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Research Article

Qualitative and Quantitative Determination of Secondary Metabolites of *Sphaeranthus indicus* and *Spathodea campanulata* Flowers Extracts

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Abstract

Since the dawn of time, humans have used plants as a natural source for healing and remedies. Among these, medicinal herbs have gained popularity due to their widespread use and lack of negative side effects. The globe has seen a rise in plant study in recent years, and a tonne of data has been gathered to demonstrate the enormous potential of the therapeutic plants employed in many traditional systems. In this work, flower extracts of *Sphaeranthus indicus* and *Spathodea campanulata* from the Madhya Pradesh region of Bhopal were evaluated for their qualitative and quantitative phytochemical composition. The well-known test procedure described in the literature was used to determine the quantitative analysis of total phenolics and flavonoids as well as the qualitative analysis of various phytochemical constituents. *Sphaeranthus indicus* and *Spathodea campanulata* both included alkaloids, carbohydrates, saponins, fixed oils and lipids, flavonoids, and phenolics, according to phytochemical analyses. By using the Folin's Ciocalteu reagent method and the aluminium chloride method, respectively, phenolic and flavonoid quantities were analysed quantitatively. The amount of total phenolics in the methanolic extract of the flowers of *Sphaeranthus indicus* and *Spathodea campanulata* was 18.23 ± 0.16 and 217.00 ± 0.916 mg/gm, respectively. Flavonoids came in second with 164.10 ± 0.52 and 79.33 ± 3.511 mg/gm. This research gave information that might be used to standardize and correctly identify this plant material. The wide range of phytochemicals found in the plant thus points to its medicinal potentials, which could be investigated in both traditional medicine and the drug manufacturing business.

Keywords: *Sphaeranthus indicus*, *Spathodea campanulata*, Qualitative analysis, quantitative analysis, Phytochemical analysis

INTRODUCTION

The majority of the world's population, between 75 and 80 percent, consists of medicinal plants, and many health care initiatives, particularly in developing nations, heavily rely on herbal medicines. An ancient Indian text that depicts all plant parts as possible sources of therapeutic compounds includes a comprehensive definition of medicinal plants¹. The primary barriers to the acceptability of alternative medicines in wealthy nations are a lack of citations and rigid quality control. Therefore, documentation is a crucial component of any research on traditional remedies². In this situation, it becomes crucial to make an effort to ensure the consistency of the plant material to be utilized as medicine. Due to the shortage of restricted synthetic medications, the WHO has also advised evaluating the physicochemical and phytochemical properties of medicinal plants for their efficacy³. The plant material can be identified and verified with the aid of these evaluation criteria. The precise identification and quality control of the raw materials are crucial for the safety and efficacy of herbal medicine. In India, *Sphaeranthus indicus* Linn (Family: Asteraceae) is a branching herb with purple blooms that is widely cultivated in rice fields. It is a naturally occurring anthelmintic utilized in the Indian medical system⁴. The herb has been used for a variety of ailments, including hemicranias, jaundice, leprosy, diabetes, fever, pectoralgia,

cough, gastropathy, hernias, haemorrhoids, helminthiasis, dyspepsia, skin conditions, and nerve tonic^{5,6}. This plant has been linked to pharmacological effects like immunomodulatory⁷, antimicrobial^{8,9}, antibacterial^{10,11}, anxiolytic⁶, and wound healing action¹². Eudesmanolides, isoflavonoids, 7-hydroxy eudesmanolides, sterol glycosides, essential oils (cadiene, ocimene, citral, p-methoxycinnamaldehyde, geraniol, eugenol, and geranyl acetate), and eudesmanolides are phytoconstituents identified from this plant¹³⁻¹⁸. The Madhya Pradesh tribal people in India utilised this plant to treat diabetes¹⁹. Bignoniaceae family member species *Spathodea campanulata* P. Beauv. Sterols, triterpenoids, tranorin, vannillic acid, ferulic acid, verminoside, pelargonidin diglycoside, maldivin, and tannin are all present in the bark. Quercetin, chlorogenic acid, and polyhydroxysterol spathodol are all found in leaves²⁰. The blooms of this plant are used as diuretics and anti-inflammatory agents, while the leaves are used as an antidote for animal poisons and to treat inflammation of the urethra and kidneys. The medicines made from stem bark are used to treat enemas, herpes, stomachaches, and diarrhoea²¹. Spathodol, caffeic acid, other phenolic acids, and flavonoids have all been produced by the leaves²²⁻²⁴. There have been several reports of medicinal properties, including flavonoids from eight bignoniaceous plants²⁵, antiplasmodial activity in leaf²⁶, analgesic and anti-inflammatory properties in stem

bark extracts^{28,29}, and hypoglycemic, anti-HIV and antimalarial activity in stem bark extracts²⁷. This research's objective was to identify the types and concentrations of bioactive chemicals in *Sphaeranthus indicus* and *Spathodea campanulata* flowers.

