

Pharmacognostical Standardization and Heavy Metal Analysis of the Brown Seaweed *Padina tetrastromatica* (Hauck)

Boovizhi K.P¹, Dr. Ravikkumar V.R², Maheswari V³, Loganayagi M⁴, Sathiya R⁵

^{1,5} PG Scholar, The Erode College of Pharmacy, Tamil Nadu, India.

²Professor, HOD, Department of Pharmacognosy, The Erode College of Pharmacy, Tamil Nadu, India.

^{3,4} Assistant Professor, Department of Pharmacognosy, The Erode College of Pharmacy, Tamil Nadu, India.

Abstract—*Padina tetrastromatica* (Hauck) is a marine brown alga that is rich in phytochemicals and shows a wide range of pharmacological activities. The present study was undertaken to establish pharmacognostical profile and to determine heavy metal content of *P. tetrastromatica* to facilitate its identification, quality control and safe medicinal use. Pharmacognostical evaluation of the drug included macroscopic, microscopic, powder microscopic and histochemical analysis. The thallus showed typical dorsiventrally flattened, polystromatic morphology with well-defined cortical and medullary regions. Powder microscopy showed diagnostic characters such as filaments, epidermal fragments, medullary cells and pigmented cell contents. The histochemical studies revealed the presence of phenolic compounds, alkaloids, starch, mucilage, lipids and absence of lignin. Heavy metal analysis was done by Atomic Absorption Spectrophotometry (AAS) and ICP-OES. Chromium (0.2646 ppm) and zinc (0.7723 ppm) were within acceptable limits but copper, cadmium, arsenic, mercury and lead were below detection limit. These findings suggest the unique pharmacognostical properties of *P. tetrastromatica* and the presence of only trace amounts of essential metals and no detectable toxic heavy metals, confirming its authenticity, quality and safety for future pharmaceutical and nutraceutical uses.

Index Terms—Marine macroalgae, Crude drug standardization, Botanical standardization, Heavy metal analysis.

I. INTRODUCTION

Marine algae are important sources of structurally diverse bioactive compounds with potential pharmaceutical, nutraceutical, and cosmetic applications. Brown algae (Phaeophyceae), in particular, contain polysaccharides, polyphenols, carotenoids, sterols, minerals, and other metabolites associated with various biological activities. *Padina tetrastromatica* (Hauck), a tropical brown alga widely distributed along the Indian coast, contains compounds such as fucoidan, alginate, phlorotannins, flavonoids, sterols, and phenolics. However, pharmacognostic standards for its identification and quality assessment remain limited.[1], [5], [6]

This study evaluated the macroscopic, microscopic, powder-microscopic, and histochemical characteristics of *P. tetrastromatica* to establish diagnostic features for its authentication and quality control. Selected heavy metals were also quantified using Atomic Absorption Spectrophotometry (AAS) and Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES), as algae may accumulate elements from their surrounding marine environment. The findings provide baseline information for the safe utilization, standardization, and future development of pharmaceutical and marine-derived products containing *P. tetrastromatica* (Hauck).

II. MATERIALS & METHODS

Collection, authentication:

The brown seaweed *Padina tetrastromatica* was sourced from the R.K. Algae Project Center, 12/172 Meenavar colony, Mandapam, Ramanathapuram District, Tamil Nadu, India (GPS coordinates: 09°12.370' N, 079°08.411' E), and authenticated by the marine botanist Mr. M. Rajendra Kumar. The collected seaweed was washed thoroughly with seawater followed by distilled water to remove adhering impurities and epiphytes, shade-dried, powdered using a mechanical grinder, and stored in an airtight container until further use. (Fig.1)



Figure 1. Brown seaweed *Padina tetrastromatica*

A. Pharmacognostical evaluation:

1. Macroscopy (PCOG-004-SOP):

External feature of test sample was documented using Nikon D-5600 Digital camera.

2. Microscopy (PCOG-005-SOP):

Sample preserved in fixative FAA for over 48 h. The preserved specimens were cut into thin transverse sections using a sharp blade, and the sections were stained with 0.8% Safranin and 0.5% Astra blue. Transversal sections were photographed using Axiolab5 trinocular microscope equipped with Zeiss Axiocam208 color digital camera with bright field light. Magnification was shown by scale bar.

3. Powder microscopy (PCOG-006-SOP):

A drop of 50% glycerol was added to a microscopic slide containing a pinch of the powdered sample, after clearing with saturated solution of chloral hydrate. The sample was treated with iodine solution to confirm the presence of starch grains. The character was observed under bright field light using Nikon ECLIPSE E200 trinocular microscope equipped with Zeiss ERc5s digital camera. Photomicrographs of the diagnostic characters were taken and recorded.

4. Histochemical tests (PCOG-008-SOP):

Plant sections were treated following the standard procedures:

1. Crystals: The section was mounted in water, and one end of the cover slip was irrigated with acetic acid. While watching thru the microscope the water under the cover slip was replaced by means of a piece of filter paper at the other end of the cover slip.

