IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2020 Vol.:18, Issue:2

Assessment on Phytochemical, Proximate, Minerals, Vitamins, Anti-Nutrient Composition and GC-MS Analysis of N-Hexane Extract of The Leaf of *Selaginella moellendorffii*

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
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Ojo, V.I.[1], Adebiyi, A.O.[1], Aluko, B.T.[2*], Tedela, P.O.[1], Oyeyemi, S.D.[1]

1. *Department of plant Science and Biotechnology, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria.*

2. *Department of Biochemistry, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria.*

Submission: 21 April 2020
Accepted: 29 April 2020
Published: 30 May 2020

Keywords: *Selaginella moellendorffii*, phytochemical, GC-MS analysis phyto-compounds

ABSTRACT

Pteridophytes are thought to be useless member of plant kingdom because there is scanty information on the nutritional and medicinal potential of the under-utilized lower green plants. This study aimed at assessing the phytochemical, proximate, minerals, vitamins and anti-nutrient composition of a fern, *Selaginella moellendorffii* using standard methods. The plant was also screened for its bioactive compounds using GC-MS technique. The phytochemical analysis of the plant showed considerable amount of total phenols, saponins, alkaloids, flavonoids and tannins while terpenoids, steroids, cardiac glycosides, anthraquinone and phlorotannins were absent. The proximate content: moisture, carbohydrate, crude protein, crude fiber, ash and crude fat contained 10.56, 56.69, 9.46, 8.53, 12.50 and 2.20 % respectively. The mineral analysis revealed the presence of minerals such as potassium (1311.35 mg/100g), calcium (397.00 mg/100g), magnesium (181.50 mg/100g), sodium (48.45 mg/100g), iron (51.05 mg/100g), phosphorus (193.75 mg/100g), manganese (5.14 mg/100g), zinc (4.38 mg/100g) and copper (1.22 mg/100g). The anti-nutrient analysis showed low concentration of phytate, oxalate and hydrogen cyanide which were lower than safe limits. The plant is a poor source of carotene (0.17 mg/100g) but rich in ascorbic acid (12.33 mg/100g). The result of the GC-MS analysis of the plant revealed thirteen bioactive compounds. The prominent phytocompounds were phenol, 4[-hydroxyphenyl)imino]methyl-2 methoxy (RT: 23.230, 23.23 %), stigmasta-4,6,22-trien-α-ol (RT: 20.325, 9.99 %), γ-sitosterol (RT: 22.768, 12.79 %), 1-heptacosanol (RT: 18.273, 6.12 %), hexadecanoic acid, methyl ester (RT: 13.463, 5.90 %), campesterol (RT: 17.878, 4.84 %) and benzene (2-nitrothyl) (RT: 6.211, 4.47 %) while other were less prominent. The present investigation suggests the plant could be a good source of important food nutrients as well as medicinal benefits. The biological activities of the bioactive compounds in the studied plant could be useful in drug development due to their therapeutic potentials.

INTRODUCTION

Modern day food and lifestyle have resulted in an increasing number of diseases and disorders. A major percentage of the population in developing countries depends on herbal medicines for their primary health care needs [1].

Ferns and their allies are one of the oldest major divisions of the Pteridophyta and comprise over 12,000 species spread among 250 different genera [2]. Recently, there has been an increase in the food uses of ferns and fern allies. Apart from their nutritive potentials, they also have medicinal applications.

For centuries, people, especially in China, have used *Selaginella* as a traditional medicine for curing various diseases including hepatitis, cancer and as an antioxidant [3]. It is a medicinal plant that has not been widely used, either traditionally or modern. Small amounts of the species are also used as ornamental plants and vegetables [4]. Pan *et al*. [5], Gayathri *et al*. [6], Sah *et al*. [7] have evaluated the active compound, biochemical and pharmaceutical functions in different species of *Selaginella*.

Selaginella moellendorffii belongs to the family Selaginellaceae. The typical *S. moellendorffii* plants used in this study contained aerial root-bearing rhizophores, which produce roots with typical characteristics such as root hairs and root cap [8]. The specie is a creeping or ascendant plant with simple, scale-like leaves (microphylls) on branching stems from which roots also arise. The stems are aerial, horizontally creeping on the substratum (as in *Selaginella kraussiana*), suberect (*Selaginella trachyphylla*) or erect (as in *Selaginella erythropus*). The vascular steles are polystelic protosteles. The stems contain no pith. Unusually for the lycopods, which generally have microphylls with a single unbranched vein, the microphylls of *Selaginella* species contain a branched vascular trace. In *Selaginella*, each microphyll and sporophyll has a small scale-like outgrowth called a ligule at the base of the upper surface [9]. The plant is heterosporous with reproductive organs represented by sporangia-bearing structures known as strobili, which are found at the tips of shoots, and contain the male micro- and the female mega-sporangia.

Generally, ferns show various economic values towards food and fodder indicators, bio-fertilizers, insect repellants, medicine and folk medicines. Selaginella contains numerous secondary metabolites, such as phenolics, alkaloids, flavonoids, lignans, selaginellins and terpenoids [10].

Selaginella moellendorffii has been used as ethnic drug for treatment of bleeding and chronic inflammation such as arthritis, gonorrhea, hepatitis and mastitis [11-12].

Previous phytochemical studies about *S. moellendorffii* focused on constituents including biflavonoids [13], lignans [14-15] and phenols [16]. It was also reported that biflavone ginkgetin from *S. moellendorffii* selectively induces apoptosis in ovarian and cervical cancer cells [17]. This traditional claim prompted many researchers to investigate its pharmacological values that include among others, its phytochemical composition and bioactivities [18].

Despite the abundance of lower green plants in Nigeria, little or no attention has been paid to the investigation of their medicinal potentials as compared to higher plants. There is no previous literature on the nutritional usefulness of *S. moellendorffii* as at the time of this documentation

Also, in recent years, GC-MS techniques have been used to screen medicinal plants as it has proved to be a reliable method for the analysis of non-polar components and volatile essential oil, fatty acids, lipids and alkaloids [19-20]. Therefore, the present investigation aimed at providing adequate information on the assessment of phytochemical, nutritional, anti-nutritional and GC-MS analysis of *S. moellendorffii* found in Ekiti State, Nigeria.

