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LARVICIDAL ACTIVITY OF *Parthenium hysterophorus* EXTRACTS, PREPARED IN HEXANE, ACETONE AND METHANOL SOLVENTS AGAINST *Aedes aegypti* MOSQUITO

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ABSTRACT

***Aedes aegypti* is responsible for higher rates of dengue, chikungunya and yellow fever in tropical and subtropical areas including India. We examined the presence of bioactive compounds and larvicidal capability of *Parthenium hysterophorus* leaf extracts, prepared in acetone methanol and hexane solvents, against *Ae. aegypti*. Fully fed *Ae. aegypti* larvae were collected from different residential areas of Gorakhpur, Uttar Pradesh (India). Four concentrations tested were 250, 500, 750 and 1000 mg L^{-1} which caused 10 to 95% mortality in 24, 48, 72 and 96 h. Phytochemical analysis revealed that methanolic extract was rich in saponin, flavonoid, terpenoid, alkaloid and phenols as compared to acetone-based extract. However, hexane-based extracts showed only terpenoid and alkaloid activities. The four concentrations of hexane-based extract showed LC_{50} and LC_{90} to be 1920.05, 1496.72, 979.83, 337.84 and 18883.78, 14213.81, 16754.35 and 1246.14 mg L^{-1} in 24 to 96 h, respectively. Acetone-based extracts also showed similar values. However, the methanolic extracts had LC_{50} and LC_{90} as 1351.14, 1029.62, 657.77, 226.29 and 5618.12, 5995.96, 2860.23, 804.75 mg L^{-1}, respectively, in 24-96 h. The study showed that leaves of *P. hysterophorus* exhibit maximum toxicity in methanol solvent followed by acetone and hexane against *Ae. aegypti* larvae.**

Keywords: *Aedes aegypti*, larvicidal activity, *Parthenium hysterophorus*, phytochemicals, plant extracts

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are one of the major organisms responsible for the spread of vector-borne diseases (Shinde *et al*., 2018). They play a key role in the transmission and spread of filariasis, malaria, dengue, zika, Japanese encephalitis, chikunguniya, yellow fever, elephantiasis, etc. (Goyal and Shinde, 2020). Every year, the World Health Organization (WHO) reports nearly a billion cases and millions of deaths caused by mosquito-borne diseases (WHO, 2017, 2022a). Dengue cases in last two decades have increased eight-fold from 0.51 million cases in 2000 to 2.4 million in 2010 followed by 5.2 million in 2019 (WHO, 2017; WHO, 2022b). More than 7.6 million cases have been reported this year worldwide including 3.4 million confirmed cases with approximately 16000 severe cases, and 3000 deaths (WHO, 2024). Recently, London School of Hygiene and Tropical Medicine has reported that malaria and dengue may affect about 8 million people worldwide by 2080 (Colon-Gonzalez *et al.,* 2021).

Over half of the world's population is affected by the deadly diseases transmitted by *Aedes* species *(Ae. aegypti)* (Yang *et al.,* 2009). *Ae. aegypti* not only spreads dengue but is also responsible for the spread of chikungunya, yellow fever and zika virus in our country. They breed mainly in rainy season

but its eggs are stored in containers and can stay alive for up to 2 years without water (Hillary *et al.*, 2024). Its management is frequently relied on mosquito repellents and synthetic insecticides, resulting in major health consequences, environmental damage, and vector resistance. Therefore, alternate methods especially natural botanicals play a key role in mosquito vector control (Sanjeev and Sushil, 2022). These botanicals are a good source of bioactive phytochemicals, due to the presence of steroids, terpenoids, flavonoids, saponins, and terpenes that function as insecticides and can kill mosquito larvae with high fatality rates (Mdoe *et al.*, 2014). So, approaches to interrupt the disease transmission by these vectors may include the eradication of mosquito's larval stages (Hemalatha *et al.*, 2015).

