E-ISSN: 2709-9423
P-ISSN: 2709-9415
JRC 2023; 4(2): 54-57

www.chemistryjournal.net
Received: 03-06-2023
Accepted: 11-07-2023

Mandavi Tripathi
Govt. Dr Shyama Prasad Mukharjee Science & Commerce College, Old Benazeer College, Bhopal, Madhya Pradesh, India

Sudhanshu Dhar Dwivedi
Govt. Degree College Women's Udhampur, Jammu & Kashmir, India

Kewal Kumar
Govt. Degree College Women's Udhampur, Jammu & Kashmir, India

Correspondence Author:
Mandavi Tripathi
Govt. Dr Shyama Prasad Mukharjee Science & Commerce College, Old Benazeer College, Bhopal, Madhya Pradesh, India

Phytochemical screening and thin layer chromatography of *Sphaeranthus Indicus* linn extract

Mandavi Tripathi, Sudhanshu Dhar Dwivedi and Kewal Kumar

Abstract
Sphaeranthus Indicus Linn Extract (Asteraceae) is widely used in Ayurvedic system of medicine to treat vitiated conditions of epilepsy, mental illness, hemicrania, jaundice, hepatopathy, diabetes, leprosy, fever, pectoralgia, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases. The aim of the present study was a preliminary phytochemical and thin layer chromatography (TLC) analysis of the various extracts of *Spheranthus Indicus* Linn. Phytochemical analysis was carried out using the standard phytochemical assays. TLC analysis of the chloroform and ethanol extract of the leaves was carried out using the solvent system Ethanol: Chloroform (9:1) and Ethanol: Hexane (3:7), respectively. The findings of the preliminary phytochemical screening revealed the presence of various chemical compounds like alkaloids, glycosides, flavonoids, carbohydrates, tannins, phenols, fixed oil and fats. The three Rf value (0.59, 0.78 & 0.94) and six Rf value (0.05, 0.21, 0.34, 0.51, 0.65 & 0.87) were found in TLC plate of chloroform and ethanol extracts, respectively. These studies provided referential information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario of lack of regulatory laws to control quality of herbal drugs.

Keywords: *Sphaeranthus Indicus* Linn, phytochemical screening, TLC

Introduction
Nature has produced medical agents since the beginning of time. The importance of herbs in treating human ailments cannot be emphasised. The plant world appears to have an unlimited supply of active chemicals that can be employed to treat a range of difficult-to-treat conditions.

Furthermore, the active components of herbal treatments have the benefit of coexisting with many other compounds that appear to be inert, yet these complementing components provide the plant as a whole with considerably greater safety and efficacy than isolated and pure active components. Plants have been found to be the source of energy for the animal kingdom. Additionally, plant synthesizes large number of chemical substances that are therapeutically effective. The chief component produce by plants are alkaloids, glycosides, flavonoids, polyphenol, saponin, steroids, tannins etc. Since last two centuries, there have been serious investigations into the chemical and biological activities of plants and these have yielded compounds for the development of synthetic organic chemistry and the emergence of medicinal chemistry as a route for the discovery of more effective therapeutic agents.

Sphaeranthus Indicus Linn is a medicinal plant widely used in Indian traditional system of medicine for curing various ailments [1]. It grows in rice fields, dry waste places and cultivated lands in tropical parts of India. It is commonly known as Gorakhmundi (In Hindi). All the parts of the *S. Indicus* have medicinal uses. The herb *S. Indicus* is much branched, strongly scented, and annual erect with branched tappering roots tap roots. Stems are cylindrical with toothed wings. Leaves are sessile, decurrent, 2-7 cm long, 1-1.5 cm wide, obovate-oblong, rounded or subacute, glandular-hairy, spinous-serrate or dentate, narrowed at the base and greenish-brown in color. Flowers are borne in terminal, solitary, globose, clusters of heads. Heads of flowers are purple, bracts are short slender and acuminate. In each head, the outer flowers are females, few or many, fertile, the central flowers bisexual, fertile or sterile, involucre narrow, bracts paleaceous, spathulate, acute, ciliate; receptacle small, naked. 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, hemicrania. The whole herb is used in *Ayurvedic* preparations to treat epilepsy and mental disorders. Leaves dried in the shade and powdered are used in doses of 20 grains twice a day in chronic skin diseases as an antisyphilitic and a nervine tonic. Thus the present investigation was aimed to investigate the pharmacognostical features and phytochemical analysis for identification and authentication of the plant.

