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Total Phenol, Flavonoid, Tannins, Vitamin C and Spectral Analysis of Ethanolic Extract of *Spilanthes filicaulis*

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Abstract: *Spilanthes filicaulis* (Compositae or Asteraceae) is a genus comprising of over 60 species that are widely distributed in tropical and subtropical regions of the world, such as Africa, America, Borneo, India, Sri Lanka and Asia. The total phenolic, flavonoid, tannins, b-carotene and lycopene content of the leaf and flower parts of *Spilanthes filicaulis* were evaluated. UV-Vis spectrophotometric scanning of leaf and flower in ethanol was also carried out. The methanolic extracts of leaf and flower of *Spilanthes filicaulis* had higher phenolic, flavonoid, tannin and ascorbic acid contents these bioactive compounds contribute to the antioxidant activity of the plant.

Keywords: Ethanolic Extract, Total Phenol, Flavonoid, Tannins, Vitamin C, Spectral Analysis, *Spilanthes filicaulis*, anti-oxidant activities.

INTRODUCTION

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as fungi, insects and herbivorous mammals. These functions include anti-oxidative activities, enzymatic activities, hormonal activities, antimicrobial effect, DNA replication, translation and transcription [1].

Plants that possess therapeutic properties or exert beneficial pharmacological effect on the human body are generally designed as medicinal plants.

Medicinal plants naturally synthesize and accumulate some secondary metabolites, like alkaloids, sterols, terpenes, flavonoids, saponins, glycosides, cyanogenics, tannins, quinines, volatile oil. Plants have been used in folk medicine in most cultures throughout the world [2].

A wide range of pharmaceutical and biological activities are exhibited by these plants, extract from leaves, stems, roots, seeds, and barks from these plants are used for the preparation of medicines, syrups from herbal remedies including digitalis, aspirin and opium, used for the treatment of injuries, oxidative related diseases and inflammations are bound [3].

Spilanthes filicaulis belongs to the family *Asteraceae*, it is distributed in tropical and subtropical regions of the world, such as Africa, America, Borneo, India, Sri Lanka and Asia [4]. It contains certain biological active and pharmacological active compounds. *Spilanthes filicaulis* contains medicinal properties and characteristics which are used for drug productions, traditionally used for the treatment of several diseases such as toothache, stomache ache, gastritis, malaria, cough, sore mouth, dysentery, ear treatment. In view of its wide use and its chemical composition, the ethanol extract of *Spilanthes filicaulis* was determined for its in vitro quantitative antioxidant properties.

MATERIALS AND METHODS

Chemicals and reagents

Sodium carbonate, potassium fericyanide, sulphuric acid, sodium nitrite, aluminium chloride; iron (iii) chroride, trichloroacetic acid, (TCA), gallic acid, quercetin, sodium hydroxide, hexane, acetone, thiourea, ascorbic acid, dinitrophenyl hydrazine, copper sulphate; were all purchased from Sigma Aldrich (England).

Plant Collection

The leaf and flowers of *Spilanthes Filicaulis* were obtained from a farm in Amassoma, Southern Ijaw Local Government Area of Bayelsa State Nigeria. The plant species (*Spilanthes filicaulis*) was authenticated by the Department of Crop/Animal Science, Faculty of

Agricultural Science and Technology, Niger Delta University.

Plant Extraction

35g of the dried leaf and 20g of the dried flower was grounded and weighed and macerated with 400ml of ethanol for the leaf and 200ml of ethanol for the flower in a sterilized conical flask for 72 hours, then the leaf and flower extract were then filtered and the filtrate evaporated.

UV-VIS Spectrophotometric analysis of extract

Preparation of 0.02 % w/v Spilanthes filicaulis 20 mg of leaf and flower were weighed into a small beaker. A small amount of ethanol was added to the beaker. The mixture was carefully transferred into a 100 ml volumetric flask. Ethanol was used to rinse the beaker and transferred to the flask. The volume in the flask was made up to 100 ml mark with ethanol. The flask was then covered and placed in a hot water bath at 50 ^{0}C with periodic shaking until all the extract was completely dissolved. The solution was allowed to cool at room temperature. Using ethanol as blank, the absorbance was read on UV-Vis spectrophotometer and the procedure was replicated at least thrice. A graph of absorbance against wavelength was plotted and lambda maximum (λm) was determined.

