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Chemical Composition of *Hypericum laricifolium* Juss. Essential Oil Collected from Mérida-Venezuela

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Abstract

Hypericum is one of nine genera forming the family *Hypericaceae* distributed mainly in temperate regions and tropical mountains. Fresh aerial parts of *H. laricifolium* Juss. collected by the Piñango road, Mérida state, at 3900 m above sea level, yielded 1.2 mL (0.24% w/v) of essential oil by hydrodistillation. **GC** and **CG/MS** analysis showed the presence of 21 components being *α*-pinene (20.2%), verticiol (13.4%), 3-methyl-nonane (12.3%), 2-methyl-octane (9.6%) and nonane (7.6%) in major proportions. To the best of our knowledge, this is the first report on the chemical composition of the *Hypericum laricifolium* essential oil.

Keywords: *Hypericum laricifolium;* Essential oil; Hypericaceae; GC-MS; *α*-pinene; Verticiol

Introduction

Hypericum is one of nine genera forming the family *Hypericaceae,* comprises about 500 species distributed mainly in temperate regions and tropical mountains being most abundant in Europe, Asia, Northern Africa and America [1,2]. Many of these species have been used in traditional medicine to alleviate a number of diseases such as antispasmodic, diuretic, antimigraine, antiepileptic and cholagougue [3], and also for diarrhea, dyspepsia, parasite, neuralgia, sciatica and rheumatism [4,5].

Phytochemical studies carried out with different *Hypericum* species have revealed the presence of bioactive substances like phloroglucinol derivatives, napthodianthrones, flavonoids, phenylpropanes, essential oils, benzophenones and xanthones, showing a wide range of biological properties related to antimicrobial, anticancer, antidepressant, antianxiety, antiviral, cytotoxic and antifungal activities [6-9].

In the present investigation, the chemical composition of *Hypericum laricifolium* Juss essential oil collected from Mérida-Venezuela has been studied and compared to several *Hypericum* species reported worldwide.

Materials and Methods

Plant material

Fresh leaves of *Hypericum laricifolium* Juss were collected in May 2012 by the Piñango road, approximately 20 km from "Collado del Condor" peak, at 3900 m above sea level. A voucher specimen (JR33) was deposited in the Luis Terán Herbarium of the Faculty of Pharmacy and Bio analysis, University of Los Andes, Mérida, Venezuela.

Isolation of essential oil

The botanical material (500 g) was cut into small pieces and subjected to hydrodistillation for 4 h, using a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulphate and stored at 4°C, until assays completed

Gas chromatography (GC)

GC analyses were performed on a Perkin-Elmer Auto System gas chromatograph equipped with flame ionization detectors. A 5% phenylmethyl polysiloxane fused-silica capillary column (AT-5, Alltech Associates Inc., Deerfield, IL), 60 m×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 [10,11].

Gas chromatography-mass spectrometry (GC-MS)

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). Source temperature 230°C; quadrupole temperature 150°C; carrier gas helium, adjusted to a linear velocity of 34 m/s; ionization energy, 70 eV; scan range 40-500 amu; 3.9 scans/s. The injected volume was 1.0 µL of a 2% dilution of oil in n-heptane. A Hewlett-Packard ALS injector was used with split ratio 1:100. The identification of the oil components was based on a Wiley MS Data Library (6th Edn.), followed by comparisons of **MS** data with published literature [11].

Results and Discussion

Fresh aerial parts of *H. laricifolium* yielded 1.2 mL (0.24% w/v) of essential oil, which showed by GC/MS (Figure 1), the presence of 21 components being *α*-pinene (20.2%), verticiol (13.4%), 3-methyl-nonane (12.3%), 2-methyl-octane (9.6%) and nonane (7.6%) in major proportions. Table 1 summarizes a complete list of identified components. According to previous reports, *α*-pinene is a common monoterpene identified in several *Hypericum* species, where it is found among the main components along with its isomer, *β*-pinene.

A study from Turkey reported for *H. triquetrifolium* essential

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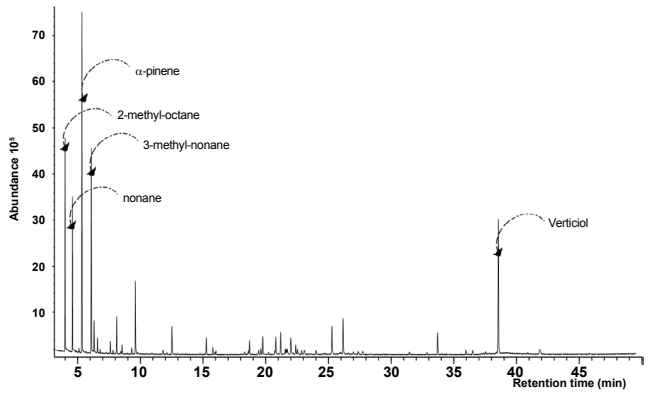

Figure 1: Gas chromatogram of *Hypericum laricifolium* Juss essential oil.

