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Research Article

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Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of *n*-hexane Leaf Extract of *Diplazium sammatii* (KUHN) C. CHR

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Abstract The present investigation was carried out to identify the bioactive compounds present in the n-hexane leaf extract of *Diplazium sammatii* by using Gas Chromatography-Mass Spectrometry (GC-MS).Fresh and matured leaves were collected from a healthy plant, rinsed, air dried and pulverized. 2 gram of the powdered sample was weighed into 250 ml conical flask and 10 ml of n-hexane was added to sonicate for two hours. It was then filtered by packing a column with silica gel and fibre glass 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 which was done using Agilent technologies Model 7890A coupled with a mass spectrometer Agilent technologies 6975. The library used for the identification of compounds was National Institute of Standards and Technology (NIST)-version Year 2014. GC-MS analysis of n-hexane leaf extract of *Diplazium sammatii* showed the presence of twenty three different bioactive compounds with varying quantities. The main compounds were Benzene, (2-nitroethyl)- (RT: 6.092; 3.94 %), n-Hexadecanoic acid (RT: 14.063; 19.64 %), Oleic Acid (RT: 15.887; 47.25 %), Octadecanoic acid (RT: 16.006; 5.87 %) and Methyl triacontylether (RT: 23.139; 6.51 %).These chemical compounds are considered biologically and pharmacologically important. Thus, they provide inspiration for further investigation in the discovery of novel herbal drugs.

Keywords *Diplazium sammatii*, GC-MS, pharmacological importance, bioactive compounds, herbal drugs

Introduction

Plant is a source of medicinal agents for a longtime. Medicinal plants have been used for years in daily life to treat various diseases [1]. Plants are used as medicine in many countries and also act as a source for many potent drugs [2]. A large number of medicinal plants and their purified constituents have shown therapeutic activities [3]. Natural remedies from medicinal plants proved as safe and effective. Medicinal plants are the source of drugs in traditional systems of medicine, modern medicines, food supplements, folk medicine, pharmaceutical intermediates and chemical entities for drug synthesis [4]. Many plant species have been used in folklore medicine to treat various ailments [5]. People have been using medicinal plants based on their acclaimed therapeutic values and till date over 85,000 medicinal plants with various therapeutic benefits have been identified and documented globally [6]. According to World Health Organization (WHO), about 80 % of the developing country populations are using traditional plant products as an alternative or complementary medicine [7].

The identification of bioactive chemical compounds from medicinal plants is an important task in the pharmaceutical industry for drug development and preparation of medicine [8]. In recent period, the GC-MS studies

have been increasingly applied for the analysis of most of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oil, fatty acids and lipids [9].

Diplazium sammatii is an erect, rhizomatous aquatic fern up to about 1 m high. It grows under shade on stream banks in the low altitude tropics. It belongs to Athyriaceae family. The stem is a rhizome. The leaves consist of erect pinnate fronds which are not fertile and are up to about 40 cm long. The leaflets are alternate, up to about 10 cm paired. They are lanceolate, 14 cm long and 2.5 cm across, glabrous, widely and finely toothed and pointed at the apex. They are sessile and have square to unequal-sided bases; and the lower surfaces have numerous, more or less closely interconnected free veins. The leaves of *Diplazium sammatii* are consumed by various people throughout the World [10]. The plant is used in the control of diarrhea by rural dwellers [11]. Also, it is a medicinal plant used to manage/treat gastroenteritis [12]. Other uses of the plant are not documented or known yet since research activities on the plant is so scanty due to its virginity in Plant Science and Pharmacognosy. Also, no study has characterized the bioactive compounds present in the leaves of *Diplazium sammatii* using the GC-MS technique.

This present study was therefore carried out to determine the major bioactive compounds present in *Diplazium sammatii* leaf n-hexane extract using GC-MS analysis.

Materials and Methods

Collection and Identification of Plant

Fresh and healthy leaves of *Diplazium sammatii* were collected from Igbara-odo – Ekiti, Ekiti South West Local Government, Ekiti State, Nigeria. It was authenticated by the curator, Mr. Felix Omotayo and was assigned a voucher specimen number of "UHAE 2019160" at the herbarium of the Department of Plant Science and Biotechnology, Ekiti State University, Ado – Ekiti. Voucher specimens were deposited in the herbarium.

