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Chemical composition and larvicidal activity of the essential oil of *Glycosmis pentaphylla* (Retz.) against three mosquito vectors

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Abstract
The larvicidal activity of essential oil extracted from *Glycosmis pentaphylla* (Retz.) was evaluated against three mosquito species *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus.* Fifty five compounds were identified through GC-MS analysis of the essential oil and showed bioactive compounds. The major compounds were biocyclo (6.1.0) non-1-ene (18.93%), benzaldehyde oxime (15.66%) caryophyllene oxide (7.47%) aromadendrene (0.30%). Five different logarithmic concentration of essential oil was evaluated. The Lethal Concentration 50 (LC_{50}) and Lethal Concentration 90 (LC_{90}) for 24 hr and 48 hr exposure against *A. stephensi* larvae were 13.79 ppm, 5.85 ppm and 45.06 ppm 30.51 ppm respectively. These properties suggest that *G. pentaphylla* oil has a potential and valuable larvicidal compound. This plant which causes high mortality at lower doses could be considered as potential plant for bioprospecting.

Keywords: *Glycosmis pentaphylla,* Essential oil, Larvicidal activity, GC-MS, *A. stephensi, A. aegypti, C. quinquefasciatus.*

Introduction
Mosquitoes constitute a major public health problem as these act as the vectors for many human diseases such as malaria, dengue fever, yellow fever, filariasis and several other diseases [11, 18]. The control of mosquito larvae worldwide depends primarily on applications of organophosphorus such a temephos, fenthion and insect growth regulators such as diflubenzuron and methoprene [45]. Moreover, vector control is facing a threat due to the emergence of resistance to synthetic insecticides. In this context, essential oils have a larger attention as potentially useful bioactive compounds against pest and as an alternative source for mosquito larval control agent [9, 28].
The application of easily degradable plant compounds is considered as the safest method in controlling insect, pest and vectors [40, 42]. Plant extracts as control agents are safer for non-target organisms including man, therefore, plant based formulations are more feasible from environmental perspective than synthetic mosquitocides [4].
Botanical pesticides and essential oils are the alternatives for chemical as they possess an array of chemicals that includes larvicidal, adulticidal and repellency activities against medically important vectors that transmit disease to humans [38].
Great deal of research have been done by number of authors around the globe and significant progress has been made in the field of management of notorious mosquito species through the utilization of botanical (essential oil) derived from aromatic medicinal plants [43, 22].
Essential oils from plants may be an alternative source of mosquito larval control agents. Since these constitute a rich source of bioactive compounds that are biodegradable into a nontoxic product and are potentially suitable for use in integrated management program. In fact, many researchers have reported on the effectiveness of plant essential oils against mosquito larval control [20].
Most of mosquito control programs target the larval stage in their breeding sites with larvicides than adulticides because it only reduces the adult population temporarily [11]. Plant essential oils in general have been recognized as an important natural resource of insecticides [15]. Their lipophilic nature facilitates them to interfere with basic metabolic, biochemical, physiological and behavioral functions of insects [31].
The ethnic people of India used the whole plant of *Glycosmis pentaphylla* for the treatment of

cancer [37]. The plant reported to be a good antifungal agent for example amides from *Glycosmis mauritiana* [17] and *G. pentaphylla* [3]. Phytochemical analysis of this species were mainly focused on hydrophobic alkaloids, including those of the quinolone, quinazoline, acridone, carbazole types of leaves; root and stem bark [5, 8, 19, 29, 35].

The insecticidal properties of *Glycosmis pentaphylla* leaf oil have not been explored. Keeping in view the recently increased interest in developing insecticides of plant origin as an alternative to chemical insecticides, due to their easy availability, low budget and less environmental impact, this study was undertaken to study the larvicidal and repellent potential of the essential oil of plant *G. pentaphylla* against larval stage of *A. stephensi*; *A. aegypti* and *C. quinquefasciatus*. The results of the present study would be useful in promoting research aiming towards the development of new agent for mosquito control based on bioactive compounds derived from indigenous plant sources.

