



MORPHOANATOMICAL, PHYSICO-CHEMICAL STANDARDISATION AND HPTLC ANALYSIS IN THE PLANT EXTRACT OF *LEPIDAGATHIS SCARIOSA* (NEES) - ACANTHACEAE.

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ABSTRACT

The objective of the study is to observe the macroscopic parameters and anatomical characterization of *Lepidagathis scariosa* (Nees) as protocol to be followed for botanical identification of the plant. Histochemical studies were made to localize different components, viz. alkaloids, phenolic compounds, flavonoids, lipids, starch and saponin in the tissues (Stem and Leaf) and to quantify flavonoid compound by HPTLC analysis with the use of standard marker compounds that is used in drug analysis. The study plant has a rich source of medicinal values. But no systematic studies is carried out for their anatomical and histochemical analysis, especially pharmacognostical studies on this plant remain unexplored. So the present attempt deals with the modest comprehensive investigation about macro morphological, micro anatomical characters and their pharmacopoeia standards perceived through physic-chemical constants. The pharmacognostic studies of *Lepidagathis scariosa* play a pivotal role in herbal technology as they lays down standardization parameters which will help and prevent adulterations.

KEYWORDS: *Lepidagathis scariosa*, morphoanatomical, pharmacognostical, physico-chemical.



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Received on: 20-06-2018

Revised and Accepted on: 01-08-2018

DOI: <http://dx.doi.org/10.22376/ijpbs.2018.9.4.p8-20>



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INTRODUCTION

Plants are the store-house of active medicines which supply remedies for all ailments among human beings. India has a rich source of aromatic and medicinal plants which act as the authentic emporium of drugs. In India the herbal medicines are well practiced since many centuries and have been incorporated into different medicinal systems such as Ayurveda, siddha and unani¹. Historically, the knowledge of drugs exists since thousands of years and these drugs have their source in plants.² The natural products are the best primary health care products because of well protective values, cogency and without side effects. Therefore, the drugs which have no side effects are the essential ones for the society.³ Standardization of herbal drugs is an essential tool for establishing their identity, pureness, inviolability and quality. The standardization of drugs is studied by macroscopic, microscopic and fluorescence analysis. Scrutinizing the microscopically characters is the cheapest method to exhibit the correct identification of plants. Morphological and microscopically parameters are used to detect the adulterations in drug production.⁴ In pharmaceutical research, extraction methods afford benefits and involve in the separation of medicinally useful compounds from plant tissues by using selective solvents. During extraction, solid plant materials with their secondary metabolites are diffused into the solvents. About 2,000 phytochemical compounds are present in the plants.⁵ These phytochemicals are aid to prevent human illness in various ways. Considering these facts about herbal medicine, a phytopharmacognostical examination was conducted in potentially important medicinal plant *Lepidagathis scariosa* species distributed in Chennimalai hill. The hill, situated in Erode district, embraces rare medicinal herbs in its lush green forests. The hill is noted for its natural flora and fauna. In the observation point of view, the commonly available *Lepidagathis scariosa* is found to be a medicinally important species in this region. For that reason this potential species has been selected for further studies. *Lepidagathis scariosa* Nees. Is a prostrate under shrub which belongs to the family Acanthaceae and it has approximately 60 species distributed throughout the tropical regions.⁶⁻⁸ It is used as herbal medicine in siddha, Ayurveda and folk medicine for treating various ailments. Generally the plants in acanthaceae members are widely used as curing agent for cough in children and for labor pains. It is a remedy for fever, cough, toothache, chest pain, vomits, diarrhea, constipation and

pile, bone fracture, jaundice, skin infection, wound healing, microbes, venereal diseases and ulcer.⁹ Therefore with the help of traditional users, the present investigation deals with the macroscopic, microscopic, histochemical localization and quality parameters including physicochemical characterization in *Lepidagathis scariosa* species. The main study was to analyze the micro morphological features of this drug source and to localize the active molecules histochemically and quantitative determination by HPTLC analysis for *Lepidagathis scariosa*.

MATERIALS AND METHODS

Chemicals and instruments

All the chemicals used were of laboratory grade. Compound microscope, watch glass, glass slides, cover slips, petridies and other common glasswares were used in this experiment. Photographs were taken with using Nikon Labophot 2 Microscopic Unit and trinocular microscope. Petroleum ether, ethanol solvent and safranin reagent used for staining different sections were procured from The Precision Scientific Co., Coimbatore, India.

Plant collection and authentication

Fresh leaves of *Lepidagathis scariosa* were collected from Chennimalai hill, Erode district. The plants were collected from the natural habitat during the season of flowering and fruiting. While collecting the samples, a thorough observation was made regarding the location, natural habitat, distribution pattern, habit, floral and fruit characteristics. The collected plant was identified with the help of the existing Floras^{10,11,12} and compared with type specimens available in the herbarium of Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore, Tamil Nadu (voucher specimen number – BSI/SRC/5/23/2017/Tech/1691) (Figure 1). The collected plant specimens were pressed properly following the method (Jain, 1970). Dried specimens were poisoned with 0.1% HgCl₂ dissolved in absolute alcohol and mounted with glue on standard herbarium sheet (42 x 28 cm). The herbaria were deposited in the Department of Botany, Vellalar College for Women, Thindal, and Erode. Photographs were also taken to supplement the herbarium. The medicinal properties of the plant were checked with the earlier reports.^{14,15} Secondary information were collected from numerous research papers, reports, records, documents, articles, books and journals.