MATERIALS AND METHODS

Study Area, Collection, Identification and Preparation of Samples

The study was carried out in Ekiti State, Nigeria. Ekiti State is located between longitudes $4^{0}5^{1}$ and $5^{0}45^{1}$ East of the Greenwich meridian and latitudes $7^{0}15^{1}$ and $8^{0}15^{1}$ north of the Equator. *S. moellendorffii* was collected from different towns in Ekiti State, Nigeria. The plant was authenticated and assigned herbarium number UHAE 2019163 at the herbarium of the Department of Plant Science and Biotechnology, Ekiti State University, Ado Ekiti, Nigeria. The voucher specimen was deposited in the herbarium. Fresh leaves of the fern were plucked from healthy plant stalks, rinsed in clean water and air-dried at room temperature to a constant weight. The plant samples were pulverized into fine powder using an electric blender (Excella QTY Ipc). The powdered samples were stored in airtight plastic containers and kept at 4ºC in a refrigerator until required for analysis.

Chemicals

Ammonium hydroxide, amyl alcohol, ethanol, sodium chloride, acetic acid, N-hexane, Folin – Denis reagent, sodium carbonate, tannic acid, diethyl ether, nitric acid, ascorbic acid and hydrochloric acid. All the chemicals used in this study were of analytical grade.

Determination of Phytochemical and Proximate Compositions

The quantitative phytochemical analysis of the leaf was done according to the procedures described by Aluko *et al.* [21]. The proximate (moisture, ash, crude fiber, crude protein and crude fat) contents were determined using the method described by AOAC [22]. The carbohydrate content was calculated by subtracting the sum of total values of moisture, ash, crude protein, and crude fat from 100. Each of the nutrient content was expressed in percentage.

Determination of Mineral Content

The modified method described by Bouba *et al.* [23] was adopted for the determination of potassium, sodium, calcium, magnesium, phosphorous, manganese, iron, zinc and copper. The powdered plant sample (5 g) was weighed and ashed at 55^0 C. The residue was dissolved in 4 mL of concentrated hydrochloric acid and filtered. The filtrate was diluted with distilled water. The resulting solution of the extract was then subjected to atomic absorption spectrophotometric analysis.

Quantification of Vitamins and Anti-Nutrients

The retinol and ascorbic acid equivalents of the sample was quantified using the methods described by Idris *et al.* [24] and Njoku *et al.*[25] respectively. Oxalate content of the sample was determined by titration method as described by Unuofin *et al.* [26]. The phytic acid content of the sample was determined using the method of Aina *et al.* [27] while the hydrogen cyanide content was analyzed according to method described by Cook [28].

GC-MS Analysis

Sample Preparation: Two (2) grams of the sample was weighed into 250 ml conical flask and 10 ml of n-hexane was added to sonicate for 2 hours. It was filtered by packing a column with silica gel and fiberglass wool. Anhydrous sodium sulphate was added to remove the

water present in the extract. The extract was then concentrated with nitrogen concentrator to 2 ml for **GC-MS analysis**

GC–MS analysis of the extract was performed using Agilent technologies model 7890A coupled with a mass spectrometer Agilent technologies 6975. The principle for the analysis was separation techniques. The mobile phase was helium gas while the stationery phase was the column of model Agilent technologies HP-5MS with length 30 m, internal diameter of 0.32 mm with thickness of 0.25 microliter. The oven temperature was programmed from 80 ^{o}C (isothermal for 2 min) with an increase of 10 ^{o}C /min to the final temperature of 240 ^{o}C and held isothermally for 6 min. The volume of sample injected was 10 microliter. The mode of analysis was split-less. The scan range was 50-550 Da. The mass spectrometer interphase temperature was 250 ^{o}C. Mass spectra were taken at 70Ev. The total GC running time was 23.154 min. The library used for the identification of compounds was National Institute Standard and Technology (NIST)-version Year 2014.

RESULTS AND DISCUSSION

The result of the phytochemical analysis of *S. moellendorffii* leaf revealed the presence of total phenols, saponins, alkaloids, flavonoids and tannins (Table 1). The leaf is rich in total phenols, flavonoids, tannins and moderate amount of saponins and alkaloids. Phytochemicals are bioactive components that are responsible for medicinal potentials of plants [21]. Polyphenols are capable of neutralizing free radicals in biological systems and combating microbes [29]. Flavonoids are hepatoprotective, antimicrobial and antitumor agents. Tannins have been reported to possess anti-diuretic and anti-diarrhoea properties [30]. The presence of these phytochemicals could justify the importance of the ferns in ethnomedical and pharmaceutical practices.

The result of the proximate analysis of the plant is presented in Table 2. The moisture, carbohydrate, crude protein, crude fiber, ash and crude fat contents in *Selaginella moellendorffii* were 10.56, 56.69, 9.46, 8.53, 12.50 and 2.20 % respectively. Result showed that the plant is very rich in carbohydrate content while it contained moderate crude protein, crude fiber and ash content. However, the crude fat and moisture content were relatively low. The high carbohydrates are known to produce energy required for the body because they are essential nutrient required for adequate diet [31] and supplies energy to cells such as brain, muscle and blood [32]. The low crude fat content (2.20 %) compares to 2.93 % reported for

an edible fern, *Diplazium sammatii* [33]. The low value of fat content in the present study is similar to the findings of many authors which showed that leafy vegetables are poor sources of fat [34]. The crude protein content (9.46 %) of the leaf was moderately available. However, it was lower than 15.31 % reported for an edible fern, *Diplazium sammatii* [33]. Protein is vital for various body functions such as body development, maintenance of fluid balance, formation of hormones, enzymes and sustaining strong immune function [31]. Crude fiber of this plant could aid absorption of trace elements in the gut and therefore, increases intestinal bowel movement [35]. The crude fiber content observed in this study is 8.53 %. This value fell below the ranges reported by previous researchers such as 12.56 – 31.50 % [36] and 10.40 – 20.10 % [37] but fell within the range of 6.70 – 11.70 % [38] in some common vegetables. The crude fiber content however, is higher than 6.27 reported by Uwem *et al.* [39] in Proximate Composition, Phytoconstituents and Mineral Contents of Soybean (Glycine Max) Flour Grown and Processed in Northern Nigeria. Incorporating this leaves in our diet could aid in managing constipation problems [40]. The moderate amount of ash content in the leaf the plant provides a measure of total amount of mineral matter present in the plant. The low moisture content (10.56 %) in *Selaginella moellendorffii* would make it less susceptible to microbial contamination [41].

