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## GC-MS Analysis of Dichloromethane Fractions of *Scutia buxifolia* Reissek Leaves and Stem Bark

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### ABSTRACT

The identification of components presents in the plants is very important to the pharmaceutical, cosmetic, fragrance and food industries. This study report, for the first time, the phytoconstituents present in dichloromethane fraction of *Scutia buxifolia* Reissek stem bark and leaves. The fractions were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and twenty-seven different compounds were identified in the fractions analyzed. In the dichloromethane (DCM) fraction of the leaves were identified sixteen compounds, totaling 36.98% and eighteen compounds were described in DCM fraction of the stem bark, representing 16.17%. The main constituents of the fractions were Spathulenol (4.28%), 3-hexen-1-ol (3.96%), ethyl-octadecenoate (3.72%), octadeca-9,12-dienoic acid (3.48%); 5, 9-heptadecadienoate (3.61%) and hexadecanoic acid (3.58%). Furthermore, several secondary metabolites with interesting biological activity, such as: thymol,  $\beta$ -cubebene, p-xilene, phytol, neophytadiene, lanost-8-en-24-al and stigmastan-3,5-diene were also identified in of *S. buxifolia* DCM fractions, these results serve as a incentive for additional studies biogued and related to biological activity of this species.

**Key words:** *Scutia buxifolia*, Rhamnaceae, dichloromethane, GC-MS analysis

### INTRODUCTION

Rhamnaceae family comprising about 58 genera and approximately 900 species, between the species highlight *Scutia buxifolia* Reissek (coronilha) that is mostly distributed in the Brazil, Argentina and Uruguay (Wasicky *et al.*, 1964; Menezes *et al.*, 1995). In these regions, an infusion of the steam bark of *S. buxifolia* has been described and widely used in folk medicine for cardiogenic, diuretic and antihypertensive purposes (Wasicky *et al.*, 1964). Some biological properties have been described in *S. buxifolia* extracts and fractions, such as, antimicrobial, antimycobacterial, antiviral and cytotoxicity activities (Morel *et al.*, 2005; Boligon *et al.*, 2012b), antioxidant capacity (Boligon *et al.*, 2009; Boligon *et al.*, 2012a) and protective effects against chromosome damage induced by H<sub>2</sub>O<sub>2</sub> (Boligon *et al.*, 2012c). In addition, the presence of phenolics acids, flavonoids, steroids, terpenoids, tannin and alkaloids has been described previously in *S. buxifolia* (Menezes *et al.*, 1995; Boligon *et al.*, 2009, 2012a). However, no study by GC-MS identified the constituents present in the nonpolar fractions of the species.

The aim of the present study was to investigate the phytoconstituents present in dichloromethane fraction of *S. buxifolia* stem bark and leaves by gas chromatography-mass spectrometry (GC-MS). The results presented here are first reported for the species.

## MATERIAL AND METHODS

**Plant material:** Stem bark and leaves of *Scutia buxifolia* Reissek (Rhamnaceae) were collected in Dom Pedrito, Rio Grande do Sul State, Brazil (coordinates 30°59'09"S, 54° 27'44" W), in October of 2007. Exsiccate was archived as voucher specimen in the herbarium of Department of Biology at Federal University of Santa Maria by register number SMBD 10919, for future references.

**Extraction:** The aerial parts of the plant were separately dried at room temperature and powdered in a knife mill (0.86  $\mu\text{m}$ ). Leaves (372.34 g) and stem bark (651.52 g) were macerated with 70% ethanol for 7 days based on the traditional methods of tincture preparation. After filtration, a portion of 100 mL of the hydroalcoholic extracts were reserved and evaporated to dryness under reduced pressure ( $\pm 40^\circ\text{C}$ ) to obtain the crude extracts (leaves and stem bark). After that, the remainder hydroalcoholic extracts were partitioned with dichloromethane (DCM), Ethyl Acetate (EA) and N-Butanol (NB), successively. The DCM fractions were subjected to analysis by GC-MS.

**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 ( $250^\circ\text{C}$ ). The transfer line temperature was  $280^\circ\text{C}$ . Helium was used as carrier gas ( $1.3 \text{ mL min}^{-1}$ ) and the capillary columns used were an HP 5 MS ( $30 \times 0.25 \text{ mm}$ ; film thickness 0.25 mm) and an HP Innowax ( $30 \times 0.32 \text{ mm i.d.}$ , film thickness 0.50 mm). The temperature programme was the same as that used for the GC analyses.

