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Essential oil composition and antichemotactic activity of *Stenachaenium* Benth. species native to South Brazil

[Composición y actividad antiquimiotóxica del aceite esencial de especies *Stenachaenium* Benth. nativas de Sur de Brasil]

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Abstract: Chemical composition of essential oils from four *Stenachaenium* species from South Brazil were established by gas chromatography coupled with mass spectrometry (GC/MS). The major compounds identified in the oil of *S. megapotamicum* were a coumarin derivative, 2H-1-benzopyran-2-one, 7-(3-methylbutoxy) (24.0%), β -bisabolene (12.8%) and thymol methyl ether (7.1%). The oil of *S. adenanthum* contained mainly pogostol (14.0%). *S. riedelli* oil showed significant presence of aliphatic compounds, with predominance of hexadecanoic acid in all samples (leaves, inflorescence and leaves collected during of inflorescence period). Hexadecanoic acid (23.8%) was also the main component in *S. macrocephalum*. Concerning antichemotactic activity, all the oil samples tested showed a significant leukocyte migration inhibition compared to chemotactic stimulant (lipopolysaccharide from *Escherichia coli* - LPS), at concentrations of 1 to 5 μ g/mL, except for *S. adenanthum*. These results suggest that the essential oils of some *Stenachaenium* species could inhibit acute inflammatory process, because the migration of neutrophils occurs mainly in the early inflammatory process.

Keywords: *Stenachaenium*, Asteraceae, essential oil, antichemotactic activity.

Resumen: Se estableció la composición química de los aceites esenciales de cuatro especies de *Stenachaenium* del Sur de Brasil mediante cromatografía de gases acoplada a espectrometría de masas (CG/EM). Los compuestos mayoritarios identificados en el aceite de *S. megapotamicum* fueron: un derivado de cumarina, 2H-1-benzopirán-2-ona, 7-(3-metilbutoxi) (24,0%), β -bisaboleno (12,8%) y éter metil timol (7,1%). El aceite de *S. adenanthum* presentó principalmente pogostol (14,0%). El aceite de *S. riedelli* mostró una significativa presencia de compuestos alifáticos, con predominio de ácido hexadecanoico en todas las muestras (hojas, inflorescencias y hojas recolectadas durante del período de la inflorescencia). También el ácido hexadecanoico (23,8%) fue el principal componente en *S. macrocephalum*. En cuanto a la actividad antichemotóxica, todas las muestras de aceites ensayadas a concentraciones de 1 a 5 μ g/ml, excepto para *S. adenanthum*, mostraron una inhibición significativa en la migración de leucocitos en comparación con agente quimiotáctico (lipopolisacárido de *Escherichia coli* – LPS). Estos resultados sugieren que los aceites esenciales provenientes de diferentes especies de *Stenachaenium* podrían inhibir procesos inflamatorios agudos, debido a que la migración de los neutrófilos se produce principalmente en el proceso inflamatorio temprano.

Palabras clave: *Stenachaenium*, Asteraceae, aceites esenciales, actividad antichemotóxica

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INTRODUCTION

Chemical compounds obtained from different plants species have been investigated for their ability to inhibit leukocyte migration as a mechanism related to anti-inflammatory activity (Andrade *et al.*, 2011; Suyenaga *et al.*, 2011). During an infection or inflammatory process, thousands of polymorphonuclear leukocytes migrate from the blood circulation or storage location to the focus of injury, in a process known as chemotaxis (Monks *et al.*, 2002). The activation of this process may be an important mechanism by which cells of the immune system are transported to the sites of inflammation.

Several studies have reported the anti-inflammatory effects of whole essential oils and of their constituents isolated. Among the botanical families characterized by the production of these substances is the Asteraceae family, which comprises about 1.500 genus and 25.000 species grouped in three subfamilies and 17 tribes, widely distributed, especially in temperate, tropical and subtropical regions (Beretta *et al.*, 2008). Species of this family are related to important biological activities, such as insecticidal, antimicrobial, antioxidant and anti-inflammatory actions that have been associated with the variety of composition of essential oils (Basile *et al.*, 2006; Đorđević *et al.*, 2007; Pavela *et al.*, 2010).

