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CHEMICAL COMPOSITION AND MOSQUITO REPELLENT ACTIVITY OF THE ESSENTIAL OIL OF PLECTRANTHUS INCANUS LINK †

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Abstract. *The chemical composition of the essential oil obtained from the leaves of* Plectranthus incanus *Link, with the yield of 0.6% (w/v), was analyzed by GC and GC/MS. A total of 16 constituents, representing 95.2% of the oil, were identified. The major components of the oil were fenchone (6.0%), piperitone oxide (32.4%), piperitenone (3.0%) and piperitenone oxide (41.5%). The repellent activity of the volatile oil at different concentrations was measured by the protection period against the bites of* Anopheles stephensi *and* Culex fatigans*. The tested oil had stronger repellent activity than citronella oil, which was used as a positive control.* LC_{50} *(30 min) for* P. incanus *oil against* An. stephensi *and* C. fatigans *were 23.8 and 19.6 µl/L air, respectively, whereas* LC_{50} *of citronella oil were 31.7 and 31.0 µl/L air. Thus,* P. incanus *may be regarded as a potential valuable source of chemicals that have strong mosquito repellent activity, and could be used for the preparation of mosquito repellent formulations.*

Key words: *Plectranthus incanus Link, essential oil, mosquito repellent activity, Anopheles stephensi, Culex fatigans*

1. INTRODUCTION

Malaria is a public health problem most especially in the tropical countries where majority bear the burden of the disease. It is one of the six killer diseases in the world today and it has been estimated that 40% of the world's population is at risk and 500 million people suffer from the disease annually. About two million children, mostly less than five years old, and pregnant women die from the malaria related illness each year and nine out of ten cases are found in Sub-Saharan Africa [1].

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Insect vectors, especially mosquitoes (e.g. *Anopheles* and *Culex*), are responsible for spreading serious human diseases like malaria, Japanese encephalitis, yellow fever, dengue and filariasis. Various synthetic products and devices designed to combat such vectors are not successful because of the increased resistance developed by various mosquito species. Moreover, most of the mosquito repellent formulations available in the market are mainly prepared with active ingredients of synthetic origin. Long term exposure of new born babies and children to parathyroid based mosquito repellents is known to cause clinical, biochemical and neurological changes [2]. Thus, there is a need to find new, effective and safe, mosquito repelling agents. It has been already shown that certain phytochemicals, produced by those species that are already traditionally used in treating numerous diseases, exhibit a significant mosquito repellent activity against variety of *Anopheles* and *Culex* species [3].

Plectranthus incanus Link (Labiatae) is an annual erect, finely pubescent, aromatic herb, about 3-4 fts. height, distributed in India, especially on hills [4]. It is known as lal-agada, basaili–rookhari, kirmar and phola [5]. Previous (phytochemical) studies on this species were focused on vernolic acid, cyclopropenoid fatty acids and volatile secondary metabolites [6, 7]. The essential oil from the dried leaves and flowering tops has been reported to possess antimicrobial [8], cardiac depressant, respiratory stimulant and vasoconstrictior activity. It exhibits relaxant activity on smooth and skeleton muscle [9, 10]. The crushed leaves of the plant are used in Chota Nagpur to stop bleeding, cure fever and repel mosquitos. However, no reports on the mosquito repellent activity and the chemical composition of the essential oil of *P. incanus* are available so far.

Having all above mentioned in mind, the aim of this study was to analyze (using GC and GC/MS) the chemical composition of the essential oil obtained from the leaves of *P. incanus* and to mutually compare the mosquito repellent activity of this and citronella oil (a renowned plant-based insect repellent, registered for this use in the United States and considered as a biopesticide with a non-toxic mode of action) against *Anopheles stephensi* (major vector of human malaria in Middle East and South Asia) and *Culex fatigans* (common house mosquito).

2. Material and Methods

2.1. Plant material

Leaves of *Plectrathus incanus* Link were collected from the Dhumka, Central India in February 2006 and identified by Dr. Kaushal Kumar, scientist, ethnobotany division. A voucher specimen (No. LKW-348521) was deposited in the Herbarium of National Botanical Research Institute, Lucknow, India.

