

Pharmacognostic Standardization of Megacarpaea Polyandra Benth Leaf



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Abstract

M. polyandra Benth. (Locally known as Chach) is a perennial medicinal herb confined to the Himalayas from Kashmir to C. Nepal. Traditionally, young leaves are cooked as vegetables; the root is used to cure fever, gastric problems, and pneumonia. Owing to the presence of analogous species in its habitat the plant often suffers from quality disputes for collectors. To maintain the plant's uniqueness and avoid adulteration, pharmacognostic approaches, and microscopic investigations were used to report its features. Tests such as physicochemical study and chemical analysis were applied. The anatomical features of the plant parts were studied and identified through light microscopy. Pharmacognostic parameters viz ash%, moisture%, extractive values, and pH showed different values; various classes of plant metabolites have been detected. Fluorescence analysis, FTIR Spectroscopy, and elemental analysis revealed unique features of this plant. Different types of tissues of stomata, midrib, and powdered leaf were illustrated and identified. The parameters indicated above are being reported for the first time in *M. polyandra* and are important in setting microscopic and pharmacopoeias criteria for future identification and authentication of adulteration in this plant species.

Keywords: Megacarpaea polyandra; Medicinal plants; Himalaya; Brassicaceae; pharmacognosy

(Figure 1)

Introduction

In recent centuries, researchers have paid close attention to complementary and alternative medicine, with a particular focus on plant-derived medicines. Alternative remedies and the use of common objects to treat, avoid, and lighten the infection have progressed for a variety of reasons, including the fact that standard pharmaceuticals have several side effects [1]. Natural substances are chosen for screening based on their traditional use, compound organization, toxicity, randomized determination, or a combination of characteristics [2]. The institutionalization of natural medications depends on thorough investigations of the plant species, phytochemical examinations assurance of following components spectroscopy, determination of functional groups and structural components found in organic compounds by Fourier transform infrared FTIR, physicochemical analysis to evaluate the quality of the herbal drugs by determining ash values, moisture content, extractive values in different solvent

systems, pharmacological activities, and so forth, to ascertain its importance in medicinal and culinary purposes [3].

The quality control of herbal medicine is currently a source of concern. The quality of these medications is dependent on the correct verification of the plant parts used and the use of established techniques to standardize herbal remedies. Due to the close likeness of several species belonging to the same genus or even the family, there is always the potential of unintentional adulteration while collecting [4]. *M. polyandra* is a high-valued wild medicinal perennial crucifer herb (Figure 1) belong to Brassicaceae family and endemic to the Himalayas in Kashmir, Pakistan, India, and Nepal [5,6] found at 3000 to 3400m in alpine and subalpine environments [7].

In several ethnobotanical reports *M. polyandra* is recognized by its use among pastoralists and local farmers, young leaves

of this plant cooked as vegetable and root is effective against pneumonia [8]. In others studies the root is reported as coolant, antidote for scorpion sting and snake bite and to cure fever [5,6]. A report regarding the growth stages of *M. polyandra* was published by Singh et al [9] they argued that the complete maturity (germination to flower/seed production) depends on a three years' time scale. The first two years are important in which vegetative growth arises. On the other hand, at the vegetative stages collection of leaves is high because of the young leaves

and tender petiole. In vegetative stage this species phenotypically resembles with its neighboring species- *Angelica archangelica* var. *himalaica* (Clarke) E. Nasir, comb., and *Heracleum candicans* Wall. ex-DC. Occasionally, collectors are confused to identify their desired species. As there are no known standards for this species with no identifying characters. Therefore, the present study was designed to standardize *M. polyandra* pharmacognostically, microscopically, and chemically to decrease the risks of adulteration of this valuable herbal drug with other species.