Classification

- Kingdom: *Plantae*
- Subkingdom: *Viridaeplantae*
- Phyllum: *Tracheophyta*
- Subphyllum: *Euphyllophytina*
- Infraphyllum: *Radiatopses*
- Class: *Magnoliopsida*
- Subclass: *Asteridae*
- Superorder: *Asteranae*
- Order: *Asterales*
- Family: *Asteraceae*
- Genus: *Sphaeranthus*
- Species: *indicus*

Materials and Methods

Plant collection & identification: Fresh *Spheranthus Indicus* Linn Plant Extract were obtained from the outskirts of Bhopal. The plant was identified by Dr. Jagrati Tripathi Professor of Botany. Unique College Bhopal. He leaves were allowed sun dried after rinsed with distilled water. The dried plant material were coarsely powdered and subjected to extraction.

Preparation of extract

The extract was done by maceration using petroleum ether, Chloroform and ethanol. The extracts obtained were evaporated in rotary evaporator to get a powdery mass. The powder extracts obtained were then subjected to phytochemical analysis to detect the chemical constituents present in each extract.

Preliminary Phytochemical studies

Preliminary phytochemical tests of various extracts of leaves powder of *Spheranthus Indicus* Linn were performed for phytochemical analysis of alkaloids, glycosides, carbohydrates, steroids, polyphenol, saponins and terpenoids

Test for alkaloids

a) **Dragendorff's test:** To 1 ml of the extract, add 1 ml of dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

b) **Mayer's test:** To 1 ml of the extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

c) **Hager's test:** To 1 ml of the extract, add 3ml of Hager's reagent (Saturated aqueous solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids.

d) **Wagner's test:** To 1 ml of the extract, add 2 ml of Wagner's reagent (Iodine in Potassium Iodide), Formation of reddish-brown precipitate indicates the presence of alkaloids.

Test for Protein

a) **Biuret test:** Added 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color was produced, and then added to the 1ml of the extract. Formation of pinkish or purple violet color indicated the presence of proteins.

b) **Ninhydrin test:** Added two drops of freshly prepared 0.2% Ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heated. Development of blue color revealed the presence of proteins, peptides or amino acids.

Test for Glycosides

a) **Legal test:** Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

b) **Baljet test:** To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

c) **Keller-Killiani test:** 1 gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10 ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5 ml of chloroform. The chloroform layer is separated in a porcelein dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

d) **Borntrager's test:** Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinone glycosides.

Test for carbohydrates and sugars

a) **Molisch's test:** To 2 ml of the extract, add 1ml of a-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

b) **Fehling's test:** To 1 ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars

c) **Benedict's test:** To 5 ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

Test for tannins and phenolic compounds

a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

b) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.

c) The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

Test for flavonoids Shinoda's test
The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

Test for steroids
a) Libermann-Burchard test: 1 gm of the test substance was dissolved in a few drops of chloroform, 3 ml of acetic anhydride, 3 ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish green colour shows the presence of sterols.
b) Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H_2SO_4. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

Test for fixed oils and fats
a) Spot Test: Press a small quantity of extracts between the filter paper. Oil stains on paper indicates the presence of fixed oils.
b) Saponification test: To 1 ml of the extract, add few drops of 0.5 N alcoholic Potassium hydroxide along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Thin layer chromatography (TLC)
The Chloroform and ethanol extracts were selected for TLC study. The extracts were prepared with the respective solvent ethanol and distilled water and made up to 10 ml in different test tubes. Then the extracts were taken in a capillary tube and it was spotted in preparative TLC plates coated with silica gel G. The plates were developed in TLC chamber previously saturated with different solvent systems. The different spots developed in each solvent system were identified by means of detecting agent and the Rf value are correspondingly calculated1,

