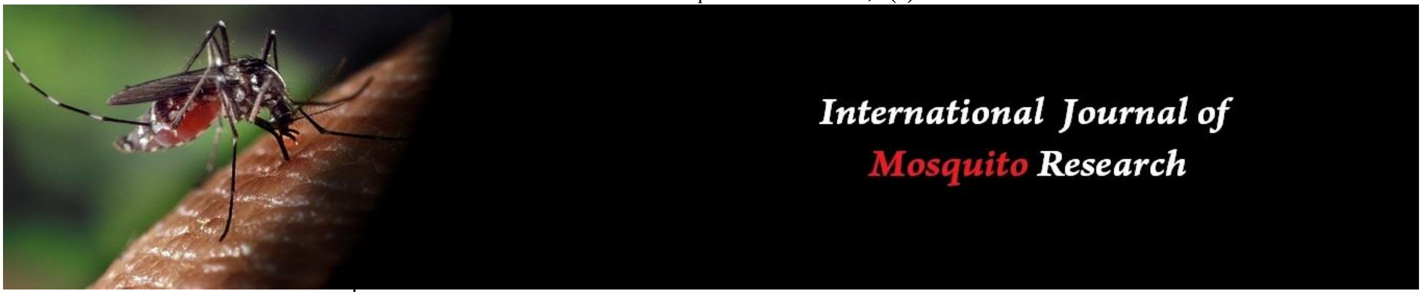

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Screening of phytochemical composition, Ovicidal and repellent activity of leaf extracts of *Sphaeranthus indicus* and *Caesalpinia pulcherrima* against the mosquito *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract
Mosquitoes contribute to main disease burden in India as they are main causative agent of many vector-borne diseases. Various methods are now being used to control the vectors that might help to stop the disease spread. In this regard, the current study was carried out to analyze the phytochemicals, ovicidal and repellent activities of acetone, chloroform and ethanol extracts of *Sphaeranthus indicus* leaves and *Caesalpinia pulcherrima* leaves against the vector *Culex quinquefasciatus*. The ovicidal potency was investigated at different concentrations ranging from of 50 ppm to 300 ppm under standard laboratory environment. The repellent activity was tested at the concentrations of 1.0 mg/cm^2, 2.5 mg/cm^2 and 5.0 mg/cm^2. The test to find out the phytochemicals present in the extract was done following the standard methods. According to the results, among the three extracts tested for their ovicidal potency, the *Sphaeranthus indicus* ethanol leaf extract showed excellent ovicidal potency and repellent potency against the vector mosquito *Culex quinquefasciatus*. Therefore, the extract was further subjected for phytochemical screening. The results of phytochemical screening showed the presence of alkaloids, phenols, sterols, terpenoids, flavonoids, tannins, and phytosterols.

Keywords: Ovicidal activity, repellent activity, phytochemical analysis, leaf extracts, mosquito

1. Introduction

Economic and social disturbances are increased due to the spreading of vector-borne diseases all across the planet. Mosquito borne diseases is said to be the main source of sickness and casualty all around the world [1]. Despite decades of efforts to control malaria, malaria continue to be the most important universal public health problem within the tropical and subtropical areas with 3.3 billion persons in danger in 106 nations [2]. Presently, no suitable preventive measure and no efficient vaccine, other than vector control is available [3]. The main targets for the disease eradicating programs are the vectors [4]. For the researchers in quest of drugs to control mosquito populations, the embryo stage and larval stages of mosquitoes are the main targets as they are present in confined spaces [5, 6, 7].

For mosquito control various chemical insecticides including pyrethroids, organophosphates and carbamates are available in the market. Synthetic pesticides usually are harmful to the environment and pollutes the planet and are harmful to untargeted organisms and are damaging to the ecosystem [5, 8, 9]. Other than this, the repeated use of harmful insecticide has caused the increase in resistance among the vector mosquito species [10, 5]. It has been reported that, against the mosquito controlling chemicals, permethrin and temephos, the larval stage mosquito of *Cx. quinquefasciatus* and *Ae. Aegypti* respectively has acquired resistance [11, 12]. Therefore, there's a necessity of combination of medicine and drugs to control population of mosquitoes.

