Bioscene

Bioscene
Volume- 21 Number- 02
ISSN: 1539-2422 (P) 2055-1583 (O)
www.explorebioscene.com

Comprehensive Evaluation of Sphaeranthus Indicus Flowers: Unveiling Pharmacognostical, Physicochemical, Phytochemical Profiling and In-Vitro Analysis for Potential Anti-Hypertensive Activity

Mohamed Insaaf P M*[1], Vadivu R[2], Mahalingam R[3], Radha R[4]
M.Pharm student[1], Assistant Professor[2], M.Pharm student[3], Head of Department[4]
[1,2,3&4] Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-03, India

Corresponding Author: **Mohamed Insaaf P M**

Abstract

Background: Sphaeranthus indicus, recurrently known as East Indian globe thistle, has been utilized in indigenous medicine for its purported ameliorative properties, including its potential in managing hypertension. However, comprehensive pharmacognostic and phytochemical investigations of its flowers, alongside in-vitro antihypertensive assessments, are lacking.**Objective:** This study focused on conducting phytochemical and pharmacognosticalevaluation of Sphaeranthus indicus flowers and evaluate their potential antihypertensive activity through in-vitro studies.Materials and Methods: Flowers of Sphaeranthus indicus were subjected to macroscopic and microscopic evaluations, as well as phytochemical screening to identify various secondary metabolites. The in-vitro antihypertensive activity was assessed using Angiotensin Converting Enzyme Inhibition Assay. Results: Pharmacognostic analysis revealed the distinctive anatomical features of Sphaeranthus indicus flowers, while phytochemical screening detected the existence of bioactive compounds such as glycosides, flavonoids, phenols and alkaloids. In-vitro studies demonstrated significant anti-hypertensive activity, suggesting the potential therapeutic utility of Sphaeranthus indicus flowers in managing hypertension.**Conclusion:** The pharmacognostic and phytochemical analysis confirmed the identity and bioactive constituents of Sphaeranthus indicus flowers. Moreover, the observed in-vitro antihypertensive activity indicates their promise as a natural remedy for hypertension. Further research, including clinical trials, is justified to vindicate these findings and explore the full therapeutic potential of Sphaeranthus indicus flowers in the management of hypertension.

Keywords: hypertension, in-vitro studies, pharmacognostic, phytochemical, Sphaeranthus indicus.

Introduction

Herbs have long been used as both recognized medications and folk cures. Long before the ancient period, plants were used for therapeutic purposes. In the global health care system, Phytomedicine has been extremely important. Although the use of plants, animals, or minerals for healing was part of traditional medicine, Phytomedicine or plant medicine, is the subject of this discussion. The World Health Organization exemplifies "Phytomedicine" as "herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration, or other physical or biological processes."

Hypertension, sometimes known as elevated or rising blood pressure, is a disorder marked by decisively higher pressure in the blood vessels. The impact of blood against the artery walls that results from the heart's pumping action is what creates blood pressure. According to WHO, 1.28 billion individuals globally, aged between 30-79, suffer from hypertension, with 2/3rd of them living in middle and low-income countries. An approximately 46% of hypertensives are nescient of their illness. Only 42% of those with hypertension are identified and alleviated. 1 in 5 persons (21%) had hypertension under control. One major goal of the world's non-communicable illness is to reduce hypertension acceptance by 33% between the year 2010 and 2030.

Herbal treatments have been utilized extensively all across the world since ancient times. Many medicines are now in use to treat the disease of hypertension. However, these medicines have been linked to serious negative effects. As a result, the emphasis these days is on natural treatments that have few or no adverse effects. Regarding the adverse effects of allopathic pharmaceuticals, it is critical to find and develop alternative drugs, among which plant-based therapies are thought to be more effective. Medicinal plants are crucial in the production of powerful medicinal medicines.

Achieving a successful treatment therapy for Hypertension with fewer side effects remains a problem, and evaluating innovative techniques to treat this condition is a top goal. The valuable benefits of plants in the management of Hypertension have been thoroughly researched and have yielded excellent results (Gunasekaranet al., 2014) (Lorenet al., 1995) (Okigbo et al., 2006).

Materials and Methods

Pharmacognostical Studies

Collection and Authentication of Plants

The plant Sphaeranthus indicus were collected from Ayyanoor Chennai, Tamil Nadu, India, on November 2023 and has been botanically recognized and authenticated.

Macroscopy

The organoleptic characteristics like color, nature, odour and taste of the flower like its surface, shape, size, thickness and fracture was evaluated. External feature of test sample was recorded using Nikon D-5600 Digital camera.

Microscopy

The sample was maintained in fixative FAA for more than 48 hours. The conserved specimens were cut into thin transverse pieces with a sharp blade, which were then dyed with 0.8% Safranin and 0.5% Astra blue. Transverse slices were taken with an Axiolab5 trinocular microscope and a Zeiss Axiocam208 colour digital camera in strong field light. Magnifications were marked by a scale bar.

Histochemical Studies

Plant sections were treated following the standard procedures:

1. Crystals: The section was mounted in water and one end of the cover slip was irrigated with acetic acid. While looking through the microscope, the water within the cover slip was replaced using a piece of filter paper at the divergent end of the cover slip

-Formation of air bubbles indicated Calcium carbonate crystals

-If no air bubbles were formed, the experiment was repeated with conc. HCl, wherein dissolution of crystal and formation of needles of Calcium sulphate indicated the presence of Calcium oxalate crystals

2. Fats, Fatty oils volatile oils and resins: About1 to 2 drops of Sudan-IV was added to the section and allowed to stand for a few minutes. Presence of fatty oil substances were indicated by orange-red/pink/red colored globules; while red coloured irregular contents indicated resin.

3. Starch: A drop of 2% iodine water solution was added - blue colour indicated starch.

4. Phenolic compounds:A drop of alcoholic ferric chloride was added; the blue black contents indicated phenolic substances such as flavonoids/tannins, etc.

5. Mucilage: A drop of ruthenium red was added - pink to red colored contents indicated mucilage.

6. Lignified cell walls: A drop of phloroglucinol was applied to the portion and left to stand for about 2 minutes, or until nearly dry. A drop of 50% HCl was poured and viewed over a cover glass; the cell walls turned pink to cherry red, confirming the presence of lignin.

7. Suberized or cuticular cell walls: A drop of Sudan red III was added and allowed to stand for a few minutes, warmed gently if necessary - cell walls-stained orange-red or red indicated suberin or cutin deposition over cell wall.

8. Alkaloids: A drop of Wagner's reagent was added - the presence of yellow to reddish brown colored contents confirmed alkaloids.

Powder Microscopy

After being cleaned with a saturated chloral hydrate solution, a pinch of powdered material were been placed on a tiny slide along with a drop of 50% glycerol solution. To confirm the detection of starch granules, the sample was treated with iodine solution. A Nikon ECLIPSE E200 trinocular microscope and a Zeiss ERc5s digital camera were used to study the characters in bright field light. Photomicrographs of diagnostic features were obtained and reported (SPI, 2008) (Kumar et al., 2011) (Krishnamurthy, 1998) (Kokate, 2002) (Divakar, 2002).

Physicochemical Studies

All the procedures of physico-chemical analysis such as determination of extractive values, ash values, swelling index, moisture content, foaming index, crude fiber content, quantitative and qualitative evaluation of inorganic elements and heavy metals was done as per World Health Organization (WHO) guidelines (API, 2001) (WHO, 1998) (Kokate et al., 2003).

Phytochemical Studies

Extraction

The phytochemical examination begins with extraction. The dried coarsely powdered sample of Sphaeranthus indicus were extracted using a Soxhlet extraction apparatus and solvents with increasing order of polarity such as ethyl acetate and ethanol. The extract was been condensed using a rotating vacuum evaporator apparatus. The consistency, % yield and colour of the extract will be noted before proceeding to more extensive phytochemical and pharmacological examination.

Qualitative estimation of phytoconstituents

The qualitative analysis was performed using the standard process outlined in phytochemical procedures. The test was done out following typical conventional techniques.

Quantitative estimation of phytoconstituents

Total phenolic content:

Using the Folin-Ciocalteu assay technique, the total phenolic compounds present in the ethanolic and ethyl acetate extract of plant sample were been measured.

Total flavonoid content:

The calorimetric technique was been used to determine the total flavonoid content present in both the extract sample was been measured.

Total alkaloid content:

The total alkaloid components in the extracts were determined using the ammonium hydroxide technique.

Fluorescence evaluation of extract and powder

Fluorescence analysis was been performed in both daylight and ultraviolet light. The fluorescence was seen during the day, as well as in long and short UV light at 365 and 254 nm, respectively. The crude powder and the extracts was been exposed to daylight and UV rays and the fluorescence effect was been noted.

Thin Layer Chromatography Method

The Thin Layer Chromatography of the ethyl acetate and ethanolic extract of the flowers of the Sphaeranthus indicus was been performed out using Toluene : Ethyl acetate (9:1) as the solvent system. The spots were been detected by placing the plates in an iodine chamber and UV chamber. The Rf value was calculated as mentioned below.

$$R_f = \frac{\text{Distance travelled by solute from the origin}}{\text{Distance travelled by solvent from the origin}}$$

HPTLC Method

The Sphaeranthus indicusethanolic extract was subjected to HPTLC analysis and the HPTLC fingerprint profile of the ethanolic extract of the flowers of Sphaeranthus indicus was observed. The acquired fingerprint may be used to monitor drug identification and purity, as well as to detect adulteration and replacement. The HPTLC method is useful in determining the identification, purity, and amount of active ingredients contained in the herbal extract (Raman, 2000) (Kokate, 2003) (Evans and Trease, 1998) (Connors, 1982) (Kokate et al., 2003) (Peach and Tracey, 1995) (Kokate et al., 1990).

In-vitro Studies

In-vitro studies on Angiotensin Converting Enzyme inhibition Activity

The activity of ACE was measured using Hippuryl-l-histidyl-l-leucine (HHL) as a substrate. The enzyme hydrolyzes HHL in a buffer with sodium chloride, producing Hippurate. The freed Hippurate was then reacted with cyanuric chloride in phosphate buffer to produce a chromogen, measured by absorbance at 382 nm. The percentage of ACE inhibition was been calculated as described below (Jabeen and Aslam, 2013).

$$\% \text{ ACE inhibition} = [(\text{OD of control} - \text{OD of test})/(\text{OD of control})] \times 100$$

