



PHYTOCHEMICAL ANALYSIS AND METAL ION DETECTION OF STERCULIA LYCHNOPHORA (MALVA NUT) BY CHROMETOGRAPHIC TECHNIQUE AAS

Ms. Amisha Raiyani ^a, Dr. Kuldeep Sharma ^b

^a Research scholar, Parul Institute of Applied Sciences And Research, ahmedabad, Gujarat, India

^b Head of Department, Faculty of Applied Science, Parul University, Ahmedabad, India,

^b Corresponding author. Parul Institute of Applied Sciences And Research,
Bopal - Ghuma Rd, opp. Kabir Enclave, South Bopal, Bopal, Ahmedabad, Gujarat 380058

ABSTRACT:

The goal of this study was to choose and identify the sample, extract Sterculia lychnophora, and analyze for the presence of phytochemicals using a preliminary test. Primary screening of phytochemicals and qualitative analysis of secondary metabolites such as tannins, saponins, phenols, terpenoids, flavonoids, alkaloids, and lipids. The primary goal of this research is to determine the current status of bioaccumulation of several heavy metals in Sterculia lychnophora. The Atomic Absorption Spectrophotometer (AAS) flame absorption technique was used to measure the quantity of heavy metals in chosen samples. Heavy metals were chosen for their importance in human health and availability in agricultural fields. Cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), nickel (Ni), arsenic (As), and mercury (Hg) have all been quantified. Amount of different metal ions detected in various samples of sterculia lychnophora are Copper (Cu) (23.31 mg/kg), Zinc (Zn) (38.34 mg/kg), iron (Fe) (47.83 mg/kg), Manganese (Mn) (139.92 mg/kg), Nickel (Ni) (0.5 mg/kg), Cadmium (Cd) (0.5 mg/kg), Arsenic (As) (1.21 mg/kg), Lead (Pb) (20.54 mg/kg) and mercury (Hg) (0.5 mg/kg) was detected in selected sample of sterculia lychnophora.

Keyword:, *sterculia lychnophora*, *Bioaccumulation*, *Atomic Absorption Spectrophotometer*, *Heavy metal*, *phytochemical*

INTRODUCTION:

Sterculia lychnophora

Plants have been used in the production of stimulant beverages such as tea, coffee, cocoa and cola. Many secondary metabolites of plant chemicals are derived biosynthetically from plant primary metabolites. The secondary metabolites can be classified into several groups on the basis of their chemical classes [1]. The seeds of *Sterculia lychnophora* are specified as boat-fruited Sterculia which is traditional Chinese drug [2]. *S. lychnophora* is also called "Niranjana phal", Malva nut or "China fruit" in India. The fruit of Malva nut is egg-shaped having a diameter of 8-15 mm and color of this fruit is green-yellow which turns to dark brown after maturation and size up to adult's fingertip. The fruit is 18-20 millimeter long, base is 5-6 millimeter wide and dark blackish-brown surface with wrinkle and fruiting season is April-June [3]



(Figure 1) : *Sterculia lychnophora*

Malva nut tree is a tree species endemic to mainland Southeast Asia in the genus *Sterculia*. Its seed is used as a "coolant," for gastrointestinal ailments, and to soothe the throat in traditional Chinese medicine

Heavy metals

Heavy metals occur naturally in the environment. Its incidence, however, has increasingly increased as industrialisation has increased. Agricultural soils, as an important component of the ecosystem, are not immune to this occurrence. Cadmium (Cd), lead (Pb), and zinc (Zn) are among the heavy metals most plentiful in agricultural soils (Förstner, 1995). When present in low quantities, nickel and zinc are critical micronutrients; nevertheless, when present in excessive concentrations, these two metals become poisonous to plants (Lester G (1997)). Zn has the capacity to occupy low symmetry sites in enzymes and disrupt enzymatic action (Olivares, Uauy 1996, Ikeda and Murakami 1995, Corn 1993). In terms of non-essential components, nickel is known to cause cancer. Lead buildup causes impaired functioning of kidney, liver, and brain cells, followed by tissue destruction. Cadmium and its derivatives are also hazardous to human health. They produce acute and chronic symptoms varying in intensity from irritation to extensive metabolic disturbances. The levels of toxic metals (Cd and Pb) were determined in seed oil sample of *sterculia lychnophora*. While the significance of cadmium and lead as nutrients is uncertain, plants rapidly collect them in their systems (Mido, Satake 2003). Iron generally harms heart and liver cells, resulting in cancer, coma, metabolic acidosis, liver failure, circulatory shock, and long-term organ damage. Heavy metals such as Cd, Cu, Ni, and Zn are phytotoxic at any quantity or above a particular threshold. Hazardous metals are naturally amplified as they go up the food chain. The purpose of this study is to look at the heavy metal content in *sterculia lychnophora* Pb, Zn, Cd, Fe, and Ni concentrations were tested for this purpose in order to estimate heavy metal contamination. The increased

