ORIGINAL ARTICLE

A comparative physicochemical and phytochemical study of whole plant of *Sphaeranthus indicus* linn collected from different geographical regions of central India

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Abstract

Background: Phytochemicals are secondary metabolite produced of all plants, where some plants have medicinal uses. *Sphaeranthus indicus* Linn is an important medicinal plant belongs to the family Asteraceae and its parts used to cure various illnesses. **Aim:** The aim of present study is to compare the physicochemical and phytochemical analysis of three *S. indicus* plants collected from Rajgarh, Nagda, and Rewa region of central India, this study is first investigation on this plant. **Materials and Methods:** The study includes physicochemical organoleptic evaluation and phytochemical screening of *S. indicus*. The physicochemical parameters assessed were within WHO limits, apart from these 17 phytochemicals were tested and the crude extracts were prepared in petroleum ether, chloroform, ethanol and chloroform water by soxhlation method**. Results and Discussion:** *S. indicus* plant extracts collected from Rajgarh region showed the highest phytoconstituents as compared to Nagda and Rewa region and the lowest were found in Nagda region. In contrast proteins, amino acids, anthraquinones/emodins, and anthocyanins were found to be completely absent in plants samples. **Conclusions:** From this study, one can collect the plants from the region where it's showing maximum concentration of phytochemicals and gives scope of investigation to extract pure compound and preparation of herbal formulations with best therapeutic value that will serve the society.

Key words: Geographical region, Organoleptic evaluation phytochemical screening, Physicochemical evaluation, *Sphaeranthus indicus* Linn

INTRODUCTION

Sphaeranthus indicus is an herb commonly known as gorakh mundi, is about 30 cm high spreading branched herb (family: Asteraceae, Compositae). It is dispersed through India, Australia, Sri Lanka, and Africa from sea level to 4000 ft. altitude,[1] especially in hills, as weed in the rice field. It grows in rice fields, dry waste places and cultivated lands in tropical parts of India. The temperature criteria for cultivation of *S. indicus* are 10°C–40°C. It needs a height of about 500–1500 m. It grows better in hot and humid conditions. Alluvial soil, red soil or lateritic soil is observed to be better for its cultivation. The pH of soil must be in the range of 6.3–7.3. It is rainfed type of plant and is propagated in monsoon season.[2] It's a medicinal plant hugely used in Indian traditional system of medicine for healing multiple ailments.[3] It is a annual, aromatic herb having lanceolate, wing toothed leaves with semi-amplexicaul base, and acutely serrate margin.[4] The herbs contain essential oil, sterols, and alkaloids.[5] Essential oil contains methylchavicol, alpha-ionone, D-cadinene, and p-methoxycinnamaldehyde as major constituents. Herb also yields the alkaloid sphacranthine beta-sitosterol, stigmasterol, beta-sitosterol-beta-D-glucoside, and hentriacontane.[6] 2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl),

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2,2,6- Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxabicyclo heptan-3-ol, 3-Hexenoic acid, 2-methyl-, methyl ester, etc.

During the past few decades, there has been increasing acceptance of natural products and therapies in the world also increase in use of Ayurvedic remedies globally. Therefore, quality control for efficacy and safety of herbal products is of main concern.[7,8] Plant phytochemical investigation is an interesting field of research, leading to the isolation of several new compounds. The pharmacological effect and therapeutic value of a drug were due to the presence of some chemical components such as various types of glycosides, resins, lipids, volatile oil and fixed oil, pectin, phenolic compounds, tannins, various kinds of alkaloids gums, and mucilage. These phytochemicals are of enormous importance for humankind.[9] The current literature revealed some physicochemical and phytochemical studies. The present study was conducted with the objectives to compare the physicochemical and phytochemical study of whole plant of *S. indicus* Linn collected from different geographical regions of central India. This study provides some valuable information with respect to its identification and standardization of *S. indicus*, which could be helpful in authenticity, purity and quality aspects.

MATERIALS AND METHODS

Procurement and Authentication of Plant Material

The taxonomic identification of the whole plant of *S. indicus* collected from different locations (Rajgarh, Nagda, and Rewa) of central India was also confirmed and authenticated by Dr. H. B Singh, Raw Materials Herbarium and Museum (RHMD) of NISCAIR, New Delhi. (Ref. letter No. of SI-1 from Rajgarh- NISCAIR/RHMD/Consult/-2017/3065-14-1), (Ref. letter No. of SI-2 from Nagda- NISCAIR/RHMD/Consult/-2017/3065-14-2), (Ref. letter No. of SI-3 from Rewa NISCAIR/RHMD/Consult/-2017/3065-14-3).

Collection and Processing of Plant Material

S. indicus L. grow as a weed in paddy field and these plant samples were collected from three different regions of India for the proposed study. Sample 1 (SI-1) collected from Rajgarh, Sample 2 (SI-2) from Nagda, and Sample 3 (SI-3) from Rewa of Madhya Pradesh. The collected plant samples were cleaned thoroughly in water to remove sticky dirt and naturally dried under shade and subjected to size reduction using a mechanical grinder. The coarsely dried, powder was stored in hermetically sealed containers for further use. These powdered samples were used for further physicochemical and phytochemical analysis.

Physicochemical Evaluation[10]

Determination of foreign matter

Approximately 50 g of all samples were weighed correctly and spread as a thin layer on separate clean papers and were thoroughly examined using a 10× magnifying lens to solve various groups of foreign matter. Foreign objects from the samples were accurately collected and weighed to record the percentage in g/100 g of air-dried sample. An analysis was performed 3 times with all samples and results were expressed as mean.

