Journal of Natural Products
Vol. 50, No. 3, pp. 503-506, May-Jun 1987

THE ESSENTIAL OIL OF *LIPPIA ADOENSIS* LEAVES AND FLOWERS

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The essential oils obtained from members of the genus *Lippia* (family Verbenaceae) have a long history of reported biological activities, particularly as medicinals. We recently reported on the composition and allelopathic nature of the volatile constituents of *Lippia nodiflora* collected in Davis, California (1). *L. nodiflora* had been valued as an antipyretic and diuretic in Indian medicine (2). We found it to also be allelopathic as measured by lettuce seedling assay. A 1938 report (3) indicates the essential oil of *Lippia adoensis* Hochst was used by natives of French N.E. Africa to treat coughs and fevers. In continuation of our studies on allelopathic plants and volatile oil containing plants of Nigeria we wish to examine the allelopathic potential and determine the composition of the essential oils of *L. adoensis* leaves and flowers.

MATERIALS AND METHODS

The leaves of *L. adoensis* were collected in Asipa, Oyo State, Nigeria in May 1984. Botanical identification was confirmed by Mr Adesakin, Department of Pharmacognosy, University of Ife. Herbarium specimens representing the collection were deposited in Faculty of Pharmacy, University of Ife. The fresh leaves were cut into small pieces and steam distilled immediately after collection using the British Pharmacopocia method (4) for 5 h. The dried (Na_2SO_4) essential oil was obtained from the steam distillate in 0.83% v/w yield. It was stored in amber colored vials at 0° until use. *L. adoensis* flowers were removed from the plants and steam distilled separately. Yield of distillate was 0.60%.

Steam distillates were subjected to gc/ms analysis using a Hewlett Packard 5985 B mass spectrometer/gas chromatograph equipped with a 0.24 mm id × 19.29 m DB-1 glass capillary column programmed to hold the initial 40° temperature for 5 min then increase at 5°/min to 275°; this final temperature was held for 8 min. Compounds were identified by comparison with published mass spectra (5). In most cases, assignments were confirmed by comparison of observed retention times with those of standards. Relative amounts of compounds were determined by instrumental integation of peak areas.

BIOASSAYS.—The effects on both leaf and flower extracts and of pure linalool of lettuce seedling root growth were tested in the range of 50 to 1000 ppm test compound or mixture in 0.5% agar (Carolina Biological Supply Company) in 10-cm Petri dishes. All test seeds were first germinated on 0.5% agar in a growth chamber set at 18°, 8-h nights, 22°, 16-h days. Germinated seedlings were transferred to the prepared Petri dishes and incubated for 72 to 113 h; at which time the control showed good growth. Two controls were run; one contained only 0.5% agar, the second contained agar plus 1% DMSO. All test plates also contained 1% DMSO as a solubilizing agent. The lengths of roots were measured to the nearest millimeter.

DATA ANALYSIS.—The results of the lettuce seedling growth bioassays were analyzed separately using a Honeywell DPS-8 mainframe computer and Statistical Package for the Social Sciences, version 9 (6). Statistical analyses included one-way analysis of variances and the Duncan multiple-range test for differences among all treatment means.

RESULTS

Gc analysis of the essential oils of both *L. adoensis* leaves and flowers showed both to be remarkably simple mixtures, completely dominated by the monoterpene alcohol linalool. *L. adoensis* leaves contained 14 components with retention times less than 40 min. Linalool comprised 81.3% of the total mixture; the remaining constituents were identified as shown in Table 1. More than 92% of the compounds with retention times less than 40 min were identified. The oil is predominantly monoterpenoid (89.1%); sesquiterpenoid constitutents constitute

TABLE 1. Composition of Steam Volatiles from *Lippia adoensis* Leaves and Flowers

Compound[a]	Composition (%)	
	Leaves	Flowers
α-pinene[b]	0.22	—
β-pinene[b]	0.79	—
unknown	0.29	0.10
1,8-cineole[b]	3.32	—
γ-terpinene[b]	0.62	0.48
linalool[b]	81.30	94.56
α-terpineol[b]	1.11	0.13
thymol[b]	1.41	—
carvacrol	0.38	—
copaene	1.36	—
unknown	2.03	0.25
unknown	—	1.02
$C_{15}H_{24}$	5.66	0.11
δ-cadinene	0.90	—
nerolidol[b]	0.61	0.73
unknowns (3)	—	2.60
Total % identified	92.02	95.90

[a]Compounds are listed in order of their elution from a 0.24 mm id × 19.29 m DB-1 glass capillary column.

[b]Denotes confirmation of identity by comparison with a gc standard.

