Chemical composition of the Schinus molle L. essential oil and their biological activities

Composición química y actividad biológica del aceite esencial de *Schinus molle L*

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ABSTRACT

Introduction: essential oils and aromatic plant extracts have been recognized for many years as a great source of pharmaceutical agents. It is important to research on the chemical composition of the essential oils of plants widely used by the population because its usability becomes safer.
Objective: to identify the chemical characterization of *Schinus molle L*. essential oils and their biological activities and to compare them with the biological activities of the main compounds found in literature.
Methods: fifty grams of leaves were used to extract the oils through distillation in a modified Clevenger apparatus. The chemical analysis of volatile oils was carried out with capillary gas chromatography using flame ionization detector for quantitative analysis of its elements and, subsequently, a mass detector for qualitative analysis.
Results: nineteen substances were separated and the major compounds were bicyclogermacrene (20.5 %), betacaryophyllene (19.7 %) and spathulenol (19.2 %).
Conclusions: given the extensive distribution of the raw material in Rio Grande do Sul state, Brazil, more studies on the chemical properties and biological activities of Schinus molle essential oil are needed.

Keywords: essential oil, biological activities, *Schinus molle*.

RESUMEN

Introducción: los aceites esenciales y extractos de plantas aromáticas han sido reconocidos desde hace muchos años como una gran fuente de agentes farmacéuticos. Es importante la investigación de la composición química de los.aceites esenciales de las plantas utilizadas ampliamente por la población porque su aplicabilidad se torna más segura.
Objetivo: identificar la composición química del aceite esencial de las hojas de *Schinus molle L*. y correlacionarlas con las actividades biológicas de los principales compuestos localizadas en la literatura.
Métodos: cincuenta gramos de hojas se sometieron a extracción a través de destilación usando un equipo *Clevenger* modificado. Para el análisis químico, los aceites volátiles se sometieron a cromatografía capilar de gases usando un detector de ionización de llama para el análisis cuantitativo de sus elementos constituyentes y, posteriormente, un detector de masas para el análisis cualitativo.
Resultados: diecinueve compuestos fueron separados y los principales eran biciclogermacreno (20,5 %), betacariofileno (19,7 %) y espatulenol (19,2 %).
Conclusiones: dada la extensa distribución del material crudo en el estado de Río Grande do Sul, Brasil, se debe incrementar los estudios sobre las características químicas y las actividades biológicas del aceite esencial de *Schinus molle.*

Palabras clave: aceite esencial, actividades biológicas, *Schinus molle*.

INTRODUCTION

Schinus molle L. commonly called "peppertree, molle and aguaribay" is tree native to South America that belongs to the Anacardiaceae family. Nowadays it is distributed through Argentina, south-eastern Brazil, Peru, Colombia, Ecuador, Uruguay, Mexico, Central and Southern of California and West Texas, United States.[1]

In folk medicine, *S. molle* has been used as antibacterial, antiviral, topical antiseptic, antifungal, antioxidant, anti-inflammatory, anti-tumoural, anti-spasmodic, astringent, digestive stimulant, tonic, diuretic, wound healing, an analgesic agents, as well as a stimulant and an antidepressant.[2-9] It has also been used in the treatment of toothache, rheumatism, menstrual disorders, and respiratory and urinary tract infection.[10,11]

The essential oil (EO) in their chemical composition might justify the therapeutic use of this plant since the essential oils (EOs) and extracts of aromatic plants have been recognized for many years as a great source of pharmaceutical agents and food additives.[12] Their antioxidant capacity for acting in metabolic response to the endogenous production of free radicals and other oxidant species has been demonstrated.[13] Furthermore, EOs have shown important in vitro antimicrobial properties against pathogens and foodborne agents causing diseases.[14] The anti-in-ammatory activity of EOs has been investigated in in-ammatory diseases such as allergy, rheumatism, arthritis and bronchitis.[15] They tend to have low mammalian toxicity, less environmental effects and wide public acceptance.[16]

In the present study, was analyzed the chemical characterization of essential oil extracted from the leaves of *S. molle L*.

METHODS

PLANT MATERIAL

The leaves of *S. molle L*. were collected on Morro do Cechella in Santa Maria (Rio Grande do Sul, State of Brazil), coordinates 29°40'35''S and 53°47'06"W. Exsiccate was identified by Pharmacobotanic Laboratory (Industrial Pharmacy Department/UFSM).

