



PHARMACOGNOSTICAL STANDARDIZATION OF YANAIKOLINCHI

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Abstract : Yanaikolinchi (Seeds of *Entada rheedii* Spreng) is an important ethnobotanical resource in the kolli hills Hotspot and has been authenticated by the CCRS institute. The present investigation focused on standardization of the seeds through macroscopy, microscopy, histochemistry, heavy metals, and physicochemical evaluation. Microscopic examination revealed the presence of cotyledon, Starch grains, Palisade, Stone cells, while histochemical tests indicated the presence of Alkaloids, Gelatin, Starch and Cutin with absence of Lignin. Physicochemical parameters including ash values and moisture content were within acceptable level, and the water-soluble extractive value was predominant. ICP-OES analysis confirmed the absence of heavy metals such as arsenic, lead, mercury and cadmium. The results confirms the purity and quality of yanaikolinchi and provide a reference for their authentication and standardization and safe utilization in preclinical research and future drug discovery.

Keywords: Seed authentication, Morphology, Anatomy study, Quality profiling.

INTRODUCTION

Pharmacognosy, which focuses on the study of medicinal substances obtained from natural origins, is crucial in this field by offering scientific methods and tools for the verification, assessment, and quality assurance of herbal medicines (Mukherjee PK, 2019). Pharmacognostic evaluation encompasses a range of analyses, including morphological, anatomical, physicochemical, and phytochemical studies. In recent years, the field has expanded to incorporate molecular markers and spectroscopic profiling techniques. Adulteration or substitution involves the use of alternative plant materials in place of authentic ones or the addition of other substances to increase the weight or effectiveness of the plant or reduce costs. The therapeutic effectiveness of medicinal plants is reliant on the quality and quantity of their chemical components. Misuse of herbal medicines or natural products often begins with incorrect identification. A frequent mistake is assigning the same common vernacular name to two or more completely distinct species (Dineshkumar C, 2007). Pharmacognostic research on medicinal plants has the potential to provide solutions to these problems. Hence, It is crucial to establish detailed pharmacognostic standards for plants that are incorporated into various pharmaceuticals.

II. METHODS & MATERIALS

The overall workflow for Pharmacognostical Standardization, including collection and authentication, macroscopic and microscopic evaluation, histochemical and anatomical analyses, physicochemical parameters, and heavy metal analysis is illustrated in (Figure.1).

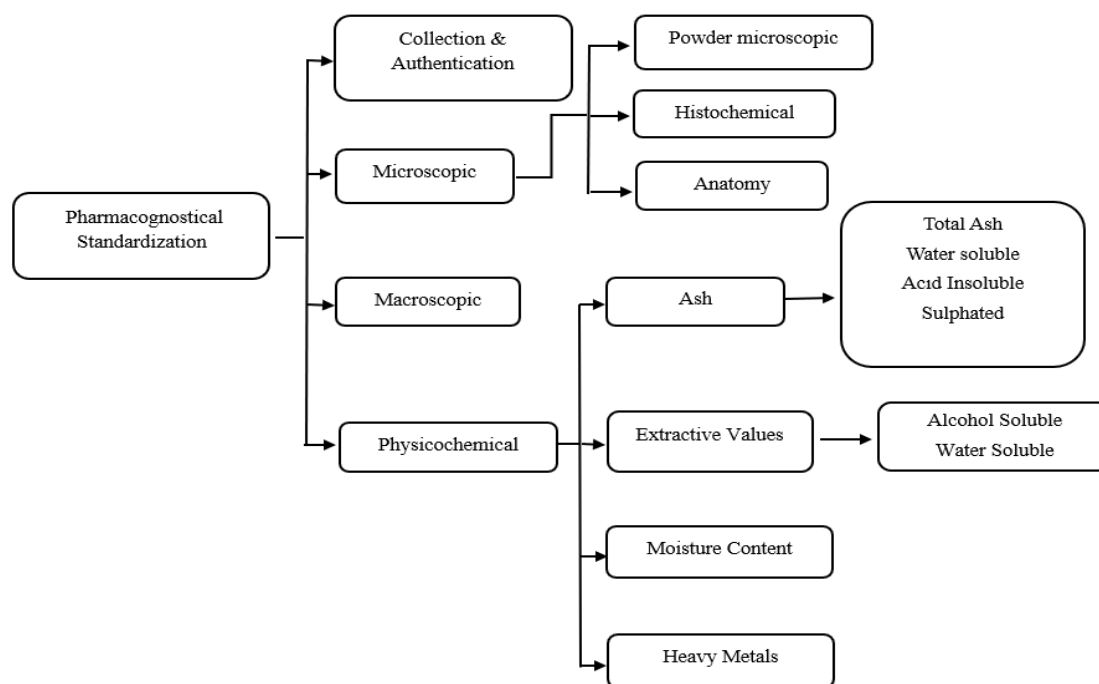


Figure 1. Workflow for the Evaluation of Yanaikolinchi

Collection & Authentication

Yanaikolinchi (Seeds of *Entada rheedii* Spreng) (Figure.2 (A-B)) were collected from Devanurnadu (GPS coordinate **Latitude:11.24917070, Longitude:78.36722390**) Kolli Hills, Namakkal District, Tamil Nadu. It was authenticated by Dr. KN Sunil Kumar Research Officer (Pharmacognosy), Sci.-II and HOD, Siddha Central Research Institute (Central Council for Research in Siddha, Ministry of AYUSH, Govt of India). The seed Voucher specimen number (E18122507R) was deposited in Department of Pharmacognosy, The Erode College of Pharmacy, Tamil Nadu, for future reference.

