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Isolation and identification of secondary metabolites from *Artemisia sodiroi* Hieron

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Abstract

Artemisia sodiroi Hieron (Asteraceae), commonly known as "Ajenjo", is used in traditional medicine in Southern Ecuador for the treatment of inflammations, stomachaches, hepatic pains, fever, kidney problems, headaches, cough and by women to regulate their menstrual cycle. In this paper we report a phytochemical screening of the aerial parts of *A. sodiroi*, which led the identification of five known secondary metabolites, 24- methylenecicloartanol, stigmasterol, sitosterol, sabinyl acetate, and methyl-L-inositol, their structure were elucidated by spectroscopic methods including GC/MS, ^{1}H NMR and ^{13}C NMR.

Keywords. *Artemisia sodiroi*, phytochemicals, 24-methylenecycloartanol, stigmasterol, sitosterol, sabinyl acetate, methyl-inositol.

Abstract

Artemisia sodiroi Hieron (Asteraceae), comúnmente conocido como "Ajenjo", se utiliza en la medicina tradicional en el sur de Ecuador para el tratamiento de inflamaciones, dolores de estómago, dolores hepáticos, fiebre, problemas renales, dolores de cabeza, tos y se utiliza por las mujeres para regular su ciclo menstrual. En este trabajo se presenta un tamizaje fitoquímico de las partes aéreas de *A. sodrioi*, lo que condujo a la identificación de cinco metabolitos secundarios conocidos, 24 - methylenecicloartanol, estigmasterol, sitosterol, el acetato de sabinyl, y metil-L-inositol, su estructura fueron determinadas por métodos espectroscópicos como GC / MS, ^{1}H NMR y ^{13}C NMR.

Palabras Clave. *Artemisia sodiroi*, fitoquímico, 24-methylenecycloartanol, stigmasterol, sitosterol, sabinyl acetate, methyl-inositol.

Introduction

The use of plants to treat and cure diseases is an ancestral practice, however the systematic study of plants for identified their chemicals constituents is still in progress.

Asteraceae family have yield several important compounds described as the active principles of medicinal plants used in traditional medicine. In the Artemisia genus of this family the most studied species are: *A. annua* employed in medicine against malaria, and source of antimalarial compound, artemisinin; *A. apiaceae* and *A. araratic* have been reported to contain flavonoids [1], and *A. albida* Willd has yield sesquiterpene lactones and flavonoids [2].

In Ecuador three species have been reported within Artemisia genus [3], *A. absinthium* L., *A. sodiroi* Hieron., and *A. annua* L. The aerial parts of *Artemisia sodiroi* commonly known as "Ajenjo" are used in traditional medicine in Southern Ecuador for the treatment of inflammations, headaches, stomachaches, hepatic pains, fever, kidney problems, he- adaches, cough and by women to regulate their menstrual cycle [4].

A GC/MS analysis of essential oil of *A. sodiroi* has been reported [5], colorimetric tests for the determination of secondary metabolites in the methanol extract showed the presence of alkaloids, tannins, steroids, and sesquiterpene lactones, cardiac glycosides [6]. However, there are no from previous reports of the isolation of phytochemicals form this species.

The purpose of this study is to identify and characterize the secondary metabolites from the aerial parts of *Artemisia sodiroi*. In this paper we report the isola-

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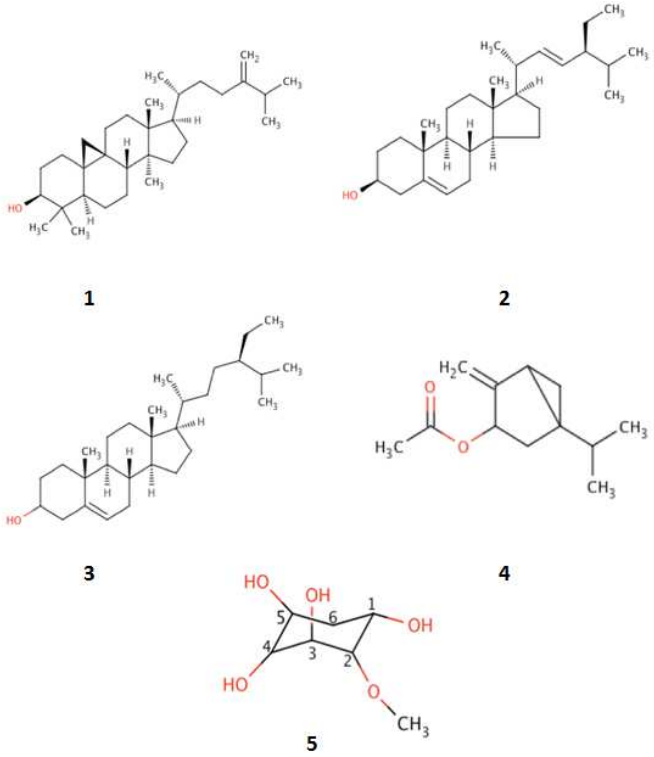

