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Volatile Components and Antioxidant Activity from some Myrtaceous Fruits cultivated in Southern Brazil

Rafaela MARIN [1], Miriam A. APEL [1], Renata P. LIMBERGER [1], Maria C.B. RASEIRA [2], José F.M. PEREIRA [2], José Â.S. ZUANAZZI [1] & Amélia T. HENRIQUES [1]*

[1] *Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, UFRGS, Av. Ipiranga 2752, 90.610-000, Porto Alegre, RS, Brazil*

[2] *EMBRAPA Clima Temperado, Caixa Postal 403, Pelotas, RS, Brazil*

SUMMARY. The volatile aroma compounds of fresh edible fruit of guabiju *(Myrcianthes pungens)*, guabiroba *(Campomanesia xanthocarpa)*, cereja-do-rio-grande *(Eugenia involucrata)*, pitanga *(Eugenia uniflora)* and araçá *(Psidium cattleyanum)*, cultivated in Southern Brazil, were analyzed by GC and GC-MS. Altogether sixty-six compounds were identified, representing 94.6-99.1% of the total oils content. The major components were limonene (10.9%) and β-caryophyllene (21.8%) for guabiroba; β-caryophyllene (32.7%), germacrene D (14.2%) and bicyclogermacrene (11.2%) for guabiju; hexadecanoic acid (11.7%) for pitanga; β-caryophyllene (22.5%), neo-intermedeol (14.2%) and β-selinene (10.1%) for araçá; and β-caryophyllene (10.1%) for cereja-do-rio-grande. An antioxidant property was tested with all the oils obtained by means of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. Except for guabiju, all samples presented interesting radical scavenging activity.

RESUMEN. "Compuestos Volátiles y Capacidad Antioxidante de Frutas de Mirtáceas Cultivadas en el Sur del Brasil". Los compuestos volátiles presentes en frutas de guabiju *(Myrcianthes pungens)*, guabiroba *(Campomanesia xanthocarpa)*, cereja-do-rio-grande *(Eugenia involucrata)*, pitanga *(Eugenia uniflora)* y araçá *(Psidium cattleyanum)*, cultivadas en el sur del Brasil, fueron estudiados por GC y GC-MS, habiéndose identificado sesenta y seis compuestos, que representan el 94,6-99,1% del total. En guabiroba se identificó limoneno (10,9%) y β-cariofileno (21,8) como los principales compuestos. En guabiju los componentes mayoritarios fueron β-cariofileno (32,7%), germacreno D (14,2%) y biciclogermacreno (11,2%), en pitanga ácido hexadecanoico (11,7%), en araçá β-cariofileno (22,5%), neo-intermedeol (14,2%) y β-selineno (10,1%) y en la cereja-de-rio-grande β-cariofileno (10,1%). La capacidad antioxidante fue estudiada a través de la prueba con 1,1-difenil-2-picrilhidrazilo (DPPH). Salvo guabiju, todas las muestras presentaron poder antioxidante.

INTRODUCTION

Many fruits of the Myrtaceae family have a rich history of use both as edibles and as traditional medicines in divergent ethnobotanical practices throughout the tropical and subtropical world. In botanical functional food ingredients these fruits may exert benefic health-related effects or reduce the risk of chronic disease beyond basic nutritional functions. The most widely used, as well as the most promising ingredients categories are those inherent to edible plants which are part of the normal human diet [1]. Among them, myrtaceous fruits, whose use was once restricted to people living in limited geographic areas, are gaining popularity worldwide not only due to exotic flavors that appeal to the consumer [2], as well as to the relative inexpensive cost, the high content of vitamins, the possibility of use in a wide range of food products [3] and the presence of secondary metabolites, as phenolic compounds and essential oils, that are often related with benefic effects on the human health [4-8]. On the basis of these considerations, this research was undertaken to determine the volatile flavor components and antioxidant activity from the following fresh and mature tropical myrtaceous fruit, cultivated in Southern Brazil:

Araçá (strawberry guava or cattley guava, *Psidium cattleyanum* Sabine) is a fruit with nu-

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* Author to whom correspondence should be addressed. *E-mail:* amelia@farmacia.ufrgs.br