The *Stenachaenium* Benth. genus, of the Asteraceae family, was described by Benth in 1873 and subsequently included in the Pluche tribe. It comprises five species: *Stenachaenium adenanthum* Krasch., *Stenachaenium campestre* Baker, *Stenachaenium macrocephalum* Benth. ex Benth. & Hook.f., *Stenachaenium megapotamicum* Baker and *Stenachaenium riedelli* Baker (Beretta *et al.*, 2008; Loeuille, 2010). Although these species belong to a botanical family known for important biological effects, few reports have described the phytochemical and pharmacological activity of the species of this genus. A previous study by our research group reported the chemical composition and antidermatophytic activity of essential oil and nanoemulsion of *S. megapotamicum* (Danielli *et al.*, 2013). The other species are cited only in floristic surveys, as a group of wide occurrence in southern Brazil (Ritter & Baptista, 2005; Barros *et al.*, 2007; Ferreira *et al.*, 2010). Only *S. campestre* was mentioned in these studies. The species is popularly

used due to its anti-inflammatory, antithrombotic, anticoagulant, antirheumatic, abortifacient activities and against accident with poisonous animals (Barros *et al.*, 2007). Thus, the aim of this study was to obtain and characterize the essential oil from four *Stenachaenium* species native to south Brazil and evaluate the antichemotactic potential of these oils.

MATERIAL AND METHODS

Plant material

Stenachaenium species were collected in May 2011 (*S. adenanthum*, *S. macrocephalum* and *S. riedelli*), in Canela/RS and in March 2012 (*S. megapotamicum*) in Nova Santa Rita/RS, and identified by Dr. Sérgio L. Bordignon. Voucher specimens were deposited in the Herbarium of the Federal University of Rio Grande do Sul (UFRGS) ICN N° 190612, ICN No 190611 and ICN N° 182557, for *S. adenanthum*, *S. riedelli* and *S. megapotamicum*, respectively.

Essential oil obtention

The essential oils were obtained from milled fresh leaves and flowers, separately, for *S. riedelli* and, together, for *S. adenanthum*, *S. megapotamicum* and *S. macrocephalum*, by hydrodistillation for 4 hours, using a Clevenger-type apparatus (Farmacopeia Brasileira, 2010). The samples were stored at 4° C, in the dark until analyzed and tested.

Essential oil analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out with a Shimadzu QP5000 system. The GC column was a DB-5 fused silica capillary with a (5% phenyl)-methyl poly siloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. Helium was used as carrier gas in a flow rate of 1 mL/min. Injector and detector temperatures were set at 220° C and 250° C, respectively, and GC oven was programmed to 60 to 300° C at a rate of 3° C/minute. The essential oil was diluted in ethyl ether to a 2:100 (v/v) ratio. Identification of compounds was carried out by comparison of their relative retention times, calculated by linear interpolation relative to the retention time of a series of n-alkenes, and their mass spectra, with authentic samples or data taken from the literature (Adams, 2001; Adams, 2009), as well by

comparison with mass spectra recorded in the database as NIST 62 and NIST 12 (National Institute of Technology and Standards).

Antichemotactic assay

Experiments were carried out according to the modified Boyden chamber method, described by Suyenaga *et al.* (2011). Prior to the chemotactic assay, the leukocytes were treated with a range of 0.5-5.0 µg/mL of essential oils dissolved in Hanks' balanced salt solution (HBSS pH 7.4) at 37° C for 1 h. The leukocyte/samples were added in the upper wells of the chamber, separated by an 8.0 µm nitrocellulose filter (Millipore, USA) from the chemotactic stimulant (LPS - lipopolysaccharide from *Escherichia coli*) present in the bottom compartment. The leucocytes migration through the filter was measured using a microscope. The distance from the top of the filter to the farthest plane of focus containing two cells, in five microscopic fields, allowed the evaluation of leukocyte migration. Indomethacin was used as a control. All experiments were carried out in duplicate.

Statistical analysis

Differences between the control and the treatments were statistically analyzed by ANOVA followed by Tukey's test ($p < 0.05$ was considered statistically significant). Data were expressed as mean \pm SEM. Data analysis was performed using the GraphPad Prism 5.0 software.

RESULTS AND DISCUSSION

Chemical composition of the essential oils

To our knowledge, this is the first report on the chemical composition and biological activity of essential oils from *S. adenanthum*, *S. macrocephalum* and *S. riedelli*. The yield of essential oils varied, ranging from 0.07% for all samples of *S. riedelli* and *S. macrocephalum*, to 0.12% for *S. megapotamicum* and 1.2% for *S. adenanthum*. An important variation among the species was also observed concerning the visual aspect of oils. Samples of *S. megapotamicum* and *S. adenanthum* produced viscous and yellow oils; however, for the different samples of the species *S. riedelli* and *S. macrocephalum*, the oil extracted was a dense, yellowish liquid. It is known that the appearance of an oil is closely related to the characteristics of the chemical composition. Despite

belonging to the same genus significant differences were observed in the chemical composition of the species investigated in this study.

