Pharmacognostical, Phytochemical and *In –vitro* Evaluation of Ethanol extract of *Abutilon guineense* for Diabetic foot ulcer Activity

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Abstract

Diabetic foot ulcer is one of the most severe complications associated with Diabetes mellitus, leading to delayed wound healing and increased risk of infection. Medicinal plants play a significant role in the management of chronic wounds due to their bioactive phytoconstituents. *Abutilon guineense* a subspecies of *Abutilon indicum* belonging to the family Malvaceae, has been traditionally used for its wound healing and antidiabetic properties. The present study aims to investigate the Pharmacognostic and phytochemical characteristics and *In vitro* Pharmacological studies of *Abutilon guineense* with special emphasis on its wound healing activity in diabetic foot ulcer conditions. Preliminary Phytochemical screening revealed the presence of important secondary metabolites such as flavonoids, tannins, alkaloids, and other phenolic compounds which are known to promote tissue regeneration and wound contraction. *In vitro* evaluation using the ethanol extract of *Abutilon guineense* showed a significant activity in scratch assay model demonstrated significant wound healing potential, thereby supporting its traditional application in the treatment of diabetic wounds.

KEYWORDS

Abutilon guineense, Pharmacognosy, Phytochemical screening, Diabetic foot ulcer.

Introduction

Abutilon guineense commonly known as Indian mallow belong to the family Malvaceae, has been extensively used in traditional systems of medicine such as Ayurveda and Siddha for the treatment of various ailments including inflammation, ulcers, diabetes mellitus, and wound healing. It comprises a large group of medicinal plants widely distributed in tropical and subtropical regions of the world. *Abutilon guineense* is considered a subspecies of *Abutilon indicum* exhibiting similar morphological and pharmacological characteristics.

Pharmacognostical evaluation plays a crucial role in the identification, authentication, and standardization of medicinal plants.These microscopic and anatomical features serve as important diagnostic markers for the authentication of the plant material used in herbal formulations. Phytochemical investigations of *Abutilon* species have revealed the presence of various secondary metabolites including alkaloids, flavonoids, tannins, glycosides, saponins, phenolic compounds, and steroids. These phytoconstituents are considered responsible for the wide spectrum of biological activities exhibited by the plant. Several pharmacological studies have demonstrated that extracts of *Abutilon guineense* possess significant biological activities such as antidiabetic, anti-inflammatory, antimicrobial, and wound healing effects. [1-3]

Methodology

Collection and Authentication

Leaves of *Abutilon guineense* were collected from the fields of Athanur Village Thanjavur district in Tamil Nadu during the month of August – 2025. The plant material was taxonomically authenticated by Dr. K.N. Sunil Kumar, Research officer, SIDDHA CENTRAL RESEARCH INSTITUTE (Central Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India) Anna Govt. Hospital Campus, Arumbakkam, Chennai - 600106. Herbarium (A17122503G) was deposited in the Department of Pharmacognosy, Dr Kalam College of Pharmacy, Avanam for future reference.

1. Pharmacognostical studies

Fresh leaves were subjected to Pharmacognostical studies which includes organoleptic and morphological studies.[4-6]

1.1Macroscopy

External feature of test sample was documented using Nikon D-5600 Digital camera.

1.2 Microscopy

Abutilon guineense leaves was preserved in fixative FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with 0.8% Safranin and 0.5% Astra blue. Transverse sections were photographed using Axiolab5 trinocular microscope attached with Zeiss Axiocam 208 color digital camera under bright field light. Magnifications were indicated by scale bar.

1.3 Powder microscopy

A pinch of the powdered sample of *Abutilon guineense* was mounted on a microscopic slide with a drop of 50% glycerol after clearing with saturated solution of chloral hydrate. Sample was treated with iodine solution to confirm the presence of starch grains. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital

camera under bright field light. Photomicrographs of diagnostic characters were captured and documented

1.4 Histochemical tests

Plant sections were treated following the standard procedures:

1.Crystals: The section was mounted in water and one end of the cover slip was irrigated with acetic acid. While looking through the microscope, the water within the cover slip was replaced using a piece of filter paper at the opposite end of the cover slip- Formation of air bubbles indicated Calcium carbonate crystals-If no air bubbles were formed, the experiment was repeated with conc. HCl, wherein dissolution of crystal and formation of needles of Calcium sulphate indicated the presence of Calcium oxalate crystals

2. Fats, Fatty oils, volatile oils and resins: About 1 to 2 drops of Sudan-IV was added to the section and allowed to stand for a few minutes. The presence of fatty oil substances was indicated by orange-red/pink/red colored globules, while red coloured irregular contents indicated resin.

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

4. Phenolic compounds: A drop of alcoholic ferric chloride was added - bluish black coloured contents indicated phenolic compounds like flavonoids/ tannins etc

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

6. Lignified cell walls: A drop of phloroglucinol was added to the section and allowed to stand for about 2 min or until almost dry. A drop of 50% HCl was added and observed over a cover-glass - cell walls-stained pink to cherry red, indicating the presence of lignin.

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

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

2. Physico-chemical paramaters

The powder sample of *Abutilon guineense* was subjected to physiochemical parameters such as loss on drying, and extractive value with different solvents in increasing order of polarity as per standard procedure.

3.PHYTOCHEMICAL STUDIES

Preparation of Ethanolic extract of *Abutilon guineense* (EEAG)

The collected leaves of *Abutilon guineense* were washed with water shade dried and powdered, extracted with ethanol (95%) by maceration technique until the complete extraction and filtered.The extract was concentrated under reduced pressure to obtain a residue.