concentration of heavy metals in soils is mirrored by greater metal concentrations in plants, and therefore in animal and human bodies. Because some plants may absorb and store heavy metals, they can be used as markers of environmental contamination.

Metal type	Importance	Disorder
Iron (Fe)	Major component of haemoglobin and myoglobin	Anemia, liver disease, may provoke diabetes, and cardiac failure. Genetic disease hemochromatosis
Copper (Cu)	Major component of enzyme ferroxidase, regulate iron transport and storage	Anemia, Genetic disorder – Wilson’s disease
Zinc (Zn)	Structural constituent of many enzymes i.e. alcohol and dehydrogenase, carbonic anhydrase, RNA’s DNA polymerase etc.	Nervous system, skin, intestine , cell growth,
Cadmium (Cd)	A commonly occurring pollutant in nature	Renal dysfunction, lung disorder, skeletal and bone defects, blood pressure
Lead (Pb)	Extensively toxic for both plant as well as human body	Mental retardation in children, slow growth, paralysis, sensor neural deafness, nervous system, liver

Material and Method:**1.1 Material Required:**

Plant Samples:

Plant Samples Sterculia lychnophora

1.2 Chemical reagent required:

- Methanol, Mercuric Chloride, Potassium Iodide , Naphthol , Sulphuric acid , Chloroform , Sodium hydroxide , Ferric chloride , Per chloric acid , Nitric acid

1.3 Instrument required:

Magnetic stirrer , Hot plate , AAS (Atomic absorption spectrometer)

2.1 Method**▶ Preparation of extract:-**

- ▶ Take 20 gm of sterculia lychnophora powder in 500 ml beaker.
- ▶ Now add 250 ml (1:1) hydro methanol in this beaker.
- ▶ Mix by stirring with a magnetic stirrer.
- ▶ 48 hour in R.T.
- ▶ And afret filter by whatmanpaper no.1 and then this solution in hot plat

Preliminary phytochemical analysis

1. Tannin test (Braymer's Test)

- ▶ 1ml of filtrate distilled water was added for diluting, followed by two drops of ferric chloride. Tannins are detected by a transitory greenish to black colour.

2. Saponin testing (Foam Test)

- ▶ 4 ml of distilled water was used to dilute the small amount of extract. The mixture was vigorously shaken. The presence of saponins is shown by the persistence of foam for ten minutes.

3. Terpenoids testing (Salkowski Test)

- ▶ The extract was combined with 0.4ml of chloroform before adding 0.6ml of concentrated H₂SO₄ to form a layer. A reddish brown colouring forms at the interface as a result of the presence of terpenoids.

4. Flavonoids testing (NaOH Test)

- ▶ A few drops of sodium hydroxide solution must be added to the extract. The presence of flavonoids is indicated by the formation of a bright yellow hue that turns colourless when acid is added.

5. Phenol screening (Ferric Chloride Test)

- ▶ A few drops of ferric chloride solution must be added to the extract. The presence of phenols is indicated by the formation of a bluish black colour.

Preparation of solution by AAS (Atomic Absorbtion Spectroscopy)

- Acid solution prepared by **H₂SO₄ (Sulfuric acid) 65% + HClO₄ (Perchloric acid).65%+Nitric acid (HNO₃)70%**
- 1:1:5+1 gm of powder that solution heat 80%^c
- Next 1hr cooling under room temperature and after filter by whatmanpaper no 1
- Suspension is prepared un to 100 ml using double distilled water
- Sample injected in a atomic absorbtion spectrometer.



Figure 2 : AAS (atomic absorbtion sperctoscopy)

3. Result and discussion

Phytochemical analysis result :- The chemical compositions of Malva nut (*sterculia lynchophora*) powder and methyl esters from Soxhlet extraction observed

The presence of tannins, saponins, flavonoids, phenols, terpenoid, alkaloids was discovered during phytochemical screening of *Sterculia lynchophora* plant extract, as indicated in the table.

