DETERMINATION OF METALS IN MEDICINAL PLANTS AND ISOLATION OF NATURAL COMPONENT FROM *Nyctanthes arbortristis* USING COLUMN CHROMATOGRAPHY

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation / Internship report entitled, "**Determination of Metals in Medicinal Plants and Isolation of Natural Component from** ***Nyctanthes arbortristis*** **using Column Chromatography**" is based on the results of investigations carried out by me in the Master of Science in Analytical Chemistry at the School of Chemical Science, Goa University under the Supervision of Dr. Prajesh S. Volvoikar and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation / internship report "Determination of Metals in Medicinal Plants and Isolation of Natural Component from *Nyctanthes arbortristis* using Column Chromatography" is a bonafide work carried out by **Ms. M.Shivani** under my supervision/mentorship in partial fulfilment of the requirements for the award of the degree of Master's in the Discipline Analytical Chemistry at the School of Chemical Science, Goa University.

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I. ABSTRACT

Plants have long been acknowledged as nature's most effective medicine, treating all illnesses. Modernization and environmental toxicity levels have been discovered to be exceeding the upper limit, which has a significant impact on the quality of medicinal plants. The aggregation of hazardous metals is one of the factors that could have an adverse effect on human health. The accessibility of modern equipment, has allowed for the identification and measurement of hazardous metals. In the following study different plant species were collected, pre-treated and characterized using Microwave-Induced Plasma Atomic Emission Spectroscopy (MP-AES). Along with evaluation of metals, a natural component was isolated from one of the medicinal plant using column chromatography and further analysed using LC-MS.

II. INTRODUCTION

Mastery of herbs has been followed from generation to generation. Most of the medicinal plants have been considered to be a significant source for human health and nutrition. This importance has been elevated by the presence of inorganic and organic substances. Metal ions play a crucial role in sustaining the lives on planet. Since ancient times, their importance in biological system has been studied and practised. [(1)] Diseases may result from their deficiency or absence. But some metal ions, especially the so-called heavy metal ions can be hazardous. Although necessary for living, essential metal ions can also be harmful if present in excess. Metals essential for biological functions are sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), molybdenum (Mo), and cadmium (Cd). [(2)] In this study only few of the million plant species have been considered to evaluate the metal concentration. Medicinal value plant species such as *Leucas aspera, Phyllanthus urinaria, Cynodon dactylon, Piper nigrum* and *Nyctanthes arbortristis,* have been analysed using Microwave-Induced Plasma Atomic Emission Spectroscopy.

Leucas aspera Linn., is a common annual plant found throughout India that is branching and erects to a height of 15 to 60 cm. It is a member of the Lamiaceae family. It includes nicotine, sterols, glucoside, diterpenes, oleanolic acid, ursolic acid, triterpenoids, and b-sitosterol. Ayurveda and Siddha, two traditional Indian medical systems, use this herb. Its pharmacological effects range from carminative to antihistaminic to antipyretic to antiseptic. [(3)]

One of the most significant groups of plants in the Phyllantaceae family is the genus *Phyllanthus* (L.). An annual perennial herb, *Phyllanthus urinaria* (L.), is found in tropical Asia, America, China, and the islands of the Indian Ocean. [(4)] In traditional medicine, *P. urinaria* is used to treat liver disorders, diabetes, malaria, and jaundice. Studies on the plant's phytochemistry show that it contains a variety of secondary metabolites, including lignans, tannins, flavonoids, phenolics, terpenoids, and others. (5)

The grass *Cynodon dactylon* is perennial. Proteins, carbohydrates, mineral components, P-sitosterol, flavanoids, alkaloids, glycosides, and triterpenoides are only a few of the metabolites that are abundant in the plant. The plant has been used in traditional medicine for a very long time to treat a variety of illnesses, including anasarca, cancer, convulsions, cough, cramps, diarrhoea, dropsy, dysentery, epilepsy, headache, haemorrhage, hypertension, hysteria, measles, rubella, snakebite, sores, stones, tumours, urogenital disorders, warts, and wounds. (6)

To the Piperaceae family of plants belongs *Piper nigrum* (black pepper). Black pepper is a nutrient and vitamin-rich food. Black pepper seeds have a high concentration of minerals like calcium, magnesium, potassium, and phosphorus and a relatively low concentration of sodium, iron, and zinc. They also contain carbohydrate, protein, and fat. These minerals are necessary for human activity on a daily basis. (7)

A well-known plant is *Nyctanthes arbortristis* Linn, sometimes known as Night jasmine or Harsinger. It is a native of India and is found growing wild in the sub-Himalayan area as well as in Indian gardens as an ornamental plant. The entire plant is frequently used to treat cancer, the root for fever, sciatica, anorexia, the bark as an expectorant, the leaf for controlling fever, diabetes, and as a cholagogue, diaphoretic, and anthelmintic. Different plant extracts are used to treat a variety of conditions, including arthritis, malaria, intestinal worms, and as laxatives with antitrypanosomal, anti-inflammatory, and antioxidant properties.

Microwave-Induced Plasma Atomic Emission Spectroscopy aids in estimating the type and amount of metal present in the above species. In this technique when an atom of a particular element is excited, as it returns to the ground state, it emits light in a distinctive range of wavelengths known as an emission spectrum. One of the sources of atomic emission is microwave plasma (MP). Temperatures in the nitrogen-fuelled microwave plasma approach 5,000 K.

Separation of components can be best achieved by chromatographic techniques. One of the chromatographic techniques used here is the column chromatography. This technique includes a stationary phase and a mobile phase. The sample is loaded onto the top of the column. The components run through the column and are isolated based on their affinity towards stationary phase and the mobile phase.

III. LITERATURE REVIEW

Pizzarno *et al.* in 2023 determined arsenic species in urine using microwave-induced plasma optical emission spectrometry. Attempt was made to quantify arsenic coupled species such as As (III), As (V), monomethylarsonic acid, and dimethylarsinic acid by using hydride generation microwave induced plasma optical emission spectrometry. accuracy of 95 % - 97 % was obtained with the intra assay precision which has been denoted by relative standard deviation fell in the range of 1.8 % - 6.2 % and 6.9 % - 15 % of inter assay precision. The limits of detection and quantification were 1.8 and 5.4 $\mu g\ L^{-1}$, respectively. Urine samples collected from wood impregnation plant worker were assessed using a validated method which was found to be effective for identifying health risk of As exposure through drinking water.