**Identification of phytoconstituents:** The identification and interpretation on mass-spectrum GC-MS was conducted using the database of mass spectra library search (NIST and Wiley) and research papers (Silverio *et al.*, 2007; Nazifi *et al.*, 2008; Rocha *et al.*, 2011).

## RESULTS AND DISCUSSION

**GC-MS analysis:** Gas chromatograms of DCM fractions are presented as Fig. 1. The mass spectra of all major peaks shown in gas chromatogram were analyzed, sixteen compounds DCM fraction leaves (Fig. 1a) and eighteen compounds for DCM fraction stem bark (Fig. 1b) were identified.

A list of the identified compounds and their quantification is presented in Table 1 according to their retention time. Compounds identified in the leaves (36.98%) includes: spathulenol (4.28%), 3-hexen-1-ol (3.96%), ethyl-octadecenoate (3.72%), octadeca-9,12-dienoic acid (3.48%); 5,9-heptadecadienoate (3.61%), hexadecanoic acid (3.58%) and thymol (3.15%). The  $\beta$ -cubebene (3.08%); octadeca-9,12-dienoic acid (2.57%), heptatriacol (2.06%) and cholesta-3,5-dien-7-one (1.88%) are present in the DCM fraction from the stem bark, in this fraction were identified 16.17% the compounds. The DCM leaves fraction had the largest amount of compounds when compared to DCM stem bark fraction. Seven compounds are present on both parts of the *S. buxifolia*, such: tetradecanoic acid, hexadecanoic acid, 5,9-Heptadecadienoate, *p*-xylene, nonane, octadeca-9,12-dienoic acid and stigmastan-3,5-diene.

Fatty acids represented the major class of nonpolar components present in the DCM fractions of the *S. buxifolia*, with tetradecanoic acid, hexadecanoic acid (palmitic acid), octadeca-9,12-dienoic

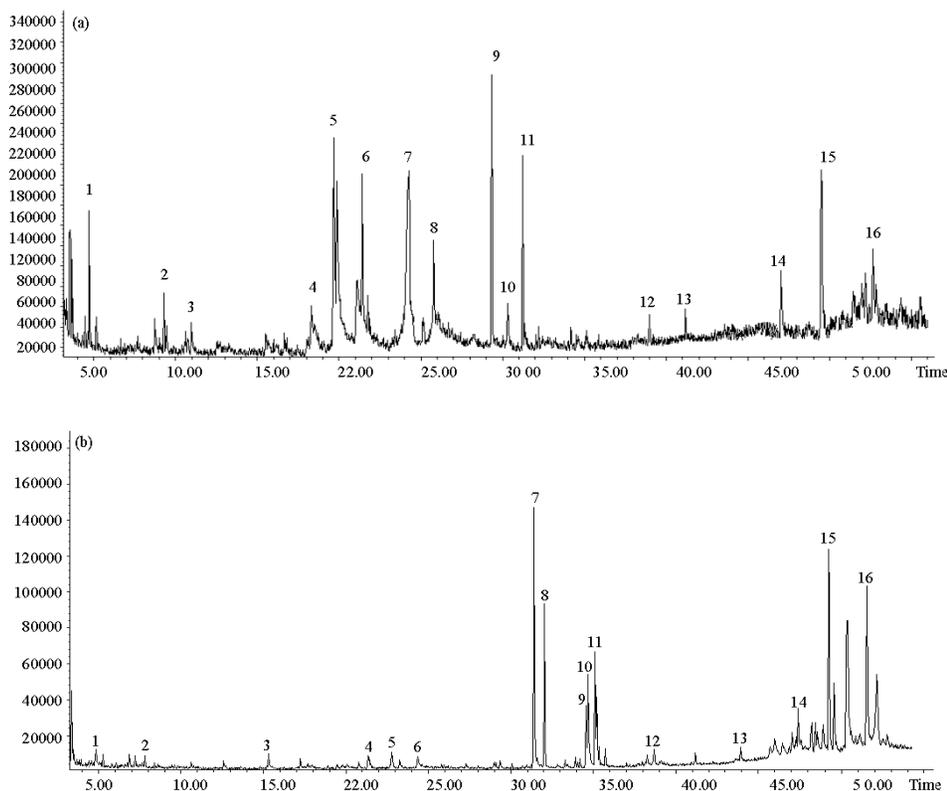


Fig. 1(a-b): GC-MS chromatogram of DCM fractions of (a) *Scutia buxifolia* leaves and (b) Stem bark

