Chapter 9

Investigation of bioactive compounds in *Sphaeranthus indicus* L. whole plant through phytochemical screening, cytotoxicity testing and chromatographic analysis

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Abstract: *Sphaeranthus indicus* L., a well-known medicinal plant in Ayurvedic and traditional medicine, is recognized for its diverse therapeutic properties including antimicrobial, anti-inflammatory, and anticancer activities. Present study aimed to investigate the bioactive constituents of the whole plant through comprehensive phytochemical screening, cytotoxicity testing, and chromatographic analysis. The results revealed significant phytoconstituents including flavonoids, alkaloids, terpenoids, and phenolic compounds, with notable cytotoxic activity, supporting the plant's potential in drug discovery and therapeutic applications.

Keywords: Antimicrobial, drug discovery, traditional medicine

Introduction

Sphaeranthus indicus L., commonly known as East Indian globe thistle, is an aromatic, erect, and branched herb belonging to the family Asteraceae (Galani et al., 2010). This is widely distributed throughout the Indian subcontinent and parts of Southeast Asia, this plant thrives in moist and marshy environments, often found near water bodies and paddy fields (Arya et al., 2022). Morphologically, it is characterized by its purple-pink flower heads and deeply lobed leaves (Figure 1). Traditionally, the whole plant comprising roots, leaves, flowers, and stem has been utilized in Ayurvedic, Unani, and Siddha systems of medicine (Makhija et al., 2011). Ethnomedicinally, it is employed for the treatment of a broad range of ailments including skin disorders, respiratory infections, epilepsy, gastrointestinal issues, and even psychological conditions such as anxiety and depression (Mahajan et al., 2015). Pharmacological studies have confirmed a wide spectrum of biological activities associated with *S. indicus*, including antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, neuroprotective, and anticancer effects (Ramachandran, 2013; Dintu et al., 2024; Selvamoorthy et al., 2024). These therapeutic properties are attributed to the plant's rich reservoir of secondary metabolites, such as flavonoids, alkaloids, sesquiterpene lactones, phenolics, and essential oils. The increasing burden of chronic diseases and the growing resistance to synthetic drugs, there is an urgent need to explore effective plant-based alternatives (Muteeb et al., 2023). *S. indicus*, with its multi-targeted bioactivity, represents a promising candidate for developing novel phytopharmaceuticals to combat pressing global health challenges like cancer, antibiotic resistance, and metabolic syndromes. Looking forward, the integration of advanced analytical techniques such as high-throughput screening, metabolomics, and molecular docking could pave the way for isolating and characterizing the most potent bioactive constituents of *S.*

indicus. Combined with in vivo validation and clinical trials, this approach could transform traditional knowledge into evidence-based therapeutic applications. The current investigation, therefore, aims to bridge the gap between ethnomedicine and modern pharmacology through systematic phytochemical screening, cytotoxicity testing, and chromatographic analysis of the whole plant of *S. indicus*.



Figure 1: Leaves and flower of *S. indicus*

Methodology

The whole plant of *S. indicus* was collected from the bank of Mahanadi River, Cuttack district of Odisha, India. The plant species was identified by the authors (Figure 1). The maceration extraction method was adopted using different solvents (n-hexane, ethanol and aqueous) for experimental analysis (Figure 2). Detection of nine secondary metabolites was conducted using standard

methods (Jena et al., 2025; Jena and Kumar, 2025; Kshirsagar et al., 2025; Verma et al., 2025). Details of phytochemical analysis is given below.



Figure 2: Filtrate extracts of *S. indicus* (whole plant) for experimental analysis

Test for tannin: 1 ml of whole plant extract was taken in a test tube, and 3-5 drops of 10% lead acetate solution was added to it. The gelatinous precipitate formation confirmed the presence of tannin.

Test for saponin: About 1 ml of distilled water was added to 1 ml of the whole plant extract and shaken well. The formation of persistent froth was observed, confirming the presence of saponin.

Test for flavonoids: About 1 ml of the whole plant extract was taken, and 2 ml of NaOH solution and 3-5 drops of dilute HCl were added to it. The color initially turned to an intense yellow color with NaOH solution and later became colorless, which confirmed the presence of flavonoids.

