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Research Article

Physicochemical and Phytochemical Studies on *Sphaeranthus indicus* Linn. with HPTLC Fingerprinting

Garg Mohit*¹, Dwivedi Neelesh²¹Sadhu Vaswani College, Bairagarh, Bhopal, M.P., India²JRD Tata Foundation of Research in Ayurveda and Yoga Sciences, Chitrakoot, Satna, M.P., India

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*Address for Correspondence:

Garg Mohit, Scan research laboratory, Sector A-109, Near JK Road, Indrapuri, Bhopal, Madhya Pradesh-462022, India.

Abstract

Ayurveda, the oldest paradigm of health and healing known to the world, grew out of the *Rishi* tradition. The medical and research community is constantly seeking for new natural agents. *Sphaeranthus indicus* Linn. (Gorakhmundi, Family: Asteraceae), is widely used in Ayurvedic system of medicine to treat various diseases. The present investigation was designed to perform physicochemical and phytochemical analysis with HPTLC fingerprints of *Sphaeranthus indicus* (leave, flower, and stem) to establish the standard parameters of this herb. Different analytical parameters like extractive values, total ash, acid insoluble ash and water-soluble ash, moisture content, pH values of different parts of the drug were performed. Preliminary phytochemical screening was done to detect and quantify different active constituents such as alkaloids, flavanoids, proteins and total poly phenol using various analytical methods. Our preliminary data of phytochemical screening of the extracts revealed the presence of steroids, carbohydrates, proteins, terpenoid, flavonoid, tannins, alkaloids, resin and saponin. In the current study we established the HPTLC fingerprint of the extract using phytochemical standards. We believe that our study provides a substantial data for identification, purification, standardization, and phytochemical characterization of novel therapeutic compounds that can be used potential drug to combat various diseases for betterment of mankind.

Keywords: *Sphaeranthus indicus*, Ayurveda, physicochemical, UV- Spectrophotometer, HPTLC

INTRODUCTION

Herbal medicines have been used by the mankind since time immemorial. Ayurveda, the oldest traditional system of India reveals that ancient Indians had a rich knowledge of medicinal value of different plant species. *Sphaeranthus indicus* Linn. is from the aroma family Asteraceae and widely used in traditional system of medicine to treat various type of diseases. The plant is commonly known as Gorakhmundi in Hindi. It is also known with other synonyms such as Munditika, Mundi, Shravana, Bhikshu, Tapodhana, Mahashravani, Shravanahva, Shravanashirshaka. It is an annual spreading herb, which grows approximately 15-30 cm in height. The plant is distributed throughout the plains and wetlands in India, Sri Lanka and Australia¹. In folk medicine, the plant is reportedly used in treating epileptic convulsions, mental illnesses and hemicranias². According Ayurvedic literature, all parts of the plant are medicinally important. It is used to treat vitiated conditions of hemicranias, jaundice, hepatopathy, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia, and skin diseases. The external application of a paste of this herb is beneficial in treating pruritus and edema, arthritis, filariasis, gout and cervical adenopathy. It also treats piles and hepatitis³.

Essential oil obtained by steam distillation of the whole herb, contains ocimene, α -terpinene, Methyl-chavicol, α -citral, geraniol, β -ionone, *s*-ionone, *d*-cadinene, *p*-Methoxy cinnamaldehyde and an alkaloid sphaeranthine^{4,5}. The alcoholic extract of powdered caputula contains stigmaterol, β -sitosterol, hentriacontane, sesquiterpene lactone, sesquiterpene glycoside, sphaeranthanolid, flavone and isoflavone glycosides⁶⁻⁹. Recently many medicinal properties have been attributed to the extracts, fractions, and isolated constituents of *S. indicus* flowers, which include hypotensive, peripheral vasodilatory and convulsions, mental illnesses and hemicranias, cathartic activity, antimicrobial activity. Alkaloidal and non-alkaloidal fractions of alcoholic extract and sesquiterpene isolated from petroleum ether extract¹⁰.