	Results
Saponins	+
Tannins	+
Terpenoid	+
Phenols	+
Flavonoids	+
Alkaloids	+

Keys: + Presence of the compounds

Heavy metal analysis:- Heavy metals accumulate in plants because they are more easily absorbed by food crops, particularly *sterculia lynchophora*. It might also be attributed to Malva nut foliar absorption of air deposits. Various nut species collect various metals. Depending on the ambient circumstances, metal species, nuts accessible, and heavy metal forms. Many studies have demonstrated that metal absorption and accumulation by different plant species are affected by a variety of conditions, which have been researched by a variety of researchers.

Apparatus:- Absorption of Atoms In this investigation, heavy metals were analysed using a spectrophotometer. All metal measurements (Cd, Ni, Fe, Pb, and Zn) were performed in an air/acetylene flame. Table shows the operating conditions for the instrumental parameters.

- ▶ The presence of copper(cu), zinc (zn), iron (fe) , manganese (mn) , arsenic (as) , lead (pb) those metal are presence below limit of quantification

Limit of quantification (ni) nickel , (cd) cadmium , (hg) mercury

Sl no.	perameters	result	LOQ
1	Copper as Cu	23.31	-
2	Zinc as Zn	38.34	-
3	Iron as Fe	47.83	-
4	Manganese as Mn	139.92	-
5	Nickel as Ni	BLQ	0.5
6	Cadmium as Cd	BLQ	0.5
7	Arsenic as As	1.28	-
8	Lead as Pb	20.54	-
9	Mercury as Hg	BLQ	0.5

Keys: BLQ : Below limit of quantification LOQ : Limit of quantification

Metal calibration curves :-

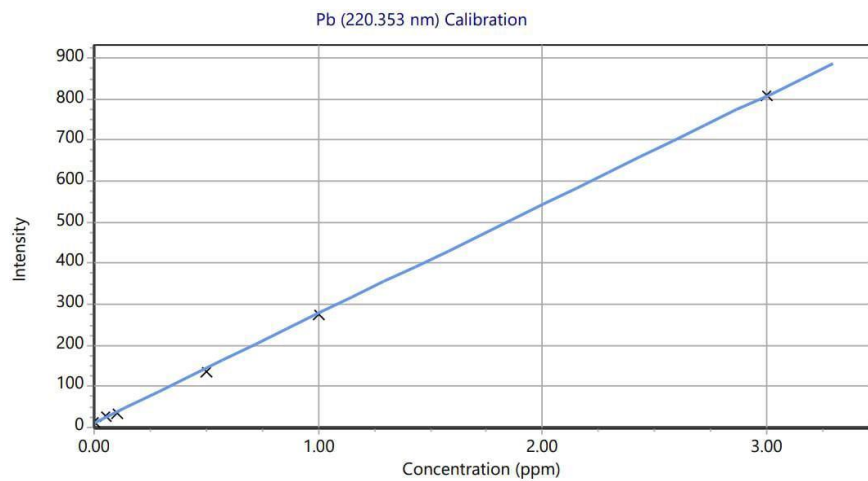
are constructed by aspirating samples of solutions containing known concentrations of metal into the flame, measuring the absorbance of each solution, and then building a graph in which the observed absorbance is plotted against the concentration of solution.

It is straightforward to calculate the concentration of relevant metal in test solution from the obtained absorbance using the calibration curve.

The calibration curves for metals are as follows.

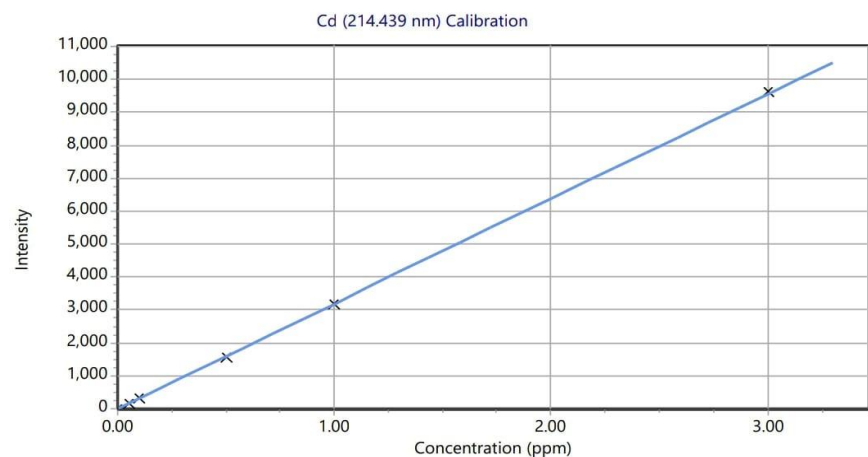
Pb (220.353 nm)

