

## Chemical Composition of the Leaves of *Ceratopteris thalictroides* (L.) Brongn

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### Abstract

### Original Research Article

This study was conducted to determine the nutritional and anti-nutritional composition of the leaves of an edible and medicinal fern, *Ceratopteris thalictroides* using standard procedures. The phytochemical screening and GC-MS analysis of the leaf extracts were also carried out. GC-MS analysis was done using Agilent technologies Model 7890A coupled with a mass spectrometer Agilent technologies 6975. The results of proximate analysis revealed that the leaves contained 13.39% moisture, 13.36% protein, 10.18 % ash, 5.54% crude fibre, 54.34% carbohydrate, 3.21% fat and energy value of 299.69 kcal/100g. Vitamin C content of 6.09 mg/100g was also observed in the leaves. Minerals analysis revealed the occurrence of minerals such as sodium (85.20 mg/100g), potassium (1341.30 mg/100g), phosphorus (215.00 mg/100g), magnesium (107.00 mg/100g), calcium (434.00 mg/100g), manganese (6.44 mg/100g), iron (58.40 mg/100g), zinc (2.76 mg/100g) and copper (1.19 mg/100g). The anti-nutritional compounds found included oxalate (0.41 mg/100g), phytate (0.36 mg/100g) and hydrogen cyanide (0.15 mg/100g) which were much lower than reported safe limits. Results of phytochemical screening revealed the presence of alkaloids (0.02 mg/g ATE), saponins (0.01 mg/g), total phenol (14.51 mg/g GAE), tannins (4.29 mg/g TAE) and flavonoids (6.23 mg/g QE) while terpenoids, steroids, cyanogenic glycosides, cardiac glycosides, anthraquinones and phlobatanins were absent. The results of the GC-MS analysis revealed the presence of twelve compounds with documented therapeutic activities. The major phytocompounds included Benzene, (2-nitroethyl)- (RT: 6.096; 40.75%), .gamma.-Sistosterol (RT: 23.154; 21.87%), n-Hexadecanoic acid (RT: 13.973; 5.96%) and Linalool (RT: 3.349; 4.04%). Others were present as minor compounds. The foregoing results revealed that the leaves of the investigated plant are good sources of essential nutrients. They also contain medicinally important compounds, having therapeutic potentials, thus they could be used as bio control agents.

**Keywords:** *Ceratopteris thalictroides*, nutrients, anti-nutrients, phytochemicals, GC-MS.

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## INTRODUCTION

*Ceratopteris thalictroides* is commonly known as swamp fern and it belongs to the family Parkeriaceae. The plant is an aquatic or semi aquatic fern, growing up to 1 meter tall, either floating in water or rooted in soil. It grows very well in swampy areas, forests, marshes, natural and man-made ponds, usually in stagnant water bodies [1]. It is widely distributed in the tropics where it prefers a temperature of 20 – 22°C and a pH of 5 – 6.5. The plant thrives in full sun to moderate shade and is sometimes massed on or around logs or other floating vegetation in the wild. It is commonly grown as an ornamental in aquariums where it is popularly called water sprite. It can be grown in water depths up to 30 cm or it can be allowed to float on water. The plant has a short life cycle of usually less than 30 days.

The young fronds are consumed as vegetable either raw or cooked. They have been reported to make excellent greens and when cooked or blanched, they can be eaten as salad and in some parts of Asia, it has been established as luxury vegetable [2]. The ethno medicinal uses of the plant have also been documented. The rhizomes and fronds are used as medicine for foetal toxins and accumulation of phlegm [2]. Both the leaves and the roots are used as poultice against skin diseases and problems [3, 4]. The whole plant parts are ground into paste and mixed with turmeric and the mixture is applied over the affected places to treat/cure skin diseases and wounds [5, 6].

The plant has been reported to have anti-inflammatory activity [7], anti-oxidant activity [8] and anti-diabetic activity [9]. The chloroform and acetone extracts of the plant have been reported to show

antibacterial activity against some human pathogens [10]. Furthermore, a novel anti-HIV (Human Immunodeficiency Virus) protein had earlier been identified from the plant [11]. An investigation on the phytochemical composition of the plant indicated the occurrence of some secondary metabolites [12].