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merous seeds of either yellow or reddish skin, rich in vitamin C and it shows a variable size and weight which in some examples can reach more than 20 g per fruit. This species is a shrub or tree which can grow from 3 to 10 m high, commonly growing in several forestry formations from south-east and south of Brazil (Espírito Santo to Rio Grande do Sul) to Uruguay [9]. It is very common in Southern Brazil. Embrapa Clima Temperado (Pelotas, Rio Grande do Sul, Brazil) has been working with this species for several years, selecting the best genotypes. Two of them have been propagated under the names of "Ya-Cy" and "Irapuã" [10]. In the folk medicine, the leaves are used for diarrhea treatment [11]. Previous study has reported the essential oil analysis of the leaves of this species, in witch β-caryophyllene was the main compound (36.8%) in the oil [12].

In the case of pitanga (Brazilian cherry, *Eugenia uniflora* L. - Syn.: *E. michelli, Stenocalyx michelli),* the climatic variations of the different cultivate regions produce roundish fruits or oblate shaped fruits with 7 or 8 grooves, with one or two seeds in general, but sometimes, it goes up to four seeds. The epidermis changes from green to purple [13]. However, some genotypes show light red, dark purple or red fruits when ripe [10]. *E. uniflora* is a tree which can grow from 3 to10 m high, commonly growing in several forestry formations in Argentina, Brazil (from Minas Gerais to Rio Grande do Sul), Paraguay and Uruguay [14]. The fruits can be eaten fresh or used on liquors, jams and jellies or also as pulp for yogurts, ice creams or juices [13]. The tea obtained from the leaves of *E. uniflora* has been used in folk medicine against fever, infections and diarrhea and to lower blood pressure [11]. Some pharmacological activities include antispasmodic [15], hypotensive [15], antimalarial [16], antihypertriglycemic [17], hypoglycemic [18], antidiarrheal [19], anti-inflammatory [19], to decrease uric acid [20] and as an antimicrobial [21], among others. Previous studies concerning leaf essential oil from this species have been published and oxygenated sesquiterpenoids have been showed to be the main class of compounds, mainly represented by furanodiene, furanoelemene, and selina-1,3,7(11)-trien-8-one and oxidoselina-1,3,7(11)-trien-8-one [22-28].

Cereja-do-rio-grande *(Eugenia involucrata* DC.) is a fruit with dark purple smooth skin, with persistent sepals at the end, edible by men and animals and can also be used for liquors, jellies and other desserts. This species is a tree which can grow from 5 to 20 m high, commonly growing in Argentina, Southern Brazil (Minas Gerais, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul), Paraguay and Uruguay [14]. The average fruits measures 2.5 cm by 2.0 cm diameter [9].

Guabiju *(Myrcianthes pungens* (O. Berg) D. Legrand) is a fruit with dark purple epidermis when ripe. This species produces pubescent fruits of oblate shape and velvet aspect. The flesh is sweet, varying from 15 to 19° Brix. The fruits are very appreciated by birds. They can also be used for human consumption. *M. pungens* is a tree which can grow to 20 m high. It occurs in forests of Southern and South Brazil [9]. In the folk medicine this species is used for its antidiarrheic property [29].The chemical composition of the leaves' oil has been reported [30-32].

Guabiroba *(Campomanesia xanthocarpa* O. Berg) is a fruit with a smooth dark yellow epidermis and with a sweet, juicy endocarp, which resembles a small guava. The average fruit measure from 1.5 cm to 3 cm. Total soluble solids are anywhere from 9 to 15° Brix. Fruit present a variable pungency. The species is a forest treelet or tree which can grow 10-20 m high. It is native to Argentina, Paraguay and Brazil [9]. Its leaves and stem bark are traditionally employed as a remedy for dysentery, stomach problems, fever, "catarrh bladder", antidiarrheic, hepatic disorders and as anti-inflammatory agent [29,33-34]. Pharmacological studies pointed out for in vitro inhibition of the enzyme xanthine-oxidase [35] and antimicrobial and antiulcerogenic activities without toxic effects in mice [36]. Chronic treatment reduces weight gain and it is hypogliceamic in mice [37]. Chemical studies of the leaves of this plant have reported the isolation of the flavonoids quercetin, myricetin, quercitrin and rutin [38], and also the presence of saponins and tanins [36]. The chemical composition of the oil from *C. xanthocarpa* leaves was analyzed and showed an important amount of *E*-nerolidol (28.8%) and linalool (17.2%) [39].

