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Determination of chemical composition, antioxidant and antimicrobial properties of *Guzuma ulmifolia* essential oil

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ABSTRACT

In this study, the antioxidant and antimicrobial activities and essential oil composition of *Guazuma ulmifolia* were investigated. The essential oil extracted by hydrodistillation (Clevenger apparatus) was investigated by GC-MS technique and thirty-one compounds were identified, consisting of a complex mixture of sesquiterpenes and monoterpenes. The main components in the oil were thymol (20.97%), carvacrol (13.76%), eugenol (10.13%), spathulenol (7.09%), β-caryophyllene (6.74%), sabinene (5.18%), globulol (5.56%), γ-terpinene (3.27) and α-copaene (3.17%). The antioxidant property of the oil was assessed by free radical scavenging (DPPH) assay. *G. ulmifolia* essential oil presented interesting radical scavenging activity (IC_{50} = 7.61 ± 0.09 µg/mL). The antibacterial activity of the oils also was tested by broth dilution method against 12 microorganisms. Essential oil showed good activity against *P. aeruginosa* and *S. aureus* (MIC = 62.50 and 125.00 µg/mL, respectively), and moderately activity against *E. coli* and *S. epidermidis* (MIC = 500 and 750 µg/mL, repectively). Investigated essential oil has a certain level of antioxidant and antimicrobial effects, which may be attributed to their chemical compounds.

Keywords: *Guazuma ulmifolia,* Essential Oil, Antioxidant, Antimicrobial.

1. Introduction

Essential oils from aromatic and medicinal plants have been known to possess biological activity, notably antibacterial, antifungal and antioxidant activities [1, 2]. Biological activity of essential oils depends on their chemical composition determined by genotype and influenced by environmental and agronomic conditions [3, 4].

In recent years, the essential oils and herbal extracts have attracted a great deal of scientific interest due to their potential as a source of natural antioxidants and biologically active compounds [4-6]. The antimicrobial and antioxidant activities of essential oils have formed the basis of many applications, including fresh and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [6, 7]. Efforts have also been made to explore the potential of some essential oils for the treatment of infectious diseases in order to substitute standard pharmaceutical remedies [8].

Sterculiaceae family is widespread in the tropics of the world, especially in America and Africa, with about 68 classes and 430 species. *Guazuma ulmifolia* Lam. known as "chico-magro" or "mutamba" occurs in all of Latin. It is popularly used for the treatment of dandruff, hypercholesterolemic and to reduce [9]. Antimicrobial properties have been described previously from *G. ulmifolia* leaves and stem bark [10], species also showed antiulcer activity that is related to the presence of several anthocyanidins isolated from stem bark of *G. ulmifolia* ethyl acetate fraction [11]. In addition, this species showed promising antioxidant capacity, being related to the presence of phenolic compounds and flavonoids [9].

The literature search did not reveal any report on the essential oil composition of *G. ulmifolia*. The aim of the present work was to determine the chemical composition and evaluate the antioxidant and antimicrobial activity of the essential oil from leaves of *G. ulmifolia*, accessed by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) analysis, 1, 1-diphenil-2-picrylhydrazil (DPPH) method and microdilution assay.

2. Materials and Methods

2.1 Plant Collection and Extractions:

Leaves of *Guazuma ulmifolia* Lam. was collected in Tangará da Serra (Mato Grosso State in Brazil) in August of 2007 (coordinates 14º37"25'S and 57º29"15'W). Exsiccate was archived as voucher specimen in the herbarium of Department of Biology at Federal University of Santa Maria by register number SMBD 7508.

2.2 Extraction of the Essential oil

One hundred grams of plant material and 500 ml water were placed in a Clevenger type apparatus. The essential oil was isolated by hydrodistillation for 3 h. The obtained essential oil was separated, dried over anhydrous sodium sulphate, and stored under argon in a sealed vial, at -20 °C before usage [2]. The yield in terms of percentage of the fresh weight of the leaves was determined.