2.2. Essential oil isolation

Fresh leaves of *P. incanus* (300g, cut into small pieces) were subjected to hydrodistillation with the appropriate amount of distilled water for 4 h using the original Clevenger-type apparatus, as given in [11]. The obtained oil was separated by extraction with diethyl ether, dried over anhydrous sodium sulphate and stored in sealed vials under refrigeration prior to analysis. The oil yield was 0.62 % (w/v).

2.3. Analysis of the essential oil

The GC/MS analysis was performed on a Thermo Fisher TRACE GC ULTRA chromatograph. The gas chromatograph was equipped with a TR 50MS column (60 m × 0.25 mm, film thickness 0.25 μm) and coupled with a DSQ II Mass Spectrometer instrument. The injector and interface were operated at 220 ^{o} and 250 ^{o}C, respectively. The oven temperature was raised from 70 ^{o} to 120 ^{o}C at a heating rate of 2 ^{o}C/min and from 120 ^{o} to 240 ^{o}C at a heating rate of 3 ^{o}C/min, and then isothermally held for 15 min. As a carrier gas helium at 1.0 mL/min was used. The sample, 1 μL of the oil solution in diethyl ether (1 : 100), was injected in a split mode (split ratio 50 : 1). Mass selective detector was operated at the ionization energy of 70 eV (ion source temperature was set at 220 ^{o}C and delay for solvent was 2 min), in the 35–650 amu range. GC (FID) analysis was carried out under the same experimental conditions using the same column as described for the GC/MS with the exception that after the temperature ramps column was held isothermally for 5 min. Detector temperature was 280 ^{o}C, while the injector was operated at 220 ^{o}C. The percentage composition was computed from the GC peak areas without the use of correction factors. Qualitative analysis of the essential oil constituents was based on several factors. Firstly, by comparison of their mass spectra with those of authentic standards, as well as those from corresponding mass spectra libraries [12, 13]. Secondly, by the comparison of the essential oil linear retention indices relative to the retention times of C_9 to C_{24} n-alkanes (Poly Science Corp., Niles, USA) on the TR 50MS column with those reported in the literature [14, 15]. Finally, wherever possible, the identification was achieved by co-injection with an authentic sample.

2.4. Mosquito repellency test

Repellency of *P. incanus* and citronella oil (Java type) was compared using a test on human subjects by exposing hands treated with different oil quantities to caged mosquitoes by following method of [16]. Hands treated with different concentrations of the oil were exposed to cages containing 100 adult female mosquitoes of *Anopheles stephensi* and *Culex fatigans* (4 day old and starved for 6 hours) for 3 minutes. Each concentration was tested against three volunteers.

$$\text{Repellency (\%)} = \frac{A-B}{A} \times 100 \qquad (1)$$

A – Number of bites on control arm, B –Number of bites on treated arm.

2.5. Protection time

Protection time was calculated by inserting treated hands in to the cages at 15 minutes intervals and observing for 3 minutes. The time between application of the repellent and landing of at least two mosquitoes, followed by confirmatory bite, was taken as a protection time.

2.6. Test for mortality

The toxicity of the oil was tested against the 3 days old adult females of *An. stephensi* and *C. fatigans* using airtight containers. Mosquitoes were released inside the containers and the filter paper impregnated with test compound was attached to the inner side of cap and thin layer of cotton was used to avoid direct contact and cap was immediately closed. The mortality of mosquitoes was observed after one hour.

Table 1. Composition of the essential oil of *Plectranthus incanus* Link

Compound[a]	Content (%)
Fenchone	6. 0
Limonene	1. 8
trans-Piperitone oxide	0. 1
Borneol	1. 4
cis-Piperitone oxide	32. 4
Piperitenone	3. 0
Hydroxyl piperitone isomer	0. 5
Piperitenone oxide	41. 5
α-Copaene	0. 8
β-Cubebene	0. 6
Caryophyllene oxide	0. 8
β-Caryophyllene	2. 9
α-Humulene	0. 8
β-Bisabolene	0. 8
α-Cadinene	1. 0
δ-Cadinene	0. 8

[a]Constituents identified by mass spectra comparison, retention index matching and/or by GC co-injection of an authentic sample.