Figure 1
Flowering twig of *Lepidagathis scariosa* Nees, the study plant

Pharmacognostical studies macroscopic analysis

Macroscopic characters of the stem and leaf such as color, texture, taste and odor were examined as per Tease and Evans (1983) and Wallis(1985).

Anatomical and histochemical studies

The leaf and stem of *Lepidagathis scariosa* were cut and removed from the plant and fixed in FAA (Formalin - 5 ml + acetic acid - 5 ml + 70 % Ethyl alcohol - 90 ml). The materials were rinsed twice in deionized water before sectioning. They were then free-hand-sectioned transversely. Micro morphological characterization studies were carried out employing standard sectioning and staining methods as per standard texts. For anatomical investigation of each specimen section, a drop of water was placed on a clean slide. The specimen section was mounted on it, and then stained with a drop of 0.1 % safran in solution. The cell arrangements, size and shape of cells, starch grains and stomatal types were localized histochemically in the leaf and stem of study plant using specific reagents and were carefully observed under an OLYMPUS (Model A X 70 TRF) light microscope at (40x and 100x) magnification. Fresh free hand sections of leaf and stem of *Lepidagathis scariosa* were taken. The fresh sections of the plant (leaf and stem) used for the histochemical study were treated with the respective reagents to localize the presence or absence of metabolites. The reagents used were: Wagner's reagents to detect alkaloids, 5 % Ferric chloride anhydrous for phenolic compounds detection, 25% Lead acetate for detection of flavonoids, Sudan-I for total lipids.¹⁸ Lugol's iodine solution was used for starch¹⁹ and concentrated sulphuric acid for saponin. The thin sections were stained with the above reagents and the images were recorded on photomicrographs with digital camera (Sony DSC-W230, China). The presences of active constituents were confirmed through color development due to the reaction of cells with specific reagents. Histochemical observations can help in identifying the major chemical constituents. This can be further confirmed by carrying out qualitative tests. These data can contribute in determining the standards for the plant drug under study and can contribute to the Ayurveda pharmacopoeia of India. Descriptive terms of the anatomical features are taken from the standard anatomy book.²⁰

Shade drying and powdering of the collected plant material

Freshly collected aerial plant parts were cleaned to remove adhering dust and then shade dried at 31°C for 15 days. The shade dried plant materials were mechanically ground to coarse powder and passed through a Willy Mill to get 60-Mesh size and used for physicochemical, phytochemical, fluorescence studies and HPTLC quantification.²¹

Soxhlet extraction

The air dried aerial plant powder (50 g) of *Lepidagathis scariosa* was extracted using Soxhlet apparatus successively with different solvents in the increasing order of polarity (Petroleum ether (60-80°C) and Ethanol (78.5°C)(50g/250 ml)) for 6-8 hrs. Finally, the material was macerated using hot water (99.98°C) with

occasional stirring for 16 hrs and the water extract was filtered. The different solvent extracts were concentrated, vacuum dried and weighed. The percentage of yields was expressed in terms of air dried sample.²² The prepared extracts were subjected to phytochemical analysis and HPTLC studies.

Physico and phytochemical studies organoleptic characters of plant powder and the extract

The organoleptic evaluation of aerial plant powder and the extracts such as color, texture, odor and taste were carried out as Tease and Evans (1983). The behavior of powder with different chemical reagents was assessed by the following methods of: Kokoshi (1958).

HPTLC analysis

5, 6, 7 and 8 µl of the test solution and 2 µl of standard solution were loaded as 6 mm band length in the 10 x 10 cm Silica gel 60F₂₅₄ (E. MERCK KGaA). TLC plate using Hamilton 100 µl syringe (stationary phase) and CAMAG Linomat V _170147" S/N 170147 (1.00.12) instrument with a dosage speed of 150 nl/s was used for application of samples. The plate containing the loaded samples was kept in TLC Twin Trough developing chamber (after saturated with solvent vapor) with its respective mobile phases (Flavonoid-Toluene-acetone-formic acid (4.5: 4.5: 1)) and the plate was developed up to 70 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo documentation chamber (CAMAG REPROSTAR 3) and images were captured at white light, short UV (254 nm) and long UV (366 nm). The developed plate was sprayed with respective spray reagent (inert gas) for color reaction and dried at 60°C in hot air oven for 5 minutes. The plate was photo-documented at day light, UV 254 nm and 366 nm mode using photo documentation (CAMAG REPROSTAR 3) chamber.

Densitometry evaluation of the chromatograms

After derivation, the plate was fixed in scanner stage (CAMAG TLC Scanner 3) and scanning was done at 500 nm.¹⁵ The peak table, peak display and peak densitogram were noted.²⁵

STATISTICAL ANALYSIS

For extractive value studies the values are expressed as mean (n = 6).