For the identification and quantification of constituents, the essential oils obtained were analyzed in gas chromatography coupled to mass spectrometry. The chromatograms for each sample are shown in Figure 1. Sixty-four compounds were identified for the essential oils of different species of *Stenachaenium* (Table 1). In general, regarding the terpenic fraction, sesquiterpenes (mainly oxygenated) were the most common compounds, and small percentages of monoterpenes were detected. In some samples, significant amounts of aliphatic compounds were also observed.

For *S. megapotamicum*, 76.2% of the total amount of compounds was identified, with prevalence of sesquiterpenes. The main terpenes identified in the oil were β -bisabolene (12.8%) and thymol methyl ether (7.1%). However, the major compound observed was a coumarin derivative, 2H-1-benzopyran-2-one,7-(3-methylbutoxy) (24.0%), besides the presence of an important amount of a ionone derivative (6-methyl- γ -(*E*)-ionone) (7.4%).

The essential oil of *S. adenanthum* was also characterized by the presence of terpenes, which were exclusively sesquiterpenes (37.7%), and small amount of aliphatic compounds (7.3%). Regarding sesquiterpenes, the main compounds identified were pogostol (14.0%), β -elemene (6.5%) and α -bisabolol (5.8%). Pogostol is a sesquiterpene oxygenated investigated in a few reports in the literature and usually related with *Pogostemon* species (Lamiaceae) was detected (Blank *et al.*, 2011). This sample also presented the coumarin derivative (7.3%).

Coumarin derivatives were found in the essential oil of different families of plants, whose quantity varies with the extraction method used. Volatile content of the aerial parts of *Melittis melissophyllum* L., obtained by solid phase microextraction and headspace showed 44.2% and 69.7% of coumarins, respectively (Maggi *et al.*, 2011). Significant quantities of different coumarin derivatives have been isolated from *Pterocaulon* species, which also belong to the Asteraceae family and the Inuleae tribe, and therefore are directly related to the *Stenachaenium* genus (Maes *et al.*, 2006; Stein *et al.*, 2006).

