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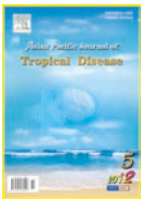

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Evaluation of the larvicidal efficiency of stem, roots and leaves of the weed, *Parthenium hysterophorus* (Family: Asteraceae) against *Aedes aegypti* L.

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ABSTRACT

Objective: To assess the larvicidal potential of various extracts prepared from the stem, roots and leaves of *Parthenium hysterophorus* (*P. hysterophorus*) against 3rd and 4th instars of *Aedes aegypti* (*Ae. aegypti*). **Methods:** The extracts from each part were prepared with four solvents; petroleum ether, hexane, acetone and diethyl ether. Each part was dried, powdered and soaked in different solvents, separately, for five days. The crude extracts thus formed were concentrated using rotary evaporator and stored as stock solution of 1 000 mg/L. **Results:** All the extracts prepared from the leaves were found ineffective against both the instars causing only 10%-40% mortality. Against 3rd instars, the hexane and petroleum ether extracts prepared from the stem of *P. hysterophorus* were found effective exhibiting LC_{50} values of 379.76 and 438.57 mg/L, respectively. Likewise the hexane and petroleum ether extracts from the *Parthenium* roots resulted in LC_{50} values of 432.38 and 562.50 mg/L, respectively, against 4th instars of *Ae. aegypti* revealing their larvicidal potential. It was further found that the hexane extracts, whether from roots or stem, were 13-28% more effective than the petroleum ether extracts. The qualitative phytochemical study of the effective extracts from the stems and roots showed the presence of alkaloids, saponins, terpenoids and flavonoids in different combinations. **Conclusions:** Our investigations demonstrated the potential of *P. hysterophorus* roots and stems against *Ae. aegypti* larvae and their benefits as new types of mosquito larvicides. Variety of types and levels of active constituents in each kind of extract may be responsible for the variability in their potential against *Ae. aegypti*. Further research is needed to identify these components.

1. Introduction

The mosquito-borne diseases, dengue fever, malaria, encephalitis, yellow fever, chikungunya, filariasis, are causing havoc in many countries, and loss in terms of human lives is irreversible[1]. *Aedes aegypti (Ae. aegypti)*, the primary vector for dengue fever, dengue haemorrhagic fever and yellow fever is widespread over large areas of the tropics and subtropics; and is reported to infect more than 100 million people every year in more than 110 countries in the tropics[2]. According to WHO[3] about two-fifths of the world's population are now at risk of dengue and the only way to prevent dengue virus transmission is to combat the disease-carrying mosquitoes. In the Indian subcontinent, cases of dengue fever are on the rise and, therefore, the control of dengue vector needs immediate attention. In 2010, a total of 28 292 cases and 110 deaths were reported because of dengue in India[4]. Over the last five decades the indiscriminate and frequent use of synthetic insecticides in agriculture and public health programs has caused multifarious problems *viz*. insecticide resistance, environmental pollution, destabilization of the ecosystem and toxic hazards to human and non-target organisms[5,6].

These problems have necessitated the need for search and development of alternative strategies using eco-friendly, environmentally-safe, biodegradable and low cost natural products as mosquito larvicides. In recent years much effort has been focused on plant extracts or phytochemicals as potential sources of mosquito control agents and as a viable component of Integrated Pest Management[7].

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The phytochemicals can also play a significant role as companions to the synthetic insecticide arsenal. Some indigenous plant-based products have been found very promising as insecticides against mosquitoes, though very few plant products have shown practical utility for mosquito control. A survey of literature indicates that most of the studies on larvicidal effects of plant products on mosquitoes included well known horticultural and commonly grown plants. A number of reports establish the mosquito larvicidal potential of the plant extracts and the essential oils obtained from the different parts of the variety of plants[8,9,10,11,12], though the insecticidal effects of plant chemicals vary not only according to plant species, mosquito species and plant parts, but also to extraction methodology. On the other hand, the larvicidal activity of weed plants that is found in vast areas on plains as well as on hilly regions is not attempted so far[13].

