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MOSQUITO REPELLENT ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF MEDICINAL AND AROMATIC PLANT, PLECTRANTHUS AMBIONICUS (LOUR) AGAINST CULEX QUINQUEFASCIATUS

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ABSTRACT

Mosquitoes are vectors for more diseases prevalent due to stagnant water in rural and urban areas. Mosquito bites can cause simple skin irritation to harmful vector borne diseases. Therefore, staying away from bites is important and challenging. The leaf extract of *Plectranthus amboinicus* (Lour) have high repellent efficacy against the vector C. *quinquefasciatus*. The natural bioactive compounds present in the plant make it an efficient alternative to the conventional insecticides used for repelling mosquito species without any skin allergies. The ethanolic leaf extract of *P. amboinicus* (Lour) provides maximum repellency against adult *C. quinquefasciatus.* It shows 100% protection up to 90 minutes at all concentrations. This is followed by 5.0 concentration of petroleum ether extract which provides 99.83% of protection at 30 minutes, 99% of protection at 60 minutes and 98% of protection at 90 minutes of exposure. Phytochemical analysis of *P. amboinicus* (Lour) leaf showed the presence of alkaloids, flavonoids, tannins, diterpenes, carbohydrates, glycosides, cardiac glycosides, saponins, phytosterols, phenols, proteins and amino acids.

KEYWORDS: Culex quinquefasciatus, repellent efficacy, Plectranthus amboinicus.

INTRODUCTION

Mosquitoes are among the best known groups of insect, because of their importance to human as pests and vectors of some of the most distressing human diseases. WHO has

declared mosquito as "public enemy number one". Mosquito borne diseases are prevalent in more than 100 countries across the world, infects 700 million people every year globally and 40 millions of the Indian population. No part of the world is free from vector borne diseases (Fradin and Day, 2002). *Culex quinquefasciatus* is the most potential vector of *Bancroftian filariasis* and it is the widely distributed mosquito in India (Rahuman, *et al.*, 2009).

Recent research efforts focus towards the development for other alternative to chemical insecticidal agents with high bio-control potentiality are naturally available but no harmful effects to environment and human health (Sharma *et al.*, 2006). Plant based products has been revived because of the development of resistance, cross-resistance and possible toxicity hazards associated with synthetic insecticides, bioaccumulation and pollution.Phytochemicals obtained from huge diversity of plant species are the major sources for safe and biodegradable chemicals, which can be screened for mosquito repellent and insecticidal activities (ICMR Bulletin, 2003).

Plants have been known to relieve various diseases in traditional medicine and Ayurveda. Secondary metabolites are responsible for medicinal activity of plants (Annapoorani and Manimegalai, 2013) In India, the juice of the leaves of *Plectranthus amboinicus* is used to treat skin allergies (Harsha *et al.* 2003). *P. amboinicus* extract has wide range of chemical diversity containing phytochemicals such as terpenes, alcohols, acetones, phenols, aldehydes, and esters is often used as component in the pharmaceutical industry (Swamy *et al.* 2015).

MATERIALS AND METHODS

The methodology adopted for the present study "Repellent activity and phytochemical screening of *Plectranthus amboinicus* (Lour) leaf and extracts against *Culex quinquefaciatus*" is discussed under the following headings.

Collection of samples

The leaves of *P. amboinicus* (Lour) were collected from Ukkadam area, Coimbatore district. The leaf was dried and homogenized to a fine powder and stored in sterile air-tight container until further use.The selected leaf sample was identified as *P. amboinicus* (Lour) (BSI/SRC/5/23/2017/Tech/3266). It was authenticated by Tamilnadu Agricultural University, Coimbatore.

Preparation of the extracts

10 g of leaf powder was subjected to extraction with 500 ml of the solvents for 8 h using a Soxhlet apparatus. Petroleum ether (60-80^{0}C) extraction was followed by chloroform extraction and ethanol extraction so that the powders were subjected to extraction with solvents of increasing polarity.

Laboratory culture of larvae

Hay infusion method was adopted in which hay was taken, cut into small pieces and boiled in 5 litres of water for 20 minutes. After cooling, this water was poured into buckets and kept in different areas where mosquitoes were abundant. After one or two days eggs were laid by female mosquitoes in clusters forming an egg raft. The egg rafts were collected and maintained in the laboratory. The third instar larvae were collected, reared in enamel trays containing culture medium and provided with powdered dog biscuits and yeast in the ratio of 3:1 as the nutrient source. Immediately after molting, the fourth instar larvae were introduced into beakers containing 200 ml of water and used for the bioassay studies.