2. Materials and Methods

Glycosmis pentaphylla plant leaves were collected from Koovathur Village in Cheyyur, Kanchipuram district, Tamilnadu, India. The *G. pentaphylla* was identified by (Voucher No: 2000) Prof. P. Jayaraman, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai-45.

2. 1 Distillation of essential oils

Fresh leaves of *G. pentaphylla* was subjected to hydro distillation using a modified Clevenger-type apparatus for 3 hours (Cheng *et al*., 2005) [7]. The yield was averaged over four experiments and calculated according to dry weight of the plant material. Essential oil was stored in air tight containers prior to analysis by Gas chromatography mass spectrometry (GC-MS).

2.2 GC-MS Analysis GC-MS Analysis

The composition of the essential oils was determined using an Agilent 7890A Gas Chromatography Mass spectroscopy instrument. Oxygen-free nitrogen was used as a carrier gas, and hydrogen was used for the flame. The GC conditions used were as follows: capillary column: fused silica (Polydimethylsiloxane 0.25µm film thickness); temperature program: 70 ºC (2 min¹), 70-230 ºC (3 min¹), 230-240 ºC (5 min¹), 270 ºC (5 min¹); carrier gas, held at 5 bar, linear velocity of 20 cm min¹; injection port split less at 250 ºC; injection volume, 0.1 µL. The MS conditions were as follows: ionization EI at 70 eV; m/z range, 30-300 ºC; scan rate 1 sec ¹; ionization chamber at 180 ºC; and transfer line at 280 ºC. The identification of the essential oil constituents was based on a comparison of their retention times, and these constituents were further identified and authenticated using MS data compared to the NIST mass spectral library.

2.3 Selection of mosquito species

The important vector species of mosquitoes such as *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* were selected for the study.

2.3.1 Procurement of eggs and rearing of mosquito larvae

The egg rafts of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* were procured from Department of Entomology, Loyola College Chennai, India The eggs of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* were collected and brought to the laboratory (Department of Zoology, Pachaiyappas College for Men, Kanchipuram-01) and kept in tray containing tap water (as culture medium) at laboratory condition (26±2 ºC). The next day, eggs were observed to hatch out into first instar larvae. Appropriate amount of nutrients (yeast powder and glucose) were added to the culture medium. On the third day after hatching, the first instar larvae moulted into second instar larvae. On the fifth day, third instar larvae were observed which moulted into fourth instar larvae on the seventh day. The durations of first to fourth instar larval periods of *C. quinquefasciatus* were observed to be similar to that of *A. aegypti* and *A. stephensi*. The fourth instar larvae which moulted on the seventh day were allowed to grow in the medium up to eighth day. The fourth instar larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* were used for the experiments in the present study.

2.3.2 Bioassays and larval mortality

Fourth instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* were exposed to test concentrations of 5, 10, 20, 40, 80, 160 and 320 ppm of essential oil for 24 hours according to standard method described by WHO (1981). In control, ethanol was applied in the water (1%) the numbers of dead larvae were counted after 24 h and 48 h of exposure and the percentage of mortality were reported from the average of five replicates. The lethal concentration (LC_{50} and LC_{90}) were calculated by probit analysis s [13].

2.4 Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95 % confidence limits of upper confidence limit and lower confidence limit, and Chi-square values were calculated using the SPSS 12.0 (Statistical Package of Social Sciences) software. Results with $P< 0.05$ were considered to be statistically significant.

3. Results

Glycosmis pentaphylla was collected from Koovathur Village in Cheyyur, Kanchipuram district, Tamilnadu, India. The yield of leaf essential oil from hydro distillation of *G. pentaphylla* was 1.0 ml /1 kg (Fig 1 and 1.1). Results of GC/MS analysis showed 55 compounds (Table 1) were present in leaf essential oil of *G. pentaphylla*. These compounds were identified through mass spectrometry attached with GC. The mass spectra of these compounds were matched with those found in NIST/NBS spectral database. The principle compounds were Bicyclo [6.1.0] non-1-ene (18.93%), Benzaldehyde oxime (15.66%), Caryophyllene oxide (7.47%), followed by 3,4-Dimethyl-2-prop-2-enyl-2,5-dihydrothiophene, 1,1-dioxide (6.43%), Bicyclo [5.1.0] octane (4.74%), 1,4-Dimethyl-8-isopropylidenetricyclo [5.3.0.0 (4,10)] decane (4.69%), 3H, Azepine, 2-methoxy (4.20%), Adamantane (4.17%), Beta Panasinsene (3.11%) and Beta Pinene (3.15%).

