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PRELIMINARY PHYTOCHEMICAL EVALUATION OF AERIAL PARTS OF *SPHAERANTHUS INDICUS* LINN. (ASTERACEAE)

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Aqeous extract, Total flavonoids, Total phenolics, Total saponins, Ayurvedic system of medicine, *Sphaeranthus indicus*

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ABSTRACT: *Sphaeranthus indicus* Linn (Asteraceae) is a popular and very useful plant used in the Indian system of medicine. It is usually found in damp areas, plains, and weeds in the rice fields. In the Ayurvedic system of medicine, the plant as a whole plant or its different parts like leaf, stem, bark, root, flower, and seed is widely used for curing many diseases. The plant is enriched with secondary metabolites such as eudesmanolides, sesquiterpenoids, sesquiterpene lactones, sesquiterpene acids, flavone glycosides, flavonoid C-glycosides, isoflavone glycoside, sterols, sterol glycoside, alkaloid, peptide alkaloids, amino acids, and sugars. The essential oil has been isolated from flowers and whole plants. In this present study, various methods are carried out to evaluate the pharmacognostic parameters of crude drug and aqeous extract of aerial part of the plant, which may form the basis for the further use of the plant and for the evaluation of its various pharmacological activities. The physicochemical evaluation confirms the quality and purity of the crude drug. Aqeous extract of *Sphaeranthus indicus* (AESI) was found to be rich in the presence of alkaloids, proteins, amino acids, saponins, flavonoids, and other phenolic compounds, while glycosides, sterols, carbohydrates, and volatile oils were found to be absent. The quantitative estimation of phytoconstituents viz. total flavonoids and total phenolics and saponins revealed that AESI was found rich in total flavonoids while less amount of total phenolic content while total saponin was present in a fair amount. This present study can be a basis of further evaluation of various pharmacological activities of the aqueous extract of *Sphaeranthus indicus*.

INTRODUCTION: *Sphaeranthus indicus* Linn. (Asteraceae) is an aromatic annual spreading herb found throughout India, at an altitude of 1500 m msl [1]. It is known by different names in different parts of India such as Gorakhmundi in Hindi, East Indian globe thistle in English, and Mahamundi, Mundi, Shravani in Sanskrit [2].

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In the Ayurvedic system of medicine, at different dosages and as different preparations, it is used in the treatment of tuberculosis, lung disorders such as bronchitis and asthma, indigestion, spleen disorders, elephantiasis, leukoderma, vomiting, urinary discharge, hemicranis, epileptic convulsions, blood purifier, as a poultice in rheumatic pain, aphrodisiac, retaining of pregnancy and looseness of the breasts [3].

It also has been pharmacologically or traditionally found to be an effective drug for the treatment of various disorders, as a hepatoprotective agent [4], antihelmintic agent [5], antigout activity [6], antitussive agent[7], antioxidant agent [8], as a renal protective

agent [9], as immunostimulant agent[10] and as antidiabetic agent [11].

MATERIALS AND METHODS:

Collection of Plant: The aerial parts of *Sphaeranthus indicus* Linn. (Asteraceae) were procured in the month of December 2018 from the village area nearby Vidisha (M.P.). This plant was identified and authenticated by Dr. G. P. Sinha, Scientist-E and Head of Office, Botanical Survey of India, CRC, Allahabad, Uttar Pradesh - 211002. A voucher specimen (Accession No. 104180) of *Sphaeranthus indicus* was deposited in the herbarium of the Institute for future reference.

Preparation of Aqueous Extracts of *Sphaeranthus indicus* (AESI): The powder drug (30 g) was soaked in 1 liter purified water and kept in dark and dry place for 48 h at room temperature. Chloroform was added in a quantity of 1% total mixture to avoid microbial growth. After 48 h, solutions were filtered by Whatman Filter paper No. 1. The filtered extracts were dried in a rotary evaporator, and percentage yield was calculated.

Standardization of Crude Drug [12]:

1. Determination of Ash Value: The determination of ash values is meant for detecting low-grade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a means of detecting the chemical constituents by using total ash and acid insoluble ash.

