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Identification of insecticidal properties in common weed - *Lantana camara* Linn by Gas Chromatography and Mass Spectrum (GC-MS-MS)

S. Murugesan, C. Rajeshkannan, D. Suresh Babu, R. Sumathi and P. Manivachakam

Division of Bioprospecting Institute of Forest Genetics & Tree Breeding, R. S. Puram, Coimbatore-641002. India.

ABSTRACT

Oil extracted from the leaves of Lantana camara by hydrodistillation was processed for bioactive molecules of insecticidal action against the teak defoliator, Hyblaea puera. Oil fraction analysed by GC/MS/MS has revealed major compounds of the said nature. About thirty six compounds were characterized from essential oil of L.camara, some of them like α- Copaene, Germacrene D & B, α – Cubebene, β – Elemene, α – Guaiene, α – humulene , Aromadendrene, β – Selinene, α – Selinene, Caryophyllene oxide, Nerolidol, Spathulenol and Delta – Cadinene, have expressed tritrophic interactions as reported by earlier findings as well as insecticidal activity in terms of larval mortality against teak defoliator.

Keywords: GC-MS-MS analysis, *Lantana camara*, essential oil and insecticidal properties.

INTRODUCTION

Lantana is a genus of about 150 species of perennial flowering and ornamental plant [1]. *Lantana camara* L. (camará) is a shrub belongs to Verbenaceae and native range of Mexico, Central America, the Greater Antilles, The Bahamas, Colombia, and Venezuela [2]. It is believed to be indigenous to the Lower Rio Grande Valley of Texas in the United States [3]. It has become naturalized in tropical and warm regions worldwide [4]. *L. camara* is a rich source of bioactive molecules and the phytochemical studies have resulted in the identification and isolation of terpenoids, flavonoids, phenylethanoid glycosides, furanonaphthoquinones, iridoid glycosides, steroids [5,6] triterpenes and flavonoids [7,8]. Leaves, have been used for the treatment of various disorders like scratching, stomachache, rheumatism, wound healing, biliary fever, tooth ache etc., [9]. It has been claimed to present activities antiprotozoal [10], antibacterial and antifungal [9,11], antioxidant [12], antiviral [13], allelopathic properties [14] and others activities. Insecticidal activity of *L.camara* oil on teak defoliator (*Hyblaea puera*) was observed where nutritional indices were conducted in the lab condition. *H. puera* ccurs in forests across southern Asia from India and Bangladesh, through Thailand and the rest of Southeast Asia to New Guinea, and north Queensland in Australia. Teak defoliator is a pest moth of teak woodlands in India and other tropical regions (e.g. Thailand) and is of major economic significance. This pest is of major concern as it is involved in complete defoliation of trees during the early part of the growing season. Outbreaks are a regular annual feature in most teak plantations in India and it is extremely difficult to predict the exact time and place of occurrence of these outbreaks. The defoliation does not kill teak trees, but it results in huge amount of timber loss. For controlling teak defoliator ample of chemical pesticides are available, due to its hazardous alternatively some of the botanicals are playing pivotal role in controlling insects and pests in edible and non-edible species. Hence in this present study, bioefficacy of the *L.camara* essential oil has been evaluated and identified some of molecules of insecticidal nature.

MATERIALS AND METHODS

Collection of plant materials
Leaves were collected from valparai located between 10º19'42.24" N and 10º19'16.32" N latitudes and 76º58'51.84" E - 76º57'17.76" E longitudes in the Anamalai range of hills in Western Ghats, Coimbatore district, Tamilnadu and situated at an altitude of 1066.8 meters above the sea level. The collected samples were processed for extraction.

Preparation of essential oil
The plant material was placed into a still (very similar to a pressure cooker) where pressurized steam passes through the plant material. The heat from the steam causes globules of oil in the plant to burst and the oil then evaporates. The essential oil vapor and the steam then pass out the top of the still into a water cooled pipe where the vapors are condensed back to liquids. At this point, the essential oil separates from water and floats to top. The top layer was collected by using separating funnel and used for GC-MS-MS analysis and bioassay studies.

