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Bioefficacy of essential oil from *Polygonum hydropiper* L. against mosquitoes, *Anopheles stephensi* and *Culex quinquefasciatus*

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ABSTRACT

The biological activity of essential oil extracted from the leaves of *Polygonum hydropiper* and a compound, confertifolin, isolated from this plant was bioassayed against larva of mosquitoes, *Anopheles stephensi* and *Culex quinquefasciatus*. The essential oil showed the LC_{50} values of 189 and 243 ppm; 217 and 242 ppm, confertifolin (6,6,9a-trimethyl-4,5,5a,6,7,8,9,9a-octahydronaphtho[1,2-*c*]furan-3(1*H*)-one) showed the LC_{50} values of 2.40 and 3.09 ppm; 4.07 and 4.18 ppm against the second and fourth instar larvae of *An. stephensi* and *Cx. quinquefasciatus*, respectively. At 10 ppm confertifolin showed ovicidal activity of 100, 98.6 and 86.4% against *An. stephensi* and 100, 100 and 75.2% against *Cx. quinquefasciatus* on 0–6, 6–12 and 12–18 h old eggs; the repellent activity persisted for 314.6 and 319.0 min; oviposition deterrent activity was 97.2 and 99% and adulticidal activity was 100 and 100% against *An. stephensi* and *Cx. quinquefasciatus*, respectively. The results were statistically significant. Confertifolin could be considered for use in the control of human vector mosquitoes.

1. Introduction

Mosquitoes are one of the haematophagous insects in the group of arthropods and they transmit parasites and pathogens which continue to have devastating impact on human beings; they also act as major vectors for many diseases such as malaria, filariasis, yellow fever, dengue, Japanese encephalitis and other fevers (Service, 1983). Among these diseases lymphatic filariasis infects 80 million people annually of which 30 million cases exist in chronic infection by *Culex quinquefasciatus* Say. *Cx. quinquefasciatus* is a worldwide vector of bancroftian filariasis in the tropical and subtropical countries. Filariasis is caused by *Wucheria bancrofti* (Cobbald), a helminth that lives in the lymph glands and vessels that provoke edemas by lymph obstruction. In India alone 25 million people harbor microfilaria (mf) and nineteen million people suffer from filarial disease manifestations (NICD, 1990). *Anopheles stephensi* is one of the major vectors of malaria in India as well as in some of the West Asian countries and has been shown to be directly responsible for about 40–50% of the annual malarial incidence. Malaria alone kills 3 million each year, including 1 child every 30 s. Although mosquito borne diseases currently represent a greater health problem in tropical and subtropical climates, no part of the world is immune to this risk (Collins and Paskewitz, 1995). According to a global estimate from The World Health Organization (WHO), 216 million people who were infected with malaria parasites in 2010 (of which approximately 81%, or 174 million cases, were reported from Sub-Saharan African) resulted in 6,55,000 malaria deaths (about 91% were in Africa) (WHO, 2011).

Global use of chemical insecticides viz., organophosphates such as temephos and fenthion and insect growth regulators such as diflubenzuron and methoprene is generally used for the control of mosquito larvae. Synthetic pesticides have created a number of ecological problems such as the development of resistant insect strains, ecological imbalance and harm to mammals. In recent years, mosquito control programs have been suffering from failures because of the ever-increasing insecticide resistance (World Health Organization, 1992). Recent studies have also stimulated the investigation of insecticidal properties of chemicals derived from plant material and many medically important plant extracts have been studied for their efficacy to kill the larvae of different species of mosquito (Maheswaran et al., 2009).

The use of botanical derivatives in mosquito control as an alternative to synthetic insecticides offers a more environment friendly method of insect control than the use of synthetic chemicals. A large number of plant essential oils may be potential sources of mosquito larvicides, since they constitute a rich source of bioactive components. Essential oils extracted from neem (Batra et al., 1998) and *Tagetes patula* (Dharmagadda et al., 2005) killed the larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*.

