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Research Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *TABERNAEMONTANA DIVERICATA*, LINN EXTRACTS

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Abstract: *Tabernaemontana divericata* used in traditional medicine for its anti-inflammatory and antimicrobial activity. The literature survey showed that this plant has not been studied yet for its pharmacognostical parameters and certain antimicrobial activity studies. We have taken up the said plant for our investigation. The pharmacognostical study includes macroscopy, histological characters, powder microscopic studies and physiochemical parameters of the plant were characterised. The methanolic and aqueous (cold and hot) extracts of *Tabernaemontana divericata*, Linn were subjected to qualitative chemical tests and antimicrobial studies. The presence of alkaloids, carbohydrates, phytosterols, saponins, phenol compounds, gums and mucilages were found in methanolic and aqueous extracts of the plant. *Tabernaemontana divericata* found to have good antimicrobial activity.The maximum zone of inhibition was produced by hot and cold extracts against gram positive organism. The significant antibacterial activity was produced by hot extracts against *Streptococcus viridians, Streptococcus pyrogens* and *Streptococcus agalactia*. Antifungal activity was not reported by *Tabernaemontana divericata.*

Key**words:** *Tabernaemontana divericata*, Antimicrobial studies, powder microscopy, active constituents.

INTRODUCTION

Traditional herbal drugs needs evaluation in the field of immunology, genetic, biochemical, biological and chemical analysis. This project study involves the investigation of *Tabernaemontana divericata* by phytochemical and pharmacological screening of active constituents for antimicrobial activity. Pharmacognostical studies are more helpful in identification of crude drugs with absolute information and provide better safety and efficacy in the formulation of herbal medicines from crude drugs. At present, due to multiple drug resistance developed by synthetic antibiotic drugs, there is a need of alternative therapeutic natural antimicrobial agents against various pathogenic microorganisms[1].

A large number of Indian medicinal plants have been screened by scientist of various disciplines viz Agriculture, Botany, Chemistry, Pharmacology, Toxicology and Clinical sciences. It revealed that physiochemical studies received maximum attention on 60.15% of the plant research. Next in order were pharmacological studies 16.31% general and cultivation aspects 8.53% and pharmacognostical / botanical studies 13.11% very few plants 1.90% reached the clinical trial stage[2].

Plant Introduction:

Tabernaemontana divariacata, Linn belongs to the family Apocynaceae. Vernacular name in Tamil known as Nantiyavattam. In English it's called as east Indian Rosebay. This plant is distributed throughout the tropical parts of the world and in Himalayas. In Kerala, this is found under cultivation in ornamental gardens. The survey of literature revealed that this plant has not been studied in detail for its pharmacognostical and antimicrobial studies on this plant extracts.

Botanical Characters:

Habit: Glabrous laticiferous shrub, 1.8-2.4m in height with silvery grey bark and milky latex. **Leaves:** Simple, glossy green, acuminate, margins way.

Flowers: White, sweetly fragrant in 1-8 flowered cymes at the bifurcations of the branches, lobes of corolla overlapping to right in the bud.

Fruits: Follicles, 2.5-7.5cm long, ribbed and curved, orange or bright red within narrowed into a slender curved beak.
Seeds: Dull brown, minutely pitted, irregular, enclosed in a red pulpy aril.

MATERIALS AND METHODS

Collection and identification of the plant material

The plant was collected in the month of October 2007 at Coimbatore. It was identified and authenticated by the botanist P.Satyanarayana, Botanical survey of India, Coimbatorre. The plants were cleaned and the aerial parts were separated from the roots and allowed for shade drying.

The extraction of the leaf parts planned with methanol for the reason that the methanol being a bipolar solvent which dissolves a major group of aqueous soluble fractions, the rural people use aqueous fractions but to find out the wide range of the phytochemical constituents we determined to use methanolic and aqueous extracts for carrying out the phytochemical and antimicrobial studies.

The pharmacological studies deals with the macroscopy, microscopy, powder characters of the leaf and stem and the physiochemical parameters. Preliminary phytochemical

investigation deals with the qualitative chemical tests for the detection of various chemical constituents. The methanolic and aqueous extracts (cold and hot) were subjected to assess the antimicrobial activity.