-Formation of air bubbles showed Calcium carbonate crystals.

If no air bubbles were formed, the experiment was repeated with conc. HCl; dissolution of crystal and formation of needles of Calcium sulfate indicated the presence of Calcium oxalate crystals.

2. Fats, Fatty oils, volatile oils and resins: About 1–2 drops of Sudan-IV were added to the section and allowed to stand for a few minutes. Fatty oil substances were present in orange-red/pink/red colored globules and resin in red colored irregular contents.

3. Starch: A drop of 2% iodine water solution was added - blue colour indicated starch.

4. Phenolic compounds: Phenolic compounds: A drop of alcoholic ferric chloride was added - bluish black colored contents indicated phenolic compounds like flavonoids/ tannins etc.

5. Mucilage: A drop of ruthenium red was added – pink to red colored contents indicated mucilage.

6. Lignified cell walls: A drop of phloroglucinol was added to the section and left to stand for about 2 min or until almost dry. Added a drop of 50% HCl and examined under cover-glass. Cell walls-stained pink to cherry red indicating presence of lignin.

7. Suberized or cuticular cell walls: A drop of Sudan red III was added and left for a few minutes, warming gently if necessary - cell walls-stained orange-red or

red indicated deposition of suberin or cutin over the cell wall.

8. Alkaloids: A drop of Wagner's reagent was added - the presence of yellow to reddish brown colored contents confirmed alkaloids. [2], [3], [9]

B. Heavy metal analysis

Procedure

The concentrations of copper (Cu), chromium (Cr), cadmium (Cd), and zinc (Zn) in the extract of *Padina tetrastromatica* was determined by Flame Atomic Absorption Spectrophotometry (AAS) (Model: AA-6880F). Standard solutions of each metal were prepared in the range of 1–3 ppm (0.5–2 for Cr) and used to generate calibration curves prior to the analysis of samples. The instrument was run in an air-acetylene flame with deuterium background correction. The analytical wavelengths used were 324.8 nm (Cu), 357.9 nm (Cr), 228.8 nm (Cd) and 213.9 nm (Zn). The prepared sample solution was aspirated into the flame, and each sample was analyzed three times. The concentrations of the metals were calculated from the respective calibration curves and expressed as ppm (mg/L).

Add 20-50 mg of sample into Teflon microwave digestion vessel, then add 1 ml of ultra-pure nitric

acid to digest about 45 min using Anton Paar microwave digestion unit. The sample is then diluted to a standard measuring flask of 50 ml. The calibration standard solution was prepared with blank and ultra-pure nitric acid for 2 µg/ml to 10 µg/ml. The instrument Agilent ICP-OES 5100 VDV was used under the following operation conditions: an RF power 1.2 kW, a plasma gas flow rate 12 L/min and a nebulizer gas flow rate 0.70 L/min. Samples are introduced into the plasma using a nebulizer and spray chamber for analysis. [4], [7], [8]

III. RESULTS & DISCUSSION

A. Pharmacognostical evaluation:

1. Macroscopy

The dried thallus is light brown to yellowish-brown in colour, thin, and membranous. It is dorsiventrally flattened and usually shows concentric bands or zonation. The margins are smooth to slightly incurved and often roll inward, while the surface appears smooth to finely velvety (Fig. 2). On drying, the thallus becomes leathery and brittle, with a distinct marine odour and a slightly saline taste.



Figure 2. Macroscopy of *Padina tetrastromatica*

2. Microscopy

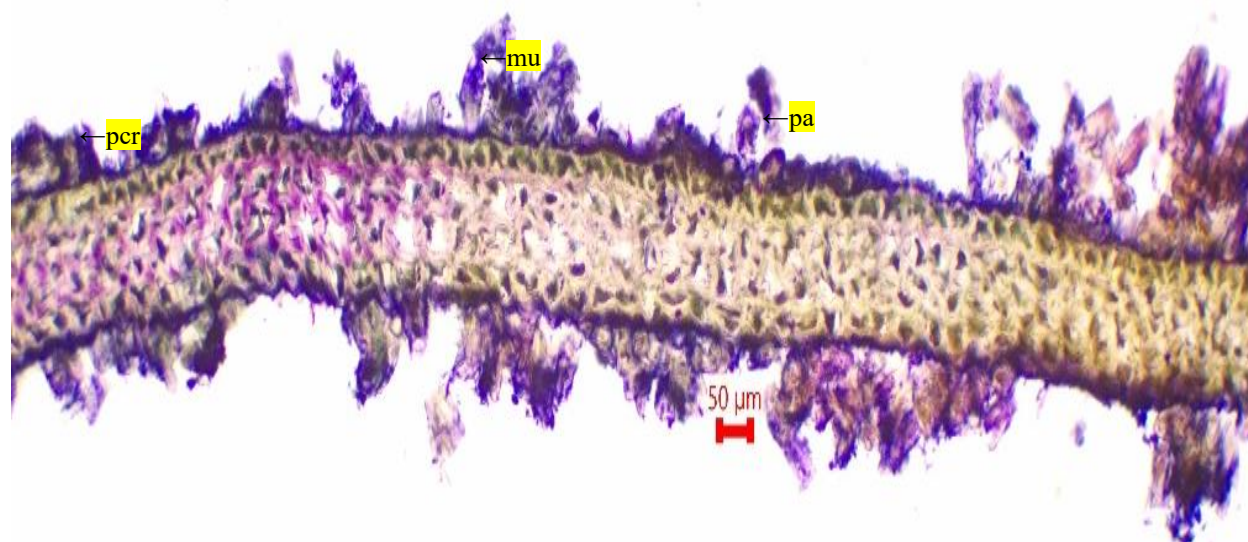
Transverse sections of the thallus show a flattened, dorsiventral, polystromatic structure. It consists of

