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Studies on chemical constituents and antifungal activity of leaf essential oil of *Lippia alba* (Mill)

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The leaf volatile oil of *Lippia alba* (Mill) has been analysed by GC and GC-MS. Fifty components have been identified accounting for about 90% of the total oil. The major constituent is geranial (15.57%) followed by a unresolvable mixture of myrthenol and myrthenal (9.89%), neral (9.44%), geraniol (7.36%), 2,6-octadien-1-ol, 3,7-dimethyl acetate (6.87%), 1-octene-3-ol (4.66%),6-methyl-5-hepten-2-one (4.60%), caryophyllene oxide (4.52%), β–caryophyllene (3.09%),citronellol (2.63%), linalool (2.20%), 3-pinene-2-ol (2.19%), β-myrcene (1.49%), farnesol (1.35%) and spathulenol. It was also found that oil vapours possessed strong antifungal activity against sugarcane pathogens. The oil was highly effective in controlling teleutospore germination of *Ustilago scitaminea* and conidial germination of *Colletotrichum falcatum* and *Curvularia lunata* at 3×10^3 µl/L concentration.The oil also proved superior in comparison to commercial fungicides in controlling plant pathogenic fungi.

Lippia alba (Mill) belongs to family Verbenaceae and is an aromatic shrub. It is found in India, Nepal, Thailand, Argentina, Brazil etc[1]. The leaf oil has already been reported to posses antimicrobial activity[2-4]. Much variation in the chemical composition of leaf oils have been reported by various workers[4-11]. Carvone, neral, geraniol, β-guiene, piperitone and β-caryophyllene are reported as major components.

Many essential oils extracted from higher plants have been investigated for their fungitoxicity against sugarcane pests but studies on spore germination inhibition properties of these oils are very scanty[12]. Hence, the GC-MS analysis and spore germination inhibition properties of leaf oil of *Lippia alba* obtained from plant material collected from Shahjahanpur, India has been reported here.

Experimental Procedure

Plant material

The plant material was collected from belts of Khannaut river, Shahjahanpur, West U.P., India. Voucher specimen has been kept in the Herbarium of the Science Faculty.

Isolation of Essential oil

The leaves were washed with distilled water and oil (yield = 1.0%) was obtained by hydrodistillation using Clevengers apparatus. It was dried over anhydrous sodium sulphate.

GC-MS analysis

The oil was analysed by Hewlett Packard HP 6890 series GC fitted with HP 5 column having 30 m length and 0.25 mm diameter (Cross linked 5% Phenyl Methyl Siloxane) coupled to a Hewlett Packard mass detector Model 5973. The oven temperature was programmed as

$$60^\circ C \xrightarrow{@\ 1.5^\circ C\ min^{-1}} 80^\circ C$$

$$80^\circ C \xrightarrow{@\ 1.0^\circ C\ min^{-1}} 160^\circ C$$

$$160^\circ C \xrightarrow{@\ 1.0^\circ C\ min^{-1}} 200^\circ C$$

Identification of components

The chemical constituents reported in Table 1 have been identified by comparing their mass spectra with the library NBS 75 K search. The major components were also authenticated by co-injection with α-thujene, α-pinene, sabinene, 1-octen-3-ol, 6-methyl-5-hepten-2-one, β-myrcene, 6-methyl-5-hepten-2-ol, 3-octanol *p*-cymene, limonene, *trans*- and *cis*-ocimene, Linalool, citronellal, terpien-4-ol, α–terpineol, nerol, citronellol, neral, geraniol, geranial, α-cubebene,

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Table 1 — Chemical Constituents of the Leaf Oil of *Lippia alba*

