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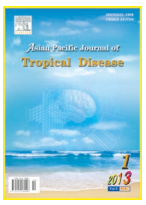

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Pupicidal and repellent activities of *Pogostemon cablin* essential oil chemical compounds against medically important human vector mosquitoes

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PEER REVIEW

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Comments

The present line of research quoted in this research article will surely focusing an insight in phytochemical research and their possible role towards the control of mosquitoes in any form, for example larval, pupal, egg or adult stages. Thus this plant essential oil and its compounds can be an alternative agent in Integrated Vector Control Programme (IVCP).
(Details on Page 30)

ABSTRACT

Objective: To determine the repellent and pupicidal activities of *Pogostemon cablin* (*P. cablin*) chemical compositions were assayed for their toxicity against selected important vector mosquitoes, *viz.*, *Aedes aegypti (Ae. aegypti)*, *Anopheles stephensi (An. stephensi) and Culex quinquefasciatus (Cx. quinquefasciatus)* (Diptera: Culicidae). **Methods:** The plants dry aerial parts were subjected to hydrodistillation using a modified Clevenger-type apparatus. The composition of the essential oil was analyzed by Gas Chromatography (GC) and GC mass spectrophotometry. Evaluation was carried out in a net cage (45 cm×30 cm×45 cm) containing 100 blood starved female mosquitoes and were assayed in the laboratory condition by using the protocol of WHO 2010. The repellent activity of *P. cablin* chemical compositions at concentration of 2mg/cm^{2}were applied on skin of fore arm in man and exposed against adult female mosquitoes. The pupicidal activity was determined against selected important vector mosquitoes to concentration of 100 mg/L and mortality of each pupa was recorded after 24 h of exposure to the compounds. **Results:** Chemical constituents of 15 compounds were identified in the oil of *P.cablin* compounds representing to 98.96%. The major components in essential oil were â-patchoulene, á-guaiene, ã-patchoulene, á-bulnesene and patchouli alcohol. The repellent activity of patchouli alcohol compound was found to be most effective for repellent activity and 2 mg/cm^2 concentration provided 100% protection up to 280 min against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, respectively. Similarly, pupae exposed to 100 mg/L concentrations of *P. cablin* chemical compositions. Among five compounds tested patchouli alcoholwas found to be most effective for pupicidal activity provided 28.44, 26.28 and 25.36 against *Ae.aegypti, An.stephensi and Cx. quinquefasciatus*, respectively. The percent adult emergence was inversely proportional to the concentration of compounds and directly proportional to the pupal mortality.**Conclusion:** These results suggest that the *P. cablin* chemical compositions have the potential to be used as an ideal eco-friendly approach for the control of mosquitoes. This is the first report on the mosquito repellent and pupicidal activities of the reported *P. cablin* chemical compositions.

KEYWORDS

Aedes aegypti, *Anopheles stephensi*, *Culex quinquefasciatus*, *Pogostemon cablin*, Repellent activity, Pupicidal activity

1. Introduction

Mosquitoes being vector for many tropical and subtropical diseases are the most important single group of insect well known for their public health importance[1-3].Mosquito-borne diseases such as malaria, filariasis, dengue, and viral encephalitis contribute to a larger proportion of health problems of developing countries. Repeated use of synthetic

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insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It also resulted in the development of resistance, undesirable effects on non-target organisms, and fostered environmental and human health concern[4]. The drastic effects of chemical insecticide-based intervention measures for the control of disease vectors have received wide public apprehension and have caused many problems like insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans, and other nontarget organisms. To alleviate these problems, major emphasis has been on the use of natural plant-based products as larvicides which can provide an alternate to synthetic insecticides[5,6].

There is an increased interest in the search for insecticides from natural sources as they are considered safer, more cost effective, biodegradable and are target specific against mosquitoes. Several studies have identified and reported plants and plant extracts effective against mosquitoes at various stages of development. The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity. The phytochemicals derived from plant resources can act as larvicides, insect growth regulators, repellents, and ovipositional attractants, having deterrent activities observed by different researchers[7,8,9,10, 11,12]. The use of scientifically proven non-chemical methods and limited use of drug is being considered as safety to environment and human health[13,14]. The *Pogostemon* is major genus of the family Lamiaceae. *Pogostemon* has been used against the common cold and as an antifungal agent in traditional medicine. Lamiaceae herb family consists of more than 252 genus and 7000 Species. Lamiaceae family is known for the wealth of species with medicinal properties, which have been used since early times and many of these species are common in Mediterranean region. Extensive cultivation of this plant is carried out in Malaysia, Indonesia, India, China, Brazil and Pakistan for its essential oil (patchouli oil). Thus, the constituents of patchouli oil have frequently been investigated, and the presence of a number of monoandsesquiterpenoids has been reported[15,16]. The aim of the study was to determine the effect of repellent and pupicidal activities of the *P. cablin* essential oil compounds against the selected vector mosquitoes.