compact parenchymatous cells differentiated into an outer cortical layer and an inner medullary region. The central portion of the thallus is clearly thicker

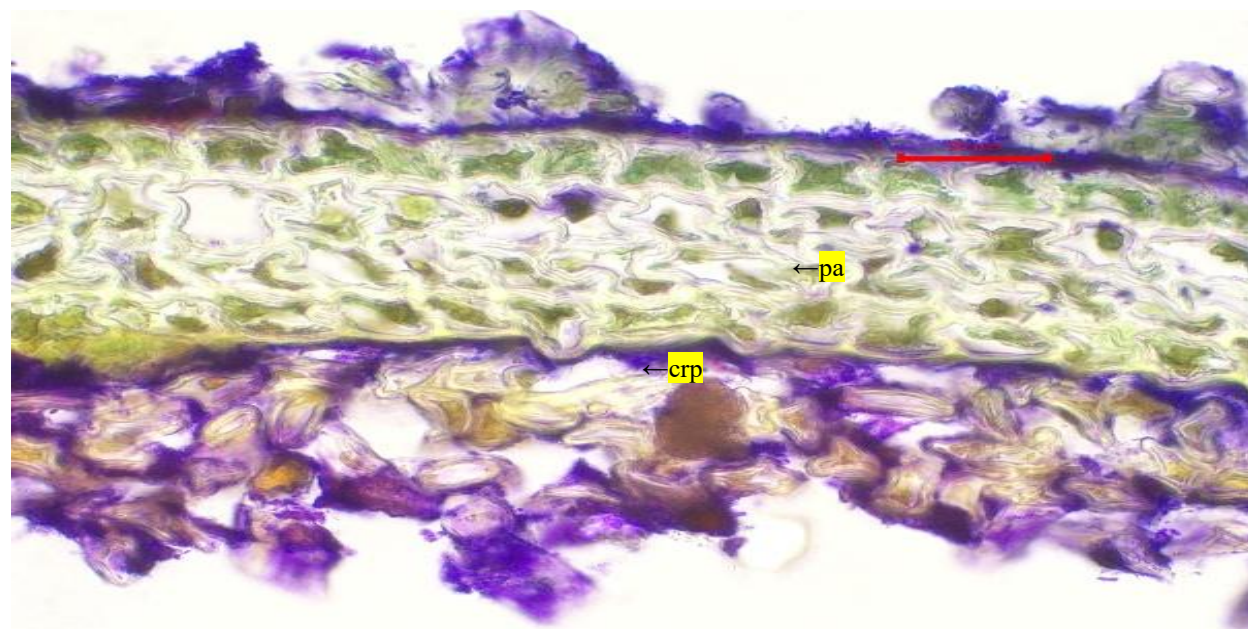
than the margins and generally contains four cell layers.

The outer surface on both sides is made up of small, closely packed cortical cells that are rectangular to polygonal in shape and contain numerous golden-brown chromatophores. The inner region is composed of comparatively larger hyaline medullary cells arranged in two or more layers. These cells are thin-walled, polygonal to rounded, highly vacuolated,

and lack dense pigmentation; this region occupies most of the thallus thickness. The central portion shows a *tetrahedral* arrangement, while the margins may become bi- or tristatose due to a gradual reduction in cell layers. The cell walls are distinctly cellulose-rich and externally covered by a thin mucilaginous layer. No vascular tissues, fibres, sclereids, stomata, trichomes, secretory structures, or lignified elements were observed (Fig. 3).



TS of Thallus



Enlarged view

crp – chromatophore; mu – mucilage; pa – parenchyma;

Figure 3. Microscopy of *Padina tetrahedralis*

3. Powder microscopy

The powder is dark brown in colour with an unpleasant odour and a characteristic marine, salty taste. Microscopic examination shows the presence of filaments, epidermal fragments, medullary cells, and cells containing dark contents (Fig. 4).