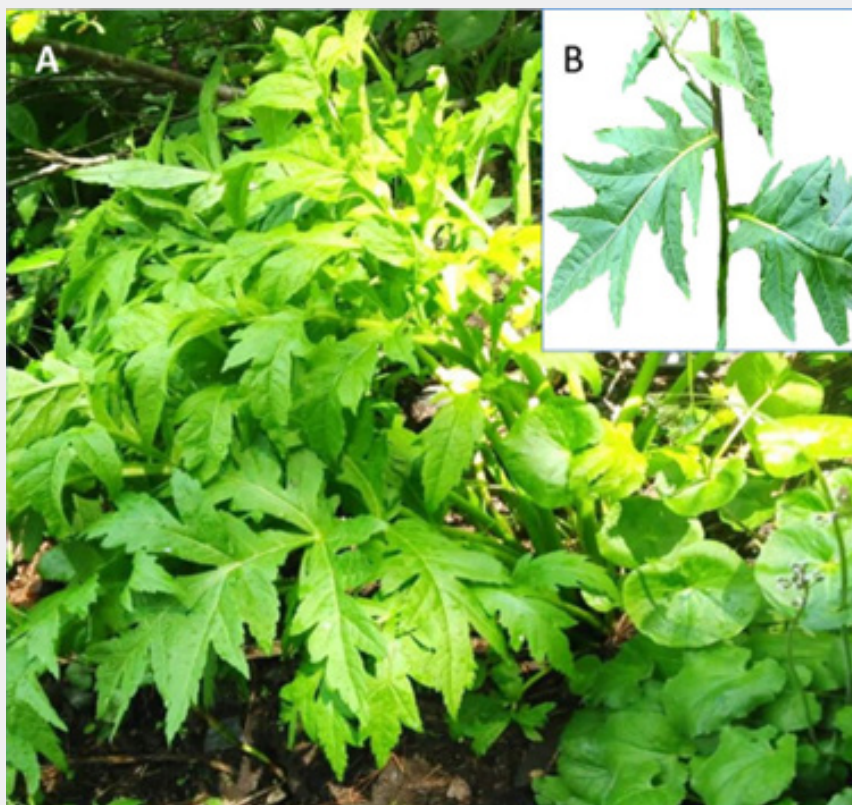


Figure 1: (A) Vegetative stage of *M. polyandra* with ground leaves, (B) Leaves (upper) on the main stem.

Methodology

Plant Collection, Identification and Submission of Specimen

Fresh and completely mature plant specimens were collected during August 2021 from Lawat, District Neelum Azad Kashmir. Plant was identified through available literature "Brassicaceae. vol. No 55, page 81-82, Flora of Pakistan" [10] and taxonomy followed "World Flora Online" [11] the plant was air dried, mounted on Herbarium sheet and assigned Herbarium number (KUH735) and deposited in the Prof. Dr. S. I. Ali Herbarium, Center for Plant Conservation, University of Karachi.

Physicochemical Analysis

Physicochemical analysis of leaf, which includes ash values

(total ash, acid insoluble, and water-soluble ash), moisture content, extractive value, and pH value was carried out following the methods of [12-14].

Morphological and Macroscopic Observations

Macromorphology of plant was carried out with the help of "Flora of Pakistan, no 55 family Brassicaceae" [10] and followed the method of Wallis [15].

Light Microscopy

Dried leaves were soaked in distilled water for 24 hours to maximum swelling and forming the shape of tissues. After 24 hours the section of the midrib (near to the leaf apex) was then cut into thin pieces and fine sections were treated with glycerin and chloral hydrate separately. For the study of stomata wet leaf

was slightly crushed then a pinch of crushed material was placed on slides. One slide was treated with chloroform and the second with glycerin. For powdered leaf study a pinch of dry powder was treated with glycerin. The sections and powder were observed under the digital microscope (B- 290TB) [16].

Phytochemical Screening

Preparation of Extracts

5g of powdered leaf material was accurately weighed. Following that, 100 mL of each solvent-acetone, chloroform, methanol, and water was filled in conical flask. The flask with leaf powder was placed on a stirrer for 48 hours. After that, the substance was filtered. The filtrate was placed in Petri dishes for complete drying. The dried extract was used to calculate the extractive value [17].

$$\text{Extractive value\%} = \frac{\text{Weight of extract}}{\text{weight of sample}} \times 100$$

Phytochemical Analysis

Extracts of four different solvents were checked to know the presence or absence of metabolites. For that purpose, 21 different standard protocols were applied. These standard protocols for preliminary phytochemical analyses have been discussed in the study of [16].