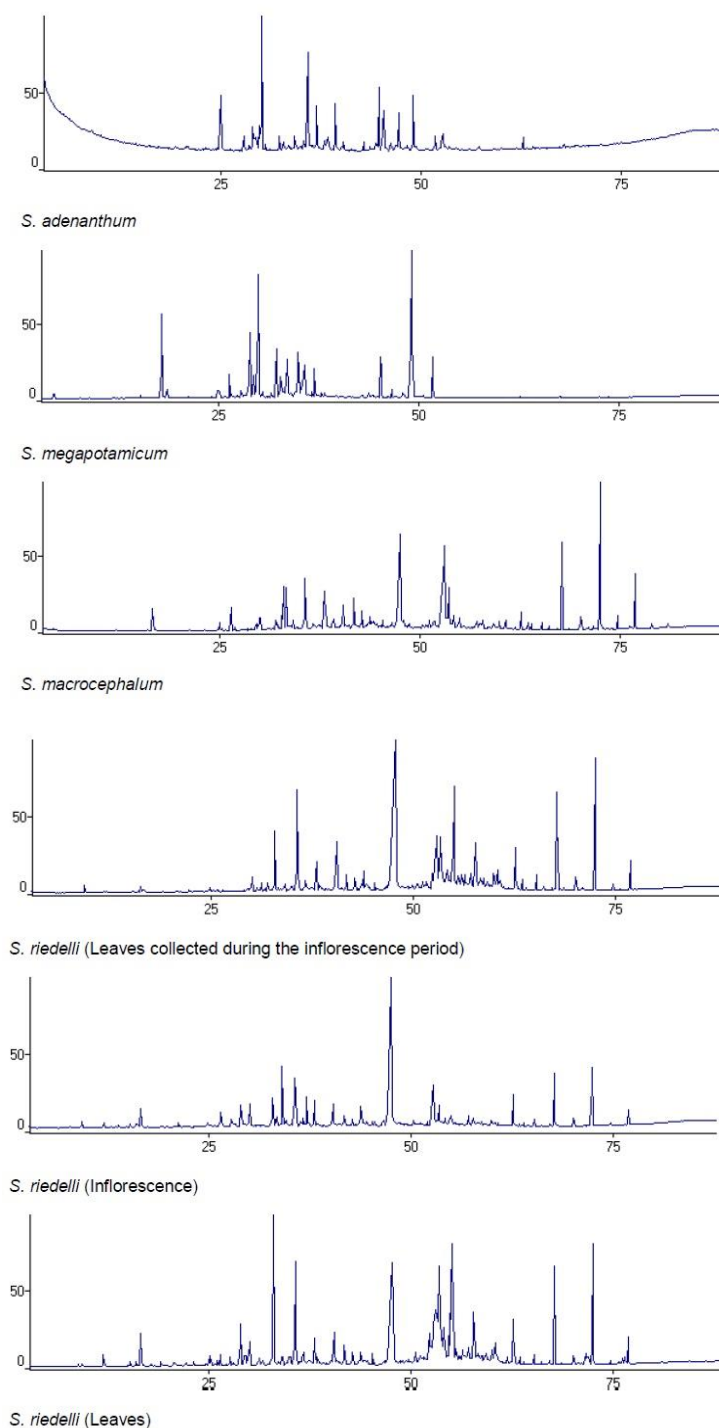


Figure 1
Chromatograms obtained by GC/MS for analysis of the chemical composition of essential oils from *Stenachaenium* species.

Table 1
Percentage composition of constituents of essential oils obtained from *Stenachaenium* species.

| Component | RI _a | RI _b | SME | SMA | SRI/LI | SRI/I | SRI/L | SAD |
|---|-----------------|-----------------|-------------|-----|------------|------------|-------------|-------------|
| Linalool | 1100 | 1098 | - | - | - | - | 0.4 | - |
| <i>n</i> -Decanal | 1204 | 1204 | - | 1.4 | - | 1.3 | 1.5 | - |
| Thymol methyl ether | 1232 | 1235 | 7.1 | - | - | - | - | - |
| β -elemene | 1381 | 1391 | 0.4 | - | - | - | - | 6.5 |
| Undecanoic acid | 1383 | - | - | 0.2 | - | - | - | - |
| Cyperene | 1387 | 1398 | 0.4 | - | - | - | - | - |
| 2,5-dimethoxy- <i>p</i> -cymene | 1416 | 1423 | 1.9 | 1.3 | - | 0.7 | 0.4 | - |
| Aromadendrene | 1449 | 1439 | 0.5 | - | - | - | - | 2.0 |
| 6-methyl- γ -(<i>E</i>)-ionone | 1474 | 1479 | 7.4 | - | - | 1.4 | 1.9 | 3.9 |
| β -selinene | 1482 | 1485 | 0.4 | - | - | - | - | - |
| α -selinene | 1483 | 1494 | 1.8 | - | - | - | - | 1.3 |
| Pentadecane | 1489 | 1500 | - | - | - | - | 0.4 | - |
| Viridiflorene | 1492 | 1493 | 0.4 | - | - | - | - | 2.7 |
| β -bisabolene | 1498 | 1509 | 12.8 | 0.7 | - | - | 0.6 | - |
| Butylated hydroxytoluene (BHT) | 1500 | 1512 | - | 0.7 | 0.5 | 1.8 | 1.1 | 16.4 |
| γ -cadinene | 1501 | 1513 | 0.5 | - | - | - | - | - |
| δ -cadinene | 1512 | 1524 | - | - | - | - | - | - |
| Ledol | 1550 | 1565 | - | 0.5 | - | - | - | 0.6 |
| (<i>E</i>)-nerolidol | 1556 | 1564 | 4.1 | - | - | - | - | 1.6 |
| Spathulenol | 1569 | 1576 | - | 0.9 | - | - | - | - |
| Caryophyllene oxide | 1572 | 1581 | - | 0.6 | 2.7 | 2.2 | 11.7 | - |
| Globulol | 1576 | 1583 | - | 2.8 | - | - | - | - |
| <i>Epi</i> -globulol | 1583 | - | - | 2.5 | - | - | - | - |
| <i>Trans</i> - β -elemenone | 1585 | 1600 | - | - | - | 0.5 | - | - |
| Fokienol | 1592 | 1596* | 3.2 | - | - | - | - | - |
| β -atlantol | 1602 | 1608* | - | - | - | 6.3 | - | - |
| Tetradecanal | 1604 | 1611 | - | 0.3 | - | - | - | - |
| Guaiol | 1606 | 1595 | - | - | - | - | - | 1.8 |
| Eremoligenol | 1621 | 1631* | 0.8 | - | - | - | - | - |
| γ -eudesmol | 1624 | 1630 | 4.1 | - | - | - | - | - |
| β -acorenol | 1631 | 1634 | - | - | - | - | - | - |
| <i>Epi</i> - α -cadinol | 1632 | 1640 | 1.2 | - | - | - | - | 0.3 |
| τ -cadinol | 1634 | - | - | - | - | - | - | - |
| β -eudesmol | 1642 | 1649 | 1.4 | 3.2 | 5.7 | 4.0 | 6.6 | - |
| α -eudesmol | 1645 | 1652 | 1.8 | - | - | - | - | - |
| Pogostol | 1646 | 1653* | 3.7 | 0.6 | 0.8 | 2.2 | 0.9 | 14.0 |
| α -cadinol | 1647 | 1653 | - | - | - | - | - | - |
| α -bisabolol oxide B | 1674 | 1655 | - | - | - | - | - | 0.6 |
| α -bisabolol | 1677 | 1683 | 2.2 | - | - | - | - | 5.8 |
| Pentadecanal | 1705 | - | - | 2.3 | 1.2 | 1.8 | 1.1 | - |
| Tetradecanoic acid | 1769 | - | - | 0.5 | 2.6 | 1.1 | 1.0 | - |
| Hexadecanal | 1806 | - | - | 1.7 | 0.6 | 0.6 | 0.8 | - |
| Heptadecanone | 1834 | - | - | 1.0 | 0.4 | - | 0.5 | 0.7 |
| Pentadecanoic acid | 1864 | - | - | - | 0.3 | 1.2 | - | - |
| NI1 | 1888 | - | - | - | - | - | - | 8.9 |

