

Variation in essential oil yield and composition of *Lippia alba* (Mill.) N.E.Br. grown in southern Brazil

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RESUMO: Variação da composição e produção de óleos essenciais de *Lippia alba* (MILL.) N.E.Br. crescendo no Sul do Brasil. O óleo essencial da *Lippia alba* coletado em Caxias do Sul (Caxias do Sul – Rio Grande do Sul) foi extraído por hidrodestilação utilizando aparelho Clevenger durante 1 hora. O rendimento médio de óleo essencial foi de 0,23%. Dezenove compostos foram identificados por análises em CG and CG-EM. Linalol foi encontrado como sendo o componente majoritário do óleo essencial (50,0% - 79,2%), seguido por 1,8-cineol (7,3% - 14,1%). Com relação ao melhor período de colheita, esta planta apresenta maior teor de linalol e maior rendimento de óleo essencial nos meses de dezembro a março.

Palavras-chave: Plantas medicinais, óleos vegetais, óleos voláteis

ABSTRACT: The essential oil of *Lippia alba* plants collected in Caxias do Sul (Rio Grande do Sul) was extracted by hydro-distillation for 1 hour using a Clevenger apparatus. The average yield was 0.23%. Nineteen compounds were identified by GC and GC-MS analysis. Linalool was the main component of the oil (50.0% - 79.2%), with a lower percentage of 1,8-cineol (7.3% - 14.1%). With relation to the collection period, the plants gave a higher yield of oil, with a higher linalool content, during December to March.

Key words: medicinal plants, plant oils, volatile oils.

INTRODUCTION

The Verbenaceae comprise 86 genera, including herbs, shrubs and trees, with a widespread tropical and subtropical distribution. The genus *Lippia* L. is comprised of over 200 species which grow wild in South and Central America, and Africa (Zoghbi *et al.*, 1998). *Lippia alba* (Mill.) N. E. Br. is native to South America (Brazil), growing in sandy soils on margins of rivers, lakes and ponds, in regions with tropical, subtropical, and even temperate climate. It grows preferentially in subtropical climates without extremes of hot or cold weather. It is sensitive to freezing, but usually re-growth starts in the springtime (Corrêa Junior *et al.*, 1994).

Lippia alba, known as "erva-cidreira-brasileira" or "falsa melissa", is one of the main popular medicinal plants in the region, and is used for sedative teas. Digestive, carminative, spasmolytic and emenagogue properties have also been characterized for the plant (Matos *et al.*, 1996). These properties are attributed to the leaf essential oils, which have been chemically and pharmacologically studied (Gomes *et al.*, 1993; Elakovich & Orguntemen, 1987).

Large variations in the composition of *L. alba* oils have been reported, depending on the origin of the plant material, the stage of development of the plant and on the parts of the plant used for the oil distillation. Some types of *L. alba* have been reported as containing citral,

carvone, γ -terpinene, 1,8-cineole, limonene, and piperitone as the main constituents (Zoghbi *et al.*, 1998). Other types have linalool as the main component (Frighetto *et al.*, 1998; Kumar *et al.*, 1999).

Linalool is one of the most frequently used fragrant substances, and is produced in large volumes. In the presence of acids, linalool isomerizes readily to geraniol, nerol and α -terpineol. It is oxidized to citral by chromic acid. Oxidation with peracetic acid yields linalool oxides, which occur in low concentrations in some essential oils and are also used in perfumery. Hydrogenation of linalool produces tetra-hydro-linalool, a stable fragrance compound with an aroma which is not as strong as, but fresher than that of linalool. Linalool can be converted into linalyl acetate by reaction with ketene or an excess of boiling acetic anhydride (Bauer *et al.*, 1997).

Linalool also is an important intermediate in the manufacture of vitamin E, and several large-scale processes have been developed for its production (Frighetto *et al.*, 1998).

In this paper we report the chemical composition of the essential oils of *L. alba* collected from October 1998 at one-month intervals up to September 1999. The aim of the present study was to investigate the variation of the oil composition collected during one-year of seasonal observation.

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MATERIAL AND METHOD

1. Sampling:

The aerial parts of *L. alba* (1 year old plants) were collected at the Experimental Farm of the Institute of Biotechnology at Caxias do Sul (Rio Grande do Sul State - Southern Brazil, S29°10.122' WO51°11.118'), from October 1998 to September 1999. The plants were identified by the botanists R. Wasun and M. Rossato of the Herbarium of the Museu de Ciências Naturais da Universidade de Caxias do Sul. A voucher specimen was deposited in the Herbarium (HUCS 13705).

2. Oil Isolation:

The oils were isolated from fresh leaves by hydro-distillation (1 hour) using a modified Clevenger-type apparatus (Mechkovski & Akerele, 1992). The separated oils were dried over anhydrous sodium sulfate (Merck, Germany). Samples were stored in a refrigerator (+ 6°C) until GC and GC/MS analysis.

3. Component Identification:

GC analyses were performed using a Hewlett Packard 6890 Series GC equipped with HP-Chemstation data processor, fitted with a HP-Innowax bonded phase capillary column (30 m x 0.32 mm, 0.50 µm film thickness; Hewlett Packard, Palo Alto, USA); column temperature program, 40°C (8 min), to 180°C at 3°C/min, 180°

- 230°C at 20°C/min, final 230°C (20 min); injector temperature 250°C; detector temperature 250°C; split ratio 1:50; carrier gas H₂ (34KPa). Volume injected, 1 µL diluted in hexane (1:10).