MATERIALS AND METHODS

Plant material

The material analyzed comprises fruits collected in November, 2003, from cultivars of the germplasm bank maintained by the Clima Temperado Research Center of Embrapa (Empresa Brasileira de Pesquisa Agropecuária, Ministério da Agricultura, Pecuária e Abastecimento), Pelotas, Rio Grande do Sul, Brazil, located at 31°40'47"S latitude, 52°26'24"SW longitude and altitude of 60 meters. Araçá fruits were obtained from populations of yellow skin plants producers collected at the Pelotas surroundings with similar characteristics to Ya-ci. The red Pitanga selections were kept by the Embrapa collection.

Isolation of the essential oils

Essential oils were obtained from whole fresh and ripped fruits (100 g) through hydrodistillation using a Clevenger-type apparatus for 4 h. The oils were dried over anhydrous sodium sulfate and kept at 4 °C in sealed brown vials until the analysis.

Qualitative and quantitative analyses

Quantitative and qualitative analyses of the oils were performed by capillary GC and GC-MS, respectively. GC analyses were carried out on a chromatograph (Shimadzu GC-17A) equipped with a Shimadzu GC 10 software, using a fused silica capillary column (30 m x 0.25 mm x 0.25 (m, coated with DB-5). Injector and detector temperatures were set at 220 °C and 250 °C, respectively; the oven temperature was programmed from 60 °C - 300 °C at 3 ºC/min and helium was employed as carrier gas (1 mL/min). The percentage compositions were obtained from electronic integration measurements using flame ionization detection without taking into account relative response factors. GC-MS analyses were performed in the same apparatus and chromatographic conditions as described above, using a quadrupole MS system (QP 5000) operating at 70 eV. The components of the oils were identified by comparison of retention indices (determined relatively to the retention times of a series of *n*-alkanes) and mass spectra with those of authentic samples and with literature data [12, 40-44].

DPPH assay

Ten microlitres of a 1:250 dilution of the essential oil in methanol were applied to TLC plates (silica gel 60 GF_{254}). The TLC plates were sprayed with a 0.2% 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in MeOH and left at room temperature for 30 min. Active compounds appear as yellow spots against a purple background [45].

RESULTS AND DISCUSSION

The yield of essential oil of the fresh fruits was 0.2% to araçá and guabiroba; and 0.1% to guabiju, pitanga and cereja-do-rio-grande. The relative amounts of each component and classes of compounds are summarized in Table 1. Altogether sixty-six compounds were identified, representing 94.6 - 99.1% of the oils content. Except for pitanga oil, all the analyzed samples were characterized by the predominance of sesquiterpene group (from 62.9% to 95.4% of the total oil), in which β-caryophyllene (from 10.1 to 32.7%) was the main compound.

Guabiroba and pitanga oil were the only species that presented important amounts of monoterpenoids in their oil composition (23.1% and 24.5% of the total oil, respectively). The former oil was characterized by the monoterpene fraction mainly by limonene (10.9%) and in the sesquiterpene fraction, β-caryophyllene (21.8%) was the major compound. In a previous work, the oil of the leaves of a native guabiroba from Rio Grande do Sul, Brazil, was characterized by *E*-nerolidol (28.8%) and linalool (17.4%) [39].

Pitanga oil presented a complex chemical composition with absence of predominant compounds. The major products detected were *(E)*-β-ocimene (7.4%), α-selinene (7.2%), β-selinene (5.2%), germacrene B (7.2%) and hexadecanoic acid (11.7%). A complex chemical composition was also observed in the essential oil from Cuban [46] and Northeastern Brazilian [2] pitanga berries. The chemical composition is fairly different from the leaves of this species, which are characterized mainly by furane sesquiterpenes.

In the cereja-do-rio-grande oil, the major components were β-caryophyllene (10.1%), spathulenol (7.8%) and β-bisabolene (7.2%). In the oil of guabiju, β-caryophyllene (32.7%), germacrene D (14.2%) and bicyclogermacrene (11.2%) were the main compounds. In previous study with native guabiju from Rio Grande do Sul, Brazil, the main components of the oil obtained from the leaves was also the sesquiterpene hydrocarbon β-caryophyllene (10.1%) [32]. The findings with this species from South Brazil, even considering to be cultivated or native, were quite different to those previously reported by other groups [30-31]. In these works, the oil was characterized by monoterpenes, especially limonene, pulegone, farnesol and 1,8-cineole.

