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Scientific Research

GC-MS Analysis of the Essential Oil and Methanol Extract of the Seeds of *Steganotaenia araliacea* Hochst

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Abstract

Background: *Steganotaenia araliacea* is a multipurpose plant and has wider applications in the folklore for the treatment of various ailments. Previously the presence of antileukemic lignan lactones and saponins was detected in the stem bark, root and leaf of *Steganotaenia araliacea.* Besides, the diuretic and antibacterial activities of the plant were reported. However, there has been no attempt to examine the constituents of the seeds of *Steganotaenia araliacea*. This paper reports the first such study of both the essential oil and methanol extract of the seeds of the plant. Methods: The seeds of the plant were shade dried, pounded and thus extracted using methanol. Besides, the essential oil of the seeds was collected using steam distillation. The components of the methanol extract were studied both by GC-MS and preliminary phytochemical studies; the essential oil was running on GC-MS for analysis. Results: The GC-MS analysis of the essential oil of the seeds identified the presence of *α*-linalool, *α*-pinene, m-cresol, *p*-menth-1-en-4-ol, *p*-menth-1-en-8-ol, myristicin and others. Besides, the methanol extract of the seeds showed the presence of falcarinol, apiol, scoparone, stigmasterol, myristicin etc. The preliminary phytochemical analysis of the methanol extract of the seeds confirms the presence of alkaloids, flavonoids, tannins, coumarines, steroids, and phenols. Conclusion: This plant contains bioactive metabolites and thus can be used as an alternative and complementary medicine in treatment of different ailments. However, further studies on the bioactivity and toxicity of the plant should be done.

Keywords

GC-MS, *Steganotaenia araliacea*, Essential Oil, Phytochemicals

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1. Introduction

Steganotaenia araliacea is a small tree up to 10 m tall, with leaves, which when crushed smell like carrots. It is exceptionally drought resistant but tolerates high rainfall. It occurs over a wide range of altitude. The plant is relatively widespread in Tropical Africa and is found in Mali, Cameroon and other regions of West Africa; Ethiopia, Somalia and Eritrea in East Africa and in a variety of locations in Southern Africa [1]. In Eritrea it is found both in open woodland and on riverbanks (1300 - 2100 m). Given the wide distribution it is unsurprising that in different areas it has been used in a number of ways in traditional medicine [2]. In Eritrea it is used in treatment of arthritis, chronic ulcer, snakebite, gynecological problem, hypoglycaemia, sore-throat, diuretic, dysentery, hypotensive and wound healing [3]. Elsewhere in Africa *Steganotaenia araliacea* has been used against malaria and other opportunistic infections resulting from AIDS [4].

The analyses of the constituents of *Steganotaenia araliacea* were dominated by the observations concerning the occurrence of cytotoxic lignans, particularly in the stem-bark, leaf and roots. In 1973 Kupchan *et al.* reported the presence of antileukemic lignan lactones in *Steganotaenia araliacea* of Ethiopian origin [5]. Over the next 20 years there was extensive activity to investigate the structure of related lignans and saponins, mostly in those from *Steganotaenia araliacea* of West African origin [6]-[15]. In addition, these structural studies stimulated a flurry of synthetic chemistry [16]-[19]. *Steganotaenia araliacea* has been reported as a multipurpose plant and has wider applications in the traditional medical practices and thus certain researches are reported. A review of the studies related to the medicinal uses of the plant include: the antioxidant and antibacterial activities [20] [21], diuretic activity of the stem bark [22], antimitotic and antitubulin activity of stegancin found in that plant [23]. Moreover, the insecticidal application of the plant, as antiplasmodial [24], larvicidal activity of the leaves [25] and anti-leishmanial activity was also published [26]. The different parts of the plant especially the stem bark have been studied; however the bioactivity and chemical constituents of the seeds of the plant were not reported. In this paper we report the first such study of the methanol extracts and essential oil of the seeds of the plant.

2. Materials and Methods

2.1. Plant Materials

The seeds of *Steganotaenia araliacea* were collected from Bet-Gerghish near Asmara Zoo, Eritrea. The collection and identification of the plant was conducted with the help of a Taxonomist, Prof. Ghebrehiwet Asghedom of Eritrea Institute of Technology (EIT). Voucher specimen of the plant was prepared and deposited in the herbarium of EIT. The seeds were thoroughly rinsed twice in running tap-water and then in sterile water before being shade dried for 3 weeks. The seeds were then powdered using an electrical blender and kept in clean vials until extraction.

