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Proximate and Phytochemical Profile of *Melanthera biflora* Leaves

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The proximate and phytochemical composition of *Melanthera biflora* was investigated, using standard methods. From the obtained results the leaves had high moisture contents (71.1± 0.2%) and crude fiber (3.91 ± 0.5) while containing moderate protein (7.0 ± 0.03%), while containing lipid (1.10 ± 4%), ash (2.8 ± 0.2%), total carbohydrate (6.09 ± 0.2%) and caloric value (62.26±0.14 kcal/100g). Eleven Phytochemical families were detected with tannin as the most abundant (27.82%) consisting 100% tannic acid. Thirteen alkaloids (13.65%) were detected consisting mainly of morphine (28.05%), methylmorphine (16.22%), dephnoline (12.02%) biflorin, (20.63%), aromoline (12.61%) homoaromaline (7.79%). Twenty-three flavonoid (5.71%) chief among which were quercetin (44.21%), kaemferol (28.94%), dandzein (7.20%), letuolin (10.17%), salvagenin (6.76%), sinensetin 8.20% were detected. The most prominent of the ten carotenoids (2.48%), is lutein (40.76%), followed by carotene (17.90%), malvidin 5.63%, zeaxanthin (16.5%), viola-xanthin (9.5%). Sixty one terpenoid including linalool (40.98%), germacrene (12.74%), Alpha-terpineal 6.40%,

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terpinen – 4-01 (5.62%), and Gamma terpine were found in the leaves Of the six phenolic acids (16.26%) the highest was ferulic acid (53.94%), followed closely by vanilic acid (45.8%). Seven phytosterol (2.25%), consisting of sitosterol (65.3%), savenasterol (14.19%) stigmasterol (12.70%), and others were detected. The leaves had very a low hydroxycinnamic acid content ($8.93x10^{-4}$%) content, consisting of eight known compounds of which caffeic acid (71.93%) and p-coumaric acid (27.91%) were the most abundant. They also had very a low allicins ($1.94x10^{-4}$%) content, consisting of daillylthiosulphunate (97.05%), and methyl thiosulphinate (2.6%) and allylthiosulphin and allylthiosulphinate (0.3%). The leaves had a very low content of glycosides consisting of eight known compounds of which quabain (78.54%) were detected and they include gitogenin (22.04%), diosgenin (20.02), neohegen (20.79%). Their rich contents of nutrients and many bioactive molecules suggest strong nutraceutical potential of these leaves, further suggesting their likely use as functional a food and therapeutic uses in the management and prevention of diseases.

Keywords: Proximate; phytochemical; vegetable.

1. INTRODUCTION

The importance and awareness of nutrition as a prerequisite for good health and longetivity has undoubtedly lead to the increase quest for knowledge about the nutritional content of food. Green leafy vegetables occupy an important place among the food crops as they provide adequate amount of vitamins and minerals for human consumption. In addition to their nutritional value, vegetables also contain phytochemicals which exhibit some protective and disease preventive effects, thus, making them serve a dual function against a number of biochemical, physiological and metabolic disorder, [1]. Green leafy vegetables constitute an indispensable constitute of human diet in Africa generally and West Africa in particular [2]. Low consumption of green leafy vegetable in the diet is one of the major factor which leads to deficiency of water soluble vitamin and iron. Nigeria is blessed with a great natural tropical rainforest that is characterized by viable soil where vegetables of high nutritional value are grown. This is even more pronounced in Southeastern Nigeria. There are edible inexpensive leafy vegetables found in this zone (Southeastern Nigeria) whose chemical, nutritional and phytochemical potentials are yet to be adequately studied and utilized. Among this vegetables is “akuwa” (*Melanthera biflora*). *Melanthera biflora*, is a tropical leafy vegetable. *Melanthera biflora* is a perenial herbaceous plant which belongs to the family of Asterecae, its common name is beach daisy, it is known among the Igbos as “akwuwa” and “akwuba” among the Efiks in Cross Rivers State Nigeria. It produces a luxurious edible leaves which is used in making soup. The present study therefore is aimed at making a comprehensive characterization of *Melanthera biflora* leaves (i.e evaluating the qualitative and quantitative phytochemistry studies of the leaves) and nutritional value so as to encourage its consumption in other to enhance healthy living as well as bring to bear the potentiality of this herbs as a possible therapeutic herb that could be used in ameliorating diseases.