ESSENTIAL OILS EXTRACTION, ISOLATION AND IDENTIFICATION

Fifty grams of leaves were subjected to extraction through distillation in a modified Clevenger[17] apparatus. After 2 hours, the fraction of oil was collected in ethyl ether, being dehydrated with sodium sulfate and concentrated on a water bath.

For chemical analysis, the volatile oils were diluted in ethyl ether in the ratio 2:100 (v / v). Subjected to capillary gas chromatography using flame ionization detector (GC/FID) for quantitative analysis of its constituents and, subsequently, to the mass detector (GC/MS) for qualitative analysis.

The separation and quantification of volatile oils constituents were performed on a gas chromatograph equipped with flow divider (spliter) with partition of 1:50. Helium was used as carrier gas at a pressure of 80 kPa and linear speed of 1 mL/min. Nitrogen, synthetic air and hydrogen were used as auxiliary gases to flame detector in 1:1:10 ratio, respectively. The quantification was obtained by electronic integration (normalization technique). For the separation of the constituents was used Durabond-DB5 column, with 30 m long and 0.25 mm internal diameter, filled with dimethyldiphenylsiloxane containing 5 % phenyl groups on a 0.25 mm thick film.

Qualitative analysis was performed using the same equipment, however coupled to a mass detector GC/MS-QP5000, equipped with cylindrical quadrupole, operated with ionization energy of 70 eV and sample partition of 1:20. The ionization was obtained by the technique of electron impact.

First of all, the chromatograms obtained by GC/FID and GC/MS of volatile crude oil were compared and the linear retention indices corresponding to each peak were calculated. A comparison was made with the retention times of the sample and a mixture of linear alkanes. The characterization of the constituents was based on Kovats indices (KI)[18] and their mass spectra, by comparison of these with authentic samples and literature data.[19]

STATISTICAL ANALYSIS

All the constituents identified in the essential oils were subjected to analysis of variance (ANOVA) and comparison of test averages (Tukey 5 %). Statistical analyses were performed by SPSS (version 10.1) and differences were considered statistically significant when $p<0.05$, $p<0.01$ and $p<0.001$.

RESULTS

The chromatogram obtained by GC/FID and GC/MS of volatile crude oil were compared and the linear retention indices corresponding to each peak were calculated. The chromatogram developed (figure) resulted in the separation of nineteen substances.

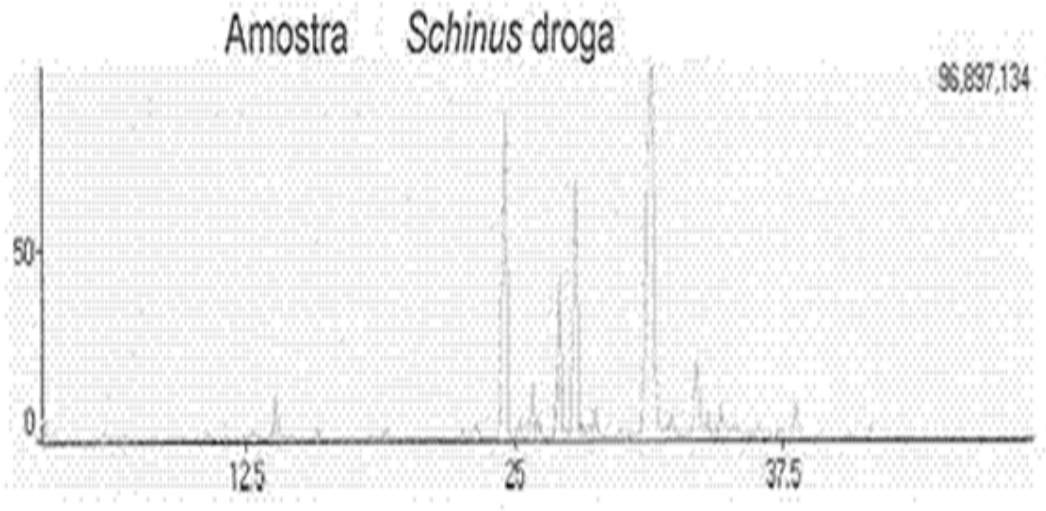

Fig. Chromatogram of EO leaves from *S. molle*.

Each peak in the chromatogram was identified by its mass spectrum, by comparison with library equipment, by consulting the literature[20, 21] and by injection of standards. Have the quantification of the relative percentage of identified constituents was obtained based on the areas of the chromatographic peaks for the method of normalization.

Statistical analysis no showed variation on the chemical composition of the EO, for the same sample.