MATERIALS AND METHODS

Plant material

The Pinnacle Biomedical Research Institute (PBRI), near the Bharat Scout and Guides Campus, Shanti Marg, Shyamla Hills Road, Depot Chouraha, Bhopal, Madhya Pradesh 462003, India, collected the flowers of *Sphaeranthus indicus* and *Spathodea campanulata*. Botanist Dr. Saba Naaz from the Saifia College of Science in Bhopal's Department of Botany carried out the plant's identification and authentication. For future use, a voucher specimen with the number 310/Saif./Sci./Clg/Bpl and 311/Saif./Sci./Clg/Bpl for *Sphaeranthus indicus* and *Spathodea campanulata* respectively was conserved in the department of botany at Saifia College of Science, Bhopal.

Chemical reagents

The Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India) provided all the chemicals used in this work. The investigation only employed analytical-grade compounds.

Hot Soxhlet Extraction Method

This technique involved gathering, correctly washing, and properly rinsing the blossoms of *Sphaeranthus indicus* and *Spathodea campanulata*. They were mechanically pulverised after being shade-dried. The plant material from *Sphaeranthus indicus* and *Spathodea campanulata*, either whole or coarsely powdered, was successively extracted using solvents such as petroleum ether, ethyl acetate, chloroform, and methanol in increasing polarity order for various lengths of time. The Soxhlet apparatus' chamber was filled with powder using a "thimble" design. The solvent used for extraction was heated in flasks, and its vapours were then condensed in a condenser. The powder is extracted by touch when the condensed extractant is dropped into the thimble holding it. The liquid inside the chamber syphon drops into the flask when the liquid level in the chamber reaches the top of the syphon tube. This procedure was continued until an evaporated drop of solvent from the syphon tube did not leave any residue. The resulting extract was filtered, dried by concentration, weighed, and stored for later use³⁰. The following formula is used to determine the extract's yield.

$$\text{Yield (\%)} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

Phytochemical screening of the extract

A variety of phytoconstituents, including alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids were qualitatively analysed in the flower extracts of *Sphaeranthus indicus* and *Spathodea campanulata*^{31,32}.

Quantification of secondary metabolites

For the purpose of estimating the quantity of phytoconstituents contained in plant extracts, quantitative analysis is a crucial instrument. TPC and TFC are established for this. TPC and TFC levels were determined using a conventional technique using extracts from the flowers of *Sphaeranthus indicus* and *Spathodea campanulata*.

Total phenolic content estimation

The Folin Ciocalteu reagent was used to calculate the total phenolic content of the extracts. Gallic acid concentration (20-100 µg/ml) was produced in methanol. Concentrations of 100 µg/ml of plant extract were also made in methanol, and 0.5 ml of each sample was added to the test along with 4 ml of 7.5% sodium carbonate and 2 ml of a 10 fold diluted folin Ciocalteu reagent. The tubes were parafilm-covered, and after 30 minutes of intermittent shaking at room temperature, the absorbance at 760 nm was measured using methanol as a blank. Gallic acid's conventional regression curve was used to compute the total phenol content, and the results were given in milligrammes per gramme (mg/g) of gallic acid³³.

Total flavonoid content estimation

Rutin (20 to 100µg/ml) was produced in methanol at various concentrations. Test samples with a polarity of 100µg/ml or close to it were created. A sample that had been diluted to 0.5 ml was combined with 2 ml of distilled water before being added to 0.15 ml of a 5% NaNO₂ solution. After waiting for 6 minutes, 0.15 ml of a 10% AlCl₃ solution was added. The combination was then given 5 minutes to stand before receiving 2 ml of a 4% NaOH solution. After reducing the final volume to 5ml with distilled water, the mixture was let to stand for an additional 15 minutes. At 510 nm, the absorbance was calculated using water as the reference. The standard regression curve of quercetin and rutin was used to compute the total flavonoid content³³.

Thin layer chromatography (TLC)

- TLC is a crucial factor in the study of natural goods. As a result, the study's *Sphaeranthus indicus* and *Spathodea campanulata* sample's TLC profile was assessed as being appropriate.
- As an adsorbent, Silica Gel G was utilized to prepare TLC plates. Different mobile phases were used to create the TLC plate.
- After experimenting with various solvent systems, the combination of chloroform and the greatest resolution was discovered: The ratio of ethyl acetate to formic acid to acetic acid is 66.6: 33.2: 0.05: 0.05.
- Spots were made visible by Iodine solution spraying.