Plant insecticides have been used to fight pests for centuries (Isman, 2006). With the introduction of synthetic insecticides, the use of botanical insecticides almost started decline. Later on, due to the adverse effect of chemical insecticides, there was revival of growing interest in the use of either plant extracts or essential oils (Peeyush *et al.*, 2011). Many studies supported the mosquito-larvicidal potential of these plant extracts and the essential oils (Lakshmi *et al.*, 2018, Sanjeev *et al.*, 2021**)**. *Parthenium hysterophorus* is a frequently grown and easily available weed that is known by various common name such as congress weed, carrot weed, star weed, feverweed, white top, chatak chandani, bitter weed, ramphool, gazar ghas, etc. It is not native but has become common and troublesome only since 1970s in India (Evans, 1997). Now it has become a destructive colonizer of wastelands and cultivated fields. Most of the research work on *P. hysterophorus* has been carried out to control and eliminate this weed because of its harmful belongings. But limited work has been carried out on its use to control mosquito population by affecting their biological characteristics (Amir, 2017). Keeping in view the harmful effects and unmanageability of *P. hysterophorus*, the beneficial aspects of the leaf's parts were explored in terms of the larvicidal potential against an Indian strain of *Ae. aegypti*. The plant parts and their secondary metabolites can be extracted with different types of solvents such as methanol, ethanol, acetone, chloroform, petroleum ether, hexane, etc. With this background, we have chosen the larvicidal activity of *P. hysterophorus* leaf extracts in the solvents of hexane, acetone and methanol based on their polarity. We also examined the bioactivity of *P. hysterophorus* phytochemicals especially saponins, flavonoids, terpenoids, alkaloids and phenols in these solvents.

MATERIALS AND METHODS

Collection of plant and preparation of leaf extracts

Fresh plant materials of *P. hysterophorus* were collected from the campus and nearby places of Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, UP (India). The identity of plant material was confirmed by a plant taxonomist in the university. The collected leaves were washed with tap water to remove all the unwanted impurities, thoroughly rinsed with sterile distilled water and dried in shade for 7-14 days at room temperature (27-37°C). The air-dried leaves were ground to a fine powder using an electric blender and the powdered materials was packed in air-lock plastic containers.

Extraction of plant materials

The extraction of any crude material with a particular solvent yields a solution containing different phytoconstituents. The ability of a solvent to dissolve different solutes was determined by the polarity of hexane, acetone, and methanol (Ricciutelli *et al.*, 2006). The plant material was extracted using the Soxhlet extraction method. For this, 20 g powder were packed in a thimble (Whatman filter paper No. 1) and placed in a Soxhlet extractor (ISKO, Boro 3.3). For extraction, hexane, acetone and methanol (Merck 99.9%), were used as selective solvent system ranging from non-polar to polar at 55°C to 70°C for 8-9 h till the solvent became decolourised (James *et al.*, 2014). The procedure was repeated 5 times to obtain enough crude extract for bioassay studies.

During each extraction operation, 250 mL solvent was introduced via a funnel and passed through the Soxhlet body (thimble) holding the sample to the Soxhlet's round bottom flask system separately. As the extraction progressed, the yellow-green coloured solution in the thimble faded, while the colour

of solution in round bottom flask turned brown. This pattern continued until the colour in thimble became colourless and the solution in flask became dark brown. The solvents had boiling point of 55, 56, and 69ºC for hexane, acetone and methanol, respectively. The extraction process for each solvent took 9 h. The extract was concentrated in a rotary vacuum evaporator to yield dark greenish, gummy extracts. The extraction processes were carried out at optimum temperature 28 ± 1ºC.

Phytochemical analysis

The phytochemical analysis of crude extracts was carried out by using standard protocol with slight modifications (Harborne, 1984). For phenol test, we added some drops of 1% ferric chloride solution to 1 mL extract. The appearance of green colour confirmed the presence of phenols. For flavonoids, 1 mL alcoholic extract with 3-5 drops of 2% lead acetate solution was added to sample and the development of orange or yellow colour indicated the presence of flavonoids. Hagger's test was done for alkaloid, in which one drop of Hagger's reagent was added to 1 mL extract and a yellow precipitate was formed. For terpenoid and saponin test, the froth formation test was done through 1 mL extract was shaken with water in a test tube, froth was developed.

Collection of experimental animals

Larvae of Culicines were collected from the stagnant water in different residential areas of Gorakhpur district. The 3rd and 4th instar larvae of *Ae. aegypti* were identified and separated by key characteristics such as comb scales with large median and stout sub-median spines; setal support plate of setae 9-12-T with prominent spine and were cross identified by Senior Entomologist of the Department (Darsie and Voyadjoglou, 1997). Larvae were identified by abdomen with presence of ventral brush (4-X) with 5 pairs of setae: seta 4-a, b X branch as well as comb scale with stout and subapical spines (Rueda, 2004). The larval colony was separated and kept in plastic and enamel trays containing tap water and fed with 3:1 ratio of baby biscuits and yeast.