The GC/MS analysis were performed in a Hewlett Packard 6890 GC using a Hewlett Packard 6890/MSD 5973 mass selective detector, equipped with HP Chemstation software and Wiley 275 spectra data library (Adams, 1995). A HP-Innowax fused silica capillary column (30 m x 0.25 mm, 0.25µm film thickness, Hewlett Packard, Palo Alto, USA) was used. The temperature program was the same one used as in the GC analysis: interface 280°C; split ratio 1:100; carrier gas He (56 KPa); flow rate: 1.0 mL/min; ionization energy 70 eV; mass range 40-350. Volume injected, 0.4 µL diluted in hexane (1:10).

Identification of the individual components was based on comparison of their GC retention indices (RI) in polar columns and comparison with mass spectra of components by GC/MS. Linear retention indices of the compounds were determined relative to *n*-alkanes (C9-C25).

RESULT AND DISCUSSION

The oil prepared from the fresh leaves of *L. alba* yielded from 0.10% - 0.37% (mL/100g of fresh leaves), where the yields were higher in the warmer months (December to March), compared to the colder period (June to August)(Table 1).

TABLE 1 – Oil yield and percentage composition of the essential oils of *Lippia alba* oils from Caxias do Sul

Month of Sampling (1998 – 1999)	Oct	Nov	Dez	Jan	Feb	Mar	April	May	June	July	Aug	Sept	
Oil Yield (%)	0.13	0.26	0.37	0.29	0.16	0.36	0.32	0.22	0.16	0.11	0.14	0.22	
Components													RI*
Sabinene	1.7	1.1	1.3	1.2	1.1	1.2	1.5	1.5	1.9	1.8	1.8	1.7	1077
Myrcene	0.3	0.3	0.3		0.2	0.3	0.3	0.3	0.4	0.4	0.4		1116
Limonene	0.3	0.3	0.2		0.2	0.2	0.3	0.3	0.3	0.3	0.3		1134
1,8-Cineole	14.1	9.2	7.8	9.2	9.2	7.3	8.9	10.0	10.9	9.8	12.0	10.0	1140
cis-β-Ocimene	0.2	0.5	1.0	0.9	0.7	1.2	1.2	1.2	1.2	1.1	1.1	1.2	1173
cis-Linalool oxide	0.4	0.1	0.1	0.1		0.1				0.1	0.2		1341
1-Octen-3-ol	2.7	1.3	1.0	1.2	1.2	1.2	1.4	2.0	2.1	2.3	3.1	2.1	1358
trans-Linalool oxide	0.4	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3		1370
Linalool	66.9	68.6	72.7	75.4	79.2	71.8	66.7	58.9	56.4	52.2	50.0	66.6	1505
α-Gurjunene	0.2	0.4	0.4	0.2	0.1	0.4	0.3	0.5	0.4	0.6	0.7	0.5	1523
β-Caryophyllene	0.6	1.4	1.1	0.7	0.3	1.1	1.0	1.7	1.4	2.2	2.2	1.7	1524
Terpinen-4-ol		0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.2		1531
Geraniol	1.7	1.2	0.7	0.8	0.5	0.7	1.7	1.8	2.6	2.9	3.0	2.6	1730
α-Murolene	0.2	0.3	0.3	0.3	0.2				0.2	0.2	0.2		1650
Germacrene B	0.3	0.3	0.3	1.1	0.4	0.3	0.3	0.4	0.4	0.5	0.5		1714
δ-Cadinene	2.3	1.8	1.8	1.1	0.8	1.7	1.3	2.6	3.7	4.2	4.3	2.5	1729
Geraniol	0.6	0.4		0.2	0.1		0.3	0.2	0.6	0.7	0.8	0.5	1750
Cuparene	0.3	1.2	1.5	1.2	1.1		1.8	1.8	1.8	2.0	1.6	1.5	1832
Caryophyllene oxide	1.1	1.3	0.7	0.3	1.2	1.0	1.0	1.9	1.4	2.2	2.3	1.9	1863
Non oxygenated	6.6	7.5	8.3	6.8	5.1	6.5	8.1	10.5	11.7	13.3	13.1	9.1	
Oxygenated	88.0	82.4	83.2	87.5	91.7	82.5	80.2	75.3	74.5	70.7	71.9	83.6	
Total	94.6	89.9	91.5	94.3	96.8	88.0	88.3	85.8	86.2	84.0	85.0	94.7	

* Retention indices in polar column

The components, the percentage of each constituent (percent integration area), and the retention index are summarized in the same Table. The components were reported according to their retention times in Innosil column. Nineteen compounds, representing ca.92% of the oil were identified. The oxygenated mono-terpenes in the oils were represented mainly by linalool (50.0% - 79.2%) and 1,8-cineol (7.3% - 14.1%). Sabinene, cis- β -ocimene, 1-octen-3-ol, β -caryophyllene, geranial, δ -cadinene and cuparene were minor constituents. Other compounds were found in traces.

The chemical composition of the essential oil obtained from plants cultivated in Caxias do Sul is very similar to the oils described by Frighetto *et al.* (1998) and Kumar *et al.* (1999), which also have high concentrations of linalool, but differ clearly from that of plants described for other regions (Dellacassa *et al.*, 1990; Matos *et al.*, 1996; Zoghbi *et al.*, 1998; Singh *et al.* 1999; Castro *et al.*, 2000).

From the results of this study, it is clear that the summer oils were higher in linalool (>72.7% - 79.2%) than the winter oils, when linalool was down to 50.0% (August) and 1,8-cineole up to 12.2%.

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