Preliminary Phytochemical screening

Preliminary Phytochemical screening were carried out by using different reagents for identify the presence of phytoconstituents as per standard Procedure. [7-8]

4. PHARMACOLOGICAL STUDIES - *In-vitro* Wound healing activity - Scratch assay method

Procedure: Detach cells from the tissue culture dish, as you would for cell passage. Prepare a 6-well culture plate with 1-2mL warmed media added to each well. Cells were seeded in 24-well plates at a density of 4000 cells/well and incubated at 37 °C for 24 h. Samples (IC_{50} 100 µg) were incubated with cells. Once at the confluence (usuallyafter18-24hours), scrape the cell layer in a straight line using a cell scriber. After a scratch, gently wash the cell monolayer to remove detached cells and then replenish with a fresh medium. Immediately, the cells were visualized using a fluorescence microscope (Olympus, CKX-53, Japan), and the percentage of dead cells was quantified in at least three random microscopic fields.[9,10]

Results& Discussion

MORPHOLOGICAL CHARACTERISTICS OF *Abutilon guineense* LEAVES

Color : Green,

Texture : Soft

Taste : Better

Odour : Pungent

Shape :Heart

Leaf is broadly cordate, with a rounded to slightly acuminate apex, a serrate to crenate-serrate margin, a green upper surface that is slightly rough and pubescent, and a pale green lower surface that is prominently pubescent with clearly visible reticulate venation; measures approximately 6 to 12 cm in length and 5 to 11 cm in width; petiole long, slender, cylindrical, and pubescent, approximately 4 to 10 cm long; texture soft, thin, and slightly velvety due to pubescence; odour: slight and characteristic, with a mildly mucilaginous and slightly bitter taste.



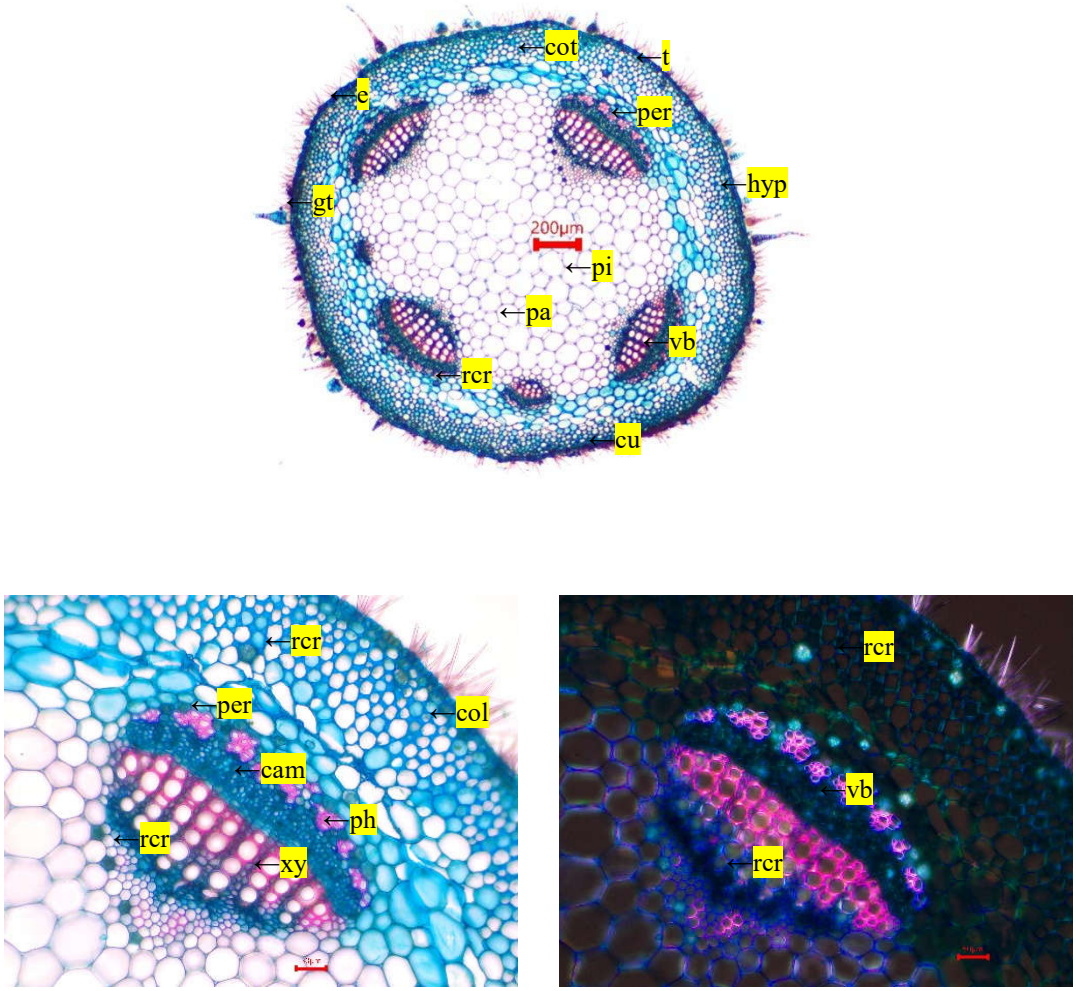
Fig 1: Macroscopy of *Abutilon guineense* Leaves

Microscopy

Petiole

The transverse section of the petiole shows a nearly circular to slightly oval outline with distinct vascular bundles arranged in a ring. The epidermis consists of a single outermost layer of closely packed rectangular to barrel-shaped cells, covered by a thin cuticle and bears numerous unicellular and multicellular, non-glandular covering trichomes and occasional glandular trichomes; hypodermis comprises 2 to 5 layers of collenchymatous cells, especially prominent at the ridges; cortex is broad and mainly made up of thin-walled, rounded to polygonal parenchymatous cells, along with mucilage cells and a few calcium oxalate crystals. There are 4 to 6 prominent collateral, open vascular bundles arranged in a ring, with xylem positioned towards the inner side, consisting of large vessels, tracheids, xylem parenchyma, and fibers.(Fig 2)

Figure 2. Microscopy of *Abutilon guineense petiole*



cam – cambium, **col** – collenchyma, **cot** – cortex, **cu** – cuticle, **e** – epidermis, **gt** – glandular trichome; **hyp** – hypodermis, **pa** – parenchyma, **per** – pericycle, **pi** – pith, **ph** – phloem, **rcr** – rosette crystal, **t** – trichome, **vb –** vascular bundle; **xy** -xylem

Leaf

The TS of the dorsiventral leaf shows a prominent midrib and a lower, prominently convex surface.Upper epidermis made up of closely packed, rectangular to barrel-shaped cells covered with a thin cuticle and bears numerous unicellular and multicellular, non-glandular and few glandular trichomes; lower epidermis made up of single-layer of comparatively smaller cells with a thin cuticle and bears more abundant trichomes than upper epidermis, , phloem present towards the outer side of the xylem composed of sieve tubes, companion cells, and phloem parenchyma; a distinct cambial strip between xylem and phloem observed; mesophyll is differentiated into one to two layers of elongated, columnar cells of palisade parenchyma located just below the upper epidermis and loosely arranged, irregular spongy parenchyma present below palisade tissue (Fig.3).

