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QUALITATIVE PHYTOCHEMICAL SCREENING AND MOSQUITO REPELLENCY OF *CHROMOLAENA ODORATA* (ASTERACEAE) LEAF EXTRACT AGAINST ADULTS OF *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE)

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Abstract:
Mosquito repellents could be one of the most effective tools for protecting humans from mosquito attack and from mosquito-borne diseases. Therefore the aim of the present study was to assess the repellent efficacy of the petroleum ether, chloroform and ethanol leaf extracts of the selected plant Chromolaena odorata against Culex quinquefasciatus mosquito and to screen the bioactive compounds present in them. The efficacy of the extracts as repellent were assessed on three day blood starved female Cx. quinquefasciatus mosquito. The results suggested that leaf ethanol extract of C. odorata showed a higher repellency on the adult of female Cx. quinquefasciatus mosquito than the other two 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 and inversely proportional to the period of exposure. The results of phytochemical screening showed the presence of bioactive compounds such as alkaloids, phenols, flavonoids, sterols, saponins, proteins and quinones in the different leaf extracts. It may be concluded from the results that ethanol extract of C. odorata leaf was effective in mosquito vector control and has an excellent potential in controlling the mosquito.

Keywords: *Culex quinquefasciatus, Mosquito, Chromolaena odorata, Leaf extracts, Repellent activity, Phytochemicals*

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INTRODUCTION:

Arthropods are dangerous vectors of deadly pathogens and parasites, which may spread as epidemics or pandemics in the increasing world population of humans and animals[1,2]. In particular, the mosquitoes are a serious threat to public health, since they are known for many life threatening diseases like malaria, filariasis, chikungunya and dengue fever, which are transmitted by the species of three mosquito genera: *Anopheles, Culex* and *Aedes*. These vector borne diseases are a major source of illness and death worldwide. Mosquitoes are one of the most important vectors that alone transmit diseases to more than 700 million people per annum[3]. Mosquito bites may also cause allergic responses including local skin reactions and systemic reactions such as urticarial[4].

The southern house mosquito, *Culex quinquefasciatus* acts as an important "urban bridge vector" which bridges different reservoir/amplifier hosts to humans because of its encounter with different vertebrates. Filariasis is a major public health hazard and remains a challenging socio-economic problem in many of the tropical countries. Thus, one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes from biting human beings. Repellency is known to play an important role in preventing the vector borne diseases by reducing man-vector contact. Synthetic chemicals and insecticides used for control of vectors are causing irreversible damage to the ecosystem, as some of them are non-degradable in nature. Some repellents of synthetic origin may cause skin irritation and affect the dermis[5]. It has been reported that these chemical repellents are not safe for public use[6,7]. Because of unpleasant smell, oily feeling to some users[8,9], some prefers to use natural insect repellent products.

Nature has been a source of medicinal agents for thousands of years and generally produces many secondary metabolites which constitute important leads for the development of new environment friendly microbicides, pesticides, herbicides and many pharmaceutical drugs[10]. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body[11]. Phytochemicals are naturally occurring in the medicinal plants and have defense mechanism to protect from various diseases[12].

Many of the herbs and shrubs are found to have promising medicinal properties, and mosquito repellent properties. The drugs used by the ancient civilization were mainly the extracts of plants, animals and few inorganic salts. Natural products of plant origin with insecticidal properties have been tried as an indigenous method for the control of a variety of insect pests and vectors in the recent past. The uses of plant parts for insect control has several appealing features, and are rich store house of chemicals of diverse biological activities[13]. As an alternative, biological control of mosquitoes could be very promising being eco-friendly as well as cost effective. Hence there is a constant need for developing biologically active plant materials as insecticides, which are expected to reduce the hazards to humans and other organisms by minimizing the accumulation of harmful residues in the environment. Natural products of plant origin are generally preferred because of their less harmful nature to non-target organisms and their innate biodegradability[14].

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 PG and Research, Department of Zoology, Nirmala college for Women, Coimbatore by Hay infusion method under laboratory 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 and extracts

Fully developed fresh leaves of the plant *C.odorata* were collected from natural habitat of Kannur 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[15,16]. Petroleum ether extraction was followed by chloroform and ethanol extraction in their increasing order of 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[18]. Three day old blood starved female *Cx. quinquefasciatus* (100) were kept in a net cage (45cm × 30 cm × 45 cm). The arms of the volunteer, only 25 cm^2 dorsal side of the skin on each arms was exposed and remaining area covered by rubber gloves. The extract was applied at 1.0, 2.5 and 5.0 mg/cm^2, separately in the exposed area of the forearm. The volunteer conducted their 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.