Fig 1: Leaves of *G. pentaphylla*

Fig 1. 1: Essential oil from *G. pentaphylla*

Table 1: Gas Chromatography Mass Spectrometry for Essential oil from leaves of *G. pentaphylla*

Sl. No	Retention Time	Area (%)	Compounds	Molecular Formula	Molecular Weight
1.	3.799	0.48	Bicycle[3.1.0]hex-2-ene,4-methyl-1-(1-methylethyl)-;	$C_{10}H_{16}$	136.23
2.	3.929	3.15	Beta-Pinene	$C_{10}H_{16}$	136.24
3.	4.176	0.06	Camphene;	$C_{10}H_{16}$	136.23
4.	4.670	0.12	Cyclohexane,4-methylene-1-(1-methylethyl)-	$C_{10}H_{18}$	138.24
5.	4.873	1.39	Santolina triene	$C_{10}H_{16}$	136.23
6.	5.149	2.27	1-Hepten-3-yne;	C_7H_{10}	94.15
7.	5.251	0.29	(+)-3-Carene	$C_{10}H_{16}$	136.23
8.	5.367	0.09	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-;	$C_{10}H_{16}$	136.23
9.	5.687	15.66	Benzaldehyde, oxime	C_7H_7NO	121.39
10.	5.774	4.74	Bicyclo[5.1.0]octane, 8-methylene-;	C_9H_{14}	122.20
11.	5.919	2.43	Tricyclo[4.1.0.0(2,7)]heptane	C_7H_{10}	94.15
12.	6.108	0.37	Gamma-Terpinene	C10H16	136.23
13.	6.616	0.34	Cyclohexane,1-methyl-4-(1 methylethylidene)-	$C_{10}H_{16}$	136.23
14.	6.776	0.11	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H_{14}O$	154.24
15.	7.052	0.29	1,4-Hexadiene, 3,3,5-trimethyl-	C_9H_{16}	124.22
16.	7.270	0.55	2,4,6-octatriene,2,6-Dimethyl-, (E,Z)-	$C_{10}H_{16}$	136.23
17.	7.487	0.06	2,4,6-Octatriene, 2,6-dimethyl-, (E, Z)-;	$C_{10}H_{16}$	136.23
18.	8.199	0.10	Butanoic acid, 3-Hexenyl ester, (Z)-;	$C_{10}H_{18}O_2$	170.24
19.	8.548	0.08	Trans-2-Caren-4-ol	$C_{10}H_{16}O$	152.23
20.	8.911	0.06	4-Methyl-1,3-pentadiene;	C_6H_{10}	82.14
21.	9.666	0.04	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans-	$C_{10}H_{16}O$	152.23
22.	10.247	0.12	4-Hexyn-3-ol	$C_6H_{10}O$	98.14
23.	10.392	0.15	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-	$C_{10}H_{14}$	134.21
24.	10.668	0.38	Alpha-Cubebene	$C_{15}H_{24}$	204.35
25.	11.046	0.25	Copaene	$C_{15}H_{24}$	204.35
26.	11.162	0.04	Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-	$C_{10}H_{16}$	136.23
27.	11.263	0.87	(E, Z)-.alpha.-Farnesene	$C_{15}H_{24}$	204.35
28.	11.539	0.36	1H-Cycloprop(e)azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta., 7b.alpha.)]-	$C_{15}H_{24}$	204.35
29.	11.757	18.93	Bicyclo [6.1.0] non-1-ene	C_9H_{14}	122.21
30	11.932	0.30	Aromandendrene	$C_{15}H_{24}$	204.35
31.	12.149	6.43	3,4-Dimethyl-2-prop-2-enyl-2,5-dihydrothiophene 1,1-dioxide	$C_9H_{14}O_2S$	186.27
32.	12.367	0.97	Cycloisolongifolene	$C_{15}H_{24}$	204.35
33.	12.541	4.20	3H-Azepine, 2-methoxy-	C_7H_9NO	123.15
34.	12.658	4.69	1,4-dimethyl-8-isopropylidenetricyclo [5.3.0.0 (4, 10)]decane	$C_{15}H_{24}$	204.35
35.	12.948	1.50	4-isopropyl-1,6-dimethyl-1, 2, 3, 4-tetrahydronaphthalene	$C_{15}H_{22}$	202.33
36.	13.122	2.23	Beta Panasinene	$C_{15}H_{24}$	204.35
37.	13.340	0.39	2-Petadecen-4-yne, (Z)-	$C_{15}H_{26}$	206.36
38.	13.413	2.23	Patchoulene	$C_{15}H_{24}$	204.35
39.	13.558	0.71	Cyclooctanemethanol	$C_9H_{18}O$	142.24
40.	13.761	7.47	Caryophyllene oxide;	C_7H_9NO	123.15
41.	13.950	0.66	Ledol	$C_{15}H_{26}O$	222.36
42.	14.037	2.25	o-Menth-8-ene	$C_{10}H_{18}$	138.24
43.	14.342	4.17	Adamantane	$C_{10}H_{16}$	136.23
44.	14.560	3.11	Beta.-Panasinsene	$C_{15}H_{24}$	204.35