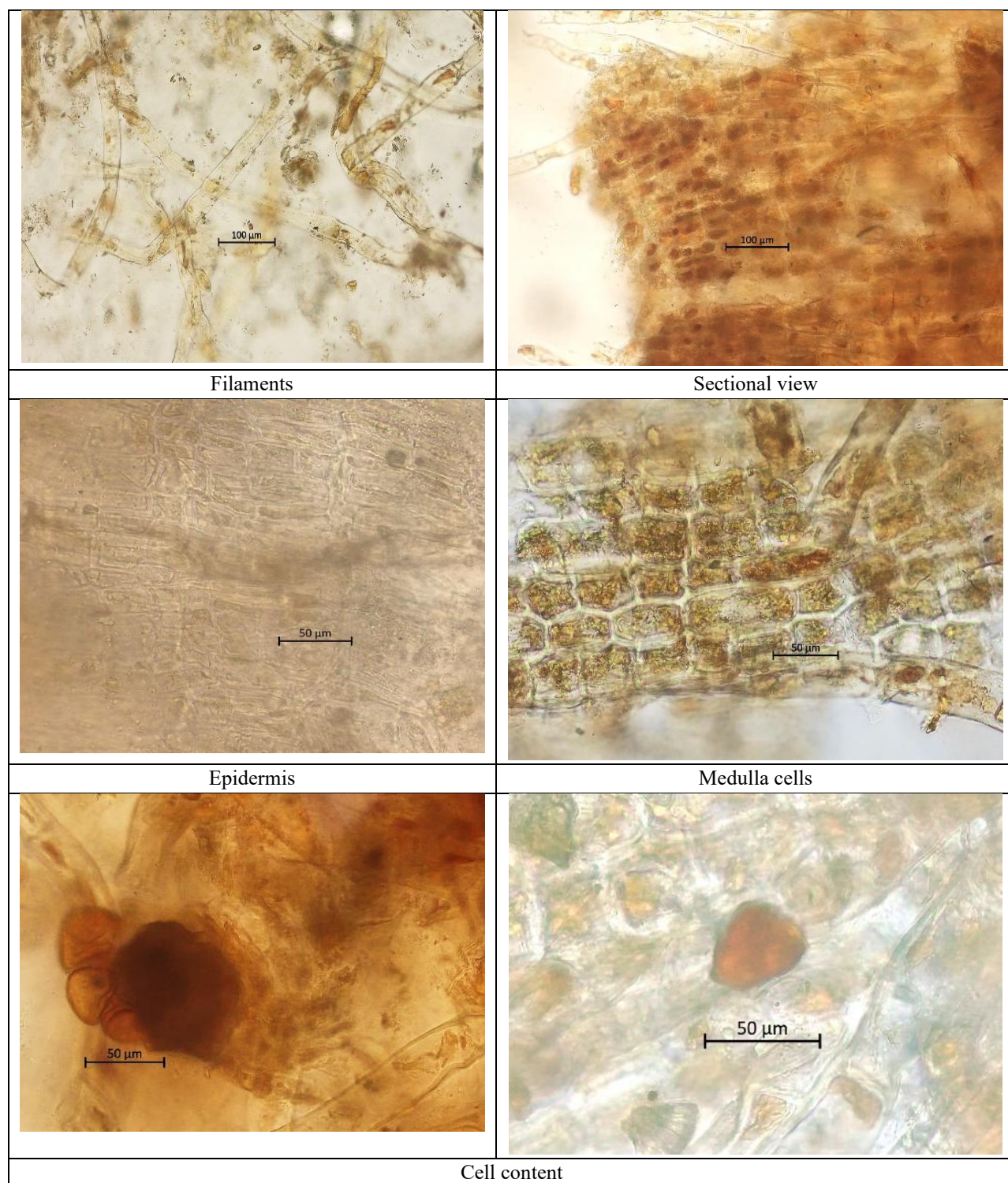
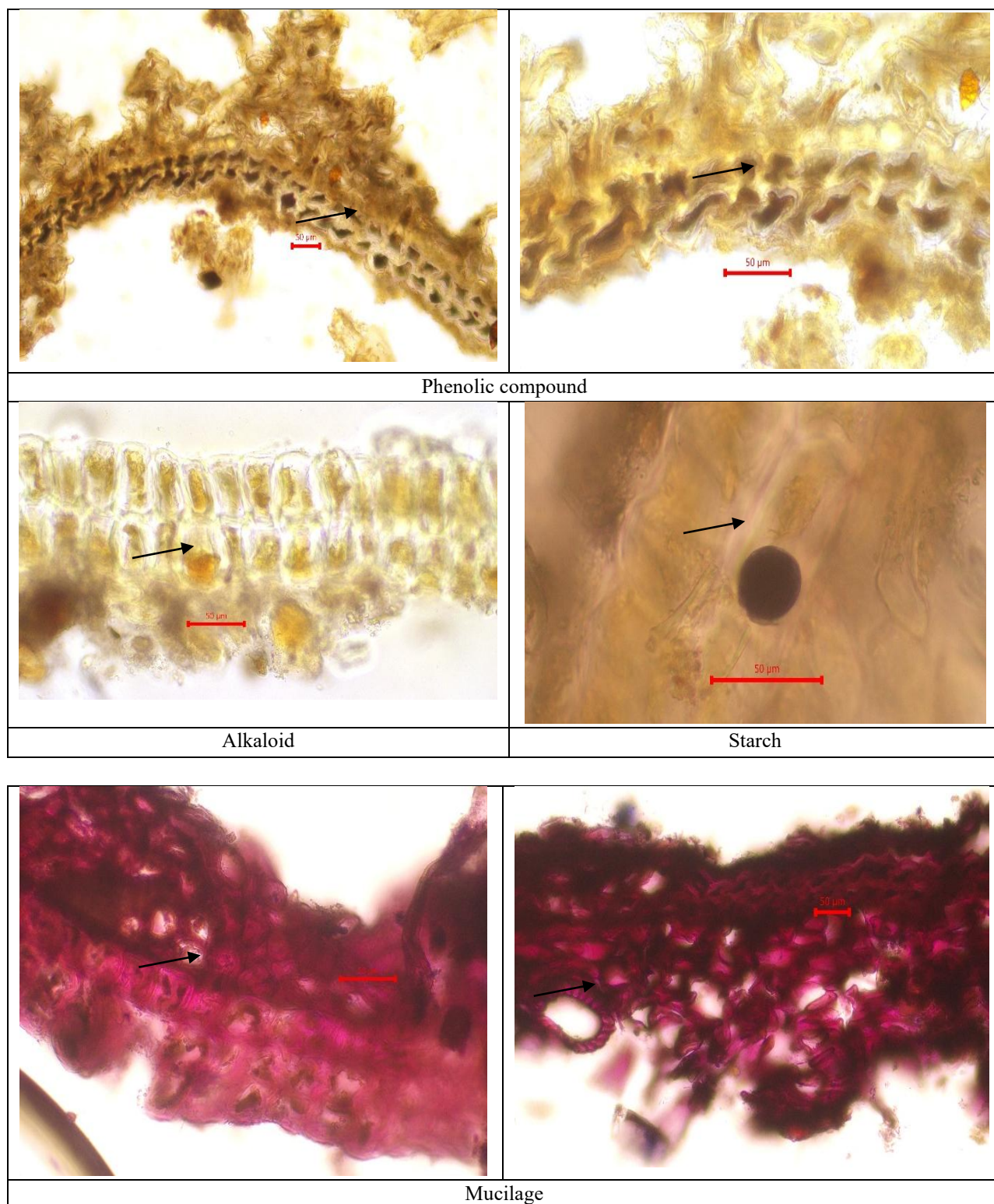