Vaikosen *et al.* in 2017 compared the presence of heavy metals in medicinal plants from wild and cultivated garden in Wilberforce Island, Niger Delta region, Nigeria. Nine different medicinal plant species and surrounding soil was collected from a medicinal garden of Nigeria to estimate the levels of Ni, Cr, Pb and Cd. Study was done by using atomic absorption spectrometry where collected samples were first dried, pre-treated and finally introduced into the instrument. Cd, Pb, Ni and Cr showed a detection frequency of 100 %, 11 %, 0 % and 0%, respectively. Concentration of Cr was found to be maximum in soil and Pb the least.

Diaconu *et al.* in 2012 analysed heavy metals in medicinal plants and their infusions. Some of the heavy metals were quantitatively determined in *Mentha piperita L., Matricaria camomilla L., Hypericum perforatum L., Achillea millefolium L., Thymus* *serpillum, Capsella-bursa-pastoris L., Urtica dioica L., Primula veris L., Plantago major L., Taraxacum officinalis L.* and their infusions, using flame absorption spectrometry. Iron and zinc were found in maximum concentration: 333.22 ±35.18 mg/Kg and 244.90 ±32.13 mg/Kg, respectively, while Pb and Cd were obtained in minute concentrations. Analysis of plant infusions revealed that heavy metals were transferred during the extraction process. Because some heavy metals in high concentrations in the body might have hazardous effects, it is crucial to monitor the mineral content of therapeutic plants and their boiling water extracts.

Rajput *et al.* in 2023 studied neuroprotective activity of novel phenanthrene derivative from *Grewia tiliaefolia.* A variety of phytochemicals found in medicinal plants are responsible for their various biological functions. These chemicals have currently been extracted from medicinal plants, characterised, and their pharmacological potential assessed. Efforts have been made in the current study to isolate the compound(s) from *Grewia tiliaefolia* Vahl., a plant recognised for its therapeutic effects on brain-related illnesses such anxiety, depression, cognitive problems, and Parkinson's disease. Using column chromatography, a plant extract was treated to the extraction of a compound or compounds, and the isolated compound was characterised using NMR, FTIR, and LCMS.

Hu *et al.* in 2022 isolated and characterized functional components from *Polygonum cuspidatum* responsible for reducing uric acid. Due to the active ingredients found in *Polygonum cuspidatum* (*P. cuspidatum*), often known as Asian knotweed, this herbaceous perennial plant is utilised in Chinese medicine. Recent studies of the uric acid-lowering activity of *P. cuspidatum* extracts have mainly focused on analysis of its crude extract or specific chemical components, with limited research about its uric acid-lowering activity in vivo. In the current investigation, monomeric chemicals with greater uric acid-lowering potential were extracted from *P. cuspidatum* extract by column and thin-layer chromatography (TLC) and evaluated in vitro using the xanthine oxidase (XOD) inhibition assay and a mouse model of hyperuricemia. Nuclear magnetic resonance (NMR) spectroscopy was used to structurally characterise the separated components, and untargeted metabolomics was used to investigate the putative mechanism of uric acid reduction. Two compounds were isolated and identified. The activity of XOD was significantly inhibited by compound 1 ($p < 0.01$), with a 49.80 % inhibition. According to metabolomics research, the fractions (compounds 1 and 2) mostly affected the metabolism of galactose in hyperuricemic mice ($p = 5.74E-4$), and their effects were strongly linked to the metabolic pathways of organic acid, carbohydrate, and lipid metabolism. These findings support the idea that *P. cuspidatum* can be used as a food and medicine, which may lead to an increase in its use as an alternative therapy for hyperuricemia.

Kumatia *et al.* in 2022 isolated Iridoids from *Morinda lucida,* (Benth.) Rubiaceae, produced analgesic and anti-inflammatory activities. This study aims to assess the analgesic and anti-inflammatory properties of iridoids from *Morinda lucida* as well as potential mechanisms behind these properties. Column chromatography was used to isolate the compounds, and NMR spectroscopy and LC-MS were used to characterise them. Using carrageenan-induced paw edoema, anti-inflammatory activity was assessed. While the hot plate and acetic acid-induced writhing assays were used to measure the analgesic efficacy. Assays using a heated plate and acetic acid to cause writhing were used to measure the analgesic activity. Pharmacological blockers, antioxidant enzyme identification, lipid peroxidation, and docking studies were all used in the mechanistic research. As a result of operating as both delta and kappa opioid receptor agonists, elevating anti-oxidant activity, and inhibiting COX-2, the results showed that ML2-2 and ML2-3 possessed extremely considerable analgesic and anti-inflammatory properties.

Kfle *et al.* in 2020, level of heavy metal contamination was evaluated in vegetables and animal grass feed in wastewater irrigated area around Asmara, Eritrea. Consuming vegetables and animal feed grasses grown on contaminated soils has been a key food chain route for human exposure to heavy metals and poses a health risk. Wastewater is typically used to irrigate soils. In three locations in and around Asmara, Eritrea, a study was carried out to evaluate the build-up of heavy metals in crops irrigated with wastewater between two and five decades. Using an inductively coupled plasma optical emission spectrophotometer (ICP-OES), the metal concentrations of soil and plants (*Medicago sativa L., Avena sativa L., Cynodon dactylon L., Corchorus olitorius L., and Cynara scholymus L.*) grown in the farms were examined. Multivariate analysis, such as principal component analysis (PCA) and cluster analysis (CA), was performed on the distribution of elements in plant species to identify the source of the heavy metals. The order of the metal concentrations in the soil samples was $Mo < Cd < Co < Cu < Pb < V < Cr \approx Zn < Mn < Fe < Al$. It has generally been $Cd < Co < V < Cr < Pb < Cu < Zn < Mn < Al < Fe$ for the metals in the various sites' plants. The study found that, with the exception of the soil sample from the Kushet region, all of the analysed areas' soil samples were dangerous for agricultural purposes in terms of Fe, Mn, and Pb. The majority of the examined heavy metals were found to be present at levels that were within FAO/WHO permitted limits in vegetation samples from all of the sites. All plant samples from the Kushet region and *M. sativa* from Paradizo were exempt from the

FAO/WHO acceptable limit for Al and Fe. Al concentrations in *C. dactylon* from Adi-Segdo and Paradizo were also below the limit of detection. Out of the five plants that were taken into account for this study, *C. olitorius* was shown to be an excellent accumulator of heavy metals and *C. dactylon* to be the least effective. The grass species *C. olitorius* should be further researched for its ability to phytoremediate contaminated soils in light of the study's findings. The multivariate analysis's findings showed that while Zn, Mo, Cu, Mn, and Cd came from an anthropogenic source, Fe, V, Al, Cr, Co, and Pb were under the control of mixed (natural and anthropogenic) sources. Studies on the build-up of heavy metals in plants growing in wastewater-irrigated farms near Asmara are extremely scarce and insufficient. As a result, it is anticipated that the study's findings will help the community understand itself and empower the city council to monitor environmental quality and take appropriate action.

