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STANDARDIZATION AND ELEMENTAL ANALYSIS OF *GALPHIMIA GLAUCA* LEAF AND STEM PARTS EMPLOYING SEM-EDAX

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ABSTRACT

The plant Galphimia glauca is well known for its varied traditional uses. The current study deals with its microscopical, physical and micro chemical (Elemental) characterization using Energy Dispersive X-ray Analysis (EDAX) detector fitted to Scanning Electron Microscope. The shrub has appealing microscopical characters. Leaf and stem parts were subjected to microscopic studies including powder microscopy and quantitative microscopy. Unicellular covering trichomes, paracytic stomata, collenchyma, lignified xylem, palisade tissue present below upper epidermis of leaf, cork cells 1-5 layers with brownish content fallowed by several layers of phelloderm, un-lignified secondary phloem and lignified secondary xylem etc. were noticed in SEM and Transverse section studies of leaf and stem. Leaf and stem powders were subjected to physical evaluation. Significant amount of variation in elemental composition was noticed in leaf and stem parts. Primary elements detected were Carbon, Oxygen, Silica, Potassium and Calcium. Other secondary elements found were Aluminium, Sodium, Magnesium and Chlorine.

KEY WORDS

Galphimia glauca; Malpighiaceae; Microscopic study; SEM-EDAX; Histological characters.

1. INTRODUCTION

India has enriched with rich source of medicinal plants diversity and vast traditional knowledge related to the use of medicinal plants against almost all reported diseases. *Galphimia glauca* (*G.glauca*) is a perennial, ever green shrub which grows well in tropical and sub- tropical habitats. It belongs to family of Malpighiaceae [1, 2] and is found across the Indian subcontinent and extensively seen in peninsular India. It has been known since pre-columbian times and it is commonly known as "*Calderona amarilla*" and "*Florestrella*" in Spanish [3, 4]. Tea infusion of *G. glauca* is used to treat diarrhoea, dysentery and gastroenteritis. In folklore medicine, the plant is known by the name "Noche buena".

The plant is used for treating allergic rhinitis and pollinosis [5]. There are no reports available on the histological characters of *G. glauca*. Hence the present study was taken up to explore the microscopic characters of this shrub. We in this publication report the light and scanning electron microscopic analysis of the plant. Singh *et al,* 2012, described the trace elements as "in-organic switches" [6]. Previous reports reveal the attempts made to correlate the mineral content of medicinal plants with therapeutic actions [7]. Hence the elemental analysis is performed to explore the elemental content keeping in view of the importance given to them in traditional system of medicine in regards to health and disease conditions of humans as this will help in prevention of nutrition related diseases and maintenance of good health of mankind [8].

The leaf and stem parts of *G. glauca* are evaluated for elemental characterization employing Energy Dispersive X- Ray analysis detector which is fitted to scanning electron microscope (SEM-EDAX) a non-destructive, highly useful technique when compared to other various methods used such as Energy Dispersive X-Ray Florescence (EDXRF), Instrumental Neutron Activation Analysis, Atomic Absorption Spectroscopy (AAS), Electro Thermal Atomic Absorption Spectroscopy (ETAAS), Energy Dispersive

X-Ray Analysis/Spectroscopy (EDAX/EX/EDS), Particle Induced X-Ray Emission, Industrially coupled Plasma Mass Spectroscopy (ICPMS), Industrially coupled Plasma Atomic Emission Spectroscopy (ICPAES).[9]

2. MATERIALS AND METHODS

2.1 Collection, identification and authentication of Plant Material

The shrub *G. glauca* was cultivated in the medicinal garden available in the School of Pharmacy, Anurag Group of Institutions. The aerial parts were collected in the time of November, 2014. The plant was identified and authenticated by taxonomist, Narsimha Murthy, Satavahana University, Karimnagar, Telangana state, India. A voucher copy is stored with the reference number No. 336, in the Department of Pharmacognosy and Phytochemistry, School of Pharmacy.