There is considerable information on the nutritive and anti-nutritive composition of most leafy vegetables [13]. However, scanty information is available on the proximate composition of lesser vegetables [14]. The use of herbal drugs has been emphasized over the last few decades due to their easy availability, therapeutic potential and minimum cost [15]. It has been reported that 80% of the world population rely on plant based drugs for their healthcare need [16]. Therefore, the need for validation of the ethno medicinal use of herbal medicine and subsequent isolation and characterization of the compounds which may be added to the potential list of drugs is sacrosanct. A detailed search through literature on the plant being investigated suggests that there are no published reports worldwide on the isolation of bioactive compounds from the plant using GC-MS technique. In fact, research activities on this plant are very scanty.

Hence, the present study was carried out to determine the nutritional, anti-nutritional and phytochemical composition of the leaves of *C. thalictroides*. The bioactive compounds in the leaves were also identified using the GC-MS technique.

## MATERIALS AND METHODS

### Collection and Identification of Sample

*Ceratopteris thalictroides* leaves used in this study were obtained from a tourist centre, Ikogosi warm spring in Ikogosi Ekiti, Ekiti State, Nigeria where they are widely distributed. The leaves were packed in a plastic container and taken to the laboratory. The samples were manually cleaned to remove all foreign materials. All the chemicals used were of analytical grade. The plant was authenticated at the herbarium of the Department of Plant Science and Biotechnology, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria by the curator, Mr. F. Omotayo and assigned a voucher specimen number 'UHAE 2019159'.

### Preparation of Plant Material

The fresh leaves of *C. thalictroides* were air dried for forty nine days and pulverized into fine powder using an electric blender ((Binatone BLG-600SMK2). The powder was then sieved through 2.0 mm mesh to remove unwanted debris. The fine powder was then stored in a clean and dry bag for further use.

### Proximate Analysis

Moisture, ash, crude fat and crude fibre were analysed in the samples in accordance with the methods described by Association of Official Analytical Chemist

[17]. Carbohydrate was however determined by difference.

## MINERAL ANALYSIS

1g of the ground sample was weighed into a pyrex beaker and 10 ml of concentrated HNO<sub>3</sub> was added and allowed to soak for 30 minutes. Then, 3ml of 60 % perchloric acid was added. The sample was placed on hot plate and heated at 350°C until frothing stopped and HNO<sub>3</sub> almost evaporated. Then, watch glass was placed on the beaker and heating continued until the sample turned light straw in colour. This was then removed from hot plate and cooled. Then, the watch glass was rinsed into the sample and sample filtered into 100 ml volumetric flask and made up to the mark with distilled water. This was analysed using flame photometry for Sodium and Potassium and atomic absorption spectrophotometer for other minerals [17].

### Determination of Vitamin C

This was carried out using 2, 6 Dichloroindophenol Titrimetric method. 5g of sample was weighed and extracted by homogenizing the sample in metaphosphoric acid /acetic acid solution. Then standard solution (1mg/ml) of L-ascorbic was prepared in H<sub>2</sub>O. The sample was filtered and sample extract was diluted appropriately to a standard concentration of 10-100 mg ascorbic acid/100ml. The filtrate and replicates each of standard and sample was titrated with dichloroindophenol solution to a pink endpoint lasting at least 10 sec. The mass of ascorbic acid equivalent to the dye was calculated using the standard:

$$\text{mg ascorbic acid/g or ml sample} = C \times V \times (\text{DF/WT})$$

Where,

C= mg ascorbic acid/ml dye

V= ml dye used for titration of diluted sample

DF= dilution factor

WT= sample weight (g)

### Analysis of Anti-nutrients

The samples were analysed for total oxalate [17], phytate [18] and hydrocyanic acid [19].

### Preparation of Crude Extracts

50 g of the powder was soaked in 250 ml of distilled water for 24 h. The extract was filtered through a sieve to remove debris. The filtrate was then filtered again using Whatman No 1 filter paper. The final filtrate was then evaporated in a water bath at 40°C to get the crude extract [20]. All the extracts were preserved in airtight bottles until further use.

