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Research Article

Pharmocognostical and Phytochemical Evaluation of Leaf of *Sphaeranthus indicus*

Dhanapal Venkatachalam, Samuel Thavamani b, Muddukrishniah

Department of Pharmacognosy, Sanjo College of Pharmaceutical Studies, Velappara, Palakkad, Kerala – 678 702.

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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 belongs to the family Asteraceae. The leaves of *Sphaeranthus indicus* (Linn.) used traditionally in Ayurveda for hyperlipidemia, epilepsy, mental illness, jaundice, diabetes, leprosy, fever cough, gastropathy, hernia, hemorrhoids, 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 hepato protective. 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 leaf, TLC and GLC analysis of the oil of the leaves and 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. The leaves are bitter in taste with pleasant odour when fresh, the aroma gradually diminishing on drying and storing. 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. No sclerenchyma cells are seen in the vascular bundle. The lamina is dorsiventral; however 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. The investigations also included leaf surface data; quantitative leaf microscopy. Physiochemical parameters such as loss on drying, extractive values and ash values were also determined. Preliminary phytochemical screening showed the presence of sterols, terpenoids, carbohydrates, flavonoids (Isoflavone), tannins and volatile oil. TLC studies reveal that the presence of isoflavone glycosides. Essential oil have been analysed by GLC and their components were identified and quantified. Conclusions: The 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 and applications.

Keywords: *Sphaeranthus indicus,* Ayurvedic system, Volatile oils, T.L.C and GLC.

INDRODUCTION

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 organization (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 inter-twined with the rich history, culture and culinary tradition of India. India has a rich and glorious ethno medical heritage[4]. Medicinal plants are also used by the codified systems of medicine such as Ayurveda, Siddha, Unani, Chinese and Tibetian systems of 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 herbal plant widely used in Ayurvedic system of medicine for treating different diseases[6]. It grows well in waste lands, paddy fields, places and it is also cultivated in tropical and subtropical parts of India. It is usually found in throughout India, some parts of Sri Lanka, Africa and Australia from

**Author for Correspondence: vddpaul@gmail.com*

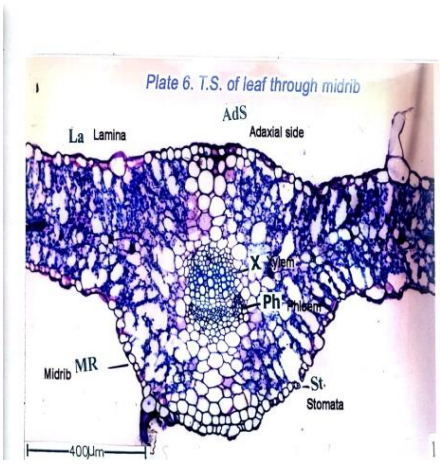


Figure 1 : T.S of leaf through midrib.

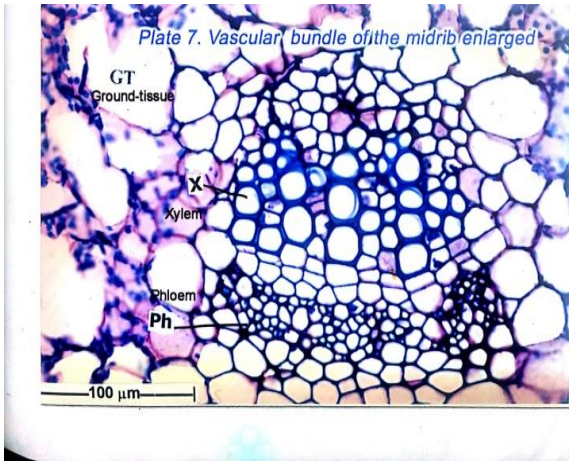


Figure 2 : Vascular bundles enlarged.

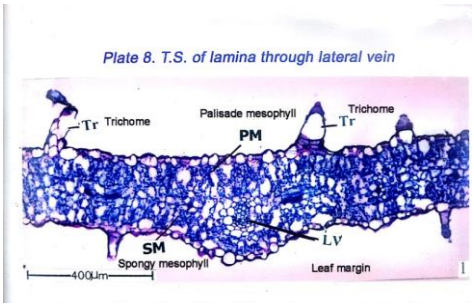


Figure 3: T. S of lamina through lateral vein.

