Identification and HPLC Quantification of an Antidiabetic Cyclitol Molecule in Selected Plant Extracts and its Isolation by an Enzyme Mediated Method

Sreeshma K.S.
13 PCH014

Thesis submitted to
Avinashilingam Institute for Home Science and Higher Education for Women, University
Coimbatore 641043

In partial fulfilment of the requirement for the
Master's degree in Chemistry
March 2015

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28.3.15
Signature of the Supervisor

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Signature of the Head of the Department

ACKNOWLEDGEMENT

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I owe my gratitude to all those who rendered their help for the completion of my work in the form of physical help and mental strength.

Every work succeeds by the blessings of **Lord Almighty**. Hence, I bow my head at the feet of the Lord Almighty for His blessings rendered with good health and clear mind throughout my work.

I extend my humble thanks to **Dr. (Thiru) T.S.K. Meenakshi Sundaram M.A.,M.Phil., Chancellor,** Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore, for permitting me to carry out my work in this eminent institution.

With a sense of deep reverence and respect I extend my thanks to **Dr. (Tmt.) Sheela Ramachandran M.Sc.,P.G.Dip.,Ph.D. (Avinashilingam) Vice Chancellor**, Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore for providing me all the facilities to carry out my work.

I owe my gratitude to **Dr. (Tmt.) Venmathi,M.Phil.,Ph.D. Registrar In-charge,** Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore, for providing me all the support to carry out my work successfully

A special note of heartfelt thanks to **Dr. (Mrs.) Saroja Prabhakaran, M.A.,Dip.Ed. (Madras), Ph.D (Mother Teresa), The Director, Hall of Residence,** Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore, for all the amenties rendered and for the support and the encouragement.

I extend a special thanks to **Dr. (Mrs.) Parvathi, M.Sc.,Dip.H.Ed., M.Phil., Ph.D., Dean., Faculty of Science,** Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore, for her kind support and facilities rendered during my work.

With great pleasure and respect I would like to extend my gratitude to **Dr.(Mrs.)R.Shymala,MSc.,Dip.Ed(Madras)M.Phil.,(Bharatiar).,Ph.D., (Avinashilingam) Professor and Head, Department of Chemistry**, Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore for her kind support, encouragement and for providing the laboratory facilities to do my work.

With deep sense of gratitude and respect I humbly extend my heartfelt thanks to my guide **Dr.(Mrs.)Shubashini K.Sripathi, M Sc.,Ph.D., Professor, Department of Chemistry**, Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore,for her care, innovative ideas, guidance, encouragement, patience, motivation throughout my thesis.

I owe my respectful thanks to **all the staff members of Deparment of Chemistry**, Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore for their care and support rendered.

I express a special word of thanks to **Mrs.Anitha, Advanced Research Laboratory** and to **Mrs.Rajeswari, Assisstant Professor, Department of Biotechnology** for their support during HPLC analysis.

I extend my thanks to **all the research scholars of Department of Chemistry** for their help and encouragement rendered during my work.

I express my deep sense of gratitude to all my **friends** for their consistent support and inspiration.

At length with deep respect and honour my gratitude highlights on **my adorable parents, to my beloved sisters and all my family members** without whom there is no glossary to my glory.

SREESHMA K S

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List of Abbreviation

HPLC	High Performance Liquid Chromatography
R_f	Retention factor
UV	UltraViolet
TLC	Thin layer Chromatography
HCl	Hydrochloric Acid
NaOH	Sodium Hydroxide
H_2SO_4	Sulphuric Acid
ppm	parts per million
RID	Refractive Index Detector

INTRODUCTION

1. INTRODUCTION

India is rich with a wide variety of medicinal plants and from the days of our ancestors plants have been used for the treatment of various ailments. Plants are an immediate source of medicines. Plants produce and contain a variety of chemical substances that act upon the body. Herbal medicines, referred to as herbalism or botanical medicine is the use of herbals for their therapeutic or medicinal value. It is the oldest form of healthcare known to mankind. Many drugs commonly used today are of herbal origin.

The medicinal qualities of plants are due to the presence of compounds that have certain chemical composition. The study of such chemical compounds in plants is a special field of chemistry and is known as phytochemistry. Phytochemistry deals with the study of the nature and properties and also isolation of such compounds. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structure of these substances. Phytochemicals are naturally occurring, biologically active chemical compounds in plants. Phytochemicals are protective and disease preventing.

One of the important stages of phytochemical investigation is the isolation of phytochemicals. Normally, isolation of phytochemicals is done by chromatographic techniques. These methods are tedious and time consuming. Hence it will be advantageous if a naturally occurring compound could be isolated by a simple method.