Results of gas chromatographic analysis of *S. molle* EO are summarised in Annex, Nineteen components were identified in leaf EO, representing 2.8 % of monoterpenes and 92.2 % of sesquiterpenes. Leaf EO was characterized mainly by bicyclogermacrene (20.5 %), beta-caryophyllene (19.7 %), spathulenol (19.2 %), globulol (9.5 %), germacrene-D (7.4 %), caryophyllene oxide (5.3 %) and terpinen-4-ol (1.2 %).

Results of this work showed that major compounds were bicyclogermacrene (20.5 %), beta-caryophyllene (19.7 %) and spathulenol (19.2 %). Furthermore, sesquiterpenes hydrocarbons were the dominant components in studied EO.

DISCUSSION

Regarding the chemical composition of EOs, a study analyzed the EO composition of leaves and its relationships within and among 11 populations of *S. molle*, collected at different locations over the distribution area of this species in State of Rio Grande do Sul (Brazil) and Uruguay. The populations analyzed were: Alto Alegre; Bagé; Caçapava do Sul; Caxias do Sul; Dom Pedrito; Erechim; Pinheiro Machado; Quaraí; Sant'Ana do Livramento; São Borja (Brazil) and Rivera (Uruguay), each population composed by 5–8 individuals. The EO composition of the 11 populations (79 samples), extracted by steam distillation and analyzed by GC–MS, identified 22 compounds, including oxygenated and non-oxygenated forms of monoterpenes and sesquiterpenes, that represented between 73.5 % and 96.9 % of the total EO extracted. Four groups were formed by the Average Linkage cluster analysis. The first group was characterized by the compound sabinene; the second, which was the largest group, was characterized by the presence of alfa and betapinene; the third group comprised the samples corresponding to the São Borja population and was characterized by the high contents of alfacadinol; and the fourth group was characterized by the high concentrations of myrcene.22

Another study, was performed to investigate the allelopathic potential of *Schinus molle* and *Schinus terebinthifolius* EOs on onion and lettuce germination and initial growth, given the well-known biological activities of several of the terpenoid compounds found in EOs. Leaves were collected in Porto Alegre City (30° 1′ 39.73″ S51°13′ 43.45″ W), State of Rio Grande do Sul, Brazil. At least six plants of each species were sampled. *S. molle* EO contained betapinene as a major compound, also containing limonene and betapinene.[23]

Twenty-two components were identified in leaf EO of *S. molle*, collected in autumn in the Évora region, in southeast Portugal; representing 69.3 % of monoterpenes and 17.0 % of sesquiterpenes. Leaf EO was characterized mainly by alfaphellandrene (25.9 %), limonene (11.7 %), betamyrcene (11.1 %), betaphellandrene (10.5 %) and elemol (9.0 %).[24]

Was observed in this study, compared with previously published studies,[22-24] there was a difference in the chemical composition of the EOs from samples collected in the same region. Differences on chemical composition suggest the presence of different chemotypes of *S. molle.*[25] In EOs, constituents and concentrations depend not only on the plant species. Among the various factors that influence the chemical composition, the most important are the origin of the plant, plant part used, the stage of plant development, climate and growing conditions such as temperature, soil and fertilizer and distillation conditions and storage.[26]

The identification of the presence of bicyclogermacrene (20.5 %), betacaryophyllene (19.7 %) and spathulenol (19.2 %) in the present leaf EO can justify the use of *S. molle* in traditional medicine.

The bicyclogermacrene is not cited as the active bactericidal, but it has demonstrated larvicidal potential through testing with the larvae of the mosquito *Aedes aegypti*. The results showed that the oil of *Cordia leucomalloides* was able to kill 98.7 % of the larvae in the concentration of 100 ppm.[27] Anti-inflammatory and

anti-ulcer activity were also tested and its inhibited 90 % of stress-induced gastric ulcers while cimetidine inhibited 70 %.[28]

Betacaryophyllene is the main volatile constituent found in large amounts in the essential oils of different plants and spices, such as oregano (*Origanum vulgare* L.), cinnamon (*Cinnamomum* spp.) and black pepper (*Piper nigrum* L.).[29,30] It is known as a potential agent anti-inflammatory, antioxidant, protector of gastric mucosal, local anesthetic, anti-acne and anticarcinogenic, due to its capacity to detoxify xenobiotics or to attack cancerous cell lines.[31,32]