RESULTS AND DISCUSSIONS

After completing each successive Soxhlet extraction step, the crude extracts were concentrated on a water bath by totally evaporating the solvents to obtain the real extraction yield. In phytochemical extraction, the percentage yield of extraction is crucial for assessing the standard extraction efficiency for a certain plant, other portions of the same plant, or various solvents utilized. Table 1 shows the yield of extracts made from plant flowers using petroleum ether, ethyl acetate, chloroform, and methanol as solvents. *Sphaeranthus indicus* samples' methanolic extracts revealed the presence of phenolics, flavonoids, and steroids. Table 2 displays the findings of an initial phytochemical analysis of a methanolic extract of *Spathodea campanulata* flowers. The methanolic extract revealed the presence of phenols, tannins, alkaloids, flavonoids, proteins, and carbohydrates. Total phenolic and total flavonoid contents were calculated as part of a quantitative phytochemical experiment. The TPC was computed using gallic acid as the reference standard, while the TFC was calculated using rutin as the reference standard. The total phenolic content of the ethyl acetate and methanol extracts of *Sphaeranthus indicus* and *Spathodea campanulata*, which is equivalent to gallic acid, were determined to be 192.73±0.503 and 217.00±0.916 mg/gm and 8.13±0.11 and

18.23±0.16 mg/gm, respectively. According to Table 3, 4 and Figures 1 and 2, the TFC in the ethyl acetate and methanol extracts of *Spathodea campanulata* and *Sphaeranthus indicus*, respectively, were determined to be 45.66±4.041 mg/gm and 79.33±3.511 mg/gm and 55.11±0.23 and 164.10±0.52 mg/gm, respectively, equal to rutin. Table 5 shows the TLC of *Sphaeranthus indicus*, including the amount of spots, their Rf value, and their colour. *Sphaeranthus indicus* was identified by the T.L.C. profile of the sample as having one spot in visible

light at Rf 0.93, two spots in UV 254 nm at Rf 0.83 and 0.92, and three spots in UV 366 nm at Rf 0.14, 0.28, and 0.87. Table 6 outlines the TLC of *Spathodea campanulata*, including the number of spots, their Rf value, and their colour. For *Spathodea campanulata*, the TLC profile of the sample showed four spots at Rf 0.10, 0.38, 0.72, and 0.84 in UV 366 nm (after derivatization) and three spots at Rf 0.28, 0.36, and 0.80 in UV 254 nm (before derivatization).

Table 1: Results of percentage yield (%) from the various extracts acquired from both the plant.

S. No.	Solvent	<i>Sphaeranthus indicus</i>	<i>Spathodea campanulata</i>
Average percentage yield value % (w/w)			
1.	Pet. Ether	2.0	3.5
2.	Ethyl acetate	0.90	10.6
3.	Methanol	6.5	17.3
4.	Chloroform	1.48	5.8

Table 2: Results of qualitative phytochemical analysis of various extracts acquired from both the plants.

Phytoconstituents	<i>Sphaeranthus indicus</i>				<i>Spathodea campanulata</i>			
	P	E	C	M	P	E	C	M
Alkaloids	-	-	-	-	-	+	+	+
Carbohydrates	-	-	-	-	-	+	-	+
Steroids	+	+	+	+	-	+	-	-
Proteins	-	-	-	-	-	-	+	+
Saponins	-	-	-	-	+	+	+	-
Fixed oils and fats	+	+	-	-	+	+	-	-
Flavonoids	-	-	-	+	-	+	-	+
Phenolics	+	+	+	+	+	-	+	+
Tannins	-	-	-	-	+	-	+	+

P= Petroleum ether, E= Ethyl acetate, C= Chloroform and M= Methanol

Table 3: Results of total phenolic and total flavonoids contents of *Sphaeranthus indicus*

Extracts	Total Phenolic Content(mg/gm equivalent to gallic acid)	Total Flavonoids contents(mg/gm equivalent to rutin)
Ethyl acetate extract	8.13±0.11	55.11±0.23
Methanolic extract	18.23±0.16	164.10±0.52

Table 4: Results of total phenolic and total flavonoids contents of *Spathodea campanulata*

