DETERMINATION OF DIFFERENT ELEMENTS PRESENT IN MEDICINAL PLANTS USING MP-AES AND ISOLATION OF NATURAL PRODUCT FROM *Psidium guajava* LEAVES EXTRACT BY COLUMN CHROMATOGRAPHY

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "**Determination of different elements present in medicinal plants using MP-AES and isolation of natural product from *Psidium guajava* leaves extract by column chromatography**" is based on the results of investigations carried out by me in the Chemistry at the School Of Chemical Sciences, 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 report "**Determination of different elements present in medicinal plants using MP- AES and isolation of natural product from *Psidium guajava* leaves extract by column chromatography**" is a bonafide work carried out by **Miss Deepti Krishna Velip** under my supervision in partial fulfillment of the requirements for the award of the degree of **Master's in the Discipline Chemistry** at the School of Chemical Sciences, Goa University.

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Although theory is heard in many ways which seems to be simple and effortless but when it comes to real ground then it matters and competes to retrospect the system in each and every way to realize the real intention processes which leads to perfection.

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ABSTRACT

The curative property of medicinal plants is due to the presence different bioactive compounds present in them and also due to the presence of various elements present in them. Plants contains essential major, minor, trace elements as well as toxic elements in them therefor it is necessary to estimate the different elements present in them. In this study different elements present in five medicinal plants was carried out using Microwave Plasma-Atomic Emission Spectroscopy (MP-AES). Also isolation of one of the non-polar natural compound present in leaves extract of *Psidium guajava* was carried out using silica gel column chromatography. Characterization of isolated fraction was done by using UV-VIS spectrophotometer and liquid chromatography - mass spectrometer. The plants that are used for the analysis of elements are *Psidium guajava, Mimosa Pudica, Cintella asiatica, Cassia tora* and *Tithonia diversifolia.*

INDEX

CHAPTERS	CONTENTS	PAGE NO.
CHAPTER 1	INTRODUCTION	8-13
	LITERATURE REVIEW	14-22
	OBJECTIVE	23
CHAPTER 2	EXPERIMENTAL WORK	24-28
CHAPTER 3	RESULTS AND DISCUSSION	30-35
CHAPTER 4	CONCLUSION	37-39
CHAPTER 5	REFERENCES	41-44

CHAPTER 1

1.1 INTRODUCTION

Plants are being used for the medicinal purposes from pre-historic period. Medicinal plants play a very important role in the prevention of diseases as they are rich in therapeutic properties. A plant is considered as medicinal plant if any part of the plant is used for treatment of disease or used as precursor for the development of drug. Around 21,000 plants are estimated, that can be used for medicinal purposes. Medicinal plants contain many minerals, vitamins, phytochemicals, etc.[1] The curative property of the plants is due to the presence of different chemical constituents in them. Each part of the plant contains different chemical constituents and each part of the plant may be used for the medicinal purpose.[2]

Plants contain a wide variety of distinct chemical constituents in them and are considered as factories of natural chemicals that can be exploited, so that these natural chemicals can be used as economically important organic compounds in pharmaceuticals and pesticides industries.

Although the medicinal plants contains majority of the organic components with therapeutic benefits, but in the formation of active components, trace elements plays a vital role. It also contains some major inorganic elements such as macro and micro elements in them. In the building block and restoration phenomena of human health, trace elements, macro as well as micro elements are very essential.[3]

In ancient times mostly plants were being used for the treatment of various disease. Natural remedies like Ayurveda were created and refined over thousands of years as a result of the daily struggles of ancient people to combat disease. As a result, they have had a positive influence on the advancement of human civilization.

Due to rising health care expenses and widespread financial austerity, natural medicines are now not just the primary source of health care for the majority of people in underdeveloped nations, but they are also gaining more and more attention in affluent nations. Recently a lot of attention has been given for the production of natural medicines and products. From the past few years the use of traditional medicine has been increased significantly. According to the estimation of WHO about 80% of the worldwide population is dependent on the traditional medicine for their primary health care needs. People use to prefer traditional medicines because it is safe to use, cost effective and has least antagonistic effect.[4] More and more reports of the medicinal plants' antibacterial qualities have surfaced in recent years from all over the world. Currently, ayurveda and homeopathic medications and almost 30% or more of modern pharmaceutical treatments are obtained directly or indirectly from plants or their extracts. Both traditional herbalists and pharmacists make use of medicinal plants. Pharmacists uses the medicinal plants as the raw material in the synthesis of drugs. With the advancement in science, it is possible to isolate the different chemical constituents present in the medicinal plants, that can be used for the synthesis of drugs. In the recent years use of natural products in the synthetic drugs has been increased a lot. Of all the clinical drugs, more than 50% of the drugs contains natural product and their derivatives.[5]

Since medicinal plants contain both toxic and medicinal content it is necessary to identify and isolate the different chemicals constituents present in them. Medicinal plants contain metals which are necessary for the human life. Medicinal plants also contain some heavy metals which might be toxic to human consumption. Therefore it is necessary to investigate the different elements present in them.

Different metals present in the medicinal plants can be identified using different technique such atomic absorption spectroscopy, flame photometry, X-ray fluorescence, energy

dispersive X-ray fluorescence, laser induced breakdown spectroscopy (LIBS), atomic emission spectroscopy, particle induced X-ray emission (PIXE).

In this report different elements present in the medicinal plants are investigated by using atomic emission spectroscopy. Microwave plasma atomic emission spectrometer is used. It has advantages over other emission spectrometric technique is that, MP-AES offers better detection limits over a wider working analytical range than flame atomic spectroscopy. It is a multielement technique. The MP-AES is ideal as it uses nitrogen gas, that can be generated from air. Different medicinal plants that are being analyzed are *Psidium guajava, Tithonia diversifolia, Cintella asiatica, Mimosa pudica and Cassia tora.*

Psidium guajava

Psidium guajava is the one of the plant that is used for the medicinal purposes. The species belongs to the family Myrataceae of order myratales. Around the world the plant is mostly grown in the subtropical and tropical regions. In many regions of the world this plant is used as the traditional medicine. It is used for the treatment of diabetes, inflammation hypertension, wounds, pain relief and diarrhea. Fruit and leaves of the plant contain some pharmacological active compounds. It also contains antioxidants, antimicrobial, anti-allergic, antimicrobial, antiplasmodial, cytoxic properties. The plant contains different elements such as calcium, magnesium, potassium, phosphorous and iron. [6]

Mimosa pudica

Mimosa pudica is an annual or perennial medicinal plant belonging to the family mimosaceae. It possesses properties such as sedative, tonic, emetic, anti-asthmatic, aphrodisiac, analgesic and antidepressant properties. It used in the treatment of the alopecia, dysentery, diarrhea, insomnia urogenital infections and tumors. The plant contains different elements such as copper, manganese, calcium, magnesium, iron, potassium, zinc, nickel, lead, chromium, cobalt, arsenic and cadmium.