2.2 Microscopic Evaluation

2.2.1 Anatomical studies

Transverse sections (T.S) of fresh tissue pieces of leaves and stems of *G. glauca* were immersed in FAA fixative (37% v/v formaldehyde, 50% ethanol and 5% acetic acid). Tissues of various parts were fixed overnight, rinsed for about three times using sodium phosphate buffer pH 7 before dehydrating in an ethanol series (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% and 100% respectively, dried for thirty min in each step) and embedded in paraffin after infiltration in a vacuum oven. Transverse sections of 10µm thickness were cut using rotary microtome. Transverse sections were stained with safranin and Fast-green. Light microscopic studies were done using Olympus Bx-51 microscope fitted with high resolution CCD camera (Charged coupled device) [10].

2.2.2 Powder microscopy

The shade dried course powder of *G. glauca* leaf and stem were subjected to powder microscopy, further the various microscopic characters were detected by staining the powders with different staining reagents. [11]

2.2.3 Quantitative microscopy

The *G. glauca* was studied for leaf constant such as stomatal number, stomatal index, palisade ratio, vein-islet number and vein- termination number according to the standard procedures [12].

2.2.4 Physical evaluation

The *G. glauca* leaf and stem powders were subjected to evaluation for various physical parameters like Total ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractive value, Water soluble extractive value, Ether soluble extractive value and Moisture content according to the standard procedures. [12, 15]

2.3 Micro chemical (Elemental) analysis

2.3.1 SEM-EDAX analysis

SEM-EDAX analysis (Energy Dispersive X- Ray analysis detector fitted to scanning electron microscope) was carried out at Electron Microscopy Centre, Indian Institute of Chemical Technology (IICT), Hyderabad, Telangan State, India. Small pieces of stem, flower (3 mm) and 5mm^2 pieces of leaves were fixed in 4% glutraldehyde in phosphate buffer (0.02 M, 6.9, pH). They were then washed with distilled water, dehydrated in graded alcohol series (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% and 100%), air dried and coated with gold in Hitachi HUS-5 GB Vacuum evaporator. SEM-EDAX analysis was carried out using INCA X-sight Oxford detector fitted to Hitachi S-520 Scanning Electron Microscope at an acceleration voltage of 20 KV. Full screen, window and spot modes were employed depending on the size of the component/plant part [13, 14].

3. RESULTS

3.1 The plant

Galphimia glauca is one among 26 species of the genus Galphimia that belongs to the family of Malpighiales, and the kingdom Plantae. The genus is characterized by bilaterally symmetrical flowers with persistent yellow to red petals. Most of the sepals lack oil glands; the fruit is a globose schizocarp which breaks apart into three small 1- seeded cocci.

3.2 Anatomical studies

G. glauca represents typical histological characters of isobilateral leaf. Transverse section (T.S) of leaf shows the presence of epidermis which is single layered covered with thick cuticle**,** it shows the presence of unicellular, conical covering trichomes. Paracytic stomata are seen more on the upper epidermal surface. Palisade tissue is present only on one side, i.e; below upper epidermis, which is elongated and compactly arranged cells. The spongy mesophyll is parenchymatous, multi-layered with loosely arranged thin walled cells. Collenchyma is multi-layered with compactly arranged cells seen above the lower epidermis. The midrib shows vascular tissue with xylem which is lignified.

Transverse section (T.S) of stem shows the presence of cork cells which are 1-5 layered with significant brownish content fallowed by several layers of phelloderm, cortex region is multi-layered. Secondary xylem is lignified and includes medullary rays, xylem vessel, tracheids, lignified fibres, un-lignified bundle

fibres and xylem parenchyma. The pith is parenchymatous.

Scanning Electron Micrographs and transverse sections of *G. glauca* leaf and stem are shown in Fig. 1 A-H; Fig. 2 A-H.

3.3. Powder microscopy

The powder microscopy of leaf showed the presence of epidermal cells, covering trichomes, palisade tissue, spongy parenchymatous tissue and lignified xylem. The powder characters of stem showed the presence of lignified fibres, un-lignified bundle fibres, cork cells with brownish matter, and lignified xylem tissue.

The powder microscopy of leaf and stem are shown in Fig. 3 A-D & Fig.4 A-H.

3.4 Quantitative microscopy

The stomatal number is 40 to 90 for upper surface and 80 to 130 for lower surface. The stomatal index is 14 to 26, 20 to 37 for upper and lower epidermis respectively, while the palisade ratio for upper and lower epidermis is 3 to 6 and 0 respectively. The vein islet-number is 23 to 29 for upper surface and 18 to 21 for lower epidermis, while the vein termination is 25 to 32 for upper epidermis and 21 to 27 for lower epidermis. The results of quantitative microscopy are tabulated in Table 1.