Compounds	%	RI
2-methyl-octane	**9.6**	**866**
nonane	**7.6**	**906**
α-pinene	**20.2**	**945**
3-methyl-nonane	**12.3**	**977**
β-pinene	1.9	986
sabinene	1.0	996
limonene	1.0	1039
(E)-β-ocymene	2.4	1058
2-methyl-decane	0.6	1073
terpinolene	0.5	1098
undecane	5.4	1311
α-terpineol	2.4	1424
2-undecanone	0.6	1430
(E)-α-bergamotene	0.5	1467
aromadendrene	1.5	1481
α-humulene	1.7	1496
β-acoradiene	2.0	1499
curcumene	0.4	1614
(E)-β-farnesene	0.5	1652
cembrene	2.0	1938
verticiol	**13.4**	**2166**

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).

Table 1: Essential oil composition of *Hypericum laricifolium* Juss.

oil α-pinene (12.3%) as one of the main components, while *H. aviculariifolium* subsp. *depilatum* var. *depilatum* showed high concentrations of α-pinene (52.1%), but β-pinene (3.6%) was present in minor proportions [12]. *H. annulatum* from Serbia also reported high concentrations of α-pinene (56.3%) and lower amount of β-pinene (4.2%) [13]. Similarly from Serbia, another investigation revealed that *H. richeri* subsp. *grisebachii* contained β-pinene (5.8%) among its components [14]. *H. elongatum* also showed a large amount of α-pinene (80.43%) and determined β-pinene (2.59%) between the minor compounds [14]. However, *H. rumeliacum* showed higher concentrations of β-pinene (26.1%) comparing to α-pinene (7.3%) [15].

On the other hand, *H. perforatum*, known as the most commercially important species within the genus *Hypericum*, showed the presence of α-pinene (8.6%) and β-pinene (3.8%), along with germacrene-*D* (22.1%), β-caryophyllene (11.3%), α-cadinol (4.4%), 2-methyl-octane (3.7%), terpinen-4-ol (3.3%), caryophyllene oxide (3.3%), α-muurolol (2.9%) and spathulenol (2.8%) [16]. Other investigation from Tajikistan showed that essential oil of *H. perforatum* was mainly composed by germacrene-*D* (13.7%), α-pinene (5.1%), trans-caryophyllene (4.7%), *n*-dodecanol (4.5%), caryophyllene oxide (4.2%), bicycle-germacrene (3.8%) and spathulenol (3.4%), while for *H. scabrum,* the main components were α-pinene (44.8%), spathulenol (7.1%), verbenone (6.0%), *trans*-verbenol (3.9%) and γ-muurolene (3.5%) [17].

A recent investigation carried out with several *Hypericum* species collected from different locations in Europe, Asia and North Africa reported for *H. uniglandulosum* 2,6-Dimethyl-3,5-heptadien-2-one (40.7%), nonacosane (3.2%), hexadecanoic acid (2.7%) and α-pinene (2.7%); for *H. scabroides,* hexadecanoic acid (17.7%), spathulenol (5.3%), nonacosane (4.4%), dodecanoic acid (4.1%), baeckeol (4.1%) and γ-muurolene (3.9%); for *H. kotschyanum*, α-pinene (14.4%), nonacosane (11.1%), hexadecanoic acid (9.2%), β-pinene (8.7%), spathulenol (6.3%) and limonene (5.1%). *H. salsugineum* showed nonacosane (42.7%), hexadecanoic acid (23.2%) and baeckeol (6.1%), and for *H. thymopsis* α-pinene (44.0%), baeckeol (32.9%), spathulenol (8.0%), limonene (7.6%) and camphene (5.2%) [18].

Hydrocarbons, such as 3-mehtyl-nonane (12.3%), 2-methyl-octane (9.6%) and nonane (7.6%) were also present among main components of *H. laricifolium* essential oil in the current investigation; related components have also been reported, previously, in high concentrations for other *Hypericum* species [12,13,15-18].

However, one particular component identified in this study caught the attention, since is very rarely detected in essential oils. Verticiol is a cembranoid diterpene that has been reported in the essential oil of several species belonging to *Burseraceae*, *Lauraceae* and Cupressaceae families [19-21]. Particularly, *B. elongata* (*Burseraceae*) oil is mainly composed by verticiol (52.4%); authors revealed that this particular compound has also been previously reported for *Boswellia elongata* collected from a different location in Yemen [19]. However, to date this component has not been identified in any *Hypericum* species. To the best of our knowledge, this is the first report on the chemical composition of the *Hypericum laricifolium* essential oil.

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