AJBS Asian Journal of Biological Sciences

OPEN ACCESS

Analysis of Free Radical Activities and Phenolic Contents of *Spilanthes filicaulis* Flower-Head and Leaf Extracts

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ABSTRACT

Background and Objective: *Spilanthes filicaulis* is a medicinal plant commonly used in traditional remedies and known for its diverse therapeutic properties, particularly its anti-inflammatory, analgesic and antimicrobial effects. However, its antioxidant potential, which plays a crucial role in combating oxidative stress linked to various chronic diseases, requires further exploration. The study aimed to investigate the antioxidant capacity of *Spilanthes filicaulis* flower-head and leaf extracts. The methanol extracts of the flower-head and leaf of *Spilanthes filicaulis* has been analyzed as well as phenolic content through different assays like DPPH, ABTS, FRAP and TAC. **Materials and Methods:** The study utilized analytical reagent-grade chemicals, including methanol and flavonoids like DPPH and ABTS. The 3 kg samples of *Spilanthes filicaulis* were air-dried, triturated and extracted with 80% methanol. The extracts were analyzed for phenolic content, antioxidant activities (DPPH, ABTS, FRAP and TAC) and phytochemical contents (Phenolics, Tannins, Flavonoids) using SPSS version 20 and ANOVA, with significance set at $p<0.05$. **Results:** The extracts' antioxidant activities the flower-head extract showed higher DPPH radical scavenging activity (EC_{50} = -0.32±0.04 µg/mL) and ABTS radical scavenging activity (EC_{50} = -2. 91±0.032). On the other hand, the identity and quantity of the phenolic and flavonoids which are related to the antioxidant potential were comparatively more significant in the leaf extract: Phenolic content had 1411.29±27.93 mg of GAE/mg of dry plant extract and flavonoids with 21.50±0.866 mg quercetin equivalent/mg dry weight. **Conclusion:** It can be concluded that extracts of *Spilanthes filicaulis*, especially the flower-head extract may be used as efficient natural sources of antioxidants. This research focuses on the pharmacological possibilities of *Spilanthes filicaulis* and opens the doors for other researcher to identify the different active compounds and analyze on how they work.

KEYWORDS

Phenolic compounds, free radical scavenging, antioxidant activity, *Spilanthes filicaulis*, flower-head, leaf extracts

INTRODUCTION

Spilanthes filicaulis or spilanthes is one plant that has been reported to possess medicinal value and has attracted interest due to its health benefits in its flower-head and the leaves of the plant[1]. *Spilanthes filicaulis* also known as Toothache plant is called as such due to its medicinal value. Originally

ISSN: 1996-3351 (Print)
https://doi.org/10.3923/ajbs.2024.730.740

Received: 22 Jul. 2024
Accepted: 10 Sep. 2024
Published: 31 Dec. 2024

from Nigeria and other African countries, it is nowadays mainly used for pain in the mouth and neck area, including toothaches. It also has flower heads and leaves that has antimicrobial as well as antifungal capabilities in treating infection[2]. Besides, for immune-boosting and antioxidant effects, *Spilanthes filicaulis* can be used in the traditional medicine for the treatment of fever, digestion disorders, dermatological diseases, etc. These current studies have concentrated on evaluating free radical activities and phenolic properties of the antioxidant compounds[3].

Free radicals are odd electron species that produce reactive oxygen species leading to oxidative stress and subsequent cellular injury. Since free radicals are detrimental to health, antioxidants work to counteract the impacts of these elements on the body[4]. The process of working with free radical activities include determine the effectiveness of *Spilanthes filicaulis* in neutralizing these radicals. Paulraj *et al*.[5] assessed the efficacy of *S. filicaulis* flower-head and the leaf extracts in the scavenging of free radicals using DPPH assay. Their research also showed that the extracts displayed considerable antioxidant properties than the leaf of the plant, with the flower-head extract being more potent. This indicates that the flower-heads harbor more effective radical scavenging phytochemical components. In the same vain, the free radical scavenging activity was also determined using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) method, according to Tai *et al*.[6]. According to Ma *et al*.[7] the flower-head extract showed superior antioxidant activity than the leaf extract in their study. The findings presented in this paper provide insight into the significance of exploring *Spilanthes filicaulis* as a resource of natural antioxidants.