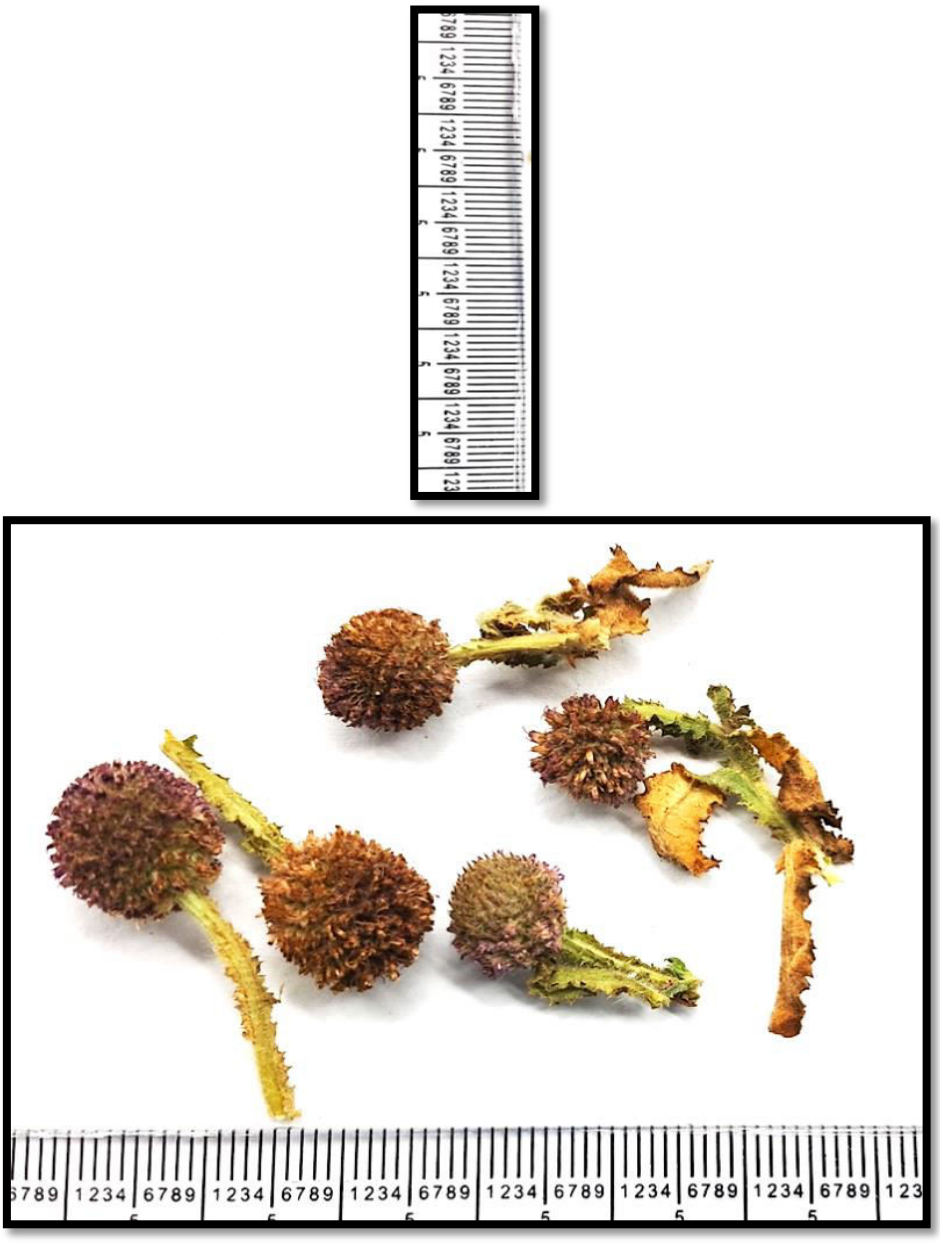
Results and Discussion

Pharmacognostical Studies

Macroscopy:

Florets are in compound heads in globose spikes, head is 1 cm across, ovoid in shape, bracteate, borne on solitary glandular peduncles with toothed wings; purple in colour, involucre of bracts spatulate, acute, and ciliate near the ends; flowers two types and tubular, outer female - all fertile, inner bisexual - fertile or sterile; corolla 5-lobed, pink; anthers sagittate at base; style filiform, ovary unilocular, single ovule on basal placentation; odour is aromatic with characteristic taste.

Figure 1: Macroscopy of Sphaeranthus indicus inflorescence



Microscopy

Longitudinal Section of inflorescence

LS of head shows center receptacle on which flowers are arranged; female flowers are seen towards periphery and bisexual florets towards inner side; center portion of the section shows inflorescence axis formed of parenchyma cells and vascular bundles can be seen traversing through it towards the florets; female flower consists of outer bracts and inner corolla whorls encircling the gynoecium; gynoecium is formed of ovary, style and stigma; bracts are elongated with numerous cilia on upper portion

Figure 2: LS of Head

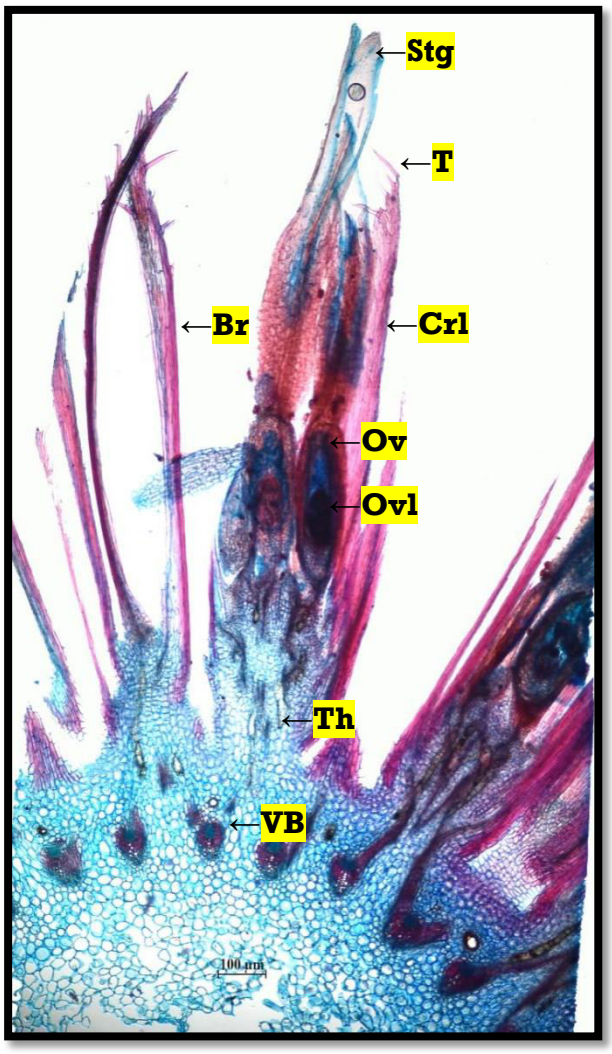
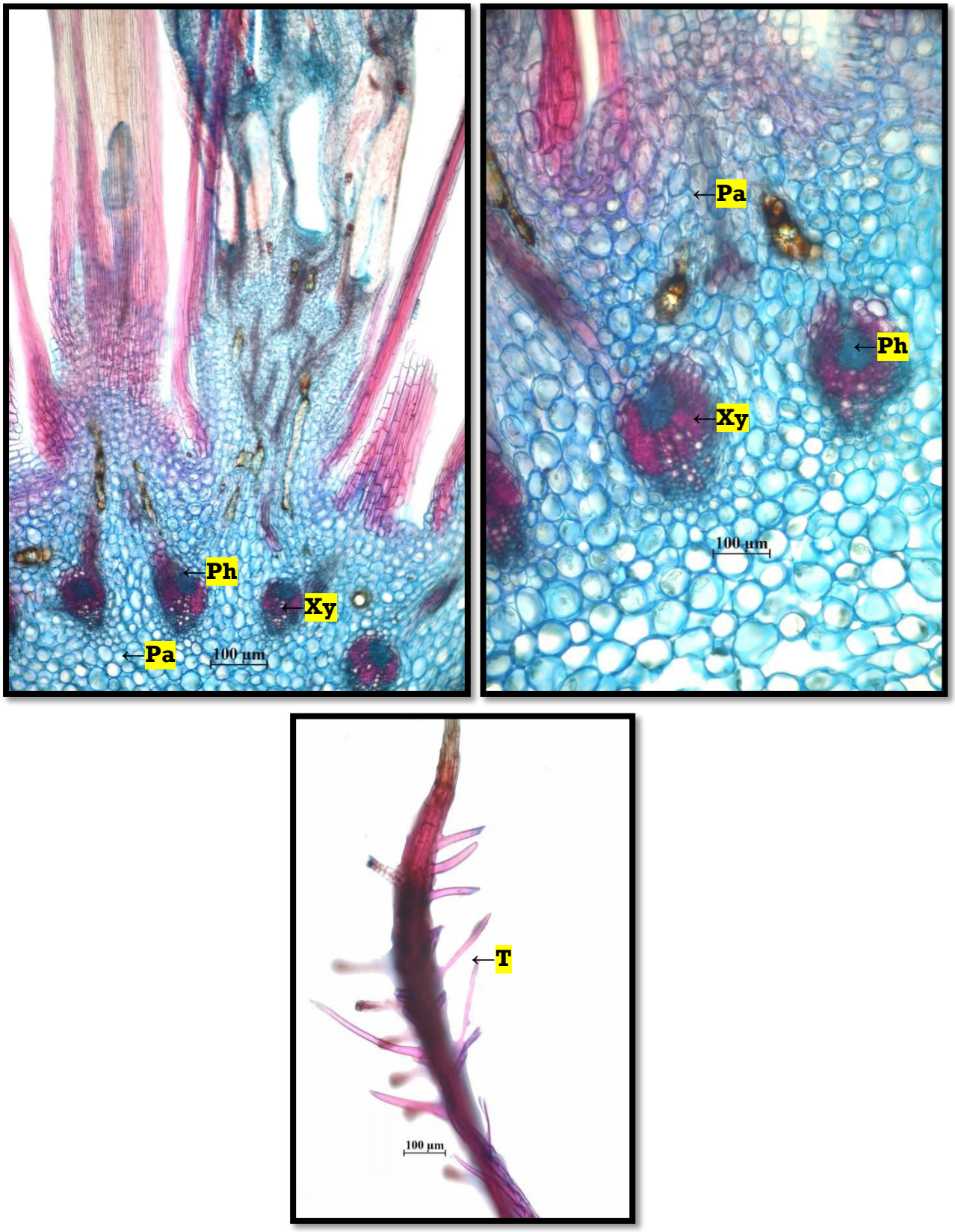


Figure 3: LS of ray florets

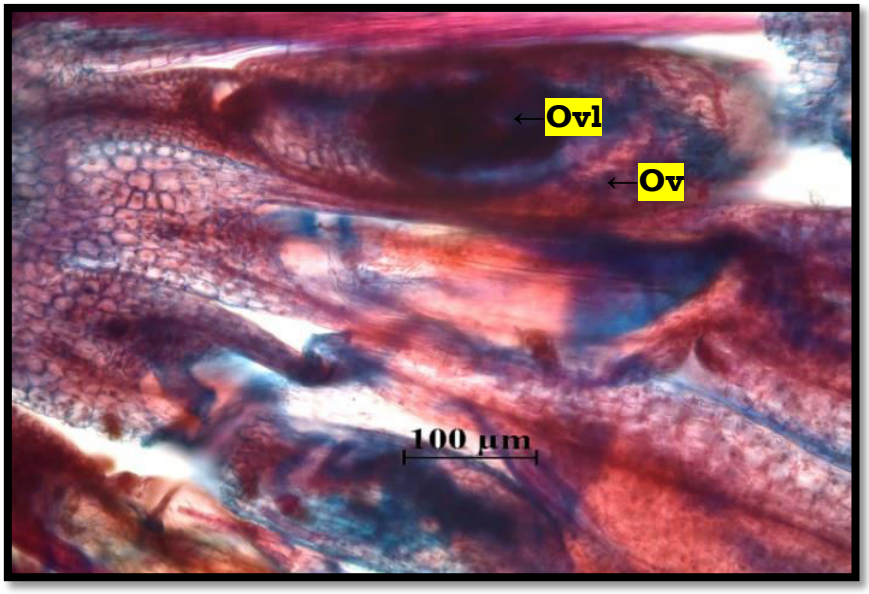
Figure 4: Enlarged view of outer whorls, vascular region and bract



LS of ovary

LS of ovary shows unilocular ovary with one single ovule on basal placentation; ovary wall is made up of parenchymatous cells.

Figure 5: LS of ovary

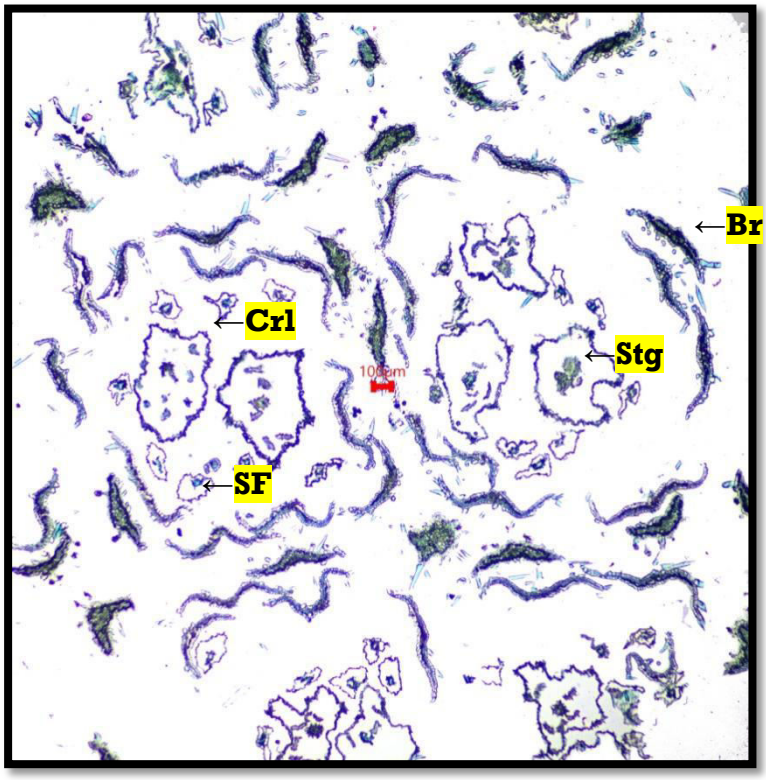


Br - bract; Crl - corolla; Ov - ovule; Ovl - ovule; Ph - phloem;Pa - parenchyma; S - style; T - trichome; Th - thalamus; VB - vascular bundle; Xy - xylem

TS of inflorescence

TS of inflorescence shows numerous bisexual florets each surrounded by outer bracts followed by corolla; inner to corolla, anther is present with abundant pollen grains inside; stigma is present at the center portion of each floret.