Cyclitols are a class of naturally occurring molecules which have good medicinal value. Pinitol is one of such cyclitol molecule which is an antidiabetic molecule. D-(+)-Pinitol, a natural product of the group of cyclitols. These compounds can also be isolated after treating the plant extract with protease enzyme, papain. This reduces the protein in the plant extract and thus makes the isolation much easier.

1.1 Papain enzyme

Papain, also known as **papaya proteinase I**, is a cysteine protease enzyme present in papaya (*Carica papaya*) and mountain papaya. Papain is one of the proteolytic enzymes, belonging to the hydrolase class and cysteine protease subclass (the hydrolysis of proteins, peptides, amides and complex ethers). (***Filatova.et.al)***

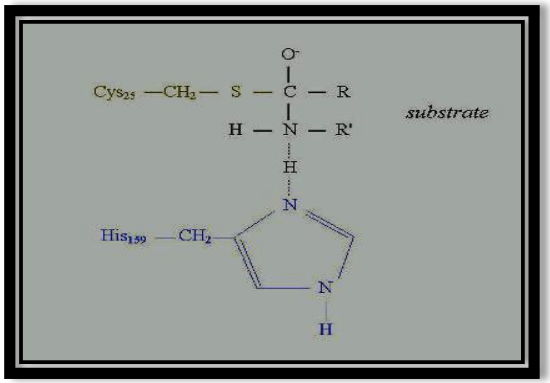

Fig.1

Papain enzyme is widely used for medical uses especially as an anti inflammatory agent and has been used for treating pain and swelling. It has also been used for the treatment for the ailments like hay fever, stomach pain also for the inflammation of throat and pharynx and for the treatment of tumours. Papain finds use in cosmetics, toothpaste, enzymatic soft contact lens cleaners, meat tenderizers, and meat products.

In the present study, thirteen antidiabetic medicinal plants were selected to identify the presence of the insulinomimetric molecule pinitol in their plant extracts. Also a simple method of isolation this molecule using papain enzyme has been attempted. The pinitol extracts has been quantified by an optimised HPLC method.

Table 1 below lists the names of the medicinal plants chosen for the study

S no	Scientific Name	Common Name	Tamil Name	Part Used
1	*Abelmoschus esculentus*	Lady's finger	Vendai	Leaves and fruit
2	*Bougainvillea glabra*	Paper flower	Paper poo	Leaves and flower
3	*Cicer arietinum*	Chickpea	Pottukadali	Seed
4	*Citrus sinesis*	Orange	Orange	Peel
5	*Magnifera indica*	Mango	Magnkai	Leaves
6	*Mirabilis jabela*	4 'O' clock plant	Nalu mani	Leaves and flowers
7	*Musa paradisica*	Plantain	Vazlai	Flower
8	*Nephelium lappaceum*	Rambutan	Rambutan	Peel
9	*Psidium guajava*	Guava (ripened and unripened)	Koiya	Peel
10	*Pithecellobium dulce*	Kodukapuli	Konapuliyanga	Peel
11	*Punica granatum*	Pomegranate	Madulai	Peel
12	*Syzygium cumini*	Blackberry	Naval	Seed
13	*Tamarindus indica*	Tamarind	Pulzi	Peel

1.2. DESCRIPTION OF PLANTS

Abelmoschus esculentus

The plant *Abelmoschus esculentus* (Family: *Malvaceae*) formally known as *Hibiscus esculentus* L. (common name okra) is widely spread from West Africa to India, Southern Europe and the Americas since it is tolerant to both (i) hot and dry, and (ii) hot and humid climates. The simplicity of cultivation techniques and dependable yields make this one of the best vegetables for the Tropics ***(Carla, et.al. 2011)***

Bougainvillea glabra

This belongs to the family of *Nyctaginaceae* (Genus: *Bougainvillea*). This is a plant that spreads and may grow to some height upto 30 ft. The flowers of this plant are brightly coloured and contain less petals. This is widely spread in tropical and sub tropical region. The leaves are dark green, variable in shape, up to 4 inches (10 cm) long

Cicer arietinum

Cicer arietinum is a legume plant (Family: *Fabaceae*) which is commonly known as chickpea. This is the first grown legumes. The plant grows up to 20-50 cm. They grow widely in tropical areas such as Syria, Turkey and India. These as consumed either as wholly or as dry seeds and can be used as sweetening agent. This is rich in carbohydrates, vitamin B and also in minerals. These are rich in proteins .i.e., about 22% ***(Fratianni et.al. 2014)***

Citrus sinesis

This plant belongs to the family of *Rutaceae*. These plants are citrus fruits. These plants grow to few meters of height. This is commonly known as orange plant. The fruits are rich in citric acid. This is the reason for the taste of the fruit. The fruit has a lot of layers in each fruit. The peel of the fruit is harder but becomes soft when ripened.