Dalvi *et al.* in 2007 used atomic absorption spectrometry to determine metals in *L. aspera*. Many mineral elements are present in plant and animal tissues in incredibly small amounts, making it impossible for older scientists to determine their precise concentration using analytical techniques that were available. Nearly all trace elements may be precisely and accurately measured in even the smallest biological samples using modern analytical techniques like atomic absorption spectroscopy. The standardisation of herbal medicines also requires that heavy metal analysis be a key component. *Leucas aspera* is an herb that primarily grows on plains and has white blooms. It has been used to treat a variety of illnesses, including diabetes, anti-inflammatory, and antioxidant properties. Atomic absorption spectroscopy was used to investigate five common heavy metals: Fe, Zn, Cr, Cu, and Pb. Fe had a higher concentration than the other four metals when they were all examined in *Leucas aspera* whole plant powder.

Rai *et al.* in 2001 studied metal accumulation in herbal drugs. Some of the significant Indian herbal medicines, including *Alpinia galanga*, *Artemisia parviflora, Butea monosperma, Coleus forskohlii, Curcuma amada, Euphorbia prostrata, Leucas aspera, Malaxis acuminata, and Pueraria tuberosa,* were found to accumulate heavy metals, including Pb, Cd, Cu, and Zn. For the purpose of estimating the presence of heavy metals, 34 samples-both real and market samples of the aforementioned plant species were obtained. The majority of the analysed samples contained Pb and Cd concentrations that were over WHO acceptable limits. It is also noteworthy that the site from which the samples were taken is related to the differential in heavy metal concentration in a certain plant species gathered from different regions. For instance, a sample of *Artemisia parviflora* leaves from Junagadh gathered more Pb and Zn than a sample from Tarikhet. This can be caused by the increased industrial activity in Gujrat State's Junagadh area. Similar to how Lucknow samples of *Alpinia galanga* and *Curcuma amada* had the highest quantities of Pb, Cd, Cu, and Zn compared to other regional samples of the same species, this city has significant levels of air pollution brought on by automobiles.

Vuong *et al.* in 2020 estimated the content of toxic elements in herbal plants from different parts of Northern Vietnam. Heavy metals from the nearby polluted environment may be present in plants. When contaminated with heavy metals like Pb, Cd, and As, the medicinal herbs and plants that are frequently utilised in Vietnam may be hazardous to the public's health. In this study, the levels of Pb, Cd, and As in five different herb plants—*Phyllanthus urinaria L., Plantago asiatica L., Eleusine indica L., Wedelia chinensis (Osbeck) Merr,* and *Artemisia vulgaris L.* were examined. The samples were taken from undeveloped areas in a few northern Vietnamese provinces.

Pb, Cd, and As concentrations were measured using the inductively coupled plasma mass spectrometry (ICP-MS) technique. The content of Pb, Cd and As was 0.247 ÷ 32.080 mg kg^{-1}, 0.000 ÷ 1.099 mg kg^{-1}, 0.000 ÷ 2.261 mg kg^{-1}, respectively. Pb, Cd, and As concentrations were lower than the World Health Organisation (WHO) allowed thresholds in 50 out of the 58 samples that were examined. The remaining samples included levels of Pb, Cd, or As that were much higher than the WHO-permitted limit.

Vaidhya *et al.* in 2017 carried on physiochemical standardization and metal analysis in *Nyctanthes arbor-tristis* linn. Native to India, *Nyctanthes arbor-tristis* Linn is widely dispersed in the sub-Himalayan areas. Due to its use in the Ayurvedic, Sidha, and Unani medical systems, different portions of *Nyctanthes arbor-tristis* Linn are recognised by tribal people of the Indian subcontinent to possess distinct illnesses. The petals are used to treat piles and as a hair tonic in addition to being stomachic, carminative, astringent to the colon, antibilious, and expectorant. The powdered stem bark is used as an expectorant, to cure malaria, and to relieve rheumatoid joint discomfort. Both bronchitis and snakebite are treated with the bark. The leaves of *Nyctanthes arbor-tristis* Linn are widely used in Ayurvedic medicine to treat a variety of ailments, including internal worm infections, rheumatism, chronic fever, and sciatica. They are also used as a laxative, diaphoretic, and diuretic. The seeds are used for alopecia and as anthelmintics. Plant physicochemical parameters provide useful data and aid in determining the sample's quality. According to WHO recommendations, the ash values, extractive values, loss on drying, and moisture content in the leaf samples were assessed. As some plants have a propensity to store heavy metals from the soil, contaminated water, and atmosphere, heavy metals are an issue in herbal medicines. Micronutrients have a critical role in the regulation and growth of plants. So, utilising inductively coupled plasma - atomic emission spectroscopy, the presence of metals was identified. The acquired results showed that the heavy metal content was within the allowable values, hence the plant was safe to be used in herbal medication compositions.

Elekes *et al.* in 2009 researched on mineral element accumulation level in some herbaceous plant species by ICP-AES method. Seven herbaceous plant species were collected and analysed from the metallurgical industry zone of Dambovita County (*Lolium perenne, Festuca pratensis, Stipa capillata, Agrostis alba, Cynodon dactylon, Luzula campestris*, and *Agrostis tenuis*) to determine the levels of heavy metal accumulation in these species. An inductively coupled plasma-atomic emission spectrometer was used to analyse the dry matter in order to determine the heavy metal concentrations (for Cr, Mn, Zn, Sr, Cu, Ba, and Sn). For the metals under study, this approach offers detection limits of 0.4–0.6 mg/kg. The concentrations of heavy metals in plants collected from the industrial area ranged from 0.99 to 113.83 mg per kg of dry matter for Sr, 0.99 to 165.89 mg per kg for Mn, 62.09 mg per kg for Zn, 29.21 mg per kg for Sr, 58.66 mg per kg for Ba, and 8.38 mg per kg for Sn. The ratio between the concentration level of the metal in plant samples and the level of the same metal in the soil, close to the radicular system for each species of plants, was used to calculate the levels of heavy metal accumulation in the analysed species of plants. *A. alba* had the highest accumulation levels for Cr (267.69%), *L. perenne* had the highest levels for Mn (51.45%), Sr (114.35%), and Ba (60.81%), and *C. dactylon* had the highest levels for Zn (136.62%), Cu (97.65%), and Sn (704.00%).