7.2% of the oil. Most of the monoterpenoid and sesquiterpenoid constituents are oxygenated. The essential oil of *L. adoensis* flowers contained almost 95% linalool. Of the ten other minor constituents eluting in less than 40 min, only three were identified: γ-terpinene, α-terpineol, and nerolidol (Table 1). A number of constituents present in *L. adoensis* leaves were not found in the flowers. These include α- and β-pinene, 1,8-cineole, thymol, carvacrol, copaene, and δ-cadinene. Two unidentified components were common to both leaves and flowers. One of these eluted shortly after β-pinene; the second, a $C_{15}H_{24}$ sesquiterpene hydrocarbon, eluted just before δ-cadinene.

Much of the previous work with steam volatiles from *Lippia* species was carried out before 1945, before modern instrumentation was available, and, therefore, the reported results must be treated with caution. One 1940 paper (7) reports 20.5% camphor from the essential oil of flowers of *L. adoensis* grown in Senegal. Three other early reports from Rabate and Laffitte, (3,8,9) describe steam distillates of *L. adoensis* flowers from French West Africa as being composed of 33 to 43% camphor. The leaves and stalks yielded little or no camphor (3,9). Rovesti (10) had earlier reported 72% carvone from steam distillation of the leaves, stems, and flowers. Our results are quite different from all of these. We observed no camphor or carvone from either leaves or flowers even though we specifically looked for these two constituents. Furthermore, linalool was the overwhelmingly dominant component of the oils of both leaves and flowers, while the flowers contained more linalool than the leaves.

Terpenes and terpenoids have been reported to allelopathic (11) and other *Lippia* species have characteristics of allelopathic plants (1). Of the compounds we found present in *L. adoensis*, the monoterpenoids 1,8-cineole and α- and

β-pinene were also among those studied by Asplund (12) and found to be inhibitory toward radish seed germinantion. Earlier work by Muller and Muller (13) identified six terpenoids as inhibitory toward cucumber seedlings (*Cucumis sativus*); these also included α- and β-pinene and cineole. A more recent paper by Weaver and Kish (14) reports these three terpenoids as well as terpineol as among the eight monterpenoids they tested and found to be inhibitory toward cucumber, *Cucumis sativus* L. var. Ashley. We could find no reports which suggest linalool is allelopathic, and we, therefore, wanted to explore the potential inhibitory nature of pure linalool and compare it to the inhibitory nature of *L. adoensis* leaf and flower essential oils.

Results of lettuce seedling growth inhibition assays are given in Table 2. Both linalool and the essential oil of *L. adoensis* flowers appear to enhance radical growth at low concentration, but this growth enhancement is not statistically significant. At 200 ppm, neither linalool nor the extract from flowers is inhibitory, while the extract of *L. adoensis* leaves is slightly inhibitory. It is only at 400 ppm that significant inhibition is observed in all three cases. At this concentration, linalool brings about a 42% reduction in radical length, *L. adoensis* leaf extract brings about a 45% reduction, and *L. adoensis* flower extract a 33% reduction. While these numbers cover a 12-percentage-point range, they represent very similar growth inhibition among all three samples. At 600 ppm, pure linalool causes an 80% growth reduction; at 800 ppm the growth reduction is not significantly greater, which suggests that we are observing maximum growth inhibition at 600 ppm of linalool.

From these data we can conclude that the essential oils of *L. adoensis* leaves and flowers have inhibitory properties, as measured by lettuce seedling growth

TABLE 2. Effect of Linalool and *Lippia adoensis* Leaf and Flower Essential Oils on Lettuce Radical Length

Sample	Concentration (ppm)	Mean root Length[a]		Incubation time (h)	Roots measured (no.)
		(cm)	% of control		
linalool	(control)	2.56 a	—	93	40
	25	2.54 a	99	93	39
	50	2.72 a	106	93	40
	100	2.73 a	107	93	40
	200	2.49 a	97	93	40
	400	1.49 b	58	93	49
	600	0.48 c	19	93	40
	800	0.45 c	18	93	24
Leaf essential oil	(control)	2.46 a	—	72	30
	25	2.40 a	98	72	30
	50	2.31 a,b	94	72	29
	100	1.77 c	72	72	30
	200	2.03 b,c	83	72	30
	400	1.36 d	55	72	30
	1000	0.66 e	27	72	30
Flower essential oil	(control)	2.66 a	—	113	40
	200	2.89 a	109	113	37
	400	1.75 b	66	113	37
	600	1.26 c	47	113	40

[a]Means associated with a given test sample with different letters are significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

bioassay, similar to their major constituent, linalool. It is difficult to say what contribution the presence of the known allelopathic terpenoids α- and β-pinene, cineole, and terpineol have on the total inhibitory nature of these essential oils. The allelopathic nature of these terpenoids was examined in other studies (12-14) in the vapor phase rather than in an agar solution as in this study, thus invalidating comparisons of inhibitory concentrations.

More information on the role of terpenoids in allelopathy will have to await further investigations of the volatile constituents of plants that appear to be allelopathic and the examination of the allelopathic nature of these essential oils and their major constituents.

ACKNOWLEDMENTS

The authors thank Dr. A.O. Adeoye of University of Lagos for assistance during the collection of the plant materials.

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Received 14 November 1986