Fig 3. Microscopy of *Abutilon guineense* leaf

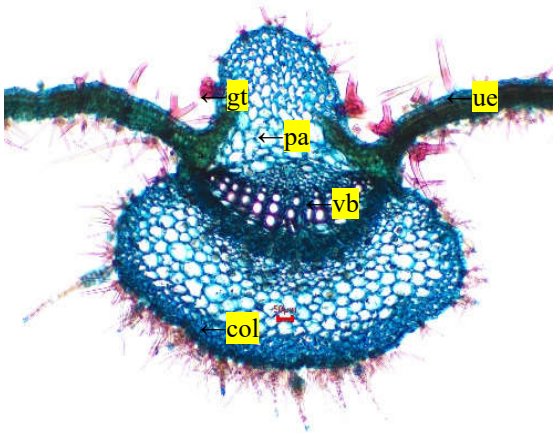


Fig 3.1 TS of lamina passing through the midrib

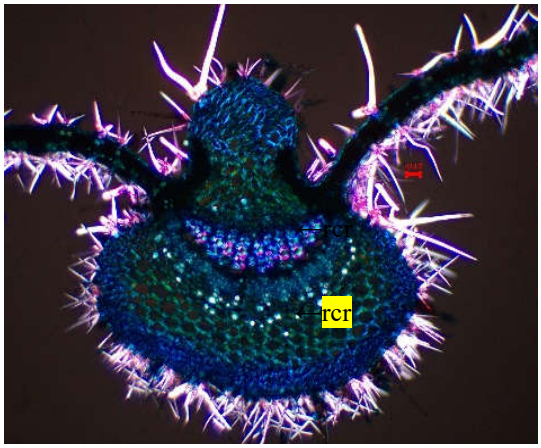


Fig 3.2 Under polarizer

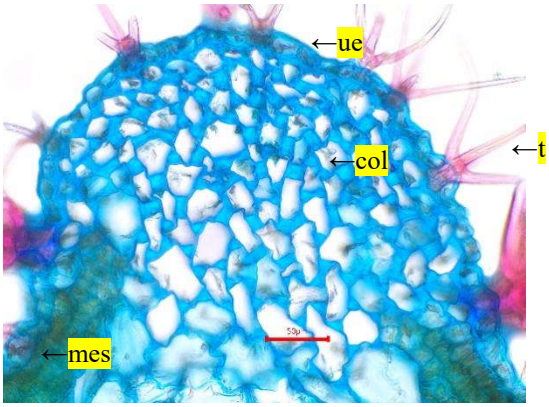


Fig 3.4 Upper region enlarged

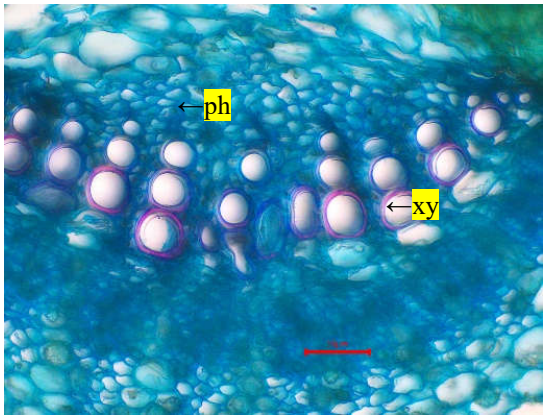


Fig 3.5 Middle region enlarged

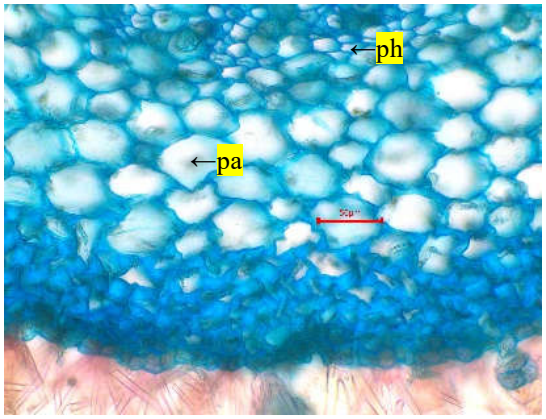


Fig 3.6 Lower region enlarged

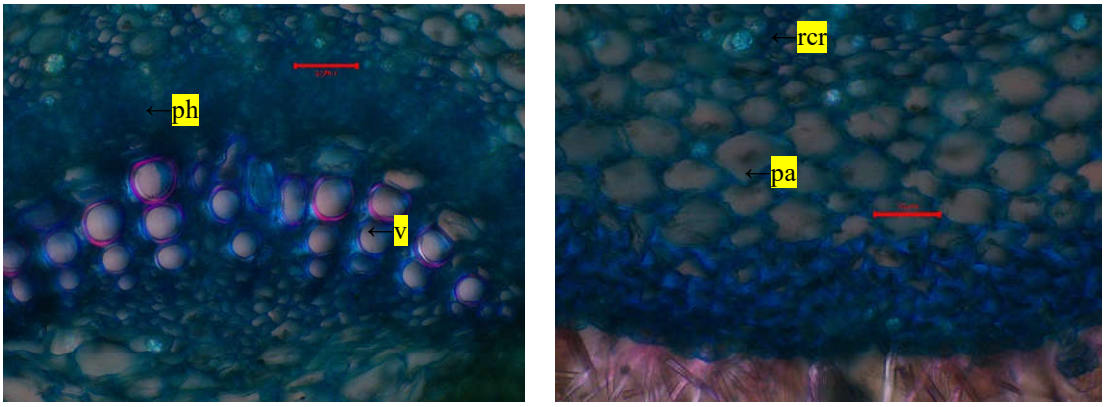


Fig 3.7 Under polariser

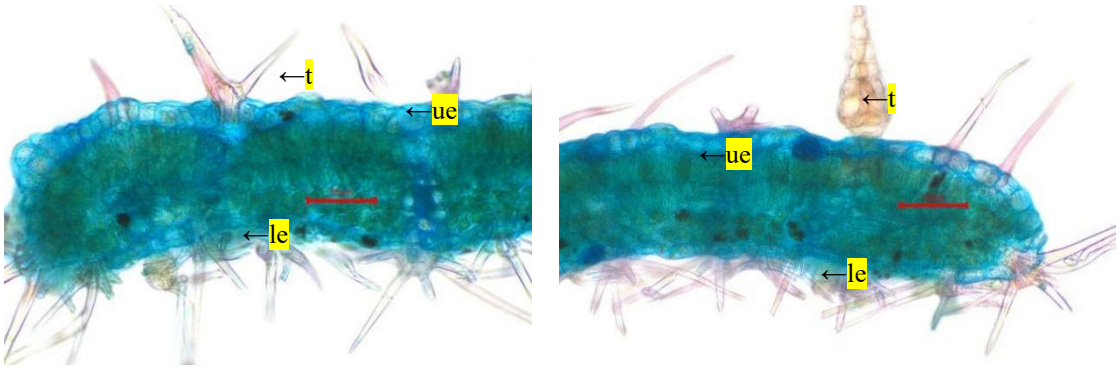


Fig 3.8. Lamina margin enlarged

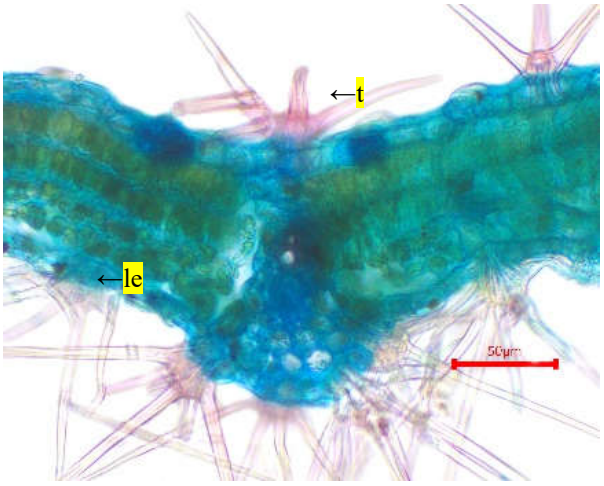


Fig 3.9.Lamina portion enlarged

col – collenchyma, **cu** – cuticle, **ue** – upper epidermis, **gt** – glandular trichome; **hyp** – hypodermis, **le**- lower epidermis, **pa** – parenchyma, **per** – pericycle, **pi** – pith, **ph** – phloem, **rcr** – rosette crystal, **t** – trichome, **vb –** vascular bundle; **xy** -xylem

Histochemistry of *Abutilon guineense* petiole and Leaf

The histochemical studies showed the presence of cutin on the epidermal surface and along the walls of trichomes in both petiole and lamina region; phenol is detected in the epidermal, hypodermal and few ground tissue cells; alkaloid is observed in the epidermal cells and the parenchymatous cells of the phloem region; lignin along the epidermal cells and walls of xylem vessels of both petiole and midrib regions; oil is detected in few parenchymatous cells of cortex; numerous starch grains crystals are observed in the mesophyll tissue of lamina and phloem parenchyma cells; abundant rosette crystals are observed in the ground tissue of petiole and lamina; mucilage is not detected (Fig. 4).

