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Composition of the essential oil of leaves and roots of *Allium schoenoprasum* L. (Alliaceae)

[Composición del aceite esencial de hojas y raíces de *Allium schoenoprasum* L.]

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Abstract
Essential oil from leaves and roots of *Allium schoenoprasum* L. (Alliaceae) were analyzed by GC/MS. The oils extracted by hydrodistillation yielded 0.02% and 0.03% for leaves and roots, respectively. Five (99.12 % leaves) and four (98.32 % roots) components were identified being bis-(2-sulfhydryethyl)-disulfide (72.06 % leaves, 56.47 % roots) the major constituent on the two oil samples while 2,4,5-trithiahexane (5.45 % leaves, 15.90 % roots) and tris (methylthio)-methane (4.01 % leaves, 12.81 % roots) were detected in lower amounts.

Keywords: *Allium schoenoprasum* L, essential oil, Alliaceae, bis-(2-sulfhydryethyl)-disulfide.

Resumen
Los aceites esenciales de hojas y raíces de *Allium schoenoprasum* L. (Alliaceae fueron analizados por CG/EM. La extracción por hidrodestilación mostró un rendimiento de 0.02% y 0.03% para las hojas y raíces, respectivamente. Cinco (99.12 % hojas) y cuatro (98.32 % raíces) compuestos fueron identificados siendo el bis-(2-sulfidietil)-disulfuro (72.06 % hojas, 56.47 % raíces) el compuesto mayoritario en los dos aceites mientras que 2,4,5-tritiahexano (5.45 % hojas, 15.90 % raíces) y tris (metilthio)-metano (4.01 % hojas, 12.81 % raíces) fueron observados en menores cantidades.

Palabras Clave: *Allium schoenoprasum* L, aceite esencial, Alliaceae, bis-(2-sulfidietil)-disulfuro.

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INTRODUCTION

Allium, the largest genus of Alliaceae, includes about 700 species, each differing in taste, form and colour but known to have close biochemical, phytochemical and neutraceutical properties (Benkeblia *et al*, 2000). These species are distributed mainly in Europe, North America, Northern Africa and Asia (Benkeblia and Lanzotti, 2007). Comprises perennial herbs with globose to piriform bulbs. The leaves are flat to cylindrical while stems are cylindrical, trigonus, or flat. The flowers are white, yellowish, pink, or purple; holding six petals that might be free or slightly connate at the base (Muoio *et al*, 2004).

For many centuries, several of these species have been used as vegetables and spices, and as folk medicines for curing various diseases (Haydar *et al*, 2005). Researches carried out in essential oil and crude extract of several *Allium* species have demonstrated antibacterial, antifungal, antixodiant and cytotoxic activities (Najjaa *et al*, 2009; Park *et al*, 2009; Pyun and Shin, 2006). On the other hand, previous investigations have reported a variety of compounds including thiosulfinates, organosulfur derivatives (Benkeblia and Lanzotti, 2007), steroidal saponins and terpenes. Studies have also revealed the health benefits of saponins, present in *Allium* genus, as lowering the cholesterol levels (Matsuura, 2001).

In the present investigation the essential oil composition of *Allium schoenoprasum* L (Alliaceae) analyzed by GC/MS, collected from Mérida-Venezuela, is being reported for the first time.

MATERIALS AND METHODS

Plant material

Leaves and roots of *Allium schoenoprasum* L. were collected in May 2010 in San Roman village, Mucuchies, at 2893 m above sea level, (754 mm of precipitation, temperature 13ºC and 72% of humidity, in average conditions). A voucher specimen (JR37) has been deposited in the Luis Terán Herbarium of the Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida, Venezuela.

Isolation of essential oil

Fresh leaves (960 g) and roots (1050 g) were cut into small pieces and submitted to hydrodistillation for 4 h, using a Clevenger-type apparatus. The oils were dried over anhydrous sodium sulfate and stored at 4ºC.