Magnifera indica

Magnifera indica is a annual plant which fruits annually (Family :*Anacardiaceae)*. The plant grows up to few meters and these trees grow mainly in tropical region like India, Pakistan.***(Martinez, et.al.,2012).*** The plant grows up to 12-14 feet. The fruits are rich antibiotic and have many medicinal values. These plants has wide spread branches and foliate leaves arranged in parallel manner. The leaves are also used for traditional cultures.

Mirabilis jabela

Mirabilis jabela is a shrub that is usually grows as flowering plant (Family: *Nyctaginaceae)*. These plants are also found in tropical region. The flowers of this plant blooms in the evening. These plants spread out and grow only up to few centimetres approximately to 90 cm. The flowers of these plants are bright coloured and tiny with five petals. These plants are potable one that can also be grown for decorative purpose. The flowers can be used for food colouring and the leaves are edible.

Musa paradisica

This belongs to *Musaceae* family. The plant grows up to 1-2 m. The plant is a terminal one which produces the fruit only once in its life time. This grows largely in the tropical region mainly in India. The flowers of the plant are edible. The leaves are long and are with parallel venation.

Nephelium lappaceum

This plant belongs to the family of *Sapindaceae*. This plant is mainly found in Malaysia and other parts of South east Asia. This is a evergreen tree that may grow upto 12-20 meters. The fruit which is 3-6 cm long is usually oval or round in shape which is edible. The skin of the fruit is reddish with spines. The fruit pulp is whitish or light pinkish in colour. This is a tropical plant that grows in a atmosphere of 20-22^0 C.

Punica granatum

These plants are found in tropical region and grow up to 5-8meters. The fruits are round in shape and the plant is thin and may spread out. These plants are mainly cultivated around Mediterranean region, South Asian Countries and in Indian Subcontinent. The leaves are tiny and have spines on the plant. The fruit has numerous seeds in it which are also edible. The peel of the fruit has many medicinal values.

Pithecellobium dulce

This plant belongs to family of *Fabaceae*. These plants grow up to 10-15 meters and the tree gives much wood. The fruit of the plant are edible and has some nature of tamarind plant. The plant is rich in antioxidant and anti diabetic properties. The leaves are tiny and arranged in parallel manner. The flowers are about 12 cm which are coiled and turns pink when ripened. This part of flower has a edible pulp which contains black shiny seeds.

Psidium guajava

Psidium guajava L. is one of the most important crops belonging to the genus *Psidium* and the *Myrtaceae* family ***(Joseph & Priya, 2011)***. *P. guajava* is naturalized in tropical and subtropical parts of the world, and is considered an invasive species in some areas. This plant is a small tree 10 m high with wide spreading branches, and leaves that are oblong or oval, 5–15cm long, with prominent pinnate veins. The skin of the fruit and flesh colour varies between cultivars depending on the type and amount of pigments. *P. guajava* is used as a traditional medicine in certain cultures. The fruits are known to possess large amounts of vitamins and minerals, and have such high levels of polyphenolic antioxidants ***(Gema Flores, .et al., 2015)***

Syzygium cumini

Syzygium cumini is a plant which belongs to *Rubus* genus (Family: *Mrytaceae*). These plants are seen widely seen in tropical regions. The plant grows upto 30 m. The fruits of these plants are anti oxidant in nature. The plant grows to several meters and can be used as wood. The seeds of the fruits are also used as anti diabetic. ***(Forbesa et.al.,2010).*** These plants have wide spread branches and the fruits are tiny. The fruits are seasonal. The size of the fruit varies depending on the place where it is cultivated. The leaves are pinkish in young and become green when matured and has high nutritional value

Tamarindus indica

Tamarindus indica Linn. Is a leguminous tree (Family: *Fabacae* Subfamily: *Caesalpiniaceae*) is a perennial tree growing widely in the tropical and sub- tropical regions like India, Pakistan, Java, Philippines, Indonesia and China. Mature fruits are black or brown in colour. The sweetening nature of the pulp is due to the combined action of tartaric acid and reducing sugar. (***Tril et.al. 2014)*** Almost every part of the tree is re- ported to possess biological activities and uses in industry