Fig 4. Histochemistryy of *Abutilon guineense* petiole and leaf

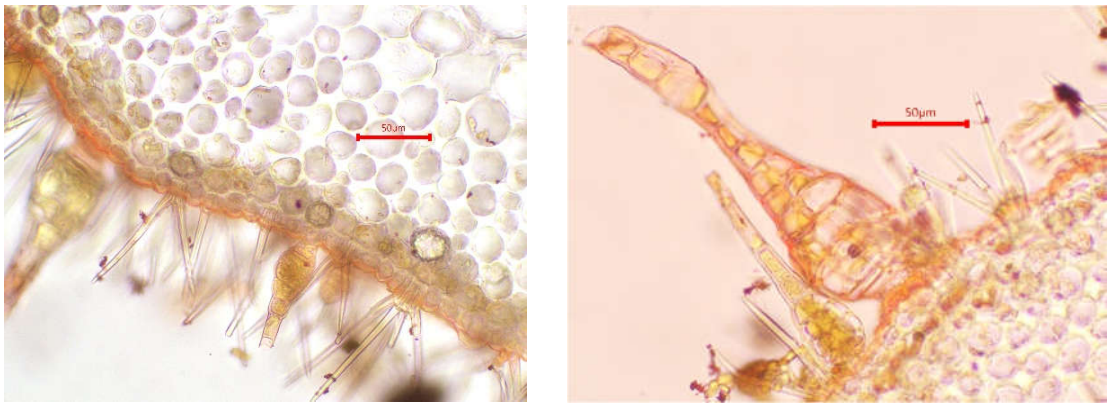


Fig 4.1Cutin

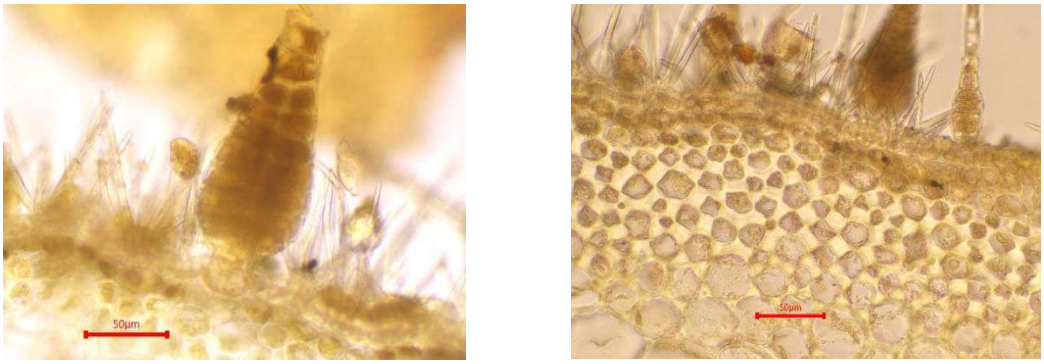


Fig 4.2.Phenol

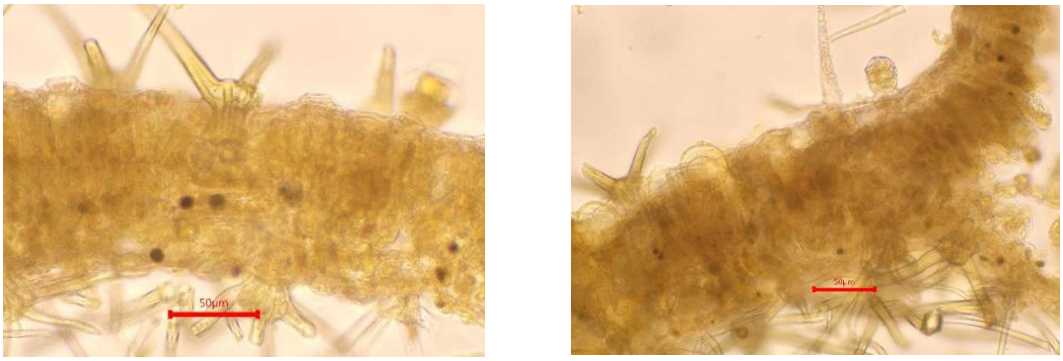


Fig 4.3Phenol

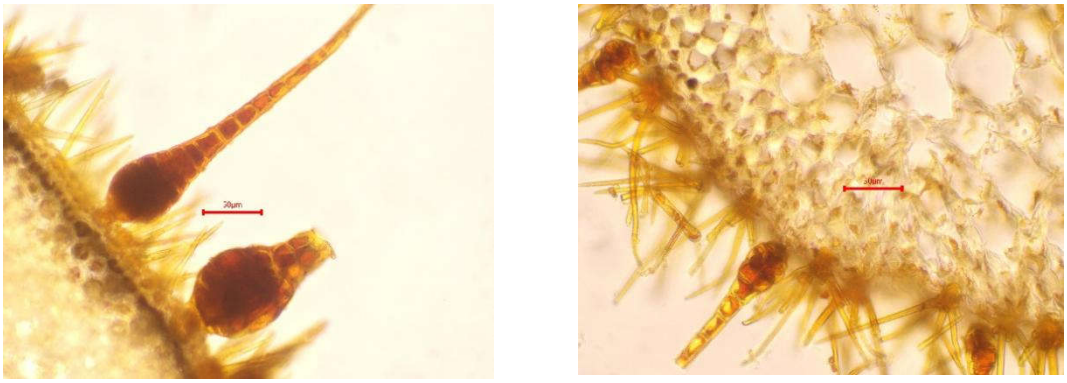


Fig 4.4Alkaloid

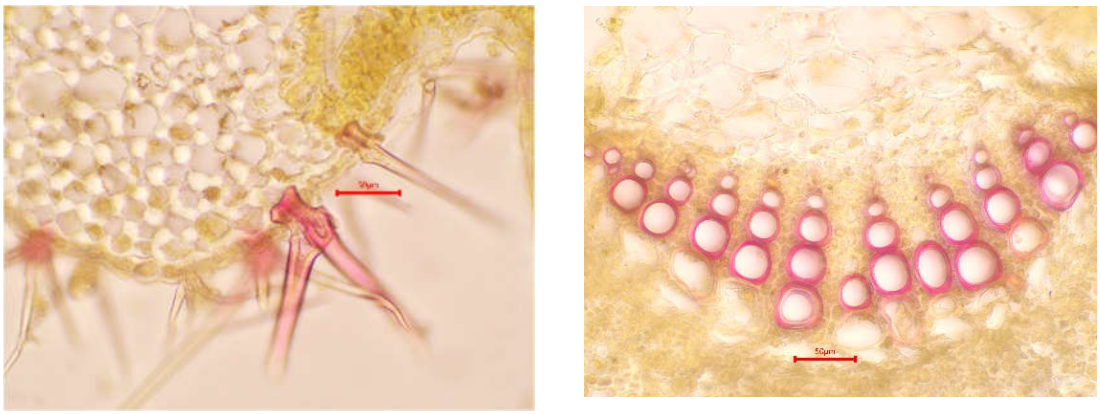


Fig 4.5.Lignin

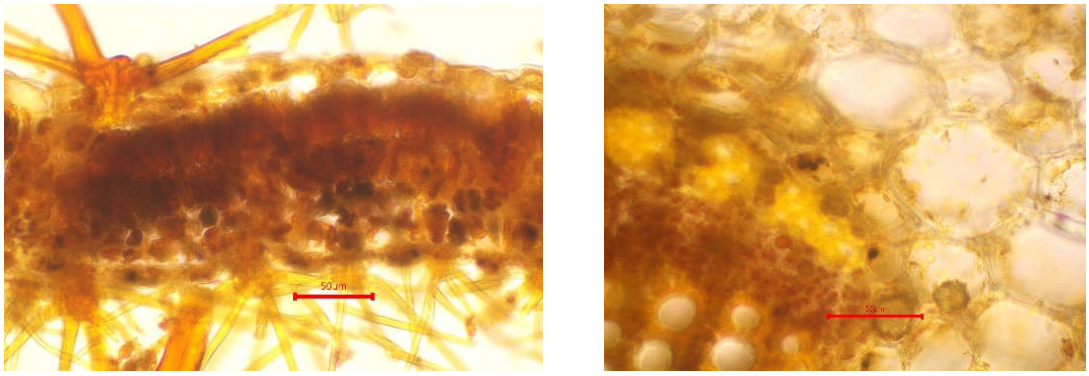


Fig 4.6.Starch

