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Research Article

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MICROSCOPICAL AND PHYSICOCHEMICAL STUDIES OF *GLOCHIDION VELUTINUM LEAF*

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Abstract

Genus Glochidion have been used for a varied of biological activities in traditional medicine and also have been using by many ethnic groups. It is a vast genus in which many plants explored chemically, but most of the species in this genus were not standardized pharmacognostically. Thus in this current study a complete pharmacognostic study was done on the leaf part. Microscopical studies have given a clear detail regarding the various cell characters and various constants.The main TDI of leaf was found to be long unicellular covering trichomes. Determination of leaf constants, qualitative physical and chemical analysis has given standard numerical values for comparison and detection of adulterants it would also be a useful for compilation of a suitable monograph. Phytochemical test like preliminary phytochemical analysis and thin layer chromatography were performed .Through chemical test presence flavonoids, saponins, tannins, steroids was revealed. From the TLC analysis presence of gallic acid was identified.

Key words: *Glochidion velutinum,* pharmacognostic, flavonoids, saponins, fluorescence analysis

INTRODUCTION:

In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still likes drugs from plants. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future[1]. Majority of crude herbs come from wild sources and it is collected to assess quality parameters by which presence of various phytochemicals can be confirmed. Standardizaton of natural products is complex task due to their heterogenous composition in form of whole plant. Authenticaton, pharmacognostic evaluation, phytochemical analysis are few basic protocols for standardization of herbals. *Glochidion velutinum* is a small monoecious tree which belongs to the family Euphorbiaceae and is distributed in India, Burma, and Pakistan. It is usually found on dry hills. The whole plant is medicinally important and many reports on claims to cure several diseases in traditional system of medicine particularly in folklore. The Chemical constituents include tannins, flavonoids, alkaloids and steroidal saponins. The folklore claim of the plant are anti-cancer, anti diabetic and anti-diarrheal activities [2]. Detailed literature review states that the plant has broad spectrum of the activities which were claimed traditionally and some are proven scientifically. Most of species in this genus were explored on the basis of the chemical constituent but not on pharmacognostical and pharmacological basis. *Glochidion velutinum* is amongst the genus which has not explored much and there were no reports

for its pharmacognostical standardization. Hence forth in the present study the pharmacognostical standardization of *Glochidion velutinum* leaf was selected.

MATERIALS AND METHODS:

The plant was collected from the forest regions of Thalakona of Chitoor dist, from Dr. K. MadhavaChetty. It was authenticated by Mr. A. Lakshma Reddy, Retired Professor, Dept. of Botany, Nagarjuna Govt. College (Autonomous) Nalgonda. A herbarium was prepared and deposited in the Dept. of Pharmacognosy for further reference. The plant was identified as *Gochidion velutinum WT*. (Euphorbiaceae) and was certified under Voucher No: NCOP-NLG/ph'cog/2009-10/003.

Instruments used:

Micro senior precision rotary microtome (latest Spencer 820 type), Sisco muffle furnace (3003137), Rotary vacuum evaporator, Stage micrometer, Eye piece micrometer.

Chemicals and reagents:

All the chemicals and reagents like chloral hydrate, phloroglucinol, hydrochloric acid, nitric acid, potassium hydroxide, picric acid, lead acetate etc used were of analytical grade.

Microscopical studies: [3-10]

Transverse section of leaf:

Microtome sectioning was done for fresh leaf to obtain a thin section. Phuloroglucinol and hydrochloric acid in the ratio 1:1 was used as a stain and mounted on a glass slide and focused under a microscope. Polychromatic stain, toluidine blue also was used to stain the section. A thin transverse section was taken and studied.

Powder microscopy:

Shade dried leaves were powdered with the help of an electric grinder till a fine powder was obtained .This fine powder was subjected to powder microscopy, as per standard procedures mentioned.

Determination of leaf constants:

Stomatal number, stomatal index, vein islet number,vein termination number and palisade ratio were determined for the leaf of the plant as per standard recommended procedures.

