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Chemical composition and Antioxidant activity of essential oil from flowers of Couroupita guianensis Aubl. from El Salvador

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Abstract: Couroupita guianensis Aubl. (Lecythidaceae) "Cannonball tree" is a tropical plant with ethnobotanical uses. The present study is focused on analyzing the essential oil composition of Couroupita guianensis (C. guaianensis) fresh flowers collected in El Salvador. Steam distillation, coupled with GC/FID and GC/MS analysis were employed to characterize the chemical composition and in vitro antioxidant activity study were carried out using DPPH assay. Eugenol was identified to be the most abundant constituent and quantified as 46.20% of the total volatiles. Other constituents were found to be 2-phenylethanol (34.82%), $(E)-(E)$-farnesol (5.53%), nerol (3.95%), geraniol (3.20%), benzyl alcohol (1.77%). $DPPH$ assay showed a dose-dependent antioxidant activity. The study demonstrated that C. guianensis essential oil from El Salvador is a rich source of eugenol, which may be responsible for its antioxidant properties. Based on these results, further studies on the antibacterial and antifungal activity of C. guianensis essential oil are of fundamental importance.

Keywords: Couroupita guianensis, essential oil, eugenol, 2-phenylethanol, $(E)-(E)$-farnesol, $DPPH$.

1. Introduction

Couroupita guianensis Aubl. "Cannonball tree", belonging to the Lecythidaceae family, is an evergreen tree high up to about 30m with cylindrical stem, which can exceed 70cm in diameter, and brownish-gray bark slightly longitudinally slotted [1]. The plant is native to tropical central and Southern America, India and the Southern Caribbean. Its leaves, of intense green color and grouped at the apex of the branches, alternate, ovate or lanceolate with an entire margin or slightly toothed, are $10-20$cm long and $4-10$cm broad. The nectarless intensely scented flowers, $8-12$cm in diameter, produced for most of the year, are carried by woody racemes up to 2m long that are born on the trunk or at the base of the main branches (see 1). The corolla is made up of six fleshy, concave, roundish petals, two of which are larger, orange, pink, internally red, often yellowish externally with a central white disk, and numerous stamens divided into two groups, one fertile around the pistil, and one of the sterile stamens, about 2cm long, gathered in a sort of cap above the pistil. A previously reported for some genera of Lecythidaceae, C. guianensis included, the flowers emit a strong sweet aroma in the early morning. Osmophores tissues are located in the corolla and the top of the filaments of the hood anthers [2]. From leaves, flowers, fruit, and stem bark of C. guianensis several compounds including fatty acids, triterpenes, sterols and indolic compounds have been isolated [3]. Maceration using solvents with different polarity (water, methanol, ethanol, acetone, dichloromethane, and benzene), is the method commonly employed for the extraction of the polar fraction of different parts of the plant [4]. Despite the importance of volatile compounds for the ecophysiological aspects of plant life, in the literature there are only a few publications concerning the chemical characterization of the volatile fraction of C. guianensis flowers collected in Malaysia, Brazil, and Ecuador, respectively. The isolation of the volatile fraction of the flowers from Malaysia was carried out by maceration in 50% aqueous ethanol for 36h at room temperature, filtration, liquid/liquid extraction with pentane, solvent concentration and distillation under reduced pressure [5]. Flowers collected in Brazil were subject to micro-distillation/extraction with pentane

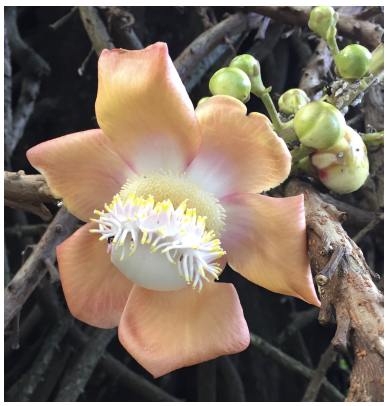

Figure 1. Flower of Couroupita guianensis collected in El Salvador (for exact site see Materials and Methods). (Photograph by Francesco Saverio Robustelli della Cuna).

[6]. A volatile fraction from flowers sampled in Ecuador was characterized in situ by dynamic sampling with Tenax and Carbotrap cartridges [7]. The present study was focused on analyzing the essential oil composition of C. guianensis, obtained by steam distillation of fresh flowers collected in El Salvador, the first ever study of plants from that country.

2. Materials and methods

2.1. Plant material

Fresh flowers of Couroupita guianensis Aubl. (Lecythidaceae) were collected from a tree growing on the campus of the University of El Salvador, San Salvador, El Salvador (latitude: $13^0 43' 5'' N$, longitude: $89^0 2' 8'' W$, elevation 650 m.a.s.l.), during the dry season in november of 2018. The plant was identified by the botanist Jenny Elizabeth Menjivar from the Natural History Museum of El Salvador, and the respective voucher (J. Menjivar *et al.*, 4930) specimen has been deposited in the herbarium at this museum.