Results
Phytochemical screening
Preliminary phytochemical investigations of the extracts of *S. Indicus* L. revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, glycosides, fats and carbohydrates. The details are presented in table 1. From the result of phytochemical screening, the petroleum ether extract of *S. Indicus* L. exhibited the presence of fats and oils. Alkaloids, flavonoids, tannins and polyphenol were found in chloroform extracts of *S. Indicus* L. Further, glycosides, carbohydrates, flavonoids, tannins and polyphenol were present in ethanol extracts of leaves of *S. Indicus* L. The maximum phytocostituents were observed in ethanol extracts of *S. Indicus* L. (Table 1). Now chloroform and ethanol extracts of *S. Indicus* L. were selected for further TLC evaluation.

TLC
The retention factors (Rf) of chloroform and ethanol extracts in different solvent systems are shown in table 2. The chromatogram revealed 3 spots and 6 spots for chloroform and ethanol extracts, respectively.

Discussions
For the pharmacological study of novel drugs, the essential information's regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts. In the present study qualitative tests of extracts showed significant indication about the presence of metabolites. Preliminary phytochemical investigations tests are useful to isolate the pharmacologically active principles present in the plant. Plant derived natural products such as polyphenol and steroids have received considerable attention in recent years due to their diverse pharmacological properties. Plant phenolics, in particular phenolic acids, tannins and flavonoids are known to be potent antioxidants and occur in vegetables, fruits, nuts, seeds, roots and barks. *S. Indicus* L. is an important medicinal plant of the world. Its usage not only fulfills the nutritive need of the human being but due to the presence of different types of bioactive constituents makes this plant medicinally very important for the human being. Different types of phytoconstituents were present in the *S. Indicus* L. like flavonoids, phenolic compounds, alkaloids and glycosides which makes this plant potent to various types of ailments

Table 1: Phytochemical Screening of crude extracts of Petroleum ether, Chloroform and 80%Ethanol from *Spharenthus Indicus Linn.*

S. No.	Tests	Observation for extracts		
		Petroleum Ether	Chloroform	Ethanol
1.	**Test for carbohydrates**			
	Fehling's Test	_	+	+
2.	**Test for Alkaloid**			
	Wagner's test	_	_	+
	Mayer's test	_	_	+
3.	**Test for Flavonoids**			
	Shinoda test	_	_	+
	Alkaline reagent test	_	_	+
4.	**Test for Terpenoids**			
	Salkowski test	+	+	_
5.	**Test for Saponins**			
	Foam test	+	_	+
6.	**Test for Proteins**			

	Biuret's Test	_	_	+
7.	**Test for C-glycosides**			
	Modified Borntrager's test	_	_	+

+ = Present, − = Absent

Table 2: TLC profile of chloroform and ethanol extract of *Sphaeranthus Indicus* Linn.

Extract	Solvent system	Detecting Reagent	No. of Spots	Rf Value.
Chloroform	Ethanol: Chloroform (9: 1)	Vanillin-sulphuric acid	04	0.59, 0.78, 0.94
Ethanol Extract	Ethanol: Hexane (3: 7)	Ceric Ammonium Sulphate	07	0.05, 0.21, 0.34, 0.51, 0.65, 0.87

TLC profiling of chloroform and ethanol extracts gives an impressive result that directing towards the presence of number of phytochemical. Various phytochemicals gives different Rf values in different solvent system. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extract can only be achieved by analyzing the Rf values of compounds in different solvent system

The TLC method is best choice for the identification of secondary metabolite present in plants. Here the different Rf values indicate the presence of different nature of phytoconstituents in single extracts. Different Rf values of the compound also reflects an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

Conclusion

The findings of study indicate that the extract of *S. Indicus* L. contained many phytochemicals as revealed by phytochemical studies and TLC analysis. The ethanol extract of Indicus L. incorporating maximum number of phytoconstituents. The present study on phytochemical screening and TLC of Indicus L. leaves will provide useful information in regard to its correct identity and help to differentiate from the closely related other species of Ficus. It is concluded from the data that extracts of *Indicus* L. plant exhibited significant role in medicinal chemistry for formulation of life saving drugs.

