ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26mar306

Phytochemical and Histochemical Analysis of *Sphagniticola trilobata*

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Publication Date: 2026/03/14

Abstract: *Sphagneticola trilobata* (Asteraceae) is a widely used medicinal plant known for its antimicrobial and anti-inflammatory properties. This study aimed to perform a detailed preliminary phytochemical screening and histochemical localization of active compounds in the leaves of *S. trilobata.* Shade-dried leaves were subjected to sequential extraction using solvents of varying polarity (Petroleum ether, Chloroform, Methanol, and Water). Qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, terpenoids, tannins, and glycosides, with methanol extract showing the highest diversity of metabolites. Histochemical investigations of leaf tissues using Sudan III and Nadi reagent confirmed the localization of lipids and terpenoids within the glandular trichomes and internal secretory canals. These findings provide baseline data for the standardization, quality control, and further isolation of active compounds from *S. trilobata.*

How to Cite: Khushabu Naresh Magar; Dr. Shahzeen Mohammad Khan (2026) Phytochemical and Histochemical Analysis of *Sphagniticola trilobata*. *International Journal of Innovative Science and Research Technology*, 11(3), 686-691. https://doi.org/10.38124/ijisrt/26mar306

I. INTRODUCTION

Botanical-based traditional medicines are increasingly recognized for their therapeutic efficacy and superior safety profiles compared to synthetic alternatives. The green kingdom continues to captivate researchers as a significant, accessible, and cost-effective reservoir of bio-active, life-enhancing compounds. For roughly 80% of the global population across Asia, Latin America, and Africa, traditional, plant-derived remedies remain the primary healthcare choice due to their minimal side effects.

Sphagneticola trilobata (L.) Pruski, belonging to the Asteraceae family and widely known as the 'Singapore daisy' or 'creeping-oxeye,' is a perennial, mat-forming herb with remarkable adaptability and ornamental value. Widely recognized in folk medicine, it is frequently utilized to treat a variety of ailments, ranging from sore throats to skin conditions.

➢ *Phytochemical Profile*

Phytochemicals are specialized secondary metabolites, synthesized naturally within various plant parts—including leaves, stems, and roots—serving as defence mechanisms and potent biochemical sources. Qualitative, comparative screenings of *S. trilobata* (also known as We Delia trilobata) demonstrate that extraction efficiency is heavily dependent on the solvent used, such as ethanol, chloroform, or water.

Scientific evaluations have revealed that *S. trilobata* possesses a rich phytochemical profile, with at least ten key compounds detected—including alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, proteins, amino acids, and carbohydrates. Notably, the ethanolic extract of the aerial parts often exhibits the highest concentration of these bioactive constituents.

➢ *Therapeutic Potential and Future Directions*

The search for natural products rich in antimicrobial, antioxidant, and anti-inflammatory properties has intensified due to their crucial role in managing chronic disorders, including hypertension, diabetes, and cancer. *S. trilobata* has shown promising results in treating wounds, infections, liver diseases, and rheumatism. Furthermore, the plant's ability to act as a natural, low-cost therapeutic agent makes it a valuable target for pharmaceutical companies seeking to produce affordable, sustainable medicine for the general population.

Coe et.al. have reported that fruits, leaves and stem are used in childbirth and in the treatment of bits and infection. leaves are used in the treatment of kidney dysfunctions, cold, wounds and annenorhaea and dysmenorrhea.

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissue. The discipline of histochemistry lies on the boundary between histology and biochemistry

II. REVIEW OF LITERATURE

Phytochemical screening of the medicinal plants is having greater significance because the therapeutic utility of the medicinal plants mainly depends up on the presence of secondary metabolites present in them. The secondary metabolites may be present or accumulated in a particular plant part or distributed throughout the plant. It is always that

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26mar306

part of the plant which contain maximum amount of secondary metabolite is designated as drug.

Nowadays more and more people concern about the healthcare- related natural products, especially the pharmacological properties of medicinal herbs and edible plants. Antitumor is one of the numerous pharmacological effects that have raised public interest. In this study, Wedelia tilobata was extracted and fractionated, and the antitumor activites of the fractions were investigated using in vitro and in vivo assays.