Phenolic compounds are part of the families of secondary metabolites in plants and they are active agents in antioxidant processes. These compounds include flavonoids, tannins and phenolic acids which are antioxidants that have various effects like: In various studies, the total phenolic content extracts have been investigated[8,9]. According to Elufioye *et al*.[10] the content of the phenolic compounds in *Spilanthes filicaulis* can be quantified through the Folin-Ciocalteu method using quantitative analysis. They described that they prepared flower-head extract with more phenolic content than the leaf extract. Phenolic content has been found to be higher in the flower-head extracts of the different *Taraxacum* species and this could be the reason for a higher antioxidant activity. In another study, Ojo *et al*.[2] used High-Performance Liquid Chromatography (HPLC) to determine and identify the individual phenolic profiles in *Spilanthes filicaulis*. Their palynological work involved the determination of various phenolic acids and flavonoids present in the fruits, including quercetin and gallic acid. The presence of these phenolics in higher amounts in the flower heads further affirms the findings made by earlier studies on higher antioxidant potential of the flower heads.

Numerous researches affirmed that there is a close connection between phenolic content and antioxidant activity. The research by Ewhea *et al*.[11] on *Spilanthes filicaulis* indicates increased phenolic content leads to increased free radical scavenging activities. In their study, Khiya *et al*.[12] used correlation value and realized that there was a direct relationship between TPC and antioxidant activity of extracts. This indicates that at least the major fraction of antioxidant activities in the plant is owed to phenolic compounds. Similar observations were made by Khiya *et al*.[12] with an indication of considerable phenolic content coupled with free radical scavenging activity. They noted that *Spilanthes filicaulis* could have confirmed its free radical scavenging properties from its phenolic content, especially from flower-heads. Considering the rich potential for their traditional use and novelty as functional foods and pharmaceutical ingredients, further research into *Spilanthes filicaulis* extracts is warranted due to their strong antioxidant activity as well as the high content of both total phenolic compounds[13]. Traditional healers in Nigeria have long used *Spilanthes filicaulis* for treating various ailments and these scientific findings provide a basis for its efficacy.

In their study on the pharmacological properties of plants used by the people of Southeastern Nigeria[14] and Senjobi *et al*.[15] described the uses of *Spilanthes filicaulis* in the traditional management of toothache, infections and inflammation. These traditional uses are supported by the claimed antioxidant effects as

measured in published research data, especially for conditions involving oxidative stress. Furthermore, there are possibilities to build nutraceuticals based on *Spilanthes filicaulis*. This high antioxidant activity was suggested that it can be used in dietary supplements that seek to increase the body's ability to counter free radical attacks. But the research into these elements is needed further to unveil the bioavailability and efficiency upon human organism. The reason why the free radical activities and phenolic contents of the flower-head and leaf extracts of the *Spilanthes filicaulis* should be studied urgently is because of the current trends in natural antioxidants and their perceived health benefits and medicinal uses. Free radicals cause oxidative damage to the body cells culminating in cancer, cardiovascular diseases and neurodegenerative disorders. Phenolic compounds-since they are antioxidants, free radicals have negative impacts and can be controlled or annulled[16].

Even though the plant *Spilanthes filicaulis* is acknowledged to possess certain pharmacological effects, there is a comparatively small amount of well-rounded investigation of the plant's bioactive compound and how it acts. Earlier works have encompassed the broad traditional healers' uses of the plant, while less emphasis has been made out of listing specific phenolic compositions and free radical scavenging capacities. Thus, there is a lack of literature that calls for subsequent research focusing on discovering the exact compounds that are involved in the performance of these roles. Also, all synthetic antioxidants are considered dangerous to the human organism, so the need for natural replacements increases daily. Knowledge of further antioxidant capability of *Spilanthes filicaulis* could help establish various new treatment and nutritional approaches for healing and preventing many diseases.

Therefore, this study is crucial in advancing our knowledge of natural antioxidants and enhancing the utilization of medicinal plants in modern healthcare. The study aimed to evaluate the antioxidant potential of methanol extracts from the leaf and flower head of *S. filicaulis* (MESF). Specific objectives included assessing their DPPH radical scavenging activity, Ferric-Reducing Antioxidant Power (FRAP) and ABTS radical scavenging ability. Additionally, the study aimed to quantify the total antioxidant capacity (TAC) and analyze the phytochemical content, including phenolic and flavonoid compounds, to understand their contribution to antioxidant properties.