3. RESULTS AND DISCUSSION

Hydrodistillation of the fresh plant material gave essential oil with a yield of 0.62% (w/v). A total of 16 constituents, representing 95.2 % of the oil, were identified and are given in Table 1. The major components were fenchone (6.0%), *cis*-piperitone oxide (32.4%), piperitenone (3.0%) and piperitenone oxide (41.5%). The mosquito repellent and fumigant activities (three different tests were performed, see sections 2.4-2.6) of this and citronella oil against *An. stephensi* (major vector of human malaria in Middle East and South Asia) and *C. fatigans* (common house mosquito) were mutually compared. Citronella oil (Java type) was selected as a positive control in all performed repellency tests because it is a renowned plant-based natural insect repellent, registered for this use in the United States and considered as a biopesticide with a non-toxic mode of action. It is used alone or in combination with cedar wood, lavender, peppermint, clove, eucalyptus, and garlic in a number of commercial insect repellent products [17]. *Plectrathus incanus* and citronella oil both showed 100% repellency against *An. stephensi* and *C. fatigans* when applied at concentrations of 10 μl/cm^2 or higher (Table 2). However, at lower concentrations *P. incanus* oil was much better repellency agent against both mosquito species. Moreover, *P. incanus* oil provided more than 300 min (5 hrs) protections against *An. stephensi* and *C. fatigans* when applied at the rate of 20 μl/cm^2, whereas citronella oil provided only 132 and 150 min (2.2 and 2.5 hrs) protection, respectively.

Table 2. Repellency of *Plectranthus incanus* oil against *Anopheles stephensi* and *Culex fatigans*

Con. μl/cm^2		% Repellency (mean ±S.E)		Protection time (min)	
		An. stephensi	*C. fatigans*	*An. stephensi*	*C. fatigans*
2	*P. incanus*	18.3±1.7	30.00±5.0	>1.0	>1.0
	Citronella	14.0±1.0	16.7±1.7	>1.0	>1.0
4	*P. incanus*	70.0±5.8	91.7±8.3	>1.0	8.3
	Citronella	56.7±6.7	86.6±6.7	0.5	5.7
10	*P. incanus*	100.0±0.0	100.0±0.0	21.8	18.3
	Citronella	100.0±0.0	100.0±0.0	15.7	15.0
20	*P. incanus*	100.0±0.0	100.0±0.0	>300.0	>300.0
	Citronella	100.0±0.0	100.0±0.0	132.0	150.0

Table 3. Toxicity of *Plectranthus incanus* oil against *Anopheles stephensi* and *Culex fatigans*

Dose (μl/L air)	Mortallity			
	Anopheles stephensi		*Culex fatigans*	
	Citronella	*P. incanus*	Citronella	*P. incanus*
5.0	10.0	0.0	6.4	0.0
12.5	26.6	25.0	23.3	40.0
25.0	50.0	50.0	53.3	50.0
50.0	60.0	70.0	60.0	82.5
75.0	66.6	100.0	70.0	100.0
Control	0.0	0.0	0.0	0.0
LC_{50} (μl/L air)	31.7	23.8	31.0	19.6
	(27.1 – 40.4)[a]	(22.2 – 27.6)	(27.2 – 38.3)	(18.5 – 22.8)

[a]Values in parenthesis are fiducially limits at 95% confidence interval

Plectrathus incanus oil, with LC_{50} (measured after 30 minutes of exposure to citronella or *P. incanus* oil) values of 23.8 (*An. stephensi)* and 19.6 μl/L air (*C. fatigans*), was more active fumigant that citronella oil, Table 3. Corresponding LC_{50} values of citronella oil were 31.72 and 30.95 μl/L air for *An. stephensi* and *C. fatigans*, respectively. This pronounced difference in the mosquito repellent activity of these two oils could be attributed to their different chemical composition. Both Ceylon and Java types of citronella oil typically contain citronellal, geraniol, and citronellol as major constituents. However, the Java-type is generally considered to be of superior quality to the Ceylon citronella oil [18]. It could be interesting to mention, based on extensive laboratory and field tests [19-21], that citronella is known to be generally less repellent to mosquitoes than dimethyl phthalate or DEET (*N*,*N*-diethyl-*meta*-toluamide), but it shows equal or greater repellency to stable flies (*Stomoxys calcitrans*).