2.2. Isolation of essential oil

Flowers of Couroupita guianensis Aubl. (490 g), to which octyl octanoate was added as internal standard (IS), were steam distilled in a Clevenger-type apparatus for 2 hours. The distillate was extracted three times with 100ml of freshly distilled diethyl ether, dried over anhydrous sodium sulfate concentrated with a rotary evaporator and finally using a stream of N_2. Samples were stored at -20^0C until analyses.

2.3. $GC-FID$ analysis

The analyses were carried out using a Hewlett Packard model 5980 GC, equipped with Elite-5MS (5% phenyl methyl polysiloxane) capillary column of ($30\ m \times 0.32 mm$ i.d.) and film $0.32\ \mu m$ thick (Agilent, Santa Clara, CA, USA). The carrier gas was *He* at a flow of 1 ml/min. One μL aliquots of essential oil were manually injected in "split" mode $(30:1)$. The oven temperature program included an initial isotherm of 40^0C for 5 min, followed by a temperature ramp to 260^0C at 4^0C/min, and a final isotherm at this temperature for 10min. Injector and detector temperatures were set at 250^0C and 280^0C, respectively. The relative amount of each component was calculated based on the corresponding FID peak area without response factor correction. Analyses were performed in triplicate.

2.4. $GC-MS$ analysis

The analyses were carried out using a *GC* Model 6890N, coupled to a benchtop MS Agilent 5973 Network, equipped with the same capillary column and following the same chromatographic conditions used for the GC/FID analyses. The carrier gas was *He* at a constant flow of 1.0mL/min. The essential oils were diluted before analysis in *n*-hexane, and $1.0 \mu l$ of the diluted solution was manually injected into the *GC* system with a split ratio of $30:1$. The ion source temperature was set at 200^0C, while the transfer line was at 300^0C. The acquisition range was $40-500$ amu in electron-impact (EI) positive ionization mode using an ionization voltage of 70 eV.

2.5. Identification of the Components of the Volatile Fractions

The components of the essential oils were identified by comparing their mass spectra with $NIST$ 98 and Wiley 5 MS libraries, as well as by comparing their Kovats retention indices (RI), relative to a $C8-C23$ homologous series of n-alkanes and calculated according to Van Den Dool and Kratz [8], with literature values [9].

2.6. $DPPH$ assay

Antioxidant properties were evaluated in terms of ROS-scavenging activity by the DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) method according to Ghadermazi et al. [10] with slight modifications. The essential oil was at first solubilized in dimethyl sulfoxide (3mg/ml) and then diluted in methanol at final concentrations of 0.5 and 1.5 μg/ml. 270μl of $DPPH$ (0.028% w/v in methanol) was mixed with 30μl of each sample, and reaction mixtures were incubated in the dark for 20 minutes at room temperature. The reaction mixture without sample was used as a negative control, while ascorbic acid was tested as a positive control at a concentration of 1.25μg/ml. The absorbance was measured at 517nm using a microplate reader (Synergy HT, BioTek, United Kingdom) and ROS-scavenging activity percentage was calculated as follows:

$$\%\text{activity} = (A-B)/A100$$

where A is the absorbance of the negative control and B is the absorbance of the tested sample. Analyses were performed in triplicate.

3. Results and discussion

Steam distillation of flowers of C. guianensis afforded 361.38 mg, of yellowish oils representing 0.0736% w/w yields on dry vegetable material. $GC-MS$ and $GC-FID$ analyses led to the identification of 20 components Figure 2, listed in order of their elution and reported as percentages of the total essential oil. Table 1 shows the results of qualitative and quantitative oil analyses on the Elite-5MS column. Major constituents of the volatile fractions were found to be benzenoids accounting for 82, 89% of the total essential oil, from which eugenol (46, 20%) 2-phenyl ethanol (34, 82%), benzyl alcohol (1, 77%) and vanillin (0.20%) are the most abundant compounds. Oxygenated monoterpenes, representing the second large class (8, 38%), from which nerol (3, 95%), geraniol (3, 20%) and a-terpineol (0, 38%) are the most representative compounds. Oxygenated sesquiterpenes accounting for 6.07% are dominated by $(E)-(E)$-farnesol (5, 53%). Monoterpenes are represented by linalool (1, 64%). Despite the extraction techniques employed in the previous works are different [5–7], it is possible to make some comparisons regarding the chemical composition of the volatile fraction of C. guianensis samples from different countries. Benzenoids are the most abundant class of volatiles in C. guianensis species, from which eugenol was the dominant compound of volatiles from El Salvador (46, 20%) followed by Malaysia (41.6%) and Brazil (18.9%), surprisingly not detected in the volatiles from Ecuador.

2-Phenylethanol is present in high concentration in the samples from El Salvador (34, 82%) followed by Malaysia (3.2%) and absent in the samples from Brazil and Ecuador. Oxygenated monoterpenes are represented by nerol, the most abundant component in all samples, with the highest prevalence in the plants collected in Malaysia (9.8%), followed by Ecuador (5.9%), Brazil (5.5%) and El Salvador (3.9%). Another compound belonging to this class was geraniol, not detected in the sample from Ecuador but present in a high concentration (8.1%) in the sample from Brazil, followed by Malaysia (5.4%) and El Salvador (3.2%).

