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Determination of Nutrient, Antinutrient Compositions and Median Lethal Dose of Leaves of *Microdesmis puberula* Grown in Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author UEU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AUU and EUD managed the analyses of the study. Author UED managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the nutrient, antinutrient, mineral element and phytochemical compositions as well as acute toxicity profile of leaf extract of *Microdesmis puberula.*
Study Design: Quantitative nutrient, antinutrient and mineral elements assessment assays, quantitative phytochemical assays as well as acute toxicity test of leaf extract of *Microdesmis puberula*.
Place and Duration of Study: Department of Chemistry, Faculty of Science, Department of Pharmacognosy, Faculty of Pharmacy, University of Uyo, Nigeria, July 2016 - September 2017.
Methodology: Standard methods were used to evaluate the concentration of ash, moisture, crude protein, crude fibre, crude lipids, carbohydrate, phytic acid and hydrocyanide in the leaf extract of

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Microdesmis puberula. Levels of calcium, magnesium, iron, potassium and zinc were also evaluated in addition to the quantitative determination of alkaloids, saponins, flavonoids and cardiac glycosides.

Results: Qualitative phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, cardiac glycosides and terpenes, while anthraquinones, phlobatannins and tannins were absent. Quantitative phytochemical determination (mg/100 g) gave the following: 12.80 ± 0.02, 5.85 ± 0.01, 2.48 ± 0.01 and 1.25 ± 0.01 for alkaloids, saponins, flavonoids and cardiac glycosides respectively. Proximate analysis indicated moisture content (48.17 ± 0.07 %) with crude protein, crude fibre and lipid were also present in appreciable quantities. Mineral elements determination (mg/100 g dry weight) showed calcium (163.50 ± 2.01), iron (188.70 ± 2.50), magnesium (168.40 ± 1.50), potassium (42.55 ± 0.55) and zinc (40.80 ± 1.01). Antinutrient analysis indicated low levels of phytic acid (18.220 ±0.030), hydrocyanide (0.002 ± 0.000) and oxalate (1.861 ± 0.002). The LD_{50} (i. p. mice) of *M. puberula* was 2872.28 mg/kg.

Conclusion: The leaf extract of *Microdesmis puberula* is rich in nutrients and mineral elements which are required for human nutritional wellbeing and development. The presence of phytochemicals in the leaves of *M. puberula* which have much pharmacological importance lends credence to the many ethnomedicinal uses of this plant. The low levels of antinutrients which are far below the lethal doses as well as the acute toxicity test value of 2872.28 mg/kg indicate low toxicity and suggest that the consumption of the leaves of as food is not harmful.

Keywords: Antinutrients; bioactive compounds; Microdesmis puberula; mineral elements; toxicity.

1. INTRODUCTION

The consumption of leafy vegetables as food has remained the cheapest and most convenient means of furnishing the body with adequate supplies of proteins, vitamins, minerals, fibre and other nutrients [1]. The nutritional value of some selected leafy vegetables of sub-Saharan Africa and their potential contribution to human health has been extensively reviewed [2-3]. These findings have given credence to the popular fact that most of these leafy vegetables are good and valuable sources of minerals, proteins, fat and oil.

One of such leafy vegetables is *Microdesmis puberula* (family: Pandaceae), a diecious plant, which grows to a height of about 6 m if not harvested or prematurely cut [4]. It is predominantly found in Eastern Nigeria, Democratic Republic of Congo and the Uganda. In Nigeria, its local names include Mkpiri or Mbugbo in Igbo; Idi-apata in Yoruba and Ntabid in the Ibibio language [5].

The leaves are edible and are used to prepare soup either pure or mixed with afang (a delicacy originally peculiar to the Efiks/Ibibios of Southern Nigeria). The leaves and tender branches make good fodder for goats; they also neutralized the effect of caterpillar sting on a fodder-eating goat. Pounded leaves with seven seeds of alligator pepper or a sizeable piece of ginger are applied on sprains, burns and bruises for healing. The small twigs and branches are used as chewing sticks. The fruits which contain one or more stone-like seeds with a thin edible pulp are eaten especially by children. The bark infusion or enema of bark extract is taken to treat worm infestation in the intestines [6].

