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Original Research Article

Phytochemical Screening and Repellent Activity of Leaf Extracts of *Ocimum basilicum* and *Albizia amara* against the Mosquito *Culex quinquefasciatus*

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ABSTRACT

Background: In recent years synthetic insecticides in mosquito control resulted in environmental hazards through persistence and accumulation of non-biodegradable chemicals in ecosystem, development of insecticide resistance among vector species and toxic effect in human health and non target organisms. These developments demand renewed alternative insecticidal agents with high biocontrol potentiality but cause little or no harmful effect to environment and human health. In accordance the present study evaluated the repellent activities of solvent extracts of *Ocimum basilicum and Albizia amara* leaves on *Culex quinquefasciatus* mosquitoes.
Methods: The repellent activity of solvent extracts of leaves of *Ocimum basilicum* and *Albizia amara* tested against the adult females of mosquito *Culex quinquefasciatus*. One hundred female mosquitoes which were starved for three days were kept on net cage and used for repellent bioassay using methods of World Health Organization. The repellency was determined at three concentration viz., 1.0, 2.5, 5.0 mg/cm^2 under the laboratory conditions.
Results: In the present study at higher concentration of 5.0 mg/cm^2, acetone extract of *O. basilicum* provided 100% protection throughout the study period and when tested showed the presence of phytochemical viz., alkaloids, flavonoids, sterols, terpenoids, phenols, tannins and proteins. In case of *A. amara* at higher concentration of 5.0 mg/cm^2, chloroform leaf extract provided 100% protection up to 150 minutes and revealed the presence of phytochemical viz., alkaloids, flavonoids, sterols, anthraquinones, phenols, tannins and quinones.
Conclusion: It may be concluded that, the repellency of different solvent extracts mat be attributed to the phytochemicals present in the solvent extracts.
Keywords: Repellent activity; Plant extracts; *Culex quinquefasciatus*; Mosquito; Phytochemical; *Ocimum basilicum*; *Albizia amara*

INTRODUCTION

Mosquito-borne disease such as malaria, dengue hemorrhagic fever, encephalitis and filariasis are the major sources of illness and death worldwide, particularly in tropics. Malaria represents 9% of the total disease burden. The major region affected by the disease is Sub - Saharan, Africa, where at least 90% of the deaths are reported. Furthermore, an estimated 300-500 million cases of infection are reported every year. It also contributes to 25-40% of all patient visits and 20 -50% of hospital admission and about 20% deaths of children under the age of 5 years at an average of one child death in every 30 seconds. The cost of its control is estimated at US$1.8 billion every year. [1]

The use of chemical insecticides in controlling mosquitoes has been

encountered by many problems due to detrimental hazards of organic synthetic pesticides to human, domestic animals, wildlife and the environment. [2] In addition to adverse environmental effects from conventional insecticides, most mosquitoes and other pest species has become physiologically resistant to many of these compounds. [3] These problems have highlighted the need for the development of new strategies for selective mosquito control. Target - specificity, non development of resistance, reduced number of applications, higher acceptability, and suitability for rural areas. [4]

Repellency is known to play an importance role in preventing the insecticides used for control of vectors are causing irreversible damage to the eco system. [5] Most common chemicals that have historically been used as mosquito repellents include dimethyl phthalate (DMP), 2-ethyl-1,3-hexanediol, dimethyl carbonate, benzyl benzoate, butyl 3,4-dihydro-2,2- dimethyl-4-0x0-2H-pyran-6-carboxylate and N,N-diethyl-mtoluarnide (DEET), that is the most widely used, effective and available mosquito repellent. Several reports on DEET toxicity, citing encephalopathy in children, anaphylaxis, urticaria syndrome, and hypotension, have intensified the initiative for developing alternative insect repellents. [6]

In recent years, many botanical insect repellents have become available on the market. However, repellent effects of plants products and synthetic repellents are more or less similar in both efficacy and duration. Although the plants have been reported to have insect repellent properties, there is no substantive data to indicate their level of effectiveness and the concentration that offer complete protection from mosquito bites. There has been a paradigm shift towards botanicals to overcome the problems associated with the use of synthetic compounds. [7] In accordance, the present investigation was aimed to investigate the mosquito repellent activities of different solvent extracts of *Ocimum basilicum* and *Albizia amara* leaf extracts against the adults of female mosquitoes *Culex quinquefasciatus.*

MATERIALS AND METHODS

Origin and laboratory maintenance of the mosquito colonies

Mosquitoes used in study were *Culex quinquefasciatus*. Individuals were reared for several generations in the Department Laboratory by Hay infusion method under standard conditions.

Adult *Cx. quinquefasciatus* mosquitoes obtained from laboratory colony were maintained at 28±2° C, 70% - 85% relative humidity with a photo period of 14:10 light and dark photo period cycle. Larvae were fed with dog biscuits and yeast powder in the ratio 3:1. Adults were provided with 10% sucrose solution and the three days blood starved female mosquitoes were used for repellent bioassay.

