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Nutraceutical Potentials of *Spilanthes Filicualis*

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I. Introduction

Africa is a continent with plants of economic and medicinal importance capable of meeting the nutrient and health needs of the populace (Josiah and Bartholomew, 2015). The current emphasis on healthy living based on antioxidant intake and the implication of oxidative stress molecules / free radicals on some diseased conditions has generated renewed interest in screening for plants with high antioxidant properties (Bouayed *et al.,* 2008). The identification and quantification of bioactive components that contribute to free radical scavenging activity are essential in the discovery of new drugs (Farombi, 2003). The basic functional units of plants are phytochemicals, which are the bioactive ingredients present in plants (Srinath and Laksmi, 2014). They include alkaloids, saponins, tannins, terpenoids, polyphenols, etc. These compounds are believed to be responsible for the medicinal properties attributed to plants, which is as result of their ability to inhibit the reactions of ROS, neutralizing free radicals by donating one of their electrons and blocking nitrosamine formation, stimulate the immune system and maintain cell membrane integrity (Sen *et al.*, 2010; Saha and Tamaraka, 2011). This study focused on exploring the nutritional compositions of leaves of *Spilanthes filicualis* and the *in-vitro* antioxidants properties of its ethanol leaf extract.*Spilanthes filicaulis* is a common plant grown in Brazil, Africa, and South America. It belongs to the family Asteraceae. The extract of the plant has been employed in the therapeutic cure of different ailments (Atawodi *et al.*, 2014). The flower heads of *Spilanthes filicaulis* are used to prevent scurvy and aid digestion. Experiments on rats suggest that cold water extract of *Spilanthes filicaulis* acts as a loop diuretic (Ratnasooriya *et al.*, 2004). Jahan *et al.* (2013) demonstrated that the ethanol extract of *Spilanthes filicaulis* showed antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes, Enterococcus faecalis*, *Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi,* and *Shigella dysenteriae. S. filicaulis* contains Spilanthol which shows activity against Plasmodium falciparum (Gasquet *et al.*, 1993). Aqueous leaf extracts of *S. filicaulis* have been reported as potent antidote for poison (Atawodi *et al.*, 2014)

Plate. 1: Spilanthes filicaulis leaves

Local names of this plant in Nigeria are: Hausa = parpehi, Igbo = osana or ósē ànì, Bayelsa (Kolokuma) = kírí ẹ̀bẹ̀dẹ̀, Common name = Toothache plant.

II. Materials and Methods

a) Materials

i. *Collection of plant material and identification*

Spilanthes filicualis leaves were harvested from a swampy field site in Emonu– Orogun, Delta State, Nigeria in April, 2017. The Department of Botany, Delta

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State University, Abraka– Nigeria identified and authenticated the plant under study.

b) Methods

i. *Preparation of plant extract*

Fresh leaves of *Spilanthes filicualis* were washed with distilled water, and air dried for two weeks and then reduced to coarse powder using a manual grinder. Then, 100g of coarsely powdered leaves was extracted with 400ml of 80% ethanol using cold maceration for 24hours. The extract was filtered through cheesecloth with a fine pore, and the filtrate filtered for the second time using Whatman No. 1 filter paper. The resulting extract concentrated at 50°C in a rotary evaporator for 2hr, and then transferred to a water bath maintained at 40°C and evaporated to dryness to yield a dark green mass. The obtained extract was put in a glass container and stored at 4°C until when required for use.

ii. *Proximate analysis*

Moisture, ash, crude fiber, crude fat, crude protein, and total carbohydrate contents were determined using standard analytical procedure (AOAC, 1990).

iii. *Determination of energy content of the leaves*

The energy content was calculated by multiplying the mean values of crude protein, crude fat and total carbohydrate by the Atwater factors of four (4), nine (9), four (4) respectively, taking the sum of the products and expressing the result in Kcal per 100g sample as reported by Onyeike and Acheru (2002).

iv. *Determination of antioxidant vitamins*

Vitamin A and C were estimated using Kirk and Sawyer (1991), while Vitamin E was determined by Futter – Mayer colorimetric method.

v. *Determination of minerals*

The Varian AA240 Atomic Absorption Spectrophotometer was used in the mineral analysis by the method of American Public Health Association (1995).