Total Phenol Estimation

The total phenol content of *Spilanthes filicaulis* leaf and flower extract was estimated using Folin-Ciocalteu reagent method as described by [5]. The plant sample 100 mg/ml, 1.0 ml was mixed thoroughly with 5 ml Folin-Ciocalteu reagent (diluted tenfold) and after 5 minutes, 4.0 ml of sodium carbonate (0.7M) was added and the mixture was allowed to stand for 1 hour with intermittent shaking; for colour development. The absorbance was measured at 750 nm in a spectrophotometer against a blank. The blank solution contained the solvent used to dissolve the plant extract. Gallic acid was used as a standard. Total phenol contained in the plant extract was calculated as Gallic acid equivalents per gram of extract (GAE/g extract).

Total Flavonoids Estimation

The total flavonoid content of *Spilanthes filicaulis* leaf and flower extract was determined using a colorimetric method described by [6] with slight modifications. Plant (100 mg) was dissolved in 10 ml of 10 % of ethanol and the resultant homogenous mixture was allowed to stand for 20 minutes at room temperature, and the aliquot of 0.4 ml was mixed with 10 ml of distilled water and 5 % $NaNO_2$ solution (0.06 ml). The mixture was allowed to stand for 5 minutes at room temperature. After 6 minutes 10 % $AlCl_3$ solution (0. 06 ml) was added to the mixture. This was immediately followed by the addition of 1M NaOH (0.5 ml) and distilled water to the mixture and allowed to stand for another 30 minutes. Absorbance of the mixture was determined at 510 nm, Quercetin was used as standard; total flavonoid content was calculated as quercetin equivalent (QE)/g of the plant extract of the leaves and flowers.

Determination of total vitamin C

Determination of ascorbic acid content was done following the method described by [7]. Briefly, the *Spilanthes filicaulis* leaf and flower extract (100 mg) was mixed with 10 ml 1% metaphosphoric acid for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 ml) was mixed with 9 ml 2, 6-dichlorophenolindophenol (DCPIP) 0.005%, and the absorbance was measured within 30 min at 515 nm against a blank. The content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (50, 100, 200 and 400 µg/ml) and the results were expressed as mg ascorbic acid/g extract.

Estimation of Tannins

A method (prussian blue) propose by Graham was utilized to quantify tannins. To about 0.1 ml of *Spilanthes filicaulis* leaf and flower extract add 6.9 ml of distilled water, 1 ml of 0.008 M potassium ferric cyanide, 1 ml of 0.2 M ferric chloride in 0.1 M HCl and mix well. The blue color formed was read at 700 nm. Tannic acid was used as standard for constructing a calibration curve.

B-Carotene and Lycopene

b-Carotene and lycopene were determined according to the method of Nagata and Yamashita [8]. The dried methanolic *Spilanthes filicaulis* leaf and flower extract (100 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm. Contents of b-carotene and lycopene were calculated according to the following equations: lycopene (mg/100 ml) =-0.0458 A663 + 0.372 A505 - 0.0806 A453; b-carotene (mg/100 ml) = 0.216 A663 - 0.304 A505 + 0.452 A453. The results were expressed as ug of carotenoid/g of extract.

RESULTS

UV-VIS spectrophotometric scan of absorption wavelength (λ) of *Spilanthes filicaulis* leaf and flower extract in ethanol

Scanning of *Spilanthes filicaulis* leaf and flower extract in ethanol was done in a spectrophotometer within the visible range (800 – 400 nm) gives a highest peaks (**λmax**) at 650 nm and Abs 0.269 for the leaf in ethanol and 450 nm and Abs 0.104 in ethanol (Fig-1 & 2).

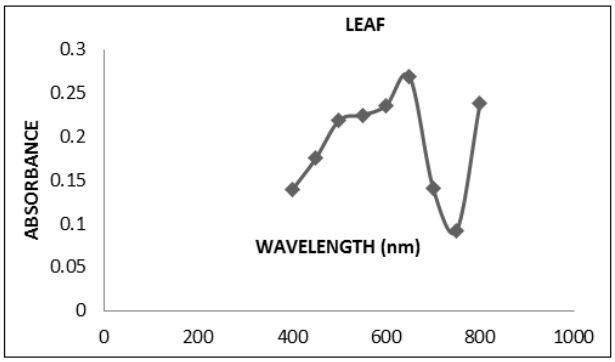

Fig-1: Absorbance vs. wavelenght of *Spilanthes filicaulis* leaf extract in ethanol.

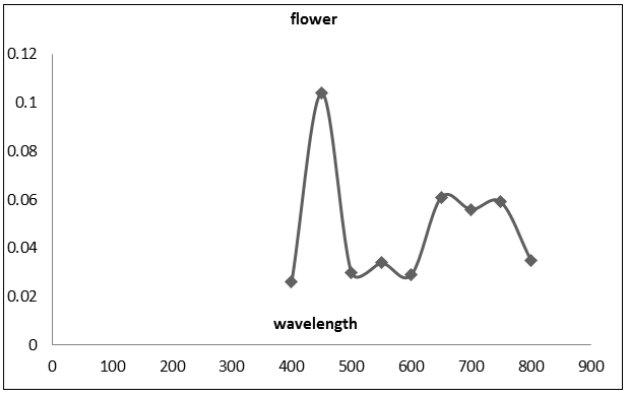

Fig-2: Absorbance vs. wavelenght of *Spilanthes filicaulis* flower extract in ethanol.

