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RESEARCH ARTICLE

Chemical characterization of the essential oils from leaves of mandarins Sunki, Cleopatra and their hybrids

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Sunki Mandarin [*Citrus sunki* (Hayata) hort. ex Tanaka] is a type of micromandarin from South China largely used as a rootstock in Brazil. Cleopatra mandarin (*Citrus reshni* hort. ex. Tanaka) is a well-formed tree, ornamental, with red-orange fruits. The main objective of this work was to analyze the chemical composition of the essential oil in leaves of accessions of Sunki and Cleopatra mandarins and hybrids. The essential oils were obtained from the leaves by hydrodistillation in a Clevenger-type apparatus and analyzed by gas chromatography/flame ionization detector (GC/FID) and GC/mass spectrometry (GC/MS). The oil yields ranged from 1.27% (BCG562 – Hybrid of 'Sunki' C12080) to 0.33% (BCG564 – Sunki from Florida) (dry weight basis), and fifty-five constituents were detected. The major constituents were β-pinene (2.5–49.9%), limonene (0–49.0%), sabinene (0.5–35.2%), linalool (0.7–27.3%), thymol methyl ether (0–22.3%), p-cymene (0.2–21.2%), γ-terpinene (0–15.1%), 1.8-cineole (0–11.0%), terpinen-4-ol (0.5–9.4%), (*E*)-β-ocimene (0.6–6.4%), α-pinene (1.1–4.4%), (*E*)-nerolidol (1.0–3.2%), α-terpineol (0.4–3.0%) and myrcene (0.7–2.4%).

Keywords: *Citrus sunki*; *Citrus reshni*; genetic resources

Introduction

The term micromandarin refers to the plants with small leaves, flowers and specially fruits, being apparently a homogenous group (1, 2). The Cleopatra (*Citrus reshni* hort. ex Tanaka) and Sunki [*C. sunki* (Hayata) hort. ex Tanaka] mandarins are well known in Brazil, both being indicated as rootstocks for oranges, tangerines and grapefruits, on light or heavy soils (3).

Sunki mandarin is a type of micromandarin from South China used as a rootstock in Brazil. It induces vigorous plants, and has precocious production when compared with the Cleopatra mandarin. Plants on this rootstock produce high-quality fruits similar to those obtained with Cleopatra mandarin, ripening by May and June. The Sunki mandarin is ornamental, resistant to scab, and tolerant to decline, saline and dry soils. It is also indicated as a female parental in citrus breeding programs via hybridization, due to the high fruit set, low polyembryony and high frequency of hybrids. Since the beginning of the last century, it has been used as a rootstock in the state of São Paulo, the largest Brazilian citrus state producer (4–7) mainly due to its compatibility with the major cultivated sweet orange, 'Pera' [*C. sinensis* (L.) Osbeck]. Currently, there is an increase in demand for Sunki mandarin aiming for diversifying rootstocks in the formation of new orchards (8).

The Cleopatra mandarin is an attractive tree, symmetrical and well formed, without spines, and with small dark green leaves. The fruit is orange-red, small, oblate and highly depressed at the apex, with thin and rough skin. The texture of the flesh is soft and juicy, and the flavor is slightly acidic but of great quality. It is considered native to India, and it is supposed to have been introduced in Florida just before 1888. It is an attractive ornamental species and fruits all year round (9). The Cleopatra mandarin has also been used as a rootstock in the state of São Paulo, Brazil, for almost 30 years. Varieties grafted on this plant develop rapidly, and are large and uniform. The initial production is slow and it takes two years to reach similar levels of Rangpur lime (*C. limonia* Osbeck) and other rootstocks. However, the Cleopatra mandarin is suitable for clay soils and is highly resistant to cold. It shows late ripening of fruits, and the fruits tend to be smaller than those obtained with other rootstocks, with a nice flavor and taste. The root system is deeper (7).

Essential oils of *Citrus* species represent the majority of the natural flavors and fragrances industry, due to the high genetic diversity of this group of plants.

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Among the types of citrus essential oils marketed, those of the sweet orange (*C. sinensis*), mandarin (several species), true lemon [*C. limon* (L.) Burm. f.], sweet limes (*C. limettioides* Tanaka, *C. limetta* Risso) and acidic lime [*C. aurantiifolia* (Christm.) Swingle], willow leaf mandarin (*C. deliciosa* Ten.) and pummelo [*C. maxima* (Burm.) Merr.] can be highlighted.

