Pest potential of *Pisonia alba* extracts and fractions against mosquito-borne disease (Diptera: *Culicidae*)

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Abstract

Mosquitocidal activity of *Pisonia alba* leaf extracts was tested against *Anopheles stephensi, Culex quinquefasciatus, Aedes aegypti*. Totally Twenty five early fourth instars larvae of *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* (*A. stephensi, C. quinquefasciatus* and *A. aegypti*) were exposed to various concentrations (50-250 $\times 10^6$) and the 24 hrs LC_{50} values of the *Pisonia alba* extracts was determined by probit analysis and ovicidal activity, determined against *A. stephensi, C. quinquefasciatus* and *A. aegypti* to various concentrations ranging from 1.0, 2.0 and 4.0 mg/cm^2 ppm under laboratory conditions. The eggs hatchability was assessed 48 hrs post treatment. The LC_{50} and LC_{90} values of *Pisonia alba* petroleum ether extract against *A. stephensi* were 100.62 and 117.80$\times 10^6$, respectively; *C. quinquefasciatus* were 98.52 and 112.75 $\times 10^6$, respectively; For, *A. aegypti* were 111.29 and 141.16 $\times 10^6$, respectively. The ovicidal activity of *Pisonia alba* exerted 100% mortality at 240, 300 and 360 $\times 10^6$ against *C. quinquefasciatus* and for repellency activity was definite against *A. stephensi, C. quinquefasciatus* and *A. aegypti* species at three concentration like 1.0, 2.0 and 4.0$\times$ 10^{-2}kg.m^{-2} under the laboratory conditions. The petroleum ether extract of *Pisonia alba* establish to more repellent than the additional extracts. A higher concentration of 4.0 $\times 10^{-2}$kg.m^{-2} provided 100% protection up to 120, 160 and 200 minutes, respectively. The outcome clearly shows that larvicidal ovicidal and repellent activity was dose reliant. From the results it can be concluded the petroleum ether extract of *Pisonia alba* was an outstanding potential for controlling the vector mosquito *C. quinquefasciatus.*

Keywords: *Anopheles stephensi, Culex quinquefasciatus, Aedes aegypti*, larvicidal, ovicidal and repellent activity, *Pisonia alba*

1. Introduction

The plant realm is considered an asset for various kinds of potential drugs. In ancient days, many of the diseases were cured using plant products, and now again, there is an increasing awareness among people about the significance of plants and their medicinal ethics[1] World Health Organization, more than 80% world population went back to traditional medicines[2]. *Pisonia alba span* (*Nyctaginaceae*) is widely spread all over India [33] and it is an evergreen commonly grown lettuce tree. Leaves, stem and root of this species are extensively used by the tribal's in the preparation of several folk medicines. It has been extensively used in Indian traditional medicine as an ant diabetic, anti-inflammatory agent, and used in the treatment of ulcer, dysentery and snake bite[4]. *Pisonia alba* has good potential therapeutic plant[5]. Mosquito-borne diseases menace the living and livelihood of millions of people worldwide. Malaria continues to be a paramount disease, blighting 300 million Africans globally, despite the advancement and achieved using indoor residuary spraying and pesticide treated bed nets. It is increasingly difficult to control due to the spread of insecticide defiance in the mosquito vectors and also defiance of the parasite to the available drugs. In terms of dengue, 2.5 billion people live at danger fever of infection with one or more serotypes of the virus, which cause an estimated 390 million infections per year, and the affected area has been increased rapidly in the past 30 years. Chikungunya breakouts in Europe have worn the notice of that western world to this disease extend by the Asian tiger mosquito, *Ae. albopictus* [6-14]. Malaria is one in all the grave scourges inflicted upon human beings. It causes human mortality and morbidity alongside giant economic loss. Roughly all tropical regions of the planet area unit expertise the recovery and reoccurrence of 1 of the world's an outsized quantity deadly diseases, ie. malaria and India is not any omission. Malaria afflicts one year of the planet folks i.e. 2020 million in 107 countries and territories placed within the tropical and semitropical regions[15]. In line with the newest estimates, there have been regarding 198 million cases of malaria in 2013 and a calculable 584,000 deaths. Most deaths occur among youngsters living in continent,

