Proximate and phytochemical profile of *Melanthera biflora*

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ABSTRACT

The proximate and phytochemical composition of *Melanthera biflora* was investigated, using standard methods, the leaves had high moisture (71.1± 0.2%) and crude fibre (3.91 ± 0.5) contents and moderate protein (70 ± 0.03%) 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%) and others in insignificant amount. 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%, and others in insignificant amount. The ten known carotenoids (2.48%), consisting of lutein (40.76%), carotene (17.90%), malvidin 5.63%, zeazanthin (16.5%), viola-xanthin (9.5%), and others in insignificant amount, were detected. Sixty one terpenoid including linalool (40.98%), germacrene (12.74%), Alpha-terpineal 6.40%, terpinen – 4-01 (5.62%), and Gamma terpine, and others in insignificant amount, were detected. Six phenolic acids (16.26%), consisting of vanilic acid (45.8%), ferulic acid (53.94%), and others in significant amount, were detected. Seven phytosterol (2.25%), consisting of sitosterol (65.3%), savenasterol (14.19%) stigmasterol (12.70%), and others were detected. The leaves had very low hydroxycinnamic acid ($8.93x10^{-4}$%) 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 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 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 food and therapeutic uses in the management and prevention of diseases.

Keyword: proximate, phytochemical, vegetable

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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 effect, thus, making them serve a dual function against a number of biochemical, physiological and metabolic disorder. (Aletor and Adeogun 1995), Green leafy vegetables constitute an indispensable constitute of human diet in Africa generally and West Africa in particular (Osagie and Offiong, 1988). Low consumption of green leafy vegetable in diet is one of the major factor which leads to deficiency of vitamin and iron. Nigeria is blessed with a great natural tropical rain-forest that is characterized with viable soil where vegetables of high nutritional value are grown. This is even more pronounced in South-Eastern Nigeria. There are edible inexpensive leafy vegetables found in this zone (South Eastern Nigeria) whose chemical, nutritional and phytochemical potentials are yet to be adequately studied and utilized. Among this vegetable is “akuwa” (*Melanthera biflora*). The present study therefore aimed at evaluating the level of mineral elements, vitamins and amino acids composition of *Melanthera biflora*, a tropical leafy vegetable found in south eastern Nigeria so as to encourage its consumption in other to enhance healthy living.

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 luxiorous edible leaves which is used in making soup. The present study therefore aimed at evaluating the level of mineral elements, vitamins and amino acids composition of *Melanthera biflora*, a tropical leafy vegetable found in south eastern Nigeria so as to encourage its consumption in other to enhance healthy living.

MATERIALS AND METHODS

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.

Sample Preparation

The harvested vegetable leaves 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 60^0C 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 air-tight container under refrigerated temperature for further use.

Determination of chemical composition

The proximate analysis (carbohydrate, fats, protein, moisture and ash) of the leaves were determined by using AOAC (1995) methods. Carbohydrate was determined by 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 (Guebel et al 1991). The Nitrogen value was converted to protein by multiplying to a factor of 6.25. The moisture and ash were determined using 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 (1978), and Ihekoronye and Ngoddy (1985). All the proximate values were reported in percentage (AOCS, 2000; Okwu and Morah, 2004).

Determination of phytochemicals profile

Phytochemicals were determined using gas chromatography after their individual extractions.

RESULTS

Table 1 The proximate composition of *Melanthera biflora* leaves

Constituent	**Composition (%)**
Protein (g)	7.00±12

Lipid (g)	1.10±0.16
Crude fibre (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 2.1 Alkaloid composition of *Melanthera biflora* leaves

Compounds	**Amounts (x 10^{-3}) (mg/100g)**	**% Composition**
Morphine	17882	28.05
Methyl morphine	10340	16.22
Papaverine	47.40	0.074
Biflorin	13154	20.63
Narcotine	7.699	0.012
Daphnoline	7664	12.02
Aromoline	8056	12.64
Homoaromoline	4914	7.71
Ambelline	2.309	0.003
6-Hydroxybuphanidine	0.981	0.002
Monocrotalline	9.025	0.001
6-Hydroxy powelline	2.012	0.003
Nitidine	1666	2.613
Total	**63751**	

