Note

ESSENTIAL OIL COMPOSITION OF FRUIT COLOUR VARIETIES OF *Eugenia brasiliensis* Lam.

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ABSTRACT: *Eugenia brasiliensis* Lam. is a variable species concerning fruit colour, with three recognized varieties. However, the definition of varieties is not easy for Myrtaceae species and not widely accepted. Two fruit colour varieties (purple and yellow) of *E. brasiliensis* had their essential oil composition analysed in order to give support to the existence of varieties for this species. Although, the major components in the leaf oil are the same monoterpenes for both varieties, α-pinene, β-pinene and 1,8-cineol, the purple fruit variety accumulates more oxygenated sesquiterpenes (33.9%) than the one with yellow fruits (3.8%). The major differences occurred in purple fruits that present as major components caryophyllene oxide (22.2%) and α-cadinol (10.4%), not found in the leaf oil, and the yellow fruit oil presented a similar composition as observed for the leaves. These fruit colour varieties of *E. brasilensis* can be considered as two distinct chemotypes, since the sesquiterpene pathway is more operant in the purple variety than in the yellow one, in which monoterpenes are mainly accumulated.
Key words: Myrtaceae, chemotypes, terpenes, pinenes, caryophyllene oxide

COMPOSIÇÃO DOS ÓLEOS ESSENCIAIS DE VARIEDADES DE COLORAÇÃO DE FRUTOS DE *Eugenia brasiliensis* Lam.

RESUMO: A espécie *Eugenia brasiliensis* Lam. apresenta a coloração dos frutos variável, sendo reconhecidas três variedades. Entretanto, a definição de variedades não é fácil para espécies de Myrtaceae e também não é amplamente aceita. Duas variedades de *Eugenia brasiliensis*, baseado na cor dos frutos (roxos e amarelos), tiveram a composição de seus óleos essenciais analisadas com a finalidade de obter indícios de variedade botânica para esta espécie. Embora, os componentes principais nos óleos das folhas fossem os mesmos monoterpenos para ambas as variedades, α-pineno, β-pineno e 1,8-cineol, a variedade com frutos roxos acumulou maior quantidade de sesquiterpenos oxigenados (33,9%) do que aquela com frutos amarelos (3,8%). As diferenças principais ocorreram nos frutos roxos que apresentaram como componente principal o óxido de cariofileno (22,2%) e o α-cadinol (10,4%), não detectados no óleo das folhas, e o óleo dos frutos amarelos apresentou uma composição similar àquela observada para as folhas. Estas variedades de coloração dos frutos de *E. brasilensis* podem ser considerados como dois quimiotipos distintos, uma vez que na variedade com frutos roxos a rota biossintética para sesquiterpenos encontra-se mais operante do que naquela com frutos amarelos, onde são acumulados principalmente os monoterpenos.
Palavras-chave: Myrtaceae, quimiotipos,terpenos, pinenos, óxido de cariofileno

INTRODUCTION

Eugenia brasiliensis Lam. is a variable species concerning fruit characters which is found in coastal Brazilian forests, commonly known as "Grumixama" or Brazilian-cherry. Three varieties were recognized by Cambessèdes (1832-1833) according to their fruit colours: 1. α-variety, with purple fruits, most common; 2. β-variety, with red fruits and 3. γ-variety, with white fruits. Not much attention has been given to these varieties this time, although more recently Mattos (1984) considered that the variety γ, described by him

as having yellowish rather than white fruits, should be recognized as a variety, and it is even called by plant breeders as "grumixama-branca" (white "grumixama"). Nevertheless, varieties are not easily definable entities in the Myrtaceae family and are not widely accepted; much of the variation that can be considered in only one species by an author may deserve not only varietal but also specific recognition by others. For instance, the delimitation of *Blepharocalyx salicifolius* (Kunth) O. Berg, as considered by Landrum (1986), encompasses not only the three varieties of this species recognized by Legrand & Klein (1978) but also what they consider as two additional distinct species, *B. suaveolens* (Cambess.) Burret and *B. picrocarpus* O. Berg.