wherever a baby dies each minute from malaria. Malaria mortality rates among youngsters in continent are reduced by Associate in nursing calculable fifty eight since 2000[16]. The dipteran *Cx. quinquefasciatus* is a crucial feature inflicting filariasis, West Nile virus, Avion malaria and St. Louis encephalitis. *Cx. quinquefasciatus*, besides known as the southern house dipterans, is extensively studied because it transmits crucial diseases[17]. In 2014, estimate is impure with lymphatic filariasis parasites and more than 20 for every penny of the planet populace is at danger of getting roundworm disease. In Asian nation it's calculable that regarding 554.2 million folk's area unit at hazard of humor disease unhealthiness in a pair of 43 districts [18]. Worldwide, twenty five million men clumsy person with sex organ sickness and over 15 million folks are afflicted with lymphoedema[19].

These diseases challenge the urbanized and rising countries of the world for irradiation. Pesticides have numerous beneficial effects. These comprise crop protection, preservation of food and material and preclusion of vector-borne diseases. Hence, there is emergency need to carry out research on the use natural plant extracts in reducing the mosquito population.

2. Material and methods

Collection of Plant

Pisonia alba was collected from the natural population in Mariyappa Nagar, Chidhambaram, Cuddalore district of Tamil Nadu, India, and identified in Department of Zoology, Annamalai University, Annamalai Nagar, Tamil Nadu, and India. The complete plant dried under darkness at room temperature for about 15 days.

Extraction

The solid leaves were washed with sterile refined water, darkness dried, and finely ground. the finely ground leaf powder (500 g/ml) was extracted with hexane, chloroform, diethyl ether, ethyl acetate and methanol exploitation Soxhlet extraction equipment, and therefore the extraction was continued until visibly no more extraction is feasible (by discerning the color of the extracted portion). The solvent from the extract area unit removed employing a rotary vacuum evaporator to gather the crude extract and keep at 4ºC. Normal stock solutions were ready at 1 % by dissolving the residues in plant product. From this stock solution, totally different concentrations were ready and this solution is employed for larvicidal activity.

Mosquito Rearing

The mosquitoes, *A. aegypti, A. stephensi* and *C. quinquefasciatus* were procured from the Centre for research in Medical entomology (ICMR), Viruddhachalam, reared within the laboratory condition, Department of zoology, Annamalai University. The larvae be gobbled dog biscuits and yeast powder within the 3:1 magnitude relation. Adults were supplied from 100 percent sucrose solution and one week previous chick for feed. Mosquitoes were control at (28±2) ºC, 70%-85% ratio (RH), with a photo amount of 14 h lightweight, 10 h dark.

Larvicidal activity

The larvicidal activity of *Pisonia alba* extract fractions were assessed according to the convention beforehand portrayed[10]. In view of the wide range and thin range tests, all concentrates tried disappearing from 10-50 ppm were ready and they were tried against the newly shed (0-6 hrs) third instar hatchlings of choosing mosquito species. The plants concentrate were disintegrated in 1 ml DMSO (Dimethyl sulfoxide) and afterward weakened in 249 ml of dechlorinated faucet water to acquire each of the fancied focuses. The control was readied utilizing 1ml of DMSO as a part of 249 ml of dechlorinated water. The total hatchlings test species (25) were obtainable in 250 ml plastic glass containing 250 ml of fluid medium (249 ml of dechlorinated water + 1ml of Dimethyl Sulfoxide) and the required to measure of the compound synthesis was incorporated. The total larval mortality was recorded after 24 h of post treatment. For each examination, five recruits were kept up at once. Percent of mortality was rectified for control mortality utilizing [11].

Ovicidal activity

The method of [27] was followed to check the ovicidal activity. The leaf extracts was diluted within the several solvent to attain completely different concentrations (60, 120, 180, 240, 300 and 360 ppm). The freshly ordered egg raft containing 100 eggs of *A. aegypti, An. stephensi* and *Cx. quinquefasciatus* were exposed to every dose of leaf extract till they hatched or died. Every concentration was replicated six times. Eggs exposed to several solvents in water served 48 h post treatment by the subsequent formula:

$$\text{Egg hatchability (\%)} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} x100$$

Toxicity on non-target aquatic organisms

The methodology developed by[16] was used to assess the fractions effect on non-target organisms. The *E. variegate* fractions was tested for toxicity against three non-target mosquito predators, namely *Diplonychus indicus, Anisops bouvieri* and G*ambusia affinis*. These organisms were collected in the field and kept separated in cement tanks (85-cm wide and 30-cm deep), containing water at 27±3° C and external relative humidity of 85%. The fractions of *E. variegata* were examined at concentrations that were 50 times higher than the LC_{50} doses for mosquito larvae. Five replicates were carried out for each concentration, accompanied by four iterations of untreated controls. In additions, non-target organisms beneath test were observed successively for ten days to investigate the post-treatment influence of the extract on their continued existence and swimming ability.

