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LARVICIDAL ACTIVITY OF *EPALTES PYGMAEA* (DC) WHOLE PLANT EXTRACTS AGAINST *CULEX QUINQUEFASCIATUS* SAY, *AEDES AEGYPTI* L. AND *ANOPHELES STEPHENSI* LISTON LARVAE

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ABSTRACT: In the present study, the larvicidal activity of successive *n*-hexane, chloroform, ethyl acetate and ethanol extracts of *Epaltes pygmaea* DC (Family: Asteraceae) whole plant was carried out against three mosquito species namely *Culex quinquefasciatus, Aedes aegypti* L. and *Anopheles stephensi*. The crude whole plant was successively extracted with *n*-hexane, chloroform, ethyl acetate, and ethanol. The larvicidal activity was studied at 62.5 ppm, 125 ppm, 250 ppm, and 500 ppm concentrations against the late third instar larvae of *C. quinquefasciatus, A. aegypti* and *A. stephensi*. Results showed that the ethyl acetate extract of *E. pygmaea* was the most effective against tested mosquito larvae. The median lethal concentration (LC_{50}) values of ethyl acetate extract were calculated as 35.79 ppm, 62.37 ppm and 29.94 ppm for *C. quinquefasciatus, A. aegypti* and *A. stephensi*, respectively. The results suggested that ethyl acetate extract of *E. pygmaea* could be used to control *C. quinquefasciatus, A. aegypti* and *A. stephensi*.

INTRODUCTION: *Epaltes pygmaea* DC is a small annual herb, 8 to 20 cm high, minutely winged branched stem with aromatic roots, leaves are alternate, linear, lanceolate to oblong, flower color is pink, solitary, terminal, heterogamous. It is found in Sri Lanka, India, Java and China and a limited extent in South India, especially towards the coast, gregarious in low lying ground by river banks and paddy field after harvesting in clayey soil [1-3].

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Epaltes is used in traditional Ayurvedic medicine in Srilanka to alleviate jaundice. A literature survey reveals that the plant of the genus has the therapeutic action of diaphoretics, diuretics, stimulant, and expectorant are used in urethral discharges and acute dyspepsia [4]. Alcohol and aqueous extract of *E. pygmaea* possesses hepatoprotective activity against paracetamol-induced liver damage in rats and also have potent diuretic activity [5].

Recent studies show that the extract of *E. pygmaea* has good inhibitory activity against the organism *Bacillus cereus, Klebsiella pneumonia* and *Staphylococcus aureus* at microgram level [6]. Microscopic, thin layer chromatographic studies [7] and quality control parameters [8] of *E. pygmaea* have been reported.

The present study was conducted to evaluate the larvicidal activity of the plant *Epaltes pygmaea* DC. whole plant extracts against the larvae of *C. quinquefasciatus, A. aegypti* and *A. stephensi*.

MATERIALS AND METHODS:

Plant Collection: Fresh whole plant of *Epaltes pygmaea* DC. was collected from Tirunelveli District in September 2016 was identified and authenticated by Prof. P. Jeyaraman, Director, Institute of Herb Botany, Plant Anatomy Research Centre, Tambaram, Chennai, India, where a voucher specimen (PARC/2014/2071) was deposited. The plant was dried in the shade and powdered in a pulverizer.

Extraction: The plant material was extracted with *n*-hexane, chloroform, ethyl acetate, and ethanol solvents in a successive manner using a Soxhlet apparatus. All the obtained extracts were filtered using sterile Whatman filter paper no. 2, dried using Rotavapor R-300 and stored separately for further use. The percentage yield of hexane, chloroform, ethyl acetate, and ethanol were 5.3, 3.7, 4.5 and 6.5 respectively.

Mosquitoes: Laboratory reared *C. quinquefasciatus, A. aegypti* and *A. stephensi* mosquito larvae were used in the bioassay experiments. The rearing conditions were: 27 ± 1 ºC, 75-85% RH and 14:10 h photoperiod.

Mosquito Larvicidal Bioassay: Larvicidal activity of the extracts was evaluated using the method recommended by the World Health Organization [9] with slight modifications. A range of concentrations *viz*., 62.5, 125, 250 and 500 ppm of each solvent extract were prepared with an emulsifier and taken in 100 ml plastic cups separately. Third instar larvae (20 larvae) of each *A. aegypti*, *C. quinquefasciatus* and *A. stephensi* were introduced into each cup that contained test solutions. Emulsifier control and water control were also maintained separately. Five replicates were maintained for each treatment and control. Larval mortality was recorded after 24 h of treatment. Larvae were considered dead when they did not move or rise to the surface of the solution.

Statistical Analysis: The lethal concentration values (LC_{50} and LC_{90}) were calculated by EPA probit analysis software (1.5 versions) [10].