Table 1: Quantitative microscopy of *G. glauca*

Leaf constants	Values	
	Upper Epidermis	Lower Epidermis
Stomatal number	40-90	80-130
Stomatal index	14-26	20-37
Palisade ratio	3-6	0
Vein-islet number	23-29	18-21
Vein -termination number	25-32	21-27

Table 2: Elemental composition [weight and atomic weight (%)] of Stem and Leaf parts of *G. glauca*

Element	Stem surface		Leaf surface	
	Element (%)	Atomic (%)	Element (%)	Atomic (%)
Carbon	41.06	49.49	32.28	41.40
Oxygen	53.54	48.45	53.16	51.17
Aluminium	0.44	0.24	2.03	1.16
Potassium	3.64	1.35	1.57	0.62
Calcium	1.31	0.47	1.65	0.63
Sodium	-	-	0.62	0.42
Magnesium	-	-	1.29	0.81
Silica	-	-	5.09	2.79
Chlorine	-	-	2.32	1.01

3.5 Physical evaluation

The results of physical parameters of leaf and stem powders were reported in Table 3.Total ash and water soluble ash values were found high in stem powder. The water, alcohol and ether soluble extractive values were found to be high in stem powder, while the moisture content was seen more in leaf powder.

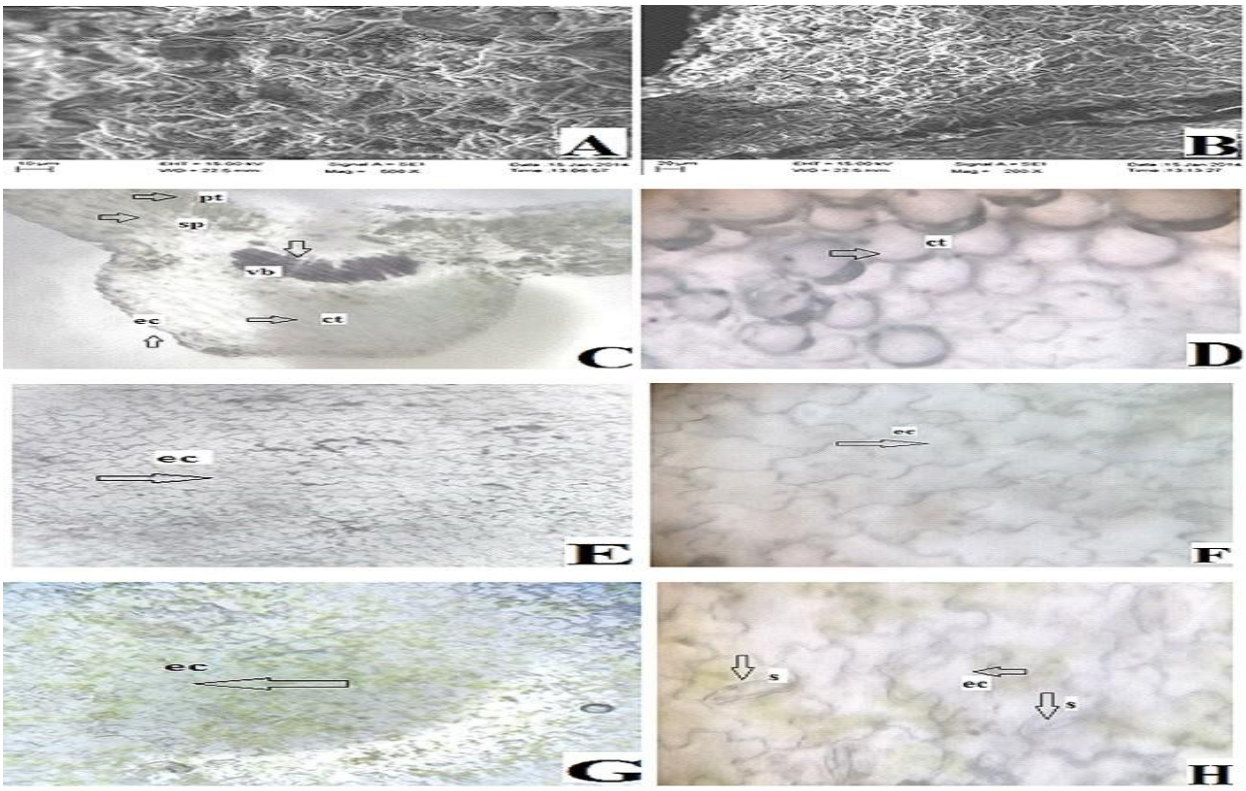
3.6 Micro chemical (Elemental) analysis

Carbon, oxygen, silica, potassium and calcium were detected in both the parts studied. However, there was a variation in their percentages. The results were tabulated in Table 2. Highest carbon, oxygen and potassium content were found on stem surface. Aluminium and calcium content were found more on leaf surface. Elements like silica, sodium, magnesium and chlorine were restricted to leaf surface.

Table 3: Physical parameters of Leaf and stem powders of *G. glauca*

Parameters	GGSP (% w/w)	GGLP (% w/w)
Total ash	8.4	7.21
Acid insoluble ash	0.4	0.61
Water soluble ash	3.1	2.56
Alcohol soluble extractive value	7.4	3.26
Water soluble extractive value	3.36	3.02
Ether soluble extractive value	4.2	3.6
Moisture content	12	19

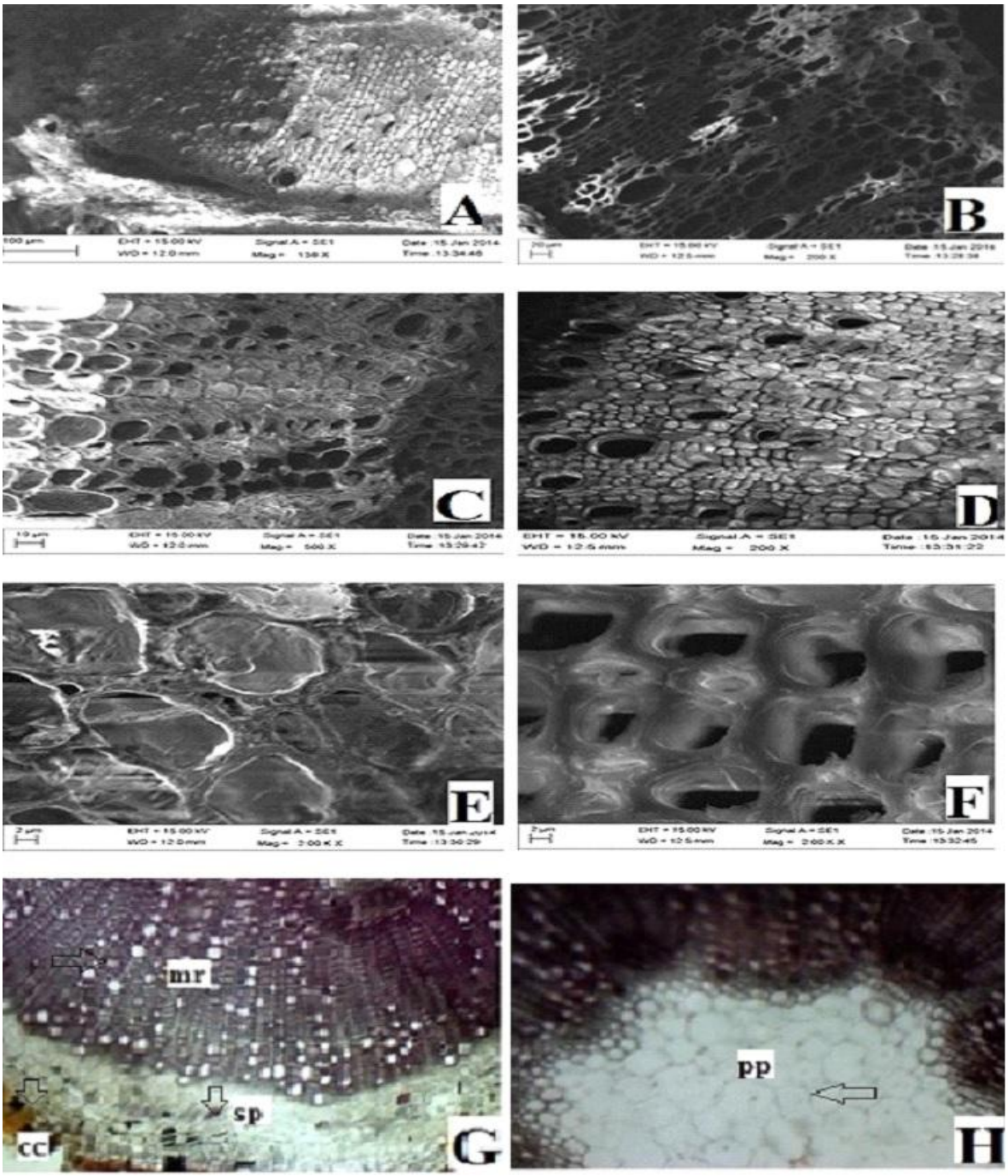
GGSP = *G. glauca* stem powder; GGLP = *G. glauca* leaf powder



