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PHYTOCHEMICAL SCREENING, NUTRITIONAL/ANTI-NUTRITIONAL AND AMINO ACID COMPOSITIONS OF NIGERIA *MELANTHERA SCANDENS*

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ABSTRACT

Dried leaves of *Melanthera scandens* were analyzed for its proximate composition and the result obtained showed that, it has ash content (14.23%), moisture (7.35%) and crude protein (25.13%), crude fibre (6.59%), fat (9.43%) and carbohydrate (37.40%). The result of the phytochemical screening shows the presence of tannins, alkaloids, saponins, flavonoids and cardiac glycoside. Steroid and phlobatanin were absent. The result for anti nutrients shows that it contains, tannic acid (0.11%), phytin phosphorous (5.34 mg/kg), phytic acid, (18.94 mg/kg), saponin (6.42%), alkaloids (8.90%), flavonoids (3.72%) and oxalate (3.06%). The leaves were also found to be rich in minerals like phosphorus (111.57 mg/100 g), sodium (22.41 mg/100 g), potassium (39.71 mg/100 g), calcium (30.26 mg/100 g), magnesium (42.55 mg/100 g) and zinc (37.83 mg/100 g). It also has some trace elements like iron 5.11 mg/100 g, manganese 0.22 mg/100 g, but elements like copper, cadmium, chromium and lead were not detected in the leaves. Amino acids composition on the leaves revealed an high value of glutamic acid (8.69 g/100 gcp), aspartic acid (8.63 g/100 gcp) and leucine (7.55 g/100 gcp). It has a low level of cystine (0.78 g/100 gcp), histidine (1.81 g/100 gcp), methionine (0.91 g/100 gcp) and serine (1.99 g/100 gcp). The values of isoleucine (4.03 g/100 gcp), glycine (3.15 g/100 gcp), threonine (4.09 g/100 gcp) and valine (3.93 g/100 gcp) were moderate. The amino acid composition showed correlation with some of the essential amino acid with respect to FAO/WHO provisional pattern. The results of this research indicates that the leaves of *melanthera scandens* plants have nutritional qualities that could provide the users and consumers with additional nutrients and the presence of phytochemicals indicated that it is a potential source of drug.

Key words: Proximate composition, Phytochemicals, Amino acid profile, Anti nutrients, Minerals, *Melanthera scanden.*

INTRODUCTION

Trees and shrubs with medicinal and nutritional potentials are abound in Nigeria[1]. The medicinal values of plants and vegetables are dictated by their phytochemicals and other chemical constituents[2]. This is the reason why efforts has been in studies aimed at elucidating their levels in many plants both in Nigeria and elsewhere[3]. *Melanthera scanden* is a scrambling or scandent herb of waste thickets, cultivation edges and forest margins, commonly dispersed in the forested areas of the region, and extending widely across tropical Africa. The plant is often confused with *Aspilia africana* and has the same uses. It provides forage for all stock in thicket edges around villages. It is a fast-growing weed of cultivation becoming troublesome in the southern Nigeria and in the western Cameroon where it is deemed a pest on oil-palm plantations. The

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leaves are a favorite's food of hares in southern and western Nigeria, and are commonly put into soups. The whole leaves are very scabrid and can cause irritation to the skin if rubbed against it. On the other hand, pulped, decocted or macerated leaves are cicatrisant. Preparations are haemostatic and are used on cuts and wounds.

Like *Aspilia africana* which are said to draw up exudations from open sores, to curb inflammation, and to promote healing. *Melanthera scanden* has varieties of use. The Ijos use either leaf-sap or the leaf reduced to ash on wounds and apply the ash to burns. They also rub the leaves with salt, and then mix them with gin to take as an antidote against poisoning. The Igbos tie on the leaf as a wound-dressing after circumcision. Leaf-sap or decoction with citron-juice added is used in Ivory coast-upper volta for eye-troubles, even for trachoma, but the treatment is irritating and it's given only with some hesitation. Akye use it for chickenpox internally and externally. Infusions made from leaves, stems and roots are emetic. The leaves are a drastic purgative, and leaf-sap is used in Ivory coast to accelerate childbirth and to treat for poisoning. In Ivory coast a leaf-decoction is used as a soothing cough-mixture and for sore-throat and in Tanganyika a 24-hour infusion of powdered leaves is taken for hiccups. The twigs also serve for teeth-cleaning, and in the Acholi-West district of Uganda bundles of sticks are burnt to make acrid smoke to drive off bees from a nest in raiding it for honey. The plant probably has superstitious attributes similar to *Aspilia africana*. In Ivory coast-upper volta, a preparation of leaves is rubbed on the patient's head for epilepsy. To the Ijaws and the Yorubas, the plant is female, the male counterpart being *Aspilia Africana*[1,4]. ***M. Scandens*** has been reported to possess antidabietic, antioxidant, anti-inflammatory and antiplasmodial activity has been reported[5-8]. Some triterpenoid saponins have been isolated from the plant[9].