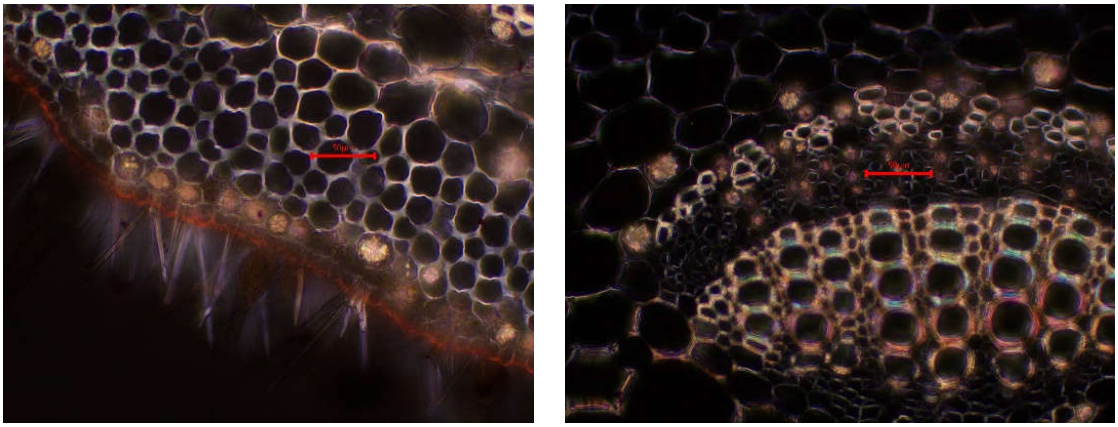


Fig 4.7.Rosette crystals

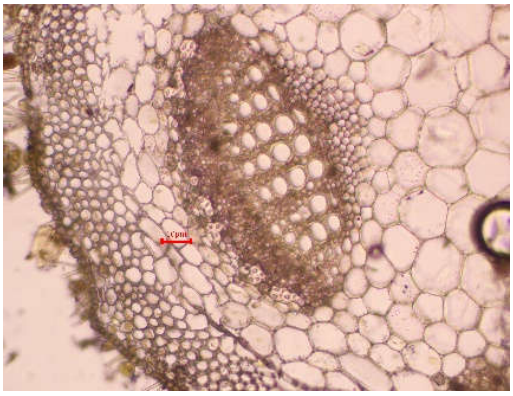


Fig 4.8.Mucilage absent

Powder microscopy of *Abutilon guineense* Leaf

The powder is green in colour, with slight characteristic odour and mucilaginous bitter taste, and shows glandular and stellate trichomes, epidermal fragment with stomata, mesophyll tissue, spiral and reticulate vessel and rosette crystals (Figure 5).