Table 2.2 Flavonoid composition of *Melanthera biflora* leaves

Compounds	**Amount X10^{-4} (mg/100g)**	**% Composition**
Catechin	0.033	1.219 x 10^{-5}
Resveratrol	1.107	4.15 x 10^{-4}

Apigenin	1880	0.705
Daidzein	19210	7.203
Butein	2.443	9.16×10^{-4}
Naringenin	6.454	2.42×10^{-3}
Biochanin	2.65	9.93×10^{-4}
Luteolin	27110	10.165
Kaempferol	77190	28.943
(-) – Epicatechin	7.979	2.99×10^{-3}
Salvagenin	18040	6.764
(-) – Epicatechin-3-gallete	5.90	2.212×10^{-3}
Gallocatechin	3.052	1.144×10^{-3}
Quercetin	117920	44.214
Isorhamnetin	36.14	1.355×10^{-3}
Myricetin	5.077	1.904×10^{-3}
Sinensatin	21860	8.19
Kaemferol-3-arabinoside	1.842	0.691
Naringerin	2.841	1.065×10^{-3}
Quercitrin	830.6	0.311
Isoquercetin	415.1	0.156
Orientin	0.409	1.534×10^{-4}
Isoorientin	278.5	0.1044
Total	**266700**	

Table 2.3 The tannic acid composition and *Melanthera biflora* leaves

Compound	**Amount (mg/100g)**
Tannic acid	129.8803

Table 2.4 The glycosides composition of *Melanthera biflora* leaves

Compound	**Amount (mg/100g) ($X10^{-6}$)**	**% Composition**
Kampferol-3-O-rhamnoside	1.490	0.268
Arbutin	6.848	1.234
Salicin	10.64	1.917
Amygdalin	71.85	12.946

Quabain	435.910	78.544
Digitoxin	3.986	0.718
Vitexicarpin	19.962	3.597
Digoxin	0.625	0.43
Costrugenin	3.952	0.712
Total	**5.5499**	

Table 2.5 The phytosterol composition of *Melathera biflora* leaves

Compound	**Retention time (min)**	**Amount (mg/100g) ($X10^{-5}$)**	**% Composition**
Cholesterol	19.488	0.0035	0.033
Cholestenol	20.521	6.834	0.64
Ergosterol	21.393	6.877	0.65
Camfesterol	21.954	84190	7.93
Stigmasterol	23.221	134700	12.70
S-Avenasterol	24.018	149900	14.10
Sitosterol	25.260	693200	63.3
Total	-	**1062000**	-

Table 2.6 Allicins composition of *Melanthera biflora* leaves

Compound	**Amount (mg/100*g*) ($X10^{-6}$)**	**% Composition**
Diallyl thiosulphinate	8.765	97.05
Metthl allyl thiosulphinate	0.234	2.591
Allyl methyl thiosulphinate	0.031	o.343
Total	**9.031**	

Table 2.7 The carotenoid composition of *Melanthera biflora* leaves

Compounds	**Amount ($X10^{-3}$)**	**% Composition**

	(mg/100g)	
Malvidin	651.4	5.627
Carotene	2080	17.968
Lycopene	1.060	0.091
Beta-cryptanxanthin	343.9	2.971
Lutein	4718	40.757
Zeaxanthin	1910	16.500
Anthera-xanthin	3.416	0.030
Asta-xanthin	4.549	0.039
Viola-xanthin	1082	9.347
Neo-xanthin	330.7	2.857
Total	**11,576**	

Table 2.8 The saponin composition *Melathera biflora* leaves

Compounds	**Amount (mg/100mg) (X10^{1})**	**% Composition**
Gitogenin	2.578	22.044
Solagenin	0.0028	0.195
Diosgenin	2.339	20.024
Tigogenin	0.00149	0.042
Neohecogenin	2.429	20.794
Hecogenin	1.764	15.101
Sapogenin	1.659	12.205
Euphol	0.055	0.471
Saponine	0.857	7.337
Total	**11.68**	