2.2. Extraction

The powdered seeds (25 g) of *Steganotaenia araliaceae* were placed in a thimble and extracted successively with methanol (250 mL) using Soxhlet extractor for 8 h. The solvent extracts were concentrated under reduced pressure and afforded a crude extract (5.6 g, 22.4%) which was preserved at 4˚C in airtight bottle container until further use. Steam distillation of the fresh seeds of *Steganotaenia araliacea* over 4 hours gave a distillate of water and a separate layer of the essential oil. The oil was separated by extracting the mixture using ether solvent and afforded 0.05% v/w. The oil droplets, placed in closed vial, were preserved at low temperature for GC-MS analysis.

2.3. Preliminary Phytochemical Analysis

The methanol extract of the seed of *Steganotaenia araliacea* was screened using chemical tests for the presence of bioactive metabolites like alkaloids, saponins, phenols, steroids, flavonoids, carotenoids, and coumarines using standard protocols [27] [28].

2.4. GC-MS Analysis

The essential oil and the crude methanol extracts of the seeds were analysed in Southampton, England on a capillary GC column and the results were presented with 5%, 1% and 0.2% cut offs of peak heights. The samples were run in duplicates and the raw data were studied and model interpretive results of the different chromatogram peaks were attempted from the GC-MS program. The identification of the compounds was made by observing their mass spectra and retention indices with the NIST library of 249,000 compounds using a search engine.

2.5. The GC-MS Conditions

The crude methanol extract and the essential oil of the seeds of *Steganotaenia araliaceae* were analyzed by GC/MS using a Thermofinnigan Trace GC/MS single quadrupole mass spectrometer with AS 800 autosampler The separations were achieved by capillary column, Phenomenex ZB5 (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 40˚C for 4 min and then at different temperatures (160˚C, 220˚C) at variable rates (10 deg/min, 2 deg/min) for 10 min. The flow rate of helium as carrier gas was 1 mL/min. MS was taken at 70 eV electron ionisation, trap current 150 µA, source temperature 200˚C.

3. Results

In this study, the methanol extract and hydro-distillated essential oil of the seeds of the plant were analyzed. During the GC-MS analyses, the excellent separations achieved by the GC (**Figure 1**) permitted the inspection of each of the peaks and the comparison of the observed spectra for each constituent with those spectra of the NIST library. The results of the GC-MS analysis of the essential oil and the preliminary phytochemical analysis are shown in **Table 1** and **Table 2**.

4. Discussion

Previously the essential oil composition from the leaflets of *Steganotaenia araliaceae*, from Cameroon, was analyzed and contains compounds like limonene, *β*-phellandrene, *α*-pinene, sabinene, *β*-caryophyllene and crypton [29]. In the present study, as indicated in **Table 1**, the GC-MS analysis of the essential oil of the seeds identified the presence of *α*-linalool, *α*-pinene, m-cresol, *p*-menth-1-en-4-ol, *p*-menth-1-en-8-ol, myristicin as the major constituents. Essential oils work at the cellular level and most have antibacterial, antifungal, insecticidal and other biological effects. Besides, there are certain essential oils that actually lower insulin needs for diabetics and reduces blood sugar fluctuations [30]. The GC-MS analysis of the methanol extract of the seeds showed the presence of falcarinol, apiol, scoparone, stigmasterol, myristicin, 1-dodecosanol, nonacosanol, 9-