| | | | | | | | | |
|--|-------------|-------|-------------|-------------|-------------|-------------|-------------|------------|
| Heptadecanal | 1905 | - | - | 0.3 | - | - | 0.4 | - |
| Hexadecanoic acid | 1976 | 1960* | - | 23.8 | 39.9 | 46.8 | 19.1 | 5.0 |
| 2H-1-benzopyran-2-one,7-(3-methylbutoxy) | 2021 | - | 24.0 | - | - | - | - | 7.3 |
| Heneicosane | 2087 | 2100 | - | 0.3 | - | - | 0.2 | - |
| Heneicosanol | 2140 | - | - | - | 4.5 | 5.8 | - | - |
| NI2 | 2142 | - | - | 10.3 | - | - | - | - |
| NI3 | 2147 | - | - | - | 5.5 | - | - | - |
| NI4 | 2151 | - | - | 12.8 | - | - | 2.7 | - |
| NI5 | 2163 | - | - | - | - | - | 6.6 | - |
| Octadecanoic acid | 2169 | - | - | 3.0 | 0.7 | 1.1 | 2.0 | - |
| Docosane | 2190 | 2200 | - | 0.5 | 0.7 | - | 0.5 | - |
| NI6 | 2218 | - | - | - | 6.0 | - | - | - |
| NI7 | 2220 | - | - | - | - | - | 8.5 | - |
| Tricosanol | 2292 | - | - | - | - | 1.0 | - | - |
| Tricosane | 2295 | 2300 | - | 0.3 | - | - | - | - |
| Tetracosane | 2393 | 2400 | - | 0.3 | 0.6 | - | 0.4 | - |
| Tetracosanal | 2422 | - | - | 0.3 | - | - | - | - |
| Pentacosane | 2490 | 2500 | - | 0.9 | 1.9 | 2.5 | 2.1 | 1.4 |
| Pentacosanal | 2522 | - | - | 0.3 | 0.3 | - | - | - |
| Hexacosane | 2592 | 2600* | - | 0.3 | 0.6 | - | 0.4 | - |
| Heptacosane | 2695 | 2700* | - | 6.2 | 5.2 | 4.3 | 5.6 | - |
| Octacosane | 2790 | 2800* | - | 0.6 | 0.5 | - | 0.2 | - |
| Nonacosane | 2896 | 2900* | - | 12.3 | 7.7 | 4.9 | 7.3 | - |
| Triacontane | 2994 | 3000* | - | 0.8 | - | - | - | - |
| Untriacontane | 3092 | 3100* | - | 3.6 | 1.3 | 1.1 | 1.3 | - |
| Total compounds identified (%) | 80.1 | | 98.1 | 90.2 | 92.6 | 82.6 | 80.8 | |