Table 2.9 Hydroxycinnamic acid composition of *Melanthera biflora* leaves

Compounds	**Amount (mg/100g) (X10^{-4})**	**% Composition**
Cinnamic acid	3.278	0.078
Coumarin	0.692	0.017
p-Coumaric acid	11.6	27.914
o-Coumaric acid	2.314	0.056
Caffeic acid	2999	71.918
Sinapinic acid	0.0856	0.002
Chlorogenic acid	0.1937	0.005
Cichoric acid	0.1735	0.004
Total	**0.417**	

Table 2.10 The phenolic acid composition of *Melanthera biflora leaves*

Compound	**Amount (mg/100g) (X10^{-4})**	**% Composition (10)**
Vanillic acid	3480	45.85
Ferullic acid	4093	63.94
Syringic acid	1.713	20.24x10^{-4}
Piperic acid	4.410	50.8 x 10^{-5}
Ellagic acid	8.444	1.111 x 10^{-4}
Rosmarinic acid	2.258	2.258
Total	**7.590**	

Table 2.11 Terpenes composition of *Melathera biflora* leaves

Compounds	**Amount (Norm. %)**
Butanol	0.083
2-Hydroxy-3-butanone	0.366
Butanoic acid	0.116
Sabinene	0.117
2-Methylbutenoic acid	0.095
2-Methylbutanoic acid	0.271
2- Methylbutanoic acid ethyl	0.290
Azulene	0.299
2-methylbutanoic acid ethyl	0.210
Alpha pinene	1.688
Beta pinene	1.788
Benzyl alcohol	0.593
Cis ocimene	3.756
Myrane	0.209
Allo ocimene	0.246
Pinene-2-ol	0.000
Alpha thujene	0.645
Gama terpinene	4.198
2,6-O-dimethyl1-5 heptanel	0.310
Citral	0.366
Camphor	0.201
Neral	0.519
Geranial	0.405
Iboartemisia	0.245
1,8-Cineole	0.592

Borneol	0.500
Linalool	40.984
Citronellal	0.196
Nerol	0.196
Alpha terpineol	6.395
Terpinen-4-ol	5.620
Citronellol	0.359
Ascaridole	0.468
Linalyl acetate	0.449
Alpha terpinenyl acetate	0.310
Ethyl cinnamate	0.583
Borneol acetate	0.733
Neryl acetate	0.2098
Geranyl acetate	0.311
Beta bisabolene	0.661
Germacrene D	12.735
Gama cadinene	1.690
Beta caryophyllene	0.968
Cyprene	0.143
Beta elemene	0.143
[6]-Shogaol	0.565
Alpha gurgunene	0.469
Alpha copane	0.211
Beta selinene	0.209
Itumulene	0.396
Vacencene	0.310
Caryophyllene oxide	3.856
Alpha selinene	0.491
[6]-Paradol	0.084
Beta selinene	0.248
Aromadendrene	0.370
Gama muurolene	0.314
Aristolone	0.310
Viridiflorol	0.304
Taraxeron	0.325
Lupeol	0.319
Total	**100**

Table 2.12 Percentage composition of group phytochemicals in *Melanthera biflora*

Phytochemicals	**Amount (mg/100g)**	**% Composition**

Alkaloids	63.75	13.654
Flavonoids	26.670	5.712
Tannic acid	129.88	27.818
Glycosides	5.55×10^{-4}	0.001
Terpenoids	100.00	21.418
Phytosterols	10.620	2.275
Allicins	9.031×10^{-6}	1.937×10^{-6}
Carotenoids	11.576	2.480
Saponins	116.81	2.502
Hydroxycinnamic acids	4.170×10^{-4}	89.3×10^{-4}
Phenolic acids	7.590	16.26
Total	**466.898**	