2. MATERIALS AND METHODS

2.1 Chemical Used

All the chemical and drugs used in this research were obtained commercially and were of analytical grade.

2.2 Sample Collection

The leaves of *Melanthera biflora* were harvested fresh from Ude plantation in Okon-Aku, in Ohafia Local Government Area of Abia State and was later identified by a taxonomist in the herbarium of the department of plant science, University of Port Harcourt. Dr. Edwin Nwosu.

2.3 Sample Preparation

The harvested vegetable leaves were destalked, washed with cold running water and divided into two. The first portion was used for proximate analysis while the other portion were dried in an oven at 60°C for 24 hours, after the drying, the leaves were ground into a fine powder using mortar and a pestle and sieved to pass through a 40 mesh sieve and stored in an airtight container under refrigerated temperature for further use.

2.4 Determination of Chemical Composition

The proximate analysis (carbohydrate, fats, protein, moisture and ash) of the leaves were determined by using AOAC methods [3].

Carbohydrate was determined by the difference method (100- (protein + fat + moisture + ash). The nitrogen value, which is the precursor for protein of a substance, was determined by micro-Kjeldah/method [4]. The Nitrogen value was converted to protein by multiplying to a factor of 6.25. The moisture and ash were determined using the weight difference method, while determination of crude lipid of the sample was done using soxhlet type and the direct solvent extraction method. Energy value was calculated using Atwater factor method [(9 x fat) + (4xcarbohydrate) + (4xprotein)] as described by Osborne and voogt [5] and [6]. All the proximate values were reported in percentage [7,8].

2.5 Determination of Phytochemicals Profile

2.5.1 Calibration, identification and quantification

Standard solutions were prepared in methanol for alkaloids, allicins, flavonoids and simple phenolics; acetone for carotenoids and lignins; methylene chloride phytosterols and simple terpenes; ethanol for hydroxycinnamic acids, glycosides and saponines. The linearity of the dependence of response on concentrations was ascertained by regression analysis. Identification was based on comparison of retention times and spectral data with standards. Quantification was performed by establishing calibration curves for each compound determined, using the standards.

2.5.2 Determination of alkaloid

The alkaloid extraction was carried out by following the modified method of Tram [9].

Three gram of the pulverized sample were extracted with 25 mL of re-distilled methanol for 6 hours at the room temperature (29±1°C), before filtration with No.44 filter paper.

The filtrate was concentrated in rotary evaporator, before drying of water using sodium sulphate, prior to gas chromatography.

2.5.3 Determination of flavoniods

The extraction was carried out according to the method of Millogo-Kone [10].

The dried extract of ethanolic and aqueous extraction, was sampled and made to be free of water by ensuring constant weight for a period of time in the laboratory. 1.00 g of the sample was weighed into the 250 ml conical flack capacity with addition of 100 ml of distilled water and boiled for 10 minutes. The flavoniods extract was obtained by pouring 100 ml of the boiling methanol;water (70:30) v. v into the materials. The mixture was allowed to macerate for about 6 hours and then concentrated to 5 ml for gas chromatography analysis.

2.5.4 Determination of hydroxycinnamic acid

The extraction was carried out by following the modified method of Romanian Biotechnological letters of Ortan [11]. The sample was pulverized and the hydroxycinnamic acids constituents extracted with methanol. The acids were removed with 10 ml of the solvent for 15 minute. The mixture was filtered and concentrated to 1 ml in the vial for gas chromatography analysis and 1 µL was injected into the injection port of GC.

2.5.5 Determination of glycoside

The extraction was carried out by following the method of Olawaniyi and Ibiyemi [12], with slight modification.

Ten grams of the pulverized sample was extracted by soaking for 2 hours with 10 ml of 70% ethanol and then filtered and concentrated. The redistilled hexane was used to replace the initial solvent and the hexane was concentrated to 1 ml in the vial for chromatography analysis.

2.5.6 Determination of phenolic acid

Phenolic extraction: The extraction was carried out by the modification of Andary [13]. Two stages extraction procedures were followed for the effective removal of the phenolic compounds as described below.