Fluorescence Study

The powdered leaf material was subjected to fluorescence analysis. Powder was treated with a number of chemicals (ether, hydrochloric acid, methanol, methyl trichloride, acetic acid, sulfuric acid, nitric acid, phosphoric acid, benzene, ammonia, water, and potassium hydroxide) and observed in ordinary daylight, under UV light- short wavelength (254 nm) and long wavelength (366 nm) [17].

FTIR Spectroscopy

Dried powdered leaf (10 mg) was subjected to FTIR analysis. After that, the sample disc was stacked in a spectrometer with an output range of 400 to 4,000 cm⁻¹ and 4 cm goals [18].

Scanning Electron Microscopy-Energy Dispersive Spectroscopy (Sem-Eds)

SEM-EDS (JSM-IT100 InTouchScope™) was used to analyze the leaf's microscopic features and element distribution. The surface areas of leaf (10 μm) (i) lamina with stomata (ii) lamina without stomata were selected for the field view and to capture elements distribution, and the microscope was operated at 20 KV accelerating voltage with the pixel size 1280 x 960μm.

Results

Morphology

Macromorphological properties of above ground parts of the plants are often employed for description and explanation of species in floras. Macromorphology is only useful for experts (plant taxonomists) when all morphological aspects of the plant (vegetative, flower, and seed) are available at the same time. On the other hand, Shinwari and Qaiser [19] says that there are few plant taxonomists/field botanists in Pakistan. Morphology of fresh and dried parts of this plant reported in this study (Table 1). Reported morphological characters are supportive for researchers during characterization of crud drug in future. Otherwise searching for plants within the flora is a difficult task for nonexperts. The macromorphology of aboveground parts (stem, leaves, flower, and seed) is summarized in Table 1. Change in morphology of fresh and dried parts was seen in size and color of leaves and flower parts other features were same in both forms.

Table 1: Morphology of *M. polyandra* Benth.

S. No.	Morphology	Fresh	Dry
1	Size of plant	Length=10 inch to 2 feet vegetative growth, 1.5-2meter (mature), width 6-13cm (base)	Leaves and stem width shrink in size
2	Leaves (basal)	Pinnatisect (basal leaves) 14-32cm long, oblong-lanceolate	Pinnatisect (basal leaves) 14-30cm long, oblong-lanceolate
3	Venation	Reticulate	Reticulate
4	Midrib	Closet mix color	Tawny brown color
5	Leaves (Uppermost)	Lanceolate or linear	Lanceolate or linear
6	Color (Leaf)	Green (upper side), lower pale green	Both surfaces brownish green
7	Leaf margin	Serrate or sinuatedentate	Serrate or sinuatedentate
8	Leaf base	Pinnatisect	Pinnatisect
9	Leaf apex	± Acuminate	± Acuminate
10	Flower color	Creamy yellow or white	Light brown
11	Pedicles size	Up to 20mm long	Up to 20mm long
12	Petals size	4.4-6mm long, 1.8-3mm broad	Shrink in size
13	Stamens size	9-16 mm long	Shrink in size
14	Seed size	1 cm in diameter	1 cm in diameter
15	Seed color	Brown	Brown

Physicochemical Analysis

Physicochemical/proximate analysis (Table 2) of the leaf was carried out which includes moisture content, ash values (total ash, water soluble ash, acid soluble ash), extractive values and pH. Physicochemical analyses are the most important tests for estimating the purity and quality of the collected crude drug material when compared to referenced values, as well as assisting in the establishment of standardization parameters and pharmacopoeial standards. The results for moisture percentage, total ash, acid soluble ash, water soluble ash and pH value were 8%, 10%, 86%, 33% and 4.5 respectively. All of the results were within the WHO's guidelines for herbal medications [13]. The existence of extraneous matter adhering to the plant part is determined by ash values, and their collection also gives a clear indication of the presence of low-grade mineral matter or inorganic contaminants in the crude medicines [16]. Moisture content measurement is an important parameter which is linked to the creation of microbial contaminants and high moisture can induce deterioration of the crude medication due to fungal/bacterial growth. Our sample's moisture content was within the specified range of 8 to 14 percent. Lower moisture content, on the other hand, ensures plant material's long-term storability and stability [13]. The pH value of the crude medication is also included