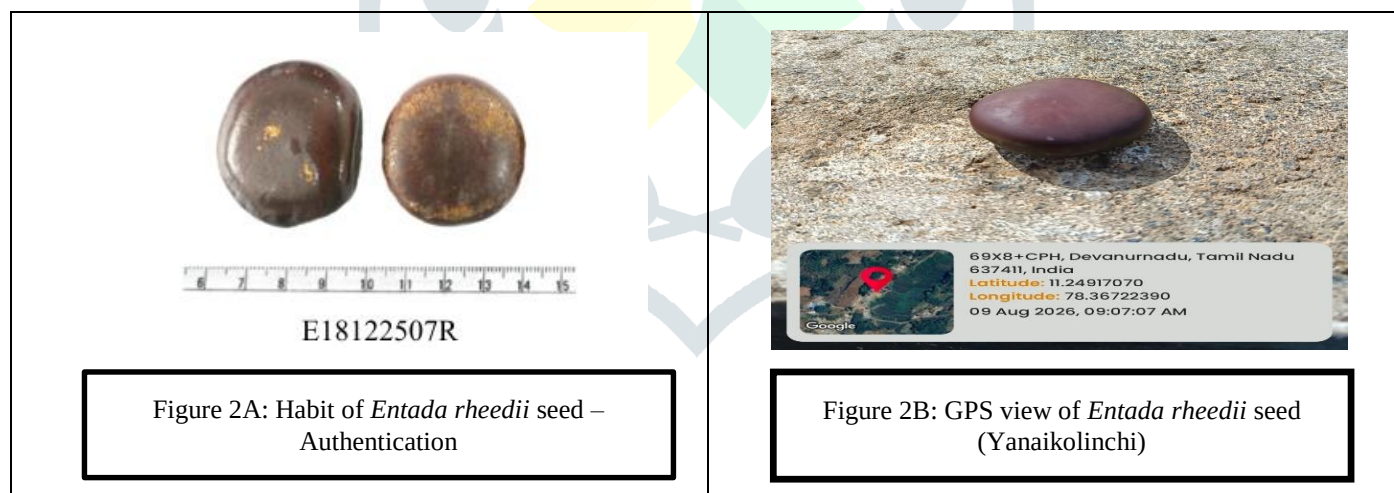


Figure 2(A-B). Collection and Authentication

Macroscopy

Nikon D-5600 digital camera is used to capture the external appearance of *Entada rheedii* seeds.

Microscopy

Testa was placed in an FAA fixative solution for over 48 hours. Thin transverse sections were prepared from these fixed samples using a blade. The sections were then stained with solutions of 0.8% safranin and 0.5% astra blue. These stained sections were observed under a microscope, specifically the Axiolab5 trinocular model, which was connected to a Zeiss Axiocam208 color digital camera, all under bright field illumination.

Powder microscopy

A powdered kernel was placed on a microscope slide, and a drop of 50% glycerol was added after clarification with a saturated chloral hydrate solution. To confirm the presence of starch granules, the sample was treated with iodine solution. The examination was carried out using a Nikon ECLIPSE E200 trinocular microscope, equipped with a Zeiss ERC5s digital camera, under bright field illumination. Photomicrographs capturing the diagnostic features were taken and documented.

Histochemical tests: Seed sections were treated following the standard procedures:

- 1. Suberized or cuticular cell walls:** Sudan red III was applied to the sample, and after a few minutes of standing, the sample was gently warmed if needed. The appearance of an orange-red or red hue on the cell walls signified the presence of suberin or cutin deposits.
- 2. Starch:** A blue color appeared, indicating starch, when a drop of 2% iodine solution was introduced to the water.
- 3. Gelatin:** Adding a drop of ruthenium red resulted in the contents changing to a pink or red color, which indicated the presence of gelatin.
- 4. Alkaloids:** Alkaloids were confirmed by the addition of a drop of Wagner's reagent, which caused the contents to turn yellow to reddish brown.
- 5. Lignified cell walls:** Phloroglucinol was applied to the section, which was then left to dry for approximately two minutes. Subsequently, 50% HCl was added, and the section was covered with a glass. The presence of lignin was confirmed if the cell walls turned a shade ranging from pink to cherry red (Wallis TE,1965; Fahn,1982; Okba MM *et al.*,2013).

Physicochemical Standardization

Following the prescribed guidelines, a series of physiochemical tests were performed, which included measuring Total ash, Acid Insoluble ash, Water soluble ash, and Sulphated ash. The study also evaluated Water soluble and Alcohol soluble Extractive, along with determining the Moisture content at 105 °C (Shinde SA *et al.*, 2023) and values summarized in (Table 1-3).

Heavy metal analysis

Seed kernels, with weights ranging from 20 to 50 mg, are placed in a Teflon microwave digestion vessel. To this vessel, 1 ml of ultra-pure nitric acid is added to facilitate digestion, which is performed for 45 minutes using an Anton Paar microwave digestion unit. After digestion, the sample is diluted to a total volume of 50 ml in a standard measuring flask. A standard solution is formulated with concentrations ranging from 2 µg/ml to 10 µg/ml, using ultrapure nitric acid, and a blank solution is also prepared. The Agilent ICP-OES 5100 VDV is utilized with the following settings: RF Power at 1.2 kW, Plasma Gas Flow Rate at 12 L/min, and Nebulizer Gas Flow Rate at 0.70 L/min. For analysis, samples are introduced into the plasma through a nebulizer and spray chamber (Castro J *et al.*,2009).

III. RESULTS AND DISCUSSION

Macroscopy

The seeds were round, disc-shaped, or elliptical, smooth in surface, dark brown in color, and shiny with fine striations. Their size ranged from 3.5 – 6 cm in breadth, 4 – 8 cm in length, and 0.5 – 3 cm thick. The testa is tough and hard to penetrate. The hilum is a pale scar in a slight depression beside the micropyle (Figure.3). The seeds have a characteristic flavor when crushed odorless.