Larvicidal bioassay

The varying concentration gradients of extracts were prepared 1 h prior to bioassay as per WHO protocol (WHO, 2005). For 1% (10,000 mg L^{-1}) stock solution, 200 mg test plant extract was dissolved in 20 mL methanol used for extraction by vigorous shaking in a capped vial. Then the desired dosages of test concentration were prepared by adding the required volumes of aliquots of stock solution to 200 mL chlorine-free tap water (Abbott, 1925). Same procedure was followed for acetone and hexane solvents separately. For control, 1 mL of each solvent was added to 200 mL water for each test.

The larvae were initially exposed to a wide range of test concentrations and control to establish the activity range of plant material under our experiments. After defining the mortality of larvae, a narrower range of 4 aliquots yielding between 10 to 95% mortality in 24, 48, 72 and 96 h were used to determine LC_{50} and LC_{90} values. Six replicates and equal number of controls were set up for each concentration simultaneously with deionized water. The bioassay was performed at an optimum temperature of 26 ± 2°C and relative humidity of 80 ± 2% and a photoperiod of 12 h light followed by 12 h dark (12L:12D) for 96 h.

Ae. aegypti larvae (20) were introduced through a dropper into a glass beaker containing 200 mL distilled water and the larvicidal activity was measured as per the standard procedure (WHO, 2005). Dead larvae were the one which did not respond to probing with a needle in siphon or cervical region. Moribund larvae were characterized by their inability to rise to the surface or exhibit typical diving response on water disturbance. Both categories were used to calculate the % mortality (Abbott, 1925).

Statistical analyses

Biostatistics were carried out for lethal concentrations (LC) values, lower (LCL) and upper confidence limits (UCL), slope value (mean ± SE), t-ratio and heterogeneity. LC_{50} and LC_{90} values were calculated using probit analysis by POLO plus programme (LeOra Software version 2.0) (Robertson *et al.*, 2007). Abbott's formula was used to correct percentage mortality where a minimal proportion of larvae (between 5 and 20%) in the control batches died during the experiment (Abbott, 1925).

RESULTS AND DISCUSSION

The revealed that 20 g *P. hysterophorus* plant material yielded 3.82 g (19.1%), 4.10 g (20.5%) and 4.30 g (21.5%) after Soxhlet extraction in hexane, acetone and methanol solvents, respectively. The bioactive component analysis revealed that the methanolic plant extract was rich in saponins, flavonoids, terpenoids, alkaloids and phenols, while acetonic extract was also rich in these phytochemicals, except flavonoid. However, hexane-based extract only exhibited terpenoid and alkaloid activities (Table 1).

Table 1: Comparison of qualitative phytochemical analysis of the leaf extracts of *Parthenium hysterophorus* in methanol, acetone, hexane solvents for various bioactive components

Test component	Saponin	Flavonoid	Terpenoid	Alkaloid	Phenol
Methanol	+	+	+	+	+
Acetone	+	_	+	+	+
Hexane	_	_	+	+	_

(+) = present, (-) = absent

Larvicidal status of plant extracts

All the test leaf *P. hysterophorus* extracts were effective against *Ae. aegypti* larvae inflicting 100% mortality at 1250 mg L^{-1} in 96 h exposure period. The toxicity values of various concentration of hexane, acetone and methanol extracts against *Ae. aegypti* larvae between 24 to 96 h exposure period is given in Table 2. The hexane-based extracts showed LC_{50} values of 1920.0, 1496.7, 979.8, and 337.8 mg L^{-1} and LC_{90} values of 18883.8, 14213.8, 16754.4 and 1246.1 mg L^{-1} in 24, 48, 72 and 96 h exposure periods, respectively. Similarly, the acetone-based extracts showed LC_{50} values of 1539.3, 1286.6, 867.0 and 269.2 mg L^{-1} and LC_{90} values of 4955.8, 5955.9, 4576.4 and 1131.9 mg L^{-1} in 24, 48, 72 and 96 h exposure periods, respectively. The methanolic extracts exhibited LC_{50} values of 1351.1, 1029.6, 657.8 and 226.3 mg L^{-1} and LC_{90} values of 5618.1, 5995.9, 2860.2 and 804.7 mg L^{-1} in the same exposure periods, respectively. The hexane, acetone and methanol solvent extracts were effective with LC_{50} values of 337.8, 269.2 and 226.3 mg L^{-1}, in above exposure periods, respectively.

In larvicidal bioassays, methanolic extracts exhibited more larvicidal potential than acetone- and hexane-based extracts as revealed by their LC_{50} values (Fig. 1**)**. The methanolic extracts were 16% more effective than acetonic extract and 33% more effective than hexane-based extracts in 96 h exposure. It was observed that acetone-based extracts were 20% more effective as larvicides than hexane-based extracts. These observations suggest that methanolic extracts were most effective larvicidal against *Ae. aegypti* followed by those extracts prepared by acetone and hexane solvents.

