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Chemical composition and variability of essential oils from the fruit peels of *Citrus medica* L. and mineral analysis of the fruit peels and soils

Tatiana de Sousa Fiuza*, Leonice Manrique Faustino Tresvenzol, Larissa Teodoro Alves Lopes, Stone de Sá, Pedro Henrique Ferri, Bruno Leite Sampaio, José Realino de Paula

Institute of Biological Sciences. Federal University of Goiás, Federal University of Goiás, CP 131, 74001-970 , Goiânia, Goiás, Brazil

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***For Correspondence**

Tatiana de Sousa Fiuza, Institute of Biological Sciences. Federal University of Goiás, Federal University of Goiás, CP 131, 74001-970, Goiânia, Goiás, Brazil.

E-mail: tatianaanatomia@gmail.com

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ABSTRACT

This study determined the chemical compositions of macro- and micronutrients in the fruit peels of *Citrus medica* collected in Pirenópolis, Jandaia and Santo Antonio do Descoberto, Goiás, Brazil, as well as the composition and variability of the essential oils and the chemical composition of the soil. High levels of iron, calcium and magnesium were found in the peels from the three sites. Twenty-four components were identified in the essential oil, and limonene was the most abundant (> 85%). No chemical variability was detected in the three oil samples analyzed. The soil analysis revealed high levels of manganese (55-146 mg/dm^3) and zinc (9.6-19.8 mg/dm^3) as well as moderate levels of potassium (87-179 mg/dm^3), calcium (3.4-6.2 cmolc/dm^3) and magnesium (1.1-1.5 cmolc/dm^3) at all three collection sites. No significant statistical correlations were verified between the chemical, organic and environmental variables.

INTRODUCTION

Citrus medica L., commonly known as citron, is a small tree (2-4 m tall) with sharp thorns (3 cm), shiny and coriaceous leaves, short petioles and a lemony odor. The fruit are fragrant with a thick albedo and pulp that is either acid or sweet. This species is originally from northeastern India and is cultivated today in domestic orchards in Brazil [1,2].

Citron peel is eaten with rice in Bangladesh, India and Indonesia. In Spain, syrup made from the peel is used to flavor unpalatable medical preparations, and in Guatemala, it is used as flavoring for soft drinks [3]. In Brazil, the peel is used to prepare jellies and other sweets (preserves and crystallized fruit). This species is also used for the treatment of various diseases in traditional Indian medicine. The ripe fruit is used to treat sore throat, cough, asthma, earache, scurvy and hemorrhoids. The water distilled from the fruit is soothing [4,5]. The seeds are used as a vermifuge, a stimulant and a cardiac tonic. In China and Japan, the fruit is used as an air freshener and is considered a symbol of happiness and prosperity.

Entezari et al. [6] detected antimutagenic and antitumorigenic activities in the ripe fruit juice of *C. medica In vitro*. Sood et al. [7] reported antioxidative, analgesic and anti-inflammatory activities of ethyl acetate and methanol extracts from the peel; in addition, Negi et al. [8] observed analgesic and anti-inflammatory activities in the ethyl acetate fraction from the peel as well as analgesic activity in the tea made from the fruit. Hypoglycemic and hypolipidemic effects were observed in the ether extract from the seeds, and the fruit juice and the ethanol extract from the roots have antimicrobial properties [9].

Essien et al. [10] verified fungi toxic activity against 14 species of fungus in the essential oil from the *C. medica* leaves, and the ether extract from the leaves presented anthelminthic activity against *Pheretima posthumad* [2,11] reported the absence of toxicity in the ethanol and benzene extracts from the seeds in mice at doses of 200 mg/kg and 400 mg/kg.

Phytochemical studies revealed the presence of flavonoids and phenolic acid in the ethyl acetate and methanol extracts from the peel of *C. medica* [7].

No data were found in the literature on the chemical compositions of either the peel fruit or the essential oil from samples collected in the state of Goiás, Brazil.