Centella asiatica

Centella asiatica is the medicinal herb that belongs to the family apiaceae. it is commonly called as Gotu kola or Brahmi which is mostly grown in the damp places, commonly found on the banks of river, ponds and in irrigated fields. It is used as culinary vegetable. Its leaves are good source of vitamins, minerals and fibers. It has antimicrobial property and it is used in the treatment of various disorders like it improves mental clarity and heals minor wounds,

skin diseases like leprocy and lupus. This species contains various elements like magnesium, sodium, zinc, iron, copper, nickel and lead.[7]

Cassia tora

Cassia tora is an edible, annual herb which mostly grows during the rainy season and flowers after monsoon. The species belongs to the family fabaceae. It mostly grows on the dry soil. The whole plant including roots, stem, leaves and seeds are used in traditional medicine in South Asia and India purpose. Its young leaves are used to make vegetable. Its roasted seeds can act as a substituent for coffee. Different elements present in it includes magnesium, sodium, zinc, iron, manganese and potassium. The plant species mostly used in the treatment of ringworm infection, ophthalmic, skin diseases like leprocy and psoriasis.[3]

Tithonia diversifolia

Thithonia diversifolia: commonly known as Mexican sunflower, belongs to family asteraceae. It is a subtropical plant. It bears yellow colour flower that are aromatic. It mostly used as a medicinal plant. Extract of its stem and leaves are taken orally as a treatment of malaria. Also used to treat diarrhea, diabetes, liver disease and stomach ache. It contains elements such as Fe, Mn, Ca and Na.[8]

This report also aim towards the isolation natural chemical component present in *Psidium guajava* leaves by silica gel column chromatography.

Although a lot of advancement has been occurred in science and technologies, isolation and purification of natural product from the plant extract is very challenging and tedious[9].For the separation and purification of desired natural compound from plant, it is necessary to extract the desired compound from plant. Therefore, the first stage that is extraction is very important.[10] Different extraction methods that can be used are maceration, percolation, Soxhlet extraction, ultrasound-assisted extraction and turbo-extraction, microwave assisted extraction. Further separation and purification of desired natural compound present in the leaves extract can be achieved using different separation techniques like thin layer chromatography, column chromatography, flash chromatography sephadex chromatography, counter current chromatography and HPLC. Characterization of isolated compound is achieved by using IR, LC-MS, NMR, etc.

1.2 LITERATURE REVIEW

Arya *et al*. in 2023, published paper entitled, "Preliminary phytochemical analysis of the extracts of *Psidium* leaves". The objective of this paper is to investigate the active phytochemicals present in the guvava leaves. Leaves extract was prepared by soaking 250 g of leaves powder in hydroalcoholic solvent for about 24 hours, followed by filtration and concentration of the extracted by evaporation at 40˚C. Phytochemical analysis was done by successively extracting the leaves powder in pet ether, chlorofom, ethanol water and hydroalcoholic solvent. Phytochemical analysis showed the presence of flavonoids, phenols, terpenes, saponins, carbohydrates, sterols, tannins. Ethanolic and hydroalcoholic extracts contains maximum amount of phyto-constituents.[11]

Kumar *et al*. in 2022, published paper entitled "Heavy metal analysis in root, shoot and leaf of *Psidium guajava* by using atomic absorption spectrophotometer". The present study focuses on estimation of heavy metals like Cd, Pb, Cr and Ni in different parts of *Psidium guajava* using AAS. Analysis shows the presence of heavy metals such as cadmium, nickel, chromium and lead. From the graph it can be concluded that the concentration of cadmium was found to be very low. The concentration of lead was found to be high in shoots than in roots and leaves. The concentration of metals found to be more in shoots than in roots and leaves.[12]

Christy *et al*. in 2022, published paper entitled "Antimicrobial activity, chemical compositions and proximate analysis of *Ixora Coccinea*". In this literature, different minerals present in the plant *Ixora coccinea* were investigated using the Oludro's (2012) method. Different elements present in *Ixora coccinea* analysed were potassium, calcium, magnesium, zinc, iron, copper, manganese and phosphorous. Among the metals estimated, the concentration of magnesium was highest.[13]

Lohoues *et al*. in 2022, published paper entitled, “Determination of trace elements and antioxidant compounds contained in the aqueous extract of leaves of *Centella asiatica* (Apiaceae)”. The objective of this report is to determine the different elements present in the plant *Centella asiatica* by using atomic absorption spectroscopy. In this paper antioxidant present in this plant is also estimated. Proposed method of International Institute of Tropical agriculture was used for the determination of trace element in the plant was used. Using atomic absorption spectroscopy, the elements contained in the sample solution was determined. Elements like sodium, iron, copper, magnesium, zinc, nickel, cadmium, lead was estimated. Zinc, magnesium, and iron is present in highest concentration. Whereas metals like copper, nickel, cadmium and lead contained in trace amounts.[14]

Omara *et al*. in 2022, published paper entitled, “Isolation and characterization of compounds in ethanolic extract of *Albizia coriaria* (Welw ex. Oliver) leaves: a further evidence of its ethnomedicinal diversity”.The objective of this paper is isolation and characterization of compounds in ethanolic extract of *Albizia coriaria* leaves. Characterization of most active ethanolic extract was done using UV-VIS spectrophotometer, FTIR spectrometer, thin layer chromatography, column homatography and by GC-MS. Leaf extract was prepared by maceration of 500 g of powdered sample in 1000 mL ethyl acetate at room temperature followed by filtration and concentrating on rotatory evaporator and further dilution in aratio of 1:10 in methanol. This extract was used for further analysis. UV-Vis spectra showed peaks at 338 nm, indicates the presence of alkaloids, peak at 663 nm indicates the presence of flavinoids. The FTIR spectrum shows the peaks at wavenumbers of 3365.73 cm−1, 2912.89 cm−1, 1659.42 cm−1, 1631.91 cm−1, 1370.12 cm−1, 1319.88 cm−1, 1065.87 cm−1 and 825.4 cm−1 are due to -OH stretch, C–H stretching, C=O bond, C=C bond, C–O, C–H, N=O and C–N stretch, respectively. The presence of O–H, C=O and aromatic–C=C indicated the presence of alcohols, carboxylic acids and aromatics in the extract. Fractions obtained from

column chromatography was analysed using GC-MS and found to be lupeol, lupenone, betulin and benzyl alcohol.[15]