Table 3 shows mineral composition of *S. moellendorffii*. The potassium, calcium, magnesium, sodium, iron, phosphorus, manganese, zinc and copper estimation in the leaf of the plant are; 1311, 397.00, 181.50, 48.45, 51.05, 193.75, 5.14, 4.38 and 1.22 kg/g respectively. Highest composition was observed in potassium while the least composition was observed in copper. The result proved that the plant could be a cheap source of dietary mineral for man and ruminant animal.

Potassium plays an important role in controlling skeletal muscle contraction and nerve impulse transmission [42-43]. It was reported by Nair *et al.*[44] that high amount of potassium is helpful for the people taking diuretics to control hypertension and it also increases the utilization of iron.

Sodium is important in the maintenance of acid-base balance in the body [45]. Na and K are important in the transport of metabolites in the human body. The Na/K ratio must be less than 1 to control blood pressure [46]. The NA/K ratio in this study is less than 1 and could serve to reduce high blood pressure in man.

Calcium is reported to be essential for blood clotting, bone and teeth formation and as a co-factor in some enzyme catalysis [47]. In humans, magnesium is required in the plasma and extracellular fluid, where it helps maintain osmotic equilibrium [48].

It can also prevent some heart disorders and lower blood pressure in humans. Iron facilitates the oxidation of biomolecules to control obesity, which predisposes an individual to various diseases. It is also essential for hemoglobin formation [48] and plays a role in energy transfer within the plant and also an essential constituent of certain enzymes and proteins.

Excessive intake of zinc has been reported to be toxic [49]. Zinc is a very important element for human growth which also increases resistance to infection [50]. It is a cofactor for the antioxidant enzyme superoxide dismutase and is required for the functioning of over 300 different enzymes, and for a number of enzymatic reactions involved in carbohydrate and protein metabolism [51]. Copper is essential for the production of enzyme in the body and plays an important role in biological electron transport [52-53]. Copper deficiency causes reduced energy production, abnormal glucose and cholesterol metabolism, and increased oxidative damage [54].

Vitamin A, C and anti-nutrient compositions of *S. moellendorffii* are shown in Table 4. The result revealed low quantity of vitamin A (0.17 mg/100g) and high amount of vitamin C (12.33 mg/100g). The phytate (0.53 mg/100g), oxalate (0.37 mg/100g) and hydrogen cyanide (0.06 mg/100g) were very low. The vitamin C in this study was lower than (15.85 mg/100g) reported by Oyeyemi *et al*. [55] in a tropical fern, *Nephrolepis cordifolia* however, the vitamin A content of the plant relatively compares to (0.25 mg/100g) by same author. The vitamin C content in this study is higher than 8.6 mg/100g reported by Islary *et al*. [56] in antioxidant activities of *Grewia sapida*. Vitamins are organic molecules that are not produced in the human body but must be supplied in the diet. Vitamins mostly present in fruits and vegetables play important specific functions in normal body metabolism. They are classified as fat and water-soluble molecules [57].

The investigated leaves contained low amount of anti-nutrients, hence they could be recommended for consumption.

The GC-MS result is presented in Table 5. In the present study, thirteen phytocompounds with their retention time and peak area (%) were identified using GC-MS technique. The GC-MS profile is shown in Figure 1 and 2. The result revealed some major compounds in the

retention time range of 6.211 to 23.230. Among these phytocompounds, phenol,4[(4-hydroxyphenylimino)methyl]-2methoxy (RT: 23.230; 23.24 %), gamma.sistosterol (RT: 22.768; 12.79 %), stigmasta-4,6,22-trien-3α-ol (RT: 20.325; 9.99 %), 1-heptacosanol (RT: 18.273; 6.12 %), hexadecanoic acid, methyl ester (RT: 13.463; 5.90 %), campesterol (RT: 17.878; 4.84 %),benzene, (2-nitroethyl)- (RT: 6.211; 4.47 %) and 11-Octadecenoic acid, methyl ester (RT: 15.163; 4.23 %) were the most abundant in occurrence while the rest phytocompounds were minor in occurrence.

Comparable to this study, various bioactive compounds were characterized through GC-MS analysis in the leaves of *Hyptis verticillata* [58] and identified fifteen bioactive compounds. Twenty three bioactive compounds were identified from n-hexane extract of the leaves of *Diplazium sammatii* using GC-MS [59]. The n-hexane extract of the leaves of *Nephrolepis cordifolia* revealed the presence of sixteen phytocompounds through GC-MS screening [60]. GC-MS technique was used to identify the presence of seventeen compounds from ethanolic extract of a fern, *Pteridium aquilinum* [61]. The presence of seven bioactive compounds in ethanolic leaves extract of *Pterocarpus mildbraedii* using GC-MS method had earlier been reported [62]. The presence of six major bioactive compounds in methanol extract of the leaves of *Melastromastum capitatum* was also reported using GC-MS technique [63].

The major bioactive compounds identified in the present study possess some significant biological potential which may be of importance for future drug development.

phenol, 4-[[(4-hydroxyphenyl)imino]methyl]-2-methoxy, which is the most abundant of all the identified bioactive compounds in this study is a derivative of phenol compound had been demonstrated to inhibit the proliferation of cancer cells [64].