The chemical composition of the araçá essential oil was characterized by the predominance of β-caryophyllene (22.5%), neo-intermedeol (14.2%), α-humulene (7.5%) and β-selinene (10.1%). It is of interest to note the presence of in high percentage of neo-intermedeol, which is distinctive in this species. In a previous work, the analysis of the volatile extract of Cuban araçá fruit [3], β-caryophyllene (10.2%) was also the main compound characterized, accompanied by minor amounts of α-pinene (6.4%), myrcene (4.0%), *(Z)*-3-hexenol (6.6%) and hexadecanoic acid (8.2%).

Compound	RI	Guabiroba	Pitanga	Cereja-do-rio -grande	Guabiju	Araçá
Monoterpene hydrocarbons		**21.8**	**24.5**	**0.0**	**0.0**	**0.8**
α-pinene	939	4.7	-	-	-	-
β-pinene	980	4.6	-	-	-	-
myrcene	988	-	1.0	-	-	-
α-phellandrene	1005	1.6	-	-	-	-
o-cymene	1018	-	2.7	-	-	-
p-cymene	1021	-	5.3	-	-	-
limonene	1031	10.9	4.4	-	-	-
(Z)-β-ocimene	1033	-	3.7	-	-	0.2
(E)-β-ocimene	1043	-	7.4	-	-	0.6
Oxygenated monoterpenes		**1.3**	**0.0**	**0.0**	**0.0**	**0.2**
linalool	1101	0.6	-	-	-	0.2
α-terpineol	1189	0.7	-	-	-	-
Sesquiterpene hydrocarbons		**61.3**	**48.5**	**42.2**	**65.1**	**63.8**
α-cubebene	1330	1.1	-	-	-	-
α-copaene	1376	2.8	-	-	1.1	1.7
β-elemene	1391	1.4	3.9	1.3	0.9	-
β-caryophyllene	1418	21.8	2.2	10.1	32.7	22.5
α-santalene	1420	-	0.4	-	-	-
β-gurjunene	1425	0.7	-	2.7	-	-
aromadendrene	1439	1.3	2.1	-	-	-
α-humulene	1454	7.6	0.3	1.6	3.3	7.5
β-chamigrene	1459	-	-	-	-	4.9
allo-aromadendrene	1461	1.4	0.6	1.7	-	-
γ-himachalene	1463	-	-	-	-	0.6
germacrene D	1480	0.2	2.9	2.4	14.2	-
β-selinene	1485	1.6	5.2	1.1	-	10.1
α-selinene	1490	2.6	7.2	4.7	-	2.1
trans-β-guaiene	1493	-	-	-	-	9.0
bicyclogermacrene	1494	5.3	5.2	-	11.2	1.2
α-muurolene	1495	5.0	1.4	-	-	-
germacrene A	1498	2.5	-	-	-	-
β-bisabolene	1500	0.3	-	7.2	-	-
γ-cadinene	1502	1.3	2.6	-	1.7	-
β-cadinene	1520	3.2	-	-	-	-
(Z)-calamenene	1521	0.6	-	-	-	-
δ-cadinene	1524	-	3.1	1.6	-	4.2
cadina-1,4-diene	1527	0.3	4.2	1.3	-	-
α-cadinene	1529	-	-	2.6	-	-
germacrene B	1552	0.3	7.2	3.9	-	-
Oxygenated sesquiterpenes		**7.7**	**14.4**	**53.2**	**21.8**	**31.3**
bulnesol	1660	1.9	-	-	-	-
(E)-nerolidol	1551	0.1	-	-	-	1.4
elemol	1549	-	-	-	3.8	-
ledol	1560	-	-	2.0	-	-
spathulenol	1576	0.5	4.4	7.8	-	-
caryophyllene oxide	1581	0.2	1.0	2.2	-	1.7
globulol	1585	-	4.7	-	1.0	-
epi-globulol	1588	-	2.8	-	3.9	-
5-*epi*-7-*epi*-α-eudesmol	1590	-	-	5.9	-	-
guaiol	1595	4.3	-	3.1	-	-
humulene oxide I	1598	-	-	2.2	-	-
humulene oxide II	1602	-	-	5.9	-	0.4
10-*epi*-γ-eudesmol	1619	-	-	5.9	-	-
iso-spathulenol	1620	-	-	4.0	-	-
1-*epi*-cubenol	1621	-	-	-	-	3.8
γ-eudesmol	1630	-	-	6.0	2.5	-
α-muurolol	1639	-	1.5	-	-	-
t-cadinol	1640	-	-	1.0	-	2.1
cubenol	1639	-	-	-	-	6.6
t-muurolol	1641	-	-	0.6	-	-
β-eudesmol	1649	0.2	-	-	8.1	-
α-eudesmol	1650	0.5	-	5.4	2.5	1.1
α-cadinol	1653	-	-	1.2	-	-
neo-intermedeol	1647	-	-	-	-	14.2
Others		**6.9**	**11.7**	**0.0**	**7.7**	**0.0**
hexadecanoic acid	1971	5.2	11.7	-	-	-
(E)-2-hexanal	841	0.2	-	-	-	-
hexanal	800	0.2	-	-	-	-
furfural	823	1.1	-	-	7.7	-
benzaldeyde	952	0.2	-	-	-	-
Monoterpenoids		**23.1**	**24.5**	**0.0**	**0.0**	**1.0**
Sesquiterpenoids		**69.0**	**62.9**	**95.4**	**86.9**	**95.1**
Others		**6.9**	**11.7**	**0.0**	**7.7**	**0.0**
Total		**99.0**	**99.1**	**95.4**	**94.6**	**96.1**

