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Chemical composition and antioxidant activity of the essential oil from leaves of *Corymbia citriodora* Hook grown in western Cuba

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Abstract
The chemical composition of the essential oil from leaves of *Corymbia citriodora* Hook. Grown in western Cuba was studied by GC-FID and GC-MS. A total of 72 volatile compounds were identified in the essential oil. The composition was dominated by oxygenated monoterpenes (92.4%), whereas citronellal (35.6%), isopulegol (27.6%), ISO-isopulegol (12.2%), and β-Citronellol (10.4%) were the most abundant constituents in the essential oil. Furthermore, we assessed the antioxidant properties by using three in vitro assays: 2,2-diphenyl-1-picrylhydrazyl test, ferric reducing antioxidant power and lipid peroxidation assays. The essential oil had in vitro antioxidant properties depending on the chosen method. In addition, this effect may be specific due to the chemical components of the oil.

Keywords: *Corymbia citriodora*; essential oil; composition; antioxidant activity

1. Introduction
Essential oils are complex mixtures of low molecular weight compounds isolated from plants usually by steam distillation. They are important products of agriculture-based industry, which are commonly used as flavoring agents in food products, perfumeries, cosmetics and pharmaceuticals [1]. Several factors including genetic variation, plant ecotype or variety, plant nutrition, agronomic practices, geographic location of the plants, seasonal variations, type of plant material, postharvest drying and storage, affect the yield and chemical composition of the essential oil, and thus determines its characteristic biological properties [2].
Eucalyptus, belonging to the Myrtaceae family, is one of the most cultivated trees in several countries. Among the species of eucalyptus used for obtaining essential oils, *Corymbia citriodora* Hook (syn *Eucalyptus citriodora* Hook) is commonly used. *C. citriodora* essential oil is extracted from dry leaves, and its major constituent is citronellal (40–95%) [3]. Studies have shown that *C. citriodora* essential oil has several properties, including antioxidant [3, 4], antibacterial [3, 5], antifungal [3, 6, 7], anti-inflammatory and analgesic [8], acaricidal [3, 9], insecticidal [10-13], herbicidal [3, 14] and anthelmintic [15-17] activities.
The present study was performed to analyze the *C. citriodora* essential oil from trees grown in Cuba, and to evaluate its antioxidant activity.

2. Materials and methods
2.1 Plant material and isolation of essential oil
Leaves of *C. citriodora* from trees, with more than 20 years, were collected at Pinar del Río, in the western region of Cuba, during March 2018 and dried in the shadefor 10 days. Authentication was performed by the Herbarium Julian Acuña at the Xiloteca Sciences Institute Agroforestry of the Ministry of Agriculture, Havana, Cuba, where a voucher specimen was deposited (accession number 276HBW).
Three samples (2.5 kg each one) from dry leaves of different plants were conventional steam distilled, at bench scale, for 1 h. Mean oil yield was 2.25% v/m. Essential oils were dried over anhydrous sodium sulfate and stored at 4 ºC in the absence of light prior to analysis.
2.2 GC−FID and GC−MS analyses
GC-FID analysis was performed using a Konik 4000A instrument (Konik, Barcelona) equipped with a 30 m × 0.25 mm i.d.× 0.25 µm DB-5ms (J & W Scientific, Folsom, CA, USA) or a 30 m × 0.25 mm i.d. x 0.25 µm HP-Inn wax (Agilent Technologies, Santa Clara,

CA, USA) columns. Initially, the oven temperature was 60 ºC, which was held isothermally for 2 min, followed by an increase to 230 ºC at 4 ºC/min and this was held isothermally for 10 min; injector and detector temperatures were 230 ºC and 250 ºC, respectively; Hydrogen at a split ratio of 1:50 and a flow rate of 1 mL/min was used as carrier gas. Pure essential oil was diluted with diethyl ether (1:10 v/v) and 0.3µL was injected with a split mode.

The essential oil was also analyzed by GC-MS on a Shimadzu QP 2010 mass spectrophotometer (Shimadzu, Kyoto, Japan) fitted with a DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; J & W Scientific, Folsom, CA, USA). Helium at a split ratio of 1:50 and a flow rate of 1 mL/min was used as carrier gas. Oven temperature program was the same as GC-FID. Mass spectral range was recorded from *m/z* 50−600 amu. The mass spectrometer source temperature was 230 ºC, and interface temperature and injector temperature were 230 ºC.

The compounds were identified by comparing their linear retention index and their mass spectra to those of commercial spectra databases (Wiley 6, NBS 75k, NIST05 and Adams 2001) and the in-house Flavorlib library created from previous laboratory studies. Some of the identifications were confirmed by the injection of the chemical standards into the GC-FID system. Linear retention indices (LRI) of the compounds were calculated using an *n*-alkane series.