The use of local indigenous food commodities to formulate local and home-based complementary foods is being practiced in many developing countries. Likewise, sustainable livestock production is dependent on the availability of various sources of nutrients that are required for the formulation of animal feed. Principal among these are protein and energy sources such as groundnut, soybeans and maize which are also important foodstuff for humans[10]. Thus, there is competition for the limited common foodstuff and hence the high cost which is ultimately translated into high cost of animal protein. With increasing global demand for livestock products, research into locally available food with potential use as additional sources of protein and energy is imperative. This research therefore aims at investigating both the nutritional and antinutritional components of *melanthera scanden* to justify its use as feedstock and in ethnomedicine.

EXPERIMENTAL

Materials and methods

The leaves of *melanthera scandens*, which was obtained in the premises of the University of Ado-Ekiti, Ekiti State, Nigeria was identified and authenticated at the herbarium of the Department of Plant Science, University of Ado Ekiti. by Mr. Omotayo. The collected plant materials were washed with distilled water and air dried in a shade for one week at room temperature. The dried sample was milled into powder using an electric blender. The powder was stored in a sample tube and placed in a refrigerator prior to analysis.

Plant extraction

100 g of dried powered sample was extracted with 500 mL of distilled water for 12 hrs. This was then filtered and phytochemical screening of the filtrate was carried out.

Proximate analysis

Proximate analysis was carried out on dried samples of leaves according to the procedure of Association of Official Analytical Chemist[11].

Phytochemical screening

Phytochemical screening procedures carried out were by the method of Sowofora et al.[12].

Mineral composition

The sample was dry ashed at 550° C and the ash boiled with 10 mL of 20% HCl in a beaker and then filtered into a 100 mL volumetric flask. It was made upto the mark with deionized solution and the mineral analysis of the resulting solution was determined using Atomic Absorption Spectroscopy (Pye, Unican SP9, Cambridge, UK).

Determination of amino acids

The amino acid was determined in the sample using ion exchange chromatography (IEC). The sample was defatted. Amount of crude protein and nitrogen determined, hydrolyzed, evaporated in a rotary evaporator and then loaded into the technician sequential multi-sample (TSM) amino acid analyzer.

Defatting

About 2 g of the sample was weighed into the extraction thimble and the fat extracted with chloroform methanol using soxhlet extraction apparatus[12]. The extraction lasted for 5-6 h.

Hydrolysis of sample

About 30-35 mg of the defatted sample was weighed into glass ampoules. 7 mL of 6 M HCI was added and oxygen was expelled by passing nitrogen gas into the ampoule (to avoid possible oxidation of some amino acids during hydrolysis). The glass ampoule was then sealed with a bunsen flame and put into an oven at 105° C for 22 h. The sample was allowed to cool before breaking open at the tip and the content was filtered to remove the residue. The filtrate is then evaporated to dryness at 40° C under vacuum in a rotary evaporator: The residue was dissolved with 5 mL of acetate buffer and stored in a plastic specimen bottle and kept in a deep freezer.

Sample analysis

The amount loaded for the sample was between 5-10 mL each. This was dispersed into the cartridge of the analyzer. The technician sequential multisampe amino acid analyzer (TSM) was designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis lasted for 76 min. for the sample.

Calculation for amino acid values from chromatogram peaks

The net height of each peak produced by the chart record of the TSM representing an amino acid measured. The half height of the peak on the chart was found and the width of the peak was then obtained by multiplying the height with the width at half height.

The norleucine equivalent for each amino acid in the standard mixture was calculated using the formula.