MATERIALS AND METHODS

Study area: The study was conducted in Anambra State, Nigeria, a region known for its diverse vegetation and rich biodiversity. This Southeastern Nigerian state provides a suitable environment for the growth of various plant species, including *Spilanthes filicaulis*. The duration of the research is between 2018 to 2020, this was done to capture the phytochemical profile of the plant under prevailing climatic conditions.

Chemicals: All chemicals used in this study were of analytical grade. Methanol, acetic acid, sulphuric acid, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical, L-ascorbic, phosphoric acid, potassium dihydrogen phosphate (KH2PO4), potassium hydroxide (KOH), TPTZ, ABTS, methionine, Ethylenediaminetetraacetic Acid (EDTA), disodium carbonate (Na_2CO_3), polyvinylpyrolidone, riboflavin, hydrated ferrous sulphate (FeSO4.7H2O), activated charcoal, gum acacia, sodium bicarbonate (NaHCO3) and Folin-Ciocalteu's reagent (FCR) were purchased from Sigma Chemical Co. (St. Louis, MO). Filtration was done Whatman No. 1 filter paper. Loperamide (Imodium), atropine and castor oil (Bell, Sons and Co., Druggist Ltd., Southport, England).

Extraction of crude plant extract: A large quantity (2736 g) of *Spilanthes filicaulis* was collected from Isuofia Village. The plant specimen was authenticated by Mr. Alfred Ozioko, a taxonomist with International Centre for Ethnomedicine and Drug Development, Nsukka, Enugu State, Nigeria. The plant's InterCEDD voucher number is InterCEDD/16291. The flower heads and leaves of the plant were selected differently, dried at room temperature for several days and then pulverized into fine powder. Pulverized

powder of *Spilanthes filicaulis* leaf and flower-head were, respectively subjected to extraction with 80% methanol. The resulting liquid extracts were filtered using Whatman No.1 filter paper and the residues were discarded. The methanol extracts were concentrated in water bath at 450°C and stored at 40°C until used.

Antioxidant activity assays

Qualitative DPPH radical scavenging assay using dot-blot: A thin-layer chromatography (TLC) based DPPH radical scavenging assay was employed to assess the antioxidant capacity of the extracts. This method, adapted from Soler-Rivas *et al.*[17], involved applying varying concentrations of the extract and a standard antioxidant to TLC plates. Subsequent exposure to DPPH reagent induced a color change, with the intensity and rate of color transition from purple to yellow correlating to the extract's antioxidant potency[13].

Quantitative DPPH radical scavenging assay: The DPPH radical test is determined on the ability of the purple coloured stable radical to decolorize when antioxidants are present[18]. Scavenging activity on DPPH radical by the extract was assessed according to the method reported by Awah *et al.*[18]. Extract solutions were prepared at varying concentrations (0-125 µg/mL) in methanol. Equal volumes of these solutions and DPPH reagent were mixed and incubated in the dark. The decrease in DPPH absorbance at 517 nm, compared to a control, indicated the extract's radical scavenging ability. The L-ascorbic acid served as a positive control. Lower absorbance of the reaction mixture showed higher radical scavenging activity. Inhibition of DPPH radical was calculated using the Equation 1:

$$\text{inhibition (\%)} = \frac{A_0 \times A_s}{A_0} \times 100 \quad (1)$$

A_0 = Absorbance of the negative control
A_s = Absorbance of the tested sample

All determinations were done in triplicate (n = 3).

ABTS radical scavenging assay: The antioxidant capacity of the plant extracts was determined using the ABTS radical cation decolorization assay[19]. This method involves generating the ABTS radical cation by reacting ABTS with potassium persulfate. The plant extracts were then added to the ABTS solution and the decrease in absorbance at 734 nm was measured to assess their radical scavenging ability. The percentage inhibition of absorbance was calculated using Equation 2:

$$\text{ABTS.+ Scavenging effect (\%)} = \frac{AB - AA}{AB} \times 100 \quad (2)$$

AB = Absorbance of ABTS radical+methanol
AA = Absorbance of ABTS radical+sample extract/standard

Butylated hydroxy toluene was used as standard substance.

