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Chemical Profiling by GC/MS Analysis of Non-Polar Extracts of *Eryngium glaziovianum* (Apiaceae)

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ABSTRACT
GC/MS was used as a tool to achieve the chemical profiling of *n*-hexane extracts from flowers, leaves and stems of plant species *Eryngium glaziovianum* L. (Apiaceae), an ornamental plant commonly found in several states of Brazil. Gas chromatograms and mass spectra of the constituents of the extracts showed significant differences among the profiles and revealed the presence of the biologically active sesquiterpene b-eudesmol and steroids g-sitosterol and sitostenone as major constituents of flowers, leaves and stems, respectively. These data allow classifying this plant as a source for further isolation of these biologically important substances.

Keywords: Apiaceae, *Eryngium glaziovianum*, terpenes, GC-MS technique.

INTRODUCTION

Eryngium comprises more than 250 plant species, being the largest genus of Apiaceae [1-3] furthermore; its wide distribution and huge chemical and morphological diversity of its species suggest a complex taxonomy. [4] This genus is widely found in different parts of the world, and approximately two-thirds of the species are found in temperate regions of North, Central and South America. [5] Although most of the species of this genus are used for ornamental, medicinal and food purposes, there are still only a few phytochemical and pharmacological reports in the literature. So far, there are records of research for 23 species, which describe isolation and structure characterization of about 120 secondary metabolites. These metabolites are, mostly, phenolic compounds, monoterpenes, sesquiterpenes, triterpenoids, saponins, flavonoids, coumarins, steroids and acetylenes. [6]

Some biological activities of extracts and isolated compounds from Eryngium species have been reported. Cytotoxic activity in different human tumor cell lines, and also antibacterial, antifungal, antimalarial, anti-inflammatory, antimutagenic, antioxidant, anthelmintic, antidote to poisonous snake and scorpion properties have been described. [7] In addition, there are reports of the role of these species in folk medicine all around the world. Extracts of aerial parts and roots of *E. campestre* are reported in Turkey as antitussive, diuretic and aphrodisiac. [8-9] In Caribbean, *E. foetidum* leaves are widely used as hypotensive, wound healing and also to heal digestive diseases. [10] In Jordan, *E. creticum* species is described for the treatment of diabetes and scorpion sting. [11] *Eryngium glaziovianum* L. is a shrub that occurs in the Brazilian states of Rio de Janeiro, São Paulo and Minas Gerais, and it is found mainly in the Atlantic Forest. [12] Its use is mostly related to ornamental purposes and there are so far no reports about phytochemical studies in the

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literature for this species. This research aimed to carry out an investigation of the chemical composition of low-polarity extracts from flowers, leaves and stems of *E. glaziovianum* by Gas Chromatography coupled with Mass Spectrometry (GC/MS), which also contributes to the search of possible bioactive compounds in the species.

MATERIAL AND METHODS

Plant material

Flowers, leaves and stems of *E. glaziovianum* were collected in Itatiaia, State of Rio de Janeiro, Brazil in October, 2012. It was identified by Dr. Marcelo Trovó of the Biology Institute of Universidade Federal do Rio de Janeiro and a voucher specimen is deposited at the herbarium of this institute under the number MLO 548 (RB).

Preparation of extracts

Fresh flowers, leaves, and stems were separated, dried and milled to obtain a fine powder. Exhaustive extraction of 1 g of the milled plant material was exhaustively extracted with *n*-hexane and further filtered through Whatmann filter paper. Solvent was removed under vacuum and it yielded 40.3 mg, 39.8 mg and 40.7 mg of the extracts from flowers, leaves and stems respectively.

Analysis by GC/MS

Analyses were performed in a Shimadzu GC-2010 instrument equipped with a DB5 MS column (25 m × 0.25 mmID × 0.25 micro mdf) and coupled with a quadrupole detector. The oven temperature was initially set at 60°C with a gradient of 60 to 290°C (3.0°C/min, kept for 5 min) and 280 to 300°C (3°C / min, hold 2 min); injector temperature 290°C. Column flow 1.00 ml / min. Helium was used as the carrier gas and ionization energy of 70 eV. The percent relative amount of each component was calculated by comparing their average peak area to total area. Analysis was performed in triplicate to achieve more accurate results.

Identification of the chemical constituents

Peaks were identified by comparison of their mass spectra with those of NIST library (version 2.0., 2011), and also by comparison with the literature. [13]

RESULTS AND DISCUSSION

Analysis of the chemical composition of the non-polar extracts of *E. glaziovianum*

Chromatograms obtained on GC analysis for the *n*-hexane extracts of flowers, leaves and stems can be seen in Figures 1, 2 and 3, respectively. Gas chromatogram of the extract from the flowers showed 5 major peaks, as the leaves extract showed 6 and from the stem was observed only 4 major peaks. Mass spectra of each one of these peaks in the chromatograms were compared with those of the compound reference library (NIST®) to assign the chemical structures of the compounds.