PHARMACOGNOSTICAL STUDIES

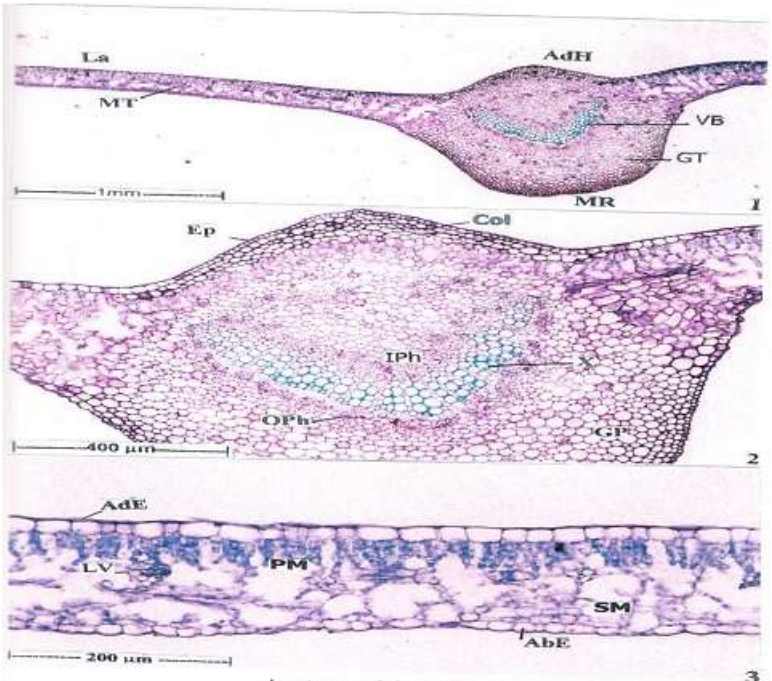
Anatomical Studies

The required samples of different organs were cut and removed from the plant and fixed in FAA (formalin-5ml+Acetic acid-%ml+70% ethyl alcohol-90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-60^{0}C) until TBA solution attained super saturation. The specimens were cast in to paraffin blocks[3].

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary microtome. The thickness of the sections was 10-12µm. Dewaxing of the sections was by customary procedure given by Johnson, 1940. The sections were stained with Toluidine Blue polychromatic stain. The staining results were remarkably good and some cytochemical reactions were also obtained . To the lignified cells dark green, violet to the mucilage and blue to the protein bodies. Wherever necessary sections were also stained with safranin and fast green and IK (for starch)[4]

For studying the stomatal morphology, venation pattern and trichomes distribution (Figure:1). Para dermal sections were done by clearing of leaf with 5% sodium hydroxide. Glycerine mounted temporary preparations were made and macerated /cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured.



Anatomy of the leaf
1.T .S of leaf through Midrib with lamina
2. T .S of Midrib enlarged
3. T .S of lamina
(Abe – Abaxial epidermis, Ade – Adaxial epidermis, Adh- adaxial hump, Col-collenchyma , EP- epidermis, GP-ground Parenchyma, IPh-inner Phloem, la-lamina, IV-lateral vein, MR-Midrib, MT- mesophyll tissue, OPh- Outer Phloem, PM- Palisade Mesophyll, SM-Spongy mesophyll, VB-vascular bundle, X-xylem).

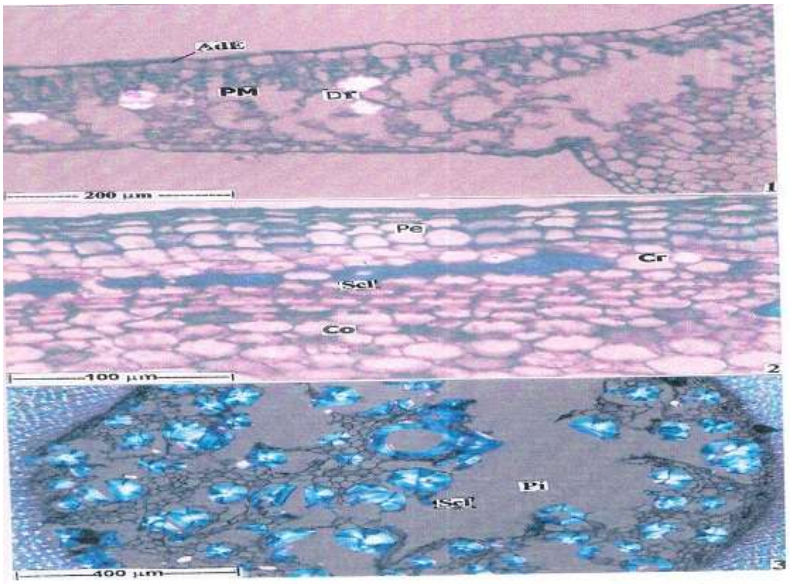
Figure- 1: Anatomy of The Leaf

PHOTOMICROGRAPHS

Microscopic descriptions of tissues are supplemented with micrographs.Photographs of different magnifications were taken with Nikon Labphot 2 microscopic units. For normal observations bright field was used. For the study of crystals, starch grain and lignified cells polarized light was employed.