The leaf has significant composition of Hexadecanoic acid, methyl ester and had been reported to act as an antioxidant, anti-inflammatory, anti-hyperlipidemic as well as antimicrobial in functions [65-66]. Campesterol, a plant sterol in nature, is known to have cholesterol lowering and anticarcinogenic effects. Methyl stearate, another major compound found in *Selaginella moellendorffii* is used as a solvent and lipid carrier in agriculture [67]. Methyl stearates also possess anti-inflammatory potentials [68]. It is worth noting that sitosterol had been reported to possess antibacterial potential [69]. It was reported that gamma. Sitosterol exerted potential anticancer activity through the growth inhibition, cell cycle arrest and apoptosis on cancer cells [70]. It has been reported that gamma. sistosterol

reduced hyperglycemia in STZ-induced diabetic rats due to increased insulin secretion and inhibition of glucogenesis, thus, it can be used in the treatment of *Diabetes mellitus* [71]. The identified phytocompounds in the investigated plant have reported beneficial activity which could implicate the medicinal potentials of the leaf.

CONCLUSION

The present findings have shown that *Selaginella moellendorffii* is rich in carbohydrate, crude fiber and moderate in other proximate contents. The mineral matters are in considerable amount. The anti-nutrients are much lower than the safe limits. The vitamins in high amount could be beneficial to health when consumed. Also, the present study revealed the presence of thirteen bioactive compounds in the n-hexane leaf extract of *Selaginella moellendorffii* using GC-MS technique. The bioactive compounds of medicinal and nutritional values have potentials of drug development and could sustain human diet.

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Table No. 1: The phytochemical constituents of *Selaginella moellendorffii* leaves

Phytochemical	Composition %
Total Phenols mg GAE/g	4.16 ± 0.04
Saponins mg/g	0.11 ± 0.01
Alkaloids mg ATE/g	0.07 ± 0.00
Flavonoids mg QE/g	2.19 ± 0.07
Tannins mg TAE/g	0.92 ± 0.02
Terpenoids	not detected
Steroids	not detected
Cardiac glycosides	not detected
Anthraquinone	not detected
Phlobatanins	not detected

The values are expressed as means ± standard deviations of triplicate analysis

Table No. 2: The proximate composition of *Selaginella moellendorffii* leaves

Content	Composition (%)
Carbohydrate	56.69± 0.09
Crude Fat	2.20± 0.03
Crude Protein	9.46± 0.36
Ash	12.50± 0.10
Crude Fiber	8.53± 0.11
Moisture	10.56 ± 0.04

The values are expressed as means ± standard deviations of triplicate analysis

Table No. 3: The mineral composition of *Selaginella moellndorffii* leaves

Element	Composition (g/kg)
Calcium	397.00 ± 1.00
Potassium	1311.35 ± 0.80
Magnesium	181.50 ± 1.50
Sodium	48.45 ± 0.45
Iron	51.05 ± 0.15
Phosphorus	193.75 ± 3.75
Manganese	5.14 ± 0.04
Zinc	4.38 ± 0.03
Copper	1.22 ± 0.02

The values are expressed as means ± standard deviations of triplicate analysis

Table No. 4: Vitamins and anti-nutrient composition of *Selaginella moellendorffii* leaves

Vitamin A (mg retinol/100g)	Vitamin C (mg ascorbic acid/100g)	Phytate mg/100g	Oxalate mg/100g	Hydrogen cyanide mg/100g
0.17 ± 0.02^{c}	12.33 ± 0.13	0.53 ± 0.01	0.37 ± 0.02	0.06 ± 0.00

The values are expressed as means ± standard deviations of triplicate analysis

Table No. 5: List of the bioactive compounds from the n-hexane leaf extract of *Selaginella moellendorffii*

S/N	Retention time	Name of compound	Peak area (%)
1	6.211	Benzene, (2-nitroethyl)-	4.47
2	13.463	Hexadecanoic acid, methyl ester	5.90
3	14.030	n-Hexadecanoic acid	1.98
4	15.163	11-Octadecenoic acid, methyl ester	4.23
5	15.411	Methyl stearate	2.69
6	17.878	Campesterol	4.84
7	18.273	1-Heptacosanol	6.12
8	19.535	Stigmasterol	0.45
9	20.197	Stigmasta-4,6,22-trien-3α-ol	9.99
10	20.325	Stigmasta-3,5-dien-7-one	0.37
11	22.673	ϒ-Sitosterol	12.79
12	22.768	ϒ-Sitosterol	12.41
13	23.230	phenol, 4-[[(4-hydroxyphenyl)imino]methyl]-2-methoxy	23.24

