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Pharmacognostic and Phytochemical Evaluation of Leaf of *Sphaeranthus indicus*

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ABSTRACT

Objective: To study detailed Pharmacognostic profile and preliminary phytochemical investigation and isolation of volatile oil, and TLC and GLC analysis of volatile oil of the leaves of *Sphaeranthus indicus* (Linn.) commonly known as Globe-thistle belonging to the family Asteraceae. The leaves of *Sphaeranthus indicus* (Linn.) are used traditionally in Ayurveda for hyperlipidemia, epilepsy, mental illness, jaundice, diabetes, leprosy, fever cough, gastropathy, hernia, helminthiasis, dyspepsia and skin diseases and AIDS. The reports showed that it is also used for hypertensive, anxiolytic, neuroleptic, immunomodulatory, antioxidant, anti-inflammatory, bronchodilator, anti-hyperglycaemic and liver disorder. It grows in rice fields, dry waste places and cultivated lands in tropical parts of India.**Methods:** Leaf of *Sphaeranthus indicus* (Linn.) was studied by Macroscopical, Microscopical, Quantitative Microscopy, Physicochemical, Phytochemical analysis of leaf powder and extracts, isolation of volatile oil from the leaves, TLC and GLC analysis of the oil of the leaves, other methods for standardization recommended by WHO.**Results**: Macroscopically leaves are simple, alternate, oblong, spatulate, spinous, surface pubescent, base decurrently forming the wings of the stem, acute, glandular, hairy and narrowed at the base up to 5.0x1.5 cm, leaf margins are coarsely serrate or dentate. Fresh leaves are dark green in colour and dried leaves are greenish black colour. No sclerenchyma cells are seen in the vascular bundle. The lamina is dorsiventral; the mesophyll tissue is not well differentiated into palisade and spongy tissues. Characteristic epidermal trichomes are abundant on the leaf. Some of the trichomes are covering-type and are multicellular, uniseriate, unbranched and whip-like others are biseriate, broad, unbranched, conical with vertically oblong cells and a few tiers of apical glandular cells. **Conclusion:** These results of the study can serve as a valuable source of information and provide suitable standards for identification of this plant material in future investigations.

INTRODUCTION:

Herbal medicine is the oldest form of health care known to mankind. Herbs had been used by all cultures throughout history. Some are made from plant extracts; others are synthesized to mimic a natural plant compound [1]. The world health organisation (WHO) estimates that about 4 billion people, 80% of the world population presently use herbal medicine for some aspect of primary health care [2]. In almost all the traditional medicine, the medicinal plants play a major role and constitute the backbone of the traditional medicine [3]. Indian Materia Medica includes about 2000 drugs of natural origin almost all of which are derived from traditional system and folklore practices. Medicinal plants are inextricably intertwined with the rich history, culture and culinary tradition of India. Our country has a rich and glorious Ethnomedical heritage [4]. Medicinal plants are also used by the codified systems of medicine such as Ayurveda, Siddha, Unani, Chinese and Tibetian systems of traditional medicine[5] with the advent in science, many of the crude drugs used in traditional system have been investigated scientifically. *Sphaeranthus indicus* Linn. is a medicinal plant widely used in Ayurvedic medicine for treating different diseases.[6] It grows well in waste lands, paddy fields, and it is also cultivated in tropical and subtropical area of India. It is usually found in throughout India, some parts of Sri Lanka, Africa and Australia from sea level to 1200 m altitude [7]. Pharmacognostic studies on leaves are not adequate necessitating the present investigation. Though chemical analysis of the volatile oil from capitulam of this plant was well documented with **GC-MS**[8] and since no detailed studies have been previously done on the leaves pertaining to the volatile oil content and chemical analysis of the same. The present study is aimed to isolate and to evaluate the volatile components from the leaves of this plant using **GLC** technique which is an ideal method for both the quantitative and qualitative analysis of the constituents of Essential oil. The novel isoflavone glycoside had been reported on leaves of methanolic extract of S. indicus.[9] So it is planned to prepare the methanolic extract and aqueous extract which is subjected to preliminary phytochemical screening and TLC studies to identify the presence of active principle.

MATERIALS AND METHODS:

Plant material

Sphaeranthus indicus leaves were collected, from in and around of Palakkad district, Kerala, India and authenticated by taxonomist and the authenticated specimen was deposited in the

Department of Pharmacognosy, Sanjo college of pharmaceutical studies, Palakkad. Authentication specimen number is SCPS/P.COG/002/2017 the fresh leaves were kept for shade drying. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Pharmacognostic studies:

Sphaeranthus indicus (Linn) is an aromatic, annual herb.

Family: Asteraceae

Systematic position[10]

Phylum : Spermatophyta

Division : Angiosperms

Class : Dicotyledons

Sub class : Sympetalae

Order : Campanulales

Family : Asteraceae

Genus : *Sphaeranthus*

Species : *indicus*

Synonym : *Sphaeranthus hirtus*

Common Names:

Baura Talam, Bodasoram, Bodataram, Chagulnadi, Ghorkmundi, Globe-thistle, Gorkhumundi, Guroli, Kamazariyus, Kamdaryus, Mundi, Mundiriki, Murmuriya, Shosimundi, Thistle, Globe.