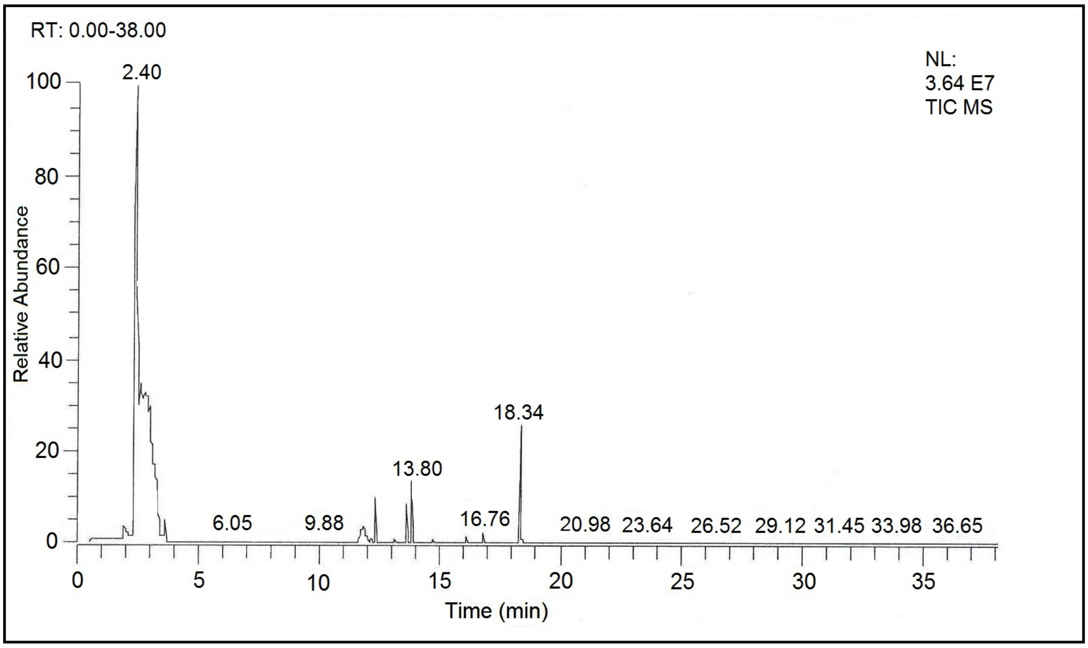


Figure 1. GC chromatogram of the essential oil of the seeds of *Steganotaenia araliacea*.

Table 1. The major constituents of the essential oil of the seeds of *Steganotaenia araliaceae*.

Compound	RT	%	SI
α-Pinene	8.69	27.25	935
m-Cresol	11.80	21.33	923
α-Linalool	12.29	14.15	919
p-Menth-1-en-4-ol	13.61	10.48	930
p-Menth-1-en-8-ol	13.80	18.72	948
p-Menth-1-en-8-ol, acetate	16.06	1.94	920
4-Allyl-1,2-dimethoxy-benzene	16.76	2.38	911
Myristicin	18.34	30.13	934

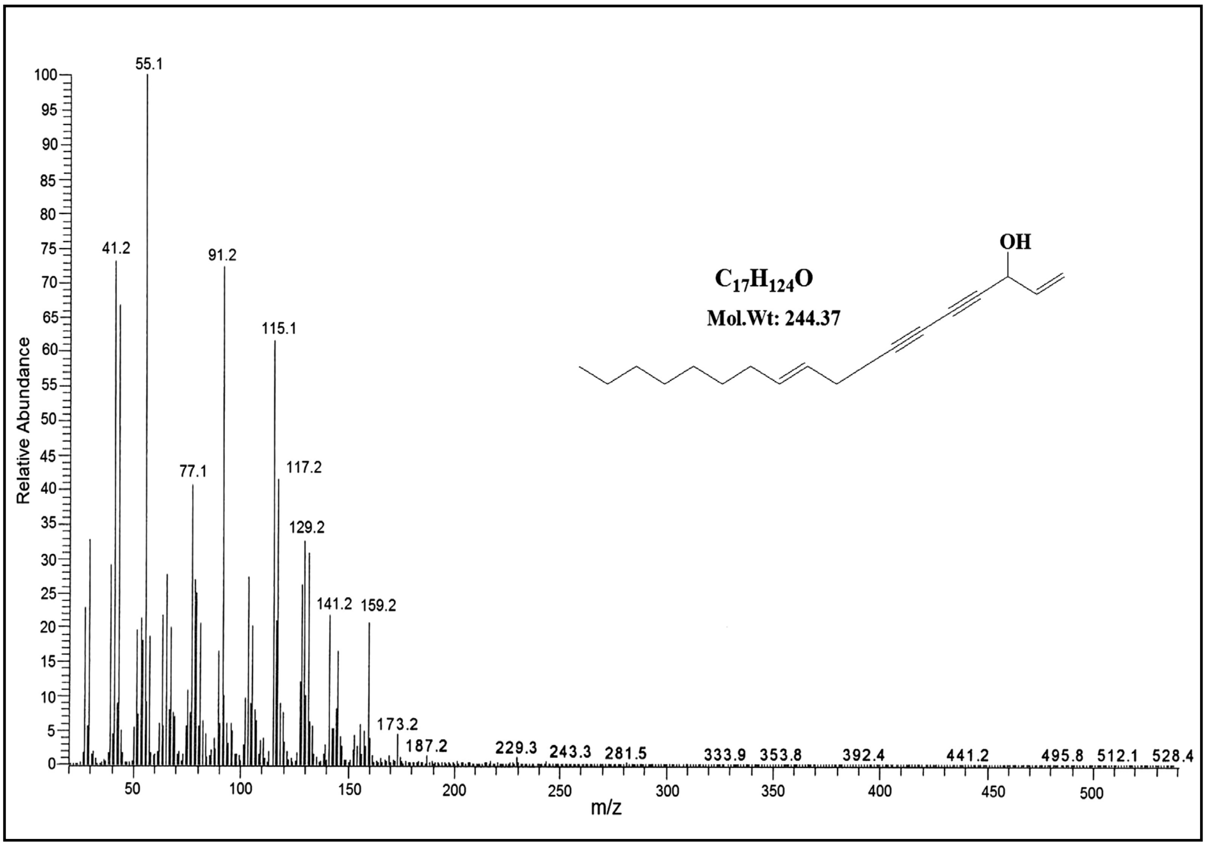
Table 2. Phytochemical studies of the methanol extract of the seeds of *Steganotaenia araliaceae*.