in the proximate analysis. The pH value of the drug determines its acidic and basic nature. The pH value of the sample was 4.5 ± 0.01 which was lower than 7, which falls in the acidic range that clearly shows that the constituents are slightly acidic. Extractive values of leaf were estimated in four solvents, which was high in water (33%), followed by methanol (7%), ethanol (6%) and chloroform (5%), these values are to Figure out the best suitable solvent for the extractive yield respectively. A solvent that produces a high extract yield is suitable and will aid in obtaining the maximum amount of components. With water as the solvent, a good yield was obtained (Table 2). Based on the nature of constituents and the solvent used, extractive values indicate distinct types of active phytoconstituents and their amounts in medicinal plants. It produces various amounts and types of phytoconstituents in response to a specific solvent and is used to detect adulterants and exhausted materials in crude drugs [20]. The active portion of a drug is separated from crude drugs and powder from various parts of the plant using different solvents, which is a significant marker of the nature of chemical ingredients in drugs [21]. Several researchers have published reports about extractive values of crude powder in which they were used variety of organic and inorganic solvents e.g Aslam et al [17]; Khan et al, [22]; Wang, et al, (2021); Nafees et al; Uza and Dastagir [23].

Table 2: Physicochemical analysis of *M. polyandra* Benth leaf (w/w) %.

S.No.	Parameters	Value
1	Moisture content	08 ± 1.08
2	Ash contents	10 ± 2.41
3	Acid (HCL) soluble ash	86 ± 0.01
4	Water soluble ash	33 ± 0.01
5	Chloroform-soluble extract	05 ± 0.03
6	Methanol-soluble extractive	07 ± 0.02
7	Ethanol-soluble extract	06 ± 0.01
8	Water-soluble extract	22 ± 1.02
9	pH value	4.5 ± 0.01

Microscopic Investigation of Midrib, Stomata and Powdered Leaf

Stomatal study revealed that the leaf of the plant bear anisocytic stomata via three companion cells that surrounds the stomata (Figure 2a, b, c). Leaf powdered showed different fragments of cells/tissues including spiral vessel (Figure 2d), fiber and vessels (Figure 2e), sclereids (Figure 2f), fibers with different colors and shapes (Figure 2g, h, i) fiber (Figure 2j), mesophyll cells (Figure 2k) and fragment of cortex cells (Figure 2, l-m).

The transverse section of the midrib showed various types of cells, midrib was covered by both the upper and lower epidermis from abaxial as well as adaxial surfaces. The shape of midrib is

more or less likely to the turtle. Vascular bundles are surrounded by inner and outer phloem and cambium (dark brown in color) is present between the outer phloem and xylem. On the abaxial side, (lower) midrib vascular bundles were arranged in oval shape and no vascular bundle was found in upper side of midrib. The adaxial side of midrib consists of two types of tissue viz epidermis and endodermis. Epidermis of the lower side of midrib consists of two layers of cells. Phloem towards the pith side was prominent with creamy white color and thick at the center, size of phloem towards cortex side was large than inner side phloem. The cortex region was brown in color with an irregular shape of tissue. The shape and size of pith tissues were not prominent; area of each xylem vessel was milky white in color (Figure 3).

