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COMPARISON OF PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF DIFFERENT PLANT PARTS OF SPHAERANTHUS INDICUS

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ABSTRACT

The different plant parts of *Sphaeranthus indicus* were extracted with different solvants in order to accommodate all the phytochemicals responsible for the antioxidant activity. Presence of tannins and sterols, triterpenes and saponins were found in all plant parts. Flavonoids were present in root methanolic extract and methanol and ethyl acetate extract of flowers principally. The quantitative estimation of total phenolic content revealed highest phenolic content in methanolic extract of stem (963.3 ± 11.2 mg tanninc acid equivalant) followed by methanol and ethyl acetate extract of flower (958.0 ± 34.7 and 704.6 ± 21.3 mg tanninc acid equivalent respectively). The antioxidant activity using DPPH radical scavenging met showed that ascorbic acid showed 92.5% antioxidant activity at 1 mg ml^{-1} concentration. In the same concentrations, the aqueous extract of root, methanolic and ethyl acetate extract of flower produced highest antioxidant activity as 70.3 ± 10.2% each. Aqueous extract of leaf, flower and methanol extract of root also produced good antioxidant activities. The study strengthens the traditional claims of use of *S. indicus* flowers as and antioxidant and hepatoprotective agent.

KEYWORDS: Medicinal plants, *Sphaeranthus indicus*, phytochemicals, antioxidants, DPPH assay.

INTRODUCTION

Herbal medicines have been used by the mankind since time immermorial. *Ayurveda*, the oldest traditional system of India, reveals that ancient Indians had a rich knowledge of medicinal value of different plants. India has been endowed with a very rich flora owing to the extreme variations in climate and geographical conditions prevalent in the country. With the advent in science, many of the crude drugs used in traditional system have been investigated scientifically.

Sphaeranthus indicus Linn. is a medicinal plant widely used in Indian traditional system of medicine for curing various ailments. It grows in rice fields, dry waste places and cultivated lands in tropical parts of India. It is distributed throughout India, Sri Lanka, Africa and Australia from sea level to 1200 m altitude.[1] All the parts of the plant have medicinal uses. In folk medicine, the plant is reportedly used in treating epileptic convulsions, mental illnesses and hemicranias. According to Ayurveda, this herb has the properties of a laxative, digestible, tonic, alterative, anthelminitic and alexipharmic. It is used to treat vitiated conditions of hemicranias, jaundice, hepatopathy, diabetes, leprosy, fever, pectoralgia, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases.[2]

The present paper investigates the phytochemical profile as well as antioxidant potential of different plant parts of *Sphaeranthus indicus* found in Central Indian region of Jabalpur. The phytochemical profile as well as antioxidant potential are the two parameters which can be used to assess the potential of different plant parts for the use as different medicaments.

MATERIALS AND METHODS

Plant material

The whole plant of *Sphaeranthus indicus* was collected from the different areas of Jabalpur region of Central India. The plants were uprooted and immediately transported to the laboratory. After washing with running tap water for 1 h, leaves, flowers, stem and roots of *Sphaeranthus indicus* were separated and dried under shade. Taxonomic identification was carried out using available literature. The different plant parts of *S. indicus* were ground and the powder obtained was sieved through a 100 µM test sieve (Sonar, India).

Phytochemical screening

For the screening, the phytochemicals were extracted from *S. indicus* powder sequentially with water, methanol, ethyl acetate and petroleum ether. For this, 10 g of dried plant powder

was first extracted with water using cold percolation method. The extract was concentrated under vacuum up to 20 ml and was kept refrigerated until use. The residue after cold percolation was dried and used for further extraction sequentially with methanol, ethyl acetate and petroleum ether using a Soxhlet extractor. The extracts were concentrated to 20 ml as described above. Qualitative tests for various secondary metabolites were performed using methods described by Trease and Evans[3] and Harborne.[4]

Total phenolic content

The total phenolic content was determined by the Folin-Ciocalteu method[5]. Plant extract from different plant parts of S. indicus (0.3 ml) was mixed with 1.5 ml of Folin-Ciocalteau reagent and 1.2 ml of 75% (w/v) sodium carbonate solution. The tubes were vortexed for 15 sec and allowed to stand for 30 min at room temperature. Absorbance was measured at 765 nm and the results were expressed as milligram of tannic acid equivalent per gram of extract weight using standard curve of tannic acid (R^2=0.976).

Antixoidant activity

DPPH assay was used to assess the antioxidant potential of different plant parts of S. indicus and their extracts.[6] For DPPH stock solution, 2.366 mg of DPPH free radical (Sigma, USA) was dissolved in 100 ml of absolute ethanol to obtain 60 µM DPPH free radical solution. For samples, 25 mg each of dried extract was redissolved in 25 ml of ethanol. Ascorbic acid (1 mg ml^{-1}) served as a positive control. The sample solution (500µl) was mixed with the same volume of DPPH solution and allowed to stand for 2 h in dark.The absorbance was then measured at 517 nm. Ethanol served as negative control. The percentage scavenging effect was calculated as shown below-

CALCULATION

$$\% \text{ antioxidant activity for DPPH} = \frac{(A-A_x)}{A} \times 100$$

Where

A- Absorbance of DPPH solution with ethanol,

A_x- Absorbance of DPPH solution with test solution

RESULTS

The different plant parts of *Sphaeranthus indicus* were extracted with different solvants in order to accommodate all the phytochemicals responsible for the antioxidant activity. Table 1

shows the screening of those phytochemicals that may exert antioxidant activity. Tannins were present in aqueous extracts in large amounts for all four plant parts. The methanol extractes showed presence of tannins in stem and flower, while ethyl acetate extract showed tannins in leaf and flower. Flavonoids were present in root methanolic extract and ethyl acetate extract of flowers. Sterols, triterpenes and saponins were present in aqueous extract principally.