III. Phytochemical Analysis

Preliminary phytochemical screening of ethanol leaf extract of *Spilanthes filicaulis* was carried out using standard methods as described by Borokini and Omotayo (2012), and Njoku and Obi (2009) to screen for the presence of various chemical constituents, while the quantitative phytochemical estimation was done using the Varian Gas Chromatograph (HRGC, DB-5MS, England).

a) Determination of antioxidant activity and free radical scavenging potentials

i. *DPPH radical scavenging assay*

The determination of free radical scavenging activity was by the1,1- diphenyl- 2- picrylhydrazyl (DPPH) radical assay (Manzocco *et al.*, 1998). A0. 2 ml of different concentrations of ethanol leaf extract of *Spilanthes filicualis* (2. 0- 10 mg/ml) was added to 2 ml of DPPH solution (0. 3 mM). After 30 min of incubation in the dark, the absorbance was measured at 517 nm. The percentage inhibition of the DPPH radical scavenging was calculated using the equation below:

$$\% \text{ inhibition of DPPH radical} = ([A_0 - A_1]/ A_0) \times 100$$

Where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance in the presence of the extract.

ii. *Nitric oxide (NO) free radical scavenging activity*

The method of Marcocci *et al.,* 1994 was used for Nitric oxide assay. Two millilitres of 10 mM sodium nitroprusside dissolved in 0. 5 ml of 10mM phosphate buffer saline (pH 7. 4) was mixed with 0. 5 ml of different concentrations of ethanol leaf extract of *Spilanthes filicualis* (2.0- 10mg/ml). The mixture was then incubated at 25°C. After 150 min of incubation, 0.5 ml of the incubated solution was withdrawn and mixed with 0.5 ml of Griess reagent [(1.0 ml sulfanilic acid reagent (0. 33% in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylenediamine dichloride (0. 1% w/v)]. The mixture was then incubated at room temperature for 30 min, and its absorbance was measured at 546 nm against blank. The percentage inhibition of the nitric oxide radical scavenging was calculated using the equation below:

$$\% \text{ inhibition of NO radical} = ([A_0 - A_1]/ A_0) \times 100$$

Where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance in the presence of the extract.

iii. *Reducing power assay (RP)*

Reducing power was assayed using the method of Oyaizu, (1986). A 2.5ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) were added to 1.0 ml of different concentrations of ethanol leaf extract of *Spilanthes filicualis* (2.0 - 10 mg/ml). The resulting mixture is incubated at 50°C for 20 min, followed by the addition of 2.5 ml of Trichloroacetic acid (10% w/v). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 ml), mixed with distilled water (2.5 ml) and 0.5 mL of $FeCl_3$ (0.1%, w/v).The absorbance was then measured at 700 nm against blank sample (that contained distilled water and sodium phosphate buffer).

iv. *Metal chelation assay*

A 150µl of freshly prepared 2 mM $FeSO_4.7H_2O$ was added to a reaction mixture containing 168 µl of 0.1 M Tris-HCl (pH 7.4), 218 µl saline and100 µl of different concentrations of ethanol leaf extract of *Spilanthes filicualis* (2.0 - 10 mg/ml). The reaction mixture was incubated at 37°C for 5 min, before the addition of 13 µl of 0.25% 1,10 -Phenanthroline (w/v). The absorbance

was subsequently measured at 510spectrophotometer against the blank (Puntel *et al.*, 2005).

The percentage inhibition of the Metal chelating radical scavenging was calculated using the equation below: % inhibition of Metal chelating radical = ($[A_0 - A_1]/ A_0$) x 100

Where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance in the presence of the extract.

v. *Inhibition of lipid peroxidation in egg homogenate.*

Ten percent egg homogenate of 0.5 ml volume and different concentrations of ethanol leaf extract of *Spilanthes filicualis* 0.1 ml (2.0 - 10 mg/ml) were mixed in a test tube, and their final volume was made to 1.0 ml by addition of distilled water. Finally 0.05 ml 0.07M FeSO4 was added to the above mixture and incubated at 37°C for 30 min to induce lipid peroxidation. After that, 1.5 ml of 20 % acetic acid and 1.5 ml of 0.8 % TBA (prepared in 1.1% sodium dodecyl sulphate) and 0.05 ml 20 % TCA was added, vortexed and heated in boiling water bath for 60 min. After cooling, 5.0 ml normal butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm (Roberto *et al.*, 2000).