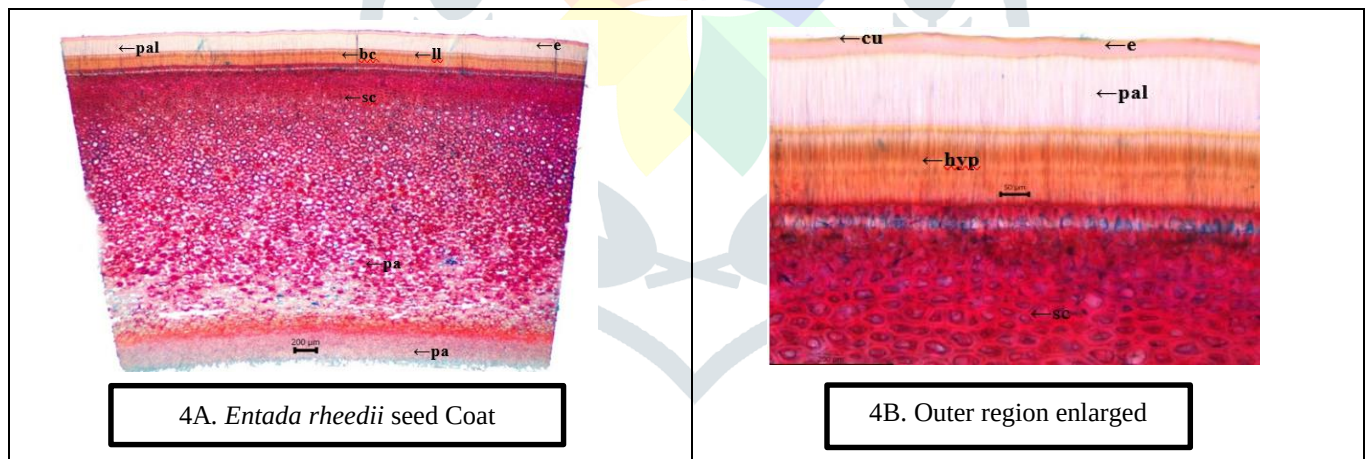


Figure 3. Morphology of *Entada rheedii* seed

Microscopical Studies of *Entada rheedii* Seed

TS of *Entada rheedii* Seed coat

The transverse section of the seed coat comprises several distinct regions. The outer epidermis consists of closely packed cells resembling a palisade, characterized by uniformly thick cell walls and small lumens, with radial elongation and the absence of intercellular spaces. This epidermal layer is covered by a thick cuticle, and a thin line, referred to as *Lenia lucida*, runs parallel to the seed surface through the center of the epidermal cells. Adjacent to this is the bearer layer, which is composed of a single layer of radially stretched cells that have large spaces between them. The cell lumens are small in the center and large peripherally due to uneven cellulose thickening. This is followed by a sclereid layer, comprising 10–15 layers of closely arranged cells with thick, pitted cell walls and no intercellular spaces. Subsequently, there is a wide stellate parenchyma layer, followed by a collapsed inner parenchyma layer composed of approximately 8–12 layers of thin-walled, flattened cells devoid of contents (Figure.4(A-D)).



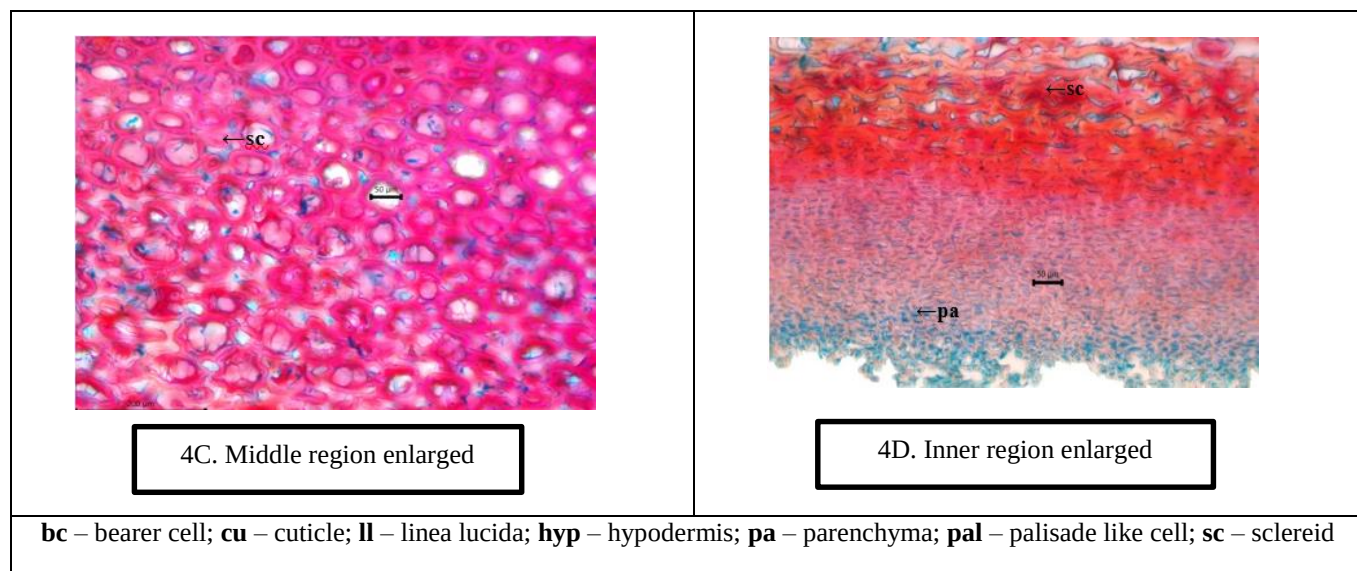


Figure 4. Transverse Section of *Entada rheedii* seed coat shows A. *Entada rheedii* seed coat, B. Outer region enlarged, C. Middle region enlarged, D. Inner region enlarged

TS of *Entada rheedii* Cotyledon

The cotyledon has a hemi circular shape and is encased by an epidermis composed of cubical cells with thin cellulose walls, which contain aleurone and starch grains. The cotyledon is primarily composed of parenchyma cells with thin walls, which are polyhedral and stretch out radially, containing both aleurone and starch grains. At the epidermis level, the cells were small and isodiametric, whereas they became large and radially elongated towards the center of the cotyledon (Figure. 5(A-C)).