Vernacular Names [11-13]

Tamil : Kottakaranthai

Sans	:	Mahamundi, Mundi, hapusa
Hindi	:	Mundi, Gorakh Mundi
Bengali	:	Mundi, Gorakh Mundi
Gujarati	:	Mundi, Gorakh Mundi
Telugu	:	Boddasoramu
Malayalam	:	Adakkamaniyam
Punjab	:	Khamadrus

Macroscopy of the leaf:

Morphological studies were done by using simple microscope to determine the shape, size, taste and odour of the leaf and sheathing leaf base. Macroscopically the leaves are simple, alternate, oblong, spatulate, spinous, surface pubescent, base decurrent forming the wings of the stem, acute, glandular, hairy and narrowed at the base up to 5.0x1.5 cm, the leaf margins are coarsely serrate or dentate. Fresh leaves are dark green in colour and dried leaves are greenish black colour. The leaves are bitter in taste with pleasant odour when fresh, the aroma gradually diminishing on drying and storing.

Microscopical study of the leaf

Materials and Methods [14]

Fresh leaf was used for microscopical examination. The cut portion of the leaf was first fixed using FAA (Formalin 5ml +Acetic acid 5ml+Ethanol 90ml.). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol then infiltration by paraffin wax (58-60^{o}c). A specimen was cast in to paraffin blocks. The paraffin embedded specimens were sectioned with the help of microtome. The sections were stained with Toluidine blue.

Quantitative microscopy and Physicochemical parameters:

The vein islet number, vein terminal number, stomatal number, stomatal index were determined on fresh leaves using standard procedure[15-17]. The physicochemical parameters

were done to evaluate the proceedings of, total ash, water soluble ash, and acid insoluble ash were calculated as per Indian Pharmacopoeia[18]. Extracts of the powdered leaf was prepared with different solvents for the study of extractive value. Fluorescence analysis was also carried out for the powder and for extract as per standard procedure.[19]

Powder analysis:

Preliminary phytochemical analysis of the powder of the leaf of *S. indicus* with different chemical reagents was carried out microscopically. [20-21]

Extraction of Plant material

For preliminary Phytochemical analysis, extract was prepared by weighing 1kg of the dried leaves powder were subjected to hot successive continuous extraction with different solvents as per the polarity, petroleum ether, benzene, chloroform, acetone, ethanol, methanol and finally with aqueous. The extracts were filtered in each step using Whatman filters paper. The filtrate was concentrated using a rotary evaporator at low temperature (40-50º) and pressure. The presence or absence of the primary and secondary phytoconstituents was detected by usual prescribed methods. [22]

Thin layer chromatography of Aqueous and Methanol Extract

Methanolic extract had been reported for the presence of a novel isoflavone glycoside. 5, 4–dimethoxy-3-prenylbiochanin –7-O-β-D-glactoside and the preliminary phytochemical screening of aqueous and methanolic extracts were revealed the presence of isoflavone glycoside. Since an attempt has been made to confirm the presence of this compound in both the extracts by (Viz aqueous and methanol) thin layer chromatography using chloroform: methanol (11:9) as mobile phase and UV light and Ammonia vapour were used as visualizing agents.

Isolation of volatile oil from the leaves of *Sphaeranthus indicus* [23]

The leaf powder was extracted with petroleum ether (40^o – 60^o). The solvent was distilled off. The extracted residue was subjected to hydrodistillation method in a volatile oil estimation apparatus and distillate collected over solvent ether. An aqueous part was rejected and the ethereal part was dried over anhydrous sodium sulphate. A solvent was dried in a weighed conical flask on a water bath at controlled temperature and kept in desiccator

overnight and weighed. Volatile oil yield comes to be 0.01 – 0.02% on fresh weight basis. Isolated oil was physically and chemically analysed. [23-26]

Thin layer Chromatography of volatile oil [27-30]

It is apparent that silica gel TLC is a choice technique for the study of essential oils because of its rapidity and simplicity. With the help of TLC hundreds of oils of different chemical races have been screened and their components were identified. Evaluate the essential oil of this plant by TLC using mobile phases in different ratios like Toluene: Ethyl acetate (93:7) and (95:5) and R_f values and colour of the components were recorded. Five reference standards were used Eugenol, Citral, Geraniol, Ionone and Geranyl acetate. To identify these constituents of this oil and that constituents were confirmed by co-TLC using the solvent system like Toluene and

Ethyl acetate (93:7) and Hexane: and Chloroform (70:30).

Chemical analysis of the essential oil of *S.indicus* by G.L.C[31]

I) Essential oil Isolated from leaves of *Sphaeranthus indicus*

II) Reference compounds - Eugenol, Geraniol, Citral, Geranyl acetate, and ionone

Chromatographic Conditions:

Stationary phase	:	Capillary glass column BPX-70 (equivalent to FFAP) 30 m long and 0.2 mm in internal diameter, the inner Surface of which is coated with a layer of 50% cyano propyl / 50% methyl silicone.
Mobile phase	:	Nitrogen gas
Flow rate	:	25ml / min
Sensitivity	:	1
Detector	:	FID (Flame Ionisation Detector)

Detector temperature: 230°C

Injector: Septum type with SGE syringe

Injector temperature: 220°C

Column temperature: 160°C

Instrument:

CHEMITTO MODEL GC – 8610, WITH ONE PACKED COLUMN AND ONE CAPILLARY COLUMN PROVISION, WITH WICHROM SOFTWARE WITH DATA COLLECTOR.

