

Total Phenolic Content and Antioxidant Activity of *Spilanthes* Species from Peninsular India

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Abstract

Spilanthes is a genus of herbs belonging to Asteraceae family commonly known as "Toothache plant". The inflorescence is used for treating sore throat, stammering and redness of gums and is known to have anti-malarial, anti-diuretic and anti-inflammatory properties. It has been reported that they show high antioxidant activity, in the present study, the antioxidant activity was measured in terms of Ferrous Reducing Antioxidant Power (FRAP) for six species of *Spilanthes* and total phenolic content. *Spilanthes ciliata* leaves were found to have highest total phenolic content (21.53 mg/g of GAE). Lowest value was recorded in *Spilanthes oleracea*. Leaves showed more phenols compared to the other parts. Inflorescence showed moderate activity with maximum recorded in *S. oleracea*. In roots, the total phenolic content was moderate with the maximum recorded in *S. ciliata* and minimum in *S. radicans*. In FRAP assay, the maximum activity was observed in *S. ciliata* followed by *S. calva* and minimum was observed in *S. radicans*. Among inflorescence, *S. calva* and *S. oleracea* showed higher activity compared to other parts of all other species. Inflorescence of all the other species showed moderate activity. In roots, the antioxidant activity was maximum in *S. ciliata* and *S. uliginosa* recorded least activity with maximum reading in *S. calva* leaves=0.381, minimum in *S. uliginosa*, root=0.23, among leaves highest frap reading was obtained in *S. calva* and in roots *S. calva* and *S. oleracea* both showed equal FRAP readings and in Inflorescence maximum was in *S. calva* followed by *S. oleracea*. This is the first report of comparison of six species of *Spilanthes* with different plant parts for total phenolic compounds and FRAP assay.

Keywords: Asteraceae, *Spilanthes*, Total Phenolic Content, Gallic Acid Equivalents, Ascorbic acid Equivalent Antioxidant Capacity, Ferrous Reducing Antioxidant Power, Antioxidant activity

Abbreviations: TPC: Total Phenolic Content; GAE: Gallic Acid Equivalents; AEAC: Ascorbic acid Equivalent Antioxidant Capacity; FRAP: Ferrous Reducing Antioxidant Power, DDW: Double Distilled Water.

Introduction

Medicinal plants are remedy sources for human diseases because they contain chemical components of therapeutic value [1]. A systematic study of medicinal plants is very important to identify find active compounds, for their use in nutraceutical, cosmetic and pharmaceutical industries [2,3]. Contemporary researchers have taken a great interest in medicinal plants for their phytochemical constituents and related total biological activities including antioxidant activity [4-6]. *Spilanthes* is a genus of herbs belonging to Asteraceae family, commonly known as toothache plant widely used in folklore medicine. *Spilanthes acmella* is a wonder drug possessing analgesic, antibiotic, antimalarial [7], diuretic [8] and anti-inflammatory activities. It is a common spice, used as salad ingredient and has been administered as traditional folk medicine for years to cure toothache [9], stammering and stomatitis.

The antifungal activity of *Spilanthes* species was recorded with *Fusarium moniliformis*, *Fusarium oxysporium*, *Aspergillus niger*, and *Aspergillus paraciticus*. The active ingredient of *Spilanthes* is an alkamide called Spilanthol presence of which gives medicinal properties to this plant. The spilanthol, inhibits the contractions of subcutaneous muscle especially facial muscles hence commercially used in the anti-aging products.

An adequate intake of natural antioxidants could protect the onset of oxidative damage in cells [10]. The term antioxidant refers to compounds which can scavenge free radical, inhibit lipid peroxidation and act as chelating agent [11]. Phenolic compounds overcome this definition as they possess a wide spectrum of biological effects including antioxidant and free radical scavenging activities [12]. Therefore, a great interest has been recently focused on the natural foods, medicinal plants and phytoconstituents due to their well-known abilities to scavenge free radicals. The

antioxidant capacity of the plant extract largely depends on both the composition of the extract and the test system. It can be influenced by a large number of factors, and cannot be fully evaluated by one single method. It is necessary to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action [13]. Hence the antioxidant activity was evaluated by FRAP assay. In this assay, the antioxidant capacity is measured on the basis of the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tripyridyl-s-triazine (TPTZ), forming an intense blue Fe^{2+} TPTZ complex with an absorption maximum at 593 nm and can be applied to both aqueous and alcohol extracts of plants.

In the present study, six species of *Spilanthes* genus collected from different locations in Peninsular India region and maintained in field gene bank at IIHR, Bangalore were analyzed for antioxidant activity in terms of total phenolic content which is reported to be directly responsible for the antioxidant activity of a species and by FRAP antioxidant assay.