Although substantial ancient literatures provide the evidence of therapeutic significance, key obstacle that has hindered the acceptance of the alternative medicines in the parallel to the modern counterparts, is the lack of their documentation and quality control parameters. Thus, it becomes extremely important to gain more insights towards standardization of the plant material to be used as medicine. The objective of the present study is to evaluate different physicochemical parameters like extractive values, total ash, acid insoluble

ash and water-soluble ash, moisture content, pH values and Phytochemical analysis to detect different active constituents with HPTLC fingerprints of *Sphaeranthus indicus* (leave, flower, and stem).

MATERIALS AND METHODS

Collection and identification of the Plant

Plant parts such as flowers leaves and stem bark of *Sphaeranthus indicus* were collected from Bagdara Ghati, Maghawan, Chitrakoot, Satna, MP, India. The plant material was identified and authenticated with the help of Herbarium specimen at Herbarium of Research Lab, Chitrakoot and with the help of available literature^{11,12}. Each of the plant parts *viz.* flower, leaf, and stem bark of *Sphaeranthus indicus* were washed, dried below 60°C in a tray drier, grounded to a fine powder and passed through sieve no 22. The drug powder was kept in an airtight container with a label showing the common name, botanical name; part used and date of powder preparation for further analysis.

Preparation of extract

Each of the dried powdered drugs (5gm) was subjected to extraction with methanol for 6 hours in a Soxhlet apparatus on water bath. The extract obtained were filtered and evaporated to dryness using water bath and stored at low temperature for further studies.

Physicochemical Analysis

Physico-chemical parameters such as moisture content (loss on drying at 105°C), water soluble extractive value, alcohol soluble extractive value, hexane soluble extractive value, total ash value, acid insoluble ash value and water-soluble ash were calculated as per the standard methods^{13,14}.

Determination of Total Ash

Total ash was determined by incinerating about 2 to 3 g accurately weighed, of the ground drug in a tared silica dish at a temperature not exceeding 450°C until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhausted the charred mass with hot water, collected the residue on an ashless filter paper, incinerated the residue and filter paper, add the filtrate, evaporated to dryness, and ignited at a temperature not exceeding 450°C. Calculated the percentage of ash with reference to the air-dried drug.

Determination of Acid Insoluble Ash

Boiled the ash obtained as total ash for 5 minutes with 25 ml of dilute hydrochloric acid, collect the insoluble matter in an ashless filter paper and wash with hot water and ignited to constant weight. Calculated the percentage of acid-insoluble ash with reference to the air-dried drug.

Determination of Water-Soluble Ash

Boiled the ash obtained as total ash for 5 minutes with 25 ml of water; collected insoluble matter in an ash less filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°C. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculated the percentage of water-soluble ash with reference to the air-dried drug.

Determination of Alcohol Soluble Extract

Macerated 5 g of the air-dried drug, coarsely powdered, with 100 ml of ethanol the specified strength in a closed flask for twenty-four hours, shaking frequently for six hours and allowing to stand for eighteen hours. Filtered rapidly, taking

precautions against loss of solvent, evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 105°C to constant weight and weighed. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Determination of Water-Soluble Extract

Proceeded as directed for the determination of Alcohol-soluble extractive, using *chloroform water* instead of ethanol.

Determination of Ether Soluble Extract (Fixed Oil Content)

Transfer a suitably weighed quantity (depending on the fixed oil content) of the air dried, crushed drug to an extraction thimble, extract with *Solvent ether* (or petroleum ether, boiling point 40°C to 60°C) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filter the extract quantitatively into a tared evaporating dish and evaporate off the solvent on a water bath. Dried the residue at 105°C to constant weight. Calculated the percentage of ether-soluble extract with reference to the air-dried drug.