Laboratory culture of adult mosquitoes

Adult mosquitoes were reared in wooden cage (30cm ×30cm×30cm) and daily provided with sponge pieces sealed with 10% of sucrose solution for a period of 3- 4 days. After emergence mosquitoes were held at 28±2° C, 70% - 85% relative humidity with a photo period of 14:10 light and dark photo period cycle. Three day blood starved *C. quinquefasciatus* mosquitoes were used for repellent bioassays studies.

Bioassay studies

A present study was carried out to access the repellent efficacy of leaf extracts of selected plants against three day old blood starved *C. quinquefasciatus* mosquitoes. Effective doses were determined first and then detailed. Investigation carried out concentration of 1.0, 2.5 and 5.0mg/cm^2 were used for determining the repellent efficacy of leaf of *P. amboinicus* (Lour).

Experimental design

The experimental setup consists of three treatments each with replications for fruit and leaf extracts. Simultaneously control was also maintained. The repellent study was followed by the method of WHO (1996). Three days old blood starved mosquito female *C. quinquefasciatus* were kept in the netcage (45cm ×30cm×45cm). The arms had no contact

with lotions, perfumes, gels, powders and soaps on the day of assays. Dorsal side of the right arms was treated with extracts, a left arm was kept as control and the remaining area was covered by rubber gloves. Crude extracts was applied at 1.0, 2.5 and 5.0mg/cm^2 separately in the exposed area of the forearm. The control and treated arms were introduced continuously into the mosquito netcage and gently tapping the sides of the cage. The mosquitoes were activated. The test was conducted at each extracts by inserting the treated and control arm into the same cage for one full minute of every five minutes. The mosquitoes that landed on the hand were recorded and then shaken off before sucking any blood and making out five minutes protection from mosquito bite.

Test for repellent activity

The percentage of repellency was calculated by the following formulae,

$$\text{Percentage of repellency} = \frac{(Ta - Tb)}{Ta.} \times 100$$

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

Statistical analysis

The data on bioassay studies were also subjects to statistical analysis. Standard deviation was calculated for the data which was obtained from the test for the repellency against *C. quinquefasciatus* mosquitoes. Each value (χ ±SD) represents the average of three replication.

Phytochemical analysis of P.*amboinicus* (Lour). Leaf extracts.

The *P. amboinicus* (Lour) leaf extracts were screened for the presence of phytochemicals, according to the method proposed by (Prashant *et al.*, 2011).

Detection of alkaloids

Mayer's test

Extracts were dissolved individually in dilute Hydrochloric acid and treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's test

Extracts were dissolved individually in dilute Hydrochloric acid and treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's test

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's test

Extracts were dissolved individually in dilute Hydrochloric acid and treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates

Molisch's test

Extracts were dissolved individually in 5 ml distilled water and filtered and the filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.\

Benedict's test

Extracts were dissolved individually in 5 ml distilled water and filtered and the filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's test

Extracts were dissolved individually in 5 ml distilled water and filtered and the filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides

Modified Borntrager's test

Extracts were hydrolysed with dil. HCl and were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia

solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal's test

Extracts were hydrolysed with dil. HCl and were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of saponins

Froth test

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam test

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phytosterols

Salkowski's test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. H_2SO_4, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burchard's test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc.H_2SO_4 was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of phenols

Ferric chloride test

Extracts were treated with 3 – 4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

Gelatin test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

Alkaline reagent test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of proteins and aminoacids Xanthoproteic test

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin test

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Detection of diterpenes

Copper acetate test

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

RESULTS AND DISCUSSION

The solvent fractions of the leaf of *P. amboinicus* (Lour) plants were evaluated for their repellent activity against the adult of *C. quinquefasciatus* mosquito. The petroleum ether, chloroform and ethanol extracts in different concentrations were tested against three day old blood starved adult female mosquitoes to test the repellent activity. The repellent efficacy was determined in three concentrations *viz.*, 1.0, 2.5 and 5.0 mg/cm^2 under laboratory conditions (Table 1). The control treatment did not provide any protection even during the first trial.