METHOD:

Stabilise the baseline for about 30 minutes with the above chromatographic conditions. About 1µl of Eugenol RS was injected using SGE Syringe and the chromatogram was recorded. The same procedure was adopted for other reference standards viz, citral, ionone, geranyl acetate and geraniol and their standard chromatograms were recorded. About 1µl of the sample of *Sphaeranthus indicus* oil was injected and the sample chromatogram was recorded. The retention time was determined for the sample and the standard. The peak area of the standard and sample were determined. The composition of the components of the Essential oil was calculated using the formula

$$\text{Percentage Composition of the components in the sample} = \frac{\text{Area of the sample peak}}{\text{Area of the standard peak}} \times 100$$

RESULTS:

Anatomy of the leaf

Leaf: A leaf has distinct midrib and uniformly thick lamina. A midrib is Plano convex in sectional view, the adaxial side is more or less flat and the abaxial side is broad and hemispherical (Fig 1). The epidermis is thin and consists of squarish or elliptical cells with thin cuticle. There is a single top-shaped, collateral vascular bundle; the bundle is surrounded by parenchymatous ground tissue. The vascular bundle has four or five parallel rows of

xylem elements and a thick are of phloem elements (Fig 2). Sclerenchyma cells are not seen in the vascular bundle.

Lamina:

The lamina has distinct, fairly thick epidermal layers which bear dense trichomes. A abaxial epidermis is stomatiferous. A mesophyll tissue consist of several layers of cubical or vertically oblong cells. A few layers of adaxial mesophyll cell appear vertically oblong palisade – like cells (Fig 3 and 4). The spongy mesophyll tissue is aerenchymatous and consists of lobed cells.

Epidermal Trichomes (Fig 5, 6)

The epidermal trichomes are characteristic. These are two types of trichomes on the leaf. One trichome was nonglandular and multicellular, uniseriate, unbranched and whip like with dilated basal cell (Fig 6). Other trichome was glandular and multicellular and biseriate. A glandular trichome has two rows of vertically oblong, thin walled cells arising from dilated basal epidermal cells (Fig 5) At the terminal part of the cells become shorter to rectangular shape and finally at the summit are two hemispherical cells. The summit cells are glandular with dense cytoplasm and prominent nuclei. (Fig 6)

Venation Pattern: (Fig 7)

The vein islets are distinct, they vary in shape and size; they are rectangular to polygonal. Vein terminations are mostly single per islet; they are simple or branched once (Fig 7). The paradermal sections, the lateral veins and veinlets have small continuous sheath cells (Fig 8).

Stomata (Fig 9)

Stomata are exclusively anomocytic; the guard cells are not surrounded by subsidiary cells distinctly differently from the neighbouring epidermal cells. The anticlinal walls of the epidermal cells are highly wavy and the epidermis become much lobed. Cell walls are thin; cuticular striations are not evident.

Microscopy of petiole (Fig 10-11)

The petiole is circular, even and smooth in cross-sectional view. That have outer aerenchymatous ground tissue, a circular vascular cylinder and central parenchymatous

ground tissue. (Fig 10) These are distinct their epidermal layer and one or two subepidermal, compact parenchyma cells; the aerenchymatous zone consists of 2 or 3 layers of wide air-chambers separated from each other by uniseriate partition (Fig 11) The cells of the aerenchyma are angular, compact and thin walled. A vascular cylinder has several wedge-shaped vascular bundles forming a circle with interfascicular parenchymatous hole (Fig 11) Vascular bundles are collateral; the xylem elements are in 3-5 radial rows; phloem occurs as thick mass on the outer part of the xylem.