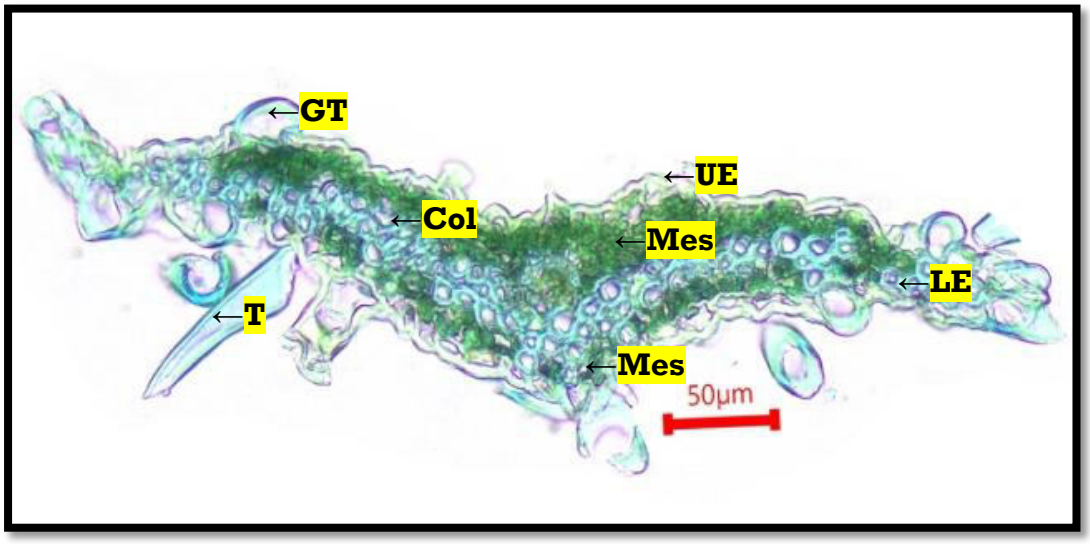
Figure 6: TS of inflorescence



TS of bract

TS of bract shows single layered lower and upper epidermis covered by cuticle and bears few covering trichomes; mesophyll cells are found below the epidermis and 2 to 3 layers of collenchymatous cells are present between mesophyll layers; vascular bundle is embedded at the center.

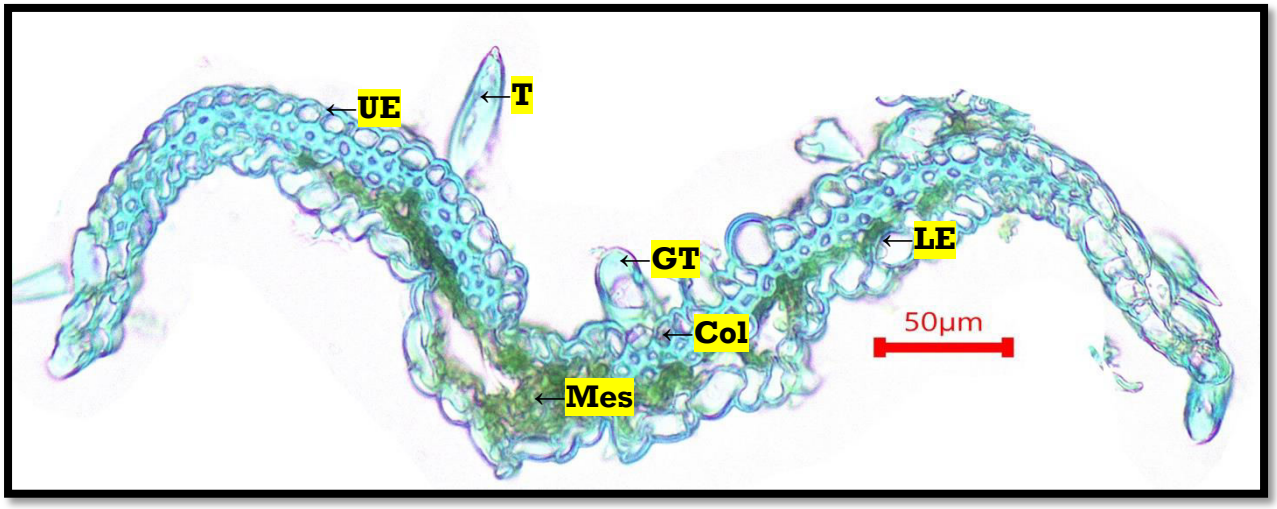
Figure 7: TS of bract



TS of corolla

TS of corolla shows single layered lower and upper epidermis covered by cuticle and bears few covering trichomes; 2 to 3 layers of collenchyma cells are found below the upper epidermis followed by few layers of mesophyll cells; vascular bundle can be seen at the center.

Figure 8: TS of Corolla



TS of bisexual flower

TS of bisexual flower shows outer whorls of paleaceous bracts and corolla; inner anther enclosing plenty of pollen grains; stigma is present at the center portion of each floret.

Figure 9: TS of bisexual floret with fertile anther

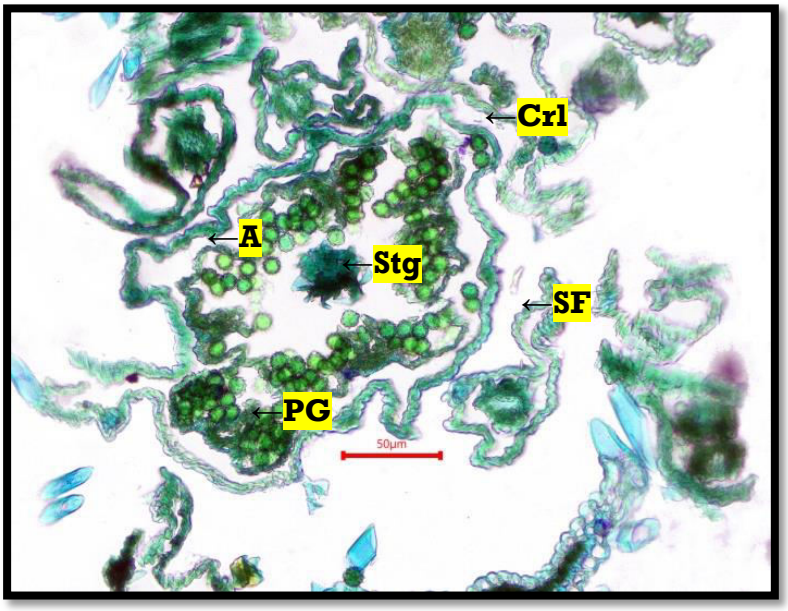
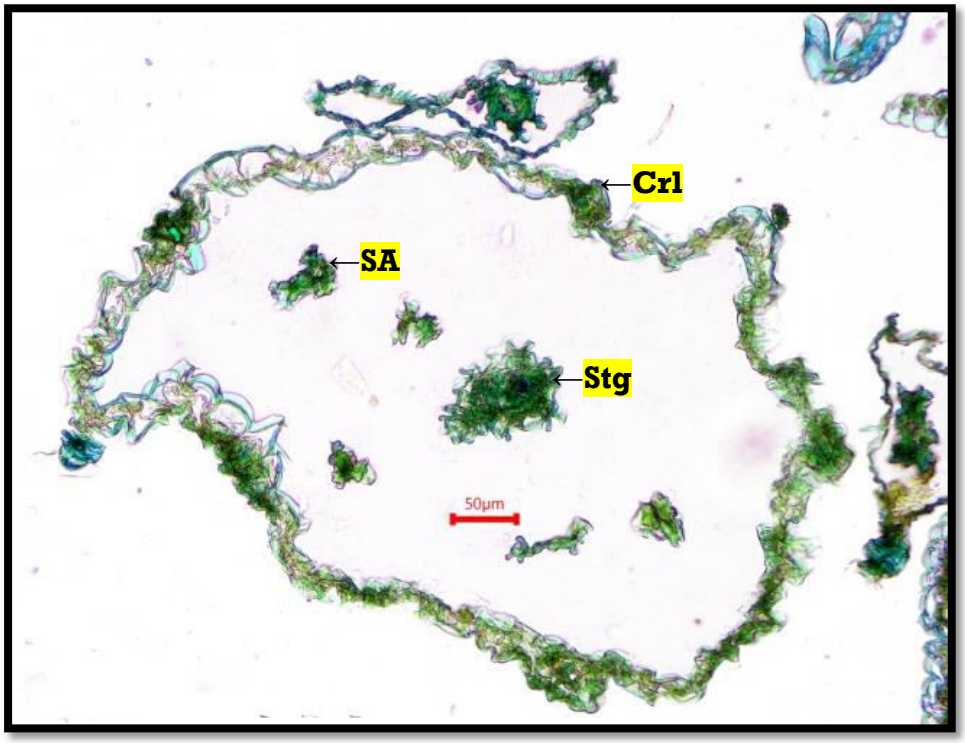


Figure 10: TS of bisexual floret with sterile anther



A - anther; Br - bract; Crl - corolla; PG - pollen grains; SA - sterile anther; SF - sterile flower; Stg - stigma; T - trichome; VB - vascular bundle; Xy - xylem

Powder microscopy:

The powder was brown coloured with aromatic odour and characteristic taste. It also shows the characters like simple unicellular trichomes and biseriate covering trichomes from bract, glandular trichomes from bract and corolla, foliar epidermis in surface view, thick-walled parenchyma, vessels with spiral thickening, and pollen grains.