STAGE 1: Fifty milligram of the sample was extracted with 5 mL of 1M NaOH for 16 hours on a shaker at ambient temperature. After extraction, the sample was centrifuged (5000xg) rinsed with water, centrifuged again and the supernatants were combined and placed in a disposable glass tube and heated at 90°C for 2 hours to release the conjugated phenolic compounds. The heated extract was cooled, titrated with 4M HCl to P_H <2 and diluted to 10 mL, with deionized water and centrifuged to remove the precipitate. The supernatant was

saved for subsequent purification and the residue was extracted further in stage 2.

STAGE 2: The residue from stage I above was extracted with 5ml of 4m NaOH, heated to 160°C in Teflon. After cooling, the mixture was filtered, supernatant collected and the residue washed with water (deionised). The supernatants were combined and adjusted to $P_{H<}$2.0 with 4M HCl. The filtrates were combined for further purification.

Purification of extracted phenolic acids: In aliquot (5-15 ml) of the various supernatants was passed through a conditioned varian (varian Assoc, Habor city. CA) Bond PPL (3-ml size with 200mg, packing) solid phase extraction tube at 5.ml with attached to a visi prep (superlco, Belletonte, PA). The tubes were then placed under a vacuum (60 kpa) until the resin was thoroughly dried after which the PAS were eluted with 1 ml of ethyl acetate into gas chromatography auto-sampler vials the PPL tubes were conditioned by first passing 2 ml of ethyl acetate followed by 2 ml water (PH 2.0).

2.5.7 Determination of terpenes

Terpenes extraction: The extraction was carried out following the modified method of R0omanian Biotechnological letters, of Ortan, [11]. The sample was pulverized and the terpenes constituents extracted with redistilled chloroform. The terpenes were removed with 10 ml of the solvent for 15 minutes. The Mixture was filtered and concentrated to 1ml in the vial for gas chromatography analysis and 1m1 was injected into the injection pot of GC.

2.5.8 Determination of tannin

The extraction was carried out by following the modified method of Luthar [14].

Five grams of the powered sample were extracted with 125 mL of redistilled methanol for 20 min at room temperature (29 ±1°C). The resultant extract was concentrated for gas chromatography analysis. The standard mixture was prepared from 1000 mg/L stock solution of tannic acid.

2.5.9 Determinations of saponin

Saponin Extraction: The extraction was carried out by the following the modified of analytical sciences according to the method of Guo [15].

Procedure: The sample was pulverized and the saponin was extracted three times with redistilled methanol. The saponins were removed with 20 ml of the solvent for 20 minutes with the aid of the sonication. The combined extracts were concentrated to syrup under reduced pressure, and then suspended in water. The suspension was extracted with petroleum ether, chloroform and 1-butanol saturated with water, successively, to give the respective extract after the removal of the solvent. The combined extract was filtered and concentrated to 1 ml in the vial for gas chromatography analysis and 1ml was injected into the injection pot of GC.

2.5.10 Determination of allicin content

The allicin extraction was carried out by following the modified method of Chahregani [16].

Five grams of the fresh samples were washed, mined and added to adequate amount of water to a concentration of 12.5% w/v respectively. The sample was grinded with a blender. The product were squeezed through gauze cloth to remove the larger particles and the extracts were passed through a 9.02 am filter (Millipore).

The process was carried out at room temperature and the filtrate was sterilized and stored at 4°C before analysis.

The extract was re-extracted with petroleum ether and later concentrated by pouring the petroleum ether extract into the round bottom flask of the rotator evaporator arrangement. It was concentrated by evaporation. Then the concentrated extract was dried of water by using anhydrous sodium sulphate before gas chromatography.

2.5.11 Determination of carotenoids

Carotenoids Extraction: The carotenoids extraction was carried out by following the modified method of Takagi [17]. The pulverized sample (5.0 g) was homogenized in 75 mL acetone and kept at room temperature for 1 hour in the dark. The homogenate was filtered through filter paper by suction. Extraction was repeated three times with the same volume of acetone. The extracts were combined and evaporated under reduced pressure and the residue was re-extracted by a mixture of diethyl ether and petroleum ether in equal ratio. The extract was poured into the round bottom flask of the rotator evaporator arrangement. It was concentrated by

evaporation. Then the concentrated extract was dried of water by using the anhydrous sodium sulphate before gas chromatography analysis.