Revival of mosquito growth and unfavorable after effects on non-targeted organisms are the main results of using chemical insecticides. The regular use of chemical repellents for the control of mosquito population has troubled the environment and resulted in the growth of resistance against the insecticides [13, 14].

In this regard, the thought of using herbal repellent products as a substitute to develop new eco-friendly repellents against the vector mosquito species could be an agreeable solution to balance the unwanted consequences on ecosystem and public health [15]. Therefore, protection against the bite of mosquito is the best technique to reduce the disease incidence.

Herbal insecticides are always useful in traditional practice throughout the generations, as a personal protection measure against several *Culex* species. Knowledge about the repellent plants may be a major resource for the development of new herbal products as a substitute to synthetic repellents. The side-effects of chemicals forced researchers to develop eco-friendly products for the management of mosquito. The literature shows sufficient research on the potential for mosquito control of plant extracts [16]. Due to the diverse chemistry of phytochemicals, their use is often a promising way to combat a variety of insect-borne diseases [17, 18, 19].

Recently, it has been reported that plant chemicals and plant solvent preparations isolated from plants have egg-killing or larval-killing activity against mosquitoes [20, 21]. In recent years, plant-based repellents have regained consciousness as they contain abundant resources of biodegradable bioactive phytochemicals as harmless, non-toxic by-products that can be studied for insecticidal and mosquito repellent properties. Numerous studies have been reported to confirm the repellent effect of plant extracts or essential oils on mosquito mediators around the world. The current systematic study was conducted to show which herbal repellents and ovicidal are reliable to provide long-term and predictable protection from mosquito species *Culex quinquefasciatus*.

2. Materials and Method

2.1 Laboratory maintenance of the mosquito colonies

Culex quinquefasciatus was the mosquito species used in the study. Individuals were reared in laboratory by Hay infusion method.

2.2 Collection of leaves and making of leaf powder and extracts

Leaves of the plant *Sphaeranthus indicus* and *Caesalpinia pulcherrima* were collected from rural habitats of Coimbatore locale, Tamil Nadu, India. Fresh leaves were collected, cleaned using water and shadow dried at room temperature for 2 to 3 weeks. It was powdered using electric mixer and fine powder was obtained by sieving.

Using balance 20 grams of the leaf powder was weighed and was subjected to extraction [22, 23]. Extraction was done using chloroform, acetone and ethanol in the order of their increasing polarity. By evaporation in a water bath, the leaf extract was dried and the residue obtained was used for further study.

2.3 Testing of ovicidal activity

Slightly modified method of Su and Mulla [24] was used to study the ovicidal activity. The eggs of *Cx. quinquefasciatus* were taken. The *Sphaeranthus indicus* and *Caesalpinia pulcherrima* leaf extracts were serially diluted in the different solvents to get concentrations from 50 ppm to 300 ppm. To each concentration of *Sphaeranthus indicus* and *Caesalpinia pulcherrima* leaf extracts 100 numbers of mosquito eggs were exposed.

The test was repeated along with control treatment three times. After 48 h of treatment hatch rates were observed and counting was done. The calculation was done using the given formula.

$$\text{Egg mortality percentage} = \frac{\text{Egg mortality in treatment} - \text{Egg mortality in control}}{100 - \text{Egg mortality in control}} \times 100$$

2.4 Testing of repellent activity

To study the repellent, the method of WHO [25] was used. Blood starved 3 day old 100 numbers, female *Culex quinquefasciatus* mosquito were introduced into cage for each experiment. The arm of the test person was cleaned using Isopropanol. After that, 25 cm^2 skin in the upper side of arms of the test person was exposed and rubber gloves was used to cover the other area of the arm. The concentrations *viz*., 1.0, 2.5 and 5.0 mg/cm^2 was applied individually in the uncovered part of the forearm. Isopropanol was used to serve as the control. The period of observation was 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes and 180 minutes. The experiment was performed by placing the treated and control arms at every five minutes in the mosquito cage for one minute. Mosquitoes that landed on the forearm were recorded before sucking blood. The experiment was replicated. From the application of leaf extracts, no skin irritation or allergy was observed. The repellency percentage calculation was done by given formula.

$$\text{Repellency percentage} = [(Ta - Tb) / Ta] \times 100$$

Ta = no. of mosquitoes in control &
Tb = no. of mosquitoes in treatment

2.5 Statistical analysis

The data on the repellent study was used to calculate standard deviation. Mean ± Standard Deviation is the value for three replicates.