RESULTS AND DISCUSSION**Macroscopically studies**

The present macroscopical investigations of aerial parts of *Lepidagathis scariosa* revealed that the species is perennial prostrate under shrub that grows up to 30-120 cm tall as shown in Table 1. The surface of the stem is covered with dense pale buff to golden stellate or dendritic hairs. Leaves are opposite decussate, usually entire, and lineolate with prominent raphides and 2-3 cm in long. The type of inflorescence is ovoid terminal cymes. The flowers are often with an expanded basal portion, sessile with congested heads, stellate pubescent, fertile and sterile floral bracts are sub-equal in size, purple-white and scarious. Fruit is capsule, 8.5-

10 mm long and shortly pubescent towards apex. Seeds - 2-4 flattened and covered with hairs. Several earlier workers have described morphological features as one of the effective parameters for the pharmacognostical identification of crude drugs.^{15, 26,}

Microscopical studies T.S of stem

Microscopic method is one of the simplest and cheapest methods to start with establishing the correct identification of the source materials²³ Botanical detection of a phyto drug involves two steps. One is identification of the plant by its floral characters and the other is diagnosis of the plant with its microscopic characters. The latter procedure is useful for identification of fragmentary plant specimens. For authentic studies, the anatomical work was carried out in *Lepidagathis scariosa*. The T.S section of the young stem shows circular in outline and it is 1.9 mm thick. It shows initial stage of secondary growth. The transverse section consists of epidermis, cortex, vascular bundle and pith. The epidermis contains single layer of elongated cells with thick cuticle. The outer epidermal cells have dense multicellular trichomes. The cortical

region is made up of 4 to 6 layers of sclerenchymatous hypodermal cells and patches of chlorenchyma cells are seen in hypodermal region. Below the hypodermal cells, 5-6 layered angular, tightly packed parenchymatous cells are present. The vascular bundles are arranged in continuous ring and it contains phloem, cambium and xylem components. Just below the cortical region secondary phloem is present in the form of bast fibers followed by cambium. Below this, inner secondary xylem is seen and it is made up of tracheids, vessels and xylem parenchyma, which is differentiated into autumn wood and spring wood later form annual rings. Patches of primary xylem is present in center and it is end arch in nature. It is followed by primary phloem. The center pith region is wide made up of circular to angular parenchymatous cells arranged compactly without intercellular spaces. The parenchymatous pith contains prismatic calcium oxalate crystals in their spaces (Figure 2). Early plant morphologist Robert Hook (1605-1703) clearly demonstrated that each kind of plant has its own distinctive structure by means of which it can be recognized.

Table 1
Macroscopic observations of *Lepidagathis scariosa*

S.No.	Macroscopic characters
1.	Habit : Prostrate under shrubs Stem: Stems with dense pale buff to golden stellate hairs Height: Much-branched, 30-120 cm tall Surface: Thick and hard Wood: Light greenish, hard Texture: Thick and hard Taste: Bitter Odour: Characteristic Color: Greenish than become light brown
2.	Leaves: Leaves are opposite decussate, usually entire, lineolate with prominent raphides, 2.8-9 × 1-3.6 cm Size: 2-3 cm in long, about 5 cm wide Surface: Hairs often long-armed; lateral veins 4-6 pairs Petiole: 1.5 cm long without stipules Texture: Rough Taste: Bitter Odour: Characteristic Color: Dark green
3.	Inflorescence: Heads terminal ovoid cyme Flowers: Small, purple-white, Calyx - 7-ribbed, deeply lobed, scarious with short glandular hairs. Corolla - 2lipped usually spotted with purple color.
4.	Fruit : Capsule
5.	Seeds : 2 or 4, flattened and hairy

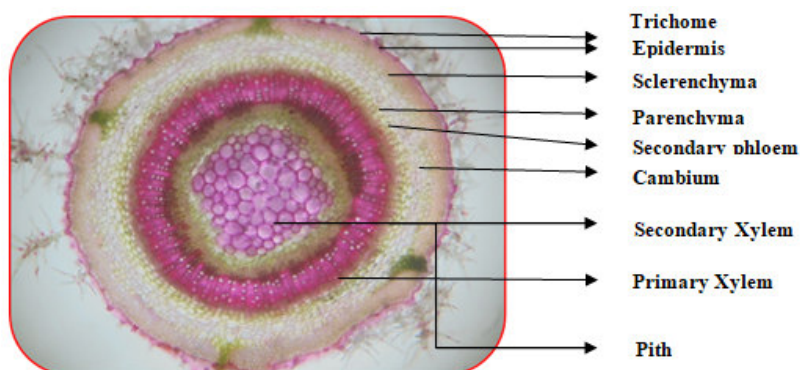


Figure 2
T.S of *Lepidagathis scariosa* stem

T.S of leaf

The transverse section of the leaf is shown in Figure. The T.S of the leaf has thick midrib region, lateral veins and thin dorsiventral lamina. The leaf is Plano convex towards lower region and towards upper region, it is slightly bulged. T.S. of midrib region is differentiated into upper and lower epidermis; it consists of multicellular stellate trichomes. Both the epidermal cells are rectangular in shape and covered by means of cuticle. In the upper epidermal region below the epidermal cells in the ridge region, 2-4 layers of compactly arranged collenchymatous cells are present followed by 2-3 layered globular, ovoid, elongated and irregularly arranged palisade cells with chloroplasts. Mesophyll region consists of patches of spongy parenchymatous cells without intercellular spaces. In the center, vascular bundle is represented by xylem and phloem present in the midrib region. The vascular bundle is collateral in nature. Large xylem and phloem cells are present towards the lower side of the midrib. There are 10-13 xylem strands are present. Protoxylem vessels exist towards the upper side of the leaves. The xylem part of the strand has several long parallel compact lines of xylem elements. The elements are narrow, elliptical and thick walled. Phloem occurs in broad spectrum and distributed in abaxial and ad axial surface of the xylem components. Below the vascular region, 5-6 layered of

