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Research Article

PHYTOCHEMICAL COMPOSITION AND LARVICIDAL ACTIVITY OF ESSENTIAL OIL FROM THE LEAVES OF PLEIOSPERMIUM ALATUM (WALL. EX WT. & ARN) SWINGLE AGAINST AEDES AEGYPTI, ANOPHELES STEPHENSI AND CULEX QUINQUEFASCIATUS (DIPTERA: CULICIDAE)

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ABSTRACT

Objective: The present study was conducted to evaluate the essential oil from the leaves of *Pleiospermium alatum* (Wall. ex Wt. & Arn) Swingle (Rutaceae) was evaluated for mosquito larvicidal activity against the larvae of *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*. **Methods:** The larval mortality was observed after 12 and 24 hours of exposure periods. GC and GC- MS analyses revealed that the essential oil contain fourteen compounds. **Results:** D-Limonene (34.02%) was identified as a major chemical compounds followed by Elemol (18.04%) and γ– Terpinene (11.83%). The results were revealed that essential oil potential larvicidal activity against the 4th instar larvae of *A. aegypti, A. stephensi* and *C. quinquefasciatus*. After 12 h of exposure period the larvicidal activities were LC50=95.2 and LC90=366.7 ppm (A. aegypti), LC50=72.6 and LC90=305.2 ppm (*A. stephensi*) and LC50=104.4 and LC90=373.6 ppm (C. quinquefasciatus) and the larvicidal activities after 24 h of exposure period was LC50=53.8 and LC90=251.0 ppm (*A. aegypti*), LC50=44.4 and LC90=213.8 ppm (A. stephensi), and LC50=143.3 and LC90=375.5 ppm (C. quinquefasciatus). **Conclusion:** The present study showed the leaves of the essential oil from the *P. alatum* and its D-Limonene major compound may have potential for use in control of *A. aegypti, A. stephensi* and *C. quinquefasciatus* and could be useful in search of new, safe, inexpensive and more effective control of vector mosquitoes.

Keywords: *Pleiospermium alatum.* GC-MS. *Aedes aegypti. Anopheles stephensi. Culex quinquefasciatus*

INTRODUCTION

Vector-borne diseases, such as dengue, malaria, filariasis, dengue haemorrhagic fever schistosomiasis, leishmaniasis, chagas disease, yellow fever, lymphatic filariasis, African trypanosomiasis and onchocerciasis [1]. They have, therefore, become a challenging problem to public health worldwide and it has a serious social and economical impact especially in tropical and subtropical countries [2]. Vector-borne diseases are endemic over 100 countries, causing mortality of nearly two million people every year and at least one million children die of such diseases each year, leaving as many as 2100 million people at risk around the world [3]. Lymphatic filariasis affects at least 120 million people in 73 countries in Africa, India, Southeast Asia, and the Pacific Islands. In India, various species of *Aedes*, *Anopheles* and *Culex* mosquitoes are important insect vectors of human diseases [4]. These diseases not only cause high levels of morbidity and mortality but also inflict great economic loss and social disruption on developing countries such as India, China, etc

Vector control has been practiced since the early 20th century. During the pre-DDT era, reduction of vector mosquitoes mainly depended on environmental management of breeding habitats, i.e., source reduction. During that period, some botanical insecticides used in different countries were Chrysanthemum, Pyrethrum, Derris, Quassia, Nicotine, Hellebore, Anabasine, Azadirachtin, D-limonene Camphor and Turpentine[5]. It has prompted researchers to look for alternative approaches ranging from provision of promoting the adoption of effective and transparent mosquito management strategies that focus on public education, monitoring and surveillance, source reduction and environment friendly least-toxic larval control [6]. However, a high level of insecticide resistance has developed through chemical control of the vector and pests threatening the control strategies. To overcome these problems, it is necessary to search for alternative methods of vector control. Extensive use of chemical insecticides for control of vector borne diseases has created problems related to physiological resistance to vectors, adverse environmental effects, high operational cost and community acceptance [7].

Several phytochemicals extracted from various botanical sources have been reported to have detrimental effects on mosquitoes [8]. Natural products are generally preferred because they are less or non toxic to non-target organisms and are easily biodegradable[5]. Much interest has, therefore, been focused on plant extracts or phytochemicals are potential sources of mosquito larvicidal agents or as lead compounds. A large number of plant extracts and essential oils have been reported to possess larvicidal activities against mosquitoes [5.9].