Moisture Content

5 g of powdered drug was weighed in a tared crucible, placed in infrared moisture balance until constant weight. The weight after drying was noted and loss on drying was calculated. The percentage was calculated on the basis of sample taken initially. The whole procedure was repeated 3 times with all samples and results were calculated as mean. The moisture content of drug was determined by the formula

$$\text{Moisture content} = (IW/DW) \times 100$$

Where, IW- Initial weight
DW- Dry weight

Determination of Ash Values

Determination of total ash

3 g of grounded dried powder material was accurately weighed in a previously tared silica crucible. The material was spread in a uniform layer and ignited until it turned white, then it was slowly heated to 500–600°C. The silica crucible was cooled in desiccator and weighed without delay. The percent of total ash content (mg/g) was calculated. The whole procedure was performed in triplicate for all samples and results were expressed as mean.

Determination of Acid – Insoluble Ash

Total ash content obtained from crud drug was boiled for 5 min in 25 ml of hydrochloride acid. After cooling the material was filtered using ashless Whatman filter paper and washed with warm water until the filtrate is neutral and then transferred the filter paper contained insoluble matter to be silica crucible, ignited cooled the residue in desiccator and weighed. The procedure was repeated many times until to get constant weight. The acid insoluble ash in mg/g of air-dried sample was then calculated. The whole procedure was performed in 3 times for all samples and results were expressed as mean.

Determination of Water-Soluble Ash

To the ash obtained as total ash 25 ml of chloroform water was added and then boiled for 5 min. The insoluble matter was collected on an ash less filter paper and washed in lukewarm water then ignited in a crucible for 15 min at a temperature of not more than 450°C after that the crucible

was cooled in desiccators and weighed. The procedure was repeated to achieve a constant weight of the insoluble matter was subtracted from the total ash weight. The differences in weight indicate the water (distilled) soluble ash percentage which was calculated with reference of air-dried drug. The whole procedure was performed in triplicate for all samples and results were expressed as mean.

Determination of Extractive Values

Determination of alcohol soluble extractives

In a conical flask added 5 g of air-dried coarse powder of drugs and 100 ml of absolute ethanol. Stoppered the conical flask with shaking regularly during the first 6 h and allowing to stand for 18 h. After that, drug containing solvent was filtered rapidly without loss of solvent. About 25 ml of filtrate was evaporated in tared shallow bottom dish to dryness on a water bath and then dried for 6 h at 105°C, cooled in a desiccator for 30 min. and was weighed without delay. The experiment was performed three times with all samples and results were expressed as mean.

Determination of water-soluble extractives

In a conical flask added 5 g of air-dried coarse powder of drugs and 100 ml of water. Stoppered the conical flask with shaking regularly during the first 6 h and allowing to stand for 18 h. After that, drug containing water was filtered rapidly without loss of water. About 25 ml of filtrate was evaporated in tared shallow bottom dish to dryness on a water bath and then dried for 6 h at 105°C, cooled in a desiccator for 30 min and weighed without delay. The experiment was performed 3 times with all samples and results were expressed as mean.

Determination of chloroform soluble extractives

In a conical flask added 5 g of air-dried coarse powder drugs and 100 ml of chloroform. Stoppered the conical flask with shaking regularly during the first 6 h and allowing to stand for 18 h. After that, drug containing solvent was filtered rapidly without loss of solvent. About 25 ml of filtrate was evaporated in tared shallow bottom dish to dryness on a water bath and then dried for 6 h at 105°C, cooled in a desiccator for 20–30 min and weighed without delay. The experiment was performed 3 times with all samples and results were expressed as mean.

Determination of petroleum ether soluble extractives

In a conical flask added 5 g of air-dried coarse powder drugs and 100 ml of petroleum ether. Stoppered the conical flask with shaking regularly during the first 6 h and allowing to stand for 18 h. After that, drug containing solvent was filtered rapidly without loss of solvent. About 25 ml of filtrate was evaporated in tared shallow bottom dish to dryness on a water bath and then dried for 6 h at 105°C, cooled in a desiccator for 20–30 min and weighed without delay. The experiment was performed 3 times with all samples and results were expressed as mean.

Organoleptic Evaluation[10]

Organoleptic evaluation of *S. indicus* was subjected to morphological studies including color, odor, and taste were examined.

Preliminary Phytochemical Screening of Plants Material

Preparation of extract by successive solvent extraction method

100 g of air-dried whole plant material of *S. indicus* Linn were extracted by successive Soxhlet extraction method for 72 h were carried using solvents with increasing polarity, namely, petroleum ether, chloroform, ethanol, and macerated to form an aqueous extraction. This process was performed with all three samples. Extracts were filtered and solvents were evaporated by distillation process under reduced pressure and the resulting extracts obtained were dried. Dried extracts were stored in well closed air tight bottles for further use. Preliminary phytochemical screening was performed as the standard protocol.

The percentage yields of Petroleum ether, chloroform, ethanol, and aqueous extract of herbs along with their color, nature, and consistency showed in Table 1.

$$\% \text{ Yield} = \text{Weight of extract (g)} / \text{Weight of dry powder(g)} \times 100$$

Qualitative Preliminary Phytochemical Analysis[11-15]

Chemical tests were carried out with three plants extract of *S. indicus*. All the extracts obtained were subjected to several qualitative chemical tests to reveal the presence or absence of common phytoconstituents.