Ferric reducing antioxidant power assay (FRAP): It is a simple, rapid and reproducible method that can provide a very useful total antioxidant concentration without measurement and summation of the concentration of all antioxidants involved. The FRAP method is based on the reduction of Fe^{3+} to Fe^{2+} by antioxidants in acidic medium. The reducing power of the crude extract and its fractions were determined

as described by Sahreen *et al.*[20]. The sample (2.0 mL) was mixed with 2.0 mL of 0.2 M phosphate buffer (PH 6.6) and 2.0 mL of 10 mg/L potassium ferricyanide (0.1% w/v) solution. The mixture was incubated in a water bath at 50°C for 20 min. Following this, 2.0 mL of 100 mg/L trichloroacetic acid solution (10% w/v) was added. An aliquot of 2.0 mL of the mixture was combined with distilled water (2.0 mL) and 0.4 mL of 0.1% (w/v) ferric chloride ($FeCl_3.6H_2O$) solution. The absorbance of the reaction mixture was measured at 700 nm after 10 min of the reaction. The ferric-reducing antioxidant power of the extract was expressed as gallic acid equivalent/g.

Total antioxidant capacity (TAC) assay: The total antioxidant capacity assay of the extract was carried out by the phosphomolybdate method[21]. A (0.1 mL) aliquot of different concentrations (25, 50, 100, 200, 250, 300 and 1000 mg/L) of the extract and ascorbic acid was mixed with 1 mL of reagent solution (600 mM sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate, 1:1:1). All the test tubes were covered using aluminum foil and incubated in a water bath at 95°C for 90 min. After the extracts were cooled to room temperature, the absorbance of the mixture was determined at 765 nm against a blank containing 1 mL of the reagent solution. Ascorbic acid was used as the standard. The assay was carried out in triplicates. The antioxidant capacity (TAC) is expressed as equivalents of ascorbic acid. The total antioxidant capacity was estimated using Equation 3:

$$\text{TAC (\%)} = \frac{\text{Abs of control - Abs of extract or sample}}{\text{Abs of control}} \times 100 \quad (3)$$

Quantitative phytochemical analysis

Determination of total phenolic contents: The total phenolic contents were determined using Folin-Ciocalteau reagent (FCR). The FCR is the yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungstic hetero-poly acids as described by Awah *et al.*[18] and Velioglu *et al.*[22] with slight modifications. The dissociation of a phenolic proton in a basic medium leads to a phenolate anion, which reduces FCR, forming a blue-coloured molybdenum oxide. The colour intensity is directly proportional to the phenolic contents[23]. To three different test tubes, 100 µL of the spice extracts dissolved in methanol (1 mg/mL) was mixed with 750 µL of Folin-Ciocalteu reagent (diluted 10-fold in H_2O) and permitted to stand at 22°C for 5 min; 750 µL of Na_2CO_3 (60 g/L) solution was then added to the mixture. After 90 min the absorbance was read at 725 nm. The results were expressed as gallic acid equivalents per mg dry weight extract.

Determination of tannin contents: The tannin contents in each spice extracts were determined using insoluble polyvinyl-polypirrolidone (PVPP), which binds to tannins as described by Makkar *et al.*[24] with slight modification . Spice extracts (1 mL of 1 mg/mL) dissolved in methanol, were mixed with 100 mg PVPP, vortexed, left for 15 min at 4°C and then centrifuged for 10 min at 3000 rpm using an Abman centrifuge (UK). In the clear supernatant the non-tannin phenolics were determined the same way as the total phenolics[22]. Tannin content was calculated as a difference between total and non-tannin phenolic content.

Determination of flavonoids: The total flavonoid content was assessed using the aluminum chloride colorimetric method adapted from Kumaran and Karunakaran[25]. Plant extracts reacted with aluminum chloride to form a colored complex. The absorbance of this complex at 415 nm was compared to a rutin standard curve to quantify flavonoid content as rutin equivalents per gram of dry extract:

$$\text{Flavonoid content} = \frac{A \times m_0}{A_0 \times m} \quad (4)$$

A = Absorbance of plant extract solution
A_0 = Absorbance of standard rutin solution
m = Weight of plant extract
m_0 = Weight of rutin in the solution

The flavonoid content is expressed in mg rutin equivalents/g plant extract.