Figure 1: Structures of 24-methylenecycloartanol (1), stigmasterol (2), sitosterol (3), sabinyl acetate (4) and methyl-inositol (5).

tion and chemical characterization of five known compounds.

Materials and methods

General experimental procedures

Uncorrected melting points were measured with a Fisher-Johns apparatus. The GC/MS data were obtained in an Agilent 5975C Series GC/MS instrument operating under electron impact conditions (EI) at 70eV. NMR spectra were recorded in $CDCl_3$ and DMSO at 25^{o}C on a Varian, operating at 400MHz for ^{1}H and 100Mz for ^{13}C spectra. All 1D and 2D spectra were acquired and processed with standard Varian software. Column chromatography was carried out using 60-230 mesh silica gel. Thin layer chromatography (TLC) was performed on aluminum plates covered with silica gel 60 F_{254} plates (0.2 mm thick, Merck).

Plant material

The aerial parts of *A. sodiroi* were collected in June 2009 in the locality of Chuquiribamba, in the Loja province of Ecuador. Voucher specimens with the code PPN-as-021 are deposited at the Herbarium of the Applied Chemistry Institute of the Universidad Técnica Particular de Loja, Loja-Ecuador.

Extraction and isolation

The air-dried plant material (400 g) was extracted with hexane (dynamic maceration for 5 h) at room temperature and filtered in vacuum, the plant residue was extracted with ethyl acetate under the same conditions described before, after de filtration process the new residue was extracted with methanol (same pressure conditions); finally the three extracts (hexane, ethyl acetate and methanol) were concentrated under reduced.

Each one of the extracts hexane (7.48 g.), ethyl acetate (12.16 g.) and methanol (34.02 g.) was fractioned by column chromatography that was packed using wet packing method in hexane, using a ratio of 1:40 extract-silica gel. The columns were run using hexane, ethyl acetate and methanol by gradient elution technique. From the hexane extract fractionation 290 fractions were collected. From the ethyl acetate extract 290 fractions and from the methanol extract fractionation 282 fractions were collected.

Compound **1** (Figure 1) was eluted in the hexane-ethyl acetate (9:1) fraction (10 mg) as a white solid. Compound **2** and compound **3** were eluted in the hexane-ethyl acetate (8:2) fraction as a white solids; this three compound were obtained from the hexane extract fractionation. Compound **4** was eluted from the ethyl acetate extract in the hexane-ethyl acetate (8:2) fraction as a white solid. Compound **5** was eluted from the methanol extract fractionation in the ethyl acetate-methanol (8:2) fraction and recrystallized from methanol as a white crystalline solid.

Spectroscopic characterization

GC/MS analysis was used to identify compounds **1** to **4**, while ^{1}H NMR and ^{13}C NMR was used to identify compound **5**.

24-methylenecycloartanol (**1**): $C_{31}H_{52}O$, the mass spectrum of compound **1** shown the molecular ion at *m/z* 440 with fragments at *m/z* (relative intensity) 440(25), 422(66.92), 407(100), 379(53.01), 300(58.81), 175(68.4), 121(77.67), 81(83.71). The results were compared with other studies previously reported[7].

Stigmasterol (**2**): $C_{29}H_{48}O$, the mass spectrum shown the molecular ion at *m/z* 412, with fragments at *m/z* (relative intensity) 412(100), 394(26.37), 369(17.34), 327 (25.43), 300(38.3), 271(48.9), 95(49.5), 69(64.16), these fragments were compared with the reported in the literature [8].