Quantification of compounds was carried out using relative percentage abundance and normalization method with correction response factors based on grouping the essential oil components by their functional groups [18]. Percentage data are the mean values of two injections per sample.

Table 1: Composition of the essential oil from *Corymbia citriodora* leaves

Compound	RI_A	RI_A^O	RI_P	RI_P^O	%
3-Methylbutanal	654	655	900	902	0.2
2-Methylpropanoic acid	785	790	1588	1591	tr
2,4-Dimethylpentan-3-one*	806	806	1043	1041	tr
3-Methylbutanoic acid	874	876	1683	1686	tr
2-Methylpropyl 2-methylpropanoate	907	906	1095	1092	0.1
α-Thujene	929	931	1020	1019	tr
α-Pinene	937	939	1032	1033	0.5
Camphene	954	956	1076	1075	tr
Sabinene	973	972	1125	1121	tr
β-Pinene	977	979	1114	1117	0.5
6-Methyl-5-hepten-2-one	984	986	1343	1338	tr
Myrcene	987	989	1162	1160	0.1
2-Methylpropyl 3-methylbutanoate	1006	1005	1195	1198	tr
δ-3-Carene	1009	1011	1155	1159	tr
3-Methylbutyl 2-methylpropanoate	1017	1021	1191	1195	tr
α-Terpinene	1016	1014	1179	1180	tr
p-Cymene	1023	1024	1270	1268	0.3
Limonene	1027	1029	1201	1203	0.3
1,8-Cineole	1016	1014	1179	1180	0.9
(*Z*)-β-Ocimene	1035	1036	1244	1240	0.2
(*E*)-β-Ocimene	1051	1052	1263	1260	tr
2,6-Dimethyl-5-heptenal	1056	1056	1355	1358	0.2
γ-Terpinene	1060	1059	1244	1242	0.3
p-Mentha-3,8-diene*	1070	1070	1267	1271	0.4
cis-Linalool oxide (furanoid)	1074	1075	1449	1449	tr
p-Mentha-2,4(8)-diene*	1085	1084	1289	1286	0.1
Terpinolene	1089	1089	1288	1291	0.2
p-Cymenene	1091	1091	1450	1452	tr
Linalool	1095	1098	1354	1355	0.3
3-Methylbutyl 3-methylbutanoate	1103	1103	1286	1285	tr
2-Phenylethanol	1106	1107	1875	1872	tr
cis-Rose oxide*	1110	1111	1335	1338	0.1
endo-Fenchol	1117	1119	1540	1543	tr
trans-Pinocarveol*	1137	1139	1665	1664	tr
Isopulegol	1148	1146	1575	1572	27.6
Citronellal	1153	1153	1487	1488	35.6
iso-Isopulegol	1160	1156	1562	1564	12.2
Borneol	1169	1171	1711	1715	tr
neoiso-Isopulegol	1172	1171	1612	1615	1.6
Terpinen-4-ol	1176	1174	1590	1592	0.4
p-Cymen-8-ol	1185	1184	1862	1864	tr
α-Terpineol	1191	1192	1698	1695	0.2
β-Citronellol	1225	1228	1764	1766	10.4
Pulegone	1235	1237	1665	1661	tr
Geraniol	1255	1256	1855	1857	0.1
Citronellylformate	1273	1275	1629	1625	tr
δ-Octalactone	1285	1288	1970	1967	tr

trans-Linalool oxide acetate (pyranoid)*	1291	1287	1620	1619	tr
Thymol	1291	1290	2167	2169	0.2
Citronellic acid*	1314	1314	2230	2232	0.1
8-Hydroxy-*neo*-menthol*	1326	1326	-	-	1.6
Citronellyl acetate	1351	1354	1667	1668	1.8
Eugenol	1356	1359	2154	2156	tr
Geranyl acetate	1382	1386	1763	1765	tr
β-Elemene	1392	1391	1602	1600	tr
(*Z*)-Jasmone	1395	1391	1956	1960	0.1
2-Phenylethyl 2-methylpropanoate	1399	1396	1899	1896	tr
α-Gurjunene	1410	1410	1535	1538	tr
(*E*)-Caryophyllene	1418	1419	1610	1612	3.0
Citronellyl propanoate	1444	1444	1697	1700	tr
cis-Muurola-3,5-diene*	1450	1450	1742	1746	tr
α-Humulene	1452	1455	1677	1678	0.1
allo-Aromadendrene	1460	1461	1660	1661	tr
Citronellyl 2-methylpropanoate	1482	1483	1720	1724	tr
2-Phenylethyl 3-methylbutanoate	1488	1489	1990	1992	tr
β-Selinene	1490	1490	1740	1742	0.1
α-Muurolene	1503	1501	1720	1725	tr
γ-Cadinene	1512	1514	1775	1776	tr
trans-Cadina-1(2),4-diene*	1535	1532	-	-	tr
Spathulenol	1577	1578	2135	2138	tr
Caryophyllene oxide	1583	1581	2005	2000	0.1
Humulene epoxide II	1608	1608	2041	2045	tr
Monoterpene hydrocarbons					2.1
Oxygenated monoterpenes					92.4
Sesquiterpene hydrocarbons					3.2
Oxygenated sesquiterpenes					0.1
Non-terpenoid compounds					0.8