Test for Alkaloids

- Mayer's Test: Mayer's reagent (potassium mercuric iodide [K_2HgI_4] solution) was prepared by 1. 36 g of $HgCl_2$ individually dissolved in 60 ml distilled water (A) and 5 g potassium iodide in 20 ml of distilled water (B). A and B were mixed together and the volume was adjusted up to 100 ml using water. Few drops of Mayer's reagent were added in 2–3 ml of each extract, a cream color precipitate indicates the presence of alkaloids

Table 1: Successive solvent extractive values of (SI-1, SI-2 and SI-3) plants selected for study under investigation. (Preliminary Phytochemical Screening of Plants Material)

Plants name with location	Solvent	Color of extracts	Average extractive values in %w/w on dry weight basis	Extraction hrs	(Consistency) nature of extract
Sphaeranthus indicus	Petroleum ether	Dark green	4.21%	72 h	Sami solid sticky mass
	Chloroform	Dark green	2.92%		Solid mass
Linn plant 1 (Rajgarh)	Ethanol	Greenish brown	3.73%		Viscous oily mass (bulky mass)
	Chloroform water	Dark brown	7.15%		Solid mass
Sphaeranthus indicus Linn plant 2 (Nagda)	Petroleum ether	Dark green	5.14%		Sami solid sticky mass
	Chloroform	Brownish black	2.01%		Semi solid
	Ethanol	Yellowish green	3.51%		Sami solid sticky mass
	Chloroform water	Blackish brown	6.79%		Sami solid sticky mass
Sphaeranthus indicus Linn plant 3 (Rewa)	Petroleum ether	Greenish brown	1.54%		Sami solid sticky mass
	Chloroform	Dark greenish black	1.02%		Solid mass
	Ethanol	Yellowish brown viscous	8.81%		Viscous oily mass
	Chloroform water	Dark brown	10.2%		Solid mass

- Dragendorff's Test: Dragendorff's reagent was prepared by taking 14 g of sodium iodide with 5.2 g of [$(BiO)_2CO_3$] basic bismuth carbonate in 50 ml of (CH_3COOH) acetic acid and boiled for a few minutes. After that, it was allowed to stand overnight and precipitate of (NaOAc) sodium acetate was filtered. To the 40 ml of red brown filtrate, 160 ml of the (EtOAc) ethyl acetate and 1 ml of water was added. 20 ml of acetate acid was added to 10 ml of stock solution and volume was made up to 100 ml with water. Few drops of Dragendorff's reagent were added to 2–3 ml of each extract. It gave a reddish-brown precipitate with alkaloids
- Wagner's Test: Wagner's reagent was prepared by taking 1.27 g of iodine and 2 g of potassium iodide (KI) in 5 ml water and the volume was made up to 100 ml with distilled water. Few drops of Wagner's reagent were added to 2–3 ml of each extract. It produced reddish brown precipitate with alkaloids
- (Hager's) picric acid test: A yellow precipitate is produced with Hager's reagent (saturated picric acid solution), indicating the presence of alkaloids.

Tests for Carbohydrates and Glycosides

Small quantities of aqueous and alcoholic extracts were dissolved separately in purified water and filtered, then used for the following test.

- Molisch's Test: Equal amount of Molisch's reagent (α- naphthol) and extract was mixed and 2 ml concentrated H_2SO_4 was added through the side of the test tube. Carbohydrate gave reddish violet color ring at the junction indicated presence of carbohydrates
- Fehling's Test: Fehling's A (copper sulfate [$CuSO_4$] in distilled water) and Fehling's B (potassium tartrate [$C_4H_4K_2O$] and sodium hydroxide [NaOH] in distilled water) reagent in equal volume were mixed along with small quantity of extract solution and boiled. Appearance of brick red precipitate of (CuO) cuprous oxide indicate the presence of carbohydrates
- Benedict's Test: Various extracts were treated with few drops of Benedict reagent and heated in boiling water bath. Reducing sugars give reddish brown precipitate with Benedict's reagent (alkaline Solution containing cupric citrate ion complex).

Test for Monosaccharide

- Barfoed's Test: For 1 ml sample filtered in the test tube, 1 ml of Barfoed's reagent (solution of cupric acetate in [CH_3COOH] acetic acid) is added and heated on a boiling water bath for 2 min. The formation of red color precipitate shows the presence of monosaccharide sugar.

Test for Hexose Sugar

- Cobalt Chloride ($CoCl_2$) test: 3 ml of plant extract was mixed with 2 ml of $CoCl_2$ solution (5 g in 100 ml of distilled water) then boiled for 2 min and cooled. 2–3 drops of 4 % NaOH solution were added and observed color formation. The formation of greenish blue, or purplish color or the upper layer part of the solution greenish blue and lower layer part purplish, which shows the presence of glucose ($C_6H_{12}O_6$) or Fructose or mixture of glucose and fructose in the sample.

Tests for Tannins

- Ferric Chloride ($FeCl_3$) Test: 0.5 g of the dried powder samples were boiled with 20 ml of distilled water in separate test tube, and then filtered. Few drops of 0.1% Iron chloride ($FeCl_3$) were added in filtrate and observed. Bluish black coloration showed presence of tannins. The formation of greenish black color showed presence of catechol-type tannins and blue color was appeared to indicated the presence of Gallic tannins
- Lead acetate test: A few drops of 10% ($Pb(CH_3COO)_2$) lead acetate solution was added in test solution and formation of white precipitate confirmed the presence of tannins.