2.3 Gas Chromatography–Mass Spectrometry (GC-MS)

The analyses of the volatile compounds were run on a Agilent Technologies AutoSystem XL GC-MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (250°C). Two columns were used: a HP 5MS (30m x 0.25 mm; film thickness 0.25 mm) and an HP Innowax (30m x 0.32mm i.d., film thickness 0.50 mm). Oven temperature was programmed as following: isothermal at 70 °C for 4 min, then increased to 180 °C, at a rate of 4 °C/min and subsequently held isothermal for 15 min (for Innowax column); isothermal at 70 °C for 2 min, then increased to 200 °C, at a rate of 3 °C/min and held isothermal for 15 min (for 5MS column). The carrier gas was helium (1.3 ml/min). The injection port temperature was 250 ºC and the detector temperature was 280 °C. Ionization of the sample components was performed in the EI mode (70 eV). Injected volume was 1 µl.

2.4 Identification of the Components

Identification of the constituents was performed on the basis of retention index (RI), determined with reference of the homologous series of n-alkanes, C_7-C_{30}, under identical experimental conditions, comparing with the mass spectra library search (NIST and Wiley), and with the mass spectra literature date Adams [12, 13] The relative amounts of individual components were calculated based on the CG peak area (FID response).

2.5 Qualitative Analysis of Antioxidant Activity

Ten microlitres of 1:50 dilution of the essential oil in hexane was applied to TLC plates (silica gel 60 GF_{254}), quercetin and ascorbic acid (Sigma-Aldrich, ≥ 98% HPLC) standards also were used. The TLC plate was sprayed with a 0.2% 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution in methanol and left at room temperature for 30 minutes. Active compounds appear as yellow spots against a purple background, indicating possible antioxidant activity [14].

2.6 Quantitative Analysis of Antioxidant Activity

The antioxidant activity of the essential oil was evaluated by monitoring their ability in quenching the stable free radical DPPH, according to a slightly modified method previously described by Boligon et al. [14]. Spectrophotometric analysis was used to measure the free radical-scavenging capacity and to determine the scavenging concentration or inhibitory concentration (IC_{50}). The DPPH quenching ability was expressed as IC_{50} (the essential oil concentration (µg/mL) required to inhibit 50% of the DPPH in the assay medium). Six different ethanol dilutions of essential oil at 250, 125, 62.5, 31.25, 15.62 and 7.81 µg/mL were mixed with 1.0 mL of DPPH 0.3 mM in ethanol solution. After 30 min, absorption was measured at 518 nm, where the radical DPPH shows maximum absorption. A solution of DPPH (1 mL; 0.3 mM) in ethanol (2.5 mL) was used as a negative control and ascorbic acid in the same concentrations used for the essential oil provided the positive control. Ethanol was used to calibrate the spectrophotometer. The test was performed in triplicate and the calculation of the antioxidant activity followed the equation: % Inhibition = $[(A_0 - A_1)/A_0] \times 100$, where A_0 was the absorbance of the control sample (without essential oil) and A_1 was the absorbance in the presence of the sample [15].

2.7 Antimicrobial Assay Determination

The essential oil was evaluated against *Candida albicans* ATCC 28967, *Cryptococcus neoformans* ATCC 2857, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 51299, *Proteus mirabilis* ATCC 7002, S*taphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis*, *Malassezia sp.*, *Aspergillus sp.*, *Aeromonas sp.* and *Escherichia coli* (clinical isolates). The minimal inhibitory concentration (MIC) of the oil against the test microorganisms were determined by the broth microdilution method M27-A2 [16]. The experiments were repeated twice and the results were determined as an average value. Six different dilutions (1000, 750, 500, 250, 125, and 62.5 µg/mL) were prepared in DMSO. Bacterial strains were cultured overnight at 37 °C in Mueller-Hinton agar. Yeasts were cultured overnight at 30 °C in Potato dextrose agar. The first column of the plate was reserved for negative control wells (without inoculants) and the last column, for the positive growth control wells (without antimicrobial agents). The MIC was considered as the lowest concentration of the essential oil inhibiting the total growth of microorganisms. MIC was detected by lack of visual turbidity (matching the negative growth control).