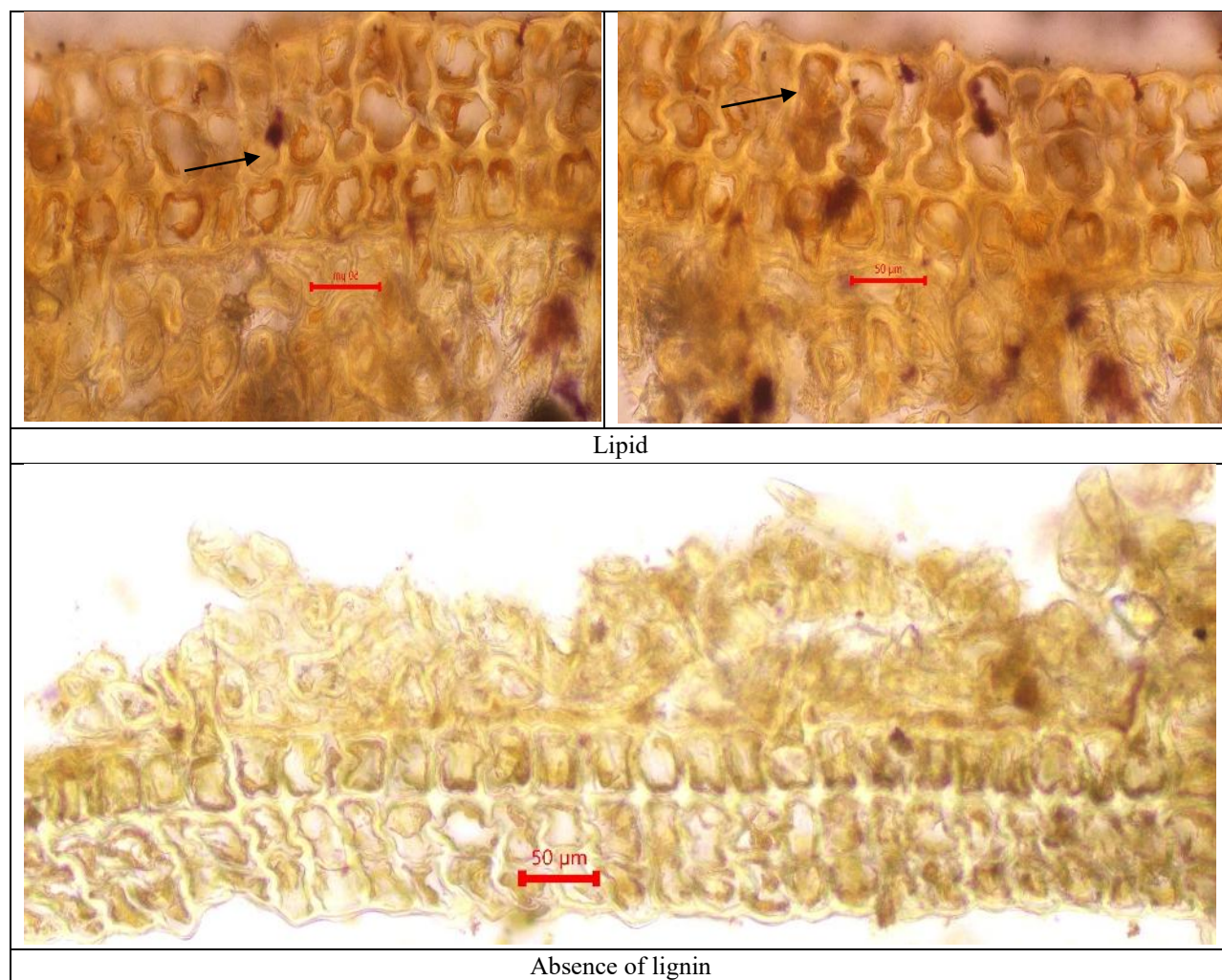