Cunico et al. extracted three essential oils from leaves, fruits and roots of *Ottonia martiana* Miq. (Piperaceae), common species in Brazilian Rain Forest, and analyzed by GC-MS and tested in an antibacterial assay against *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Pseudomonas aerogenes* (ATCC 27853) and *Escherichia coli* (ATCC 25922). Spathulenol was the major component of the oil extracted from the roots (17.83 %) and fruits (17:37 %). The oils showed an activity against *S. aureus* with the MIC of 5 mg mL^{-1} (fruit oil) and 5 µg mL^{-1} (root oil). This oils also showed an activity against *S. epidermidis* with the MIC of 5 µg mL^{-1} (fruit oil) and 5 µg mL^{-1} (root oil).[33]

The methanol extract of *Salvia mirzayanii* has shown an immunomodulatory effect on peripheral blood lymphocytes in study developed by Ziaei et al. Fractionation of the methanol extract and purification of the components using normal column chromatography and preparative thin layer chromatography resulted in identification of the bioactive compound, spathulenol, with an immunoinhibitory effect. Treatment of activated lymphocytes with a concentrated fraction containing 62 % of spathulenol showed a decrease in the proliferation of lymphocytes with an IC_{50} of 85.4 ± 11.08 µg/mL. Spathulenol showed the capacity to inhibit proliferation in the lymphocytes and to induce apoptosis in these cells possibly through a caspase-3 independent pathway.[34]

It is demonstrated that the chemical characteristics of the *S. molle* essential oil encourages more studies regarding biological activities, becoming economically viable, because, the raw material is widely distributed in the state of Rio Grande do Sul, Brazil

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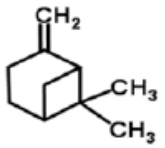
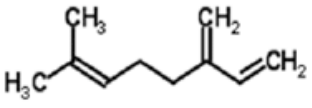
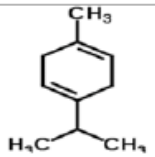
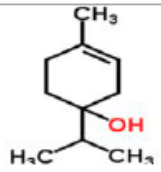
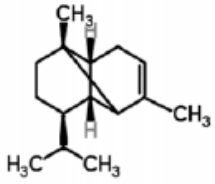
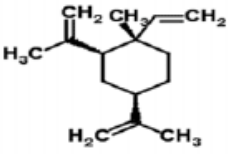
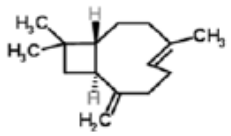
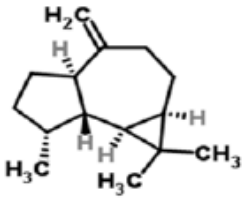
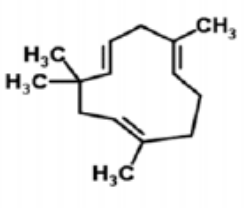
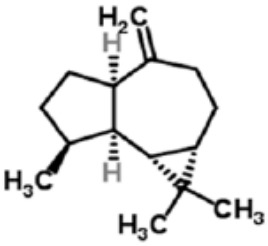
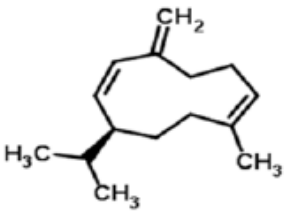
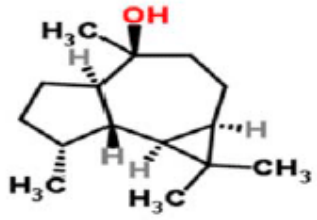
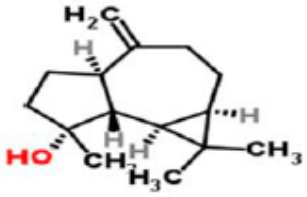
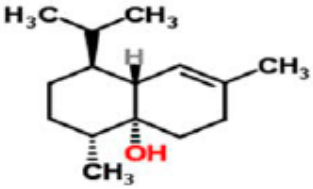
Annex. EOs isolated from the leaves of Schinus molle *L*. by gas chromatography.

Peak	KI	KI (Adams, 1995)	%	Structure
1	964	980	1.1	 *β pinene*
2	981	991	0.2	 *Myrcene*
3	1048	1062	0.3	 *γ terpinene*
4	1167	1177	1.2	 terpinen-4-ol
5	1363	1376	0.3	 αcopaene
6	1380	1391	0.5	 βelemene

7	1416	1418	19.7	 β caryophyllene
8	1428	1439	0.7	 Aromadendrene
9	1443	1454	1.5	 α humulene
10	1447	1461	0.4	 Alloaromadendrene
11	1474	1480	7.4	 germacrene-D

17	1594	1564	1.7	 Epiglobulol
18	1633	1619	2.0	 Isospathulenol
19	1639	1642	2.1	 Cubenol