% inhibition of Lipid peroxidation = ([1– E]/C) x 100

Where C = absorbance of fully oxidized control and E = absorbance in the presence of extract.

vi. *Statistical analysis*

All data were subjected to statistical analysis. Values were reported as Mean ± Standard deviation while one way ANOVA was used to test for differences. The results were considered significant at p-values of less than 0.05 ($p<0.05$).

IV. Results

The results of the proximate composition of the leaves of *Spilanthes filicualis* are in Table 1. Total carbohydrate (64.9%), crude protein (6.30%), crude fat (2.00%), Fibre (4.50%), Moisture (17.8%) and Ash (4.55%). The energy content of the leaves of *Spilanthes filicualis* was 303Kcal/100g sample.

Table 1: Proximate composition of *Spilanthes filicualis* leaves

Parameters	Composition (%)
Total carbohydrate	64.9 ± 0.89
Crude protein	6.30 ± 0.30
Crude fat	2.00 ± 0.10
Fibre	4.50 ± 0.26
Moisture	17.8 ± 0.14
Ash	4.55 ± 0.07
Energy content (Kcal/100g sample)	303

Values are means ± standard deviations of triplicate determinations.

Results from the evaluation of the antioxidant vitamin composition are in Table 2. Antioxidant vitamins of A, C and E, were determined with the highest value of these vitamins being vitamin A (239mg/100g).

Table 2: Antioxidant vitamins composition of *Spilanthes filicualis* leaves.

Parameters	Composition (mg/100g)
Vitamin A	239 ± 2.90
Vitamin C	7.03 ± 0.17
Vitamin E	0.23 ± 0.02

Values are means ± standard deviations of triplicate determinations.

Mineral composition of *Spilanthes filicualis* leaves is presented in Table 3. Calcium (20.9 mg/kg) recorded the highest concentration, followed by Magnesium (19.8 mg/kg) and the lowest was Cobalt (0.13 mg/kg).

Table 3: Mineral concentration of *Spilanthes filicualis* leaves

Mineral	Concentration (mg/kg)
Chromium	0.81 ± 0.10
Magnesium	19.8 ± 0.71
Cobalt	0.13 ± 0.02
Iron	17.5 ± 0.37
Copper	0.87 ± 0.12
Manganese	2.06 ± 0.03
Zinc	18.5 ± 0.33
Selenium	0.59 ± 0.09
Calcium	20.9 ± 0.06
Sodium	14.2 ± 0.19
Potassium	8.33 ± 0.19

Values are means ± standard deviations of triplicate determinations.

Phytochemical screening of ethanol leaf extract of *Spilanthes filicaulis* is in Table 4. Bioactive components of saponins, tannins, flavonoids, Alkaloid, and phenol were found to be present.

The results of the quantitative phytochemical analysis of leaves of *Spilanthes filicualis* are presented in Table 5. Bioactive components of the flavonoids family were obtained. Highest concentrations were rutin (27.6 μg/ml) followed by kaempferol (26.0 μg/ml), and the lowest was anthocyanin (0.69 μg/ml).

Table 4: Qualitative phytochemical screening of ethanol leaf extract of *Spilanthes filicualis*

Phytochemi	Concentrations
Saponin	+
Tannin	+
Terpene	–
Flavonoid	+
Phlobatannin	–
Alkaloid	+
Glycoside	–

Key: + Present, - Absent

Table 5: Quantitative phytochemical analysis of Spilanthes filicualis leaves

Phytochemicals	Concentration (µg/ml)
Anthocyanin	0.69
Tannin	7.77
Rutin	27.6
Phenol	7.08
Epicatechin	3.59
Lunamarine	8.77
Saponin	21.3
Sapogenin	20.0
Phytate	0.91
Kaempferol	26.0
Catechin	20.2

