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Research Article

GC-MS Analysis of Bioactive Compounds from N-Hexane Leaf Extract of a Tropical Fern, *Nephrolepis cordifolia* (L) C. Presl

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Abstract: The present investigation was carried out to identify the bioactive compounds contained in n-hexane the leaf extract of *Nephrolepis cordifolia* by using the GC-MS machine. 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 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. Results provided different peaks, determining the presence of sixteen bioactive compounds. The main compounds were n-Hexadecanoic acid (RT:14.149; 24.42%), cis-13-Octadecenoic acid (RT:15.735; 10.31%), Octadecanoic acid (RT:15.963; 11.05%), Bis(2-ethylhexyl) phthalate (RT:19.216; 8.72%), 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (RT:21.839; 18.35%) and Squalene (RT:23.187; 5.82%). Others were present as minor compounds. The occurrence of these bioactive compounds in the plant may be responsible for its biological activities in traditional medicine. Therefore, it is recommended as a plant of pharmaceutical importance. However, further research on toxicological aspects of the medicinal potentials of these compounds is recommended for safe drug development.

Keywords: Fern, bioactive compounds, *Nephrolepis cordifolia*, GC-MS, drug development.

INTRODUCTION

Nephrolepis cordifolia is an important medicinal fern with rich source of phytochemicals (Oloyede *et al*., 2013). It is widely distributed particularly in the subtropical and tropical regions where they grow in the soil, among rocks or as an epiphyte (Cheng *et al*., 2001; Segarra Moragues, 2001). It has bright green fronds that are from 40-80 cm long and 10 cm wide at their widest points. It has short rhizomes and small scally tubers. They spread aggressively by windblown spores or by accidental movement of tubers and rhizomes. It is commonly called Boston fern, erect sword fern, fish-bone fern, herring bone fern, ladder fern, lemon butter fern, narrow sword fern, southern sword fern, sword fern, tube ladder fern and tuber sword fern (Schenk *et al*., 2001). In Nigeria, it is called nma ozo among the Ibo tribe.

Nephrolepis cordifolia has been reported to be of immense ethnobotanical importance. The juice of the root tubers is taken to treat fever, cough and hematuria (El-Tantawy *et al*., 2015). Traditionally, it is used as diuretic, contraceptive and in the treatment of liver disorders and skin diseases (Dhiman, 1998). Upreti and Gyawali (2015) reported the traditional use of *Nephrolepis cordifoilia* in asthma, immunity and biliousness.

Aqueous and non-aqueous extracts of *Nephrolepis cordifolia* have been reported to have antimicrobial activity (Bernejee and Sen, 1980; Rani *et al*., 2010; Adebiyi, 2019). The antidiuretic potential of *Nephrolepis cordifolia* rhizome juice has also been reported (Rajasekaran and Sivakumar, 2009). The chemical composition and cytotoxic activities of the volatile constituents from the subterranean organs of the plant in Egypt have been reported by El-Tantawy *et al*.

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(2015). No information is available on the essential oils of the plant grown in Nigeria.

Nigeria is known for its rich diversity of medicinal plants and from time immemorial, these plants have been utilized as therapeutic agents with various biological activities (Monier, 2016). Akinmoladun *et al*. (2007) reported that the phytochemical constituents, natural bioactive compounds, nutrients and fibres present in medicinal plants, fruits and vegetables defend the consumers from various ailments.

Many medicinal plants particularly angiosperms are currently being screened for bioactive compounds and subsequent biological activities owing to the natural origin, cost effectiveness and lesser side effects (Ahmad *et al*., 2008; Chellaram and Edward, 2009). Very less work has been done on pteridophytes in this regard despite the fact that their ethno medicinal importance has been studied and documented (Sathiyaraj *et al.*, 2015). Suvarnalathat *et al*. (2015) reported that pteridophytes have been successfully used in different systems of medicine such as Ayurvedi, Unani, Homeopathic and other systems of medicine.

The present study was therefore aimed at investigating the chemical composition of *Nephrolepis cordifolia* by subjecting it to GC-MS analysis.

MATERIALS AND METHODS

Collection and Identification of Sample

The plant (*N. cordifolia*) was collected from the bark of a palm tree in the premises of Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria. It was authenticated at the herbarium of Ekiti State University, Ado Ekiti, Ekiti State, Nigeria by the curator, Mr. F. Omotayo and assigned a voucher specimen number 'UHAE 2019162'.