Determination of Moisture Content (Loss on Drying)

Placed about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish. After placing the above said amount of the drug in the tared evaporating dish dry at 105°C for 5 hours and weighed. Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25%. Constant weight is reached when two consecutive weightings after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

Preliminary Phytochemical analysis

Preliminary qualitative phytochemical analysis of aqueous, alcoholic and petroleum ether extract of *Sphaeranthus indicus* was carried out by employing standard protocols^{15,16} for determining the presence and/or absence of phytochemical. Dragendorff's test and Wagner test was performed for analysis of alkaloids. Dragendorff's test was performed by Dissolving a few ml of alcoholic or aqueous extract of drug in 5 ml of distilled water, followed by addition of 2 ml diluted HCl until an acid reaction occurs, then added 1ml of Dragendorff's reagent, an orange or orange red precipitate is produced immediately. Wagner test was performed by mixing Acidified 1 ml of alcoholic extract of the drug with 1.5% v/v of HCl and added a few drops of Wagner reagents, a yellow or brown precipitate is formed. Flavonoid content was determined by Shinoda by adding 5-10 drops of dilute HCl followed by small pieces of magnesium in a test tube containing 0.5 ml of alcoholic extract of the drug. In the presence of flavonoid, pink, reddish pink, or brown color is produced. Tri- terpenoids in the extract was assessed using Libermann Burchard test by Adding 2ml of acetic anhydride solution to 1ml of petroleum ether extract of the drug in chloroform followed by 1 ml of conc. H₂SO₄, a violet-colored ring is formed indicate the presence of triterpenoids. Carbohydrate content in the extract was detected by performing Anthrone test and Fehling test by adding 2ml of anthrone test solution added 0.5 ml of aqueous extract of the drug, a green or blue color indicates the presence of carbohydrate, for anthrone test and by adding 2 ml of aqueous extract of drug added 1 ml of mix of equal part of Fehling solution A and B and boiled the content of the test tube for few minutes, to obtain red color for Fehling test.

Protein content was determined using Biurate test by adding 1ml of hot aqueous extract of drug, added 5-8 drops of 10% w/v NaOH solution followed by 1-2 drops of 3% w/v CuSO₄ solution to obtain a purple color solution. Resins were detected by dissolving the aqueous extract in 1 ml of acetone and poured the solution into 5ml of distilled water; the turbidity indicates the presence of resin. To detect the presence of Saponins, Foam test was performed by adding drops of sodium bicarbonate in test tube containing about 5ml of an aqueous extract of the drug, add, vortexing it vigorously. A visible honeycomb like structure is the confirmation for presence of saponins. Tannins in the extract was detected by adding a few drops of 5% FeCl₃ in 1-2ml of extract of the drug. Presence of greenish color is an indication the presence of gallotannin, and white, brown color for tannin. Steroids were detected by performing Salkowski test by adding 1ml of conc. H₂SO₄ to 2ml of chloroform extract of the drug carefully from the side of test tube. Presence of a red color is in the chloroform layer is an indication for steroids.

Spectrophotometric analysis

The fluorescence properties of the powdered samples T1, T2 and T3 were studied under UV radiations at 254 nm and 365 nm wavelengths as per the standard procedure^{17,18}. A small quantity of root powder was placed on a clean microscopic slide and one to two drops of freshly prepared reagent solution was added, followed by gentle mixing. The behavior of the sample with different chemical reagents was studied and fluorescence characters were observed on short and long UV light after 1-2 minutes.

HPTLC Fingerprinting¹⁹⁻²¹

It is apparent that silica gel TLC is a choice technique for the study of phytochemicals because of its versatility and simplicity. With the help of TLC hundreds of oils of different chemical races have been screened and their components were identified. The methanolic extract of the powdered samples T1 (leaf) T2 (flower) and T3 (stem) of *Sphaeranthus indicus* were subjected to HPTLC fingerprint analysis to check the presence of phytoconstituents. Chromatography was carried out on pre-coated silica gel HPTLC aluminum plates 60F254 (20 cm×20 cm, 0.2mm thickness, 5-6 μm particle size, E-Merck, Germany) by using CAMAG HPTLC system (Switzerland). 5μl of each of the extract was spotted as bands of 8 mm width by using a LINOMAT 5 Sample Applicator fitted with a 100 μl Hamilton syringe. The plates were developed using Chloroform: methanol (9:1) and Toluene: ethyl acetate (9:1) as a mobile phase in CAMAG twin-trough chamber lined with filter paper and pre-saturated with 10 ml mobile phase. The resulted plates were dried in air and photo documentation was done at ultraviolet light 254nm and 366nm and at day light using CAMAG REPROSTAR 3 equipped with WINCATS Software. Numbers of bands, colors of separated compound and R_f values were recorded.