1.3. OBJECTIVES OF STUDY

- To identify the presence of cyclitols (pinitol) in the extracts of chosen plants
- To quantify pinitol in the plant extracts by HPLC
- To isolate cyclitols by a simple method using protease enzyme papain

Fig 2-14 represents the photographs of the plants selected

Fig.2 *Abelmoschous esculentus*

Fig.3. Bougainvillea glabra

Fig.4. *Cicer arientum*

Fig.5.*Citrus sinensis*

Fig.6. *Magnifera indica*

Fig.7. *Mirabilis jalapa*

Fig.8. *Musa paradisica*

Fig. 9 *Nephellium lappacum*

Fig.10.*Pithecellebuce.dulce*

Fig.11.*Punica granatum*

Fig.12.*Psidium gujava*

Fig.13. *Syzygium cumini*

Fig. 14.*Tamarindus indica*

Table 2

Details of plant materials selected for study and their designated code

S no	Chosen plant material	Sample code
1	*Abeimoschous esculentus* leaf	AEL
2	*Abeimoschous esculentus* fruit	AEF
3	*Bougainvillea glabra* leaf	BGL
4	*Bougainvillea glabra* flower	BGF
5	*Cicer arientum* leaf	CAL
6	*Cicer arientum* seed	CAS
7	*Citrus sinensis* peel	CSP
8	*Magnifera indica* leaf	MIL
9	*Mirabilis jalapa* leaf	MJL
10	*Mirabilis jalapa* flower	MJF
11	*Musa paradisica* flower	MPF
12	*Nephellium lappaceum* peel	NLP
13	*Punica granatum* peel	PAP
14	*Pithecellobium dulce* peel	PDP
15	*Psidium guajava* leaf	PUL
16	*Psidium guajava ripened* peel	PUP_R
17	*Psidium guajava unripened* peel	PUP_U
18	*Syzgium cumini* seed	SCS
19	*Tamarindus indica* peel	TIP

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Objectives

Review of literature has been done for the knowledge on the previous studies pertaining to the present work from recent time to 2009

The review comprises of recent reports on

- Medicinal potential of chosen plants
- Activity of papain enzyme on protein degradation
- Isolation of pinitol from various plant extracts

2.2. Medicinal potential of the selected plants

Abelmoschus esculentus

The plant *Abelmoschus esculentus* (L.) Moench, formally known as *Hibiscus esculentus* L. (common name okra) is widely spread from West Africa to India, the plant is found to possess very good antioxidant potential

Biological activities

- The anti-diabetic activity of *Abelmoschus esculentus* fruit extract was reported and it showed reduced blood glucose levels with standard drug Metformin. **Subrahmanyam et al.,** (2011). The hypoglycaemic activity of aqueous extract and alcoholic extracts of *Abelmoschus esculentus* were reported by **Dibyajyoh Saha *et al.,* (2010).** The hypoglycaemic activity of *Abelmoschus esculentus* fruits when soaked in water for overnight was reported by **John Ray *et al.,* (2013)**

- The fresh water extracts of *Abelmoschus esculentus* pods were found to possess antibacterial properties. Due to the presence of palmitic and stearic acids, the proteins of polysaccharides do not show antimicrobial activity. **Carla *et al.,* (2011)**

Phytochemical constituents

- **Dibyajyoh Saha *et al.,* (2010)** showed the presence of carbohydrates, gums and mucilages, proteins, phytosterols, flavanoids, tannins, phenolics compounds in the alcoholic and aqueous extracts of *Abelmoschus esculentus*

Bougainvillea glabra

Bougainvillea glabra, a flowering plant generally used as ornamental plant and seen mostly in areas with warm climate. Apart from its use as ornamental plant, the leaf of *B.glabra* is reported to have various medicinal values.

Biological activities

- The in vitro anthelmintic activity of the hydroethanolic extracts of leaves of *Bougainvillea glabra* has been reported by **Hari *et al.,* (2012).** The same activity was also reported on the petroleum ether, methanol, ethyl acetate and aqueous extract of Bougainvillea glabra leaves using Albendazole as standard reference by **Eswaraiah *et al.,*(2012)**

- The antioxidant activity of *Bougainvillea glabra* of chloroform extract was reported by **Jancyrani *et al*.,(2013)** using various assays like DPPH radical scavenging assay, Hydrogen peroxide assay and reducing power assay

