Boligon et al., Pharmaceut Anal Acta 2012, 3:10
DOI: 10.4172/2153-2435.1000199

Pharmaceutica Analytica Acta

Research Article Open Access

Chemical Composition, Antioxidant and Antimicrobial Activities of the Essential Oil of *Scutia buxifolia* Reissek Leaves

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Abstract

The chemical composition, antioxidant and antibacterial activities of essential oil isolated by hydrodistillation from the leaves parts of *Scutia buxifolia* were evaluated. The chemical composition was analyzed by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Twenty-five substances were identified, consisting of a complex mixture of sesquiterpenes (73.69%) and monoterpenes (18.74%). The main components in the oil were spathulenol (27.09%), β-cubebene (11.26%), germacrene D (9.81%), carvacrol (7.01%), globulol (5.36%), α-copaene (4.17%), γ-eudesmol (3.59%), thymol (3.27%), 1,8-cineol (3.08%), *p*-cymene (2.56%), α-eudesmol (2.34%), β-elemene (2.04%), butylated hydroxytoluene (2.00%) along with eugenol acetate, n-hexanol, α-pinene, α-humulene, eugenol, humulene epoxide, phytol as minor constituents. The antioxidant property of the oil was assessed by free radical scavenging (DPPH) assay. *S. buxifolia* essential oil presented interesting radical scavenging activity (IC_{50}=13.62 ± 0.17 μg/mL). The antibacterial properties of the oils also was tested by broth microdilution method and was effective only against *S. aureus* and *E. coli* (MIC=500 and 750 μg/mL, respectively). To the best of our knowledge, this is the first study of the composition, antioxidant and antimicrobial activities of essential oil from the *S. buxifolia* collected from Brazil.

Keywords: *Scutia buxifolia;* Essential oil; Antioxidant; Antimicrobial

Introduction

Essential oils in plant are complex volatile mixtures exist at low concentrations and are commonly found in aromatic plants [1,2]. Studies have demonstrated beneficial properties of essential oils in the prevention and treatment of cancer, cardiovascular diseases including atherosclerosis and thrombosis, as well as their bioactivity as antibacterial, antiviral, antioxidants, antidiabetic, anti-inflammatory agents, local anaesthetic and immunomodulatory [1,3-5].

Biologically, the essential oils perform the function of adaptation of the plant to the environment, acting in the defense against the attack of predators, attraction of pollination agents, protection against water loss and temperature increase and as inhibitors of germination [6,7]. Economically, they are employed in food, cosmetic and cleaning products industries, as well as in alternative medicine due to their many therapeutic properties.

Scutia buxifolia Reissek belong to the Rhamnaceae family and is popularly known as "coronilha". It is native tree from South America, with a dispersion area that comprises Rio Grande do Sul State in Brazil, and the countries Argentina and Uruguay. The root bark infusion is popularly used as cardiotonic, antihypertensive and diuretic [8]. Antimicrobial activities of some cyclopeptide alkaloids isolated from the root bark of this species were reported by Morel et al. [9] using the bioautography method. Cytotoxicity of extracts from leaves, twigs and stem bark of the plant was evaluated by the *Artemia salina* assay, as well as the antimicrobial activity against a panel of microorganism strains [10]. Extracts from the leaves and stem bark of *S. buxifolia* were effective inhibitors of TBARS production and also presented DPPH scavenger activity [11].

The literature search did not reveal any report on the essential oil composition of *S. buxifolia*. 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 *S. buxifolia*, 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 material

Scutia buxifolia (Rhamnaceae) leaves were collected in Dom Pedrito, State of Rio Grande do Sul, Brazil, on June of 2011 (coordinates 30°59'09"S and 54°27'44"W). It was identified and archived as voucher specimens in the herbarium of Department of Biology at Federal University of Santa Maria by register number SMBD 10919.

Extraction of the essential oil

The fresh material (250 g) of the plant leaves was extracted using a hydrodistillation process in a Clevenger apparatus for 4 hours. Oil was dried over anhydrous sodium sulphate and, after filtration, stored at -4°C until test and analysis. The yield in terms of percentage of the fresh weight of the leaves was determined.

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Received November 22, 2012; **Accepted** December 09, 2012; **Published** December 13, 2012

Citation: Boligon AA, Schwanz TG, de Brum TF, Frohlich JK, Nunes L, et al. (2012) Chemical Composition, Antioxidant and Antimicrobial Activities of the Essential Oil of *Scutia buxifolia* Reissek Leaves. Pharmaceut Anal Acta 3: 199. doi:10.4172/2153-2435.1000199

Gas chromatography (GC-FID)

The gas chromatography (GC) analyses were carried out using an Agilent. Technologies 6890N GC-FID system, equipped with DB-5 capillary column (30 m×0.25 mm; film thickness 0.25 mm) and connected to an FID detector. The injector and detector temperatures were set to 280°C. The carrier gas was helium, at a flow rate of 1.3 mL/min. The thermal programmer was 50-300°C at a rate of 5°C/min. Two replicates of samples were processed in the same way. Component relative concentrations were calculated based on GC peak areas without using correction factors. The injection volume of the oil was 1 µL [4,12].