- Identify – Family Asteraceae /composite sphagneticloa trilobata
- Scientific Name- *Sphagneticola trilobata*
- Common Name- Pruski Singapore daisy wedelia *sphaneticola trilobata* commonly known as the bay Biscayne creeping oxeye.

- *Classification-*

✓ Kingdom: - plantae
✓ Phylum:-Tracheophyte/aspematophyla
✓ Sub-phylum: - Angiospermae
✓ Class: - Magnoliopsida
✓ Order:- Asterales
✓ Family: - Asteraceae
✓ Genus: - Sphagneticola
✓ Species:- *Sphagneticola trilobata*

- *Morphological Characteristics: -*

It is native to Mexico, central America and the caribben, but now grows throughout the neotropics.

✓ Habit: - Mat forming perennial herb with rounded stems.
✓ Root: - rooting at the nodes, adventitious.
✓ Leaves: - Leaves are fleshy, hairy and dark green above and lighter green below.
✓ Stem:- has rounded stem up to 40cm long. Solid with few hairs less green and Redish.

- *Medicinal Uses: -*

Its therapeutic effect for ulcer, sore throat, varicose, headache, fever, epilepsy, amenorrhea, snakebite, wounds, kidney dysfunction, hepatitis, cold and indigestion.

It is reported that Singapore daisy is used to indigestion due to sluggish liver, white tools burning in urine/stopping of urine and for infections.

The levees are used in dyeing grey hair and in promoting the growth of hair.

III. MATERIAL & METHODOLOGY

A. Collection of Plant Material -

The leaves of Sphagneticola trilobata (L.) Puru ski. Collected leaves were shade dried and was ground using m molten and pestle to obtain a fine powder. The powder was further passed through a 2mm sieve to obtain finer particles. The powdered samples were stored in a clean glassware container until needed for analysis.

B. Histochemical Investigation

- *Methodology –*

These sections were observed under a microscope and they photographed with a help of a digital camera. The different tests performed were as follows:

- *Starch Detection (Iodine Test):*

To identify the location of starch, thin tissue sections were treated with a working iodine solution. This reagent was prepared by dissolving 0.3 grams of iodine and 1.5 grams of potassium iodide into 100 ml of distilled water. A small amount of this iodine-potassium iodide [KI2]solution was applied directly to the specimen, followed by a water rinse to remove excess reagent. Under microscopic observation, areas containing starch reacted with the iodine to produce a distinct blue-black colour, allowing for the precise mapping of starch granules.

- *Protein Detection (Picric Acid Method):*

For the localized detection of proteins, a saturated aqueous solution of picric acid was used, which acts as a powerful protein-precipitating agent. This method stains proteinaceous structures an intense yellow. The tissue sections were soaked in the reagent for a duration of 24 hours to ensure thorough penetration and reaction. Afterward, the tissues were examined under a microscope to visualize the yellow-stained, precipitated protein structures.

- *Tannin Detection:*

To localize tannins, sections were submerged in a dilute acidic ferric chloride solution (prepared with 0.5%–1% $FeCl_3$ in 0.1N HCl). After treatment, the samples were mounted in clove oil. The presence of tannins was confirmed by the development of a blue-green coloration under microscopic view.

- *Saponin Analysis:*

For saponin localization, specimens were initially soaked in a saturated barium hydroxide solution for a 24-hour period. Following a wash with calcium chloride, the sections were transferred to potassium dichromate. A yellow hue served as a positive indicator for saponins.

- *Lipid/Fat Staining:*

A staining solution was prepared by dissolving 0.5g of Sudan III or Sudan IV dye in 70% ethanol. The sections were immersed in this dye for 20 minutes, briefly rinsed with 50% alcohol, and then mounted in glycerine. The appearance of blue, red, or pink precipitates signaled the presence of fats.

- *Glucoside (Grignard's Test):*

To test for glucosides, sections were first soaked in 1% aqueous picric acid for half an hour. After rinsing with water, they were treated with 10% sodium carbonate. The

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emergence of a red color upon the addition of hydrochloric acid confirmed the presence of glucosides.