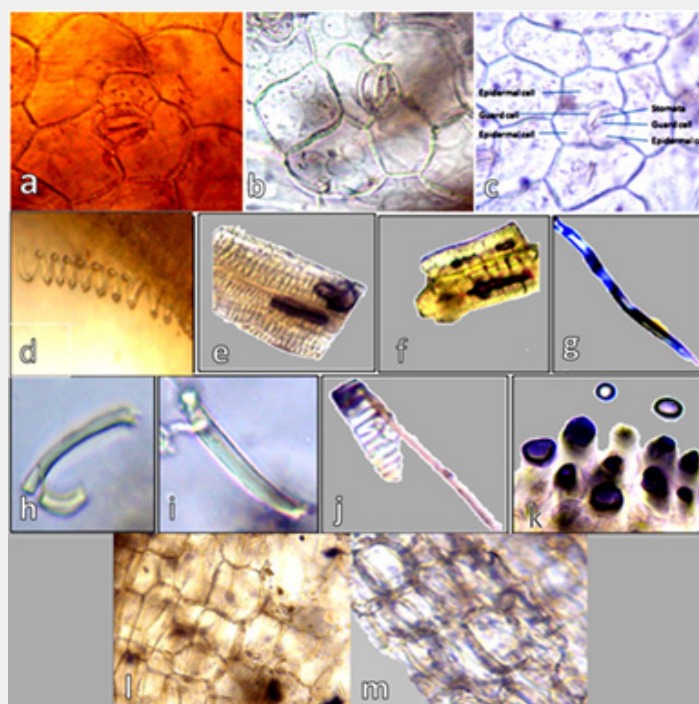


Figure 2: Powdered leaf microscopy (a) Anisocytic stomata under chloral hydrate, (b and c) Stomata treated with glycerin, (d) Spiral vessel, (e) fragment of vascular bundle with spiral vessels (f) Sclereids, (g, h, i) Different types of fibers, (j) Fiber, (k) Mesophyll cells (l, m) fragment of cortex cells.

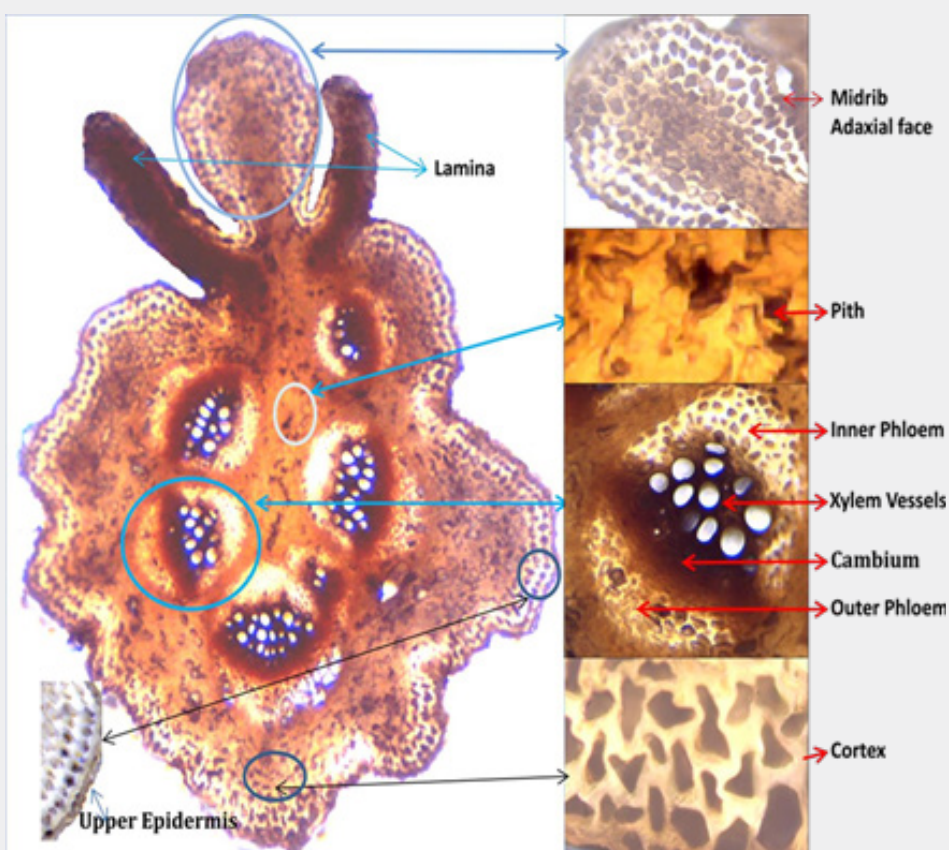


Figure 3: Transverse section of midrib of *M. polyandra*.

Midrib is an important part of studying tissues and have been reported in many studies such as Nafees, Ullah, & Ikram, [23] carried out the midrib studies of *Buddleja asiatica*. Mahmud et al, [24] studied *Holoptelea integrifolia* leaves, and they explained that the leaf anatomy is the foundation for standardizing and correctly identifying plants, as well as distinguishing them from closely related species.

Fluorescence and Phytochemical Analysis

Fluorescence analysis of the powdered leaf of *M. polyandra*

showed vast variation in color, when was treating with 13 different types of reagents under low (254nm) Visible light and high (366 nm). The most popular approaches identifying chemical substances include labeling with fluorescent chemicals and assessing color changes for [13]. The powder was treated with different reagents and different colors were observed under both visible and ultraviolet light according to the different contents of the powder (Table 3). This method can quickly verify the validity of medicinal materials and determine their quality in a tentative manner.