Kumar *et al*. in 2021, published paper entitled, "Extraction of bioactive compounds from *Psidium guajava* leaves and its utilization in preparation of jellies." This paper mainly focuses on the extraction of bioactive compounds from the leaves of guavava plant and utilisation of this bioactive compound for the preparation of jellies. Guvava leaf extract was prepared by boiling 20 g of leaves powder in a 100 mL double deionisd water at 90˚C for 30 mins. After centrifugation of mixture at 4000 rpm for 10 mins. the supernatent was used for the analysis antioxidant and microbial activity, proximate and nutritional. Characterization of phytochemicals was done using Agilent 1100 LC-MS system. Hand refractrometer was used for the analysis of total soluble solid. phytochemical analysis revealed the presence of glycosides, flavonoids, phenols, terpenes.[16]

Dawurung *et al*. in 2021, published paper entitled, "Isolation of bioactive compounds from medicinal plants used in traditional medicine: Rautandiol B, a potential lead compound against *Plasmodium falciparum*". This paper mainly focuses on isolation and characterization of different bioactive components present in selected plants using chromatographic method. Sample preparation was done by extracting the leaves powder n DCM and ethanol. Using flash column chromatography the phytochemicals in ethanol extract was isolated. Gradient elution was done by increasing the polarity of solvent from MeOH/DCM (15:85) to 100% methanol. All together 12 fractions were obtained. Using column chromatography, fraction 2 was purified with 100% MeOH. The extracts obtained and their pure phytochemicals were evacuated using in vitro models for their inhibitory activities.[17]

Shah *et al*. in 2020, published paper entitled, "Assessment of some heavy metals in selected vegetables, fruits and their respective soil". In this literature presence of heavy metals were

determined in plant *Psidium guajava* and *Colocasia esculanta, Abelmoschus esculentus* using atomic emission spectroscopy. The part of the plant used for the analysis of heavy metal was fruit. The analysis shows heavy metals such was chromium, lead, magnesium, copper, iron and nickel. Of all the metal estimated in *Psidium guajava* plant the concentration of magnesium was found to be highest. As compared to other plants that are being analysed, *Psidium guajava* contains more amount of lead and iron and the concentration of magnesium is found to be lowest.[18]

Singh *et al*. in 2020, published paper entitled "Investigation on major, minor and trace elements in some medicinal plants using particle induced x-ray emission". The present report aims towards the estimation of major, minor and trace elements in plants, using Particle Induced X-ray Emission. In this paper the analysis was done in plants such as *Psidium guajava, Hibiscus rosa-sinensis, Annona reticulate*. Leaves of the plant *Psidium guajava* was analyzed for the elemental determination. Results obtained shows that the elements present in *Psidium Guajava* are phosphorous, sulphur, chlorine, potassium, calcium, manganese, iron, copper, zinc. Elements such as manganese, zinc and copper are present at trace level in *Psidium guajava*. Whereas it contains phosphorous, sulphur, chlorine and iron in minor concentrations and as measure components it contains potassium and calcium.[19]

Djama *et al*. in 2020, published paper entitled "Elemental contents of some medicinal plants using Energy Dispersive X-Ray Fluorescence (EDXRF)". About 16 elements were determined in the plant *Psidium guajava*. This report aimed towards the investigation of presence of different elements in guava plant using EDXRF technique. They also have estimated antimicrobial potency in this plant. The elements determined were copper, iron, zinc, calcium, titanium, vanadium, manganese, chromium, nickel, bromine, chlorine, cobalt, rubidium, strontium, lead. Among all the plants that were analysed, *Psidium guajava contains*

highest concentration of zinc, iron and copper and it also contains highest amount of lead among the plants analyzed which is a toxic metal.[20]

Olumide *et al*. in 2020, published paper entitled, "Evaluation of chemical and elemental constituents of *Centella asiatica* leaf meal". In this literature different elements present in the plant *Centella asiatica* was identified. The technique used was using Atomic absorption spectroscopy. Also Using standard procedures, presence of phytochemicals and proximate composition were determined. Elements like magnesium iron and calcium were determined by using Atomic absorption spectroscopy and for the determination of sodium atomic emission spectroscopy was used. The analysis revealed that *Centella asiatica* contains elements such as calcium, magnesium, iron, phosphorous and sodium. Since the plant contains about 24.38% of calcium, it can be considered as the good source of food supplement for the people who is deficient of calcium.[21]

Shaheena *et al*. in 2019, published paper entitled, "Extraction of bioactive compounds from *Psidium guajava* and their application in dentistry". This study mainly focuses on the extraction of bioactive compounds with antimicrobial, antioxidant, anticancer, and antitumor properties present in *Psidium guajava* using GC-MS. Extract was prepared by suspending leaf powder in ethyl acetate for 24 hrs. The filtrate obtained was analysed for determination of bioactive compound. The compounds were identified based on retention time and mass spectra.[22]

Afrin *et al*. in 2018, published paper entitled "Proximate and elemental analysis of three medicinal plants: *Cuscuta reflexa*, *Cassia tora* and *Cassia fistula* by using atomic absorption spectroscopy and flame photometry". In this literature different macro, micro and heavy metals elements present in the plant *Cassia tora* and other two plants were investigated. Technique used for the analysis is atomic absorption spectroscopy. The part of the plant

Cassia tora analysed was stem. Sample preparation was done using wet digestion method. Presence sodium and potassium was identified using flame photometry and other metals were estimated using absorption spectroscopy. It was found that *Cassia tora* plant is rich in sodium, potassium, calcium, magnesium and iron. Some trace elements such as copper, manganese, zinc, nickel, chromium cadmium, lead and arsenic was identified in very minute concentrations.[23]

Mohamed *et al*. in 2018, published paper entitled, "Determination of heavy elements content of some sudanese medicinal Cassia species using X-RAY Fluorescence Spectroscopy". The objective of this paper was to estimate different heavy metals present in selected plant species. Heavy metals such as titanium, chromium, manganese, iron, copper, zinc, lead strontium, rubidium and zirconium were found in Cassia tora plant. The concentration of heavy metals present in Cassia tora were less than that of other Cassia species that are been analysed.[24]

Islam *et al*. in 2018, published paper entitled, "Isolation of organic compounds from medicinal plant (*Mimusops elengi*) and their chemical analysis".This study aims towards the isolation of organic compounds from the *Mimuscops elengi* plant and also the chemical analysis of the compounds. For analysis of chemicals present in this plant the leaves were succesively extracted with pet ether (40-60), ethyl acetate and methanol. Isolation and chemical analysis revealed that, this plant contains aldehyde, ketones, carboxylic acid, phenols, alkaloid, tannins, carbohydrates and ester.[25]

Parvathay et al. in 2017, published paper entitled, "ICP-MS assisted heavy metal analysis, phytochemical, chemical proximate and antioxidant activities of Mimosa pudica L." In this report different elements present in the plant *Mimosa pudica* was identified by by using ICP-MS technique. This paper focuses on the evaluation of the presence of heavy metals and trace

elements in the plant *Mimosa pudica*. By using soxhlet extraction method at 40-80˚C crude plant extract was prepared using methanol. Perkin-Elmer SCIEX Wlan 9000 ICP-MS technique was used for the measurements of heavy metals after calibration and optimization. Different metals analysed are copper, potassium, calcium, potassium, magnesium, iron, manganese, zinc, nickel, lead, cadmium, chromium and arsenic.[26]