RI_A^O and RI_P^O, linear retention indices from standard or literature on DB-5ms and HP-Innowax column, respectively
*tentatively identified compound by comparison with literature data.tr: <0.1%

2.3 Antioxidant capacity assays

The free-radical scavenging capacity of the essential oil from leaves of *C. citriodora* was determined [19]. For it, an ethanolic solution of 130 mM 2, 2-diphenyl-1-picrylhydrazyl (DPPH•, Sigma, USA) was mixed with the extracts (2-1000 µg/mL). Eugenol (0.1-1000 µg/mL) (Sigma) was employed as standard. The reaction mixtures were incubated in the dark at room temperature for 30 min and the absorbance was measured at 515 nm. The inhibition percent of DPPH• radical was calculated by: Inhibition (%) = (D.O. control– D.O. sample)/D.O. control) × 100. The concentration required to scavenge 50% of DPPH• (IC_{50}) was determined.

The reducing capacity of the essential oil from leaves of *C citriodora* was measured according to a reported method [20]. Briefly, acetate buffer (300 mM, pH=3.6), TPTZ (2, 4, 6-tripyridyl-s-triazine; Sigma, USA) 10 mM in 40 mMHCl and $FeCl_3 \cdot 6H_2O$ (20 mM) were mixed in the ratio of 10:1:1 to obtain FRAP reagent. The essential oil (20 µL) was mixed with 900 µL of FRAP reagent. The mixtures were incubated at room temperature for 4 min and absorbance was measured at 593 nm. Ascorbic acid (100 µM) was used as standard.

To evaluate the inhibition of brain phospholipid peroxidation, rat brains were excised after decapitation, weighed and washed with 0.9% NaCl ice cold solution. Tissue homogenates were prepared in a ratio of 1 g of wet tissue to 9 mL of phosphate buffer (50 mM, pH 7.4), by using a tissue homogenizer (Qiagen). The homogenates were centrifuged at 800 g in a Sigma centrifuge at -4 °C for 15 minutes and the supernatants were kept at -70 °C until analysis. Thiobarbituric reactive substances (TBARS) measurement assay was carried out by a modification of the procedure previously described [21]. Brain homogenates (25 mL) were incubated with different concentrations of the essential oil from leaves of *C. citriodora* (40-5000 µg/mL). Incubations were stopped by the addition of 350 mL of cold acetic acid 20% pH 3.5. Malondialdehyde (MDA) levels were determined by the addition of 600 mL of TBA 0.5% in acetic acid 20% pH 3.5. The mixtures were incubated at 90 °C for 1 h. Then, 50 mL of sodium dodecyl sulfate (SDS) were added, and samples were centrifuged at 500 g during 15 min at room temperature. The absorbance was measured at 532 nm. Eugenol (0.1-1000 µg/mL) was used as standard. The concentration which is needed to achieve the 50% of inhibition of lipid peroxidation (IC_{50}) was calculated. All the determinations were conducted at least three times. The statistical mean and IC_{50} (the concentration required to obtaining antioxidant activity 50% from the dose-response curves) value were calculated with ± SD using Graph Prism ver. 5 (Microsoft Corporation, Redmond, WA, USA).

Table 2: Antioxidant activity of essential oil extracted from *Corymbia citriodora*

		FRAP IC_{50} (µg/mL)	**DPPH IC_{50} (µg/mL)**	**Lipid peroxidation inhibition IC_{50} (µg/mL)**
Essential oil		91.98 ± 2.5	343.20 ± 3.4	314.30 ± 7.4
Eugenol		15.40 ± 1.2	4.26 ± 1.3	49.83 ± 1.7

Values represent the mean ± SEM of the antioxidant activity of essential oil from leaves of *C. citriodora*. IC_{50} values were calculated as the concentration required for obtaining antioxidant activity 50% in each assay performed. Eugenol

was employed as standard of the experiments. Three independent experiments were done and samples were analyzed by triplicate.