Pleiospermium alatum (Wall. ex Wt. & Arn) Swingle (Rutaceae) a medicinal plant commonly called "Kurunthumul thazhai", distributed in India (mainly in the states of Punjab, Bihar, Orissa, Assam, Madhya Pradesh, Bombay, Mysore, and Tamil Nadu), Burma, Thailand, South Western China, Indochina and Ceylon[10]. The leaves and bark are used in the treatment of inflammation and pain management. The folklore claim suggests that the leaf is showing wound healing property [11]. The juice extracted from fresh leaves of *P. alatum* and fresh leaves of lemon grass (*Cymbopogon citratus* Stapf) is boiled in neem oil in a low flame and this oil is applied on the joints, shoulders and the other affected parts for the pain relief. Hot water is sprinkled to get relief from rheumatic complaints by the *Kanikkar* tribals of Kalakad - Mundanthurai Tiger Reserve, Western Ghats, Tamil Nadu [12].

The leaves and bark are used for the fomentation of rheumatic pain; the dried fruit is useful in malignant and pestilent fevers and is used as and for poisons [13]. The stem bark of *P. alatum* along with that of *Azadirachta indica* are boiled in water and the decoction is given orally for post-natal complaints [14]. Leaves and bark of *P. alatum* was used as fomentations in rheumatic pain, Juice as a Nasya in *Peenasa* (chronic rhinitis) and it has been described in Telugu verses of Basavarajeeyam are considered to be an elite contribution to the

Indian system of medicine [15]. Hence, the present investigations report the chemical composition and larvicidal activity of leaves from the *P. alatum* essential oil.

MATERIALS AND METHODS

Plant Material

The fresh leaves of *Pleiospermium alatum* (Wall ex. Wt. & Arn.) Swingle (Rutaceae) was collected from Silambur (Lat, 11.35 ºN; Long, 79.31ºE), Ariyalur District, Tamil Nadu, India during the month of April 2015. The voucher specimen (AUBOT#262) is deposited at the herbarium, Department of Botany, Annamalai University.

Isolation of essential oil

The fresh leaves of *P. alatum* was cut into small pieces and subjected to hydro-distillation using Clevenger X77 type of apparatus for eight hours. The obtained essential oil was dried over anhydrous sodium sulphate and the purified essential oil was stored in amber colour vial (sealed with parafilm) at 4 °C for further analysis and larvicidal assay.

Chemical Analysis of Essential oil

GC analysis was carried out using Thermo GC-Trace Ultra Ver: 5.0, Thermo MS DSQ II. The chromatograph was fitted with DB 5-MS capillary non-polar column. The injector temperature was set at 300 ºC and the oven temperature initially at 80 °C then programmed to 200 °C at the rate of 5 °C/min and held at 200 °C for 10 min. Then the temperature was increased to 300 °C at the rate of 20 °C /min, finally held at 300 °C for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 ml/min. The sample was injected in the split mode in the ratio of 1:100. The percentage of compositions of the essential oil was calculated by the GC peak areas.

GC-MS analysis

GC and GC-MS analyses of essential oil were performed by using Varian 3800 Gas chromatography equipped with Varian 1200-L single quadrupole mass spectrometer. GC conditions were same as reported for GC analysis and the same column was used. The mass spectrometer was operated in the Electron Impact (EI) mode at 70 eV. Ion source and transfer line temperature was kept at 300 °C. The mass spectrum was obtained by centroid scan of the mass range from 40-800 amu. Identification of components of the essential oil was matching their recorded spectra with the data bank mass spectra of NIST and WILEY library provided by the instrument software and the components were confirmed by comparing with previous literature.

Mosquito Larvicidal Bioassay

The eggs of *A. aegypti* and *A. stephensi* were received from the Field Station, Centre for Research in Medical Entomology (ICMR-Government of India), and Virudhachalam, and the egg rafts of *C. quinquefasciatus* was collected from drainage of local residential area of Annamalai Nagar (11° 23′ 17 N, 79° 42′ 57 E) and reared in the laboratory (29±3 °C, 75 to 85 % RH). The larvae were fed with Brewer's yeast/dog biscut (1:3). The larvicidal effect of leaves essential oil from the *P. alatum* against *A. aegypti, A. stephensi* and *C. quinquefaciatus* were studied with the standard procedures recommended[16]. The essential oil was dissolved in 1 ml of acetone and prepared in to different concentrations *viz.,* 12.5, 25, 50, 100, 200 and 400 ppm with distilled water. Twenty larvae (in a 100 ml beaker) of early fourth instars stage were used for Larvicidal assay and five replicates were maintained for each concentration. During this experiment, no food was offered to the larvae. The larval mortality was calculated after 12 and 24 h of the exposure periods. All moribund mosquito larvae were considered as dead. The larval mortality was also checked for water and DMSO individually.