GC-MS-MS analysis
GC-MS-MS analysis was performed on a Varian 4000 MS coupled with a Varian 3800 GC, equipped with a cross linked 5% Phenyl 95% dimethyl polysiloxane VF-5MS capillary column (30 m x 0.25 mm i.d, film thickness, 250nm) and operating under the conditions as mentioned below: The oven temperature was programmed as 60°C (10 min), 60°C - 220°C (4°C/min), 220°C (10 min) and 220°C -240°C (1°C/min). Injector and detector temperatures were maintained at 60°C and 240°C respectively. The amount of the sample injected was 1.0 µl in the splitless mode. Helium was used as carrier gas with a flow rate of 1ml/min.

Identification of phytocompounds
Interpretation on mass-spectrum of GC-MS-MS was conducted using the database of National institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight, molecular formula, retention time and retention indices of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Lantana camara leaves yielded 0.8% of essential oil, and thirty six components were identified as listed in Table 1; Fig.1. The constituents are listed in the order of their elution from VF-5MS column, α- Copaene, Germacrene D, B,α – Cubebene, β – Elemene, α – Guaiene, α – humulene , Aromadendrene, β – Selinene, α – Selinene, Caryophyllene oxide, Nerolidol, Spathulenol and Delta – Cadinene were the major components of the essential oil of *L.camara* in high altitude. The major components found to have insecticidal, insect repellent, attractant, anti-microbial agent and its other uses are mentioned in the Table. 2. The present study on the bioactivity potential of *L.camara* oil confirms the insecticidal activity in the nutritional indices experiments conducted on teak defoliator.

Bioassay studies in the laboratory condition
Among the concentrations tested ranging from 2500 ppm to 10000 ppm have brought larval mortality of 20%-50% at 24 hrs of bioassay experiment on 3rd instar larvae; 90 – 100% during 7th day (Fig.2). Similarly, it was reported that [16] *L.camara* essential oil was brought about maximum larval mortality of *Aedes aegypti* on 24h at the concentration of 1mg/ml. Most of the ethnobotanical species were involved to control insect and pest, [17] was reported that *M. dubia, M. koenigii, A.vasica, B .scandens, V. negundo, S. nuxvomica* were the potential biopesticide for teak defoliator (*H.puera).* One of the major volatiles reported from GC-MS-MS analysis of *L.camara* essential oil is caryophyllene. It was reported that it acts as kairamones and attracted towards predators and parasites, and as an effective biocontrolling agent against most of the lepidopteran pests. The predators *Chrysoperla carnea* and *Collops vittatus* are attracted by caryophyllene, a terpenoid released by damaged cotton leaves [18], and the eastern yellow jacket, *Vespula maculifrons,* is thought to use plant released substances to locate leaf feeding insects [19], the predaceous dolichopodid fly, *Medetera bistriata,* is attracted to its bark beetle prey only when both frontalin, the beetles aggregation pheromone, and tree odours are present [20]. Pine trees under attack liberate volatile terpenes attractive to numerous bark beetle predators [21,22]. Herbivores may counter such plant responses by dispersing [23] or by severing damaged leaves so that they fall to the ground [24]. The aphid parasitoid, *Diaeretiella rapae* M'Intosh, was attracted, in an olfactometer, to collards leaves and to allyl iso thiocyanate (mustard oil), which is found in collards [25]. Caryophyllene from cotton plants similarly attracted adult Chrysopa [18]. *Eucelatoria Sp.,* a