Parthenium hysterophorus (*P. hysterophorus*) is a common and easily available weed which is also known as congress weed, carrot weed, star weed, feverweed, white top, chatak chandani, bitter weed, ramphool or gajarghas. It is a poisonous, pernicious and aggressive weed and is reported to have pharmacological properties against rheumatism, hepatic amoebiasis, tumours, *etc*. and has also been reported to possess muscle relaxant and hypoglycemic[14,15]. There has also been an epidemic of hundreds of cases of *Parthenium* weed dermatitis in India[16]. Most of the research work on *Parthenium* is carried out to control and eliminate this weed because of its deleterious properties. Limited work has been carried out, however, to assess its potential to control mosquito population by affecting their biological characteristics. Keeping in view the harmful effects and unmanageability of *Parthenium*, the beneficial aspects of the different parts of *P. hysterophorus* were explored in terms of the larvicidal potential against an Indian strain of *Ae. aegypti*. The assessment of larvicidal potential of this weed, besides its management may help in the formulation of effective strategies for reduction of mosquito population.

2. Materials and methods

2.1. Mosquito culture

The present investigations employ the third and the early fourth instar larvae of *Ae. aegypti* originated from field-collected engorged female adults from Delhi. The colony was maintained in an insectary without any insecticide exposure at (28±1) ℃ , 80%±5% RH and 14L: 10D photoperiod[17]. Wet cotton was kept on the top of each cage to provide water for the mosquitoes. Water-soaked split raisins were kept in the cage, mainly as a source of the food for the male mosquitoes. Female mosquitoes were provided with blood meal by keeping a restrained albino rat in the cage for 1-2 h during day time. On the day following blood meal, an ovitrap consisting of an enamel bowl (10 cm diameter) lined with Whatman filter paper strips on all the sides and half-filled with de-chlorinated tap water was kept in the cage for collection of the eggs. The filter paper strips with laid eggs were taken out on every alternate day and kept dipped in water for two days to allow hatching of the larvae. The newly hatched larvae were reared in enamel trays (25 cm×30 cm×5 cm) containing de-chlorinated water. The larvae were provided daily with food consisting of finely ground dog biscuits and yeast in the ratio of 3:2 by weight. Care was taken to prevent formation of any scum on the surface of water. Pupae formed thereafter were transferred to the cage for adult emergence. Blood meal was provided to the females after two days of emergence.

2.2. Plant collection

For the larvicidal bioassays, different parts of the *P. hysterophorus* plant, *i.e.* roots, stem and leaves, were collected from the surrounding areas in New Delhi, India. The collected parts were thoroughly washed with tap water and dried under shade at room temperature of (27±2) ℃ separately for about 20 days to dry them completely. The dried parts were then crushed, powdered and sieved thoroughly to get fine powder.

2.3. Preparation of the extract

The powdered plant parts, *i.e.* roots, stems and leaves, were weighed separately. The 200 g of each powdered material was soaked in 1 000 mL of acetone, hexane, diethyl ether and petroleum ether, separately, resulting in four sets of each part. The soaked materials were left undisturbed for five days. The crude extracts, thus formed, were concentrated using a vacuum evaporator at 45 ℃ under low pressure. After complete evaporation of the solvent the concentrated extracts were collected and stored in a refrigerator at 4 ℃ as the stock solution of 1 000 mg/L for further use. This stock solution was used to prepare the desired concentrations of the extracts for investigating the cidal effects against mosquito.

2.4. Screening of extracts for their larvicidal efficacy against *Ae. aegypti*

The larvicidal bioassay was performed at (28±1) ℃ on the third and early fourth instars of *Ae. aegypti* larvae in accordance with the procedure described by WHO with slight modifications[18]. For experimental treatment, 1 mL of 1 000 mg/L plant extract was added to 99 mL of distilled water in a 250 mL beaker. The mixture was shaken lightly to ensure a homogeneous test solution. The early fourth instar larvae of *Ae. aegypti*, in batches of 25, were taken in plastic bowls containing 99 mL of distilled water and transferred to glass jar containing distilled water-extract mixture. Controls were exposed to the particular solvent alone. Three replicates were carried out simultaneously for each extract. During

the treatment period, the larvae were not provided with any food. The dead and moribund larvae were recorded after 24 h. Similar tests were carried out with each extract with third as well as early fourth instars of *Ae. aegypti* to assess the larval efficiency of *P. hysterophorus*.

2.5. Evaluation of larvicidal potential of selected extracts

The extracts which could not result in 80%-100% larval mortality at 1 000 mg/L were considered ineffective and not tested further for larvicidal efficacy. Other extracts causing 80%-100% larval mortality at 1 000 mg/L were evaluated further for larvicidal potential. The bioassays were performed as described earlier and the larval mortality was recorded after 24 h. Three replicates were carried out for each assay.

2.6. Statistical analysis of data

The tests with more than 20% mortality in controls and pupae formed were discarded and repeated again. If the control mortality ranged between 5%-20%, it was corrected using Abbott's formula[19]. The data were subjected to regression analysis using computerized SPSS 11.5 Programme. The LC_{50} and LC_{90} values with 95% fiducial limits were calculated in each bioassay to measure difference between the test samples. The results obtained with different extracts were analyzed using Student's *t*-test with statistical significance considered for $P \leq 0.05$.