Roopa *et al*. in 2017, published paper entitled, "Comparative analysis of phytochemical constituents, free radical scavenging activity and GC-MS analysis of leaf and flower extract of *Tithonia diversifolia*". This report aims towards the estimation of concentration of secondary metabolite and evaluation of potential of antioxidant in the leaves and flower extrat of *Tithonia diversifolia* plant. Characterization of presence of phytochemicals was done using GC-MS. The analysis revealed that the leaf contains 16 bioactive compounds, whereas flower contains 13 bioactive compounds. Quantification of phytochemical shows that leaf extract contains phenols and flavonoids in highest concentration and flower extract contains alkaloids in highest concentration.[27]

Jahan *et al*. in 2015, published paper entitled "Elemental and fatty acid content of four medicinal plants: *Kaiempferia rotunda, Cuscuta reflexa, Centella asiatica* and *Asparagus racemosus*". In this literature, using the flame photometry and atomic absorption spectroscopy technique elemental analysis was done on the plant *Centella asiatica* and other three medicinal plants. Beside the elemental determination, fatty acid content present in these medicinal plants were also investigated. Ash content was determined by using Furnace PLC/MBC/W1/32 by using Premnath *et al*. method. The collected ash was then subjected for the determination of elements present using atomic absorption spectrometry and flame photometry. The literature shows that the plant contains elements such as sodium, potassium, iron, copper, cadmium and chromium. It was observed that among the plants analysed *Centella asiatica* contains the highest percentage of sodium, magnesium, manganese.

Chromium and cadmium were found in the lowest concentration in the plant *Centella asiatica*.[28]

Dhiman *et al.* in 2011, published paper entitled "*Metal analysis in citrus sinensis fruit peel and psidium guajava leaf*". This report aims towards the analysis of heavy metals in *Citrus Sinensis* fruit peel *and Psidium guajava* leaf and determination of accumulation level of heavy metal and mineral ion in medicinal plant species. Sample preparation was done using wet digestion method and AAS was used for metal analysis. From the analysis it was found that *Psidium guajava* contains metals such as copper, zinc, cadmium, magnesium and calcium, which are in acceptable limit of human consumption. Also, heavy metals like selenium, arsenic and mercury were estimated in very minute concentrations. For *Psidium guajava* among the metals that are been estimated magnesium was found in highest concentration.[29]

Rai *et al.* in 2007, published paper entitled "*Role of LIBS in elemental analysis of Psidium guajava responsible for glycemic potentia*l". The objective of this report is to detect the elements responsible for glycemic potential of ripe and unripe fruit peel of *Psidium guajava* leaves aqueous extract. For elemental determination, Laser Induced Breakdown Spectroscopy has been used. Different elements that were analyzed are magnesium, potassium, sodium. In unripe fruit it was found that the concentration of magnesium was higher than in ripe fruit. But in ripe fruit concentration of potassium was found to be more. Aqueous extract of unripe fruit shows a fall in glucose level.[30]

1.3 OBJECTIVE

- The objective of this study is to investigate different elements present in medicinal plants using Atomic Emission Spectroscopy (AES) .
- To isolate the natural compound present in leaves extract using column chromatography.
- To characterize theisolated compound using UV-VIS spectrophotometer, LC-MS, NMR .

CHAPTER 2

EXPERIMENTAL WORK

2.1 Materials and Methods

All the reagents and chemicals used such as chloroform, pet ether, methanol, ethyl acetate, nitric acid is of analytical grade. Sample preparation for LC-MS was done using HPLC grade acetonitrile. All sample solutions for AES were made using deionised water.

2.2 Plants sample collection

Five distinct plants having medicinal benefits were gathered. (*Psidium guajava, Mimosa pudica, Cassia torra, Cintella asiatica, Tithonia diversifodia)* The plant's leaves are the component that is analysed. The plant leaves were properly washed with tap water and allowed to sun dry for 7 days. Each plant's leaves were finely ground individually in a mechanical grinder and stored in an air tight plastic container.

2.3 Elemental determination in medicinal plants using MP-AES

2.3.1 Sample preparation

0.5 g powdered sample was taken in a 100 mL beaker to that 4 mL of distilled water was added followed by addition of 5 mL of conc. nitric acid. This solution was then heated on hot plate for 20 mins at 100˚C. the solution was then cooled and filtered using Whatman filter paper in a 250 mL standard volumetric flask. The solution was then made upto the mark with distilled water. Blank solution was prepared by taking 5 mL of distilled water in 250 mL volumetric flask and made upto the mark with distilled water. Elemental composition in the digested sample were analysed in triplicates using MP-AES.

Series of standard solutions of 0.05 ppm, 0.10 ppm and 0.50 ppm were prepared for each element to plot a standard calibration curve. Each experimental sample curve was then approximated using this standard calibration curve.

