

## *Stachytarpheta gesnerioides* Cham.: chemical composition of hexane fraction and essential oil, antioxidant and antimicrobial activities

[*Stachytarpheta gesnerioides* Cham.: composición química de la fracción hexánica y aceite esencial, actividades antioxidante y antimicrobiana]

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### Abstract

In the present study we investigated the chemical composition of hexane fraction and essential oil of *Stachytarpheta gesnerioides* (Verbenaceae) by GC-MS, total phenol and flavonoid contents. The antioxidant capacity and antimicrobial activity were investigated in five extracts of leaves of *S. gesnerioides*. Aqueous and 100% ethanol extracts were prepared by dynamic maceration. Hexane, ethyl acetate and methanol extracts were prepared by Soxhlet extraction. The essential oil (EO) and hexane fraction (HF) are mainly composed by guaiol. Moreover, the HF is also rich in the monoterpene  $\alpha$ -pinene. The total phenol content ranged from 0.85 to 22.74 mg gallic acid equivalent /100mg dry extract at Folin-Ciocalteu's reagent method. The total flavonoid concentration ranged from 0.68 to 13.65 mg rutin equivalent /100mg dry extract, detected using 8% aluminium chloride. The ethyl acetate extract (IC<sub>50</sub>=9.41  $\mu$ g/ml) showed the highest antioxidant activity. The extracts were found to be effective to inhibit the microorganisms tested.

**Keywords:** Verbenaceae; *Stachytarpheta gesnerioides*; chromatography-mass spectrometry; flavonoids; total phenol.

### Resumen

Se han investigado la composición química de la fracción hexánica (FH) y aceite esencial (AE) de *Stachytarpheta gesnerioides* (Verbenaceae) por GC-MS, el contenido de fenoles totales y flavonoides. La capacidad antioxidante y actividad antimicrobiana fueron investigadas en cinco extractos de hojas de *S. gesnerioides*. Extractos acuosos y etanólico fueron preparados por la maceración dinámica y extracción continua en Soxhlet con hexano, acetato de etilo y metanol. Las fracciones AE y FH están compuestas principalmente por guaiol. La fracción FH es también rica en  $\alpha$ -pineno. El contenido de fenoles totales varió desde 0,85 hasta 22,74 mg de ácido gálico/100 mg de extracto seco (Folin-Ciocalteu). La concentración total de flavonoides varió desde 0,68 hasta 13,65 mg en equivalentes de rutina/100 mg de extracto seco, que se detectó mediante reacción con cloruro de aluminio al 8%. El extracto de acetato de etilo (CI<sub>50</sub>=9,41g/ml) enseñó la más grande actividad antioxidante. Los extractos se encontraron eficaces para inhibir los microorganismos ensayados.

**Palabras Clave:** Verbenaceae; *Stachytarpheta gesnerioides*; Espectrometría cromatografía de masa; flavonoides; Fenoles totales.

Recibido | Received: April 9, 2012.

Aceptado en versión corregida | Accepted in revised form: July 13, 2012.

Publicado en línea | Published online: November 30, 2012.

Declaración de intereses | Declaration of interests: The authors thank FAPEMIG, CNPq and CAPES for financial support.

Este artículo puede ser citado como / This article must be cited as: Pâmela Souza Silva, Érika Yoko Suzuki, Ana Paula Moreira, Nádia Rezende Barbosa Raposo, Tânia Maria Almeida Alves, Lyderson Facio Viccini. 2012. *Stachytarpheta gesnerioides* Cham.: chemical composition of hexane fraction and essential oil, antioxidant and antimicrobial activities. *Bol Latinoam Caribe Plant Med Aromat* 11(6): 542 – 548.

## INTRODUCTION

Verbenaceae is included in the order Lamiales and is widely found in practically every terrestrial ecosystem. This family includes 1035 species and 36 genera mainly distributed throughout the tropics with few species in the temperate areas (Judd *et al.*, 1999). Several genera have been investigated due to their medicinal properties, especially *Stachytarpheta*, *Lippia* and *Lantana* (Salimena, 2000).

The genus *Stachytarpheta* includes nearly 113 species distributed in tropical and subtropical America (Stevens, 2001). Some species are traditionally used in folk medicine as purgative, vermifuge, expectorant, diuretic, emmenagogue, sore-throat gargle and general tonic (Sanders, 2001). Previous studies have revealed the occurrence of the iridoids lamiide and ipolamiide in some species including *S. jamaicensis*, *S. cayennensis*, *S. indica*, *S. australis*, *S. mutabilis*, *S. urticifolia* and *S. glabra* (Tantisewie and Sticher, 1975; Jawad, 1977; De Luca, 1980; Akisue *et al.*, 1981; Rodriguez and Castro, 1996; Futuro and Kaplan, 1998; Schapoval *et al.*, 1998; Ganapaty *et al.*, 1998; Roengsumran *et al.*, 2002; Chowdhury *et al.*, 2003; Viccini *et al.*, 2008). These constituents have been shown to possess several biological activities such as antimicrobial, antitumoral, anti-inflammatory, antinociceptive, hepatoprotective and laxative actions (Roengsumran *et al.*, 2002). *Stachytarpheta gesnerioides* is a tropical and subtropical America widespread shrub (Munhoz, 2003) that can be found in Minas Gerais, Bahia and Goiás States, Brazil.