2.7. Phytochemical analysis

All the plants extracts were subjected to phytochemical analysis and the components in each extract were identified using standard procedures as described by Harborne and Harborne[20].

Alkaloids; Mayer's test: A drop of Mayer's reagent was added to 2 mL of each extract along the side of the test tube. The formation of a creamy or white precipitate indicated the positive test for alkaloids.

Carbohydrates; Benedict's test: A mixture of 0.5 mL of Benedict's reagent and 0.5 mL of each extract was prepared separately. Each mixture was heated for 2 min in a boiling water bath. The development of a characteristic red-coloured precipitate indicated the presence of carbohydrates.

Saponins; Foam test: 2 mL of the extract was diluted with distilled water and made up to 20 mL. The suspension thus formed was shaken in a graduated cylinder for about 15 min. The formation of about two centimetre layer of foam indicated the presence of saponins.

Phenolic compounds; Ferric chloride test: 1 mL of the extract was diluted to 5 mL with distilled water to which a few drops of neutral 5% ferric chloride solution were added. Change of the colour of solution to a dark green colour indicated the presence of phenolic compounds.

Tannins; Ferric chloride test: About 0.5 mg of dried and powdered sample was boiled in 20 mL of water in test tubes and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colouration as positive test for tannins.

Flavonoids; Ammonia test: A portion of the aqueous extract, as prepared in the previous test was added to 5 mL of the dilute ammonia solution. Subsequently, a few drops of concentrated sulphuric acid were added to this mixture of aqueous extract and ammonia solution. Appearance of yellow colouration in the solution indicated the presence of flavonoids.

Terpenoids; Salkowski test: Five mL of the extract was mixed with 2 mL of chloroform. Concentrated sulphuric acid was added to the prepared solution along the sides of the tube in order to form a layer. A reddish brown colour at the interface showed the presence of terpenoids.

Phlobatannins; Acid test: Formation of red precipitate on boiling aqueous extract of plant sample with 1% aqueous hydrochloric acid indicated the presence of phlobatannins.

3. Results

3.1. Screening of extracts for their larvicidal efficacy against Ae. aegypti

The results of the larvicidal tests performed against third and fourth instars of *Ae. aegypti* with 1 000 mg/L of different extracts prepared from root, leaf and stem of *P. hysterophorus* are presented in Table 1. The results clearly revealed that all the extracts prepared from the leaves of *P. hysterophorus* were not found significantly effective against both the third and fourth larval instars of *Ae. aegypti* causing only 10%-40% mortality (Table 1).

Table 1.
Larvicidal activities of different extracts prepared from the stems, leaves and roots of *P. hysterophorus against* III and early IV instars of *Ae. aegypti*.

Part of the plant	Solvent	% Mortality		
		Control	III instar	IV instar
Stem	Acetone	0	0	10
	Hexane	0	80	0
	Petroleum ether	0	100	20
	Diethyl ether	0	0	40
Leaves	Acetone	0	20	30
	Hexane	0	10	40
	Petroleum ether	0	20	20
	Diethyl ether	0	10	20
Roots	Acetone	0	10	0
	Hexane	0	0	100
	Petroleum ether	0	40	100
	Diethyl ether	0	20	40

The extracts that resulted in significant mortality of 80%-100% in III instars of *Ae. aegypti* at 1 000 mg/L were found to be hexane and petroleum ether extracts prepared from the stems of *P. hysterophorus*. However, against early IV instars, though the hexane and petroleum ether extracts

Table 2.
Larvicidal activities of extracts prepared from the stems and roots of *P. hysterophorus against* different instars of *Ae. aegypti*.

Part of the plant used	Extract	LC_{50} (mg/L)	95% fiducial limits	LC_{90} (mg/L)	95% fiducial limits	SE	$\chi^2(df)$	Regression coefficient
Stem against III larval instars	Hexane	379.76[a]	253.20–585.04	1314.40c	789.22–4060.29	0.42	2.25 (4)	1.66
	Petroleum ether	438.57[a]	320.16–579.59	870.59d	645.44–1613.38	0.99	1.25 (3)	4.30
Root against IV larval instars	Hexane	432.38[a]	299.62–614.58	1118.50c	749.48–2792.95	0.73	4.39 (3)	3.10
	Petroleum ether	562.50[b]	425.74–741.58	1232.11c	887.85–2777.8	2.58	1.55 (4)	3.76

Figures in the column followed by the same letter are not significantly different at P=0.05.