Data analysis

Mortality data were subjected to probity analysis. LC_{90} (LD_{90}) and LC_{50} (LD_{50}) were estimated via Finney methodology[23]. ANOVA analysis, followed by Tukey's HSD test ($P<0.05$) were in employment to investigate ovicidal data. The Suitability Index (SI) was used to assess biotoxicity on no-target organisms; the Index was calculated through the following formula[20].

$$SI = \frac{LC50 \text{ of non - target organisms}}{LC50 \text{ of target vector species}}$$

Data analysis was carried out using the SPSS Statistical Software Package version 16.0. The significance of differences between values was assessed at the 0.05 probability level.

3. Results

Medicinal plants are produce on vector control. These plants can be used to develop environmentally it present terpenoids compound present in primary screening (Table 1) safe vector and pest administration agents. The regression equation (Based on Probit Analysis) between the concentrations of petroleum ether, acetone, benzene and hexane solvent extracts against *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* after 24 h exposure are represented in (Table 2) and the petroleum ether extract of *Pisonia alba* reported in the present study showed the larvicidal exploit in the plant betokening their utilize in mosquito population control (Table. 2-4). The result clearly stimulated that petroleum ether and benzene solvent plant extract at very low concentrations was toxic against the entire three mosquito species tested when compared to acetone and hexane extracts. The LC_{50} and LC_{90} value for petroleum ether extract of *Pisonia alba* against *Cx. quinquefasciatus* at 24 h post treatment was 98.95 $\times 10^6$ and 112.75 $\times 10^6$ respectively. Petroleum ether extracts were also effectual against *An. stephensi* larvae with LC_{50} and LC_{90} value of 100.62 $\times 10^6$ and 117.80 $\times 10^6$, respectively. The LC_{50} and LC_{90} value of petroleum ether against *A. aegypti* after 24 h post treatment were 111.29 $\times 10^6$ and 141.16 $\times 10^6$, respectively. Among the extracts tested for ovicidal activity against *Cx. quinquefasciatus*, the petroleum ether extract of *Pisonia alba* exerted 100% mortality (i.e., no hatchability was recorded this research) at 240, 300 and 360 ppm, respectively

4. Discussion and conclusions

In our results outcomes demonstrated that, the chemical composition of *Pisonia alba* leaf have noteworthy larvicidal, ovicidal movement against human vector malarial mosquito*, An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. The investigated that the larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* against *An. stephensi, A. aegypti* and *Cx. quinquefasciatus*. The most noteworthy concentrations of 450 $\times 10^6$ provided over 180 and 150 min. protection in ethanol extracts of *Citrus sinensis* against *Cx. quinquefasciatus*. Among three vectors tested, the highest adulticidal activity was observed in high mortality followed by *An. stephensi, A. aegypti* and *Cx. Quinquefasciatus*[33]. The larvicidal, oviposition deterrent and repellent activity of *Annona squamosa* against *A. aegypti, An. stephensi* and *Cx.. quinquefasciatus*. The LC_{50} and LC_{90} values of 219.41 and 394.87$\times 10^6$ severally. In oviposition deterrent activity the best concentration of 0.1%, *Annona squamosa* manufacture 92.4% against *A. aegypti*. Skin repellent check at 0.02 ppm concentration of *Annona squamosa* offers the entire protection time renges from 50.4 to 271 minutes. The *Annona squamosa* exerted the best protection time of 126.2 minutes [38-34] examination that the larvicidal potential of *Murraya exotica* essential oil against *A. aegypti, An. stephensi* and *Cx. quinquefasciatus*. After 12 h of introduction period, the larvicidal action are LC_{50}= 74.7 and LC_{90}= 152.7 ppm; after 24 h presentation period were LC_{50}= 35.8 and LC_{90}= 85.4 ppm, respectively against *A. aegypti*. The most noteworthy mortality was found in acetone extract against *Ae. aegypti* with LC_{50} and LC_{90} estimations of 4.1783 and 9.3884 g/L individually. Smoke poisonous quality was seen at 10 min interim for 40 min, and the mortality information was recorded [25]. The investigated that the larvicidal, ovicidal and repellent activity of *Polygala arvensis* benzene and methanol extracts tested against *A. aegypti, An. stephensi* and *Cx. quinquefasciatus* with maximum LC_{50} and LC_{90} values of methanol extract of *Polygala arvensis* were 58.21, 46.37 and 42.68 $\times 10^6$ 208.45, 189.82 and 130.44$\times 10^6$, respectively. The maximum ovicidal activity of methanol extracts against *A. aegypti, An. stephensi* and *Cx. quinquefasciatus* at