- Antibacterial activity of *Bougainvillea glabra* plant was reported by **Diaz *et al.,* (2012)**. The same work was also reported by **Gupta (2009)** on their comparative study of antibacterial activity of Bougainvillea glabra ‘snow white’ and Bougainvillea glabra ‘choicy plant’ extracts with petroleum ether and hydro alcohol

- The antidiabetic activity of aqueous extracts of Bougainvillea glabra using the standard Gilbenclamide has been reported by **Edwin and Sheeja (2009)**

Phytochemical analysis

- Phytochemical analysis was carried out in various extracts of *Bougainvillea glabra* flowers and it showed the *presence* of alkaloids, flavanoids, phenolic compounds, tannins, glycosides, steroids and proteins **Neha *et al.*,(2012)**

Cicer areinutum

Among legumes, chickpea (*Cicer arietinum* L.) ranks third worldwide, and its seeds contain >20% protein that is an important energy source for human. The nutritional and health values of legumes are affirmed by their increasing use in dietetic formulations. Several studies have reported the antidiabetic potential of legumes, which is significant in the current situation of the increasing prevalence of diabetes due to modern lifestyles.

Biological activities

- The antioxidant activities of common legumes were calculated and chickpea *(Cicer arinutum)* found to have low antioxidant capacity compared to other legumes. **Marathe *et al*., (2011).** The antimicrobial activity of proteins from *Cicer arienutum* has been reported by **Kumar *et al*.,(2014)**

Isolation of compounds

- **Kumar *et al*.,(2014)** has reported the isolation of antimicrobial proteins from *Cicer arienutum,* C-25, which is an antifungal protein was isolated and found to be effective antimyotic and antipoliferative agent against human oral cancer
- **Sagratini *et al*., (2013)** in their research has quantified soyasaponins I and βg in various legumes including *Cicer arienutum* by solid phase

extraction (SPE) coupled with LC-MS. These soyasaponins was found to be effective in lowering exogenous cholesterol.

Other activities

- **Hithamani *et al.,*(2014)** reported the polyphenol content in various cereals and legumes including *Cicer arienutum* after various stages like sprouting and heat processing

- **Nithyanantham *et al.,*(2012)** has reported that the rich source of proteins in legumes was found to be increased during dry heating process and also found to have more antioxidant activity in such a stage

Citrus sinensis

Citrus fruits are grown widely and hs a richer source of bioactive compounds which has beneficial activities for human by containing vitamin C, flavanoids, linminoids, alkaloids, essential oils, vitamin B complexes. These fruit peels have a very good antioxidant and anti-inflammatory activities.

Biological activities

- The effect of dried *Citrus sinensis* peel on gastrointestinal microbial population was reported by **Zohreh *et al.,*(2012**

- The antidiarrhoel activity of methanolic extract of *Citrus sinensis* peels in castor induced diarrhoea was reported by **J. Anbu *et al.,*(2009)**

- The methanolic extracts of *Citrus sinensis* shows the antibacterial and antifungal activities using agar well diffusion assay and was active against *Colletrotrichum capsici* fungi **Madhura S *et al*.,(2014).** The in vitro antibacterial activity of *Citrus sinensis* peels extracted by hot and cold methods for various pathogens was reported by **Najmu *et al.,*(2011)**

- **Omodamiro.O.D and Umekur.J.C (2013)** has reported the antioxidant, anti-inflammatory and antibacterial properties of ethanolic extracts of *Citrus sinensis* peels and leaves using various assays. The antioxidant activity of methanolic extracts of *citrus sinensis* peel superior to that of other extracts was reported by **Swapna Rekha and Bhaskar(2013)**

- The presence of antiradical activity for the extracts of leaves and peels of *citrrus sinensis* has been identified by **Benamrouchi *et al.,*(2013)**

Other activities

- The essential oil present in the *Citrus sinensis* peels i.e., **Limonene,** were use as an eco-friendly weed controller i.e., as allellochemical that can be produced from the residues of citric industry **Ribeiro *et al.,*(2012)**
- **Benamrouchi *et al.,*(2013)** has identified the presence of phenolic compounds in peels of *Citrus sinensis* extracts and also has shown the presence of antiradical activity for the extracts of leaves and peels

Mangifera indica

Mango (*Mangifera indica* L.) is one of the most important tropical plants in the world. It grows in the tropical and subtropical regions and its parts are commonly used in folk medicine for a wide variety of remedies. Many phenolic compounds have been detected in mango peels mango bark, mango puree concentrate mango pulps and seed kernels. Several pharmacological activities of mango extracts have been reported including anti-inflammatory, antioxidant antiallergic and antihelmintic and antiamoebic.