Intensity = 265.03201156 * Concentration + 11.82184032 Correlation coefficient: 0.99992



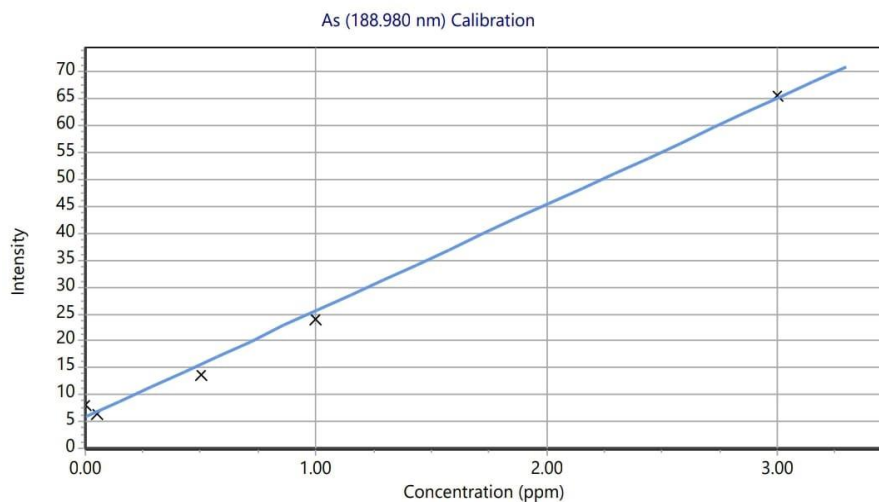
Cd (214.439 nm)

Intensity = 3185.14826953 * Concentration + 5.14540918 Correlation coefficient: 0.99999



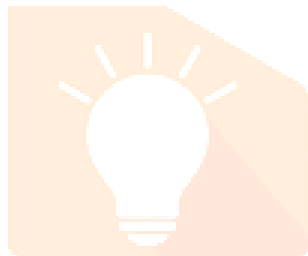
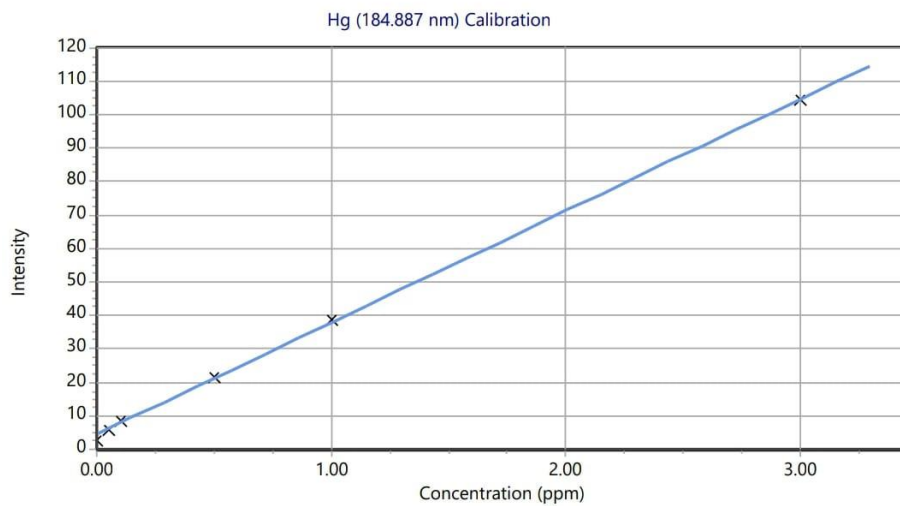
As (188.980 nm)

Intensity = 19.78236950 * Concentration + 5.75328775 Correlation coefficient: 0.99762