The present study evaluated mosquito larvicidal activity of *P. hysterophorus* among hexane, acetone and methanol solvent against *Ae. aegypti*. The phytochemical analysis of crude extracts revealed that methanolic extract was rich in saponin, flavonoid, terpenoid, alkaloid and phenols, followed by acetone-based extract. The hexane-based extracts showed only terpenoid and alkaloid activities. The polarity of solvents i.e. methanol, acetone and hexane were 0.762, 0.355 and 0.009, respectively (Reichardt and Welton, 2010). These qualitative findings suggest that higher polar solvents conserve not only the crude extracts bioactivity but also conserve the phytochemicals, which develop unique toxic effects on insects in extreme environments, leading to the biosynthesis of diverse bioactivities. Phytochemical analysis of *P. hysterophorus* indicated the presence of bioactive constituents such as alkaloids, saponins, tannins, flavonoids, terpenoids, and phenolics which exhibit vigorous biocidal and larvicidal activities (Bilal and Hassan, 2012). The plants with high mosquito larvicidal activity are endowed with secondary plant metabolites like carbohydrates, protein, glycosides, alkaloids, tannins, saponins, flavoides, lignin and terpenes (Ghimire *et al.*, 2014; Yu *et al.*, 2014).

The present study showed that *P. hysterophorus* leaves exhibited maximum toxicity in methanol solvent followed by acetone and hexane against *Ae. aegypti* larvae. The assessment of this weed's larvicidal potential, in addition to its management, may aid in the development of successful mosquito

Table: 2: Toxicity (LC values) of different concentration of hexane, acetone and methanol leaf extracts of *Parthenium hysterophorus* against *Ae. aegypti* larvae at 24-96 h exposure period

Exposure period (h)	Tested material	LC values	LCL-UCL	Slope value	t ratio	Hetero-geneity
24	Hexane	LC_{50}= 1920.05 LC_{90} =18883.78	1279.58-5237.0 6373.01-32346.23	1.291 ± 0.297	4.35	0.26
	Acetone	LC_{50} = 1539.26 LC_{90} =4955.80	1226.96-2339.95 3010.95-13118.67	2.524 ± 0.423	5.964	0.40
	Methanol	LC_{50} = 1351.14 LC_{90} =5618.12	1085.46-1980.58 3293.60-15422.54	2.071 ± 0.332	6.237	0.17
48	Hexane	LC_{50} =1496.72 LC_{90} =14213.81	1072.29-3209.91 5345.98-16600.11	1.311 ± 0.291	4.506	0.24
	Acetone	LC_{50} =1286.62 LC_{90} =5955.89	1027.71-1937.39 3322.17-19232.38	1.926 ± 0.335	5.744	0.23
	Methanol	LC_{50} =1029.62 LC_{90} =5995.96	842.67-1440.54 3316.01-19152.76	1.675 ± 0.283	5.921	0.35
72	Hexane	LC_{50} =979.83 LC_{90} =16754.35	735.52-1885.45 5297.97-52648.59	1.039 ±0.267	3.893	0.14
	Acetone	LC_{50} =867.02 LC_{90} =4576.38	730.80-1123.02 2724.99-12466.03	1.774 ±0.295	6.011	0.16
	Methanol	LC_{50} =657.77 LC_{90} =2860.23	574.53-768.98 1988.88-5318.84	2.008 ±0.279	7.118	0.57
96	Hexane	LC_{50} =337.84 LC_{90} =1246.14	277.29-389.96 1011.85-1718.67	2.261 ±0.283	7.988	0.55
	Acetone	LC_{50} =269.20 LC_{90} =1131.92	200.85-324.92 909.84-1612.97	2.055 ±0.291	7.051	0.79
	Methanol	LC_{50} =226.29 LC_{90} =804.75	166.67-275.39 680.93-1030.02	2.326 ±0.313	7.442	0.66

Batches of twenty larvae were exposed to the test concentrations. Each experiment was replicated 6 times. The mortality was noted at 24 to 96 h exposure time. Regression analysis showed significant p values < 0.05 with confidence limits (LCL, lower confidence limit; UCL, upper confidence limit), slope value (± SE) & t-ratio and was applied to locate significant changes with control. LC_{50} and LC_{90} mean lethal concentration that kill 50 and 90% larvae.