These *Citrus* essential oils have been the most popular sources of perfume and fragrance essences, due to their pleasant and refreshing aroma, and the capacity to grow in extensive areas of the world. Besides the diversity among species, there is a variety of compounds and uses of citrus essential oil. They have numerous compounds and odor properties, as well as deodorizing, antimicrobial and antihypertensive activities. They are also antioxidants, helping in the prevention of diseases associated with oxidative damage (10). Among the compounds usually present in citrus essential oils, limonene, sabinene and β-pinene should be specifically mentioned. *Citrus* essential oils can be extracted from the peel of the fruits by cold pressing (cold-pressed oils) or by distillation (distilled lime oils), flowers (neroli), leaves and fruitlets (petitgrain) (11).

There is very little information on the chemical composition of essential oils in citrus leaves, since the vast majority of recent studies refers to essential oils from the fruit peel. Furthermore, plant material from many *Citrus* species is of industrial interest in the fields of pharmaceuticals, agricultural, cosmetics and food industries. The aim of this study was to analyze the chemical composition of the leaf essential oils of accessions of Sunki and Cleopatra mandarins and hybrids.

Experimental

Plant material

Accessions of Sunki and Cleopatra mandarins and hybrids (Table 1) were grown and harvested in the Active Germplasm Bank of Citrus (Citrus-AGB) located at Embrapa Mandioca e Fruticultura in the town of Cruz das Almas, Bahia state, at 12° 40’ 19” S and 39° 06’ 22” W.Gr., between September and November 2011. Plants were multiplied by grafting resulting in clones, grown under the same climatic conditions and similar agricultural practices. The average annual temperature is 24.1°C (12).

Extraction of essential oil

Mature and healthy leaves were collected from all quarters of the crown of two plants per accession. The samples were placed in paper bags, taken to a circulating air oven for four days at 38°C and dried to a constant weight. Dried leaves (70 g) of each sample were extracted by hydrodistillation in a 2-L flask for 2 hours. The essential oil was dried with anhydrous sodium sulfate, stored in amber glass sealed containers and kept refrigerated at +5°C.

The yield of essential oils was expressed as a percentage (g EO/100 g of dried plant material), calculated

Table 1. Accessions of mandarine, denomination, origin and essential oil percentage in leaves harvested at the Citros Germplasm Bank from Embrapa Mandioca e Fruticultura, Brazil.

Scientific name[a]	Accessions	Denomination[b]	Origin	% (w/w) Essential oil
Citrus sunki (Hayata) hort. ex Tanaka				
	BGC562	Hybrid of ‘Sunki’ C12080	University of California, Riverside, USA	1.27
	BGC563	‘Sunki’ common	Centro de Citricultura Sylvio Moreira, SP, Brazil	0.37
	BGC564	‘Sunki of Flórida’	Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, Brazil	0.33
	BGC565	‘Sunki Maravilha’	Embrapa Mandioca e Fruticultura, Brazil	0.48
	BGC566	‘Sunki Maravilha Clone 02’	Embrapa Mandioca e Fruticultura, Brazil	0.79
	BGC567	‘Sunki Tropical’	Embrapa Mandioca e Fruticultura, Brazil	0.39
Citrus sunki × *Citrus macrophylla* Wester				
	BGC568	‘Sunki’ × ‘Alemow’	Texas, EUA	0.43
Citrus reshni hort. ex Tanaka				
	BGC 188/189	‘Cleópatra’[c]		0.86
Citrus reshni × *Citrus reticulata Blanco*				
	BGC192	Hybrid ‘Cleópatra’ × ‘Cravo’ (*C. limonia* Osbeck)	United States Department of Agriculture, California, USA	0.35

Notes: [a]Scientific name based on GRIN (20). [b]Denomination as cited by Passos et al. (21). [c]Origin not available.

using the formula: YEO (%) = WEO (g) × 100/WDL (g), where WEO is the weight of oil extracted and WDL the weight of dry leaves used in the extraction. The amount of each compound per kg of dried leaves was estimated according to the formula YC (g/kg leaf) = (YEO × *C* × 0.1), where YC is the yield in grams of each compound per kg of dry leaves and *C* is the relative percentage of each chemical compound (Table 2).

