Essential Oil Composition, Antioxidant and Antimicrobial Activities of Guazuma Ulmifolia from Brazil

Aline Augusti Boligon*, Andriéli Cassel Feltrin, Amanda Leitão Gindri and Margareth Linde Athayde
Phytochemical Research Laboratory, Department of Industrial Pharmacy, Federal University of Santa Maria, Build 26, room 1115, Santa Maria, RS 97105-900, Brazil

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 (IC50 = 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, respectively). 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; Nitoxidant; Antimicrobial

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 antitoxic activity that is related to the presence of several anthocyanids 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 [11].

The literature search did not reveal any report on the essential oil composition of G. ulmifolia. However, in a study conducted by Reid et al. [12] with five species of Sterculiaceae family, was verified the presence of essential oils in three species. Chemical composition of essential oil of Helicteres guazumifolia leaves (Sterculiaceae) was investigated, and the main components essential oil this species are non-terpenoids volatile secondary metabolites (30.28%) [13].

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.

Materials and Methods

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 14°37’25’S and 57°29’15’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.

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.

Gas chromatography–mass spectrometry (GC-MS)

The analyses of the volatile compounds were run on an Agilent Technologies AutoSystem XL GC-MS system operating in the EI mode.

*Corresponding author: Aline Augusti Boligon, Phytochemical Research Laboratory, Department of Industrial Pharmacy, Federal University of Santa Maria, Build 26, room 1115, Santa Maria, RS 97105-900, Brazil; Tel: +55 55 3220 9618; Fax: +55 55 3220, 8248; E-mail: alineboligon@hotmail.com

Received June 11, 2013; Accepted June 28, 2013; Published July 01, 2013


Copyright: © 2013 Boligon AA, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
at 70 eV, equipped with a split/splitless injector (250°C). Two columns were used: a HP 5MS (30 m×0.25 mm; film thickness 0.25 mm) and an HP Innowax (30 m×0.32 mm 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°C 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.

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, Cn-C30, under identical experimental conditions, comparing with the mass spectra literature data Adams [14,15]. The relative amounts of individual components were calculated based on the CG peak area (FID response).

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 [16].

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. [16]. Spectrophotometric analysis was used to measure the free radical-scavenging capacity and to determine the scavenging concentration or inhibitory concentration (IC50). The DPPH quenching ability was expressed as IC50 (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 scavenging activity followed the equation: % Inhibition=[(A0−A1)/A0]×100, where A0 was the absorbance of the control sample (without essential oil) and A1 was the absorbance in the presence of the sample [17].

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, Staphylococcus aureus ATCC 29213, Staphylococcus epidermidis, Malassezia sp., Asperillus 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 M2-A2 [18]. 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).

Statistical analysis

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

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 α-Humulene (6.74) were major components. The two main classes of compounds were monoterpenes (84.88%) and sesquiterpenes (15.12%). The major monoterpenes were thymol (20.97%), carvacrol (13.76%) and γ-Terpinene (10.85). The main sesquiterpenes were α-Humulene (6.74), germacrene D (4.30), and α-Patchoulene (3.27). The relative proportions of the essential oil constituents were expressed as percentages. Rt=Retention time according their order on MS.

Relative proportions of the essential oil constituents were expressed as percentages. Rt=Retention time according their order on MS.

Retention indices experimental (based on homologous series of n-alkane Cn-C30).

Retention indices from literature (Adams, 1995).

Table 1: Chemical compounds present in Guazuma ulmifolia essential oil.
The essential oil of *G. ulmifolia* leaves was tested also against 12 microorganisms; the antimicrobial screening is summarized in Table 2. 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 [23-25]. Aliphatic alcohols (e.g. linalool) were reported to possess strong to moderate activities several bacteria [20]. The antimicrobial effects of linalool, β-caryophyllene and carvophyllene oxide [26] were also reported.

The analysis of *G. ulmifolia* essential oil chemical composition, antioxidant and antimicrobial activities is the first work described in 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.

**Acknowledgments**

The authors would like to thank the professors from NAPO (Center for Analysis and Organic Research at Federal University of Santa Maria) for providing the GC/MS chromatograms and spectra and A.F. Morel (Department of Chemistry at Federal University of Santa Maria) for the assessment of the n-alkane series. The authors thank the financial support of CAPES and CNPq (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Conselho Nacional de Desenvolvimento Científico e Tecnológico/Brazil).

**References**


<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Essential oil (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>62.50</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>125.00</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>500.00</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>750.00</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>1000.00</td>
</tr>
<tr>
<td><em>C. neoformans</em></td>
<td>1000.00</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>&gt; 1000.00</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>&gt; 1000.00</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>&gt; 1000.00</td>
</tr>
<tr>
<td>Malassezia sp.</td>
<td>&gt; 1000.00</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>&gt; 1000.00</td>
</tr>
<tr>
<td>Aeromonaas sp.</td>
<td>&gt; 1000.00</td>
</tr>
</tbody>
</table>

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