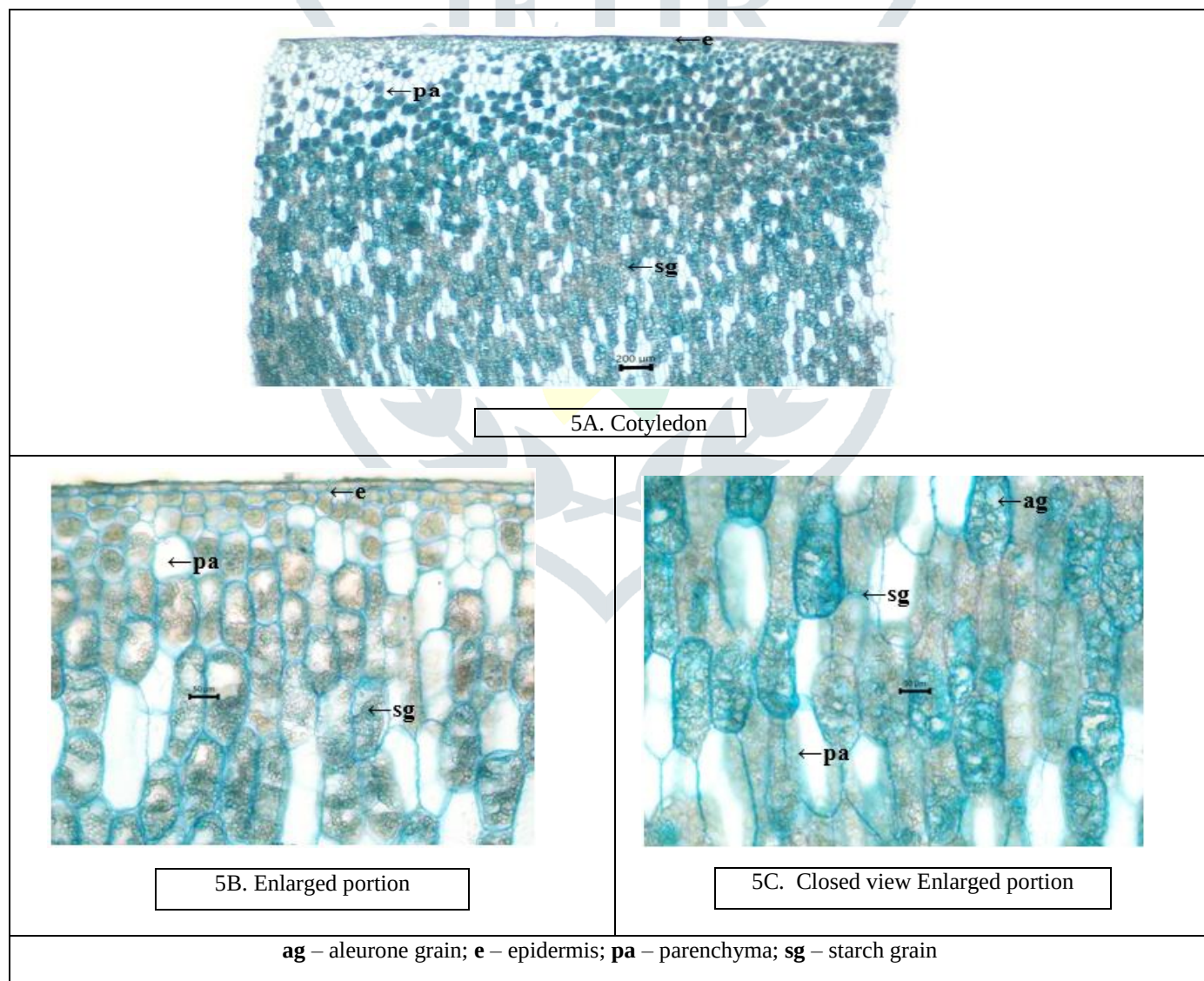


Figure 5. Transverse Section of *Entada rheedii* seed shows A. Cotyledon, B. Enlarged Portion, C. Closed view of Enlarged portion

Powder Microscopy of *Entada rheedii* seed

The powder was characterized by its dark brown color and distinctive smell and taste. It contained fragments that exposed the testa, palisade-like cells, stone cells, and parenchyma cells, along with starch and aleurone grains (Figure. 6(A-G)).