tachinid parasitoid of *Heliothis* spp., was shown to be attracted to volatile chemicals from cotton, corn and okra [26,27]. Hubbell et al. (1983)[28] argue that caryophyllene epoxide is repellent because it is toxic to the Alimentary fungus. Similarly, azadirachtin lies in effects on deterrent and other chemoreceptors resulting in antifeedancy and direct effects on most other tissues studied resulting in an overall loss of fitness of the insect [29,30]. Hubbell and Howard (1984) [31] showed further that there is seasonal variability to the chemical repellency of 42 plant species to these ants in the dry forest of Santa Rosa National Park, Costa Rica. Seventy five percent of the species tested demonstrated non polar extractible repellents (terpenoids, steroids and waxes) chemicals often associated with fungicidal properties. Insects, including those that are plant pests, have natural enemies, which are often other insects. These enemies may be parasites, predators, or parasitoids. A parasite is usually much smaller than its host, and a single individual usually does not kill the host. A predator is a free living organism that is usually larger than its prey, kills the prey, and requires more than one prey during its development. A parasitoid is a special kind of predator, is often the same size as its host, kills the host, and requires only one host (prey) for development into a free living adult. One can distinguish between naturally occurring biological control, which involves the human use and manipulation of natural enemies of pests, although the term ‘biological control’ has itself traditionally been used for the latter. Biological control is intimately linked with the allelochemical web involving plant, pest, parasitoid, and predator, resulting in a tritrophic or sometimes a tetratrophic level of interaction. For effective manipulation of the communication systems involved in this complex allelochemical relationship, an understanding of the direct or indirect, beneficial or detrimental effects of plant secretions on phytophagous insects and their natural enemies is important.

Table : 1 Biologically active components of *Lantana camara*

S.No	Retention Time	Retention indices	Name of the compound	Peak Area	Molecular weight	Molecular formula
1	26.486	802	Bicycloelemene	0.970	204	$C_{15}H_{24}$
2	27.005	818	α – Cubebene	4.226	204	$C_{15}H_{24}$
3	28.017	830	α – Copaene	1.187	204	$C_{15}H_{24}$
4	28.487	879	β – Elemene	2.404	204	$C_{15}H_{24}$
5	29.653	900	Bicyclo [5.2.0] Nonan, 2-Methy	4.509	204	$C_{15}H_{24}$
6	29.826	850	Germacrene B	3.179	204	$C_{15}H_{24}$
7	29.992	877	α – Guaiene	4.268	204	$C_{15}H_{24}$
8	30.704	855	α – Humulene	3.463	204	$C_{15}H_{24}$
9	30.820	906	Aromadendrene	6.257	204	$C_{15}H_{24}$
10	31.290	877	Napthalene	1.472	204	$C_{15}H_{24}$
11	31.499	905	Germacrene D	4.109	204	$C_{15}H_{24}$
12	31.754	912	β – Selinene	1.635	204	$C_{15}H_{24}$
13	31.839	884	Epi – Bicyclosesquiphellandren	0.977	204	$C_{15}H_{24}$
14	31.976	873	α – Selinene	6.747	204	$C_{15}H_{24}$
15	32.575	770	1-Hydroxy-1, 7-dimethyl-4-iso	2.119	222	$C_{15}H_{26}O$
16	32.636	911	β – Cadinene	3.600	204	$C_{15}H_{24}$
17	33.662	845	- Caryophyllene oxide	1.127	220	$C_{15}H_{24}O$
18	33.988	887	Nerolidol	4.841	222	$C_{15}H_{26}O$
19	34.924	833	Salvial – 4 (14) – en – 1 – one	2.862	220	$C_{15}H_{24}O$
20	34.980	877	Veridifloral	0.764	222	$C_{15}H_{26}O$
21	35.444	761	12-Oxabicyclo [9.1.0] dodeca – 3	2.296	220	$C_{15}H_{24}O$
22	35.574	777	1 – Napthalenamine, 4 – bromo	1.321	220	$C_{15}H_{24}O$
23	35.932	796	(-) – Spathulenol	4.087	220	$C_{15}H_{24}O$
24	36.064	839	Isospathulenol	1.632	220	$C_{15}H_{24}O$
25	36.208	830	Tetracyclo [6.3.2.0 (2,5) .0 (1,	1.188	220	$C_{15}H_{24}O$
26	36.303	856	Delta – Cadinene	2.399	204	$C_{15}H_{24}$
27	36.450	860	1-Napthalenol, 1, 2, 3, 4, 4a, 7	2.596	222	$C_{15}H_{26}O$
28	36.707	831	1R, 4S, 7S, 11R-2, 2, 4, 8 – Tetrame	3.211	204	$C_{15}H_{24}$
29	36.816	776	Alloaromadendrene Oxide – (2)	1.220	220	$C_{15}H_{24}O$
30	37.158	808	Aromadendrene Oxide– (2)	3.592	220	$C_{15}H_{24}O$
31	37.675	768	6-isopropenyl-4,8a-dimethyl-	1.407	220	$C_{15}H_{24}O$
32	38.122	750	4,4 – Dimethyl – 3 – (3 – methyl but -	5.599	202	$C_{15}H_{22}$
33	38.229	770	1H – Cycloprop [e] azulen – 7 – ol,	4.970	220	$C_{15}H_{24}O$
34	39.806	734	6-isopropenyl-4,8a-dimethyl-	0.952	220	$C_{15}H_{24}O$
35	41.920	948	Phthalic acid, butyl hexyl e	0.958	306	$C_{18}H_{26}O_4$
36	47.777	853	2-Hexadecen – 1 – ol, 3, 7, 11, 15-	1.859	296	$C_{20}H_{40}O$
				100.00		