Analysis of the essential oil

The essential oil was diluted in dichloromethane in a proportion of 1%, and then 1.0 μL of the solution was injected (split 1:20) into an Agilent 7890A gas chromatograph equipped with a flame ionization detector (GC/FID) and a HP-5MS (5% phenyl–95% methylpolysiloxane) fused silica capillary column (30 m × 0.25 mm × 0.25 μm). Hydrogen was used as carrier gas at a flow rate of 1.0 mL/minute. The oven temperature was programmed from 60 to 240°C at 3°C/minute. The injector temperature was kept at 250°C and the detector temperature at 280°C. The percentage composition was obtained by area normalization. The procedure was performed in triplicate.

Analyses by GC/MS were performed on an Agilent 5973N mass selective detector coupled to an Agilent 6890 gas chromatograph fitted with a HP-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as carrier gas at 1.0 mL/minute. The mass detector was operated in electronic ionization mode (70 eV), at 3.15 scans/second, with mass range from 40 to 450 u. The transfer line was kept at 260°C, the ion source at 230°C and the analyzer at 150°C. The oven temperature program and injection procedure were the same as above.

Identification of the essential oil components was performed by comparison of their mass spectra with those from the Wiley Registry of Mass Spectral Data (1994) or the NIST databases (2012), as well as their linear retention indices (LRI), after the injection of a homologous series of hydrocarbons (C_7–C_{26}), in the same conditions as above, and compared with literature data (13, 14).

Statistical analysis

From the sum of each constituent of all samples, those with values greater than ten were considered for principal component analysis (PCA) using the method of factorization HJ-symmetric (15) in order to measure the inter-relationship among the accessions and constituents. The R program, public domain, was used to develop the statistical analyses (16).

Results and discussion

The accessions showed that the essential oil yield ranged from 1.27% (BCG562 – hybrid of Sunki C12080) to 0.33% (BCG564 – Sunki from Florida). The high value of BCG 562 essential oil yield compared with the other *C. sunki* accessions can be probably due to their genetic differences, since they have been obtained from clones in a similar condition at the germplasm bank. On the other hand, the accession BCG192, which is a hybrid of *C. reshni* and *Citrus reticulata*, had one of the lowest values, showing no effect in essential oil yield.

Also, the levels of essential oil found in accessions BGC 188/189 (Cleopatra) with 0.86% and BGC 566 (Sunki Maravilha Clone 2) with 0.79% (Table 1) can be highlighted. The essential oil yield reported in the literature for different genotypes of citrus, extracted by steam distillation of fresh leaves, ranged between 0.05% and 0.60% (17, 18). Although they should not be compared, as they have been extracted in different processes, the essential oil yields obtained at Citrus-ABG showed a reasonable content.

Among the accessions, BGC192 (a hybrid of Cleopatra mandarin with Rangpur lime) showed the highest percentage of total identification of the essential oil (98.7%), distributed in thirty-two constituents, followed by accession BGC567 (Sunki tropical mandarin) with 87.3% of essential oil components identified divided into thirty-eight constituents. Sabinene was the major compound in both accessions, with 30.5% and 31.5%, respectively.

Table 2 presents the percentages of the chemical constituents of the essential oil of each accession studied. A total of fifty-five components were identified, ranging from 87.3% to 98.7% of the constituents detected. The main constituents were: β-pinene (0–49.9%), limonene (0–49.0%), sabinene (0.5–35.2%), linalool (0.7–27.3%), thymol methyl ether (0–22.3%) p-cymene (0.2–21.2%), γ-terpinene (0–15.1%), 1,8-cineole (0–10.9%), terpinen-4-ol (0.5–9.4%), (*E*)-β-ocimene (0.6–6.4%), α-pinene (1.1–4.4%), (*E*)-nerolidol (1.0–3.2%), α-terpineol (0.4–3.0%) and myrcene (0.7–2.4%) (Figure 1).