Table 3.
Comparison of qualitative phytochemical analysis of the root, stem and leaf extracts of *Parthenium* in different solvents for various components.

Tested component	Root				Stem				Leaves			
	PE	Hexane	DE	Acetone	PE	Hexane	DE	Acetone	PE	Hexane	DE	Acetone
Alkaloids	–	–	–	–	+	+	–	–	–	+	–	–
Carbohydrates	–	–	–	–	–	–	–	–	–	–	–	–
Saponins	+	–	–	–	+	–	–	+	+	–	–	+
Phenolic compounds and tannins	–	–	–	–	–	–	–	–	–	–	–	–
Tannins	–	–	–	–	–	–	–	–	–	–	–	–
Flavonoids	–	+	–	+	–	–	–	+	+	–	–	–
Terpenoids	+	+	–	+	–	+	–	–	–	+	–	–
Phlobatamins	–	–	–	–	–	–	–	–	–	–	–	–

DE: Diethyl ether; PE: Petroleum ether.

of *P. hysterophorus* established their larvicidal potential resulting in 100% mortality, but they were obtained from roots. The extracts prepared from stems and roots in diethyl ether and acetone proved to possess insignificant larvicidal potential (10%–40% mortality) in order to be considered for further trials and evaluations (Table 1).

3.2. Evaluation of larvicidal potential of selected extracts

When the larvicidal bioassays were carried out with the potential extracts against *Ae. aegypti*, it was found that the hexane extracts exhibited more larvicidal potential than the petroleum ether extracts, irrespective of whether these were prepared from roots or stems of *P. hysterophorus* (Table 2). Against third instars, the hexane extracts prepared from the stem were 14% more effective than the petroleum ether extracts exhibiting LC_{50} of 379.76 and 438.57 mg/L, respectively. Likewise, the hexane root extracts of *P. hysterophorus* (LC_{50}-432.38 mg/L) were proved to be 23% more effective than petroleum ether extracts with LC_{50} of 562.50 mg/L against early fourth instar larvae of *Ae. aegypti*. It was also observed that though the stem and root extracts were found effectual against different instars, the extracts prepared from the stems of *P. hysterophorus* were more effective larvicides than the extracts prepared from the roots. This confirmed the hexane extract prepared from the stems as the most effective larvicide against *Ae. aegypti* followed by that prepared from the roots (Table 2).

3.3. Phytochemical analysis

The qualitative phytochemical study of each extract prepared from different parts of *P. hysterophorus* revealed that the diethyl ether extracts of all the three parts did not show the presence of any component tested. The acetone extracts had combinations of saponins, terpenoids and flavonoids. The hexane and petroleum ether extracts showed the presence of alkaloids, saponins, terpenoids and flavonoids in different combinations (Table 3).

4. Discussion

The various problems associated with the use of synthetic chemicals and the growing incidence of resistance in the mosquitoes has highlighted the need for the development of new strategies for mosquito control[21]. Bio-pesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans and other advantages[22]. In addition, increasing documentation of negative environmental and health impact of synthetic insecticides and increasingly stringent environmental regulation of pesticides have resulted in renewed interest in the development and use of botanical insect management products for controlling mosquitoes and other insect pests.

During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides. Plants are the chemical factories and rich source of bioactive chemicals, some of which have medicinal and pesticidal properties[23]. The complex mixtures of these compounds can be used to develop environmentally-safe vector and pest-managing agents. The botanical extracts from the plant leaves, roots, seeds, flowers and bark in their crude form have been used as conventional insecticides for centuries. In fact, many researchers have reported the effectiveness of plant extracts or essential oils against mosquito larvae[8,9]. The preliminary

screening of these extracts is a good mean of evaluation of the potential mosquiticidal activity of plants popularly used for this purpose.

P. hysterophorus being an aggressive and toxic weed invading all disturbed land, including farms, pastures, and roadsides has caused havoc in human life. Despite of its pharmacological properties against a few diseases, it is known for causing dermatitis and respiratory malfunction in humans, cattle and domestic animals. Since, no major research work has been carried out to explore the potential of *P. hysterophorus* as an agent of mosquito control, the present investigations were carried out to assess the prospective use of *P. hysterophorus* as the larvicidal agent in mosquito management programs.

