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Repellency of Essential Oil and Sesquiterpenes from Leaves of *Chloroxylon swietenia* DC against Mosquito Bites

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ABSTRACT

Chloroxylon swietenia DC. is a medicinal as well as aromatic tree of tropical dry deciduous forests native to India and Srilanka. Analysis of essential oil from leaves isolated by hydrodistillation was carried out by GC and GC-MS. The essential oil was found to be rich in limonene, trans-β-ocimene, pregeijerene, geijerene and germacrene D. Based on an arm-in-cage test, the oil and the isolated sesquiterpenes namely, germacrene D, pregeijerene and geijerene were screened for repellent activity against *Aedes aegypti* and *Anopheles gambiae* and compared favourably with a positive control sample, DEET. The crude oil exhibited potent repellency with RD_{50} of 22.2 & 25.1 x 10^{-5} mg cm^{-2} respectively for two mosquito species followed by germacrene D, pregeijerene and geijerene. However, the crude oil was found to be equally potent as DEET, a widely used insect repellent which showed RD_{50} of 20.6 & 22.4 x 10^{-5} mg cm^{-2}. The results indicate the potential of oil and sesquiterpene compounds for future field trials in various mosquito eradication programmes.

Keywords: *Essential oil, Chloroxylon swietenia, Germacrene D, Repellent activity, Aedes aegypti and Anopheles gambiae.*

INTRODUCTION

It is estimated that every year at least 500-600 million people suffer from one of the infections like malaria, filariasis, dengue fever, yellow fever etc which are caused and spread by mosquitoes [1-3]. One of the approaches for management of these diseases is the regulation of disease transmission through mosquito eradication or prevention of mosquito bites. People used to depend on herbal products earlier but the discovery of synthetic products overshadowed the plant derived compounds as they are proved to be more efficient. In this connection, the cosmetics industry developed a formulation DEET (N,N-diethyl toluamide) for use on human skin to provide protection against blood feeding insects for a prolonged period of time [4-5]. However, the most widely used compound DEET is not pleasant smelling and causes many allergic reactions [6-7]. This has led to the searching of safe, effective plant derived repellents as an alternative to DEET. Essential oils isolated from aromatic plants that are complex mixture of odorous, volatile compounds have received much attention in recent years as potent repellents against various species of mosquitoes [8-11].

Chloroxylon swietenia DC. (*C. swietenia*) belonging to the family Rutaceae, is a tropical medicinal as well as aromatic tree of dry deciduous forests commonly known as East Indian Satinwood. The medicinal properties of the tree have long been known where the bark is used as an astringent and leaves are applied to wounds and prescribed in rheumatism [12]. Various tribal groups living in the forest areas of Andhra Pradesh, India hang leaf garlands of this tree in their houses to repel mosquitoes. The potential use of essential oil and sesquiterpenes against mosquito bites and also in mosquito management has not been investigated in detail. Therefore, this study was undertaken to screen the mosquito repellent activity of leaf essential oil and isolated sesquiterpenes against *Aedes aegypti* (*A. aegypti*) and *Anopheles gambiae* (*An. gambiae*) using arm in cage method.

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Materials and Methods

Plant material

C. swietenia leaves were collected from the Nallamala forests, near Narsapur village, Andhra Pradesh, India. A voucher specimen has been deposited in the Department of Botany, Osmania University, Hyderabad, India (OU BOT 4793).

Extraction of essential oil

The leaves were air dried and hydrodistilled in a Clevenger apparatus for 4 hr. The distilled oil was dried over anhydrous sodium sulphate and stored under nitrogen atmosphere until further use.

GC analysis

Varian-gas chromatograph equipped with Flame Ionization Detector and BP-1 capillary column was used for the analysis of essential oil. Helium was used as a carrier gas with a flow rate of 1.0 ml/min and 8 p.s.i inlet pressure. Temperature programming employed was from 60 °C to 220 °C at a ramp rate of 5 °C/min and a final hold time of 6 min. The temperatures of injector and detector were maintained respectively at 250 °C and 300 °C. The sample (0.2 µl) was injected with 1:20 split ratio.

GC-MS analysis

Agilent 6890 GC equipped with 5973 N mass selective detector and capillary column (HP-5 MS) was used for the analysis by GC-MS. The oven temperature was programmed from 50 °C to 280 °C at a ramp rate of 4 °C/min and held at this temperature for 5 min. Inlet and interface temperatures were 250 °C and 280 °C respectively. Helium at a flow rate of 1.0 ml/min (constant flow) was employed as a carrier gas. Sample (0.2 µl) was injected under split of 20:1. EIMS: electron energy, 70ev. Ion source and quadrupole temperatures were maintained respectively at 230 °C and 150 °C.