Test for terpenoids: To 1 ml of the whole plant extract, 5-6 drops of chloroform were added. Then, 4-6 drops of concentrated sulphuric acid were added to it. The reddish-brown interface confirmed the presence of terpenoids.

Test for phenols: About 1 ml of the whole plant extract was taken. A few drops of 5% ferric chloride solution were added. The dark bluish-black appearance confirmed the presence of phenolic compounds.

Test for reducing sugars: About 1 ml of the whole plant extract was taken. 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept in the water bath for a few minutes. The presence of red-orange precipitate confirmed the presence of reducing sugars.

Test for steroids: To 1 ml of the whole plant extract, 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid were added. The appearance of upper red and lower yellow with green fluorescence indicated the presence of steroids.

Test for alkaloids: About 1 ml of the whole plant extract was taken, and 3-4 drops of Dragendroff's reagent were added to it. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

Test for carbonyl compounds: About 1 ml of whole plant extract was taken, and 3-4 drops of 2,4-Dinitrophenylhydrazine (DNPH) reagent were added. The formation of yellow crystals confirmed the presence of carbonyl compounds.

Cytotoxicity analysis: For cytotoxicity test, hatching of Brine shrimp cysts was the initial process. Standardization of the optimum salinity was done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*A. salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5 % saline water with proper aeration, room temperature or between (28-35^{o}C) and light for 72 hours. Proper hatching could be observed within 24-72 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (15.62 mg ml^{-1}, 31.25 mg

ml^{-1}, 62.5 mg ml^{-1}, 125 mg ml^{-1} and 250 mg ml^{-1}) of the *S. indicus* whole plant extracts were taken and 3.5% saline water were used to make the volume 2 ml in each test tube. 1 % DMSO was used to dissolve the crude extracts. Ten nauplii were selected and introduced to five test tubes of the extracts. Positive and negative controls were prepared using 3.5% saline water and vincristine sulphate (5 mg ml^{-1}) respectively with a volume of 2 ml. The live larvae are highly mobile and thus differentiates from the unhatched cysts. The survivor nauplii were counted and analyzed the death % (Nayak et al., 2021).

Chromatographic analysis: TLC plates were prepared on 9 cm glass slides using silica gel powder. Slides were washed with clinical laboratory detergent and dried. Clean and dried slides were wiped with ethyl acetate for removing surface adherents. 3 g of silica was taken in 20 ml of distilled water and slurry was prepared by constantly stirring and finally poured over the slides. The slides were left undisturbed till the drying of silica layer. Then the slides were activated at 50 ^{o}C for 20 minutes before running the TLC (Mishra and Bhatnagar, 2024). Methanol: chloroform (1:1), ethyl acetate: chloroform (1:9) and ethyl acetate: water: acetic acid (8:1:1) were used as mobile phases. Then spots were visualized under UV chamber and recorded the RF (Retention Factor) values (Kumar and Jena, 2014).

Results and discussion

Preliminary phytochemical screening of *S. indicus* whole plant extracts revealed the presence of various bioactive components, including tannin, saponin, phenolic compounds, reducing sugars and alkaloids (Table 1; Figures 3-5).

The phytochemical analysis of *S. indicus* whole plant revealed the presence of various bioactive compounds, including tannins, saponins, flavonoids, alkaloids

and reducing sugars (Table 1: Figures 4-6). These compounds were found to be present in different extracts having various ethnomedicinal uses and pharmacological potential. The presence of wide range of phytoconstituents suggests that *S. indicus* s may possess medicinal properties.