$NE_{std} = NH_{nor} \times W_{nor}$

NHAA x WAA

$Sstd = NEstd \times Mol.wgt_{AA} \times 0AA_{std}$

The amount of each amino acid present in the sample was calculated in g/100 g protein using the following formula;

Conc. (g/100 g protein) = NH x width at NH/2 x S_{std} x C

Note: Nor = Norleucine

AA = Amino Acid $AA_{std} = 0.025\ mm^2$

Dilution factor = 5

% N = 6.42

Volume loaded = (10 basic) or 5 µL (acidic/neutral)

Std = Standard

RESULTS AND DISCUSSION

The proximate composition of *melanthera scanden* is presented in Table 1. A low moisture content of 7.24% was observed which an indication that it is not liable to microbial spoilage. The result obtained here is higher compared to that earlier reported for *aerva lanata* (6.38%) by the ash content of 14.22% was obtained which is high compared to that reported for *F. asperifolia* and *F. sycomorus* with a value of 11.25 and 10.24%, respectively, but low compared to that reported by Omoyeni and Adeyeye[13] for *A. lanata* (31.2%). Crude protein value is high with a value of 22.58%, this shows that the plant is a good source of protein and can be used as feed stock in animals, with rabbit being a good consumer of the plants. The value obtained is low compared to *F. asperifpolia* (20.27%) Nkafamiya, et al.[14].

The result obtained can also be compared to protein rich foods like soybeans, cowpeas, melon and pumpkin (23.10% - 33.00%)[15]. The crude fat content of 9.43% was obtained which is comparable to the result obtained by Adeyeye and Fagbohun[16] for *solanum gilo* (9.0%). It is thus, a good source of oil. The crude fibre content is 6.59%, which is found to have hypocholesterolemic properties[17]. The results were compared to the result obtained for *F. asperifolia* (28.68%) and *F. sycomorus* (31.54%). Okon et al.[18] reported that a diet low in fibre is undesirable and could cause constipation, hence many diets are associated with disease of colon like piles, appendicitis and cancer. Conversely, nutritionally, this is of high benefits since it had been reported that food fibre aids absorption of trace elements in the gut and reduce absorption of cholesterol. The high content of carbohydrate (37.4%) in *melanthera scanden* shows that it is a good source of energy and also needed for efficient oxidation of fats[13]. This is beneficial since carbohydrate constitutes a major class of naturally occurring organic compounds that are essential for the maintenance of plant and animal life and also provide raw materials for many industries[19].

Table 1: Mean proximate composition (%) of *melanthera scandens*

Parameter	Concentration (%)
Ash	14.23 ± 0.01
Moisture content	7.24 ± 0.01
Crude protein	25.13 ± 0.43
Fat	9.43 ± 0.02
Crude fibre	6.59 ± 0.01
Carbohydrate	37.40 ± 0.42

The Mineral composition (mg/100 g) of *melanthera scandens* is presented in Table 2.

Generally, minerals from plants sources are less bio-available than those from animal sources[20]. In the present study, it was observed that *melanthera scandens* were rich sources of most essential minerals as shown in Table 2 (mg/100 g) with Na (22.41), K (39.71), Ca (30.26), Mg (42.55), Zn (37.82) and P (111.57). Sodium and potassium are involved in body water balance and acid-base balance and is the major extra cellular and intracellular mineral respectively, failure to consume sufficient sodium when fluid and sodium losses are high can lead to hyponatremia. They are also involved in the transport of some non-electrolytes. The maintenance of osmotic equilibrium by Na can lead to serious disorders, like headache, tiredness, muscle cramps in the body[14]. Calcium and magnesium are majorly found in the skeleton. Ca helps in forming and maintaining bone, blood clotting and muscle contraction. In plants, magnesium is absorbed as the Mg^{2+} ion, it is a constituent of chlorophyll molecule and thus considered indispensable in metabolism and for maintaining the electrical potential in nerve and muscle cells. When associated with widespread malnutrition, especially in alcoholics, a magnesium deficiency results in tremors and convulsions. Magnesium also forms part of the skeleton.

Table 2: Mineral composition mg/100 g of *melanthera scandens*

Minerals	Composition
Na	22.41
K	39.71
Ca	30.26
Mg	42.55
Zn	37.82
Fe	5.11
P	111.57
Mn	2.08
Cu	ND
Cd	ND
Cr	ND
Pb	ND
Na/K	0.56
Ca/P	0.27
Ca/Mg	0.71

ND = Not detected

Zinc helps to form a large number of enzymes, many of which function in energy metabolism and in wound healing[21]. It also helps in DNA synthesis, storage, release, and function of insulin and also in the development of sexual organs and bones. Fe is essential for heamoglobin formation. Iron-deficiency, anemia is characterized by poor oxygen-carrying capacity, a condition that causes endurance problems in athletes. Phosphorus is essential in bone and teeth formation, Manganese is a trace mineral involved in bone formation, immune function, antioxidant activity, and carbohydrate metabolism. Its deficiency may result in paralysis and convulsion[21]. It also activates enzymes involved in the transfer of phosphate and hydroxyl groups as well as some hydrogenation reactions. The absence of heavy metals like Cu, Cd, and Pb makes it safe for human consumption; the metals are of no use to human. This indicates that these minerals are beneficial to consumers, since it has been reported that some of these minerals like lead, cobalt and cadmium are highly toxic even at low concentrations[22,23].