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

Table 2: Physico chemical 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

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 of capitulam of this plant was well documented with **GC-MS**[8] and since no detailed studies seems to have been previously done on the leaves pertaining to the volatile oil content and chemical analysis of the same. so the present study is aimed to isolate and evaluate the volatile oil 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.A novel isoflavone glycoside have 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 principles.

MATERIALS AND METHODS

Plant material

Sphaeranthus indicus leaf was collected, from in and around of Palakkad district, Kerala, India and authenticated by taxonomist and the plant authenticated specimen is 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 : Sphaeranthus indicus
Synonym**:** Sphaeranthus hirtus

Common Names

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

Table 4: Extractive values of leaf of *shaeranthus indicus* with 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

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
Punjabi : 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). The specimens were 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 Physico chemical parameters

The vein islet number, vein terminal number, stomatal number, stomatal index were determined on fresh leaves using standard procedure[15-17]. The parameters were done to evaluate the proceedings of vein islet number, vein termination number; stomatal number, stomatal index, 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 leaf 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 (40-45^{o}C) 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 have 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 hydro distillation in a volatile oil estimation apparatus and distillate collected over solvent ether. The aqueous part was rejected and the ethereal part was dried over anhydrous sodium sulphate. The solvent was dried in a weighed conical flask on a water bath at controlled temperature and kept in vaccum desiccator overnight and weighed. The yield comes to be 0.01 –

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.

Table 6: Physical parameters of oil.

Wt.per ml(gm/ml)	Refractive index	Optical rotation
0.9935	1.5055	± O°

Table 7: Chemical analysis of oil.

Acid Value	Ester value
5.8	75.8

0.02% on fresh weight basis. The isolated oils are 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 vice Eugenol, Citral, Geraniol, Ionone and Geranyl acetate to identify these constituents of this oil and these constituents were confirmed by co-TLC using the solvent system like Toluene:Ethylacetate (93:7) and Hexane: Chloroform (70:30).

Chemical analysis of the essential oil of s.indicus by gas liquid chromatography [31]

Essential oil Isolated from leaves of *S.indicus*

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: 230ºC

Injector: Septum type with SGE syringe

Injector temperature: 220ºC

Column temperature: 160ºC

Instrument:

Chemitto model gc – 8610, with one packed column and one capillary column provision, with wichrom software with data collector.

METHOD

Stabilize 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 *S.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.

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

The leaf has distinct midrib and uniformly thick lamina. The 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). No sclerenchyma cells are seen in the vascular bundle.

Lamina

The lamina has distinct, fairly thick epidermal layers which bear dense trichomes. The abaxial epidermis is

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)	Silicagel -G (activated at 110° 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° for 30mts)	5% Vanillin sulphuric acid	0.1	Rose
			0.17	Violet
			0.6	Pink
			0.95	Blue

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 for 30mts)	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

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	-	-

stomatiferous. The mesophyll tissue is consists of several layers of cubical or vertically oblong cells; A few layers of adaxial mesophyl cells 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. There are two types of trichomes on the leaf. One is nonglandular and is multicellular, uniseriate, unbranched and whip like with dilated basal cell (Fig 6). The other trichome is glandular and multicellular and biseriate. The glandular trichome has two rows of vertically oblong, thin walled cells arising from dilated basal epidermal cells. (Fig 5) At the terminal part 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; generally they are rectangular to polygonal. The vein terminations are mostly single per islet; they are simple or branched once (Fig 7). In 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 epidermals become much lobed. The cell walls are thin; cuticular striations are not evident.

