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Phytochemical, Nutrient, Anti-Nutrient Compositions and the GC-MS Analysis of n – Hexane Extract of the Leaf of *Drynaria quercifolia.*

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ABSTRACT

Ferns plants that are underutilized because of paucity of information about their medicinal and nutritional importance. This study focused on the assessment of phytochemical, nutrient, anti-nutrients and bioactive constituents of *Drynaria quercifolia* using the standard analytical techniques. The phytochemical analysis of the plant revealed appreciable concentrations of phenolics, saponins, alkaloids, flavonoids and tannins. The proximate analysis showed high carbohydrate but low crude fat content. The study revealed high concentration of calcium (818.00 g/kg) with appreciable levels of potassium, sodium, phosphorous and magnesium. Traces of phytate, oxalate and hydrogen cyanide were found in the leaf. The estimated retinol and ascorbic acid equivalents in *D. quercifolia* leaves were 0.27 and 14.60 respectively. The principal bioactive compounds found in the leaf were Octadec-9-enoic, 11-Octadecenoic methyl ester, n- Hexadecanoic acid, Hexadecanoic acid methyl ester and phytol. The research reveals that *D. quercifolia* leaves may be used for medicinal purposes and could also be supplemented in the diet.

Keywords: phytochemical, proximate composition; mineral, vitamins and bioactive compounds

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INTRODUCTION

Pteridophytes are ancient vascular plant species that do not produce flowers or seeds but constitutes an important part of the plant kingdom. They are often referred to as "ferns and fern allies" and are distributed in a wide range of habitats. They are widely distributed in Africa, Asia, Europe and America. Ferns are used in the production of insect repellents, aromatic oil, perfume, cosmetics and dyes [1 – 3]. They are popularly employed as ornamental plants and foliage for grazing animals. Their application in phytoremediation processes has also been established [4-6]. In Nigeria, ferns are underutilized and are referred to as useless plants because of paucity of information on their economic, medicinal and nutritional properties.

Drynaria quercifolia (L.) belongs to the family of Polypodiaceae. It is commonly known as "Oak Leaf Fern" and is found in India, Pakistan, North America and Africa [7]. The rhizome is popularly used as a traditional soup condiment and analgesics by the people of Eastern Ghats, Tamil Nadu, India [8]. It has also been used in Bangladesh by traditional healers for the treatment of jaundice, hepatitis, diabetes, gonorrhea and malaria [9-10]. In Southeast Asia, rhizome decoction of *D. quercifolia* is used in antipyretic preparation and as poultice on swellings [11]. Although different workers have tried to substantiate the nutritive and medicinal qualities of this plant in Asia, the information pertaining to these claims is scanty in Nigeria.

Therefore, this study is aimed at elucidating the phytochemical, proximate, minerals, anti-nutrients and bioactive components of this plant. The outcome of this research will provide information on the possible nutritional and medicinal values of *D. quercifolia* grown in Nigeria.

MATERIALS AND METHODS

Collection, Identification and preparation of sample

D. quercifolia was collected from different towns in Ekiti State, Nigeria. The plant was authenticated and assigned herbarium number UHAE 2019161 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 ferns were plucked from healthy plant stalks, rinsed in clean water and air-dried to a constant weight. The plant samples were pulverized into fine powder using an electric blender. The powdered samples were stored in an air tight plastic container and kept at 4^0C in a refrigerator until it was required for analysis.

Chemicals

Ammonium hydroxide, amyl alcohol, ethanol, sodium chloride, acetic acid, N – hexane, Folin – Denins reagent, sodium carbonate, tannic acid, diethyl ether, nitric acid, ascorbic acid and hydrochloric acid were purchased from Merck USA. All 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.* [12]. The proximate (moisture, ash crude protein and fat) contents were determined using the method described by AOAC [13].The carbohydrate content was calculated by subtracting the sum of total values of moisture, ash, protein, and fat from 100. Each of the nutrient content was expressed in percentage.

Determination of Mineral content

The modified method described by Bouba *et al.* [14] 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 550 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.* [15] and Njoku *et al.* [16] respectively. Oxalate content of the sample was determined by titration method as described by Unuofin *et al.* [17]. The phytic acid content of the sample was determined using the method of Aina *et al.* [18]. While the hydrogen cyanide content was analyzed as described by [19].