The present studies revealed that the leaf extracts of *P. hysterophorus* were not significantly effective against *Ae. aegypti* as they caused only 10%-40% mortality. Our results are contrary to the works published elsewhere according to which the hexane and acetone extracts formed from the leaves of *P. hysterophorus* were effective against *Ae. aegypti* larvae exhibiting LC_{50} values of 47.69 and 72.34 mg/L, respectively. Raj Mohan and Ramaswamy[13]found the leaf extracts of the weed *Aegeratina adenophora* moderately effective against 4th instars of *Ae. aegypti* and *Cx. quinquefasciatus* reporting an LC_{50} value of 256.70 and 227.20 mg/L, respectively and suggested its use for mosquito control in stagnant water bodies. The leaf and flower extracts of the weed *Lantana camara* are also reported to exhibit larvicidal activity against third and fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* with maximum mortality at 3.0 mg/mL[24]. The larvicidal activity of crude leaf extracts of five species of cucurbitaceous plants against *Ae. aegypti* and *Cx. quinquefasciatus* was found in the range of 74.57 to 554.20 mg/L[11]. Rajkumar and Jebanesan[25]also proved that the leaf extract of *Centella asiatica* has larvicidal properties and is an inhibitor for adult emergence against *Cx. quinquefasciatus*.

In the present investigations at 1 000 mg/L, the hexane and petroleum ether extracts prepared from the stems of *P. hysterophorus* were found to be effective against III instars of *Ae. aegypti* while those from the roots were proved to be efficient against early IV instars. In 2009, Rahuman *et al*[12] have reported that the leaf, stem-bark, and flower extracts of *Acacia arabica* Willd. Sans, *Cedrus deodara* Roxb, *Hibiscus rosa-sinensis* L., *Mangifera indica* L., *Nerium indicum* Mill., *Nicotiana tabacum* Linn., *Pongamia pinnata* (L.) Pierre, and *Solanum nigrum* Linn showed moderate larvicidal effects against mosquitoes, with LC_{50} value ranging from 76.27 to 709.51 mg/L after 24 h of exposure. However, the petroleum ether extract of *Euphorbia tirucalli* was found significantly effective against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus* with LC_{50} values of 4.25 mg/L and 5.52 mg/L[26]. Further, the more larvicidal potential of hexane extracts as compared to the petroleum ether extracts, and that too prepared from stems than roots suggested that the chemical constituents present in the hexane extract from stem arrested the metabolic activities of the larvae.

Earlier studies have showed that phytochemicals play a major role in mosquito control programme. Gopieshkhanna and Kannabiran[27] have observed the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins in the plant extract having mosquito larvicidal activity. The phytochemical analysis of hexane and petroleum ether extracts of *P. hysterophorus* exhibiting larvicidal potential showed the presence of alkaloids, saponins, terpenoids and flavonoids in different combinations. However, as no single phytochemical component was found common in the effective extracts, the larvicidal potential of selective extracts might be because of the synergistic effects of other compounds present in them, identified or unidentified in the present study. Earlier Oudhia[28]has suggested that *Parthenium* may possess the larvicidal and pupicidal property against *Ae. aegypti* and *Cx. quinquefasciatus* because of the combined effect of phenolic acids such as caffeic acid, vanillic acid, ansic acid, p-ansic acid, chlorogenic acid and parahydroxy benzoic acid and parthenin. Recently Kumar *et al*.[29] has reported that *Parthenium* may possess the ovicidal and oviposition deterrent property against *Ae. aegypti*.

Sathish kumar and Maneemegalai[24] reported the presence of flavonoids and cardiac glycosides in methanol extract of both leaf and flower of *Lantana camara* whereas saponin in leaf and terpenoid in the methanol extract of flower. They also reported saponin and cardiac glycosides in ethanol extract of both leaf and flower samples, while flavonoid in leaf and terpenoid in flower of ethanol extract. Rawani *et al*.[30-35] established the larvicidal properties of crude extracts of three plants, *viz. Carica papaya, Murraya paniculata* and *Cleistanthus collinus* against *Cx. quinquefasciatus* and revealed the presence of many bioactive principles such as steroids, alkaloids, terpenes, saponins, *etc*. that may be responsible for their biocontrol potentiality.

Although our investigations demonstrated and emphasized the potential of *P. hysterophorus* roots and stems against *Ae. aegypti* larvae and their benefits to developing new types of larvicides used for mosquito control, the mechanism causing mortality of mosquito larvae is still unknown and needs to be studied further. Variety of types and levels of active constituents in each kind of extract may be responsible for the variability in their potential against *Ae. aegypti*. Our investigations need further exploration to find out and identify the bioactive constituent, qualitatively as well as quantitatively, present in the *P. hysterophorus* extracts with larvicidal potential.

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