2.8 Statistical Analysis

The obtained antioxidant and antimicrobial results were stated in mean ± standard deviation of three replicates.

3. Results and Discussion

The yield of essential oil of the fresh leaves of *G. ulmifolia* obtained by hydrodistillation was 0.53%. Using GC/MS analyses a total of thirty-one volatiles compounds were identified, representing 99.85% of the total composition (Table 1). Thymol (20.97%), carvacrol (13.76%) and eugenol (10.13%) were found as the major compounds. Other important compounds were spathulenol (7.09%), β-caryophyllene (6.74%), sabinene (5.18%), globulol (5.56%), γ-terpinene (3.27) and α-copaene (3.17%). Thymol and carvacrol, the most abundant components of this oil, has also been reported in the oil of other species such as *Satureja cuneifolia* (66.60%) and *Thymus vulgaris* (46.40%) [17, 18]

Many *in vitro* studies have addressed the antioxidant and radical-scavenging properties of essential oils [1, 3, 14, 15]. In particular, DPPH radical is widely used for quickly assessing the ability of antioxidants to transfer labile H atoms to radicals [18]. Following a similar line of thought, the essential oil was subjected to a preliminary test in order to verify the antioxidant activity using the DPPH free radical scavenging assay. Therefore, the anti-scavenging ability of the essential oil applied on silica gel TLC

plate was performed. One sample yellow spot could be observed immediately after spraying DPPH reagent on the TLC plate, suggesting some antioxidant activity for this oil, with intensity and color similar to quercetin and ascorbic acid used as standards. However, in order to get relevant data, a single method for testing antioxidant activities of essential oils is not recommended due to their complex composition. So, this test was the first step in the screening of the potential activity of this essential oil and DPPH test quantitative also was performed.

In the DPPH assay quantitative, antioxidants are typically characterized by their IC_{50} value, concentration necessary to reduce 50% of DPPH radical, lower IC_{50} value indicates higher antioxidant activity [17]. The efficiency of the essential oil of *G. ulmifolia* and ascorbic acid standard were evaluated for this method, and presented IC_{50} values of 7.61±0.09 and 15.98±1.30 µg/mL, respectively; compared to *Scutia buxifolia* (IC_{50} = 13.62 µg/mL), *Acacia Senegal* (IC_{50} = 17.89 µg/mL), *Thymbra capitatus* (IC_{50} = 19.27 µg/mL) and *Satureja cuneifolia* (IC_{50} = 65.10 µg/mL) [14, 17, 20, 21]. Also, DPPH scavenging abilities of the *G. ulmifolia* oil was more than that of synthetic antioxidant BHT (IC_{50} = 23.17 µg/mL) [17] these results proved that the essential oil from *G. ulmifolia* leaf possess significant antioxidant properties.

Essential oils rich in phenolic compounds such as carvacrol, thymol, spathulenol and eugenol are widely reported to possess high levels of antioxidant activity [1, 17, 22]. These compounds are also present in the essential oil of *G. ulmifolia*, and may account, in part, the good antioxidant potential reported here. The results presented here may contribute to the knowledge of the antioxidant potential of the essential oil and provide some information for its uses.

In addition, essential oil of *G. ulmifolia* leaves was tested also against 12 microorganisms; the antimicrobial screening is summarized in Table 2. The essential oil showed good activity against *P. aeruginosa* and *S. aureus* (MIC = 62.50 and 125.00 µg/mL, respectively), and moderately activity against *E. coli* and *S. epidermidis* (MIC = 500 and 750 µg/mL, respectively). Essential oils rich in carvacrol, thymol, spathulenol, 1, 8 cineole and eugenol demonstrated activity against Gram-positive and Gram-negative bacteria [20, 23, 24]. Aliphatic alcohols (e.g. linalool) were reported to possess strong to moderate activities several bacteria [17]. The antimicrobial effects of linalool, β-caryophyllene and caryophyllene oxide [25] were also reported.