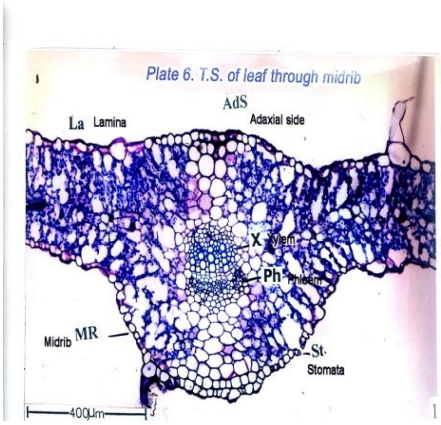


Fig 1: T.S of leaf through midrib

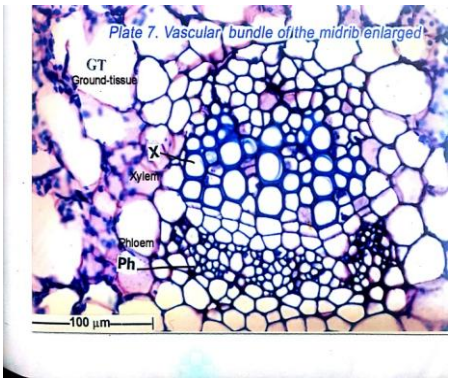


Fig 2: Vascular bundles

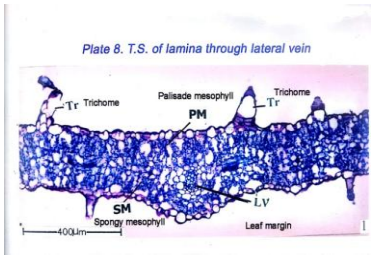


Fig 3: T.S of lamina

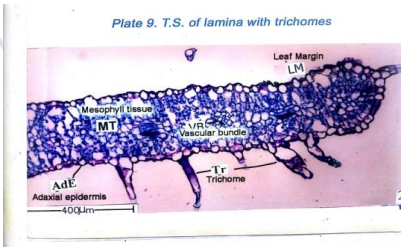


Fig 4: T.S of lamina with trichome

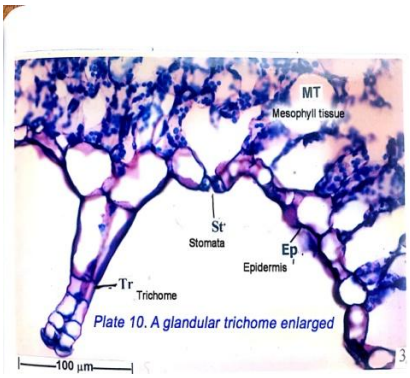


Fig 5: Glandular trichome enlarged

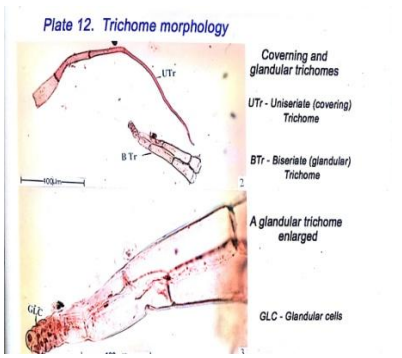


Fig 6: Trichome morphology

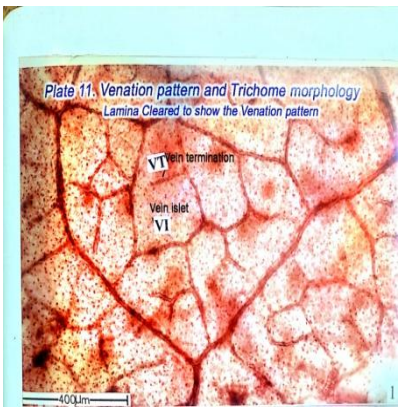


Fig 7: Venation

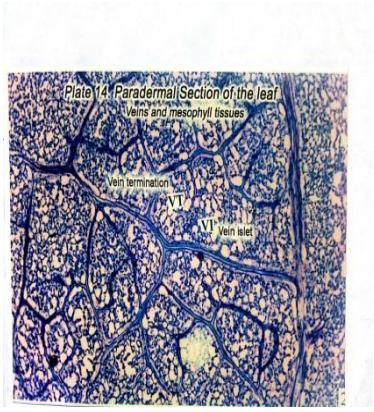


Fig 8: Paradermal section

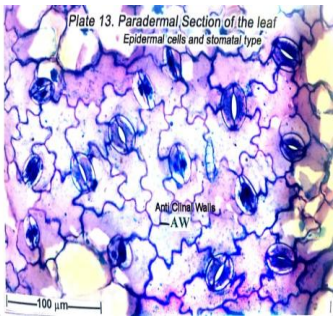


Fig 9: Stomata

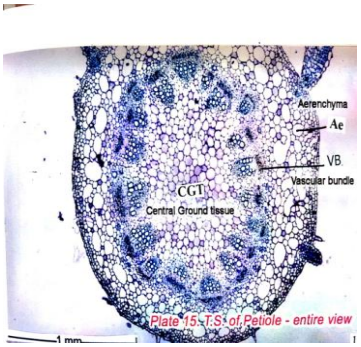


Fig 10: T.S of Petiole

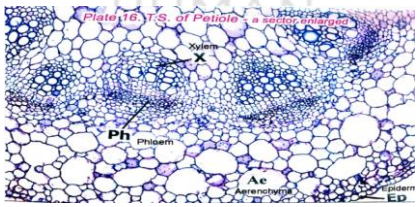


Fig 11: T.S. of petiole enlarged

Powder Microscopy

The organoleptic evaluation of the leaf powder shows that it was coarse, green with aromatic odour having slightly bitter taste. Piece of parenchyma cells, aerenchyma cells are seen. Glandular trichomes have two rows of vertically oblong thin walled cells. Non glandular trichomes are multicellular, uniseriate, unbranched, whip like with dilated basal vessel. When stained with toulidine and anomocytic stomata were observed when stained with aniline blue and vascular bundles were observed, when stained with phloroglucinol and concentrated hydrochloric acid.

Quantitative microscopy:

The quantitative microscopy such as vein- islet number, vein- terminal number, stomatal number and stomatal index were determined and the results were tabulated. (Table 1)

Table 1: Quantitative evaluation of the crude drug of leaf of *Sphaeranthus indicus*

S. No	Plant constants	Values
1.	Vein islet no	14.4
2.	Vein termination no	17.5
3.	Stomatal number (upper)	18.8
4.	Stomatal number (lower)	50.6
5.	Stomatal index (upper)	24.5
6.	Stomatal index (lower)	35.4

Physicochemical features:

Powdered drug was evaluated for its physicochemical parameters like total ash values, acid insoluble ash, water soluble ash and loss on drying, and the results were tabulated (Table 2).