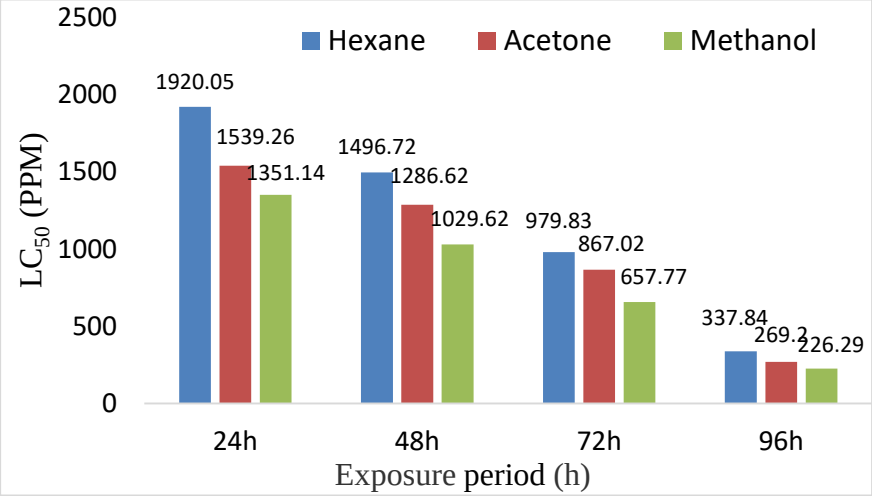

Fig. 1: Toxicity (LC_{50}, ppm) of hexane, acetone and methanol solvent leaf extracts of *P. hysterophorus* against *Ae. aegypti* larvae between 24 to 96 h exposure period

control measures. Our recent work showed that the aqueous leaf extract of *P. hysterophorus* was promising larvicidal agent for *Ae. aegypti* larvae with sublethal dose of 5.88 g L^{-1} (Sanjeev and Sushil, 2024). This inspired us to check the plant extract activity in various solvents. The present study showed that the mortality of larvae after exposure to *P. hysterophorus*

increased with increase in the concentration of extracts as well as the time of exposure which is supported by Obomanu *et al.* (2006). Similar to our findings, Tarekegn (2021) found that *P. hysterophorus* solvent extract used against 4th instar larvae of *Anopheles arabiensis* exhibited LC_{50} values of 478, 393, and 368 ppm in acetone, petroleum ether, and hexane solvents, respectively. In another study, the leaf extracts were found ineffective against both instars causing only 10-40% mortality; however, hexane- and petroleum ether-based extracts prepared from *P. hysterophorus* stem were effective against 3rd instar larvae of *Ae. aegypti*, with LC_{50} values as 379.7 and 438.6 mg L^{-1}, respectively (Sarita Kumar *et al.,* 2022). This finding supports our choice of selecting leaf as better extracts for larvicidal activity. *P. hysterophorus* leaf extracts have been found active against 3rd and 4th instars of *Culex quinquefasciatus* causing 70-90% mortality (Bansode *et al.*, 2016). The highest larvicidal potential has previously been found in the methanolic extract of *P. hysterophorus* (Jundare *et al.,* 2020; Kumar *et al.,* 2023). In another study, two green seaweed extracts tested for toxicity against 3rd instar of *A. aegypti*, in petroleum ether, chloroform, acetone and methanol solvent were in concordance with our findings and their respective LC_{50} values were 144.5, 184.9, 183.3, and 155.6 mg L^{-1} for *Caulerpa racemosa* and 173.2, 166.0, 193.2 and 178.6 mg L^{-1} for *Ulva fasciata,* (Selvi, 2024).

The insecticidal activity of plant extracts differs based on the plant parts, plant species, mosquito species, regional variance, extraction methods, and solvent polarity (Shaalan *et al.,* 2005). It is reported that mosquito control programs can easily be carried out by targeting the larval stages as they are confined to water bodies which are mainly manmade and can be located in urban areas (Srivastava, 2008). More studies on larger scale, semi-field and field settings against mosquito-borne diseases are required to confirm their efficacies. The screening of plant's bioactive compounds with larvicidal activity is recommended. Also, the possible mechanisms responsible for the activity need to be explored.

Conclusion: The use of *P. hysterophorus* extracts in mosquito vector control is an alternative method for minimizing the persistence, bioaccumulation and toxicity caused by chemical compounds used as insecticides. The study suggests the promising effect of plant based larvicides. The methanol extract of *P. hysterophorus* possessed higher larvicidal activity than other solvent extracts. The methanol polarity also increased the larvicidal potential against *Ae. aegypti* due to the presence of bioactive compounds and they supported the toxicity effects followed by acetone and hexane extracts. *P. hysterophorus* can serve as a valuable new-generation mosquito larvicide. There is also need to promote the use of plant-based larvicides through community-based vector control programs.

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