Yashvanth *et al.* in 2013 analysed micro chemical of *Leucas aspera* using SEM-EDAX. Leucas aspera (Willd) Link, a plant, is well known for its several medicinal applications. The current work uses a scanning electron microscope equipped with an Energy Dispersive X-ray Analysis (EDAX) detector to characterise its microchemical (elemental) makeup. The form of the plant is quite intriguing. On the leaf and stem surfaces as well as within the crystals, there were inclusions and exudates of various shapes. Stem surface, stem sections, stem inclusions, blebs on stem hairs, crystals of various shapes, root sections, abaxial and adaxial surfaces, flowers, seeds, and seed caruncles were some of the plant elements investigated. The elemental composition of different plant sections varied greatly. Aluminium, calcium, silica, carbon, oxygen, and carbon were the main elements found. Iron, sodium, potassium, phosphorus, and chlorine were additionally discovered.

Vásquez-Londoño *et al.* in 2022 evaluated whether *S. incarnata* roots' chemical components have any neuroprotective effects against C2-ceramide-induced cell death in Cath.-a-differentiated (CAD) cells. Infrared spectroscopy, mass spectrometry, and nuclear magnetic resonance spectroscopy were used to clarify the structures of the chemicals obtained from the fractionated *S. incarnata* root ethanol extract. CAD cells were used to evaluate the cytotoxic and neuroprotective effects of *S. incarnata* root extract, fractions, and isolated chemicals against C2-ceramide. Although *S. incarnata* root extract and its n-butanol fraction did not cause cytotoxicity in CAD cells, they did exhibit neuroprotective properties against C2-ceramide toxicity. Additionally, the phenylethanoid glycosides stachysoside C (12.5, 25 and 50 g/mL) and incarnatoside (first identified) from *S. incarnata* roots shielded CAD cells from C2-ceramide without causing cytotoxic effects. The extracted phenylethanoid glycosides and the neuroprotective properties of *S. incarnata* root extract on CAD cells give an ethnopharmacological basis for the long-standing usage of this species for disorders of the central nervous system in Colombia.

Perumal *et al.* in 2022 isolated Phlorizin from seagrass *Syringodium isoetifolium* responsible for inhibition of diethylnitrosamine and carbon tetrachloride-induced hepatocellular carcinoma in BALB/c mice. Natural compounds with marine origins are secondary metabolites with several medicinal uses. In the current work, the bioactive component from the seagrass *Syringodium isoetifolium* was isolated, and the anticancer activity against hepatocellular carcinoma was investigated in vitro, in silico, and in vivo. By using column chromatography, the bioactive substance was extracted from an aqueous ethanol extract of *S. isoetifolium*. Utilising 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Acridine Orange/Ethidium Bromide (AO/EtBr), DNA fragmentation, flow cytometry, and western blotting against HepG2 cell lines, researchers examined the isolated compound's in vitro anticancer effects. Additionally, hepatocellular cancer in BALB/c mice was produced by diethyl nitrosamine and carbon tetrachloride, as well as molecular docking. In the current study, liver morphology, haematological and biochemical indicators, as well as a histological investigation, were also carried out. The isolated compound's structure was verified by ^{1}H NMR, ^{13}C NMR, FT-IR, and mass spectral data. Phlorizin-treated isolated HepG2 cells underwent apoptosis, as shown by the in vitro anticancer activity. The isolated molecule had a better binding cavity against the SIRT3 protein, according to docking studies of the chemical into the NAD+-dependent deacetylase's binding site. The malignant tumour was greatly reduced by phlorizin's in vivo anticancer action, and the haematological and biochemical parameters were returned to normal. The histological analysis of mice with HCC generated by DEN+CCl4 showed encouraging anticancer efficacy.

According to the findings of the current study, phlorizin, an isolated substance from *Syringodium isoetifolium*, may have anticancer properties against hepatocellular carcinoma.

Asadipour *et al.* in 2023 extracted, identified and determined molecular structure of two natural compounds along with phytochemical study of *Hymenocrater calycinus* plant of the lamiaceae family collected from Iran. *Hymenocrater calycinus* (Boiss) Benth., an Iranian native plant from the Lamiaceae family, was evaluated and its natural constituents in the essential oil and extracts were analysed phytochemically. The plant was taken from the southern mountains of Khorasan Razavi. Sesquiterpene hydrocarbons were found to have the largest concentration in the essential oil of this plant, according to the results of GCMS spectra. The two monoterpenes with the greatest percentages in this essential oil were 1,8-cineole (12.54 %) and -cadinene (11.27 %). The content of flavonoids (Shinoda method), tannins (Ferric chloride method), alkaloids (Mayer test), glycosides (Keller-Kiliani test), and saponins (Froth method) was investigated in the plant extract. The results showed that the plant extract is devoid of alkaloids but contains significant amounts of flavonoids (red colour), saponins (foam layer), glycosides (reddish-brown colour), and tannins (dark green for catechol tannin). After being soaked in a solvent, the plant was degreased to separate the long-chain hydrocarbons. The components were then analysed and purified using thin-layer chromatography (TLC), column chromatography, and plate chromatography methods. Utilising techniques from IR spectroscopy, ^{1}H and ^{13}C NMR, and lastly DEPT spectroscopy, natural chemicals extracted from the extract were interpreted and identified. Santin and ermanin, two flavanols, were found. The findings of this study offer an important report on the phytochemical activities of *H. calycinus* because there have only been a few numbers of studies published on its therapeutic properties.

Anjani *et al.* in 2022 extracted bioactive compounds from fruits and vegetables waste. Due to being able to destroy germs, plants and their by-products have been used extensively against terrible infections from ancient times. Due to their medicinal qualities like antipathogenic activity, waste from fruits and vegetables is attracting increased interest in research. The antibacterial and cytotoxic effects of a herbal mixture made from the peels of *Allium cepa, Cucumis sativus, Citrus reticulata,* and *Mangifera indica* were examined in the current study. With regard to *Streptococcus mutans, Bacillus licheniformis, Lactobacillus plantarum, Escherichia coli, Pseudomonas aeruginosa,* and *Citrobacter freundii*, the herbal combination was tested for broad spectrum antibacterial action. Additionally, researchers looked into the existence of phytochemical indicators such phenolics and flavonoids. According to the findings, the herbal mixture had antimicrobial activity against *S. mutans, B. licheniformis, L. plantarum, and E. coli,* and only modest activity against *P. aeruginosa* and *C. freundii*. Additionally found were phenolics (7981.52 g/g) and flavonoids (355.32 g/g). With the use of column chromatography, high-performance liquid chromatography (HPLC), fourier-transform infrared spectroscopy (FTIR), and nuclear magnetic resonance spectroscopy (NMR) investigation, it was also shown that the herbal mixture contained flavonoids like quercetin and rutin. The MTT experiment confirmed that the herbal mixture aids in the growth of L929 cells without affecting their viability. The current study's findings point to the potential for using herbal combinations as a source for plausible antibacterial agents that could be isolated and used as a lead candidate for the development of antibacterial drugs that help to prevent or lessen the spread of infectious diseases brought on by various pathogenic microbes.