rounded parenchymatous cells are present with large intercellular spaces. Maximum cells contain prismatic calcium oxalate crystals in their spaces. The leaves contain numerous paracytic stomata. From the anatomical point of view, the foliar epidermal studies are one of the most noteworthy taxonomic studies (Bhatia, 1984; Jones, 1986). However, some aspects concerning trichomes, cuticle characters, patterns and occurrence of stomata on the leaf surface are of considerable taxonomic value and may help to distinguish one species from another.^{29, 30, and 31} Transverse section of the leaf through lamina region shows the same upper epidermis as shown in the midrib region, covered by cuticle and some of the epidermal cells elongate to form elongated multicellular trichomes. The palisade cells are arranged in 2-3 layers. The cells are wide, cylindrical and compactly arranged. Lamina region also consists of clustered calcium oxalate crystals. The lower region consists of stomata and of paracytic type. Hence these characteristics could be used to identify and to know the adulterants if any with this species.³² Uno *et al.* (2001) stated that ergastic substances in plants are known to be objects of defensive mechanism for warding off or discouraging herbivores. The presence or absence of micro-characters in plant system like calcium oxalate crystals has been used to understand the evolutionary relationships of plant species.³³

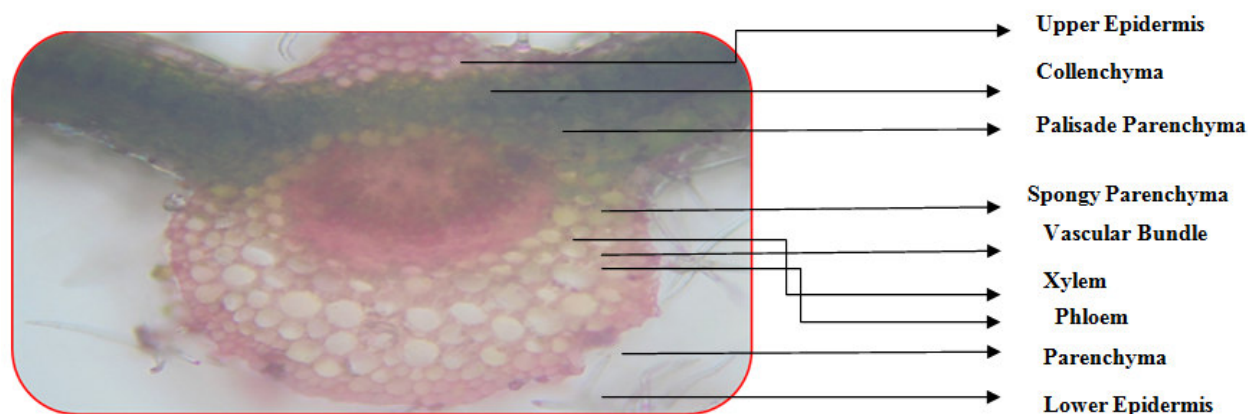


Figure 3
T.S of *Lepidagathis scariosa* leaf

Histochemical studies

In the recent times, pharmacognosists have started (Figure 4 a-f) histochemical features in solving taxonomic problems and also the identification and characterization of plant drug. The present study screens the presence of alkaloids, phenols, flavonoids, lipids, starch and saponin in stem and leaf of the *Lepidagathis scariosa* which could be used as a diagnostic tool for this plant drug. The presence of phenols, flavonoids, lipids, starch and saponin were confirmed through colour development due to the reaction of cells with specific reagents provided chemical markers that could be used in identification. Alkaloids, phenols, flavonoids and saponin are compounds which are present commonly in plants, have been reported to have a wide range of biological activities including antioxidant properties. The presence of alkaloids, phenols, flavonoids and saponin in this study were also confirmed by the qualitative analysis of

Lepidagathis scariosa and hence supported the therapeutic potentials of the plant drug under study. Transverse stem section of *Lepidagathis scariosa* treated with Wagner's reagent showed the presence of alkaloids (Brown color) in the outer epidermal region and in vascular bundles (Figure 4 - a). Figure 4 - b revealed the presence of phenolic (brown to black) treated with Anhydrous FeCl₃ in the region of epidermis and internal cortical region. Figure 4 -c shows the presence of flavonoids in the region of secondary xylem (pink color to yellow color) when treated with Lead acetate. The section shows the presence of Lipids (yellow color) in the secondary xylem when treated with the Sudan-I (Figure 4 - d) reagent. The presence of starch (pink patches) occur when treated with Lugol's reagent (Figure 4 - e) in the region of vascular traces and cortical regions. Figure 4 - f shows the presence of saponin in the epidermis (bluish black) when treated with concentrated sulphuric acid. The transverse section

of leaf concealed the presence of alkaloid in the mesophyll region (dark brown) when treated with Wagner's reagent (Figure 5 - a). Figure 5 - b - shows the presence of phenol (Brown to black precipitate) when treated with the Anhydrous $FeCl_3$ reagent in the region of vascular bundles. The flavonoid is present in the lower part of mesophyll region treated with the Lead acetate (yellow color) (Figure 5 - c). Figure 5 - d discloses the presence of Lipids (yellow) when treated with Sudan-I reagent in the region of lamina. For detection of starch, the sections were treated with Lugol's iodine solution which shows positive result (pink patches) in the epidermis (Figure 5 - e). Figure 5 - f proclaim the presence of saponin (bluish black) in the xylem region when treated with concentrated sulphuric acid. These results are in accordance with the results of Kathiresan(2011).He studied the histochemical studies of *Viburnum punctatum* that showed the presence of

tannins in phloem region, mucilage in mesophyll region and lignin in vascular bundle. In case of root, all ergastic cell content such as lignin, starch grains, saponins and tannins were confined within xylem region of the organ in *Viburnum coriaceum*.³⁴ The histochemical colour reactions were carried out through transverse sections of leaf, stem and root of *Sven Sonia hyderbadensis* studied.³⁵ In the present investigation, the data obtained on histochemical studies on *Lepidagathis scariosa* leaf and stem concluded the presence of alkaloids, polyphenols, flavonoids, lipid, starch and saponin which are of great medicinal value and find extensive use in the drug preparation and pharmaceutical industry. Results from this work therefore supports that the *Lepidagathis scariosa* could be used as an alternative in the management of various ailments such as anticancer and wound healer.