### Phytochemical Screening

The extracts were subjected to preliminary phytochemical screening for the presence of bioactive constituents [21].

## Qualitative phytochemical analysis

### Test for Flavonoids

#### Alkaline Reagent Test

The extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour which became colourless on addition of dilute acid indicated the presence of flavonoids.

### Test for Saponin

#### Froth Test

The extract was diluted with distilled water and shaken in a graduated cylinder for 15 min. The formation of layer of foam indicated the presence saponin.

### Test for Reducing Sugars

#### Fehling's test

To 1 ml of the extract, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added in a test tube and heated on a water bath for 10 min. Formation of red precipitate indicated the presence of reducing sugar.

### Tests for Alkaloids

To the extract, dilute hydrochloric acid was added, shaken well and filtered. With the filtrate, the following tests were performed.

#### Mayer's reagent test

To 3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of creamy precipitate indicated the presence of alkaloids.

#### Wagner's test

To 2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate indicated the presence of alkaloids.

### Test for Cardiac glycosides

#### Keller-Killiani test

To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Carefully 0.5 ml of concentrated sulphuric acid was added by the sides of the test tube. Formation of blue color in the acetic acid layer indicated the presence of cardiac glycosides.

### Tests for Tannin

#### Ferric chloride test

A small amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet colour indicated the presence of tannin.

### Test for Phenolic Compounds

A small amount of extract was dissolved in distilled water. To this solution 2 ml of 10% folin reagent was added. Then 2 ml of saturated sodium carbonate was added. Formation of blue, blue black colour indicated presence of phenol.

### Test for Steroids

The sample was extracted with chloroform and the extract treated with few drops of acetic anhydride. The treated extract was boiled and cooled. Then, conc. sulphuric acid was added. Formation of a bluish green colour solution confirmed the presence of phytosterols.

### Test for Anthraquinone

The sample was extracted with ether and aqueous ammonia was added to the filtrate. Red, pink or violet colour produced indicated the presence of anthraquinone.

### Test for Terpenoids

The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layer turns red, steroid is present. Presence of golden yellow layer at the bottom indicated the presence of terpenoids.

### Quantitative phytochemical analysis

The amounts of phytochemicals in the sample were further determined using standard procedures [22, 23].

### Preparation of sample for GC-MS analysis

2 g of the powdered sample was weighed into 250 ml conical flask and 10 ml of n-hexane was added to sonicate for 2 h. 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 stationary 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°C (isothermal for 2 min) with an increase of 10°C/min to the final temperature of 240°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°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

The results of the proximate and vitamin C analyses of the leaves of *C. thalictroides* are presented in Table-1. The results indicated that the vegetable is a

good source of protein, carbohydrate and crude fibre. However, the contents of crude fat, vitamin C and energy values were low. The mineral analysis of the investigated vegetable revealed that the vegetable contained different quantities of essential minerals as shown in Table-2. The results of the anti-nutrient analysis of the leaves of *C. thalictroides* are presented in Table-3. The results revealed very low levels of all the anti-nutrients in the vegetable. Phytochemical screening revealed the presence of various secondary metabolites in various quantities such as alkaloids (0.02 mg/g ATE), tannins (4.29 mg/g TAE), total phenols (14.51 mg/g GAE), saponins (0.01 mg/g) and flavonoids (6.23 mg/g QE) (Tables 4 and 5). Terpenoids, steroids, cyanogenic glycosides, cardiac glycosides, anthraquinones and phlobatanins were however absent. Gas Chromatography – Mass Spectrometry (GC-MS)

analysis of n-hexane extract of the leaves of *C. thalictroides* revealed the presence of twelve bioactive compounds as shown in Table 6. The GC – MS profile is shown in Fig. 1. Among these bioactive compounds, Benzene, (2-nitroethyl)- (RT: 6.096; 40.75%), gamma.-Sistosterol (RT: 23.154; 21.87%), n-Hexadecanoic acid (RT: 13.973; 5.96%) and Linalool (RT: 3.349; 4.04%) were the most abundant in occurrence (Figs. 2-5) while Hexadecanoic acid, methyl ester (RT: 13.463; 2.16%), cis-13-Octadecenoic acid (RT:15.644; 2.10%), 8-Octadecenoic acid, methyl ester (RT: 15.163; 1.79%), Phytol (RT: 15.287; 1.00%), Methyl stearate (RT: 15.406; 1.04%), Oleic acid (RT:15.682; 1.79%), Ergost-5-en-3-ol, (3.beta.)- (RT: 17.940; 0.12%) and Cyclooctadecane, ethyl- (RT: 19.568; 0.76%) were the least.