2. Materials and methods

2.1. Plant material and essential oil extraction

The plants fresh aerial parts of *P. cablin*, collected from the forest region of Ooty, Uthagamandalam District, Tamilnadu, India. the plant material were collected in the month of February 2010, were air dried and essential oil was obtained by hydrodistillation of 5kg of fresh leaves in a clavenger apparatus for 4 h. the distilled oil was dried over anhydrous sodium sulphate and stored under nitrogen atmosphere until further use. The plant material was identified and the voucher specimens were deposited at the department of Zoology, Annamalai University, Annamalai Nagar, Tamilnadu, India.

2.2. Gas Chromatography Analysis

Analysis was carried on a varian-gas chromatograph equipped with a flame ionization detector and a BPI (100% dimethyl polysiloxane) capillary column. Helium at a flow rate of 1.0 mL/min and 8 psi inlet pressure was employed as a carrier gas. Temperature was programmed from 60 to 220 at 5 °C min-1with a final hold time of 6 min. The injector and detector temperatures were maintained at 250 and 300 °C , respectively. The sample (0.2 μL) was injected with 1:20 split ratio.

2.3. Gas Chromatography – Mass Spectrometry Analysis

Gas chromatography – mass spectrometry (GC-MS) analysis was performed on an Agilent 6890 GC equipped with 5973 N mass selective detector and an HP-5 (5% phynylmethylpolysiloxane) capillary column. The oven temperature was programmed from 50 to 280 °C at the rate of 4 /min and held at this temperature for 5 min. The inlet and interface temperatures were 250 and 280 °C, respectively. The carrier gas was helium at a flow rate of 1.0 mL/min (constant flow). The sample (0.2μL) was injected with a split of 20:1. Electron impact mass spectrometry was carried at 70 eV. Ion source and quadrupole temperatures were maintained at 230 and 150 °C respectively.

2.4. Mosquito rearing

The mosquitoes, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, were reared in the Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 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.

2.5. Repellent activity

The repellent study was following the methods of WHO[17]. 3-4 days old blood-starved female of selected mosquitoes (100) were kept in a net cage (45 cm×45 cm×40cm). 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 *P. cablin* major chemical compositions were dissolved in 1 mL DMSO and this DMSO served as control. The selected essential oil chemical compositions at 2 mg/cm^2 concentration were applied. The control and treated arms were introduced simultaneously into the cage. The numbers 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 chemical

compositions. The percentage protection was calculated by using 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.

2.6. Pupicidal activity

Batches of ten early emerged pupae were introduced into 500 mL of the test medium containing 100mg/L concentration of essential oil chemical compositions in a plastic cups in five replications. In control, the same number of pupae was maintained in 500 mL of dechlorinated water containing appropriate volume of DMSO. All containers were maintained at room temperature with naturally prevailing photoperiod (12: 12h / L: D) in the laboratory. Any pupa was considered to be dead if did not move when prodded repeatedly with a soft brush. Mortality of each pupa was recorded after 24 h of exposure to the selected concentration of chemical compositions following the Abbott formula Abbott[18].

2.7. Statistical Analysis

The data were calculated by using the software statistical package of social science (SPSS) version 13.0 for windows, significance level was set at $P<0.05$.

3. Results

3.1. Chemical constituent of essential oil

The chemical constituents of the essential oil of *P. cablin* the retention indices and the percentage of the individual components are summarized in Table 1. The essential oil was obtained from fresh aerial parts of *P. cablin* hydrodistilled in a clavenger apparatus and was analysed by GC-MS. A total of 15 compounds were detected representing to 98.96%. The major components in essential oil were β-patchoulene (12.88%), α-guaiene (15.44%), γ-patchoulene (11.72%), α-bulnesene (19.49%) and patchouli alcohol (22.62%). The percentage compositions of remaining 10 compounds ranged from 0.22% – 3.58%.

Table 1
Chemical constituents of the essential oil from aerial parts of *P. cablin*.