Figure 4. Powder Microscopy of *Padina tetrastromatica*

4. Histochemistry

Histochemical analysis revealed the presence of phenols, alkaloids, starch, and mucilage throughout the thallus. Lipids were detected in the outer medullary layers, while lignin was absent (Fig. 5).



Figure 5. Histochemistry of *Padina tetrastromatica***B. Heavy metal analysis:**

AAS analysis showed the presence of chromium and zinc in the sample. Copper, cadmium, arsenic, lead, and mercury were below the detectable range of the calibration curve. The measured concentrations are presented in Table 1.

Table 1: Heavy metal analysis of Cu, Cr, Cd, Zn, Hg, As, Pb.

Heavy Metal	Wavelength (nm)	Calibration Range (ppm)	Sample Concentration (ppm)
Cu	324.8	1–3	BDL *
Cr	357.9	0.5–2	0.2646
Cd	228.8	1–3	BDL *
Zn	213.9	1–3	0.7723
As	188.980	2–10	BDL
Hg	194.164	2–10	BDL
Pb	220.353	2–10	BDL

*The reported negative concentrations for Cu (-0.5537 ppm) and Cd (-0.4745 ppm) indicate that the sample absorbance was below the calibration blank and should be reported scientifically as Below Detection Limit (BDL) or Not Detected (ND) rather than as negative values.

