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Abstract

Bioprospecting molecules of native Brazilian flora enable the discovery of active principles that help enterprises participate in the competitive market often with limited innovation. This study investigated the chemical composition of the essential oil from leaves of the species Campomanesia phaea (O. Berg.) Landrum (cambuci) is from the botanical family Myrtaceae. The cambuci species is an endemic species in Brazil's São Paulo State located in Atlantic Forest biome. The studied sites are located in the Tropical Rain Forest (TRF) in the micro region of Paraibuna-São Paulo/ Brazil and in the Seasonal Semi deciduous Forest (SSF) in the micro region of Limeira-São Paulo/Brazil, both belonging to the Atlantic Forest biome. We studied the possibility of developing new products with essential oils for industries of food, beverages, pharmaceuticals, and cosmetics. Essential oils were extracted in the pilot plant of steam distillation under controlled conditions of pressure and temperature from fresh and dry leaves of plant materials. The average yield of essential oils from leaves of Campomanesia phaea collected in the microregion of Limeira - SP (SSF) showed higher values compared with those from leaves collected in the microregion Paraibuna - SP (TRF) but no significant difference was found at a significance level of 5%. The chemical composition analysis of the essential oils extracted from Campomanesia phaea proved the presence of 29 chemical substances. The main compounds found were trans-caryophyllene, bicyclogermacrene, trans-β-ocimene, δ-cadinene, trans-nerolidol and linalool. The drying process did modify the composition of essential oils. On the other hand, the same chemical compounds were found in fresh leaves from both locations. The use of Campomanesia phaea (cambuci) species could be a good alternative from a social, economic and environmental perspective, as it potentially may raise employment opportunities and income in the regional communities (including the indigenous ones) allowing a sustainable use of inputs from the Brazilian biodiversity.

Keywords: Atlantic Forest biome, Cambuci, *Campomanesia phaea*, Essential oil.
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Introduction

Throughout history, mankind has used the essences of herbs in medicine, cooking and religious ceremonies. With technology development, the aromas acquired new and important functions such as improving the sensory quality of many products offered in the market.

Synthesized by plants, essential oils are defined as a group of chemically complex natural substances of varied composition and flavoring properties extracted from different parts of the plants (roots, flowers, fruit, seeds, wood and bark) by specific processes, mainly hydro distillation and by steam-dragging distillation (Bakkali et al., 2008; Marques et al., 2012; Perrin and Colson, 1987; Regnault-Roger et al., 2011). Essential oils are related to various functions necessary for plant survival, and play a fundamental role in the defense against microorganisms (Fischer et al., 2005; Magina et al., 2009; Marques et al., 2008; Murbach Teles Machado and Fernandes Junior, 2011; Siani et al., 2000; Silva et al., 2010).

Each vegetable family offers a wide range of aromas; some families are recognized by a characteristic aroma, as the Myrtaceae, whose leaves and fruit are intensely aromatic (Rynal and Aubaile-Sallenave, 1988).

The species *Campomanesia phaea* (O. Berg.) Landrum, popularly known in Brazil as "cambuci" or "cambucizeiro” belongs to the subfamily Myrtoideae. The Cambuci tree naturally occurs in Brazil’s Atlantic Coastal Forest in the states of São Paulo (mainly in the Serra do Mar). It is thus a fine choice for tropical to subtropical climates with abundant rainfall, also, it may be attractive to some because of its history and rarity (Lorenzi, 2014).

The fruit of the cambuci tree (a single in *Campomanesia* genre) has an intense sweet aroma, but with extremely acid taste such as lemons. The fruit of *C. phaea* is very much valued by the local people and is generally used as a flavoring ingredient in alcoholic beverages and its pulp is used in the preparation of juices, jellies, ice-creams and sweets (Mathias and Andrade, 2011).

The leaves, barks and fruit of the cambuci tree have medicinal uses according to Donado-Pestana et al., 2015 because they reduce the risk of developing obesity and other metabolic diseases characterized by a chronic, low-grade inflammation.