Microscopic Features (Leaf)

The leaf is bifacial with smooth ever lamina and prominent dorsiventral with midrib. Midrib has broadly conical adaxial hump and semicircular abaxial part. The epidermal layer of midrib is comprised of small circular cells. A narrow bond of two to four layers of collenchymas cells occurs beneath the adaxial epidermis. Similar types of small thick walled cells are seen on the lower part of the adaxial midrib. Remaining region of the midrib consists of circular, walled, less compact parenchymal cells. The vascular system consists of a single strand of wide, shaped bicollateral xylem and phloem. Xylem band has 3 to 5 elements which are arranged in radial fibre. Phloem occurs on both and outer sides of the xylem. The mid rib is 900µm thick and 1mm broad (Figure:2).



Crystal distribution in the leaf and stem (polarized light microscope)

1. Drugs in the mesophyll adjacent
2. Prismatic crystals to the cortical sclerenchyma
3. Sclereids in the pith.

(AdE- Adaxial Epidermis, Co- cortex, Cr- crystals, Dr- Drases, Pe-Periderm, Pi- Pith, PM- Palisade mesophyll, Scl- Sclerid).

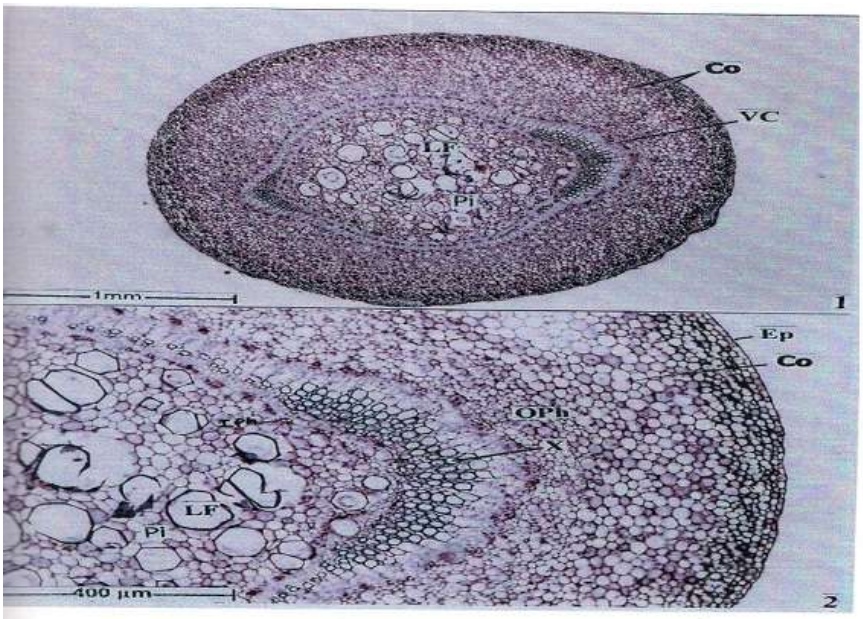
Figure-2: Crystal Distributions in Leaf And Stem

Lamina

The lamina is 200 µm thick .It is hypostonatic. The adaxial epidermis is thick compraising of wide squarish cells. The adaxial epidermis is 20 µm thick and abaxial epidermis is 10 µm thick. The palisade zone is 40 µm in height and has single layer of this, loosely arranged cylindrical cells. The spongy mesophyll has 4 or 5 layers of small lobed cells large drases are fairly common in the messophyll tissue.

Young stem

The young stem is circular in sectional view measuring 2mm in diameter(Figure:3). It has a thin epidermal layer wide, homogemous, parenchymatous cortical cells and a thin vascular cylinder. The vascular cylinder has thick lateral, opposite xylem segments are only one or two in each row. The pith has wide, circular or angular laticifers which are thin walled. Pholem occurs in small groups both outer and minor portions of xylem cylinder. Thus the vascular cylinder is bicollateral[5].



