



Chemical composition, antimicrobial and antioxidant potential of the essential oil of *Guarea kunthiana* A. Juss

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Abstract

The essential oils are extracted from plant compounds and can present activities antimicrobial and antioxidant properties. The goals of the present study were: (a) to determine the chemical composition of the essential oil of *Guarea kunthiana* A. Juss using the method of gas chromatography coupled to mass spectrometry (GC-MS); (b) to evaluate the antimicrobial potential of this oil using the broth microdilution method against different microorganisms: five Gram-negative bacteria, four Gram-positive bacteria and a yeast and (c) to determine the antioxidant activity of the oil using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical assay. The GC-MS analyses allowed identifying 13 constituents, representing 96.52% of the essential oil composition. The main compounds identified were α -zingiberene (34.48%), β -sesquiphellandrene (22.90%), and α -curcumene (16.17%). With respect to the antimicrobial activity, the essential oil was effective against all the microorganisms tested, except for the bacteria *E. coli* and *K. pneumoniae*, which were resistant to the action of the oil. From a general point of view, Gram-positive bacteria were more susceptible to the action of the essential oil than Gram-negative bacteria. The essential oil exhibited antioxidant potential.

Keywords: *Guarea kunthiana*, essential oil, chemical composition, antimicrobial activity, antioxidant.

Composição química, potencial antimicrobiano e antioxidante do óleo essencial de *Guarea kunthiana* A. Juss

Resumo

Os óleos essenciais são compostos extraídos de plantas e podem apresentar propriedades antimicrobianas e antioxidantes. O objetivo deste trabalho foi (a) determinar a composição química do óleo essencial de *Guarea kunthiana* A. Juss pelo método de cromatografia gasosa acoplada à espectrometria de massas (CG-EM); (b) avaliar o potencial antimicrobiano deste óleo pelo método de microdiluição em caldo frente a diferentes micro-organismos, sendo cinco bactérias Gram-negativas, quatro Gram-positivas uma levedura e (c) por fim, determinar a atividade antioxidante do óleo pelo método de captura do radical livre 2,2-difenil-1-picril hidrazil (DPPH). As análises de CG-EM resultaram na identificação de 13 constituintes, representando 96,52% da composição do óleo essencial. Os principais compostos identificados foram α -Zingibereno (34,48%), β -Sesquifelandreno (22,90%) e α -Curcumeno (16,17%). Em relação à atividade antimicrobiana, o óleo essencial foi efetivo frente a todos os micro-organismos testados exceto para as bactérias *E. coli* e *K. pneumoniae*, as quais se apresentaram resistentes à ação do óleo. Em geral, as bactérias Gram-positivas foram mais suscetíveis à ação do óleo essencial em relação às Gram-negativas. O óleo essencial apresentou potencial.

Palavras-chave: *Guarea kunthiana*, óleo essencial, composição química, atividade antimicrobiana, antioxidante.

1. Introduction

Brazil has the largest plant biodiversity in the world, with about 20% of the number of species on the planet. Essential oils with medicinal properties are among the main products of plant origin (Sartoratto et al., 2004) that have stood out in the industrial sector as ingredients in

food, cosmetic and sanitizers formulations, as well as in alternative medicine and natural therapies (Ceyhan et al., 2012; Scherer and Godoy, 2009). In addition, essential oils may have antimicrobial and insecticidal properties. Studies relating to these properties are of extreme relevance,

especially those addressing plants with unknown biological potential (Krifa et al., 2011).

Essential oils are chemically characterized as complex mixtures of low molecular weight compounds and, some of them, are highly volatile and capable of generating flavors and/or aromas (Trombetta et al., 2005). Scientific studies have shown the role of essential oils in biological interactions among plants and their potential therapeutic including anti-inflammatory, analgesic, anti-tumor, antifungal, and antibacterial activities (Siani et al., 2000; Silva et al., 2003; Sousa et al., 2004; Osei-Safo et al., 2010; Kaileh et al., 2007).

