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Limonia acidissima, A RICH SOURCE OF β-PINENE, FROM THE WESTERN GHATS OF INDIA

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Limonia acidissima L., syn. *Feronia elephantum* Correa is a multipurpose tree species belonging to Rutaceae family.

Different parts of this tree, fruits, seeds, and leaves, have been reported to possess many medicinal properties and are widely used in folk medicine. The fruits and leaves are prescribed for vomiting, hiccups, dysentery, indigestion, and slight bowel affections in children [1]. The fruits are considered as tonic, refreshing, cardiacal, astringent, carinative, antiscorbutic, and alexipharmic [2]. Indole alkaloids, acidissimin, a tyramine derivative, limonoids, and coumarin derivatives were reported earlier from the fruit [3–5]. Rahman and Alexander [6] reported the antimicrobial constituents from the stem bark of *F. limonia.* An antitumor pectic polysaccharide [7] and *n*-hexadecanoic acid, a potent anti-mosquito larvicide, were also reported from this plant [8].

Previous investigations on leaves indicate that they have aromatical smell of anise seed and yield essential oil. Few investigations on the chemical examination of the *L. acidissima* leaf essential oil have been reported earlier [9, 10]. The antimicrobial and antifungal activities of the essential oil have also been discussed extensively [11, 12]. Bhati and Deshpande [13] reported that *L. acidissima* leaf oil is a good source of methyl chavicol (90%). In another sample it was observed that methyl chavicol (72.7%) and anethole (26.2%) were the major components of the oil [3]. Garg in 2003 reported *trans*-anethole (4.7%), methyl eugenol (3.6%), and anisaldehyde (4.4%) along with methyl chavicol (68.3%) [11], whereas, Ahmad et al. reported *trans*-anethole (10.9%), methyl chavicol (27.2%), and thymol (24.4%) as the major constituents from the sample collected from central India [10]. These results indicate wide variations in the content of methyl eugenol.

We have collected *L. acidissima* leaf samples from three different places in the Western Ghats area and also two samples from nearby plains in Karnataka State.

The aerial parts of *L. acidissima* used in the present investigation were collected from Karanthai malai, Tamil Nadu (sample DST-79, September, 2005), Sersi-Dhavngeri road (sample DST-214, August, 2006), and Veerahosanahalli, Karnataka (sample DST-277, September, 2006), located all in the southern region of Western Ghats and also from the plains in Mysore (sample DST-Mysore) and Bangalore (sample DST-GKVK) (September, 2006) in Karnataka State of India. The voucher specimens were deposited at the herbarium in CIMAP, Research center, Bangalore. The aerial parts collected were packed properly and distilled within 24 h of collection by hydrodistillation using a Clevenger-type apparatus for 5–6 hrs for its essential oil. Essential oil yields (v/w) are shown in Table 1. The oil samples after drying over anhydrous Na_2SO_4 were stored at 4–5°C before being subjected to GC and GC/MS analysis. The oils obtained were analyzed by GC using methylpolysiloxane and carbowax columns.

In our present collection the leaf collected from Karanthai malai from Tamil Nadu State gave the highest yield of essential oil (0.5%) and showed that methyl chavicol is the major constituent (91.2%). The other important minor constituents are α-pinene, myrcene, limonene, linalool, anisaldehyde, and *p*-methoxycinnamaldehyde. Interestingly, the samples collected locally from Bangalore and Mysore showed a low percentage of oil, and β-pinene as the major compound. This prompted us to collect more samples from different regions for the investigation. All the other four samples from the central part of the Western Ghats and nearby plains, which are in the Karnataka State region, gave from 0.3 to 0.2% as the oil yield and β-pinene as the major compound, ranging from 63.9–76.9%. The other major compounds that contribute to these samples are α-pinene (4.7–6.1%), sabinene (4.4–6.0%), and limonene (4.5–6.2%) (Table 1). The other important minor constituents are camphene, myrcene, *E*-β-ocimene, linalool, α-terpineol, methyl chavicol, β-caryophyllene, δ-cadinene, and glubulol.