Note: Retention indices on DB-5 capillary column. RI_a: Retention Index calculated; RI_b: Retention Index literature (Adams, 2001 and *2009); SME: *S. megapotamicum*; SMA: *S. macrocephalum*; SRI/LI: *S. riedelli* (Leaves collected during the inflorescence); SRI/I: *S. riedelli* (Inflorescence); SRI/L: *S. riedelli* (leaves); SAD: *S. adenanthum*. NI: notidentified. NI1: m/z (rel. int.): 41(100), 55(36), 65(27), 77(41), 91(56), 105(42), 121(38), 131(23), 145(32), 161(26), 171(50), 176(36), 199(6), 217(51), 232(23). NI2: m/z (rel. int.): 41(100), 55(75), 69(31), 81(45), 95(47), 107(26), 119(18), 135(10), 135(4), 150(2), 280(3). NI3: m/z (rel. int.): 41(100), 55(57), 67(46), 79(52), 95(23), 108(15), 121(5), 135(4). NI4: m/z (rel. int.): 41(100), 55(49), 67(52), 79(65), 93(26), 108(19), 121(7), 135(5), 149(2), 222(2). NI5: m/z (rel. int.): 41(100), 55(23), 69(77), 81(31), 93(25), 107(13), 119(13), 133(6), 147(4), 161(4), 187(2), 203(2). NI6: m/z (rel. int.): 41(100), 55(75), 69(31), 81(45), 95(47), 107(26), 119(18), 135(10), 150(9), 161(4), 187(3), 203(2). NI7: m/z (rel. int.): 41(100), 55(73), 67(32), 81(45), 95(45), 107(26), 119(22), 133(10), 150(7), 187(3), 202(1).

Stenachaenium riedelli was the only species that allowed obtaining the essential oil from different parts of the plant separately (leaves – SRI/L; leaves collected during the inflorescence period – SRI/LI and inflorescence – SRI/I). All these samples presented a significant amount of aliphatic compounds. Thus, SRI/LI exhibited predominantly these compounds (69.6%), with hexadecanoic acid as major component (39.9%), followed by nonacosane (7.7%) and heptacosano (5.2%). Only 9.3% of the oil constituents were identified as terpenes: β -eudesmol

(5.7%), caryophyllene oxide (2.7%) and pogostol (0.8%). In the sample collected during inflorescence (SRI/I), aliphatic compounds were the major constituents (74.1%), with predominance of hexadecanoic acid (46.8%). The terpenoidic fraction was only represented by sesquiterpenes, of which 0.7% corresponded to hydrocarbons and 15.4% to oxygenated, with β -atlantol (6.3%) as the most representative constituent. Aliphatic compounds (45.6%) were predominant in oil obtained from the leaves (SRI/L), and hexadecanoic acid was the major

constituent, but to a lesser amount (19.1%). Terpenoids were represented mainly by sesquiterpenes, where caryophyllene oxide (11.7%) and β -eudesmol (6.6%) were the most representative. Similarly, the chemical composition of *S. macrocephalum* presented aliphatic compounds as major constituents, with predominance of

hexadecanoic acid (23.8%), followed by nonacosane (12.3%) and heptacosano (6.2%). β -eudesmol was the most representative constituent in the terpenoidic fraction (3.2% of the total 11.4%). The molecular structures of the major constituents are schematically represented in Figure 2.