Until now, a sole paper has been published on the essential oil from the leaves of this species, in the city of Ubatuba, São Paulo-Brazil, by Adati (2006). The essential oils showed as major constituent’s linalool (11.1%), caryophyllene oxide (11.8%), β−cariofileno (6.3%), β−selineno (6.3%) and α−cadinol (1.9%), substances that have commercial value for the pharmaceutical and cosmetic industries. Therefore, the essential oils extracted from the leaves of the cambuci tree can be considered a rarity with a high economic value and environmental impacts, capable of influencing the whole business chain and contributing to the sustainable development of society (Adati, 2001; Adati and de Oliveira Ferro, 2006).

Essential oils are the result of the specialized metabolism of plants, and their chemical phenotype and production are influenced by genetic factors and genotype interaction with the environment.

Thereby, the objective of this paper was to evaluate the yield and chemical composition of the essential oil extracted from fresh and dry leaves of the cambuci tree in two different microregions of the Atlantic Forest in Brazil with the purpose to make possible the economic use of this emblematic Brazilian species.

Experimental

Plant Material

Fresh leaves of Campomanesia phaea (O. Berg.) Landrum (cambuci) were collected in the Spring of 2011, from nine trees in each cultivated stand (commercial stands) in two different areas in the State of São Paulo (SP) proposed for this study: the cities of Paraibuna (23°27'50.77"S 45°42'37.37"O) with voucher nº ESA 114-275 and Limeira (22°34'33.71"S 47°27'41.04"O) with voucher nº ESA 114-276, herbarium at the Department of Botany, at the College of Agriculture "Luiz de Queiroz" (ESALQ/USP) in Piracicaba, State of São Paulo.

These locations were classified as a Tropical Rain Forest (TRF) in the microregion of Paraibuna-SP and in the Seasonal Semideciduous Forest (SSF) in the microregion of Limeira-SP, both belonging to the Atlantic Forest biome.

Part of the fresh leaves was dried at 40◦C in an air-circulating oven to constant weight (about 48 h).

Extraction of Essential Oil

The essential oils of fresh and dry leaves (each of the homogeneous mixture of the nine samples for each cultivated area) were extracted by a semi-pilot plant, during a total time of 4h at 110ºC and 0,5 kgf/cm^2 (8 psi), according to the methodology established by the Laboratory of the Natural Products at the Agronomic Institute (IAC).

Chemical Composition of Essential Oil

The essential oils were solubilized in ethyl acetate (1 mg of essential oil/1 mL of solvent) and injected in a volume of 1 μL for both analyses (GC-FID and GC-MS).

The quantitative analysis (area normalization method) of substances in the oil extracted from the leaves of *C. phaea* was performed in gas chromatographer with a flame ionization detector (GC-FID, Shimadzu®, GC-2010/AOC-20i), equipped with a fused silica capillary column DB-5 (30 m x 0.25 mm, i.e., coating thickness 0.25 μm) using helium as gas carrier (1.0 mL min-1) and splitted ratio 1/20. The GC oven temperature was heated at 60 °C up to 180°C at a rate of 3°C min-1, and then increased to 240°C at a rate of 10°C min-1.

The qualitative analysis of the chemical composition of the essential oils was performed in a gas chromatographer coupled to a mass spectrometer (GC-MS, Shimadzu® QP-5000), equipped with a fused silica capillary column OV-5 (30 m x 0.25 mm, i.e., coating thickness 0.25 μm) operating by an electron impact (70 eV.), with helium as a gas carrier (1.0 mL min-1), injector 240 °C, detector at 230 °C, splitted ratio 1/20 and under the same operating conditions of GC / FID.

The compounds were identified by comparing the mass spectra with the database GC-MS system (62 Nist. lib.) and retention indices (Adams, 2007). The retention indices (RI) of the compounds were obtained by injecting a mixture of n-alcanes (C_9H_{20}–$C_{25}H_{52}$, Sigma Aldrich, 99%), performed at the same conditions used in the GC/MS analysis for the essential oils, and using the equation proposed by Vandendool and Kratz's equation (Van Den Dool and Dec. Kratz, 1963).

Statistical Analysis

The data yield extraction evaluation used a completely randomized design (CRD). This study adopted the significance level = α = 0.05. All inference procedures were preceded by tests of the assumptions of non-parametric analysis of variance of Tukey's method.