Preparation of Plant Material

The fresh leaves of the plant 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 (Model: Excella QTY IPC with 3.5.5 jars). The powdered sample was stored in an air tight plastic container and kept at 4 ^{o}C in a refrigerator until required for 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 fibre glass 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

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

In the present investigation, the GC-MS analysis of the bioactive compounds present in the n-hexane extract of the leaves of *D. sammatii* showed twenty three peaks. The phyto-compounds are shown in Table 1 with their retention

time (RT) and peak area (%). The GC-MS chromatogram of n-hexane extract of the leaves of *D. sammatii* revealed the presence of various compounds with corresponding peaks area at different retention time (min) (Figure 1). The result showed five prominent peaks in the retention time range of 6.092 to 23.139. Their GC-MS spectra are shown in Figures 2-6. The peak at 15.887 retention time had the highest peak area of 47.25 % and was due to the presence of oleic acid. The second less prominent peak at 14.063 retention time had the peak area of 19.64 %. This was due to the presence of n-Hexadecanoic acid. The third, fourth and fifth less prominent peaks indicated the compound Methyl tricontyl ether (6.51 %), Octadecanoic acid (5.87 %) and Benzene,(2-nitroethyl)- (3.94 %) with retention times of 23.139, 16.006 and 6.092 respectively. The other less prominent peaks at other retention time of various compounds included Neophytadiene, Cyclopropanecarboxylic acid, 2-[4- (1,1-dimethylethyl)phenyl]-, ethylester-, Hexadecanoic acid, methyl ester, 2,2'-Biphenylylenephosphorous acid chloride, 11-Octadecenoic acid, methyl ester, Pentyldotriacontyl ether, Methyl stearate, Henicos-1-ene, 1-Hexacosene, 3-Methyl-1,5-diphenylpenta-2,4-dien-1-one, Methyl 18-methylnonadecanoate, 1,2-Benzisothiazole, 3-(hexahydro- 1H-azepin-1-yl)-, 1,1-dioxide, Cholestan-3-one, 4,4-dimethyl-, (5α)-, Hexadecane, 1-iodo-, Cholesta-6,22,24-triene, 4,4-dimethyl-, 17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-ol, 1-Hexacosene and 1-Nonadecene.

Table 1: List of the bioactive compounds from the n-hexane leaf extract of *Diplazium sammatii*

S/N	Retention time (min)	Name of compound	Peak area (%)
1	6.092	Benzene, (2-nitroethyl)-	3.94
2	12.530	Neophytadiene	0.27
3	13.173	Cyclopropanecarboxylicacid, 2-[4- (1,1-dimethylethyl)phenyl]-, ethylester-	0.48
4	13.458	Hexadecanoic acid, methyl ester	1.06
5	13.601	2,2'-Biphenylylenephosphorous acid chloride	2.69
6	14.063	n-Hexadecanoic acid	19.64
7	15.158	11-Octadecenoic acid, methyl ester	0.88
8	15.287	Pentyldotriacontyl ether	0.31
9	15.406	Methyl stearate	0.99
10	15.492	Henicos-1-ene	0.51
11	15.558	1-Hexacosene	0.46
12	15.887	Oleic Acid	47.25
13	16.006	Octadecanoic acid	5.87
14	16.192	3-Methyl-1,5-diphenylpenta-2,4-dien-1-one	2.50
15	17.206	Methyl 18-methylnonadecanoate	0.27
16	17.635	2-Benzisothiazole, 3-(hexahydro- 1H-azepin-1-yl)-, 1,1-dioxide	0.48
17	18.920	Cholestan-3-one, 4,4-dimethyl-, (5α)-	0.30
18	19.225	Hexadecane, 1-iodo-	2.03
19	20.316	Cholesta-6,22,24-triene, 4,4-dimethyl-	1.24
20	21.916	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta [a]phenanthren-3-ol	0.09
21	22.935	1-Hexacosene	1.67
22	22.949	1-Nonadecene	0.24
23	23.139	Methyl tricontyl ether	6.51