The purposes of this study were as follows: to determine the composition and variability of the chemical components of the essential oils in the fruit peel; to perform a mineral analysis of the peel of *C. medica* and the soil collected in three cities in Goiás, Brazil; and to ascertain whether there is a correlation between the mineral nutrients found in the soil and in the fruit peel and the main chemical components of the essential oil.

MATERIAL AND METHODS

The *C. medica* fruit were collected in Pirenópolis (829 m altitude, 15º 00' 14.5" South, 49º 54'16.4" West), Jandaia (560 m altitude, 17º 03'20.0" South, 50º16'40.3" West) and Santo Antônio do Descoberto (912 m altitude, 15º 56'24" South, 48º15'18" West) in the state of Goiás. The plants were identified by Dr. José Realino de Paula at the Federal University of Goiás, and the voucher specimens were deposited in the herbarium of that institution under registration numbers UFG/41405 (Santo Antônio do Descoberto), UFG/41493 (Pirenópolis) and UFG/41496 (Jandaia).

For this study, the fruit were peeled and the pericarp (flavedo) was utilized both fresh (for extraction of essential oil - volatile compounds) and dry (for chemical analysis- non-volatile compounds).

Essential oils analysis

Fresh plant material was triturated and submitted to hydrodistillation in a Clevenger-type apparatus for two hours. At the end of each distillation the oils were collected, dried with anhydrous Na_2SO_4, measured, and transferred to glass flasks and kept at a temperature of -18°C for further analysis.

Chromatographic analyses of *C. medica* fruit peel essential oil were performed on a Varian gas chromatograph (FID) equipped with a DB-5 (J&W) fused silica capillary column (30 m x 0.25 mm; 0.25 μm film thickness), and the temperature program was as follows: 60-240°C at 3°C/min, then to 280°C at 10°C/min, ending with 10 min at 280°C. The carrier gas was N_2 with a flow of 1.0 mL/min; the temperatures of the injector port and detector were 220°C and 240°C, respectively. Samples were injected by splitting, and the split ratio was 1:20. GC/MS analysis was performed on a Shimadzu QP5050A using a CBP-5 (Shimadzu) fused silica capillary column (30 m x 0.25 mm; 0.25 μm film thickness, composed of 5% phenylmethylpolysiloxane), and the temperature was programmed as outlined above. The carrier gas was He with a flow rate of 1.0 mL/min, and the split ratio was 1:20. The injection port was set at 220°C. Significant quadrupole MS operating parameters were as follows: the interface temperature was 240°C and the electron impact ionization was 70 eV with a scan mass range of 40–400 m/z at a sampling rate of 1.0 scan/s. Compounds were identified by computer search using digital mass spectral data libraries (NIST, 1998) and by comparison of their retention indices and authentic mass spectra [1] relative to the C_8–C_{32} n-alkane series [12] in a temperature-programmed run.

Fruit peel and soil mineral analyses

For the mineral analysis, 15 g of fruit peel powder were collected from each sample. For the soil analysis, four samples were collected around each *C. medica* specimen at a 0-20 cm depth. Later, the four samples were mixed and left to dry.

The mineral composition of the fruit peel was analyzed according to the following procedure [13]: The samples first underwent nitric-perchloric digestion to determine levels of potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn). The samples were then digested with sulfuric acid and catalysts to determine the nitrogen level (N). The elements were later quantified by atomic absorption (Ca, Mg, Cu, Fe, Mn, and Zn), turbidimetry (S), calorimetry (P), flame photometry (K) or distillation (N).

The soil samples were analyzed according to the procedure described by Silva [13]. For pH determination, a water-soil volume at a 1:1 ratio and a potentiometer with combined electrodes were utilized. The Ca, Mg and Al ions were extracted with KCl (1 mol/L), and Mehlich solution was used to extract phosphorus (P), potassium (K), zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn). The elements were then quantified by titration (Ca, Mg and Al), spectrophotometry (P), flame photometry (K) or atomic absorption spectrophotometry (Zn, Cu, Fe and Mn).