Table 2 : Physicochemical evaluation of the crude drug of leaf of *Sphaeranthus indicus*

S. No	Physical Evaluation	%w/w
1.	Total Ash	20.21
2.	Acid Insoluble Ash	6.10
3.	Water Soluble Ash	7.56
4.	Loss on Drying	0.64

Fluorescence analysis of the extracts:

The extracts were prepared as per their polarity in hot successive extraction technique, and they were treated with reagents and the colour changes were observed under Ultra Violet light and the results were tabulated (Table 3).

Table 3: FluorescencE analysis of leaf of *Sphaeranthus indicus*

S. No	Sample	Colour in Day Light	Colour in UV Light
1.	Petroleum ether extract	Pale Yellow	Yellow
2.	Benzene Extract	Yellow	Orange Red
3.	Acetone Extract	Green	Red
4.	Chloroform Extract	Yellowish green	Yellow
5.	Methanolic Extract	Green	Light blue
6.	Ethanol Extract	Green	Dark Green
7.	Aqueous Extract	Yellow	Blue

Extractive values

The extracts were prepared according to the polarity and they were concentrated and their values were calculated with reference to air dried drug and the results were tabulated (Table 4).

Table 4: Extractive values of leaf of *sphaeranthus indicus* in different solvents

S. No	Sample	Extractability (%)
1.	Petroleum ether extract	9.96
2.	Benzene Extract	1.20
3.	Chloroform Extract	0.64
4.	Acetone Extract	0.98
5.	Methanolic Extract	4.20
6.	Ethanol Extract	5.82
7.	Aqueous Extract	2.84

Preliminary phytochemical analysis

The powdered leaf and various extracts such as petroleum ether extract, benzene extract, chloroform extract, ethanol extract and aqueous extract were subjected to preliminary phytochemical screening for their presence or absence of the constituents and the results were tabulated (Table 5).

Table 5: Preliminary phytochemical tests for drug powder and various extracts of leaf of *Sphaeranthus indicus*

S.NO	Test	Drug Powder	Petroleum Ether Extract	Benzene Extract	Chloroform Extract	Acetone Extract	Methanol Extract	Ethanol Extract	Aqueous Extract
1.	Sterols	+	+	+	+	+	+	+	-
2.	Terpenoids	+	+	+	+	+	+	+	-
3.	Carbohydrates	+	-	-	-	+	+	+	+
4.	Flavanoids	+	-	-	-	+	+	+	+
5.	Proteins	-	-	-	-	-	-	-	-
6.	Alkaloids	-	-	-	-	-	-	-	-
7.	Glycosides	-	-	-	-	-	-	-	-
8.	Saponins	-	-	-	-	-	-	-	-
9.	Tannins	+	-	-	-	+	+	+	+
10.	Mucilages	-	-	-	-	-	-	-	-
11.	Volatile Oil	+	-	-	-	-	-	-	-

+ indicates positive reaction, -indicates negative reaction.

Thin layer chromatography of Aqueous and Methanolic Extract:

A yellow colour spot was obtained with both the extracts indicate the presence of isoflavone glycoside. (Fig 12, 13) The phytochemical tests and TLC studies reveals the presence of Isoflavone compound in both the extract

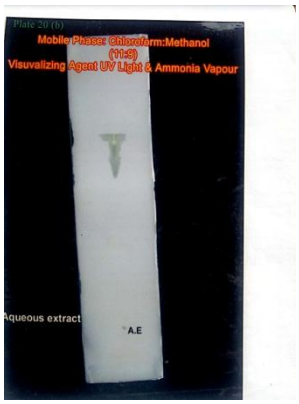


Fig 12: T.L.C OF AQUEOUS EXTRACT Fig13: T.L.C OF METHANOLIC EXTRACT

ANALYSIS OF VOLATILE OIL

Physical analysis

Colour	:	Very deep cherry red
Odour	:	Aromatic and pleasant
Taste	:	Bitter
Solubility	:	Soluble in water, alcohol, acetone, chloroform, Toluene, benzene and ether

The weights per ml, refractive index and optical rotation are some important distinctive criteria for the oils were determined and tabulated.(Table 6)

Table 6: physical parameters of oil

Weight /ml (gm/ml)	Refractive index	Optical rotation
0.9935	1.5055	$\pm O^{o}$

Chemical Analysis

The essential oil was analysed chemically by its acid value, and ester value, and tabulated.

(Table 7)

Table 7: Chemical analysis of volatile oil

Acid Value	Ester value
5.8	75.8

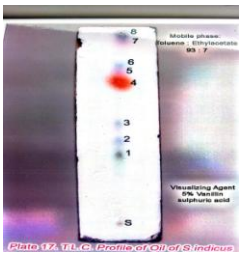
Thin layer chromatography of volatile oil

The essential oil of this plant confirmed by TLC using mobile phases in different ratios and R_f values and colour of the components were recorded and tabulated. (Table 8)

Five reference standards were used Eugenol, Citral, Geraniol, Ionone and Geranyl acetate to identify the constituents of this oil and the constituents were confirmed by co-TLC.