Due to the large number of compounds that may be present in the chemical composition of essential oils, the data were subjected to a multivariate analysis by the Principal Component Analysis with bi plot graphic (PCA) and hierarchical cluster analysis (HCA) in order to understand the similarity between essential oils as the levels of the chemical constituents. The PCA was used to characterize and establish links between the different culture locations and processing of the leaves (dried or fresh).

The Hierarchical Cluster Analysis (HCA) evaluated the phytochemical profile of essential oils in fresh and dry leaves in both locations: Paraibuna and Limeira.

HCA by the average method with the Euclidean distance with a similar coefficient with cutting 0.75 had two main groups. It elucidates that the phytochemical profile essential oils of fresh leaves from Limeira and Paraibuna have chemical similarities. The statistical software used was the SAS 9.3 (SAS Institute, 2010).

Results and Discussion

Yield of Essential Oil

In order to evaluate the oil potential application from both regions, we needed to know the average yield of each and then establish future studies of oil exploitation.

The data shows there was no significant difference at a significance level of 5% between leaf conditions from each site. An average yield of essential oil was found for Paraibuna of 0.06% (fresh leaves) and 0.09% (dry leaves), for Limeira of 0.12% (fresh leaves) and 0.14% (dry leaves).

Chemical Composition of Essential Oil from Campomanesia phaea (Cambuci)

Table 1. *Average Chemical Composition (%) of Essential Oil Obtained from Fresh and Dry Leaves of Campomanesia phaea (Cambuci) in the Sampling Sites*

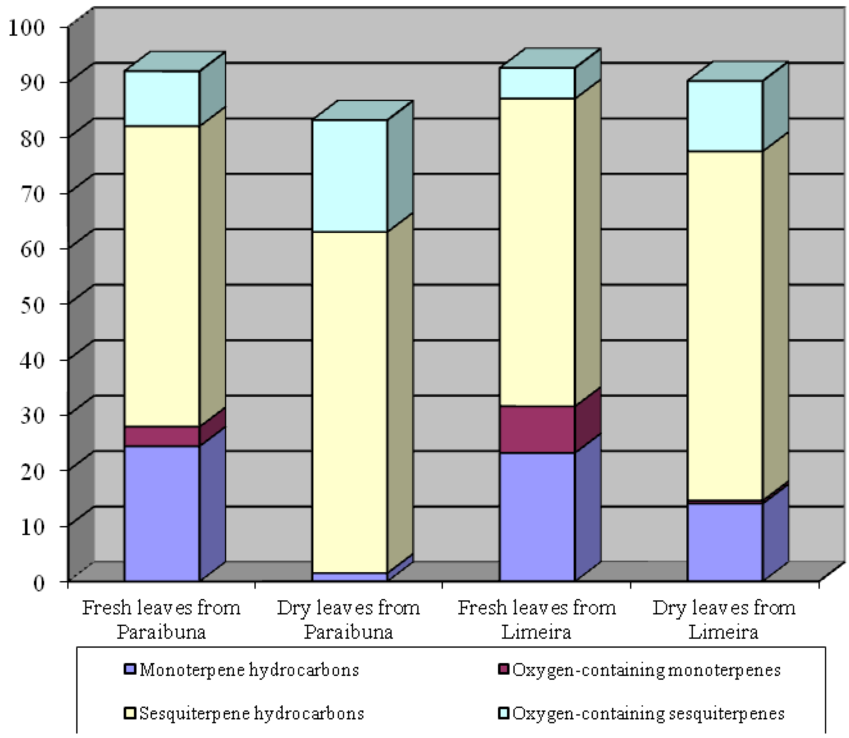
SUBSTANCE	RI* calc	RI** lit	PARAIBUNA		LIMEIRA	
			Fresh leaves	Dry leaves	Fresh leaves	Dry leaves
α-pinene	932	939	3.5	tr	5.3	2.6
β-pinene	975	979	3.1	tr	4.4	1.9
limonene	1027	1029	1.6	tr	2.4	1.4
1.8-cineol	1029	1031	0.5	tr	0.7	tr
trans-β-ocimene	1045	1050	16.2	1.5	11.2	8.1
Linalool	1098	1096	3.0	tr	7.7	0.5
σ-terpineol	1183	1188	tr	tr	tr	tr
α-copaene	1375	1376	3.2	5.3	2.2	3.4
β-elemene	1391	1390	0.9	tr	2.4	1.5
trans-caryophyllene	1418	1419	17.0	16.0	13.8	16.9
β-copaene	1428	1432	1.1	1.2	0.8	0.9
aromadendrene	1438	1449	1.5	1.5	1.7	2.5
α-humulene	1452	1454	3.9	4.0	3.1	3.5
allo-aromadendrene	1459	1461	2.2	2.3	2.5	2.6
γ-muurolene	1475	1477	3.0	4.8	3.5	4.4
germacrene D	1480	1480	2.7	1.6	1.8	1.2
β-selinene	1485	1485	2.3	4.7	4.7	6.4
bicyclogermacrene	1495	1494	6.3	6.8	9.2	9.8
germacrene A	1503	1503	1.3	1.3	1.4	1.5
γ-cadinene	1512	1513	1.5	2.4	1.0	1.7
δ-cadinene	1522	1523	5.7	8.2	3.9	5.6
germacrene B	1555	1561	1.5	1.5	3.4	1.1
trans-nerolidol	1563	1563	4.6	12.1	3.1	6.2
spathulenol	1576	1578	1.0	1.2	0.6	1.0
caryophyllene oxide	1583	1583	2.1	2.2	1.3	2.7
10-*epi*-γ-eudesmol	1621	1619	tr	tr	tr	0.6
1-*epi*-cubenol	1627	1627	0.9	1.7	tr	0.8
cubenol	1640	1642	1.3	2.4	0.6	1.4
α-muurolol	1645	1645	tr	0.7	tr	tr