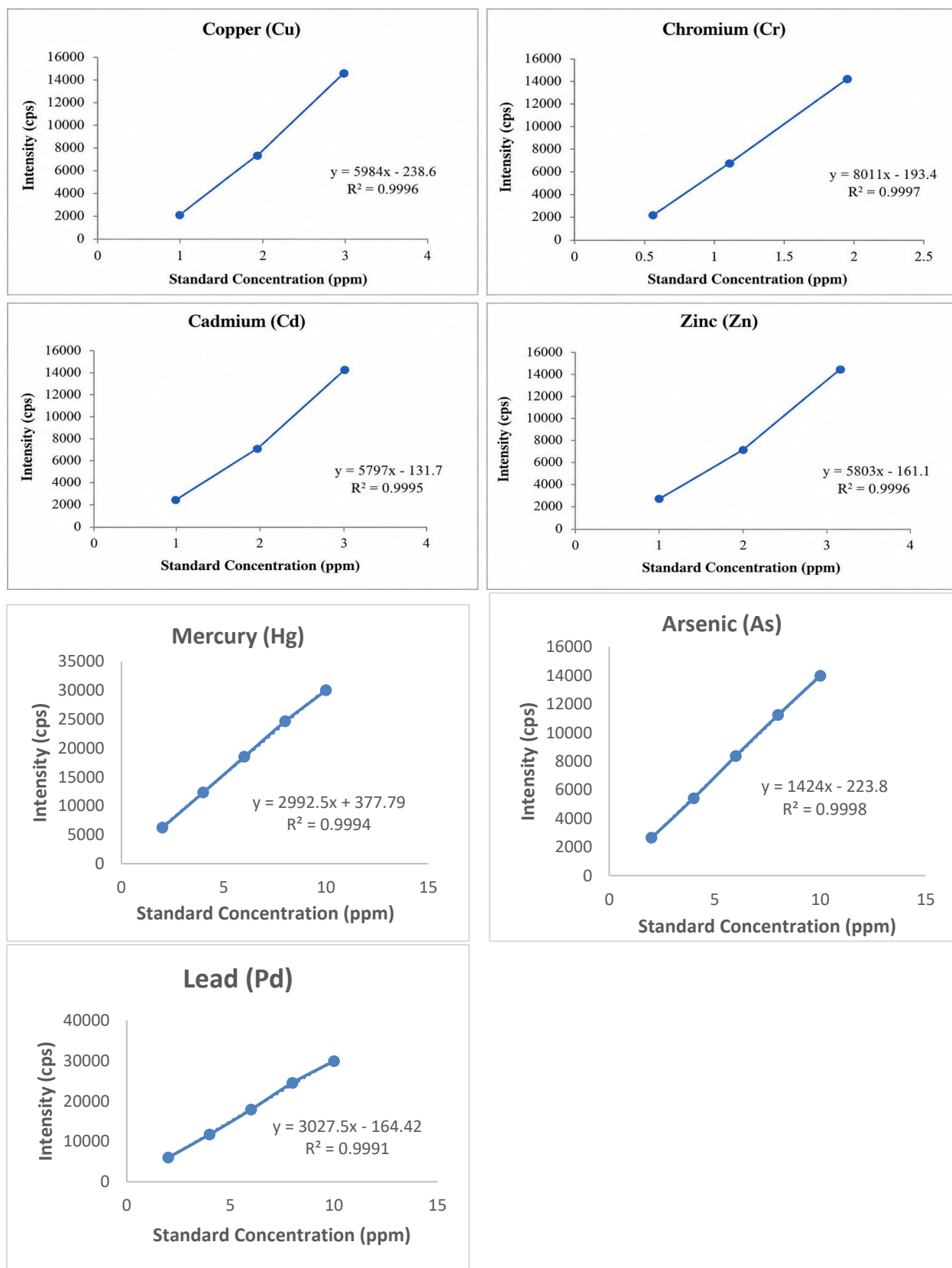


Figure 6: Calibration curve graph for Cu, Cr, Cd, Zn, Hg, As, Pb.