"Figure 1: Scanning Electron Micrographs; Transverse section of *G. glauca* leaf".

A. SEM view of leaf upper surface, **B.** SEM view of leaf upper surface, **C.** Transverse section of leaf, **D.** Collenchyma tissue, **E.** Leaf upper epidermis, **F.** Upper epidermal cells, **G.** Leaf lower epidermis, **H.** Lower epidermal cells; **pt:** palisade tissue, **sp:** spongy parenchyma, **vb:** vascular bundles, **ep:** epidermal cells, **ct:** collenchyma tissue, **ec:** epidermal cells, **s:** stomata

"Figure 1: Scanning Electron Micrographs; Transverse section of *G. glauca* leaf".



"Figure 2: Scanning Electron Micrographs; Transverse sections of *G. glauca* stem".

A. SEM view of stem, **B.** Cortex, **C.** Medullary rays, **D.** Xylem and phloem tissues, **E.** parenchyma cells, **F.** vacuoles, **G.** Transverse section view of stem, **H.** Parenchymatous pith; **mr**: medullary rays, **sp**: secondary phloem, **cc**: cork cells, **pp**: parenchymatous pith.

"Figure 2: Scanning Electron Micrographs; Transverse sections of *G. glauca* stem".

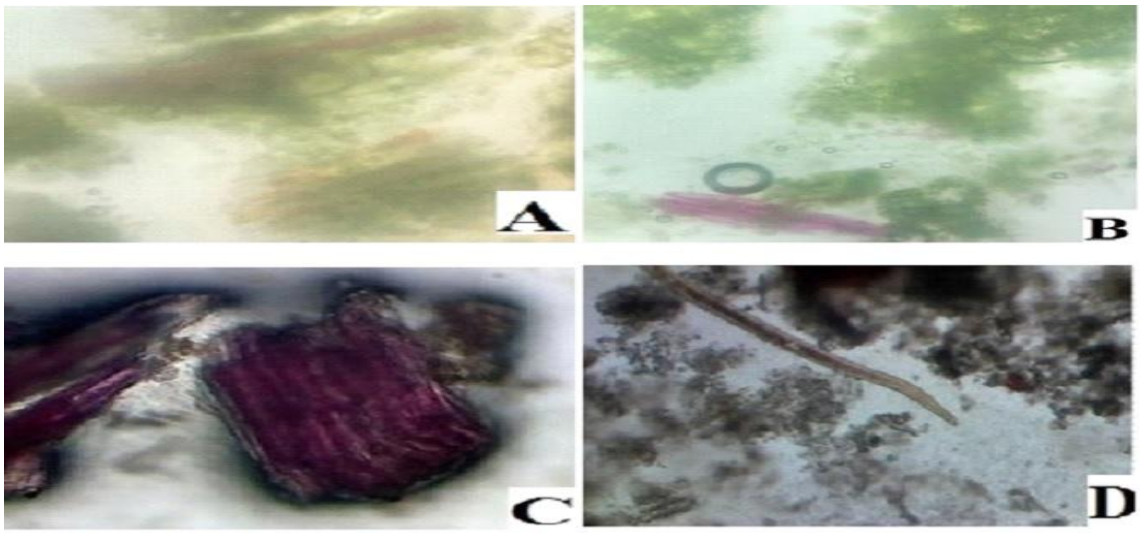


Fig. 3 Powder microscopy of *G. glauca* leaf

A & B. Lignified xylem tissue and chlorophyll leaf tissue, **C.** Lignified xylem tissue **D.** Fibres in leaf powder.