Although several studies reported the importance of the *Stachytarpheta* genus in folk medicine, some species such as *Stachytarpheta gesnerioides* have never been studied phytochemically. As part of an ongoing program to evaluate compounds with biological activities from this genus, in this paper we determined the chemical composition of hexane fraction and essential oil of *S. gesnerioides* using gas chromatography-mass spectrometry (GC-MS) analysis, total phenol and flavonoid tests. The antioxidant capacity and antimicrobial activity were also evaluated for five *S. gesnerioides* extracts.

## MATERIALS AND METHODS

### Plant Material

Leaves of *Stachytarpheta gesnerioides* Cham. were collected at Serra do Cipó (794m; 19°27'47''S 43°33'10''W), Espinhaço Range, Minas Gerais State,

Brazil. The plants were identified by Dr. Fátima Regina Gonçalves Salimena (Department of Botany, UFJF) and the vouchers were deposited at the CESJ Herbarium of Federal University of Juiz de Fora.

### Extraction

The essential oils (EO) from fresh leaves were obtained by hydrodistillation using a Clevenger-type apparatus for 2 h. The chemical composition of EO was analyzed by GCMS.

The extracts were prepared by dynamic maceration (5 g) in aqueous (30.00%) and 100% ethanol (25.52%) for 72 h. The leaves (15 g) were submitted to Soxhlet extraction with hexane (10.72%), ethyl acetate (5.06%) and methanol (11.31%) during 72 h. The solutions were concentrated in vacuo at 40° C using a rotary evaporator (Pereira *et al.*, 2005).

### Phytochemical Screening

Fresh leaves (at about 3 g) were transferred to Falcon™ 50 ml conical tube with ethanol P.A. (30 ml). The tubes were kept for one week at room temperature. After filtration, an aliquot of 100 µL of the solution was taken and mixed to the same quantity of MilliQ water and twice partitioned with hexane. The chemical composition of the hexane fraction (HF) was analyzed by GCMS.

The analysis was performed on an Shimadzu gas chromatograph-mass spectrometer model QP5050A equipped with a FID detector and a DB-5 fused silica capillary column (35m×0.2 mm, film thickness 0.10 µm), using helium as a carrier gas (1.0 ml min<sup>-1</sup>). The injector temperature was 200° C and the column oven program was 50° C to 200° C at 4° C min<sup>-1</sup>. The mass spectra were obtained by electronic impact 70 eV and the range from 50 to 500 *m/z* was scanned. Data acquisition and handling was done via CLASS 5000 Shimadzu software. Retention Index (RI) using the range from 900 to 3000 was generated from a standard mixture containing C<sub>9</sub> to C<sub>30</sub> hydrocarbons. Oil constituents were identified by comparison of their mass spectra with those in a Shimadzu spectral database and RI (Adams, 1995).

Total phenol constituents were determined using the Folin–Ciocalteu reagent and gallic acid as the standard (Bonoli *et al.*, 2004). The reaction mixture contained sample (50 µl), Folin–Ciocalteu reagent (250 µl), 20% sodium carbonate (500 µl) and distilled water (4.2 ml). The calibration curve was prepared using gallic acid solutions ranging from 0 to

7 µg/l. Total phenol of the extract, as gallic acid equivalent, were determined using the absorbance of the extract measured at 765 nm. Results were expressed as mg gallic acid equivalents per 100 mg dry extract. Tests were carried out in triplicate (n=3).

Total flavonoid content of each extract was determined using the aluminium chloride reagent and rutin as a standard. This colorimetric method was based on the formation of a flavonoid-aluminium complex that can be measured in a spectrophotometer at a wavelength of 405 nm (Vennat *et al.*, 1992). To quantify the flavonoid content, aliquots of 1 ml of chloroform and 1.5 ml of distilled water were added to 2.5 ml of previously obtained samples. The resulting solutions were mixed and centrifuged during 3 min at 3500 rpm at room temperature. The aqueous phase (20 µl) was mixed with distilled water (99 µl), 8% aluminium chloride (25 µl), pyridine: methanol solution (100 µl) and glacial acetic acid (6 µl). After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 405 nm. The calibration curve was prepared with rutin solutions ranging from 2 to 30 µg/l. Results were expressed as mg rutin equivalents per 100 mg dry extract. Tests were carried out in triplicate (n = 3).