In addition to the abovementioned properties, many essential oils have been confirmed to possess antioxidant activity. They are extremely important for disease prevention, since they inhibit and delay the oxidation of biomolecules by preventing the initiation or propagation of chain oxidation reactions (Kaur and Kapoor, 2001; Bamoniri et al., 2010; Quariachi et al., 2011). Due to the harmful effects that synthetic antioxidants may cause, such as toxicity and carcinogenicity, the interest in the discovery of natural antioxidants has increased considerably (Losso et al., 2007).

The growing interest in natural bioactive compounds has led to conduct further studies addressing the replacement of synthetic chemical agents in the industrial sector, since natural products are less harmful to health (Gao et al., 2011), in addition to being biodegradable and usually exhibiting low toxicity in mammals (Figueiredo et al., 2008).

The family Meliaceae has pantropical distribution, including about 50 genera and 600 species. In Brazil, there are six genera and about 100 species (Lorenzi and Matos, 2008). The members of this family have a wide diversity of chemical compounds, including limonoids, triterpenes, steroids, diterpenes, sesquiterpenes and coumarins (Cortez et al., 2000; Lago et al., 2000, 2002; Soares et al., 2012; Scur et al., 2016). Limonoids are the most abundant compounds and, probably, the largest representatives of the class of terpenes with insecticidal activity (Luo et al., 1999). In addition to these properties, these compounds can also perform as antitumor, antifungal, bactericidal, antiviral (Champagne et al., 1992), antioxidant (Jayaprakasha and Patil, 2007), leishmanicidal (Lima, 2006), and antimalarial (Kaur et al., 2009) agents.

One of the species belonging to the family Meliaceae is *Guarea kunthiana* A. Juss, popularly known as *jatuauba*, *figo-do-mato*, *peloteira*, and *jitó*. It is a tree native to Brazil and has wide geographic distribution throughout the national territory (Lorenzi, 2009). Studies conducted on extracts from the roots of this plant have reported antiparasitic (Lima, 2006; Mesquita et al., 2005) and insecticidal (Coelho, 2006) activities. Phytochemical studies have indicated the occurrence of diterpenes and sesquiterpenes in ethanolic extract obtained from the leaves of *G. kunthiana* (Garcez et al., 2004) and a limonoid eucadorin isolated from the dichloromethane extract of the leaves (Mootoo et al., 1992). However, the antimicrobial and antioxidant potential and the chemical composition of the essential oil of *G. kunthiana* have not been reported in the literature.

Thus, the goal of the present study was to determine the chemical composition of the essential oil of *G. kunthiana* and its antimicrobial potential against different microorganisms (five Gram-negative bacteria, four Gram-positive bacteria, and yeast *Candida albicans*), in addition to assessing its antioxidant activity.

2. Material and Methods

2.1. Collection and identification of plant material

The leaves of *G. kunthiana* were collected from September to December 2014 in a rural property of the western region of the State of Paraná, Brazil (Latitude 24°31' S, Longitude 53°44' W, altitude of 442 m). Drying of the leaves was held in an oven at 35 °C for subsequent milling in a knife mill until obtaining the crushed plant material with particle size less than 0.42 mm. An exsiccate specimen was sent to the Herbarium of the State University of Oeste do Paraná (UNOP) for botanical identification, deposited under number 7843 Pandini, J. A.

2.2. Obtaining essential oil

Aerial parts of *G. kunthiana* (60 g) were submitted to extraction by hydrodistillation in 700 mL of distilled water for four hours using a Clevenger-type apparatus. The essential oil were collected, dried over anhydrous sodium sulphate (~1 g), and stored at 4 °C until analyzed. The essential oil yield was 0.35% (w/w) dry weight.

2.3. GC-MS analysis

The analyses of the essential oil compounds was performed using a FOCUS GS gas chromatograph (Thermo Electron) coupled to a DSQ II mass spectrometer (Thermo Electron) and a detector with electron ionization impact at 70 eV and quadrupole-type mass analyzer. Chromatographic separation was carried out using a DB-5 fused-silica capillary column (30 m x 0.25 mm inner diameter, film thickness 0.25 µm) and 5% phenyl/95% dimethylpolysiloxane stationary phase.