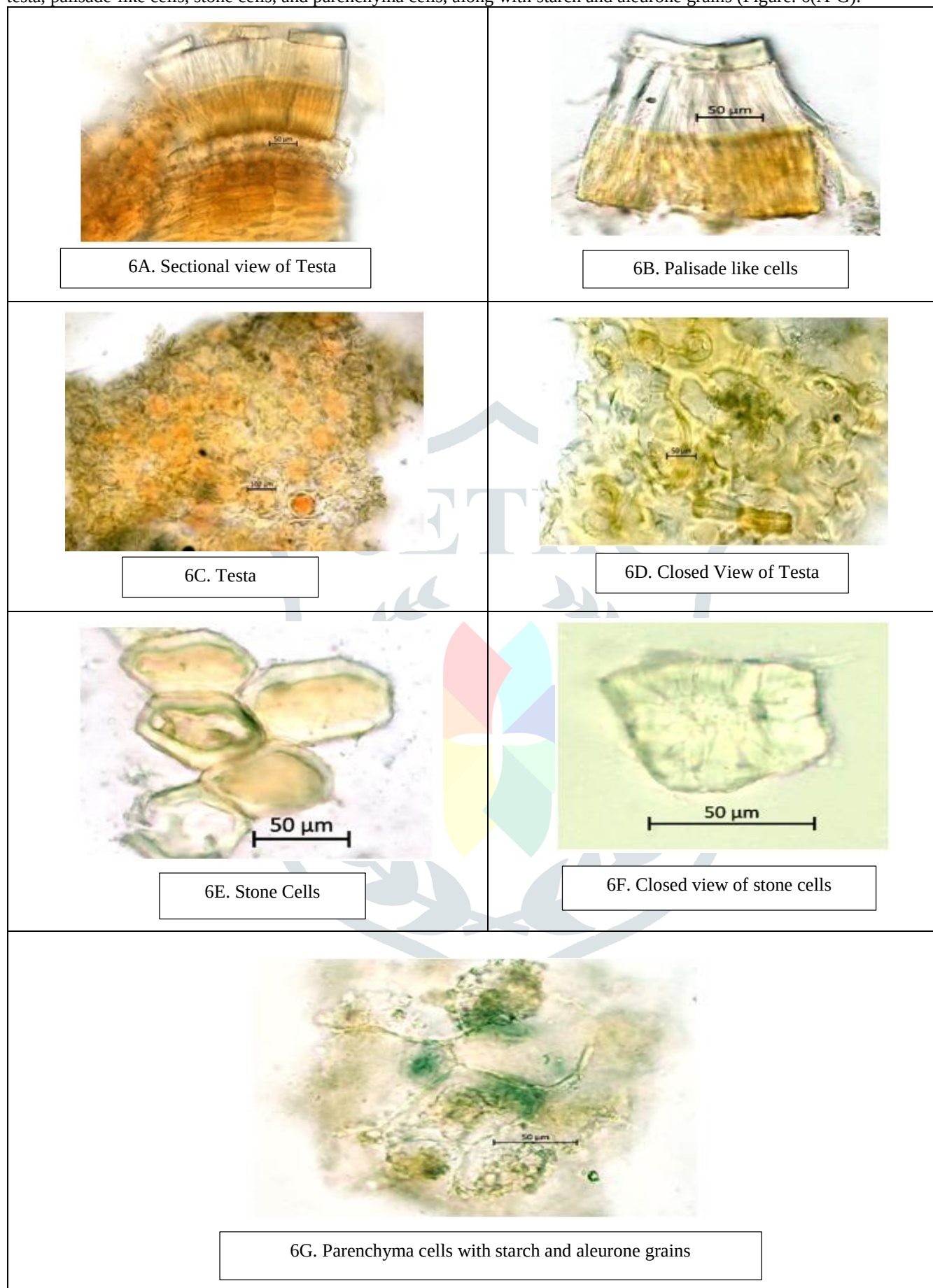
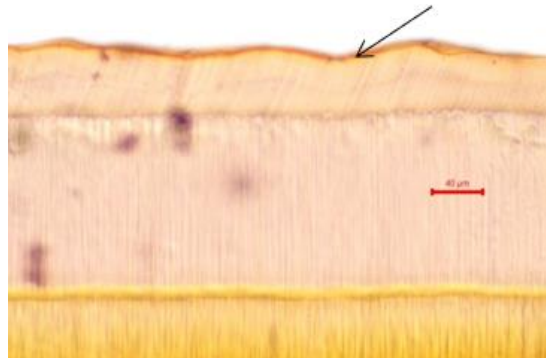
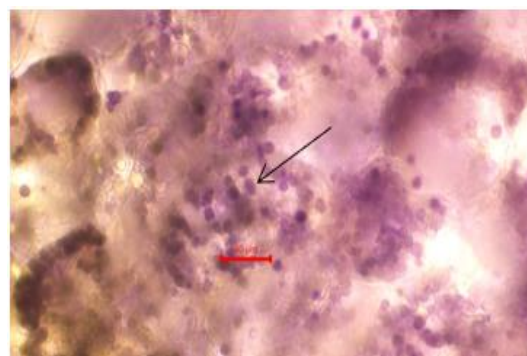


Figure 6. Powder Microscopy of *Entada rheedii* seed shows A. Sectional view of testa, B. Palisade like cells, C. Testa, D. Closed View of Testa, E. Stone cells, F. Closed view of stone, G. Parenchyma cells with starch and aleurone
Histochemistry of *Entada rheedii* seed

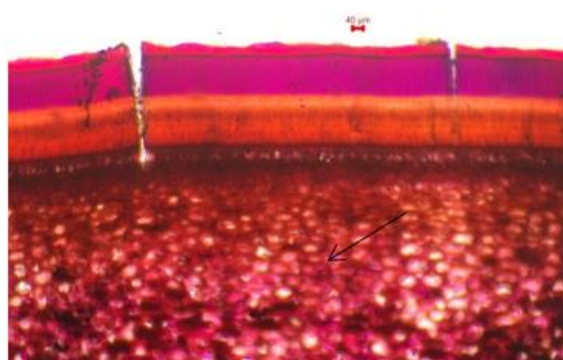
Histochemical analysis revealed cutin on the epidermal wall, gelatin within the subepidermal layers, and alkaloids in the sclereidal layers. Starch grains were found in the parenchyma cells, while lignin was absent (Figure.7(A-G)).