Statistical analysis

The results are expressed as the mean ± SD. All statistical analyses were performed using SPSS version 11.5 statistical software (SPSS Inc., Chicago, IL, USA). The average larval mortality data probit analysis calculating LC_{50}, LC_{90} and other statistics, 95 % confidence limits and chi-square values were calculated.

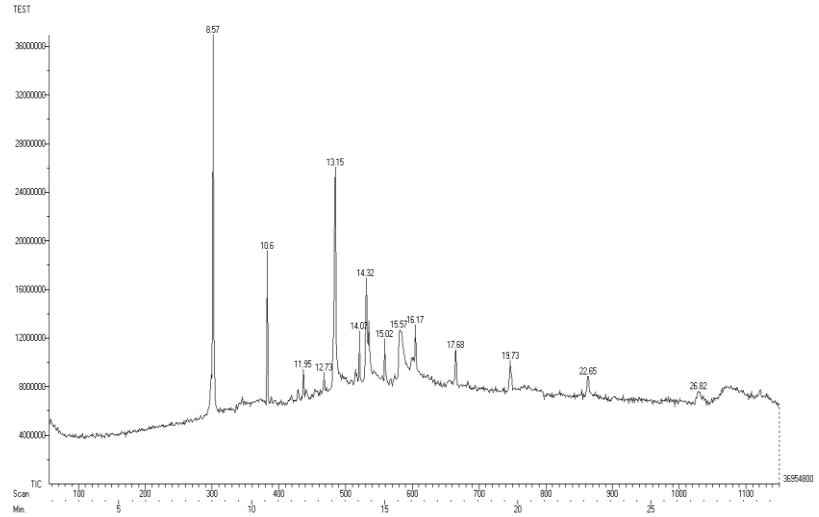

Fig. 1: Gas chromatogram of essential oil from the leaves of *Pleiospermium alatum.*

RESULTS AND DISCUSSION

Yield and Chemical composition of essential oil

The essential oil extracted from the fresh leaves of *P. alatum* yielded 0.02 % v/w of pale greenish yellow colour essential oil after the extraction time of 8 hours and the oil is soluble in acetone. GC chromatogram is presented in Fig 1. The chemical compounds were identified from the essential oil and the major chemical compositions were D-Limonene (34.02%) followed by Elemol (18.04%) and *γ*–Terpinene (11.8%) (Table1). Sethuraman *et al.* [14] (2011) reported that the *P. alatum* major components in the leaf oil was elemol (12.5%) followed by pregeijerene (10.5%), a-cadinol (8.5%) and geijerene (8.5%). The stem oil contained elemol (12.4%), a-cadinol (11.1%) and epi-a-muurolol (9.4%) as the major components, while the root oil had a-cadinol (27.9%), a-bisabolol (11.5%), a-santalene (11.0%) and elemol (9.8 %) as the major components.

Table 1: Chemical Composition of essential oil from the leaves of *Pleiospermium alatum*

Peak No.	Retention Time (min)	Chemical Constituents[a,b]	%
1	8.57	D-Limonene	34.02
2	10.60	γ Terpinene	11.83
3	11.95	Terpinen-4-0l	2.36
4	12.73	Linalool	1.77

5	13.15	Elemol	18.04
6	14.07	*trans*-Geraniol	5.32
7	14.32	α-Humulene	7.69
8	15.02	Geraniol	3.28
9	15.57	E-Citral	3.84
10	16.17	α Eudesmol	4.47
11	17.68	Elemol	3.55
12	19.73	Geranyl acetate	2.07
13	22.65	Germacrene D-4 ol	1.18
14	22.82	Blunesol	0.59
Total			**100**

[a]Compounds listed in order of elution from DB 35-MS Capillary Standard non-polar column., [b]Components identified based on computer matching of the mass peaks with WILEY and NIST Library

Fourteen compounds were identified by GC-MS and represent about 100 % of essential oil, where as DL-Limonene (34.02%) was identified as the major chemical compound. Thirugnanasampandan and David [17] reported that the various constituents as the major ones. Variation, presence and absence of chemical constituents in the essential oil may be due to various factors [18]. However, the variation of each component amount depended on several parameters including ripeness of fruits, vegetative stage of plant, storage condition and extraction method [19]. The chemical profile of the essential oil from peels and fruits differ not only in the number of molecules but also in the sterochemical types of molecules extracted. This observed differences in the chemical composition may be attributed to occurrence of chemotypes, geographical locations, season at the time of collection, stage of development, culture climate and other culture conditions, which may affect biological activities [20].