Figure 11: Unicellular covering trichomes and biseriate trichomes

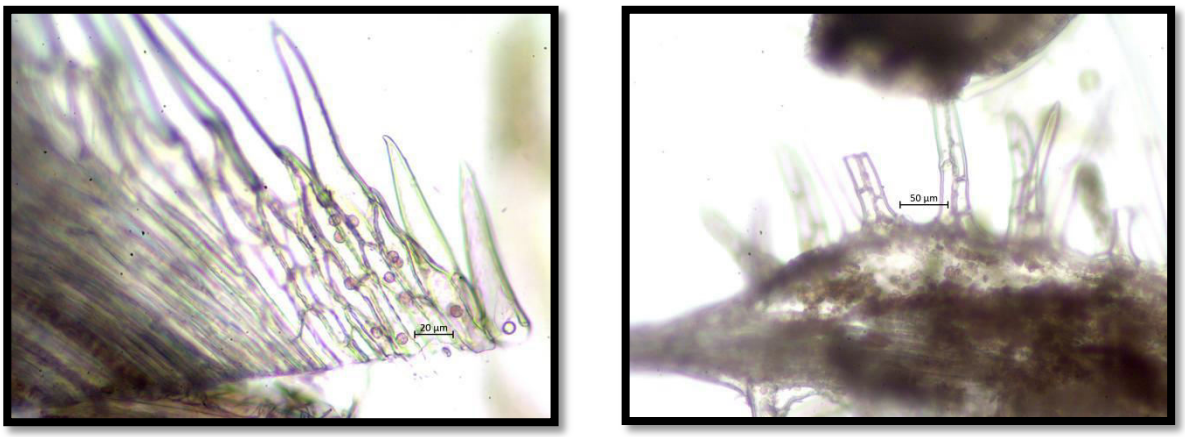


Figure 12: Glandular trichomes

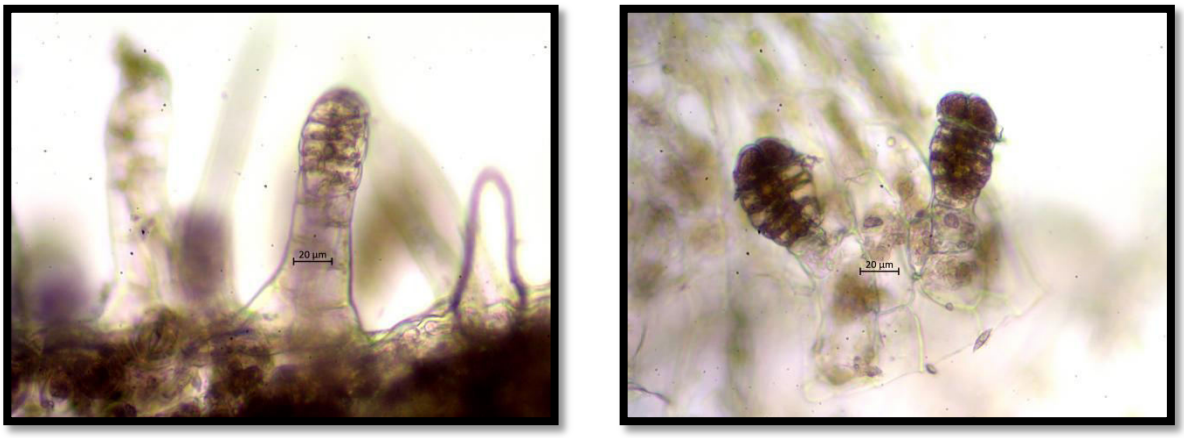


Figure 13: Floral epidermis

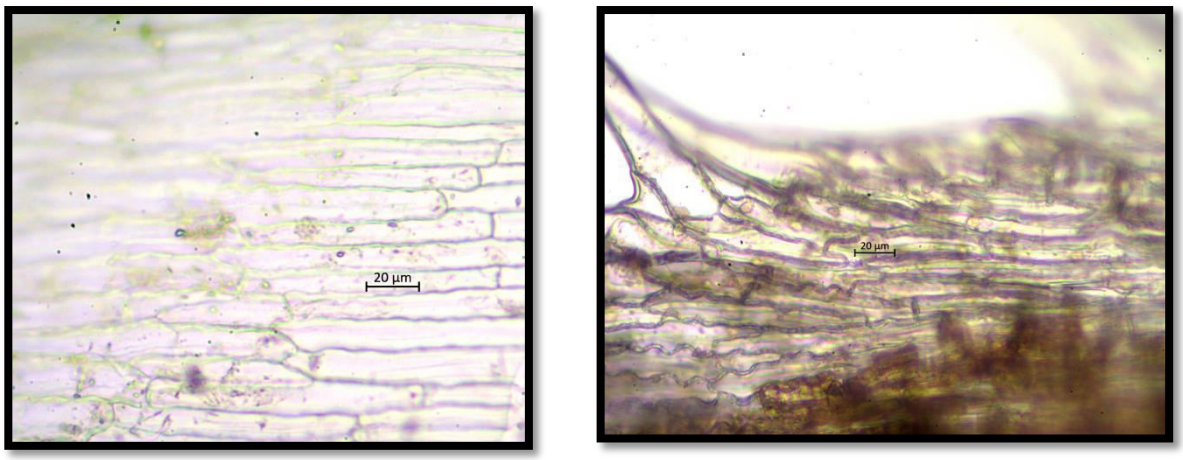


Figure 14: Thick walled parenchyma and spiral vessel

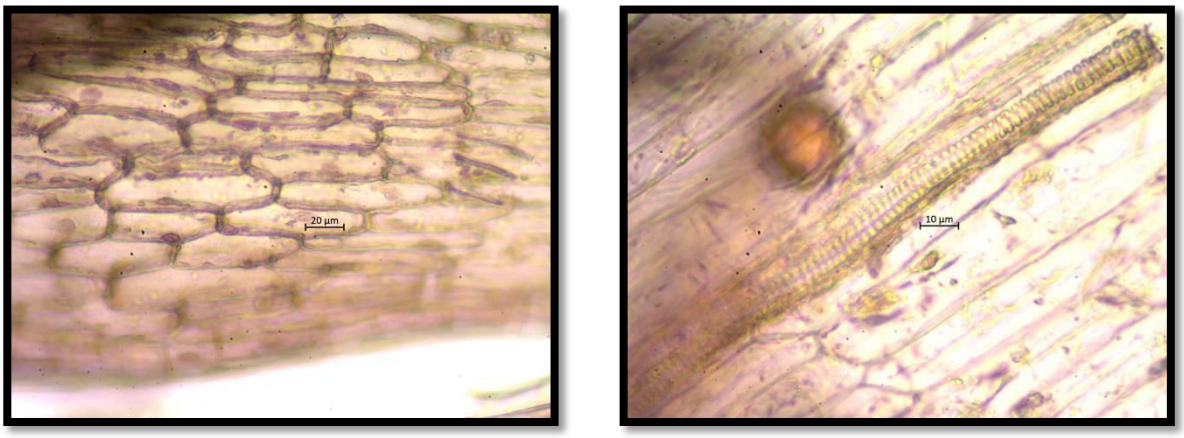
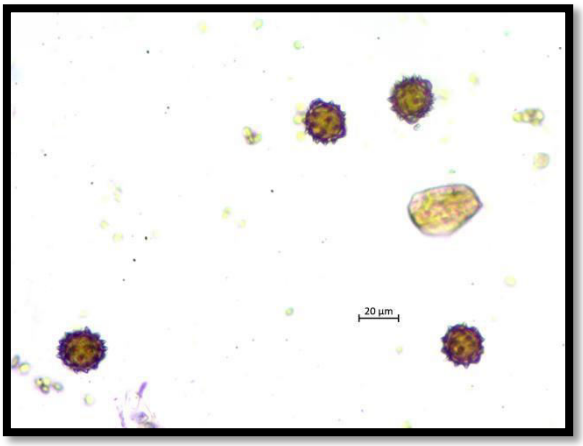


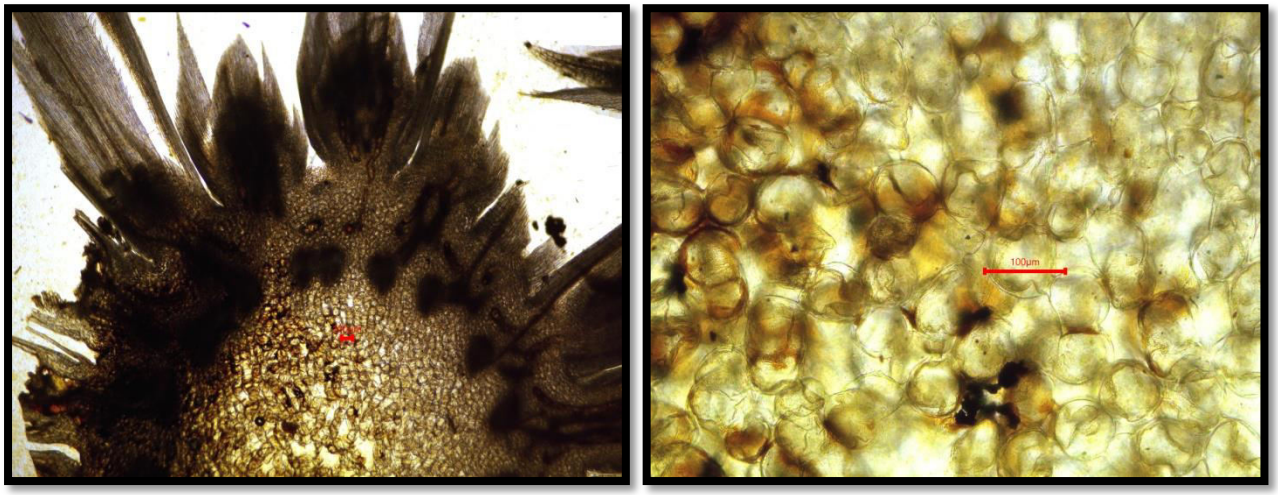
Figure 15 : Pollen grains



Histochemical Studies:

Phenolic compounds found in inflorescence; alkaloids and mucilage observed in receptacle; lignin detected in xylem and bracts; starch grains, oil globules, cutin and resin absent in inflorescence.