total identified			92.0	83,2	92.6	90.2
monoterpene hydrocarbons			24.4	1.5	23.2	14.1
oxygen-containing monoterpenes			3.5	tr	8.4	0.5
sesquiterpene hydrocarbons			54.2	61.5	55.5	63.0
oxygen-containing sesquiterpenes			9.9	20.2	5.5	12.7

RI* calculated retention index; RI** retention index from literature (Adams, 2007); tr – traces (≤ 0.05)

This study detected a single chemotype of the essential oil of the cambuci tree. The chemical composition was characterized by many chemical substances: twenty-nine compounds, representing average at 91.52% of essential oil extracted from C. phaea (Table 1) with an economic potential value and positive environmental impacts. These results are in accordance with the considerations described by Adati & de Oliveira Ferro (2006), which reports the existence of more than 35 compounds representing 94.9% of the total oil contents. In order to identify the main compounds, we considered those present in a percentage amount higher than 5%. Thus, we also noticed the following major compounds: trans-caryophyllene, bicyclogermacrene, trans-b-ocimene, d-cadinene, trans-nerolidol and linalool. The major compounds of all essential oils were the trans-caryophyllene that might represent important tools for the management and/or treatment of inflammatory diseases, and bicyclogermacrene (Martin et al., 1993; Fernandes et al., 2007).

Figure 1. *Separation of Chemical Constituents of the Essential Oils in Substances Classes*



We observed a large variety of different substance classes in essential oils from the cambuci tree (Figure 1).

At both sampling sites (Paraibuna-SP and Limeira-SP) the sesquiterpene hydrocarbons represented the most abundant class in the essential oils.

The relative proportion of hydrocarbons monoterpenes in essential oils significantly reduced with the leaves by the drying process: 24.4% (fresh leaves) to 1.5% (dry leaves) from leaves of Paraibuna; and 23.2% (fresh leaves) to 14.0% (dry leaves) from leaves from the site of Limeira. The same behavior was observed for the oxygen-containing monoterpenes.

Due to the high complexity of essential oils we used the Analysis of the Main Component (AMC) and the Hierarchical Cluster Analysis (HCA), in order to elucidate characteristics relating to the results of the chromatographic analysis, correlating and grouping the data of chemicals over their percentage in their essential oils. The Analysis of Main Component (AMC) described 88.87% of the data with 61.3% of the total variance described by the first principal component.