Determination of physico chemical properties:

Total ash, acid insoluble ash and water soluble ash of *Gochidion velutinum* was determined by standard method and the results are tabulated in table. The crude fibre content, moisture content, alcohol soluble extractive value, water soluble extractive value, chloroform soluble extractive value and petroleum ether soluble extractive values were determined by standard method and the results obtained are tabulated in table

Table-1: Measurement of trichomes

Unicellular	130.4µm--233-456µm
Multicellular	104 µm--211.8--521.6 µm

Table 2 Leaf Constants:

Parameter	**value**
Stomatal number	2-2.5-3 3-5.5-6
Stomatal index upper lower	 17.3 21.4
Vein islet number	1-4-7
Vein islet termination	1-1.75-3
Palisade ratio	22

Determination of Fluorescence analysis:

Powdered leaf was subjected to analysis under ultra violet light after treatment with various chemical and organic reagents.

PARAMETER	VALUES(%w/w)
ASH VALUE	
Total ash Acid insoluble Water soluble	10.5% 1% 0.7%
EXTRACTIVE VALUES	
Ether soluble Chloroform soluble Water soluble Alcohol soluble	4.8% 25.1% 16% 34.4%
Moisture content	9.2%
Crude fiber Content	53%

Table: 3-Proximate Analysis of Leaf

Behaviour of powdered drug with different chemical reagents:

Small quantity of the powdered drug sample is taken in a watch glass and mixed with different reagents. The change in the colour was observed.

Successive Solvent Extraction:

The powdered leaf material was subjected to soxhlet extraction using petroleum ether, chloroform, n-butanol, methanol, by successive solvent extraction method based on the increasing order of polarity of solvent. All the extracts were subjected to preliminary chemical tests and TLC analysis for identifying the best solvent.

Preliminary chemical screening: The extract obtained was subjected to various chemical tests as per the procedure mentioned in the standard reference books.

Thin Layer Chromatographic Analysis :[11, 12]

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures of chemical compounds. It is the most basic method of confirming the presence of a phytochemical compound.

Absorbent: Precoated TLC Silica GEL $60F_{254}$. Solvent Systems used:

Ethylacetate:formic:acid:glacialaceticacid:n-butanol(flavonoids) (100:11:11:26)
N-butano:glacial:acetic:water (5:3:2)saponins
Chloroform:ethylacetate:aceticacid (6:4:4)(tannins)

Detection system: UV chamber

RESULTS:

Transverse Section of Leaf:

T.s of the leaf showed the dorsiventral condition .The tissues present lamina and midrib region are as follows:

Lamina Region:

Upper epidermis: Consist of single layered rectangular cells with a thin cuticle covering it.

***Mesophyll**:*

Differentiated into palisade and spongy parenchyma. Single layer of elongated palisade cells with chlorophyll were observed in the upper epidermis. Below the palisade region there were 3-4 layers of circular spongy parenchyma cells(Figure 3).