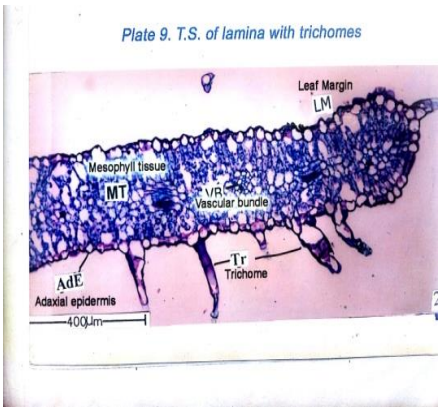


Figure 4: T.S of lamina with trichome

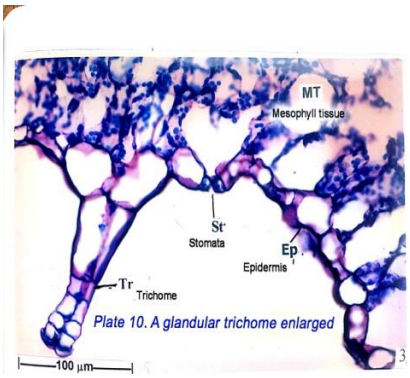


Figure 5: Glandular trichome enlarged

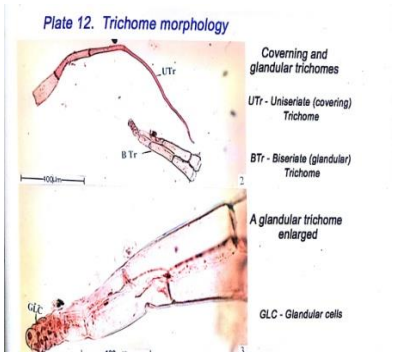


Figure 6: Trichome morphology

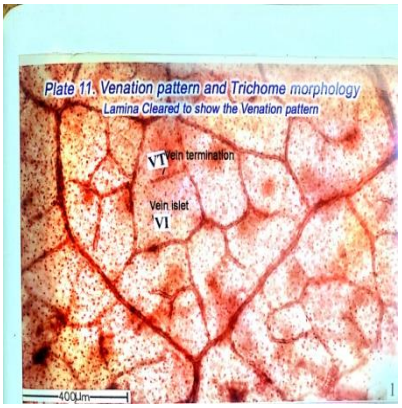


Figure 7: Venation patteren

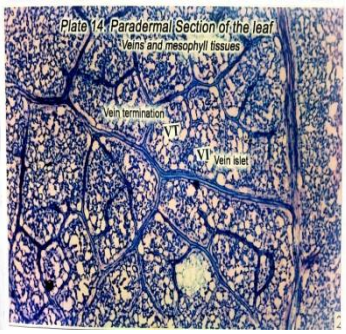


Figure 8: Paradermal section of the leaf

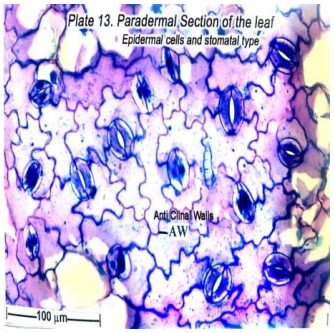


Figure 9: Stomata

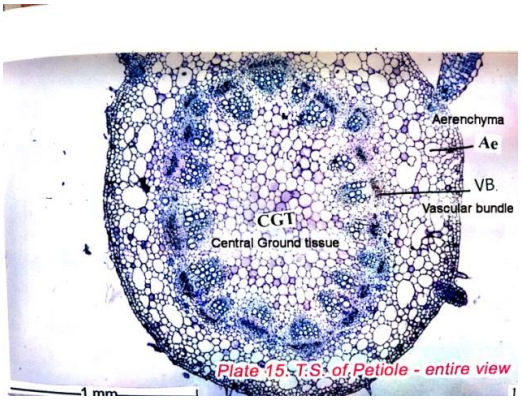


Figure 10; T.S of petiole

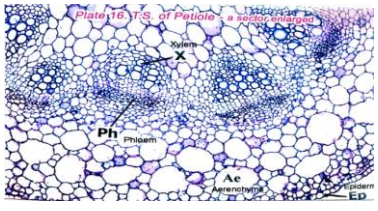


Figure 11: T.S of petiole enlarged

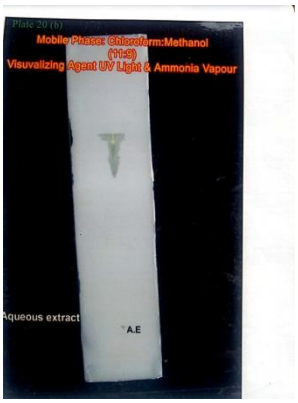


Figure 12: T.L.C of aqueous extract

Figure 13: T.L.C of methanolic extract

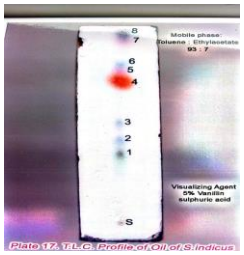


Figure 14: T.L.C of volatile oil (1)

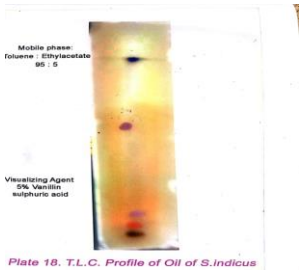


Figure 15: T.L.C of volatile oil (2)