2.6 Analysis of Phytochemicals in Extracts

Phytochemical analysis was done using standard protocols.

2.6.1 Alkaloids

- **Mayer's test [26]:** Along the sides of test tube, one ml of the extract was mixed with one or two drops of Mayer's reagent. White or cream coloured precipitate generation was observed.
- **Wagner's test [27]:** Through the sides of the test tube, Wagner's reagent was added and mixed with one millilitre extract and observed for the appearance of a reddish-brown precipitate.

2.6.2 Flavonoids

- **NaOH test [28]:** 1 ml of the extract was mixed in water and then 2 ml of 10% sodium hydroxide was noted for the formation for yellow colour. If colour changes from yellow to colourless with the addition of diluted hydrochloric acid, it means that flavonoids is present.
- **Lead acetate test [29, 30]:** 1 ml of lead acetate solution were mixed with 1 ml of the extract taken in a test tube and noted for the appearance of yellow precipitate.

2.6.3 Sterols

Liebermann-Burchard test [31]: Two millilitre of acetic anhydride and one or two drops of H_2SO_4 was mixed with 1ml of extracts taken in a test tube and noted for along the side of the test tube and observed for a series of colour changes.

2.6.4 Terpenoids

Liebermann-Burchard test [32]: Ethanol was used to dissolve 1 ml of the extract taken in a test tube. One millilitre of acetic anhydride and few drops of concentrated sulphuric acid was added to it and observed for the change of colour from pink to violet that shows the presence of terpenoids.

2.6.5 Anthraquinone
Borntrager's test [32]: 5 ml of benzene was mixed with 0.1 gram of extract and then filtered. To this filtrate, 3 ml of the 10% ammonia solution was added. The formation of violet, red or pink colour in the ammoniacal phase was checked.

2.6.6 Phenols
- **Ferric chloride test [33]:** One millilitre of water was added to 25 mg of extract and treated with few drops of 5% ferric chloride and noted for the appearance of dark green colour.
- **Lead acetate test [29, 30]:** 25 mg of extract was mixed with three millilitres of water and three millilitres of 10% lead acetate solution and observed for the appearance of white precipitate.

2.6.7 Saponins
Foam Test [34]: 25 mg of extract was diluted with water and made up to ten millilitres. It was then shaken for 15 minutes and observed for the development of thick foam.

2.6.8 Tannins
Ferric chloride test [28]: Two millilitres of the extract was dissolved in 5 ml of distilled water and filtered. To 2 ml of this filtrate add 1 ml of 1% ferric chloride solution, and checked for the blue-green, green or blue-black precipitate.

2.6.9 Cardiac Glycosides
- **Keller Kelliani's test [35]:** One milliliter of extract was taken in a test tube to that one milliliter of glacial acetic acid, 2 drops of ferric chloride solution and concentrated sulphuric acid was mixed. The presence of cardiac glycosides will be confirmed on the appearance of yellowish green colour.

2.6.10 Reducing Sugar
- **Fehling's test [36]:** Fehling's A and Fehling's B solution was mixed together and from that 2 ml was taken and mixed with 2 ml of extract and kept in water bath for at 40° C. presence of reducing sugars is confirmed by the appearance of brick red colour precipitate at the lower bottom of the test tube.

3. Results and Discussion
3.1 Test results of ovicidal activity
The acetone, chloroform and ethanol extracts of *Caesalpinia pulcherrima* and *Sphaeranthus indicus* were tested for their ovicidal efficacy. The leaf extracts were tested against the eggs of the mosquito *Cx. quinquefasciatus*. Ethanol extract of *S. indicus* displayed maximum ovicidal efficacy, at concentrations from 150 – 300 ppm in that egg hatchability was inhibited. Following that acetone extract of *S. indicus* exhibited moderate ovicidal activity providing non hatchability of eggs from concentration ranging from 200 to 300 ppm. A similar study was carried out by Rajiv Gandhi *et al*., [37] to check the ovicidal efficacy of solvent extracts of, *Elytraria acaulis, Rubia cordifolia, Scilla peruvina* and *Gymnema sylvestre* roots against the eggs of *Aedes aegypti* mosquito and *Culex quinquefasciatus* mosquito. Results displayed that when compared to other extacts, the most effective extract was *R. cordifolia* root methanol extract.