Phytochemical	Type of test	Inference/Result
Alkaloids	Dragendroff's test/Mayer's test	+
Flavonoids	Shinoda test/Lead acetate tests	+
Tannins	Gelatin test/Ferric chloride test	+
Steroids	Salkowski tests/Lieberman Burchardt tests	+
Saponins	Foam test/Froth test	+
Phenols	$FeCl_3$ test/Liebermann's test	+
Cardenolides	Keller Kiliani's test	+
Coumarines	Alcoholic sodium hydroxide	+
Anthraquinone	Borntragers test	−

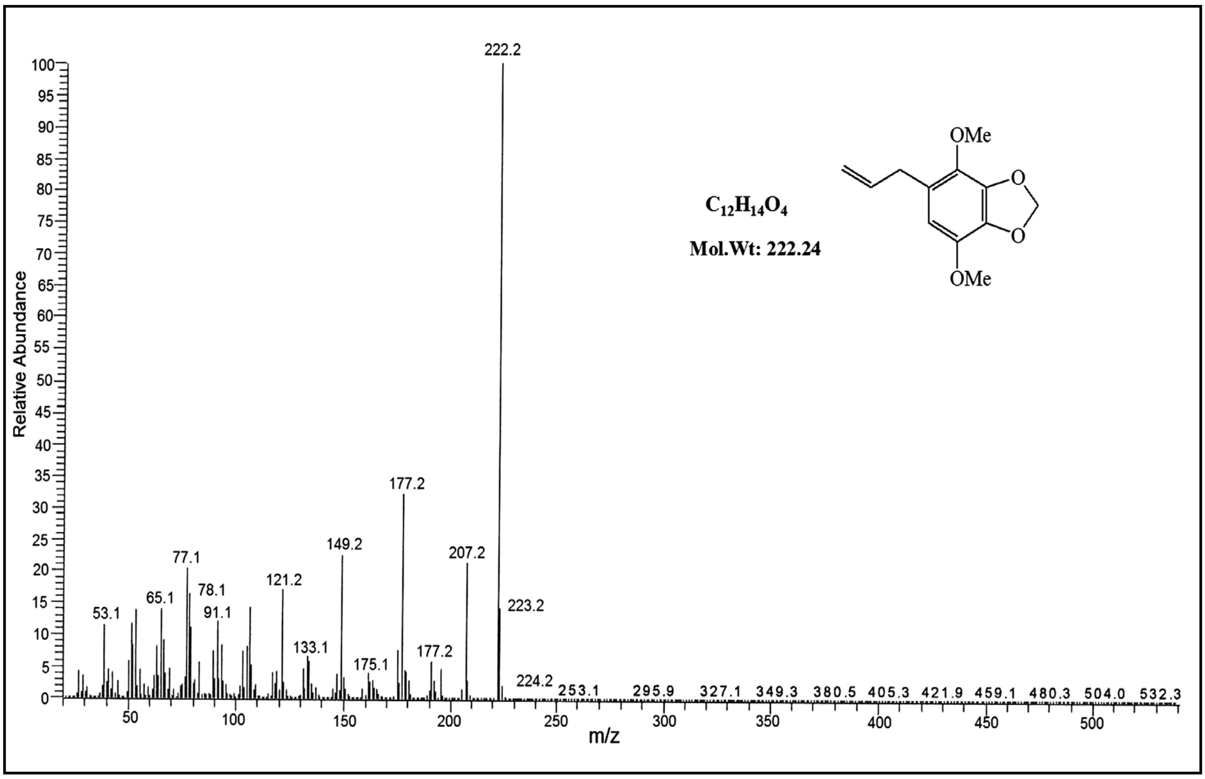
Note: (+) = positive result; (**−**) = negative result.