- *Alkaloid Identification:*

Plant transverse sections were exposed to Mayer's Reagent (a mixture of 13.55g $HgCl_2$ and 50g KI in 1000ml distilled water). The formation of a greyish tint within the tissue sections was used to identify the presence of alkaloids.

➢ *Mayer's test (Evan, 1997)-*

- *Sample Preparation of Plant Material -*

1 gram of each sample was taken and extracted in Soxhlet apparatus successively with ethanol, acetone, chloroform, and water. After extraction, the extracts were rotary shaker used Shaked using filtered through Whatman No.1 filter paper and stored for further phytochemical investigations

- *Preliminary Phytochemicals Investigation -*

➢ *Qualitative Phytochemical Screening*

- *Test for Alkaloids*

✓ Mayer's Test: A small volume of filtrate was treated with Mayer's reagent (prepared by mixing 1.358g mercuric chloride in 60ml water with 5.0g potassium iodide in 10ml water, diluted to 100ml). The reagent was added carefully along the side of the test tube. The formation of a creamy or white precipitate confirmed the presence of alkaloids.
✓ Wagner's Test (Wagner, 1993): A few drops of Wagner's reagent (1.27g iodine and potassium iodide solution adjusted to 100ml) were added to the filtrate. A reddish-brown precipitate served as a positive result.
✓ Dragendorff's Test (Walfi, 1965): The filtrate was treated with 1–2ml of Dragendorff's reagent. The appearance of a distinct yellow precipitate indicated a positive reaction.

✓ *Note: The reagent was prepared by boiling bismuth carbonate and sodium iodide in glacial acetic acid, filtered after 12 hours, and stabilized with ethyl acetate.*

- *Detection of Carbohydrates and Glycosides (Ramakrishnan et al., 1994)*

✓ Barfoed's Test: 1ml of the filtrate was mixed with an equal volume of Barfoed's reagent. The mixture was then heated in a boiling water bath for approximately two minutes. The development of a red precipitate indicated the presence of monosaccharides/reducing sugars.

Barfoed's reagent-copper acetate,30.5 is dissolved in 1.8 ml of glacial acetic acid.

➢ *Benedict's Test-*

To 0.5ml of filtrate 0.5ml of Benedict's Reagent is added. The mixture heated on a boiling water bath for 2 min. A characteristic-coloured precipitate indicates the presence of sugar.

- *Saponin & Glycoside Detection (Lokate, 1999)*

✓ Froth Test: To screen for saponins, 100mg of the extract was diluted in 20ml of distilled water. This mixture was vigorously agitated in a graduated cylinder for 15 minutes. The formation of a persistent foam layer (approximately 2cm high) confirmed the presence of saponins.
✓ Hydrolysis: For further characterization, 50mg of the extract underwent hydrolysis using concentrated hydrochloric acid (HCl) on a water bath for a duration of 2 hours before further testing.

- *Analysis of Proteins and Amino Acids (Fisher, 1968; Ruthmann, 1960)*

✓ Millon's Test (Tasch & Swift, 1960): 2ml of the filtrate was treated with a few drops of Millon's reagent. The emergence of a white precipitate indicated the presence of protein. (Reagent Prep: 1g of mercury dissolved in 9ml fuming nitric acid, then diluted with an equal volume of water.)
✓ Ninhydrin Test (Yasuma & Ichikawa, 1953): Two drops of Ninhydrin solution (0.005% in acetone) were added to 2ml of the aqueous filtrate. The development of a distinct purple coloration served as a positive result for free amino acids.

- *Phenolic Compounds and Tannins*

✓ Ferric Chloride Method (Mace, 1963): 50mg of the extract was dissolved in 5ml of distilled water. Upon adding a few drops of neutral 5% ferric chloride, the appearance of a dark green tint indicated the presence of phenolic constituents.
✓ Lead Acetate Method: 50mg of extract was dissolved in water, followed by the addition of 3ml of 10% lead acetate solution. The formation of a heavy white precipitate confirmed the presence of phenolic compounds.