Peak	Compounds	RT(min)*	Concentration (%) leaves
1	α-pinene	938	0.46
2	δ-elemene	1327	1.32
3	β-patchoulene	1380	12.88
4	β-caryophyllene	1421	2.53
5	α-guaiene	1440	15.44
6	γ-patchoulene	1441	11.72
7	α-patchoulene	1456	3.58
8	eremophilene	1486	1.36
9	α-bulnesene	1505	19.49
10	β-bisabolene	1509	0.22
11	t-β-elemenone	1601	2.74
12	patchouli alcohol	1659	22.62
13	farnesol	1713	1.55
14	aromadendrene oxide	1749	1.57
15	nonadecane	1902	1.48

*RT= Retention time (min).

3.2. Repellent activity of chemical compounds

The results of the repellent activity of chemical compounds of *P. cablin* against selected important vector mosquitoes, *viz.*, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, are presented

Table 2
Repellent activity of *P. cablin* essential oil compounds tested against selected vector mosquitoes.

Compounds	Mosquitoes	Concentration 2mg/cm^2, % of repellency							
		Time post application of repellent (min)							
		40	80	120	160	200	240	280	320
γ-patchoulene	*Ae. aegypti*	100±0.0	100±0.0	100±0.0	100±0.0	94.3±2.4	88.5±2.2	83.2±2.4	80.4±1.2
	An. stephensi	100±0.0	100±0.0	100±0.0	100±0.0	92.6±1.3	86.3±1.7	79.2±1.8	77.2±1.6
	Cx.quinquefasciatus	100±0.0	100±0.0	100±0.0	100±0.0	91.6±1.2	84.4±1.6	76.4±1.6	71.8±1.8
patchouli alcohol	*Ae. aegypti*	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	98.3±2.2
	An. stephensi	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	96.7±1.4
	Cx.quinquefasciatus	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	91.4±2.6
α-bulnesene	*Ae. aegypti*	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	98.5±2.0	94.3±2.5
	An. stephensi	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	95.2±2.2	91.6±2.2
	Cx.quinquefasciatus	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	91.2±2.3	89.3±1.8
β-patchoulene	*Ae. aegypti*	100±0.0	100±0.0	100±0.0	100±0.0	98.3±2.5	93.3±2.4	88.4±1.6	85.9±2.8
	An. stephensi	100±0.0	100±0.0	100±0.0	100±0.0	94.8±1.9	88.5±2.8	82.2±2.5	79.4±1.6
	Cx.quinquefasciatus	100±0.0	100±0.0	100±0.0	100±0.0	92.2±1.3	84.3±1.7	80.5±2.2	75.8±1.4
α-guaiene	*Ae. aegypti*	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	95.8±2.2	88.2±2.6
	An. stephensi	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	91.2±2.8	84.2±2.3
	Cx.quinquefasciatus	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	88.4±1.6	82.6±2.6

Each value mean±SD represents mean of six values.

Table 3
Pupicidal activity of *Pogostemon cablin* essential oil compounds tested against selected vector mosquitoes.

Compounds	Mosquitoes	Concentration 100mg/L, 24 h of exposure period			
		Mortality*		Adult emergence	
		Pupal mortality	% Mortality	Adult	% Emergence
γ-patchoulene	*Ae. aegypti*	24.25±1.52^{i}	80.83	5.75±1.86^{i}	19.16
	An. stephensi	22.46±1.46^{f}	74.86	7.54±1.64^{l}	25.13
	Cx.quinquefasciatus	21.22±1.82^{c}	70.73	8.78±1.22^{o}	29.26
patchouli alcohol	*Ae. aegypti*	28.44±1.35^{p}	94.80	1.56±1.48^{b}	5.20
	An. stephensi	26.28±1.33^{m}	87.60	3.72±1.62^{e}	12.40
	Cx.quinquefasciatus	25.36±1.42^{l}	84.53	4.64±1.66^{f}	15.46
α-bulnesene	*Ae. aegypti*	27.25±1.83^{o}	90.83	2.57±1.32^{c}	9.16
	An. stephensi	24.64±1.44^{j}	82.13	5.36±1.52^{h}	17.86
	Cx.quinquefasciatus	23.86±1.65^{h}	79.53	6.14±1.64^{j}	20.46
β-patchoulene	*Ae. aegypti*	25.18±1.22^{k}	83.93	4.82±1.66^{g}	16.06
	An. stephensi	21.83±1.37^{e}	72.76	8.17±1.72^{m}	27.23
	Cx.quinquefasciatus	20.24±1.62^{b}	67.46	9.76±1.45^{p}	32.53
α-guaiene	*Ae. aegypti*	26.38±1.24^{n}	87.93	3.62±1.36^{d}	12.06
	An. stephensi	23.22±1.83^{g}	77.40	6.78±1.84^{k}	22.60
	Cx.quinquefasciatus	21.64±1.75^{d}	72.13	8.36±1.63^{n}	27.86
Control group	Selected mosquitoes	0.0±0.0^{a}	0.0	0.0±0.0a	0.0