200 ppm concentration. The highest repellent activity of methanol extracts provided 100% protection against *A. aegypti, An. stephensi* and *Cx. quinquefasciatus* for 280 minutes[18]. Among the different extracts of the plants screened the hexane extract of *Pisonia alba*recorded the highest ovicidal activity of 79.2% at 500 ppm concentration against the eggs of *Cx. quinquefasciatus*. Among the *Aegle marmelos, Limonia acidissima, Sphaeranthus indicus, Sphaeranthus amaranthaides* and *Chromolaena odorata* extract screened, the hexane extract of *Pisonia alba* noted the 100% oviposition deterrent activity at tested concentrations against *Cx. quinquefasciatus* and *A. aegypti* adult females [34]. The highest lethal activity was recorded against *Gnetum ula* extract in the experimental larvae of *An. stephensi* (LC_{50} = 82.86 ppm). Ovicidal activity revealed that *Spermacoce hispida* showed more than 50% activity against *A. aegypti, An. stephensi* and *Cx. quinquefasciatus*. Notably, at 200 ppm concentration of all the plants showed 100% ovicidal activity against *An. stephensi*, followed by *A. aegypti* and *Cx. quinquefasciatus*. The selected two plants, *Gnetum ula, Spermacoce hispida* extract offers 100% protection against *An. stephensi, A. aegypti* and *Cx. quinquefasciatus* adult female mosquitoes as far as repellency up to 120 minutes of presentation periods[19]. Hence this study clearly reveals that the extracts of *S. indicum* seem to be made in phytochemicals, ovicidal and repellent activity, wide utilized in traditional drugs to combat and cure varied ailments [27-32]. The antispasmodic, anti-inflammatory, antidiuretic drug and antianalgesic is attributed to their high steroids, terpenoids, tannins and saponins and extraction of *S. indicum* has efficiency to manage the eggs and adults of the mosquito, *Cx. quinquefasciatus*.

In conclusion, the present study results that medicinal plant *Pisonia alba* have the potential for the development of new and safe control products and exhibits larvicidal and ovicidal activity against important mosquitoes. Furthermore, the results of the present study may donate to a diminution in the relevance of synthetic insecticides, which in turn increases the opening for nature control of various medically significant pests by botanical pesticides. Also, our results open the opportunity for further investigations of the efficacy of larvicidal, ovicidal and repellent activity of natural product extracts.

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Conflict of Interest:

The authors declare that they are no conflict of interest regarding this manuscript.

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Table 1. Phytochemical screening of plant extract of *Pisonia alba*

S.no	Phyto constituents	Methanol	Ethyl acetate	Acetone	Benzene
1	Alkaloids	--	+	+	--
2	Flavonoids	++	++	--	+
3	Saponins	+	--	--	--
4	Steroids	--	+	--	--
5	Tannins	+	--	+	--
6	Terpenoids	+	++	+++	++
7	Tri-terpenoids	++	+++	++	+
8	Anthraquinones	++	++	--	+
9	Amino acid	--	--	--	--
10	Phenol	+	--	+	--
11	Glycosides	--	--	--	--
12	Carbohydrate	--	+	+	--
13	Protein	+++	++	+	+
14	Phytosteroids	+++	+	--	+

"+++" Strongly positive phytochemical group, "++"Positive phytochemical group, "+"Trace phytochemical group, "-" Absence of phytochemical group

Table 2
Larvicidal activity of the *Pisonia alba* extracts against third instar larvae of *An. stephensi Cx. quinquefasciatus and Ae. aegypti.*