GC – MS analysis of n – hexane extract of *D. quercifolia* leaves

The powdered leaf sample was extracted with HPLC-grade n-hexane. During the extraction process, the sample was shaken several times to increase the extraction efficiency. The extract was filtered and the filtrate was concentrated to dryness on rotary evaporator. The analysis was performed using a GC-MS system (7890A-5975C, Agilent Technologies Inc., Santa Rosa, CA, USA) equipped with an HP-5 MS capillary column (30 m 0.25 mm, 0.25 mm, Agilent Technologies Inc., Santa Rosa, CA, USA [20]. The separated constituents were identified by comparing their mass spectra with those in the National Institute of Standards and Technology, Gaithersburg, MD, USA [21]. Each constituent was quantified based on the comparison of its peak area with that of the standard.

Statistical analysis

Data were analyzed using the descriptive statistical analyses where means and standard deviation (SD) were obtained using Microsoft Excel 2013 version. Results were expressed as mean ± SD.

RESULTS AND DISCUSSION

The result of the phytochemical composition of *D. quercifolia* leaves revealed the presence of phenolics, saponins, alkaloids, flavonoids and tannins (Table 1). The leaf is rich in total phenol, flavonoids, and tannins with minute amount of saponins and alkaloids. Phytochemicals are bioactive components that are responsible for medicinal potentials of plants [12]. Polyphenols are capable of neutralizing free radicals in biological systems and combating microbes [22]. Flavonoids are hepatoprotective, antimicrobial and antitumor agents. Tannins have been reported to possess anti-diuretic and anti-diarrhoea properties [23]. The presence of these compounds in the leaves of *D. quercifolia* is an indication that it could be used in traditional medicine for various medicinal purposes.

Table 2 depicts the proximate composition of *D. quercifolia.* The results demonstrated that the leaves contain significantly higher amount of crude protein, crude fat, carbohydrate and ash compared with some convectional vegetables [12]. Proximate analysis are important for the determination of quality and microbial safety of food products [24]. Digestible carbohydrates and fats are sources of fuel in the diet. The leaves can be incorporated to diet to produce energy. *D. quercifolia* is also a rich source of protein (14.01%). The ash content is an indication of the mineral in the leaves. The ash content could be the reason for the high mineral level of the leaf. Previous studies reported that crude fibers can lower the blood cholesterol and sugar levels, thus reducing the risk of diabetes [25]. It can be suggested that the leaves of *D. quercifolia* leaf could be an important source of essential nutrients to supplement human diet. The shelf life of natural products depend mainly on their moisture content. It has been reported that plant material with a level of moisture level higher than 15% stands the risk of microbial contamination [26]. The moisture content of this plant (9.78 %) suggests that it and could be preserved with little risk of microorganism invasion.

The most abundant macro element in the leaves of *D. quercifolia* is calcium (Table 3). Calcium is responsible for the building of strong bones and teeth. The high concentration of calcium in the leaves suggests that it can be a good source of nutrients for the elderly who are majorly predisposed to osteoporosis [27]. Potassium and sodium were also present in appreciable amounts (681.25 and39.55) in the leaves. These are vital elements which are required for the normal functioning of the nervous system and maintenance of blood pressure [28]. Magnesium, phosphorous, iron, zinc and copper essential components of immune system. The concentrations of these minerals in *D. quercifolia* leaves are higher than those reported for some vegetables [29]. This study implies that *D. quercifolia* leaf is a good source of vital elements.

The vitamin and the anti-nutrient compositions of the plant sample were determined and presented in Table 3. The estimated retinol and ascorbic acid equivalents in the sample were 0.27 and 14.60 respectively. 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 [30]. Data from this study suggested that *D. quercifolia* leaves can be added as ingredient to supplement human diet since it is rich in ascorbic acid. The percentage phytate (0.71), oxalate (0.35) and hydrogen cyanide (0.05) compositions of the leaf were found to be lesser than those reported for some vegetables [31]. This is beneficial because anti-nutrients are known to prevent the absorption of certain nutrients in the body system [32].

The identified chemical components of the n – hexane extract of *D. quercifolia* leaves is shown in Figures 1 and 2. The name and percentage composition of the compounds are presented on Table 5 proper identification. The principal compounds found in the leaf are Octadec-9-enoic 11-Octadecenoic methyl ester acids which are known to possess antimicrobial activities [33]. Also, n- Hexadecanoic acid and Hexadecanoic acid, methyl ester which have biosurfactant properties [34]. The leaf has significant composition of phytol which have been reported to have antimicrobial [35], antioxidant [36] and cytotoxic [37] effects. Methyl stearate also possess anti-inflammatory potentials [38]. The identified compounds in *D. quercifolia* leaves have reported beneficial biological activities which may enhance the medicinal potentials of the leaf.

Table 1: The phytochemical constituents of *Drynaria quercifolia* leaves.