Statistical analysis: All data were appropriately expressed as Mean±SD. The results were analyzed using Statistical Package for Social Sciences (SPSS) version 20 for windows using One-way Analysis of Variance (ANOVA) to test for significance at $p<0.05$. Group mean obtained after each treatment was compared with controls and difference was considered significant at $p<0.05$.

RESULTS

Scavenging activity of MESF leaf and flower-head on DPPH radical: The extracts investigated significantly scavenged DPPH radical by changing the extract spot from purple to yellow on a piece of thin layer chromatography (TLC) plates. The ability of the methanol extract of *S. filicaulis* to scavenge DPPH radicals was further determined quantitatively. The extracts showed a significant dose-dependent DPPH radical scavenging capacity (Table 1). The MESF flower-head showed higher DPPH radical scavenging activity (EC_{50} = -0.32±0.04 µg/mL) than MESF leaf (EC_{50} = 3.43±0.08 µg/mL) compared to the ascorbic acid standard (EC_{50} = -15.86±0.12 µg/mL).

Ferric reducing antioxidant power (FRAP) of MESF leaf and flower head: All extracts showed a dose-dependent ferric-reducing ability. The MESF flower-head was more efficient with EC_{50} value of 934.72±17.16 µg/mL than MESF leaf (EC_{50} = 1923.19±131.13 µMFe^{2+}/g) compared to that of standard Gallic acid (EC_{50} = 84.7±112.63 µMFe^{2+}/g) as shown in Table 2.

MESF leaf and flower-head scavenging activity on ABTS radical: As shown in Table 3, MESF flower-head showed higher ABTS scavenging activity (EC_{50} = -2.91±0.032 µg/mL) than MESF leaf (EC_{50} = 15.30±0.21 µg/mL) compared to that of the standard, butylated hydroxytoluene (EC_{50} = 48.67±0.31 µg/mL).

Table 1: MESF scavenging activity on DPPH radical at different concentrations

Concentration (µg/mL)	Inhibitory activity (%)-leaf	Inhibitory activity (%)-flower-head	Inhibitory activity (%)-ascorbic acid
62.5	73.72±4.81	30.43±40.22	63.02±0.78
125	73.21±1.18	32.70±23.62	63.30±0.68
250	76.36±0.72	66.15±7.85	64.13±0.72
500	68.61±0.30	83.43±12.36	62.78±0.96
1000	49.39±1.21	84.17±9.54	64.39±0.50
EC_{50}	3.43±0.08	-0.32±0.04	-15.86±0.12

Data represented as Mean±SD (n = 3)

Table 2: Ferric reducing antioxidant power (FRAP) of MESF at different concentration

Concentration (µMFe^{2+}/g)	Reducing activity (%)-leaf	Reducing activity (%)-flower-head	Reducing activity (%)-gallic acid
15.63	0.035±0.0036	0.068±0.000	0.055±0.004
31.25	0.039±0.0015	0.067±0.0021	0.097±0.029
62.5	0.039±0.0010	0.068±0.0021	0.074±0.019
125	0.044±0.003	0.073±0.0026	0.084±0.018
250	0.05±0.006	0.088±0.0015	0.125±0.030
500	0.064±0.0006	0.12±0.00610	0.122±0.015
1000	0.088±0.006	0.176±0.0032	0.188±0.025
EC_{50}	1923.19±131.13	943.72±17.16	84.7±112.63

Data represented as Mean±SD (n = 3)

Table 3: MESF scavenging activity on ABTS radical at different concentration

Concentration (µg/mL)	Inhibitory activity (%)-leaf	Inhibitory activity (%)-flower-head	Inhibitory activity (%)-BHT
15.63	85.53±1.07	72.98±0.733	92.13±0.57
31.25	95.57±0.50	79.01±1.16	90.15±2.41
62.5	96.28±0.30	84.72±0.73	90.90±0.57
125	95.29±0.30	90.57±0.70	88.21±4.49
250	93.97±0.08	89.11±0.43	89.87±0.99
500	90.80±0.09	88.07±0.16	89.86±0.57
1000	80.63±1.12	84.68±0.94	90.24±0.00
EC_{50}	15.30±0.21	-2.91±0.032	48.67±0.31