Table 1: Compounds present in the DCM fractions of *S. buxifolia* leaves and stem bark

Leaves			Stem bark		
Compounds	RT	%	Compounds	RT	%
Tetradecanoic acid	4.36	3.23	Tetradecanoic acid	4.39	0.22
6,10 Dimethyl-2-pentadecanone	9.35	1.3	4,7 Dimethyl-2-pentadecanone	7.85	0.18
Tetracloro ethylene	11.08	0.79	Cyclohexane ethyl	15.4	0.24
Methyl palmitate	19.54	1.06	Hexadecanoic acid	21.43	0.30
3, hexen-1-ol	20.03	3.96	Pentadecanoic acid	22.87	0.26
Hexadecanoic acid	21.5	3.58	5,9-Heptadecadienoate	24.49	0.21
5,9-Heptadecadienoate	24.45	3.61	$\beta$ -Cubebene	31.26	3.08
Thymol	26.00	3.15	Heptatriacol	32.08	2.06
Spathulenol	29.57	4.28	Butylated hydroxytoluene	34.55	0.71
9-Octadecenoic acid	30.84	0.90	Octadec-9-enoic acid	34.68	1.14
Ethyl-octadecenoate	31.61	3.72	Methyl-7-octadecenoate	35.42	1.43
p-Xilene	39.07	0.69	p-Xilene	38.93	0.23
Neophytadiene	41.28	0.73	Phytol	44.01	0.20
Nonane	47.12	1.24	Nonane	47.53	0.46
Octadeca-9,12-dienoic acid	49.27	3.48	Octadeca-9,12-dienoic acid	49.25	2.57
Stigmastan-3,5-diene	52.21	1.26	Cholesta-3,5-dien-7-one	50.61	1.88
-	-	-	Lanost-8-en-24-al	-	51.70
-	-	-	Stigmastan-3,5-diene	52.17	0.84

RT: Retention Time (min)

acid (linoleic acid), and octadec-9- enoic acid (oleic acid). These results also showed that the DCM fractions of *S. buxifolia* have in their chemical constitution the terpenoids (spathulenol, thymol,  $\beta$ -cubebene) and esterified sterols such as cholesta-3,5-diene-7-one, lanost-8-en-24-al and stigmastan-3, 5-diene. Silverio *et al.* (2007) describes the presence of esterified steroids in three *Eucalyptus* species, these compounds may be responsible for antibacterial and antifungal activity described above for the *S. buxifolia* (Boligon *et al.*, 2012b), since, steroids and terpenoids reduce the synthesis of ergosterol fungal cell membrane component and causes defective cell wall formation and leakage of cellular contents (Pinto *et al.*, 2009). Terpenes also increase the permeability of bacterial and mammalian cell by inserting themselves into the lipid layer of the cell membrane thus influence the selective permeability of the cell to foreign substance (Villani *et al.*, 1972).

Several species like *Ornithogalum cuspidatum* Bert, *Centroceras clavulatum*, *Eucalyptus urograndis*, *Eucalyptus urophylla*, and *Eucalyptus camaldulensis* have the same compounds described in dichloromethane fraction (Silverio *et al.*, 2007; Nazifi *et al.*, 2008; Rocha *et al.*, 2011). Compounds described in this study are reported in essential oils of several species (Silva *et al.*, 2010; Souza *et al.*, 2011) an explanation may be no polarity of dichloromethane fraction, which has compounds similar to those extracted by hydrodistillation (Woerdenbag *et al.*, 1993).

## CONCLUSIONS

This work report describes for the first time the identification and quantification of a large number of lipophilic components present in the DCM fractions of *Scutia buxifolia* leaves and stem bark. The presence of these compounds in studied species is important phytochemically and pharmacologically. However, the potential chemical species must be investigated and biological properties evaluated.

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