The major compounds of each accession evidence a large variation of the Citrus-ACG mandarin collection. Among the *C. sunki* accessions, a high level of β-pinene was observed in BCG 563 and 564 (45.5% and 49.9%), and also in BCG 565 and 566 – these last two with the presence high values of p-cymene and γ-terpinene. BCG 562, which is a hybrid, showed a different profile, with a predominance of thymol methyl ether (22.3%), p-cymene and γ-terpinene. BCG 567 also showed a distinct profile, with high values of sabinene (31.5%), similar to the *C. reshni* accessions.

Table 2. Relative percentage of essential oil constituents of leaves from accessions of *Citrus sunki*, *Citrus reshni* and hybrids from Embrapa Citros Germplasm Bank, Brazil.

			Accessions[b]								
Peak	Constituents	LRI[a]	BGC 562	BGC 563	BGC 564	BGC 565	BGC 566	BGC 567	BGC 568	BGC 192	BGC 188/189
1	α-Thujene	926	1.9	0.4	0.3	1.3	1.3	0.9	0.1	0.4	0.6
2	α-Pinene	933	4.3	3.2	4.1	4.3	4.4	2.4	1.1	1.8	1.5
3	Camphene	948		0.2	0.3	0.1	0.1	0.1	0.1		
4	Sabinene	974	0.5	8.9	9.5	5.6	5.1	31.5	2.5	30.5	35.2
5	β-Pinene	977	4.6	45.5	49.9	25.3	24.1	9.4	16.0	8.5	2.5
6	6-Methyl-5-hepten-2-one	988							0.9		
7	Myrcene	991	1.2	0.7	0.8	1.0	1.1	2.4	1.2	2.2	2.4
8	α-Phellandrene	1006	0.1	0.2				0.3		0.1	0.1
9	δ-3-Carene	1011		0.2				0.2			
10	α-Terpinene	1017	0.3	0.1		0.2	0.2	0.7		0.6	1.4
11	*p*-Cymene	1025	15.0	0.8	0.9	21.2	20.8	2.7	0.2	0.7	0.7
12	Limonene	1028	3.6	1.9	2.2	3.1	3.3	1.6	49.0	24.9	
13	1,8-Cineole	1031	0.2	10.9	4.7	7.8	6.3	0.2		0.4	
14	(*Z*)-β-Ocimene	1037	0.3	0.3	0.1	0.2	0.2	0.3	0.1	0.2	0.2
15	(*E*)-β-Ocimene	1047	6.4	1.7	1.3	3.4	4.6	3.3	0.6	4.9	4.3
16	γ-Terpinene	1058	14.0	0.3	0.1	12.7	15.1	1.4		1.3	2.7
17	*cis*-Sabinene hydrate	1070	0.1	0.5	0.6	0.6	0.5	2.2	0.1	1.0	1.4
18	*cis*-Linalool oxide	1074	0.2			0.1	0.1	0.2	0.2		0.1
19	Terpinolene	1089	2.8	0.2	0.1	0.8	0.9	0.5	0.1	0.4	0.8
20	*trans*-Linalool oxide	1091							0.1		
21	Linalool	1106	12.3	1.0	1.0	0.8	0.7	5.7	1.2	1.8	27.3
22	*cis*-*p*-2-Menthen-1-ol	1125	0.1	0.3	0.1	0.2	0.2	0.8	0.2	0.3	0.6
23	*cis*-Limonene oxide	1134	0.2	0.3	0.4	0.2	0.2	0.5	0.3	0.2	0.3
24	*trans*-Limonene oxide	1139	0.3			0.2	0.3				
25	Nopinone	1140		0.2	1.2				0.2		0.5
26	*trans*-Pinocarveol	1144		0.4	1.2		0.1	0.4		0.5	
27	Citronellal	1155							1.5	0.3	
28	Sabinone	1165					0.2		0.2		
29	Terpinen-4-ol	1181	0.5	1.9	1.4	1.4	1.2	9.2	0.6	5.1	9.4
30	α-Terpineol	1197	0.4	3.0	1.3	1.5	1.1	1.3	0.6	0.9	1.0
31	Myrtenol	1199	0.1	0.4	0.4	0.2	0.2	0.4	0.2		
32	Thymol methyl ether	1237	22.3		0.1						
33	Nerol	1244			0.2				2.3	0.2	0.1
34	Neral	1247						0.3	3.4	2.0	
35	Geraniol	1256	0.1				0.1	0.2	1.0	1.3	
36	Geranial	1274	4.3						3.0	0.7	
37	α-Cubebene	1337			0.2		0.1				0.2
38	Citronellyl acetate	1356							0.3		
39	Neryl acetate	1367							1.5	0.3	
40	Geranyl acetate	1387		0.1			0.2	0.2	1.0	2.1	
41	β-Elemene	1392		0.4	0.7	0.1	0.2	0.4	0.2		0.1
42	(*E*)-β-Caryophyllene	1418	0.1	0.6	0.8	0.5	0.4	1.0	0.6	2.9	0.4
43	α-Humulene	1453		0.2	0.4	0.2	0.1	0.3	0.2	0.3	0.1
44	Germacrene D	1481									0.4
45	Elemol	1549		1.7	2.5	0.3	0.1	1.2	0.3		0.6
46	Germacrene B	1560	0.2								
47	(*E*)-Nerolidol	1569	1.2	1.3	3.2	1.8	1.8	1.8	2.2	1.0	0.1
48	Spathulenol	1583	0.4	0.5	1.0	0.7	0.7	1.3	1.2	1.0	0.1
49	Caryophyllene oxide	1595		0.1							
50	Humulene epoxide II	1608		0.1	0.2	0.1	0.1	0.3	0.1		
51	iso-Spathulenol	1630		0.6	1.2	0.9	0.4	0.6	0.2		
52	α-Muurolol	1637		0.9	1.2			0.7			
53	α-Cadinol	1648		0.4	0.8	0.3	0.3	0.2	0.3		0.1
54	β-Sinensal	1708		0.1	0.2	0.2	0.1	0.3	0.3		
55	α-Sinensal	1758		0.1	0.2	0.2	0.2	0.2			
	Monoterpenes		54.8	54.8	64.5	69.5	79.2	81.2	57.5	71.1	76.5