Phytochemical	Composition (%)
Total phenols mg GAE/g	22.73 ± 0.13
Saponins mg/g	0.06 ± 0.00
Alkaloids mg/ ATE/g	0.12 ± 0.00
Flavonoids mg QE/g	12.25 ± 0.17
Tannins mg TAE/g	7.29 ± 0.09
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 2: The proximate composition of *Drynaria quercifolia* leaves.

Content	Composition (%)
Carbohydrates	56.13 ± 0.14
Crude Fat	2.63 ± 0.13
Crude Protein	14.01 ± 0.04
Ash	11.38 ± 0.08
Crude Fiber	6.08 ± 0.04
Moisture	9.78 ± 0.08

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

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Table 3: The mineral composition of *Drynaria quercifolia* leaves

Element	Composition (g/kg)
Calcium	818.00 ± 2.00
Potassium	681.25 ± 1.25
Magnesium	332.50 ± 2.50
Sodium	39.55 ± 0.05
Iron	26.65 ± 0.15
Phosphorous	225.00 ± 1.00
Manganese	16.15 ± 0.15
Zinc	3.57 ± 0.03
Copper	2.66 ± 0.06

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

Table 4: Vitamins and anti-nutrient composition of *Drynaria quercifolia* leaves

Vitamin A (mg retinol/100g)	Vitamin C (mg ascorbic acid/100g)	Phytate	Oxalate	Hydrogen cyanide
0.27 ± 0.02	14.60 ± 0.02	0.71 ± 0.01	0.35 ± 0.01	0.05 ± 0.00

The values are expressed as means ± standard deviations (n = 3)

Table 5: List of the bioactive compounds from the n-hexane extract of *Drynaria quercifolia*

Peak	Retention time	Name of compound	Area (%)
1	6.377	Benzene, (2-nitroethyl)-	1.12
2	12.539	Neophytadiene	2.21
3	13.468	Hexadecanoic acid, methyl ester	12.96
4	13.963	n-Hexadecanoic acid	23.31
5	15.111	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	1.21
6	15.158	11-Octadecenoic acid, methyl ester	9.39
7	15.287	Phytol	7.15
8	15.406	Methyl stearate	5.96
9	15.649	Octadec-9-enoic acid	29.17
10	15.892	Oleic Acid	1.79
11	22.563	β-Sitosterol	0.68