The injector temperature was 250 °C and the carrier gas flow was kept constant at 1 mL.min⁻¹. The sample and the alkane standards C7-C28 were injected at a split-ratio of 1:25. The temperature program was: initial temperature of 50 °C/2 min; followed by an increase to 180 °C/2 °C min⁻¹, and 290 °C/5 °C min⁻¹. The interface between the GC and the MS was kept at 270 °C, and the temperature of the ionization source for the mass spectrometric analysis was 250 °C. The identification of the compounds was accomplished by comparing their retention times with the retention times obtained from the literature (Adams, 2007) and through their Retention Indices.

2.4. Antimicrobial activity

2.4.1. Microorganisms and test conditions

The essential oil of the plant was tested against different microorganisms: five Gram-negative bacteria (*Escherichia coli* ATCC 25922; *Salmonella enterica* subsp. *Enterica* ATCC 14028; *Pseudomonas aeruginosa* ATCC

27853; *Proteus mirabilis* ATCC 25933; and *Klebsiella pneumoniae* ATCC 13883); four Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923; *Enterococcus faecalis* ATCC 19433; *Staphylococcus epidermidis* ATCC 12228; and *Bacillus subtilis* CCD-04); and yeast *Candida albicans* ATCC 10231 (American Type Culture Collection, USA).

For the test, the microorganisms were grown in brain heart infusion enrichment broth (BHI) and incubated at 36 ± 0.1 °C for 24 hours. After this period, microbial strains were standardized in saline solution (0.85%) until they reached the final concentration of 1×10^5 UFC.mL⁻¹, with the exception of yeast *C. albicans* that was diluted at the final concentration of 1×10^6 UFC.mL⁻¹ to serve as inoculum.

2.4.2. Determination of minimal inhibitory concentration (MIC)

Minimal inhibitory concentration (MIC) was determined as the lowest oil concentration able to inhibit microbial growth. The microdilution test was performed according to the standards proposed by the Clinical Laboratory Standards Institute – CLSI (2007).

The essential oil was diluted with methanol and Mueller-Hinton broth for testing with the bacteria, and methanol and RPMI 1640 broth for *C. albicans* at a proportion of 1:10 until reaching the concentration of 7000 µg.mL⁻¹. A total of 150 µl of Mueller-Hinton broth for the bacteria and RPMI 1640 broth for *C. albicans* were distributed from the second column in 96-well microtiter plates. The first columns received 300 µl of oil of *G. kunthiana* and, thereafter, dilutions of 7000-3.4 µg.mL⁻¹ were performed. Finally, 10 µL of inoculum were added to each well and the plates were incubated at 36 ± 0.1 °C for 18-24 hours. Subsequently, an aliquot of 10 µL 10% triphenyltetrazolium chloride (TTC) was added and the plates were again incubated at 36 ± 0.1 °C for three hours. The presence of red color was considered a negative evidence of inhibitory effect of the essential oil.

2.4.3. Determination of the minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC)

An aliquot of 10 µL was withdrawn from the wells where there was non-visible bacterial growth before the addition of TTC and inoculated on the surface of the Mueller-Hinton broth. The plates were incubated at 36 ± 0.1 °C for 24 hours and, after this period, the MBC and MFC were defined as the lowest concentration of essential oil capable of causing the death of the inoculum (Santúrio et al., 2007).

Methanol was used as negative control, and gentamicin for bacteria and nystatin for *C. albicans* were used for positive control. Both of them were tested at concentrations of 100-0.78 µg .mL⁻¹

2.4.4. Antioxidant activity

The antioxidant activity was determined using the method of reducing the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) based on the method proposed by Scherer and Godoy

(2009), Rufino et al. (2007), and Weber et al. (2014). Hundred microlitres of various concentrations of the samples (11,71 - 6000 µg.mL⁻¹) were added to 3.9 mL of DPPH methanolic solution (0.2 mM) and slightly homogenized in a tube agitator. After agitation, the tubes were left to stand for 30 minutes in the dark. After the reaction time, the absorbance of samples was measured at 515 nm. An aliquot of 0.1 mL of control solution (methyl alcohol, acetone, and water) was used for the negative control and the synthetic antioxidant butylhydroxytoluene (BHT) was used for the positive control under the same conditions of the negative control. Methyl alcohol was used as blank for the calibration of the spectrophotometer. The ability of free radical sequestration was expressed by the equation $I\% = [(Abs_0 - Abs_1) / Abs_0] \times 100$, where Abs_0 is the absorbance of the control and Abs_1 is the absorbance of the sample. The IC_{50} (amount of antioxidant substance required to reduce by 50% the initial DPPH concentration) was calculated on the basis of the equation of the line obtained from the calibration curve. The tests were carried out in triplicate.