Peak No.	RT	Percentage	Compd. Identified	CAS
1	9.18	0.28	3-hexen-1-ol-(z)	000928-06-4
2	14.65	0.08	α-phellandrene	0002867-05-2
3	15.09	0.56	α-pinene	000080-56-8
4	19.02	0.08	sabinene	003387-41-5
5	20.39	4.66	1-octen-3-ol	003391-86-4
6	21.03	4.60	5-hepten-2-one,6-methyl	000110-93-0
7	21.25	1.49	β-myrcene	000123-35-3
8	21.78	0.12	5-hepten-2-ol,6-methyl	001569-60-4
9	21.98	0.27	3-octanol	000589-98-0
10	24.19	0.10	*p*-cymene	000099-87-6
11	24.57	0.62	limonene	000138-86-3
12	26.12	0.08	1,3,6-octatriene,3,7-dimethyl-(E) [trans-ocimene]	003779-61-1
13	27.12	0.13	1,3,6-octatriene-3,7-dimethyl-(Z) [*cis*-ocimene]	003779-61-1
14	32.49	2.20	linalool	000078-70-6
15	36.18	0.15	verbenol, *cis*	018881-04-4
16	36.54	1.96	terpene mw-152*	
17	37.87	0.18	6-octenal-3,7-dimethyl [citronellal]	000106-23-0
18	39.72		3-cyclohexene-1-ol-4-methyl-1-(1-methyl ethyl)	000562-74-3
19	40.07	0.59	unidentified mw 166*	
20	41.20	0.19	3-cyclohexene-1-methanol,α,α, 4-trimethyl-(s)[terpinen-4ol]	010482-56-1
21	41.45,	9.89*	myrthenal	000564-94-3
	41.71		myrthenol	000515-00-4
22	42.91	0.27	verbenone	018309-32-5
23	44.67	0.49	unidentified, mixture of two compounds	
24	45.25		nerol	000106-25-2
25	45.54	2.63	citronellol	000106-22-9
26	46.44	9.44	neral	000106-26-3
27	47.26	0.66	2-cyclohexene-1-one-3-methyl-6-(1-methyl ethyl)	000089-81-6
28	48.06	7.36	geraniol	000106-24-1
29	49.43	15.57	geranial	000141-27-5
30	50.11	0.87	monoterpene mw 152	
31	50.31	0.11	unidentified terpene	
32	52.31	0.55	2,6-octadien-1-ol,3,7-dimethyl formate (E) [geranyl formate]	
33	54.09	2.19	monoterpene mw 152 (3-pinen-2-ol, *cis* or *trans*)	
34	56.05	0.11	α-cubebene	017699-14-8
35	56.96	0.31	eugenol	000097-53-0
36	57.28	0.32	sesquiterpene mw 204	
37	58.02	0.22	unidentfied	
38	59.80	6.87	2,6-octadien-1-ol, 3,7-dimethylacetate(z) [neryl acetate]	000141-12-8
39	61.48	0.15	benzene, 1,2-dimethoxy-4-(2-propenyl)-5 ($C_{11}H_{14}O_2$) [*trans*-methyl isoeugenol]	000093-15-2
40	61.89	3.09	β-caryophyllene	000087-44-5
41	62.91	0.20	unidentified	
42	64.79	0.31	α-caryophyllene	006753-98-6
43	65.84	0.25	*trans*-β-farnesene	
44	70.22	0.91	sesquiterpene mw 222	
45	70.73	0.53	sesquiterpene	
46	74.58	1.35	1,6,10-dodecatrien-3-ol-3,7,11-trimethyl-s-(2) [fornesol]	000142-50-7
47	75.11		spathulenol	077171-55-2
48	75.43	4.52	caryophyllene oxide	001139-30-6
49	81.69	1.18	sesquiterpene mw 218	
50	82.56	0.48	sesquiterpene mw 220	

(* = unresolved mixture)

eugenol, neryl acetate, caryophyllene, *trans* β-fornesene, fornesol, caryophyllene oxide, spathulenol.

Vapour action of oil of Lippia alba on the spore-germination inhibition

For spore germination inhibition study, conidia of *Colletotrichum falcatum, Curvularia lunata* and teleutospores of *Ustilago scitaminea* syd. were collected separately from culture tubes and infected sugarcane plants in the fields, respectively. For this study, empty glass vials (10 mL) were kept inverted on a glass-slide bearing a drop of water with requisite concentration of conidia/teleutospores.The vacuum grease was applied on the surface of the mouth of vial to make it air tight.

Required quantity of volatile oil diluted with acetone was placed on a piece of filter paper on the bottom of the vial. The space inside vial was measured for calculation of dose of oil. The dilution of conidia/teleutospores were made as such to get 20-30 conidia/teleutospores per microscopic field. In control sets, requisite amount of acetone alone was placed instead of diluted oil. The closed vial was kept air tight for 4-5 h at room temperature. Then the slides were removed from top of the vial and observed under compound microscope for germination of conidia/ teleutospores in treated and control sets, and replicated five times. Spore germination inhibition was calculated, using the formula gc-gt/gc × 100, where gc = germination of conidia/ teleutospores in control sets, gt = germination of conidia/ teleutospores in treated sets and results are reported in Table 2.

Comparative fungitoxic efficacy of Lippia alba oil with synthetic fungicides

The fungitoxic efficacy of *L.alba* leaf oil was compared with some commercial synthetic fungicides viz., Bavistin , Blitox-50, Dithane M-45 using the poison food technique (Grover and Moore, 1962).Different concentration of each of synthetic fungicides viz., 1000, 2000,3000 and 4000 ppm were prepared and tested for their minimum inhibitory concentration (MIC) against *Colletotrichum falcatum, Fusarium moniliforme* ,*Curvularia lunata*,and *Ceratocystis paradoxa* . The data are presented in Table 3.

