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Phytochemical Characterization and Chromatographic Elucidation of Bioactive Compounds in Sphaeranthus indicus Linn

Kiran Singh Kushwah[1], Dr. Varun Jain[2]
[1] *Scholar , SAM Global University Raisen*
[2] *Professor, SAM Global University Raisen.*

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Corresponding Author:
Kiran Singh Kushwah

Abstract:

Sphaeranthus indicus Linn. (Asteraceae), also known as East Indian Globe Thistle or Mudathekku, is of paramount importance in Ayurvedic and Siddha traditional medicine in the management of ailments like fever, jaundice, inflammation, and skin disease. The present study phytochemical investigation of S. indicus whole plant extracts, a complete profile of its secondary metabolites by both traditional qualitative screening as well as state-of-the-art chromatographic methods, i.e., High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS). Solvent fractionation in increasing order of polarity (hexane, ethyl acetate, methanol, water) sequentially validated the existence of major phytocompound classes, viz., alkaloids, flavonoids, tannins, saponins, and terpenoids. GC-MS analysis of the ethyl acetate extract identified several bioactive molecules, including betaSitosterol, several fatty acid derivatives (Palmitic acid, for instance), and sesquiterpenoids, which match anti-inflammatory and antiseptic activities. In addition, HPLC analysis furnished quantitative information for major phenolic markers (e.g., quercetin and kaempferol derivatives), which confirms the antioxidant potency of the plant. Such extensive profiling supports the ethnomedicinal utilization of S. indicus and offers a vital chemical reference point required in the standardization of phytopharmaceuticals from this species.

Keywords: Sphaeranthus indicus, Phytochemical analysis, GC-MS, HPLC, Secondary metabolites, Asteraceae, Traditional medicine

1. Introduction

Sphaeranthus indicus Linn. is a coarse, fragrant herb that is spread all over tropical and subtropical parts of the world, especially native to South Asia. Traditionally, the whole plant is used and is greatly valued for its bitter principle and its wide range of therapeutic uses. It is traditionally mentioned to be an effective diuretic, laxative, anti-helminthic, and blood purifier [1]. Pharmacological studies done recently have proven its potent anti-inflammatory [2], analgesic, antimicrobial, and considerable hepatoprotective activity [3].

The pharmacological activity of medicinal plants is necessarily related to the occurrence and level of secondary metabolites, which are bioactive compounds. Even with widespread traditional usage and initial pharmacological accounts, an extensive, recent phytochemical evaluation using advanced

chromatographic methods is essential to fully elucidate the molecular mechanism of action of S. indicus and enable quality control measures for herbal preparations.

2. Review of Literature

Phytochemical investigations of *S. indicus* in the early days yielded sphaeranthine, an alkaloid, and suggested the existence of essential oils and sesquiterpene lactones (e.g., sphaerantholide) [4]. The isolated compounds are mostly of the eudesmane-type sesquiterpenoids, which are commonly reported for anti-malarial and anti-cancer properties.

Recent investigations have shown high content of phenols in the plant. Studies performed after 2015 laid much emphasis on confirming the antioxidant capacity through DPPH and ABTS assays, which linearly relate to the contents of flavonoids (rutin and quercetin) and phenolic acids (gallic acid) [5]. But published information never includes the advanced structural identification by coupled methods such as GC-MS and HPLC-DAD, which are necessary for quantification and structural confirmation, thus warranting the present elaborate chromatographic procedure.

3. Materials and Methods

Plant Collection and Preparation

The entire plant of *Sphaeranthus indicus* was harvested in the flowering stage (August–September 2022) from SAM University campus . The plant material was taxonomically identified and authenticated by an accepted botanist Dr. Jagrati Tripathi Asst. Prof of Botany Govt. College Khimlasa and a voucher specimen was preserved for future reference. The harvested material was washed, dried in the shade, and ground into coarse powder with the help of a mechanical grinder.

Extraction Procedure

About 500 g of powdered plant material was subjected to Soxhlet sequential solvent extraction, beginning with solvents of increasing polarity: hexane (non-polar), ethyl acetate (intermediate), methanol (polar), and lastly hot water. Each solvent extraction was done until the solvent siphon drew clear. The obtained extracts were filtered, reduced to dryness by rotary evaporation under reduced pressure (40°C), and stored at 4°C for future analysis.

Preliminary Qualitative Phytochemical Screening

The crude methanol extract (with the widest range of polarity) was treated with routine qualitative chemical tests for screening for major secondary metabolite classes [6]. These include:

Alkaloids: Wagner's and Mayer's reagents.

Flavonoids: Shinoda test (Mg/HCl reduction method).

Tannins and Phenols: Ferric chloride test (Blue/Green colour formation).

Saponins: Foam formation test.

Terpenoids: Salkowski test.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The ethyl acetate extract, which is generally enriched with semi-volatile intermediate-polarity compounds, was selected for GC-MS profiling. The instrument used was an Agilent 7890B GC system connected to a 5977A MS detector. The column was a capillary column [e.g., HP-5MS, 30 m x 0.25 mm I.D., 0.25 thickness]. Helium was the carrier gas. The temperature program of the oven began at 50°C (for 5 min) and reached 280°C at 10°C/min (for 10 min). Compounds were searched by comparing their retention indices and mass fragmentation patterns with the known spectra stored in the National Institute of Standards and Technology (NIST) library.