There are reports on the use of various parts (stem bark, leaves and roots) of *Microdesmis puberula* for various medicinal purposes [4,7]. The roots of this plant are used in the treatment of gonorrhea and erectile dysfunction [8]. The analgesic and anti-stress properties of *M. puberula* in addition to the identification of several polyamine derivatives have also been reported [7,9]. The phytochemical composition of the root extract of *M. puberula* and its effect on some hematological and biochemical parameters have also been reported [10].

The effects of two alkaloids, keayanidine B and keayanine from *Microdesmis keayana* on vascular parameters of erectile dysfunction has been reported [11]. Also, the effects of *Microdesmis keayana* roots on the sexual behavior of male rats have also been reported [12]. There is also a report on the characterisation and identification of spermine and spermidine derivatives in *Microdesmis keayana* and *Microdesmis puberula* roots by electrospray ionisation tandem mass spectrometry and high-performance liquid

chromatography/electrospray ionisation tandem mass spectrometry [13].

Fig. 1. *Microdesmis puberula*

Two new quinolines and tris (4-hydroxycinnamoyl) spermine derivatives from *Microdesmis keayana* roots have been isolated and their structures determined using spectroscopic analyses [14]. Three new N1, N5, N10- tris (4-hydroxycinnamoyl) spermidines have been isolated from a methanolic root exact of *Microdesmis keayana* and their structures established using spectral techniques [15]. The vasoactivity and antioxidant properties of *Microdesmis keayana* roots showed that *M. keayana* roots had significant hypotensive and vasorelaxing properties [16].

The effect of enzyme supplementation on the performance of broiler finisher fed with *Microdesmis puberula* leaf meal has been reported. The result suggests that 0.10% of the enzyme supplementation in diets containing 12.5% *Microdesmis puberula* leaf meal did not improve the performance of finisher broilers [17]. Also, the performance, nutrient utilisation and organ characteristics of broilers fed with *Microdesmis puberula* leaf meal has been reported [18]

In the present study, the phytochemical, proximate, mineral elements, antinutrient composition as well as the toxicity profile of the leaves of *Microdesmis puberula* from Nigeria have been evaluated using standard analytical methods.

Fig. 2. *Microdesmis keayana*

2. MATERIALS AND METHODS

2.1 Plant Material

The fresh leaves of *Microdesmis puberula* were collected from a forest edge in Ikono Local Government Area of Akwa Ibom State, Nigeria in the month of July 2016. Plant identification, authentication and specimen referencing were done at the Faculty of Pharmacy, University of Uyo, Uyo, Nigeria. A voucher specimen has been deposited at the Herbarium of Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

2.2 Sample Preparation

The leaves of *M. puberula* were thoroughly washed with distilled water to remove any trace of dirt sticking to the surfaces. The leaves were

chopped into small pieces and air-dried for seven (7) days. They were later homogenized to the fine powder using an electric blender (Binatone BLG-402, China) and stored in an air-tight plastic bag, properly labelled prior to analyses.

2.3 Extraction Procedure

The dried pulverized plant leaves (500 g) were thoroughly macerated with 80% ethanol (5 L) for 7 days at room temperature. The sample mixture was filtered and the filtrate concentrated using a rotary evaporator at 40°C to afford a green colored extract. The extracts were stored in a sealed container and kept in a refrigerator at 4°C until use. All reagents and chemicals used in this work were of analytical (AnalaR) grade and were sourced from Sigma-Aldrich Chemical Company, United Kingdom.