Table 1: Qualitative phytochemical analysis of *S. indicus* (whole plant) using different extracts

Bioactive compounds	n-hexane	Ethanol	Aqueous
Tannin	Absent	Absent	Present
Saponin	Present	Present	Present
Flavonoids	Absent	Absent	Absent
Terpenoids	Absent	Absent	Absent
Phenolic compounds	Absent	Absent	Present
Reducing sugars	Present	Present	Present
Steroids	Absent	Absent	Absent
Alkaloids	Absent	Absent	Present
Carbonyl compounds	Absent	Absent	Absent

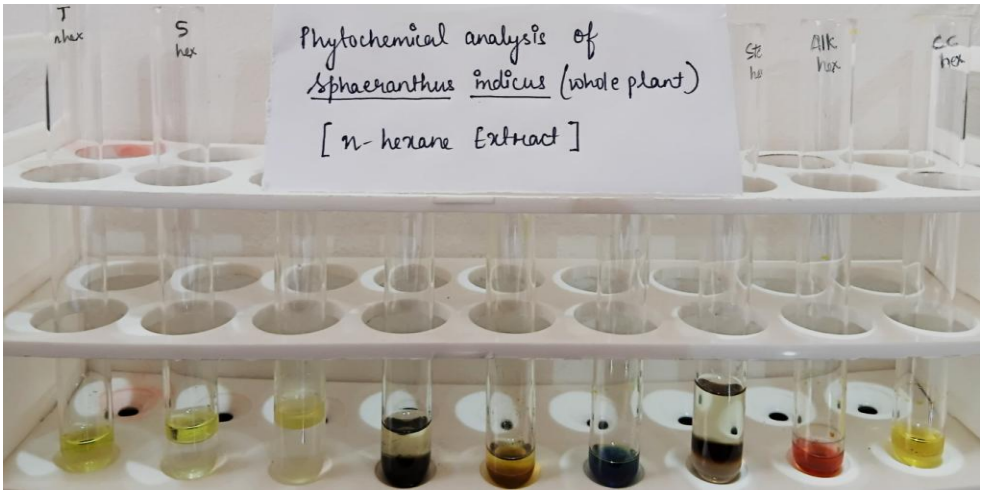


Figure 3: Detection of secondary metabolites of *S. indicus* (whole plant) using n-hexane extract

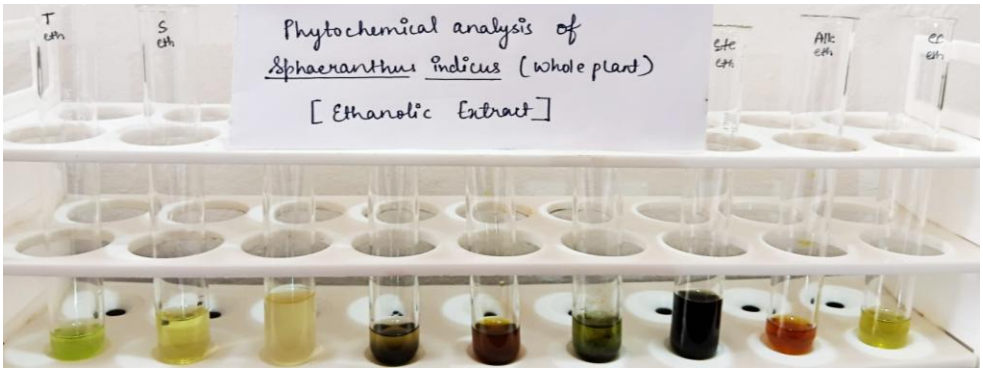


Figure 4: Detection of secondary metabolites of *S. indicus* (whole plant) using ethanolic extract