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were performed 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). The transfer line temperature was 280°C. Helium was used as carrier gas (1.3 mL/min) and the capillary columns used were an 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). The temperature programmer was the same as that used for the GC analyses. The injected volume was 1 µL of the essential oil.

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 [13]. 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 methanol 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].

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 Mensor 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=$[(A_0-A_1)/A_0]\times100$, 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 [11].

Antimicrobial assay determination

The essential oil was evaluated against *Candida albicans* ATCC 28967, *Cryptococcus neoformans* ATCC 2857, *K. pneumoniernaae* ATCC 700603, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 51299, *P. mirabilis* ATCC 7002, *S. aureus* ATCC 29213, *Malassezia sp*., *Aspergillus sp*., *Aeromonas sp*., *S. aureus* and *E. coli* (clinical isolates). The minimal inhibitory concentration (MIC) of the oil against the test microorganisms were determined by the broth microdilution method M27-A2 [15]. 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 pale yellowish essential oil of the fresh leaves of *S. buxifolia* was obtained by hydrodistillation in the yield of 0.4%. Essential oil was analyzed by GC-FID and GC-MS systems and the oil components were identified both quantitatively and qualitatively. Twenty-five components, representing 98.38% of the total composition, were identified, of which 73.69% are sesquiterpenes and 18.74% are monoterpenes (Table 1).

The main components in the oil were spathulenol (27.09%), β-cubebene (11.26%), germacrene D (9.81%), carvacrol (7.01%), globulol (5.36%), α-copaene (4.17%), γ-eudesmol (3.59%), thymol (3.27%), 1,8-cineol (3.08%), *p*-cymene (2.56%), α-eudesmol (2.34%), β-elemene (2.04%), butylated hydroxytoluene (2.00%) along with eugenol acetate, n-hexanol, α-pinene, α-humulene, eugenol, humulene epoxide, phytol as minor constituents. In addition, *p*-cymene showed a maximum when carvacrol was at its minimum, which is in agreement with the literature, reporting that *p*-cymene is the precursor of carvacrol [16].

Spathulenol, the most abundant component of this oil, has also been reported in the oil of other species such as *Baccharis uncinella* (16.41%), *Stevia rebaudiana* (15.41%), *Origanum vulgare* (11.67%) and *Baccharis dracunculifolia* (9.54%) [17-19]. The second major component of the oil, β-cubebene, has also been found in the oils of *Dendropanax morbifera* [20] and *Cinnamomum osmophloeum* [21] in about 4.19% and 59.4%, respectively. Germacrene D, the third major compound present, has also been found in the oil of *Artemisia annua* (15.64%), *Baccharis uncinella* (14.87%), *Campomanesia adamantium* (11.82%), *Tagetes minuta* (10.00%) and *Origanum vulgare* (8.11%) [19,22].

Many *in vitro* studies have addressed the antioxidant and radical-scavenging properties of essential oils [1,23]. In particular, DPPH

Rt (min)	Compounds	(%)	RI[a]	RI[b]	Mol. Formula
	Monoterpenes				
3.261	α-Pinene	1.27	939	939	$C_{10}H_{16}$
3.880	β-Pinene	0.42	981	980	$C_{10}H_{16}$
10.625	*p*-Cymene	2.56	1026	1026	$C_{10}H_{14}$
11.381	1,8-Cineol	3.08	1029	1033	$C_{10}H_{18}O$
16.153	Thymol	3.27	1288	1290	$C_{10}H_{14}O$
16.947	Carvacrol	7.01	1298	1298	$C_{10}H_{14}O$
18.042	Eugenol	1.13	1357	1356	$C_{10}H_{12}O_2$
	Sesquiterpenes				
19.511	Thymol acetate	0.92	1357	1355	$C_{12}H_{16}O_2$
23.760	α-Copaene	4.17	1378	1376	$C_{15}H_{24}$
25.934	β-Cubebene	11.26	1400	1390	$C_{15}H_{24}$
28.486	β-Elemene	2.04	1390	1391	$C_{15}H_{24}$
28.627	Methyl eugenol	0.49	1400	1401	$C_{11}H_{14}O_2$
29.002	α-Humulene	1.21	1454	1454	$C_{15}H_{24}$
30.165	Germacrene D	9.81	1477	1480	$C_{15}H_{24}$
31.931	Butylated hydroxytoluene	2.00	1509	1512	$C_{15}H_{24}O$
32.471	Eugenol acetate	1.55	1537	1536	$C_{12}H_{16}O_3$
33.502	Spathulenol	27.09	1577	1576	$C_{15}H_{24}O$
35.099	Globulol	5.36	1583	1583	$C_{15}H_{26}O$
35.710	Humulene epoxide	1.08	1598	1606	$C_{15}H_{24}O$
36.420	γ-Eudesmol	3.59	1630	1630	$C_{15}H_{26}O$
40.015	Cubenol	0.78	1643	1642	$C_{15}H_{26}O$
43.127	α-Eudesmol	2.34	1651	1652	$C_{15}H_{26}O$
	Other				
5.618	n-Hexanol	4.43	867	867	$C_6H_{14}O$
34.701	Hexadecanol	0.32	1871	1879	$C_{16}H_{34}O$
47.095	Phytol	1.00	1957	1949	$C_{20}H_{40}O$
54.32	α-tocoferol	0.24	2960	-	$C_{29}H_{50}O_2$
Total identified (%)		98.38			