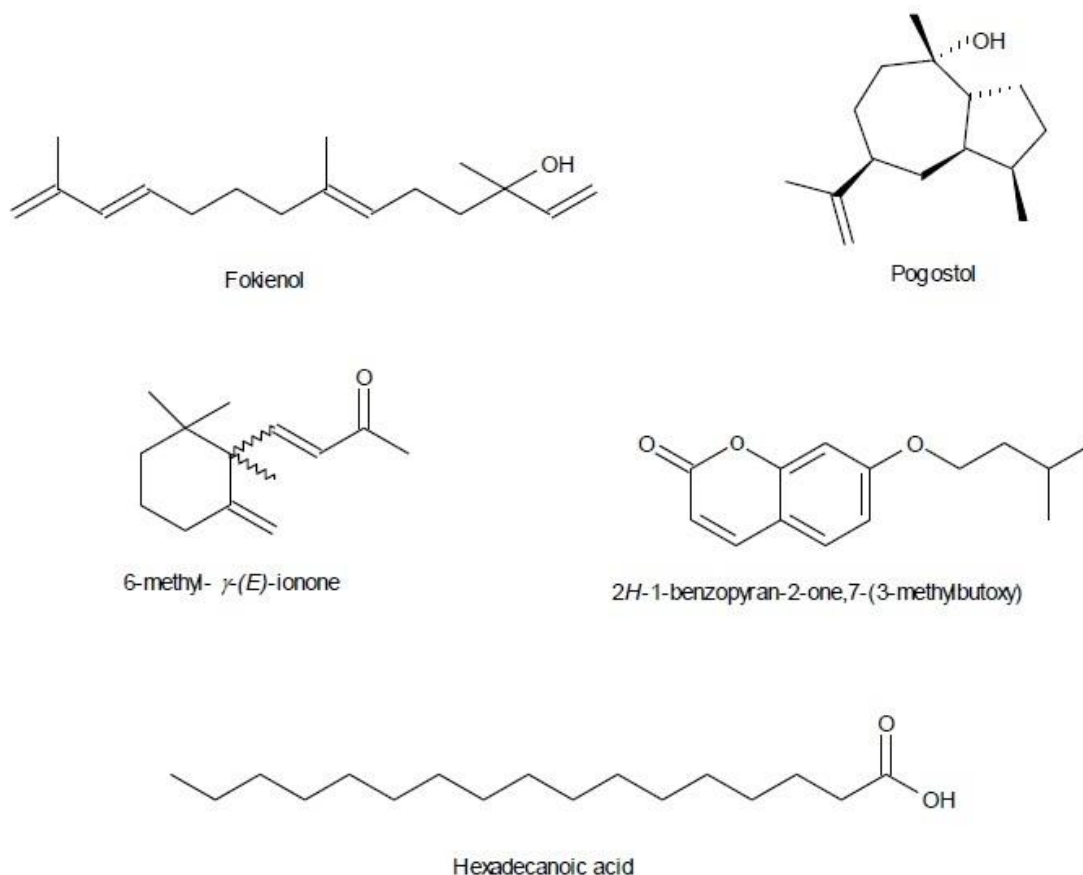


Figure 2

Chemical structure of the major compounds identified in the essential oils from *Stenachaenium* species.

Frequently, the chemical composition of essential oils is directly related with their aspect. As described, *S. megapotamicum* and *S. adenanthum* exhibited oil with viscous aspect. However, *S. macrocephalum* and the samples of *S. riedelli* presented dense liquid oil, probably due to the high quantities of aliphatic compounds, especially hexadecanoic acid. Aliphatic compounds, in general, are commonly observed in essential oils.

Hexadecanoic acid is often reported in different amounts, and it was described as the major component in some species (Tabanca *et al.*, 2007; Saïdana *et al.*, 2008; Rosselli *et al.*, 2009). Authors describe this compound as a constituent of plant waxes and for this reason it could be obtained during the extraction process of the essential oils (Reverchon & Senatore, 1994; Pansarin *et al.*, 2008).

Antichemotactic activity

The antichemotactic activity assay was used to evaluate the potential of essential oil to inhibit the polymorphonuclear neutrophil migration through a filter in the Boyden chamber method. Samples of essential oils from *S. riedelli* were tested at concentrations from 0.5 to 5 µg/mL, whereas for *S. adenanthum*, *S. megapotamicum* and *S. macrocephalum* only concentrations of 2.5 and 5 µg/mL were tested due to the reduced amount of oil obtained. The results of antichemotactic activity are shown in Table 2, expressed as maximum percentage of chemotaxis, compared to control (LPS). Indomethacin was used as a positive control and showed 55% inhibition of leukocyte migration at concentration of 10 µg/mL.

only concentrations of 2.5 and 5 µg/mL were tested due to the reduced amount of oil obtained. The results of antichemotactic activity are shown in Table 2, expressed as maximum percentage of chemotaxis, compared to control (LPS). Indomethacin was used as a positive control and showed 55% inhibition of leukocyte migration at concentration of 10 µg/mL.