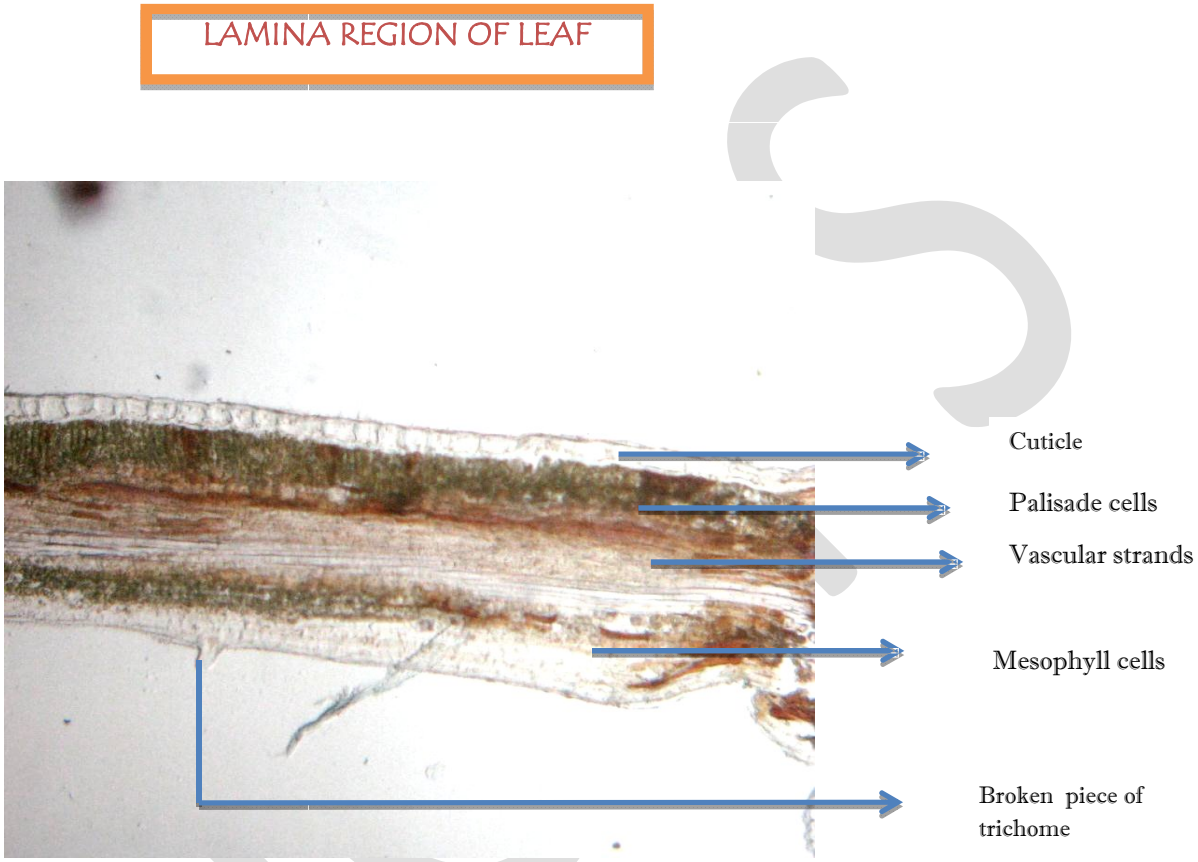

Figure-3: Lamina of Leaf

Midrib Region:

In the midrib region below the upper epidermis and above the lower epidermis there was an irregular arrangement of collenchyma and parenchyma cells. In upper epidermis 1-2 layers of compactly arranged circular collenchyma cells were observed below the cuticle. Bellow the collenchyma there were 4-5 layers of irregularly arranged

circular parenchyma cells with inter cellular spaces were present. Tannin containing parenchyma cells were prominently observed. The major portion of the mid rib was occupied by the vascular tissue. Arc shaped vascular bundles with protoxylem radiating towards upper epidermis was noticed. Arrangement of the vascular bundles was alternate. A sheath of pericyclic fibre was observed below the vascular tissue (Figure 1,2).