**Table-1: Proximate composition, vitamin C content and energy value of leaves of *C. thalictroides***

Moisture (%)	Fat (%)	Ash (%)	Protein (%)	Crude fibre (%)	Carbohydrate (%)	Vitamin C (mg/100g)	Energy (kcal/100g)
13.39 ±0.07	3.21 ±0.01	10.18 ±0.04	13.36 ±0.16	5.54 ±0.12	54.34 ±0.14	6.09 ±0.04	299.69

\*Results are expressed as means of three replicates ± standard deviation

**Table-2: Mineral composition (mg/100g) of leaves of *C. thalictroides***

Mineral elements	Contents
Sodium	85.20 ± 0.40
Potassium	1341.30 ± 0.80
Phosphorus	215.00 ± 3.00
Magnesium	107.00 ± 1.00
Calcium	434.00 ± 4.00
Manganese	6.44 ± 0.06
Iron	58.40 ± 0.40
Zinc	2.76 ± 0.06
Copper	1.19 ± 0.01

\*Results are expressed as means of three replicates ± standard deviation

**Table-3: Anti-nutrient composition (mg/100g) of the leaves of *C. thalictroides***

Anti-nutrient	Composition
Oxalate	0.41 ± 0.01
Phytate	0.36 ± 0.00
Cyanide	0.15 ± 0.01

\*Results are expressed as means of three replicates ± standard deviation

**Table-4: Qualitative phytochemical composition of leaves of *C. thalictroides***

Phytochemical	Occurrence
Alkaloids	+
Tannins	+
Phenols	+
Saponins	+
Flavonoids	+
Terpenoids	-
Steroids	-
Cardiac glycosides	-
Cyanogenic glycosides	-
Anthraquinones	-
Phlobatanins	-

(+): present, (-): not detected

**Table-5: Quantitative phytochemical composition of leaves of *C. thalictroides***

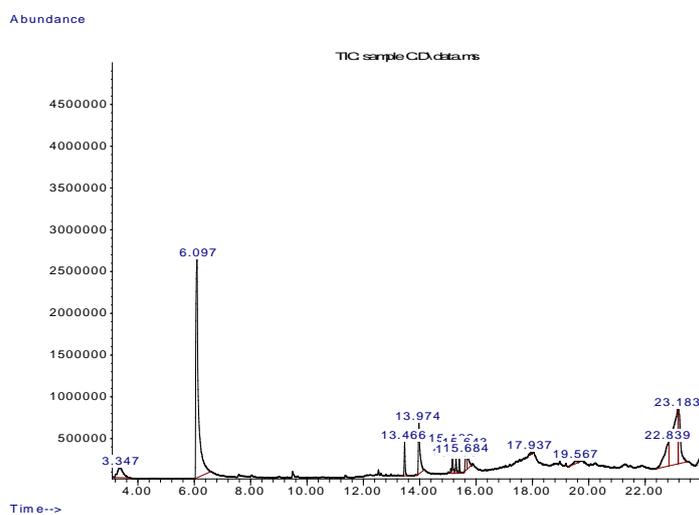
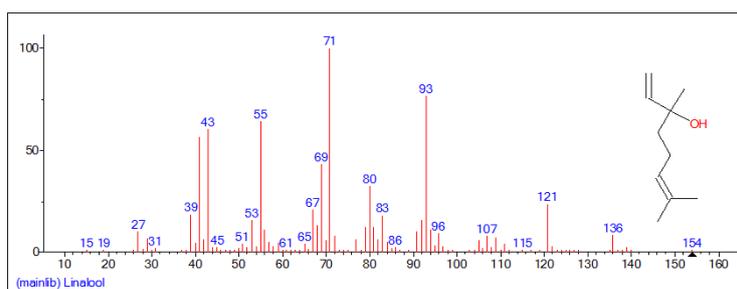
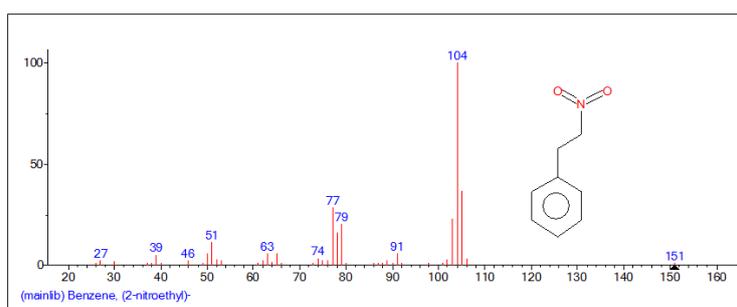
Phytochemical	Composition	±S.D.
Alkaloids	0.02 mg/g ATE	0.00
Tannins	4.29 mg/g TAE	0.09
Total Phenols	14.51 mg/g GAE	0.09
Saponins	0.01 mg/g	0.00
Flavonoids	6.23 mg/g QE	0.03