The essential oil composition of *E. brasiliensis* has previously been investigated in specimens collected in southern and south eastern Brazil. In the southern specimens, the main components were α- and β-pinene (10.3% and 10.4%, respectively), spathulenol (7.7%) and τ-cadinol (7.1%) (Vérin, 1996; Apel et al., 2004) and in a specimen collected in São Paulo the major constituents were α- and β-selinene (14.8 and 17.3%) (Fischer et al., 2005). In these previous investigations, there was no indication of the fruit colour.

In order to correlate the varietal concept to chemical composition, the essential oil composition of the two fruit colour varieties from both, leaves and fruits, were analysed.

MATERIAL AND METHODS

The leaves of both purple and yellow fruit *Eugenia brasiliensis* Lam., were collected from six specimens for each variety cultivated in Martinho Prado, Moji-Guaçu, SP, between 22°10'43'' and 22°18'19''S; 47°8'5'' and 47°11'34''W, in October 2000 during the morning. The identification was performed by Marcos Sobral and voucher specimens (Fischer 13 and 14, respectively) were deposited at the Herbarium of the Universidade Federal do Rio Grande do Sul (ICN).

The essential oils were obtained from a pool of leaves and fruits of the six specimens for each variety by hydro-distillation during 5h using a Clevenger-type apparatus. The oil from the purple fruit specimens presented a yield of 0.17 % for leaves and 0.002% for fruits while the yellow fruit specimens yielded 0.34% for leaves and 0.15% for fruits.

For component identification, the essential oils were submitted to Gas Chromatography (GC) and Mass Spectrometry (GC/MS) analysis. GC analysis was performed in a Shimadzu GC-17A chromatograph equipped with Shimadzu GC 10 software, using a fused silica capillary column (30 m × 0.25 mm × 0.25 μm, coated with DB-5), and a flame ionization detector. Injector and detector temperatures were set at 220ºC and 250ºC, respectively; the oven temperature was programmed from 60º-300ºC at 3ºC min^{-1} and helium was employed as carrier gas (1 mL min^{-1}). The percentage compositions were obtained from electronic integration measurements using flame ionization detection without taking into account relative response factors.

GC/MS analysis was performed using a Shimadzu GC-quadrupole MS system (QP 5000), EI operating at 70 eV; GC carried out in the same conditions as described above. The compound identification was performed by comparing retention indices (Kóvats Index (KI), determined relatively to the retention times of a series of *n*-alkanes) and mass spectra with those of authentic samples and with literature data (Adams, 1995; Jennings & Shibamoto, 1980).

RESULTS AND DISCUSSION

The chromatographic analysis of the oils from both specimens identified (Table 1) fifty-nine compounds representing 93.0 - 100% of the total oil components. Clear differences in the pattern of terpenes were observed for both fruit colour varieties. Although the major components in the leaf oil are the same for both varieties (α-pinene, β-pinene and 1,8-cineol), in the leaf oil from the purple fruit variety a higher amount of oxygenated sesquiterpenes (33.9%) was observed as compared to the yellow fruit variety, in which the majority of the identified compounds were monoterpene hydrocarbons (55.6%) and oxygenated monoterpenes (32.6%) and only 3.8% of oxygenated sesquiterpenes. In the fruit oils, this difference can be seen more clearly. The purple fruit oil contained almost exclusively sesquiterpenes (57.3% oxygenated and 34.1% hydrocarbons) while the yellow fruit oil was composed in the majority by monoterpenes (42.9% hydrocarbons and 18.5% oxygenated). The essential oil composition of the oil from fruits and leaves of the yellow fruit variety were very similar while in the purple fruit variety the fruit oil composition was quite different from that observed in the leaves. In this variety, the major fruit oil components were caryophyllene oxide (22.2%) and α-cadinol (10.4%), these compounds were not observed in the leaves oil. In this oil, the major component was the sequiterpene globulol (6.7%), not found in fruit oil, and very low amount of monoterpenes were detected.