The percentage of repellency was calculated by the following formula.

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

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

Statistical analysis

Standard deviation was calculated for the data obtained. Each value ($\chi \pm SD$) represents average of three replications.

Phytochemical screening

Qualitative analysis

The preliminary qualitative phytochemical analysis has been attempted in *C.odorata* leaf extracts to find out the presence or absence of certain bioactive compounds. The preliminary screening was carried out by using standard procedures described by Raman[17].

Test for Alkaloids

- **Mayer's test**

A fraction of extract was treated with Mayer's test reagent (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml of water) and observed for the formation of cream coloured precipitate.

- **Wagner's test**

A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and observed for the formation of reddish brown colour precipitate.

- **Hager's test**

A few ml of extract was treated with Hager's reagent (saturated aqueous solution of picric acid) and observed for the formation of prominent yellow precipitate.

Test for Tannins

- **Acetic Acid Test**

The extract was treated with acetic acid solutions and observed for the formation of red colour solution.

- **Dilute HNO3 Test**

The extract was treated with dil. HNO3. The extract turns from reddish to yellow colour which indicates the presence of tannins.

Test for Phenols

- **Ferric chloride test**

The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour

- **Liebermann's test**

The extract was heated with sodium nitrite, added H2SO4 solution diluted with water and excess of dilute NaOH was added and observed for the formation of deep red or green or blue colour.

Test for Flavonoids

- **NaOH test**

A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour.

- **H2SO4 test**

A fraction of the extract was treated with concentrated H2SO4 and observed for the formation of orange colour.

Test for Sterols

- **Liebermann-Burchard test**

Extract (1ml) was treated with chloroform, acetic anhydride and drops of H2SO4 was added and observed for the formation of dark pink or red colour.

Test for Terpenoids

- **Liebermann-Burchard test**

Extract (1ml) was treated with chloroform, acetic anhydride and drops of H2SO4 was added and observed for the formation of dark green colour.

Test for Saponins

- **Foam Test**

The extract or dry powder was vigourosly shaken with water and observed for the formation of persistent foam.

Test for Anthraquinones

- **Borntrager's test**

About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia and observed for the formation of pink or deep red colouration of aqueous layer.

Test for Proteins

- **Ninhydrin test (Aqueous)**

The extract was treated with aqueous ninhydrin and observed for the presence of blue colour, indicating the presence of amino acid or purple colour indicating the presence of protein.

- **Ninhydrin (Acetone)**

Ninhydrin was dissolved in acetone and the extract was treated with ninhydrin and observed for the formation of purple colour.

- **Biuret test**

The extract was heated in distilled water and filtered. The filtrate was treated with 2% copper sulphate solution, 95% ethanol and potassium hydroxide and observed for the formation of pink ethanolic layer.

- **Test for Quinones**

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

RESULTS AND DISCUSSION:

Repellent activity

In the present study, the repellent efficacy of petroleum ether, chloroform and ethanol extracts of the leaf of *C.odorata* were analyzed against *Cx. quinquefasciatus* mosquito. The data were recorded and were statistically analysed. The results of the skin repellent activities of *C.odorata* leaf extracts against the three days blood starved female *Cx. quinquefasciatus* mosquitoes were summarized in the Table 1.

Table 1: Repellent activity of *C. odorata* leaf extracts against adults of *Cx. quinquefasciatus*

Solvent used	Concentration 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
Petroleum ether	1.0	99±0.81	98±0.81	97±0.81	96.6±0.47	94±0.47	90±0.81
	2.5	100±0	99±0.81	98±0.81	97±0.81	95.3±0.81	93±0.81
	5.0	100±0	100±0	99.6±0.47	99±0.81	96.3±0.47	95.3±0.81
Chloroform	1.0	100±0	99.3±0.47	98±0.81	97±0.81	96.6±0.47	96±0.81
	2.5	100±0	100±0	99.3±0.47	99±0.81	97.6±0.47	97±0.81
	5.0	100±0	100±0	100±0	100±0	99.6±0.47	98±0.81
Ethanol	1.0	100±0	100±0	100±0	99±0.81	98±0.81	97±0.81
	2.5	100±0	100±0	100±0	100±0	99±0.81	98.6±0.47
	5.0	100±0	100±0	100±0	100±0	100±0	99±0.81