The quantitative estimation of total phenolic content revealed highest phenolic content in methanolic extract of stem (963.3 ± 11.2 mg tanninc acid equivalant) followed by methanol and ethyl acetate extract of flower (958.0 ± 34.7 and 704.6 ± 21.3 mg tanninc acid equivalent respectively) and aqueous extract of flower of *S. indicus* (Table 2).

The antioxidant activity was assessed using DPPH radical scavenging method. The control, ascorbic acid showed 92.5% antioxidant activity at 1 mg ml^{-1} concentration. In the same concentrations, the aqueous extract of root, methanolic and ethyl acetate extract of flower produced highest antioxidant activity as 70.3 ± 10.2 % each. Aqueous extract of leaf, flower and methanol extract of root also produced good antioxidant activities (Table 3).

Table 1: Phytochemical screening of potential antioxidant compounds from different plant parts of *Sphaeranthus indicus.*

#	Test	Aqueous extract				Methanolic extract				Ethyl acetate extract				Petroleum ether extract			
		Stem	Root	Leaf	Flower	Stem	Root	Leaf	Flower	Stem	Root	Leaf	Flower	Stem	Root	Leaf	Flower
1.	Tannins	+	++	+	+	++	-	-	++	-	-	++	+	-	-	-	-
2.	Flavonoids	+	+	-	-	-	++	-	-	-	-	-	++	-	-	-	-
3.	Sterols	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-
4.	Triterpenes	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
5.	Saponins	+	+	+	++	-	-	-	-	-	-	-	-	-	-	-	-
6.	Resin	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	++

Table 2: Total phenolic content of different parts of *S. indicus.* Data is presented as mean ± standard deviation (n=3) and as mg equivalent of tannic acid, using standard curve of tannic acid.

#	Extracts	Total phenolic Content (mg tannic acid equivalant)			
		Stem	Root	Leaf	Flower
1.	Aqueous extract	95.3 ± 12.3	326.3 ± 23.1	255.6 ± 2.9	542.6 ± 3.4
2.	Methanolic extract	963.3 ± 11.2	340.3 ± 4.5	17.0 ± 1.2	958.0 ± 34.7
3.	Ethyl acetate extract	85.2 ± 2.1	237.0 ± 1.1	132.0 ± 1.3	704.6 ± 21.3
4.	Petroleum ether extract	74.7 ± 0.8	23.7 ± 0.3	3.4 ± 0.4	22.7 ± 0.5

Table 3: Antioxidant activity (%) of different plant parts of *Sphaeranthus indicus* extracted with different solvents, as compared with ascorbic acid in 1 mg ml^{-1} concentration, which provided 92.5% antioxidant activity during the experiment. The data are presented as mean ± standard deviation (n=3).

S. No.	Plant parts	Antixodant Activity (%)			
		Aqueous	Methanol	Ethyl Acetate	Petroleum ether
1.	Stem	13.8 ± 1.4	50.5 ± 3.2	38.61 ± 2.7	50.41 ± 9.4
2.	Root	70.3 ± 10.2	64.35 ± 6.4	39.60 ± 5.9	26.73 ± 2.1
3.	Leaf	64.3 ± 8.6	0.99 ± 0.1	41.58 ± 2.8	2.97 ± 0.2
4.	Flower	58.4 ± 3.6	70.29 ± 12.3	70.29 ± 11.1	32.67 ± 1.5

DISCUSSION

All the parts of the *S. indicus* have medicinal uses. In *Ayurvedic* system of medicine, the whole herb is used in insanity, tuberculous glands, indigestion, bronchitis, spleen diseases, elephantiasis, anaemia, pain in the uterus and vagina, piles, biliousness, epileptic convulsions, asthma, leukoderma, dysentery, vomiting, urinary discharges, pain in the rectum, looseness of the breasts, hemicranias.[7]

Most of the studies have used the whole plant for assessing the *in vitro* antioxidant activity of *S. indicus*. In an *in vitro* study, ethanolic extract of *S. indicus* showed maximum scavenging of the radical 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS), 1,1-diphenyl, 2-picryl hydrazyl (DPPH), superoxide and nitric oxide radical. In an *in vivo* study, methanolic extract of *S. indicus* exhibited a significant antioxidant effect showing increasing levels of superoxide dismutase, catalase, and glutathione peroxides by reducing malondialdehyde levels in rats. Further, The aqueous and methanolic extracts of flower head of *S. indicus* L. were previously shown to have hepatoprotective and antioxidant effect on mouse.[8]

Sphaeranthus indicus Linn (family Asteraceae), is abundantly distributed in damp areas in plains and also as a weed in the rice fields. In the Indian system of medicine, the plant as a

whole plant or its different anatomical parts viz., leaf, stem, bark, root, flower and seed are widely used for curing many diseases. The whole plant and its anatomical parts have been reported with different types of secondary metabolites which include eudesmanolides, sesquiterpenoids, sesquiterpene lactones, sesquiterpene acids, flavone glycosides, flavonoid C-glycosides, isoflavone glycoside, sterols, sterol glycoside, alkaloid, peptide alkaloids, amino acids and sugars.[9] The present study supported the traditional claims and different plant parts, especially flowers, showed higher antioxidant activity, well supported by total phenolic content and the flavonoids contents.

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