2.4.5. Analysis of data

The data obtained by calculations of DPPH radical sequestration capacity and the IC_{50} were assessed using T test, at 5% significance, employing the Sisvar software (Ferreira, 2011).

3. Results and Discussion

The GC-MS analysis identified 13 compounds, representing 96.52% of the total composition of the essential oil. The major compounds were α -zingiberene (34.48%), β -sesquiphellandrene (22.90%), and α -curcumene (16.17%) (Table 1).

The compounds identified were mostly sesquiterpenic hydrocarbons (84.37%), followed by kaurane diterpenes (9.95%), and oxygenated sesquiterpene spathulenol (2.20%). The occurrence of sesquiterpenes has been well evidenced in the essential oils of different *Guarea* species, such as: *G. convergens*; *G. humaitensis*; *G. scabra*; and *G. silvatica*, which exhibited mainly the presence of sesquiterpenic and oxygenated hydrocarbons, and kaurane diterpenes. Lago et al. (2000) assessed the compounds present in the essential oil of *G. guidonia* and only reported the occurrence of sesquiterpenes. Studies on the species *G. macrophylla* reported the occurrence of oxygenated sesquiterpenes, hydrocarbons, diterpenes, and also fatty acids exhibiting variations in different times of the year. In the summer, the sesquiterpene guai-6-en-10 β -ol was the major compound. On the other hand, γ -cadinene was the major compound in spring (Lago et al., 2000, 2006).

The variation of the chemical compounds present in essential oils can be attributed to different factors: seasonality; circadian rhythm; age and development of the plant; temperature; water availability; ultraviolet radiation; nutrient content; altitude; atmospheric pollution; and attack by pathogens (Gobbo-Neto and Lopes, 2007). Changes in some of these factors significantly influence the yield and composition of essential oils (Dudareva et al., 2004), which

reflects the differences found when comparing different studies and different species.

With respect to the antimicrobial potential, the essential oil of *G. kunthiana* exhibited activity against all the microorganisms tested, except for the bacteria *E. coli* and *K. pneumoniae*, which were resistant to the action of the oil. MIC values ranged from 13.6 to 3500 $\mu\text{g}\cdot\text{mL}^{-1}$ and MBC values from 54.6 to 3500 $\mu\text{g}\cdot\text{mL}^{-1}$ for Gram-positive bacteria, and MIC and MBC of 7000 $\mu\text{g}\cdot\text{mL}^{-1}$ for Gram-negative bacteria. For *C. albicans*, the MIC and MBC values were 1750 and 3500 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively (Table 2).

Gram-positive bacteria were more susceptible to the action of the essential oil than Gram-negative bacteria. There are no reports in the literature on the antimicrobial potential of the essential oil of *G. kunthiana* and studies on

antimicrobial testing with essential oils of plants from the family Meliaceae are scarce. Data similar to those found in the present study were reported by Sairam et al. (2000) who assessed the antibacterial and antifungal potential of the essential oil of *Azadirachta indica* (Meliaceae) and found greater antimicrobial activity of the essential oil against Gram-positive bacteria than against Gram-negative bacteria. Aromdee and Sriubolmas (2006) assessed the antimicrobial potential of the mentioned plant and observed moderate antimicrobial activity against *B. subtilis* and *C. albicans*. On the other hand, Parthasarathy and Thombare (2013) reported activity only for *Staphylococcus auricularis* using the essential oil of this species.

The mechanisms of action of essential oils in bacterial cells involve different targets. One of the most important

Table 1. Volatile components of the essential oil of *Guarea kunthiana* obtained by GC-MS analysis.