Quantitative estimation of phytochemicals

Determination of Alkaloids

Alkaloids content was measured by following the protocol described by Harborne²². A suspension was prepared by dispersing 5 grams of the dried leaves in 10% acetic acid solution in ethanol and kept at 28°C for 4hrs which was further filtered through Whatman No. 42. Thereafter alkaloid was precipitated by concentrating the filtrate to one-quarter of its original volume and drops of conc. aqueous NH₄OH

were added. Finally, the precipitate was washed with 1% ammonia 70 solution and dried at 80°C in the oven. The content of alkaloid was calculated and expressed as mg/g of sample.

Determination of Flavonoids

The aluminum chloride colorimetric method was used for flavonoids determination²³. Each plant extract (0.5 ml of 1:10 gm/ml in methanol) were separately mixed with 1.5ml of methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It was kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 418 nm. The percentage of total flavonoids were calculated from the calibration curve of Rutin (200-1000μg) plotted by using the same procedure and total flavonoids was expressed as Rutin equivalents in mg/gm sample.

Determination of total phenols

Accurately 0.5gm powdered drug was taken and extracted with 75ml of 50% methanol by cold maceration for 2hrs with intermittent shaking. Solution was filtered and volume was made up to 100ml. From the above extract, 0.1ml was pipetted out into a 25ml volumetric flask and 10ml of distilled water was added followed by 1.5 ml of Folin-Ciocalteu reagent. After 5 mins 1ml of 20% sodium carbonate solution was added and volume was made up to 25 ml with distilled water and incubated for 30mins. After 30min absorbance was recorded at 765nm. 10mg of standard Gallic acid was weighed and dissolved in 10ml distilled water in a volumetric flask (1000μg/ml of stock solution) and then pipette out 0.5 to 2.5ml of aliquots into 25ml volumetric flasks from the above prepared stock solution. Then 10 ml of distilled water and 1.5ml of Folin-Ciocalteu reagent. After 5 min, 1ml of 20% sodium carbonate was added, and then distilled water was added to make the volume up to 25ml. After 30 min, absorbance at 765nm was recorded and calibration curve of absorbance v/s concentration was plotted. Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass). The total phenolic contents of the methanolic extract were calculated by using standard calibration curve^{24,25}.

Protein Estimation by Lowry's Method^{26,27}

Extraction is carried out with buffers used for the enzyme assay. Weighted 500mg of the sample and grind well with a pestle and mortar in 5-10ml of the buffer. Centrifuged and used the supernatant for protein estimation. Pipetted out 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard into a series of test tubes. Pipetted out 0.1ml and 0.2ml of the sample extract in two other test tubes. The volume was made to 1ml in all the test tubes. A tube with 1ml of water serves as the blank. Added 5ml of Alkaline copper solution to each tube including the blank mix well and allow standing for 10 minutes. Then added 0.5ml of Folin - Ciocalteu reagent, mix well and incubate at room temperature. Kept in the dark for 30 min. blue colour is developed. Absorbance was recorded at 660 nm and unknown protein concentration was calculated using standard curve.

RESULTS AND DISCUSSION

Sphaeranthus indicus is a highly reputed drug used in Ayurveda. The powdered drugs colors are greenish brown, odour-slightly aromatic when fresh, aroma disappearing on long storage and taste are bitter in all the samples (Figure 1).