Results and Discussion

GC-MS analysis showed more than fifty components which comprises about 90 per cent of the total oil. A list of the identified components alongwith their percentage in the oil and *CAS* are given in Table 1. Major components of the oil are geranial (15.57%), are unresolvable mixture of myrthenol and myrthenal (9.89%), neral (9.44%), geraniol (7.36%), 2,6-octadien-1-ol, 3,7-dimethyl acetate (6.87%), 1-octen-3-ol (4.66%), 6-methyl-5-hepten-2-one (4.60%), citronellol (2.63%), linalool (2.20%), 3-pinen-2-ol (2.19%), β-myrcene (1.49%), caryophyllene oxide (4.52%), fornesol (1.35%) and β-caryophyllene

Table 2 — Effect of vapour of *L. alba* leaf oil on conidial and teleutospore germination of sugarcane fungal pathogens

Fungus	Conc^{n} of oil vapour (in μL/L)	* Percent spore germination inhibition
CF	1×10^{3}	100.0
	2×10^{3}	100.0
	3×10^{3}	100.0
CL	1×10^{3}	70.0
	2×10^{3}	92.0
	3×10^{3}	100.0
US	1×10^{3}	64.0
	2×10^{3}	100.0
	3×10^{3}	100.0

CF = *Colletotrichum falcatum,* CL = *Curvularia lunata,* US = *Ustilago scitaminea,* *= Average of 5 replications

Table 3 — Iso-conductance (K^{m}_{iso}) and counter-ion diffusion coefficient (D^{m}_{i}) values for ion-exchange membranes in different electrolytic solutions

Electrolyte	Thickness (mm)	CEM			AEM		
		C_{iso} (*M*)	K^{m}_{iso} (S/m)	$D^{m}_{i}\times10^{11}$ (m^2/sec)	C_{iso} (*M*)	K^{m}_{iso} (S/m)	$D^{m}_{i}\times10^{11}$ (m^2/sec)
NaCl	0.130	0.065	0.625	14.200	0.018	0.165	6.520
	0.180	0.036	0.355	9.600	0.013	0.135	5.917
KCl	0.130	0.045	0.535	12.130	0.010	0.065	2.573
	0.180	0.030	0.340	9.204	0.008	0.040	0.661
Na_2SO_4	0.130	0.018	0.285	6.463	0.007	0.039	1.542
	0.180	0.015	0.225	6.091	0.005	0.032	1.403

(3.09%). Variation in the chemical composition of *L. alba* leaf oil has also been reported from different places by various workers[4-11]. Carvone, nerol, geraniol, β-guiene, piperitone and β-caryophyllene are reported as major components. Recently, Singh *et al.*[13] observed myrcene (26.4%) as the major component of *L. alba* leaf oil from North India. The two morphologically identical samples of *L. alba*, collected in different regions with possible difference in the soil properties, might have a significant influence on the oil composition. It is interesting that in the previously published work[13], the *L. alba* oil composition was completely different from these results, as myrcene (26.4%) and geranial (9.8%) were the main components. These distinct chemical compositions point to a species with a great variability in their chemical compositions and further research is necessary to verify whether this is due to geological or pedological conditions of the collection sites or due to the existence of different chemo- types.

Results recorded in Table 2 show that the oil vapours of *Lippia alba* are highly effective in controlling the teleutospore germination of *U. scitaminea* and conidial germination of *C. falcatum* and *Curvularia lunata*. Spore germination of all the tested fungi was completely inhibited at 3×10^3 µL/L concentration. However, the oil vapours were found to check completely the conidial germination of *C. falcatum* at 1×10^3 µl/l concentration and teleutospore germination of *U. scitaminea* at 2×10^3 µL/L concentration. At other tested concentrations, the oil showed partial inhibitory nature towards germination inhibition. *Lippia alba* oil has already been reported to posses antimicrobial properties. Recently Singh *et al.*[1] have reported that this oil is fungistatic against *Colletotrichum falcatum* and *Curvularia pallescens* at 700 ppm concentration, and fungicidal at higher concentrations against many fungal pathogens of sugarcane. In the present investigation, the strong spore germination inhibition property of *L. alba* oil has proved more significant in controlling fungal pathogens. The results suggests that *L. alba* oil should be evaluated for large scale antimicrobial trials against many important agricultural pests to explore its potential as a commercial natural pesticide.

Results presented in Table 3 reveal that leaf oil of *L. alba* was found to be more efficacious than some commercial fungicides. The oil was found as nearly two times more effective than Blitox-50, Dithane M-45 *against C. falcatum* and *C. lunata* , eight times more effective than all the tested commercial fungicides against *C. paradoxa*. The oil was also found 1.5 to 2 times more effective in controlling *F. moniliforme* and *C.lunata* in comparison with all the tested fungicides. The superiority of *L.alba* leaf oil over commercial fungicides, further adds to its merit as a promising natural pesticide against many plant pathogenic fungi of agricultural crops.

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