(*Continued*)

Table 2. (*Continued*).

Peak	Constituents	LRI[a]	Accessions[b] BGC 562	BGC 563	BGC 564	BGC 565	BGC 566	BGC 567	BGC 568	BGC 192	BGC 188/189
	Oxygenated monoterpenes		41.1	41.1	19.1	12.6	12.9	11.3	21.6	18.8	16.9
	Sesquiterpenes		0.3	0.3	1.2	2.1	0.8	0.8	1.7	0.9	3.2
	Oxygenated sesquiterpenes		1.5	1.5	5.8	10.3	4.5	3.8	6.5	4.7	2.0
	Total		97.7	90.6	94.4	97.3	96.9	87.3	95.4	98.7	95.1

Notes: [a]Linear retention indices on DB-5. [b]Accessions: BGC 562 (Sunki hybrid C12080), BGC 563 (common Sunki), BGC 564 (Florida Sunki), BGC 565 (Sunki Maravilha), BGC 566 (Sunki Maravilha Clone 02), BGC 567 (Sunki Tropical), BGC 568 (Sunki × Alemow), BGC 188/189 (Cleopatra) and BGC 192 (Cleopatra × Cravo Hybrid).

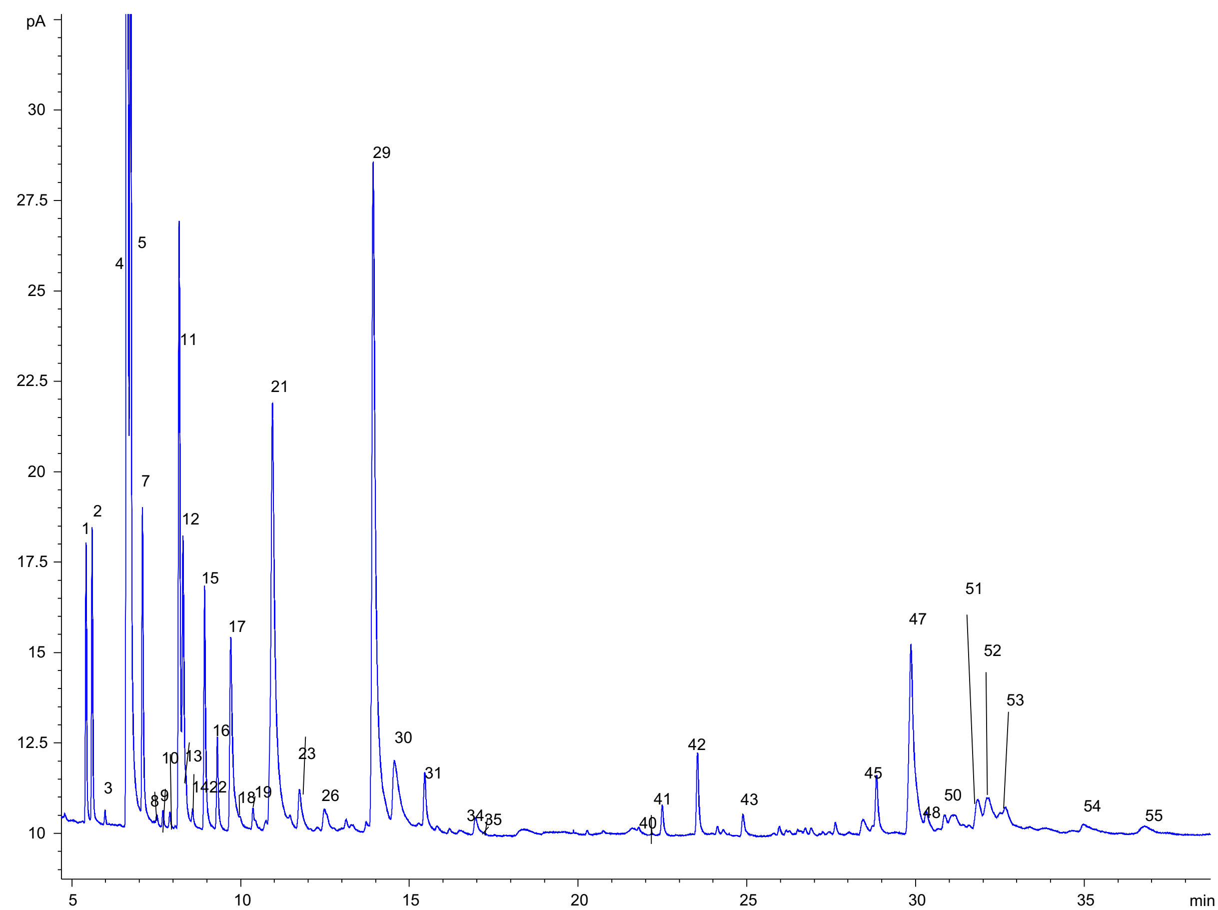

Figure 1. Chromatogram of *Citrus sunki* accession BGC 567 and its constituents.

In the Cleopatra accession BCG 568 and its hybrid with *C. reticulata* (BCG192), a high content of limonene was observed (49% and 24.9%), followed by β-pinene and sabinene, respectively. The accession BCG 188/189 showed the highest value for linalool (27.3%), but also presented some levels of sabinene. It a high percentage of monoterpenes was observed in all accessions, with the predominance of oxygenated monoterpenes in the accessions of *C. sunki* BCG 562 and 563 (Table 2).