Gas chromatography

GC analyses were performed on a Perkin-Elmer Autosystem Gas Chromatograph equipped with flame ionization detectors. A 5% phenylmethyl polysiloxane fused-silica capillary column (AT-5, Alltech Associates Inc., Deerfield, Il), 60 m x 0.25 mm, film thickness 0.25 µm, was used for the GC analysis. The initial oven temperature was 60°C; this was then raised to 260°C at 4°C/min, and the final temperature maintained for 20 min. The injector and detector temperatures were 200°C and 250°C, respectively. The carrier gas was helium at 1.0 mL/min. The sample was injected using a split ratio of 1:100. Retention indices were calculated relative to C8-C24 n-alkanes, and compared with values reported in the literature (Adams, 1995; Davies, 1990).

Gas chromatography-Mass spectrometry

The GC-MS analyses were carried out on a Hewlett Packard GC-MS system, model 5973, fitted with a 30 m long, cross-linked 5% phenylmethyl siloxane (HP-5MS, Hewlett Packard, USA) fused-silica column (0.25 mm, film thickness 0.25 µm). The source temperature was 230°C, the quadrupole temperature 150°C, and the carrier gas helium, adjusted to a linear velocity of 34 m/s. the ionization energy was 70 ev, and the scan range 40-500 amu at 3.9 scans/s. The injected volume was 1.0 µl of a 2% dilution of oil in n-heptane. A Hewlett-Packard injector was used with a split ratio of 1:100. The identification of the oil components was based on a Wiley MS data library (6^{th} ed), followed by comparisons of ms data with published literature (Adams, 1995).

RESULTS AND DISCUSSION

Essential oil of leaves (0.2 mL; 0.02%) and roots (0.3 mL; 0.03%) of *Allium schoenoprasum* L. analyzed by GC/MS showed the presence of five (99.12% leaves) and four (98.32% roots) components. A list of identified compounds, along with their percentages of the total oil, is given in Table 1. The major constituent on the two oil samples was bis-(2-sulfhydryethyl)-disulfide (72.06% leaves, 56.47% roots) while 2,4,5-trithiahexane (5.45% leaves, 15.90% roots) and tris (methylthio-methane (4.01% leaves, 12.81% roots) were detected in lower amounts (Figure 1). Some differences were observed in the composition of the two oils; compounds such as 2,4,5-trithiahexane and tris-(methylthio)-methane were detected in minor concentrations on the leaves but in major amounts on the roots, while bis-(2-sulfhydryethyl)-disulfide was present as the major component in both samples.

However, two compounds on this study could not be identified. One of these gave a mass spectrum (MS) [m/z (rel. int.): M+ 217.9(5); 93 (33); 138.9 (71);

61 (100)], while another produced a MS [m/z (rel. int.): M+ 232(10); 139(19); 124(20); 107(37); 61(100). Unfortunately, we were unable to find a mass spectrum corresponding to these compounds during analysis on both the polar (HP-5MS) and non-polar (AT-WAX) columns, and these were not comparable with any of the compounds listed in either the library data base or the literature consulted (Adams, 1995; Davies, 1990).

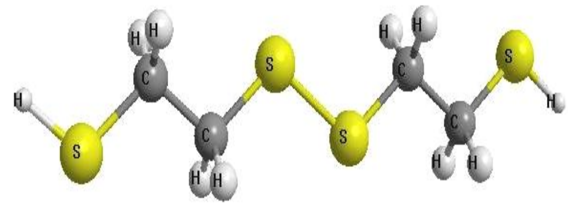
Table 1.-Composition of the essential oil of leaves and roots of *Allium schoenoprasum* L

Components	RI	Leaves (%)	Roots (%)
2,4,5-trithiahexane	1130	5.45	15.90
tris-(methyltio)-methane	1365	4.01	12.81
bis-(2-sulfhydryethyl)-disulfide	1527	72.06	56.47
217.9(5); 93 (33); 138.9 (71); 61 (100)	1755	15.26	13.14
232(10); 139(19); 124(20); 107(37); 61(100)	1903	2.34	-----