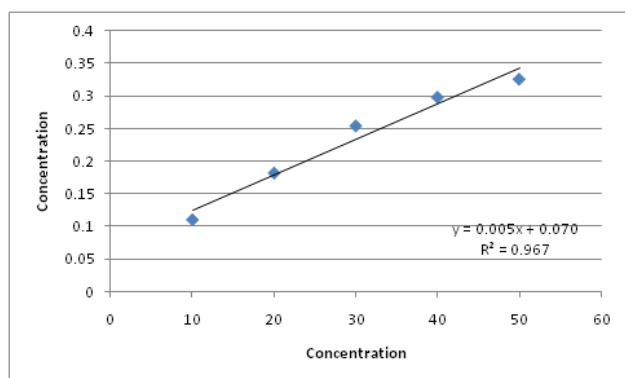
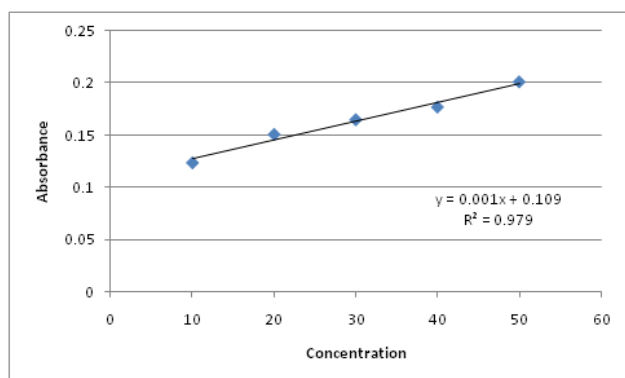
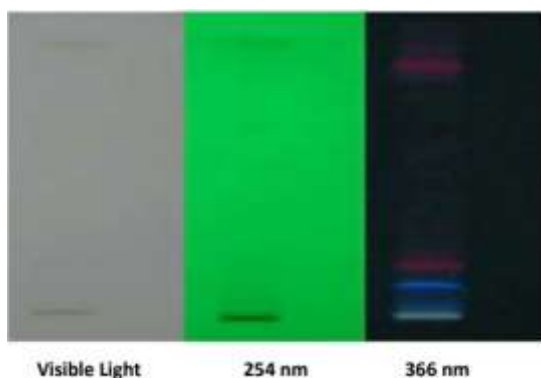
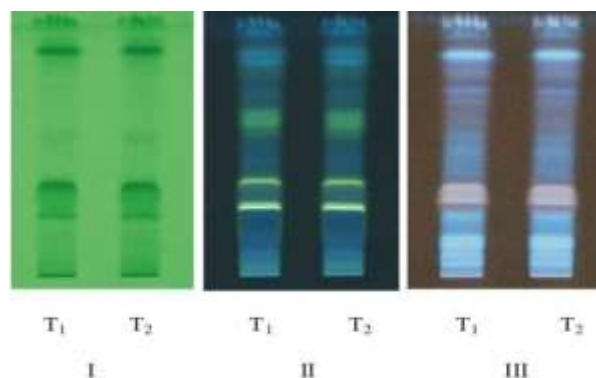
Extracts	Total Phenolic Content(mg/gm equivalent to gallic acid)	Total Flavonoids contents(mg/gm equivalent to rutin)
Ethyl acetate extract	192.73±0.503	45.66±4.041
Methanolic extract	217.00±0.916	79.33±3.511

Table 5: Thin layer chromatography of *Sphaeranthus indicus*

S. No.	UV 254 nm		UV 366 nm		White light	
	Color	Rf	Color	Rf	Color	Rf
1	Green	0.83	Blue	0.14	Pale green	0.93
2	Pale green	0.92	Pink	0.28	-	-
3	-	-	Pink	0.87	-	-

Table 6: Thin layer chromatography of *Spathodea campanulata*

Rf Values	254 nm (Before Derivatization)	366 nm (Before Derivatization)	366 nm (After Derivatization)
Rf1	0.28(Black)	0.38(Yellow)	0.10(Sky white)
Rf2	0.36(Black)	0.36(Yellow)	0.38(Sky blue)
Rf3	0.80(Black)	0.80(Sky blue)	0.72(Blue)
Rf4		0.84(Orange)	0.72(Blue)

**Figure 1:** Graph represent standard curve of Gallic acid**Figure 2:** Graph represent standard curve of Rutin**Figure 3:** TLC Profile of *Sphaeranthus indicus***Figure 4:** TLC finger print of *Spathodea campanulata* (I= 254 nm; II =366 nm before derivatization; and III= after derivatization under 366 nm)**Figure 4:** TLC finger print of *Spathodea campanulata*

CONCLUSION

The preliminary phytochemical investigation study of *Sphaeranthus indicus* and *Spathodea campanulata* flowers produced a set of standards that can be used as a crucial basis of evidence to establish the identity and to establish the quality and purity of the plant material in light of its potential uses in the future; it can be concluded from the current investigation. The phytochemical investigation provided important details about the various phytoconstituents found in the plant, which will aid future researchers in choosing a specific extract for additional research isolating the active principle. It also provided insight into the different phytochemicals that have been discovered to have a variety of activities. Methanolic floral extract was found to have the highest total phenolic and flavonoid content of any of the extracts. When it comes to the presence of phytoconstituents, methanolic extracts produce positive findings; as a result, these plants may be used directly to make medicines or to create new treatments for a variety of pathological conditions. It may be necessary to conduct more research on the potential health benefits of this plant's phytochemicals.

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