RESULTS AND DISCUSSION: Larvicidal effect of *E. pygmaea* whole plant extracts on the third instar larvae was recorded and the results are presented in **Table 1**. The ethyl acetate extract of *E. pygmaea* was very effective against all three mosquito larvae. The ethyl acetate extract of *E. pygmaea* was most effective against the larvae of *A. stephensi* with LC_{50} and LC_{90} values of 29.94, 141.0 ppm. The LC_{50} and LC_{90} values of ethyl acetate extract of *E. pygmaea* were 35.79, 160.47 ppm and 62.37, 313.81 ppm against the larvae of *C. quinquefasciatus* and *A. aegypti*, respectively.

The LC_{50} and LC_{90} values of *E. pygmaea* hexane extract were 247.72 and 1599.42 ppm against *C. quinquefasciatus* and 495.28 and 4288.45 ppm against *A. aegypti* larvae and 168.50 and 1640.68 ppm against *A. stephensi* larvae, respectively **Table 1**. The LC_{50} and LC_{90} values of *E. pygmaea* chloroform extract were 40.99 and 197.81 ppm against *C. quinquefasciatus*, 69.12 and 433.221 ppm against *A. aegypti* and 32.623 and 174.110 ppm against *Anopheles stephensi* larvae, respectively **Table 1**. The LC_{50} and LC_{90} values of *E. pygmaea* ethanol extract were 350.25 and 2912.24 ppm against *C. quinquefasciatus*, 485.12 and 4937.64 ppm against *A. aegypti* larvae and 244.55 and 1994.81 ppm against *A. stephensi* larvae, respectively **Table 1**.

The active ethyl acetate extract of *E. pygamea* extract contained terpenoids, flavonoids, phenol, tannin, steroid group of phytochemicals; the mortality may be due to these phytochemicals. *Culex quinquefasciatus* control was mainly conducted through the use of neurotoxic insecticides belonging to the organochlorines (OC), the organophosphates (OP) and the pyrethroids [11].

Aedes aegypti is the main vector of Dengue and Dengue hemorrhagic fevers [12, 13]. *Anopheles stephensi* larvae is a major malaria vector [14]. Hence researchers continuously study to control these larvae with herbal extracts and found *Tridax procumbens* L. [15] and *Leucas aspera* L. [16] with positive results. Results from the present study also suggested *E. pygmaea* was found effective in controlling of *C. quinquefasciatus, A. aegypti*, and *A. stephensi* and successive ethyl acetate extract as most active than other extracts.

TABLE 1: LETHAL CONCENTRATION (IN PPM) OF VARIOUS EXTRACTS OF *E. PYGMAEA* AGAINST THREE MOSQUITO LARVAE

Mosquito species	Treatment	LC_{50} (ppm)	95% confidence limit LL	95% confidence limit UL	LC_{90} (ppm)	95% confidence limit LL	95% confidence limit UL	Slope ± SE	Intercept ± SE	χ^2
Culex quinque fasciatus	Hexane	247.72	189.47	350.91	1599.42	869.086	5538.65	1.58 ± 0.28	1.21 ± 0.66	0.1*
	Chloroform	40.99	17.43	61.04	197.81	147.60	327.77	1.87 ± 0.40	1.97 ± 0.84	1.9*
	Ethyl acetate	35.79	13.27	54.86	160.47	120.57	254.70	1.96 ± 0.44	1.94 ± 0.92	1.9*
	Ethanol	350.25	253.32	611.18	2912.24	1276.08	19685.18	1.39 ± 0.29	1.45 ± 0.67	0.1*
Aedes aegypti	Hexane	495.28	336.59	1093.27	4288.45	1647.23	45487.46	1.36± 0.30	1.31 ± 0.70	0.2*
	Chloroform	69.12	38.68	95.52	433.221	294.112	926.839	1.60± 0.31	2.04 ± 0.68	3.0*
	Ethyl acetate	62.37	36.05	84.97	313.81	227.77	560.07	1.82± 0.34	1.72 ± 0.73	5.6*
	Ethanol	485.12	323.76	1152.39	4937.64	1743.16	74921.77	1.27 ± 0.29	1.58 ± 0.68	0.1*
Anopheles stephensi	Hexane	168.50	117.37	237.61	1640.68	802.33	8806.38	1.29 ± 0.27	2.11 ± 0.63	0.1*
	Chloroform	32.62	9.942	52.950	174.110	127.582	293.164	1.76 ± 0.41	2.33± 0.87	1.8*
	Ethyl acetate	29.94	8.18	49.19	141.00	103.65	223.53	1.90± 0.47	2.18 ± 0.97	1.5*
	Ethanol	244.55	181.52	364.69	1994.81	972.12	9872.57	1.40 ± 0.28	1.64± 0.65	0.6*

LC_{50}: Lethal concentration to kill 50% of the exposed larvae; LC_{90}: Lethal concentration to kill 90% of the exposed larvae; P=≤0.05-chi-square values were significant, LL: Lower Limit, UL: Upper Limit.

CONCLUSION: In conclusion, the ethyl acetate extract of *E. pygmaea* was the most potent treatment against the three tested mosquito vectors. Based on these results, the ethyl acetate extract of *E. pgymaea* could be used in vector mosquito control and maybe further investigated to isolate the active constituent responsible for the larvicide.

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CONFLICTS OF INTEREST: There is no conflict of interest.

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