\*Results are expressed as means of three replicates ± standard deviation

**Table-6: Bioactive compounds identified in n-hexane leaf extract of *C. thalictroides***

S/no	RT (min)	Name of compound	PA (%)
1	3.349	Linalool	4.04
2	6.096	Benzene, (2-nitroethyl)-	40.75
3	13.463	Hexadecanoic acid, methyl ester	2.16
4	13.973	n-Hexadecanoic acid	5.96
5	15.163	8-Octadecenoic acid, methyl ester	1.79
6	15.287	Phytol	1.00
7	15.406	Methyl stearate	1.04
8	15.644	cis-13-Octadecenoic acid	2.10
9	15.682	Oleic acid	1.79
10	17.940	Ergost-5-en-3-ol, (3.beta.)-	0.12
11	19.568	Cyclooctadecane, ethyl-	0.76
12	23.154	.gamma.-Sitosterol	21.87

RT = Retention time; PA = Peak area

**Fig-1: GC-MS profile of n-hexane leaf extract of *Ceratopteris thalictroides*****Fig-2: GC-MS spectra of Linalool (RT: 3.349; 4.04%) from *C. thalictroides* leaf extract****Fig-3: GC-MS spectra of Benzene, (2-nitroethyl)- (RT: 6.096; 40.75%) from *C. thalictroides* leaf extract**

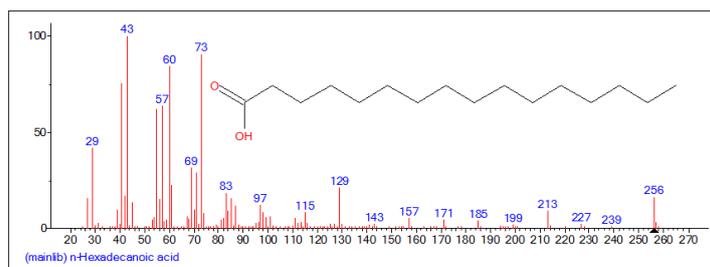


Fig-4: GC-MS spectra of n-Hexadecanoic (RT: 13.973; 5.96%) acid from *C. thalictroides* leaf extract

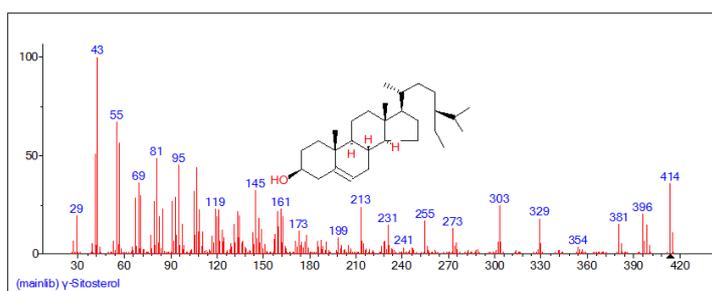


Fig-5: GC-MS spectra of gamma-Sitosterol (RT: 23.154; 21.87%) from *C. thalictroides* leaf extract