TABLE 8: T.L.C OF VOLATILE OIL

Mobile phase	Adsorbent	Visualizing Agent	R_f values of the spots	Colour
Toluene : Ethyl acetate (93:7) (Fig 14)	Silica Gel-G (activated at 110^o for 30 mts)	5% Vanillin sulphuric acid	(i) 0.34	Green
			(ii) 0.4	Blue
			(iii) 0.46	Greenish Blue
			(iv) 0.7	Reddish brown
			(v) 0.71	Pink
			(vi) 0.73	Violet
			(vii) 0.84	Light Pink
			(viii) 0.92	Greenish Black
Toluene: Ethyl acetate (95:5) Fig (15)	Silica gel- G (activated at 110^o for 30 mts)	5% Vanillin sulphuric acid	0.1	Rose
			0.17	Violet
			0.6	Pink
			0.95	Blue



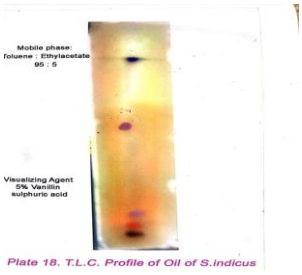


Fig 14: T.L.C OF VOLATILE OIL

Fig 15 T.L.C OF VOLATILE OIL (2)

TABLE 9: Co T.L.C OF VOLATILE OIL

Mobile phase	Adsorbent	Visualizing Agent	Standard		Sample Rf Values	Colour
			Name of the Standard	Rf Values		
Toluene: Ethyl acetate (93:7) (Fig 16)	Silica gel-G (activated at 110º c for 30 mts)	5% vanillin sulphuric acid	Eugenol	0.7	0.7	Reddish brown
			Geraniol	0.84	0.84	Light pink
			Ionone	0.46	0.46	Greenish blue
			Geranyl Acetate	0.5	-	Greenish black
Hexane: chloroform (70:30) (Fig 17)	Silica gel -G	UV light	Citral	0.92	0.92	Bluish green

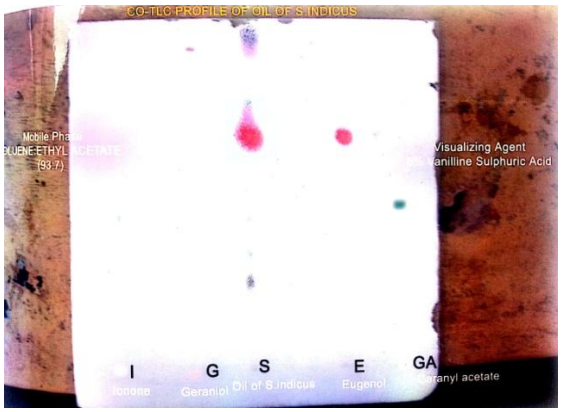


Fig 16: co T.L.C OF VOLATILE OIL

Fig 17 co- T.L.C OF VOLATILE OIL

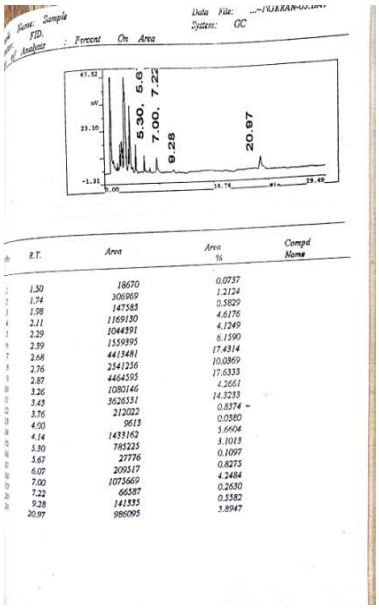
Analysis of oil of *Sphaeranthus indicus* by GLC:

Essential oil had been analysed by GLC and their components were identified and quantified. five standards Eugenol, geraniol, citral, Geranyl acetate, and ionone were used and the standard chromatograms were recorded. A sample chromatogram also recorded with the oil of *Sphaeranthus indicus*. Various parameters of the GLC of the oil such as retention time and area of the peak were considered for standards and sample. A retention time for the reference standard geraniol was 1.62 minutes corresponding to the area of the peak was 11529021, and in the sample the retention time for Geraniol was 1.74 minutes corresponding to the area of the peak 306969 and the percentage of Geraniol in the sample was calculated as 2.6. The retention time for the reference standard Eugenol was 3.86 minutes corresponding to the area of the peak 29408069, but in the sample retention time for Eugenol was 3.76 minutes corresponding to the area of the peak 212022 and the percentage of Eugenol in the sample was calculated as 0.72. The retention time for the reference standard Citral was 1.88 minutes corresponding to the area of the peak 8257500 and in the sample retention time for Citral was 1.98 minutes corresponding to the area of the peak 147583 and the percentage of citral in the sample was calculated as 1.7.The retention time for the reference standard ionone was 2.54 minutes, corresponding to the area of the peak 32005243, and in the sample retention time for ionone was 2.68 minutes corresponding to the area of the peak 4413481 and the percentage of ionone in the sample was calculated as 13.78. The retention time for the reference standard Geranyl acetate was 1.44 minutes corresponding to the area of the peak 15676144 whereas the sample retention time did not correlate the standard retention time. So it did not contain geranyl acetate. The retention time and area of the peaks are tabulated. (Table 10)