Table 1. Percentage composition of the essential oils obtained from hydrodistillation of fresh and ripped myrtaceous fruits: (RI) retention indices on DB5 column, (Guabiroba) *Campomanesia xanthocarpa,* (Pitanga) *Eugenia uniflora* selection 03/03, (Cereja-do-rio-grande) *Eugenia involucrata,* (Guabiju) *Myrcianthes pungens* and (Araçá) *Psidium cattleyanum* selection Ya-Cy.

The essential oils were subjected to screening for their possible antioxidant activity by DPPH free radical scavenging. It is well known that antioxidants can seize the free radical chain of oxidation and form stable free radicals, which would not initiate or propagate further oxidation, and DPPH has been used extensively as free radical to evaluate reducing substances. The antiscavenging ability of the fruit's essential oils applied on silica gel TLC plates and the main zones were determined for each oil. Except for guabiju, on all samples yellow spots could be observed immediately after spraying DPPH reagent on the TLC plates, suggesting interesting antioxidant activity for these fruits.

Considering that some isolated terpenes have been previously tested individually in order to determine the antioxidant nature of the oils, such as beta-pinene, terpinen-4-ol and alpha-pinene, but none exhibited antioxidant activity [47], the results presented herein pointed out for the possible synergism that might occur involving the main components with each other or with other minor components, since the essential oils comprehend a complex matrix and support the renowned pantropically as both food and medicine, since these fruits have long been incorporated into traditional health systems as potentially beneficial dietary components.

Taking together, our results showed that several known constituents of the myrtaceous leaf oils and extracts were also found in the essential oils from fruit, suggesting that the fruit may display functional properties similar to those of the leaf counterpart, such as anti cough and antimicrobial [48], antioxidant and radical-scavenging [49-51], antiviral [52], antidiarrhoeic [53], hypoglycemic [54-55], spasmolytic [56], mutagenic and anti-mutagenic potential [57], citotoxic [58], antihypertensive [59] and cardioprotective [60] activities, affording a likeness health benefits to the consumers.

CONCLUSION

In the recent years, more attention has been paid to functional ingredients contained in fruits because epidemiological studies revealed that high fruit intake was associated with reduced mortality and morbidity, especially of cardiovascular disease, some types of cancer and CNS degeneration that are related with oxidative damage in the human body. Brazil has a natural abundance of tropical fruits with distinctive exotic flavors appealing to the foreign consumer; however, the chemical profile of the majority of these fruits has not yet been characterized, in this way, our findings can contribute to the chemical knowledge of the Brazilian tropical fruits and stimulate the thriving and consumption of these, not only by their nutritional and tasty aspects, but also due to the possibility of linking the chemical contents with particular functional properties.

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