Species	Extracts	LC_{50} (ppm)	LCL-UCL	LC_{95}(ppm)	Slope	Regression
An. Stephensi	Petroleum ether	100.62[a]	97.62-103.71	117.80[a]	2.029896	Y=2.029x+0.508
	Acetone	107.41[c]	103.90-111.03	129.23[c]	2.16047	Y=2.160x-0.185
	Benzene	104.81[b]	101.47-108.25	124.96[b]	1.969739	Y=1.969x+0.358
	Hexane	111.52[d]	107.53-115.67	137.71[d]	2.424203	Y=2.424x-0.934
Cx. quinquefasciatus	Petroleum ether	98.52[a]	95.95-101.16	112.75[a]	2.274699	Y=2.274x+0.179
	Acetone	103.92[c]	100.58-107.37	123.92[c]	2.00234	Y=2.002x+0.358
	Benzene	101.03[b]	98.21-103.93	117.15[b]	2.159682	Y=2.159x+0.213
	Hexane	108.44[d]	104.65-112.36	132.45[d]	2.002549	Y=2.002x+0.106
Ae. aegypti	Petroleum ether	111.29[a]	106.80-115.98	141.16[a]	1.724065	Y=1.724x+0.609
	Acetone	122.48[c]	116.44-128.84	168.06[c]	1.852355	Y=1.852x+0.017
	Benzene	115.60[b]	110.77-120.64	149.13[b]	2.071039	Y=2.071x+0.300
	Hexane	127.76[d]	120.74-135.19	183.71[d]	1.626349	Y=1.626x+0.409

Value represents mean of five replications. Mortality of the after 24 h of exposure period LC_{50}= Lethal concentration brings out 50% mortality and LC_{95}= Lethal concentration brings out 95% mortality. LCL= lower confidence limit; UCL= upper confidence limit; values in a column with a different superscript alphabet are significantly different at $P<0.05$ level DMRT Test.

Table 3

Ovicidal activity of the *Pisonia alba* extracts against third instar larvae of *An. stephensi Cx. quinquefasciatus and Ae. aegypti.*

Species	Extract	Percentage of egg hatch ability					
		Concentration used (ppm)					
		60	120	180	240	300	360
An. stephensi	Petroleum ether	71.3±2.5[a]	53.8±2.5[a]	32.5±3.1[a]	11.6±2.3[a]	NH	NH
	Acetone	92.1±2.2[c]	71.5±2.3[c]	59.1±2.1[c]	38.6±2.5[c]	20.8±2.4[c]	8.6±2.3[c]
	Benzene	88.5±3.8[b]	67.8±3.0[b]	43.3±2.9[b]	21.6±2.6[b]	9.8±2.4[b]	NH
	Hexane	97.5±1.7[d]	78.1±2.5[d]	59.8±2.1[d]	43.5±1.7[d]	28.8±2.1[d]	16.3±1.8[d]
Cx. quinquefasciatus	Petroleum ether	66.1±2.7[a]	39.5±2.5[a]	10.5±2.1[a]	NH	NH	NH
	Acetone	89.3±1.6[c]	67.3±2.3[c]	54.1±2.9[c]	31.6±1.0[c]	12.1±1.9[c]	NH
	Benzene	80.1±2.0[b]	59.8±1.7[b]	31.3±2.0[b]	10.3±1.8[b]	NH	NH
	Hexane	94.5±1.7[d]	79.8±1.3[d]	61.5±2.0[d]	50.6±1.8[d]	34.3±2.3[d]	11.5±1.7[d]
Ae. aegypti	Petroleum ether	85.3±1.8[a]	60.8±2.4[a]	43.8±2.1[a]	25.8±1.6[a]	8.1±1.3[a]	NH
	Acetone	95.3±1.7[c]	77.8±2.5[c]	64.6±2.7[c]	42.8±2.3[c]	27.3±1.8[c]	12.6±1.7[c]
	Benzene	92.5±1.7[b]	76.1±2.1[b]	61.6±1.8[b]	43.6±1.9[b]	22.3±2.7[b]	5.6±1.5[b]
	Hexane	98.8±0.9[d]	82.3±1.6[d]	67.1±2.3[d]	34.5±2.3[d]	20.8±1.9[d]	10.5±2.1[d]

NH- No hatchability; values are mean of six replicates ±SD. of five replications. Different alphabets in the column are statistically significant at $P \leq 0.05$ level DMRT Test.