Volumetric analysis using potassium dichromate was performed to determine the organic matter (OM). The usual methods were applied to determine the cation exchange capacity (CEC), potential acidity (H+Al), base saturation (V), Al (m) saturation and soil texture.

Statistical analysis

ANOVA was applied where values of $p \leq 0.05$ were considered to be statistically significant for verification of the chemical variability of the compounds in the essential oils identified in the samples.

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The data were obtained from the chemical analyses of both the fruit peels and soil pre-treated prior to statistical analysis. The chemical organic variables (metabolites), organic matter (OM), aluminum saturation (m) and base saturation (V) (expressed in %) were transformed by arcsine (x/100)1/2, and the remaining environmental variables (soil/fruit peel analysis), except pH, were transformed by log (x+1). The statistical programs applied were Conoco for Windows Version 4.5 with Cano Draw for Windows 4.1, SYSTAT 10 and STATISTICA 7. The Distendency Correspondence Analysis (DCA) was applied to measure the environmental gradient. The Canonical Redundancy Analysis (CRA) was applied to evaluate the variable inflation and the metabolite environmental correlation, and then it was used to select the relevant environmental variables. The significance for the Canonical Redundancy analysis was determined by the Monte Carlo test (with 999 permutations under a reduced model). The values were considered to be significant when $p < 0.05$.

RESULTS

Twenty-four chemical compounds were identified in the essential oil of the *C. medica* peels, and monoterpenic hydrocarbonates were predominant (>90%). Ten of these compounds were present in all the samples (α-pinene, limonene, β-myrcene, Z-β-ocimene, E-β-ocimene, citronellol, nerol, geraniol, trans-cadine-1(6), 4-diene and β-bisabolene), and limonene was the most abundant (> 85%). The sample collected in Jandaia presented only ten components, and limonene was the most abundant (90.14%) (Table 1). There were no significant differences across the chemical compounds in the essential oil samples from the *C. medica* peels collected in Pirenópolis, Jandaia and Santo Antônio do Descoberto/GO (Tables 2 and 3). The essential oil yield was approximately 0.1 to 0.2%.

Table 1. Percentage of chemical components in the essential oils from the fruit peels of *C. medica* collected in three different regions in the countryside of Goiás.

Constituents	RI	***Citrus medica* essential oils**		
		Pirenópolis	**Jandaia**	**Santo Antonio Descoberto**
		Fruit peel	**Fruit peel**	**Fruit peel**
Tricyclene	926	0.32	-	-
α-Pinene	939	-	0.29	0.41
Sabinene	975	0.14	-	-
β-Pinene	979	0.36	0.27	0.18
Myrcene	990	1.97	1.94	2.41
Limonene	1029	85.35	90.14	87.49
Z-β-Ocimene	1037	0.91	0.58	0.84
E-β-Ocimene	1050	1.29	0.81	1.16
Linalool	1096	0.24	-	0.35
n-Nonanal	1100	0.15	-	-
Citronellal	1153	0.22	-	-
α-Terpineol	1188	-	-	0.19
Citronellol	1225	0.51	1.27	0.22
Nerol	1229	1.70	0.91	1.43
Geraniol	1252	0.32	1.52	2.08
Geranial	1267	2.40	1.36	
Undecanone	1294	0.20	-	-
Neryl acetate	1361	1.10	-	0.22
Geranyl acetate	1381	0.29	-	0.20
E-Caryophyllene	1419	0.91	-	0.52
α-trans-Bergamotene	1434	0.34	-	0.47
Trans-Cadina-1(6),4-diene	1476	0.78	0.40	1.01
β-Bisabolene	1505	0.51	0.50	0.81
Monoterpene hydrocarbons		90.02	94.03	92.49
Oxygenated monoterpenes		6.78	5.06	4.69
Sesquiterpene hydrocarbons		2.54	0.90	2.81
Oxygenated sesquiterpenes		-	-	-
Others		0.35	-	-
Total identified (%)		99.69	99.99	99.99
RI: Retention Indices; (-): Not Detected.				