There are only few studies on the literature reporting the essential oil composition of *C. sunki* and *C. reshni*. In 2000 and 2001, Lota and collaborators (17, 18) have reported the composition of leaf essential oils of mandarins, including Cleopatra (*C. reshni*) and Sunki varieties.

The essential oil of mandarins leaves have been reported in the literature as containing fifty-eight constituents, with sabinene (0.1–57.3%), γ-terpinene (0.1–67.4%), linalool (traces–59.3%) and methyl *N*-methyl-anthranylate (traces–78.7%) as major constituents (18). The highest values obtained for sabinene (35.2%), γ-terpinene (15.1%) and linalool (27.3%) were lower than those presented in the literature, and no methyl *N*-methyl-anthranylate was detected in the Citrus-ACG. Furthermore, the quantitative values obtained can be affected by the environmental conditions observed in the places where the plants were collected. However, the qualitative profiles seem quite consistent, considering that the plants analyzed were harvested at similar environmental conditions.

In the essential oil from the leaves of a variety of Cleopatra, 97.2% of the compounds were identified, and 89.6% of these were also observed in two accessions of Citros-AGB (BGC192 and BGC188/189). The compounds that were present in both studies were α-thujene, α-pinene, sabinene, myrcene, (*Z*)-β-ocimene, (*E*)-β-ocimene, γ-terpinene, terpinolene, linalool, terpinen-4-ol, (*E*)-β-caryophyllene and α-humulene (18).

