Anais da Academia Brasileira de Ciências
ISSN: 0001-3765
aabc@abc.org.br
Academia Brasileira de Ciências
Brasil

BOLIGON, ALINE A.; PIANA, MARIANA; DE BRUM, THIELE F.; FROEDER, AMANDA L.F.; BELKE, BIANCA V.; SCHWANZ, THIAGO G.; MARIO, DÉBORA N.; ALVES, SYDNEY H.; ATHAYDE, MARGARETH L.

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Anais da Academia Brasileira de Ciências, vol. 86, núm. 3, enero-septiembre, 2014, pp. 1463-1469
Academia Brasileira de Ciências
Rio de Janeiro, Brasil

Available in: http://www.redalyc.org/articulo.oa?id=32731840039

Anais da Academia Brasileira de Ciências (2014) 86(3): 1463-1469
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201420120034
www.scielo.br/aabc

Scutia buxifolia Reissek essential oil: *in vitro* antioxidant and antimicrobial activities

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Manuscript received on December 19, 2012; accepted for publication on December 10, 2013

ABSTRACT

The volatile oil from the stem bark of *Scutia buxifolia* (Rhamnaceae) has been obtained by hydrodistillation and analyzed by GC-MS. Twenty-one components were identified representing 99.93 % of the total oil composition, spathulenol (35.87%), β-cubebene (17.26%), germacrene D (6.43%), linalool (5.19%), carvacrol (4.05%) were the main components of *S. buxifolia* essential oil. Antioxidant and antimicrobial properties of the essential oil were evaluated by free radical scavenging (DPPH) assay and micro broth dilution method, respectively. *S. buxifolia* essential oil presented interesting radical scavenging activity (IC_{50} = 15.03 ± 0.11 μg/mL). The antibacterial assay showed that *S. buxifolia* stem bark essential oil was moderately active against the *Staphylococcus aureus* and *Micrococcus sp.* (MIC = 500 μg/mL) and *Escherichia coli* (250 μg/mL). To the best of our knowledge, this is the first study on the composition, antioxidant and antimicrobial activities of essential oil from the *S. buxifolia* collected from Brazil.

Key words: *Scutia buxifolia,* essential oil, antioxidant, antimicrobial.

INTRODUCTION

Scutia buxifolia Reissek belongs to the Rhamnaceae family and is commonly known in Brazil as coronilha, being a native tree from of Southern Brazil, Uruguay and Northern Argentina (Wasicky et al. 1964). In these regions, an aqueous infusion prepared with stem bark and leaves of *Scutia buxifolia* has been described and widely used in folk medicine for cardiotonic, diuretic and antihypertensive purposes (Boligon et al. 2009, Da Silva et al. 2012). Phytochemical screening of fractions of *Scutia buxifolia* bark revealed the presence of cyclopeptide alkaloids (Maldaner et al. 2011), polyphenols and flavonoids in fractions of *Scutia buxifolia* leaves and stem bark (Boligon et al. 2009, 2012a, b). Antimicrobial activities of some cyclopeptide alkaloids isolated from the root bark of this species were reported by Morel et al. (2005) using the bioautography method. Cytotoxicity of extracts from leaves, twigs and stem bark of the plant were evaluated by the *Artemia salina* assay, as well as the antimicrobial activity against

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a panel of microorganism strains (Boligon et al. 2012b). Extracts of the leaves and stem bark of *S. buxifolia* were effective against lipid peroxidation by inhibiting the production of thiobarbituric acid reactive substances and also presented DPPH scavenger activity (Boligon et al. 2009).

Considering that the infusion and decoction of this plant are used in folk medicine, we were interested in detecting the volatile constituents that get into water. This paper represents the first report on the oil composition, antioxidant and antimicrobial activity of the essential oils of *S. buxifolia* stem bark.

MATERIALS AND METHODS

Plant Material

Scutia buxifolia (Rhamnaceae) stem bark was 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). They were identified and archived as voucher specimens in the herbarium of the Department of Biology at the Federal University of Santa Maria by register number SMBD 10919.

Extraction of the Essential Oil

The fresh material (250g) of the plant stem bark 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 stem bark was determined.

Gas Chromatography (GC-FID)

The gas chromatography (GC) analyses were carried out using an Agilent Technology 6890N GC-FID system, equipped with DB-5 capillary column (30m x 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 (Verma et al. 2010, Nazemiyeh et al. 2011).

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analyses were performed on an Agilent Technology 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 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). 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 (1995). 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 were applied to TLC plates (silica gel 60 GF_{254}), quercetin and ascorbic acid (Sigma-Aldrich, ≥ 98% HPLC) standards were also 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 appeared as yellow spots against a purple background, indicating possible antioxidant activity (Mensor et al. 2001).

Quantitative Analysis of Antioxidant Activity

The antioxidant activity of the essential oil was evaluated by monitoring its ability in quenching the stable free radical DPPH, according to a slightly modified method previously described by Mensor et al. (2001). 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 showed 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 (Boligon et al. 2009).