The level of some of these elements are high, consumption of these leaves will help to alleviate symptoms of magnesium and zinc deficiency such as weakness, cardial arrhythmia, poor growth, impairment of sexual development and poor wound healing often observed in the study area where majority are alcohol addicts. A ratio of less than one is recommended to prevent high blood pressure[24]. The processing method produced Na/K ratio less than one, which is in accordance with the recommended ratio. The Ca/P ratio is 0.27. The Ca/ Mg ratio is 0.71, which is good for healthy living.

In Table 3 the anti-nutrients results of *M. scandens* are presented, *Melanthera scandens* contains low level of anti-nutrients like saponin (6.42%), flavonoids (3.72%), oxalate (3.06%), alkaloids (8.90%) and phytin phosphorus (5.34 mg/kg). It is very low in tannic acid (0.11%), but a bit high in phytic acid (18.94 mg/Kg). The anti-nutrient content of the leaves listed are compounds that limit the wide use of many plants due to their ubiquitous occurrence of them as natural compounds capable of eliciting deleterious effect in man and animals[25]. The result as shown in Table 3 indicated that *melanthera scandens* is not detrimental to humans and animals who are the major consumers. Some of these anti-nutrients can be reduced by processing and cooking, for example, boiling can reduce the soluble oxalate content of a food, if the water used for boiling is discarded[26].

Table 3: Anti-nutrients values (%) of *melanthera scandens*

Anti-nutrients	Composition
Tannic acid (%)	0.11
Phytin phosphorous (mg/kg)	5.34
Phytic acid (mg/kg)	18.94
Saponin (%)	6.42
Alkaloids (%)	8.90
Flavonoids (%)	3.72
Oxalate (%)	3.06

Tannins have been reported to bring about their anti-nutritional influences largely by precipitating dietary proteins and digestive proteins and digestive enzymes to form complexes which are not readily digestible[13]. Saponin can either be deleterious or beneficial. There are suggestions that saponin consumption be encouraged because of their hypocholesterolaemic activity, forage saponins have been reported to cause toxic and anorexia effects in the rats and swine thereby limiting the feeding value of high saponin animal feed[27]. Alkaloids are a group of naturally occurring chemical compounds which mostly contain basic nitrogen atoms. It has been reported to have analgesic properties.

Oxalate level (3.06%) in *M. scanden* is low, and it is known to cause great risk of renal absorption. Heat treatment (cooking) has been found to be an effective measure in reducing the oxalate levels in leafy vegetable, thus, making the food prepared from these accessions safe for human consumption[28]. This is particularly beneficial because oxalic acid and its salts can have deleterious effects on human nutrition and health, mainly by decreasing calcium absorption and aiding the formation of kidney stones[28].

The Phytochemical screening results of *melanthera scandens* are presented in Table 4.

The screening showed the presence of alkaloids, tannins, saponins, flavonoid and cardiac glycoside while steroid, terpenoid and phlobatanin were found absent.

Table 4: Phytochemical screening of *melanthera scandens*

Tests	Observation
Tannins	+
Akaloids	+
Saponins	+
Steroid	-
Terpenoid	-
Flavonoid	+
Phlobatanins	-
Cadiac glycoside	+

Key: +ve = Presence of constituents
-ve = Absence of constituents

The presence of these phytochemicals has been attributed to the bioactive principles responsible for ethnopharmalological activities of most medicinal plant[3]. This dictates why efforts have been expanded in studies aimed at elucidating their levels in medicinal plant[3]. The medicinal values of plants and vegetables are dictated by their phytochemicals and other chemical constituents[2]. The importance of alkaloids, saponins, tannins and inulins in various antibiotics used in treating common pathogenic strains has recently been reported by Kubmarawa et al.[25] Ayitey- Smith[29] had earlier recorded that bitter leaf contains an alkaloid, vernomine, which is capable of reducing headaches associated with hypertension. The therapeutic relevance of bitter leaf has also been reported[26,30]. Ocimum species are also important sources of alkaloids with medicinal properties and are used in the management of cold, chronic catarrh, persistent headaches and migraine[30]. The use of celosia which contains alkaloids, and relatively higher concentrations of sodium and potassium ions, in the treatment of diarrhoea by local inhabitants has also been documented by Odugbemi[26] among the Yorubas of Western Nigeria. The antibacterial properties of tannins have been documented by Banso and Adeyemo[31]. *M. scandens* is thus an important medicinal plant, and it may be used to treat several diseases.