Babalola *et al.* in 2022 extracted, purified and characterized papain cysteine-proteases from the leaves of *Carica papaya*. One polypeptide chain with three disulphide bridges and a sulfhydryl group required for proteolytic action makes up the single polypeptide chain that makes up papain, a member of the globular cysteine-protease family. It has numerous applications in medical and food industries. This work aims to isolate, purify, and characterise papain enzymes from *Carica papaya* leaves. Using 70 % $NH_4(SO_4)_2$, DEAE-Cellulose column chromatography, and Sephadex G-25 column chromatography, crude extracts containing the enzymes were obtained from papaya leaves at Mountain Top University in Nigeria. This purification process resulted in a purification fold of 1.6424. On the enzymes' activity, the effects of temperature, pH, substrate concentration, and Mg^{2+} concentrations were identified. This is the first study to describe the presence of five cysteine proteases with varying degrees of binding affinity and catalytic efficacy towards the casein substrate in papaya leaves. The ideal temperature range and pH range for the pure papaya enzymes are 50 °C to 59 °C and 4.5 to 6.6. In general, they perform better when Mg^{2+} is present. The enzyme's K_m and Vmax values in the different pools ranged from 1.47 mg/ml to 8.70 mg/ml and 0.42 µmol/ml/min to 0.4167 µmol/ml/min, respectively. When compared to the isolated enzymes in this work, papain E had the best catalytic efficiency (K_{cat}/K_m 59.776 - pmL/mg.min) and binding affinity (K_m - 0.83 mg/mL) at pH 5.5 and 50 °C in the presence of Mg^{2+}. The industry might use the values of the parameters evaluated from this study to produce papain and to store the product for usage in homes and hospitals.

Lin *et al.* in 2022 performed structural characterisation and anti-osteoporosis effect of an arabinomannan from *Anemarrhena asphodeloides* Bge. Using column chromatography, an arabinomannan (PAAP-1B) with a molecular weight of 14.0 kDa was extracted from *Anemarrhena asphodeloides* Bge. to identify the polysaccharide with anti-diabetic osteoporosis (DOP) activity and define its structure. It has a 6:3:1 molar ratio of arabinose, mannose, and galactose. The backbone of PAAP1B is made up of 1,5-Araf, 1,4-Manp, and 1,6-Galp residues, with the Araf and Galp residues branching at C3. T-Araf, T-Manp, T-Galp, and 1,6-Galp are the side chains. In mice exposed to alloxan, PAAP-1B attenuated DOP and decreased ferroptosis in the femurs and tibias. By lowering the levels of 4-hydroxynonenal, malondialdehyde, mitochondrial reactive oxidative species, and lipid peroxidation and reversing the downregulation of solute carrier family 7 membrane 11 and glutathione expression, it also prevented ferroptosis in advanced glycation end product-induced osteoblasts.

Anita *et al.* in 2022 used deoxylapachol obtained in *Tectona grandis* wood as a catalyst for delignifcation and carbohydrate protection during the kraft cooking of eucalyptus wood. The pulp and paper sector's leading technology is kraft cooking. However, it must be made more efficient and productive. By using catalysts, kraft cooking can produce more pulp while using less energy and less lignin in the pulp fibres. In this study, natural 2-methylanthraquinone (2-MAQ) and deoxylapachol were extracted by column chromatography from acetone extracts of Indonesian Jepara Tectona grandis (teak) wood and employed as alkaline cooking catalysts. Deoxylapachol, iso-deoxylapachol, and 2-MAQ were all present in the extract, according to gas chromatography-mass spectrometry. Lapachol was also included in the Indonesian Gunung Kidul teak wood extract. Deoxylapachol's capacity to speed up delignification and preserve carbohydrates was demonstrated by the kraft cooking test of *Eucalyptus globulus* wood at 145 ^{o}C and charged with 17 % active alkali. It was found that the cooking pulp made with kraft-deoxylapachol had less lignin than the control pulp made without a catalyst. The addition of deoxylapachol accelerated lignin degradation, according to the molecular weights of the dissolved lignin in black liquor, but 86 % of the carbohydrates were still present after the kraftdeoxylapachol cooking procedure. Deoxylapachol was applied, and the result was a pulp yield that was roughly 0.8–1.2 % higher than it would have been without the catalyst. This study shown that natural deoxylapachol derived from teak wood can be a promising cooking catalyst for the pulp and paper industry and may have positive social and environmental effects.

IV. EXPERIMENTAL WORK

1. MATERIALS AND METHODS:

Reagents and chemicals such as chloroform, Pet ether, methanol, nitric acid were of analytical grade. Samples analysed by LC-MS and AES were prepared in HPLC grade solvents and distilled water.

2. DETERMINATION OF METALS IN MEDICINAL PLANTS USING MP-AES

Medicinal plants such as *Leucas aspera*, *Nyctanthes arbortristis*, *Piper nigrum, Cynodon dactylon* and *Phyllanthus urinaria* were collected and washed with water to eliminate dust particles and sun dried until complete dryness. Dried sample leaves were stored and protected from any contamination.

2.a. Sample Preparation:

0.5 gram of each dried leaves were weighed and digested using 5 mL of concentrated nitric acid and 4 mL of distilled water on hot plate for 20 mins at around 100º C. Distilled water was added in minute quantity to avoid complete dryness and the prepared test solution was filtered using Whatmann filter paper in a 250 mL standard volumetric flask and diluted using distilled water.

2.b. Instrumentation:

Estimation of elements were performed on Microwave-Induced Plasma Atomic Emission Spectroscopy (Agilent 4210 MP-AES) and a calibration curve was constructed using series of standards of 0.05 ppm, 0.1 ppm and 0.5 ppm.