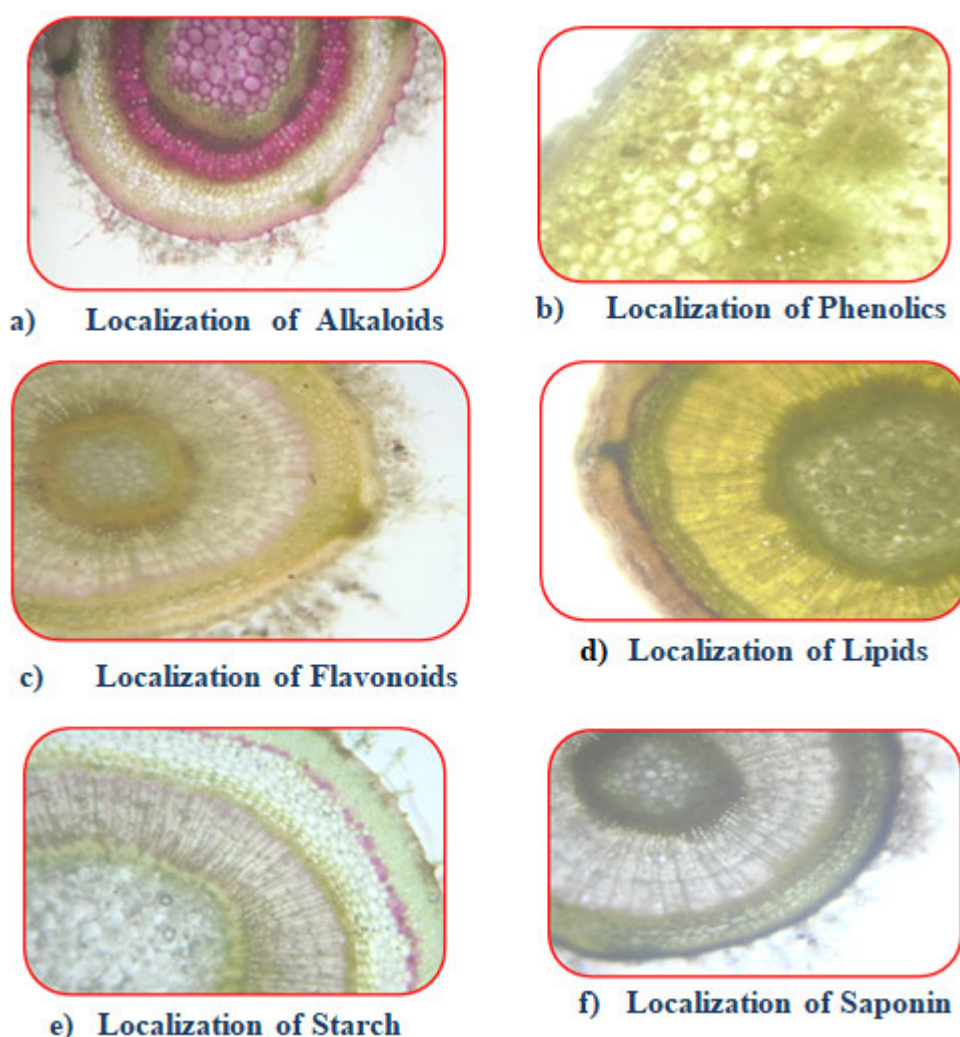


Figure 4
Histochemical localization of *Lepidagathis scariosa* – stem

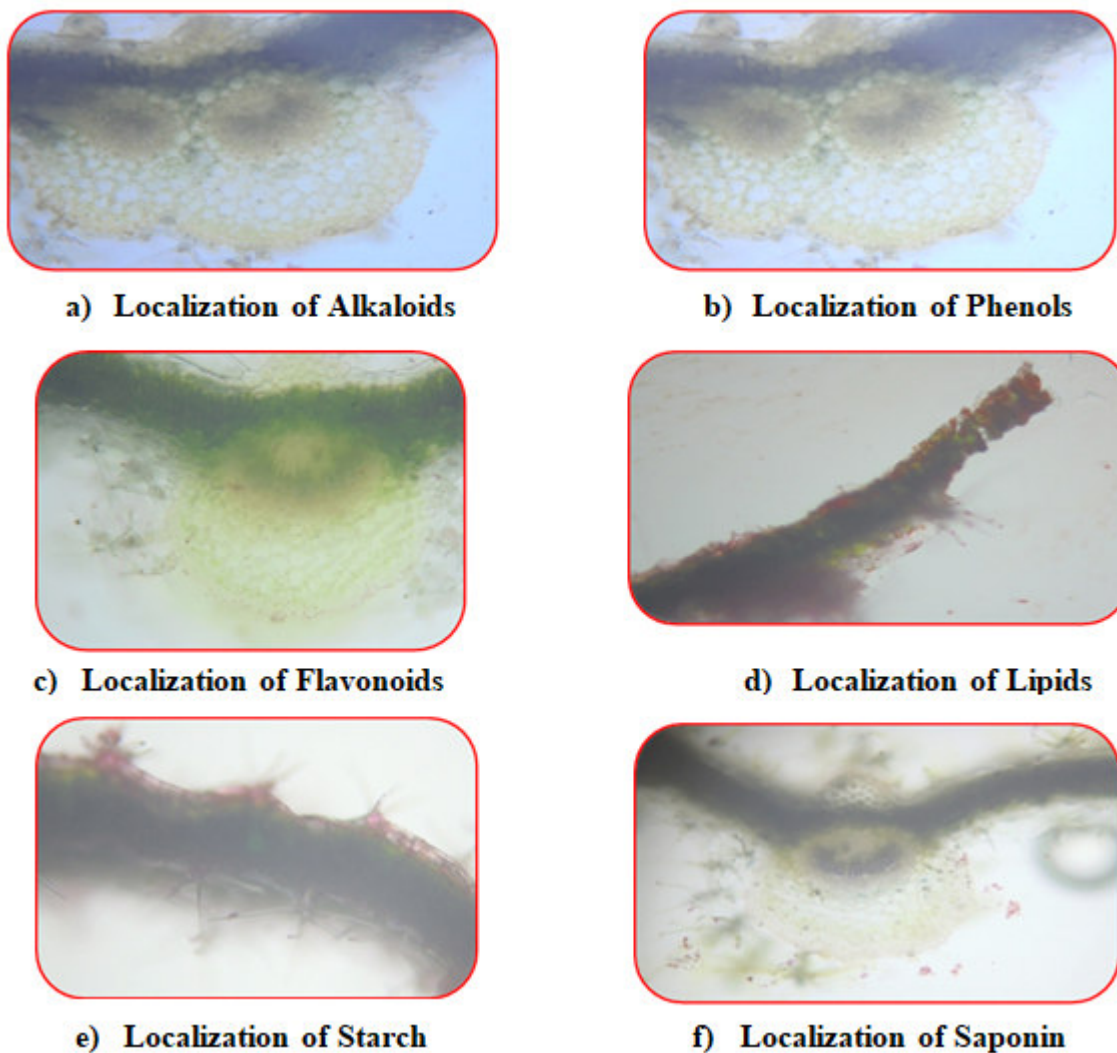


Figure 5
Histochemical localization of *Lepidagathis scariosa* – leaf

Powder microscopical study

The microscopic study of powder revealed the presence of bunch of raphides and fiber. The lengths of fiber are nearly 2mm. The multicellular stellate trichomes are

slightly curved and numerous paracytic stomata are seen in the powder. The raphides are short, cylindrical blunt bundles comprising several thin pointed needles (Figure6).



Figure 6
Stomata and powder studies

Physico-chemical studies

Organoleptic characters of plant powder and the plant extract

The plant powder showed characteristic odor and bitter taste. Upon drying and powdering, the color of the powder changed from dark green to greenish yellow as

shown in Table 2. The organoleptic characters such as color, consistency and odor were noted in the petroleum ether, ethanolic and aqueous aerial plant extracts of *Lepidagathis scariosa* (Table3). Organoleptic profile is one of the many diagnostic parameters in the proper identification of raw materials.^{36-38.}

Behavior of plant powder with different chemical reagents

The behavior of plant powder with various reagents were observed and presented in Table 4. Powder treated with acetone, benzene, 5 % Ferric chloride and

methanol showed greenish shades. Where as in powder reacts with NaOH + water, NaOH + methanol and glacial acetic acid showed brown shades. Powder treated with 40 % NaOH + Lead acetate yield milky white shades.

Table 2
Organoleptic characters of *Lepidagathis scariosa* plant powder

Characters	Observations
Color	Greenish yellow
Texture	Coarse
Taste	Bitter