Table 1: Ovicidal activity of *C. pulcherrima* and *S. indicus* extracts

Plants	Solvents	Egg hatchability %						
		Control	50 ppm	100 ppm	150 ppm	200 ppm	250 ppm	300 ppm
S. indicus	Acetone	100.0±0.0	58.2±1.3	43.9±0.8	34.8±1.7	NH	NH	NH
	Chloroform	100.0±0.0	63.8±1.7	55.7±1.9	40.4±1.3	29.5±1.5	NH	NH
	Ethanol	100.0±0.0	51.6±0.8	32.2±1.3	NH	NH	NH	NH
C. pulcherrima	Acetone	100.0±0.0	65.8±1.5	56.1±0.8	44.4±1.5	37.2±1.7	NH	NH
	Chloroform	100.0±0.0	71.4±0.8	66.2±0.8	50.4±0.8	42.4±1.9	33.5±1.3	NH
	Ethanol	100.0±0.0	57.6±1.5	41.5±1.9	28.3±0.8	NH	NH	NH

The value (χ ±SD) = mean of replication; NH = Non hatchability or 100% death

The extracts of *C. pulcherrima* leaves when tested for its ovicidal activity showed that ethanol extract caused no hatchability of egg (100% mortality) at concentrations of 200, 250 and 300 ppm. Following that acetone extract showed non hatchability of eggs at the concentration of 250 and 300 pm. Thus, according to the results, *S. indicus* leaf ethanol extract showed more efficacy as an ovicide than any other extract of *C. pulcherrima*. Similar results were conducted by Reegan *et al.*, [38] in that ovicidal activities of solvent extracts of *Cliona celata* was tested in different concentrations and found that against *Cx. quinquefasciatus*, 100% activity was recorded in methanol extract, and against *Ae. Aegypti*, 72% ovicidal activity was recorded at 500 ppm. Another study by Gaurav *et al*., [39] showed the activity of leaves of *Calotropis procera* aqueous extract against *Culex tritaeniorhynchus* and *Culex gelidus*.

3.2 Test results of repellent activity
The results for the repellent activity test against the *Cx. quinquefasciatus* with the leaf extracts of *Sphaeranthus indicus* and *Caesalpinia pulcherrima* are given in Table 2 & 3 respectively. The repellent activities of *S. indicus* leaf extract tested at 5.0 mg/cm^2 concentration revealed that ethanol extract provided good repellency throughout the entire study. Protection time was seen to get reduced, when the concentration was reduced to 2.5 mg/cm^2, thereby providing 100% repellency up to 150 minutes of the study period. At low concentration a security till 120 minutes was obtained from mosquito bite.

Acetone extract at higher concentration (5.0 mg/cm^2) gave full protection from mosquito bite till 150 minutes of the study period and lower concentration (2.5 mg/cm^2) gave protection till 90 min of the study after that repellency percentage came down to 81.2%. Similar work was done by Effiom *et al*., [40] in that, extracts of five citrus fruit species was tested for its repellency. According to the study, extracts of citrus fruit species hold great promise for topical mosquito repellent in general.

Table 2: Repellency of *S. indicus* leaf extracts

Solvents	Conc. (mg/cm^2)	30 minutes	60 minutes	90 minutes	120 minutes	150 minutes	180 minutes
Acetone	1.0	100.0±0.0	100.0±0.0	88.4±0.6	73.2±1.4	68.3±1.2	61.3±1.9
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	81.2±0.7	74.7±1.2	66.5±1.8
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	74.4±0.9
Chloroform	1.0	100.0±0.0	100.0±0.0	82.2±1.7	76.7±1.4	68.3±1.3	62.9±1.4
	2.5	100.0±0.0	100.0±0.0	94.3.0±1.8	82.4±1.9	76.3±1.5	69.2±1.2
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.7±1.2	77.5±0.8
Ethanol	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	89.5±1.2	85.7±1.5
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.3±1.8
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

The value (χ ±SD) is the mean of replicates

The repellent activities of *C. pulcherrima* leaf extract against *Cx. quinquefasciatus* was tested at varying concentrations. The results revealed that at a high concentration, the ethanol extracts exhibited highest repellency by providing100% protection till 150 minutes of the study period. The extract at 2.5 mg/cm^2 gave security till 120 minutes and after which the repellency percentage came down to 90.6%. The lower concentration of 1.0mg/cm^2 only provided protection up to 90 minutes after which repellency was lowered to 92.6%. Similar study was carried out by Govindarajan and Sivakumar, [41] to analyse the repellent activity against *Anopheles stephensi* by *Andrographis paniculata* and *Eclipta Alba* leaves extracts. According to results, methanol extract was more potential.