Acknowledgements

Authors are thankful to the Director General MPCST, Bhopal and Principal Govt. Dr. Shyama Prasad Mukharjee Science & Commerce College (Old Benazeer College) Bhopal. M.P. to carry out this work.

Conflict of interests

No conflict of interest among all authors of this work

References

1. Gujjeti PR, Mamidala E. Phytochemical Analysis and TLC Profile of *Madhuca indica* Inner Bark Plant Extract. International Journal of Scientific & Engineering Research. 2013;4(10):1507-1510.
2. Pandey A, Singh P. Antibacterial activity of *Syzygium aromaticum* (clove) with metal ion effect against food borne pathogens. Asian J Plant Sci. Res. 2011;1(2):69-80.
3. Prabakaran M, Chandrakala N, Panneerselvam A. Antimicrobial activity of *Indigofera glandulosa* (wild). Asian J Plant Sci Res. 2011;1:18-25.
4. Hassan IA, Nasiru, Malut AM, Abdulkadir I, Ali AS. Phytochemical studies and thin layer chromatography of leaves and flower extracts of *Senna siamea* lam for possible biomedical applications. Journal of Pharmacognosy and Phytochemistry. 2015;7(3):18-26.
5. Namukobea J, Kaseneneb JM, Kiremere BT In. Amukama M, Kamatenesi-Mugisha S, Krief V, Dumontet DJ, Kabasa JD. Traditional plants used for medicinal purpose by local communities around northern sector of Kibole National park, Uganda. J. Ethnopharmacol. 2011;136:236-255.
6. Roja G, Rao PS. Anticancer compound from tissue cultures of medicinal plant. J Herbs Spices Med. Plants. 2000;7:71-102.
7. Kirtikar KR, Basu BD, ICS. Indian medicinal plants. In: Blatter E, Caius JF, Mhaskar KS, editors. 2nd ed. Vol-2. Allahabad India: Lalit Mohan Basu; c1981. p. 1346–8. [Google Scholar]
8. Chatterjee A, Pakrashi SC. 1st ed. Vol-5. New Delhi: National Institute of Science communication and information resources. The treatise on Indian medicinal plants; c2003. p. 177. [Google Scholar]
9. Gogte MG, Ananthasubramanian L, Nargund KS, Bhattacharyya SC. Some interesting sesquiterpenoids from *Spharanthus indicus* Linn. (compositae) Indian J Chem. 1986;25B:233–8. [Google Scholar]
10. Singh SK, Tripathi VJ, Singh RH. ß-D-glucoside of (24S)-24-ethylcholesta-4, 22-dien-3- ß-ol from *Sphaeranthus Indicus* L. Indian drugs. 1989;26:317–8. [Google Scholar].
11. Mishra BB, Yadav SB, Singh RK, Tripathi V. Novel flavonoid C-glycoside from *Sphaeranthus Indicus* L. (Family Compositae). Molecules. 2007;12:2288–91. [PMC free article] [PubMed] [Google Scholar]
12. Yadava RN, Kumar S. A novel isoflavone glycoside from the leaves of Spharanthus indicus. Fitoterapia. 1999;70:127–9.
13. Chughtai MI, Khokhar I, Ahmad A. Isolation, purification and structural determination of alkaloids from the flowers of Spharanthus indicus. Sci Int (Lahore). 1992;4:151–4.
14. Nanda BK, Jena J, Rath B, Behera BR. Analgesic and Antipyretic activity of whole parts of *Sphaeranthus Indicus* Linn. J Chem Pharma Res. 2009;1:207–12.
15. Jha RK, Garud N, Nema RK. Excision and incision wound healing activity of flower head alcoholic extract of *Sphaeranthus Indicus* Linn.in albino rats. Global J Pharmacol. 2009;3:32–7.
16. Vijaya K, Anathan S. Microbiological screening of Indian Medicinal plants with special reference to enteropathogens. J Altern Comp Med. 1997;3:13-20. [PubMed] [Google Scholar]
17. Garg SC, Kasera HL. *In vitro* antibacterial activity of the essential oil of *Spharanthus indicus* L. Fitoterapia. 1983;54:37–9. [Google Scholar]