hexacosene etc. The mass spectra of four common bioactive molecules found in the methanol extract are shown in **Figure 2**. These results justify the following comments: 1) Apiol occurs in other members of the Apiaceae-family and has had a significant role in the termination of pregnancy by provoking abortions [31]. 2) Scoparone (6, 7-dimethoxycoumarin) is recognised as a vasodilator, smooth muscle relaxant and ananti-inflammatory agent. It is an anti-asthmatic and has anti-hypertensive activity [32]. 3) Falcarinol, theanti-cancer compound found in carrots appears omnipresent in *Steganotaenia araliaceae* [33] [34]. 4) Stigmasterol is used in food supplements and is associated with a lowering of the risk of cancer [35]. The GC-MS results establish a number of candidates and show that the seeds of *Steganotaenia araliaceae* are rich in bioactive compounds. As shown on **Table 2**, the preliminary phytochemical analysis of the methanol extract of the seeds confirms the presence of alkaloids, flavonoids, carotenoids, coumarines, steroids, and phenols.

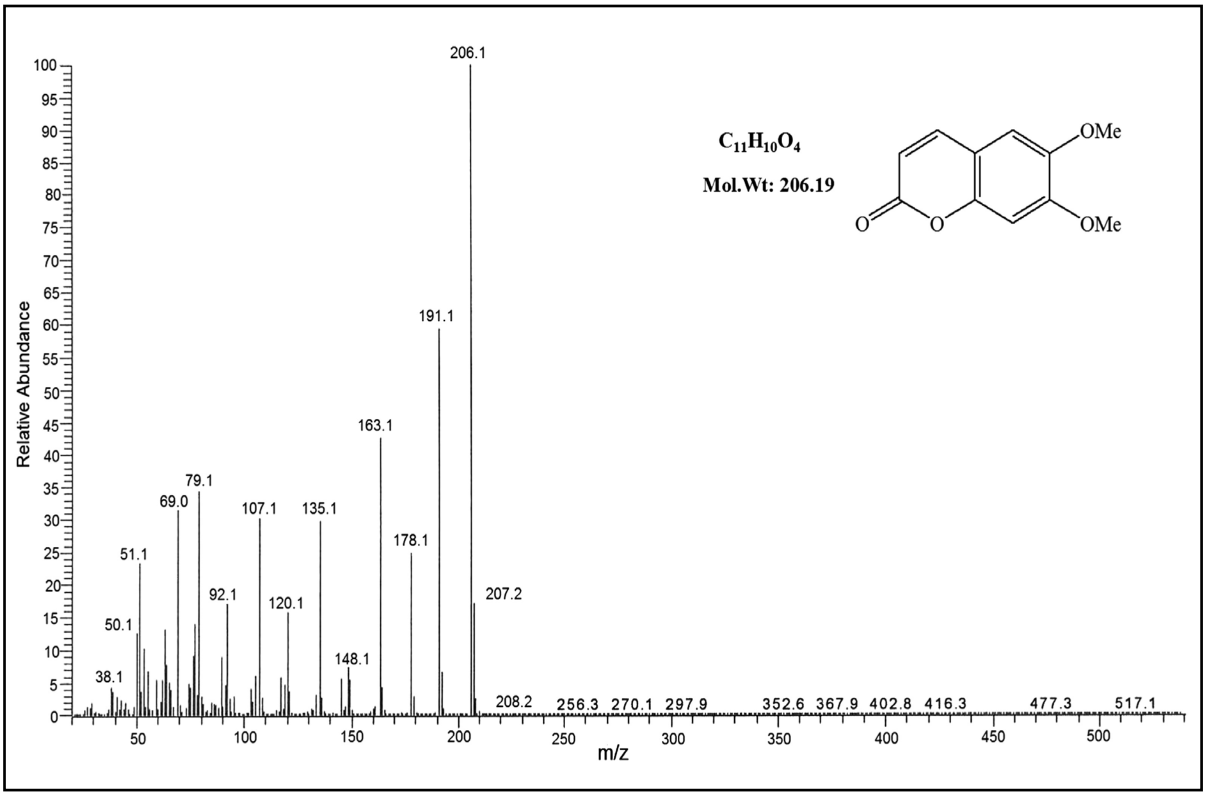
This plant has been recommended for different ailments including diabetes and hypertension by different herbalists. The preliminary results obtained confirm our starting hypothesis that aside from the well-established anti-cancer activity of the lignans from the roots of *Steganotaenia araliaceae,* this plant has other bioactive constituents, which on the one hand justify the interest in the plant of traditional healers and on the other merit a more detailed investigation of the seeds in order to elucidate the structure of the different compounds responsible