Table 2
Effect of the essential oil from different *Stenachaenium* species on the polymorphonuclear neutrophil chemotaxis *in vitro* compared to LPS.

| Sample | Concentration (µg/mL) | Migration (µm) | Migration inhibition (%) |
|---------------|-----------------------|----------------|--------------------------|
| SMA | 5.0 | 52.6 ± 1.9* | 33.7* |
| | 2.5 | 57.7 ± 3.4* | 27.1* |
| SME | 5.0 | 30.1 ± 2.4* | 62.0* |
| | 2.5 | 52.5 ± 3.8* | 33.7* |
| SRI/I | 5.0 | 5.4 ± 0.8* | 93.15* |
| | 2.5 | 26.2 ± 1.7* | 67.0* |
| | 1.0 | 50.6 ± 3.1* | 36.2* |
| | 0.5 | 59.6 ± 3.5* | 24.8* |
| SRI/L | 5.0 | 9.7 ± 1.01* | 87.8* |
| | 2.5 | 7.8 ± 0.7* | 90.2* |
| | 1.0 | 37.4 ± 2.9* | 52.8* |
| | 0.5 | 39.1 ± 3.1* | 50.7* |
| SRI/LI | 5.0 | 0.0 ± 0.0* | 100.00* |
| | 2.5 | 3.0 ± 0.4* | 96.21* |
| | 1.0 | 43.1 ± 1.8* | 45.6* |
| | 0.5 | 66.5 ± 1.7 | 16.00 |
| Control (LPS) | - | 79.2 ± 6.4 | 0 |
| Indomethacin | 10 | 35.6 ± 3.0 | 55.0 |

Chemotaxis is represented as mean ± SEM of leukocyte migration. * $P < 0.05$ indicates a significant difference compared to reference chemoattractant (LPS) (ANOVA–Tukey's test). SMA (*S. macrocephalum*); SME (*S. megapotamicum*); SRI/I (*S. riedelli* – inflorescence); SRI/L (*S. riedelli* – leaves); SRI/LI (*S. riedelli* – leaves collected during inflorescence).

All the oil samples tested showed a significant leukocyte migration inhibition compared to LPS ($p < 0.05$), at concentrations of 1 to 5 µg/mL, except *S. adenanthum*, which showed no activity in the tested concentrations. For oils that showed a significant effect, inhibition ranged from 27% to 100%. *S. riedelli* samples presented higher antichemotactic profile, in relation to the others,

where SRI/LI exhibited 100% inhibition of migration at 5 µg/mL. The same concentration for SRI/L and SRI/I inhibited leukocyte migration by around 90%. All concentrations of the oil extracted from *S. macrocephalum* oil presented low activity, inhibiting only 27.1% and 33.7% of the leukocytes migration for 2.5 and 5 µg/mL, respectively. The differences found in activity were probably associated with

variations in the chemical composition of the samples. This result demonstrate a better effect when compared with indomethacin. In a concentration of 5 µg/ml, this compound was not able to inhibit leukocyte migration.

According to Miguel (2010), the anti-inflammatory activity of the essential oils may be related to signaling cascade involving cytokines and transcription factors. Some compounds isolated from essential oils have also been described with activities related to inhibition of inflammatory processes by various mechanisms, such as thymol, carvacrol, *trans*-cinnamaldehyde, myrcene, limonene, α -humulene, *trans*-caryophyllene, citral, 1,8-cineole, camphene, borneol, among others (Takaki *et al.*, 2008; Ballabeni *et al.*, 2010; Mulyaningsih *et al.*, 2010; Tavares *et al.*, 2010; Tumen *et al.*, 2011). Neutrophils are cells with an important role in host defense and collaborate in primary acute inflammatory processes through several mechanisms, such as release of chemotactic factors. Inhibition of process of leukocyte migration may be related to some mechanisms of control of the inflammatory response (Rioja *et al.*, 2000; Barros *et al.*, 2013). Whereas neutrophils are present in the acute phase of this process, the results suggest that the essential oils of different *Stenachaenium* species could act in this inflammation phase, inhibiting their migration to the focus of the lesion.

CONCLUSION

The analysis of the essential oil from different *Stenachaenium* species showed important variations in the chemical composition, mainly related to terpenic and aliphatic fractions. This difference is directly related to the biological effect of the samples. *S. riedelli* exhibited predominance of aliphatics compounds and few terpenes, and showed a higher capacity to inhibit leukocyte migration. However, others studies should be conducted to identify the compounds responsible for this activity.

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