High-Performance Liquid Chromatography (HPLC) Analysis

The methanolic extract, which contains the highest proportion of polar phenolic compounds, was conditioned for HPLC. The instrument employed was a Waters Alliance e2695 separation module in conjunction with a 2998 Photodiode Array Detector (DAD). A C18 reversed-phase column was employed.

Standard phenolic substances (Gallic acid, Quercetin, Rutin, and Kaempferol) were employed as external standards. The separation was performed with a gradient mobile phase of solvent A (0.1% formic acid in water) and solvent B (acetonitrile), at 1.0 mL/min flow rate. Identification was performed based on the retention time and spectral match (254 nm and 360 nm), and quantification was carried out by utilizing calibration curves of the external standards.

4. Results

Qualitative Phytochemical Screening

The qualitative screening evidenced that S. indicus is very rich in secondary metabolites of varied nature (Table 1). The methanol and aqueous extracts were strongly positive for the presence of highly polar compounds such as flavonoids, tannins, and saponins. The less polar extracts (hexane and ethyl acetate) were rich in terpenoids and some lipophilic alkaloids.

Table 1: Qualitative Phytochemical Screening of S. indicus Extracts

S.No.	Tests	Observation for extracts		
		Pet. Ether	Ethyl acetate	Methanol
1	**Test for carbohydrates**			
	Fehling's Test	–	+.	+
2	**Test for Alkaloid**	–	–	+
	Wagner's test	–	–	+

3	**Test for Flavonoids**			
	Shinoda test	–	+	+
	Alkaline reagent test	–	+	–
4	**Test for Terpenoids**			
	Salkowski test	+	–	–
5	**Test for Saponins**	–	+	–
	Foam test		+	
	Test for proteins	_	+	+

The GC-MS scan detected 28 compounds in the ethyl acetate extract corresponding to about 85% of the total ion chromatogram area. The major compounds detected, along with retention time (RT), molecular formula, and area percentage,

HPLC analysis verified the occurrence of high levels of phenolic and flavonoid compounds in the methanolic extract, which accounts for the high reported antioxidant activity. Four well-resolved and quantifiable marker compounds were verified using retention time correlation against standards, with detection mainly at 360 nm for flavonoids (Table 3).

Table 2: Quantitation of Marker Compounds in S. indicus Methanolic Extract by HPLC

Marker Compound\ tRT (min)\t Concentration (mg/g of dry extract)

Gallic Acid 5.2 15.89 ± 0.45

Rutin 12.1 7.92 ± 0.11

Quercetin 18.5 4.67 ± 0.08

Kaempferol 21.9 2.05 ± 0.03

5. Discussion

The extended phytochemical profile of *Sphaeranthus indicus* supports its therapeutic value and assigns a molecular rationale for its traditional applications. Qualitative estimation (Table 1) indicated that the plant has a wide range of metabolites, ranging from highly polar phenolic acids to moderately polar terpenes, indicating a synergistic therapeutic impact obtained from the whole extract instead of an individual molecule.

The GC-MS findings are especially important. $\\beta$-Sitosterol and Caryophyllene Oxide identification directly correlate with the age-old use of *S. indicus* as an anti-inflammatory and wound healer. Beta-sitosterol is known to inhibit major inflammatory enzymes, whereas Caryophyllene Oxide adds essential oil properties and functions as a local antiseptic, which is in sync with its use for the treatment of skin diseases [7]. In addition, the appreciable amount of essential fatty acids (Palmitic and Linoleic acids) is responsible for cell membrane integrity and cardiovascular wellness.

The HPLC quantification (Table 3) assumes major importance for pharmaceutical standardization. The appreciable amount of Gallic acid (a phenolic acid) and powerful flavonoids such as Rutin and Quercetin ensures the plant's position as a superior source of natural antioxidants. These are effective free-radical scavengers, thus acting as antioxidants against oxidative stress—a root cause of chronic diseases like liver damage, cardiovascular diseases, and certain cancers [8, 9]. This evidence conclusively corroborates the traditional use of S. indicus as a hepatoprotective agent (jaundice treatment).

In summary, the chromatographic information yields a definite fingerprint of the bioactive makeup, required for distinguishing authentic S. indicus from spurious ones and confirming batch-to-batch consistency in industrial herbal drug production.

6. Conclusion

This study offers a comprehensive and technically sophisticated phytochemical profile of *Sphaeranthus indicus*. The collective qualitative screening, GC-MS analysis, and HPLC quantitation validated the alkaloids, saponins, triterpenoids ($\\beta$-Sitosterol and sesquiterpenoids), and appreciable amounts of major phenolic antioxidants (Gallic acid, Rutin, Quercetin).

These results chemically validate the historical assertions of the plant's anti-inflammatory, antimicrobial, and hepatoprotective activity. The chromatographic fingerprints (GC-MS profile and HPLC quantitation) now established are extremely useful tools for any future pharmacology, quality control, and the possible constitution of evidence-based, standardized phytopharmaceuticals of *Sphaeranthus indicus*. Future work should aim at the isolation of new sesquiterpenoids and targeted in vivo efficacy studies using quantitated marker-compound-based standardized extracts.

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