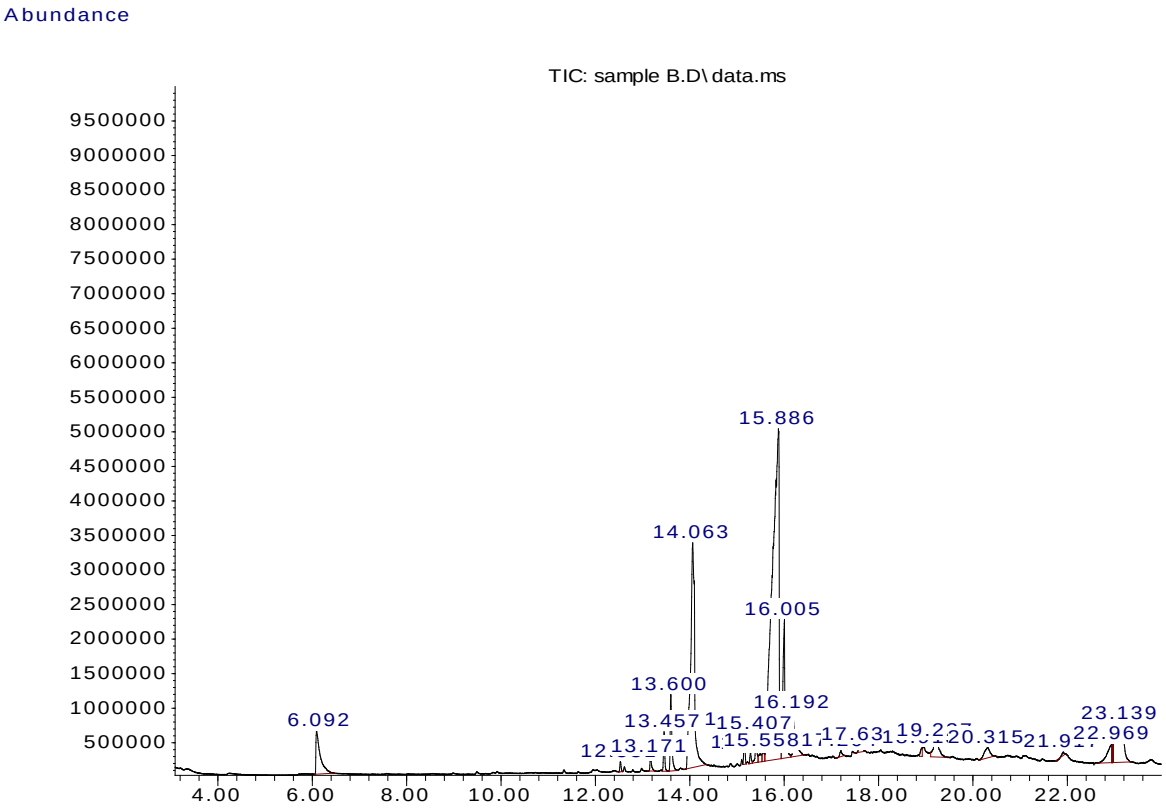

Figure 1: GC-MS chromatogram of n-hexane leaf extract of D. sammatii

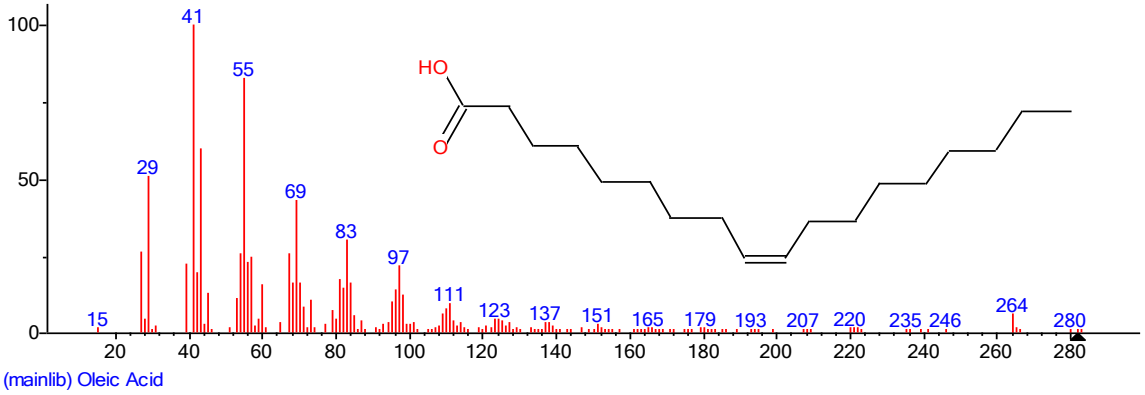

Figure 2: GC-MS spectra of Oleic acid (47.25%; RT: 15.887) from n-hexane leaf extract of D. sammatii