2.4 Proximate, Mineral and Phyto-chemical Analyses

These analyses were carried out to determine the moisture, ash, crude fibre, fat, crude protein content in the leaves of *Microdesmis puberula*. The moisture content was determined by drying the leaves in an oven (Gallenkamp OV-330) at 105°C until a constant weight was obtained [19] (method 14:004). The percentage moisture content was calculated from the equation:

$$\text{Moisture } (\%) = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad (1)$$

Where,

W_1 = Initial weight of empty crucible,

W_2 = Weight of crucible + sample before drying,

W_3 = Final weight of crucible + sample after drying

Total ash was determined by furnace incineration described by [19] (method 14:006) using about 1.0 g of oven-dried sample. This analytical method is based on the vaporization of water and volatiles with burning organic substances in the presence of oxygen in the air to CO_2 at a temperature of 550°C for 5 hours. The percentage ash content was calculated as:

$$\text{Ash } (\%) = \frac{\text{Weight of Ash}}{\text{Weight of Original Sample}} \times 100 \quad (2)$$

Crude fibre was determined using the method of [19] (method14:020). The percentage crude fiber was calculated as per the formula:

$$\text{Crude fibre } (\%) = \frac{\text{Weight after drying}}{\text{Weight of Sample}} \times 100 \quad (3)$$

Crude protein content was calculated by converting the nitrogen content determined by Kjeldahl's method (6.2 N). Fat was determined by the method of AOAC [19] using the Soxhlet system. The carbohydrate content was estimated as the difference obtained after subtracting the values of the organic protein, fat, ash and fiber from the total dry matter. The calorific value of the sample was obtained by multiplying the values of the crude protein, lipid and carbohydrate by 4, 9 and 4 respectively and taking the sum of the products.

Mineral digestion was done following the method earlier reported [20]. The concentrations of calcium, magnesium, iron and zinc were determined using an atomic absorption spectrophotometer (AAS Unicam 919) in conjunction with reference mineral standards from Unicam Limited, United Kingdom. The flame photometer (Jenway Limited, UK) was used for determination of potassium concentration in the extract.

Phytochemical tests to identify the constituents of the extract were performed using standard procedures outlined by previous workers [21-22]. Precisely, screening of alkaloids was carried out using Dragendroff's and Mayer's reagents and saponins by Frothing and Fehling's tests. Cardiac glycosides were detected by Liebermann's and Keller-Killiani's tests, tannins by the Ferric chloride test and phlobatannins by hydrochloric acid test. Flavonoids were detected by the magnesium metal/hydrochloric acid test, triterpenes by the chloroform/acetic anhydride/sulfuric acid test and anthraquinones by the benzene/ammonia solution test.

Quantitative determination of alkaloids and flavonoids was by the method of Harborne [21], saponin by the method used by Obadoni and Ochuko [23] and cardiac glycosides was done using the Buljet's reagent as described by El-Olemy et al. [24].

2.5 Antinutrient Analysis

The composition of oxalate was determined using the method outlined by Sanchez-Alonso and Lachica [25] and hydrocyanic acid by that of

AOAC [19]. Phytic acid was determined by the method of McCane and Widdowson [26]. The Folin-Denis Spectrophotometric method described by Pearson [27] was used for determination of tannins.

2.6 Animals

Albino mice (21-24 g) of either sex were obtained from the University of Uyo Animal House. They were kept under standard conditions in the animal house and maintained on standard animal pellets and water *ad libitum*. The animals were handled by the National Institute of Health (NIH) guidelines for the care and use of laboratory animals. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo, Nigeria.

2.7 Acute Toxicity Test

The median lethal dose (LD_{50}) of the extract was estimated using albino mice by intraperitoneal (i. p.) route using the method of Lorke [28]. This involved the administration of different doses (1000, 2000, 2500, 2750, 3000 mg/kg) of the extract to five groups of three mice each. The animals were observed for the manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each group within 24 hours was recorded. The LD_{50} was calculated as the geometrical means of the maximum dose producing 0 % mortality and the minimum dose producing 100 % mortality. That is

$$LD_{50} = \sqrt{D_0\% \times D_{100}\%} \quad (4)$$

Data handling: All analyses were done in triplicate and values were expressed as mean ± standard error of mean (i.e. Mean ±SEM).

3. RESULTS AND DISCUSSION

The results of the nutrient, antinutrient compositions and median lethal dose of leaf of *Microdesmis puberula* are shown as follows.