Polygonum hydropiper L. (Polygonaceae) is an erect herb; it is known as kalatadi, galpudi, gahurunia and gotkinamaru in different

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tribal areas. It is a weed generally occurring in muddy or swampy places of canal sides and riverbanks in eastern India (Jena, 2000). Juice of leaves is used to treat headache, toothache, liver enlargement, gastric ulcers, dysentery, loss of appetite and dysmenorrheal; roots are used as a stimulant; juice is applied to wounds, skin diseases, and painful carbuncles (Ghani, 1998); insecticidal activity (Kundu et al., 2007) has also been reported. The objective of this study was to investigate the mosquitocidal effect of essential oil and confertifolin isolated from the leaves of *P. hydropiper* against *An. stephensi* and *Cx. quinquefasciatus.*

2. Materials and methods

2.1. Plant material

The matured leaves of *P. hydropiper* were collected from Jarkand district, India, in 2005. The plant specimen was identified by Dr. Narasimhan, Department of Botany, Madras Christian College, Chennai, India. The voucher specimen (ERIH-75) was deposited at the herbarium of Entomology Research Institute, Loyola College, Chennai, India, for future reference.

2.2. Isolation of essential oil

Thirty kilogram of fresh leaves were macerated and steam distilled using a clavenger apparatus with condenser. Distillation continued for 3–5 h at 100 °C, and the volatile compounds containing the water-soluble fractions were allowed to settle for 30 min. The essential oil layer was separated and purified through microfiltering. Finally 180 ml of essential oil was obtained and stored at 4 °C for further analysis.

2.3. Gas chromatography–mass spectroscopy (GC–MS) analysis of essential oil

The GC–MS analysis of the essential oil was carried out on a Shimadzu OP-2010 instrument. Column length, 30.0 m; i.d., 0.25 mm; coated material, polysilane; injection temperature, 180 °C; carrier gas, helium; flow rate, 1.4/min. MS condition: ion source temperature, 200 °C; interface temperature, 240 °C start *m/z* 40, end 650.

2.4. Isolation of the active constituent

The essential oil of *P. hydropiper* was chromatographed over silica gel Acme's (100–200 mesh) in hexane and eluted with hexane and ethyl acetate solvents and their mixtures (100% hexane, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 100% ethyl acetate) and 117 fractions (each 50 ml) were collected. Finally sixteen fractions were obtained based on similar TLC pattern. Fraction 8 showed a crystal.

2.5. Identification of active compound

The crystal was subjected to crystallographic analysis. The X-ray data for crystal were recorded using Bruker-AX, X-ray diffractometer in Indian Institute of Technology, Chennai, India. A needle shaped crystal was cut to a size of $0.3 \times 0.2 \times 0.4$ mm. It was tested for single crystalline using Leica-LSP polarizing microscope. The single crystal was mounted on a BRUKER AXS kappa apex diffractometer. The structure was solved through the divert methods using the programme SIR 92 (WINGX) least squares refinement with anisotropic thermal parameter.

2.6. Microscope

Motic Digital Microscope attached with computer (DMWB Series, B1), Pal System, Japan and Nikon Digital Microscope (Optiphot 2) (40× magnification), Japan, were used for ultra structural studies of treated larvae.

2.7. Mosquito culture

Larvae of *An. stephensi* and *Cx. quinquefasciatus* were derived from various places with stagnant water bodies within Chennai, India. They were colonized and eighteen generations were maintained at 27 ± 2 °C, 75–85% RH under a photoperiod of 14:10 h (light/dark) continuously in the laboratory free of exposure to pathogens, insecticides or repellents. Under these conditions, full development from egg to adult lasted for about 3–4 weeks. Larvae were fed on finely ground dog biscuit and yeast extract in the ratio of 3:1. Water was changed every day to avoid scum formation, which might create toxicity. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages ($30 \times 30 \times 30$ cm dimension) for adult emergence. The adults were reared in respective glass cages ($30 \times 30 \times 30$ cm dimension). The adult colony was provided with ten percent sucrose solution and it was periodically blood-fed on restrained rats. After three days, ovitrap was kept in the cages for egg laying and the eggs were collected and transferred to enamel trays. Two developmental stages, larvae and adult females, were continuously available for the experiments. They were maintained at the same condition.