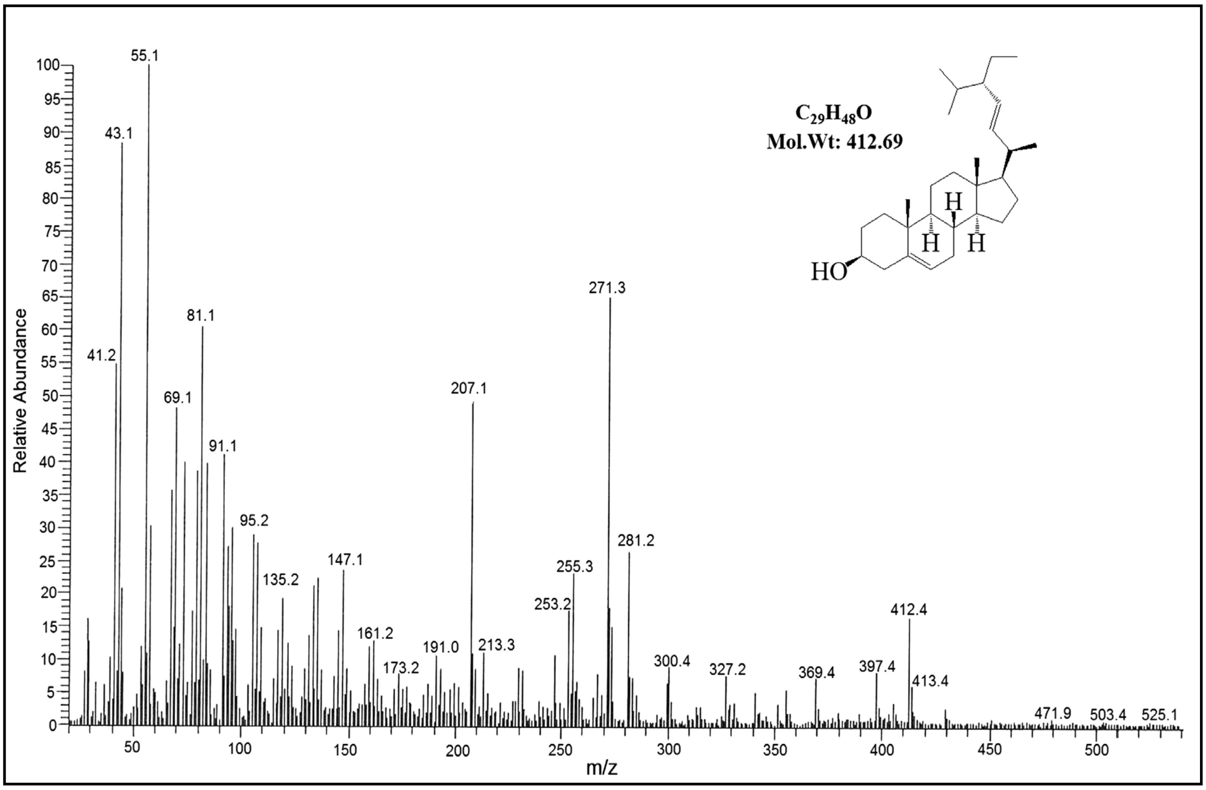
Mass spectrum of Falcarinol



Mass spectrum of Apiol



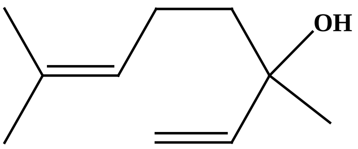
Mass spectrum of Scoparone



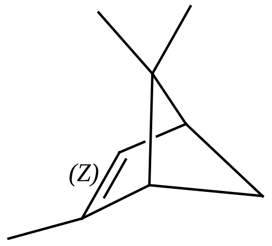
Mass spectrum of Stigmasterol

Figure 2. Mass spectra of the main bioactive molecules found in the methanol extract of the plant.