The chemical analysis of the data by the HCA and AMC techniques allowed grouping the samples into three groups that have similar characteristics and similar main compounds, as shown below (Figures 2 and 3).

Figure 2. *Graph Biplot PCA for Score for Essential Oils of Fresh and Dry Leaves of Campomanesia phaea (O. Berg.) Landrum (Cambuci) from Paraibuna and Limeira*

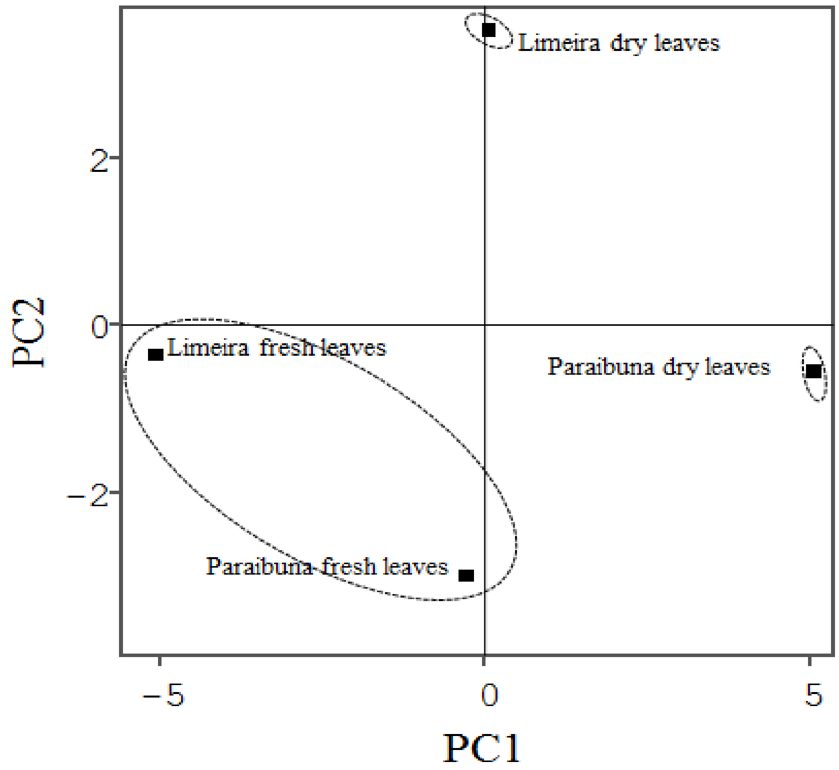
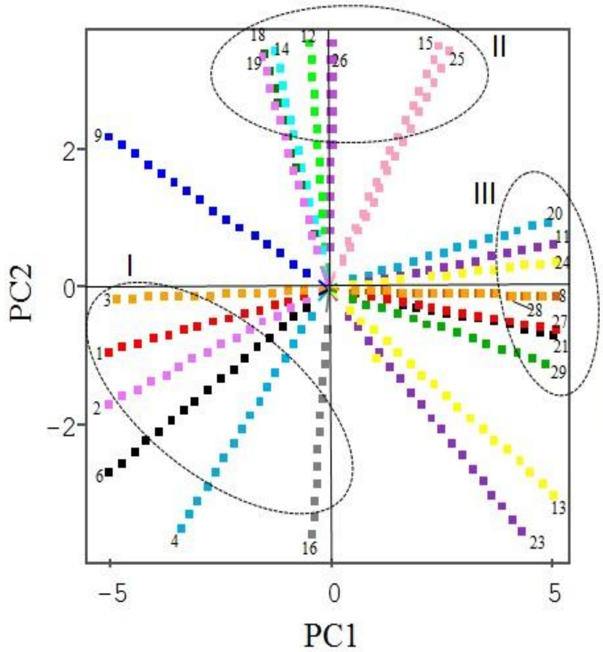


Figure 3. *Graph Biplot PCA for Loadings and Compounds in Essential Oils of Fresh and Dry Leaves of Campomanesia phaea (O. Berg.) Landrum (Cambuci) from Paraibuna and Limeira*