➢ *Quantitative Estimation of Biochemical Components –*

- *Reducing Sugar-*

0.5 gm plant material crushed it with hot 80% ethanol. Then centrifuge it and pour that supernatant in Petri dish and kept it for evaporated. After evaporated add 10 ml water in Petri dish this is extract. 1ml extract was taken add 2 ml water + 3ml DNS Reagents, kept it in water bath for 5 min then add 1 ml 40% Rochelle salt and cooled it then Reading was taken at 510 nm.

- *Alkaloid –*

2.5 g plant material,50 ml 10% acetic acid was taken in conical flask & cover it with aluminium foil. allow it to stand for 3-4hrs. filter the extract using filter paper. then, extract reduce to 1/4th to original in water bath add conc. Nh4oh dropwise unit, ppt form. weight the Whatman no1 filter paper

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26mar306

& filtered the extract. Dry the filter paper weight the alkaloid content.

- *Total Carbohydrate Estimation (Anthrone Method)*

To quantify total carbohydrates, a series of standard sugar solutions were prepared and adjusted to a 1.0 ml volume with distilled water. Unknown samples were similarly diluted. Each tube received 5 ml of anthrone reagent, followed by vigorous vortexing. The mixture was incubated at 90°C for 17 minutes (or 10 minutes in a boiling water bath) and subsequently cooled to room temperature. Absorbance was recorded at 620 nm against a reagent blank. The sugar concentration in the samples was determined using a standard calibration curve.

- *Total Phenolic Content (Folin-Ciocalteu Assay)*

The phenolic concentration was determined using the Folin-Ciocalteu reagent (FCR). 1 mL of the extract was combined with 0.4 mL of diluted FCR (1:10 v/v). After 5 minutes, 4 mL of sodium carbonate solution was added, and the total volume was adjusted to 10 mL with distilled water. The mixture was left to react for 90 minutes at room temperature. The absorbance was then measured at 750 nm. Results were calculated via a catechol standard curve and expressed as mg catechol equivalents per gram of dry weight.

- *Total Flavonoid Content*

Flavonoid levels were assessed using the aluminum chloride colorimetric method. 1 mL of extract was mixed with 3 mL of water and 0.3 mL of 5% NaOH2.After 5 minutes, 0.3 mL of 10% AICL3 was added. Following another 5-minute interval, the solution was treated with 2 mL of 1 M NaOH and diluted to 10 mL. Absorbance was read at 510 nm. A quercetin calibration curve (r^2=0.999) was utilized to express the results in mg quercetin equivalents per gram.

- *Vitamin C Determination (Titrimetric Method)*

Ascorbic acid content was determined through titration against a dye. Initially, 5 ml of the working standard in 4% oxalic acid was titrated until a persistent pink endpoint was achieved (V1ml). For the samples, 0.5–5g of material was extracted in 4% oxalic acid, centrifuged, and made up to 100 ml. A 5 ml aliquot of the supernatant was then titrated (V2 ml) under the same conditions. The dye volume consumed was used to calculate the ascorbic acid concentration.

- *Protein Estimation (Lowry's Method)*

One gram of plant tissue was homogenized in 80% ethanol and centrifuged at 5000 rpm. The resulting residue was treated with 10 ml of 5% Trichloroacetic acid (TCA) and incubated at 80°C for 20 minutes. After further washing and centrifugation, the residue was incubated in Lowry's Solution A at 30°C for one hour. The collected supernatant (sample) was mixed with Lowry's Solution C and Folin-Ciocalteu reagent. The absorbance was measured at 660 nm using Bovine Serum Albumin (BSA) as the reference standard.

- *Total Amino Acid Estimation (Ninhydrin Method)*

Following the method by Krishnamurthy et al. (1989), 500 mg of plant material was homogenized in cold 80% ethanol. The mixture was diluted with distilled water and boiling ethanol, then centrifuged at 10,000 rpm. A 1 ml aliquot of the supernatant was reacted with 3 ml of alcoholic ninhydrin at 60°C for 20 minutes. After cooling and the addition of 50% ethanol, the optical density was measured at 420 nm. Glycine was employed as the standard for quantification.