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TABLE 1. Essential Oil Yield and Composition* of *L. acidissima* Samples Collected from Western Ghats

Compound	RRI**	RRI***	DST-79	DST-214	DST-277	DST-Mysore	DST-GKVK
Percentage of oil, v/w			0.50	0.30	0.20	0.20	0.16
α-Thujene	931	1025	–	0.2	0.1	0.1	–
α-Pinene	935	1025	0.3	5.7	6.1	4.7	5.2
Camphene	947	1070	–	0.4	0.4	0.4	0.4
Sabinene	970	1112	0.3	5.6	5.9	5.4	4.4
β-Pinene	976	1124	3.5	73.5	76.9	63.9	65.9
Myrcene	984	1161	–	0.4	0.3	0.1	–
1:8-Cineole	1012	1168	–	0.2	0.2	0.2	0.3
p-Cymene	1014	1274	–	–	–	0.1	–
Limonene	1025	1206	0.4	6.2	6.0	5.4	4.5
Z-β-Ocimene	1039	1210	–	0.3	0.1	–	–
E-β-Ocimene	1053	1215	–	0.5	0.4	0.5	0.5
γ-Terpinene	1058	1249	–	0.1	0.1	0.1	–
Terpinolene	1082	1285	–	0.2	0.2	0.2	0.2
Linalool	1085	1547	0.2	0.3	0.2	0.3	0.2
Z-β-Terpineol	1127	1616	–	–	–	0.3	–
Terpinene-4-ol	1164	1604	–	–	0.6	1.2	1.5
α-Terpineol	1176	1731		1.0		1.0	1.2
Methyl chavicol	1180	1676	91.3	0.7	0.4	–	–
Anisaldehyde	1235		0.2	–	–	–	0.3
Methyl eugenol	1371	2000	1.1			0.2	0.2
β-Caryophyllene	1431	1618	0.3	1.2	0.6	0.8	0.4
(*E*)-*iso*-Eugenol	1445		0.2	–	–	–	–
α-Humulene	1464	1690	–	0.2	0.1	–	–
Eugenol acetate	1485		–	0.3	0.3	0.1	–
α-Bisabolene	1510		0.3	–	–	–	–
δ-Cadinene	1524	1734		0.3	0.3	0.3	0.3
Spathulenol	1572	2089	–	–	–	0.4	0.2
Caryophyllene oxide	1583	1989	–	0.1	0.3	0.5	0.2
Glubulol	1586	2056	–	–	–	4.8	3.1
β-Eudesmol	1628	2235	–	–	–	–	1.9
Total % of compounds identified			98.1	97.1	99.4	90.6	90.3

*Percentage composition of components based on methylpolysiloxane column.
**RRI: dimethylpolysiloxane column. ***RRI: wax column.

In the Karanthi Malai sample, the contents of α-pinene (0.3%), β-pinene (3.5%), sabinene (0.3%), and limonene (0.4%) were even less, and these were observed in relatively larger quantities in all the other samples where β-pinene is the major compound. Methyl eugenol (1.1%) was observed in the sample from Karanthai malai and in very small amounts (0.2%) in the samples collected from the plains of Mysore and Bangalore. It is totally absent from the samples collected from the Western Ghat areas in Karnataka. Globulol was observed only in the samples collected from the plains (3.2–4.8%). Methyl chavicol was not observed in other samples collected in the plains and in very small amounts in hill areas (0.4–0.7%) in Karnataka. *Ocimum basilicum* L., *Ochrosperma lineare* (C. T. White) Trudgen, and *Artemisia dracunculus* L. oil are some of the rich natural sources of methyl chavicol.

GC-FID Analysis. Gas Chromatographic analysis of the essential oil sample was done on a Varian CP-3800 gas chromatograph fitted with two flame ionization detectors and split/splitless capillary injectors and Star workstation software. 100% dimethylpolysiloxane column CP-Sil 5 CB 50 m × 0.32 mm I.D., film thickness 0.25 μm from Chrom-Pack and CP-Wax 52 CB column 60 m × 0.25 mm I. D., film thickness 0.25 μm from Varian were used with nitrogen as carrier gas at a pressure of 16 and 17 psi, respectively. A 0.2 μL sample was injected in the split mode with split ratio 1:100. The column was initially held at 60°C for 5 min, then heated to 220°C at 5°C per min, held for 3 min, and heated to 250°C at a rate of 5°C per min and held at 250°C for 4 min. Injector and detector temperatures were kept at 250°C and 300°C respectively. The 0.2 μL of sample injections were repeated thrice under similar experimental conditions. The relative percentages of individual components of the oils in the Table 1 were determined by averaging the GC-FID peak area percentages.

GC/MS Analysis. GC/MS analysis was carried out on a Perkin–Elmer Turbo mass Auto XL instrument at 70 eV using a column (50 m × 0.32 mm PE-5) coated with 5% phenyl 95% dimethylpolysiloxane of thickness film 0.33 μm. Oven temperature was programmed from 100–280°C at 3°C/min with an initial hold of 2 min. Helium was used as the carrier gas at 10 psi. Mass spectra were obtained in the electron impact EI mode at 70 eV. Injector and detector temperatures were kept at 220°C and 300°C, respectively.

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