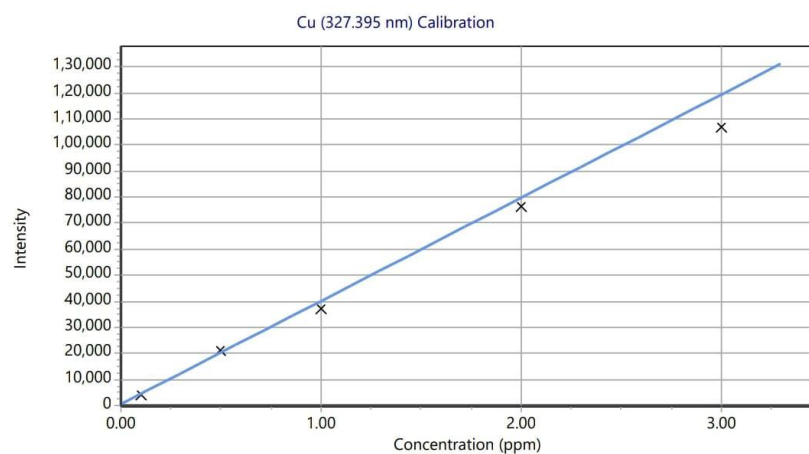
Hg (184.887 nm)

$$\text{Intensity} = 33.35877262 * \text{Concentration} + 4.46457887 \quad \text{Correlation coefficient: } 0.99967$$



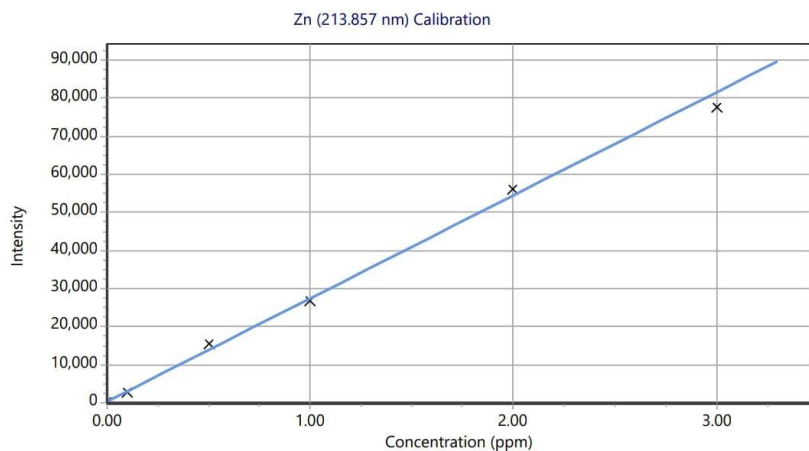
Cu (327.395 nm)

$$\text{Intensity} = 39679.30938942 * \text{Concentration} + 358.42301012 \quad \text{Correlation coefficient: } 0.99885$$



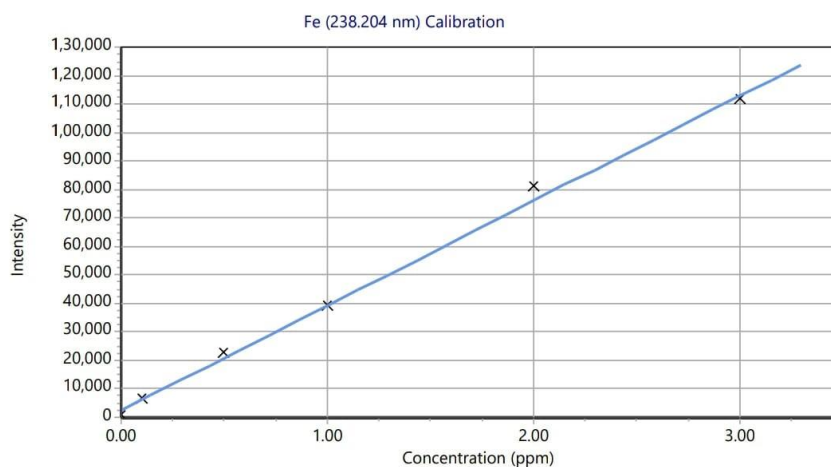
Zn (213.857 nm)

$$\text{Intensity} = 27113.09406658 * \text{Concentration} + 249.53564269 \quad \text{Correlation coefficient: } 0.99851$$