As the fruit colour alone is a weak character for the establishment of a variety or the recognition of two different species, our results might support the varietal concept for this species due to the remarkable

Table 1 - Volatile oil composition of leaves and fruits from two fruit colour varieties of *Eugenia brasiliensis*.

Compound	IK*	Plant Part			
		Leaves		Fruits	
		Purple Variety	Yellow Variety	Yellow Variety	Purple Variety
ethyl acetate	790	2.7		0.9	0.2
tricyclene	905	0.2		0.2	
α-pinene	914	18.8	33.5	15.4	0.6
α -fenchene	925			0.2	
β-pinene	953	11.0	14.4	9.3	0.2
myrcene	966	0.6	5.0	10.7	
p-cymene	1002	0.4		0.7	
limonene	1006	8.6	2.7	4.4	0.7
1,8-cineol	1009	9.6	28.2	7.5	0.3
(*Z*)- β -ocimene	1022			0.3	
(*E*)- β -ocimene	1033			0.2	
terpinolene	1061			0.8	
linalool	1072	1.5			
perilene	1073			0.9	0.3
exo-fenchol	1086			0.4	
(*E*)-sabinol	1111			0.4	
terpinen-4-ol	1147	0.8			
α -terpineol	1161	5.4	4.4	10.2	0.5
phenyl ethyl 2-acetate	1224				0.6
α -copaene	1344	0.4		1.1	1.6
β -elemene	1360				2.0
β -caryophyllene	1389	2.1	1.7	5.4	9.2
α -humulene	1422	0.7		1.4	2.1
(*E*)- β -farnesene	1425				0.2
allo-aromadendrene	1429				0.4
γ-muurolene	1445				1.8
germacrene D	1450				0.5
ar-curcumene	1452				0.3
β-selinene	1456				0.9
viridiflorene	1466	1.0		0.5	1.7
α-muurolene	1471			0.4	1.4
β-bisabolene	1479	0.6			9.6
γ -cadinene	1483	0.5			
δ-cadinene	1494	1.8		4.3	
(*Z*)-calamenene	1496				2.4
(*E*)-nerolidol	1538	0.3			1.6
ledol	1539	0.9			
hidroxy-caryophyllene	1543				1.0
spathulenol	1548	5.2		4.4	
NI[1]	1552			5.4	
caryophyllene oxide	1555		0.9		22.2
globulol	1557	6.7	1.2		

Continue...

Table 1 - Continuation.

epi-globulol	1564	2.4			1.7
5-*epi*-7-*epi*-α-eudesmol	1572	1.1			1.7
$C_{15}H_{26}O$ (eudesmol)[2]	1575	1.6			
humulene oxide II	1578				1.5
1,10-di-*epi*-cubenol	1593	0.3		2.4	
10-*epi*-gama-eudesmol	1595	0.9			
1-*epi*-cubenol	1596	2.8	1.7		3.1
γ-eudesmol	1599				0.6
iso-spathulenol	1603	0.4		0.5	
τ-cadinol	1611	6.8		3.2	9.9
cubenol	1612	1.4		1.0	
α-muurolol	1617	3.1		2.5	2.2
α-cadinol	1630				10.4
$C_{15}H_{22}O$ (calemenenol)[3]	1638			0.9	
$C_{15}H_{22}O$ (calemenenol)[4]	1643			0.6	0.8
β-bisabolenol	1783				0.6
ethyl hexadecanoate	1968				0.6
Total		100.0	93.5	93.0	95.3
Aliphatic compounds		2.9	0.0	1.1	1.4
Monoterpene hydrocarbons		39.4	55.6	42.9	1.8
Oxygenated monoterpenes		17.3	32.6	18.5	0.8
Sesquiterpene hydrocarbons		7.1	1.7	13.1	34.1
Oxygenated sesquiterpenes		33.9	3.8	17.8	7.3