Preparation of sample for GC–MS analysis

Fresh and matured leaves of the plant were taken to the laboratory where they were manually cleaned to remove all foreign materials. They were then air dried for forty nine days and pulverized into fine powder using an electric blender (Model Excella QTY LPC). The powder was then sieved using sieve number 20 mesh to remove unwanted debris. 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.

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 80^0C (isothermal for 2 min) with an increase of 10^0C /min to the final temperature of 240^0C 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 250^0C. Mass spectra were taken at 70Ev. The total GC running time was 23.187 min. The library used for the identification of compounds was National Institute Standard and Technology (NIST)-version Year 2014.

RESULTS

The list of all the bioactive compounds identified in the n-hexane extract of the leaves of *N. cordifolia* is given in Table 1. The chromatogram (Figure 1) showed six prominent peaks in the retention time range of 14.149 to 23.187. The peak at 14.149 retention time had the peak area of 24.42 % and was due to the presence of n-hexadecanoic acid. The second less prominent peak at 21.839 retention time had the peak area of 18.35. This was due to the presence of 1,4-benzenedicarboxylic acid, bis (2-ethylhexyl) ester. The third less prominent peak of octadecanoic acid (11.05 %) had the retention time of 15.963. The fourth less prominent peak indicated the compound cis-13-octadecanoic acid (10.31 %) and the retention was 15.735. The fifth and sixth less prominent peaks indicated the compounds Bis (2-ethylhexyl) phthalate (8.72 %) and squalene (5.82 %) with retention times of 19.216 and 23.187 respectively. The GC-MS spectra of the identified major compounds are shown in Figures 2-7. The other less prominent peaks at other retention time of various compounds are also shown in Figure 1 and Table 1. These compounds included hexadecanoic acid, methyl ester, 9-Octadecenoic acid, methyl ester, methyl stearate, Docosyl pentafluoropropionate, 1-Nonadecene, 2-Methyl-Z,Z-3,13-octadecadienol , docosanoic acid, tetracosane, heptacosane and nonadecane, 1-chloro-.

DISCUSSION

In the present study, sixteen bioactive compounds have been identified in the n-hexane leaf extract of *N. cordifolia* using GC-MS analysis. Similar to this study, various bioactive compounds were characterized through GC-MS analysis in the medicinal fern *Drynaria quercifolia* (Prasunna and Chirta, 2015). Six major bioactive compounds have been identified from methanol extract of the leaf of *Melastomastrumm capitatum* by Gas Chromatogram Mass Spectrometry (GC-MS) (Ukwubile *et al*., 2019). GC MS analysis of the methanol extracts of the leaf and stem of an aquatic fern *Marsilea quadrifolia* led to identification of 39 and 29 compounds respectively. (Gopalakrishnan and Udayakumar, 2014). The ethanolic leaf extract of

Macrotyloma uniflorum were subjected to chemical analysis using GC-MS method and this confirmed the occurrence of phytocompounds with various biological activities (Das *et al*., 2014). Janakiraman *et al*. (2012) reported that there is growing awareness in correlating the phytochemical compounds and their biological activities.

The major compounds identified in the present study possess some significant biological potential which may be useful for future drug development. Among the bioactive compounds identified in this study, n-Hexadecanoic acid which was the most abundant compound has been reported to have antimicrobial, antioxidant (Bodoprost and Rosemayer, 2007) and larvicidal activities (Falodun *et al.*, 2009). n-Hexadecanoic acid has biological activities such as antifungal, antioxidant, hypocholesterolenic, nematicidal, anti-androgenic flavour, haemolithic-5-alpha reductase inhibitor and anti-malarial potentials (Hema *et al*., 2011; Pietro *et al*., 2010). Arora *et al*. (2017) had earlier reported the therapeutic uses of another identified major compound, cis-13-Octadecanoic acid in the medicine, surgery. Squalene which is another major compound identified in the present study has also been reported to possess cardioprotective effect (Farvin *et al*., 2006) and detoxifying property (Kelly, 1999). Scientific research has also shown that squalene reduces skin damage by UV radiation and also has anti-tumour and anti-cancer effects against ovarian, breast, lung and colon cancer (Smith, 2000; Rao *et al*., 1998). Octadecanoic acid and heptacosane have also been reported to possess antibacterial activities (Danta da Silva *et al*., 2002; Mihailovi *et al*., 2011). Another major compound identified in the present study was Bis (2-ethylhexyl) phthalate. It is a phthalate ester that is the bis (2-ethylhexyl) ester of benzene 1,2-dicarboxylic acid. It has a role as apoptosis inhibitor, an androstane receptor agonist and a plasticizer (Kim *et al*., 2019). The antibacterial and antifungal activities of another compound identified in the present study (hexadecanoic acid) have also been reported (Chandrasekaran *et al*., 2011). Tetracosane which was also identified in the present study has been reported to show significant cytotoxicity against HT-29 colon cancer cells. It has also shown some toxicity against the oestrogen-dependent breast cancer (MDA-MB-231) CELLS (IC_{50}>250µM) (Uddin *et al*., 2012).