3. ISOLATION OF NATURAL COMPONENT FROM *Nyctanthes arbortristis* USING COLUMN CHROMATOGRAPHY

3.a. Extraction:

Around 5 grams of dried *Nyctanthes arbortristis* leaves were crushed using mortar and pestle and transferred to 100 mL round bottom flask. Extraction was done using 60 mL of 80% ethanol: water solvent mixture. Powdered leaves were extracted by refluxing at 70 ^{0}C in an oil bath for 1 hour. TLC spotting was done for extracted solvent using 3 different solvent system: 100% PET ether; 10% EtOAc: PET ether and 20% MeOH: $CHCl_3$. The 20% MeOH: $CHCl_3$ approach worked best for separating the components. The extracted solvent was concentrated and collected using 4-5 mL of $CHCl_3$ and TLC spotting was carried out with 100% $CHCl_3$, 50% PET ether: $CHCl_3$ and 5% MeOH: $CHCl_3$. Components were isolated well using 5% MeOH: $CHCl_3$ solvent mixture.

3.b. Isolation:

The residue obtained was adsorbed onto silica gel and loaded onto the column of silica gel (100-200 mesh size) prepared using 100 % $CHCl_3$. Elution was done using 100 % $CHCl_3$, 1% MeOH: $CHCl_3$, 2 % MeOH: $CHCl_3$, 4 % MeOH: $CHCl_3$ and 10 % MeOH: $CHCl_3$. Set of fractions acquired were reduced using rotatory evaporator and TLC spotting was done. The fraction was further elucidated using UV spectrometer and LC-MS. Experimental set up is shown in Fig. 1 below.

3.c. Instrumentation:

Isolated fraction was diluted using acetonitrile and around 10 µL of sample was injected into Shimadzu LC-MS. Shimadzu Shim-pack C18 column (150 mm × 2.0 mm, 5 µm) was used as stationary phase was used for separation. Mobile phase consisting of acetonitrile:

water (50:50) was pumped at a gradient flow rate of 1.0 mL/min for 20 mins. Column temperature was maintained at 30^0C.

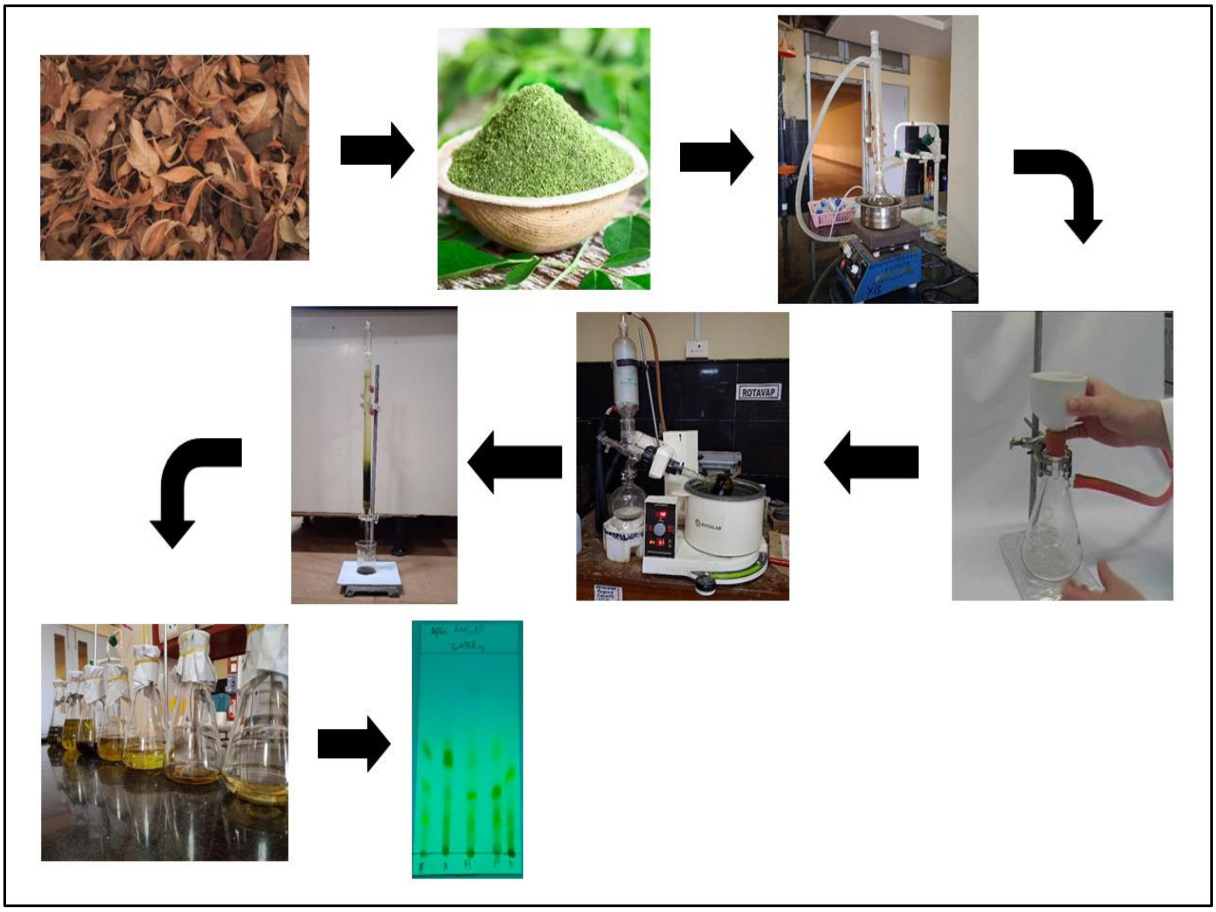


Fig. 1: Experimental images

V. RESULTS AND DISCUSSION

1. Metal Determination:

Metals analysed using MP-AES were Al, As, Ba, Cd, Co, Cr, Cu, K, Mn, Ni, Pb and Zn.

Fig 1.: Emission wavelength of analysed metals.