Antimicrobial Assay Determination

The essential oil was evaluated against *Candida albicans* ATCC 28967, *Cryptococcus neoformans* ATCC 2857, *Klebsiella pneumoniernaae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 51299, *Proteus mirabilis* ATCC 7002, *Staphylococcus aureus* ATCC 29213, *Micrococcus sp.* ATCC 7468, *Malassezia sp*., *Aspergillus sp*., *Aeromonas sp*., *S. aureus* 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 (2002). 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. The first dilution was made in DMSO and further dilutions in the culture medium. Bacterial strains were cultured overnight at 37 °C in Mueller-Hinton broth. Yeasts were cultured overnight at 30 °C in Potato dextrose broth. 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 stem bark of *S. buxifolia* was obtained by hydrodistillation in the yield of 0.57%. Essential oil was analyzed by GC-FID and GC-MS systems and the oil components were identified both quantitatively and qualitatively. Twenty-one components, representing 99.93% of the total composition, were identified, of which 82.81% were sesquiterpenes and 17.12% were monoterpenes (Table I).

The main components in the oil were spathulenol (35.87%), β-cubebene (17.26%), germacrene D (6.43%), linalool (5.19%), carvacrol (4.05%), α-copaene (3.56%), cubenol (2.80%), γ-Eudesmol (2.75%), 1,8-Cineol (2.73%), Thymol acetate (2.54%), Butylated hydroxytoluene (2.49%), cedrene, α-eudesmol, globulol, cyclosativene, thymol, among others, as minor constituents. Spathulenol, the most abundant component of this oil, has also been reported in the oil of other species

TABLE I
Chemical compounds present in *Scutia buxifolia* stem bark essential oil.

Rt (min)	Compounds	(%)	RI[a]	RI[b]	Mol. Formula
		Monoterpenes			
3.273	α-Pinene	0.97	935	939	$C_{10}H_{16}$
3.692	β-Pinene	1.06	981	982	$C_{10}H_{16}$
11.450	1,8-Cineol	2.73	1029	1029	$C_{10}H_{18}O$
12.045	Linalool	5.19	1078	1081	$C_{10}H_{18}O$
12.629	Pulegol	0.85	1213	1209	$C_{10}H_{18}O$
16.250	Thymol	1.36	1288	1290	$C_{10}H_{14}O$
16.948	Carvacrol	4.05	1296	1298	$C_{10}H_{14}O$
18.041	Eugenol	0.91	1354	1356	$C_{10}H_{12}O_2$
		Sesquiterpenes			
19.531	Thymol acetate	2.54	1357	1355	$C_{12}H_{16}O_2$
20.920	Cyclosativene	1.87	1365	1368	$C_{15}H_{24}$
23.760	α-Copaene	3.56	1378	1376	$C_{15}H_{24}$
25.934	β-Cubebene	17.26	1400	1390	$C_{15}H_{24}$
28.486	β-Elemene	0.47	1390	1391	$C_{15}H_{24}$
28.491	Cedrene	2.03	1405	1409	$C_{15}H_{24}$
29.012	α-Humulene	1.21	1454	1454	$C_{15}H_{24}$
30.245	Germacrene D	6.43	1477	1480	$C_{15}H_{24}$
31.871	Butylated hydroxytoluene	2.49	1519	1512	$C_{15}H_{24}O$
33.502	Spathulenol	35.87	1576	1576	$C_{15}H_{24}O$
35.127	Globulol	1.59	1583	1583	$C_{15}H_{26}O$
37.458	γ-Eudesmol	2.75	1633	1630	$C_{15}H_{26}O$
40.125	Cubenol	2.80	1643	1642	$C_{15}H_{26}O$
42.113	α-Eudesmol	1.94	1656	1652	$C_{15}H_{26}O$
	Total identified (%)	99.93			

Relative proportions of the essential oil constituents were expressed as percentages. Rt = Retention time according to 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).

such as *Baccharis uncinella* (16.41%), *Stevia rebaudiana* (15.41%), *Origanum vulgare* (11.67%) and *Baccharis dracunculifolia* (9.54%) (Al-Ja'fari et al. 2011, Muanda et al. 2011, Xavier et al. 2011). The second major component of the oil, β-cubebene, has also been found in the oils of *Dendropanax morbifera* (Chung et al. 2010) and *Cinnamomum osmophloeum* (Lin et al. 2007) in about 4.19% and 59.4%, respectively. Germacrene D, the third major compound present, has also been found in the oils of *Artemisia annua* (15.64%), *Baccharis uncinella* (14.87%), *Campomanesia adamantium* (11.82%), *Tagetes minuta* (10.00%) and *Origanum vulgare* (8.11%) (Ghiasvand et al. 2011).

Many *in vitro* studies have addressed the antioxidant and radical-scavenging properties of essential oils (Edris, 2007, Gourine et al. 2010, Fabri et al. 2012). In particular, DPPH radical is widely used for quickly assessing the ability of antioxidants to transfer labile H atoms to radicals (Brand-Williams et al. 1995). 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.