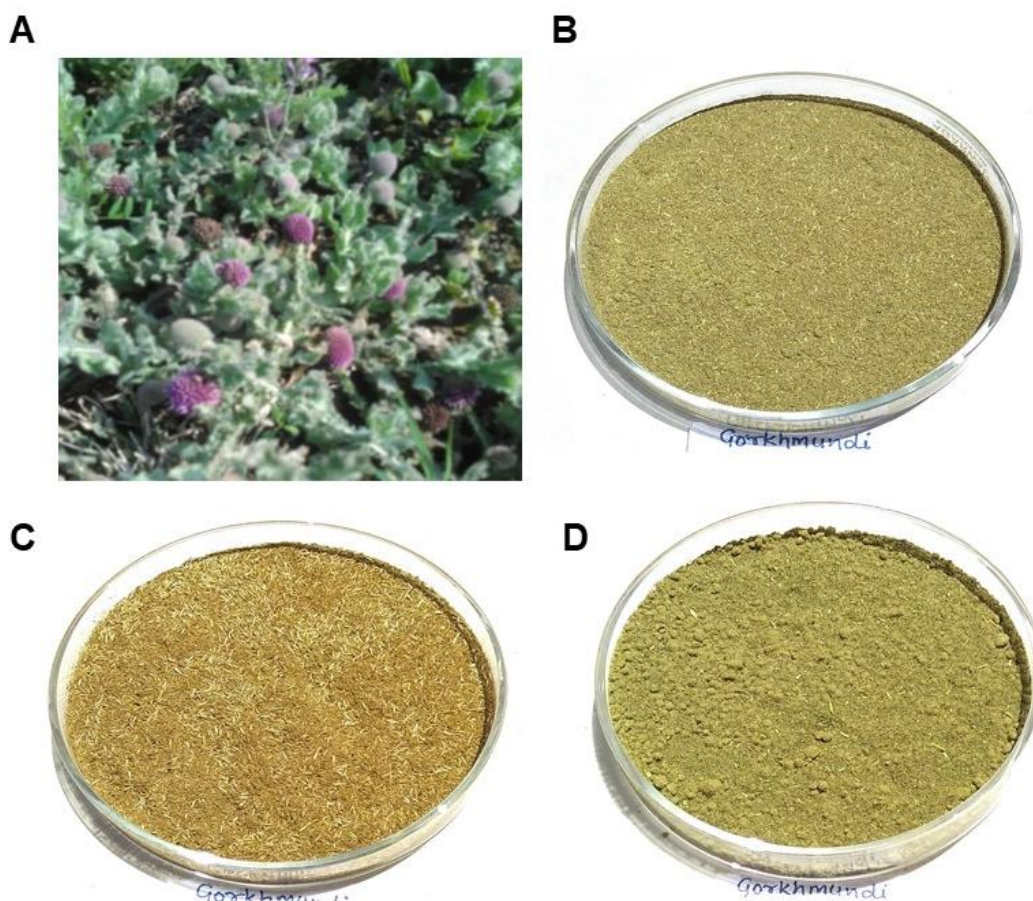


Figure 1: *Sphaeranthus indicus* specimen. The image in the Fig 1 shows the different sample of *Sphaeranthus indicus* plant. Panel A shows the typical greenish brown flowers of *Sphaeranthus indicus*. Panel B, C, and D shows the powder obtained from stem, flower and leaf of *Sphaeranthus indicus*, respectively. Each of these samples were collected, dried and crushed using motor pestle and directly used for study.

Phytomedicine represents one of the most important fields of traditional medicine all over the world and are of prime importance to the health of individuals and communities. To promote the proper use of phytomedicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way²⁸. Standardization is an important modern tool of analysis used to ensure the quality of herbal

drugs. There are various physicochemical parameters used for the quality evaluation of the herbal drugs. In the present study, physicochemical parameters include extractive values, total ash value, acid insoluble ash value and water-soluble ash value, moisture content, loss on drying, pH values (1% and 10% solutions) were determined to check the purity of the drug. The results of physicochemical parameters are summarized in Table 1.

Table 1: Physicochemical parameters of *Sphaeranthus indicus* Linn.

Sr.No.	Parameters	<i>Sphaeranthus indicus</i> Linn			API Limits
		T ₁ (Leaf) % w/w	T ₂ (Flower) % w/w	T ₃ (Stem) % w/w	
1	LOD at 105° C	7.84	5.83	7.3	
2	Total Ash	16.92	8.39	8.63	NMT 5
3	Acid-insoluble ash	4.52	0.91	2.03	NMT 2
4	Alcohol-soluble extract	3.7	12.58	6.03	NLT 13
5	Water soluble extract	19.86	21.71	22.35	
6	Ether soluble extract	9.96	18.36	13.76	
7	Volatile oil	2.1	1.9	2.0	NLT 1.5
8	pH (10% aqua solution)	7.24	7.09	7.6	4 - 5

This study of physicochemical parameters such as moisture content and ash values are useful as it determines the physiological and non physiological state of ash, this will help to determine the possibility of microbial growth and lastly contaminant or impurities. The ash values were used to detect the presence of any foreign matters e.g., sand and soil, water soluble salts adhering to the surface of the drugs. There is always a considerable difference in the ash values of different drugs but mostly the difference varies within narrow limits in case of the same drug. The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthy materials.