Biological activities

- **Bharti (2013)** concluded that among various extracts of *Magnifera indica* leaves hexane-ethyl acetate showed significant role in antimicrobial activity of pathogenic organisms and these were characterized by Gas Chromatography

- The aqueous extracts of leaves of *Mangifera indica* were found to have an active effect over gastro intestinal disorder **Pintu.K.D and Arna Pal (2014)**

- The antioxidant capacity of the phenols extracted from *Mangifera indica* leaves using methanol – acetone mixture as the excellent solvent was reported by **Ruth Mactinez *et al*.,(2012).** The efficient antioxidant capacity of mango leaves in its methanolic extract was reported by **Joona *et al.,*(2013)**

- **Joona *et al.,*(2013)** has shown and was also identified that the methanolic extracts has potential against the cytotoxicity of adenocarcinoma.

Phytochemical constituents

- **Pintu.K.D and Arna Pal (2014)** has identified the presence of tannins, flavanoids, steroids, cardiac glucoside, alkaloids and carbohydrates in the aqueous extracts of leaves of *Mangifera indica*

Mirabilis jalapa

Mirabilis jalapa .L is a perennial herbal medicinal plant, has a long traditional uses. Various constituents are found in different parts of the plants. The plant is used in treatment of skin diseases, carminative, cathartic, purgative, stomachic, tonic and vermifuge properties, anti-dysenteric, anti-parasitic, wound healer, digestive stimulant. Its various extracts reported number of pharmacological activities such as anti-diabetic, anti-inflammatory, anti-oxidative, antibacterial, anti-microbial, anti-fungal, anti-spasmodic, antinociceptive, anti-viral, diuretic, anthelmentic and urinary tract disorder.

Biological activities

- The anti arthritic activity on the hydro ethanolic extract of *Mirabilis jalapa* flowers in formaldehyde has been identified by **Augustine *et al.,*(2012)**

- Ethanolic extract of *Mirabilis jalapa* roots has shown the antidiabetic activity as well as shows reduced LDL cholesterol, urea, creatine but shows increased serum insulin, HDL cholesterol and protein **Piyali *et al.*,(2011)**

- **Walker *et al*.,(2008)** has reported that the hydroethanolic extract of *Mirabilis jalapa* leaves were more effective to induce antinocicephon in mice. It was reported by **Subin *et al*.,(2012)** that the methanolic extracts of the ariel parts of the same plant shown in vitro anthelmintic activity which required less time for the death of worms compared with other extracts.

- **Nowshin *et al*.,(2008)** has concluded that the petroleum ether, chloroform and methanolic crude leaves extracts of *Mirabilis jalapa* showed cytotoxicity and antioxidant activity using DPPH free radical scavenging assay.

- The antibacterial activity of methanolic, acetone, chloroform and ethanolic extracts of leaves of Mirabilis jalepa against Gram positive and gram negative bacteria by disc diffusion method was reported by **Kaladhar *et al*((2010)** and **Oladunmoye *et al*(2007)**

Other activities

- **Sharmila Shaik *et al.,*(2012)** has reported the phytochemicals present in roots, seeds, leaves and flowers and also various TLC analysis provided an evidence for the presence of various compounds like alanine, arabinose, campesterol, daucosterol and dopamine, d-glucan, hexacon-1-ol, indicaxanthin, isobetanin, 6-methoxyboeravinone, C methylabronisoflavones, miraxanthins, n-dotriacontane, n-nonacosane, n-pentacosane, n-triacontane and also has isolated three new phenolic compounds from organic extract of cell mass from manipulated plant cell

culture of *Mirabilis jalapa* leaves and among these two compounds shown inhibitory activity against the fungi, Candida albicane.

Musa paradisica

Musa paradisiaca is a monoecious herb. Its leaves can be used in the treatment of cough and bronchitis. Roots are used to arrest hemoptysis, possess strongly astringent and as an Anthelmintic. Fruits can increases the renal activities, reduces the risk of kidney cancer. It contains antioxidizer and counteracts the noxious effects of the free radicals. *Musa Paradisiaca* can be used as antidote for snake bite, asthma, burns, diabetes, dysentery, excessive menstrual flow, fever.