2.5.12 Determination of phytosterols

The oil were extracted according to AOAC method 999.02 [3], while the sterols were analyzed according to the modified AOAC method 994.10 and AOAC 970.51 official methods.

Extraction of Oil: After preparation and installation of the extraction cell and collection vessel in the SFE instrument, 3.0 g of the pulverized sample was placed in the extraction cell and extracted for 60 mins with CO_2 and 15% ethanol, under the following conditions; 51.7 kpa, 100°C, nominal fluid flow rate, 2.1 g/min; and restrictor temperature such that CO_2 evolve at temperature between 80-100°C.

Analysis of Sterols: The aliquot of the extracted oil was added to the screw-capped test tubes. The sample was saponified at 95°C for 30 minutes, by using of 3 ml of 10% KOH in ethanol, to which 0.20 ml of benzene had been added to ensue miscibility. Deionised water (3 mL) and 2 ml of hexane was used in extracting the non-saponifiable materials (sterol, etc). Three extractions each with 2 ml of hexane, were carried out for 1 hour, 30 minutes and 30 minutes respectively, to achieve complete extraction of the sterols. The hexane was concentrated to 1 ml in the vial for gas chromatography analysis and 1 µL was injected into the pot of gas chromatogram.

Chromatographic conditions: The gas chromatograph was an HP 6890 (Hewlett Packard, wellington, DE, USA), GC apparatus, fitted with flame ionization detector (FID), powered with HP Chemstation Rev. A 09.01(1206) software, to identify and quantify compounds. The column was a capillary DB-5MS (30 mx 0.25 mm x 0.25 µm thickness). The inlet and detection temperatures were 250°C and 320°C. Split injection was adopted with a split ratio of 20:1. The Carrier gas was nitrogen gas .The compressed air and hydrogen pressures were 38 psi and 28 psi. The oven programmed was; initial temperature at 60°C for 5 mins. First ramping at 10°C/min for 20 min was followed by a second ramping at 15°C /min for 4 min.

3. RESULTS

Table 1. The proximate composition of *Melanthera biflora* leaves

Constituent	Composition (%)
Protein (g)	7.00±12
Lipid (g)	1.10±0.16
Crude fibers (g)	3.91±0.01
Ash (g)	2.80±0.14
Moisture (g)	71.10±0.03
Total carbohydrate (g)	6.09±0.12
Total caloric content (kcal)	62.26±0.14

Results are means ±S.D of triplicate determination

Phytochemical profile of *Melanthera biflora* leaves.

Table 2. Alkaloid composition of *Melanthera biflora* leaves

Compounds	Amounts (x 10^{-3}) (mg/100g)	% Composition
Morphine	17882	28.1
Methyl morphine	10340	16.2
Papaverine	47.40	0.1
Biflorin	13154	20.6
Narcotine	7.699	0.0
Daphnoline	7664	12.0
Aromoline	8056	12.6
Homoaromoline	4914	7.7
Ambelline	2.309	0.0
6-Hydroxybuphanidine	0.981	0.0
Monocrotalline	9.025	0.0
6-Hydroxy powelline	2.012	0.0
Nitidine	1666	2.6
Total	63751	

Table 3. Flavonoid composition of *Melanthera biflora* leaves

Compounds	Amount $X10^{-4}$ (mg/100g)	% Composition
Catechin	0.033	1.2×10^{-5}
Resveratrol	1.107	4.2×10^{-4}
Apigenin	1880	0.7
Daidzein	19210	7.2
Butein	2.443	9.2×10^{-4}
Naringenin	6.454	2.4×10^{-3}
Biochanin	2.65	9.9×10^{-4}
Luteolin	27110	10.2
Kaempferol	77190	28.9
(-) – Epicatechin	7.979	3.0×10^{-3}
Salvagenin	18040	6.8
(-) – Epicatechin-3-gallete	5.90	2.2×10^{-3}
Gallocatechin	3.052	1.1×10^{-3}
Quercetin	117920	44.2
Isorhamnetin	36.14	1.4×10^{-3}
Myricetin	5.077	1.9×10^{-3}
Sinensatin	21860	8.2
Kaemferol-3-arabinoside	1.842	0.7
Naringerin	2.841	1.1×10^{-3}
Quercitrin	830.6	0.3
Isoquercetin	415.1	0.2
Orientin	0.409	1.5×10^{-4}
Isoorientin	278.5	0.1
Total	266700	