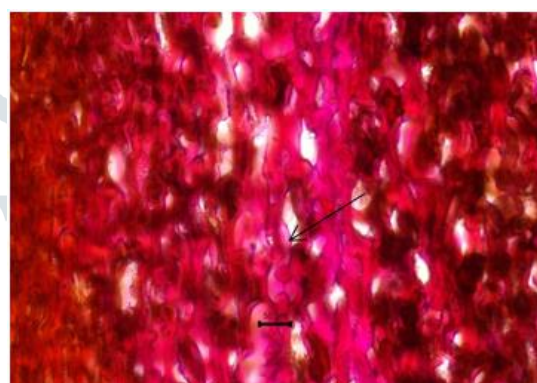
7A. Presence of Cutin



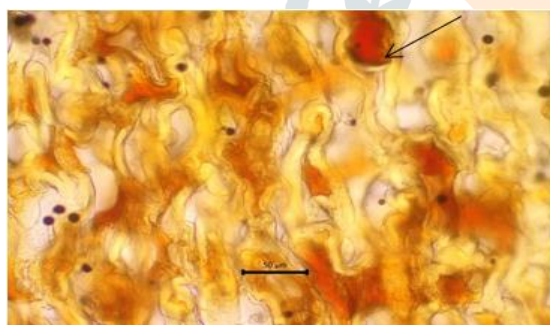
7B. Presence of Starch



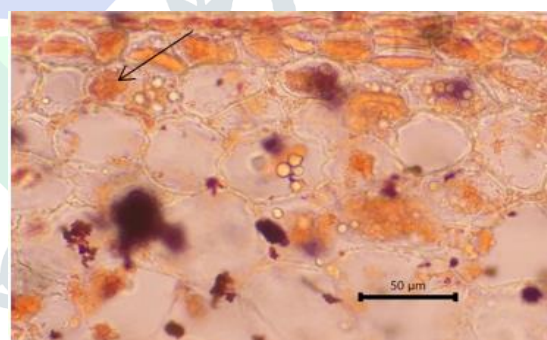
7C. Presence of Gelatin



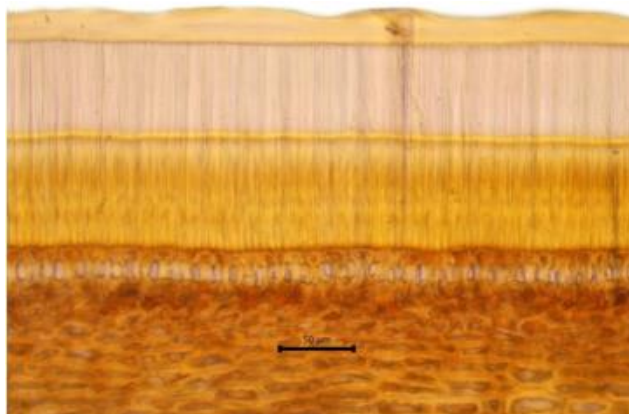
7D. Closed view of Gelatin



7E. Presence of Alkaloid



7F. Closed view of Alkaloid



7G. Absence of Lignin

Figure 7. Histochemical characteristics of *Entada rheedii* seed shows A. Presence of cutin, B. Presence of Starch, C. Presence of Gelatin, D. Closed view of Gelatin, E. Presence of Alkaloid, F. Closed view of Alkaloid, G. Absence of Lignin

Physicochemical Standardization

Determination of Ash Values

Table 1. Ash Values

Ash Values	Mean value (%w/w) n=3
Total ash	2.8 % w/w
Acid insoluble ash	0.4 % w/w
Water Soluble ash	2.2 % w/w
Sulphated ash	2.6 % w/w

Determination of Extractive Values

Table 2. Extractive Values

Extractive Values	Mean value (%w/w) n=3
Alcohol Soluble	6.8 % w/w
Water Soluble	25.2 % w/w

Determination of Moisture content

Table 3. Moisture Content

Moisture content	% w/w
<i>Entada rheedii</i> seed	6% w/w

Heavy metal analysis

To assess the linearity of this method, multi-elemental standard solutions with concentrations ranging from 2 to 10 µg/ml to all metals under investigation with $R^2 > 0.999$ (Table 4). Arsenic (188.980 nm), cadmium (214.439 nm), mercury (194.164 nm), and lead (220.353 nm) showed strong linear relationships between concentration and emission intensity at their respective wavelengths with correlation coefficients of 0.9998, 0.9997, 0.9994, and 0.9991 respectively. In (Figure. 8A), the calibration curves demonstrate a linear relationship between signal intensity and concentration. The method's high precision is highlighted by the overlapping of the standards spectra, which had sharp peaks without any spectral overlap (Figure. 8B). ICP-OES spectral analysis (Figure. 8C), reveals that seeds of *Entada rheedii* (Yanaikolinch) contain all heavy metals such as arsenic, cadmium and lead with respect to mercury were **Below Detection Limit (BDL)** which was confirmed by indicating the non-contaminated level from detectable contamination of these toxic trace elements.

Table 4 . Standard linearity for Heavy metals As, Cd, Hg, Pb using ICP-OES

S.No	Element	Wavelength	R ² Value
1	Arsenic [As] (µg/ml)	188.980 nm	0.9998
2	Cadmium [Cd] (µg/ml)	214.439 nm	0.9997
3	Mercury [Hg] (µg/ml)	194.164 nm	0.9994
4	Lead [Pb] (µg/ml)	220.353 nm	0.9991