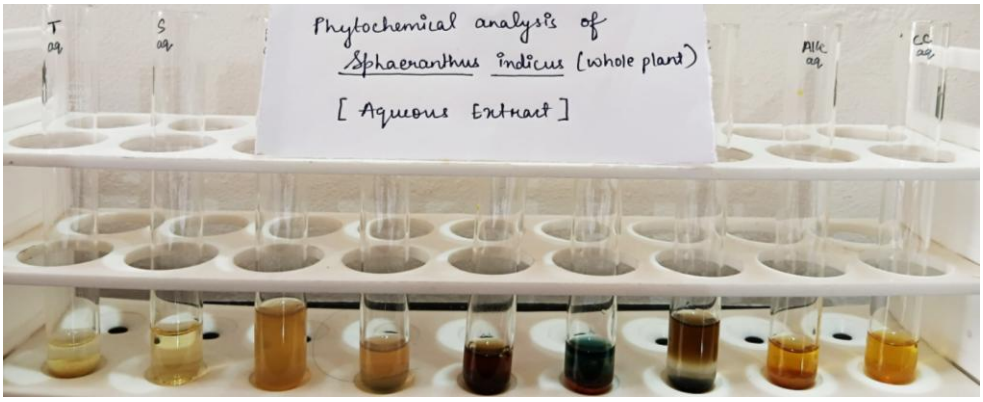


Figure 5: Detection of secondary metabolites of *S. indicus* (whole plant) using aqueous extract

The cytotoxicity analysis of *S. indicus* whole plant extracts using brine shrimp nauplii showed significant mortality rates for n-hexane and ethanol extracts, indicating potential anticancer or antimicrobial properties (Table 2; Figures 6-8; Krishnaraju et al., 2005). The observed cytotoxicity may be attributed to the presence of bioactive phytochemicals, which are known to interfere with cancer cell proliferation and induce apoptosis. Further studies involving mechanism-based assays and in vivo evaluations are needed to the pathways involved in its anticancer activity.

Table 2: Cytotoxicity analysis of *S. indicus* (whole plant) using Brine shrimp nauplii

Extracts	Concentration (in mg/ml)	Initial number of Nauplii	Number of Deaths of Nauplii (after hours)				Death rates (%)
			1	2	3	4	
n-hexane	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Ethanol	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Aqueous	15.62	10	0	0	0	0	0
	31.25	10	0	3	3	3	30
	62.5	10	7	10	10	10	100
	125	10	10	10	10	10	100
	250	10	7	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100

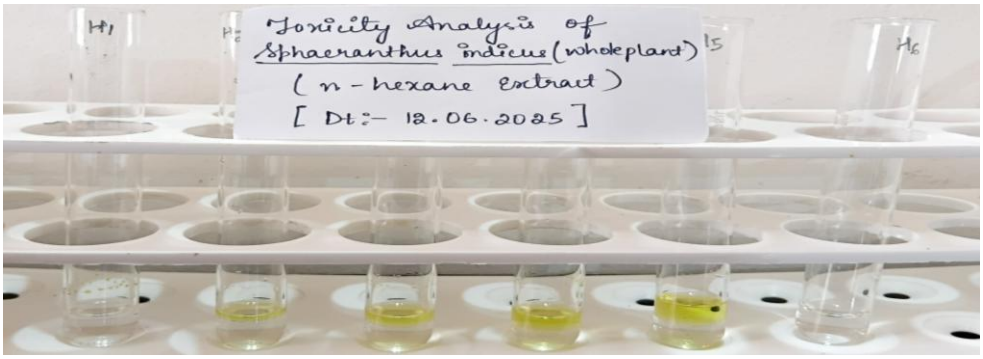


Figure 6: Toxicity analysis using Brine shrimp nauplii of n-hexane extract of *S. indicus* (whole plant)



Figure 7: Toxicity analysis using Brine shrimp nauplii of ethanolic extract of *S. indicus* (whole plant)

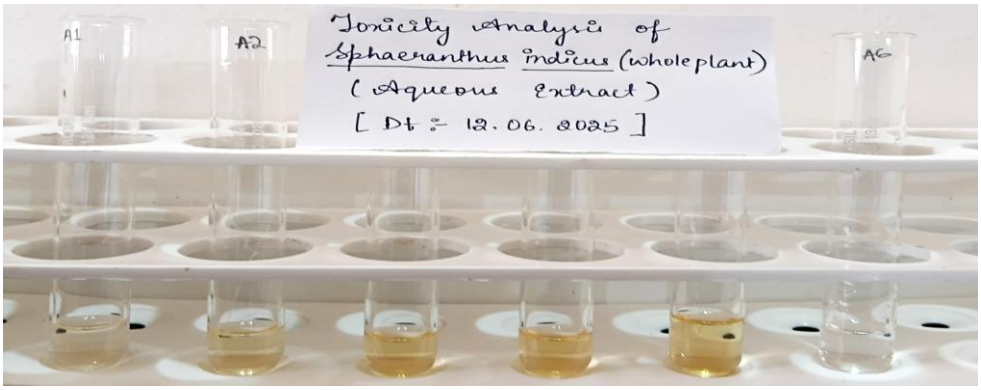


Figure 8: Toxicity analysis using Brine shrimp nauplii of aqueous extract of *S. indicus* (whole plant)