Odor	Characteristic smell

Table 3
Organoleptic characters of successive extracts of *Lepidagathis scariosa*

Extraction medium	Color	Consistency	Odor
Petroleum ether	Light green	Semi solid	Characteristic smell
Ethanol	Blackish green	Semi solid	Characteristic smell
Aqueous	Light brown	Semi liquid	Characteristic smell

Table 4
Behavior of *Lepidagathis scariosa* plant powder with different chemical reagents

S. No.	Powder + Reagents used	Color of the powder
1.	Acetone	Light green
2.	Benzene	Pale green
3.	5 % Ferric chloride	Light green
4.	Methanol	Dark green
5.	NaOH + Water	Brown
6.	NaOH + Methanol	Light brown
7.	Glacial acetic acid	Yellowish brown
8.	40 % NaOH + Lead acetate	Milky white

Fluorescence behavior of plant powder

Analytical tests based on fluorescence behavior in day light and UV light can be used to check identity of powdered drugs.²³ Fluorescence behavior of *Lepidagathis scariosa* plant powder after treating with different chemical reagents was observed in daylight as well as UV light at short (250-270) and long (365-390) wavelengths and the observations are presented in Table 5. In visible light the powder as such gives different green shades. The same powder under short UV light at 250-270 nm shows dark green shades and in

long UV at 365-390 nm shows black shades. The powder treated with 1N NaOH in water, 1N NaOH in ethanol, 1 N HCl, 50 % H₂SO₄ and 50 % HNO₃ shows slight color differences like dark green, bluish green to yellowish green under different wavelengths of UV light and pale green to dark green and brown shades under visible light. In the present examination, the 365-390 nm (long) shows black observations in maximum treated reagents. There are no distinct color differences in short and long UV lights.