Table-1: Total phenol flavonoid and vitamin C content of flower and leaf extract of *Spilanthes filicaulis*

	Phenol	Flavonod	Vitamin C
Leaf	5.03 ± 0.17 mg GAE/g extract	6.05 ± 0.34 mg QE/g extract	7.14 ± 1.24 mg AE/g extract
Flower	4.25 ± 1.45 mg GAE/g extract	7.23 ± 0.67 mg QE/g extract	3.97 ± 2.47 mg AE/g extract

Table-2: Total tannin beta carotene and lycopene content of flower and leaf extract of *Spilanthes Filicaulis*

	Tannin	beta-corotene	Lycopene
Leaf	6.25 ± 0.76 mg TE/g extract	0.61 ± 0.11 ug b-carotene/g extract	0.02 ± 0.77 ug lycopene/g extract
Flower	9.5 ± 0.76 mg TE/g extract	0.30 ± 0.65 ug b-carotene /g extract	0.05 ± 0.97 ug lycopene/g extract

DISCUSSIONS

Phenolics are the most abundant secondary metabolite in the plant kingdom. These diverse groups of compounds have received much medical attention as antioxidants in terms of their ability to act as both efficient ROS scavengers and metal ion chelator. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers [9]. Flavonoids are a class of secondary plant phenolics with powerful antioxidant properties. Total phenolic were estimated to be equivalents to 5.03 ± 0.17 mg GAE/g extract and 4.25 ± 1.45 mg GAE/g extract of leaf and flower of *Spilanthes filicaulis*, respectively. The flavonoid content were estimated to be equivalents to 6.05 ± 0.34 mg QE/g extract and 7.23 ± 0.67 mg QE/g extract of leaf and flower of *Spilanthes filicaulis*, respectively.

Vitamin C is a 6-carbon lactone ring structure with 2, 3–enediol moiety. The antioxidant activity of ascorbic acid comes from 2, 3–enediol. L–Ascorbic acid first changes to semi-dehydroascorbic acid through donating 1 hydrogen atom, and then L–dihydroascorbic acid by donating a 2nd hydrogen atom. Both L–ascorbic acid and L–dihydroascorbic acid retain the vitamin C activity. Ascorbic acid is highly susceptible to oxidation in the presence of metal ions such as Cu^{2+} and Fe^{3+} [10]. Total Vitamin C in the present study were estimated to be equivalents to 7.14 ± 1.24 mg AE/g extract and 3.97 ± 2.47 mg AE/g extract of leaf and flower of *Spilanthes filicaulis*, respectively.

Tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose,

hemicellulose, pectin, etc.), alkaloids, nucleic acids and minerals, etc [11]. Total tannins in the present study were estimated to be equivalent to 6.25 ± 0.76 mg TE/g extract and 9.5 ± 0.76 mg TE/g extract of leaf and flower of *Spilanthes filicaulis*, respectively.

Carotenoids are a group of natural pigments that are synthesized by plants and microorganisms but not by animals. The carotenoid hydrocarbons known as the carotenes contain specific groups like lycopene and b-carotene; and the oxygenated carotenoids known as xanthophylls, like zeaxanthin and lutein. Total beta-carotene in the present study depicted in table 2 were estimated to be equivalents to 0.61 ± 0.11 ug b-carotene/g extract and 0.30 ± 0.65 ug b-carotene /g extract of leaf and flower of *Spilanthes filicaulis* respectively. The main antioxidant property of carotenoids is due to singlet oxygen quenching which results in excited carotenoids that dissipate the newly acquired energy through a series of rotational and vibrational interactions with the solvent, thus returning to the unexcited state and allowing them to quench more radical species. This can occur while the carotenoids have conjugated double bonds within [12]. Total lycopene in the present study were estimated to be equivalent to 0.02 ± 0.77 ug lycopene/g extract and 0.05 ± 0.97 ug lycopene/g extract of leaf and flower of *Spilanthes filicaulis* respectively.

CONCLUSION

The methanolic extract of leaf and flower of *Spilanthes Filicaulis* had higher phenolic, flavonoid, tannin and ascorbic acid contents these bioactive compounds contribute to the antioxidant activity of the plant.

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