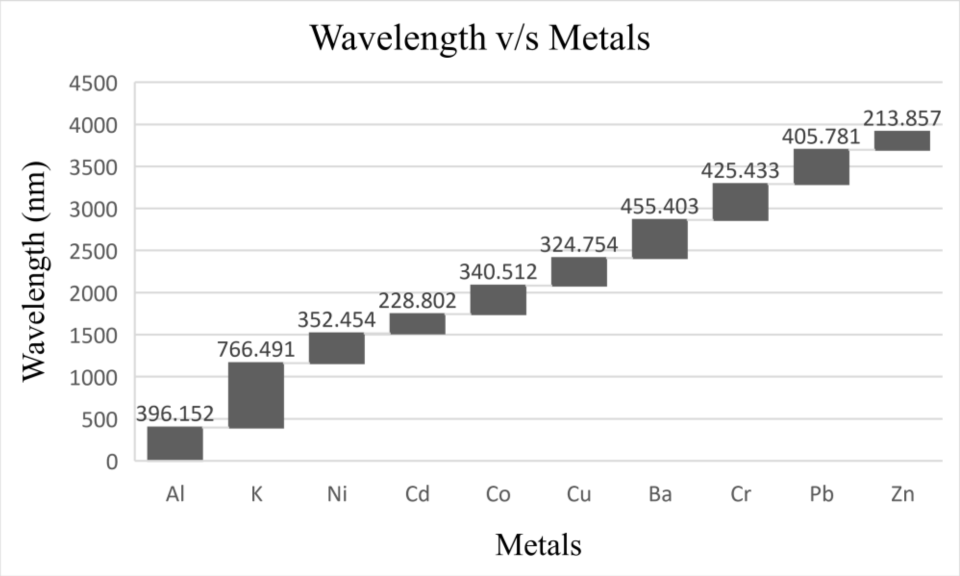
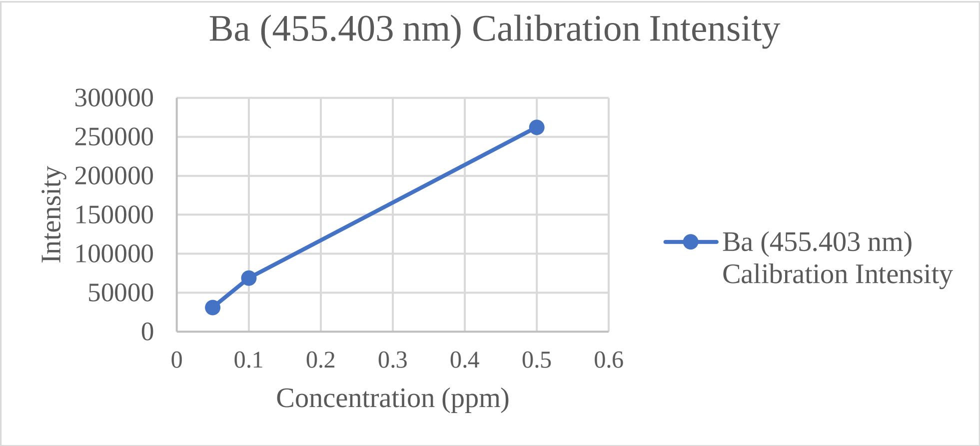
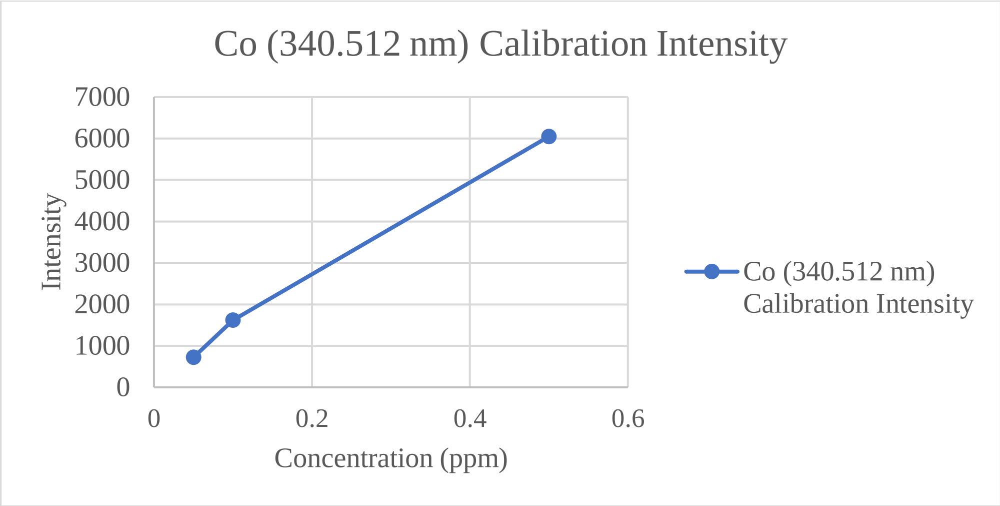


Fig 2: Calibration curve of some of the metals using standards of 0.05 ppm, 0.1 ppm and 0.5 ppm.

a.)

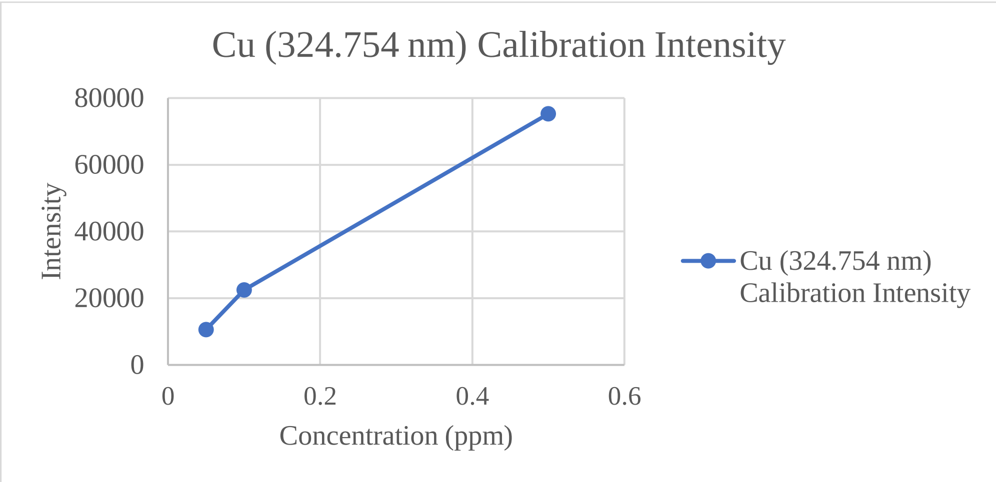


b.)