Higher moisture content in the drug sample may causes hydrolysis of active ingredients of the drug and decreases its quality and efficacy. The final dryness of the drug and rate of moisture removal are equally important, and it was observed that the moisture content in the samples T₁, T₂ and T₃ were found to be 7.84, 5.83, 7.3% (w/w) which showed that the drug was well dried and stored. This result complies with the standards established by the International Pharmacopoeia, because this water content rate, prevent oxidation reaction fermentation and give less chance to microbial growth and contamination in drugs²⁹. The extractive values were used to find out the number of active

principles. The higher percentage yield of *Sphaeranthus indicus* Linn extracts for T₁, T₂ and T₃ (leaf, flower & stem bark) were found to be 9.96%, 18.36 & 13.76 % (w/w) respectively in petroleum ether. This indicated that flower of *Sphaeranthus indicus* contain higher concentration of fatty constituents. The alcohol soluble extractive of the extracts for T₁, T₂ and T₃ (leaf, flower & stem bark) were found 3.7, 12.58, 6.03% (w/w) and water-soluble extractives were found 19.86, 21.71 & 22.35% (w/w) respectively. The pH values of the *Sphaeranthus indicus* extracts (1% and 10% solutions) was also investigated using digital pH meter as per the standard method. The pHs of the drug (1% and 10% solutions) were found 7.24, 7.09 and 7.6 respectively for T₁, T₂ and T₃. The pH of the extracts reveals the concentration of acidic and basic compounds.

All the extracts of the drug were subjected to different tests for detecting the presence of various phytoconstituents present in the drug, which revealed the presence of steroids, carbohydrates, proteins, terpenoid, flavonoid, tannins, alkaloids, resin and saponin etc. all the active constituents have specific pharmacological effects and often used as medications^{30,31}. The results of phytochemicals screening are represented in Table 2.

Table 2: Phytochemical Analysis in aqueous and methanol extracts of *Sphaeranthus indicus*

S.No.	Constituent	Results		
		T ₁ (Leaf)	T ₂ (Flower)	T ₃ (Stem)
1	Alkaloids			
	Wagner's test	+	+	+
	Dragendraff's	+	+	+
2	Flavonoids (Shinoda test)	+	+	+
3	Resins	+	+	+
4	Saponins (Foam tests)	+	+	+
5	Tannins	+	+	+
6	Carbohydrates	+	+	+
	Anthrone's test	+	+	+
	Fehling's test			
7	Proteins test (Biuret test)	+	+	+
8	Triterpenoids	+	+	+
	Libarmann Burchard test			
9	Steroids (Salkowski tests):	+	+	+

The quantification of total phenols, flavonoids, alkaloids, and proteins were carried out using UV-spectrophotometer (UV-1800, Shimadzu). The results were tabulated in Table 3. The phenolic compounds act as antioxidants. These compounds are also reported to have anticancer, antimicrobial, anti-inflammatory, and anti-allergic activities etc. Phenolic compounds are most widely occurring groups of phytochemicals and derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants. These compounds are secondary metabolites which have vital role in reproduction and growth, gives protection against harmful predators and pathogens³². Therefore, quantitative analysis of these compounds is very important to check the quality of drug. The phenolic content expressed as gallic acid

equivalent per gram (GAE/gm) and was found 13.64±0.008, 11.4±0.007, 9.6±0.004 mg GAE/gm for samples T₁, T₂ and T₃ respectively. The standard calibration curve was used to calculate the total phenolic contents in the methanolic extract of the drugs (standard curve equation: $y = 0.00219x + 0.12646$ R²= 0.99897). Flavonoids are the most common group of polyphenolic compounds in the human diet and are found in plants³³. Total flavonoids were measured by aluminum chloride method and are expressed in terms of rutin equivalent (RE) the standard curve equation: $y = 0.686x - 0.246$, R²=0.927. All the samples exhibited good quantity of flavonoids and were found to 18.5±0.051, 17.4±0.046, and 16.4±0.070 mg/gm for T₁, T₂ and T₃, respectively.