Results of the i*n-vitro* antioxidant and free radical scavenging activities of ethanol leaf extract of *Spilanthes filicualis* are embodied in Table 6. It revealed that plant activity against DPPH radical, reducing power and lipid peroxidation (LPO) was significantly increased with increasing concentrations ($p<0.05$), while metal chelating activity recorded a statistical decrease with increasing dose (24.36 $\pm$ 1.41 to -32.31 $\pm$ 3.60 % inhibition) ($p<0.05$). Nitric oxide scavenging activity recorded a statistical increase from 2mg/ml to 6mg/ml (1.87 $\pm$ 0.20 to12.68 $\pm$ 2.1 % inhibition) and conversely statistical decrease in from 8mg/ml to 10mg/ml (-6.64 $\pm$ 0.62 to -29.80 $\pm$ 2.15 % inhibition) ($p<0.05$).

Table 6: In-vitro antioxidant activity of ethanol leaf extract of *Spilanthes filicualis*

Conc.(mg/ml	% Inhibition				700nm
	DPPH	NO	MC	LPO	RP
2	31.03 $\pm$ 1.44^{a}	1.87 $\pm$ 0.20^{a}	24.36 $\pm$ 1.41^{a}	42.32 $\pm$ 2.73^{a}	0.37 $\pm$ 0.04^{a}
4	46.15 $\pm$ 1.87^{b}	12.26 $\pm$ 0.73^{b}	17.84 $\pm$ 1.47^{b}	61.20 $\pm$ 1.59^{b}	0.54 $\pm$ 0.16^{b}
6	52.87 $\pm$ 1.67^{c}	12.68 $\pm$ 2.17^{b}	7.26 $\pm$ 0.30^{c}	63.22 $\pm$ 3.27^{b}	0.61 $\pm$ 0.05^{b}
8	57.54 $\pm$ 1.23^{d}	-6.64 $\pm$ 0.62^{c}	2.76 $\pm$ 0.19^{d}	68.67 $\pm$ 0.74^{c}	0.67 $\pm$ 0.03^{c}
10	61.81 $\pm$ 1.86^{e}	-29.80 $\pm$ 2.15^{d}	-32.31 $\pm$ 3.60^{e}	85.35 $\pm$ 1.44^{d}	0.74 $\pm$ 0.02^{c}

Values are means $\pm$ standard deviations of triplicate determinations. Values not sharing common superscript on the same column differ significantly ($p < 0.05$).

DPPH =1, 1-diphenyl -2-picryl hydrazyl, NO = Nitric oxide, MC = Melating Chelating, LPO = Lipid peroxidation, RP = Reducing power

V. Discussion

Proximate analysis of *Spilanthes filicualis* leaves reveals that the plant is rich in carbohydrate (64.9%). Carbohydrate is a source of fuel in living cells which is required for the production of energy and maintenance of general body function (Adesuyi *et al.*, 2012). Proteins play an essential role in information transmission in the body, serving as a neurotransmitter and genetic transmission of traits, tissue repair and general growth (Voet *et al.,* 2008). The presence of moisture content in food aids digestion. High values of moisture content result in short shelf life of food (Shukla *et al.*, 2015). Dietary fiber is essential in aiding digestion, removal of cholesterol, detoxification of carcinogens etc. (Dhingra *et al.,* 2012), while the level of ash content is an indication of the mineral concentration of the plant leaf. Thus with a crude protein content of 6.30%, a dietary fiber of 4.50%, an ash content of 4.55% and crude fat content of 2.00%, implies that if the leaves of *Spilanthes filicualis* supplemented with another nutrient-rich plant, it could be of great nutritional benefits.

Quantitative analysis of vitamin A, C and E are indicative of an enhanced free radical scavenging

capacity of the plant. Vitamin A composition of 239 mg/100 was the highest value recorded for selected antioxidant vitamins determined in this study. Vitamin A is not only responsible for neutralizing the effect of singlet oxygen but also contributes to the immunostimulatory properties of *S. filicaulis* and for better vision (Pham-Huy *et al*., 2008). The concentration of vitamin C is higher than that of vitamin E. Vitamin C potentially regenerates vitamin E and renews its potency. The presence of vitamin E in the leaves of this plant suggests its antioxidant activity which is responsible for stabilization of biomembrane structure. The vitamin constituents of *S.filicaulis* may establish, in part, the efficient regulation of reactive oxygen species and scavenging activity observed in the leaves extract investigated in addition to maintaining membrane fluidity and integrity (Niki *et al.,* 1995).