Test for Saponins

- 200 mg of water and alcoholic extracts were boiled in two individual test tubes using 20 ml of (d/w) distilled water in a water bath and filtered it. 10 ml of filtrate solution along with 5 ml of distilled water was mixed and shaken for a stable continuous foaming. 3–5 drops of olive oil were added in foaming and shaken immediately, then emulsion formation was observed
- Foam test: The extracts were diluted using distilled water. After continuous shaking for 10 min, foam is produced, which indicating the presence of saponin.

Test for Terpenoids and Steroids

- Salkowski Test: 200 mg of each extract (petroleum ether, chloroform, and ethanol) was separately dissolved in 2 ml chloroform ($CHCl_3$) and 2 ml of concentrated sulfuric acid was poured from the side wall of test tube then shaken for few min. Red color appearance in chloroform layer indicates the presence of terpenoids and acidic layer show greenish yellow color (fluorescent) which show the presence of sterols
- A few drops of conc. (H_2SO_4) sulfuric acid added in plant extracts solution (petroleum ether, chloroform, and ethanol). The formation of reddish-brown color of the interface was shows the presence of steroids and formation of yellow color in a lower layer confirmed the presence of triterpenoids.

Test for Flavonoids: Four Tests were performed for the determination of flavonoids.

- Shinoda Test: To dry extract (petroleum ether, chloroform, ethanol, and water extracts) 5 ml of 65% ethanol was added with few drops of conc. HCI and Mg metal. Appearance of pink color indicated the presence of flavonoids
- 5 ml of diluted ammonia (NH_4OH) and a few drops of saturated H_2SO_4 were added in each plant extracts. The appearance of yellow color shows the presence of flavonoids
- 10 ml of ethyl acetate (EtOAc) was added in each plant extract, and then heated for 3 min on water bath. The above mixed solution was filtered and 4 ml of filtrate was shaken vigorously with 1 ml of NH_4OH solution. The appearance of yellow color shows the presence of flavonoids
- Alkaline reagent test: Each plant extract was treated with a few drops of 2% NaOH solution. Formation of yellow color, which turned to colorless when added 2–3 drops of HCl, shows the presence of flavonoids.

Fixed Oil and Fats Test

- Filter paper test: Pressed a small amount of plant extracts between filter papers. Formation of oil stains on the filter paper shows fixed oil
- Saponification test: 5–6 drops of 0.5 n alcoholic KOH was added in a small quantity of petroleum ether extract, and then added a 1–2 drop of phenolphthalein. Mixed solution was heated for 1 h. Then observed the formation of soap and partial neutralization of alkali for the confirmation of fixed oil and fats.

Volatile Oil Test

- 50 g of powdered material was taken in a volatile oil estimation apparatus and subject it to hydro distillation, for the detection of volatile oil.

Test for Proteins and Free Amino Acids

100 mg of each extract (petroleum ether, chloroform, ethanol, and water) was taken in a test tube and dissolved with 10 ml distilled water, then filtered using Whatman No. 1 filter paper. The filtrate was used for tests of amino acid and proteins.

- Millon's Test: 2–3 drops of millon's reagent were added in 2 ml of filtrate and heated for a few minutes. A white precipitate turns red after heating, which confirms protein and free amino acids
- Biuret Test: 3 ml of extract was taken in a test tube. After that 1 ml of 4 % NaOH solution and a few drops of 1% Copper sulfate (CuSO4) solution was added. The formation of violet or pink color confirms the protein

- Xantho Protein Test: 3 ml of plant extract was treated with 1 ml of concentrated H_2SO_4. The formation of white precipitate turns to yellow after boiling, then 1 ml of ammonium hydroxide was added in a test tube. Yellow precipitate turn to orange color, which confirms proteins (tyrosine tryptophan) and free amino acids
- Ninhydrin Test: Few drops of ninhydrin solution (0.005 g of ninhydrin dissolved in 100 ml of acetone) were added in 2 ml of aqueous filtrate and then boiled for a few minutes. Formation of blue color shows the presence of amino acid.

Test for Cardiac Glycosides

- Keller-Kiliani Test: 5 ml of (petroleum ether, chloroform, ethanol, and water extracts) each extract was treated with 2 ml of CH_3COOH and few drops of $FeCl_3$ solution. Then slowly 1 ml of conc. H_2SO_4 was added in the side wall of test tube. The appearance of brown color ring on the interface shows a deoxy sugar characteristic of cardenolides compound. A violet color ring may be appeared below brown ring. While in the acetic layer, a greenish colored ring may form gradually around the thin layer.

Detection of Gum and Mucilage

- 100 mg of plant extract (ethanolic and water extract) was dissolved with 10 ml of water then 25 ml of absolute alcohol was added in this solution with constant stirring. The appearance of white and cloudy precipitate shows the presence of gums and mucilage.

Test for Anthraquinones Glycosides/(Emodins)

- Borntrager's test: 0.5 g of various solvent extracts are mixed with equal volume of H_2SO_4. After that, benzene was added to the above mixture solution and shaken. The organic layer was separated and half volume of 10% ammonia solution ((NH_4OH)) was added. The formation of pink, reddish, or violet color in the (NH_3) ammonia phase show the presence of anthraquinone
- Modified Borntrager's test: 2 ml of plant extract was mixed with equal volume of dilute H_2SO_4 then boiled for 5 min and filtered. An equal amount of chloroform was mixed well in the filtering solution. Subsequently, the organic layers were separated and NaOH solution was added. The appearance of pinkish red color in the ammonia phase shows the presence of anthraquinone glycosides.