Isolation of compounds

The major sesquiterpene compounds, Germacrene D, Pregeijerene and Geijerene were isolated by preparative gas chromatography.

Preparative GC

Preparative GC was carried out on a Hewlett Packard GC equipped with a Thermal conductivity detector (TCD). A stainless steel column packed with 5 % OV 101 on Chromosorb W-HP 100/ 120 mesh was used for the analysis. Injector and detector temperatures were maintained respectively at 250 °C and 260 °C. The column temperature was 150 °C for isothermal analysis and 200-240 °C at 20 °C/min for the programmed analysis. Nitrogen was employed as a carrier gas at a flow rate of 100 ml/min.

Identification of compounds

GLC facilitated the identification of individual components by comparing the retention indices (RI) of the peaks on BP-1 column determined with reference to a saturated mixture of C_8-C_{22} n-alkanes with linear interpolation and also with those reported in literature [13-15]. Further identification of components was accomplished by GC-MS by comparing their mass spectra with those in Wiley and NIST mass spectral databases.

Mosquito repellent activity

Laboratory bioassays were performed by using arm-in-cage test [16-17] against *Aedes. aegypti* (*A. aegypti*) and *Anopheles gambiae* (*An. gambiae*) (obtained from Toxicology Division, Vimta Laboratory, Hyderabad, India). The tests were performed using six human volunteers who showed mild or no allergic reactions to mosquito bites or oil and the compounds. A series of concentrations ranging from 10^{-2} to 10^{-5} g/ml of dissolved oil (in Acetone, HPLC grade) and isolated compounds were prepared and six replicates were run for each concentration. An arm was covered with a rubber glove with a 3x10 cm window and 0.2 ml of test solutions was applied. The treated arm was exposed to hungry female mosquitoes of 5-7 days old in the cage and kept for 3 min. Observations were made on the number of mosquitoes that landed on the arm. The arm treated with acetone served as control and the control arm was exposed before the start of each assay. Protective efficacy (PE) at each dose was calculated using the formula, PE = (% control mean - % test mean) / % control mean [18]. Probit analysis [19-20] was conducted to determine the RD_{50} representing the concentration of dose in g/ml that caused 50 % repellency along with 95 % confidence intervals.

Results

Composition of essential oil

The essential oil obtained by hydrodistillation of leaves of *C. swietenia* was analyzed by GC, GC-MS and mass spectroscopy and the results were presented in Table-1. In the present study, the oil obtained was pale yellow colour with a yield of 0.30 % and a total of 27 compounds have been identified amounting to 92 %. Five monoterpene hydrocarbons identified were -pinene (3.5 %), myrcene (1.0 %), limonene (23.8 %), cis-ocimene (2.4 %) and trans-ocimene (4.8 %). Sesquiterpenes were found to be present in large quantities o the total oil (49.6 %) and the major compounds identified were germacrene D, geijerene, pregeijerene, -caryophyllene and bicyclogermacrene. However, the oxygenated derivatives of monoterpenes and sesquiterpenes were also noted as minor constituents.

Mass Spectra of isolated compounds

Pregeijerene : EIMS (70ev). M^+ 162 (32.4), 147 (100), 133 (2.9), 119 (11.8), 105 (10.3), 91 (10.3), 77 (4.4), 65 (2.9), 55 (2.9), 41 (5.9).

Geijerene : EIMS (70ev). M^+ 162 (1.4), 147 (5.6), 133 (1.4), 120 (2.8), 106 (3.5), 94 (55.6), 79 (100), 65 (4.2), 53 (9.7), 39 (20.8).

Germacrene D : EIMS (70 ev). M^+ 204 (12.5), 161 (100), 147 (6.9), 133 (23.6), 119 (45.8), 105 (70.8), 91 (54.2), 81 (36.0), 67 (12.5), 55 (15.3), 41 (23.6).

Mosquito repellent assays

The essential oil and the isolated compounds were evaluated for repellent activity against *A. aegypti* and *An. gambiae* mosquitoes which are anthropophilic in comparison with that of DEET. As evident from table-2, the pure oil showed potent repellent activity with RD_{50} 22.2 and 25.1 x 10^{-5} mg cm^{-2} respectively for *A. aegypti* and *An. gambiae* indicating that the oil was equally efficient with that of a positive control sample, DEET. Among the three sesquiterpenes, germacrene D (RD_{50} 29.9 and 33 x 10^{-5} mg cm^{-2}) was found to be more active followed by pregeijerene (43.5 and 48.7 x 10^{-5} mg cm^{-2}) and geijerene (61.8 and 64.4 10^{-5} mg cm^{-2}). However, DEET gave RD_{50} 20.6 and 22.4 x 10^{-5} mg cm^{-2} respectively for the two vector species under same conditions.