#### **Antioxidant Activity**

The radical scavenging activity of plant extracts was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Sreejayan and Rao, 1997). An aliquot of ethanol solution of the extracts (50 µl - 0.97-250 µg/ml) was added to a 0.05mM ethanol DPPH solution (150 µl) in a 96 well microplate and incubated at room temperature for 30 min. A blank (consisting of the extract and ethanol) was used to remove the influence of sample color. An ethanol solution of DPPH was used as negative control. Butylated hydroxytoluene (BHT) was used as antioxidant reference compound, at the same concentration used for the sample. Results were expressed as mean of inhibiting concentration (IC<sub>50</sub>) which was calculated using the following equation:

$$IC_{50}(\%) = 100 \times (A_0 - A_s) / A_0(1)$$

where A<sub>0</sub> and A<sub>s</sub> are the values for the absorbance of the negative control and the absorbance of the sample, respectively. Tests were carried out in triplicate.

#### **Antimicrobial Screening**

Antimicrobial activity was determined by turbidimetric method (Candan *et al.*, 2003). The microorganisms tested were *Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 14028, *Shigella sonnei* ATCC 25931, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231. Inhibition of microorganism growth was determined using a micro dilution assay in a sterile 96 well microplate. Each well contained 100 µl of each extract (0.625 to 10 mg/ml) and 100 µl of Mueller Hinton broth with the bacteria or yeast representing, approximately, 4 x 10<sup>3</sup> colony-forming units (CFU)/ml. The microplates were incubated at 37° C for 24h (bacteria) and 25° C for 48h (yeast). The results were based on visible growth or inhibition. Tests were carried out in triplicate. The minimum inhibitory concentration (MIC) was defined as the lowest concentration able to inhibit any visible bacterial or yeast growth. In addition, cloranphenicol (0.025 – 250 µg/ml) and nistatin (0.2 – 2000 U/ml) served as drug reference.

#### **RESULTS AND DISCUSSION**

GC/MS analysis of the essential oil and hexane fraction from leaves of *Stachytarpheta gesnerioides* allowed the identification of approximately 68.50 and 75.75% of their components, respectively. Out of eighteen peaks (representing 96.87% of the EO), ten components were identified of the total oil composition. The chromatogram of HF showed nine peaks (representing 98.9%) and six constituents were identified. The compounds identified in the EO and HF, their relative content and also Kovat's indices are presented in Table 1. The components that could not be identified had IKC higher than 1633.20. The yield of the EO (w/w) was 0.01 %, showing that the plant contains little essential oil. On the other hand, the yield of the HF was 0.22%, about twenty times than EO.

EO and HF of the *S. gesnerioides* are mainly composed of guaiol. This oxygenated sesquiterpene, which comprises more than 50% of the EO and HF, is one of the major compounds found in plants that possess antioxidant and antimicrobial activities (Al-Howiriny, 2002; Cuca *et al.*, 2009; Simionatto *et al.*, 2007; Trovati *et al.*, 2009). Moreover, the HF of *S. gesnerioides* is also rich in the monoterpene α-pinene (16.09%).

Phenolic compounds constitute one of the most widely investigated groups of substances that possess a broad biological activities spectrum including antioxidant and radical scavenging

properties (Gülçin, 2004). Flavonoids are chemical constituents that contribute for plants antioxidant effects due to their hydroxyl groups (Ebrahimzadeh, 2008). The consumption of antioxidants has been associated to an incidence reduction of many oxidative diseases such as cardiovascular disorders, cancer and diabetes mellitus (Lai, 2009). It was suggested that polyphenolic compounds may have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g are daily ingested mainly from fruits and vegetables (Gülçin, 2004).

As a preliminary test to evaluate antioxidants and radical scavengers, the DPPH assay has been considered an easy, rapid and convenient method (Nickavar *et al.*, 2007). According to Table 2, the extracts obtained from Soxhlet and maceration, were used to evaluate the antioxidant activity of *S. gesnerioides*. The IC<sub>50</sub> values ranged from 9.41 to 305.93 µg/ml. Lower IC<sub>50</sub> value indicates higher antioxidant activity. The ethyl acetate extract showed

higher antioxidant activity (IC<sub>50</sub> = 9.41 µg/ml) and also contains higher amount of total phenolic and flavonoid constituents than other extracts. Lower antioxidant activities were exhibited by ethanol (IC<sub>50</sub> = 24.64 µg/ml) and methanol extracts (IC<sub>50</sub> = 31.82 µg/ml). The lowest radical scavenging activity was observed with hexane extract (IC<sub>50</sub> = 305.93 µg/ml) that contained lower amount of phenolic and flavonoid compounds. Butylated hydroxytoluene (BHT) produced 11.82 µg/ml of IC<sub>50</sub> value.