Test for Anthocyanins

- 2 ml of extract solution was added in ammonia containing tube and added 2–3 drops of 2 N HCl. Formation of Pinkish-red color which turns into blue violet color indicates presence of anthocyanins.

Test for Coumarins

- Coumarin Glycosides: Small amount of the extract was taken into a glass tube and then the test tube was covered with a filter paper that was moistened with diluted NaOH solution. The test tube was covered for a while in a water bath. The filter paper was then removed and exposed under ultraviolet light. The filter paper shows green fluorescence, which confirms the presence of coumarins
- 3 ml of 10% sodium hydroxide solution was mixed with 2 ml of water extract. The formation of yellow color in the solution shows the coumarins glycosides.

Test for Fatty Acids

- 0.5 ml of extract solution gently mixed with 5.00 ml of ether was poured on filter paper to perform evaporation process. Transparent spots indicated occurrence of fatty acids.

RESULTS

These studies enable the identification of the plant material for future investigation and form an important aspect of drug studies. Various Physicochemical Evaluation (foreign matter, moisture content, ash value, and extractive value) of SI plant 1, SI 2, and SI 3 are given in the Table 2. The foreign matter of SI sample 1, SI 2, and SI 3 was evaluated. Foreign matter in SI sample 1, SI 2, and SI 3 was found to be 0.70 ± 0.05, 0.74 ± 0.04, and 0.70 ± 0.005% w/w and all found to be under their specified limits. The moisture content of all three samples was determined as 4.625 ± 0.2, 5.26 ± 0.23, and 3.53 ± 0.47% w/w, respectively. The all plants showed less moisture content; it was range from 3.5% to 5.5%. The total ash, water soluble ash, and acid insoluble ash value were recorded from all three samples of *S. indicus* Linn. Where, the total ash values from all three samples are 10.15 ± 0.15, 12.13 ± 0.13, and 9.07 ± 0.07, water-soluble ash values 5.79 ± 0.15, 5.15 ± 0.43, and 4.84 ± 0.78, and acid insoluble ash values 0.77 ± 0.03, 1.84 ± 0.29, and 1.04 ± 0.06 has received. Comparatively, the total ash content was the highest for SI-2 and the lowest for SI-3. Water-soluble ash was highest in SI-1 and lowest in SI-3. Acid insoluble ash is highest in SI-2 and lowest in SI-1.

The extractive values of all samples were determined in different polar or non-polar solvents obtained by the cold maceration process. Extractive value of samples SI-1, SI-2, and SI-3 was evaluated. Water soluble extractive value of samples SI-1, SI-2, and SI-3 was found to be 29.65 ± 0.03, 24.3 ± 0.5, and 27.19 ± 0.35% w/w. The higher percentage yield of *S. indicus* Linn extracts was found in water extracts.

Table 2: Physical analysis of (SI-1, SI-2, and SI-3) plants selected for study (**Physicochemical Evaluation**)

Parameter	***Sphaeranthus indicus* Linn plant 1 (Rajgarh)**	***Sphaeranthus indicus* Linn plant 2 (Nagda)**	***Sphaeranthus indicus* Linn plant 3 (Rewa)**
	(%w/w)	***(%w/w)***	***(%w/w)***
Foreign matter (%)	0.70±0.05	0.74±0.04	0.70±0.005
Moisture content (%)	4.625±0.2	5.26±0.23	3.53±0.47
Total ash (% dry wt)	10.15±0.15	12.13±0.13	9.07±0.07
Water soluble ash (% of total ash)	5.79±0.15	5.15±0.43	4.84±0.78
Acid insoluble ash (% of total ash)	0.77±0.03	1.84±0.29	1.04±0.06
Water soluble extractive value (% dry wt)	29.65±0.03	24.3±0.5	27.19±0.35
Alcohol soluble extractive value (% dry wt)	13.7±0.12	9.91±0.10	11.98±0.10
chloroform soluble extractive value (% dry wt)	2.15±0.11	1.53±0.11	1.88±0.08
Pet. ether soluble extractive value (% dry wt)	1.08±0.09	0.72±0.09	0.99±0.04

All values are expressed as Mean±SD of 3 observations

Alcohol soluble extractive value of samples SI-1, SI-2, and SI-3 was found to be 13.7 ± 0.12, 9.91 ± 0.10, and 11.98 ± 0.10% w/w. Chloroform soluble extractive value of samples SI-1, SI- 2, and SI- 3 was found to be 2.15 ± 0.11, 1.53 ± 0.11, and 1.88 ± 0.08% w/w. While, petroleum ether soluble extractive value of samples SI-1, SI -2, and SI-3 was determined as 1.08 ± 0.09, 0.72 ± 0.09, and 0.99 ± 0.04% w/w, as shown in Table 2. All the parameters for all samples were compared with their respective standards given in the literature and were found satisfactory. The powder analysis of all three samples was performed and the results are shown in Table 3. The powdered drugs colors of SI-1 and SI-3 were found to be dark green, odor-slightly aromatic where the aroma disappears on fresh, long storage, and the taste is slightly sweet. Whereas, the powder of SI-2 was found in Light green color, characteristic odor, and pungent taste. Results, obtain from the all three samples, were varied due to collection areas, soil, environmental factors, and other factors.