Table 4. The Tannic acid composition and *Melanthera biflora* leaves

Compound	Amount (mg/100g)	% Composition
Tannic acid	129.8803	100

Table 5. The glycosides composition of *Melanthera biflora* leaves

Compound	Amount (mg/100g) ($X10^{-6}$)	% Composition
Kampferol-3-O-rhamnoside	1.490	0.3
Arbutin	6.848	1.2
Salicin	10.64	1.9
Amygdalin	71.85	12.9
Quabain	435.910	78.5
Digitoxin	3.986	0.7
Vitexicarpin	19.962	3.6
Digoxin	0.625	0.4
Costrugenin	3.952	0.7
Total	5.5499	

Table 6. The phytosterol composition of *Melathera biflora* leaves

Compound	Retention time (min)	Amount (mg/100g) ($X10^{-5}$)	% Composition
Cholesterol	19.488	0.0035	0.0
Cholestenol	20.521	6.834	0.6
Ergosterol	21.393	6.877	0.7
Camfesterol	21.954	84190	7.9
Stigmasterol	23.221	134700	12.7
S-Avenasterol	24.018	149900	14.1
Sitosterol	25.260	693200	63.3
Total	-	1062000	-

Table 7. Allicins composition of *Melanthera biflora* leaves

Compound	Amount (mg/100*g*) ($X10^{-6}$)	% Composition
Diallyl thiosulphinate	8.765	97.1
Methyl allyl thiosulphinate	0.234	2.6
Allyl methyl thiosulphinate	0.031	0.3
Total	9.031	

Table 8. The carotenoid composition of *Melanthera biflora* leaves

Compounds	Amount ($X10^{-3}$) (mg/100g)	% Composition
Carotene	2080	18.0
Lycopene	1.060	0.1
Beta-cryptanxanthin	343.9	3.0
Lutein	4718	40.8
Zeaxanthin	1910	16.5
Anthera-xanthin	3.416	0.0
Asta-xanthin	4.549	0.0
Viola-xanthin	1082	9.3
Neo-xanthin	330.7	2.9
Total	11,576	

Table 9. The saponin composition *Melathera biflora* leaves

Compounds	Amount (mg/100mg) ($X10^{1}$)	% Composition
Gitogenin	2.578	22.0
Solagenin	0.003	0.2
Diosgenin	2.339	20.0
Tigogenin	0.00149	0.0
Neohecogenin	2.429	20.8
Hecogenin	1.764	15.1
Sapogenin	1.659	12.2
Euphol	0.055	0.5
Saponine	0.857	7.3
Total	11.68	

Table 10. Hydroxycinnamic acid composition of *Melanthera biflora* leaves

Compounds	Amount (mg/100g) ($X10^{-4}$)	% Composition
Cinnamic acid	3.278	0.1
Coumarin	0.692	0.0
p-Coum aric acid	11.6	27.9
o-Coumaric acid	2.314	0.1
Caffeic acid	2999	71.9
Sinapinic acid	0.0856	0.0
Chlorogenic acid	0.1937	0.0
Cichoric acid	0.1735	0.0
Total	0.417	

Table 11. The Benzoic acid derivatives composition of *Melanthera biflora leaves*

Compound	Amount (mg/100g) ($X10^{-4}$)	% Composition (10)
Vanillic acid	3480	45.9
Ferullic acid	4093	63.9
Syringic acid	1.713	$20.2x10^{-4}$
Piperic acid	4.410	50.8×10^{-5}
Ellagic acid	8.444	1.1×10^{-4}
Rosmarinic acid	2.258	2.3
Total	7.590	