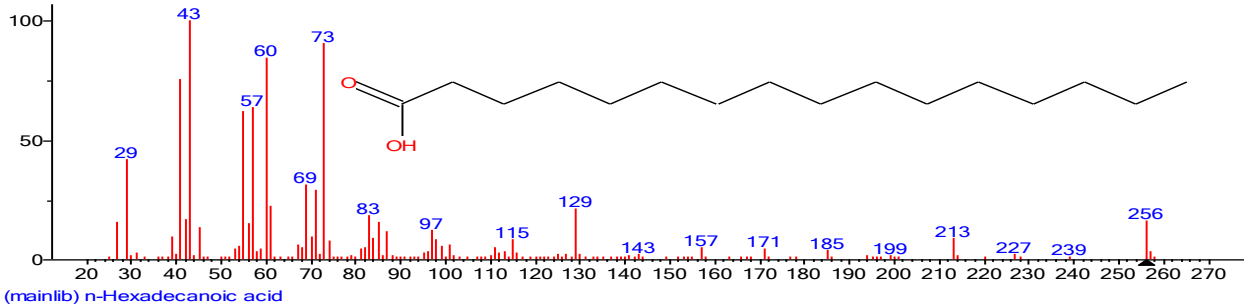

Figure 3: GC-MS spectra of n-Hexadecanoic acid (19.64%; RT: 14.063) from n-hexane leaf extract of D. sammatii

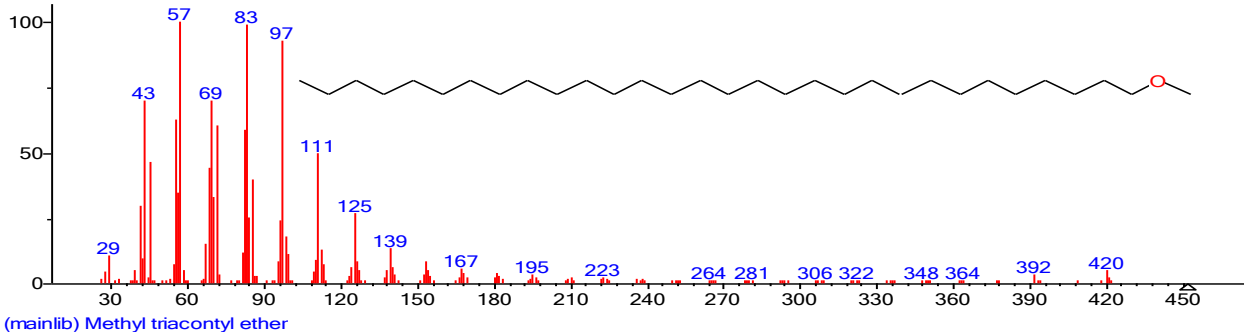

Figure 4: GC-MS spectra of Methyl tricontyl ether (6.51%; RT: 23.139) from n-hexane leaf extract of D. sammatii

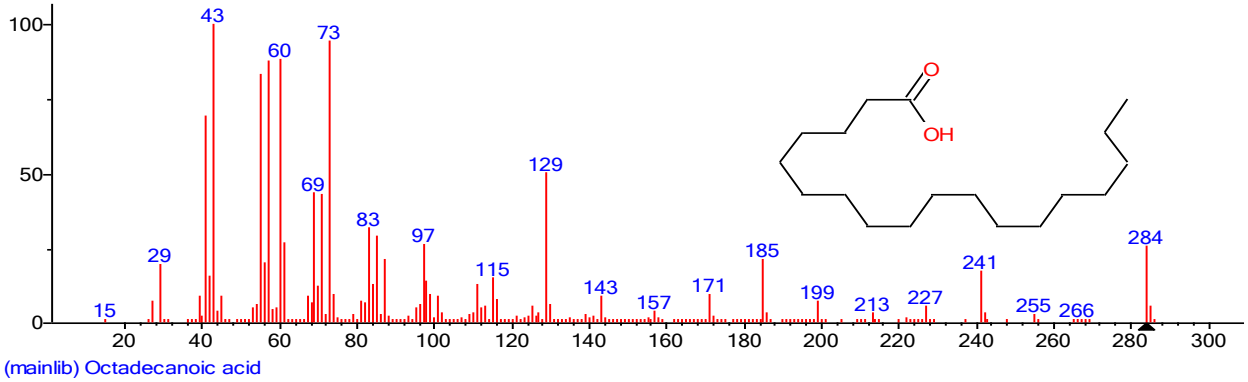

Figure 5: GC-MS spectra of Octadecanoic acid (5.87%; RT: 16.006) from n-hexane leaf extract of D. sammatii