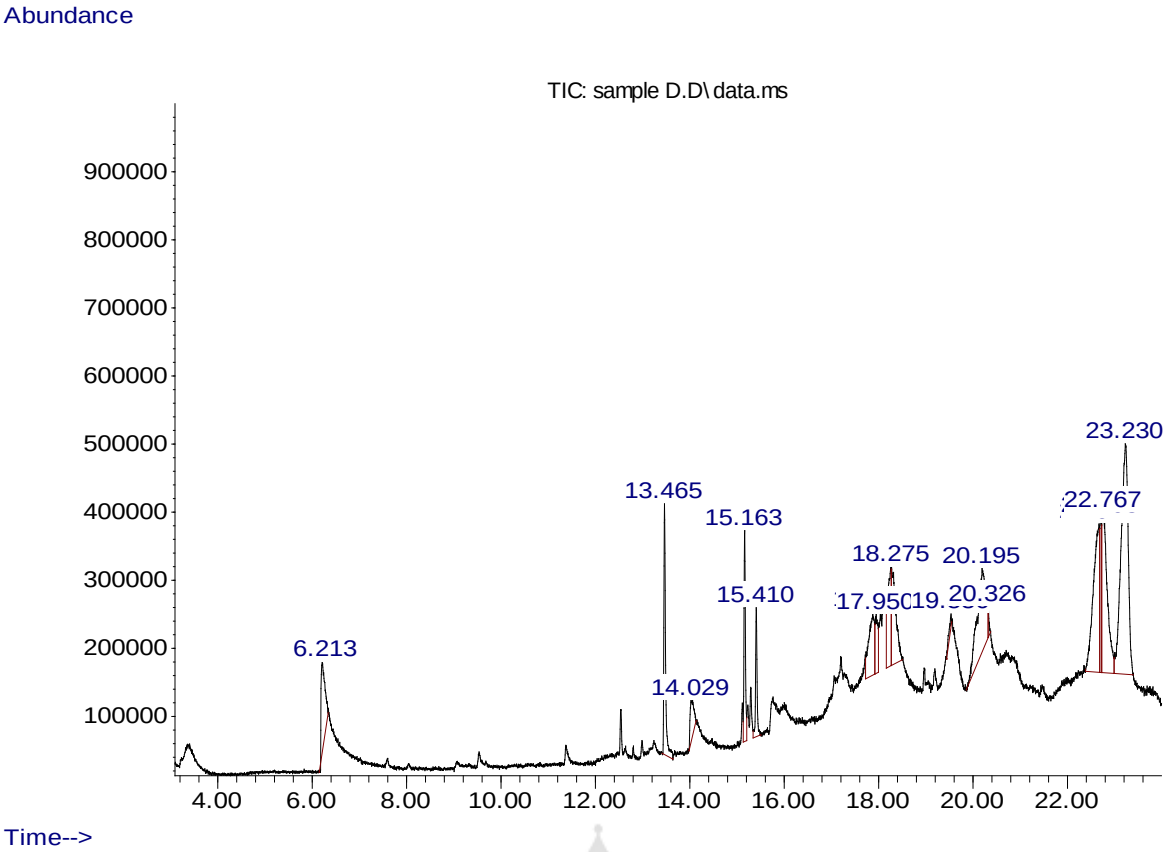
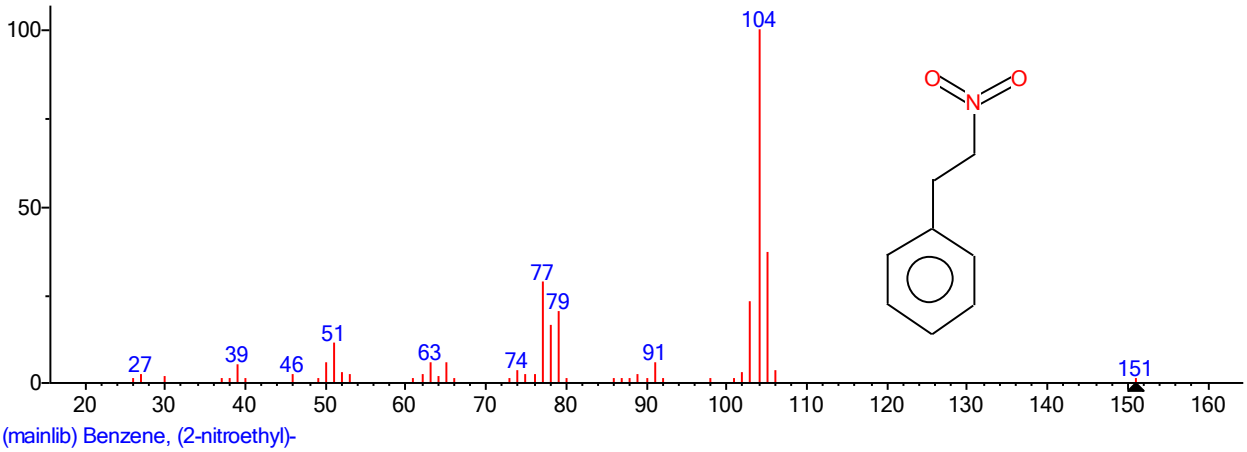
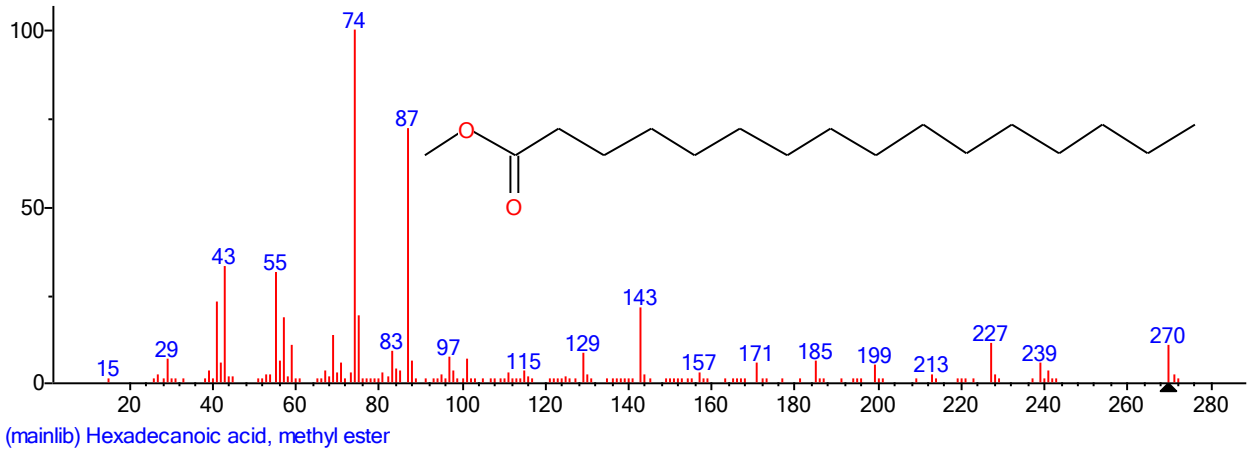
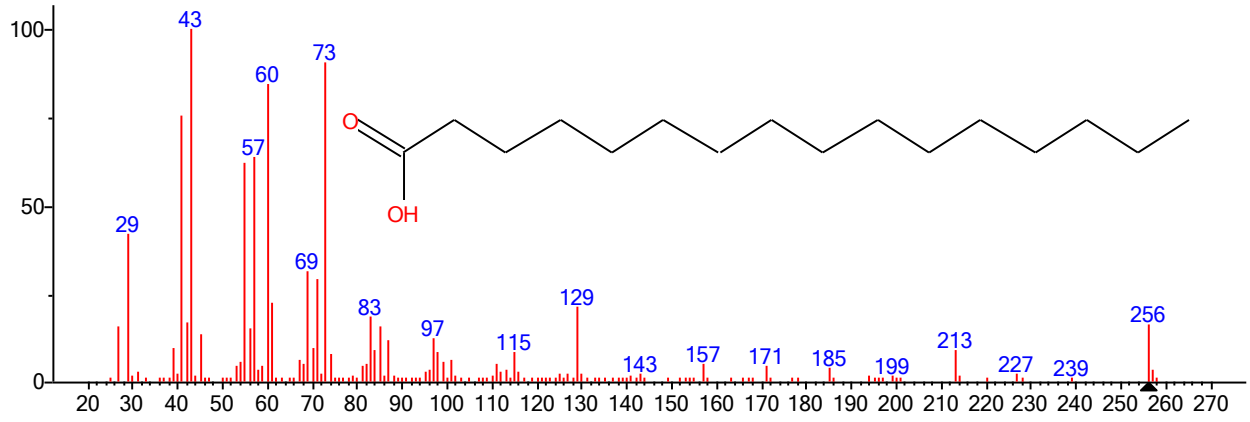