Figure 16: Phenolic compound in inflorescence



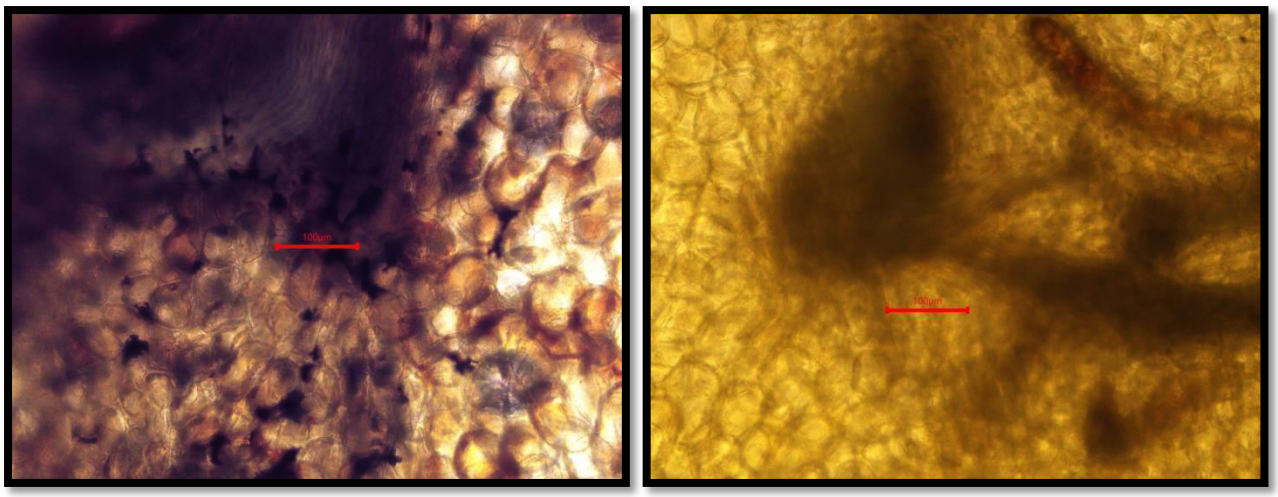


Figure 17: Mucilage in receptacle

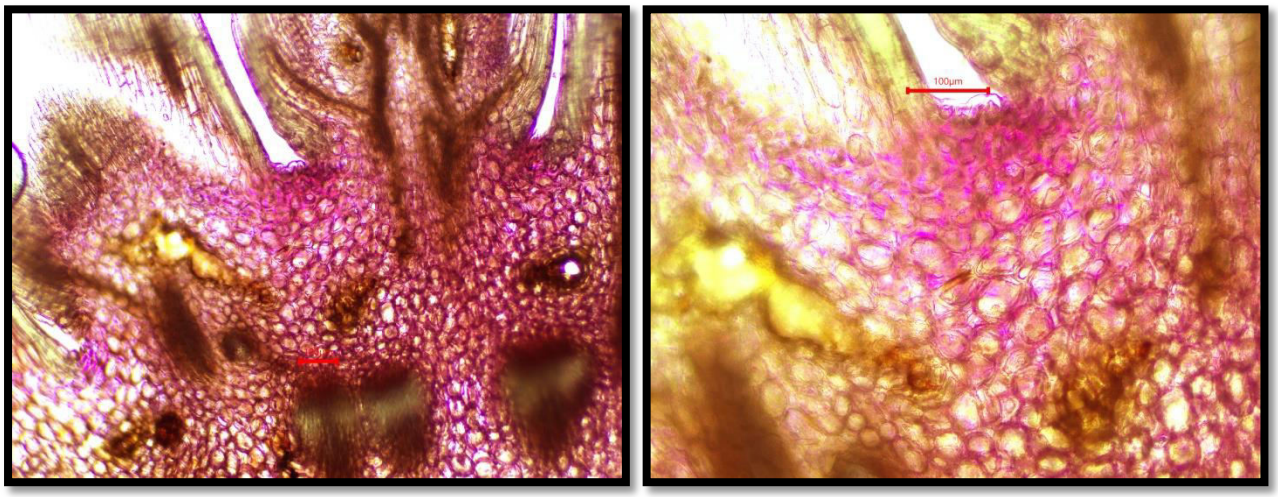


Figure 18: Alkaloid in receptacle

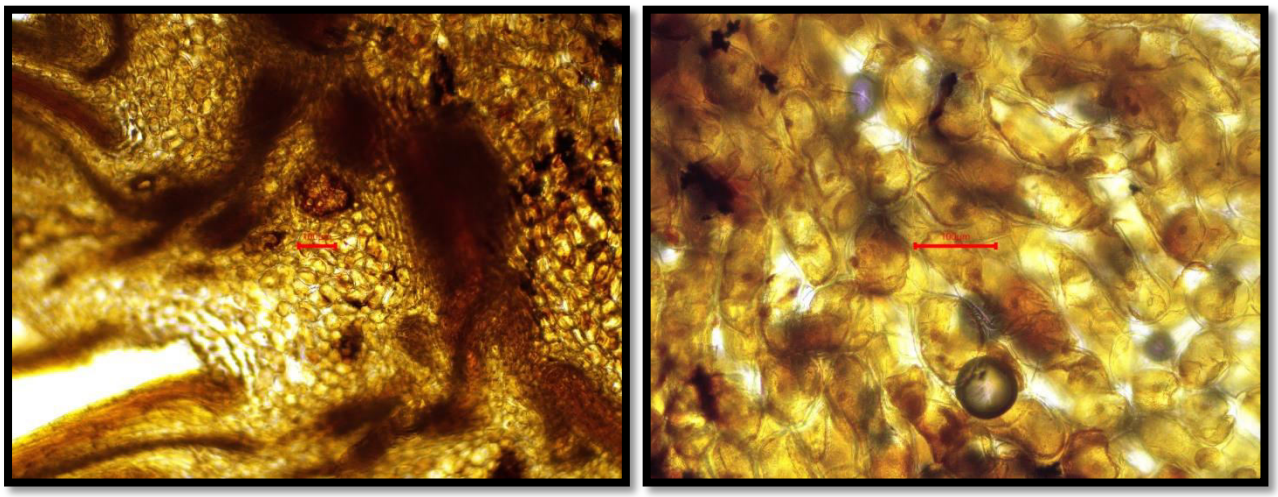


Figure 19: Lignin in xylem

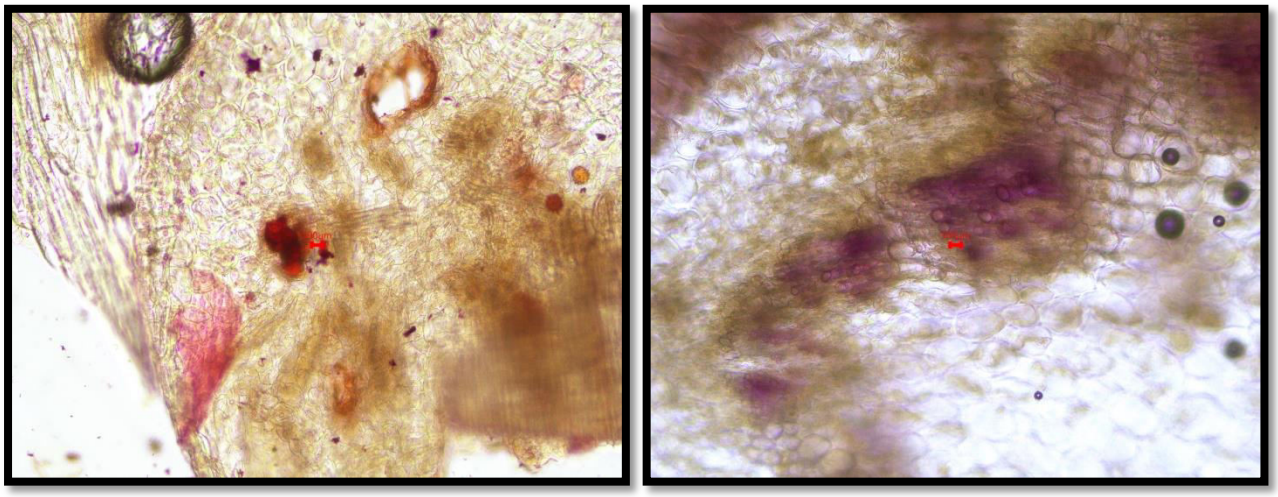
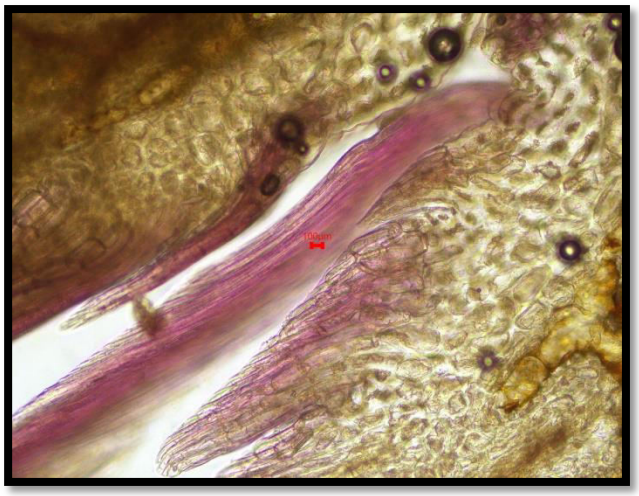


Figure 20: Lignin in bract



Physicochemical Studies

The flowers of Sphaeranthus indicus Linn., powder was studied and reported for the physicochemical properties as per Ayurvedic Pharmacopoeia of India and other reference books.

Table 1: Physicochemical parameters of flowers of Sphaeranthus indicus Linn.,

S.NO.	PARAMETERS	RESULTS (%W/W)
I	Ash Values	
1.	Total ash	6.4% ± 0.24
2.	Acid Soluble ash	2.4% ± 0.31
3.	Sulphated ash	7.1% ± 0.27
4.	Water insoluble ash	5.6% ± 0.18
II	Extractive Values	
1.	Water soluble extractive	8% ± 0.16
2.	Alcohol soluble extractive	11.2% ± 0.35
III	Foaming index	<100
IV	Loss on drying	1.1% ± 0.12
V	Swelling index	NIL
VI	Crude fiber content	24.55 ± 0.04

Note: Each value in the table represents mean ± standard deviation (n = 5)

Qualitative analysis of heavy metals and inorganic elements:

Qualitative Analysis of inorganic elements and heavy metals was analyzed in powder sample of Sphaeranthus indicus. The reports of qualitative analysis are given in the table.

Table 2 : Qualitative analysis of heavy metals and inorganic elements

Heavy metal and In-organic element Tested	Observation	Inference
Arsenic	Green precipitate formed which gives red precipitate of cupric oxide upon boiling.	Arsenic is absent
Borate	No burning flame tinged with green observed	Borate is absent
Copper	No blue precipitate or deep blue coloured solution formed	Copper is absent
Iron	No intense blue precipitate was formed	Iron is absent
Lead	No white precipitate was formed	Lead is absent
Mercury	No yellow precipitate was formed	Mercury is absent
Sulphates	No white precipitate was formed	Sulphate is absent
Silver	No cream coloured precipitate	Silver is absent

Quantitative Analysis of Heavy Metals:

Instrument used : Atomic Absorption Spectroscopy. The results are been interpreted in the following table.

Table 3 : Quantitative estimation of Heavy metals

HEAVY METALS	CONCENTRATION (ppm)	SPECIFICATION (ppm)
Arsenic	0.032	not more than 3.0 ppm
Lead	NIL	not more than 10 ppm
Cadmium	0.035	not more than 0.3 ppm
Mercury	NIL	not more than 1.0 ppm

Phytochemical Studies

Percentage yield of ethyl acetate extract of flowers of Sphaeranthus indicus Linn.,

Plant name : Flowers of Sphaeranthus indicus
Solvent : Ethyl acetate
Method of Extraction : Soxhlet extraction
Physical nature : Semi-solid
Colour : Yellowish brown
Yield : 4.76% w/w

Figure 21: Ethyl acetate extract of flowers of Sphaeranthus indicus

Percentage yield of ethanolic extract of flowers of Sphaeranthus indicus Linn.,

Plant name : Flowers of Sphaeranthus indicus
Solvent : Ethanol
Method of Extraction : Soxhlet extraction
Physical nature : Semi-solid
Colour : Brown
Yield : 6.46% w/w

Figure 22: Ethanolic extract of flowers of Sphaeranthus indicus

Qualitative estimation of Phytoconstituents

The qualitative identification of phytoconstituents from the extracts of flowers of Sphaeranthus indicus was performed and reported.

Table 4: Qualitative estimation of phytoconstituents

S.NO.	PHYTOCHEMICAL TESTS	POWDERED DRUG	ETHYL ACETATE EXTRACT	ETHANOLIC EXTRACT
1.	Carbohydrates	+	+	+
2.	Flavonoids	+	+	+
3.	Alkaloids	+	+	+

4.	Glycosides	+	+	+
5.	Tannins and Phenols	+	+	+
6.	Proteins	-	-	-
7.	Saponins	-	-	-
8.	Terpenoids	+	+	+
9.	Steroids	+	-	+
10.	Fats and fixed oils	-	-	-
11.	Resins	-	-	-

Note: The symbol + indicates presence, - indicates absence

The preliminary phytochemical investigation of powdered drug and both the extracts were been performed which indicated the presence of Tannins, Flavonoids, Phenols, Alkaloids, Carbohydrates, Glycosides, Terpenoids and Steroids.

Quantitative estimation of Phytoconstituents

The phytocompounds such as phenolic, flavonoid and alkaloid compounds were quantitatively evaluated and tabulated.

Table 5 : Quantitative estimation of phytoconstituents

Extract	Parameters	Values (mg/g)
Ethyl acetate extract	Total phenolic content	11.03 ± 0.19
	Total flavonoid content	13.93 ± 0.25
	Total alkaloid content	5.97 ± 0.41
Ethanolic extract	Total phenolic content	13.28 ± 0.16
	Total flavonoid content	19.37 ± 0.27
	Total alkaloid content	8.23 ± 0.34

Note: Each value expressed in the table represents mean ± standard deviation (n = 5)

Fluorescence Analysis

Table 6: Fluorescence analysis of powder

S.NO.	Treatment	Short Uv (254 nm)	Long Uv (366 nm)	Visible Light
1.	Powder	Brown	Dark Blue	Light Brown
2.	Powder + Iodine water	Blue	Dark Blue	Brown
3.	Powder + 50% KOH	Green	Brown	Light Brown
4.	Powder + 1N NaOH in Methanol	Dark Green	Light Blue	Light Brown
5.	Powder + Acetic acid	Dark Green	Brown	Light Green
6.	Powder + 50% HNO_3	Green	Blue	Light Green
7.	Powder + Conc. H_2SO_4	Dark Green	Brown	Green
8.	Powder + 1N HCl	Dark Green	Blue	Light Green
9.	Powder + 1N NaOH	Light Green	Dark Blue	Green
10.	Powder + 50% H_2SO_4	Light Green	Light Blue	Light Green

Table 7: Fluorescence characteristics of extract

S.NO.	Extract	Short Uv (254 nm)	Long Uv (366 nm)	Visible Light
1.	Ethyl acetate extract	Light Green	Brown	Yellowish Brown
2.	Ethanolic extract	Light Brown	Blue	Brown

The results of fluorescence analysis of Sphaeranthus indicus was essential for the first
line standardization of crude drug. Fluorescence was not observed.

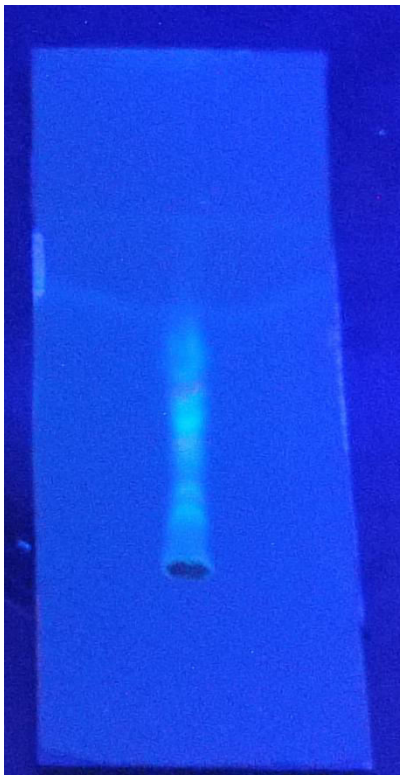
Thin Layer Chromatography

The TLC studies of flower extract of Sphaeranthus indicus was performed and tabulated.