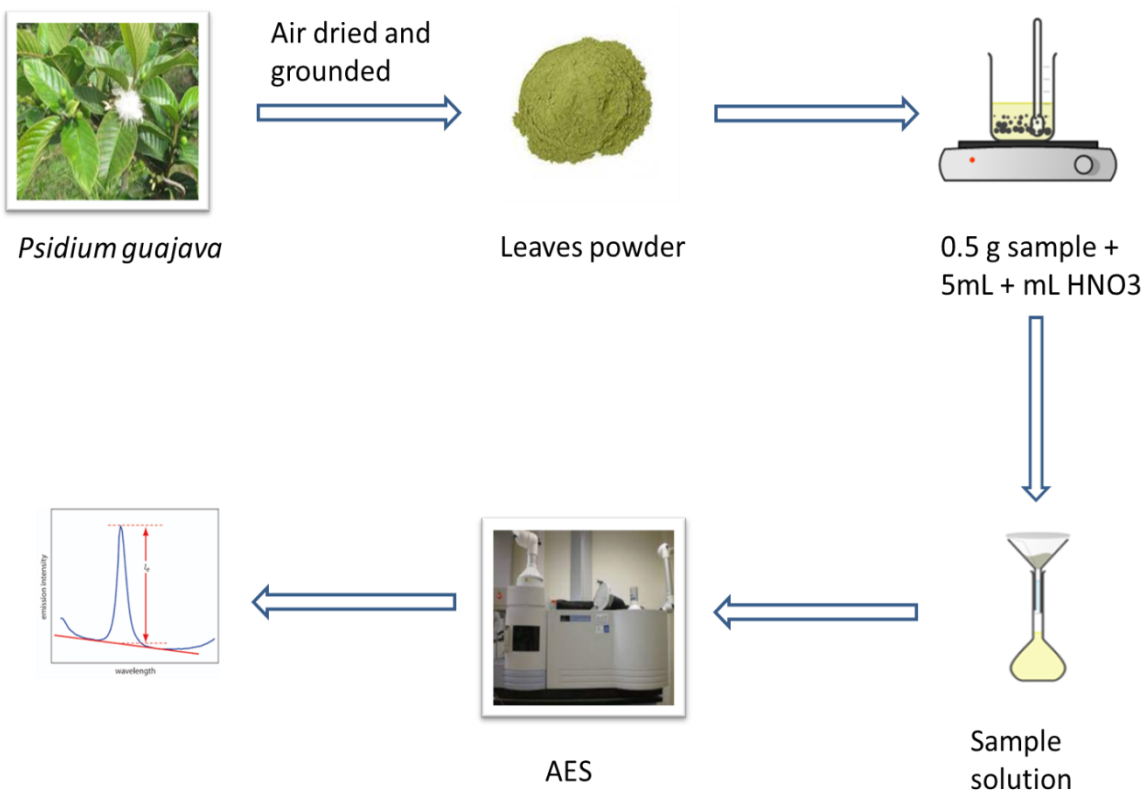


Fig 2.3 Schematic diagram for elemental analysis procedure

2.4 Isolation of natural compound present in *Psidium guajava* plant leaves

2.4.1 Preparation of guava leaves extract

Accurately weighed 5 g of powdered sample was taken in a clean 100 mL round bottom flask containing 60 mL of 80% ethanol and water mixture. The mixture was then refluxed for 1 hour in an oil bath maintained at 70 ˚C. After cooling the flask at room temperature, the extract was filtered using Buckner's funnel under vacuum. The filtrate collected was then concentrated using rotatory evaporator.

2.4.2 Isolation of natural product by column chromatography

In a 5 ml of chloroform, the resulting dried extract was dissolved. A small amount of the solution, carefully spotted using a capillary to the TLC plates. Assorted ratios of prepared solvent systems (100% pet ether, 10 % pet ether/ethyl acetate, 20 % methanol/chloroform, 100% chloroform, and 50% chloroform/pet ether) were used to develop the plates. After the development of plates, the visualisation of spot done using UV chamber. The solvent in which the best separation was seen, was used as a mobile phase for column chromatography.

In order to prepare the sample for column chromatography, a little amount of silica was added to the extract that had been dissolved in chloroform. A free flowing powdered sample was obtained by heating this in a steam bath. The sample was treated to silica gel normal phase column chromatography using the optimised solvent ratios. To prepare a column, silica (100-200 mesh) was mixed with 10 % chloroform /pet ether solvent system to get the homogenous slurry. The slurry was poured into the column with continuously tapping the column to prevent formation of air bubbles. The sample introduced onto the column and eluted first with solvent established through TLC profiling. The elution was gradient elution from 10 % chloroform/pet ether to 20% methanol/chloroform. Different fractions were

collected and then concentrated on rotatory evaporator. TLC plates were developed for each fraction and visualized under UV chamber.

2.5 Characterization

2.5.1 Liquid chromatography – mass spectrometry analysis

Fraction 1 obtained was dissolved in acetonitrile, filtered through filter and transferred to 2 mL vials for LC-MS analysis. Shimadzu LC-MS was used with Lab Solutions as Software. Shim-pack GIST C-18 column was used, mobile phase used was acetonitrile/water. Elution was gradient with the flow rate 0.5 mL/min. Detection was done at 254 nm with UV detector.

2.5.2 Ultraviolet – visible spectrometry scanning

After concentrating the fractions obtained, solid formed was dissolved in 5 mL chloroform, scanned from 200-800 nm on Shimadzu ultraviolet spectrophotometer using chloroform as a blank.