Most of the compounds showed a significant similarity index (>90%), showing strong correlation between the compared spectra. The identified compounds of all the *n*-hexane extracts, together with their molecular formula (MF), molecular weight (MW), retention time (RT), percentage of the peak area (PA) and Similarity Index with the spectra in the NIST® library (SI), obtained by the GC/MS analysis, are described in Table 1.

Analysis by GC/MS allowed a preliminary study of the composition of non-polar extracts of *E. glaziovianum* as the major chemical constituents of these were identified. Chromatographic profiles of the extracts were different, and they revealed that the major components of each one are different.

Sesquiterpene β-eudesmol was the major metabolite present in the *n*-hexane extract from flowers, and showed a significant concentration (45.5%) compared to the other identified compounds. In Japan, β-eudesmol is one of the constituents in traditional medicinal products for relief of muscle aches. [14] In addition, studies using mice demonstrated that this terpene assists the enhancement of neuromuscular blockers, such as paeoniflorin and glycyrrhizin, and also succinylcholine and decamethonium. Nicotinic receptors have been shown to block channel acetylcholine at the neuromuscular junction. [15]

β-eudesmol has a potential for drug development in the treatment of angiogenic diseases, since it was shown to inhibit angiogenesis *in vitro* and *in vivo*, being more potent than thalidomide, which is widely used in this treatment. [16] It also exhibits excellent antimicrobial and antifungal activity in woods in deterioration process. [17] Recently, studies revealed that the β-eudesmol presented chopped activity against two species of mosquitoes, showing activity similar to DEET (*N, N*-diethyl-*m*-toluamide), commonly used as an insect repellent. [18]

Another constituent found in this extract which features description in the literature to biological activity was the sesquiterpene guaiol, which, like β-eudesmol, presented activity as insect repellent. [18-19]

Stigmasterol is a common steroid, which is also in the stem of *n*-hexane extract and shows prominent antimicrobial activity against various microorganisms, being even more powerful than the drug Fluconazole. [20] Antioxidant, hypoglycemic, inhibition of thyroid, antimicrobial, anticancer, anti-inflammatory and diuretic properties are also attributed to this steroid. [21]

Extract of the leaves showed that its major constituent is the triterpene sitostenone, which presents description for treatment against tuberculosis, hypoglycemia, antiarrhythmic, antiemetic, vasodilator and anti-inflammatory activity. [22-23] The second major compound, triterpene α-spinasterone shows antifungal action. [21]

The main constituent in the extract of the stems was the steroid γ-sitosterol, which was also found in the extract of the leaves, but in a low amount. γ-Sitosterol is described as an assistant to reduce hyperglycemia, and

in vitro studies showed potential in the treatment of breast and lung cancers. [24] Furthermore, it was observed antimicrobial, antioxidant, antibacterial and antifungal activity for this steroid. [25]

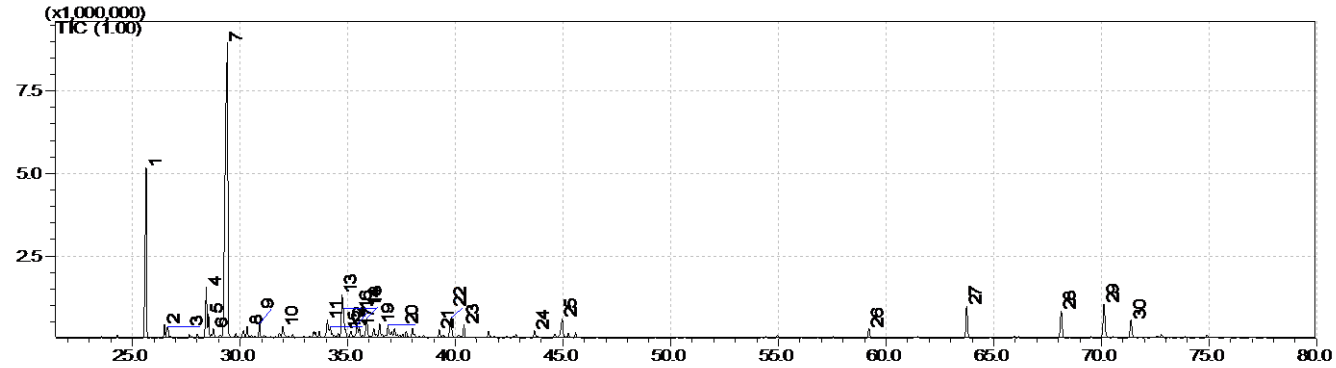

Fig. 1: Chromatogram from the *n*-hexane extract of flowers.