Materials and Methods

Plant Material

The six species of *Spilanthes* viz. *S. calva* (DC.) R.K, *S. ciliata* (Kunth) Cassini, *S. oleracea* Linnaeus R.K. Jansen, *S. paniculata* Wallich ex DC, *S. radicans* (Jacquin) R.K. Jansen and *S. uliginosa* (Swartz) Cassini were collected from different parts of Peninsular India region during 2011-2013 and the voucher specimens are deposited in cryo-bank of division of Plant Genetic Resources, Indian Institute of Horticultural Research, Bangalore, Karnataka. The total phenolic assay was carried out in all plants during the full flowering stage. Three types of plant material namely leaves, inflorescence and roots were collected from the plants grown in the field gene bank of Division of Plant Genetic Resources, Indian Institute of Horticultural Research, Bangalore. The

methanolic extract of the sample was prepared by dissolving 5 g of the plant material in 20 ml of methanol and made up to 50 ml using DDW.

Determination of Total Phenolic Content

The total phenolic content in the *Spilanthes* species extracts was determined spectrophotometrically following the Folin-Ciocalteu's method [14] using gallic acid as a standard (the concentration range: 0.025 to 0.5 mg/ml). Acidic methanol extract was mixed with Folin-Ciocalteu Reagent (Merck Co. Ltd., Darmstadt, Germany), and the color was developed using 20% sodium carbonate reagent. Intensity of color developed was read by measuring the absorbance at 700 nm using a spectrophotometer (Beckmann DU64, Beckmann Instruments International, SA, Nyon, Switzerland). Results were expressed as milligrams of gallic acid equivalents per 100 g dry weight.

Ferric Reducing Antioxidant Potential (FRAP)

Antioxidant capacity was measured as FRAP using a modified method of Benzie and Strain [15]. At low pH, reduction of ferric tripyridyltriazine (Fe III-TPTZ) complex to the ferrous form by antioxidants present in the sample results in an intense blue color that was measured at 593 nm to estimate antioxidant capacity. The FRAP assay mixture, containing 200 μ l of the extract and 1.8 ml of FRAP reagent, was incubated at room temperature for 40 min and absorbance was measured at 593 nm. The standard curve was prepared using ascorbic acid as an antioxidant. Antioxidant capacity was expressed as milligrams ascorbic acid equivalent antioxidant capacity (AEAC) per 100 g dry weight. The statistical analysis was carried out with MS Excel where the graph was developed for each species total phenol estimation and the comparison was carried out depending on which the results were interpreted.

Results and Discussion

Determination of Antioxidant Activity by TPC

The TPC accounts for the antioxidant potential of plant species. The antioxidant assay indicated that *S. ciliata* leaves produced high TPC amounting to 21.53 mg g⁻¹ of GAE; the lowest value was recorded in *S. oleracea* (4.53 mg g⁻¹). Leaves produced more TPC as compared to the other plant parts. The inflorescence showed moderate content; high content (10.48 mg g⁻¹) was recorded in *S. oleracea* and least (6.81 mg g⁻¹) in *S. ciliata*. The TPC in root was less; high content (13.7 mg g⁻¹) was recorded in *S. ciliata* and least (3.50 mg g⁻¹) in *S. radicans* (**Figure 1**).

Determination of Antioxidant Activity by FRAP Assay

The FRAP assay expressed antioxidant potential in terms of ACAE. The maximum antioxidant activity was detected in *S. ciliata* (58.59 mg g⁻¹) followed by *S. calva* and least in *S. uliginosa* (46.18 mg g⁻¹). The inflorescence of *S. calva* and *S. paniculata* (~73.30 mg g⁻¹) had highest activity. The antioxidant activity in root was highest (58.61 mg g⁻¹) in *S. ciliata* and lowest (43.16 mg g⁻¹) in *S. uliginosa* (**Figure 2**).

The ferrous reducing antioxidant potential was expressed in terms of ascorbic acid equivalents/g⁻¹⁰⁰ in different plant parts of *Spilanthes*.

The comparative study of antioxidant potential of species of *Spilanthes* by TPC and FRAP has not been documented in previous studies. The Mantel test carried out to find the correlation among TPC and FRAP exhibited positive correlation (0.6695) and the p value (<0.025) showing significant correlation. Similar, this study established the antioxidant property of compounds in *Spilanthes* species. Phenolics are compounds possessing aromatic rings with hydroxyl groups [16], and react as hydrogen donors and neutralize the free radicals [17,18]. Although, reports are documented in *Spilanthes calva* and *S. paniculata*. This study gives comparison of six species of *Spilanthes* from peninsular India region. The documentation of antioxidant potential of phenolic and other compounds in *Spilanthes* species supported the previous recommendation of the plant species for their medical application.