After the analyses, it was possible to establish a relationship among antioxidant activity and phenols and flavonoids contents of extracts. A positive correlation was observed between flavonoids and antioxidant activity (R = 0.844, p < 0.05) and also between phenols and antioxidant activity (R = 0.930, p < 0.05). In addition another correlation between phenols content and flavonoids (R = 0.589, p < 0.01) was also observed.

**Table 1**  
**Chemical composition of essential oil and hexane fraction of *Stachytarpheta gesnerioides***

Compounds	KI	Essential Oil			Hexane Fraction		
		KIC	RI	%	KIC	RI	%
α-pinene	0939	-	7.375	0.31	-	7.375	16.09
β-pinene	0980	-	-	-	-	8.767	1.46
1-octen-3-ol	0978	-	8.992	0.46	-	-	-
3-octanol	0993	1002.32	9.508	0.23	-	-	-
Isocaryophyllene	1404	1431.27	24.517	1.51	1430.74	24.500	1.71
Bicyclogermacrene	1494	1511.09	27.050	1.26	1510.26	27.025	1.42
β-sesquiphellandrene	1524	1539.77	27.917	4.72	-	-	-
Espatulenol	1576	1597.95	29.675	1.28	-	-	-
Caryophyllene oxide	1581	1601.92	29.792	0.68	-	-	-
Guaiol	1595	1625.97	30.483	56.50	1616.71	30.217	53.52
Total identified				68.50			75.75

KI: Kovat's index; KIC: Kovat's index calculated; RI: retention index; % relative percentage

The antioxidant activity of phenolic compounds was reported to be probably a consequence of their redox properties. They can act adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Basile *et al.*, 2005).

Food poisoning is still a concern for both consumers and food industry despite the use of various preservation methods. Due to pathogens resistance against antibiotics, there is a growing interest to use natural antibacterial products for food preservation, such as plant extracts and spices (Duros-Meot *et al.*, 2008).

The antimicrobial activity results are shown in Table 3. Plant extracts were effective on inhibiting the microorganisms when hexane, ethyl acetate, methanol, ethanol and aqueous extracts were tested. According to Nyiligira *et al.* (2008), plant extracts with a MIC value lower than 8.00 mg/ml are considered to have significant antibacterial activity. Based on this value, the hexane extract showed an antimicrobial effect on *Candida albicans* (MIC=2.5 mg/ml).

In conclusion, this work demonstrated for the first time the chemical composition of the essential oil and hexane fraction from leaves of *S. gesnerioides*, both mainly composed by guaiol. For the five *S.*

*gesnerioides* extracts, the hexane and ethyl acetate extracts showed the highest antimicrobial and antioxidant activity, respectively. However, further

studies are necessary to identify the compounds responsible for these activities.

**Table 2**  
**Total phenol and flavonoid content and antioxidant activity of *S. gesnerioides* extracts**

Extracts	Phenol content (mg/100mg)	Flavonoids (mg/100mg)	IC <sub>50</sub> (µg/mL)
Hexane	0.85 ± 0.10	0.68 ± 0.11	305.93
Ethyl Acetate	22.74 ± 0.09	13.65 ± 0.07	9.41
Methanol	9.54 ± 0.25	2.11 ± 0.38	31.82
Ethanol	9.75 ± 0.15	4.59 ± 0.19	24.64
Aqueous	8.82 ± 0.03	2.78 ± 0.34	73.56
BHT	-	-	11.82

Data are expressed as mean ± standard deviation (n=3).

**Table 3**  
**Minimal Inhibitory Concentrations (MIC) of *S. gesnerioides* extracts.**

Microorganisms	MIC (mg/ml)					Drug reference	
	Hex	EtOAc	MeOH	EtOH	Aq	Cloranphenicol (µg/ml)	Nistatin (U/ml)
<i>Staphylococcus aureus</i> ATCC 25923	-	5	-	-	-	2.5	NT
<i>Pseudomonas aeruginosa</i> ATCC 9027	-	10	-	10	-	2.5	NT
<i>Salmonella typhimurium</i> ATCC 14028	-	5	10	10	5	2.5	NT
<i>Shigella sonnei</i> ATCC 25931	-	5	10	10	5	2.5	NT
<i>Escherichia coli</i> ATCC 8739	-	10	-	-	5	2.5	NT
<i>Candida albicans</i> ATCC 60193	2.5	-	-	-	-	NT	20

Hex=hexane extract; EtOAc=ethyl acetate extract; MeOH=methanol extract; EtOH=ethanol extract; Aq= aqueous extract; (-) not detected at all tested concentrations; NT=not tested.

#### ACKNOWLEDGMENTS

The authors thank FAPEMIG, CNPq and CAPES for financial support.

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