Table 3: Fluorescence analysis of *M. polyandra* Benth powdered leaf.

S.No	Used chemicals	Visible light	Wavelength 366nm	Wavelength 254nm
1	Powder of leaf	Grayish-green	Silver white	Silver brown
2	Powder + Ether	Grayish-yellow	Brown	Light brown
3	Powder + 2N Hcl	Light golden	Golden brown	Dark brown
4	Powder + Methanol	Light yellow	Silver	Grayish-white
5	Powder + CHCL ₃	Brownish-green	Pink	Light yellow
6	Powder + CH ₃ COOH	Yellowish-brown	Silver white	Grayish-white
7	Powder + H ₂ SO ₄	Yellowish-green	Aqua green	Whitish green
8	Powder + Nitric acid	Brownish Red	Light brown	Yellowish-brown
9	Powder + H ₃ PO ₄	Brownish-black	Grayish green	Brown
10	Powder + Benzene	Yellowish-brown	Dark pink	White
11	Powder + Ammonia	Yellowish-green	Grayish-white	Dark brown
12	Powder + H ₂ O	Greenish Brown	Grayish-white	Light brown
13	Powder + KOH	Greenish yellow	Yellowish-green	Light brown

The leaves of the aforementioned plant contained a variety of bioactive chemicals. The existence of active secondary phytoconstituents such as flavonoids, saponins, alkaloids, terpenoids, and phenols determines the medicinal potential of the plant (Table 4). Qualitative phytochemical screening must be responsible for the detection of secondary metabolites in plant crude materials, pharmacological amplification of crude pharmaceuticals, and the provision of genuine drugs for businesses [17]. Flavonoids have been shown to have anti-anaphylactic, anti-inflammatory, antioxidant, anti-allergic, antimicrobial, and anticancer properties, as well as anti-inflammatory, antioxidant, anti-allergic, anti-allergic, antimicrobial, and anticancer properties [25]. The presence of high amounts of steroids, terpenoids, and saponins in plants is thought to be responsible for their astringent characteristics. Tannins are used to treat bacterial, viral, and fungal infections, as well as burns, inflammation, and wound healing [27]. Saponins and glycosides must be utilized as immune regulators, anti-cancer agents, and in the treatment of the majority of heart illnesses. Phenolic constituents have been shown to have cytotoxic, anti-mutagenic, anti-oxidative, and astringent actions against pathogens such as bacteria [27]. Metabolites like anthraquinones are employed as laxatives, antimalarials, and anticancer drugs [28].

To determine the presence of different novel compounds in the plant 20 different tests were performed. We identified seven different classes of bioactive compounds (except fixed oil) in the leaves of *M. polyandra*, which show that leaves are also a rich source of therapeutic compounds. Similar work was carried out by various workers; for example, Aslam et al. 2019 carried out the phytochemical's investigation of *Caralluma edulis*; Uza and Dastagir (2022) *Astragalus scorpiurus*; Nafees et al. [23] authenticated of *Buddleja asiatica* for correct identification, authentication and quality assurance of the preliminary resources is an important requirement to make sure the reproducible quality of phytomedicine which will show the safety and effectiveness of herbal products. Pharmacognosy is an easy step to discriminate adulterated drug from an unadulterated one.

SEM-EDS and FTIR Analysis

SEM-EDS (scanning electron microscopy with energy dispersive X-ray spectrometry) is an elemental microanalysis technique used in a variety of fields including physical and biological sciences, engineering, technology, and forensic investigations [29]. X-ray peaks give identification and quantification for all elements of the periodic. To gather, analyze, and measure X-ray spectra, modern SEM-EDS X-ray microanalysis systems are often equipped with a

robust, flexible, and extremely helpful collection of software tools [30]. This technique was used by Boi et al, [31] for element detection in the root part of *Helichrysum microphyllum* subsp *Tyrrenicum*. By rastering a focused electron beam across the surface and detecting secondary or back scattered electron signals, SEM offers detailed high-resolution pictures of the material. The elemental identification and quantitative compositional information are also provided by an Energy Dispersive X-Ray analyzer. The elements

detected by SEM-EDS from *M. polyandra* dry leaf (lamina with stomata) (Figure 4a) are C, O, K, Ca, S, Cu, P, Co, Ni, Na, and Cr with the mass percentage of 52.01, 37.59, 4.34, 3.03, 1.17, 0.71, 0.42, 0.34, 0.23, 0.14, and 0.02 respectively. The detected elements with percentages from the lamina (10 μm) without stomata were C (49.70%), followed by O (35.31%), K (8.81%), Ca (3.84%), S (1.30%), Zn (0.74%), P (0.14%), Co (0.10%) and Cr (0.06%) (Figure 4b).

