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Larvicidal and repellent properties of *Adansonia digitata* against medically important human malarial vector mosquito *Anopheles stephensi* (Diptera: Culicidae)

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ABSTRACT

Background & objectives: Development of plant-based alternative compounds for mosquito control has gained importance now-a-days, in view of increasing resistance in mosquito vectors to existing insecticides. The larvicidal and repellent activities of benzene, chloroform, hexane and methanol leaf extracts of Indian medicinal plant, *Adansonia digitata* were investigated against malarial vector, *Anopheles stephensi.*

Methods: In all, 25 III instar larvae of *An. stephensi* were exposed to various concentrations (30–180 mg/l) in the laboratory by using the standard protocol described by WHO (2005). The larvae were exposed for 24 h and mortalities were subjected to log-probit analysis. Repellent activity of crude leaf extract at the dosages of 2, 4 and 6 mg/cm^2 was evaluated in a net cage (45 × 30 × 45 cm) containing 100 blood starved female mosquitoes of *An. stephensi* using the protocol of WHO (1996).

Results: Preliminary phytochemical analysis of *A. digitata* showed the presence of triterpenoids and saponins. The LC_{50} and LC_{90} values of hexane, benzene, chloroform, and methanol extracts of *A. digitata* against *An. stephensi* larvae in 24 h were 111.32, 97.13, 88.55, 78.18 and 178.63, 176.19, 168.14, 155.42 mg/l, respectively. The repellent activity of methanol extract was found to be most effective and at higher concentration of 6 mg/cm^2 benzene, chloroform hexane and methanol extracts provided 100% protection up to 150, 180, 120 and 210 min against *An. stephensi*, respectively.

Conclusion: The preliminary study indicated that *A. digitata* showed larvicidal and repellent activities against *An. stephensi* and could be used for controlling mosquitoes. Further studies are indicated to purify the active compounds from these plants for developing larvicide and repellents.

Key words *Adansonia digitata*; *Anopheles stephensi*; larvicidal activity; phytochemistry; repellent activity

INTRODUCTION

Insect-transmitted diseases remain a major cause of illness and death worldwide[1]. Vector and vector-borne diseases have become a challenging problem to public health in these days as it has social and economical impact especially in subtropical and tropical countries[2,3]. Mosquitoes are vectors of several diseases affecting humans and domestic animals worldwide. Mosquitoes are the major vectors for the transmission of malaria, dengue fever, yellow fever, filariasis, Japanese encephalitis, *etc*., causing millions of deaths every year[4]. Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema[5]. *Anopheles stephensi* is the primary vector of malaria in India and other west Asian countries and malaria remains one of the most prevalent diseases in the tropical world. With 200 to 450 million infections annually worldwide, it causes up to 2.7 million deaths. The disease remains endemic in more than 100 developing tropical countries, and its control is a major goal for improved worldwide health. On a global scale, malaria causes 300–500 million cases and results in 1.5–3 million deaths annually. In India, malaria is one of the most important causes of direct or indirect infant, child, and adult mortality. About 2 million confirmed malaria cases and 1000 deaths are reported annually, although 15 million cases and 20,000 deaths are estimated by WHO South East Asia Regional Office. India contributes 77% of the total malaria cases in southeast Asia[6].

Insect pests have mainly been controlled with synthetic insecticides in the last 50 years. However, one major drawback with the use of these chemical insecticides is that these are non-selective and could be harmful to other organisms in the environment[7]. Plant products have been used traditionally by human communities in many parts of the world against the vectors and the species of insects. The

phytochemicals derived from plant sources can act as larvicides, insect growth regulators, and repellents[8-10]. Arivoli *et al*[11] reported the larvicidal activity of seven plant extracts against the III instar larvae of *An. stephensi.*

Adansonia digitata belongs to the family Bombacaceae, which has traditionally been used as immunostimulant[12], anti-inflammatory, analgesic[13] and pesticide[14]. In addition, antibacterial, antiviral and antitrypanosomal activities of the plant extracts have been reported[15–19]. Insecticidal and repellency effects of *A. digitata* leaves against *An. gambiae* have been first time studied in Nigeria by Denloye *et al*[20]. Hence, the present study was undertaken to assess the mosquitocidal activities with special reference to larvicidal and repellent potential of the extracts from the *A. digitata* against malaria vector, *An. stephensi*.

MATERIAL & METHODS

Plant material

Plant sampling was carried out during the growing season of (January–June 2011) from different places of Rangapuram village (located in 12°54′40″ N 79°8′10″E), Vellore district, Tamil Nadu. Bulk samples were air-dried in the shade and after drying, these were ground to fine powder. At the time of collection, two pressed voucher herbarium specimens were prepared and identified with the help of Plant Taxonomist, Department of Botany, Govt. Arts College, Nandanam, Chennai, whenever possible, flowering or fruiting specimens were collected to facilitate taxonomic identification.