Anatomy of the young stem

1. T.S of the stem (ground plan.)
2. T.S of the stem a sector enlarged.

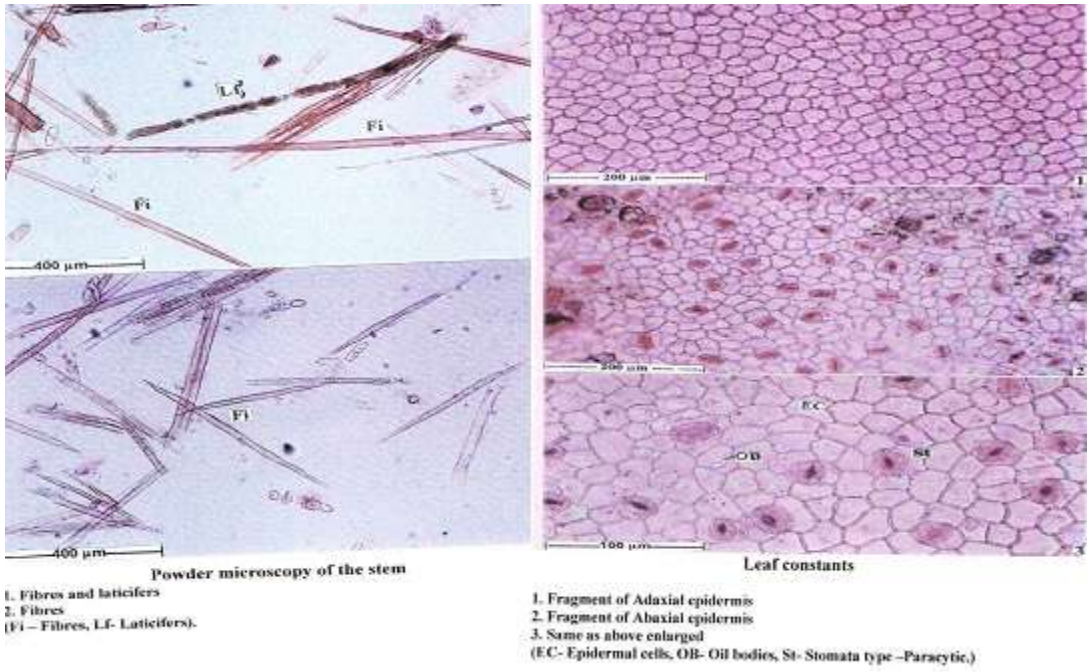
(Co-cortex, Ep- Epidermis, LF- Laticifers, OPh- Outer phloem, Pi- Pith, VC- vascular cylinder, X- Xylem).

Figure- 3: Anatomy of The Stem

POWDER MICROSCOPY

Leaf Powder[6]

Fragments epidermal layer are frequently seen in the powder adaxial epidermal fragments are appostomatic. Cell are polydermal with 4 to 8 faces. The cells are random in orientation. Their anticlinal walls are fairly thick and straight. The cells are 30 X 50 µm in size. The lower epidermis is stomatiferrous. The stomata are parasitic. The stomata surrounded with 2 less distinct lateral subsidery cells. The stomata are elliptical and measure 25 X 30 um the stomatal number 70/mm^2. The abaxial epidermal cells are similar both adaxial side cells. They are random in orientation, the anticlinal walls are thin, straight, or slightly wavy (Figure: 4). Spherical translucent oil bodies are sometimes seen in the cells of the abaxial epidermis.



Powder microscopy of the stem

1. Fibres and laticifers
2. Fibres
(Fi – Fibres, Lf- Laticifers).

Leaf constants

1. Fragment of Adaxial epidermis
2. Fragment of Abaxial epidermis
3. Same as above enlarged
(EC- Epidermal cells, OB- Oil bodies, St- Stomata type –Paracytic.)