Total ash- The air-dried powder drug (4 g) was weighed accurately in a previously ignited and tarred Silica dish. The material was spread evenly and ignited in a muffle furnace at 600ºC until it is white, indicating the absence of carbon. Then the dish was cooled in a desiccator and weighed. If this manner cannot obtain carbon-free ash, the residue on the cool dish was moistened with about 2 ml of water or a saturated solution of ammonium nitrate, dried on a water bath, and then ignited in the muffle furnace upto constant weight. The percentage of total ash was calculated using the following formula

Percentage of Total ash = (Weight of ash) / (Weight of sample taken) × 100

Acid insoluble ash- Total ash of the sample as described above was determined first. To the dish containing the total ash, 45 ml of 1:5 hydrochloric acid in three portions of 15 ml was added each time, boiled gently for 5 minutes, and filtered. The insoluble matter was collected on an ashless filter paper (Whatman No. 41), and washed with distilled water until the residue was free from acid. The filter paper containing the insoluble matter was transferred to the original dish, dried, and ignited to constant weight. After cooling the dish in desiccators, the weight was taken. The percentage of acid-insoluble ash was calculated using the following formula-

Percentage of Acid insoluble ash = (Weight of acid-insoluble residue) / (Weight of sample taken) × 100

2. Determination of Extractive Value: Plant materials are water, mineral and organic compounds (primary and secondary metabolites). These compounds are extracted in different solvents in various proportions. Extractive value is the amount of active constituents extracted with solvents from a given amount of medicinal plant material.

a) Alcohol soluble extractive- The air-dried powder drug (3 g) was weighed accurately in a glass stoppered flask. To this 100 ml of distilled ethanol (approx. 95%) was added and shaken occasionally for 6 h. After keeping for 18 h, it was filtered (rapidly, taking care not to lose any solvent).

Filtrate (25 ml) was pipette out in a pre-weighed 100 ml dish and evaporated to dryness in a water bath, after which it was kept in hot air oven at 105°C for 6 h. After cooling the flask in a desiccator, weight was taken. The percentage of alcohol-soluble extractives was calculated using the following formula-

Alcohol soluble extractive (%) = (Weight of the extract) / (25 × Weight of sample taken) × 100 × 100

b) Water-soluble extractive- The air-dried powder drug (3 g) was weighed accurately in a glass stoppered flask. 100 ml of distilled water was added and shacked occasionally for 6 hours. After keeping for 18 hours it was filtered. Filtrate (25 ml) was pipette out in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath, after which it was kept it in hot air oven at 105°C for 6 h. After cooling the flask in a desiccator, weight was taken.

The percentage of water-soluble extractive matter was calculated using the following formula

Water soluble extractive (%) = (Weight of the extract) / (25 × Weight of sample taken) × 100 × 100

3. Determination of Loss on Drying at 105°C: Loss on drying is the loss in weight in % w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions. The air-dried powder drug (2 g) was weighed accurately in a previously weighed dish. Then it was heated in an oven at 105 ºC for 5 h. After cooling the dish in a desiccator, the weight was taken. The procedure was repeated till constant weight is obtained. Loss on drying was calculated using the following formula-

Percentage of Loss on drying at 105°C = (Loss in weight of the sample) / (Weight of sample taken) × 100

4. Determination of pH Value: The pH value of an aqueous liquid may be defined as the common logarithm of reciprocal of the hydrogen ion concentration expressed in gram per liter. The pH value of liquid is determined potentially using the glass electrode and a suitable pH meter. One gram of coarsely powdered drug was added in 100 ml of water and the solution was filtered in a beaker and pH was checked at 25°C.

Thin Layer Chromatography (TLC) Analysis [13, 14]: One gram of powder of *Sphaeranthus indicus* aerial part was subjected to extraction in 25 ml methanol for 18 h at room temperature. The extract was filtered and used for TLC. Ten µl of the filtrate of *Sphaeranthus indicus* and geraniol was applied on Merck aluminum TLC plate and the plate was developed to a distance of 8 cm using toluence: ethyl acetate (7:3) as mobile phase.

After development, the plate was allowed to dry in air and examined under ultraviolet light (254 and 366 nm) for the presence of spots. After this, the plate was sprayed with an anisaldehyde-sulphuric acid reagent followed by heating at 105 °C for about 10 min. Spots were examined under visible light. TLC photos were captured at visible light, UV 254 nm and UV 366 nm and after derivatization. The number of spots, colors of spots and their Rf values was calculated.

Rf = (Distance travel by solute) / (Distance travel by solvent)

Preliminary Phytochemical Screening [15, 16]: The aqeous extract of the *Sphaeranthus indicus* (AESI) was tested for the presence of carbohydrates, proteins, alkaloids, flavonoids, glycosides, saponins, tannins, and essential oils using standard procedures.

Quantitative Estimation of Phytoconstituents:

Total Phenolic Content [17]: The extract's total phenolic content was determined spectrometrically. One ml of Folin-Ciocalteu's reagent, previously diluted (1:20), was added to 1 ml of sample (AESI) (1000 µg/ml), tannic acid (10-100 µg/ml) mixed thoroughly. To the mixture, add 4 ml of sodium carbonate (75 g/l) and total volume was made up to 10 ml with distilled water in a volumetric flask and mix well. The mixture was allowed to stand for 2 h at room temperature. Contents were then centrifuged at 2000 rpm for 5 min, and the absorbance of the supernatant was read at 760 nm. A standard curve was obtained using various concentrations of tannic acid. Results were expressed as mg of tannic acid equivalents (TAE) per gram of extract.