3.1 Phytochemical Analysis

The result of phytochemical analysis of leaves of *Microdesmis puberula* indicates the presence of alkaloids, cardiac glycosides, flavonoids, saponins and terpenes, while tannins, anthraquinones and phlobatannins are found to be absent. The medicinal properties of these bioactive compounds are quite numerous and have been well documented [29-33]. The result of the quantitative phytochemical determination shows that the leaves of *M. puberula* are richer in alkaloids compared to other phytochemicals like saponins, flavonoids and cardiac glycosides. The presence of these bioactive compounds in the leaves of *M. puberula* corroborates the various pharmacological activities of this plant and supports its widespread use in traditional medicine.

3.2 Proximate Composition

The result of the proximate composition of the leaves of *M. puberula* shows moisture content of 48.17% which is not high but quite appreciable. High moisture content tends to promote microbial contamination and chemical degradation [34]. The protein content was found to be 17.56%, while ash content was 5.08%. Crude fibre level was found to be 18.16%, while the crude lipid content was found to be 6.51%. Lipids provide very good sources of energy and aid in the transport of fat-soluble vitamin, insulate and

Table 1. Contents of phytochemicals in the leaf extract of *Microdesmis puberula*

Phytochemical	Test/reagents	Detection
Alkaloids	Dragendroff's and Mayer's	Present
Anthraquinones	Benzene/ammonia solution	Absent
Flavonoids	Magnesium metal, HCl	Present
Cardiac glycosides	Libermann's, Keller-Killiani's	Present
Phlobatannins	HCl acid solution	Absent
Saponins	Frothing, Fehling's test	Present
Tannins	Ferric chloride solution	Absent
Terpenes/steroids	Chloroform, H_2SO_4 acid	Present
Protein	Biuret solution	present
Carbohydrate	Molish	present

protect internal tissue and contribute to important cell processes [35]. The level of carbohydrate present in the leaves of *M. puberula* (4.52%) was quite low and the calorie was found to be 146.91 Kcal.

Table 2. Quantitative determination of some bioactive compounds in *Microdesmis puberula* leaf extract

Plant constituents	Concentration (mg/100 g)
Alkaloids	12.80 ± 0.02
Saponins	5.85 ± 0.01
Flavonoids	2.48 ± 0.01
Cardiac glycoside	1.25 ± 0.01

Each value represents mean ± SEM of three determinations analyzed individually in triplicate.

Table 3. Results of proximate composition of *Microdesmis puberula* leaf extract

Parameter	Mean ± SEM
Moisture (%)	48.17 ± 0.03
Ash (%)	5.08 ± 0.01
Crude fibre (%)	18.16 ± 0.02
Crude lipid (%)	6.51± 0.01
Crude protein (%)	17.56 ± 0.02
Carbohydrate (%)	4.52 ± 0.01
Calorie (Kcal/KJ)	146.91/614.67

Each value represents mean ± SEM of three determinations on dry weight (DW) basis.

Table 4. Levels of minerals in *Microdesmis puberula* leaf extract

Mineral (mg/100 g DW)	Mean ± SEM
Calcium	163.50 ± 2.01
Iron	188.70 ± 2.50
Magnesium	168.40 ± 1.50
Potassium	42.55 ± 0. 55
Zinc	40.80 ± 1.01

Values are mean ± SEM calculated as mg/100 g dry weight analyzed individually in triplicate

Table 5. Levels of antinutrients in *Microdesmis puberula* leaf extract

Antinutrients (mg/100 g DW)	Mean ± SEM
Phytic acid	18.220 ±0.030
Hydrocynate	0.002 ± 0.000
Oxalate	1.861 ± 0.00

Values are mean ± SEM calculated as mg/100 g dry weight analyzed individually in triplicate.

