu (India). Both larvae were kept in separate plastic trays having deionized water and maintained in our research laboratory. The larvae were fed with the combination of dog nutrition and yeast solution, before being used in the experiment and the III and IV instar larvae were used for all the experiments (Kamaraj et al., 2009). The larvicidal activity was carried out in the lab at ~28 ± 2 °C, 70–80% relative humidity (RH) and photoperiod of