Figure -4: Leaf Constants

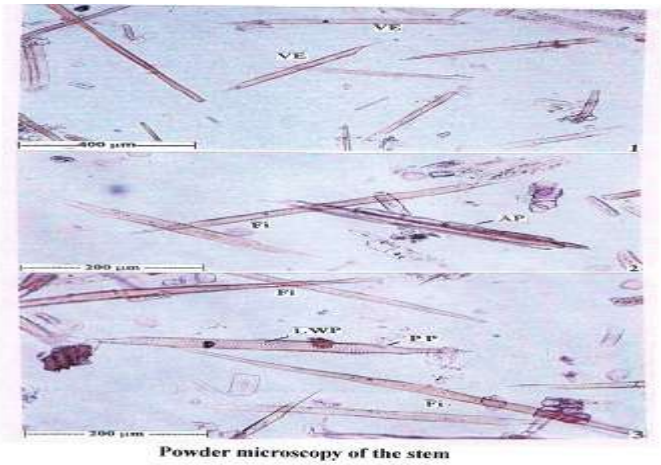
Stem Powders

In the stem powder, three types of cells are seen a) laticifers b)fibers c)vessel elements

Laticifers: These are latex containing tubes .They are long branched and septate. They posses dark, granular contents. The laticifers are 20µm wide.

Fibres: Xylem fibres are abounded in the powder, they are liberiform type. The fibres have their walls and wide lumen. The wide lumen fibres have slit like pits and also possess some inclusions some of the fibres are narrow lumened; these fibres have no inclusion or pits. The fibres are 350-500um long. The wide fibres are up to 450 micrometer long.

Vessel elements: The vessel elements are long, narrow and cylindrical they have tapering ends. They have simple, circular oblique perforations. The lateral wall pits are minute, circular and multiserriate. The vessel elements are 430µm long[7]



Powder microscopy of the stem

1. Vessel elements
2. Fibers and Axial parenchyma
3. Fibers and vessel elements
(AP - Axial parenchyma, Fi- Fiber, LWP- Lateral wall piths, PP- perforation plate, VE- Vessel element).

Figure- 5: Powder Microscopy Of The Stem

DETERMINATION OF PHYSIOCHEMICAL PARAMETERS[8, 9]

Ash Values

Ash values are helpful in determining the quality and purity of the crude drugs in the powder form. Standard Indian pharmacopeia procedure were used to determine the different ash values such as total ash, acid insoluble ash, water soluble ash and sulphated ash. The ash values determined separately for leaves and entire plant powders.

Determination of total ash

About 3gms of the powdered drug was accurately weighed and taken in a silica crucible, which were previously ignited and weighed. The powdered drug was spread as a fine even layer on the bottom of the crucible. The crucible was incinerated gradually by increasing the temperature to make it red hot until free from carbon. The crucible was cooled and weighed. The percentage of the total ash was calculated with reference to the air dried drug.

Determination of acid insoluble ash

The ash obtained as described in the above method was boiled with 25ml of 2NHCL for 5 min, the insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was calculated with reference to the air dried drug.

Determination of water soluble ash

The ash obtained as described in the determination of total ash was boiled for 5mins with 25ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred in a silica crucible and ignited for15 mins and weighed. The percentage of water soluble ash was calculated with reference to the air dried drug.

Determination of sulphated ash

3gms of the powdered drug was accurately weighed and taken in a silica crucible which was previously ignited and weighed. The drug was moistened with sulphuric acid, ignited gently and again moistened with sulphuric acid and reignited. The percentage of sulphated ash was calculated with reference to the air dried drug.

Table 1: Data showing the different ash values of *Tabernaemontana diveriacata* linn.

S.No	Type of ash	% Yield (W/W)
1.	Total ash	24.33
2.	Acid insoluble ash	15
3.	Water soluble ash	25
4.	Sulphated ash	11.33

Extractive values[10]

Extractive values of the crude drugs are useful for their evaluation especially when the constituients of a drug cannot be readily estimated by other means. These values indicate the nature of the constituients present in the crude drug.

Determination of alcohol soluble extractive value

About 5gms of accurately weighed coarsely powdered air dried leaf was macerated with 100ml of alcohol (90%) in a stoppered flask for 24hrs. 25 ml of the alcoholic extract was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105^0C. The percentage yield of alcohol soluble extractive value was calculated with reference to the air dried drug.

Determination of water soluble extractive value

5gms of accurately weighed coarsely powdered air dried leaf powder with 100ml of chloroform in a stoppered flask for 24hrs and subsequently 25ml of chloroform extract to dryness at 105^0C. The percentage w/w of chloroform (90%) soluble extract was calculated with reference to the air dried drug.