Fig.1. GC-MS-MS Chromatogram of *L.camara* leaves essential oil

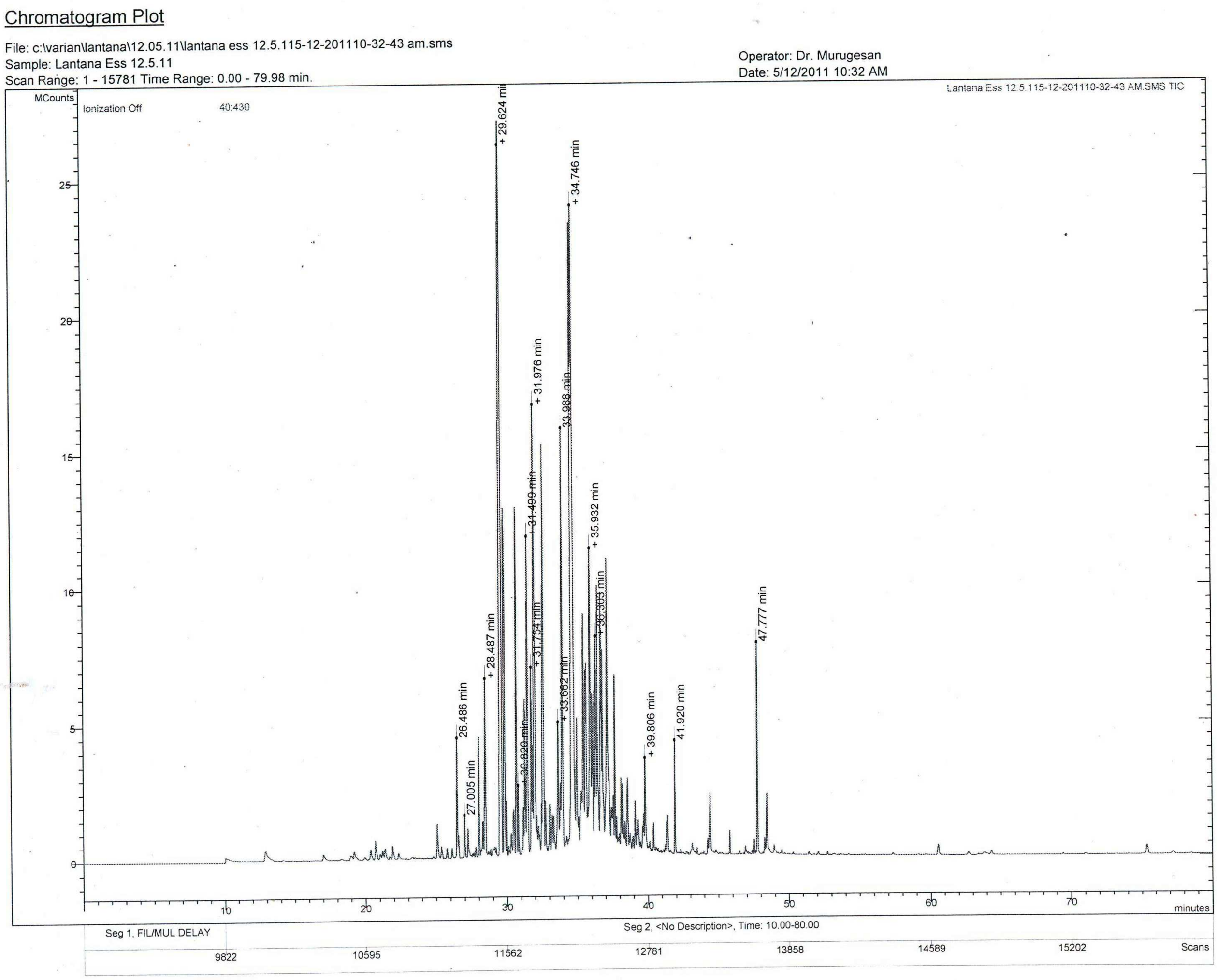
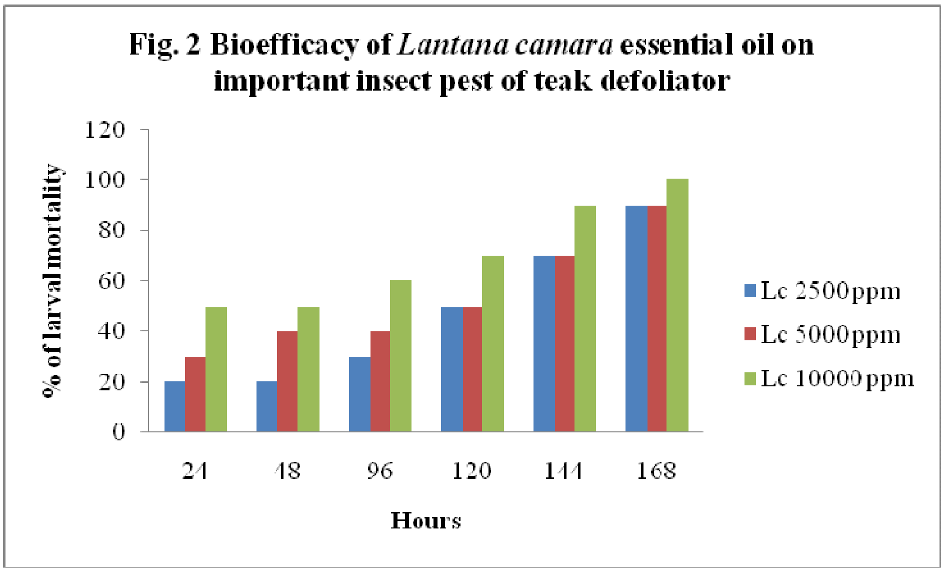