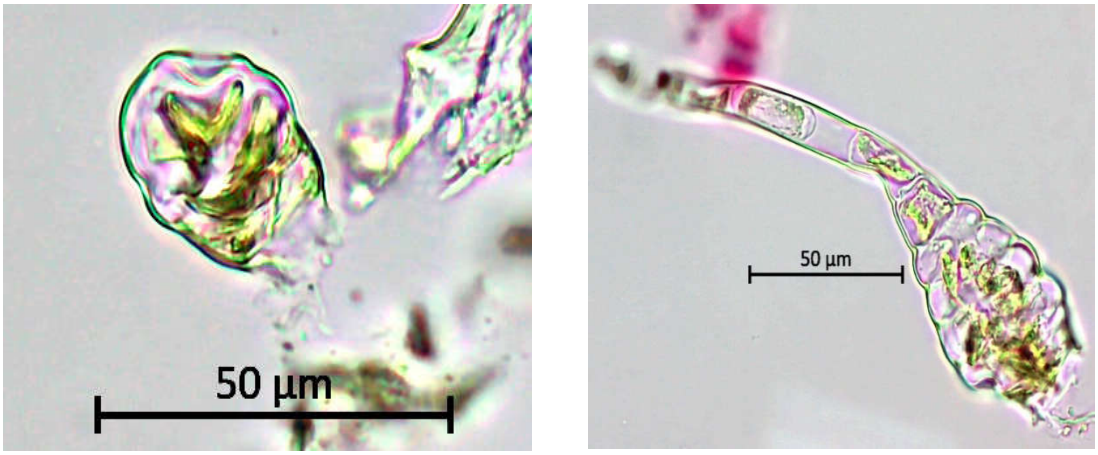


Fig 5.1 Glandular trichome

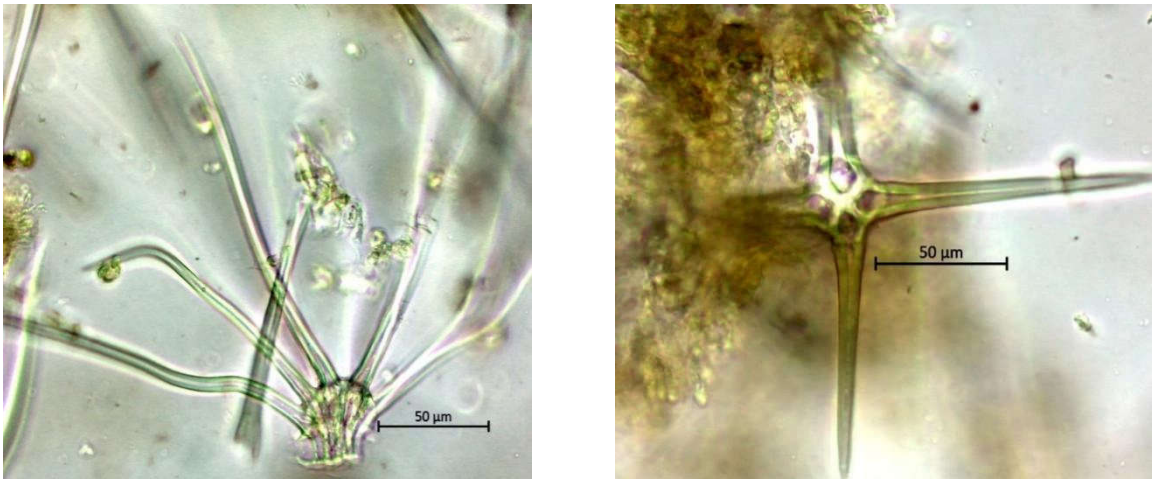


Fig 5.2.Stellate trichome

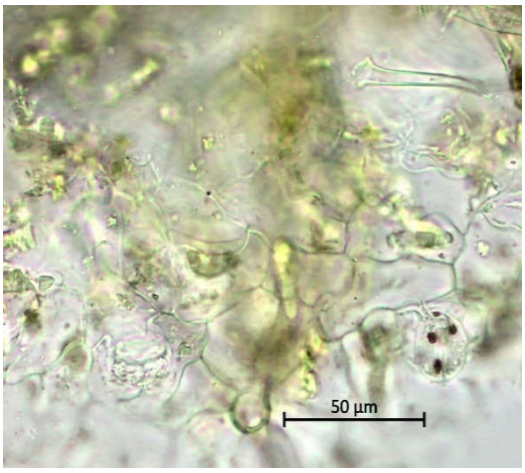


Fig 5.3.Epidermis with stomata

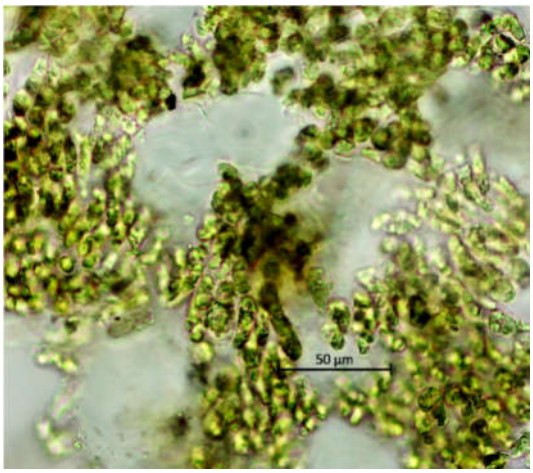


Fig 5.4.Mesophyll tissue

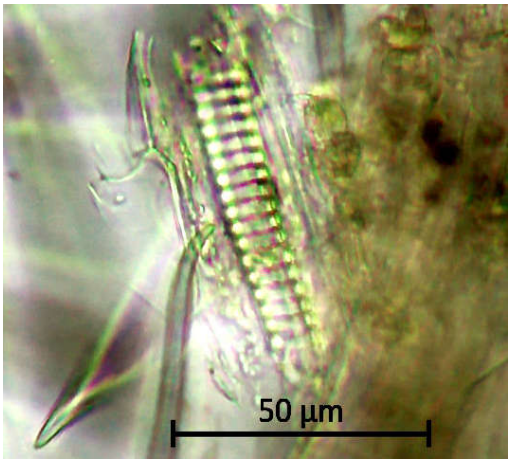


Fig 5.5 Spiral vessel

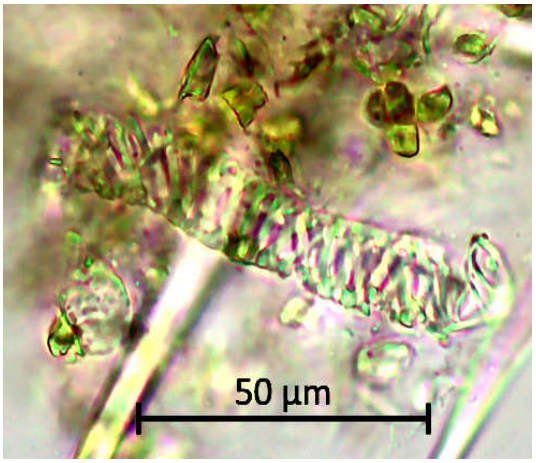


Fig 5.6 Reticulate vessel

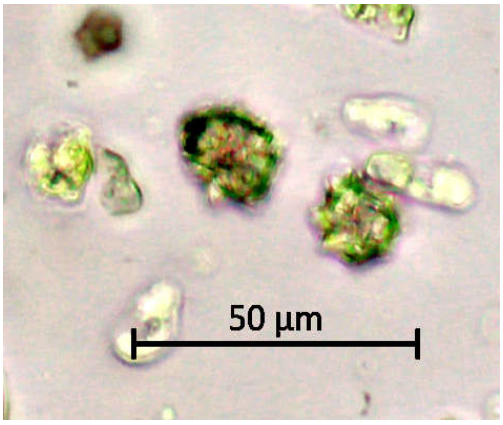




Fig 5.7 Rosette crystals

2.DETERMINATION OF PHYSICOCHEMICAL CONSTANTS

Table:1. Determination of physicochemical constants

Parameters	**Solvents**	**Yield%(w/w)**
Extractive value	Ethanol	5%(w/w)
	Petroleum ether	2%(w/w)
	Ethyl acetate	1%(w/w)
	Chloroform	3%(w/w)
Moisture content		3%(w/w)

PHYTOCHEMICAL STUDIES of *Abutilon guineense* Leaf Extract

3.1Extraction

Table:2 Ethanol extract of *Abutilon guineense*

S.no	Extract	Method of extraction	Physical nature	Colour	Yield%(w/w)
1.	Ethanol	Maceration	Semisolid	Dark green	20%(w/w)

3.2 Qualitative phytochemical screening of EEAG:

Table:3 Qualitative phytochemical screening of EEAG

S.NO	Chemical test	Presence (or) Absence
1.	Alkaloid	+
2.	Flavonoid	+
3.	Carbohydrate	+
4.	Tannins	+
5.	Phenolic compound	+
6.	Anthroquinone	_
7.	Fixed oil	+
8.	Protein	_
9.	Glycosides	+

(+) indicates presence **(-) indicates absence**

PHARMACOLOGICAL STUDIES - *In-Vitro* Wound scratch assay

In-Vitro Wound scratch assay

The scratch assay is typically utilized to quantify cellular migration on two-dimensional (2-D) surfaces over time upon different treatments. It is one of the most used in vitro wound-healing assays, allowing the determination of the optimal dose of the agents being tested. Cells are grown to confluency in a monolayer, and a scratch is made with a pipette tip to create an incision-like gap. The "wounded" area is photographed immediately after wounding and at defined time points. Cell migration is quantified and expressed as the average percentage of closure of the scratch area. For longer-term wound-healing assays (> 24 h), mitosis inhibitors such as mitomycin C treatment are required to eliminate the contribution of cell proliferation to gap closure, allowing the assay to precisely assess the migration of the given cell population. Scratch assay is compatible with time-lapse microscopy, including live cell imaging, imaging specific cell migration behaviour patterns, and a single-cell movement .