➢ *Spectroscopy*

Spectroscopy is technique of scientific study using many fields of science and medicine. It is used of the fact that different absorb or emit electromagnetic radiations in different ways. These different are observed in spectra of substances by means of an instrument called spectrometer. The sample often liquid is contained in optical container called cuvette. The cuvette is placed into the spectrophotometer into light beam of varying wavelength. Many spectroscopic methods are based upon the exposure of sample substance of electromagnetic radiation. Measurement is made of the intensity of radiation absorbed, emitted or scattered by the sample and of how the intensity changes function of the energy wavelength or frequency of radiation.

IV. RESULT

➢ *Histochemical Investigation* -

Histochemical localization in different organs of the taxa under study was made, using methods described elsewhere. The initial presentation gives details about the occurrence of ergastic content or secondary metabolites, viz. starch, proteins, fat, tannin, saponin, glucosides and alkaloids in leaves.

- *Starch* -

Starch has an ordinary arrangement of molecules and therefore, shows optical anisotropy and double refraction. In starch granules, the molecules are radially arranged, therefore, in polarized light a cross pattern is seen. The morphometric variation of starch grain is so extensive that they may be taxonomically and pharmacognostically up to a limit. In the present work, for the taxa under study, starch was present in leaves which was indicated starch-by blue coloured deposits. It was present in lower amounts scattered in upper and lower epidermis, palisade, vascular bundle, pith, spongy palisade and in xylem and phloem, upper epidermis.

- *Tannin*-

Tannin is a heterogeneous group of phenol derivatives, usually related to glucosides. Sometimes tannins containing cells are conspicuously associated with a vascular tissue terminates beneath storage tissue or secretary cells of nectaries. The transverse section of leaflet of Bombax ceiba showed tannins in abundant amounts. They were observed in upper and lower epidermis, pith, palisade cells, vascular bundle and xylem indicated by blue green colure.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26mar306

- *Glucoside-*

In the present plant glucosides were restricted to the xylem, spongy palaside, vascular bundle, pith, upper epidermis indicated by reddish colour.

- *Alkaloid –*

Alkaloids are complex organic molecules containing a heterocyclic nitrogen ring, which have been widely exploited for their diverse pharmacological properties. in Bombax ceiba leaflet they were the most abundant. Alkaloids were present in almost all parts of the leaflet as tested by Mayer's reagent indicated by grey colour. Alkaloids were present in upper epidermis, palisade cells, spongy palisade, pith, vascular bundle pith, secondary xylem, secondary phloem, upper epidermis.

➢ *Histrochemical Test for Leaflet of Shpagnitocola trilobata*

Table 1 Histrochemical Test for Leaflet of *Shpagnitocola trilobata*

Sr. No.	Content	Reaction	Localization
1	Starch	+ve	Lower epidermis, spongy palisade, palisade, vascular bundle, pith, secondary xylem, secondary phloem, upper epidermis.
2	Glycoside	+ve	Spongy palisade, vascular bundle, pith, secondary xylem, upper epidermis.
3	Tannin	+ve	Lower epidermis, spongy palisade, palisade, vascular bundle, pith, secondary xylem, upper epidermis.
4	Alkaloid	+ve	Lower epidermis, spongy palisade, palisade, vascular bundle, pith, secondary xylem, secondary phloem, upper epidermis.