Thin Layer Chromatography (TLC) profiling of the *S. indicus* whole plant extracts provided a preliminary chemical fingerprint, revealed the presence of multiple phytoconstituents. Using different mobile phases of varying polarity, distinct bands were observed in UV chamber. In methanol: chloroform mobile phase, maximum bands are shown (Table 3; Figure 9).

Table 3: TLC analysis of *Sphaeranthus indicus* (whole plant) using different mobile phases

	Mobile Phases	Rf value of Spots				
		S1	S2	S3	S4	S5
Ethanolic aqueous	Methanol: Chloroform (1:1)	0.61	0.65	0.7	0.77	0.88
	Ethyl acetate: Chloroform (1:9)	0.68	0.88	-	-	-
	Ethyl acetate: Water: Acetic acid (8:1:1)	0.8	0.82	-	-	-

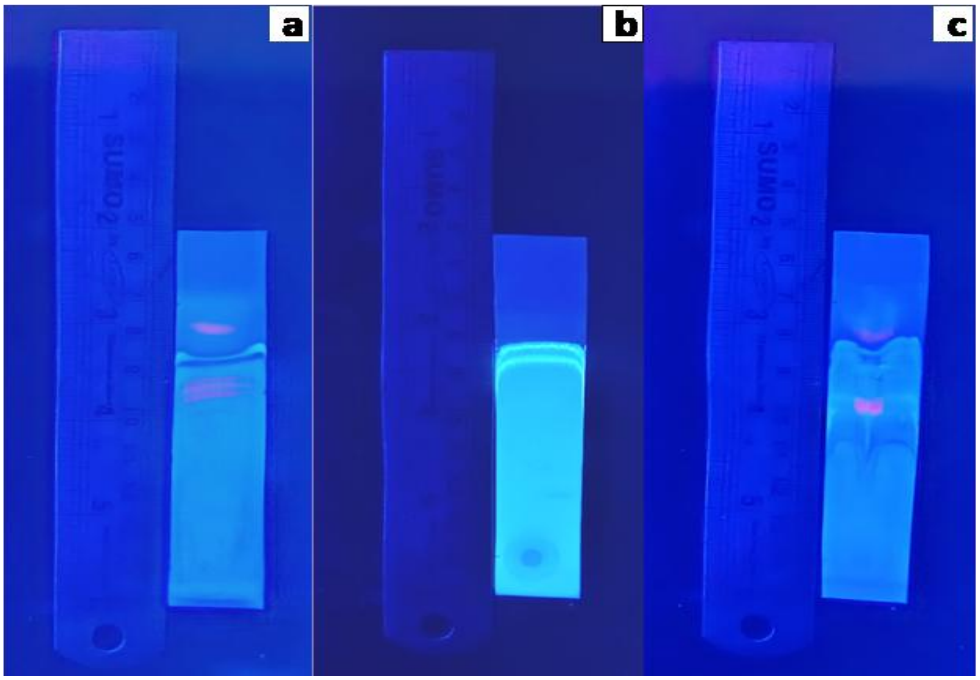


Figure 9: TLC analysis of hydroethanolic extract of *S. indicus* (whole plant) using a) Chloroform: Methanol b) Ethyl acetate: Water: Acetic acid and c) Ethyl acetate: Chloroform

Conclusion

Current research has effectively identified a range of bioactive compounds present in the whole plant of *S. indicus* L. reinforced the traditional medicinal applications of the plant. The cytotoxicity findings suggested encouraging anti-cancer properties, necessitating further exploration. In vivo pharmacological investigations and molecular docking techniques may assist in confirming the mechanisms of action of these compounds which could facilitate the advancement of innovative plant-derived therapeutics.

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