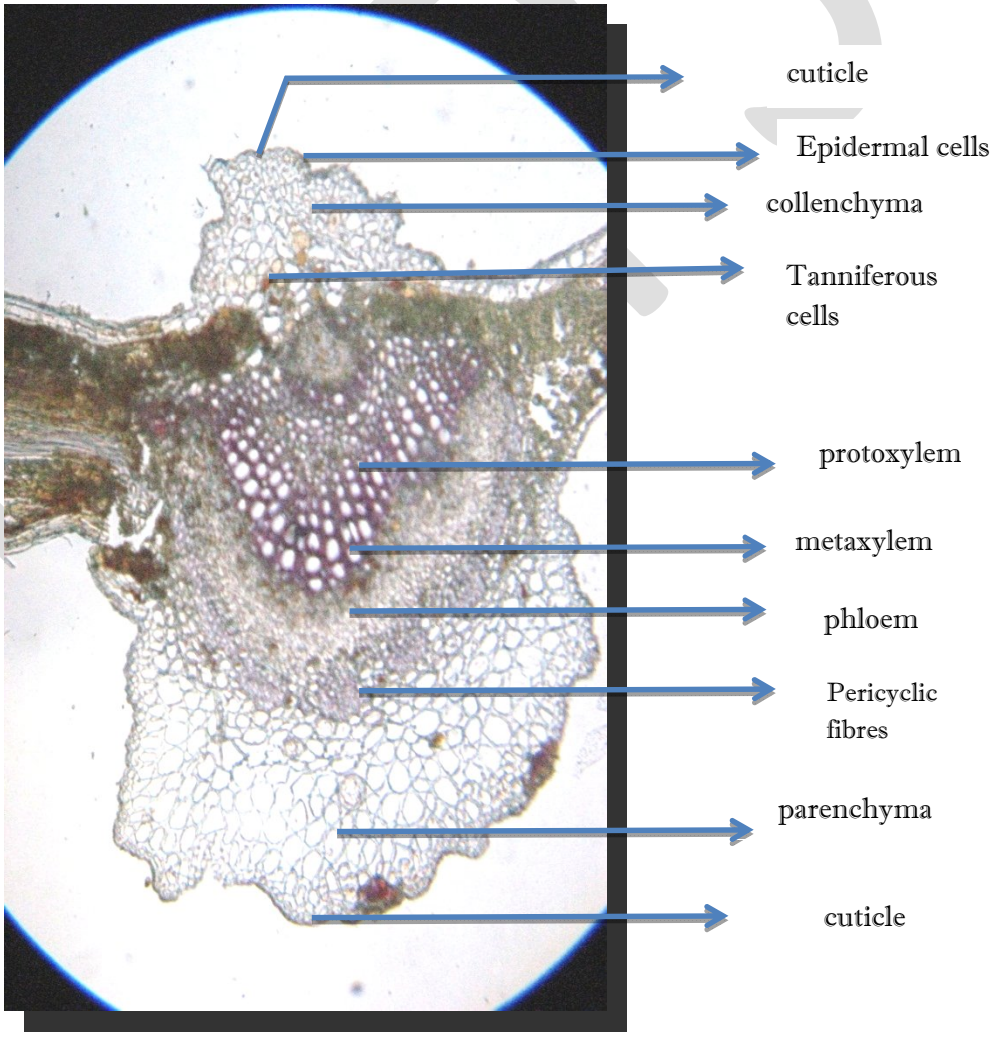

Figure 1. Stained with phluroglucinol-HCl

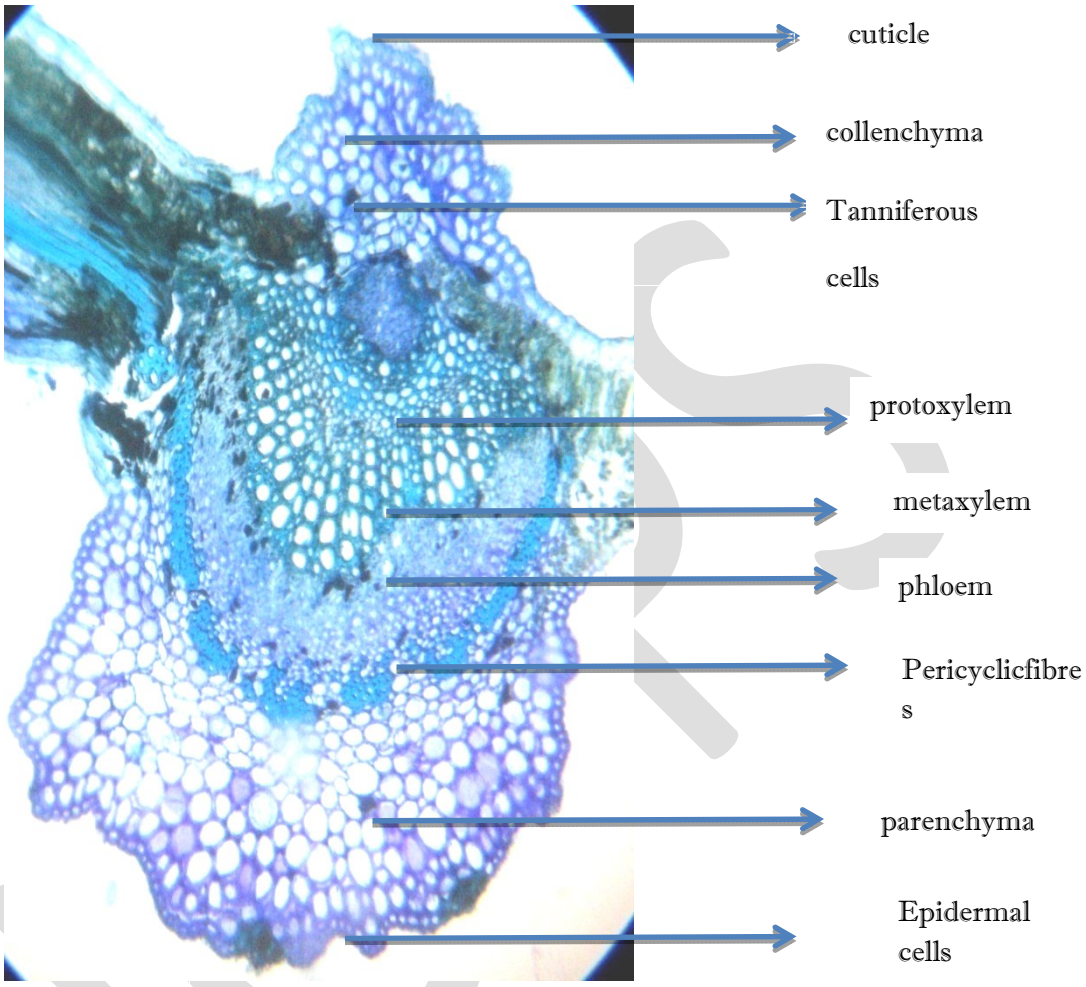

Figure 2. Section stained withToluidein Blue

Lower Epidermis:

In lower epidermis above 1-2 layers of compactly arranged circular collenchyma cells were observed below the cuticle. Bellow the collenchyma there were 8-11 layers of irregular arranged circular parenchyma cells with inter cellular spaces were present Surface preparation of the leaf showed the well-defined rectangular epidermal cells with occasional paracytic stomata

(Figure 7).