Table 1. Compounds identified in the n-hexane leaf extract of *N. cordifolia* by GC- MS

S. No	Retention Time (min)	Name of compound	Peak area (%)
1	13.468	Hexadecanoic acid, methyl ester	3.13
2	14.149	n-Hexadecanoic acid	24.42
3	15.173	9-Octadecenoic acid, methyl ester, (E)-	2.60
4	15.416	Methyl stearate	1.50
5	15.735	cis-13-Octadecenoic acid	10.31
6	15.963	Octadecanoic acid	11.05
7	17.668	Docosyl pentafluoropropionate	0.72
8	18.630	1-Nonadecene	1.29
9	18.982	2-Methyl-Z,Z-3,13-octadecadienol	2.11
10	19.216	Bis(2-ethylhexyl) phthalate	8.72
11	19.535	Docosanoic acid	2.12
12	19.701	Tetracosane	1.92
13	20.997	Heptacosane	3.30
14	21.839	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	18.35
15	22.635	Nonadecane, 1-chloro-	2.63
16	23.187	Squalene	5.82

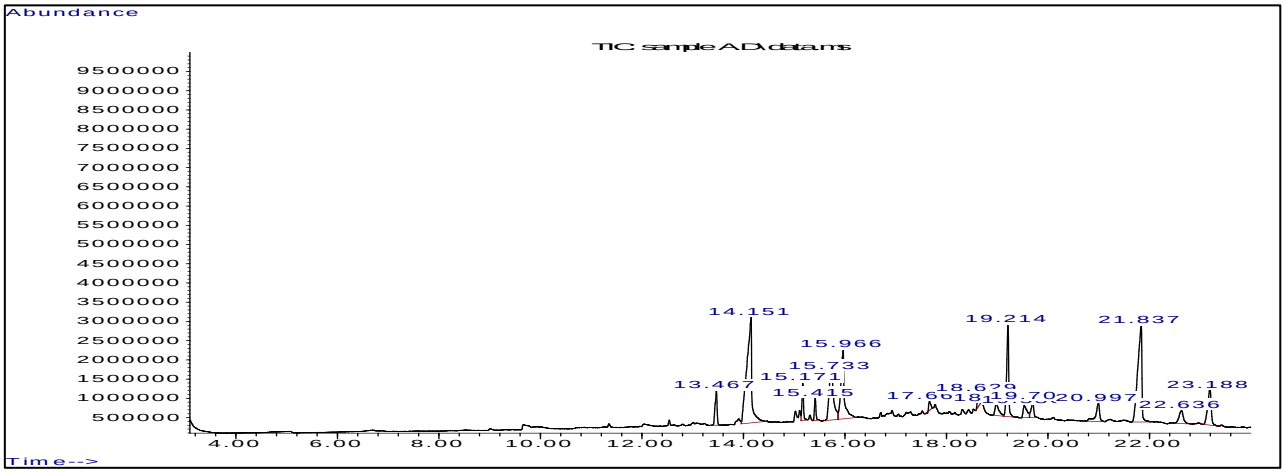

Figure 1: GC-MS Chromatogram of n-hexane leaf extract of *N. cordifolia*

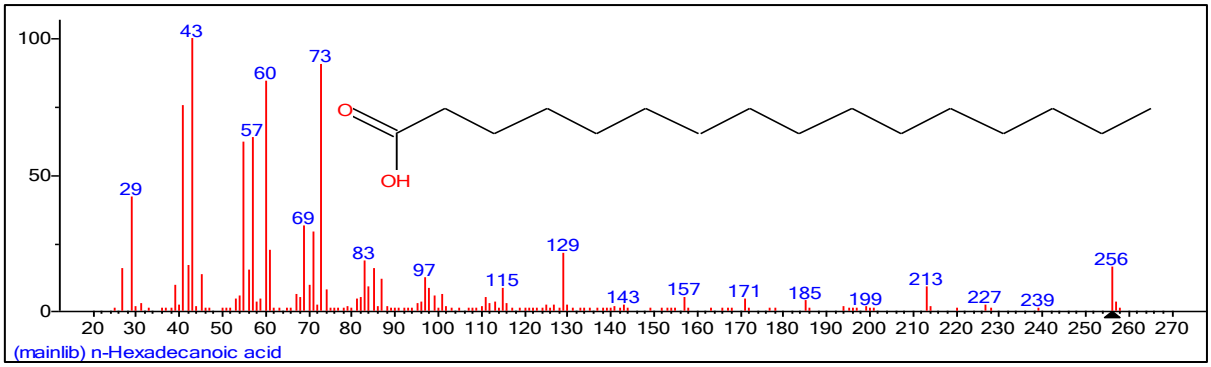

Figure 2: GC-MS spectra of n-Hexadecanoic acid (24.42%; RT: 14.149) from n-hexane leaf extract of *N. cordifolia*

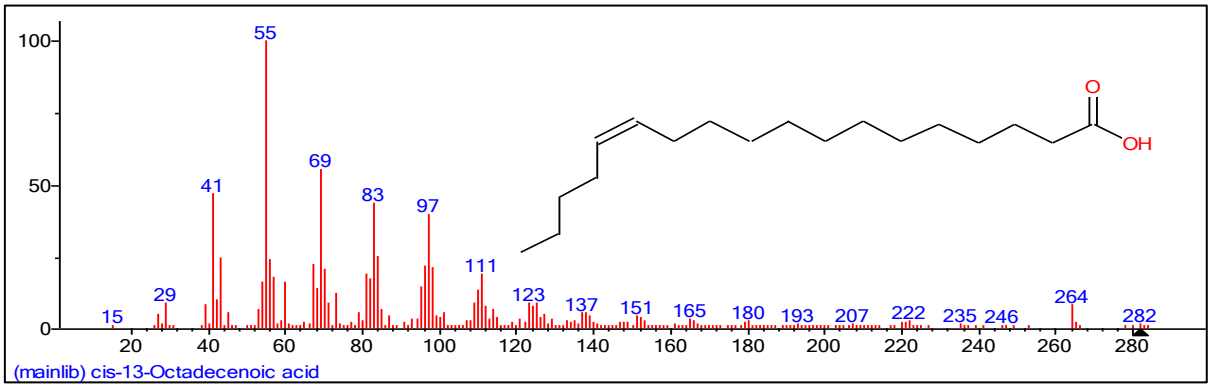

Figure 3: GC-MS spectra of cis-13-Octadecenoic acid (10.31%; RT: 15.735) from n-hexane leaf extract of *N. cordifolia*

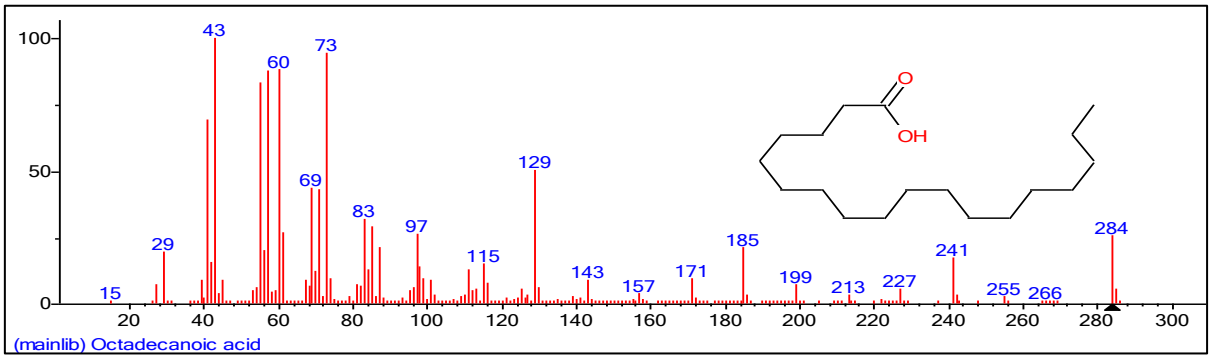

Figure 4: GC-MS spectra of Octadecanoic acid (11.05%; RT: 15.963) from n-hexane leaf extract of *N. cordifolia*