## DISCUSSION

The result of proximate analysis indicated that the studied plant is a good source of protein, carbohydrate and crude fiber. The crude protein content of *C. thalictroides* is higher than protein content of *Momordica foecide* (4.6%) leaves consumed in Nigeria and Swaziland [24, 25] but lower than those of *Brassica oleracea* (24.32%) [26]. *C. thalictroides* meets the requirement of providing more than 12% of its calorific value from protein and thus can be considered a good source of protein [27]. The carbohydrate content of *C. thalictroides* is higher than values reported for some leafy vegetables commonly consumed in Lagos, Nigeria [28] but comparable to 52.59, 55.42, 51.04 and 57.41% reported for *Solanum macrocarpon*, *Ocimum gratissimum*, *Solanum nigrum* and *Cnidioscolus aconitifolius* respectively [26]. The leaves of *C. thalictroides* are poor sources of lipids. The observed crude fat content is low compared to reported values (9.05, 11.04 and 14.02%) in some vegetables consumed in Nigeria [29] but compares favourably to some as reported by the same authors. It has been reported that vegetables are generally deficient in fats hence they are good for health. The low energy value observed in the present study is in accord with the general assertion that vegetables have low energy levels and this is due to their low fat contents [30]. The observed crude fibre content in the present study is low compared to reported ranges (6.6-11.24%, 4.69-19.45% and 4.02-12.08%) in some leafy vegetables consumed in Nigeria [26, 28, 29]. Dietary fibre is known to provide bulking thereby easing defecation [31]. Also, it hinders the absorption of cholesterol in the gastrointestinal tract thus helping in the therapy of hypercholesterolemia [32]. The ash indicates the mineral contents of the leaves. Thus high ash content in *C. thalictroides* as compared to reported values [26,

28, 29] in some vegetables showed high level of minerals in the vegetable.

The importance of minerals in human diet cannot be overemphasized given their roles in metabolic reactions, rigid bone formation, osmoregulation among others [33]. Results of the present revealed that minerals are well represented in the vegetable. This is in agreement with reports of previous researchers that vegetables are good sources of minerals [34, 35]. Sodium and potassium are important intracellular and extracellular cations respectively. Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction [36]. The Na/K ratio of the vegetable is less than 1 and this can help to lower blood pressure [37]. Phosphorus is important in the energy transfer of nucleic acids while calcium helps in the regulation of muscle contractions, transmission of nerve impulse and in bone formation. The Ca/P ratio of the studied vegetable is greater than 1. This is an advantage when the fern is consumed because a diet is considered balanced if the Ca/P ratio is greater than 1 and unbalanced if it is less than 0.5 [38]. Magnesium is an obligate co-factor of DNA synthesis and the proportion supplied by vegetables can be used to supplement low magnesium based staple food in Nigeria. Iron is important in human diet to prevent anaemia and other related diseases.

The content of vitamin C obtained in the present study is lower than values reported for some leafy vegetables in Nigeria [39, 40]. Ascorbic acid is an anti-oxidant which helps to protect the body against cancer and other degenerative diseases such as arthritis and Type II Diabetes mellitus [41].

The compositions of anti-nutrients in many plants have limited their use for food because the anti-nutrients may cause dangerous effects on both man and animals [42]. The levels of oxalate and phytate in the investigated vegetable are much lower than those reported for cereals, beans, nuts, buckwheat, beetroot greens, rhubarb and Purslane leaves [43-46]. Phytate has strong binding affinity for essential minerals such as zinc, iron and calcium which leads to the formation of insoluble precipitates which are far less absorbable in the intestines thus reducing their bioavailability [47, 48]. Oxalate can however combine with divalent metallic cations to form crystals of the corresponding oxalates which may then be excreted in urine. The oxalate crystals may form larger kidney stones that can obstruct kidney tubules and may lead to kidney diseases [49]. The cyanide level in the vegetable was found to be much lower than levels that can cause acute and chronic toxicity [50].