45.	14.705	1.47	Z-3-Hexadecen-7-yne	$C_{16}H_{28}$	220.393
46.	15.010	0.75	Cedrene-V6	$C_{15}H_{24}$	204.35
47.	15.112	0.25	Cyclohexane, 1,5-diethenyl-3-methyl-2-methylene-, (1.alpha., 3.alpha.,5.alpha)-	$C_{12}H_{18}$	162.27
48.	15.170	0.80	Farnesol isomer a;	$C_{15}H_{26}O$	222.36
49.	15.417	0.46	Bicyclo[3.2.0]heptane, 6-methylene	$C_{18}H_{24}$	110.19
50.	15.751	0.54	1-(3,3-dimethyl-1-yl)-2,2-dimethylcyclopropene-3-carboxylic acid	$C_{12}H_{16}O$	192.25
51.	16.114	0.15	Caryophyllene;	$C_{15}H_{24}$	204.35
52.	16.201	0.19	3-Undecyne	$C_{11}H_20$	152.27
53.	16.739	0.21	1,5,5-trimethyl-6-(3-methyl- buta-1,3-dienyl)-cyclohexane	$C_{14}H_{22}$	190.32
54.	16.739	0.07	Benzoic acid, 2-propenyl ester	$C_{10}H_{10}O_2$	162.18
55.	18.293	0.04	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-,	$C_{15}H_{26}O$	222.36

The essential oil extracted from the leaves of *Glycosmis pentaphylla* had a remarkable larvicidal activity (Table 2) against *A. stephensi* LC_{50} = 13.79 and LC_{90} = 45.06 ppm (after 24 hrs) LC_{50} = 5.85 and LC $_{90}$ = 30.51 ppm (after 48 hrs): *C. quinquefasciatus* LC_{50} = 29.74 and LC_{90} = 65.95 ppm (after 24 h) LC_{50} = 19.40 and LC $_{90}$ = 42.70 ppm (after 48 h) and *Aedes aegypti*, LC_{50} = 32.48 and LC_{90} = 108.19 ppm (after 24 h). LC_{50} 21.45 and LC_{90} 53.37 ppm (after 48 h). The oil showed 100% mortality against all the tested species at 100 ppm.