Table 5
Fluorescence behavior of *Lepidagathis scariosa* plant powder

S. No.	Reagents	Behavior of plant powder		
		Visible light	UV light	
			Short (250-270nm)	Long (365-390nm)
1.	Powder as such	Light green	Deep green	Black
2.	Powder + 1 N NaOH in water	Deep green	Brownish green	Dark black
3.	Powder + 1 N NaOH in ethanol	Dull green	Green	Bluish green
4.	Powder + 1 N HCl	Pure Green	Yellowish green	Dark green
5.	Powder + 50 % H ₂ SO ₄	Dark green	Blackish green	Black
6.	Powder + 50 % HNO ₃	Brown	Greenish yellow	Black

Successive solvent extraction**Percentage yield**

The air dried, powdered sample was extracted with different solvents for the phytochemical and HPTLC chemical compound examination studies. The yield of different solvent extracts during successive solvent

extraction was calculated and presented in Table 6. The percent yield was maximum in ethanol extract (8.2 %) followed by petroleum ether extract (7.5 %). The aqueous extract shows minimum yield (6.4 %). Earlier similar studies have revealed the extractive value profile in several medicinal plants.^{39, 40, 41}

Table 6
Extractive values of *Lepidagathis scariosa* powder in various solvents

Method of extraction	Solvents used	Yield (%)
Continuous hot percolation using Sox let apparatus	Petroleum ether	7.5
	Ethanol	8.2
Hot and cold maceration	Aqueous	6.4

HPTLC analysis

According to WHO, it has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards.⁴²The result analysis of HPTLC profile for flavonoid of *Lepidagathis scariosa* petroleum ether extract is given in Table 7. Yellow-brown coloured zones were detected in the TLC plate, confirms the presence of flavonoids. Further, two different flavonoids with RF value 0.71 and 0.92 were recorded in the chromatogram after derivatization. Among the two flavonoids, the maximum peak area is occupied by Flavonoid 2 (0.92).

The TLC procedure for *Senna alata* flowers with a view to separate the compounds and to identify one of the phytochemical flavonoids in the ethanolic extract.^{43, 44}This newly developed and validated HPTLC method provides a new approach to estimate major plant flavonoids like quercetin, rutin, luteolin and vitex in extract of plants like *A. racehorses*, *Withania somnifera*, *Vitex negundo*, *Plumbago zyleneica*, *B. monosperma* and *T. purpurea*.⁴⁵ Figure 7 shows the HPTLC chromatogram of flavonoid. Densitogram peak and baseline peak scanned at 254 nm and 366 nm for the standard and test sample with regard to flavonoid are displayed in Figure 8 and 9.

Table 7
HPTLC profile of petroleum ether extract of *Lepidagathis scariosa* for Flavonoids and unknown compounds

Track	Peak	Rf	Height (mm)	Area (AU)	Assigned substance
Petroleum ether extract of <i>Lepidagathis scariosa</i> (5 µL)	1	0.02	27.5	5.90	Unknown
	2	0.04	17.6	3.17	Unknown
	3	0.12	14.3	7.14	Unknown
	4	0.55	19.2	2.71	Unknown
	5	0.65	23.1	15.47	Unknown
	6	0.78	0.7	11.88	Unknown
	7	1.02	286.7	53.72	Unknown
Petroleum ether extract of <i>Lepidagathis scariosa</i> (6 µL)	1	0.04	5.9	13.60	Unknown
	2	0.48	12.8	2.88	Unknown
	3	0.56	43.5	30.59	Unknown
	4	0.69	12.0	48.32	Unknown
	5	0.94	0.1	4.61	Unknown
Petroleum ether extract of <i>Lepidagathis scariosa</i> (7 µL)	1	0.02	35.8	3082.6	Unknown
	2	0.05	11.1	445.9	Unknown
	3	0.53	52.5	2714.8	Unknown
	4	0.56	51.8	992.0	Unknown
	5	0.66	13.4	4056.5	Unknown
	6	0.71	5.3	385.9	Flavonoid 1
	7	0.91	0.3	784.1	Unknown
	8	0.97	4.2	340.5	Unknown
Petroleum ether extract of <i>Lepidagathis scariosa</i> (8 µL)	1	0.02	33.9	3022.0	Unknown
	2	0.05	10.5	371.7	Unknown
	3	0.59	49.9	2871.9	Unknown
	4	0.65	65.7	1457.0	Unknown
	5	0.74	26.7	3691.9	Unknown
	6	0.79	23.6	1124.9	Unknown
	7	0.92	68.3	2912.0	Flavonoid 2

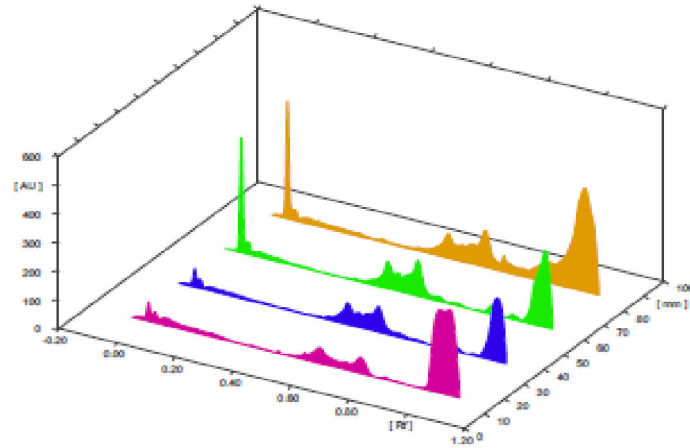


Figure 7
3D display of HPTLC chromatogram of petroleum ether Extract of *Lepidagathis scariosa*