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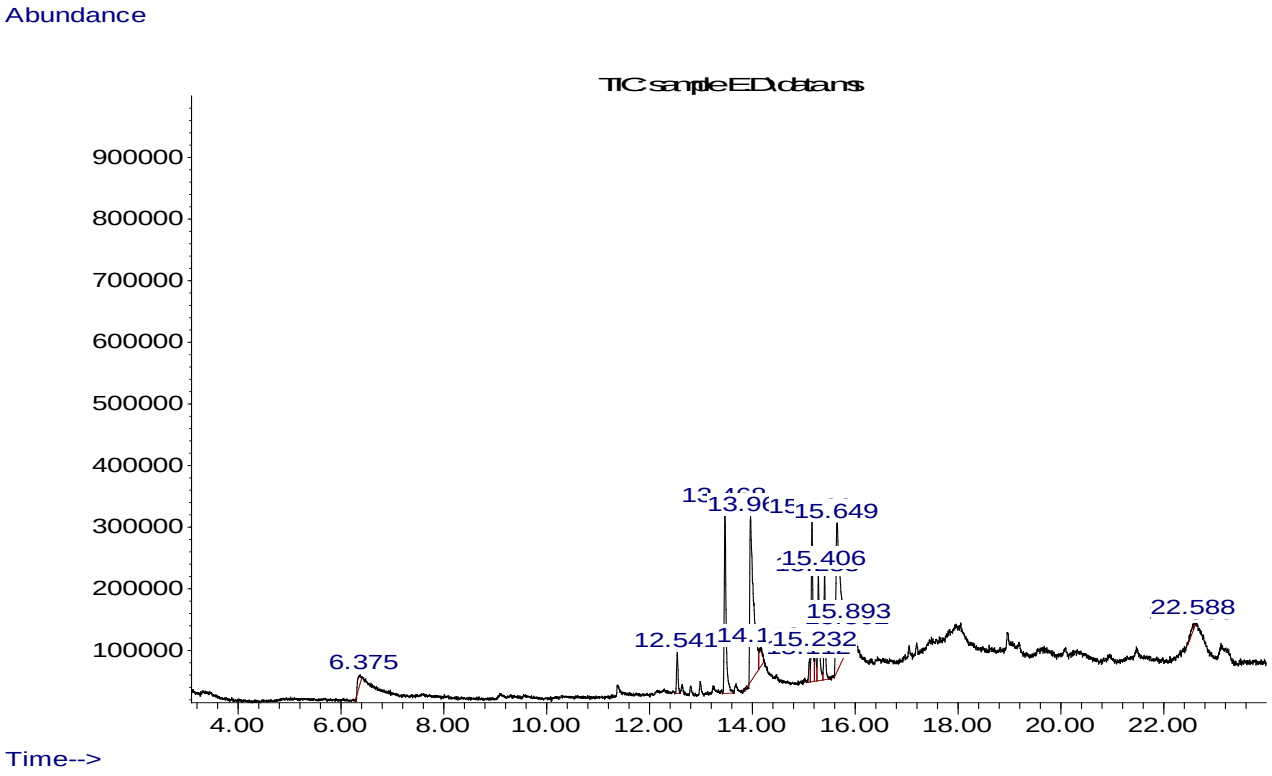
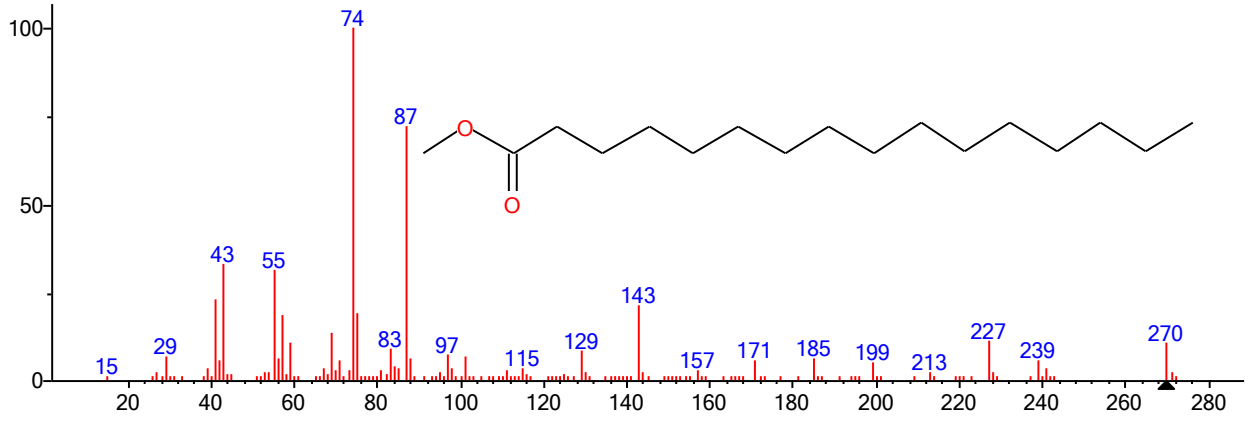
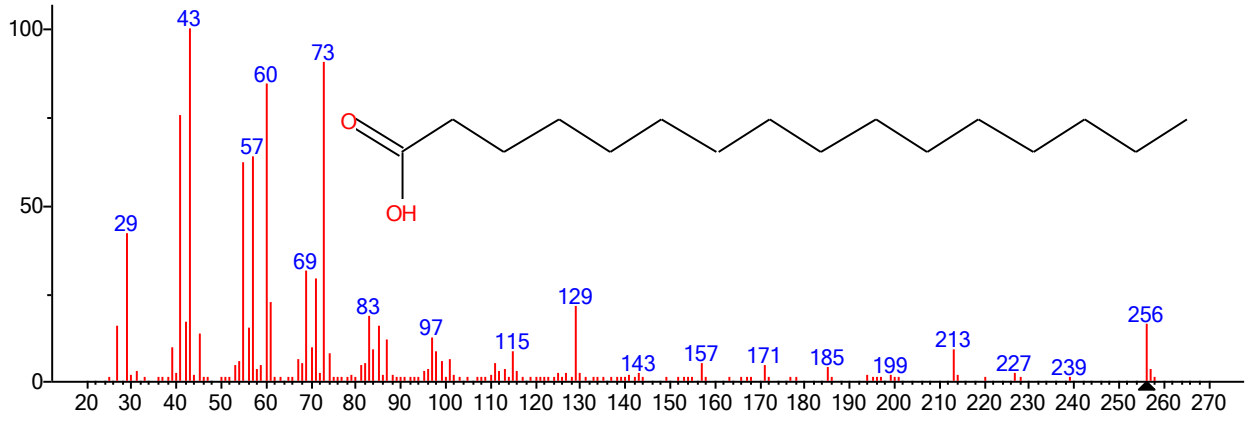