Table 1: Seasonal variations of essential oil composition of leaves of *Chloroxylon swietenia* DC

Compound	RI	%Composition	Method of Identification
α-pinene	937	3.5	a, b
myrcene	984	1.0	a, b
limonene	1024	23.8	a, b
cis-β-ocimene	1035	2.4	a, b
trans-β-ocimene	1040	4.8	a, b
linalool	1085	1.1	a, b
geijerene	1143	16.4	a, b
geraniol	1240	0.3	a, b
pregeijerene	1285	10.2	a, b
delta-elemene	1337	0.4	a, b
geranyl acetate	1358	1.3	a, b
β-cubebene	1378	0.4	a, b
β-bourbonene	1386	1.2	a, b
β-elemene	1389	0.6	a, b
methyl eugenol	1403	1.0	a, b
β-caryophyllene	1421	2.2	a, b
α-humulene	1446	0.5	a, b
germacrene D	1480	13.6	a, b
bicyclogermacrene	1493	1.5	a, b
delta cadinene	1536	0.5	a, b
caryophyllene oxide	1574	0.4	a, b
T- cadinol	1642	0.6	a, b
β-bisabolol	1672	0.9	a, b

a- Retention indices, b- Mass spectra

Discussion

The oil from the leaves of *C. swietenia* was studied earlier [21]. The study revealed that the main constituents in the oil were limonene, -pinene, (E)-ocimene, -caryophyllene, -humulene and germacrene D with a total yield of 0.05 %. But germacrene D was reported as being present only up to 2.5%. Further studies on the essential oil of the same tree were carried out by Garg and Oswal [22] wherein, the major constituents identified include methyl-heptenone, Terpenen-4-ol and caryophyllene oxide. Umer Sharief and Jagadishchandra [23] identified 29 constituents from the leaf essential oil of *Chloroxylon swietenia* and reported that the oil was rich in limonene. In the present study, the leaves collected from Narsapur area of Andhra Pradesh have displayed an altogether different composition than those of published reports which clearly indicates the marked influence of environmental conditions on the production of phytochemicals. Sesquiterpenes germacreneD, pregeijerene and geijerene were isolated by preparative GC and their mass spectra were presented (Figure 1).

Evaluation of repellent activity was carried out using human subjects, as testing repellents on animals or artificial membranes may not give representative data of how the repellent when applied to human skin will perform [24]. Dose response studies carried out by application of compounds to human skin indicate that the pure oil is equally potent and comparable to DEET and the sesquiterpenes are less potent than those of parent oil and DEET. According to the recommendations of US Environmental Protection Agency [25], laboratory studies of repellent activities using *A. aegypti* along with mosquitoes from genera *Anopheles*, a representative human biting species can provide information on the difference in response of the main vector genera of mosquitoes. The patterns of sensitivity to repellent compounds varied between mosquito genera [26]. Furthermore, *A. aegypti*, the traditional test species, was an exceptionally poor predictor for the responses of *Anopheles* species to repellents. Curtis et al., [27] showed that *Anopheles* mosquitoes were less sensitive to DEET and other repellent chemicals than *A. aegypti*. The present results showed that the pure oil and sesquiterpenes were found active against *A. aegypti* than *An. gambiae*. The sesquiterpenes isolated are the major active principles responsible for the repellent activity, and however, the synergistic action of other constituents of the oil cannot be disregarded as the whole oil was more active than the individual compounds. The sesquiterpenes, germacrene D, pregeijerene and geijerene are being reported for the first time as repellents against the two vector species of mosquitoes.

Conclusions

The present study indicates that the essential oil and sesquiterpenes have repellent properties and compared favourably with DEET which lends support to the practice of local tribal population in using leaf garlands as mosquito repellents. Further studies are needed to develop an appropriate