3.3 Mineral Element Composition

The composition of mineral elements in the leaves of *Microdesmis puberula* is presented in Table 4. This result indicates that *M. puberula* leaves contain calcium, magnesium, potassium, iron and zinc. The calcium content was found to be 163.50 mg/100 g. This value is higher than 100 mg/100g earlier reported for Indian *Solanum tubirosam* [36] and 101 mg/100 g in Vietnamese *Ipomea aquatia* leaves [37]. The iron content was found to be 188.70 mg/100 g. The concentration of iron in *M. puberula* leaves is higher than 34.92 mg/100 g reported for *Telfairia occidentalis* leaves grown in South West Nigeria [38] but lower than 210.30 mg/100 g reported for *Ipomea aquatic* forsk leaves [39]. The magnesium content *of M. puberula* leaves was found to be 168.40 mg/100 g. This value is higher than 61.08 mg/100 g reported for *veronia amygdalina*, *Gongronema latifolium* (92.51 mg/100g), *Ocimium grastissimum* (88.25 mg/100 g) but lower than 249.92 mg/100 g obtained for *Amaranthus hybridus* [38]. The potassium content in the leaves of *M. puberula* was found to be 42.55 mg/100 g, while the zinc content was 40.80 mg/100 g. Earlier findings have implicated mineral elements in many significant health-promoting functions within the human body [40-41] and thus, consumption of *M. puberula* leaves might play useful roles in optimizing their bioavailability and utilization.

3.4 Antinutrient Composition

Levels of hydrogen cyanide, oxalate and phytic acid in the ethanol extract of *Microdesmis puberula* were generally low and are given in Table 5. The content of hydrogen cyanide (0.002 mg/100 g DW) was far below the lethal dose of 35 mg/100 g [42]. The quantity of total oxalate in the extract (1.861 mg/100 g DW) was below the toxic level of 2-5 g/100 g [43]. This value is very low when compared to 10.0 mg/100 g obtained for older leaves of *Telfairia occidentalis* [44]. Oxalates are known to complex with calcium to form calcium crystals which get deposited as renal stones which are associated with blockage of renal tubules [45]. The level of phytic acid was found to be 18.220 mg/100 g. Phytic acid is the major phosphorus storage compound in African leafy vegetables [46]. Although phytic acid is an antioxidant, it has been shown to inhibit absorption of minerals. Phytic acid chelates multivalent metal ions such as zinc, calcium and iron, thus it is a strong inhibitor of iron-mediated free radical generation [47]. The disadvantage of this is that a diet high in phytate content reduces the bioavailability of zinc, iron and calcium and has adverse effects on the digestion of proteins and starches [48]. Tannins

were however not found to be present. It is an established fact that most of these toxicants are eliminated during processing and cooking [44].

3.5 Acute Toxicity Test

The acute toxicity test showed that the administration of the ethanol crude leaf extract at a dose of 3000 mg/kg resulted in 100% mortality in group five, while no mortality was recorded in groups one to four administered with 1000, 2000, 2500 and 2750 mg/kg of the sample respectively. The LD_{50} value of 2872.28 mg/kg indicates slight toxicity. In general, the smaller the LD_{50} value, the more toxic the sample is and the larger the LD_{50} value, the lower the toxicity with respect to the same route of administration. It can be inferred courtesy of the findings of this study that a very large quantity of the material will be required to cause a toxic response.

4. CONCLUSION

The result of this study has shown that the leaves of *Microdesmis puberula* contain an appreciable amount of protein, fibre and lipid in addition to some mineral elements. The amount of carbohydrate was low. The low levels of anti-nutrients – phytic acid, oxalates and hydrocynides suggest that the consumption of leaves of *M. puberula* is not harmful and therefore not expected to produce any adverse health effects. The presence of some phytochemicals which have great pharmacological significance supports the ethnomedicinal use of this plant in the treatment of diseases. This study, therefore, concludes that *M. puberula* leaves can contribute significantly to the human nutritional requirements, while also offering adequate protection against some diseases.

The World Health Organisation (WHO) has indicated that as many as eighty per cent of all people living in the developing countries make use of herbal medicine as their main source of healthcare. In recent years, many people living in industrialized countries have begun taking a second look at herbal medicines due to the rising cost of medicine and healthcare in their own nations. The alternative health industry has become a billion dollar a year business, and this is driving the quest to find new plants or chemicals which are useful.