Various extracts were prepared using petroleum ether, chloroform, ethanol, and chloroform water, the powdered drug (100 g) was used in Soxhlet apparatus. Then, the marc left after the petroleum ether extract was dried and extracted with chloroform. This procedure was followed by ethanol as well as aqueous extract. The successive solvent extractive value of all plants sample and its consistency and color of extract was examined. The extractive values were used to find out the number of active principles. The petroleum ether soluble extractive of the extracts for SI-1, SI-2, and SI-3 was found 4.21, 5.14, and 1.54% (w/w). The chloroform soluble extractive of the extracts for SI-1, SI-2, and SI-3 was found 2.92, 2.01, and 1.02% (w/w) and alcohol soluble extractives were found 3.73, 3.51, and 8.81% (w/w), respectively. The higher percentage yield of *S. indicus* Linn extracts for SI-1, SI-2, and SI-3 was found to be 7.15%, 6.79, and 10.2% (w/w), respectively, in water extracts. The results are shown in Table 1. The results of preliminary phytochemical analysis of

Table 3: Organoleptic evaluation of (SI-1, SI-2, and SI-3) plants selected for study. (Organoleptic Evaluation)

Crude drug	Color	Odor	Taste
Sphaeranthus indicus Linn plant 1 (Rajgarh)	Dark green	Aromatic	Slightly sweet
Sphaeranthus indicus Linn plant 2 (Nagda)	Light green	characteristic odor	pungent taste
Sphaeranthus indicus Linn plant 3 (Rewa)	Dark green	Aromatic	Slightly sweet

the different extracts of sample SI-1, SI-2, and SI-3 are shown in Tables 4-7. Preliminary phytochemical testing of all three-plant extract of *S. indicus* revealed the presence of phenolic groups, alkaloids, flavonoids, carbohydrate, glycoside, terpenoid/steroid, tannin, fixed oil fat, and saponins. By a qualitative reaction of phyto-constituents, present or absent is shown in all three plants as the following: Significantly presently (+++), moderately present (++), slightly present (+), and absent (–).

The present study has shown remarkable variations in the number of phytochemicals present in the plants collected from different region of central India. The maximum solubility of the phytochemicals from the plants of all the sites was in petroleum ether, ethanol, and water. The results from the present study are as follows;

Results of preliminary qualitative phytochemical screening of petroleum ether extracts of samples SI-1, SI-2, and SI-3, it was found to contain steroids/terpenoids, flavonoids compound, fixed oils and fats, cardiac glycosides, coumarins, and fatty acids. The results are shown in Table 4. But by comparative study of all three plant samples, it was found that terpenoids/

Table 4: Preliminary phytochemical screening of petroleum ether extracts of SI-1, SI-2, and SI-3

Constituent's test	Pet. ether extracts of SI		
	SI 1	SI 2	SI 3
Alkaloids			
Mayer's test	–	–	–
Dragendorff's test	–	–	–
Wagner's test	–	–	–
Hager's test	–	–	–
Carbohydrate's test			
Fehling test	–	–	–
Benedict test	–	–	–
Molisch test	–	–	–
Test for monosaccharide's			
Barfoed test	–	–	–
Test for hexose sugar			
Cobalt chloride test	–	–	–
Tannins and Phenolic compounds			
Ferric chloride test	–	–	–
Lead acetate test	–	–	–
Test for saponins			
Foam test	–	–	–
Test	–	–	–
Steroids and Triterpenoids			
Salkowski test	++	++	+++
Terpenoid test	++	++	+++
Flavonoids			
Shinoda test	–	–	–
Test	+	+	+
Test	–	–	–
Alkaline reagent test	+	+	+
Fixed oil and fats			
Filter paper test	++	+	++
Saponification test	++	+	++
Volatile oil test	+++	+	+++
Protein and Amino acid			
Millon's test	–	–	–
Biuret test	–	–	–
Xanthoproteic test	–	–	–
Ninhydrin test	–	–	–
Test for cardiac glycosides			
Killer–kiliani test	++	++	+++
Mucilage and Gum	–	–	–
Test for anthraquinone/emodin			
Borntrager's test	–	–	–
Modified Borntrager's test	–	–	–
Test for Anthocyanins	–	–	–
Test for coumarins	+	+	+
Fatty acid test	++	+	++

(+++) = significantly present, (++) = Moderately present, (+) = Slightly present, (–) = Absent

Table 5: Preliminary phytochemical screening of Chloroform extracts of *S. indicus* samples SI-1, SI-2, and SI-3 (*Sphaeranthus indicus* Linn plant 1 (Rajgarh), *Sphaeranthus indicus* Linn plant 2 (Nagda), *Sphaeranthus indicus* Linn plant 3 (Rewa)

Constituent's test	Chloroform extracts of SI		
	SI 1	SI 2	SI 3
Alkaloids			
Mayer's test	–	–	–
Dragendorff's test	–	–	–
Wagner's test	–	–	–
Hager's test	–	–	–
Carbohydrates test			
Fehling test	–	–	–
Benedict test	–	–	–
Molisch test	–	–	–
Test for monosaccharide's			
Barfoed test	–	–	–
Test for hexose sugar			
Cobalt chloride test	–	–	–
Tannins and Phenolic compounds			
Ferric chloride test	–	–	–
Lead acetate test	–	–	––
Test for saponins			
Foam test	–	–	–
Test	–	–	–
Steroids and Triterpenoids			
Salkowski test	+	++	++
Terpenoid test	+	++	++
Flavonoids			
Shinoda test	–	–	–
Test	+	+	+
Test	–	–	–
Alkaline reagent test	+	+	+
Fixed oil and fats			
Filter paper test	–	–	–
Saponification test	–	–	–
Volatile oil test	–	–	–
Protein and Amino acid			
Millon's test	–	–	–
Biuret test	–	–	–
Xanthoproteic test	–	–	–
Ninhydrin test	–	–	–
Test for Cardiac glycosides			
Killer–kiliani test	+	+	+
Mucilage and Gum	–	–	–
Test for anthraquinone/emodin			
Borntrager's test	–	–	–
Modified Borntrager's test	–	–	–
Test for Anthocyanins	–	–	–
Test for coumarins	+	+	+
Fatty acid test	–	–	–