SUBSTANCE			
1	α-pinene	17	β-selineno
2	β-pinene	18	bicyclogermacrene
3	limonene	19	germacrene A
4	1.8-cineol	20	γ-cadinene
6	linalool	21	δ-cadinene
8	α-copaene	23	*trans*-nerolidol
9	β-elemene	24	spathulenol
11	β-copaene	25	caryophyllene oxide
12	aromadendrene	26	10-*epi*-γ-eudesmol
13	α-humulene	27	1-*epi*-cubenol
14	*allo*-aromadendrene	28	Cubenol
15	γ-muurolene	29	α-muurolol
16	germacrene D		

Figure 3 presents the data of the chemical constituents from cambuci essential oils and correlate them into three groups. It is observed that the essential oil samples of Group I was closer due to the similarities of the levels of the components as α-pinene, β-pinene, limonene, 1.8-cineol, linalool, germacrene D. In the essential oil of Group II there was a similarity in the content of the components as bicyclogermacrene, germacrene A, *allo*-aromadendrene, aromadendrene, caryophyllene oxide, 10-epi-γ-eudesmol, γ-muurolene. In relation to the essential oil of Group III there was a similarity with the contents of the components γ-cadinene, β-copaene, spathulenol, α-copaene, cubenol, 1-epi-cubenol and α-muurolol.

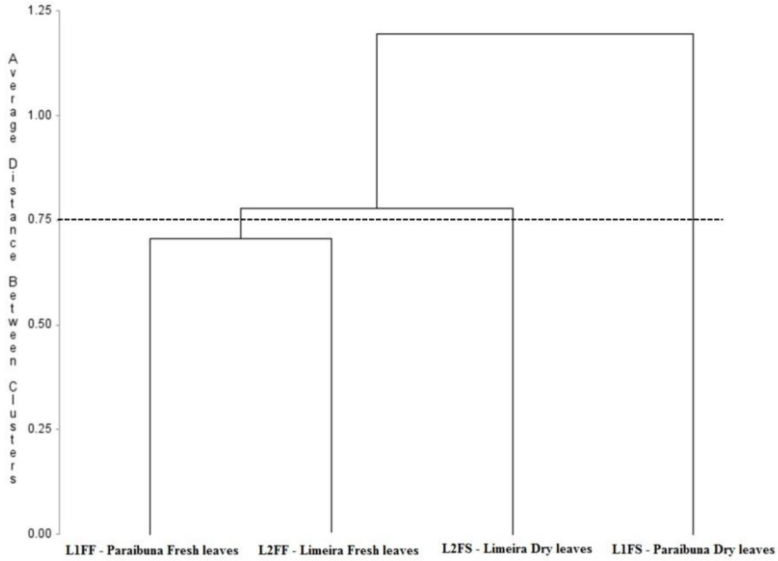
The essential oils dendrogram are in Figure 4. It shows that the phytochemical profile of essential oils extracted from fresh leaves from Paraibuna and Limeira have chemical similarities.

The essential oils were classified into three groups using the Analysis of Main Component analysis (AMC) and Hierarchical Cluster Analysis (HCA) (Figure 3).

- **Group I -** Fresh leaves from Paraibuna and Limeira: Due to their similarity levels of α-pinene, β-pinene, limonene, 1.8-cineol, linalol, germacrene D.;
- **Group II** – Dry leaves from Limeira: Due to their similarity levels of bicyclogermacrene, germacrene A, allo-aromadendrene, aromadendrene, caryophyllene oxide, 10-epi-γ-eudesmol, γ-muurolene;

- **Group III** - Dry leaves from Paraibuna: Due to their similarity levels of γ-cadinene, β-copaene, spathulenol, α-copaene, Cubenol, 1-epi-cubenol and α-muurolol.

Figure 4. *Hierarchical Cluster Analysis (HCA) of Essential Oils from Dry and Fresh Leaves from Campomanesia phaea (O. Berg.) Landrum (Cambuci)*



Conclusions

The major compound of the essential oils from Paraibuna and Limeira, for both treatments of leaves of *Campomanesia phaea* (O. Berg.) Landrum was the *trans*-caryophyllene, which might represent an important tool for the management and/or treatment of inflammatory diseases.

Furthermore, it was evident that the drying process modified the composition of essential oils; on the other hand, for fresh leaves the same chemical compound was observed for both locations.

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