However, it is worthy of note that the Nigerian rainforest is fast disappearing as a result of a continuous increase in the demand for forest lands and forest products. In order to retain its benefits to the population and ensure a stable environment, conservation of the remaining part of the ecosystem is necessary. Efforts geared towards forest conservation in the country are faced with many obstacles. Despite these obstacles, several strategies could still be adopted to ensure the conservation of the rainforest ecosystem.

The main strategy is to successfully tackle the human elements responsible for de-reservation, deforestation and destruction of forests by getting government policymakers and the general public to appreciate the uses of, and need for the sustenance of forests as an important component of man's habitat. Forest resources conservation education should also be injected into primary and secondary schools Agricultural science syllabus, while public enlightenment should be through all available media of information dissemination, for instance, radio and TV discussions, jingles, newspapers, handbills, personal contacts and demonstration by officials. The annual tree planting exercise is one of such examples except that it is still too ceremonial in many parts of the country. Development planners should always take into consideration the ecological consequences of such developments instead of being primarily socio-politically motivated. Forest resources conservation programs in Nigeria require the support of policymakers and lawmakers nationwide and should be entrenched in our infrastructural development system with sufficient legal backing by the government.

ETHICAL APPROVAL

All authors hereby declare that "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Akan J, Abdulrahman F, Ogugbuaja V, Ayodele J. Heavy metals and anion levels

in samples of vegetable grown within the vicinity of Challawa industrial areas, Kano State, Nigeria. American Journal of Applied Sciences. 2009;6(3):534-542. http://thescipub.com/html/10.3844/ajassp.2009.534.542.