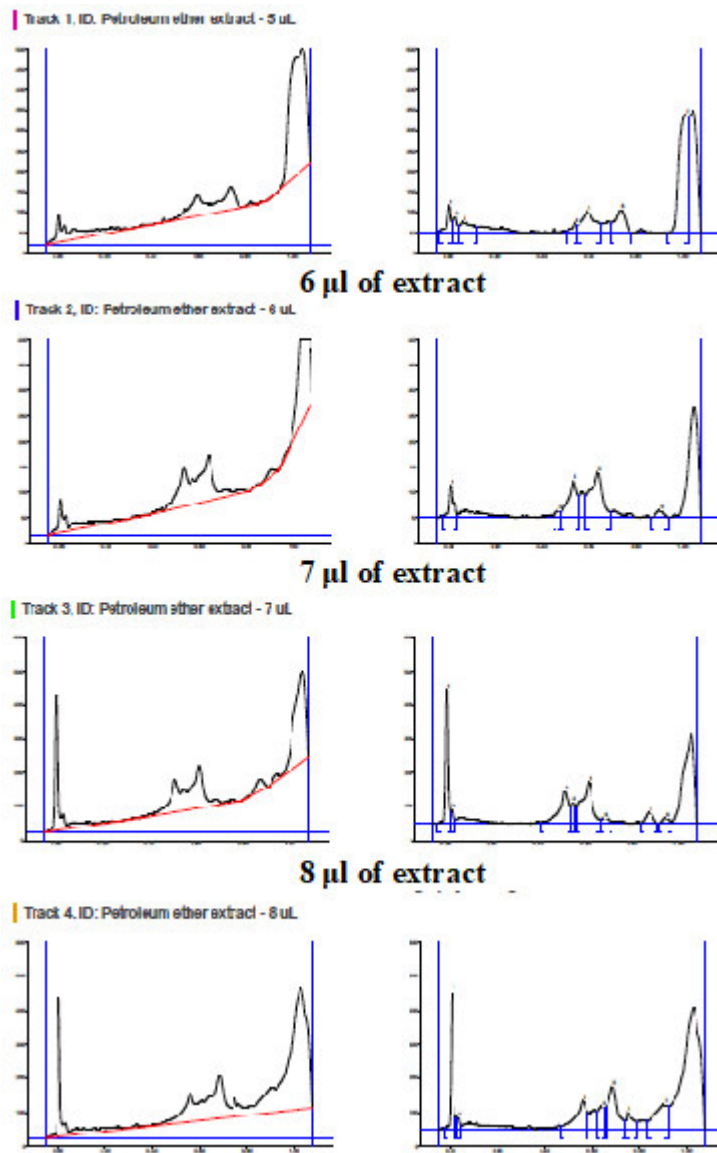


Figure 8
Densitogram and base line for flavonoid of petroleum ether Extract of *Lepidagathis scariosa*

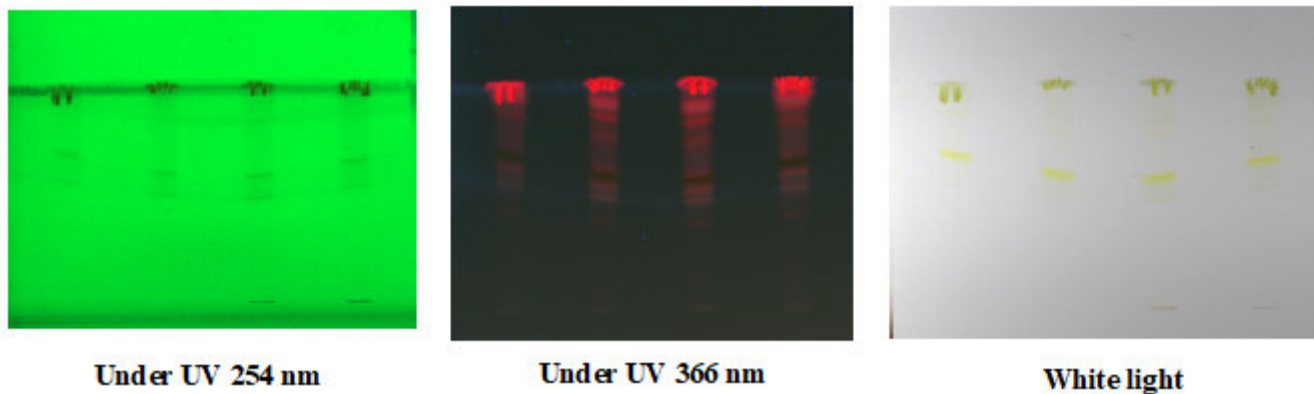


Figure 9
Chromatogram after derivatization for flavonoid of petroleum ether
Extract of *Lepidagathis scariosa*

CONCLUSION

The outcome of study strongly supports the usage of this straggling shrub to human society. Further, the isolation, identification, purification, characterization and structural elucidation of bioactive compounds from this species are taken under investigation. The current study may be of great use and interest to the researchers, pharmaceutical industries and medical practitioners. The plant species selected for the study can be used as a potential source of useful drug. It also justifies the folklore medicinal use and claims therapeutic values of study plant as a curative agent. All characters obtained from the results are also required for database

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preparation in this digital world by which further experiments or research can be done.

AUTHOR CONTRIBUTION STATEMENT

A to investigate and supervised the findings of this work. B, C and D performed to take plant section, characterization, phytochemical screening and to the writing of the manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

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