The medicinal values of *C. thalictroides* may be due to the presence of chemical compounds such as alkaloids, tannins, total phenols, saponins and flavonoids in the plant as revealed in the present study. Alkaloids and flavonoids are the sources of antimicrobial activities; tannins have the potential values as cytotoxic agents [51] while phenolic compounds are source for antimicrobial and insecticidal activities. Saponins have also been implicated as bioactive antibacterial agents [52]. The presence of these compounds suggests that the plant may be useful as antimicrobial and anti-cancer agents.

Previous researchers have characterized several bioactive compounds in different medicinal plants using GC – MS analysis. Five major compounds were characterized through GC – MS analysis in ethanol extract of *Polygonum chinese* [53]. Also, eighteen bioactive constituents were identified from the ethanolic extract of the leaves of *Desmodium gyran* using GC–MS [54]. The presence of ten bioactive compounds in ethanolic leaf extract *Phyllodium pulchellum* using GC–MS was also reported [55]. The identified major compounds in the present study possess some important biological potential which may be useful in the future for drug development. It has been reported that these bioactive compounds play crucial roles in disease management and general human metabolism. For instance, it was reported that  $\gamma$ -Sistosterol exerted potential anticancer activity through the growth inhibition, cell cycle arrest and the apoptosis on cancer cells [56]. It has also been reported that  $\gamma$ -Sistosterol reduced hyperglycemia in STZ-induced diabetic rats due to increased insulin secretion and inhibition of gluconeogenesis, thus, it can be used in the treatment of *Diabetes mellitus* [57]. Also, n-Hexadecanoic acid functions as an anti-inflammatory agent [58], anti-oxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic flavour [59] and a potent mosquito larvicide [60]. Linalool, a much sought

after compound in the flavour and fragrance industry was also identified in the present study. It is known to exhibit biological activities such as antimicrobial, anti-inflammatory, anti-cancer and anti-oxidant properties and many *in vivo* studies have confirmed various effects of linalool on the central nervous system [61]. A detailed search through literature suggests that the biological activity of Benzene, (2-nitroethyl)- which was the most abundant compound in the plant being investigated was either not known or documented. This may be due to the fact that the compound has not been identified in large quantities in many plants [62]. Hence, it is recommended that the biological activity of this compound should be investigated. The compound was however identified as the most abundant component (32.55%) of the flower scent of the Japanese loquat *Eriobotrya japonica* which is an evergreen fruit tree that blooms during winter [62]. Though, it is generally thought that compound(s) with nitro as the functional group(s) are uncommon as natural products, the compound, Benzene, (2-nitroethyl)- which has a powerful sweet floral, warm spicy odour reminiscent of cinnamon has been reported to be widely distributed as a constituent of many flowers such as many species of orchids [63], fruits of tomato [64] and the Nigerian spicy medicinal plant, *Dennetia tripetala* [65]. The identification of Benzene, (2-nitroethyl)- (40.75%) in the present study is an example of a major distribution of nitro-derivatives as the natural products throughout the animal [66, 67] and plant kingdoms [68, 69] apart from nitrophenanthrene derivatives in plants and Lepidoptera [70]. The biological activities of other minor compounds identified in the present study have also been reported. For instance, Hexadecanoic acid, methyl ester has been reported to have anti-oxidant, anti-inflammatory and antimicrobial properties [71]. The therapeutic uses of cis-13-Octadecenoic acid in medicine, surgery have been documented [72]. Methyl Stearate is used as a solvent and lipid carrier in agriculture [73] while oleic acid has been reported to possess antibacterial potential [74].

## CONCLUSION

The present study has shown that the vegetable examined is rich in protein and essential minerals but low in fat and energy values. The anti-nutrients in the vegetable are much lower than the safe limits. The results suggest that if consumed in sufficient amount, it would contribute significantly towards meeting the human nutritional requirement for normal growth. Also, the present study revealed the occurrence of twelve bioactive compounds in the n-hexane leaf extract of *Ceratopteris thalictroides* using GC-MS technique. These bioactive compounds have various documented biological activities which may be responsible for the usage of the plant in traditional medicine. The identification of Benzene, (2-nitroethyl)- in the present study is an example of a major distribution of nitro-derivatives as natural products throughout the

animal and plant kingdoms other than nitrophenanthrene derivatives in plant and Lepidoptera.

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