Evaluation of the repellent efficacy of selected leaf extracts on *C. quinquefasciatus*

All the three extracts *viz..*, petroleum ether, chloroform and ethanol showed dose dependent repellent activity. The ethanolic extract was found to be effective against *C. quinquefasciatus* and 100% protection time was obtained at the concentration of 5.0 mg/cm^2. The repellent activity was very high at the initial stage of exposure.

Table 1: Repellent activity of *P. amboinicus* (Lour). leaf extracts against *C. quinquefasciatus.*

S. no	Solvent used	Concentration Mg/ cm^2	% of repellency		
			30mins	60mins	90mins
1	Control	0±0	0±0	0±0	0±0
2	Petroleum Ether	1.0	93.83±0.89	93.50±0.76	93.33±0.47
3		2.5	97.33±0.74	96.50±0.95	95.00±0.81
4		5.0	99.83±0.37	99.00±0.37	98.oo±0.81
5	Chloroform	1.0	95.33±0.74	93.83±0.89	93.16±0.37
6		2.5	95.33±0.74	94.83±0.68	93.66±0.47
7		5.0	96.16±0.89	95.83±0.68	95.33±0.74
8	Ethanol	1.0	100±0.0	100±0.0	100±0.0
9		2.5	100±0.0	100±0.0	100±0.0
10		5.0	100±0.0	100±0.0	100±0.0

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

Repellent activity of petroleum ether extract of *P. amboinicus* (Lour)

The petroleum ether leaf extract of *P. amboinicus* (Lour) showed 93.83% repellency for first 30 minutes at 1.0 concentration and 93.50% of repellency was observed at 60 minutes. The results recorded as more or less same up to 90 minutes in the same concentration.

The extract gives 97.33% of protection in 2.5 concentration at 30 minutes of exposure. 96.50% of repellency was observed at 60 minutes and it provides 95% of protection at 90 minutes of extract applied.

In 5.0 concentration, the petroleum ether extract provides 99.83% of protection followed by 99% of protection at 60 minutes and 98% of protection at 90 minutes of exposure.

Repellent activity of chloroform extract of *P. amboinicus* (Lour)

The chloroform extracts of leaf of *P. amboinicus* (Lour) showed 95.33% of repellence in the first 30 minutes and the repellency was reduced to 93.16% at 90 minutes in the concentration of 1.0. In 2.5 concentration, it showed 95.33%, 94.83% and 93.66% response at 30 minutes, 60 minutes and 90 minutes of exposure respectively.

The maximum repellency (96.16%) was obtained at 30 minutes in 5.0 concentration. 95.83% of protection was obtained in that 60 minutes of exposure and 95.33% repellency was observed at 90 minutes of extracts applied.

Repellent activity of ethanol extract of *P. amboinicus* (Lour)

The ethanolic leaf extract of *P. amboinicus* (Lour) provides maximum repellency against adult *C. quinquefasciatus.* It shows 100% protection up to 90 minutes at all concentrations.

Phytochemical screening of leaf extract of *P. amoinicus* (Lour).

Qualitative analysis

Preliminary phytochemical screening of plants part is very useful for determination of the active constituents in different solvents and their yields. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds. The phytochemical screening of *P. amboinicus* (Lour) in petroleum, chloroform and ethanolic extract was analysed (Table 2).

Table 2: Phytochemical screening of leaf extracts of *Plectranthus amboinicus* (Lour).

TESTS	PETROLEUM ETHER	CHLOROFORM	ETHANOL
Alkaloids			
Mayer's Test	-	-	-
Wagner's Test	+	+	+
Dragendroff's Test	+	-	+
Hager's Test	-	+	+
Carbohydrates			
Molisch's Test	+	-	+
Benedict's Test	+	-	+
Fehling's Test	-	+	+
Glycosides			
Modified Borntrager's Test	+	-	+
Legal's Test	-	-	+
Saponins			
Froth Test	-	+	+
Foam Test	+	-	+
Phytosterols			
Salkowski's Test	-	-	+
Libermann Burchar's Test	+	-	-
Phenols			
Ferric Chloride Test	+	-	+
Tannins			
Gelatin Test	-	-	+
Flavonoids			

Alkaline Reagent Test	-	-	+
Lead acetate Test	-	+	+
Proteins and amino acid			
Xanthoproteic Test	+	+	+
Ninhydrin Test	+	-	+
Diterpenes			
Copper acetate Test	-	-	-

(+) Detected; (-) Not dedected.