Table 2: Lethal Concentration of plant oil extract *G. pentaphylla* against *A. stephensi*, *A. aegypti* and *C. quinquefasciatus*

Species	Hr	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation		95% Confidence limits				Chi-sqare		*P*-Value	
						UCL (ppm)		LCL (ppm)					
				LC_{50} (ppm)	LC_{90} (ppm)	LC_{50} (ppm)	LC_{90} (ppm	LC_{50} (ppm)	LC_{90} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)
Anopheles	24	13.797	45.064	Y=-0.724+0.052X	Y=-0.800+0.046X	14.946	49.526	12.796	41.832	28.60	32.15	0.194	0.097
	48	5.855	30.513	Y=-0.580+0.099X	Y=-0.873+0.070X	6.472	33.130	5.167	28.636	15.59	59.36	0.872	0.000
Aedes	24	32.481	108.194	Y=-1.067+0.032X	Y=-0.406+0.015X	35.299	136.532	30.173	92.380	31.814	33.187	0.104	0.078
	48	21.451	53.371	Y=-0.700+0.032X	Y=-0.907+0.041X	24.315	58.511	18.638	49.642	65.49	70.731	0.000	0.000
Culex	24	29.749	65.951	Y=-1.174+0.039X	Y=-0.693+0.029X	31.357	74.890	28.326	59.681	29.164	46.738	0.175	0.002
	48	19.405	42.700	Y=-0.546+0.028X	Y=-0.536+0.042X	21.192	45.852	17.565	40.148	15.636	33.559	0.870	0.072

LC_{50}- lethal concentrations 50; LC90-lethsl concentration 90; ppm-parts per million; UCL=upper concentration level; LCL-lower concentration level

4. Discussion

Essential oils may be an alternative source for mosquito larval control, since they have a rich source of bioactive compounds that are biodegradable into nontoxic products and are potentially suitable for use in integrated pest management programs. The high volatile of essential oil and its constituent strongly suggest that the residue problem will be minimal when they are applied in the field [32].

In fact, many researchers have reported the effectiveness of plant essential oils of *Juniperus macropoda* and *Pimpinella anisum* as larvicidal, adulticidal, ovicidal, oviposition-deterent and repellent activity against three mosquito species; *An. stephensi, Ae. Aegpti,* and *Cx. quinquefasciatus* [34].

The high sesquiterpene content of the oil may be responsible for the larvicidal activity, which is in agreement with other reports [21, 27] myristicin, which has insecticidal activity against *Spilarctiaovliua* [41] and larvicidal activity against *A. aegypti* [26]. could be playing a major role in the bioactivity of this oil. The larvicidal activity of essential oil may also be due to the presence of major chemical constituents such as biocyclo-non-1-ene, benzaldehyde oxime, caryophyllene oxide and aromandendrene.

Plant essential oils, in general, have been recognized as an important natural resource of insecticides [15, 1]. The compound gamma terpinene from the essential oil of *Blumea martiniana* was found to possess potent larvicidal activity against *A. anthropophagus* [24]. Gamma terpinene also shows potent larvicidal activity against larvae *A. aegypti* and *A. albopictus* with LC_{50} 30.7 and LC_{50} 29.8 µg/ml [6, 16, 33, 30, 2, 36]. Caryophyllene present in the essential oils of different plants possess significant insecticidal activities against different species of mosquitoes [25, 39, 10, 23]. The larvicidal activity of essential oil of *G. pentaphylla* could be attributed to the presence of caryophyllene oxides, terpinene and beta pinene compounds.

The present studies suggest the use of the essential oil from the leaves of *G. pentaphylla* as a mosquito larvicide. The essential oils are easily available and the cost constraint may be overcome by low LC_{50} value. The essential oil of this plant could be utilized by public for controlling mosquito larvae in small water bodies recognized as breeding sites. Future scope of this investigation is to develop a suitable formulation with appropriate synergistic agents and field evaluation of the product for determination of toxicology effect and bioefficiency.

5. Conclusion

In conclusion, an attempt has been made to evaluate the possible role of *G. pentaphylla* essential oil to control mosquitoes. As natural products are generally preferred in vector control measures due to their less deleterious effect on non-target organisms, their innate biodegradability and keeping in view the resistance developed by the mosquito's larvae against chemical insecticides, it is worthwhile to

identify new bioactive compounds from natural products against larvicidal activity. Hence the results reported here open the possibility of further investigation of the efficacy *G. pentaphylla* essential oil in terms of its larvicidal activity.

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