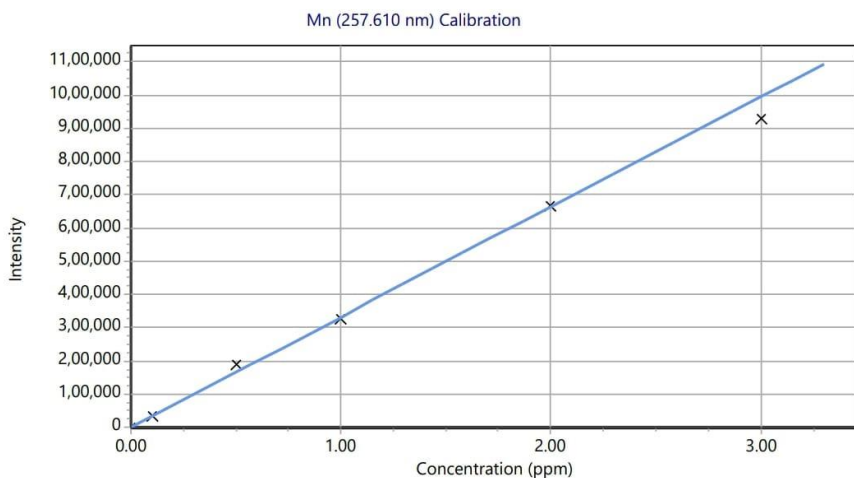
Fe (238.204 nm)

Intensity = 36802.70735783 * Concentration + 2392.57796287 Correlation coefficient: 0.99853



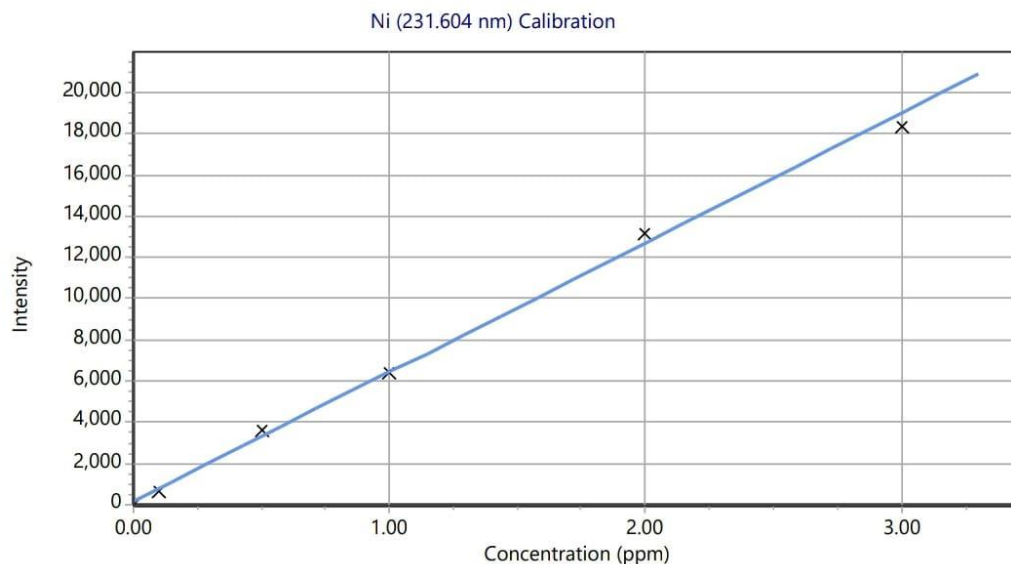
Mn (257.610 nm)

Intensity = 331572.56941793 * Concentration + 395.33494243 Correlation coefficient: 0.99848