2. Aletor VA, Adeogun OA. Nutrient and antinutrient components of some tropical leafy vegetables. Food Chemistry. 1995; 53(4):375-379. http://dx.doi.org/10.1016/0308-8146(95)99830
3. Uusiku NP, Oelofse A, Duodu KG, Bester MJ, Faber M. Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. J. Food Comp. Anal. 2010;23(6): 499-509. DOI: 10.1016/j.jfca.2010.05.002
4. Dounias E. *Microdesmis puberula* Hook. F. ex Planch. In: Plant resources of tropical Africa Medicinal Plants. Schmeizer GH and Gurib-Fakim A (eds). Prota Foundation. Wageningen, Netherlands. 2008;11(1): 382-384.
5. Esonu BO, Azubuike JC, Ukwu HO. Evaluation of *M. puberula* leaf meal as feed ingredient in laying hen diets. Int. J. Poult. Sci. 2004;3(2):96-99.
6. Etukudoh I. Ethnobotany: Conventional and traditional uses of plants. The Verdict Press, Uyo, Akwa Ibom State, Nigeria; 2003.
7. Okany CC, Ishola IO, Ashorobi RB. Evaluation of analgesic and antistress potential of methanolic stem wood extract of *M. puberula* Hook .f. ex. Planch (Pandaceae) in mice. Int. J. Appl. Res. Nat. Prod. 2012;5(3):30-36.
8. Ajibesin KK, Ekpo BA, Bola DN, Essien EE, Adesanya SA. Ethnobotanical survey of Akwa Ibom State of Nigeria. J. Ethnopharmacol. 2008;115:387-488.
9. Roumy V, Hermebelle T, Zamble A, Quare S, Sahpaz S, Bailleul F. Characterization and identification of spermine and spermidine derivatives in *Microdesmis. keayana* and *Microdesmis Puberula* roots by Ionisation tandem mass spectrometry and high-performance liquid chromatography/ electrospray ionisation tandem mass spectrometry. Eur. J. Mass Spectromet. 2008;14(2):111-115.
10. Akpanyung EO, Ita SO, Opara KA, Davies, KG, Ndem JI, Uwah AF. Phytochemical screening and effect of ethanol root extract of *Microdesmis puberula* on some haematological and biochemical parameters in normal male albino Wistar rats. Journal of Medicinal Plants Research. 2013;7(31):2338-2342. DOI: 10.5897/JMPR2013.5080
11. Zamblé A, Martin-Nizard F, Sahpaz S, Reynaert ML, Staels B, Bordet R, Duriez P, Gressier B, Bailleul F. Effects of *Microdesmis keayana* alkaloids on vascular parameters of erectile dysfunction. Phytotherapy Research. 2009; 23(6):892-895.
12. Zamblé A, Sahpaz S, Brunet C, Bailleul F. Effects of *Microdesmis keayana* roots on sexual behavior of male rats. Phytomedicine. 2008;15(8):625-629.
13. Roumy V, Hennebelle T, Zamblé A, Yao JD, Sahpaz S, Bailleul F. Characterisation and identification of spermine and spermidine derivatives in *Microdesmis keayana* and *Microdesmis puberula* roots by electrospray ionisation tandem mass spectrometry and high-performance liquid chromatography/electrospray ionisation tandem mass spectrometry. European Journal of Mass Spectrometry. 2008; 14(2):111-115.
14. Zamble A, Hennebelle T, Sahpaz S, Bailleul F. Two new quinoline and tris (4-hydroxycinnamoyl) spermine derivatives from *Microdesmis keayana* roots. Chemical and Pharmaceutical Bulletin. 2007;55(4):643-645.
15. Zamble A, Sahpaz S, Hennebelle T, Carato P, Bailleul F. N1, N5, N10-Tris (4-hydroxycinnamoyl) spermidines from *Microdesmis keayana* roots. Chemistry and Biodiversity. 2006;3(9):982-989.
16. Zamblé A, Yao D, Martin-Nizard F, Sahpez S, Offoumou M, Duriez P, Brunet C, Bailleul F. Vasoactivity and antioxidant properties of *M. keayana* roots. J. Ethnopharmacol. 2006;104:263-269.
17. Esonu BO, Azubuike JC, Emenalom OO, Etuk EB, Okoli IC, Ukwu H, Nneji CS. Effect of enzyme supplementation on the performance of broiler finisher fed *Microdesmis puberula* leaf meal. International Journal of Poultry Science. 2004;3(2):112-114.
18. Esonu BO, Iheukwumere FC, Emenalom OO, Uchegbu MC, Etuk EB. Performance, nutrient utilisation and organ characteristics of broilers fed *Microdesmis puberula* leaf meal. Livestock

Research for Rural Development. 2002; 14(6):14-19.