Extraction method

The dried leaves (100 g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with benzene, chloroform, hexane and methanol (500 ml, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure of 22–26 mm Hg at 45°C by 'Rotavapour' and the residue obtained was stored at 4°C in an amber vial.

Mosquito rearing

The *An. stephensi* mosquitoes were reared in the Department of Zoology, Govt. Arts College, Nandanam, Chennai. The larvae were fed on dog biscuits and yeast powder in 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at 28 ± 2°C, 70–85% relative humidity (RH), with a photo period of 14 h light : 10 h dark.

Phytochemical analysis

Qualitative analyses of the phytochemicals present were carried out using methods described by Harbone[21].

Larvicidal activity

The larvicidal activity of crude extract was evaluated as per the protocol previously described by WHO[22]. From the stock solution, six different test concentrations (30, 60, 90, 120, 150 and 180 mg/l) were prepared and tested against the freshly moulted (0–6 h) III instar larvae of *An. stephensi*. The test medium (500 ml plastic cups) was prepared by adding 1 ml of appropriate dilution of test concentrations and mixed with 249 ml of dechlorinated water to make up 250 ml of test solution. The larvae were fed with dry yeast powder on the water surface (50 mg/l). The control (without plant extracts) experiments were also run parallel with each replicate. For each experiment, six replicates were maintained at a time. A minimum of 25 larvae per concentration was used for all the experiments. The larval mortality was observed and recorded after 24 h post-treatment. The LC_{50} value was calculated by using probit analysis[23].

Repellent activity

The repellent study was done following the method of WHO[24]. Blood-starved, 3–4 days old female of selected mosquitoes (n=100) were kept in a net cage (45 × 30 × 45 cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of the test person were cleaned with isopropanol. After air-drying the arm only 25 cm^2 of the dorsal side of the skin on each arm was exposed, the remaining area being covered by rubber gloves. The plant extract was dissolved in isopropanol and this alcohol served as control. The leaf extract at 2, 4 and 6 mg/cm^2 concentration was applied. These concentrations were derived after thorough analysis in preliminary wide range test. Subsequently, in narrow range test, the above concentrations were confirmed as effective concentration to assass repellent activity of *A. digitata.*

The control and treated arms were introduced simultaneously into the cage. The number of bites were counted over 5 min every 30 min. The experiment was conducted five times. It was observed that there was no skin irritation from the plant extract. The percentage protection was calculated by using the following formula:

$$\% \text{ Repellency} = [(T_a - T_b)/T_a] \times 100$$

Where, T_a is the number of mosquito bites in the control group, and T_b is the number of mosquito bites in the treated group.

Statistical analysis

The larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit and chi-square values were calculated using the SPSS software package 17.0. Results with $p < 0.05$ were considered to be statistically significant.

RESULTS

Phytochemical analysis of *A. digitata* clearly showed the presence of triterpenoids and saponins in benzene and chloroform extracts whereas, hexane extract showed the presence of saponins alone. Besides, methanol extract of *A. digitata* showed the presence of steroids, alkaloids, saponins and tannin (Table 1). Larvicidal activity of benzene, chloroform, hexane and methanol crude leaf extracts of *A. digitata* are shown in Table 2. As evidenced from the table, generally increased larval mortality was observed with increased concentration of the extracts tested against *An. stephensi*. Above all, among the extracts tested against *An. stephensi* methanol extract showed strong larvicidal activity, with LC_{50} value of 78.18 ppm and LC_{90} value of 155.42 ppm. Chloroform extract showed LC_{50} and LC_{90} values of 88.55 ppm and 168.14 ppm respectively; benzene extract showed LC_{50} and LC_{90} of 97.13 and 176.16 ppm respectively; and hexane extract showed LC_{50} and LC_{90} of 111.32 and 178.63 ppm respectively. Data pertaining to the repellent activity of four solvent extracts of *A. digitata* are shown in Table 3. Benzene extract provided complete protection (100%) up to 150 min at 4 and 6 mg/cm^2. Cent percent repellency of *An. stephensi* was noticed up to 180 min with chloroform extract at all concentrations. But, hexane extract showed 100% repellency up to 120 min, whereas methanol extract showed strong repellency up to 210 min except the minimal concentration (2 mg/cm^2).

Table 1. Phytochemical analysis of various extracts of *A. digitata*

Phytochemicals	Benzene	Chloroform	Hexane	Methanol
Steroids	–	–	–	+
Triterpenoids	+	+	–	–
Alkaloids	–	–	–	+
Saponins	+	+	+	+
Tannin	–	–	–	+
Flavonoids	–	–	–	–
Quinone	–	–	–	–

+ indicate presence; and – indicate absence.