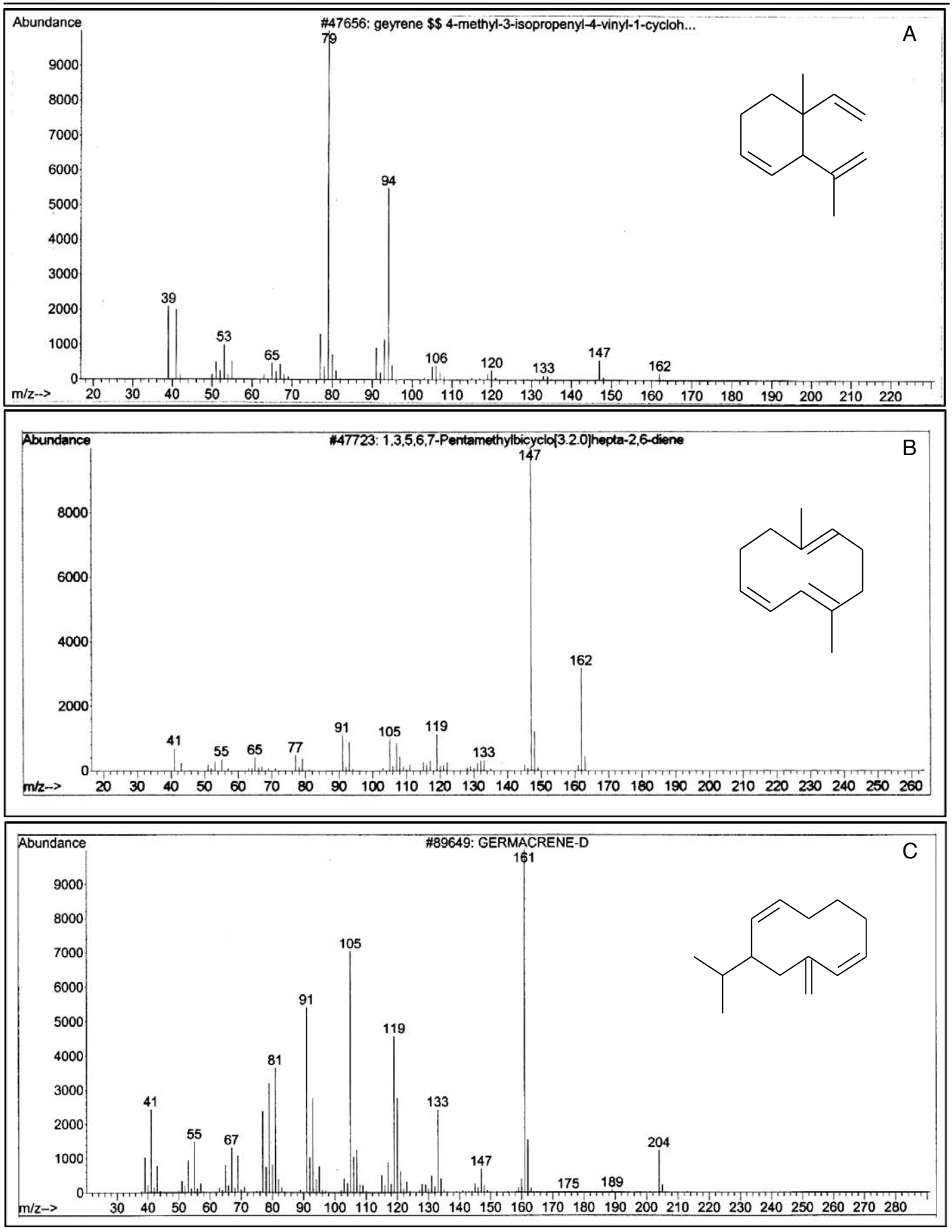

Figure 1: Mass spectra of (A) geijerene; (B) pregeijerene; (C) germacrene D

Table 2: Repellent assay of oil and isolated compounds

Compound	Species	% mean P.E Concentration g/ml			$RD_{50} \times 10^{-5}$ (mg cm^{-2})
		10^{-1}	10^{-3}	10^{-5}	
Pure oil	*A. aeg..*	98.1±1.24	72.2±2.21	51.6±2.16	22.2 (18.165-26.168)
	An. gam.	97.0±1.10	70.3±1.70	49.9±2.68	25.1 (20.412-29.721)
Germacrene D	*A. aeg.*	90.1±1.84	58.9±2.67	40.1±2.18	43.5 (38.360-48.573)
	An. gam.	88.2±1.65	53.6±2.47	38.2±1.86	48.7 (43.565-54.002)
Pregeijerene	*A. aeg.*	86.2±1.37	49.9±2.46	36.3±1.90	61.8 (56.004-67.523)
	An. gam.	83.0±1.50	46.6±2.30	32.3±1.28	64.4 (55.087-73.713)
Geijerene	*A. aeg.*	80.0±2.12	43.6±2.66	29.5±1.44	88.3 (81.490-95.110)
	An. gam.	78.4±2.28	40.8±2.98	25.4±1.54	91.5 (84.034-98.366)
DEET	*A. aeg.*	99.0±1.00	77.9±1.77	54.1±1.73	20.6 (14.561-25.439)
	An. gam.	98.6±1.12	74.4±2.13	50.1±1.31	22.4 (17.278-27.522)

Mean ± S. E, N = 6; An. gam. = Anopheles gambiae; A. aeg. = Aedes aegypti; Numbers in parentheses are 95 % cl.

formulation of oil and the compounds of this plant which would increase their efficacy and cost effectiveness.

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