TABLE 10: GLC ANALYSIS OF VOLATILE OIL

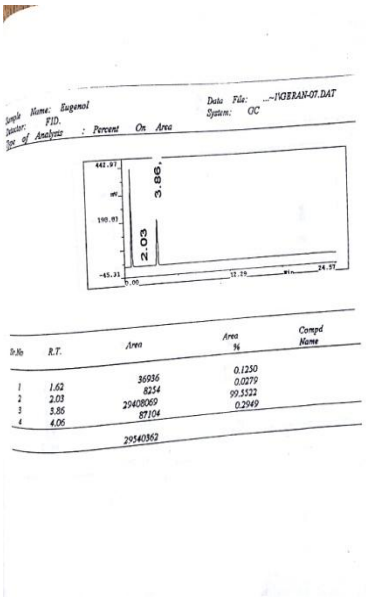
Name of the Reference standard	Retention Time		Area of the Peak		Percentage composition of components in the sample
	Standard	Sample	Standard	Sample	
Eugenol	3.86	3.76	29408069	212022	0.72
Citral	1.88	1.98	8257500	147583	1.7
Geraniol	1.62	1.74	11529021	306969	2.6
Ionone	2.54	2.68	32005243	4413481	13.78
Geranylacetate	1.44	-	15676144	-	-

G.L.C of the oil of Sphaeranthus indicus



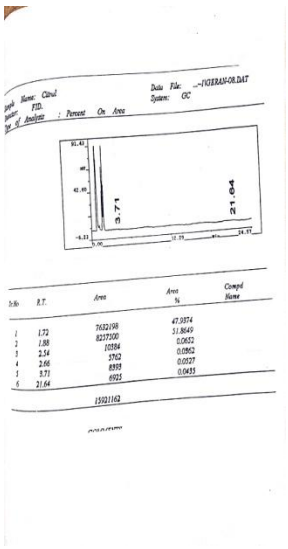
Sr.No	R.T.	Area	Area %	Compd Name
1	1.50	18670	0.0737	
2	1.74	306969	1.2124	
3	1.98	147583	0.5829	
4	2.11	1169130	4.6176	
5	2.29	1044391	4.1249	
6	2.39	1559393	6.1590	
7	2.68	4413481	17.4314	
8	2.76	2541256	10.0369	
9	2.87	4464595	17.6333	
10	3.26	1080146	4.2651	
11	3.43	3626531	14.3233	
12	3.76	212022	0.8374	
13	4.00	9618	0.0380	
14	4.14	1433162	5.6604	
15	5.30	785225	3.1013	
16	5.67	27776	0.1097	
17	6.07	209517	0.8275	
18	7.00	1073669	4.2484	
19	7.22	66587	0.2630	
20	9.28	141335	0.5582	
21	20.97	986095	3.8947	

G.L.C of Eugenol



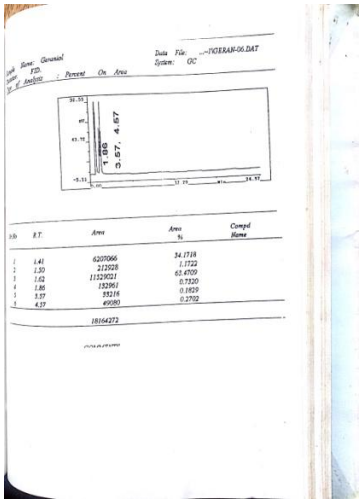
Sr.No	R.T.	Area	Area %	Compd Name
1	1.62	36036	0.1250	
2	2.03	8254	0.0279	
3	3.86	29408269	99.5322	
4	4.06	87104	0.2949	
		29540362		

G.L.C of Citral



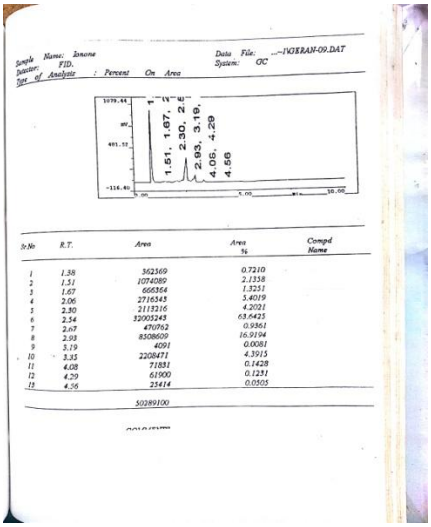
Sr.No	R.T.	Area	Area %	Compd Name
1	1.72	7632198	47.3874	
2	1.88	8217100	51.0649	
3	2.54	10504	0.0652	
4	2.66	5762	0.0862	
5	3.71	8595	0.0537	
6	21.64	6925	0.0433	
		15921162		