Figure No. 1: GC-MS chromatogram of n-hexane extract of *S. moellendorffii* leaves

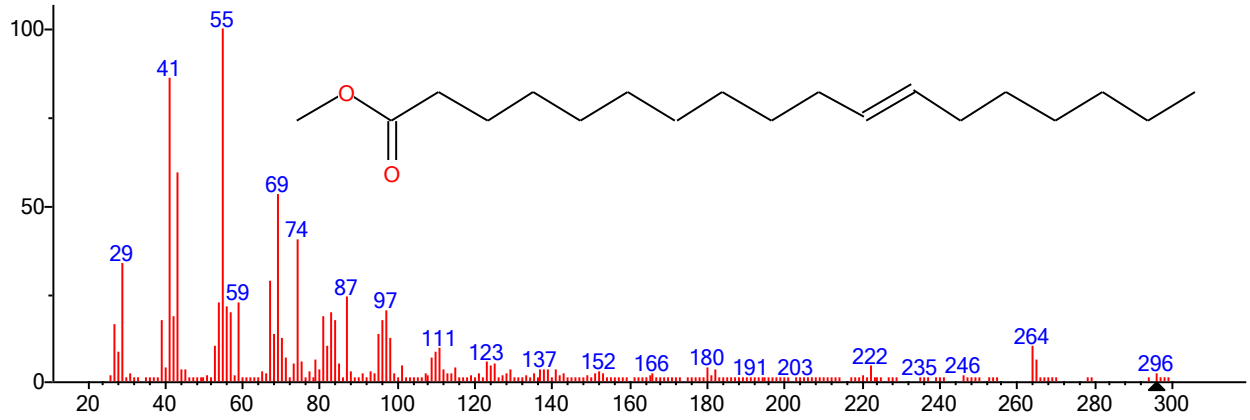




(mainlib) Hexadecanoic acid, methyl ester



(mainlib) n-Hexadecanoic acid



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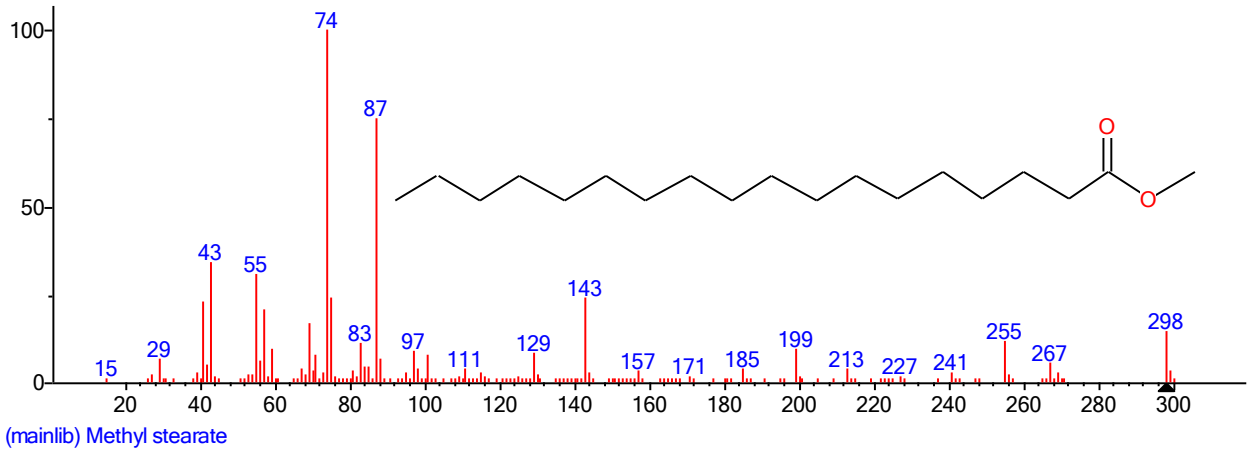
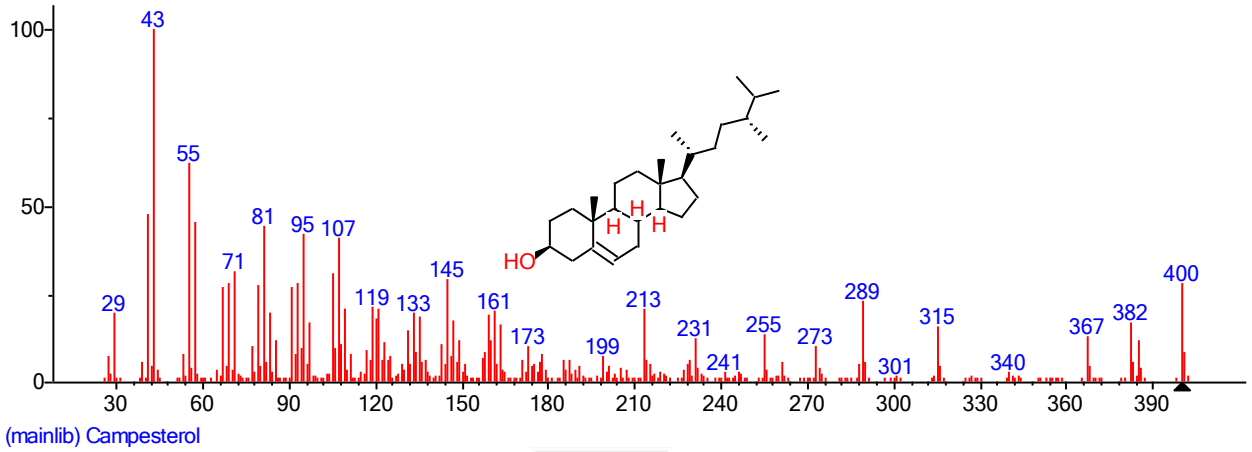
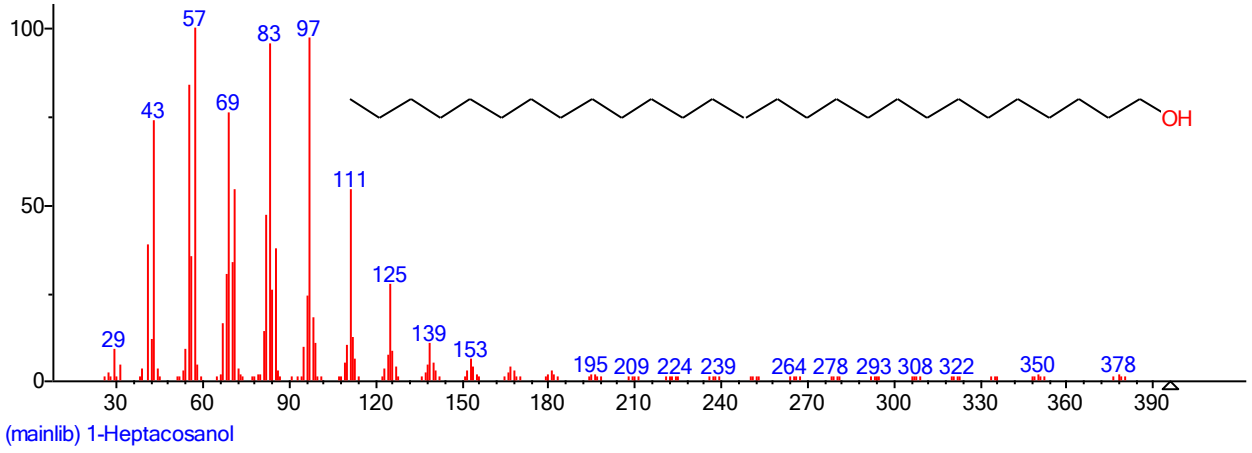