Collection of test materials and preparation of leaf powder

Fully developed fresh leaves of the plant *P. amboinicus* were collected from natural habitat of Coimbatore locale. Fresh leaves were collected, washed in water and left to shade dry at room temperature for 2 to 3 weeks and finely powdered separately using an electric pulverizer. These powders were subjected to extraction. [8-9] Chloroform (60-80°C) extraction was followed by acetone and ethanol extraction, so that the powders were subjected to extraction with solvents in the order of increasing polarity. The leaf extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath. The residue thus obtained was used for further bioassays.

Repellent Bioassay

The repellent study was following the method of WHO. [10] Three day old blood starved female *Cx. quinquefasciatus* (100) were introduced in a net cage (45cm × 30 cm × 45 cm) during each trail. Isopropanol was used to clean the arms of the test person. After drying, only 25 cm^2 dorsal side of the skin on each arms was

exposed and remaining area covered by rubber gloves. The leaf extract was dissolved in isopropanol and was applied at 1.0, 2.5 and 5.0 mg/cm^2, separately in the exposed area of the forearm. The arm treated with ethanol served as control. The observation period was 30, 60, 90, 120, 150 and 180 minutes. The test person conducted the test by inserting the control and treated arms simultaneously into the same mosquito cage for one full minute for every five minutes. Mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood making out a 5 minute protection from 17.00 hrs to 20.00 hrs. Each experiment was conducted five times. It was observed that there was no skin irritation or no signs of allergy from the application of leaf extracts. The percentage of repellency was calculated by the following formula.

% Repellency = [(Ta – Tb) / Ta] ×100

Where Ta is the number of mosquitoes in the control group and Tb is the number of mosquitoes in the treated group.

Statistical analysis

The data on bioassay studies were also subjected to statistical analysis. Standard deviation was calculated for the data which was obtained from the test for repellency against *Cx. quinquefasciatus* mosquito. Each value (Mean±Standard Deviation) represents average of three replications.

Phytochemical Screening

The preliminary qualitative phytochemical analysis has been attempted in *P. amboinicus* leaf extracts to find out the presence or absence of certain bioactive compounds. The preliminary screening was carried out by using standard procedures.

Test for Alkaloids

- **Mayer's test:** [11] One ml of extract was treated with a drop or two of Mayer's test reagent along the sides of test tube and observed for the formation of white or cream coloured precipitate.
- **Wagner's test:** [12] One ml of extract was treated with Wagner's reagent along the sides of the test tube and observed for the formation of reddish brown colour precipitate.
- **Hager's test:** [13] One ml of extract was treated with 1 or 2 ml of Hager's reagent and observed for the formation of prominent yellow precipitate.

Test for Tannins

- Ferric chloride test: [14] 0.5 g extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate, and observed for the blue-black, green or blue-green precipitate.

Test for Phenols

- **Ferric chloride test:** [15] The extract (50mg) was dissolved in 5 ml of distilled water and treated with few drops of 5% ferric chloride and observed for the formation of dark green colour
- **Lead acetate test:** [16-17] The extract (50 mg) was dissolved in 5 ml of distilled water and 3 ml of 10% lead acetate solution was added and observed for the formation of bulky white precipitate.

Test for Flavonoids

- **NaOH test:** [14] 1 mg of extract was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.
- **Lead acetate test:** [16-17] Test extract (50 mg) was taken in a test tube and few drops of lead acetate solution was added to it and observed for yellow coloured precipitate.

Test for Sterols

- **Liebermann-Burchard test:** [18] The extract (50 mg) is dissolved in 2 ml of acetic anhydride. To this one or two drops of Conc. $H_2SO_4$is added along the side of the test tube and observed for an array of colour changes.

Test for Terpenoids

- **Liebermann-Burchard test:** [19] 50 mg of extract was dissolved in ethanol. To it

1 ml of acetic anhydride was added followed by the addition of Conc. H_2SO_4. A change in colour from pink to violet showed the presence of terpenoids.

Test for Saponins

- **Foam Test:** The extract (50 mg) or dry powder was diluted with distilled water and made up to 20 ml. The suspension is vigorously shaken in a graduated cylinder for 15 minutes and observed for the formation of 2 cm layer thick foam.

Test for Anthraquinones

- **Borntrager's test:** [19] 0.2 g of extract to be tested was shaken with 10 ml of benzene and then filtered. 5 ml of the 10% ammonia solution was then added to the filtrate and thereafter shaken and observed for the appearance of a pink, red or violet colour in the ammoniacal (lower) phase.