Table 2. Larvicidal activity of crude extracts of *A. digitata* against *An. stephensi*

Extracts	LC_{50} (mg/l)	95% confidence limits (mg/l)		LC_{90} (mg/l)	$\div^2$
		LCL	UCL		
Hexane	111.32	94.56	131.19	178.63	22.450
Benzene	97.13	88.53	108.83	176.16	11.616
Chloroform	88.55	72.41	108.55	168.14	15.294
Methanol	78.18	62.16	97.86	155.42	22.827

LCL—Lower confidence limit; UCL—Upper confidence limit.

DISCUSSION

Our results showed that crude extracts of *A. digitata* have significant larvicidal and repellent activities against *An. stephensi* mosquitoes. The leaf extract of *Citrullus vulgaris* with different solvents was tested for larvicidial and repellent activities against *An. stephensi*. The LC_{50} values were 18.56, 48.51, 49.57 and 50.32 ppm respectively[25]. Insecticidal and repellency effects of *A. digitata* leaves against *An. gambiae* have been first time studied in Nigeria. According to Denloye *et al*[20], pellets were made from pulverized waste wood, palm-kernel cake, dried "Kuka" (*A. digitata*) leaves and d-allethrin 90 EC and different pellets were prepared. All the pellets grades caused mortality of *An. gambiae* and *Musca domestica* when produced smoke. The hexane extract of *Andrographis paniculata* was more effective in exhibiting the repellent action against the mosquitoes as compared with *A. lineate* extract and the complete protection was observed for 150 min in hexane extract of *A. paniculata* at 500 ppm against *Cx. tritaeniorhynchus* bites[26]. Phukan & Kalita[27] reported that *Litsea salicifolia* recorded 70 and 50% repellency for 3 and 4 h, respectively against *Ae. aegypti* but failed to show much activity against *Cx. quinquefasciatus.* The hexane extract at 2000 ppm exhibited 70% repellent activity for 3 h and 50% activity for 4 h against *Ae. aegypti* and 46% activity for 3 h against *Cx. quinquefasciatus.* Karunamoorthi *et al*[28] have also reported that the leaves of *Echinops* sp (92.47%), *Ostostegia integrifolia* (90.10%), and *Olea europaea* (79.78%) were also effective and efficient to drive away mosquitoes and the roots of *Silene macroserene* (93.61%) exhibited the significant repellency by direct burning. Traboulsi *et al*[29] showed that extracts of *F. vulgare* leaves were toxic against *Cx. pipiens* larvae, and terpineol and 1,8-cineole were the most effective components in repellency tests. Arunachalam & Kadarkarai[30] reported that *Toddalia asiatica* leaf extracts has larvicidal activity against all the larvae and pupae of *Ae. aegypti* with LC_{50} and LC_{90} ranging from 47.90 to 61.28 and 93.98 to 116.22 ppm, respectively. This is the

Table 3. Repellent activity of crude extracts of *A. digitata* against *An. stephensi*

Extracts	Concentration (mg/cm^2)	% Repellency							
		Time post-application of repellent (min)							
		30	60	90	120	150	180	210	240
Benzene	2	100±0	100±0	100±0	100±0	96.3±1.2	76.2±1.3	64.5±1.6	58.3±1.4
	4	100±0	100±0	100±0	100±0	100±0	89.6±1.7	82.3±1.4	73.4±1.9
	6	100±0	100±0	100±0	100±0	100±0	98.4±1.5	95.2±1.2	87.6±1.6
Chloroform	2	100±0	100±0	100±0	100±0	100±0	100±0	81.5±1.5	62.3±1.7
	4	100±0	100±0	100±0	100±0	100±0	100±0	94.2±1.7	78.2±1.3
	6	100±0	100±0	100±0	100±0	100±0	100±0	98.2±1.3	92.3±1.4
Hexane	2	100±0	100±0	100±0	100±0	84.6±1.7	63.3±2.8	51.5±1.3	38.6±1.7
	4	100±0	100±0	100±0	100±0	89.2±1.2	77.2±1.3	68.6±1.9	52.4±1.6
	6	100±0	100±0	100±0	100±0	97.6±1.5	88.3±1.4	82.5±1.2	71.3±1.4
Methanol	2	100±0	100±0	100±0	100±0	100±0	100±0	88.2±1.3	75.4±1.2
	4	100±0	100±0	100±0	100±0	100±0	100±0	100±0	84.6±1.1
	6	100±0	100±0	100±0	100±0	100±0	100±0	100±0	96.3±1.7

Each value (mean ± S.D.) represents mean of six values.

first hand report on the mosquitocidal activities of the different solvent leaf extracts of *A. digitata* against the selected mosquitoes. Further, purification, characterization and structural elucidation of the bioactive fraction of *A. digitata* are underway in our laboratory.

CONCLUSION

An attempt has been made to evaluate the role of *A. digitata* extracts for their larvicidal and repellent bioassay against *An. stephensi*. The results reported in this study open the possibility for further investigations of the efficacy of larvicidal and repellent properties of natural product extracts as a potential agent for combating mosquitoes.

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