RT (min) ^a	Components ^b	Area (%)	RI ^c
35.27	α -copaene	0.67	1373
36.36	β -elemene	5.19	1386
37.85	E-Caryophyllene	0.48	1414
39.05	α -bergamoten	1.91	1434
40.51	β -farnesene	0.44	1459
41.59	Germacrene D	0.52	1477
41.93	α -curcumene	16.17	1482
42.72	α -zingiberene	34.48	1495
44.18	Calamenene	1.27	1520
44.33	β -sesquiphellandrene	22.90	1523
47.18	Spathulenol	2.20	1573
52.58	Cadalene	0.34	1671
69.66	Kaurene	9.95	2033
Total		96.52	-
Sesquiterpenic hydrocarbons		84.37	-
Oxygenated sesquiterpenes		2.20	-
Diterpenes		9.95	-

^aRetention time in minutes; ^bCompounds identified in the DB-5ms capillary column; ^cLiterature retention indices.

Table 2. Minimal inhibitory concentration, minimal bactericidal concentration, and minimal fungicidal concentration of the essential oil of *Guarea kunthiana* against microorganisms tested.

Microorganisms	MIC/MBC (MFC) ^{a,*}		
	Essential oil ^b	Gentamicin ^c	Nystatin ^d
<i>E. coli</i>	Na	.78/.78	Nt
<i>P. aeruginosa</i>	7000/7000	.78/.78	Nt
Gram - <i>S. enterica</i>	7000/7000	.78/.78	Nt
<i>P. mirabilis</i>	7000/7000	.78/.78	Nt
<i>K. pneumoniae</i>	Na	.78/.78	Nt
<i>S. aureus</i>	13.6/54,6	.78/.78	Nt
Gram + <i>E. faecalis</i>	437.5/875	.78/.78	Nt
<i>S. epidermidis</i>	3500/3500	.78/.78	Nt
<i>B. subtilis</i>	875/875	.78/.78	Nt
Yeast <i>C. albicans</i>	1750/3500	Nt	.78/.78

^aMinimal inhibitory concentration (MIC)/minimal bactericidal concentration (MBC)/minimal fungicidal concentration (MFC);

* $\text{mg}\cdot\text{mL}^{-1}$; ^bTested at a concentration of 7000-3.4 $\mu\text{g}/\text{mL}$; ^cTested at a concentration of 100-0.78 mg/mL ; ^dTested at a concentration of 100-0.78 $\mu\text{g}\cdot\text{mL}^{-1}$; Na = no activity; Nt = Not tested. Methanol showed no activity.

features is the hydrophobicity of their components, which allows the partition of lipids of the bacterial cell membrane and mitochondria, disrupting the structures and leading to the leakage of the cell content (Burt, 2004). The greatest resistance of Gram-negative bacteria to the action of essential oils can occur due to the complexity of the double membrane of these microorganisms, which limits the diffusion of hydrophobic compounds through the lipopolysaccharide component (Burt, 2004; Holley and Patel, 2005).

The antimicrobial activity of essential oils depends on the chemical composition of the plant, which can vary according to the period of the year (Yesil-Celiktas et al., 2007). Among the compounds of the essential oil of *G. kunthiana* is α -zingiberene. It has antimicrobial properties (Croteau et al., 2000) as reported for *Zingiber officinale* (ginger). This compound is the most prevalent in the essential oil of *Z. officinale* and has exhibited significant activity against some Gram-positive and Gram negative bacteria (Andrade et al., 2012). Antibacterial and antifungal properties are attributed to the other compounds, such as E-caryophyllene and germacrene-D (Costa et al., 2010; Veiga-Júnior and Pinto, 2002). On the other hand, spathulenol has antibacterial properties (Pacciaroni et al., 2000), which can explain the activity found.

Although some of these compounds feature low concentrations, they can have an effect on the overall efficiency of the antimicrobial activity of the essential oil through synergistic interaction with the other constituents (Burt, 2004; Vagionas et al., 2007; Giles et al., 2010). Although the major components many times account for more than 85% of the chemical characterization of essential oils, their proportions are not related to their great activity, which may be fundamental for the pharmacological action of the compounds with very small proportions (Galindo et al., 2010).