Table-2: Data showing the different extractive values of *Tabernaemontana diveriacata* Linn (Aerial part of powder)

S.No	TYPE OF EXTRACTIVE VALUES	% YIELD (W/W)
1.	Alcohol soluble extractive value	17.8%
2.	Water soluble extractive value	12.6%

PRELIMINARY PHYTOCHEMICAL SCREENING[11]

Collection and preparation of the plant material

The plants were cleaned and the aerial parts were separated from the roots and allowed for shad drying. It was then milled into a coarse powder by a mechanical grinder and used for further studies.

Extraction

The aqueous (cold and hot) and methanolic extracts were subjected to qualitative chemical tests for the detection of various chemical constituents such as alkaloids, steroids, tannins, carbohydrates, fixed oils, fats and phenolic compounds etc.

Table-3: Qualitative phytochemical tests of *Tabernaemontana divariacata* linn

S.No	Chemical constituents	Methanol extracts	Aqueous (cold extracts)	Aqueous (hot extracts)
1.	Alkaloids	+	+	+
2.	Carbohydrates	+	+	+
3.	Glycosides	+	+	+
4.	Phytosterols	+	+	-
5.	Gums & mucilage	+	+	+
6.	Phenolic compounds	+	+	+
7.	Proteins & Amino acids	-	-	-
8.	Saponins	+	+	+

ANTIMICROBIAL STUDIES [12, 13]

The aqueous (cold and hot) and methanolic extracts of *Tabernaemontana divariacata*, Linn were screened for antimicrobial activity according to the standard procedures28-30. The antimicrobial assay was performed by Kirby-Bauer Disc diffusion method. A concentration of 200µg plant extracts were prepared by dissolving the extracts in DMSO Solvent. For bacterial and fungal growth blood agar medium and sabourd dextrose medium were prepared. In case of gram positive microorganism the standard erythromycin and penicillin was utilized where as in case of detection of antimicrobial activity against gram negative microorganism Gentamycin and Ampicillin were used as the standard drug. Amphotericin-B and Clotrimazole are used as standard to determine the antifungal activity.

Table-4 (a): Zone of inhibiton against gram positive organisms with extracts of *Tabernaemontana divariacata*

S.No	Gram Positive Organism	Methanol extracts (mm)	Aqueous Cold extracts (mm)	Aqueous hot extracts(mm)	Erythromycin (mm)	Penicillin (mm)
1.	*Steptococococus viridians*	8	10	15	30	10
2.	*Streptococous pyrogens*	-	-	11	17	20
3.	*Streptococococus agalactiae*	-	7	13	20	15

Table-4 (b): Zone of inhibiton against gram negative organisms with extracts of *Tabernaemontana divariacata*

S.No	Gram Negative Organism	Methanol extracts (mm)	Aqueous Cold extracts (mm)	Aqueous hot extracts(mm)	Gentamycin (mm)	Ampicillin (mm)
1.	*Klebsiella pneumonia*	-	-	-	-	-
2.	*Citrobacter freundi*	-	-	-	17	-
3.	*Proteus vulgaris*	-	-	-	16	-

Table-4(c) : Zone of inhibiton against fungus with extracts of *Tabernaemontana divariacata*

S.No	Fungal Organism	Methanol extracts (mm)	Aqueous Cold extracts (mm)	Aqueous hot extracts(mm)	Amphotericin-B (mm)	Clotrimazole (mm)
1.	*Candida albicans*	-	-	-	18	20

RESULTS AND DISCUSSION

In histological studies anatomy of leaf shows adaxial epidermis, abaxial epidermis, collenchymas, midrib, parenchyma, inner phloem, xylem, vascular bundle, palisade and spongy mesophyll and lamina. Anatomy of young stem shows cortex ,sclerides, periderm,secondary phloem and xylem. Crystal distribution of leaf and stem shows adaxial epidermis, crystals, periderm, pith, sclerites and palisade mesophyll. Leaf constants of leaf shows epidermal cells, oil bodies and paracytic type of stomata. Powder microscopy of stem shows fibers, laticifers,vessel elements axial parenchyma, lateral pith and perforation plate. The antimicrobial activity of hot aqueous extracts was found to be more significant when compared tothe cold aqueous and methanolic extracts. Antifungal activity was not produced by the extracts of *Tabernaemontana divariacata.* Our studies on *Tabernaemontana divariacata* showed a good antimicrobial activity compared to the standards.

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