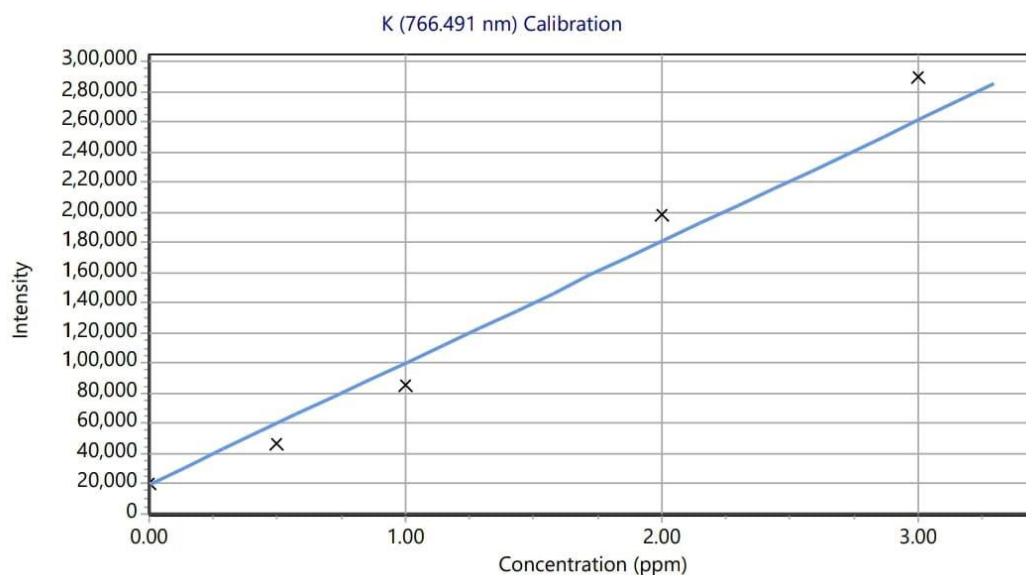
Ni (231.604 nm)

Intensity = 6300.69132995 * Concentration + 131.34984453 Correlation coefficient: 0.99857



K (766.491 nm)

Intensity = 80344.07249398 * Concentration + 19725.99642470 Correlation coefficient: 0.99515



The use of an HNO₃/HCL combination in the digestion of a nut sample allows for the measurement of the total concentration of heavy metals examined in a malva nut powder sample.

For metal analysis, place a corresponding metal hollow cathode lamp in the working position, adjust the current, pick the suitable resonance line, and modify the operating circumstances to produce a fuel-lean air-acetylene flame.

Beginning with the least concentrated solution, aspirate sequentially the standard solution of metals into the flame, followed by the test solution, with absorbance recorded in each case.

All the metal concentrations were determined on dry weight basis.

The majority of laboratory research on heavy metal biosorption found that no one mechanism is responsible for metal absorption. In general, two mechanisms are known to occur: 'adsorption,' which refers to the binding of

materials on the surface, and 'absorption,' which refers to metal penetration into the interior matrix. According to the findings, the cadmium and lead concentrations are too high. Cadmium buildup is caused by the fact that cadmium is rapidly absorbed by food crops. The concentration of lead in the sample varies with traffic volume; high concentrations of lead indicate high traffic flow near the place where the malva nut has grown. As a result, the plant can be recommended as a bioindicator for determining environmental contamination levels.

References

- [1] Angela A. Salim, Young-Won Chin and A. Douglas Kinghorn. Drug Discovery from plants. Bioactive molecules and medicinal plants 2008; 1-23
- [2] Pancharee Surapanthanakorn. Evaluation of analgesic, antipyretic and anti-inflammatory activities of the ethanol and dichloromethane extract from *Scaphium lychnophora* fruit in experiment animals. M.Sc. Thesis Prince of Songkla University, 2010.
- [3] Qian Xin-Zhong and Xin Zhin Qiang, An illustrated atlas of the commonly used Chinese Materia Medica, People's Medical Publishing House, 1st Edition, 2: 406-40
- [4] Mohammadpourfard, Issa, et al. "Determination of heavy metals in apricot (*Prunus armeniaca*) and almond (*Prunus amygdalus*) oils." *Iranian Journal of Health Sciences* 3.1 (2015): 18-24.
- [5] Orisakeye OT and Olugbade T.A. Studies on antimicrobial activities and phytochemical analysis of the plant *Sterculia tragacantha* lind. *Middle-East Journal of Scientific Research*.2012; 11(7): 924-927.
- [6] Sharma Beena, Gupta Shraddha and Sharma Kavita. Natural folk remedy for menorrhagia. *Journal of information, knowledge and research in humanities and social service*, 2011; 2(1): 86-88.
- [7] Shetty P, Palve A, Jadhav RN, Shinde P, Pimpliskar Mukesh. In- Silico docking analysis of *S.lychnophora* compounds against protein causing Alzheimer's disease. *Int.J.Engineering Sci and Innovative technology* 2014; Vol3(4):158-164.
- [8] Ru-Feng Wang , Xiu-Wen Wu and Di Geng. Two cerebrosides Isolated from the seeds of *Sterculia lychnophora* and their Neuroprotective effect. *Molecules* 2013; 18: 1181-1187 <http://dx.doi.org/10.3390/molecules18011181>
- [9] Tiwari Prashant, Kumar Bimlesh, Mandeep Kaur, Gurpreet Kaur and Harleen Kaur. Phytochemical screening and Extraction: A Review. *Int. Pharmaceutica Scientia*, 2011; 2(1): 98-106.
- [10] . Lijun Wang and Curtis L. Welle, Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology*, 2006; 17: 300–312. <http://dx.doi.org/10.1016/j.tifs.2005.12.004>
- [11] Margaret F. Roberts and Michael Wink. *Alkaloids: Biochemistry, Ecology, and Medicinal Applications*. Springer, 1998; 1-2.
- [12] Makkar HPS, Francis G and Becker K., Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. *Res. J. of the Animal Consortium*.2007; 1(9): 1371-1391.