Table 8: TLC of ethyl acetate extract and ethanolic extract of flowers of Sphaeranthus indicus

S.NO.	Extract	Solvent System	No.of Spots	R_f Value
1.	Ethyl acetate extract	Toluene : Ethyl acetate (9:1)	3	0.24
				0.39
				0.75
2.	Ethanolic extract	Toluene : Ethyl acetate (9:1)	2	0.36
				0.54

Figure 23: TLC of ethanolic extract and ethyl acetate extract of flowers of Sphaeranthus indicus

TLC of ethyl acetate extract of Sphaeranthus indicus	TLC of ethanolic extract of Sphaeranthus indicus
	

HPTLC Fingerprint Profile

HPTLC finger printing were been carried out with the ethanolic extract of Sphaeranthus indicus Linn.,

The HPTLC profiling was done with different concentrations of Sphaeranthus indicus extract at 366 nm and 254 nm. The HPTLC finger print profile for Sphaeranthus indicus extract at 366 nm and 254 nm was shown in the figures. The values given in the table indicates the presence of numerous phytoconstituents in the extract and was observed at 366 nm.

Solvent system for Alkaloids = Ethyl acetate : Methanol : Water [10: 1.35: 1]

Figure 24 : HPTLC fingerprint profile for Alkaloids

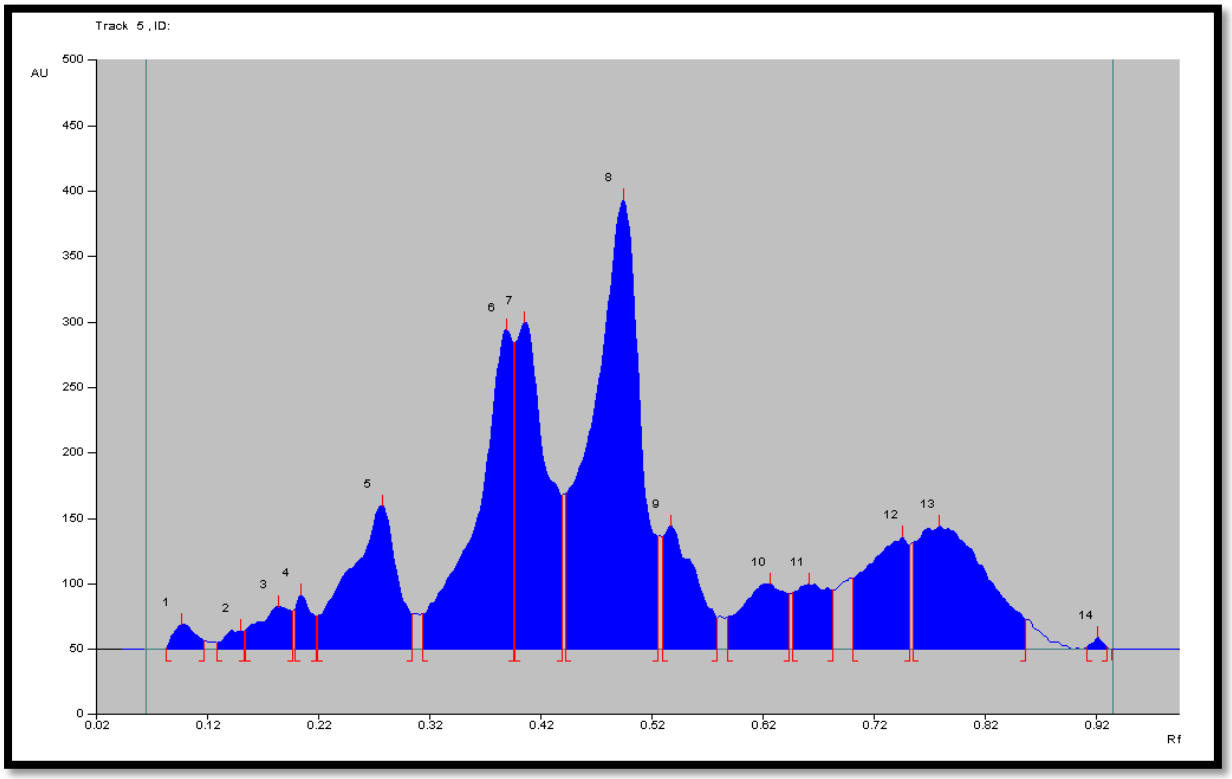


Table 9: HPTLC fingerprint data for Alkaloids

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.09 Rf	0.5 AU	0.10 Rf	18.8 AU	1.31 %	0.12 Rf	6.3 AU	353.4 AU	0.72 %	unknown *
2	0.13 Rf	4.8 AU	0.15 Rf	14.5 AU	1.01 %	0.16 Rf	3.2 AU	232.4 AU	0.48 %	unknown *
3	0.16 Rf	14.0 AU	0.19 Rf	32.9 AU	2.29 %	0.20 Rf	9.0 AU	906.4 AU	1.86 %	unknown *
4	0.20 Rf	30.2 AU	0.21 Rf	41.3 AU	2.87 %	0.22 Rf	5.6 AU	562.0 AU	1.15 %	unknown *
5	0.22 Rf	25.7 AU	0.28 Rf	109.8 AU	7.64 %	0.31 Rf	6.8 AU	4352.7 AU	8.93 %	unknown *
6	0.32 Rf	27.4 AU	0.40 Rf	243.8 AU	16.97 %	0.40 Rf	4.1 AU	7472.2 AU	15.32 %	unknown *
7	0.40 Rf	234.2 AU	0.41 Rf	249.2 AU	17.35 %	0.45 Rf	7.5 AU	6539.1 AU	13.41 %	unknown *
8	0.45 Rf	119.1 AU	0.50 Rf	343.5 AU	23.91 %	0.53 Rf	6.3 AU	3752.0 AU	28.20 %	unknown *
9	0.54 Rf	86.3 AU	0.54 Rf	94.3 AU	6.56 %	0.58 Rf	3.9 AU	2555.6 AU	5.24 %	unknown *
10	0.59 Rf	24.6 AU	0.63 Rf	49.6 AU	3.45 %	0.65 Rf	2.0 AU	1844.4 AU	3.78 %	unknown *
11	0.65 Rf	43.0 AU	0.67 Rf	49.9 AU	3.48 %	0.69 Rf	4.8 AU	1405.3 AU	2.88 %	unknown *
12	0.71 Rf	54.3 AU	0.75 Rf	85.9 AU	5.98 %	0.76 Rf	9.6 AU	3073.1 AU	6.30 %	unknown *
13	0.76 Rf	81.5 AU	0.78 Rf	94.1 AU	6.55 %	0.86 Rf	2.8 AU	5637.9 AU	11.56 %	unknown *
14	0.92 Rf	2.0 AU	0.93 Rf	8.7 AU	0.61 %	0.94 Rf	1.9 AU	79.0 AU	0.16 %	unknown *

Solvent system for Glycosides = Ethyl acetate : Ethanol: Water [8: 2: 1.2]

Figure 25: HPTLC fingerprint profile for Glycosides

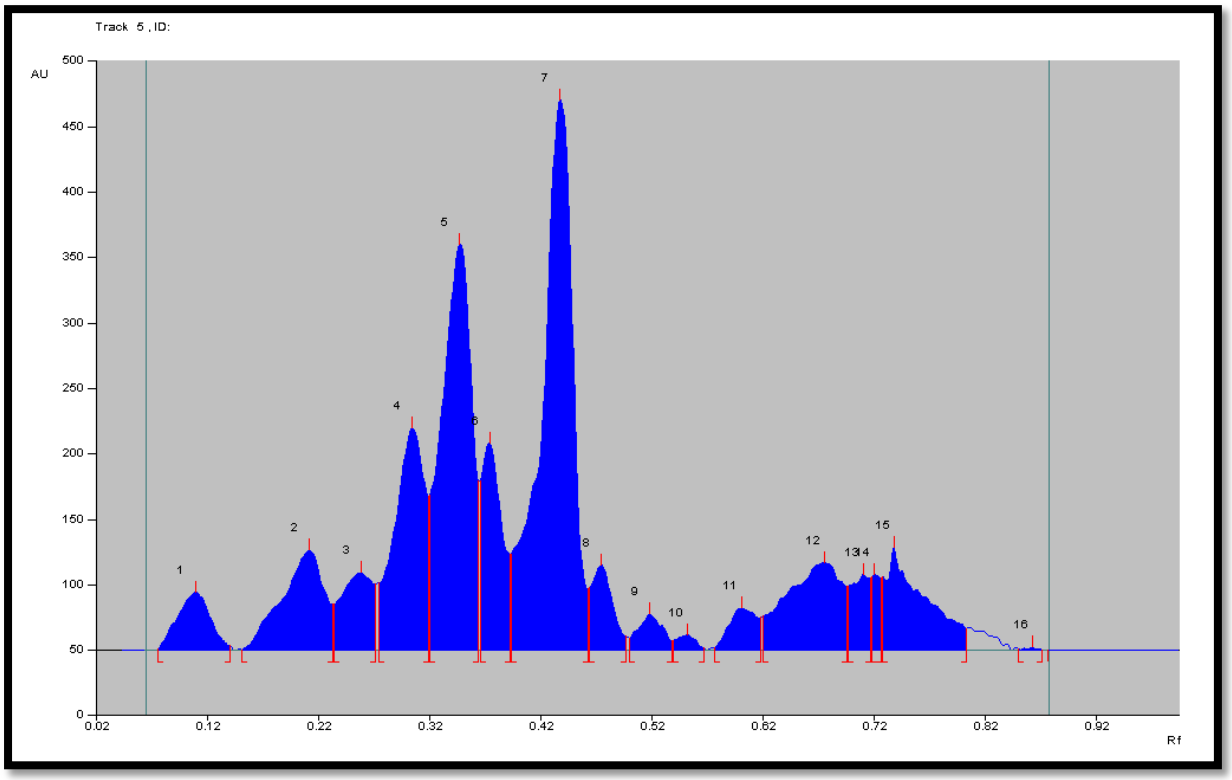


Table 10: HPTLC fingerprint data for Glycosides

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.08 Rf	0.6 AU	0.11 Rf	44.6 AU	2.72 %	0.15 Rf	3.2 AU	1244.4 AU	2.99 %	unknown *
2	0.16 Rf	0.3 AU	0.22 Rf	76.5 AU	4.67 %	0.24 Rf	5.1 AU	2699.7 AU	6.48 %	unknown *
3	0.24 Rf	35.2 AU	0.26 Rf	59.3 AU	3.62 %	0.28 Rf	0.5 AU	1605.6 AU	3.85 %	unknown *
4	0.28 Rf	51.4 AU	0.31 Rf	170.2 AU	10.38 %	0.32 Rf	7.8 AU	4406.3 AU	10.57 %	unknown *
5	0.33 Rf	118.7 AU	0.35 Rf	310.3 AU	18.93 %	0.37 Rf	9.2 AU	7937.0 AU	19.04 %	unknown *
6	0.37 Rf	129.4 AU	0.38 Rf	157.8 AU	9.63 %	0.40 Rf	3.5 AU	2792.9 AU	6.70 %	unknown *
7	0.40 Rf	73.7 AU	0.44 Rf	420.7 AU	25.67 %	0.47 Rf	6.6 AU	1210.3 AU	26.89 %	unknown *
8	0.47 Rf	47.5 AU	0.48 Rf	64.9 AU	3.96 %	0.50 Rf	0.7 AU	1157.0 AU	2.78 %	unknown *
9	0.50 Rf	9.3 AU	0.52 Rf	27.6 AU	1.68 %	0.54 Rf	7.0 AU	580.5 AU	1.39 %	unknown *
10	0.55 Rf	7.4 AU	0.56 Rf	11.8 AU	0.72 %	0.57 Rf	1.5 AU	188.8 AU	0.45 %	unknown *
11	0.58 Rf	1.6 AU	0.61 Rf	32.1 AU	1.96 %	0.62 Rf	4.9 AU	764.1 AU	1.83 %	unknown *
12	0.63 Rf	26.3 AU	0.68 Rf	66.9 AU	4.08 %	0.70 Rf	8.6 AU	3125.4 AU	7.50 %	unknown *
13	0.70 Rf	48.8 AU	0.72 Rf	58.1 AU	3.54 %	0.72 Rf	5.6 AU	906.1 AU	2.17 %	unknown *
14	0.72 Rf	56.1 AU	0.73 Rf	57.5 AU	3.51 %	0.73 Rf	5.2 AU	451.4 AU	1.08 %	unknown *
15	0.73 Rf	55.7 AU	0.74 Rf	78.3 AU	4.78 %	0.81 Rf	6.4 AU	2596.0 AU	6.23 %	unknown *
16	0.85 Rf	1.0 AU	0.87 Rf	2.4 AU	0.14 %	0.88 Rf	0.0 AU	20.2 AU	0.05 %	unknown *