Figure 3: Powder microscopy of *G. glauca* leaf

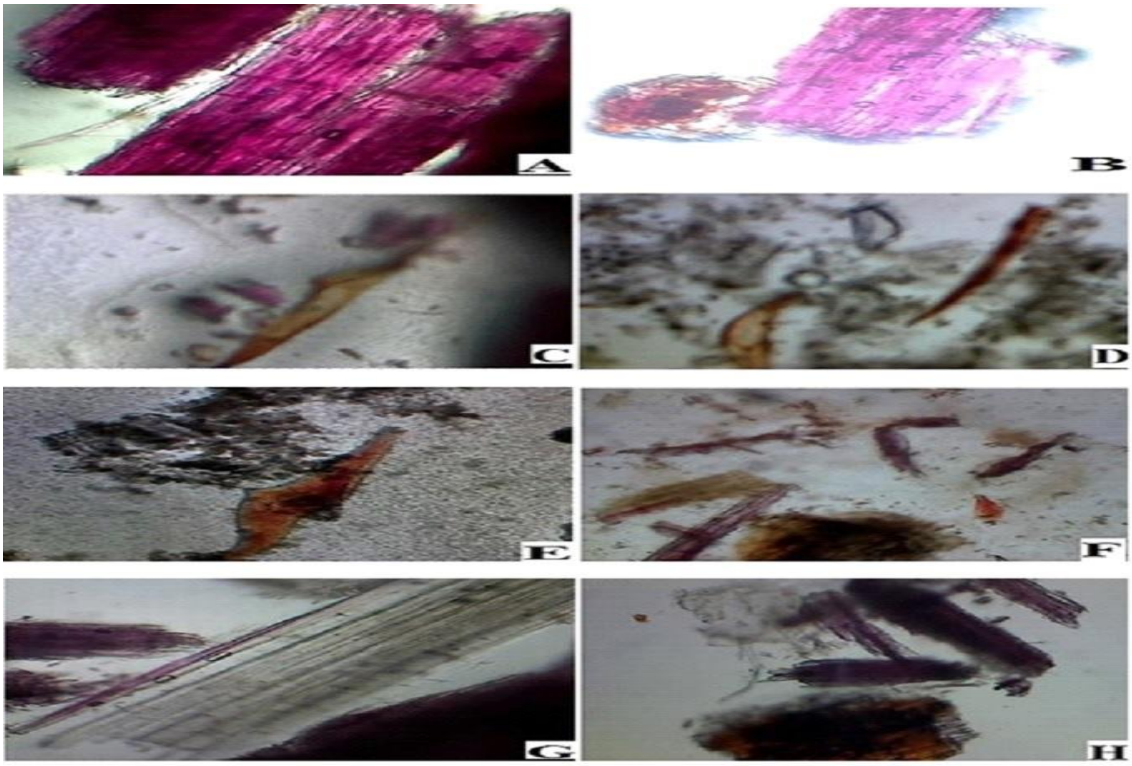


Fig. 4 A-H: Powder microscopy of *G. glauca* stem

A. Lignified bundle fibres; **B.** Cork cells with brownish matter; **C, D & E.** Fibres with brownish content; **F.** Lignified bundle fibres; **G.** Un-lignified bundle fibres; **H.** Cork cells with brownish content.

Figure 4: A-H: Powder microscopy of *G. glauca* stem

4. DISCUSSIONS

With the ever increasing use of herbs as medicines and the global expansion of herbal market safety has become a major concern among the people and the health authorities. The first important step in this process is to control the quality of medicinal plants. WHO has developed guidelines relating to safety and quality assurance of medicinal plants and herbal material. These guidelines include quality control/ standardization of medicinal plant materials. Standardization of the crude drug is important to ensure the proper quality of crude drugs, [15] it is achieved by evaluating various parameters of the crude drug.