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

Discussions

The moisture content of *Melanthera biflora* is higher than that of *Talinum triangulare* and *Telferia occidentalis* (Oguntana, 1988), but less than *Pennisetum purpureum* (Okaraonye and Ikewuchi, 2009). The moisture content of any food is an index of its water activity (Olutiola et al., 1991) and it is used as a measure of stability and susceptibility to microbial contamination (Uriah and Izuagbe, 1990). 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*. 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* is greater than that of *Pennisetum purpureum* (Okaraonye and Ikewuchi, 2009), *Amarantus hybridus*, *T. occidentalis* and *T. triangulare* (Oguntona, 1998). The leaf protein is rich in essential amino acids. These amino acids serve as an alternative source of energy when carbohydrate availability in the body is impaired. A 100 g of this sample can meet the daily protein requirement of 23-56 g (FAO/WHO/UNU, 1991; Chaney, 2006a). Regular uses of plant food rich in protein make an invaluable addition to a diet (Wardlaw, 1999). The ash content of *Melanthera biflora* was greater than that reported for *T. occidentalis*, *T. triangulare* (Oguntona, 1998) and *P. purpureum* (Okaraonye and Ikewuchi, 2009), but less than *A. hybridus* (Oguntona, 1998). The ash composition of a food is the amount of minerals substances left after the carbon material must have been burnt of (Onyeike and Osuji, 2013).

Melanthera biflora leaves contain comparable lipid content to *P. purpureum* (Okaraonye and Ikewuchi, 2009) and *A hybridus* (Oguntona, 1998), but greater one than *T. occidentalis, T. Triangulare* (Oguntona, 1998) and *Sansevieria liberica* (Ikewuchi et al., 2010).

The total carbohydrate content of *Melanthera biflora* was less than those reported for *A. hybridus* (Oguntona, 1998) and *P. tuberregium* sclerotia (Ikewuchi and Ikewuchi, 2009), but more than *P. purpureum* (Okaraonye and Ikewuchi, 2009). 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* (Oguntona, 1998) and *P. purpureum* (Okaraonye and Ikewuchi, 2009).

Results from epidemiological studies reveal that increased fibre 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 (Walker 1978; Food and Agriculture Organization; Eriyamremu and Adamson, 1994; Scientific Advisory Committee on Nutrition, 2008). Dietary fibre 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 (Dillard and German, 2000). This is one benefit derivable from the consumption of *Melanthera biflora*.

The total caloric content of *Melanthera biflora* was higher than *P. purpuerum* (Ikewuchi and Okaraonye, 2009), but less than *P. tuberregium* sclerotia (Ikewuchi and Ikewuchi, 2009). This result shows that Melanthera is a good source of nutrient.

Phytochemical composition of the leaves of *Melanthera Bifola* 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 (Liu et al 2005) Tannin is used in the treatment of inflamed or ulcerated tissues. *Melanthera Biflora* is rich in alkaloid, prominent

which is morphine used as n analgesic, local anaesthetic and anti-leishmanial agent (Carrol nd starmer 1967). Flavonoid are of a particular importance in the human diet as there are evidence that they act as antioxidants, antiviral and anti-inflamentory agent. (Soetan 2008) and are associated with reduced risk of cancer and cardiovascular diseases. (Middleton et al 2000). Terpenes are used as flavor enhancers in food, frangrances in perfuming and in traditional and alternative medicines such as aromatherapy (Kappers et al 2005). They have anticancer (Dewick 2004) Antimicrobial (Islam et al 2003) and anti-oxidant Dillard and German 2000).

The leaves have low saponin, very low glycoside and moderate allicin content. Saponins are reported to have broad range of pharmcological properties (Soetan 2008). Allicin is reported to have an anti-inflammatory, antimicrobial, anti oxidation, anti-thrombotic, ` anti-ulcer, cardioprotective, hypolipidemic, hypotenisve and insecticidal properties (Elilat et al, 1995; Elkayam et al 2003).

Melathera Biflora has moderate phytosterol content. Phytosterol reduce cholesterol levels by competing with cholesterol absorption in the gut of humans (Tilvis and Miethinen 1986). The sample has phenolic acid, which are important for cell structure, signaling and pigmentation (Adyanthaya, 2007). They are known to act as allelochemicals (Yoshioka et al, 2004), protect plant against environmental and biological stress such as high energy radiation, bacterial infection or fungal attacks (Tuzen and Ozdemir, 2003), cold, stress

hyperthermia and oxidation stress (Dillard and German, 2000). Thus their presence in *melanthera biflora* may suggest a likely allelopathic potential of the plant.

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 potential pharmaceutical which will help to alleviate some certain kind of diseases and infections such as cancer, cardiovascular diseases, type 2 diabetics, cough, hypertension, piles, asthma, malaria etc.

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