Table 2. Summary of the analysis of variance of essential oils from the fruit peels of *C. medica.*

Comparison between the components of the citron fruit peel oils			
Sources of variation	**GL**	**SQ**	**QM**
Treatments	2	12.0 e-06	60.0 e-07
Error	66	21.8 e+03	330.421
F=0.0000			
(p)=0.9999			

Table 3. Summary of the analysis of variance of the major components of essential oils from the fruit peels of *C. medica.*

Comparison of the major components of the fruit peels of *C. medica*			
Sources of variation	**GL**	**SQ**	**QM**
Treatments	2	1.994	0.997
Error	12	17.9 e+03	14.9 e+02
F=0.0007			
(p)=0.9992			

The mineral analysis of the fruit peels showed that there was practically no variation in the concentrations of the macronutrients magnesium, potassium or sulfur. The nitrogenous macronutrients varied from 1.46 to 1.96 dag/kg, and calcium varied from 0.4 to 0.8 dag/kg (Table 4). The macronutrients in the samples collected in Pirenópolis and Jandaia presented very similar levels of copper, iron and zinc, while the sample from Santo Antonio do Descoberto presented lower concentrations of copper, iron and manganese (Table 4).

The chemical analysis of the soil samples showed a large variation in the concentrations of the macronutrients containing phosphorus (1.7 to 107.9 mg/dm^3), potassium (87 to 179 mg/dm^3) and calcium (3.4 to 6.2 cmolc/dm^3) and in the micronutrients containing copper (1.8 to 3.3 mg/dm^3), manganese (55 to 146 mg/dm^3) and zinc (9.6 to 19.8 mg/dm^3) (Table 5).

With regard to the fertility indices, the potential acidity (H + Al) varied from 1.2 to 2.5, and in the organic matter (O.M.), it varied from 2.4 to 3.3 (Table 6).

Table 4. Concentration of nutrients (N, P, K, Ca, Mg, S, Cu, Fe, Mn and Zn) in the fruit peels of *C. medica* collected in Pirenópolis, Jandaia and Santo Antônio do Descoberto.

Samples	**N**	**P**	**K**	**Ca**	**Mg**	**S**	**Cu**	**Fe**	**Mn**	**Zn**
	dag/kg	**dag/kg**	**dag/kg**	**dag/kg**	**dag/kg**	**dag/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**
1	1.46	0.291	1.08	0.80	0.10	0.03	28.0	336.0	38.0	7.7
2	1.96	0.279	1.04	0.4	0.10	0.03	25.0	354.0	60.0	7.8
3	1.88	0.341	1.14	0.70	0.10	0.04	1.0	57.0	7.0	11.4
1: Pirenópolis; 2: Jandaia; 3: Santo Antônio do Descoberto. 0.1 dag=1 g										

Table 5. Concentrations of macronutrients (P and K in mg/dm^3, Ca and Mg in cmolc/dm^3), pH ($CaCl_2$) and micronutrients (Cu, Fe, Mn, Zn in mg/dm^3) in the soil collected in the three sites.

Samples	**P (Mehl)**	**K**	**Ca**	**Mg**	**pH**	**Cu (Mehl)**	**Fe (Mehl)**	**Mn (Mehl)**	**Zn (Mehl)**
1	14.6	127.0	6.2	1.4	6.1	1.8	10.5	146.4	9.6
2	1.7	87.0	3.4	1.1	5.4	3.3	34.7	119.2	19.8
3	107.9	179.0	5.8	1.5	5.7	3.1	18.3	55.0	9.9
1: Pirenópolis; 2: Município Jandaia; 3: Santo Antônio do Descoberto									

Table 6. Concentrations of fertility indices (H+Al, CEC in cmolc/dm^3, M.O., V, Ca/CEC, Mg/CEC, K/CEC in percentage. H+Al: Potential Acidity; CEC: Cation Exchange Capacity in the Soils, O.M.: Organic Matter; V: Base Saturation Potential of the CEC at pH 7.0; Ca/CEC, Mg/CEC and K/CEC: Percentage of Exchangeable Cations.