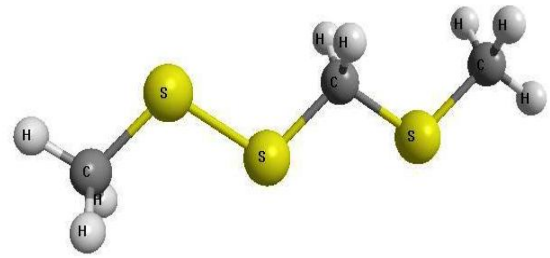
*The composition of the essential oil was determined by comparison of the MS of each component with Wiley GC/MS library data and also from its retention index (**RI**).

Furthermore, the lack of pure reference samples made it difficult to have complete identification of these compounds. According to the references consulted, it is well known that *Allium* species, are characterized by their rich content of thiosulfinates and other organosulfur compounds, such as the lachrymatory factor. However, depending on the species, and under differing conditions, thiosulfinates can decompose to form additional sulfur constituents, including diallyl, methyl allyl, and diethyl mono-, di-, tri-, tetra-, penta-, and hexasulfides, vinyldithiins, and (E)- and (Z)-ajoene. (Benkeblia and Lanzotti, 2007). Thus, the two unidentified compounds might be closely related to these.

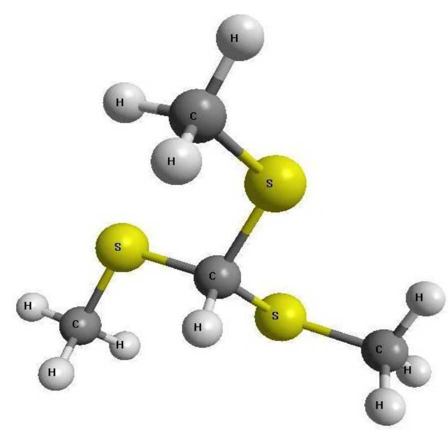
Many *Allium* species are mainly composed by sulfide type components that are biosynthesized from alliin, a non-protein sulphur aminoacid. (Benkeblia and Lanzotti, 2007). From *Allium tuberosum* Rottler growing in China and *Allium ascalonicum* L (Thailand) a series of diallyl mono, di, tri and tetrasulfide and some derivatives components were identified (Rattanachaikunsopon and Phumkhachorn, 2009; Yabuki *et al*, 2010). Similar components were reported for *Allium schoenoprasum* L. collected from China where only sulfide derivatives were observed (Rattanachaikunsopon and Phumkhachorn, 2008).



bis-(2-sulfhydryethyl)-disulfide



2,4,5- trithiahexane



tris-(methylthio)-methane

Figure 1.-Sulfur type constituents observed in the essential oil of leaves and roots of *Allium schoenoprasum* L.

On the other hand, *Allium roseum* L. collected from Tunisia showed a wide variety of compounds including thiophene, methyl disulfide and terpene (monoterpene and sesquiterpene) (Najjaa *et al*, 2007). Comparative studies on the essential oil of *Allium sativum L.*, *Allium cepa* L. and *Allium fistulosum* L. revealed the presence of methyl thiirane, dimethyl thiophene, methyl propyl disulfide, methyl *cis/trans* propenyl disulfide, ethyl thio methyl propene, di-2-propenyl disulfide along with limonene, γ-terpinene, linalool and other monoterpene and sesquiterpenes components (Yabuki *et al*, 2010).

Comparing previous investigations to the results observed in the present study, the composition of the essential oil of *Allium schoenoprasum* L collected from Merida-Venezuela is somehow similar to those reported for *Allium tuberosum* Rottler, *Allium ascalonicum* L and *Allium schoenoprasum* L, as the oil analyzed is composed of sulfide type components. Even though important differences might be observed, since in the present study di and tri straight chain sulfur compounds were identified that have not been reported previously.

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