Sitosterol (**3**: $C_{29}H_{50}O$, the mass spectrum showed the molecular ion at *m/z* 414 with fragments at *m/z* (relative intensity) 414 (100), 396 (48.16), 329 (40.6), 303 (36.02), 273 (17.02), 255 (20.23), 213 (23.07), 145 (25.36), 107 (24.9), 95 (22.66), 81 (22.7), 69 (16.69), these data was compared with the reported in the literature for this compound [8].

Sabinyl acetate (**4**): $C_{12}H_{18}O_2$, the mass spectrum shows the characteristic fragments at *m/z* 150[M-44], 134[M-60], 91[$C_7H_7^+$].

Methyl-inositol (**5**): colorless crystal (methanol), $C_7H_{14}O_6$, mp 190-193^{o}C. ^{1}H NMR (D_2O, 400 Mz): δ 3.08 (dd, 1H); 3.26 (m, 1H); 3.31 (s, 3H, H-OMe); 3.39 (m, 1H); 3.44 (m, 1H); 3.66 (q, 1H); 3.84 (q, 1H); 4.29 (d, H-O); 4.43 (dd, 2H-O); 4.63 (dd, 2H-O). ^{13}C NMR (D_2O, 100 Mz): δ 56.81 (Me); 68.062 (C1); 70.501 (C5); 72.059 (C6); 72.236 (C4); 73.322 (C3); 81.113 (C2).

Results and discussion

From the hexane extract of *A. sodiroi* three triterpenes were identified using GC/MS analysis. The weak molecular ion of compound **1** was given at *m/z* 440 and the characteristic peaks at *m/z* 422 [M-H_2O], 407 [-CH_3], 300 [M-$C_{10}H_{20}$]. The molecular ion and the fragmentation pattern indicate that the compound is 24-methylenecycloartanol. The fraction eluted in hexane-ethyl acetate (8:2) shown two compounds with molecular ions at *m/z* 412 and 414, the characteristic peaks are given at *m/z* 271 [M-139] and 273 [M-141] due to the loss of $C_{10}H_{19}$ and $C_{10}H_{21}$. The molecular weights and the fragmentation pattern indicate that the compounds present in this sample are stigmasterol (**2**) and sitosterol (**3**) respectively. The unique difference between these two structures is the presence of a double bond in the side chain. In traditional medicine in southern Ecuador the specie *A. Sodiroi* is used by woman to treat menstrual discomforts, use that may be associated with the presence of sitosterol in the plant; previous studies report the activity of this compound as a phytoestrogen [9].

From the ethyl acetate extract the fraction eluted in hexane-ethyl acetate (8:2) was analyzed in GC/MS, the mass spectrum did not show the molecular ion, but the structure was confirmed as sabinyl acetate (**4**) by comparison with the spectra reported in the literature [10] which shows the characteristic fragments at *m/z* 150[M-44], 134[M-60], 91[C7H7 +].

Methyl-inositol (**5**) was isolated as a colorless crystal (methanol) from the methanol extract in the ethyl acetate-methanol (8:2) fraction, this structure was elucidated by NMR. The ^{13}C NMR spectrum showed seven carbon signals with chemical shifts within the region of heteroatom-linked carbon. The ^{1}H NMR spectrum showed signals between 3.08 and 4.63, the signal at δ 3.31 was assigned to the three-methoxyl protons, and the signal at δ 3.08, 3.26, 3.39, 3.44, 3.66, 3.84 were atributed to the methine protons. The assignments of its ^{1}H and ^{13}C NMR resonances have been done involving two-dimensional experiments COSY, HMQC, and HMBC. NMR analysis of this compound was previously reported [11] and it is fully consistent with our study.

Conclusions

Phytochemical study of the extracts of *A. sodiroi* allowed the identification of five secondary metabolites three triterpenes (24-methylenecycloartanol, stigmasterol and sitosterol), an ester of a monoterpene alcohol (sabinyl acetate), and a cyclitol (methyl-inositol). The compounds were characterized using spectral techniques.

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