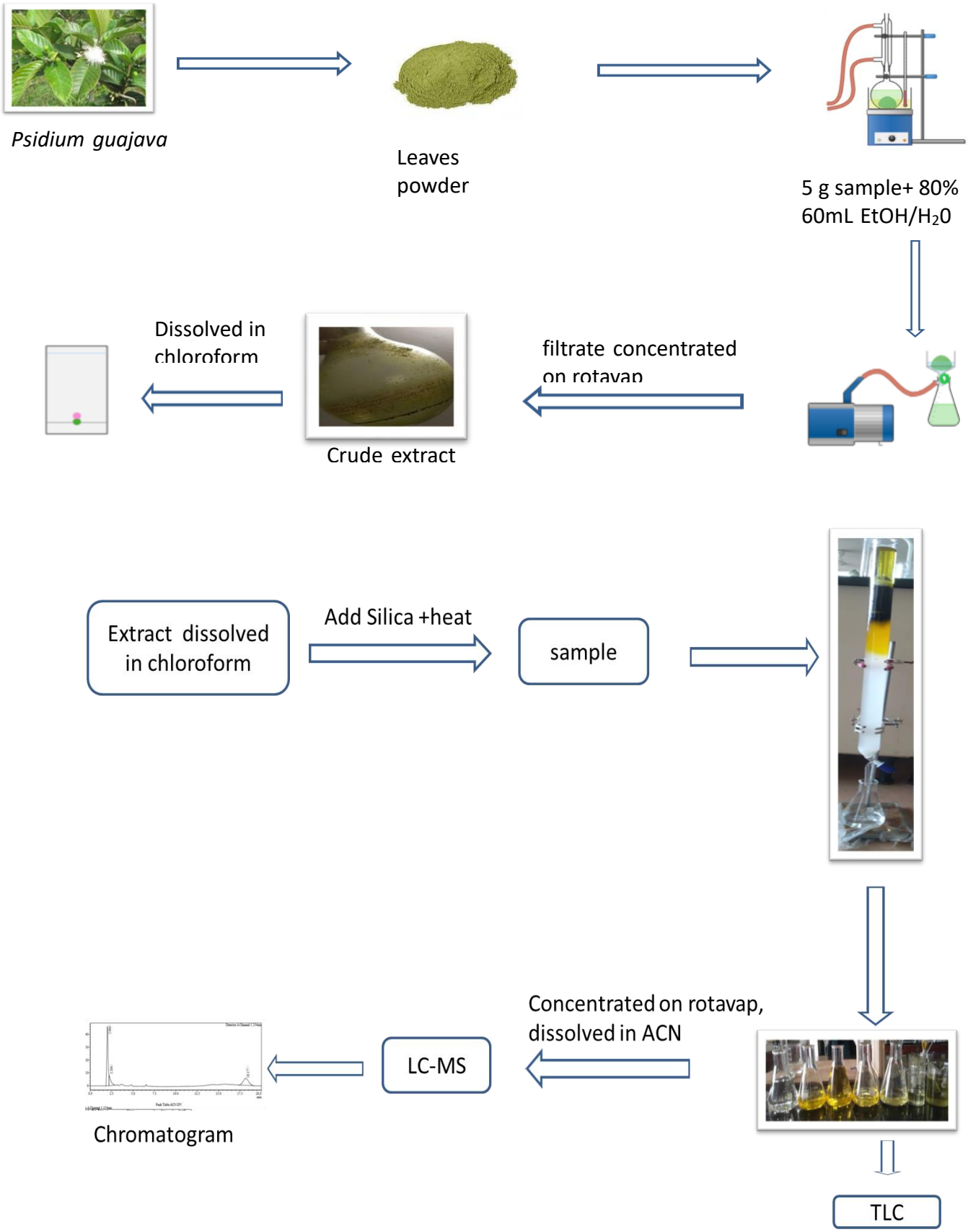


Fig. 2.4 Schematic diagram for isolation process

CHAPTER 3

Results And Discussion

3.1 Elemental analysis

Using AES elemental analysis of Zn, Cd, Ba, Cu, Ni, Co, Pb, K, Mn, Cr, Al, As in the medicinal plants was done. Elemental composition of plant *Psidium guajava*, *Mimosa pudica Cassia torra*, *Cintella asiatica*, *Tithonia diversifolia* are summarized in Table. (3.1.c).

Elements	Wavelength in nm	Elements	Wavelength in nm
Zn	213.857	Pb	405.781
Cd	228.802	K	766.491
Ba	455.403	Mn	403.076
Cu	324.754	Cr	425.433
Ni	352.454	Al	396.152
Co	340.512	As	193.695

Table. 3.1a. Instrumental parameters for MP-AES

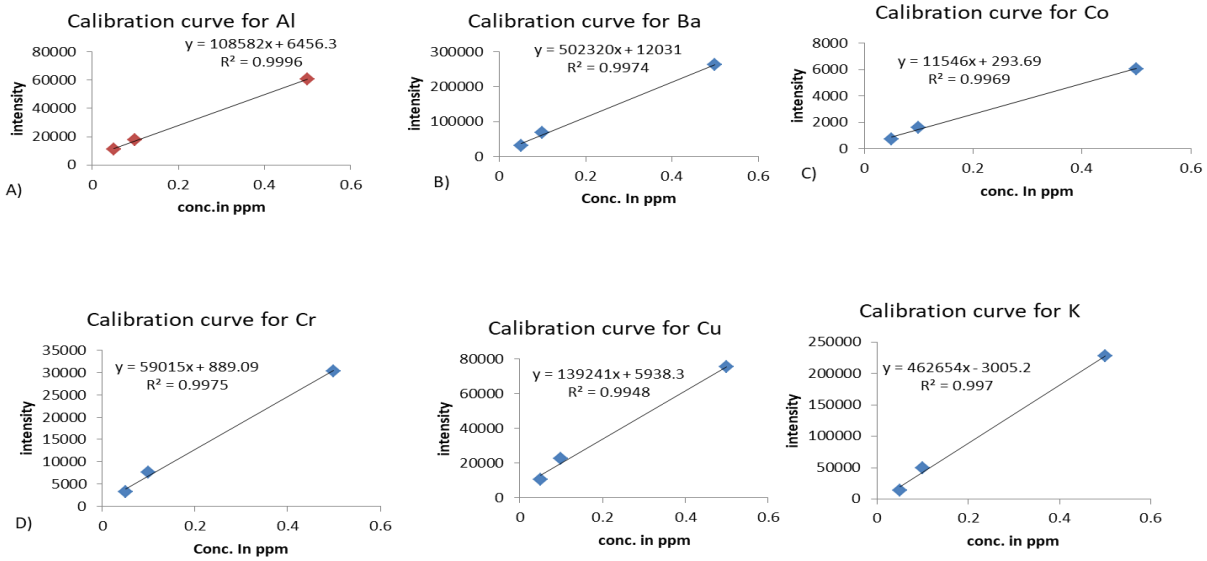


Fig. 3.1 b. calibration curve for metals

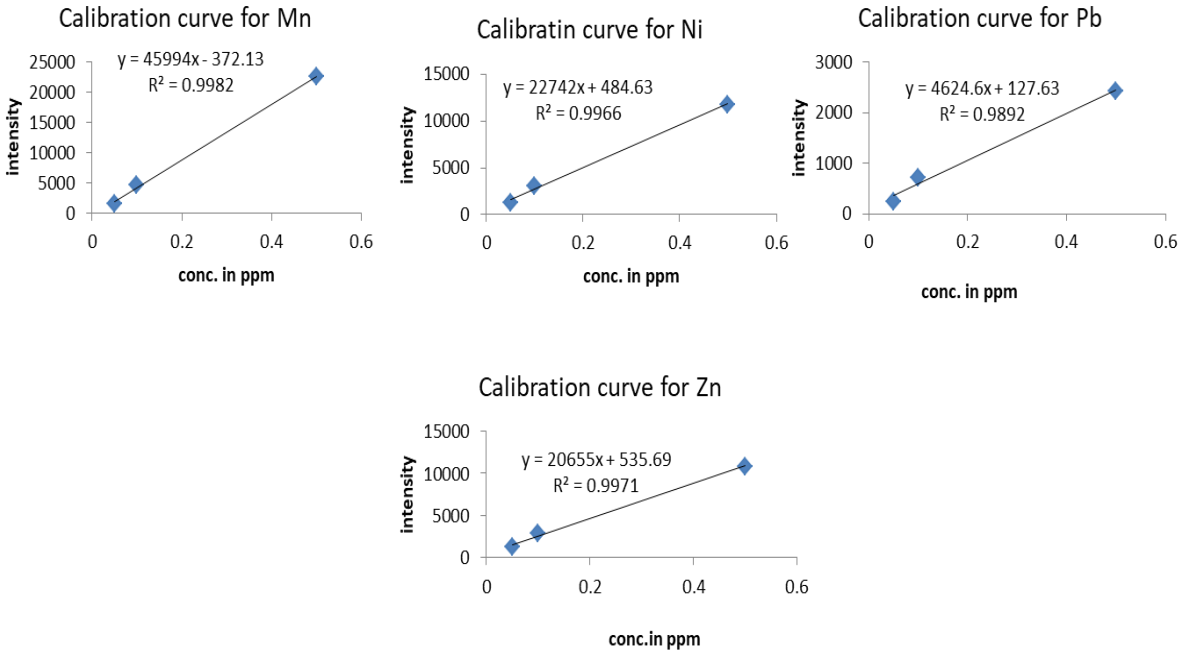
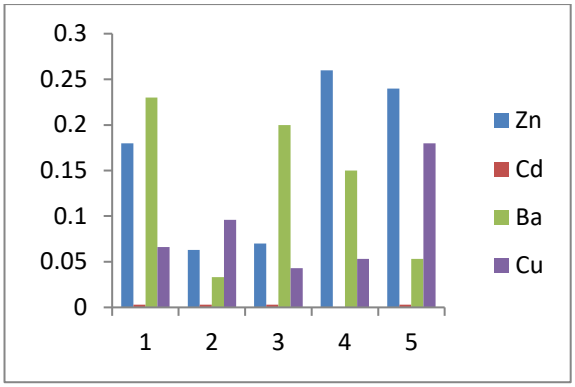