Minerals are essential component necessary for optimal functions of the body as they play an immense role in energy production, defense against disease, bone formation, blood coagulation, hormonal regulation, transportation of fluid, muscle contraction and nerve transmissions (Delvin, 2006). Zinc, copper, and manganese are cofactors of superoxide dismutase (SOD), and iron is a component of hemoglobin and also serves as a cofactor for catalase, Selenium is a component of the prosthetic group of glutathione peroxidase (GPx). Calcium plays an essential role in bone formation and regulation of vitamin D, while Potassium and sodium serve as the main cation of the internal and external cellular fluid respectively, and also aid intracellular membrane transport system (Na^+ - K^+ ATPase transporter). Cobalt is vital for DNA synthesis, cell division, and cell growth. Chromium is believed to have an implicating role in upholding the configuration of the RNA molecule (Delvin, 2006; Soetan *et al.,* 2010). Thus, *Spilanthe filicualis* leaves could be a useful supplementary source of mineral nutrients to humans.

Phytochemicals are the important component of plants, and they play a vital role that is beneficial to human health (Batta, 2016). Qualitative phytochemical screening of ethanol leaf extract of *Spilanthes filicualis* showed the presence of saponins, tannins, flavonoids, alkaloids, and phenol. Ndam *et al*. (2014) reported the presence of tannins and steroids, while Ilondu *et al*. (2014) reported the presence of the enlisted phytochemicals found in this study and also detected the presences of terpenes in leaf extracts of *Spilanthes filicualis*. This variation could be as a result of soil and climatic conditions. Quantitative phytochemical analysis showed that plant contained flavonoids class of phytochemicals of anthocyanins, catechins epicatechin and kaempferol; phenol, glycosides class of saponins and rutin, alkaloid, and steroid glycoside compound sapogenin. Phytate was found to be 0.91 $\pm$ 0.02 µg/ml (0.91ppm). Phytate is well known for decreasing the bioavailability of minerals such as zinc, iron, calcium, magnesium, manganese, and copper (Kumar *et al.,* 2010). Wreesman (2014) reported that the levels of phytate more than 1000ppm affects mineral absorption. Saponin has anti-inflammatory effects, hemolytic activity, and cholesterol binding properties. Flavonoids are known to have antimicrobial, anti-inflammatory and antioxidant properties (Tijjani *et al.,* 2013; Myha *et al.,* 2014; Batta, 2016). Phenols play an active role in free radical scavenging by acting as a quencher to free radicals reactions (Tijjani *et al.,* 2013).

The *in-vitro* antioxidant study revealed that ethanol leaf extract of *Spilanthes filicualis* possesses antioxidant activity in a dose-dependent manner against DPPH, reducing power and lipid peroxidation activity ($p<0.05$). The extract showed a high level of percentage inhibition against lipid peroxidation than DPPH. Antioxidant activities of plants relates to the presence of phytochemicals. DPPH assay is based on scavenging activities of free radicals which serve as good criteria for measuring scavenging activities of the plants, the levels of total phenols and flavonoids are determinant to DPPH scavenging activity of plants (Boni *et al.,* 2014). Antioxidants activity against reducing power are attributed to the presences of reductones. Reductones mechanism of action is by the disintegration of free radical chains (Boni *et al.,* 2014).

VI. Conclusion

The study showed that *Spilanthes filicualis* leaves are rich in carbohydrate as shown in the result of the proximate composition, the presences of bioactive component of phenol, flavonoids, alkaloids, tannins, and saponins were detected from the qualitative phytochemical screening. The quantitative phytochemical analysis showed that rutin had the highest concentration, while the lowest was anthocyanin. Calcium concentration recorded the highest value and cobalt was the lowest from the mineral composition determination, whiles the highest vitamin, was recorded for vitamin A. The *in-vitro* antioxidant activities of ethanol leaf extract of *Spilanthes filicualis* showed scavenging activities against DPPH, reducing power and lipid peroxidation. The leaves of S. filicaulis possesses both good nutrient and medicinal properties.

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