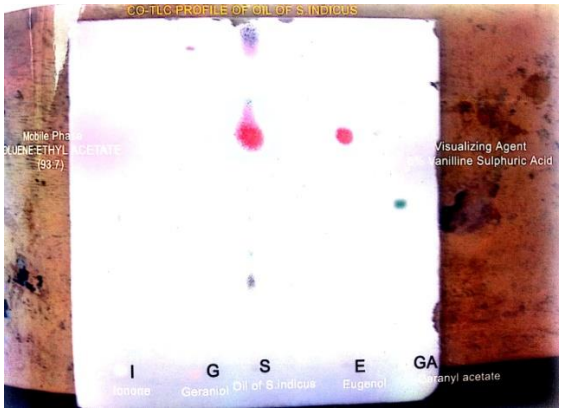


Figure 16: co T.L.C of volatile oil

Figure 17: co- T.L.C of volatile oil

Microscopy of petiole (fig 10,11)

The petiole is circular, even and smooth in cross-sectional view. It has 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 partitions. (Fig 11) The cells of the aerenchyma are angular, compact and thin walled. The vascular cylinder has several wedge-shaped vascular bundles forming a circle with interfascicular parenchymatous gap. (Fig 11) The 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.

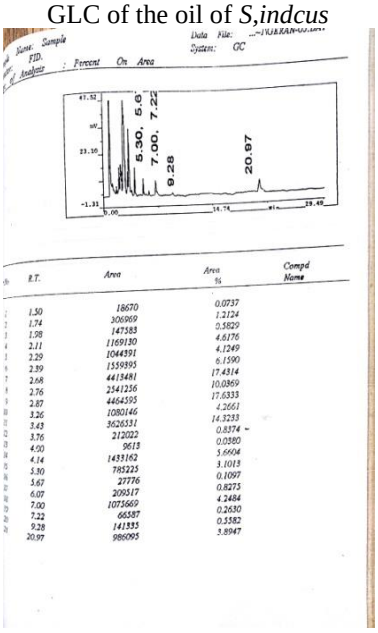
Powder Microscopy

The organoleptic evaluation of the leaf powder shows that it was coarse, green with aromatic odour having slightly bitter taste. Fragments of parenchyma cells, aerenchyma cells and collenchymas cells. 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 phluroglucinol and concentrated hydrochloric acid.

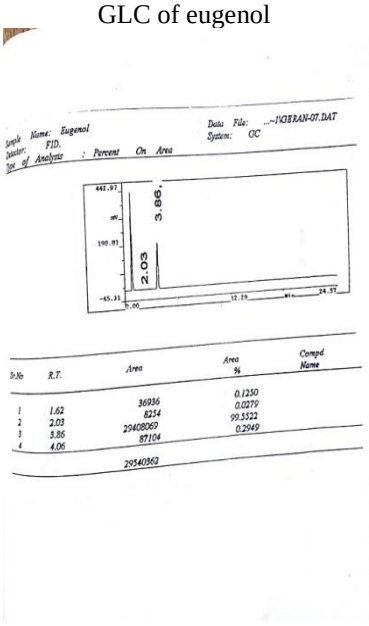
Quantitative microscopy

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)