Generally speaking, high activity of *P. incanus* oil in mosquito repellent tests was not surprising as essential oils obtained from some other species belonging to the same genera were found to be strong insect repellents [22-25]. The toxic, repellent and reproduction inhibitory effects of the essential oil extracted from *P. glandulosus* and one of it major compounds, fenchone, were recently evaluated against *Prostephanus truncatus* and *Sitophilus zeamais* [22]. The results suggest that fenchone, though a major constituent of *P. glandulosus* oil (and also identified in the present study on *P. incanus* oil (6.0%)), may only be a minor component of its bioactivity. Repellency of essential oil of another member of *Plectranthus* genera (*P. longipes*) against *Anopheles gambiae* was also studied [23]. The individual components of the oil or their partial synthetic blends were screened for repellency, and the obtained results of this assays suggested possible synergetic/antagonistic effects of tested compounds. In another study, *P. marruboides* essential oil showed high level of fumigant toxicity against *An. gambiae* [24]. Chemical composition and larvicidal activity of the essential oil of *P. amboinicus* against *An. stephensi* were also recently studied. The results of this study suggested that mentioned essential oil, as an inexpensive and eco-friendly source of natural mosquito larvicidal agents, could be used to control/reduce the population of malarial vector mosquito [25]. It must be stressed, however, that the chemical compositions of *P. incanus* and essential oils obtained from other *Plectrathus* species [22-25] were quite different, suggesting that a number of different plant volatiles are strong insect repellents. In fact, some previous studies have shown that quite different plant secondary metabolites, especially those that are volatile under hydrodistillation conditions (essential oils), express significant repellent effects against different insects [26]. As an example, high repellent activity of *Cymbopogan nardus* oil against *Ades aegypti* could be used [27].

To summarize, it is not only that *P. incanus* showed good mosquito repellent activity in performed tests, but it is also strong mosquitocidal agent. Hence, *P. incanus* essential oil, alone or in combinations with those obtained from other mosquito repellent plant species, could be potentially used for the preparation of mosquito repellent products. These could be in form of spray, cream, liquidator, coil, candle and sticks, and could be prepared using suitable carries/solvents/diluents, to get better protection from mosquito bites. Such formulations could help in reducing the harmful effects of synthetic mosquito repellents on human health [28].

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ODBOJNO DEJSTVO PREMA KOMARCIMA I HEMIJSKI SASTAV ETARSKOG ULJA BILJNE VRSTE *PLECTRANTHUS INCANUS* LINK

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Hemijski sastav etarskog ulja izolovanog iz listova biljne vrste Plectranthus incanus *Link, sa prinosom od 0,6% (v/m), analiziran je pomoću gasne hromatografije i gasne hromatografije sa masenom detekcijom (GC i GC/MS). Uspešno je identifikovano ukupno 16 jedinjenja, koja su sačinjavala 95,2% ulja. Glavni sastojci su bili fenhon (6,0%), piperiton oksid (32,4%), piperitenon (3,0%) i piperitenon oksid (41,5%). Testirano je odbojno dejstvo dobijenog ulja (pri različitim koncentracijama) prema komarcima, merenjem perioda zaštite od ugriza* Anopheles stephensi *i*

Culex fatigans. *Analizirano ulje pokazalo je jače odbojno dejstvo prema komarcima od "citronela" ulja, koje je korišćeno kao pozitivna kontrola. Pored toga, dobijene LC_{50} vrednosti (merenje je vršeno nakon 30 minuta) za* An. stephensi *i* C. fatigans *su bile niže u slučaju* P. incanus *(23,8 i 19,6 µl/L vazduha) u odnosu na "citronela" ulje (31,7 i 31,0 µl/L vazduha). Prema tome, biljna vrsta* P. incanus *bi se mogla smatrati potencijalnim vrednim izvorom jedinjenja sa jakim odbojnim dejstvom prema komarcima, te bi se mogla koristiti za pripremu odgovarajućih zaštitnih preparata.*

Ključne reči: *Plectranthus incanus, etarsko ulje, odbojno dejstvo prema komarcima, Anopheles stephensi, Culex fatigan*