Table 3: Quantitative estimation of phytochemicals (mg/gm) in *Sparanthus indicus* (mg/gm)

Samples	Alkaloids	Flavonoids	Phenols	Proteins
T ₁	9.46±0.10	18.5±0.051	13.64±0.008	3.20±0.25
T ₂	8.45±0.35	17.4±0.046	11.4±0.007	4.05±0.24
T ₃	7.65±0.45	16.4±0.070	9.6±0.004	3.78±0.35

*Experimental results were mean ± S.D of three parallel measurements.

Alkaloids are produced by large variety of organisms, plants, and animals. They almost uniformly invoke bitter taste. They have therapeutic potential and often used as medications and recreational drugs³⁴. The alkaloids content was quantitatively estimated and was found 9.46 ± 0.10 , 8.45 ± 0.35 and 7.65 ± 0.45 mg/gm, respectively. Similarly, Protein content of samples T₁, T₂ and T₃ were found 3.20 ± 0.25 , 4.05 ± 0.24 and 3.78 ± 0.35 mg/gm, respectively. The standard

curve equation: $y = 0.0058x - 0.00442$, $r^2 = 0.99445$. The finding specifies that the samples contain a significant amount of alkaloid, flavonoids, phenolic, protein content. Fluorescence analysis of the powdered samples T₁, T₂ and T₃ were treated with different chemicals and color change were observed under Ultraviolet light at 254 nm and 366 nm wavelengths and the results were tabulated in Table 4.

Table 4: Fluorescence characteristics of the drugs with different reagents

S. No.	Treatment	254 nm			366 nm			Ordinary light		
		T ₁	T ₂	T ₃	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃
1.	Powder as such	G	W	W	G	W	W	G	LB	LG
2.	Powder + Iodine water	LG	B	B	Lb	Lb	G	B	B	WG
3.	Powder + 50% KOH	B	LB	B	DB	B	G	DB	DB	YB
4.	Powder + 1N NaOH in Methanol	B	B	B	R	LB	B	G	G	DB
5.	Powder + Acetic acid	B	B	B	G	SB	SB	G	B	G
6.	Powder + 50% HNO ₃	B	B	B	DB	DB	G	B	B	YB
7.	Powder + Conc.H ₂ SO ₄	B	Db	Db	Sb	Lb	blk	DB	DB	blk
8.	Powder + 1N HCl	B	B	B	B	LB	G	B	blk	LB
9.	Powder + 1N NaOH	B	B	W	R	B	G	G	G	G
10.	Powder + 50% H ₂ SO ₄	LB	LB	LB	G	B	G	LB	LB	B

Abbreviations: G=green, W=white, LB=Light brown, B=brown, GB=greenish brown, DB=dark brown, Db=dark blue, YB= Yellowish brown, Gb= greenish black, SB=sky blue, LG=light green, WG= white, green, Lb= light blue, Db= dark blue, SB= sky blue, b= black, R= red

HPLTC is an important analytical tool in the separation, detection, and estimation of different classes of natural products and chromatographic fingerprint is a rational approach for more strong and efficacious quality control of the natural products³⁵. HPTLC fingerprinting of methanolic extracts was performed using Camag-HPTLC system (Switzerland) with a Linomat 5 sample applicator. TLC was

done on pre-coated silica gel HPTLC aluminum plates 60F²⁵⁴, E-Merck, Germany. The plates were developed using Toluene: Ethyl acetate (9:1) and Ethyl acetate-methanol-water (100:13.5:10) as mobile phases. The colors of the resolved bands and R_f values were calculated. (Figure 2, Table 5).