Phytochemical screening of leaf extract of *P. amboinicus* (Lour).

The petroleum ether extract of *P.* amboinicus (Lour) leaf showed the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, proteins and amino acids.

The chloroform extract of *P. amboinicus* (Lour) revealed the presence of alkaloids, carbohydrates, saponins, flavonoids, proteins and amino acids.

Alkaloids, carbohydrates, glycosides, cardiac glycosides, saponins, phytosterols, tannins, phenols, flavonoids, diterpenes, proteins and amino acids were found in the ethanolic leaf extract of *P. amboinicus* (Lour).

Repellent activity of leaf extract of *P. amboinicus* (Lour).

The ethanolic leaf extract of *P. amboinicus* (Lour) shows higher repellency towards adult female *C. quinquefasciatus* mosquito than the petroleum ether and chloroform extracts. The repellent activity was found to be dose dependent and the percentage of protection was found to be directly proportional to the concentration of extract (Dhivya and Manimegalai, 2013). Tikar *et al.* (2008) reported that the development of insecticide resistance in the population of *C. quinquefasciatus* against temephos, fenthion, cypermethrin and cyhalothrin indicating the need of search for safe, effective and alternative safe control measures.

Kolli *et al.* (2013) proposed that the incidence of mosquito bites significantly reduced after usage of plant products and the natural products from plants of insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their repellent and toxicity.

The results of repellent activity of leaf extracts of *P. amboinicus* (Lour) were comparable with earlier reports. In accordance to the results of the present study similar observations were reported by Govindarajan *et al.* (2011) in which the methanol leaf extract of *Ervatamia coronaria* showed remarkable repellent properties at the higher concentration of 5.0 mg/cm^2

which provided 100% protection up to 150 minutes against female mosquitoes of *C. quinquefasciatus*.

Pushpanathan *et al.* (2006) showed that the skin repellent test at 1.0, 2.5 and 5.0 mg/cm^2 concentration of *Cymbopogon citratus* essential oil against the filarial mosquito *C. quinquefasciatus* showed 100% protection upto 3 hrs, 4 hrs and 5 hrs protections respectively. The total percentage of protection of the essential oil was 49.64% at 1.0mg/cm^2, 62.19% at 2.5mg/cm^2 and 74.03% at 5.0mg/cm^2 for 12 hrs.

The present investigation is an attempt to screen effective botanicals for the management of adult female *C. quinquefasciatus.* The leaf of *P. amboinicus* (Lour) extracts showed significant repellency against *C. quinquefasciatus* and gave protection against mosquito bites without any allergic reaction to the tested persons. The repellent activity was found to be dependent on the strength of the extracts applied. Therefore, these plant extracts are recommended for use in the management of mosquitoes.

Phytochemical screening

Qualitative analysis

The phytochemical study of the petroleum ether extract of *P. amboinicus* and the chloroform extract revealed the presence of alkaloids, carbohydrates, saponins, flavonoids, proteins and amino acids.

Ethanolic extract of both samples showed the presence of all the components such as, alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, protein and amino acids where as the di-terpenoids were found to be absent in the *P. amboinicus* leaf ethanolic extract.

The results of the phytochemical screening of the extracts showed that the leaf is rich in most of the secondary metabolites analyzed using different solvents as (Okwu, 2001), and it has been reported that several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens.

Other compounds like saponins also have antifungal properties (Aboaba and Efuwape, 2001). It can therefore be assumed that the repellent activity *P. amboinicus* may be due to the

presence of these metabolites (glycocides). Above results showed in line with the present investigation.

CONCLUSION

The findings of this study help to assess appropriate and possible strategies to repel and control mosquito species. The leaf extract of *P. amboinicus* (Lour) have higher repellent efficacy against the vector *C. quinquefasciatus.* However toxicity tests of the leaf and fruit extract did not caused any irritation to human skin which ascertained the safety in its usage. Hence, in the present study it could be concluded that leaf of *P. amboinicus* showed repellent activity against *C. quinquefasciatus* without any allergic reaction and it could be recommended for synthetic repellence.

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