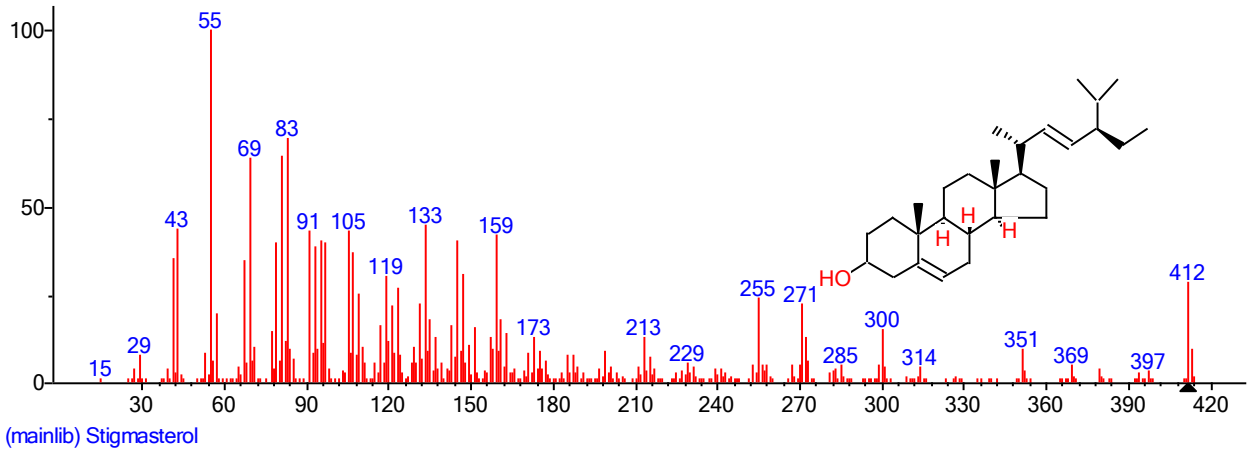
(mainlib) Methyl stearate



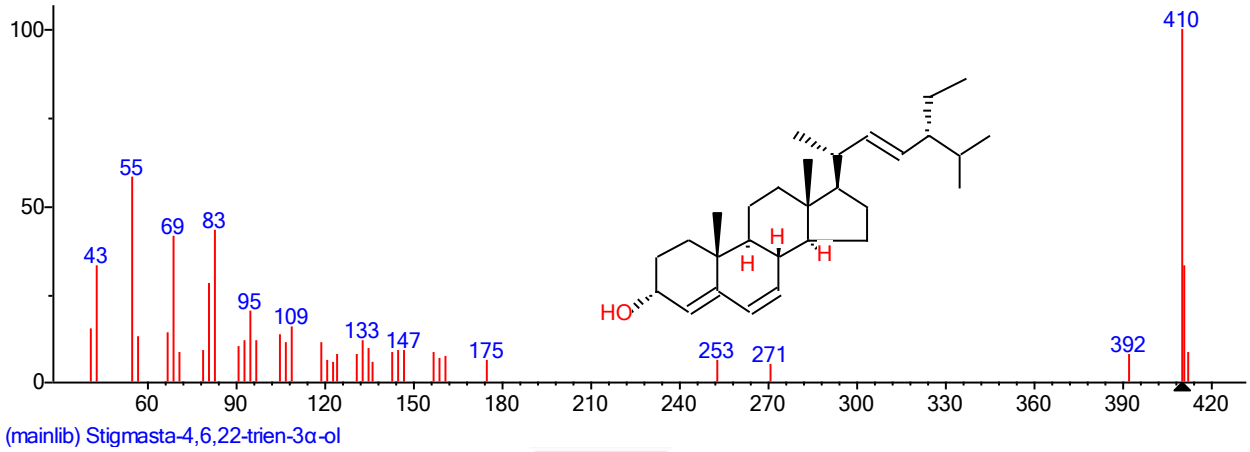
(mainlib) Campesterol



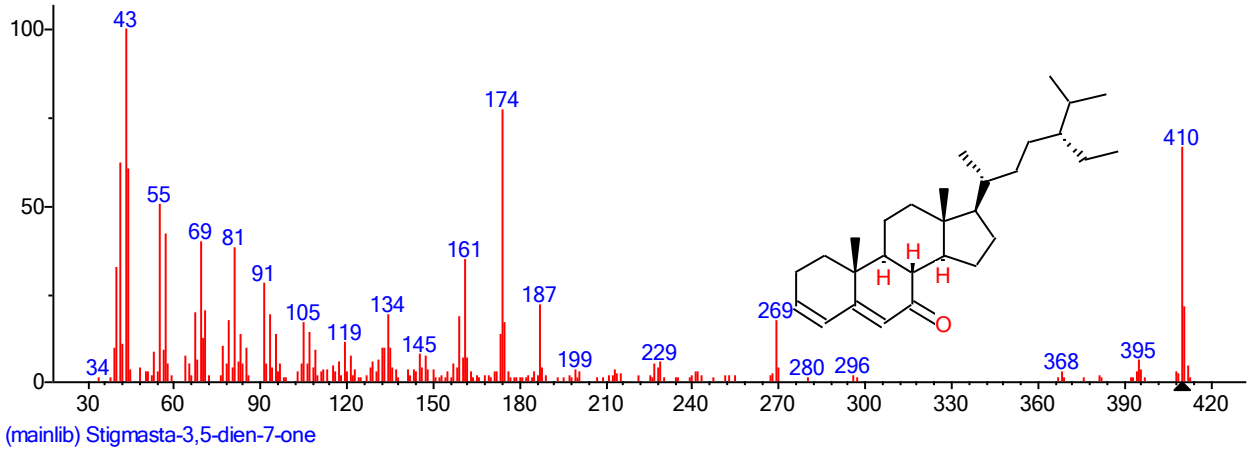
(mainlib) 1-Heptacosanol



(mainlib) Stigmasterol