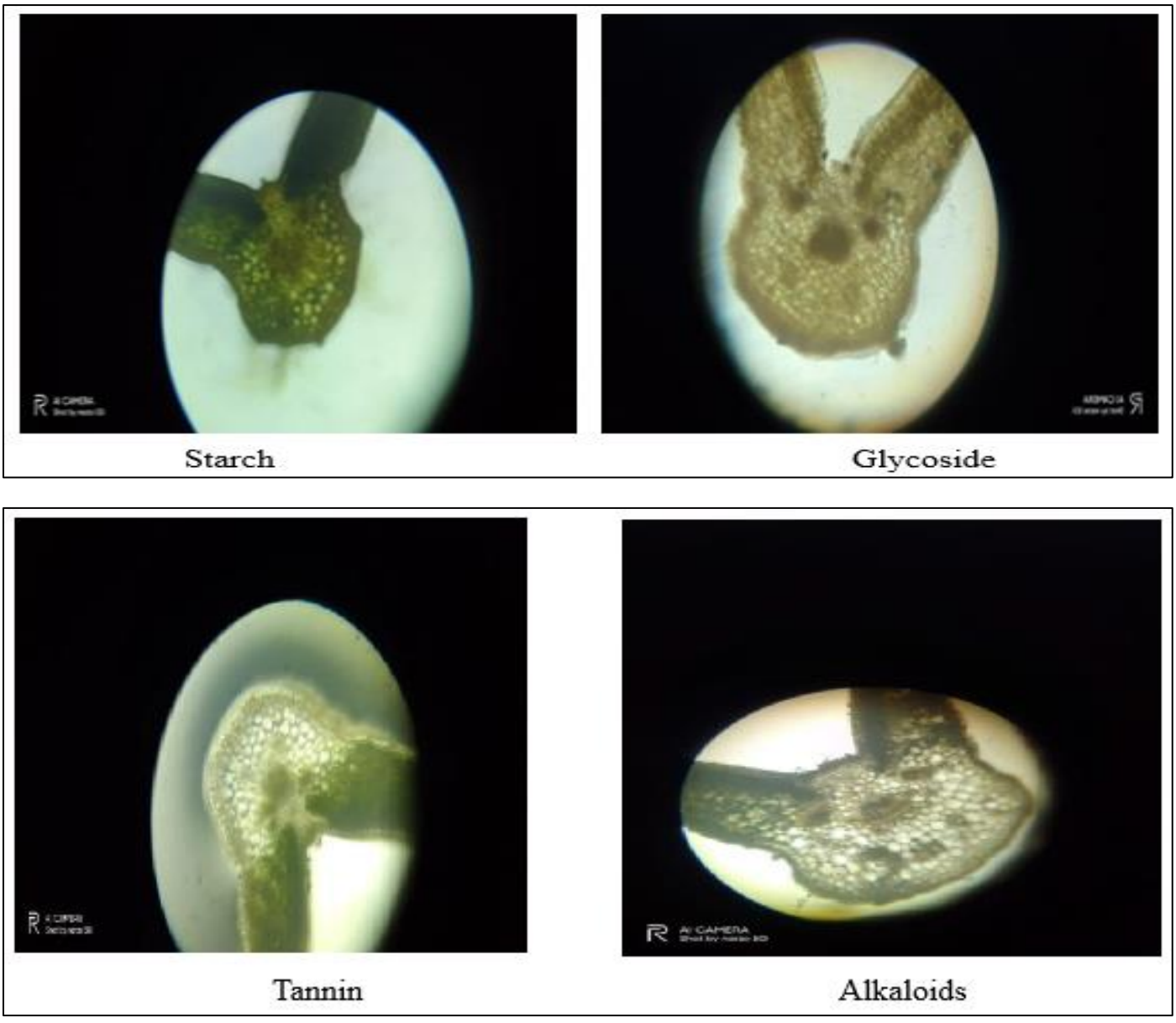

Fig 1 Histochemical Investigation Transverse Section of *Shpagniticola trilobata.*

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26mar306

➢ *Qualitative Phytochemical Screening*

Table 2 Qualitative Phytochemical Screening

Sr. No.	Phytochemical Test	D.W	Ethanol	Acetone	Chloroform
1	Alkaloids				
A	Mayers Reagent	-	-	-	+
B	Wagners Reagent	-	-	-	+
C	Dragendorffs Reagent	+	+	+	+
2	Protein and amino acid				
A	Millon's Reagent	-	+	-	+
B	Ninhydrin Reagent	_	-	-	-
3	Phenolic Reagent				
A	Ferric Chloride Test	-	-	-	-
B	Lead Acetate Test	-	-	-	+
4	**Result**	-	-	-	-
5	Benedict's Test	+	+	-	+
6	Saponins	+	-	-	-
7	Coumarin	-	+	+	-
8	Glycoside's	-	+	+	+
9	Steroids	-	+	+	+

V. CONCLUSION

This study reveals a rich phytochemical profile in *sphagneticola trilobata* ,with histochemical evidence of flavonoid accumulation in leaves, underscoring its pharmacological promise .These finding validate ethanomedicinal uses and pave the way for targeted isolation of bioactive compounds in drug development, warranting further toxicological studies.

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