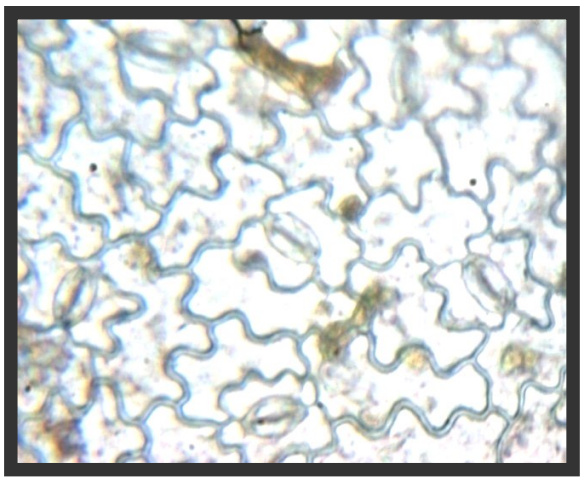

Figure: 7 Stomata

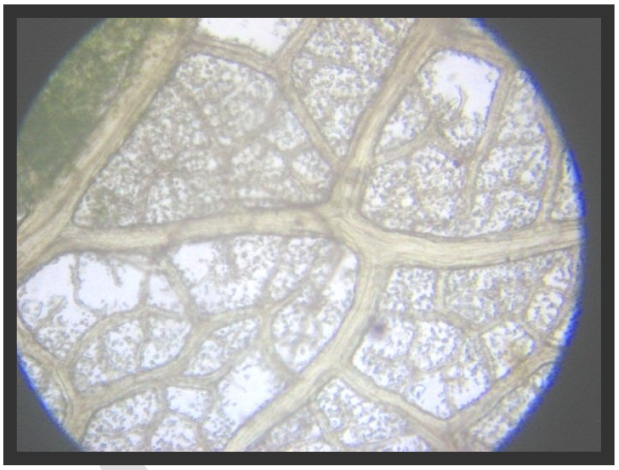

Figure: 8
Vein islets and termination

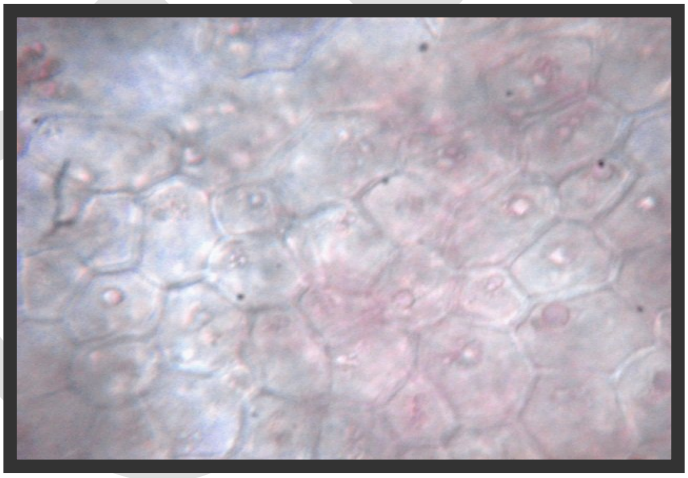

Figure: 9 Palisade cells

Powder Microscopy: Powder microscopy was done according to the standard procedure mentioned. The powder microscopy revealed the presence of the following parts:

Epidermal cell (Figure 4): small polygonal shaped epidermal cells were seen

Unicellular covering Trichomes (Figure 5): unicellular covering trichomes were found to be long, slender with bent at the base and with pointed apex

Multicellular covering trichome:

short uniseriate multicellular trichomes with pointed tip were observed (Figure 6) the length of the trichomes were measured and are tabulated in Table 1.

Determination of Leaf Constants:

Leaf constants aid to determine the adulteration and substitution of the drug, because these parameters were fixed to the particular plant (Figure 7, 8, 9, Table2).

Proximate Analysis

Proximate analysis of *Glochidion velutinum leaves* were determined by standard method and the results were tabulated in Table-3

Fluorescence Analysis:

Powdered leaves were subjected to analysis under ultra violet light after treatment with various chemical and organic reagents. The findings were tabulated in the (Table 4).

Behavior of Leaf Powder with Different Chemical Reagents:

The results obtained are tabulated in table .The behavior studies were found to be matching with that of the chemical tests performed (Table 5).

Preliminary chemical screening:

The chemical tests performed for the extracts obtained showed the presence of the following constituents.

Petroleum ether extract: alkaloids:

Cloroform extract: carbohydrate, alkaloids, proteins, tannins, steroids, flavoinoids, saponins

n-Butanol: carbohydrates, flavonoids, saponins

Methanol: carbohydrates, saponins

TLC analysis of leaf:

Thin layer chromatographic analysis showed that spots for all the solvent systems. The spots obtained for the tannins were found to match with reference drug Gallic acid. The colour of the spot obtained and the Rf value were found to be similar to that of Gallic acid. Hence it can be assumed that the extract may contain Gallic acid or its derivatives (Table 6).