Samples	**H+Al**	**CTC**	**M.O.**	**V**	**Ca/Mg**	**Mg/K**	**Ca/K**	**Ca/ CTC**	**Mg /CTC**	**K /CTC**
1	2.5	10.4	3.3	76.0	4.4	4.3	19.1	59.5	13.4	3.1
2	2.8	7.5	2.4	62.8	3.1	4.9	15.3	45.2	14.6	3.0
3	1.2	9.0	2.4	86.6	3.9	3.3	12.7	64.7	16.7	5.1
1: Pirenópolis; 2: Município Jandaia; 3: Santo Antônio do Descoberto										

The chemical analysis of the fruit peels and soil showed that, according to the CRA, the compounds are satisfactory and the data analyzed are not tendentious. The CRA showed a 57.7% relationship between the chemical organic and environmental variables, but a statistically significant correlation was not found between the variables (p>0.05). A new CRA was then performed

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using the main chemical compounds from the essential oil samples (limonene, myrcene and nerol) as chemical variables, and again no statistically significant correlation was found between the chemical organic and environmental variables (Figure 1).

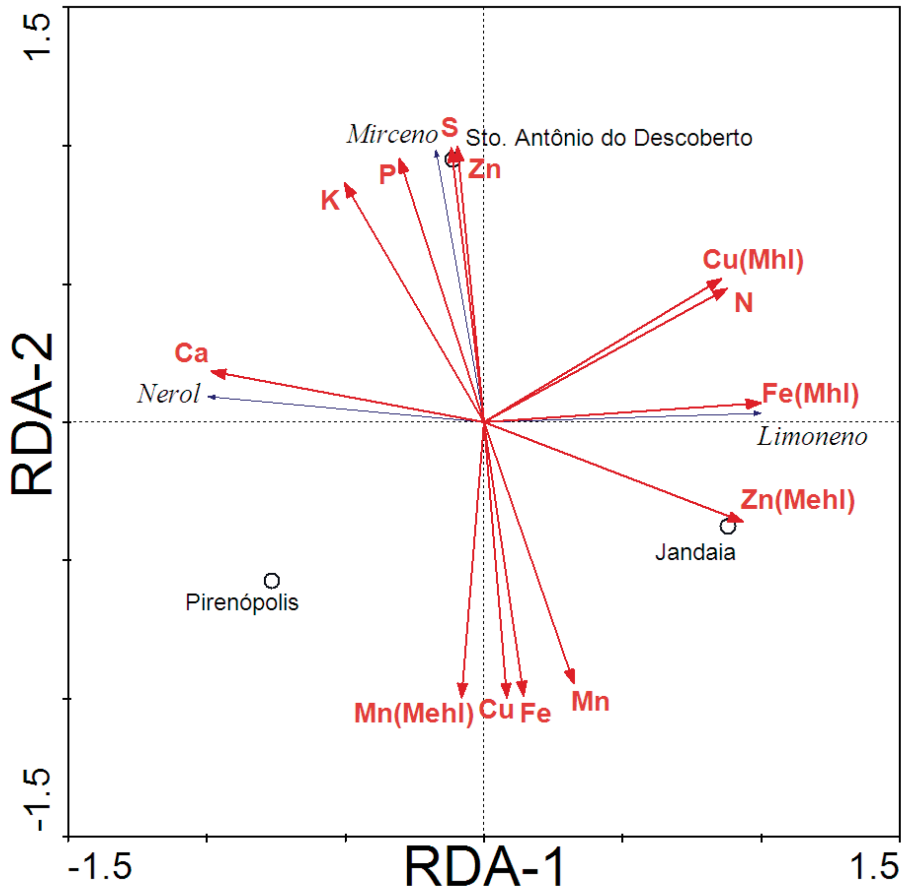

Figure 1. *C. medica* Distribution tendency of mineral nutrients in the soil and fruit peels, content of major chemical compounds myrcene, limonene and nerol in relation to the first two axes of the CRA (CRA-1 and CRA-2). The dots represent the sample scores, while the discriminant variables are represented by the vectors.