Table 3: Repellency of *C. pulcherrima* leaf extracts

Solvents	Conc (mg/cm^2)	30 minutes	60 minutes	90 minutes	120 minutes	150 minutes	180 minutes
Acetone	1.0	100.0±0.0	100.0±0.0	79.3±1.7	71.7±1.9	65.4±0.9	60.8±1.7
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	80.2±0.9	72.7±1.1	65.2±0.5
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	80.1±1.4	69.1±0.2
Chloroform	1.0	100.0±0.0	89.8±1.7	80.7±1.2	74.5±2.3	66.2±1.0	61.6±2.0
	2.5	100.0±0.0	100.0±0.0	85.3±2.1	77.5±1.6	69.8±1.5	62.1±0.2
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	80.5±1.5	74.2±1.3	67.7±1.2
Ethanol	1.0	100.0±0.0	100.0±0.0	100.0±0.0	92.6±0.9	87.3±0.2	76.4±1.6
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	90.6±1.1	89.3±2.4
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.4.0±2.1

The value (χ ±SD) is the mean of replicates

Acetone leaf extract showed the moderate repellent activity showing 100% security from bite of mosquito at 5.0 mg/cm^2 concentration until 120 minutes of the experiment. When the concentration was reduced to 2.5 mg/cm^2 the repellency was seen only up to 90 minutes of the study there after the repellency came down to 80.2% at 120 minutes. Therefore, it's noted that, *S. indicus* ethanol extract showed excellent ovicidal and repellent activity.

3.3 Screening of Phytochemicals in leaf extract

Phytochemical analysis is carried out in ethanol extracts of *S. indicus* leaves that displayed highest ovicidal and repellent activity. The result of phytochemical analysis of *S. indicus* leaf extract is presented in Table 4.

Table 4: Phytochemicals in *Sphaeranthus indicus* leaf ethanol extract

Sl. No.	Constituents	Leaf Extract of Acetone
1	Alkaloids	+
2	Phenols	+
3	Sterols	+
4	Cardiac Glycosides	-
5	Anthraquinones	-
6	Flavonoids	+
7	Saponins	-
8	Terpenoids	+
9	Tannins	+
10	Reducing sugar	+

"+" Presence "-" Absence

Ethanol leaf extract of *S. indicus* leaves displayed good repellency in the study and displayed the presence of flavonoids, alkaloids, phenols, terpenoids, sterols, tannins, and reducing sugar. Similar results were noted by Idris *et al*., [42] in that screening of phytochemicals and repellent activity was done in *Euphorbia balsamifera* stem bark extracts. Results revealed that the chloroform extract had good repellent potency and showed the presence of many secondary metabolites. Gaurav *et al*., [39] determined the phytochemical composition of *Calotropis procera* aqueous leaf extract.

Phytochemicals that were present were alkaloids, phenols, flavonoids, saponins, tannins, reducing sugar and glycosides.

4. Conclusion

In the past, the use of ovicides and insect repellents to protect people against mosquitoes was accepted as a component of an integrated programme to prevent insect-borne diseases. Public health experts have deemed synthetic repellents to be hazardous. It should therefore be used with caution due to its detrimental effects on synthetic fabric and plastic. Additionally, they exhibit hazardous reactions that have often been documented following improper application, such as dermatitis, allergies, and cardiovascular and neurological adverse effects. The current study's findings emphasized the efficacy of an ethanol extract of S. indicus leaves in controlling mosquito populations and their potential role in developing a natural insecticide for the control of *Cx. quinquefasciatus*. Finally, the current study deduces the mosquito-controlling potential of S. indicus ethanol extract and its potential application in the future to develop effective and safer formulations.

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