(mainlib) Stigmasta-4,6,22-trien-3α-ol



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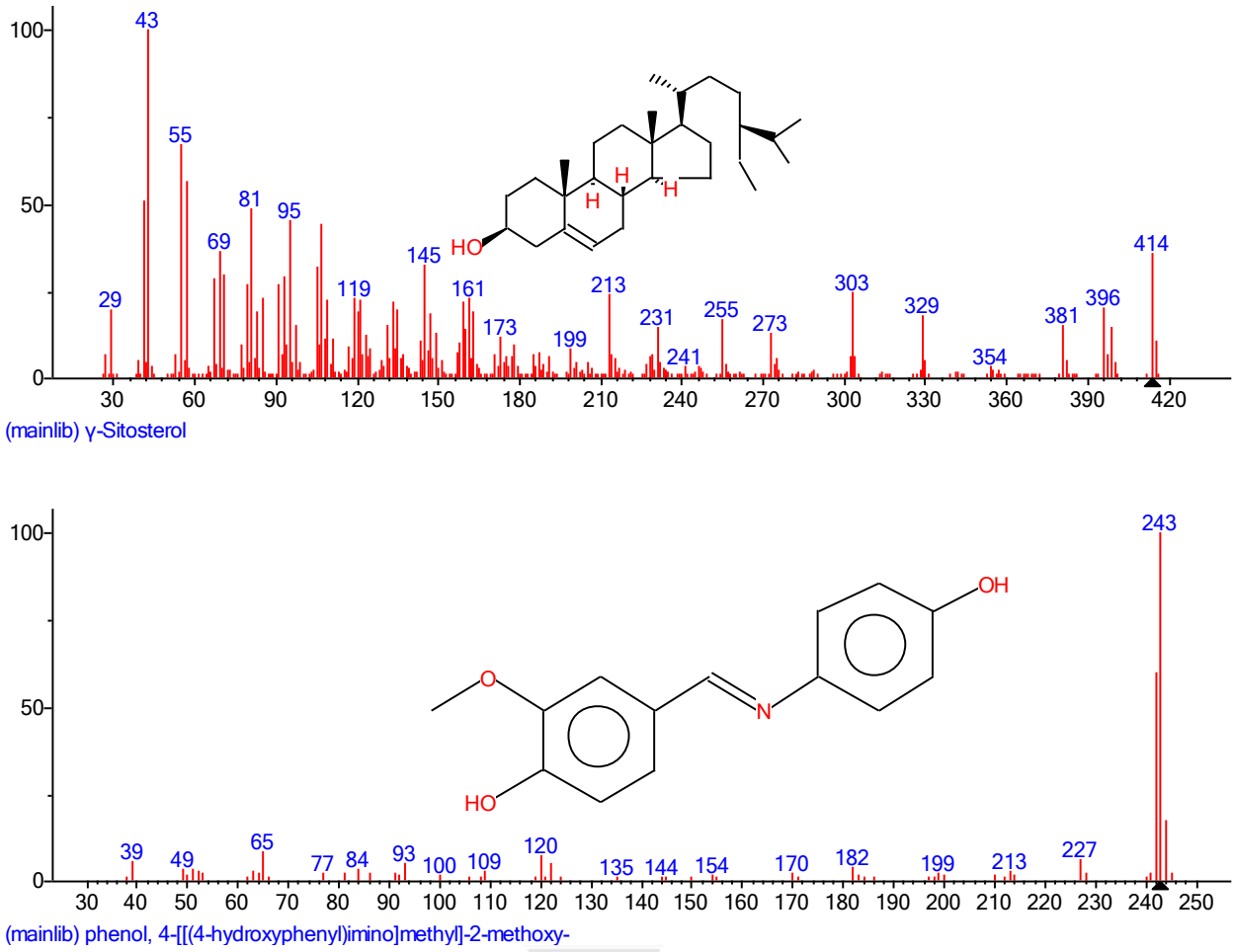

Figure No. 2: GC-MS spectra of bioactive compounds from n-hexane extract of *S. moellendorffii* leaves