3. Results and Discussion

The essential oil from leaves of *C. citriodora* was investigated by means of GC-MS and GC-FID to determine its qualitative and quantitative profile (Table 1). The identified compounds are listed in order of elution. A total of 72 constituents were identified, which represent approximately 99% of the total composition. Data reported in Table 1 are expressed as relative percentage of the peak areas, with correction response factors based on grouping the essential oil components by their functional groups. Means of three replicates are given, in which CV% values were always lower than 3% for their petitions.

The essential oil was dominated by oxygenated monoterpenes (92.4%). Citronellal (35.6%), isopulegol (27.6%), *ISO*-isopulegol (12.2%), and β-citronellol (10.4%) were the most abundant constituents identified in essential oil.

The data presented in Table 1show qualitative and quantitative variability in the chemical composition in comparison to previous papers [10-16]. In those reports, citronellal is always the main constituent with high content (>40%), but the other accompanying major compounds can be different to those found in the present research or they are the same at different concentrations. Based on the literature, the composition of the essential oils can vary according to several factors, including Geobotanical conditions of the environment, plant age, harvest period, cultivation procedure, drying procedure prior to extraction, among others [3, 22, 23].

The screening of the antioxidant activity of the substances may require a combination of different methods to describe the background about their antioxidant properties. Here, the antioxidant potential of essential oil from leaves of *C. citriodora* grown in western in Cuba was determined by using three methods, which previously have been used to predict the *in vitro* antioxidant capacity of several compounds (DPPH free radical scavenging assay, FRAP assay and the determination of lipid peroxidation in brain rat homogenates).

The model of scavenging the stable DPPH radical has been used to evaluate the free radical scavenging ability of one substance [24, 25].The antioxidant effect of the analyzed sample on DPPH radical scavenging may be due to their hydrogen donating ability and it reduces the stable violet DPPH radical to the yellow DPPH-H. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [26]. On the other hand, FRAP assay is based on the ability of antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of tripyridyltriazine (TPTZ), forming the intense blue Fe^{2+}–TPTZ complex with an absorption maximum at 593 nm; the absorbance increase is proportional to the antioxidant content [27].

DPPH test showed a free radical-scavenging activity of the essential oil from leaves of *C. citriodora* (Table 2). The percentage inhibition of DPPH activity of essential oil was in the order of 88% up to 1250 µg/mL, with ^{IC50} values of 343.2 ± 3.4 µg/mL. The second antioxidant criterion used was the measure of the reductive capacity of the tested sample (FRAP). The FRAP, expressed as µM of ascorbic acid equivalents (AAE) or known Fe^{2+} concentration, the $IC_{50}$was found to be in the order of 91.98 ± 2.5µg/mL of the essential oil. The positive control, eugenol, showed the expected effect in both assays performed.

In the last years, lipid peroxidation has received renewed attention from the viewpoints of nutrition and medicine [28]. It is the accumulated result of reactive oxygen species and a chain reaction that causes the dysfunction of biological systems [29]. The data show that essential oil inhibits lipid peroxidation against brain phospholipid peroxidation concentration manner, showing IC_{50} values of 314.3 ± 7.4 µg/mL and it is being able to reduce the lipid peroxidation in the order of 85% at the highest concentrations evaluated (2500 and 5000 µg/mL) (Table 2).

Previous studies explored antioxidant properties of *C. citriodora* essential oil in terms of total antioxidant activity, FRAP assay, ferrous ion chelating activity and scavenging of hydrogen peroxide, and DPPH radicals and inhibition of lipid peroxidation [4].The essential oil and the major monoterpenes exhibited moderate to strong antioxidant activity in terms of TAA and FRAP chelating, DPPH and H_2O_2 scavenging, and lipid peroxidation inhibition. This study confirmed that *C. citriodora* essential oil contains monoterpenoids with antioxidant activity.

Here, we evaluated the antioxidant properties of essential oil from leaves of *C. citriodora* grown in western in Cuba by using these *in-vitro* methods, as above was mentioned. Our results are in line with the observed by Singh *et al.* [4], which remarks about the antioxidant capacity of this species and suggesting the potential use of *C. citriodora* as source of new foods, cosmetic or pharmaceuticals.

4. Conclusions

Essential oil composition of *Corymbia citriodora* leaves from western Cuba show the presence of 72 volatile constituents, of which the most prominent were citronellal (35.6%), isopulegol (27.6%), *ISO*-isopulegol (12.2%) and β-citronellol (10.4%). The essential oil had in vitro antioxidant activity by using the DPPH, the ferric reducing antioxidant power and lipid peroxidation assays.

5. Conflict of interest

The authors declare no conflict of interest.

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