- [13] Cook NC and Samman S, Flavonoids Chemistry, metabolism, cardio protective effects, and dietary sources. *J. of Nutritional Biochemistry*, 1996; 7(2): 66-76. [http://dx.doi.org/10.1016/0955-2863\(95\)00168-9](http://dx.doi.org/10.1016/0955-2863(95)00168-9)
- [14] Asaph Aharoni, Maarten A. Jongsma, Tok-Yong Kim, Man-Bok Ri, Ashok P. Giri, Francel W. A. Verstappen, Wilfried Schwab and Harro J. Bouwmeester, *Metabolic Engineering of Terpenoid Biosynthesis in Plants*. Springer publishers, 2006; 5: 49-58,.
- [15] Gupta Ankit, Naraniwal Madhu and Kothari Vijay, Modern extraction methods for preparation of bioactive plant extracts. *Int. J. of Applied and Natural Sciences*, 2012; 1(1): 8-26.
- [16] Thamaraiselvi, P. Lalitha and d P. Jayanthi, Studies on phytochemicals and antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.)Solms. *Asian Journal of Plant Science and Research*, 2012; 2 (2): 115-122.
- [17] Singh Dharmendra, Poonam Singh Abhishek Gupta, Shikha Solanki, Ekta Sharma and Rajeev Nema, Qualitative Estimation of the Presence of Bioactive Compound in *Centella Asiatica* An Important Medicinal Plant. *International Journal of Life Science and Medical Science*, 2012; 2(1):5-7.
- [18] Judith Laure Ngondi, Emile Joachim Djiotsa, Zephyrin Fossouo and Julius Oben. Hypoglycaemic effect of the methanol extract of *Irvingia gabonensis* seeds on streptozotocin diabetic rats. *AfricanJournal of Traditional*, 2006; 3(4): 74-77. <http://dx.doi.org/10.4314/ajtcam.v3i4.31179>
- [19] Peter C. H Hollman, Evidence for health benefits of plant phenols: local or systemic effects. *Journal of the Science of Food and Agriculture*, 2001; 81(9): 842–852, <http://dx.doi.org/10.1002>
- [20] Sven O. Dahms, Sandra Hoefgen., Dirk Roeser, Bernhard Schlott, Karl-Heinz Gührs, Manuel E Than. Structure and biochemical analysis of the heparin –induced E1 dimer of the amyloid precursor protein. *Proceeding of the National Academy of Sciences*,2010.107(12): 5381-5386.
- [21] Wagner H., Blatt S. *Plant drug analysis: A thin layer chromatography Atlas*. Germany:Springer –Verlog Berlin Heidelberg:1996. <http://dx.doi.org/10.1007/978-3-642-00574-9>
- [22] Giby Abraham. Pharmacognostical and phytochemical studies of the plant Sahadevi [*Vernonia cinerea*(L.)Less.]. *Int. J. Res. Ayurveda Pharm.*2015; 6(1):47-54.
- <http://dx.doi.org/10.7897/2277-4343.06112>
- [23] Pimpliskar M.R., Jadhav R.N and Jadhav B.L. Study on antimicrobial principles of *Rhizophora* Species Along Mumbai Coast. *J.Aqua. Biol.* 2011;26(1): 6 – 11.
- [24] Omeje, Kingsley O., et al. "Quantification of heavy metals and pesticide residues in widely consumed Nigerian food crops using atomic absorption spectroscopy (AAS) and gas chromatography (GC)." *Toxins* 13.12 (2021): 870.
- [25] 25. Kumar, P., et al. "Substitution of antibiotic growth promoter with locally available plant derivatives: in-vitro study." *Indian Journal of Animal Health* (2022): 2