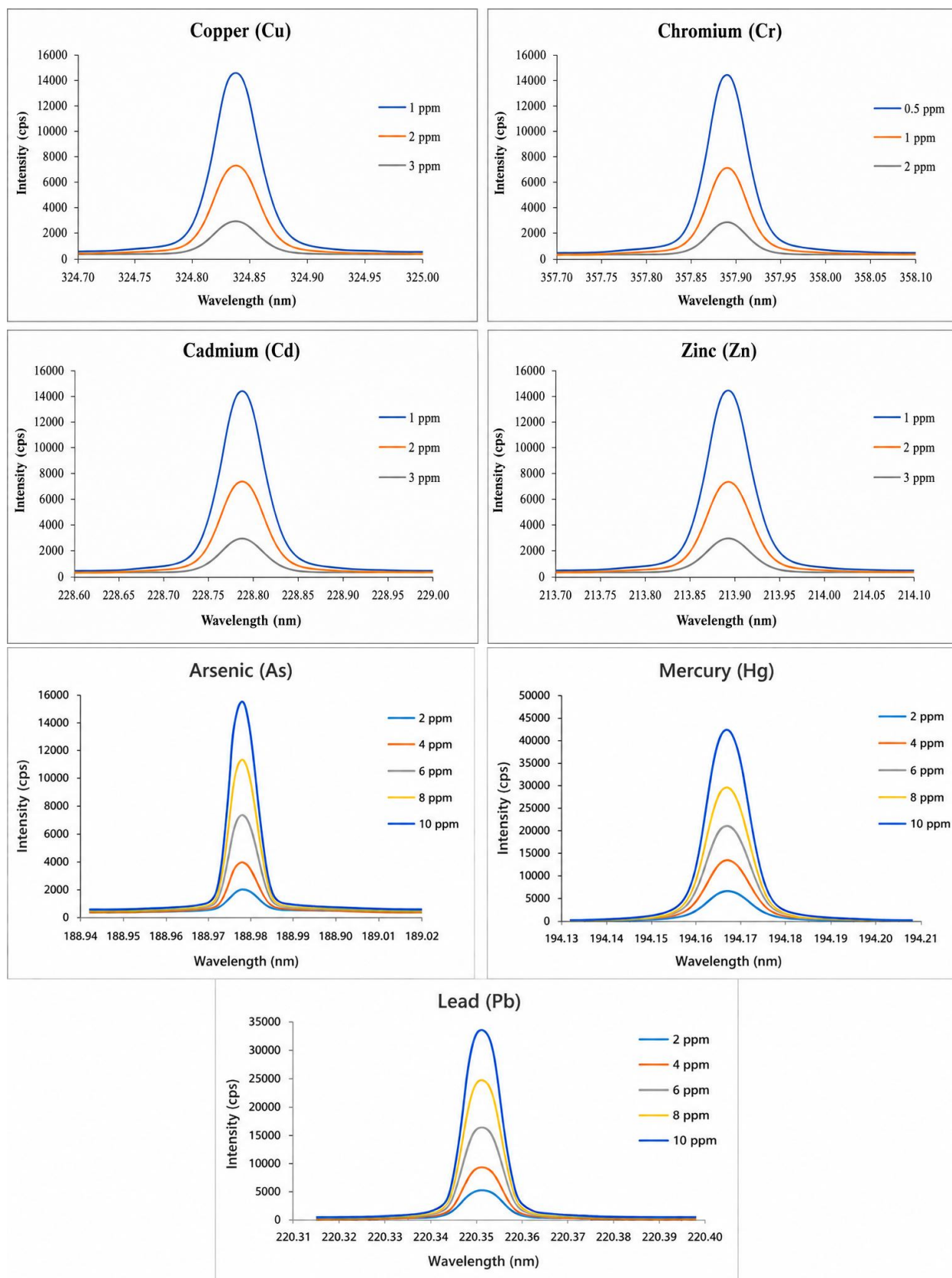


Figure 7: Standard Overlaid Graph for Cu, Cr, Cd, Zn, Hg, As, Pb.

IV. CONCLUSION

The present study established pharmacognostic parameters and evaluated the heavy-metal profile of *Padina tetrastromatica* (Hauck). The macroscopic, microscopic, powder-microscopic, and histochemical characteristics provided distinctive diagnostic features useful for the identification and authentication of the species. Histochemical analysis confirmed the presence of important phytochemical constituents, including phenolic compounds, alkaloids, starch, mucilage, and lipids, whereas lignin was not detected. These findings are consistent with the characteristic anatomical features of brown algae. Heavy-metal analysis revealed trace levels of chromium and zinc, with concentrations of 0.2646 and 0.7723 ppm, respectively. Copper, cadmium, arsenic, mercury, and lead were not detected within the limits of the analytical method. The low levels of detectable heavy metals suggest minimal contamination in the investigated sample. However, confirmation of its suitability for medicinal use should be based on comparison with relevant regulatory safety limits. Overall, the findings provide valuable baseline information for the pharmacognostic evaluation and quality control of *P. tetrastromatica*. They may support the future standardization and authentication of this species for use in herbal formulations, while also highlighting the need for further research on its bioactive constituents, therapeutic potential, and possible applications in marine-derived pharmaceutical products.

REFERENCE

- [1] Baweja P, Kumar S, Sahoo D, Levine I (2016) Biology of seaweeds. In: *Seaweed in Health and Disease Prevention*. Academic Press, pp 41–106. <https://doi.org/10.1016/B978-0-12-802772-1.00003-8>
- [2] Fahn A (1980) *Plant Anatomy*. 3rd edition. Pergamon Press, Oxford.
- [3] Jha B, Reddy CRK, Thakur MC, Rao MU (2009) *Seaweeds of India: The Diversity and Distribution of Seaweeds of the Gujarat Coast*. Springer, Dordrecht.
- [4] Manikandan R, Annamalai, A., Kolandhasamy, P., & Rajendran, R. (2026) Bioaccumulation and Health Risk Assessment of Heavy Metals in Seaweeds from the Gulf of Mannar, Southeast Coast of India. *Biological Trace Element Research*. DOI: 10.1007/s12011-025-04855-z.
- [5] Naqvi SWA, Kamat SY, Fernandes L, Reddy CVG. (1980) Screening of marine plants for biologically active substances. *Bot Mar* 24(1):51–56.
- [6] Prakash Yoganandam G, Gowrishankar J, Gopal V (2020) Review of *Padina tetrastromatica*. *Int J Pharm Pharm Res (IJPPR)* 17(4):711–722.
- [7] Rashida Qari (2015) Heavy metals concentration in brown seaweed *Padina pavonia* (L.) and *Padina tetrastromatica* at different beaches of Karachi coast. *Indian J Geo-Mar Sci* 44(8): 1200-1206.
- [8] Siddique MAM, Hossain MS, Islam MM, Rahman M, Kibria G (2022) Heavy metals and metalloids in edible seaweeds of Saint Martin's Island, Bay of Bengal, and their potential health risks. *Mar Pollut Bull* 181:113866. <https://doi.org/10.1016/j.marpolbul.2022.113866>
- [9] Wallis TE (1965) *Analytical Microscopy: Its Aims and Methods in Relation to Foods, Water, Spices and Drugs*. 3rd edition. Little, Brown and Company, Boston.