DISCUSSION

Limonene was the most abundant chemical compound in the essential oil from the fruit peel of *C. medica* collected at the three sites, varying from 85.35% to 90.14%. It was also the most common compound in *C. medica* var. cedrate fruit peels collected in Iran (56.6%) [14], while the essential oil from *C. medica* peels collected in Bangladesh presented isolimonene (39.37%) as the most common, followed by citral (23.12%) and limonene (21.78%) [3]. Limonene was also the most abundant compound in the fruit peels from other Rutaceae species, such as *Citrus maxima* (J. Burman) Merrill (93.2%) collected in Northeastern India [15], *Citrus sinensis* var. Valencia (90-93%) collected in northern of Santander, Colombia [16] and *Citrus reticulata* Blanco (92.4%) collected in New Delhi, India [17].

The chemical analysis performed on the *Citrus medica* fruit peels revealed high levels of iron in the samples collected in Pirenópolis (336 mg/kg) and in Jandaia (354 mg/kg) and slightly lower levels in those collected in Santo Antonio do Descoberto (57 mg/kg). The samples collected in Santo Antonio do Descoberto presented a higher iron content than was detected by Gondim et al. (2005) in the peels of pineapples (7.1 mg/kg), bananas (12.6 mg/kg), papayas (11.0 mg/kg), passion fruit (8.9 mg/kg), melons (4.0 mg/kg) and tangerines (47.7 mg/kg). The calcium content in the *C. medica* fruit peels varied from 0.4 to 0.8 dag/kg, proving higher than the levels noted in the peels from pineapples (0.076 dag/kg), bananas (0.066 dag/kg), papayas (0.055 dag/kg), passion fruit (0.044 dag/kg), melons (0014 dag/kg) and tangerines (0.47 dag/kg) [18]. The quantity of Mg in the three *C. medica* samples was 0.100 dag/kg, and the content of this element in the fruit mentioned above varied from 0.013 to 0.029 dag/kg except in tangerine peels (0.159 dag/kg).

The chemical analysis of the soil revealed high levels of the micronutrients Mn and Zn in the three sites and of Cu in the samples collected in Jandaia and Santo Antônio do Descoberto; moderate levels of Cu and Fe were detected in the soil collected in Pirenópolis and Jandaia, respectively. The Fe levels were considered low in the samples from Pirenópolis and Santo Antonio do Descoberto. The levels of macronutrients containing K, Ca and Mg were considered very high or high in the three samples, and the level of P was very high in the sample from Santo Antonio do Descoberto, average in the sample from Pirenópolis and very low in the sample from Jandaia.

A good level of organic matter (OM) was noted in the three samples. There were low levels of potential acidity (H+Al) in the samples collected in Pirenópolis and Santo Antônio do Descoberto and an average level in the Jandaia sample. The effective cation exchange capacity (CEC) presented high or very high levels, and the soil presented average or low levels of acidity with satisfactory pH in all the samples. The base saturation (V) values were considered good or very well [19].

According to Hardisson et al. [20-24] minerals play a vital role in the development and health of the human body, and fruit is considered the main source of minerals necessary in a healthy human diet. The chemical characterization of the peel of this fruit is not sufficient to consider it as being of high nutritional value because the bioavailability of the nutrients was not investigated, but the high levels of iron, calcium and magnesium detected encourages more studies so that this fruit is better utilized as a food.

CONCLUSIONS

The data obtained show that the fruit peel of *C. medica* contains essential oils with compounds of commercial interest. Limonene was the most abundant chemical compound in the essential oil from the fruit peel of *C. medica* collected at the three sites and has pharmacological properties. The high concentrations of minerals detected encourage its cultivation and use as food.

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