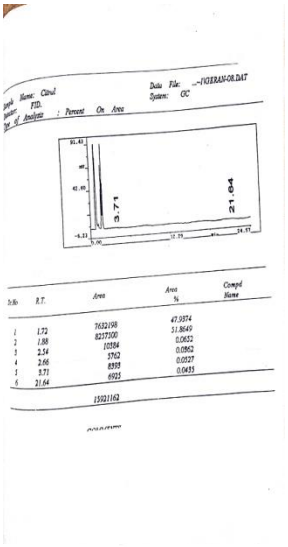
GLC of the oil of *S,indcus*



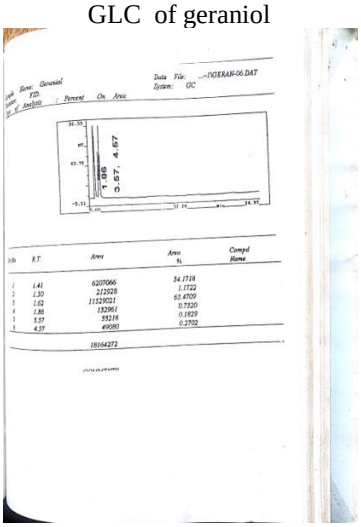
GLC of eugenol



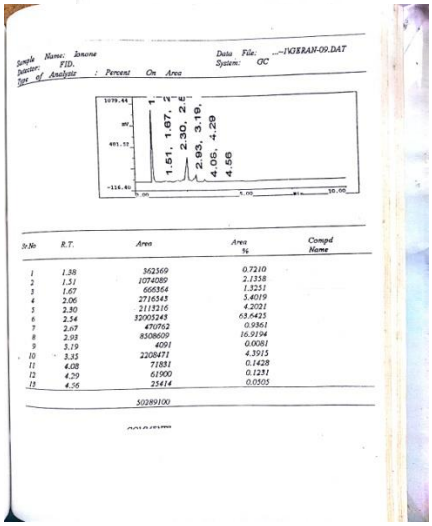
GLC of citral



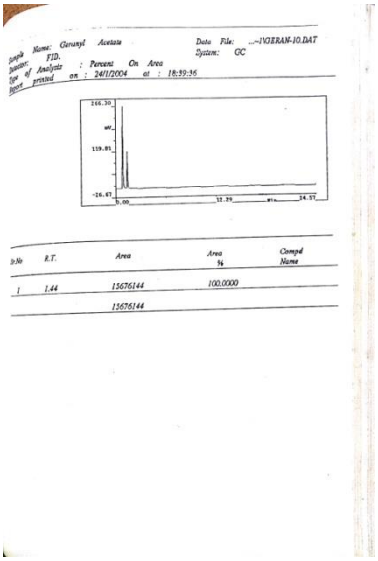
GLC of geraniol



GLC of ionone



GLC of geranyl acetate



Physico chemical features

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

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).

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).

Preliminary phytochemical analysis

The leaf powder 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).

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 extracts

Analysis of volatile oil

Physical analysis

Colour : Very deep sherry 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 when where determined and tabulated (Table 6)

Chemical Analysis

The essential oil is analysed chemically by its acid value, and ester value which were determined and tabulated. (Table 7)

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

Analysis of oil of Sphaeranthus indicus by GLC

Essential oil have been analysed by GLC and their components were identified and quantified. Five standards viz Eugenol, geraniol, citral, Geranyl acetate, and ionone were used and the standard chromatograms were recorded. The sample chromatogram also recorded with the oil of *Sphaeranthus indicus*.The various parameters of the GLC of the oil such as retention time and area of the peak were considered for standards and sample. The retention time for the reference standard geraniol was 1.62 minutes corresponding to the area of the peak 11529021, where as 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 where as 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 where as 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, where as 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)

DISCUSSION

Our study has focused on examining Pharmacognostic and Preliminary phytochemical and T.L.C, G.L.C studies of Sphaeranthus indicus leaves. Normalization of the macroscopic and microscopic characteristics of the *S,indicus*. 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. No sclerenchyma cells are seen in the vascular bundle. The lamina is dorsiventral; however, 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 characteristics are important in drugs because they play a role in the detection of adulterated or substituted drugs[32].Thus leaves dark green in colour, emit a very fragrant and aromatic mintyodor 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, wavy epidermal cells, the anomocytic type of stomata,Glandular,Nonglandular trichomes, are diagnostic substances for drugs of plant origin. These diagnostic

elements are consistent with botanical standards and WHO guidelines[33-34]. The 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 reactions, 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,terpenoids, flavanoids, and tannins. Preliminary phytochemical analysis indicated a high percentage of quercetine and flavonoids and this may be one of the reasons behind the hypolipidemic activity of the plant. plant. TLC profile of aqueous and methanolic extracts showed yellow colour spots under UV, indicates the presence of isoflavonoids, T.L.C analysis of volatile oil of *S.indicus* showed seven spots, these were compared with co TLC, it indicates that the presence of Eugenol, Geraniol, Ionone, and Citral. GLC analysis of volatile oil obtained from *S.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. Though Sphaeranthus indicus is a weed, it is a highly reputed drug used in Ayurveda. Barring the anatomical details and preliminary phytochemical screening, rest of the pharmacognostical parameters, 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. *Sphaeranthus indicus L*. is often confused with *S. amaranthoides* and other members of Asteracea. When the specimens are in fragmentary condition, to identify the crude fragmentary plant materials, anatomical characters are often helpful, this research paper covers an extensive study on the leaves of *Sphaeranthus indicus*. The Pharmacognostic, Phytochemical profile 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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