G.L.C of Geraniol

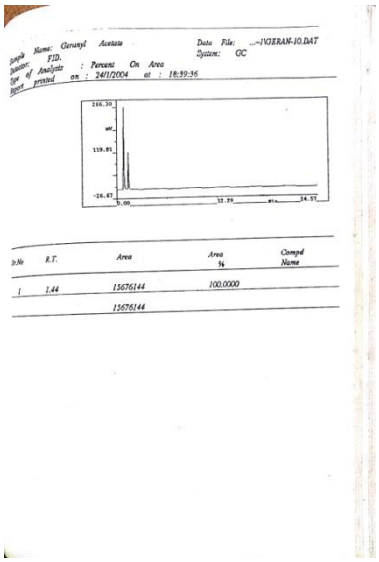


Sr.No	R.T.	Area	Area %	Compd Name
1	1.41	6207066	34.1718	
2	1.50	212928	1.1722	
3	1.62	11529021	63.4709	
4	1.86	132961	0.7320	
5	3.57	55216	0.3039	
6	4.57	49080	0.2702	
		18164272		

G.L.C of Ionone



G.L.C OF Geranyl acetate



DISCUSSION:

Our study has focused on examining Pharmacognostic and Preliminary phytochemical and T.L.C, G.L.C studies ofleaves of *Sphaeranthus indicus*. Normalization of the macroscopic and microscopic characteristics of the leaf.Drug remains essential in other to identify and avoid falsification. The leaf has distinct midrib and thick, soft lamina. The midrib is plano-convex in cross-sectional view with single top-shaped collateral vascular bundle surrounded by parenchymatous cells. Sclerenchyma cells are not seen in the vascular bundle. The lamina is dorsiventral.The mesophyll tissue is not well differentiated into palisade and spongy tissues. Characteristic epidermal trichomes are abundant on the leaf. Some of the trichomes are covering-type and are multicellular, uniseriate, unbranched and whip-like others are biseriate, broad, unbranched, conical with vertically oblong cells and a few tiers of apical glandular cells. Stomata are anomocytic; anticlinal walls of the epidermal cells are highly wavy. Vein islets are distinct, with one, simple or branched vein terminations. Petiole is circular in sectional view with aerenchymatous outer ground tissue, broad central tissue and is open ring of discrete collateral vascular bundles. Organoleptic characters are important in drugs because they play a role in the detection of adulterated or substituted drugs[32]. Theleaves dark green in colour, emit a very fragrant and aromatic minty odour when bruised. The powdery appearance of the crushed leaves, with a coarse texture. The micrograph performed on the powder has highlighted a number of characteristic elements namely: the polygonal epidermal cells, the anomocytic stomata. Glandular trichomes, are diagnostic

characters for drugs of plant origin. These diagnostic elements are consistent with botanical standards and WHO guidelines [33-34]. This study of physicochemical parameters such as moisture content and ash values are useful as it determines the physiological and nonphysiological state of ash, this will help to determine the possibility of microbial growth and lastly contaminant or impurities. The moisture content of the drug studied had a rate of 0.68 ± 0.1. which is below 10%. This result comply with the standards established by the International Pharmacopoeia, because this water content rate, prevent oxidation reaction fermentation and give less chance to microbial growth and contamination in drugs[35]. Therefore, for proper conservation of drugs made from the leaves of *S. hirta* it would be desirable to use those whose water content is less than or equal to 10%..The determination of total ash gave us a rate of 20.21 ± 0.03. This value indicates the level of minerals in drugs. Insoluble ash in hydrochloric acid gave a rate of 6.10 ± 0.02. Indeed, the ash insoluble in hydrochloric acid tells us about the contamination of the drug by siliceous elements[36]. This result is in agreement with Srikanth *et.al*[37] who found rate of 0.97% and 0.5% respectively. The maximum extractive value was found in distilled water (12.84%) followed by Petroleum ether (9.96%), Ethanol (5.82%), methanol (4.20%) benzene (1.20%), acetone (0.98%),Chloroform (0.64%). All the extracts of the drug was subjected to different tests for detecting the presence of various phytoconstituents present in the drug, which revealed the presence of sterols, terpenoid flavonoid,and tannins. Preliminary phytochemical analysis indicated a high percentage of flavonoids and this may be one of the reasons behind the hypolipidemic activity of the medicinal plant. TLC profile of Aqueous and methanolic extract showed yellow colour spot under UV, indicates the presence of isoflavonoid..T.L.C analysis of volatile oil of *Sphaeranthus indicus* showed seven spots, these were compared with co-T.L.C these indicates that the presence of Eugenol, Geraniol Ionone and Citral. GLC analysis of volatile oil obtained from *Sphaeranthus indicus* indicates that the presence the above volatile substances. These parameters, which are being reported for the first time in this plant, are significant towards establishing the pharmacognostic standards for future identification and authentication of genuine plant material. *Sphaeranthus indicus* is a highly reputed drug used in Ayurveda. Barring the anatomical details and preliminary phytochemical screening, rest of the pharmacognostical parameter gives us the clue that it can be cashed economically as well to improve the standard of health in the developing countries.

CONCLUSION

Microscopic method is one of the simplest and cheapest methods to start with, for establishing the correct identity of the source materials. *S .indicus* is often confused with *S. amaranthoides* and other members of asteraceae family.This research paper covers an extensive study on the leaves of *Sphaeranthus indicus*. The Pharmacognostic, Phytochemical studies including preliminary phytochemical tests, TLC and GLC analysis of essential oil obtained from the leaves. Pharmacognostic parameters have been determined for leaf in order to substantiate and identify the plant for future work. It gives us the clue that it can be cashed economically as well to improve the standard of health in the developing countries.

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