*IK = Kóvats Retention Index
[1]NI (non-identified) – m/z (rel. int., 70 eV): 41(100), 53(21), 67(14), 91(38), 105(28), 117(17), 131(30), 145(19), 159 (3).
[2]Correct isomer not determined – m/z (rel. int., 70 eV): 41(52), 59(100), 81(31), 93(29), 107(38), 149(33), 161(19), 204(9).
[3]Correct isomer not determined – m/z (rel. int., 70 eV): 41(100), 43(62), 55(37), 91(27), 105(17), 131(15), 187(7), 202(3).
[4]Correct isomer not determined – m/z (rel. int., 70 eV): 41(100), 43(61), 55(37), 91(26), 105(18), 131(16), 187(8), 202(1).

difference in the chemical composition of the essential oils for both fruit colour varieties of *E. brasiliensis*, mainly in the fruits. These results indicate that these fruit colour varieties have a distinct biochemical regulation or even a different genomic of their secondary metabolism, since in the purple fruit variety the sesquiterpene pathway is more operant than in the yellow one, in which mainly monoterpenes are accumulated. Additionally, the phenolic pathway seems to be different in these two varieties, in the purple variety this biochemical pathway leads to the accumulation of anthocyanidins, noticed by the dark purple colour of the fruits, whether in the yellow one these metabolites are apparently not present. These observations together with the essential oil analysis may indicate that these two fruit colour varieties are indeed two chemotypes of this species. A more detailed taxonomic study, such as DNA analysis, is required to define whether we are dealing with two taxonomic varieties or two distinct species.

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REFERENCES

ADAMS R.P. **Identification of essential oil components by gas chromatography/ion trap mass spectrometry**. Carol Stream: Allured Pub., 1995. 469p.

APEL, M.A.; SOBRAL, M.; MENUT, C.; BESSIERE, J.-M.; SCHAPOVAL, E.E.S.; HENRIQUES, A.T. Essential oils from *Eugenia* species. Part VII: Sections Phyllocalyx and Stenocalyx. **Journal of Essential Oil Research**, v.16, p.135-138, 2004.

CAMBESSÈDES, J. Myrtaceae. In: SAINT-HILAIRE, A. (Org.) **Flora Brasiliae Meridionalis**. Paris: A. Belin, 1832-1833. v.2, p.277-384.

FISCHER, D.C.H.; LIMBERGER, R.P.; HENRIQUES, A.T.; MORENO, P.R.H. Essential oils from leaves of two *Eugenia brasiliensis* specimens from Southeastern Brazil. **Journal of Essential Oil Research**, v.17, p.499-500, 2005.

JENNINGS, W.; SHIBAMOTO, T. **Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography**. New York: Academic Press, 1980, 184p.

LANDRUM, L.R. *Campomanesia, Pimenta, Blepharocalyx, Legrandia, Acca, Myrrhinium and Luma*. **Flora Neotropica**, v.45, p.1-178, 1986.

LEGRAND, C.D.; KLEIN, R.M. Mirtáceas - *Myrciaria, Pseudocaryophyllus, Blepharocalyx*, Espécies suplementares, espécies cultivadas, generalidades. In: REITZ, R. (Org.) **Flora Ilustrada Catarinense**. Itajaí: Herbário Barbosa Rodrigues, 1978. p.731-876.

MATTOS, J.R. Myrtaceae do Rio Grande do Sul. **Roesslória**, v.6, p.3-394, 1984.

VÉRIN, P. Étude chimique des constitutants volatils de plantes aromatiques du Brésil. Montpellier: Université de Montpellier II, 1996. 312p. (Ph.D. Thesis.).

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