Similarly, in the leaf essential oil of a variety of Sunki mandarin reported in the literature (18), 90.7% had a similar composition to the seven samples of Sunki accessions studied (BGC 562, 563, 564, 565, 566, 567 and 568), and the following compounds were presented in all samples when compared with the literature: α-thujene, α-pinene, sabinene, myrcene, p-cymene, limonene, (*Z*)-β-ocimene, (*E*)-β-ocimene, linalool and terpinen-4-ol (18).

The essential oil from leaves of 113 samples of various citrus genotypes reported in the literature presented almost the same number of compounds, among which the most important were: methyl *N*-methyl-anthranylate (<0.1–85.2%), linalool (<0.1–76.4%), γ-terpinene (<0.1–63.4%), sabinene (<0.1–57.2%), limonene (1.7–55.8%), (*E*)-β-ocimene (0.1–20.6%), ethyl *N*-methyl-anthranylate (0–16.2%), thymol (<0.1–13.0%), δ-3-carene (<0.1–11.1%) and citronellal (<0.1–11.7%) (19).

Regarding the performance of the main chemical constituents of the essential oil (in grams of each constituent per kilogram of dry leaves), the following accessions can be highlighted: Cleopatra (BGC188/189), with the presence of 3.03 g/kg of sabinene, 0.21 g/kg of myrcene and 2.35 g/kg of linalool; the hybrid Sunki C12080 (BGC562) with a high content of γ-terpinene (2.8 g/kg), nerol (2.8 g/kg) and p-cymene (1.92 g/kg). For the compound β-pinene, accession BGC566 (Sunki Maravilha clone 2) had the highest yield of 1.91 g/kg followed by accession BGC 568 (*C. sunki* × *Citrus macrophylla* Wester) with 2.11 g/kg.