Figure 1: GC-MS chromatogram of n-hexane extract of *Drynaria quercifolia*



(mainlib) Hexadecanoic acid, methyl ester



(mainlib) n-Hexadecanoic acid

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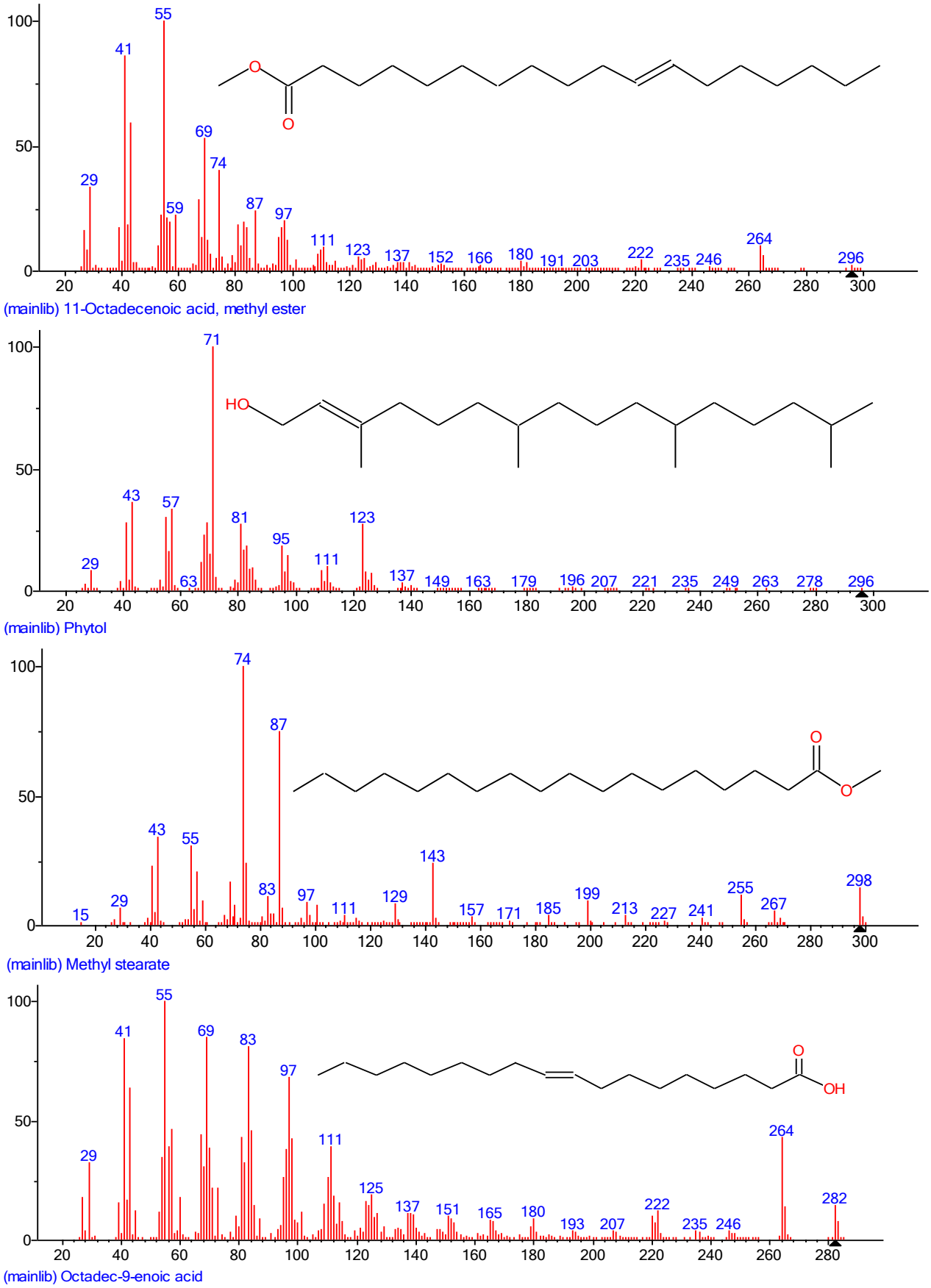

Figure 2: Mass spectrum and structure of bioactive components identified by GC-MS in the n - hexane extract of *Drynaria quercifolia*

CONCLUSION

The results obtained from this study indicate that the plant is mainly constituted by carbohydrates and then followed by protein, ash, moisture and fat. The high level of ash revealed the presence of high mineral composition in the plant which could be harnessed to combat micronutrient deficiencies. The leaf also contains high amount of retinol and ascorbic acid which are good sources of dietary antioxidant. The presence of vital bioactive compounds of medicinal and nutritional importance in the leaf shows that *D. quercifolia* is a potential source natural medicine and complimentary diet.

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