Table.2: Biological properties of some of the major componenets [15]

S.No	Identified components	Biological uses
1	α- Copaene	Strongly attracting to an agricultural pest.
2	Germacrene D, B	Antimicrobial and insecticidal properties.
3	α – Cubebene	Insecticide
4	β – Elemene	Anti-carcinogenic agents
5	α – Guaiene	Flavor and fragrance. It acts as insect repellent.
6	α – humulene	Anti-carcinogenic agents
7	Aromadendrene	Antimicrobial agents
8	β – Selinene	It acts against to bacteria.
9	α – Selinene	It acts as many pharmacological uses
10	Caryophyllene oxide	Antifungal, anti inflammatory, analgesic agents and attractants to predators.
11	Nerolidol	It is one of the chemical raw materials.
12	Spathulenol	Immunoinhibitory molecule.
13	Delta – Cadinene	Insecticide



CONCLUSION

The result indicates that the leaf essential oil, *L.camara* is a potential source of producing volatile profiles like Caryophyllene oxide, Germacrene D, B, α – Guaiene and α – Cubebene attract beneficial organisms and may be cognizable to nearby vegetation complex as an induced plant defensive chemicals.The emergence of chemotypes in plants as well as of races or biotypes in insects is a response to selection pressure exerted by insects and plants on each other; plants develop new chemicals against insects and insects develop detoxification systems, enabling each to enter a new 'adaptive zone' and resulting in diversity.

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