The present study is focused on microscopic evaluation, which serves as one of the important diagnostic feature in evaluating crude drug. Type of cells and cell content study will provide the diagnostic character of individual crude drug. Many plant drugs were evaluated using light and scanning electron microscopy [16]. The need for the enhanced use of electron microscope in characterization of medicinal plants is emphasized [9]. Several micro morphological features of *G. glauca* were unfolded employing scanning electron microscope (SEM), which would not have been possible only with light microscope.

Qualitative histological evaluation of types and arrangement of tissues in leaf and stem parts of the plant *G. glauca* was evaluated according to the WHO parameters for standardization [15]. *G. glauca* leaf showed the presence of unicellular conical covering trichomes, paracytic stomata, palisade tissue, spongy mesophyll, collenchyma and lignified xylem. The stem showed the presence of cork cells with significant brownish content, phelloderm, cortex, lignified secondary xylem, medullary rays, xylem vessel, tracheids, lignified fibres, un-lignified bundle fibres and parenchymatous xylem. This study is also focused in its uniqueness in bringing out the ultramicroscopic diagnostic characters of *G. glauca* plant parts. Quantitative assessment of stomatal number, stomatal index, palisade ratio, vein-islet number and vein- termination number were done as a part of standardization of *G. glauca* leaf and stem parts.

Physical evaluation of powdered leaf and stem is done as a routine standardization. The results showed higher amount of silica/earthy matter/in-organic content in stem when compared to leaves through ash values. Ash values are performed to judge the identity and purity of crude drugs. While the extractive values are the indicative weights of the extractable chemical constituents of crude drugs under different solvent conditions. The study results shows higher extractive values with stem powder indicating complete exhausting of the stem powder which in-turn directly depends on the powder material drying/storage conditions. The moisture content was noticed low for stem powder compared to leaf signifying its stability against degradation.

Many plant species exhibited phylogenic variations. Elemental composition varies from part to part. Yashvanth *et al*, 2013 [17] has discussed the significance of various elements with respect to human physiology. SEM-EDAX data collected from stem and leaf parts showed the variation in elemental composition. Major elements detected by SEM-EDAX study in stem and leaf parts include Aluminium, Calcium, Carbon, Chlorine, Magnesium, Oxygen, Potassium, Sodium and Silica.

In plant stem, carbon and oxygen are found in high levels. The biological role of oxygen is involved in photosynthesis and cellular respiration, while carbon through the biological carbon cycle in plants is used in the production of carbohydrates like starch, sugars and cellulose which are the principle compounds that are required to build up biomass for plants or the bodies of the humans.

Calcium was also found in quite high amounts in stem (1.31%) and in leaf (1.65%). In humans and animals, calcium functions as a constituent of bones and teeth, regulation of nerve and muscle function, conversion of prothrombin to thrombin in blood coagulation, activation of many enzymes which are involved in muscle contraction, in neuromuscular excitability. Potassium was found more in stems (3.64%). It functions in acid base balance, regulation of osmotic pressure, conduction of nerve impulse, cardiac muscle contraction, cell membrane functions and Na^+/k^+-ATPase. Diarrhoea and metabolic alkalosis is seen with hypokalaemia while hyperkalaemia is seen in conditions of Addison's disease, chronic renal failure shock and dehydration [8].

The element silica was present in high amounts on leaf surface (5.09%) which could be the reason for its roughness. Aluminium was found more in leaf (2.03%) when compared to stem (0.44%). Aluminium is capable of altering the membrane functions at blood brain barrier [8]. Chlorine was restricted to leaf surfaces, it is involved in fluid and electrolyte balance. It is the chief anion in extracellular fluids which regulates extra cellular osmotic pressure, participates in acid production in the stomach. Depletion of chlorine through diet leads to alkalosis [8]. Magnesium is seen with 1.29% in leaf. In humans it is also a

constituent of bones, teeth and enzyme co-factor. Depletion of magnesium levels in body leads to excessive vomiting and diarrhoea and vasodilation [8]. The above elements present in the various plant parts of *G. glauca* may have a role in therapeutic actions of the plant.

5. CONCLUSION

The present study constitutes the first report and description of the significant diagnostic features of the plant *G glauca* to be published. It includes anatomical features, leaf constants, powder characters and physical parameters. The elemental analysis for stem and leaf parts was performed to correlate their role in the therapeutic actions of the plant. The above discussed pharmacognostic parameters will help in proper identification and standardization of the plant *G. glauca*.

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