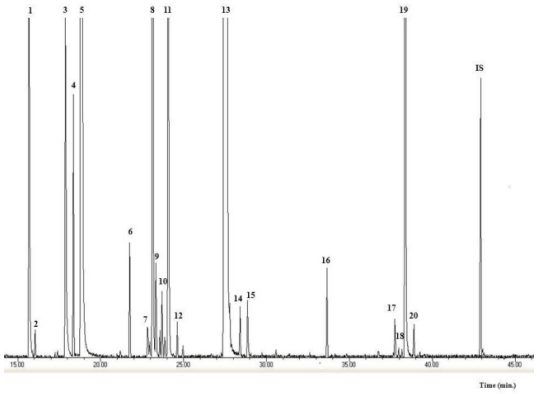

Figure 2. GC/MS trace of the essential oil from flowers of Couroupita guianensis. The main compounds are indicated by a number; for peak identification see Table 1. IS: internal standard (octyl octanoate).

Table 1. Percentage composition of the essential oil from flowers of Couroupita guianensis. RI^a: Retention Indices from the literature [9], RI^b: Retention Indices calculated by GC/FID using n-alkane series (from C_8 to C_{23}) under the same analytical conditions as for samples, ^{c}mean + SD of three determinations.

#	Compound	RI^a	RI^b	$\%^c$
1	Benzyl alcohol	1032	1034	1.77 ± 0.3
2	Phenylacetaldehyde	1042	1043	0.09 ± 0.1
3	Linalool	0.2955	1085	1.64 ± 0.8
4	α-terpinolene	1102	1100	0.87 ± 0.2
5	2-Phenylethanol	1107	1114	34.82 ± 0.7
6	α-terpineol	1189	1191	0.38 ± 0.5
7	Citronellol	1226	1221	0.13 ± 0.3
8	Nerol	1230	1230	3.95 ± 0.5
9	Isogeraniol - (Z)	1232	1235	0.36 ± 0.6
10	Isogeraniol-(E)	1244	1245	0.24 ± 0.4
11	Geraniol	1253	1256	3.20 ± 0.9
12	Geranial	1267	1272	0.10 ± 0.8
13	Eugenol	1359	1361	46.20 ± 1.2
14	Geranyl acetate	1381	1385	0.15 ± 0.4
15	Vanillin	1394	1399	0.20 ± 0.2
16	Nerolidol	1554	1556	0.30 ± 0.7
17	$(Z)-(E)$ - Farnesol	1701	1701	0.12 ± 0.3
18	$(Z)-(Z)$ - Farnesol	1718	1718	0.02 ± 0.6
19	$(E)-(E)$ - Farnesol	1725	1725	5.53 ± 0.9
20	$(E)-(Z)$ - Farnesol	1746	1745	0.10 ± 0.6
		Benzenoid		82.89
		Fatty acid derivatives		0.15
		Monoterpenes		2.51
		Oxygenated monoterpenes		8.38
		Oxygenated sesquiterpenes		6.07

Table 2. Antioxidant activity (mean ± SD) of C. guaianensis essential oil.

Sample	Concentration (ţg/ml)	DPPH radical scavenging (%)
C. guianensis essential oil	0.5	47.20 ± 1.25
	1.5	96.73 ± 1.08

Oxygenated sesquiterpenes are dominated by farnesol isomers, in detail $(E)-(E)$-farnesol is ubiquitous in all samples with different distributions (16.1% Brazil, 10.3% Malaysia, 5.53% El Salvador and 2.3% Ecuador). Interestingly, the sample from El Salvador is the only one in which four isomers of farnesol have been detected.

Given the volatile extracts composition, which included *ROS*-scavenging compounds, the antioxidant activity has been evaluated by *DPPH* method. The sample demonstrated good antioxidant activity (2) which is related to the presence of eugenol, the major component responsible for the *DPPH* free-radical scavenging activity, as previously reported from essential oil from Cinnamon bark [11], followed by benzyl alcohol, as previously reported for essential oils from Fagopyrum species, Anacamptis pyramidalis and Ophrys holosericea [12,13] and to a lesser extent by vanillin [14].

4. Conclusion

The present study showed that C. guianensis essential oil from El Salvador is a rich source of eugenol, which may be responsible for its antioxidant properties. Based on these results, further studies on the antibacterial and antifungal activity of C. guianensis essential oil are of fundamental importance. This project, which is in its initial phase, shows promising potential for future research in terms of bioactivity analysis as antimicrobial, anthelmintic, neuropharmacological, antihyperglycemic, antidiabetic and antiulcer [15], noting the importance of this plant for pharmaceutical formulations.

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Conflicts of Interest: "The authors declare no conflict of interest."

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