Data represented as Mean±SD (n = 3)

Table 4: Total antioxidant capacity of MESF at different concentrations

Concentration (mg/TE/g)	Inhibitory activity (leaf)	Inhibitory activity (flower-head)
15.63	211.33±23.71	179.33±17.90
31.25	206.33±5.5	224.67±12.50
62.5	201.33±21.01	228.00±39.95
125	217.00±18.68	233.67±4.73
250	241.00±28.58	250.33±3.06
500	289.67±8.96	276.67±10.12
1000	362.67±60.87	369.67±7.095
EC_{50}	-0.43±0.07	-0.35±0.007

Data represented as Mean±SD (n = 3)

Table 5: Phytochemical composition of *Spilanthes filicaulis* leaf and flower-head extracts

Plant extract	Total flavonoid (mg‡ quercetin equivalents/ mg dry weight)	Total phenolic* content (mg gallic acid equivalents/ mg dry weight)	Non-tannins (mg gallic acid equivalents/mg dry weight)	Tannins (mg gallic acid equivalents/mg dry weight)
Leaves	21.50±0.866	1411.29±27.93	1400.2±27.56	11.09±0.37
Flower head	0.013±0.002	0.166±0.312	0.098±0.08	0.069±0.23

Data represented as Mean±SD (n = 3), ‡Expressed as mg quercetin equivalents/mg dry weight plant extract and *Expressed as mg gallic acid equivalents/mg dry weight plant extract

Total antioxidant capacity (TAC) assay of MESF leaf and flower-head: The total antioxidant capacity (TAC) assay was dose-dependent. At the concentration of 1000, TAC was higher in MESF flower head (369.67±7.095 mg/TE/g) when compared to MESF leaf (362.67±60.87 mg/TE/g) whereas it was lower at the concentration of 500, but higher in other concentrations except at 15.63 mg/TE/g when compared together as shown below (Table 4). The total antioxidant capacity (TAC) is expressed as equivalents of ascorbic acid.

Phytochemical analysis

Total phenol and flavonoid contents: Table 5 shows that phenolic compounds were a major class of bioactive compounds in the extracts. The total phenolic content in MESF leaf (1411.29±27.93 mg GAE/mg dry plant extract) was found to be more than the total phenolic content in MESF flower-head (0.166±0.312 mg GAE/mg dry plant extract). The total flavonoid content of MESF leaf and flower-head were 21.50±0.866 and 0.013±0.002 mg quercetin equivalents/mg dry weight plant extract.

DISCUSSION

The study investigated the antioxidant properties of *Spilanthes filicaulis* leaf and flower-head extracts. Results showed significant antioxidant activity in both, measured by DPPH, FRAP and ABTS assays. The flower-head extract generally exhibited higher antioxidant capacity than the leaf extract. Total phenolic and flavonoid content analysis revealed that these compounds likely contributed to the observed antioxidant effects. Scavenging of DPPH radical model is broadly used to efficiently evaluate antioxidant actions of extracts[26]. The total phenol content of the extracts suggested that phenolic compounds could be responsible for the observed DPPH radical scavenging since phenols can readily donate hydrogen

atoms to the radical, thereby quenching the radicals[27]. The ability of the crude methanol extract of *Spilanthes filicaulis* leaf and flower-head to scavenge radicals were determined quantitatively (Table 1). When the extracts were added to DPPH solution, there was a rapid decrease in the optical density at 518 nm indicating good scavenging capacity of the extracts. The extracts showed substantial antioxidant activity in a dose-dependent manner similar to that of the ascorbic acid standard. The minimal effective concentration of the extract that could scavenge fifty percent of the radical, (EC_{50}) was found to be higher in the flower-head extract (-0.32±0.04 µg/mL) followed by the leaf extract (3.43±0.08 µg/mL). The DPPH assay measured the hydrogen atom or electron donating ability of the extract to the stable radical DPPH produced in solution[28].