2.8. Larvicidal activity

Bioassays were performed using World Health Organization (1996) with second and fourth instar larvae of *An. stephensi* and *Cx. quinquefasciatus. P. hydropiper* leaf essential oil was tested at 100, 50, 25, 12 and 6.25 ppm and the confertifolin was tested at 10, 5, 2.5, 1.25 and 0.625 ppm concentrations respectively. A minimum of twenty larvae per concentration was used for all the experiments with 99 ml dechlorinated tap water and 1 ml of the desired concentration of test solution. Essential oil was dissolved in water with emulsifier (0.1% Tween 80). Tween 80 was used as a negative control. The experiment was replicated five times. Mortality and survival rate were recorded after 24 h of the exposure period. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within a reasonable period of time) or showing the characteristic diving reaction when the water was disturbed. Larvae were also observed for discoloration, unnatural positions, uncoordination, or rigor. Considering the mortality of the larvae at the experimental concentrations the LC_{50} was calculated using a Probit analysis programme developed by SPSS 11.5 version.

2.9. Ovicidal activity

Ovicidal activity was evaluated by following the method of Su and Mulla, (1998) with slight modifications. Ten freshly laid (0–6, 6–12 and 12–18 h old) eggs of *An. stephensi* and *Cx. quinquefasciatus* were treated with confertifolin at 10, 0.5, 2.5, 1.25 and 0.625 ppm concentrations. Each treatment was replicated five times. Water was used as a negative control. Temephos was used as a positive control. Ovicidal activity was observed under the microscope. The ovicidal activity was assessed up to 120 h post treatment and the results were calculated and analyzed with Duncan's multiple range test (DMRT) using software of SPSS 11.5 version. The following formula was used:

$$\text{Ovicidal activity} = \frac{\text{number of unhatched eggs}}{\text{total number of eggs in treated water}} \times 100$$

2.10. Repellent activity

The protection time (minutes) in relation to dose was used to assess repellent activity (World Health Organization, 1996). Three-day-old blood-starved female *An. stephensi* and *Cx. quinquefasciatus* mosquitoes (100) were kept in a net cage ($45 \times 30 \times 45$ cm). The arms of the test person were cleaned with unscented soap and distilled water. After air drying the arm, only 25 cm^2 of the dorsal side of the skin in each arm was exposed simultaneously; the remaining parts of the arms were covered with rubber gloves. Confertifolin dissolved in ethanol at 10, 0.5, 2.5, 1.25 and 0.625 ppm concentrations was applied on one arm and 1 ml of ethanol was applied on the other arm as a control. The experiment was replicated five times. Skin irritation of treated arm was also observed. All the experiments were conducted at a temperature of 27 ± 2 °C and relative humidity of 80 ± 2%. The maximum protection time was statistically analyzed by Tukey's test of multiple comparison using software of SPSS 11.5 version.

2.11. Oviposition deterrent activity

The oviposition deterrent activity was assessed using the method of Rajkumar and Jebanesan (2004a) with slight modifications. Ten gravid females of *An. stephensi* and *Cx. quinquefasciatus* (10 days old, 4 days after blood feeding) were transferred to each mosquito cage ($45 \times 45 \times 45$ cm) covered with a plastic screen, with a glass top and a muslin sleeve to access separately. A ten percent sucrose solution was available at all times. Serial dilutions of confertifolin were tested at 10, 5, 2.5, 1.25 and 0.625 ppm; temephos (1 ppm) was also tested as the positive control. Two enamel bowls holding 100 ml of tap water for *Cx. quinquefasciatus* and 100 ml of rainwater for *An. stephensi* were placed in opposite corners of each cage; one bowl was treated with the test material and the other bowl was without treatment. Four replicates were run for each treatment, with cages placed side by side for each bioassay. All experiments were at ambient temperature (27 ± 2 °C) with relative humidity of 75–85%. After 24 h, the number of eggs laid in treated and control bowls was recorded.