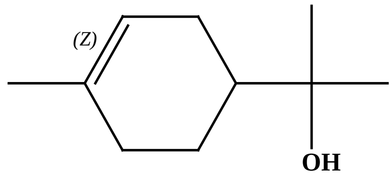
for the observed activities. Based on the GC-MS analyses, some of the structures of the compounds present both in the essential oil and methanol extract of the seeds of the plant include:



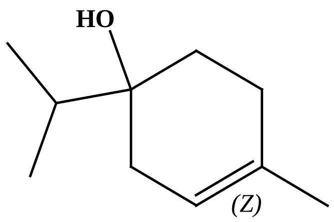
alpha-limonene



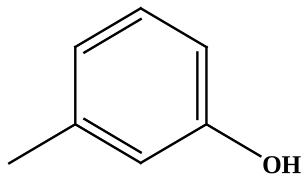
alpha-pinene



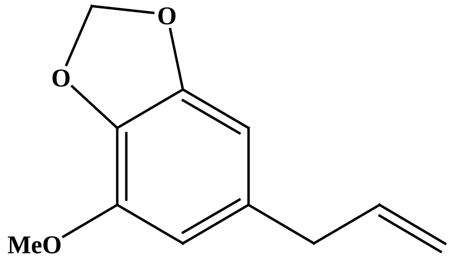
p-menth-1-en-8-ol



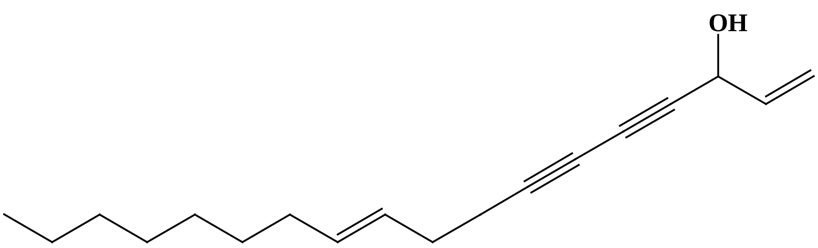
p-menth-1-en-4-ol



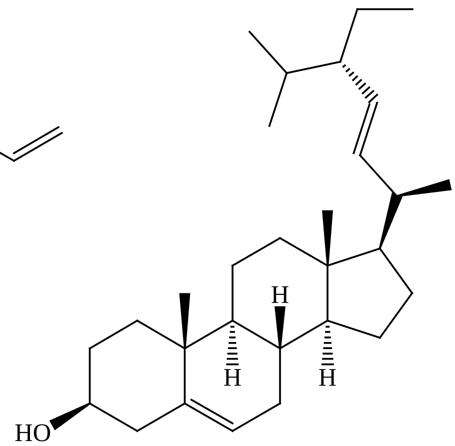
m-cresol



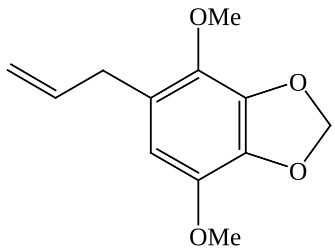
myristicin



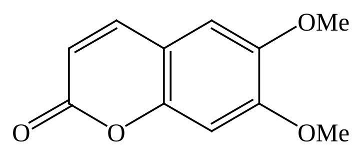
Falcarinol (Octadeca-1,10-diene-4,6-diyn-3-ol)



Stigmasterol



Apiol (5-Allyl-4,7-dimethoxy-benzo[1,3]dioxole)



Scoparone (6,7-dimethoxycoumarine)

5. Conclusion

Steganotaenia araliaceae is traditionally used for the treatment of various ailments and its wider application can easily be noticed by the presence of the different bioactive molecules. Further studies on the efficacy and toxicity of the plant would be required to ensure its use for different ailments.

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