Value represents mean±SD of five replications. *Mortality of the pupae observed after 24 h of exposure period. Values in the column with a different superscript alphabet are significantly different at $P<0.05$ level DMRT Test.

in Tables 2. Among five compounds tested, the maximum repellent activity was observed in patchouli alcohol at 2mg/cm2concentration provided 100% protection up to 280min against *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus*,respectively.

3.3. Pupicidal activity of chemical compounds

The pupicidal activity of *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus*, were tested with five different compounds of *P. cablin* at 100 mg/L concentration, and the results are listed in Table 3. The percent adult emergence was inversely proportional to the concentration of compounds and directly proportional to the pupal mortality. Among the five different compounds tested for pupicidal activity against selected vector mosquitoes, the patchouli alcohol was found to be most effective for pupicidal activity provided 28.44, 26.28 and 25.36 against *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus*, respectively.

4. Discussion

In our results showed that, chemical constituents of the essential oil of *P. cablin* have significant repellant and pupicidal activity against *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus* mosquito. The results are comparable with an earlier report by Eliningaya *et al* reported that mortality of *Cx. quinquefasciatus* ranged from 0.50% to 96.75% while for An. gambiaes. It was from 13.75% to 97.91%[19]. In the semi-field experiments, the mortality rates observed varied for both species with time and concentrations. The LC_{50} and LC95 value in the laboratory was similar for both species while in the semi- field they were different for each. In wild, adult mosquitoes, the KT_{50} for *S. terebinthifolia* was 11.29 min while for alphacypermethrin was 19.34 min. The 24 h mortality was found to be 100.0% for *S. terebinthifolia* and 75.0% for alphacypermethrin which was statistically significant.The larvicidal activity of petroleum ether, ethanolic, aqueous extracts of dried leaves, and fixed oil from the seeds of *Caesalpinia bonduc* showed 100% mortality in 1% concentration of petroleum ether and ethanolic extract of leaf, whereas it was 55% in 2.5% concentration of aqueous extract and 92.6% in 2.5% concentration of fixed oil against the fourth instar larvae of *Cx. quinquefasciatus*[20]. The methanol extract of *Ervatamia coronaria* showed promising larvicidal and ovicidal activity agains *An. stephensi*. The direct and indirect contributions of such effects to treatment efficacy through reduced larval feeding and fitness need to be properly understood in order to improve the use of botanical insecticides for management of *An. Stephensi*[21]. The petroleum ether extract of Solanum xanthocarpum was observed to be the most toxic with LC_{50} of 1.41 and 0.93 mg/L and LC_{90} of 16.94 and 8.48 mg/L at 24 and 48 h after application, respectively, against *An. Stephensi*[22]. Govindarajan and Karuppannan[23] reported that The LC_{50} values of benzene, hexane, ethyl acetate, methanol and chloroform extract of *E. alba* against early third instar larvae of *Ae. Aegypti*, respectively. Maximum larvicidal activity was observed in the methanol extract followed by chloroform, benzene, ethyl acetate and hexane as well as most ovicidal activity was fond in methanol extract of *E. alba* against eggs of *Ae. Aegypti* 100% mortality recorded.