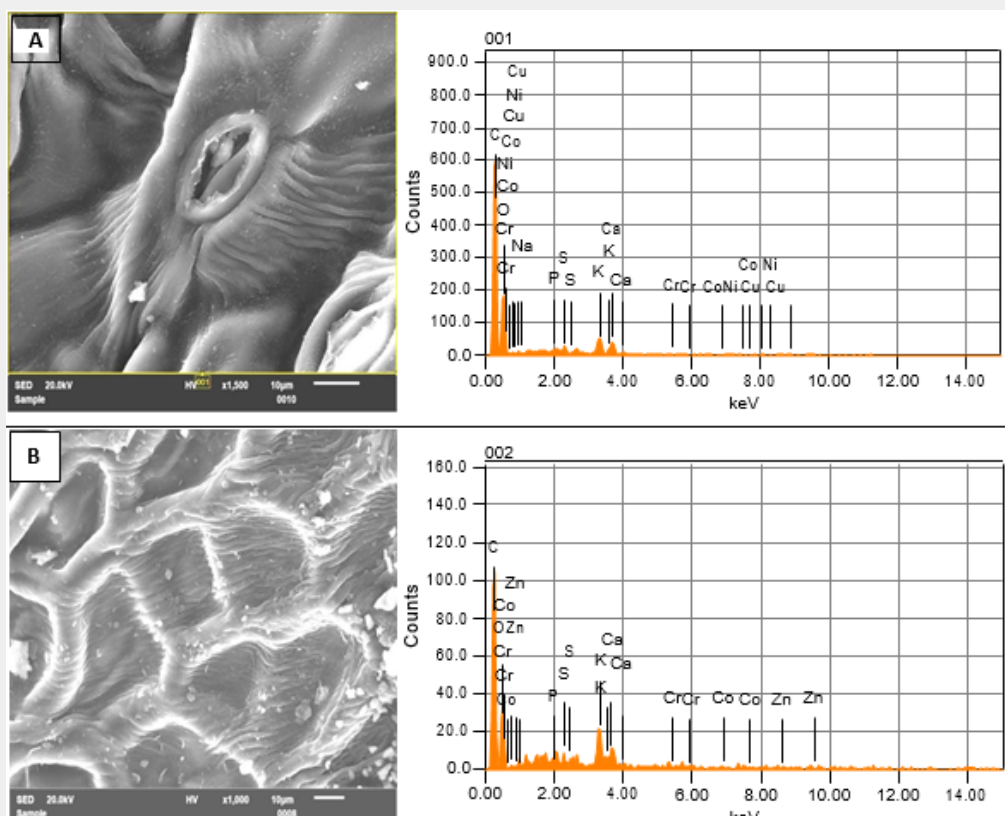


Figure 4: SEM-EDS analysis of *M. polyandra* dry leaf (A) field view of lamina with stomata and detected elements, (B) Field view of lamina without stomata, and detected elements.

The FTIR analysis was performed for the powdered leaf. The transmittance scan was run over 4,000- 1000 cm^{-1} (Figure 5). There were 27 sharp bends observed in the IR spectrum. First peak was at 3493.42 cm^{-1} which indicates the presence of O-H bonds. Other values such as 3286.63 indicates OH alcohol stretch, 2915.83 indicates O-H aliphatic stretch, 2849.57 show CH_3 group, 2365.26 C-H aromatic bond, 2336.82 $\text{O}=\text{C}=\text{O}$ stretching, 2323.64 $\text{O}=\text{C}=\text{O}$, 2234.38 C=C Alkyne, 2201.16 $\text{C}\equiv\text{C}$ medial alkyne, 2161.55 terminal alkyne, 1981.56 C=C=C alkane, 1731.23 Six-membered ring lactone, 1602.06 N=N, 1552.73 aliphatic nitro compounds, 1525.90 N-O starching, 1416.84, peaks such as 1237.56, 1135.87, 1102.66, 1030.32 represents -C-O stretching sulphate ion, 855.62 P-O-C stretch, 829.20, 812.39, three peaks 787.96, 758.57 and 701.68 shows C-Cl stretch, 659.09 represents thioether and $\text{CH}_2\text{-S}$