Mosquito control at the larval stage is effective procedure because they are localized in space and time[21] resulting in less-dangerous out comes to non-target organisms, while the fight against adult is temporary and unsatisfactory. During the last three decades, mosquito controls were directed to the use of insecticide of plant origin. Environmental safety of insecticides is of first and foremost criterion for mosquito control programmes [22]. Natural pesticides derived from plants are promising tool especially for and *C. quinquefaciatus* with LC_{50} 52.08 and LC_{90} 124.33 ppm (after 12 h); LC_{50} 22.49 and LC_{90} 60.90 ppm (after 24 h) respectively.

Table 2: Larvicidal properties of *Pleiospermium alatum* leaves of essential oil against the larvae of *A. aegypti, A. stephensi* and *C. quinquefasciatus* after 12 and 24 h of exposure periods

Name of the mosquito species	Time	Concentration (ppm)	% mortality ± SE	LC_{50} (LCL- UCL)[a]	LC_{90} (LCL- UCL)[a]	$\chi 2$ (*df*=4)[b]
A .aegypti	After 12h	12.5	24 ± 0.36	95.24 (11.65 – 167.87)	366.7 (259.6 – 713.6)	15.8
		25	33 ± 0.25			
		50	45 ± 0.32			
		100	62 ± 0.57			
		200	75 ± 0.60			
		400	88 ± 0.50			
	After 24h	12.5	31 ± 0.50	53.88 (34.1 – 71.29)	251.0 (217.8 – 299.5)	5.59
		25	44 ± 1.50			
		50	52 ± 0.45			
		100	68 ± 0.56			
		200	85 ± 0.52			
		400	98 ± 0.36	72.65 (15.15 –119.33)	305.2 (229.4 – 487.9)	10.3
	After 12h	12.5	27 ± 0.51			
		25	38 ±0.52			
		50	49 ± 0.50			
		100	65 ± 0.75			
		200	78 ± 0.23			
		400	94 ± 0.55			
	After 24h	12.5	33 ± 0.50	44.4 (25.9 – 59.8)	213.8 (187.7 – 276.4)	5.03
		25	48 ± 1.12			
		50	56 ± 0.46			
		100	69 ± 0.23			
		200	86 ± 1.0			
		400	100± 0.0			
C.quinquefasciatus	After 12h	12.5	18 ± 0.51	104.4 (33.8 – 172.1)	373.6 (271.0 – 668.62)	13.2
		25	28 ± 0.28			
		50	42 ± 0.50			
		100	58 ± 0.11			
		200	68 ± 0.42			
		400	76± 0.32			
	After 24h	12.5	21 ± 0.68	143.3 (66.0 – 253.7)	375.5 (261.4 – 796.4)	5.45
		25	34 ± 0.50			
		50	46 ± 0.28			
		100	55± 0.40			
		200	74 ± 0.12			
		400	88 ± 0.82			

Control-Nil activity; SD standard deviation, LCL- lower confidence level, UCL- upper confidence level; [a] 95 % confidence interval; [b] Degrees of freedom ; $\chi 2$ - -Chi-square value

In the present study, the essential oil of the fresh leaves of *P. alatum* had DL-Limonene (33.02%) as the major constituents and this reason for the highest larvicidal activity. Rajkumar and Jebanesan [21] demonstrated that borneol and sabinene, which were the major components of *Clausena dentate* essential oil, showed larvicidal activity against *A. aegypti*. In another investigation, Park *et al.* [27] reported that residues of some Myrtaceae essential oil of their components, such as allyl isothiocyanate, (E)-nerolidol, limonene, p-cymene and c-terpinene showed strong larvicidal activities against *A. aegypti.* Kovendan *et al.* [28] suggest that the terpene hydrocarbon constituents (β-myrcene, sabinene, (+)-limoene and p-cymene) are potential natural mosquito larvicides.

Cheng *et al.* [29] reported that a-phellandrene, limonene, p-cymene, c-terpinene, terpinolene and a-terpinene examined in this study exhibited great larvicidal performance. Vector control is one of the most powerful weapons in the process of managing vector

populations to reduce/interrupt the transmission of disease. As a result, vector control remains considered to be a cornerstone in the vector-borne disease control program due to lack of reliable vaccine, drug resistance parasites and insecticide resistance of insect vectors disease[30].

The results obtained in this studies support that the fresh leaves *P. alatum* essential oil secondary metabolites generally display remarkable larvicidal properties which are useful for preserving foods from decay, contamination and preventing living tissues from various diseases. Further investigations for the mode of the constituents' actions, effects on non-target organisms and field evaluation are necessary. These results obtained are useful in search of more selective, biodegradable, new, safe and more naturally produced larvicidal compounds.

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