Sample – AG

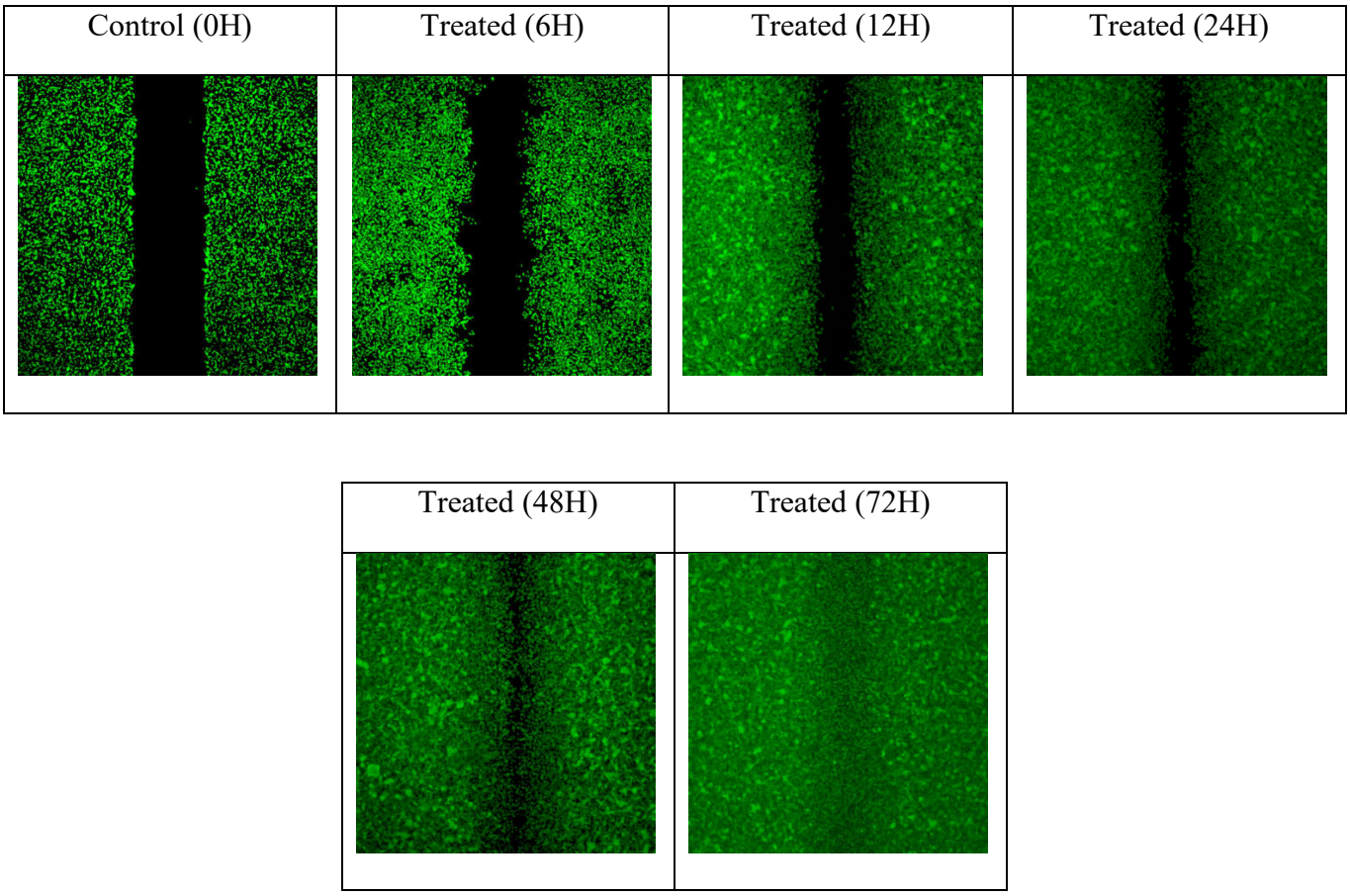


Fig 6. Cell migration

OBSERVATION FOR WOUND HEALING ACTIVITY

Table4. Wound healing activity observation for Ethanol Extract of *Abutilon guineense*

S.no	Time (hrs)	Observation	Conclusion
1	0H(Control)	Wide scratch gap with no cell Migration	Initial wound area before Treatment.
2	6H	Slight movement of cells toward the scratch region	Early stage of cell migration begins.
3	12H	Increased number of cells moving into the scratch area.	Moderate migration indicating treatment response.
4	24H	Noticeable reduction in scratch width	Significant cell migration and wound closure starts
5	48H	Most of the scratch area covered by migrating cells	Strong wound healing activity observed.
6	72H	Scratch area almost completely closed	**Maximum** cell migration and effective wound healing potential.

Conclusion

The present investigation confirms that *Abutilon guineense* possesses significant pharmacological potential in promoting wound healing activity, particularly in diabetic foot ulcer conditions. This can be justified on the basis of results obtained from *In vitro* evaluation of scratch wound healing assay .

The presence of bioactive phytoconstituents such as flavonoids, tannins, and alkaloids supports its traditional medicinal usage in chronic wound management. Hence, *Abutilon guineense* may serve as a promising natural therapeutic agent for the development of plant-based formulations for the treatment of diabetic foot ulcers. Further experimental and clinical studies are recommended to validate its efficacy and safety.

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