Fig.3.1.b.Calibration curve for metals

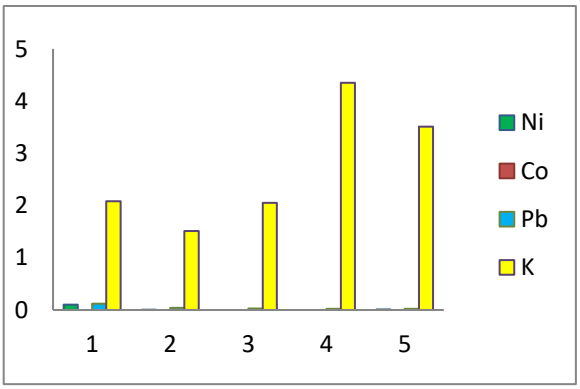
Sample code	Zn (ppm)	Ba (ppm)	Cu (ppm)	Ni (ppm)	Pb (ppm)	K (ppm)	Mn (ppm)	Cr (ppm)	Al (ppm)
1	0.18	0.23	0.066	0.1	0.12	2.08	0.12	0.01	1.47
2	0.063	0.033	0.096	0.003	0.043	1.51	0.42	0.04	0.02
3	0.07	0.2	0.043	0	0.03	2.05	0.18	0.01	0
4	0.26	0.15	0.053	0	0.023	4.35	1.74	0.01	0.213
5	0.24	0.053	0.18	0.01	0.023	3.51	0.25	0.06	0.043

Table:3.1.c. Elemental composition in medicinal plants. 1-*Psidium guajava*, 2- *Mimosa pudica*, 3-*Cintella asiatica*, 4- *Cassia tora*, T-*Tithonia diversifolia*

A)



B)



C)

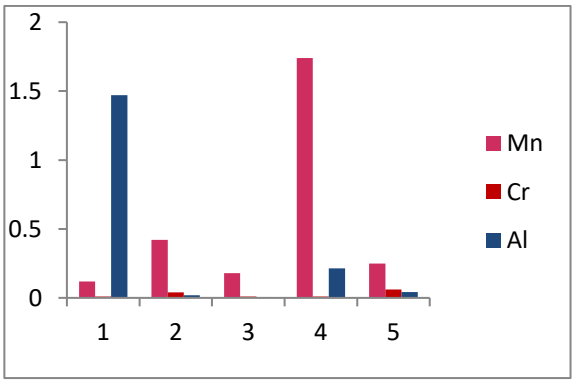


Fig 3.1.d A), B), C) shows the conc. of elements present in each plant in ppm per 0.5 g of sample

Zinc: Among these five plants, plant that contains highest amount of zinc was found to be *Cintella asiatica* (0.26 ppm) followed by *Tithonia diversifolia*, *Psidium guajava*, *Cassia tora* and *Mimosa pudica* plant.

Barium: *Psidium guajava*, *cassia tora* and *Cintella asiatica* contains highest amount of Barium whereas other plants contains Barium between 0.03- 0.05pm.

Copper: All the plants that are being analysed contains copper in the range of 0.04 ppm - 0.19 ppm.

Nickel: Nickel is found in plant *Psidium guajava* and *Tithonia diversifoia***.**

Lead: *Psidium guajava* contains lead in highest concentration that is 0.12 ppm among all other plants.

Potassium: potassium found to be in greatest among all the detected elements in all five plants. *Cintella asiatica* and *Tithonia diversifolia* contains potassium in largest quantity.

Manganese: all the plants contain manganese in considerable amount that is between 0.1 ppm - 1.8 ppm.

Chromium: trace amount of chromium content is present in the plants.

Aluminium: *Psidium guajava* contains Aluminium in large amount as compare to other plants, whereas Cintella asiatica does not contains aluminium at all.

The concentration of cobalt, and arsenic was found to nil indicating absence of these elements in the medicinal plants. Cadmium was found in very trace amount which is almost negligible.

3.2 Isolation of natural product from *Psidium guajava* leaves

3.2.1 Selection of best solvent system for elution.

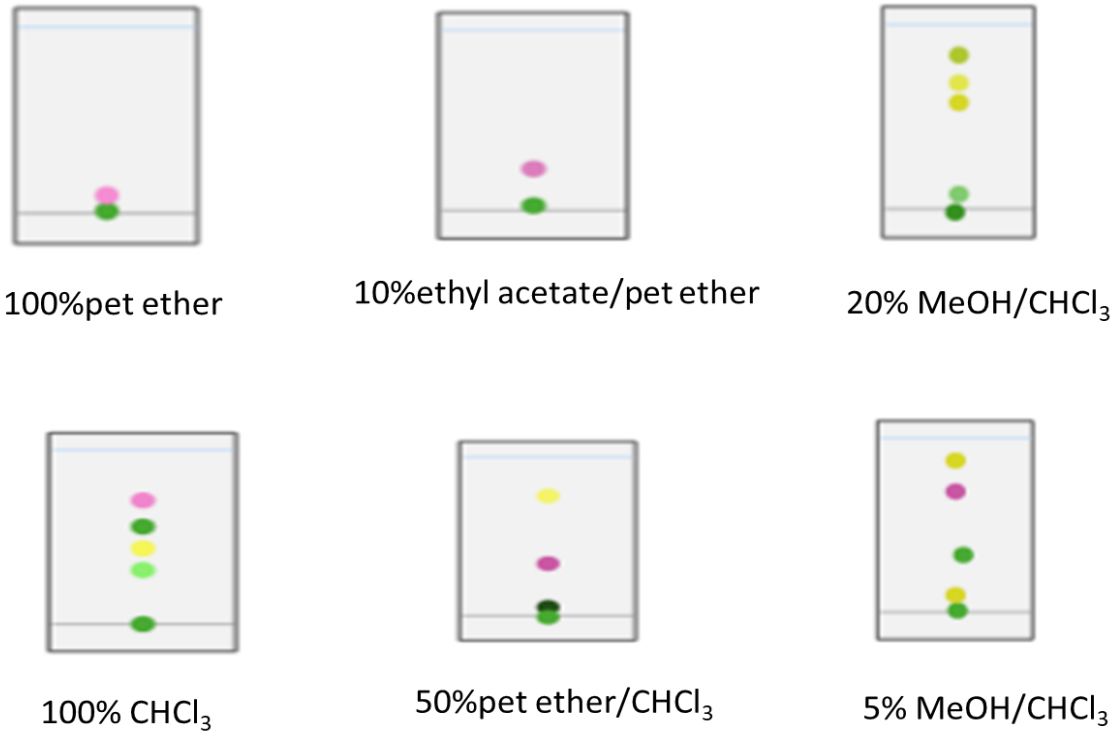


Fig 3.2.1. a) TLC plates of leaves extract in different solvent system.