(+++) = significantly present, (++) = Moderately present, (+) = Slightly present, (–) = Absent

Table 6: Preliminary phytochemical screening of the ethanol extracts of *S. indicus* samples SI-1, SI-2, and SI-3

Constituent's test	Ethanol extracts of SI		
	SI 1	SI 2	SI 3
Alkaloids			
Mayer's test	+	+	+
Dragendorff's test	++	+	++
Wagner's test	++	+	++
Hager's test	–	–	–
Carbohydrates test			
Fehling test	++	+	++
Benedict test	+	–	+
Molisch test	++	+	+
Test for monosaccharide's			
Barfoed test	++	–	++
Test for hexose sugar			
Cobalt chloride test	++	+	++
Tannins and Phenolic compounds			
Ferric chloride test	+++	–	++
Lead acetate test	+	+	+
Test for saponins			
Foam test	+++	+	++
Test	+++	+	++
Steroids and triterpenoids			
Salkowski test	+	–	++
Terpenoid test	++	+	+++
Flavonoids			
Shinoda test	–	–	–
Test	+++	+	++
Test	+++	+	++
Alkaline reagent test	++	+	+++
Fixed oil and fats			
Filter paper test	++	–	++
Saponification test	++	–	++
Volatile oil test	–	–	–
Protein and Amino acid			
Millon's test	–	–	–
Biuret test	–	–	–
Xanthoproteic test	–	–	–
Ninhydrin test	–	–	–
Test for cardiac glycosides			
Killer–kiliani test	–	–	+
Mucilage and Gum	–	–	–
Test for anthraquinone/emodin			
Borntrager's test	–	–	–
Modified Borntrager's test	–	–	–
Test for anthocyanins	–	–	–
Test for coumarins	++	+	+
Fatty acid test	++	–	++

(+++) = significantly present, (++) = Moderately present, (+) = Slightly present, (–) = Absent

Table 7: Preliminary phytochemical screening of water extracts of *S. indicus* samples SI-1, SI-2, and SI-3

Constituent's test	Water extracts of SI		
	SI 1	SI 2	SI 3
Alkaloids			
Mayer's test	+	+	+
Dragendorff's test	+	+	+
Wagner's test	+	+	+
Hager's test	+	+	+
Carbohydrates test			
Fehling test	+++	+	++
Benedict test	+++	–	++
Molisch test	+++	+	++
Test for monosaccharide's			
Barfoed test	++	–	+
Test for hexose sugar			
Cobalt chloride test	+++	+	++
Tannins and Phenolic compounds			
Ferric chloride test	–	–	–
Lead acetate test	–	–	–
Test for saponins			
Foam test	++	++	+
Test	+	+	+
Steroids and Triterpenoids			
Salkowski test	–	–	–
Terpenoid test	–	–	–
Flavonoids			
Shinoda test	–	–	–
Test	+	+	+
Test	–	–	–
Alkaline reagent test	+	+	+
Fixed oil and Fats			
Filter paper test	–	–	–
Saponification test	–	–	–
Volatile oil test	–	–	–
Protein and Amino acid			
Millon's test	–	–	–
Biuret test	–	–	–
Xanthoproteic test	–	–	–
Ninhydrin test	–	–	––
Test for cardiac glycosides			
Killer killani test	–	–	–
Mucilage and Gum	+++	+	++
Test for anthraquinone/emodin			
Borntrager's test	–	–	–
Modified Borntrager's test	–	–	–
Test for Anthocyanins	–	–	–
Test for coumarins	–	–	–
Fatty acid test	–	–	–

(+++) = significantly present, (++) = Moderately present, (+) = Slightly present, (–) = Absent

steroids and cardiac glycosides are significantly present in SI-3, while moderately present in SI-1 and SI-2 samples. Flavonoids and coumarins are slightly present in petroleum ether extracts of all three plants. Fixed oil and fat (fatty acid) moderately present in SI samples 1 and 3 of petroleum ether extract, while slightly present in SI-2. Volatile oil was found significantly in petroleum ether extracts of SI-1 and SI-3, while a slight presence of volatile oil was found in SI-2. Alkaloids, carbohydrates, monosaccharides, hexose sugar, phenolic compounds, saponin, gum, and mucilage were not found in petroleum ether extracts of all three plants samples.

Chloroform extracts of the samples SI-1, SI-2, and SI-3 revealed the presence of steroids/terpenoids, flavonoids, cardiac glycosides, and coumarins, as shown in Table 5. Comparatively, terpenoids/steroids were found to be moderately in chloroform extracts of SI-2 and SI-3, while slightly found in SI-1. Whereas, flavonoids, cardiac glycosides, and coumarin were found to be slightly in chloroform extracts of all three plants. Alkaloids, carbohydrates, monosaccharides, hexose sugar, phenolic compounds, saponin, fixed oil, fatty acid, volatile oil, gum, and mucilage were not found in chloroform extracts of all three plants.