Biological activities

- A review work on anti ulcer activity, wound healing activity, antidiabetic activity, antiurolithiatic activity, skeletal muscle contraction activity and antidote activity for Crotalidae venoms for all parts of Musa paradisiaca plant has been performed by **Swathi *et al.,*(2011)**

- The antidiabetic activity of ethanolic extract as well as the hexane and chloroform fraction produced by maceration method of *Musa paradisica* leaves and fruit peels was reported by **Vijai *et al.,*(2014)**

- The methanolic extracts of *Musa paradisica* leaves and stalks were identified to have very effective antifungal agent and kill the growth of many spoilage fungi and hence was used for food preservation **Okorondu *et al.,*(2012)**

- The hypoglycemic activity of chloroform extracts of *Musa paradisica* flowers was reported which also provided an increase in total haemoglobin. It was also reported that these flowers also used for curing dysentery and menorchagia in the review article by **Imam and Akter (2011)**

- The daily intake of *Musa paradisica* significantly reduces the oxidative stress, hyperglycemia and inflammatory reactions **Nisha P and Mini S (2011)**

Nephelium lappaceum

Rambutan (*Nephelium lappaceum*) which belongs to the family of Sapindaceae, is a potential fruit to be commercialized. The red coloured peel of rambutan has high antioxidant activity (Okonogi *et al*., 2006). Thus, rambutan peel, which usually is thrown away as waste, may serve as a source of useful antioxidant for extraction. However, rambutan has a short shelflife, where the quality of the fruits will drastically decrease if they are not well handled.

Biological activities

- The methanolic extracts of the peels of *Nephelium lappacum* were found to have rich antioxidant activity **Thitiertdecha *et al.,*(2008).** The ethyl acetate fraction of *Nephelium lappacum* peels has a potential novel antioxidant activity than that of butylated hydroxyl toluene and vitamin E. These activities were due to the presence of high polyphenol content. **Khonkarn *et al*.,(2010)**

- The extracts like ether, methanol and aqueous of *Nephelium lappacum* shown antibacterial activity against five pathogens **Thitiertdecha *et al.,*(2008)**

Other activities

- The antioxidant activity and the phenolic contents does not vary by water or steam blanching for 5 to 15 minutes but there is an significance increase in the level of anthocyanin for 2.5 minutes and decreased after 5

minutes treatment and also there was a significant reduction in residual peroxidise (POD) and polyphenoloxidase (PPO) activities. **Nuruhuda *et al.,*(2013)**

Pithecellobium dulce

Pithecellobium dulce Benth. (Leguminosae) is a small to medium sized, evergreen, spiny tree up to 18 m height. The bark of the plant is reported to be used as astringent in dysentery, febrifuge and is useful in dermatitis and eye inflammation. Roots have been reported to possess estrogenic activity **(Saxena and Singal, 1998)**. The fruits have been shown to have anti-inflammatory activity **(Bhargvakrishna *etal.,* 1970)** and leaves have been reported to be a folk remedy for ear ache, leprosy, peptic ulcer, tooth ache, and venereal disease. It also acts as astringent, emollient, abortifacient, antidiabetic, anodyne and larvicidic in folk medicines.

Biological activities

- *Pithecellobium dulce* fruit extract posses significant antidiabetic activity and also that these are non toxic and can maintain normoglycemia and also showed reduced blood glucose levels, glycosylated haemoglobin, urea and creatine **Pradeepa S *et al*.,(2013).** The antidiabetic activity was reported in the ethyl acetate extract of *Pithecellobium dulce* fruit peel compared to that of methanolic and aqueous extracts. **Sukantha T.A *et al*.,(2011)**

- The methanolic extracts of *Pithecellobium dulce* leaves has shown the analgesic and anti-inflammatory activities in comparative to standard drugs **Muthukumaran *et al.,*(2011)**

- It was concluded that ethyl acetate, methanolic and aqueous extracts of *Pithecellobium dulce* fruit peel have antioxidant activity by their phenolic content and DPPH free radical scavenging by **Sukantha T.A *et al*.,(2011)**

Punica granatum

Punica granatum (pomegranate) is native to the region from northern India to Iran Pharmacological effects of pomegranate represent a long history and have been mentioned in the Greek and Egyptian documents studies have shown that pomegranate has many potential effects including: bacteriocidal, antifungal, antiviral, immune modulation, vermifuge, stimulant, refrigerant, astringent, stomachic, styptic, laxative, diuretic and anthelmintic. Moreover, it serves to decrease the adverse effects of cardiovascular diseases, diabetes, diarrhea, dysentery, asthma, bronchitis, cough, bleeding disorders, fever, inflammation, acquired immune deficiency syndrome, dyspepsia, ulcers, bruises, sores, mouth lesions, skin lesions, malaria, prostate cancer, atherosclerosis, hypertension, periodontal diseases, hyper lipidemia, denture stomatitis, male infertility, vaginitis, erectile dysfunction, alzheimer, obesity and infant brain ischemia