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

The results revealed that the ethanol extract of *C.odorata* leaf was found to be more repellent against *Cx. quinquefasciatus*. Maximum repellent activity was observed in ethanol extract. A lower concentration of 1.0 mg/cm² provided 100% protection up to 90 min and a 1% decrease in repellency was observed in 120 min and showed 99% protection. A decrease in 1% repellency was observed when there was increase in time. Concentration of 2.5 mg/cm² provided 100% protection up to 120 min and showed the non-repellency of 1% in 150 min. At higher concentration of 5.0 mg/cm², the ethanol extract of *C.odorata* leaf provided 100% protection up to 150 min of the experimental period against adults of *Cx. quinquefasciatus*.

Ethanol extract was followed by chloroform extract which shows moderate range of repellency against *Cx. quinquefasciatus*. Lower concentration of 1.0 mg/cm² showed 100% protection from mosquito bites up to 30 min and 2.5 mg/cm² showed 100% protection up to 60 minand repellency decreased with increase in the period of exposure. At a higher concentration of 5.0 mg/cm² 100% protection was recorded up to 120 min. Chloroform extract was followed by petroleum ether which showed minimum repellency against the mosquitoes. At a higher concentration of 5.0 mg/cm² 100% protection was observed only up to 60 min and it gradually decreased with increase in time. At lower concentrations like 1.0 and 2.5 mg/cm² the percentage of repellency was very low.

Phytochemical analysis

Phytochemical screening of ethanol, petroleum ether and chloroform extract of *C.odorata* leaf was carried out to test the presence of secondary metabolites such as alkaloids, tannins, phenols, flavonoids, sterols, terpenoids, saponins, anthraquinones, proteins and quinones by using standard procedures described by Raman[17]. The result of phytochemical screening is presented in Table 2.

Table 2: Phytochemicals present in the extracts of *C. odorata* leaf

Sl. No.	Constituents	*Chromolaena odorata leaf*		
		Petroleum ether extract	Chloroform extract	Ethanol extract
1	Alkaloids	-	-	+
2	Tannins	-	-	-
3	Phenols	-	+	+
4	Flavonoids	+	-	+
5	Sterols	+	-	+
6	Terpenoids	-	+	-
7	Saponins	+	+	+
8	Anthroquinones	-	-	-
9	Proteins	-	+	+
10	Quinones	+	+	+

(+) Presence (-) Absence

In the present study maximum repellent activity was exhibited by ethanol extract of *C.odorata* and the phytochemical screening showed the presence of secondary metabolites such as alkaloids, tannins, phenols, flavonoids, sterols, saponins, proteins and quinones. Minimum repellent activity was found in petroleum ether extract of the leaves of *C.odorata* and the phytochemical such as flavonoids, sterols, saponins and quinones were found to be present.

Mosquito control represents an important strategy for prevention of disease transmission and epidemic outbreaks. Mosquitoes are a serious threat to public health transmitting several dangerous diseases for over two billion people in the tropics. However a high level of insecticide resistance has developed through chemical control of the vector and pests, threatening the control strategies. Insecticides residues in the environment, as a result of chemical insecticide usage, have turned the researcher's attention towards natural products[19]. The plant kingdom synthesizes a variety of secondary metabolites which play a vital role in defense of plants against insects/mosquitoes. Plants may be alternative source for mosquito repellent agents since they constitute a rich source of bioactive chemicals[20].

In the present study, the ethanol extract of *C. odorata* leaf was found to be more effective against *Cx. quinquefasciatus*. A higher concentration of 5.0 mg/cm² provided 100% protection up to 150 min against *Cx. quinquefasciatus*. The repellent activity was very high at the initial stage of exposure. In accordance to the results of the present study similar observations were reported by Govindarajan *et al*[21] in which the methanol leaf extract of *Erratamia coronaria* showed remarkable repellent properties at the higher concentration of 5.0 mg/cm² which provided 100% protection up to 150 min against *Cx. quinquefasciatus* mosquitoes.