Table 6: Thin Layer Chromatographic analysis

PHYTO CONSTITUENTS	RF VALUES	Colour of Spots
Flavonoids	Leaf:0.22	Pale brown
Saponins	Leaf:0.91	Orange yellow
Tannins &Gallic acid	Leaf:0.75 Gallic acid(std):0.7	Brown &Dark Brown

TABLE-4: Fluorescence Analysis:

Reagent	Observation	Inference
powder+ iodine	Black colour observed	Presence of starch
powder+ $HgCl_2$	Bulecolour observed	Presence of Alkaloids
powder+ Ammonia	No pink colour observed	Absence Of Cardiac Glycosides
powder+ $AgNO_3$	No ppt formed	Absence of Protiens
powder+ Picric Acid	Colour change	Presence of Alkaloids
powder+ Water (shaking)	Foam appeared	Presence of Saponins
powder+ conc H_2SO_4	Black	Presence of starch
powder+$FeCl_3$	Bluish black	Presence of tannins
powder+ Conc HNO_3	Orange yellow	Presence of tannins

Table: 5 Behavior of Leaf Powder with Different Chemical Reagents:

Reagent	Long (365)	Short (256nm)	Day
50% H_2SO_4	Black	Green	Olive green
50%HNO_3	Dark Green	Green	Light Brown
5% NaOH	Black	Dark Green	Dark Brown
1N Me NaOH	Brick Red	Dark Green	Light Olive Green
1N KOH	Brick Red	Green	Dark Brown
5% KOH	Black	Bottle Green	Brown
$FeCl_3$	Black	Dark Green	Black
Methanol	Brown	Green	Light Olive Green
ConcHCl	Black	Green	Olive Green
Conc H_2SO_4	Flo.green	Black	Dark Brown
Ammonia	Black	Green	Brown
Conc HNO_3	Black	Green	Brown

DISCUSSION:

Detailed microscopy of the plant specimen divulged the valuable information regarding the microanatomy of the *Glochidion velutinum*. Section of leaf revealed the dorsiventral nature. Fundamental regions like upper epidermis, mesophyll, midrib, and lower epidermis were observed. It consists of thin cuticle on the surfaces, collenchyma, parenchyma. In the mesophyll region palisade cells were present only on the upper epidermis. Powder microscopy of the leaf powder showed the presence epidermal cells, trichomes. From the detailed microscopy of the leaf, unicellular and multicellular trichomes were considered as tissue of diagnostic importance. Extractive values play a vital role for the evaluation of the crude. Alcohol and water soluble extractive values indicate the presence of the adulterants, faulty processing and poor quality of the drug. While petroleum ether soluble extractive value indicates percent of lipid content present in the crude drug. Ash values were used to detect the presence of any siliceous contamination and presence of any water soluble salts and incorrect preparation. Crude fibre content is useful technique for differentiation of the similar drugs and for the detection of adulteration. Moisture content is an inevitable content in the crude drug. It should be eliminated as far as practicable. While processing drying of plant material plays crucial role. It helps to fix the constituents, aid in preservation. The values obtained aids to establish the suitable monograph of the plant. Fluorescence analysis of the powdered drugs were performed and tabulated which helps to detect the adulteration, because phyto constituents exhibits characteristic fluorescence under ultraviolet light when they got mixed with the reagents. The fluorescence exhibited by the mixture was attributed to the chemical constituents present in the crude drug [13,14]. Prior to the phyto chemical screening a rough

estimation of phytoconstituents was done by the behaviour of powder drug with different chemical reagents which powdered drug showed different colours when it got mixed the particular reagents which reflects the presence phytochemicals in accordance with the colours obtained. Phyto chemical evaluations like preliminary phytochemical screening was performed according to the standard procedures. The investigation revealed the presence of various active phyto constituents like alkaloids, tannins, flavonoids, saponins. Preliminary phytochemical screening was further quantified by TLC, in which TLC performed for the detection of flavonoids, saponins and tannins. For tannins gallic was taken as standard, the Rf values of the extracts were quite closer to the Rf values of the gallic acid. From results it may be conclude that the extracts may contains gallic acid and its derivatives.The preliminary chemical tests confirmed that chloroform was a suitable solvent for extraction of the active principles from the leaves of *Glochidion velutinum*. The detailed phytochemical investigation strengthens the resourcefulness of the extracts for the further pharmacological evaluations. All these results put together will help in filing a suitable monograph for the leaf of *Glochidion velutinum*.

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