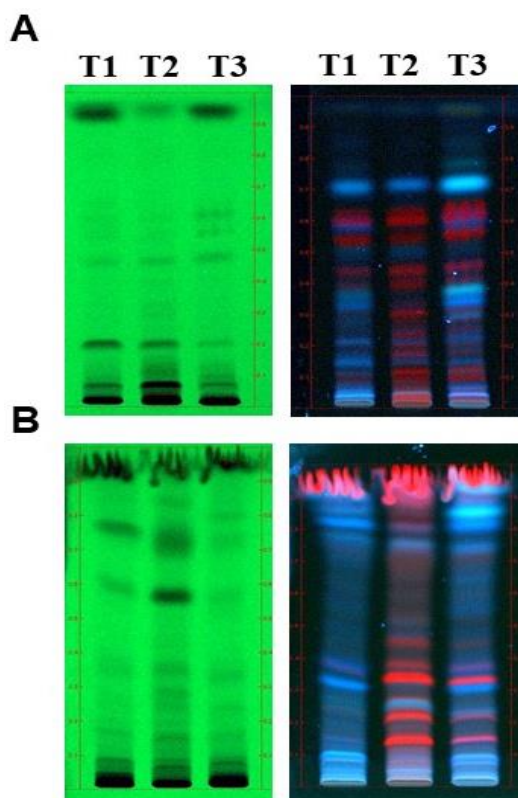


Figure 2: HPTLC chromatograms of *Sphaeranthus indicus*. Panel A and B of the Figure 2 shows the chromatogram of the extracts from *Sphaeranthus indicus* separated using solvent system 1 (toluene: ethyl acetate; 9:1) and solvent system 2 (ethyl acetate-methanol-water; 100:13.5:10). Tracks T₁, T₂ and T₃ indicate samples from leaf, flower, and stem, respectively.

Table 5: R_f values and color of the resolved bands of test solutions of *Sphaeranthus indicus*

Mobile phase: Toluene: Ethyl Acetate (9:1)			
	T₁	T₂	T₃
254nm	0.7, 0.20,0.47,0.97	0.7, 0.20, 0.97	0.7, 0.20, 0.97
366nm	0.9 (red), 0.15 (blue), 0.22 (blue), 0.32 (blue), 0.39 (red), 0.45 (red), 0.54 (red), 0.58 (blue), 0.60 (pink), 0.71 (blue)	0.9 (red), 0.12 (red), 0.14 (blue), 0.22 (blue), 0.23 (red), 0.30 (red), 0.40 (red), 0.44 (red), 0.54 (red), 0.60 (red), 0.72 (blue)	0.9 (red), 0.12 (red), 0.14 (blue), 0.22 (blue), 0.29 (blue), 0.34 (blue), 0.37 (sky blue), 0.45 (red), 0.54 (red), 0.61 (red), 0.72 (blue)
Mobile phase: Ethyl acetate-methanol-water (100:13.5:10)			
	T₁	T₂	T₃
254nm	0.9,0.18,0.60,0.78	0.7,0.17,0.36,0.57,0.64	0.8,0.17,0.25,0.34,0.57,0.73
366nm	0.11 (sky blue), 0.32 (blue), 0.34 (pink), 0.82 (blue)	0.15 (red),0.22 (red), 0.26(sky blue), 0.27 (red), 0.34 (red), 0.37 (red), 0.44 (red) 0.76 (blue), 0.82 (pink)	0.15 (pink),0.22 (pink),0.30 (blue), 0.32 (red), 0.36 (pink), 0.44 (light red), 0.72 (red),0.74 (blue), 0.82 (blue)

CONCLUSION

Standardization of crude drugs has become very important for identification and authentication of a drug of natural origin. The present study was developed to establish the preliminary phytochemical analysis with other various physicochemical standards for the development of quality parameters of highly valuable drug. The presence of various phytochemicals like-steroids, carbohydrates, proteins, terpenoid, flavonoid, tannins, alkaloids, resin and saponin in all the samples supports the traditional use of this plant by the rural population of India. The investigated parameters, which are being reported for the present study, are most significant and relevant towards establishing the standards for quality, purity and strength in future identification and authentication of genuine plant material. Thus, this drug may provide novel or lead compounds that could become the starting material for the synthesis of a new cheap drug.

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