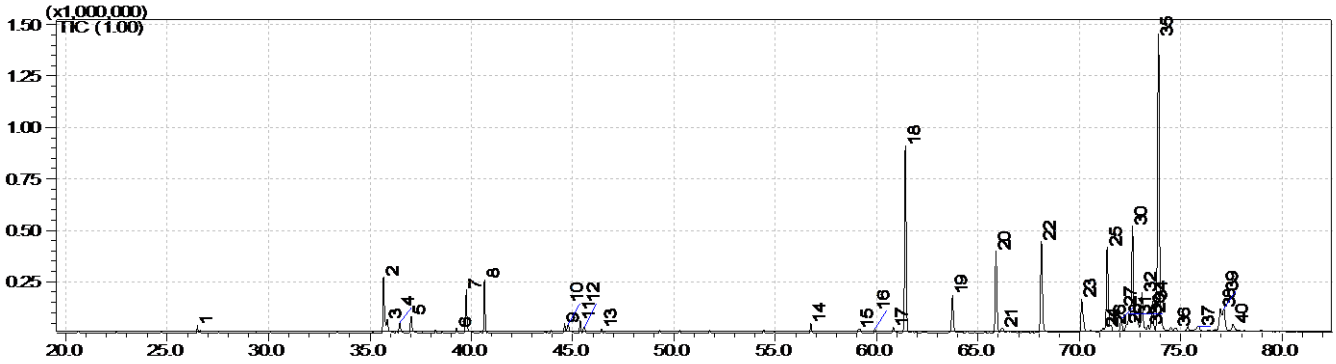

Fig. 2: Chromatogram from the *n*-hexane extract of leaves.

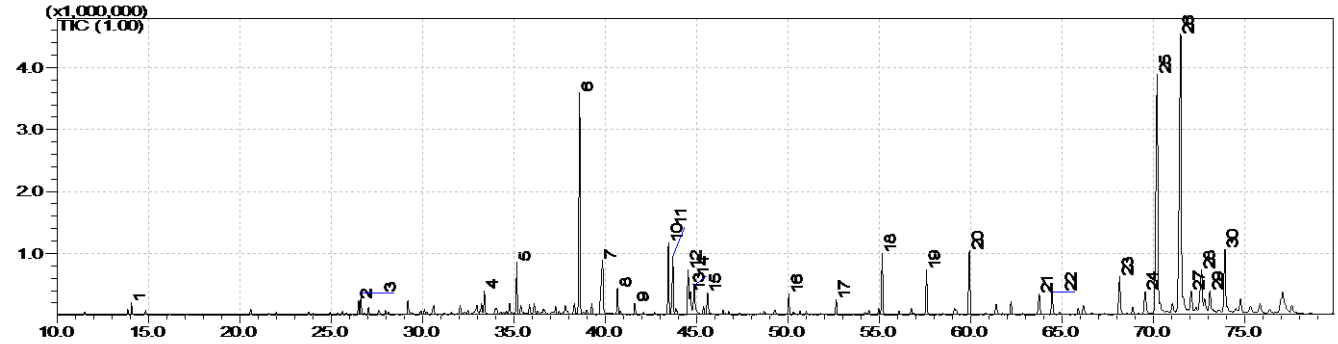

Fig. 3: Chromatogram from the n-hexane extract of stems.

Table 1: Major components of the *n*-hexane extracts of *E. glaziovianum*

Peak	Compound	MF*	MW**	RT***(min)	PA**** (%)	SI**** (%)
		FLOWERS				
7	β-Eudesmol	$C_{15}H_{26}O$	222	29.417	45.53	96
1	Elemol	$C_{15}H_{26}O$	222	25.642	14.73	96
29	Stigmasterol	$C_{29}H_{48}O$	412	70.125	3.67	91
4	Guaiol	$C_{15}H_{26}O$	222	28,433	3.66	92
27	Tetratriacontane	$C_{44}H_{90}$	618	63.750	3.07	97
		LEAVES				
35	Sitostenone	$C_{29}H_{48}O$	384	73.900	23.21	91
18	Octadecanoic acid ethyl ester	$C_{20}H_{40}O_2$	312	61.409	11.92	90
30	α-Spinasterone	$C_{29}H_{46}O$	410	72.617	8.15	86
22	Tetratriacontane	$C_{34}H_{70}$	478	68.125	7.00	92
25	γ-Sitosterol	$C_{29}H_{50}O$	414	71.367	6.84	94
20	Docosanoic acid ethyl ester	$C_{18}H_{36}O_2$	368	65.883	5.53	87
		STEMS				
26	γ-Sitosterol	$C_{29}H_{50}O$	414	71.483	23.12	94
25	Stigmasterol	$C_{29}H_{48}O$	412	70.183	18.64	91
6	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270	38.575	10.90	97
7	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	39.842	4.14	94

MF*= Molecular Formula, MW**= Molecular weight, RT***= Retention Time, PA****= Percentage of the peak area, SI*****= Similarity index with the NIST® library

The fourth major compound was the fatty acid ester hexadecanoic acid methyl ester, which has antioxidant activity. [26]

Other major constituent found in the extract of the stems is the fatty acid *n*-hexadecanoic acid, which has antioxidant, anti-inflammatory, antibacterial, hypocholesterolemic, nematicides, pesticides, antiandrogenic and lubricant activities. [27-30]

Thus, literature shows that the major constituents found in different organs of the species *E. glaziovianum* show biological activity and in some cases more effective than the commonly used drugs for the treatment of certain diseases.

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