Ethanolic extracts of samples SI-1, SI-2, and SI-3 revealed the presence of alkaloids, carbohydrates, monosaccharides, hexose sugar, Phenolic compounds, saponins, steroids/Triterpenoids, flavonoids, cardiac glycosides, coumarins fixed oil and Fats (Fatty acid), as shown in Table 6. Comparatively, alkaloids and carbohydrates were found to be moderately present in the ethanolic extracts of SI-1 and SI-3, while slightly found in SI-2. Monosaccharides, oils, and fats (fatty acid was found to be moderately present in SI-1 and SI-3), whereas no reaction was found in SI-2 for monosaccharide, oil, and fat. Flavonoids and phenolic compounds were found significantly present in SI-1 and moderately present in SI-3, while slightly in SI-2 sample. Hexose sugar was found to be moderately present in SI-1 and SI-3, while slightly in SI-2. Saponin was found significantly in SI-1 and was moderately present in SI-3, whereas slightly present in SI-2. Terpenoids were found to be moderately present in SI-1 and slightly found in SI-3, while significantly present in the ethanolic extracts of SI-3. Cardiac glycosides were found slightly only in SI-3 and no response was found in SI-1 and SI-2. Coumarin was found moderately in SI-1 and slightly in SI-2 and SI-3. Volatile oil was not found in ethanolic extracts of all three plants.

Aqueous extracts of samples SI-1, SI-2, and SI-3 revealed the presence of alkaloids, carbohydrates, monosaccharides, hexose sugar, phenolic compounds, saponins, steroids/terpenoids, flavonoids, and mucilage and gum, as shown in Table 7. Comparatively, carbohydrates, hexose sugar, mucus, and gum were found to be sufficiently present in SI-1 and SI-3, while little was found in aqueous extracts of SI-2. Whereas, alkaloids and flavonoids were found slightly in aqueous extract of all three plants. Monosaccharides were found to be moderately present in SI-1and slightly found in SI-3 while, absent in SI-2. Saponin was found moderately in SI-1 and SI-2 while slightly present in SI-3. Phenolic compounds, steroids/terpenoids, fixed oil, fatty acid, volatile oil, cardiac glycosides, and coumarin were not found in aqueous extracts of all three plants.

DISCUSSION

The plant was selected carefully on the basis of extensive literature review on reported pharmacognostic and phytochemical profile. The plants material was collected and authenticated followed by physicochemical analysis that was further helpful in the study of the drug. The active constituents were also estimated qualitatively as well as quantitatively. Various physicochemical parameters were analyzed for identification and purity confirmation. The moisture content is a mandatory component of crude drugs, while its excess can stimulate microbial growth and hydrolytic deterioration and that is why the moisture content was determined for every crude drug. Ash values are also very useful to determine the purity and quality of crude drug, especially in powdered form. The total percentage of ash values, acid insoluble ash, water soluble ash, and percentage yield of plant extracts in various polar and non-polar solvents are constant characteristic of a part of the medicinal plant which may constitute individual drug. These reports would be of much significance in finding out the genuineness of the drug sample. In physicochemical parameters, ash values and extraction values are used as reliable aid in the detection of adulteration and in the identification of plants. Ash values give an idea about the earthy matter, inorganic formation, and various other impurities present with the drug, while extractive values are very useful in determining of adulterated drug material. Preliminary phytochemical screening of the various extracts of *S. indicus* revealed some differences in their constituents.

From the above study found that the ethanol and water extracts of the three plants have higher phytochemicals, while chloroform extracts showed less number of phytochemicals. Protein, amino acids, anthraquinone/emodine, and anthocyanins were not found in all extracts of SI-1, SI-2, and SI-3. Significantly, all the extracts of the SI-1 plant showed the highest amount of phytoconstituents compared to the SI-2 and SI-3 plant extracts and the lowest was found in the SI-2 plant extracts, as shown in Table 4-7.

This study has shown that the presence of various medicinal important phytoconstituents in good amounts in various *S. indicus* plant samples, which therefore justify the use of this plant species as an herbal remedy. Similarly, other authors have compared the phytochemical between different solvent extracts of other plant such as *Andrographis paniculata*.[16] The phytochemical study of this plant has been done several times by other authors and they concluded that the ethanolic and water extract of this plant showed the maximum number of components and the present research agreed with the authors research.[17-21]

The presence of various phytochemicals in the plant tested shows that it can be a good source of new drug production for various diseases. The qualitative phytochemical study provided valuable information on the various phytocomponents found in the plant, which helps the future investigators regarding the selection of the particular extract for further investigation of isolating the active principle. Industries are deeply interested in using plant tissue culture technology to produce these substances on a large-scale level because of pharmacological importance and high economic value of secondary metabolites.[22]

CONCLUSIONS

It may be concluded that the comprehensive and accurate physicochemical values of this study are useful for the identification and authentication of *S. indicus* plant. The current work has been undertaken to determine the standardization parameter that reveals the authenticity, purity and quality of this medicinally crude drug.

Thus, from the present study on comparison of phytochemical screening in plant *S. indicus* collected from three different geographical areas shown noteworthy and remarkable observations. From the above study, it is clear that the plant produces abundant phytochemicals as secondary metabolites under various climatic and soil conditions which can be used in the production of a potent drug. The plant taken from Rajgarh site 1 was found to have maximum phytochemical presence as compared to the other two sites. Production of some phytochemicals in one site and absence of the same in another site may be due to environmental induced production of certain phytochemicals or may be due to activation or suppression of certain genes producing the phytochemicals in particular environmental conditions and which gives scope for further investigation.

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