19. AOAC. Official methods of analysis. 15th Edition, Helrich, K. (ed) Association of Official Analytical Chemists, Washington DC, USA; 1990.
20. Egan H, Kirk RS, Sawyer R. Pearson's chemical analysis of food.8th edition, Churchill Livingstone, Edinburg, United Kingdom; 1981.
21. Harborne JB. Phytochemical methods. 2nd edition, champman and Hall, London; 1984.
DOI:10.1007/978-94-009-5570-7Print
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22. Trease GE, Evans WC. Pharmacognosy. Thirteen edition, Balliere Tindall, London; 1996.
23. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta states of Nigeria. Global Journal of Pure and Applied Sci. 2001; 8:203-208.
24. El-Olemy MM, Al-Muhtadi FJ and AFA Afifi. Experimental phytochemistry: A laboratory manual. King Saud University Press, Saudi Arabia. 1994:21-27.
25. Sanchez-Alonso F, Lachica M. Seasonal trends in the elemental content of plum leaves. Commun. Soil Sci. Plant Anal. 1987;18:31-43.
DOI:10.1080/00103628709367801
26. McCance RA, Widdowson EM. Phytin in human nutrition. Biochemic J. 1953; 29(12):2694-2699.
PMCID: PMC1266816
27. Pearson D. Chemical analysis of foods. (7th Ed), Henry Edward company, Churchchill, Livingstone, London. 1976: 218-336.
28. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54:275–286.
29. David H. The new holistic herbal.3rd Edition. Findhorn Press, USA. 1983;241.
30. Frantisek S. The natural guide to medicinal herbs and plants. Tiger Book International, plc, Twickers, London. 1998;8-20.
31. Price KR, Johnson IT, Fenwick GR. The chemical and biological significance on saponins in food and feeding stuffs.CRC Crit. Rev. Food Sci. Nutr. 1987;26(1):27-135.
PMID: 3308321
32. Banso A, Adeyemo SO. Evaluation of antimicrobial properties of tannins isolated from *Dichrostachys cinerea*. African Journal of Biotech. 2007; 6(15):1785-1787.
http://dx.doi.org/10.5897/AJB2007.000-2262
ISSN: 1684-5315.
33. Akpan MM, Odeomena CS, Nwachukwu CN, Danladi B. Antimicrobial assessment of ethanol extract of *Costus afer* leaves. Asian J. Plant Sc. Research. 2012;2(3): 335-341.
34. Hussain K, Ismail Z, Sadikun A, Ibrahim P. Proximate and qualitative analysis of different parts of *Piper sarmentosum,* and quantification of total amides in various extracts. Phcog. Res. 2009;1:113-119.
35. Pamela CC, Richard AH, Denise RF. Lippincotts illustrated reviews in biochemistry. 3rd edition, Lippincott Williams and Wilkins, Philadelphia. 2005; 335-388.
ISBN-13: 978-1451175622.
36. Okaka JC, Akobundu EN, Okaka CA. Food and nutrition, an integrated approach. O. J. C. Academic Pub. Enugu. Nigeria; 2006.
37. Ogle BM, Grivetti LE. Legacy of the chameleon: Edible wild plants in the Kingdom of Swaziland, Southern Africa. A cultural, ecological nutritional study. Part IV: Nutritional analysis and conclusion. Ecol. Food Nutr. 1985;17:41-64.
38. Asaolu SS, Adefemi OS, Oyakilome Ajibulu IG, Asaolu MF. Proximate and mineral composition of Nigerian leafy vegetables. Journal of Food Research. 2012;1(3):214-218.
DOI: http://dx.doi.org/10.5539/jfr.v1n3p214
39. Umar KJ, Hassan LG, Dangoggo SM, Ladan MJ. Nutritional composition of water spinach (*Ipomoea aquatica* Forsk.) leaves. Journal of Applied Sciences. 2007; 7(6):803-809.
40. Aremu CY, Udoessien EI. Chemical estimation of some inorganic elements in selected fruits and vegetables. Food Chemistry. 1989;37:227-234.
41. Gbolahan D. Lesson note on medicinal importance of trace elements. Center for Natural Health Studies, Surulere, Nigeria. 2001;67.
42. Munro A, Bassir O. Oxalate in Nigerian vegetables. W. A. J. Biol. Appl. Chem. 1969;12:14-17.

43. Onwuka GI. Food analysis and instrumentation: Theory and practice. Naphtalic Prints, Surulere, Lagos. Nigeria. 2005;219-230.
44. Akwaowo EU, Ndon BA, Etuk EU. Minerals and antinutrients in fluted pumpkin (*Telfairia occidentalis* Hook f.). Food Chemistry -Analytical, Nutritional and Clinical Methods Section. 2000;70(2):235-240. http://dx.doi.org/10.1016/S0308-8146(99)00207-1
45. Banso A, Adeyemo SO. Evaluation of antimicrobial properties of tannins isolated from *Dichrostachys cinerea*. African Journal of Biotech. 2007; 6(15):1785-1787. http://dx.doi.org/10.5897/AJB2007.000-2262 ISSN: 1684-5315
46. Champ MM. Non-nutrient bioactive substances of pulses. British Journal of Nutrition. 2002;88:307-319.
47. Schlemmer U, Frølich W, Prieto RM, Grases F. Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis. Molecular Nutrition and Food Research. 2009; 53:330–375.
48. Reddy NR, Pierson MD. Reduction in antinutritional and toxic components in plant foods by fermentation. Food Research International. 1994;27:281–290.

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