50% pet ether/ $CHCl_3$ was selected as mobile phase for elution, since this solvent system shows better separation.

3.2.2 UV-Vis spectrum of the isolated fraction

The maximum absorption was recorded at wavelengths of 738 nm and 661 nm.

3.2.3 LC-MS analysis of isolated fraction

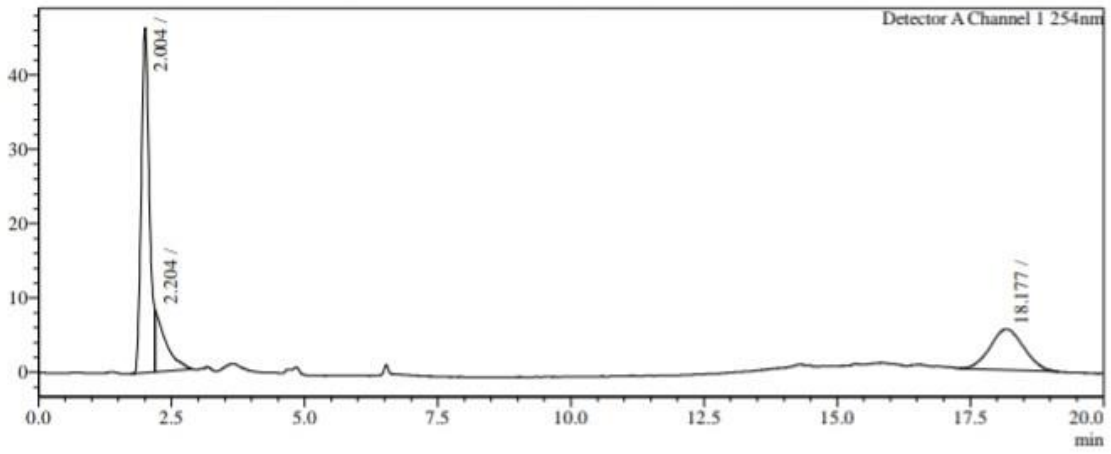


Fig 3.2.3.a. chromatogram of fraction 1

In LC chromatogram (fig 3.2.3.a) for isolated fraction shows a peak at a retention time of 18.17 mins. this indicates that the compound present in this fraction is non-polar since the compound has more affinity towards the stationary phase it elutes out slowly. Broad peak is obtained due to improper method development. A peak at retention time of 2.004 mins. is also observed which is due to blank.

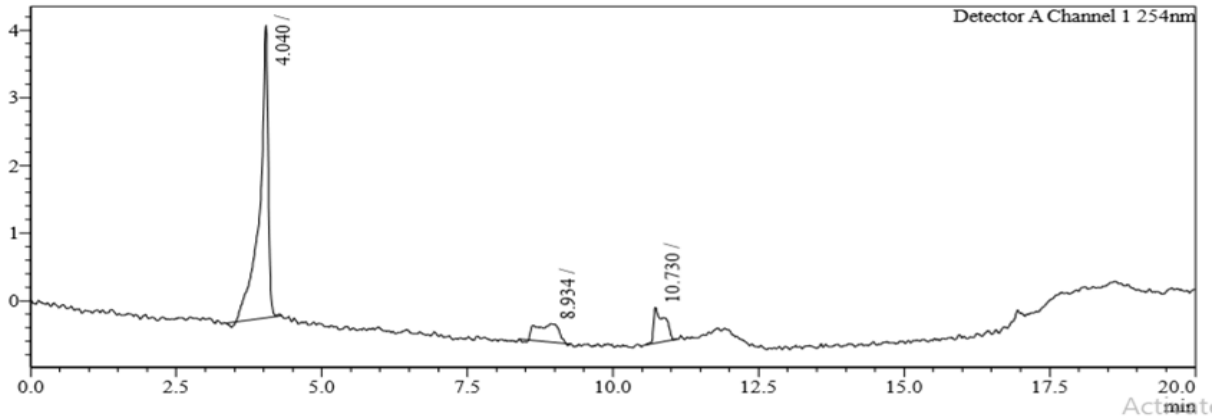


Fig.3.2.3.b Chromatogram of fraction 3

LC Chromatogram of fraction 5 (fig 3.2.3.b) shows 3 peaks at retention time of 4.0 mins, 8.9 mins and 10.7 mins. respectively. A sharp peak is obtained at the retention time of 4.0 mins.

CHAPTER 4

CONCLUSION

4.1 Elemental analysis

The elemental determination in medicinal plants, *Psidium guajava, Mimosa pudica Cassia tora, Centella asiatica, Tithonia diversifolia* shows that, these plants contain major, trace elements and ultra trace elements. These elements are very essential for our body for the proper functioning. Different medicinal plant contains different macro, micro and trace elements in different concentrations. The analysis shows the presence of major elements like potassium. among all the elements estimated, potassium found to be in highest concentration. Essential trace elements like Cu, Zn, Cr, Mn are also found in these plants. These elements are required for metabolic, oxygen transport, red blood cell enzymatic function and cellular immune support. These medicinal plants contain non-essential elements like Ba and Al as well toxic elements such as lead. Uptake of these non-essential metals is harmful to the human life. *Centella asiatica* and *Cassia tora* are considered are consumed as vegetable. Among these five plants that are being analysed *Centella asiatica* contains most of the essential trace elements and major elements in highest amount. Since mineral elements cannot synthesise by living organism, they need to be acquired by food intake. The people who are deficient of these essential elements, *Centella asiatica* and *Cassia tora* are rich in these minerals therefore one can consume it.

4.2 Isolation of natural product by column chromatography

From the LC-MS chromatogram (Fig.3.2..3a.) it is concluded that the fraction 1 obtained through column chromatography contains a single non - polar compound. Further characterization of this isolated compound could not be done due to breakdown of LC-MS and NMR instruments.

Since LC chromatogram of fraction 5 (Fig.3.2.3.a.). shows a sharp peak at retention time of 4.0 mins along with two small peaks at retention time of 8.9 mins and 2.7 mins, indicates that the fraction 5 contains impure compound. Hence proper separation and purification of desired natural compound from mixture using column chromatography was found to be difficult. Proper separation and purification of desired natural products can be achieved using either flash chromatography or counter current chromatography.

CHAPTER 5

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