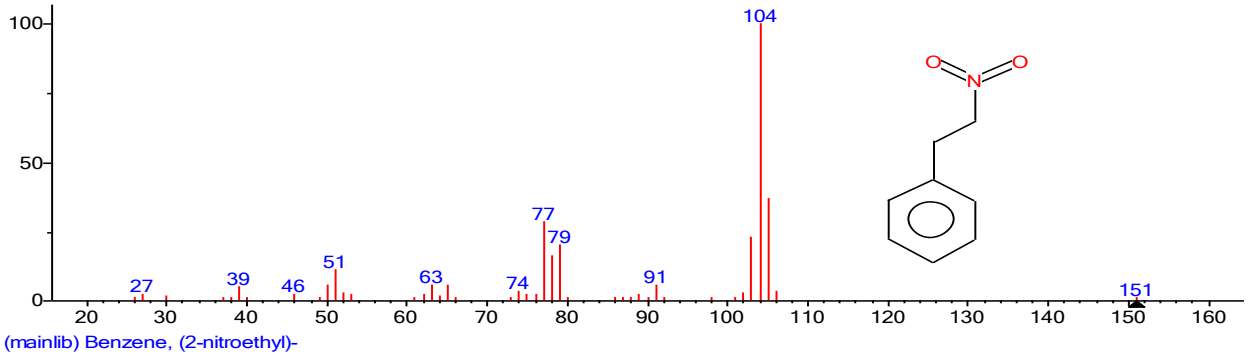

Figure 6: GC-MS spectra of Benzene, (2-nitroethyl)- (3.94%; RT: 6.092) from n-hexane leaf extract of D. sammatii

Discussion

In the present study, twenty three bioactive compounds have been identified in the n-hexane leaf extract of *Diplazium sammatii* using GC-MS analysis. Comparable to this study, various bioactive compounds were characterized through GC-MS analysis in the medicinal fern *Drynaria quercifolia* (Prasunna and Chirta, 2015). Eight phytocompounds were identified from methanolic extract of the leaf of *Azolla caroliniana* using GC-MS [14]. The presence of sixteen bioactive compounds in n-hexane leaf extract of *Nephrolepis cordifolia* using GC-MS technique had earlier been reported [15]. GC-MS analysis of methanol extracts of the leaf and rachis of *Acrostichum aureum* led to the identification of nineteen and fifteen compounds respectively [16]. The ethanolic leaf extract of *Pergularia daemia* was subjected to chemical analysis using GC-MS technique and identified the existence of fifteen compounds with their various biological activities [17]. GC-MS technique was used to identify the presence of seventeen compounds from ethanolic extract of a fern, *Pteridium aquilinum* [18]. The presence of six major bioactive compounds in methanol extract of the leaf of *Melastomastum capitatum* was reported using GC-MS

technique [19]. Also, GC-MS method was used to detect the presence of six compounds in acetone extract of the fern, *Nephrolepis cordifolia* [20].

The major compounds identified in the present study possess some biological potential which may play crucial roles in diseases and general metabolism of humans. Oleic acid was the most abundant identified bioactive compound in the present study. It is an aliphatic carboxylic acid. It has antimicrobial activity, antibacterial activity and antitumour activity [21]. It lowers heart attack risk and arthrosclerosis and aids in breast cancer prevention [22]. Oleic acid, the main monounsaturated fatty acid of olive oil, suppressed expression and synergistically enhances the growth inhibitory effects of trastuzumab (Herceptin) in breast cancer cells [23]. Also, oleic acid (Omega-9) is used to flavor baked goods, candy, ice cream and sodas [24]. N-hexadecanoic acid (Palmiticacd) was the second most abundant identified bioactive compound had been reported to have antioxidants, hypochloresterolenic, nematicide, pesticide, lubricant, antiandrogenicflavor, hemolytic properties [25]. Hexadecanoic acid, methylester, one of the identified compounds in the present study had been reported to act as an antioxidant, anti-inflammatory, antihyperlipidemic as well as antimicrobial in functions [26; 27]. Methyl stearate is used as a solvent and lipid carrier in agriculture [28]. Octadecanoic acid is useful in drug system [29]. It is also used to produce dietary supplements [30]. The present study is the first step towards understanding the nature of active principles in this edible and medicinal fern and this may be helpful in further studies.

Conclusion

In the present investigation, twenty three bioactive compounds were identified in the n-hexane leaf extract of *Diplazium sammatii* using GC-MS analysis. The presence of these bioactive principles might be responsible for the acclaimed biological activities of *D. sammatii* in traditional medicine. However, further investigation is recommended on toxicological aspect to ensure safety in drug development.

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