Test for Proteins

- **Ninhydrin test:** [20] Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added to 2 ml of aqueous filtrate and observed for the presence of characteristic purple colour.
- **Biuret test:** [21] An aliquot of 2 ml of filtrate is treated with one drop of 2% copper sulphate solution. To this 1 ml of 95% ethanol was added followed by excess of potassium hydroxide pellets and observed for the formation of pink ethanolic layer.

Test for Quinones

- **H_2SO_4 test:** [17] To 1 ml of extract, 1 ml of Conc. H_2SO_4 was added and was observed for the formation of red colour.
- **HCl test:** [22-23] To 1 ml of the extract 5 ml of HCl was added and observed for the presence of yellow colour precipitate

RESULT AND DISCUSSION

Different solvent extracts of leaves of the plant *Ocimum basilicum* and *Albizia amara* was effectively compared with each other. The results of the repellent activities of *O. basilicum* and *A. amara* leaf extracts against the female *Cx. quinquefasciatus* mosquito are summarized in the Table 1 & 2.

The repellent activity was very high at initial hours of exposure. In the present study at higher concentration of 5.0 mg/cm^2, acetone extract provided 100% protection throughout the study period. Even the lower concentration of 2.5 mg/cm^2 and 1.0 mg/cm^2 provided protection from mosquito bite up to 150 and 120 min respectively. Thus in the study maximum repellent activity was exhibited by acetone extract. Similar observation was reported by Govindarajan *et al* [24] whose findings showed that crude extract of *Sida acuta* showed significant repellent activity against *Cx. quinquefasciatus*, at a higher concentration of 5.0 mg/cm^2 providing 100 % protection up to 120 minutes. The repellent efficacy of acetone leaf extract was followed by chloroform leaf extract which provided 100 % protection up to 150 minutes at a higher concentration of 5.0 mg/cm^2 followed by 2.5 mg/cm^2 that which provided a protection up to 90 min against adults of *Cx. quinquefasciatus*. Thereafter in both the concentrations the repellent activity was noted to fall down gradually with increase in time. Increase in the exposure period showed reduction of the repellent activity.

Swathi [25] reported that the ethanolic extract of the *Pongamia pinnata* leaves provided complete protection of 99.96, 141.35 and 144.73 minutes against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* mosquitoes. The control did not provide any protection even during in the first trial. Parallel to this in the present study ethanol extract of *O. basilicum* leaf at higher concentration of 5.0 mg/cm^2 which provided protection from bites of adults of *Cx. quinquefasciatus* up to 90 min. Thereafter the repellency percentage fell down by 3.34 % thereby providing a protection of 96.66 %. The concentration of 2.5 mg/cm^2 provided repellency only up to 60 minutes.

Table 1: Repellent activity of *Ocimum basilicum* leaf extracts against adults of *Cx. quinquefasciatus*

Solvent used	Conc in mg/cm^2	% of repellency					
		30 min	60 min	90 min	120 min	150 min	180 min
Control	-	0±0	0±0	0±0	0±0	0±0	0±0
Acetone	1.0	100±0.0	100±0.0	100±0.0	100±0.0	99±0.81	98.33±0.47
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	97.66±0.49
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0
Ethanol	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0
	2.5	100±0.0	100±0.0	98.66±0.47	97.66±0.47	96.66±0.81	95±0.81
	5.0	100±0.0	100±0.0	100±0.0	96.66±0.81	96.33±0.47	95.33±0.47
Chloroform	1.0	100±0.0	100±0.0	100±0.0	96.66±0.47	95.33±0.47	93±0.0
	2.5	100±0.0	100±0.0	100±0.0	99±0.81	98.33±0.49	97.66±0.49
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	95.33±0.47

Each value ($\chi \pm$ SD) represents average of three values

Table 2: Repellent activity of *Albizia amara* leaf extracts against adults of *Cx. quinquefasciatus*

Solvent used	Conc in mg/cm^2	% of repellency					
		30 min	60 min	90 min	120 min	150 min	180 min
Control	-	0±0	0±0	0±0	0±0	0±0	0±0
Acetone	1.0	100±0.0	100±0.0	99.33±0.47	99±0.81	98.33±0.47	97.33±0.47
	2.5	100±0.0	100±0.0	100±0.0	99.33±0.47	99±0.81	97.66±0.47
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	99.0±0.47	98.66±0.47
Ethanol	1.0	100±0.0	99.66±0.81	99.33±0.47	99±0.81	97.66±0.47	96.66±0.47
	2.5	100±0.0	100±0.0	99.33±0.81	97±0.81	96±0.47	95.33±0.47
	5.0	100±0.0	100±0.0	100±0.0	97.66±0.47	95.33±0.47	94.33±0.47
Chloroform	1.0	100±0.0	100±0.0	100±0.0	99±0.81	99.33±0.47	98.33±0.47
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	99±0.81	99.33±0.47
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	94.33±0.47

Each value ($\chi \pm$ SD) represents average of three values

In the present study, higher concentration of 5.0 mg/cm^2, chloroform leaf extract of *A. amara* provided 100% protection up to 150 minutes and at 2.5 mg/cm^2 the repellency percentage was recorded as 100% up to 120 minutes against *Cx. quinquefasciatus*. The repellent activity was therefore noted to be dose dependent. The repellent efficacy of chloroform leaf extract was followed by acetone leaf extract. Mandal [26] and Dhivya and Manimegalai [27] has also observed that the repellent action of plant extract depends on the dose rate and nature of the solvent used and exposure time.