Bagavan *et al*[24]have reported that peel chloroform extract of *Citrus sinensis*, leaf ethyl acetate extracts of *Ocimum canum* and *Ocimum sanctum*, and leaf chloroform extract of *Rhinacanthus nasutus* against the larvae of *An.subpictus* (LC_{50} 58.25, 88.15, 21.67, and 40.46 mg/L; LC_{90} 298.31, 528.70, 98.34, and 267.20 mg/L) and peel methanol extract of *Citrus sinensis*, leaf

methanol extract of *Ocimum canum*, and ethyl acetate extracts of *Ocimum sanctum* and *Rhinacanthus nasutus* against the larvae of *Cx. tritaeniorhynchus* (LC_{50} 38.15, 72.40, 109.12, and 39.32 mg/L; LC_{90} 184.67, 268.93, 646.62, and 176.39 mg/L), respectively. The efficacy shown by Schinustere binthifolia for knock down time and 100% mortality after 24 hours to adult mosquitoes from wild resistant population warrants further investigation of these compounds for IRS small scale whether singly or in blends. This essential oil may be of great value in complementing other compounds which are losing efficacy[25]. Kamaraj *et al*[26] reported that the highest larval mortality was found in leaf petroleum ether, flower methanol extracts of *Cryptocoryne auriculata*, flower methanol extracts of *Leucas aspera* and *Rhinacanthus nasutus*, leaf and seed methanol extracts of Solanum torvum, and leaf hexane extract of Vitex negundo against the larvae of An. subpictus (LC_{50} 44.21, 44.69, 53.16, 41.07, 35.32, 28.90, and 44.40 mg/L; LC_{90} 187.31, 188.29, 233.18, 142.66, 151.60, 121.05, and 192.11 mg/L, respectively) and against the larvae of *Cx. tritaeniorhynchus* (LC_{50} 69.83, 51.29, 81.24, 71.79, 44.42, 84.47, and 65.35 mg/L; LC_{90} 335.26, 245.63, 300.45, 361.83, 185.09, 351.41, and 302.42 mg/L, respectively).The highest repellency was observed in *Zingiber officinale*, a higher concentration of 5.0 mg/cm^2 provided 100% protection up to 150 and 180 min against *Cx. tritaeniorhynchus* and An. subpictus, respectively[27]. Wandscheer *et al*[28] reported the naturally occurring insecticides may play a more prominent role in mosquito control programs in the future. Earlier, Georges *et al*[29] reported that the n-hexane, ethyl acetate, and methanol extracts of *Cassia nigricans* showed 100% larval mortality against Ochlerotatus triseriatus. This is because larval habitat treatment is more localized in time and space resulting in effective control. In tropical countries, plants are known to possess larvicidal, ovicidal and adulticidal activities[30]. Since there is no previous record of literature available about the mosquitocidal activity of the selected chemical compounds of *P. cablin* these present investigations serve as first hand information. The finding of the present investigation revealed that the chemical compounds of *P. cablin* possessed remarkable repellent and pupicidal activity against selected vector mosquitoes.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Mosquitoes are well known for their public health importance since they cause major health problems and diseases. Indiscriminate use of several mosquitocidal agents caused various side effects. Thus there is a need to develop an alternative strategies to control vector mosquitoes. One such strategy is utilizing the plant and their secondary metabolites. In india, nearly 8000 plant species have been reported so far towards their insecticidal properties. In these, context, the present study was aimed to investigate the mosquitocidal activity of *Pogostemon cablin* essential oil against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* mosquitoes.

Research frontiers

The data obtained from the present experiments are in close agreement with the earlier reports of Saravanan *et al* (2007), Bagavan *et al* (2008) and Eliningala *et al*., (2011).

Related reports

In this present investigation, they have followed standard protocols to assess the mosquitocidal actions of selected plant essential oil. Their results showed that, The percent adult emergence was inversely proportional to the concentration of compounds and directly proportional to the pupal mortality. Among the five different compounds tested for pupicidal activity against selected vector mosquitoes, the patchouli alcohol was found to be most effective for pupicidal activity provided 28.44, 26.28 and 25.36 against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, respectively. These findings are in close with the earlier findings of Saravanan *et al* (2007), Bagavan *et al* (2008) and Eliningala *et al*., (2011).

Innovations & breakthroughs

Since, there is no previous record of literature available about the mosquitocidal activity of the selected plant essential oil and its components, the present report serve as first hand information on repellent and pupicidal activities against the adult and pupae of the selected vector mosquitoes.

Applications

Plants are always considered as vast repository of natural compounds, the exploration of research leading to their possible utilization certainly pave the way for search of new phytochemical compounds and their proper role in the near future as eco-friendly natural pesticides. These plant secondary metabolites can be used in nature as they are easily biodegradable, and safer to human beings.

Peer review

The present line of research quoted in this research article

will surely focusing an insight in phytochemical research and their possible role towards the control of mosquitoes in any form, for example larval, pupal, egg or adult stages. Thus this plant essential oil and its compounds can be an alternative agent in Integrated Vector Control Programme (IVCP).

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