The ferric-reducing antioxidant power of the extracts can be seen in Table 2 which shows the reducing power of the extracts compared to their standard, gallic acid. The reducing capability was determined by measuring the Fe_{3+}-Fe_{2+} transformation in the presence of the leaf and flower-head extracts. The reducing power is linked with the occurrence of reductants, which exert antioxidant action by offering a hydrogen atom, thereby breaking the free radical chain. The result in Table 2 shows that the flower-head extract showed higher reducing ability (EC_{50} = 943.72±17.16) followed by the leaf extract (EC_{50} = 1923.19±131.13). The antioxidant activity of plant extracts has been recognized to have various mechanisms of action, such as binding of heavy metal ion catalysts, breakdown of peroxides, inhibition of chain initiation, reductive capacity of metals, among others[29]. Nevertheless, the reducing power of the gallic acid was relatively more effective than the extracts.

The ABTS assay effectively measures the antioxidant capacity of compounds that donate hydrogen atoms or interrupt oxidative chains. Previous research by Pietta *et al.*[30] on commonly used medicinal plants established that phenolic compounds are key contributors to the neutralization of ABTS radicals. Table 3 shows that methanol leaf and flower-head extract of *Spilanthes filicaulis* exhibited strong scavenging activity against ABTS radicals. The flower-head extract (EC_{50} = -2.91±0.032) showed higher ABTS radical scavenging activity, followed by the leaf extract (EC_{50} = 15.30±0.21) compared to that of the standard, BHT (EC_{50} = 48.67±0.31). The result (Table 3) indicates that the extracts could stop the oxidation course by reducing free radicals. This could be attributed to the presence of a high content of phenols in the extracts[31].

The total antioxidant capacity (TAC) assay measures the antioxidant potential of a sample by quantifying its ability to reduce phosphomolybdic acid into a green-colored complex[32]. Table 4 shows the antioxidant capacity of methanol leaf and flower-head extract of *Spilanthes filicaulis*. The leaf extract (EC_{50} = -0.43±0.07) showed higher antioxidant capacity followed by the flower-head extract (EC_{50} = -0.35±0.007). This is due to the high phenolics and flavonoid content in the leaf extract.

Phenols present in plant extracts exhibit a broad range of physiological properties such as anti-inflammatory, anti-allergic, anti-atherogenic, anti-microbial, antioxidant, cardioprotective and vasodillatory effect[33]. They are effective hydrogen donors which make them good antioxidants. The high content of phenol(s), as observed in this study could be responsible for some of the pharmacological properties of *Spilanthes filicaulis* since it contains phenol containing compounds which are reducing agents[27]. The methanol leaf extract showed higher total phenolic content (1411.29±27.93 mg GAE/g) than the flower-head extract (0.166±0.312 mg GAE/g).

Flavonoids are naturally occurring polyphenolic compounds that are one of the most prevalent classes of compounds in nuts, fruits, vegetables and beverages[34]. In this study, the highest amount of total flavonoid was observed in the methanol leaf extract (21.50±0.866 mg/g) followed by the methanol flower head extract (0.013±0.002 mg/g). Therefore, the results suggest that phenolic acid and flavonoids may be the major contributor for the antioxidant properties and inhibitory actions toward the oxidative reaction *in vitro* and *in vivo*.

CONCLUSION

This study reveals that both the flower-head and leaf extracts of *Spilanthes filicaulis* possess significant antioxidant properties, with the flower-head extract demonstrating superior free radical scavenging activity across multiple assays, despite its lower phenolic content. Conversely, the leaf extract contains higher concentrations of phenolic and flavonoid compounds. Future research should focus on identifying the specific active fractions responsible for these activities and exploring their detailed mechanisms of action, which could have valuable pharmacological applications.

SIGNIFICANCE STATEMENT

This study aimed to evaluate the antioxidant potential of *Spilanthes filicaulis* leaf and flower head extracts. By quantifying phenolic compounds and assessing free radical scavenging activities, the study demonstrated that both plant parts possess substantial antioxidant properties. Notably, the flower-head extract exhibited superior antioxidant capacity, while the leaf extract contained higher levels of phenolic compounds. These findings contribute to the growing body of knowledge on this plant species and underscore its potential as a natural source of antioxidants.

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