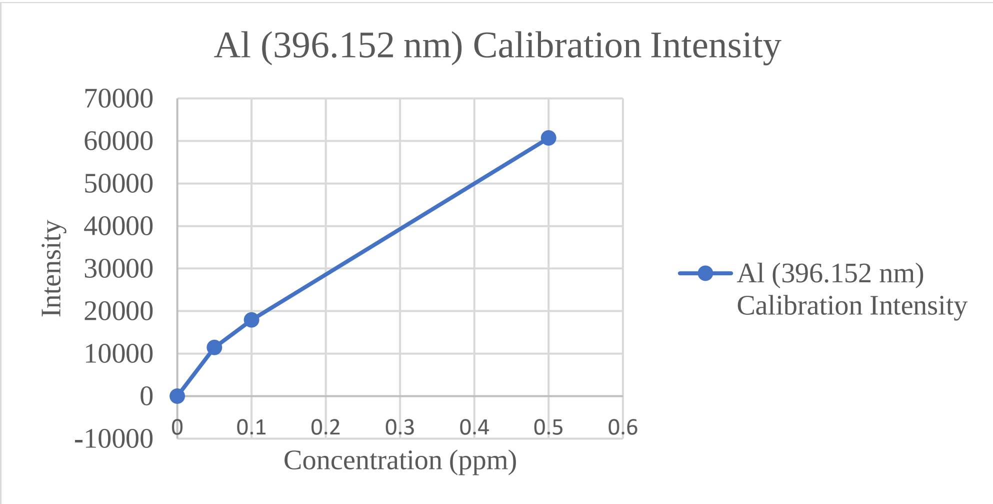
Co (340.512 nm) Calibration Intensity
7000
6000
5000
4000
3000
2000
1000
0
Intensity
0
0.2
0.4
0.6
Concentration (ppm)
Co (340.512 nm) Calibration Intensity

c.)



Cu (324.754 nm) Calibration Intensity
80000
60000
40000
20000
0
Intensity
0
0.2
0.4
0.6
Concentration (ppm)
Cu (324.754 nm) Calibration Intensity

d.)



Al (396.152 nm) Calibration Intensity
70000
60000
50000
40000
30000
20000
10000
0
-10000
Intensity
0
0.1
0.2
0.3
0.4
0.5
0.6
Concentration (ppm)
Al (396.152 nm) Calibration Intensity

Serial No.	Sample	Zn (ppm)	Ba (ppm)	Cu (ppm)	Ni (ppm)	Pb (ppm)	K (ppm)	Mn (ppm)	Cr (ppm)	Al (ppm)
1	*L.aspera*	0.15	0.04	0.09	0.0	0.04	4.26	0.14	0.01	0.10
2	*P.urinaria*	0.20	0.05	0.09	0.04	0.08	2.90	0.42	0.01	0.42
3	*C.dactylon*	0.16	0.05	0.05	0.04	0.03	2.59	0.10	0.01	0.27
4	*N.arbortristis*	0.33	0.07	0.04	0.03	0.08	1.82	0.21	0.0	0.12
5	*P.nigrum*	0.28	0.05	0.09	0.01	0.04	3.53	0.29	0.0	0.01

Table 1.: Metal concentration in plant samples at ppm level.

Regression (R^2) for all the metals was obtained as 0.99.

Estimation of metals such as arsenic (As), cobalt (Co) and cadmium (Cd) was also done. These metals showed negative results indicating their absence in the selected plant species and concentration of potassium (K) was found to be highest in all plant species. Concentration of nickel (Ni), copper (Cu), lead (Pb), barium (Ba) and chromium (Cr) were in minute quantity in all the medicinal plant species.

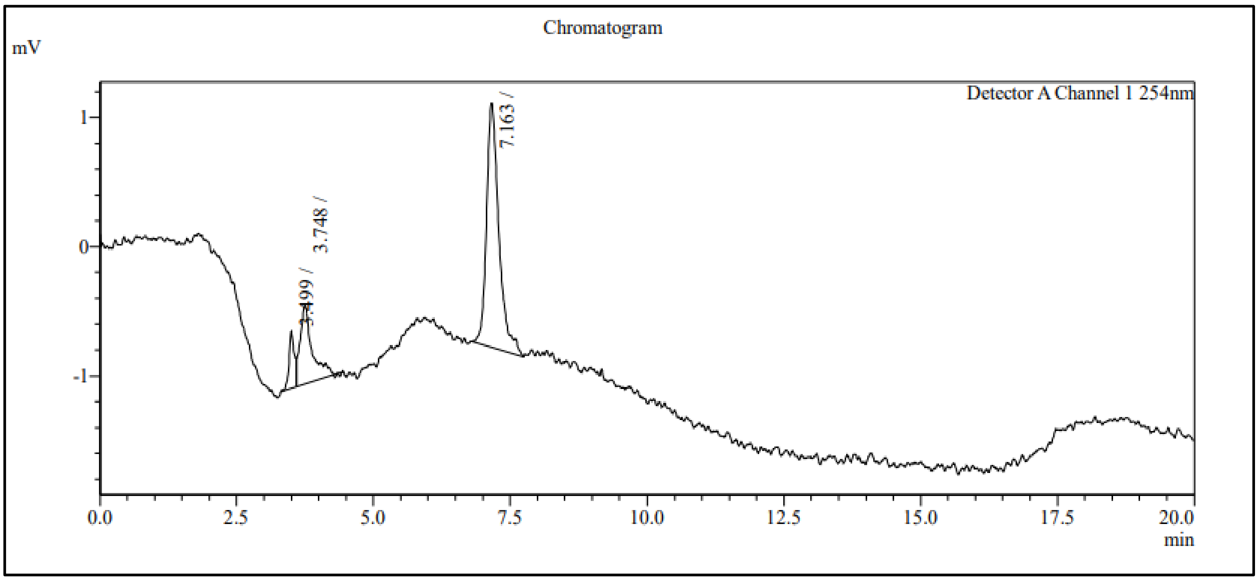
2. Isolation using Column Chromatography:

2.a. UV-Visible Spectrometry: -

UV results showed maximum absorption at 427 nm.

2.b. Liquid Chromatography: -

Three peaks were obtained from one of the fractions. Two peaks obtained at 3.499 min and 3.748 min is due to the blank which was confirmed by injecting HPLC grade acetonitrile. A sharp peak obtained at retention time 7.163 min is the isolated compound which could be slightly polar and hence eluting out early.



VI. CONCLUSION

Presence of some metals in human body can be both, a boon and a bane. When these elements are found in biological system they can be characterized as micro and macro nutrients. Some of the essential elements required to carry out the process in a living body are potassium (K) and sodium (Na). As the concentration exceeds the permissible limit of these vital minerals it can cause adverse effects to human health. Similarly, existence of heavy metals can also infect the human body.

Our studies based on estimation of metals in medicinal plants helps in determining the extent of usage of these plants. As these plants were collected from residential area, they contained less concentration of the harmful heavy metals and required concentration of the necessary metals making it possible of their intake as a fruitful therapeutic.

Isolation of natural component from medicinal plant helps in identifying the healing property of that particular isolated compound. The organic components in human body are evenly important as metals and best away to consume these medications are from natural habitat i.e., medicinal plants. In the above experiment of isolation of natural component, chromatogram obtained ensured segregation of one of the constituents. Further characterization of this separated content was not possible as a result of breakdown of Mass Spectrometer and NMR and necessity of advanced instruments such as flash chromatography and preparative HPLC.

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