Table 12. Terpenes composition of *Melathera biflora* leaves

Compounds	Amount	% Composition
Butanol	0.083	0.0
2-Hydroxy-3-butanone	0.366	0.5
Butanoic acid	0.116	0.1
Sabinene	0.117	0.1
2-Methylbutenoic acid	0.095	0.1
2-Methylbutanoic acid	0.271	0.3
2- Methylbutenoic acid ethyl ester	0.290	0.3
Azulene	0.299	0.3
2-methylbutanoic acid ethy ester	0.210	0.2
Alpha pinene	1.688	1.7
Beta pinene	1.788	1.8
Benzyl alcohol	0.593	0.6
Cis ocimene	3.756	3.8
Myrane	0.209	0.2
Allo ocimene	0.246	0.2
Pinene-2-ol	0.000	0.0
Alpha thujene	0.645	0.6
Gama terpinene	4.198	4.2
2,6-O-dimethyl1-5 heptanel	0.310	0.3
Citral	0.366	0.4
Camphor	0.201	0.2
Neral	0.519	0.5
Geranial	0.405	0.4
Iboartemisia	0.245	0.2
1,8-Cineole	0.592	0.6
Borneol	0.500	0.5
Linalool	40.984	41.0
Citronellal	0.196	0.2
Nerol	0.196	0.2
Alpha terpineol	6.395	6.4
Terpinen-4-ol	5.620	5.6
Citronellol	0.359	0.4
Ascaridole	0.468	0.5
Linalyl acetate	0.449	0.5
Alpha terpinenyl acetate	0.310	0.3
Ethyl cinnamate	0.583	0.6
Borneol acetate	0.733	0.7
Neryl acetate	0.2098	0.2
Geranyl acetate	0.311	0.3
Beta bisabolene	0.661	0.7
Germacrene D	12.735	12.7
Gama cadinene	1.690	1.7
Beta caryophyllene	0.968	1.0
Cyprene	0.143	0.1
Beta elemene	0.143	0.1
[6]-Shogaol	0.565	0.6
Alpha gurgunene	0.469	0.5
Alpha copane	0.211	0.2
Beta selinene	0.209	0.2
Itumulene	0.396	0.4
Vacencene	0.310	0.3
Caryophyllene oxide	3.856	3.9
Alpha selinene	0.491	0.5
[6]-Paradol	0.084	0.1

Compounds	Amount	% Composition
Beta selinene	0.248	0.2
Aromadendrene	0.370	0.4
Gama muurolene	0.314	0.3
Aristolone	0.310	0.3
Viridiflorol	0.304	0.3
Taraxeron	0.325	0.3
Lupeol	0.319	0.3
Total	100	

Table 13. Percentage composition of group phytochemicals in *Melanthera biflora*

Phytochemicals	Amount (mg/100g)	% Composition
Alkaloids	63.75	13.7
Flavonoids	26.67	5.7
Tannic acid	129.9	27.8
Glycosides	5.55×10^{-4}	0.0
Terpenoids	100.0	21.4
Phytosterols	10.62	2.3
Allicins	9.031×10^{-6}	2.0×10^{-6}
Carotenoids	11.58	2.5
Saponins	116.8	2.5
Hydroxycinnamic acids	4.170×10^{-4}	89.3×10^{-4}
Phenolic acids	7.590	16.3
Total	466.898	

End Note: Percentages are based on the weight of the compounds per the total extract of its family

4. DISCUSSION

The moisture content of *Melanthera biflora* was higher than that of *Talinum triangulare* and *Telferia occidentalis* [18], but less than *Pennisetum purpureum* [19]. The moisture content of any food is an index of its water activity [20] and it is used as a measure of stability and susceptibility to microbial contamination [9,21]. The higher moisture content provides for greater activity of water soluble enzymes and coenzymes needed for metabolic activities of leafy vegetables. The implication of this is that, the leaf will have higher shelf life than *Pennisetum purpureum*, but a lower one than *Talinum triangulare* and *Telferia occidentalis* and will aid in enzymatic activities in the body better than *Pennisetum purpureum*. This suggests that the leaves will not be stored for a long time as higher water content enhances microbial action.