With respect to the results of antioxidant activity, it should be noted that IC_{50} values were inversely related to the percentage of DPPH scavenging capacity, i.e., the higher the scavenging rate, the lower the IC_{50} value. T test was used to assess the difference between the means, considering p -value less than 0.05 ($p < 0.05$) statistically significant (Table 3).

The analysis of the results of antioxidant activity of the essential oil demonstrated that there were significant differences between the essential oil and the commercial synthetic antioxidant (BHT). However, the difference in IC_{50} values between BHT values and the essential oil was small (9.27 for BHT and 17.54 for the essential oil). The same

fact can be observed when comparing the percentage of DPPH radical scavenging capacity (95.85% for the BHT and 91.52% for the essential oil), which demonstrates that the essential oil exhibited greater antioxidant activity at this concentration. At the other concentrations assessed, the essential oil exhibited low DPPH radical scavenging capacity (Table 3).

The free radical scavenging capacity of the essential oil of *G. kunthiana* can be attributed to the presence of some compounds, such as α -zingiberene that plays an important role in defending some plants against oxidation (Rice-Evans et al., 1997). Other compounds—such as E-caryophyllene—have recognized antioxidant activity, and they can have this capacity increased by the synergistic effect with other compounds (Shahidi et al., 1992; Morais et al., 2006).

The results of the antioxidant activity reported in the literature are difficult to compare, since it is strongly influenced by the determination method. Several methods have been described for assessing the antioxidant activity of chemicals present in essential oils and plant extracts. Some authors propose tests that rely on reducing free radicals generated *in vitro*, resulting from the antioxidant activity of substances assessed, especially the DPPH method, because it is a quick and feasible alternative (Molyneux, 2004).

The products derived from plants serve as a prototype in the control of growth of pathogenic microorganisms and production of less toxic and more effective medicines (Ahmad and Beg, 2001; Kelmanson et al., 2000). There is a growing concern on the part of industries to use less aggressive compounds and the non-inclusion of synthetic raw materials for the preservation of products (Packer and Luz, 2007). As a result, studies have been conducted on essential oils for the discovery of new compounds with antimicrobial and antioxidant potential, since these two mechanisms increase significantly the use of the products (Guleria and Kumar, 2006; Quariachi et al., 2011).

The present study is the first report in the literature addressing the chemical composition and antimicrobial and antioxidant potential of the essential oil of *G. kunthiana*, and it can serve as the basis for conducting further studies on plants that exhibit unknown biological potential. This way, it is worth noting the importance of phytochemical studies, since they may reveal the presence of active compounds that can explain the biological potentials found.

The essential oil of *G. kunthiana* has the potential to be used in the food, cosmetics, and pharmaceutical industries, since it exhibits antimicrobial and antioxidant properties.

Table 3. Index of DPPH (2,2-diphenyl-1-picrylhydrazyl) (scavenging %) and IC_{50} of the different concentrations of essential oil of *Guarea kunthiana* tested.

Test solutions	DPPH scavenging capacity (%)	IC_{50} $\mu\text{g.mL}^{-1}$
BHT	95.85 \pm 0.04 ^a	9.27 \pm 0.08 ^a
<i>G. kunthiana</i> oil	91.52 \pm 0.09 ^b	17.54 \pm 0.18 ^b

BHT = commercial synthetic antioxidant; DPPH = 2,2-diphenyl-1-picrylhydrazyl; IC_{50} = half maximal inhibitory concentration; the values correspond to the mean and standard deviation of triplicates; values followed by the same letter in the column do not differ between them according to T test ($p < 0.05$).

It is important to stress the importance of further studies, mainly for determining the mechanism of action of this essential oil, as well as the action of its compounds tested in isolation and synergy.

4. Conclusion

The GC-MS analysis of the essential oil of *G. kunthiana* revealed the presence of 13 compounds, among which α -zingiberene, β -sesquiphellandrene, and α -curcumene were the major components. With respect to testing the antimicrobial activity, the essential oil was effective against all the microorganisms tested, except for *E. coli* and *K. pneumoniae*. From a general point of view, Gram-positive bacteria were more susceptible to the action of the essential oil than Gram-negative bacteria. Regarding the antioxidant activity, the oil was effective exhibiting values close to those of the synthetic antioxidant.

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