Standard linearity

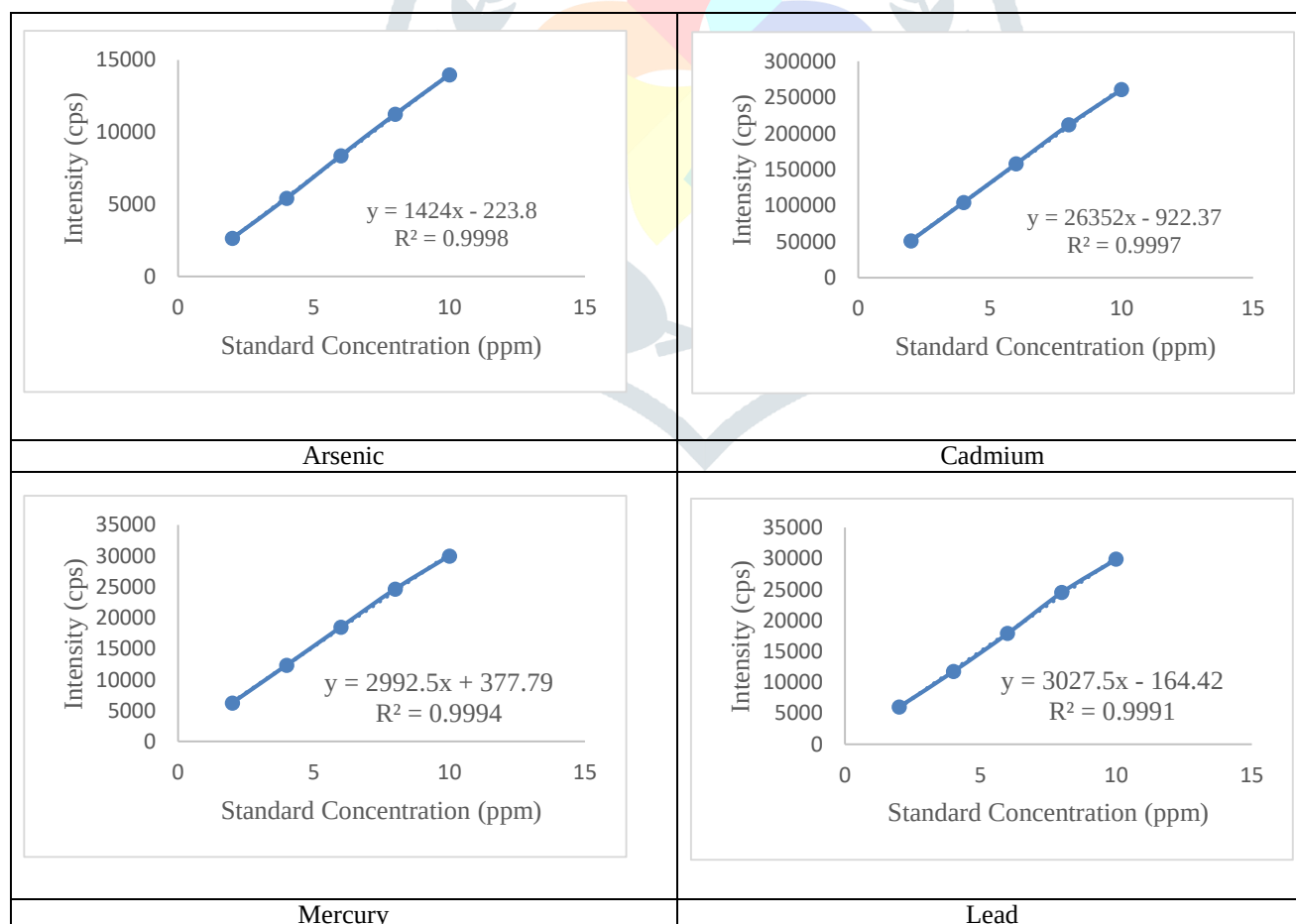


Figure 8A. Calibration Curve Standard for Heavy metals As, Cd, Hg, Pb

Standard Overlaid Graph

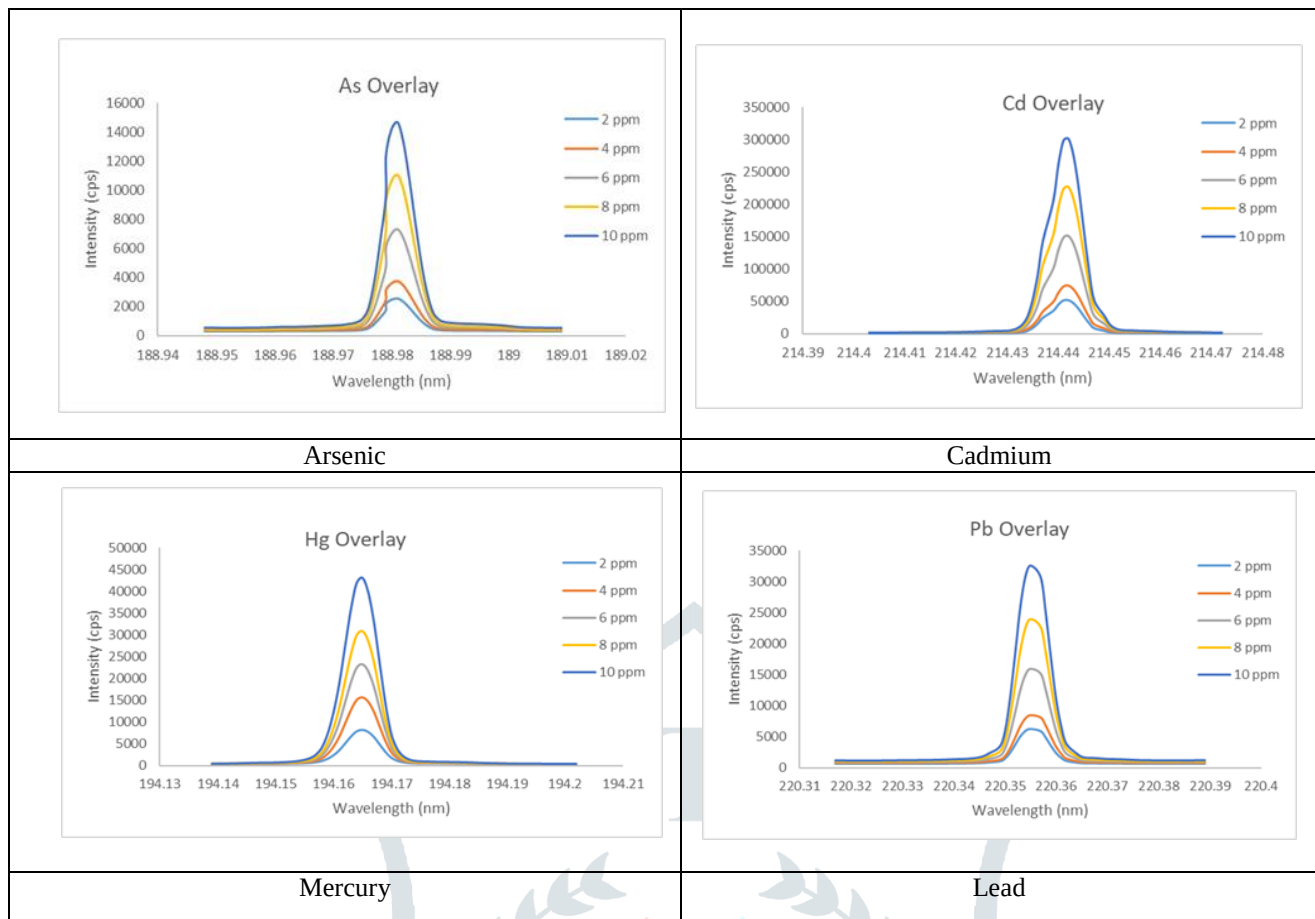
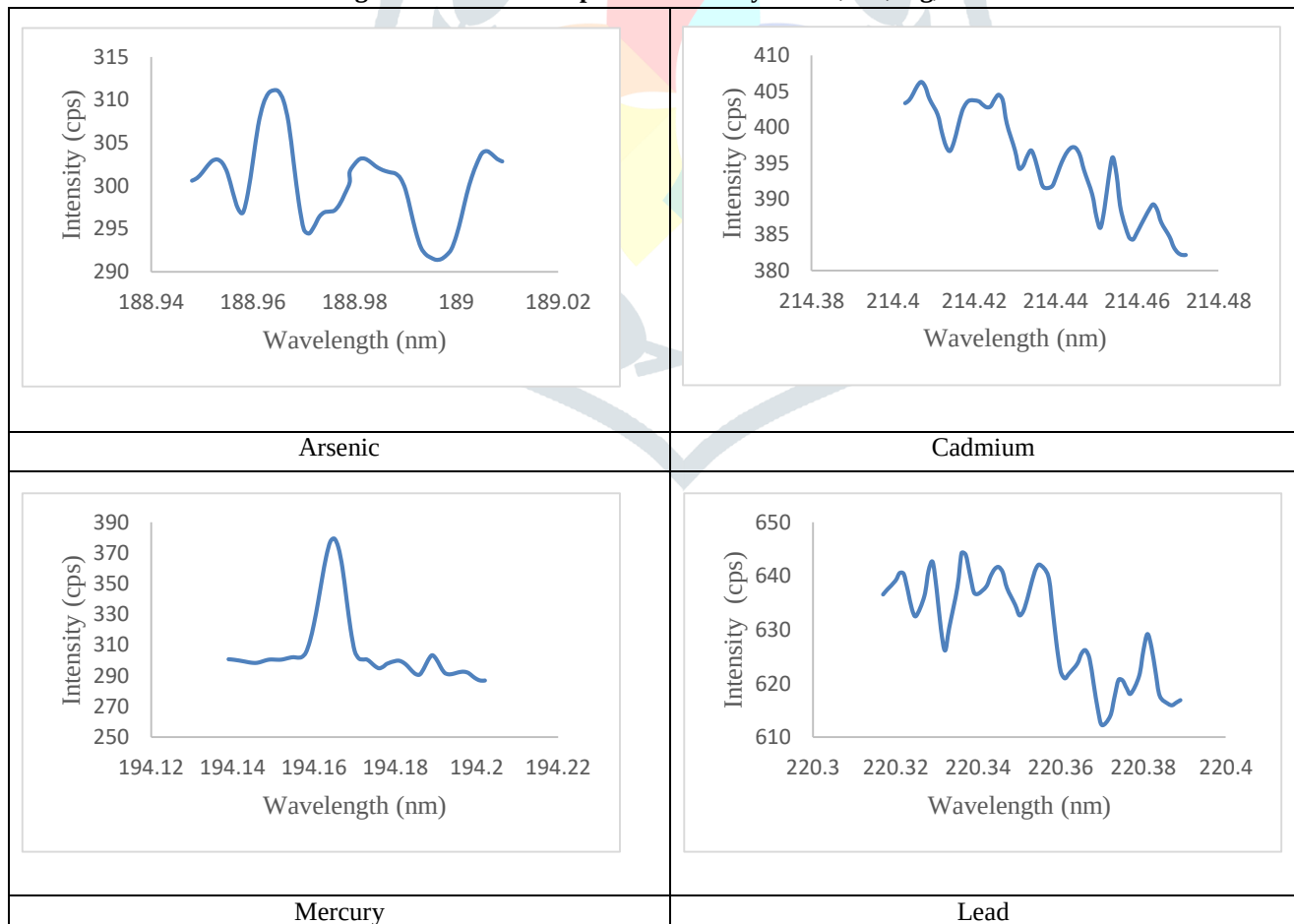


Figure 8B. ICP-OES spectrum overlay for As, Cd, Hg, and Pb

Figure 8C. ICP-OES spectral analysis of *Entada rheedii* seed

IV. CONCLUSION

In the present study, Yanaikolinchi (Seeds of *Entada rheedii* Spreng) collected from the Kolli Hills hotspot were comprehensive pharmacognostical standardized, which exhibited macroscopy, microscopy, powder, and histochemistry characteristics that may be helpful for authentication. The seed quality and purity are confirmed by its physiochemical parameters, which are within the acceptable limit. ICP-OES analysis confirmed the absence of heavy-metal (As, Cd, Hg, Pb) contamination. These results provide a reference for identification and quality control as well as the future application for preclinical research and drug discovery.

IV. REFERENCES

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