Solvent system for Flavonoids = Toluene : Acetone: Formic acid [4.5: 4.5: 1]

Figure 26: HPTLC fingerprint profile for Flavonoids

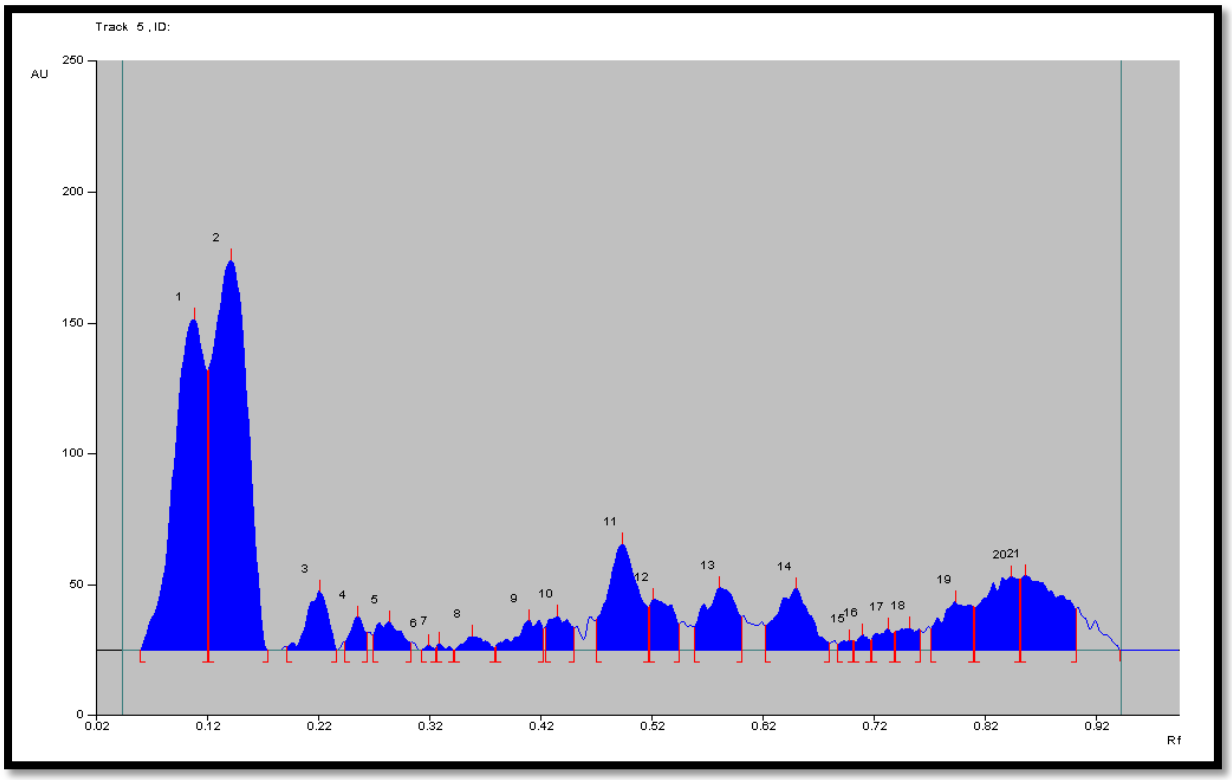


Table 11 : HPTLC fingerprint data for Flavonoids

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.06 Rf	0.0 AU	0.11 Rf	126.4 AU	22.38 %	0.13 Rf	6.5 AU	398.4 AU	23.73 %	unknown *
2	0.13 Rf	107.2 AU	0.15 Rf	149.0 AU	26.39 %	0.18 Rf	0.0 AU	259.1 AU	29.74 %	unknown *
3	0.20 Rf	1.5 AU	0.23 Rf	22.6 AU	4.00 %	0.24 Rf	0.2 AU	389.5 AU	2.72 %	unknown *
4	0.25 Rf	3.2 AU	0.26 Rf	12.8 AU	2.27 %	0.27 Rf	6.6 AU	150.2 AU	1.05 %	unknown *
5	0.27 Rf	5.8 AU	0.29 Rf	11.0 AU	1.94 %	0.31 Rf	2.9 AU	218.7 AU	1.53 %	unknown *
6	0.32 Rf	0.0 AU	0.32 Rf	1.9 AU	0.33 %	0.33 Rf	0.4 AU	10.7 AU	0.07 %	unknown *
7	0.33 Rf	1.1 AU	0.33 Rf	2.7 AU	0.47 %	0.35 Rf	0.2 AU	16.3 AU	0.11 %	unknown *
8	0.35 Rf	0.0 AU	0.36 Rf	5.3 AU	0.93 %	0.38 Rf	1.1 AU	92.3 AU	0.64 %	unknown *
9	0.39 Rf	1.5 AU	0.41 Rf	11.5 AU	2.04 %	0.43 Rf	8.2 AU	238.7 AU	1.67 %	unknown *
10	0.43 Rf	8.4 AU	0.44 Rf	13.0 AU	2.30 %	0.45 Rf	8.6 AU	242.8 AU	1.70 %	unknown *
11	0.48 Rf	12.0 AU	0.50 Rf	40.5 AU	7.16 %	0.52 Rf	6.3 AU	021.3 AU	7.13 %	unknown *
12	0.52 Rf	16.8 AU	0.53 Rf	19.4 AU	3.43 %	0.55 Rf	9.9 AU	382.7 AU	2.67 %	unknown *
13	0.56 Rf	8.7 AU	0.59 Rf	23.9 AU	4.23 %	0.61 Rf	2.9 AU	642.9 AU	4.49 %	unknown *
14	0.63 Rf	9.3 AU	0.65 Rf	23.6 AU	4.19 %	0.69 Rf	2.8 AU	656.5 AU	4.58 %	unknown *
15	0.69 Rf	2.0 AU	0.70 Rf	3.7 AU	0.65 %	0.71 Rf	3.5 AU	37.1 AU	0.26 %	unknown *
16	0.71 Rf	3.5 AU	0.71 Rf	5.8 AU	1.02 %	0.72 Rf	3.6 AU	57.4 AU	0.40 %	unknown *
17	0.72 Rf	4.0 AU	0.74 Rf	8.1 AU	1.44 %	0.74 Rf	6.9 AU	113.9 AU	0.80 %	unknown *
18	0.75 Rf	7.2 AU	0.76 Rf	8.5 AU	1.51 %	0.77 Rf	7.7 AU	147.4 AU	1.03 %	unknown *
19	0.78 Rf	8.2 AU	0.80 Rf	18.5 AU	3.27 %	0.81 Rf	6.5 AU	474.6 AU	3.31 %	unknown *
20	0.82 Rf	16.5 AU	0.85 Rf	28.1 AU	4.98 %	0.86 Rf	7.0 AU	800.1 AU	5.59 %	unknown *
21	0.86 Rf	27.0 AU	0.86 Rf	28.6 AU	5.06 %	0.91 Rf	5.7 AU	969.8 AU	6.77 %	unknown *

IN-VITRO STUDIES

In-vitro Angiotensin Converting Enzyme Inhibition Activity:

The results of in-vitro studies of flower extract are as follows:

Figure 27: Different concentrations of Standard (Captopril)

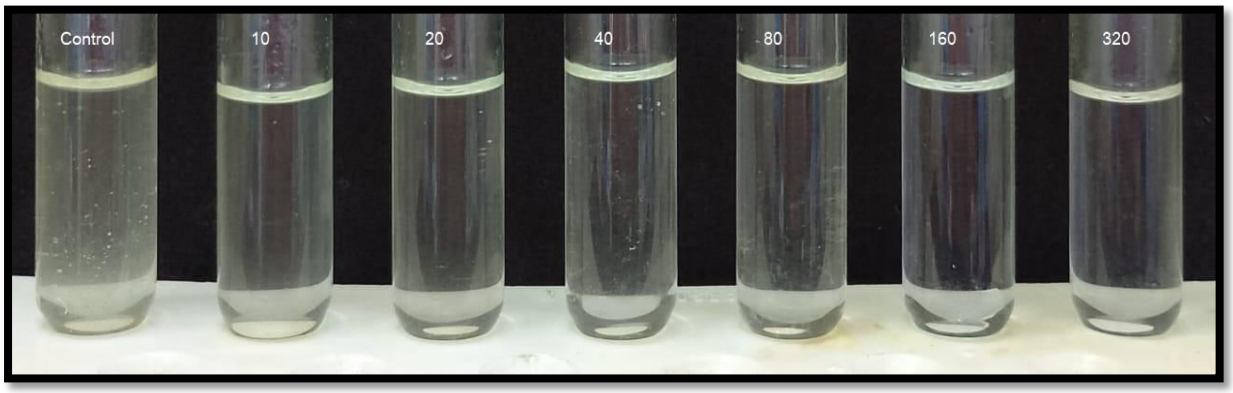


Figure 28: Different concentrations of Ethanolic extract of Sphaeranthus indicus

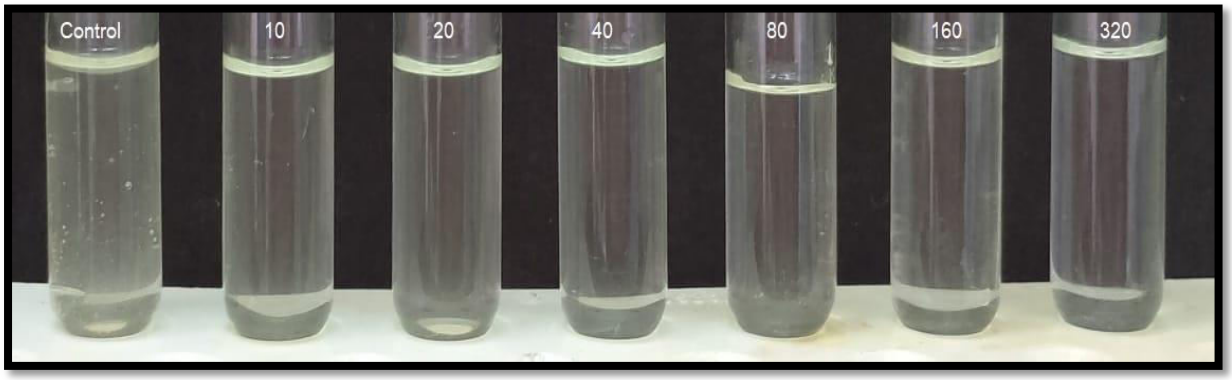


Figure 29: Different concentrations of ethyl acetate extract of Sphaeranthus indicus