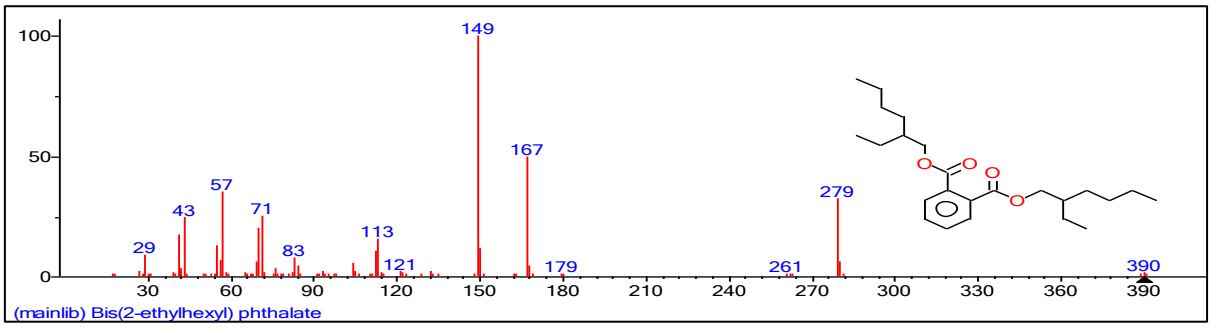

Figure 5: GC-MS spectra of Bis(2-ethylhexyl) phthalate (8.72%; RT:19.216) from n-hexane leaf extract of *N. cordifolia*

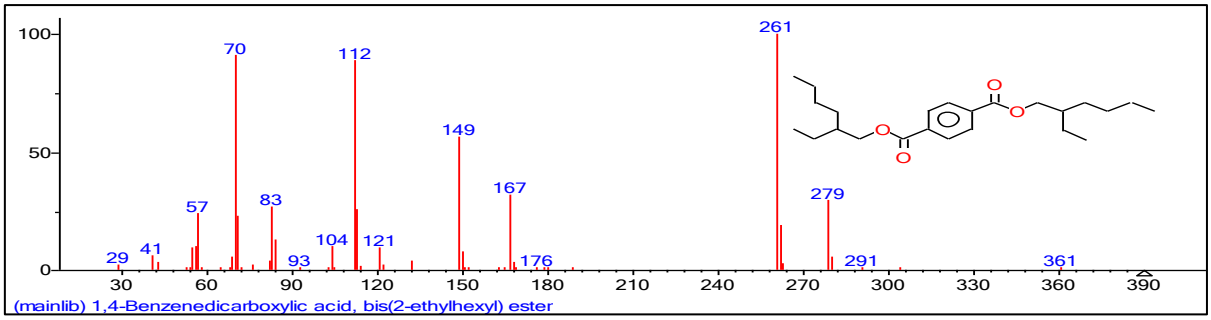

Figure 6: GC-MS spectra of 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (18.35%; RT: 21.839) from n-hexane leaf extract of *N. cordifolia*

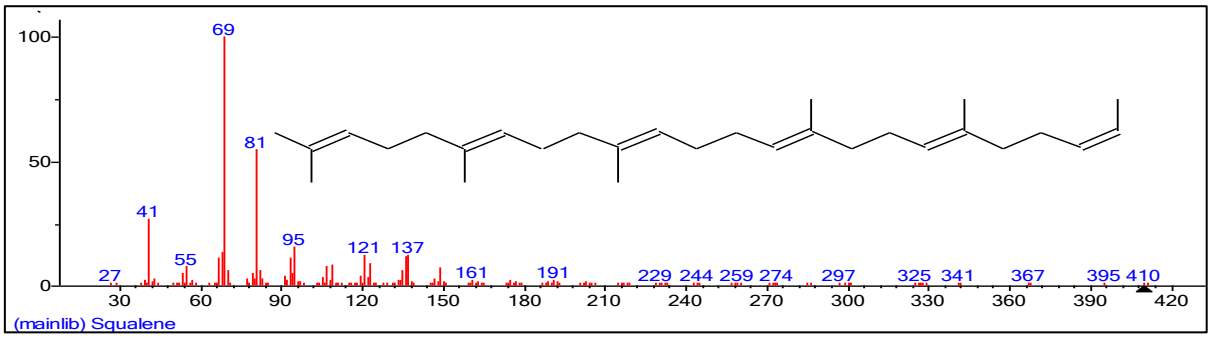

Figure 7: GC-MS spectra of squalene (5.82%; RT: 23.187) from n-hexane leaf extract of *N. cordifolia*

CONCLUSION

In the present study, sixteen bioactive compounds have been identified in the n-hexane leaf extract of *Nephrolepis cordifolia* using GC-MS analysis. Each of these compounds has documented therapeutic potentials. The presence of these bioactive compounds might be responsible for the use of the plant for the treatment/ management of various ailments by traditional practitioners. However, the medicinal potential of these components needs further research on toxicological aspects to develop safe drugs.

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