(Figure 5) [32]. The FTIR analysis has great importance which indicates the functional groups/ compound variety of the raw material during authentication of medicinal plants [33-36].

Conclusion

Raw material collected from most therapeutic plants loses its morphological characteristics. Sometimes only a single part of the plant is collected and used, or occasionally one common name is given to different species. That is why it is difficult to find out the correct scientific name of raw material by searching flora. Pharmacognostic study is a tool that can be used for the correct identification of raw materials of medicinal plants and also useful for specific differentiation among closely related species. It can be used to gain complete information about a crude drug and to

promote traditional herbal medicine knowledge in a meaningful way. Accurate identification of the plants paves the way for further research such as pharmacological activities, isolation of therapeutic bioactive compounds and drug preparation.

The parameters that have been studied in this study provides monograph for *M. polyandra* plant. These characteristics will aid in recognizing the species from its co-species and preventing the plant from becoming adulterated.

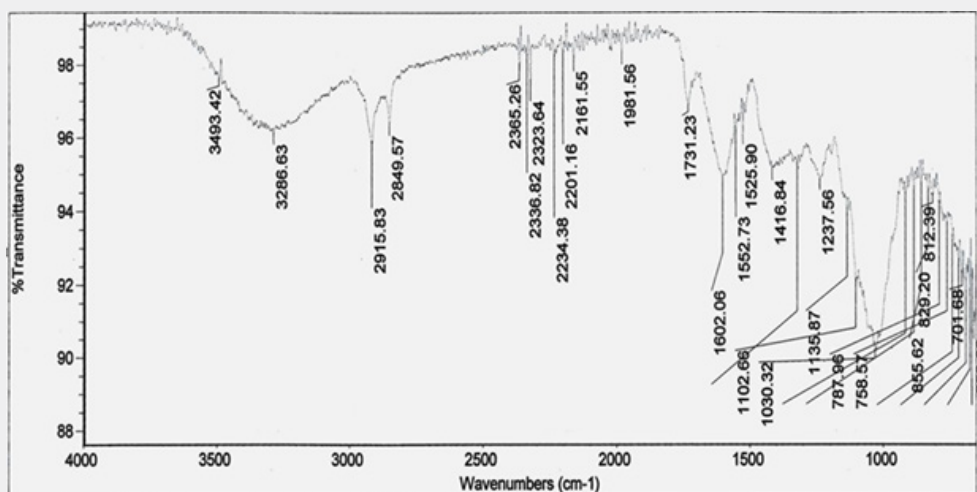


Figure 5: FTIR spectrograph of *M. polyandra* powdered leaf.

Table 4: Preliminary phytochemical analysis of *M. polyandra* Benth leaves.

S.No.	Class	Test applied	Extracts			
			Chloroform	Ethanol	Methanol	Water
1	Alkaloids	Mayer's test	+	+	+	+
		Hager's test	+	+	+	+
		Wagner's test	+	+	+	+
		Dragendorff's test	+	+	+	+
2	Proteins	Ninhydrin test	+	+	+	+
		Millon's test	+	+	+	+
		Biuret test	+	+	+	+
3	Carbohydrates	Barfoed's test	+	+	+	+
		Molisch's test	+	+	+	+
		Benedict's test	+	+	+	+
4	Saponins	Foam test	-	-	-	+
		Bromine water test	-	-	-	+
5	Fats and Fixed oil	Spot test	-	-	-	-
		Saponification test	-	-	-	-
6	Terpenoids	Salkowaski test	+	+	+	+
7	Glycosides	Bromine water test	+	+	+	+
		Keller-killani test	+	+	+	+
		Legal's test	+	+	+	+
8	Flavonoids	Ferric Chloride test	+	+	+	+
		Alkaline reagent test	+	+	+	+

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