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 15.03 ± 0.11 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), *Mycobacterium peregrinum* (IC_{50} = 13.48 μg/mL) and *Mitracarpus frigidus* (IC_{50} = 38.00 μg/mL) (Bounatirou et al. 2007, Gourine et al. 2010, Muanda et al. 2011, Fabri et al. 2012), these results proved that the essential oils from *S. buxifolia* stem bark possess significant antioxidant properties. The antioxidant activity of essential oils has often been attributed to the presence of phenolic constituents, especially spathulenol, carvacrol and thymol (Bounatirou et al. 2007, Hazzit et al. 2009, Muanda et al. 2011, Viuda-Martos et al. 2011). 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) and butylated hydroxytoluene (BHT) (Yanishlieva et al. 1999, Gülçin et al. 2004, Jirovetz et al. 2006, Lin et al. 2007); these compounds are also present in the essential oil of *S. buxifolia*, and may account, in part, for the good antioxidant potential reported here. The results presented here may contribute to the knowledge of the antioxidant potential of the essential oils and provide some information for its uses.

The essential oil of *S. buxifolia* stem bark was also tested against 12 microorganisms; the antimicrobial screening is summarized in Table II. The essential oil showed only moderate activity against *S. aureus* and *Micrococcus sp.* (MIC = 500 μg/mL) and *E. coli* (250 μg/mL), previous study describes the activity of *S. buxifolia* against *S. aureus* (Boligon et al. 2009). 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 spathulenol (Chinou et al. 2004) and carvacrol (Burt 2004) have been reported to present notable antimicrobial activity against bacterial infections. Spathulenol also showed a decrease in the proliferation of lymphocytes demonstrating immunomodulatory effects (Ziaei et al. 2011).

The antimicrobial activity of thymol (1.36% in the essential oil of *S. buxifolia*) has been confirmed on bacteria such as *E. coli* (Rivas et al. 2010). Thymol has been shown to cause disruption of the cellular membrane, inhibition of ATPase activity, and release of intracellular ATP and other constituents (Raybaudi-Massilia et al. 2006, Viuda-Martos et al. 2011). The spathulenol, major compound described in the essential oil of *S. buxifolia* stem bark (35.87%), evidenced a high activity against fungi strains dermatophytes such as *Trichophyton mentagrophytes* and *Microsporum gypseum* with MIC values ranging from 32 to 64 μg/ml. Furthermore, the MIC value against *Candida lactis-condensi* and *Penicillium purpurogenum* for the spathulenol was 32 μg/ml (Al-Ja'fari et al. 2011). 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 antioxidant and antimicrobial activity is the first work described in

the literature for this species, indicate that the data obtained here inspire more studies supporting the possibility of linking the chemical contents with particular biological properties.

TABLE II
Minimal inhibitory concentrations (MIC) of essential oil of the *S. buxifolia* stem bark.

Microorganisms	Essential oil (μg/mL)
Candida albicans	> 1000.00
Cryptococcus neoformans	> 1000.00
Klebisiella pneumoniernaae	> 1000.00
Pseudomonas aeruginosa	> 1000.00
Enterococcus faecalis	> 1000.00
Proteus mirabilis	> 1000.00
Malassezia sp.	> 1000.00
Aspergillus sp.	> 1000.00
Aeromonas sp.	> 1000.00
Staphylococcus aureus	500.00
Micrococcus sp.	500.00
Escherichia coli	250.00

ACKNOWLEDGMENTS

The authors would like to thank the professors from NAPO (Center for Analysis and Organic Research at the 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 Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

RESUMO

O óleo volátil das cascas do caule de *Scutia buxifolia* (Rhamnaceae) foi obtido por hidrodestilação e analisados por CG-EM. 21 componentes foram identificados representando 99,93% da composição total de óleo, espatulenol (35,87%), β-cubebene (17,26%), germacreno D (6,43%), linalol (5,19%) e carvacrol (4,05%) foram os principais componentes do óleo essencial de *S. buxifolia*. Propriedades antioxidantes e antimicrobianas do óleo essencial foram avaliadas através do ensaio do DPPH e do método de micro diluição em caldo, respectivamente. O óleo essencial de *S. buxifolia* apresentou interessante atividade de radical de limpeza (IC_{50} = 15,03 ± 0,11 μg/mL). O ensaio antibacteriano mostrou que *S. buxifolia* possui moderadamente atividade frente a *S. aureus* e *Micrococcus* sp. (CIM = 500 μg/mL) e *E. coli* (250 μg/mL). Tanto quanto pudemos avaliar, este é o primeiro estudo sobre a composicão química, atividade antioxidante e antimicrobiana do óleo essencial da cascas do tronco de *S. buxifolia* coletadas do Brasil.

Palavras-chave: *Scutia buxifolia*, óleo essencial, antioxidante, antimicrobiana.

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