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 1: Chemical compounds present in *Scutia buxifolia* essential oil.

radical is widely used for quickly assessing the ability of antioxidants to transfer labile H atoms to radicals [24]. 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. The efficiency of the essential oil of *S. buxifolia* and ascorbic acid standard were evaluated for this method, and presented IC_{50} values of 13.62 ± 0.17 and 15.98 ± 1.30 μg/mL, respectively; compared to *Thymbra capitatus* (IC_{50}=19.27 μg/mL), *Pistacia atlantica* (IC_{50}=18.95 μg/mL), *Stevia rebaudiana* (IC_{50}=19.26 μg/mL), *Acacia Senegal* (IC_{50}=17.89 μg/mL), *M. peregrinum* (IC_{50}=13.48 μg/mL) [16,18,23], these results proved that the essential oil from *S. buxifolia* leaf possess significant antioxidant properties.

For this oil, the medicinal benefit derived from their use may include prevention of oxidative damage and subsequent disease progression. Essential oils are complex mixtures and the determination of the component(s) responsible of the activity is difficult. Antioxidant activity of essential oils has often been attributed to the presence of phenolic constituents, especially spathulenol, carvacrol and thymol [16,18,25,26]. This association was confirmed in our study, but other compounds also seem to play an important role such as eugenol (IC_{50}=1.26 μg/mL by DPPH method), β-cubebene (IC_{50}=19.3 μg/mL), butylated hydroxytoluene (BHT) and *p*-cymene [21,27-29]; these compounds are also present in the essential oil of *S. buxifolia*, 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.

The essential oil of *S. buxifolia* leaves was tested also against 11 microorganisms; the antimicrobial screening is summarized in table 2. The essential oil showed only moderately activity against *S. aureus* and *E. coli* (MIC=500 and 750 μg/mL, repectively), previous study describes the activity of *S. buxifolia* leaves against *S. aureus* [11]. Sesquiterpenoids spathulenol, β-cubebene, germacrene D and carvacrol were the main components identified in this essential oil and may be responsible, in part, for the antimicrobial activity described, since that spathulenol [30] and carvacrol [16,31] have been reported to present notable antimicrobial activity against bacterial infections. Spathulenol showed also a decrease in the proliferation of lymphocytes demonstrating immunomodulatory effects [5].

The antimicrobial activity of thymol (3.27% in the essential oil of *S. buxifolia*) has been confirmed on bacteria such as *E. coli* [32]. Thymol has been shown to cause disruption of the cellular membrane, inhibition of ATPase activity, and release of intracellular ATP and other constituents [26,33]. The spathulenol, major compound described in the essential oil of *S. buxifolia* leaves (27.09%), evidenced a high activity against the fungi strains dermatophytes as *T. mentagrophytes* and *M. gypseum* with MIC and MFC values ranging from 32 to 64 μg/ml. Furthermore, the MIC value against C. *lactis-condensi* and the MIC and MFC values against *P. purpurogenum* for the spathulenol were 32 μg/ml [17]. However, in our work that was not observed, since the essential oil of the *S. buxifolia* showed no activity against strains of fungi.

In conclusion, the analysis of the chemical composition of the essential oil of this plant and the preliminary evaluation of its

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

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

antioxidant and antimicrobial activity is the first work described in the literature for this species, and, taken together, the data obtained here inspire more studies supporting the possibility of linking the chemical contents with particular biological properties.

Acknowledgments

The authors would like to thank the professors from NAPO (Center for Analysis and Organic Research at Federal Universtity of Santa Maria) for providing the CG/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.

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