Table 5 shows the amino acid composition of the leaves of *melanthera scandens.* The concentration of each amino acid was calculated in g/100 gcp. From the result, glutamic acid was found to be the highest, with concentration of (8.69), aspartic acid (8.63); following in the order is leucine (7.55) and cystine (0.78) is considerably low in the leaves.

Aspartic acid moves the coenzyme nicotinamide adenine dinucleotide (NADH) molecules from the main body of the cell to its mitochondria, where it is used to generate adenosine triphosphate (ATP), and the fuel that powers all cellular activity. The GLU of the sample is comparable to the value reported for *P. mildbraedii* 10.38 g/100 gcp[32], which followed the same trend in both fermented and unfermented cocoa nibs[33]. All the amino acid contents found in the leaves were comparable with those found in *S. indicum* and *B. aegyptiaca*[25] and also with the leaves of *F. asperifolia* and *F. sycomorus*[14]. The Total Amino Acid contents (TAA) is 66.00 g/100 gcp. It is lower to the value reported for *J. curcas* (78.7 g/gcp)[34]. The total essential amino acid is 28.81 g/100 gcp, which is lower compared with *limicolaria sp* (428.0 mg/gcp), and melon oil seeds (534.4 mg/gcp)[15]. The nutritive value of protein depends on its capacity to satisfy the needs for nitrogen and essential amino acids. The percentage of TEAA in *M. scandens* is 43.65 which is an indication that it is a good source of essential protein. The value is comparable to that of *P. mildbraedii*[32]. The majority of the amino acids found in the leaves are neutral with % TNAA being 47.16 (Table 6). A similar trend occurred in *P. mildbraedii*[32].

Table 5: Amino acid concentration (g/100 g crude protein) of *melanthera scandens*

Amino acid	Concentration (g/100 g crude protein)
Lysine * -	3.40
Histidine * -	1.81
Arginine * -	4.24
Aspartic acid +	8.63
Threonine *	4.09
Serine x	1.99
Glutamic acid +	8.69
Proline x	2.33
Alanine x	3.47
Cystine x	0.78
Valine * x	3.93
Methionine x	0.91
Isoleucine * x	4.03
Leucine x	7.55
Tyrosine x	3.21
Phenylalanine * x	3.79

Key: *Essential amino acid, x Neutral amino acid, +Acidic amino acid, ¯Basic amino acid

Table 6: Classification of essential, non-essential, acidic and basic amino acids (g/100 gcp)

Amino acids	Value (g/100 gcp)
Total amino acid (TAA)	66.00
Percentage total amino acid (% TAA)	100.00
Total essential amino acid (TEAA)	28.81
Percentage total essential amino acid (% TEAA)	43.65
Total non-essential acid amino acid (TNEA)	37.19
Percentage total non-essential amino acid (% TNEA)	56.35
Total neutral amino acid (TNAA)	31.13
Percentage total neutral amino acid (% TNAA)	47.16
Total basic amino acid (TBAA)	9.45
Percentage total basic amino acid (% TBAA)	14.32
Total acidic amino acid (TAAA)	17.32
Percentage acidic amino acid (% TAAA)	26.24

Leucine is an essential amino acid, which cannot be manufactured in the body and is part of the three branched-chain-amino-acids. Supplements and protein powders that contain leucine are used extensively by body builders and other athletes to promote muscle recovery, although it has not produced significant changes in body composition[35]. *M. scandens* is recommended for athlethes due to its high contents of

leucine (7.55 g/100 gcp). This is high compared to the one reported for *J. curcas*[34]. *M. scanden* has low cysteine contents (0.78 g/100 gcp). Lysine value is moderate but lower to the value for Hasta La asta (*C. pepo*).[36] Methionine has the least value of EAA (0.91 g/100 gcp), this is lower compared to the values reported for flesh of the three-land snails *Limicolaria sp* (35.6 mg/gcp), *Arachatina archatina* (29.6 mg/gcp) and *Archatina marginata* (47.00 mg/gcp)[37].

CONCLUSION

From the present study, we can deduce that, these leaves may serve as constituents of animal and human diet supplying the body with minerals, proteins and energy. The presence of secondary metabolites that are biologically important contributes to its medicinal value and thus can be potential sources of useful drugs.

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