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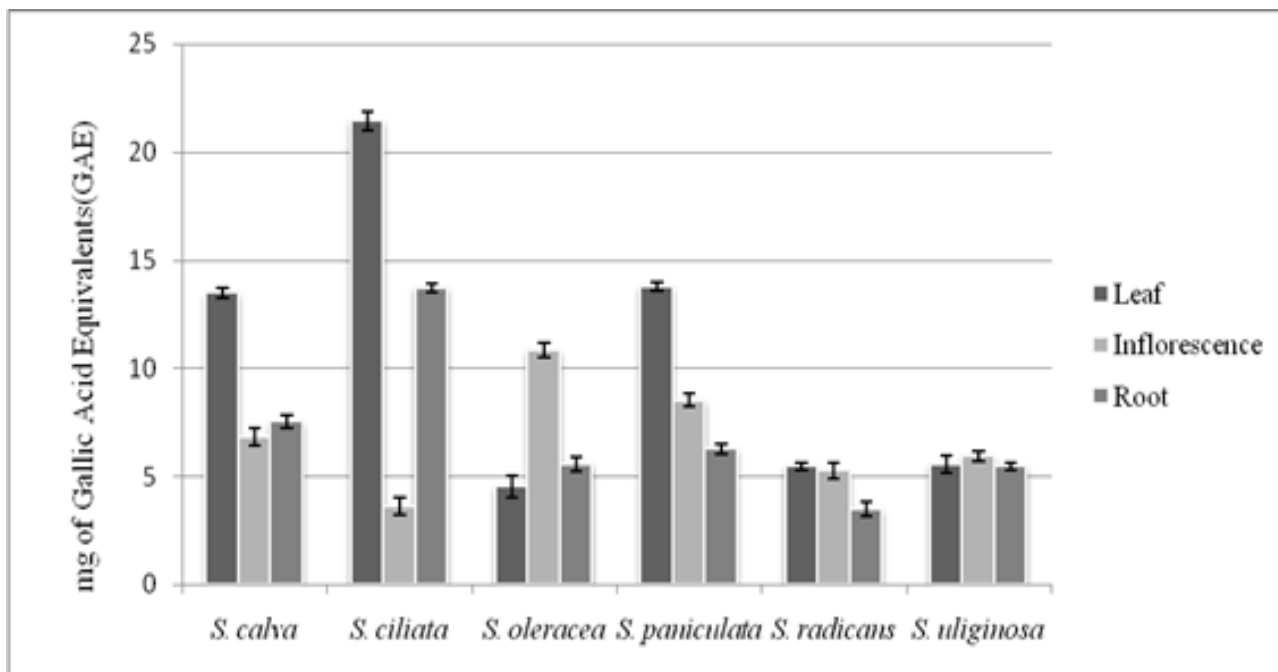


Figure 1: Total phenolic content in leaf, inflorescence and root extracts of *Spilanthes* species viz., *Spilathes calva*, *S. ciliata*, *S. oleracea*, *S. paniculata*, *S. radicans* and *S. uliginosa* concentration of extracts (2 g/ 50 ml).

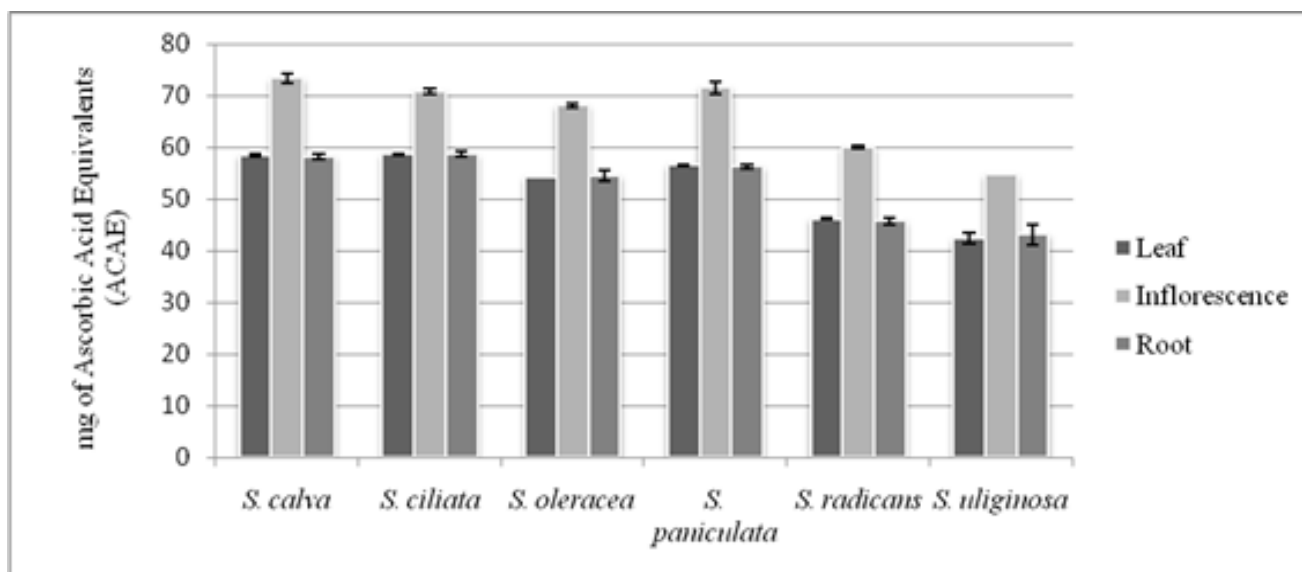


Figure 2: The antioxidant potential of leaf, inflorescence and root extracts of *Spilanthes* species viz., *Spilathes calva*, *S. ciliata*, *S. oleracea*, *S. paniculata*, *S. radicans* and *S. uliginosa* concentration of extracts (2 g/ 50 ml by FRAP assay).

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