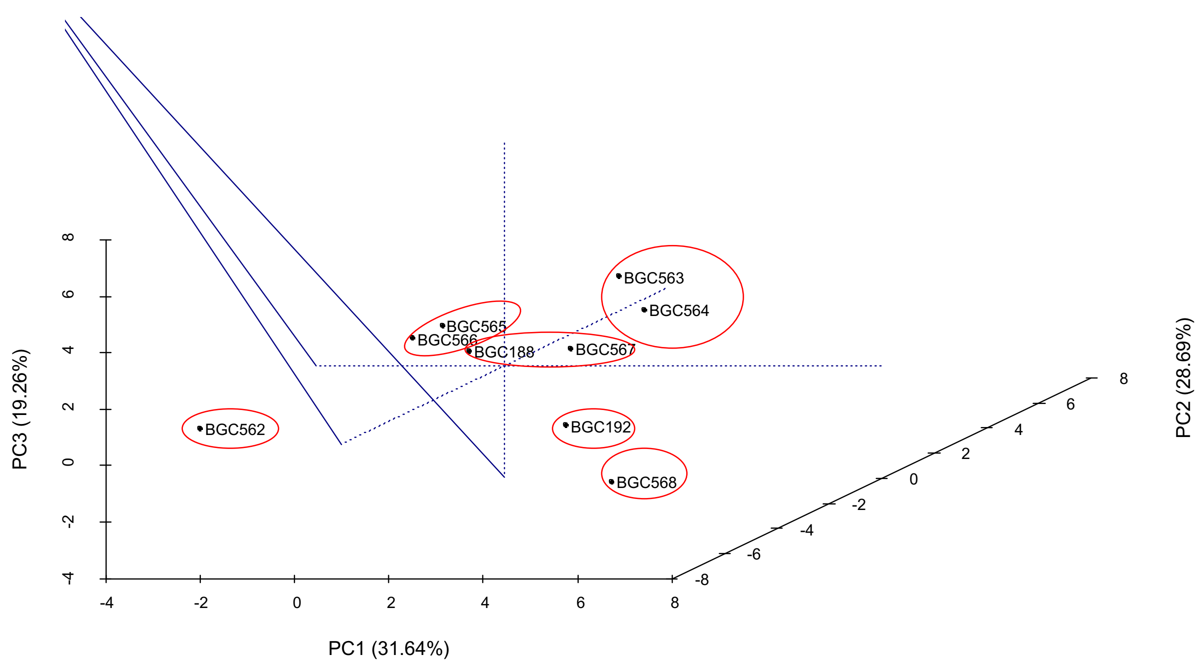

Figure 2. Principal component analysis of essential oil chemical constituents from leaves of accessions of *Citrus sunki* (Hayata) hort. ex Tanaka, *Citrus reshni* hort. ex Tanaka and hybrids from the Citros Germplasm Bank, Embrapa Mandioca e Fruticultura, Brazil. *Accessions: BGC 562 (hybrid of Sunki C12080), BGC 563 (Sunki common), BGC 564 (Sunki from Florida), BGC 565 (Sunki Maravilha), BGC 566 (Sunki Maravilha Clone 02), BGC 567 (Sunki Tropical), BGC 568 (Sunki × Alemow), BGC 188/189 (Cleopatra) and BGC 192 (hybrid Cleopatra × Cravo).

PCA was based on twenty-two major constituents, with sum values above 5% showing the highest expression to discriminate accessions. PCA allowed accession separation into three components (Figure 2). The first component (PC1) accounted for 31.64% of the existing variability, showing as major discriminant constituents terpinolene, α-thujene, thymol methyl ether, α-pinene, γ-terpinene and p-cymene, which separated accession BGC562 (hybrid 'Sunki' C12080) from the rest of the group. The second component (PC2) allowed an explanation of the 28.69% variation between accessions and had as major constituents myrcene, (*E*)-nerolidol, 1,8-cineole, linalool and β-pinene. PC2 discriminated accession BGC188/189 (Cleopatra) from BGC563 (common Sunki mandarin) and BGC564 (Sunki from Florida mandarin). Finally, the third component (PC3) allowed an explanation of the 19.26% existing variation and had as major constituents neral, geranial and limonene, which allowed separation of BGC568 [Sunki × Alemow (*C. macrophylla*)] from the rest of the group.

In Figure 2, a separation of six types of chemical profiles based on PCA analysis can be observed. The first type is characterized by accession BGC562 (Sunki hybrid C12080), whose main chemical components are thymol methyl ether, terpinolene and geranial. The second type is characterized by accession BGC563 (common Sunki mandarin) and BGC564 (Sunki from Florida mandarin), with the main differential element β-pinene (45.5% and 49.9%, respectively), but with influences from elemol and α-terpineol. The values obtained for β-pinene are quite high compared with the other accessions.

The third group is characterized by accession BGC565 (tangerine 'Sunki Maravilha') and BGC566 (Sunki Maravilha mandarin clone 2), and the main element was p-cymene, with influences of 1,8 cineole and β-pinene. γ-terpinene also influences the formation of this type, as it is present in concentrations of 12.67% and 15.14% in its constituents, two of the largest concentrations found in the samples analyzed. The fourth type is formed by BGC567 (Sunki Tropical mandarin) and BGC188/189 (Cleopatra), which differ from the other accessions by the chemical constituents *cis*-sabinene hydrate, myrcene, terpinen-4-ol and sabinene. They also present a consistent difference in linalool value.

Accession BGC192 (hybrid of Cleopatra mandarin with Rangpur lime) had influences of chemical compounds such as neral, (*E*)-β-caryophyllene and limonene, being considered the fifth type.

The sixth type consists of accession BGC568 (Sunki × Alemow) and has limonene as the major compound (49%), and influences of spathulenol and (*E*)-nerolidol. The accessions BGC567, 188/189 and 192 have values close to sabinene but differ in the quantities of the other secondary constituents. In BGC568, the highest value was limonene, showing a large variation among the essential oils of citrus leaf analyzed.

In conclusion, the leaf essential oil of different accessions of *C. sunki*, *C. reshni* and hybrids showed a large variability of constituents, mostly recognized also in other citrus leaves in literature. Limonene, sabinene and β-pinene were the major constituents. The observed amount of essential oil was also variable, but some accessions have showed a yield superior to 1% w/w. PCA was able to separate different accession groups based on essential oil composition. A further study on olfactory evaluation and its potential associated with *Citrus* disease resistance could be interesting to better evaluate the potential of these germplasms for industry, as this specific group of *Citrus* has a good agronomical feasibility.

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