Acetone extract of *A. amara* leaf provided moderate repellency thereby providing 100% protection up to 120 minutes at a higher concentration of 5.0 mg/cm^2. Repellency was noted to be 1% less in the following exposure period of 150 minutes. At 2.5 mg/cm^2 and 1.0 mg/cm^2 showed 100 % repellent activity up to 90 minutes and 60 minutes respectively. Repellency percentage was found to be inversely proportional to the exposure period of the solvent extract. In accordance Dhivya and Manimegalai [28] stated that ethanol extract of *C. gigantea* flower produced 100% protection against *Cx. quinquefasciatus* mosquito up to 90 min, even at the lower concentration of 1.0 mg/cm^2. The repellency percentage was found to fall down thereafter as the exposure period increased. Similar to this, in the present study, at 2.5 mg/cm^2 and 1.0 mg/cm^2 100 % repellent activity was recorded up to 90 minutes and 60 minutes respectively.

The results of phytochemical analysis of *O. basilicum* leaf extract are presented in Table 3. The qualitative phytochemical analysis of acetone extract of *O. basilicum* showed the highest repellent activity against the mosquito and when tested for the phytochemicals showed the presence of 7 phytochemicals viz., alkaloids, phenols, terpenoids, sterols, tannins, flavonoids and proteins. The chloroform extract of the *O. basilicum* extract that showed the moderate repellency against the adults of mosquito of *Cx. quinquefasciatus* was analysed for the presence of phyto constituents and revealed the presence of the following phytochemical constituents such as alkaloids, sterols,

phenols, saponins and proteins. The repellency of the extract can be attributed to the phytochemical constituent and were retained for further studies. Similar observations were recorded by Muhammed and Umair [29] in which potential repellent activity and maximum protection was shown by *Stachytarpheta indica* extracts against *Aedes aegypti* mosquitoes. Plants display great protection against mosquito bites and the repellent activities of botanicals on mosquitoes have been reported by several authors.

Table 3: Phytochemicals present in *Ocimum basilicum* and *Albizia amara* leaf extracts

Sl. No.	Constituents	*Ocimum basillicum* leaf			*Albizia amara* leaf		
		Acetone	Chloroform	Ethanol	Acetone	Chloroform	Ethanol
1	Alkaloids	+	+	-	+	+	-
2	Flavonoids	+	-	-	+	+	-
3	Sterols	+	+	-	-	+	-
4	Terpenoids	+	-	+	+	-	-
5	Anthraquinones	-	-	-	-	+	+
6	Phenols	+	+	+	-	+	+
7	Saponins	-	+	+	+	-	-
8	Tannins	+	-	-	-	+	+
9	Proteins	+	+	-	+	-	+
10	Quinones	-	-	+	-	+	+

"+" Presence "-" Absence

In the present study the chloroform extract of *A. amara* that showed the highest repellency activity against the *Cx. quinquefasciatus* mosquito revealed the presence of 7 phytochemical viz., alkaloids, flavonoids, sterols, anthraquinones, phenols, tannins and quinones. The acetone extract of the *A. amara* leaf presented only moderate repellency against *Cx. quinquefasciatus* and was investigated for the presence of following phytochemicals viz., alkaloids, flavonoids, terpenoids, saponins and proteins. Similarly Selvamani and Balamurugan [30] revealed that leaves of *Acalypha indica* reported the presence of alkaloids, terpenoids, flavonoids and phenolic compounds in its acetone leaf extract. In harmony to this in the present study acetone extract of *A. amara* leaf showed the presence of secondary metabolites such as phenols, tannins, quinones and proteins when screened using standard protocols.

CONCLUSION

The extracts of *O. basilicum* and *A. amara* was confirmed to have a broad mosquito repellent and should be employed as insect repellent because it was found to be non-toxic to the human volunteers. However, further investigations leading to identification of the repellent activity is recommended. Formulations like this could help in reducing the harmful effects of synthetic mosquito repellents on human health. It may be concluded from the results of the study that medicinal plants can be used alone or combined for effective protection against mosquito bites and also can be used for control of mosquito breeding under integrated vector control programme in various situations. They also offer alternative to synthetic chemicals and can be easily obtained by individuals and communities at a very low cost.

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