The analysis of *G. ulmifolia* essential oil chemical composition, antioxidant and antimicrobial activities is the first work described in the literature for this species. According to the results of this study, the essential oil exhibited remarkable antioxidant and antimicrobial properties, may be suggested as a new potential source of natural antioxidant and antimicrobial for food industry.

Table 1: Chemical compounds present in *Guazuma ulmifolia* essential oil.

Rt (min)	Compounds	(%)	RI[a]	RI[b]	Mol. Formula
3.261	α-Pinene	1.07	939	939	$C_{10}H_{16}$
3.880	β-Pinene	0.35	981	980	$C_{10}H_{16}$
5.174	Sabinene	5.18	990	990	$C_{10}H_{16}$
10.625	*p*-Cymene	1.56	1025	1026	$C_{10}H_{14}$
10.851	α -Phellandrene	0.43	1028	1027	$C_{10}H_{16}$
11.381	1,8-Cineol	2.68	1029	1033	$C_{10}H_{18}O$
13.054	γ-Terpinene	3.27	1279	1251	$C_{10}H_{16}$
16.153	Thymol	20.97	1288	1290	$C_{10}H_{14}O$
16.947	Carvacrol	13.76	1298	1298	$C_{10}H_{14}O$
18.042	Eugenol	10.13	1357	1356	$C_{10}H_{12}O_2$
19.511	Thymol acetate	0.21	1357	1355	$C_{12}H_{16}O_2$
23.760	α-Copaene	3.17	1378	1376	$C_{15}H_{24}$
25.934	β-Cubebene	0.26	1400	1390	$C_{15}H_{24}$
28.486	β-Elemene	2.04	1390	1391	$C_{15}H_{24}$
28.627	Methyl eugenol	0.51	1400	1401	$C_{11}H_{14}O_2$
28.951	Bicycloelemene	1.93	1439	1435	$C_{15}H_{24}$
28.975	β-Caryophyllene	6.74	1443	1451	$C_{15}H_{24}$
29.002	α-Humulene	1.21	1454	1454	$C_{15}H_{24}$
30.165	Germacrene D	2.81	1477	1480	$C_{15}H_{24}$
31.931	Butylated hydroxytoluene	0.39	1509	1512	$C_{15}H_{24}O$
32.116	Camphor	0.96	1526	1521	$C_{10}H_{16}O$

32.471	Eugenol acetate	1.55	1537	1536	$C_{12}H_{16}O_3$
32.381	Linalool	2.12	1563	1557	$C_{10}H_{18}O$
33.502	Spathulenol	7.09	1577	1576	$C_{15}H_{24}O$
35.099	Globulol	3.56	1583	1583	$C_{15}H_{26}O$
35.710	Humulene epoxide	0.18	1598	1606	$C_{15}H_{24}O$
36.420	γ-Eudesmol	1.59	1630	1630	$C_{15}H_{26}O$
40.015	Cubenol	0.78	1643	1642	$C_{15}H_{26}O$
43.127	α-Eudesmol	0.34	1651	1652	$C_{15}H_{26}O$
5.618	n-Hexanol	2.73	867	867	$C_6H_{14}O$
34.701	Hexadecanol	0.28	1871	1879	$C_{16}H_{34}O$
Total identified (%)		99.85			

Relative proportions of the essential oil constituents were expressed as percentages. Rt = Retention time according their order on MS. [a]Retention indices experimental (based on homologous series of *n*-alkane C_7-C_{30}). [b]Retention indices from literature (Adams, 1995).

Table 2: Minimal inhibitory concentrations (MIC) of essential oil of the *G. ulmifolia* leaves.

Microorganisms	***Essential oil (µg/mL)***
P. aeruginosa	*62.50*
S. aureus	*125.00*
E. coli	*500.00*
S. epidermidis	*750.00*
C. albicans	*1000.00*
C. neoformans	*1000.00*
K. pneumoniae	*> 1000.00*
E. faecalis	*> 1000.00*
P. mirabilis	*> 1000.00*
Malassezia sp.	*> 1000.00*
Aspergillus sp.	*> 1000.00*
Aeromonas sp.	*> 1000.00*

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