The crude protein of *Melanthera biflora* was greater than that of *Pennisetum purpureum* [19], *Amarantus hybridus*, *T. occidentalis* and *T. triangulare* [18] Kalu et al. [22] had earlier reported that the leaf was rich in essential amino acids. These amino acids serve as an alternative source of energy when carbohydrate availability in the body is impaired when consumed. A 100 g of this sample when supplemented with other vegetables can meet the daily protein requirement of 23-56 g (FAO/WHO/UNU, [23,24]. Regular uses of plant food rich in protein make an invaluable addition to a diet (Wardlaw, [25]). The ash content of *Melanthera biflora* was greater than that reported for *T. occidentalis*, *T. triangulare* [18] and *P. purpureum* [19], but less than *A. hybridus* [18]. The ash composition of a food is the amount of minerals substances left after the carbon material must have been burnt off [26]. The leaves of *Melanthera biflora* is rich in soluble mineral as reported by Kalu et al. [22].

Melanthera biflora leaves contain comparable lipid content to *P. purpureum* [19] and *A hybridus* [18], but greater one than *T. occidentalis, T. Triangulare*, [18]) and *Sansevieria liberica* [27].

The total carbohydrate content of *Melanthera biflora* was less than those reported for *A. hybridus*, [18]) and *P. tuberregium* sclerotia [27], but more than *P. purpureum* [19]. A 100 g of the leaves can provide 6-10% of the recommended daily allowance for carbohydrate. *Melanthera biflora* contains higher fibre content than *A. hybridus*, *T. triangulare*, *T. occidentalis* [18] and *P. purpureum* [19].

Results from epidemiological studies reveal that increased fiber consumption may help in the reduction of certain diseases such as diabetes, coronary heart diseases, colon cancer, obesity, high blood pressure and various digestive disorders [28,29,30,31]. Dietary fiber has been associated with alternations of the colonic environment that protect against colorectal diseases. It provides protection by increasing faecal bulk, which dilates the increased colonic bile concentration that occurs with a high-fat diet [32]. This is one benefit derivable from the consumption of *Melanthera biflora*.

The total caloric content of *Melanthera biflora* was higher than *P*. *purpuerum* [19], but less than *P*. *tuberregium* sclerotia [33]. This result shows that *Melanthera biflora* is a good source of nutrient.

Phytochemical composition of the *Melanthera biflora* leaves as determined by gas chromatography: The phytochemical screening revealed that *Melanthera Biflora* is rich in tannic acid. Tannic acid is an antioxidant, hepatoprotective, hypocholesteromic and hypoglycemic agent [34] Tannin is used in the treatment of inflamed or ulcerated tissues. *Melanthera Biflora* is rich in alkaloid, prominent which is morphine used as an analgesic, local anaesthetic and anti-leishmanial agent [35], this shows that, the leaves can be used in curbing pain related symptoms. Flavonoid are of a particular importance in the human diet as there are evidence that they act as antioxidants, antiviral and anti-inflammatory agent. [36] and are associated with reduced risk of cancer and cardiovascular diseases [37]. Terpenes are used as flavor enhancers in food, fragrances in perfuming and in traditional and alternative medicines such as aromatherapy [38]. They have anticancer [39] Antimicrobial [40] and anti-oxidant [41] properties.

The leaves had low saponin, very low glycoside and moderate allicin content. Saponins are reported to have broad range of pharmcological properties [36]. Allicin is reported to have an anti-inflammatory, antimicrobial, anti-oxidative, anti-thrombotic, anti-ulcer, cardioprotective, hypolipidemic, hypotenisve and insecticidal properties [42,43].

Melathera Biflora had moderate phytosterol content. Phytosterol reduce cholesterol levels by competing with cholesterol absorption in the gut of humans [44]. The sample had phenolic acid, which are important for cell structure, signaling and pigmentation [45]. They are known to act as allelochemicals [46], protect plant against environmental and biological stress such as high energy radiation, bacterial infection or fungal attacks [47], cold, stress hyperthermia and oxidation stress [41]. Thus their presence in *Melanthera biflora* may suggest a likely allelopathic potential of the plant.

5. CONCLUSION

These results suggest strong nutraceutical potential of this plant and suggest further research in it therapeutic uses in the management and prevention of disease as a result of its rich phytochemical composition.

It is a potent pharmaceutical which will help to alleviate some certain kind of diseases and infections such as cancer, cardiovascular diseases, diabetics mellitus, cough, hypertension, piles, asthma, malaria etc.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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