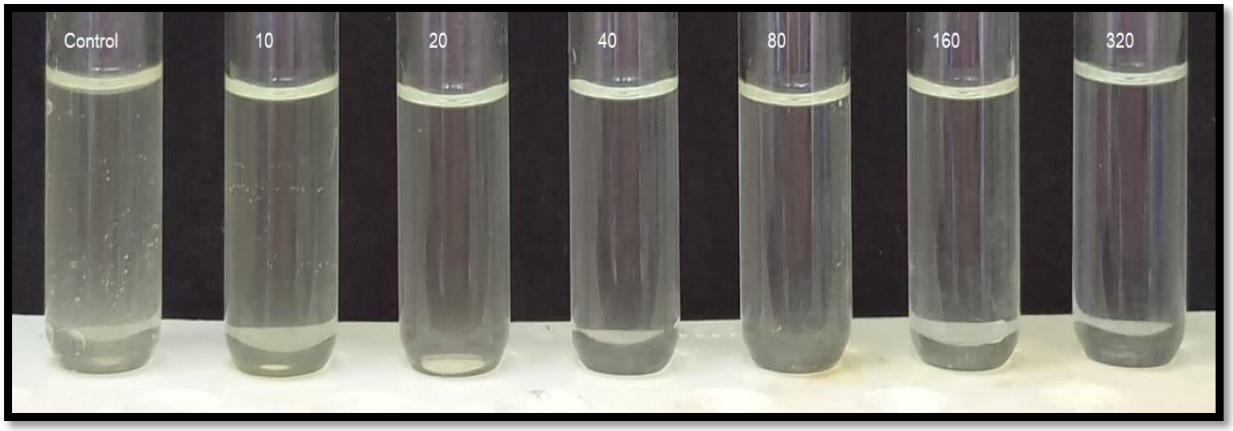
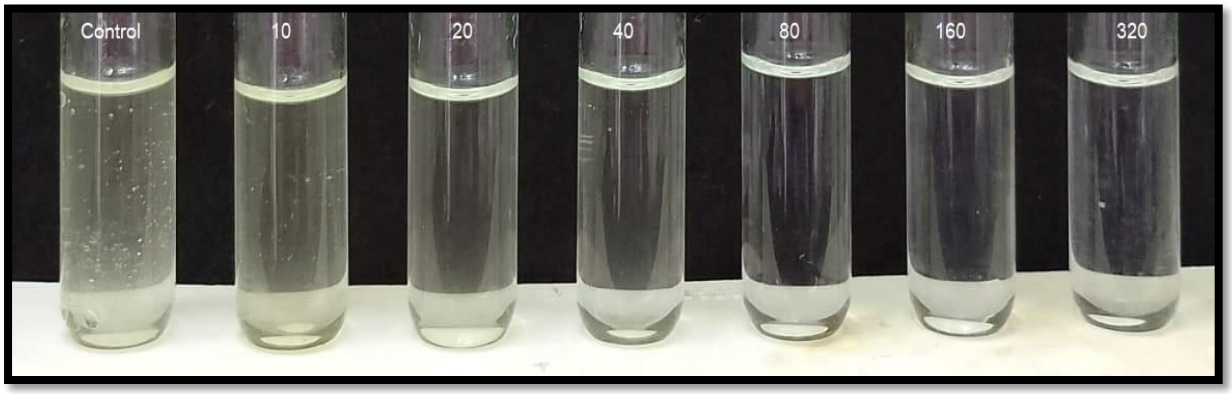


Figure 30: Different concentrations of water soluble extract of Sphaeranthus indicus



Control	0.72	0.78	0.73	0.74

Table 12: OD value of Standard (Captopril)

			OD value	
Samples	Conc.(µg)	Singlet	Duplicate	Triplicate
	10	0.41	0.42	0.42
	20	0.30	0.32	0.32
Captopril	40	0.24	0.22	0.21
	80	0.11	0.12	0.12
	160	0.07	0.09	0.09
	320	0.02	0.03	0.03

Table 13: % of Inhibition of Standard (Captopril)

		% of Inhibition			
Singlet	Duplicate	Triplicate	Mean	SD	IC50
44.94	42.93	43.60	43.83	1.02	
59.10	56.43	56.70	57.41	1.47	
67.79	70.20	70.86	69.62	1.61	12.24
84.89	82.89	83.69	83.83	1.00	
90.11	87.83	87.30	88.41	1.49	
96.25	95.18	95.85	95.76	0.54	

Table 14: OD value of Ethyl Acetate extract of Sphaeranthus indicus

Samples	Conc.(µg)	Singlet	Duplicate	Triplicate
	10	0.66	0.67	0.65
	20	0.54	0.55	0.54
EA	40	0.48	0.47	0.46
	80	0.35	0.32	0.38
	160	0.29	0.29	0.30
	320	0.21	0.22	0.20

Table 15: % of Inhibition of ethyl acetate extract of Sphaeranthus indicus

Singlet	Duplicate	Triplicate	Mean	SD	IC50
11.40	9.39	11.93	10.91	1.33	
27.03	25.43	27.57	26.68	1.11	
35.59	35.99	37.86	36.48	1.21	85.11
52.29	56.16	48.68	52.38	3.74	
60.31	60.04	59.24	59.86	0.55	
71.80	70.20	72.33	71.44	1.11	

Table 16: OD value of ethanolic extract of Sphaeranthus indicus

Samples	Conc.(µg)	Singlet	Duplicate	Triplicate
ETH	10	0.55	0.54	0.55
	20	0.44	0.45	0.45
	40	0.32	0.32	0.33
	80	0.26	0.27	0.26
	160	0.16	0.15	0.16
	320	0.12	0.11	0.11

Table 17: % of Inhibition of ethanolic extract of Sphaeranthus indicus

Singlet	Duplicate	Triplicate	Mean	SD	IC50
25.56	26.90	26.36	26.28	0.67	
40.26	39.46	39.73	39.82	0.40	
56.30	57.10	55.63	56.34	0.73	35.02
64.58	62.85	64.05	63.83	0.88	
78.21	78.75	78.48	78.48	0.26	
83.83	84.23	84.76	84.27	0.46	

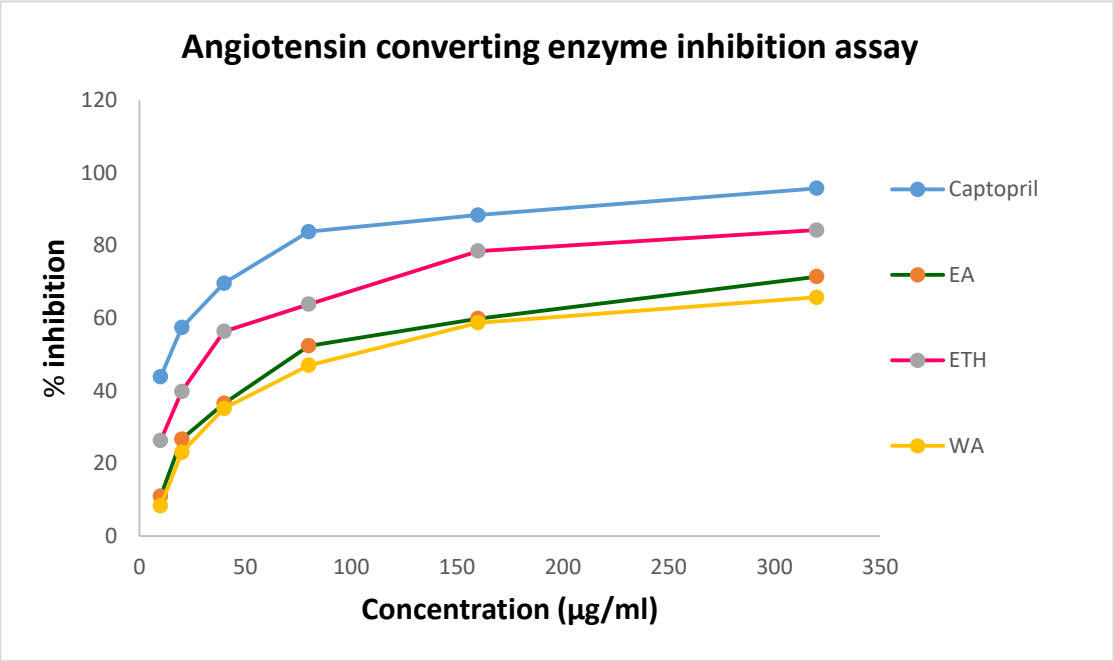
Table 18: OD value of water soluble extract of Sphaeranthus indicus

Sample	Conc.(µg)	Singlet	Duplicate	Triplicate
WA	10	0.68	0.69	0.67
	20	0.57	0.58	0.56
	40	0.49	0.48	0.47
	80	0.39	0.39	0.39
	160	0.30	0.31	0.31
	320	0.26	0.25	0.24

Table 19: % of Inhibition of water soluble extract of Sphaeranthus indicus

Singlet	Duplicate	Triplicate	Mean	SD	IC50
8.06	7.52	9.26	8.28	0.88	
23.16	21.95	23.96	23.02	1.00	
34.25	34.92	36.25	35.14	1.02	104.96
47.08	47.21	46.68	46.99	0.27	
59.64	58.44	58.04	58.70	0.83	
64.85	65.38	66.99	65.74	1.11	

Figure 31: % of Inhibition of Captopril, ethyl acetate extract, ethanolic extract and water soluble extract



The IC_{50} value of the given samples EA (Ethylacetate), ET (Ethanol), WA (water) was found to be 85.11 µg/ml, 35.02 µg/ml, 104.96µg/ml and the standard drug (Captopril) was 12.24 µg/ml, respectively.

Discussion

Phytomedicine has emerged as a vital component of world healthcare due to its better therapeutic effect and reduced side effects compared to conventional medicines. The combination of nanotechnology and herbal science has emerged as a solution to overcome the limitations of herbal drugs. Extensive research has demonstrated the positive effects of plant-based treatments for Hypertension, offering promising results. Hence the goal of the contemporary study is to scrutinize the potential of the selected plant Sphaeranthus indicus Linn., for treating Hypertension and also the study focused on formulating a nanoparticle loaded capsule loaded with extract obtained from the flowers of Sphaeranthus indicus and evaluating its impact on experimentally induced Hypertension.

Plant material authentication has a crucial role in the identification of plant species. The collected specimens of flowers of Sphaeranthus indicus was identified and authenticated by Dr. Sunil Kumar K. N., Research Officer and HOD, Pharmacognosy.

Pharmacognostical studies like microscopy, macroscopy and determination of physicochemical constants of the flowers of Sphaeranthus indicus were carried out to establish the data for proper authentication.

Macroscopical study showed that the flowers are buff coloured, aromatic odour with characteristic taste.

Transverse section of sprouts indicated the presence of ovary, the corolla, and the inflorescence.

Physicochemical parameters like extractive values, crude fibre content, foaming index and swelling index were carried out. These estimates will aid in verifying the authenticity and quality of the plant by confirming its identity and purity and also ensures its contrast from its adulterants and substitutes.

The first step involved in phytochemical analysis is extraction. The extraction of flowers of Sphaeranthus indicus was done by soxhlet extraction method using ethyl acetate as a solvent. The percentage yield of ethanolic and ethyl acetate extract of the flowers of Sphaeranthus indicus were been found to be 6.46% and 4.76% w/w. Qualitative preliminary phytochemical analysis and fluorescence analysis were carried out in phytochemical studies.

The investigation of preliminary phytochemical screening of the extracts specified the presence of diverse potential bioactive phytoconstituents such as flavonoids, alkaloids, steroids, carbohydrates and glycosides.

Fluorescence analysis of powder and extracts were accomplished to discover the presence of chromophore in the extract.

HPTLC is a standardization tool which develop quality control of a drug one step forward. The fingerprint of a drug would be the same under indistinguishable conditions which makes chromatographic method as a ideal method for authentication and identification of herbal drugs.

The peaks observed could be accredited to the Glycosides, Alkaloids and Flavonoids present as vital constituents in flowers of Sphaeranthus indicus.

In-vitro studies of different extracts of flowers of Sphaeranthus indicus unveiled that the ethanolic extract of flowers of Sphaeranthus indicus exhibited eminent Angiotensin inhibition activity when compared with the standard.

Conclusion

This study results could be utilized for standardization of Sphaeranthus indicus flowers as the study sets a distinct specific protocol. The physicochemical investigation exhibited the purity of the sample. The qualitative evaluation of phytoconstituents along with the HPTLC analysis performed in ethanolic extract of flowers of Sphaeranthus indicus indicated the presence of numerous therapeutically important phytocompounds.

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Acknowledgement

The authors are grateful to Madras Medical College, Faculty of Pharmacy for their valuable guidance and support and also thankful to Centre Council for Research in Siddha, Chennai, for providing the facilities to carry out this research work.

Conflicts of Interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.