The repellent efficacy of ethanol extract was followed by chloroform leaf extract which provided 100% protection up to 120 min at a higher concentration of 5.0 mg/cm². Increase in the exposure period showed reduction in the repellent activity. Govindarajan[22] reported similar findings from his study in which the crude extracts of *Sida acuta* showed significant repellent activity against *Cx. quinquefasciatus* at a higher concentration of 5.0 mg/cm² providing 100% protection up to 120 min. In the present study 100% repellency was observed up to 60 min and 30 min at a concentration of 2.5 mg/cm² and 1.0 mg/cm² respectively.

In the present study, petroleum ether extract, chloroform extract and ethanol extract showed varying repellency percentages against adults of *Cx. quinquefasciatus* mosquitoes. Petroleum ether extract provided a protection of 100% at the higher concentration of 5.0 mg/cm² up to 60 min. Similar observations were reported by Venkatachalam and Jebanesan[23] in which they reported that the methanol extract of *Feronia elephantum* leaves at 1.0 and 2.5 mg/cm² concentrations gave 100% protection up to 2.14±0.16h and 4.00±0.24h respectively against *Ae.aegypti* mosquitoes and the total percentage protection was 45.8% at 1.0 mg/cm² and 59.0% at 2.5 mg/cm² for 10h.

In the present study the highly active ethanol extract of *C.odorata* leaf showed more phytochemical constituents. The results showed the presence of phytochemical compounds such as alkaloids, flavonoids, sterols, saponins, proteins, quinones and phenol in the leaf extract. Whereas terpenoids and anthraquinones were found to be absent. The results of the present study were in accordance to the observations reported by Dhivya and Manimegalai[24] in which ethanol extract of *Calotropis gigantea* flower extract showed the presence of phytochemical compounds like alkaloids, tannins, phenols, flavonoids, sterols, anthraquinone, proteins and quinones where as terpenoids and saponins were found to be absent. Parallel to the present study Mgbemena *et al*[25] reported the phytochemical screening of the methanol extracts of *Moringa oleifera* and *Stachytarpheta indica* and revealed the presence of alkaloids, tannins, flavonoids and saponins in both plants. Anthraquinones, terpenes and steroids were found in oils from *S.indica* but were absent in oils from *M.oleifera*.

Results of the present study showed that the repellent activity was dose dependent. Similar trend were reported in the work by Rajkumar and Jebanesan[26] in which both oviposition deterrent and skin repellent activity of *Solanum trilobatum* against the malaria vector *An.stephensi* were dose dependent. Govindarajan and Sivakumar[27] tested the repellent activities of crude hexane, ethyl acetate, chloroform and methanolic leaf extracts of *Eclipta alba* and *Andrographis paniculata* at three concentrations of 1.0, 2.5 and 5.0 mg/cm² against *Ae.aegypti* and suggested that the plant extracts have the potential to be used as an ideal-ecofriendly approach for the control of mosquitoes. The seed extracts of *Tribulus terrestris* exhibited 100% repellent protection at 1.0 mg/cm² against *An.arabiensis* as reported by Sheikh *et al*[28]. The health giving properties of medicinal plants are due to the presence of various complex

chemical substances of different composition which occur as secondary metabolites. In the present study also the repellent potential of the leaf extracts might be due to the presence of certain chemicals that are able to irritate the olfactory senses of the mosquitoes.

CONCLUSION:

The findings of the present study suggested that the ethanol leaf extracts of *C.odorata* have higher repellent efficacy against the adults of mosquito vector *Cx. quinquefasciatus*. In accordance with the results of the present study it is clear that repellent activity is dependent upon the concentration of the extract and period of exposure, when time increases repellency decreases. That is the percentage of repellency is directly proportional to the concentration of the extract and inversely proportional to the period of exposure. The extract of *C.odorata* was confirmed as an effective mosquito repellent because it was found to be non-toxic to the human beings. No skin rash, irritation, no hot sensations on the arms of the treated volunteer was found throughout the period of the study which ascertained the safety in its usage.

Hence *C.odorata* leaf could be potentially used for the preparation of mosquito repellent products. Such formulations could help in reducing the harmful effects of synthetic mosquito repellents on human health. It may be concluded from the results that the leaf extracts of selected plant can be used alone or combined for effective protection against mosquito bites and also can be used for control of mosquito breeding under integrated disease vector control programme in various situations. They also offer safer alternative to synthetic chemicals and can be easily obtained by individuals and communities at a very low cost. Further characterization and isolation of bioactive molecules from the extracts of *C.odorata* leaf will provide further clarity about the nature of these bioactive compounds which could become an alternative to the conventional insecticides used for repelling annoying mosquito species.

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