Total Flavonoids Content [18]: The aluminum chloride colorimetric assay measured total flavonoid content. One milliliter of sample (AESI) (1000 µg/ml) or standard solution of quercetin (25-1000 µg/ml) were added to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5% NaNO2 was added. After 5 minutes, 0.3 ml of 10% AlCl3 was added. At 6th min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against the prepared reagent blank at 510 nm. The total flavonoid content of the extracts was expressed as milligrams of quercetin equivalents per gram of extract.

Total Saponins Content [19]: The samples were ground and 3 g of each were put into a conical flask and 50 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered, and the residue was re-extracted with another 100 ml 20% ethanol. The concentrate was combined extract was reduced to 20 ml over a water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel then 10

ml ether was added and shaken well. The ether layer was discarded. The purification process was repeated. Thirty milliliter of n-butanol was added and shaken well. n-butanol layer was collected. The combined n-butanol extracts were washed twice with 5 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The content was calculated as mg saponins per 100 g of powdered material.

Total Saponins Content = (Weight of extract) / (Weight of sample) × 100

RESULTS AND DISCUSSIONS: Results of the physic-chemical studies *viz.* ash content, extractive value, moisture content, and pH were as per the pharmacopoeial standard [20] and previous reports. This indicated that the crude drug has the prescribed limits of all the stated parameters and suggested the appropriateness of the drug quality **Table 1**.

TABLE 1: PHYSICO-CHEMICAL EVALUATION OF *SPHAERANTHUS INDICUS* AERIAL PARTS

01	Ash analysis	
	❖ Ash content (Total ash)	9.407±0.07623
	❖ Acid insoluble ash	6.692±0.2866
02	Extractive value (Maceration process)	
	❖ Alcohol soluble	17.08±0.4543
	❖ Water soluble	26.46±0.9702
03	Moisture content (Loss on drying)	11.89±0.1158
04	pH (1% aqueous solution)	7.830±0.06028

Values are expressed as mean±SEM; n=3

TLC of methanol extract of the aerial parts of the *Sphaeranthus indicus* was run and compared with standard geraniol (active constituent of *Sphaeranthus indicus*). The TLC chromatogram revealed the presence of geraniol in the extract and thus authenticated the plant **Table 2**. The phytochemical screening of the plant extracts revealed the presence of alkaloids, proteins, amino acids, saponins, flavonoids and other phenolic compounds, while glycosides, sterols, carbohydrates, and volatile oils were absent. The results were in accordance with the several previous reports mentioned earlier [1, 2, 5, 21, 22, 23]. The quantitative estimation of phytoconstituents viz. total flavonoids and total phenolics and saponins revealed that AESI was found rich in total flavonoids (187.9±10.32 quercetin equivalents mg/g of AESI) while less amount of total phenolic content (37.23±0.70 tannic acid equivalents mg/g of AESI) while total saponin (3.33 g/100g powder drug) was present in fair amount. *S. indicus* Linn. is widely distributed throughout India. The plant appears to have a broad spectrum of activity on several ailments. Traditionally, various parts of the plant have been found effective for anxiolytic activity, neuroleptic activity, immunomodulatory activity, anti-inflammatory activity, mast cell stabilizing action, antihyperglycemic activity, hepatoprotective activity, larvicidal action, bronchodilatory effect, antihyperlipidemic activity, renoprotective effect and many other miscellaneous activities. It is reported to contain eudesmanoids, eudesmanolides, sesquiterpene lactone, sterol glycoside, flavanoids and essential oil.

TABLE 2: TLC PROFILE OF *SPHAERANTHUS INDICUS*

S. no.	366 nm	
	Colour	R_f
1	Light pink	0.15
2	Light pink	0.19
3	Pink	0.27
4	Light pink	0.54
5	Pink	0.58
6	Light pink	0.62
7	Light pink	0.68
8	Light Blue (geraniol)	0.73
9	Light pink	0.85
10	Pink	0.93

CONCLUSION: The pharmacological studies reported in this study confirm Sphaeranthus indicus Linn's therapeutic value. However, less information is available regarding this plant's clinical, toxicity, and phytoanalytical properties. Several phytochemical studies have been reported, but still, it needs to progress. With the availability of primary information, further studies can be carried out like clinical evaluation, psychoanalytical studies, and toxicity evaluation. The plant is preclinically evaluated to some extent; if these claims are scientifically evaluated clinically, it can provide good remedies and help mankind with various ailments.

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CONFLICT OF INTEREST: None

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