Biological activities

- The antioxidant, anti carcinogenic and anti-inflammatory activities of extracts of peels of *Punica granatum* and also the effectiveness of this on the treatment of cancer, cardiovascular disease, diabetes, dental conditions, bacterial infection and antibiotic resistance and ultra violet radiation –induced skin damage was reported by **Dipak *et al.*,(2012)**. The antioxidant and immunomodulatory properties, cytotoxicity in human cancer cells through the induction of apoptosis and antitumor activity was also reported for the same fruit by **Manu *et al*.,(2013)**

- The antimicrobial activity of the methanolic extracts of *Punica granatum* peels of all concentrations of 4 mg/ml, 8mg/ml and 12 mg/ml against Lactobacillus acidophilus, Streptococcus mutans and Streptococcus salivarius was reported by **Abdollahzadeh *et al.*,(2010)**

Isolation of compounds

- **Manu *et al*.,(2013)** has reported the isolation of Galactomannan polysaccharide (PSP001) from *Punica granatum* fruit.

Phytochemical analysis

- **Dipak *et al.,*(2012)** has done the phytochemical analysis on *Punica granatum* and analysed that it contain ellagic acid ellagitannins (including punicalagins), punicic acid, flavonoids, anthocyanins, estrogens flavonols and flavones

Psidium guajava

Psidium guajava is a evergreen shrub that belong to the native of America. All most all parts of this plant has been used for the treatment of various human ailments. The leaves of the plant have been used for the treatment of diahorrea and stomach problems. The fruits of the plant contain vitamin C, iron, calcium and phosphorus

Biological activities

- The methanol, acetone and N-N-dimethylformamide (DMF) fractions of leaves of *Psidium guajava* showed pronounced antibacterial activity against gram positive bacteria and moderate activity against gram negative bacteria and also showed antifungal activity against nstatin and fluanazole fungi **Rathish *et al*.,(2007).** The comparative study among the various extracts like ethanolic, methanolic, ethyl acetate and hot water of leaves, fruits and stems of *Psidium guajava*, stems showed good antibacterial activity which was tested using agar well diffusion assay and concluded that ethanolic and hot water extract had low antibacterial activity. **Pandey and Shweta (2012).** The ethanolic and methanolic leaf extracts of *Pisidum guajava* have better antibacterial activity than n-hexane and water. But these extracts have no active compounds against

gram negative bacteria and concluded that these extracts are effective against infections and diseases caused by *Bacillus cereus* and *Staphylococcus aureus*. **Biswas *et al.*,(2013)**

- The ability of aqueous extract of *Pisidum guajava* fruit peel to reduce oxidative stress in pancreas of diabetic rats and has identified that it may play major role in reducing the development of diabetic complicationswas reported by **Poundin *et al*.,(2013)**

- The highest antioxidant activity and anti-inflammatory in ethyl acetate fraction of Costa Rica guava compared to hexane, chloroform and n-butane fraction. **Flores *et al*.,(2013)**

Isolation of compounds

- It was reported that among various extracts like ethanolic, methanolic, ethyl acetate and hot water of leaves, fruits and stems of *Psidium guajava*, the antibacterial compounds present were reducing sugar, tannins, phcobatannins, saponins, terpenoids, alkaloids and poly phenols. **Pandey and Shweta (2012)**

- The presence of nine compounds were found which were phenolic compounds 1-O-trans-cinnamoyl-b-D-glucopyranose, ellagic acid, myricetin, quercitrin, and quercetin were identified using standard compounds or literature reports from related species. Some compounds were tentatively identified as 1,5-dimethyl citrate, sinapic aldehyde 4-O-b-D-glucopyranose, 3,30,4-tri-O-methylellagicacid-40-O-D-glucopyranoside, and 1,3-O-diferuloylglycerol and all these compounds were reported for the first time in Costa Rican guava **Flores *et al*.,(2013)**

Other activities

- **Flores *et al*., (2013)** has identified that the compounds and the antioxidant activity in the *Psidium guajava* fruit varied according to the

nature of the colour of the pulp and the cultivators. Pink pulp showed higher activity compared to white pulp according to DPPH assay and ABTS assay and also 21 compounds were characterized in those fruits

Syzygium cumini

Syzygium cumini (L.) Skeels (synonym *Eugenia jambolana*), popularly known as “jambolão”, belonging to *Myrtaceae* family is one of the most commonly medicinal plants used to treat *diabetes mellitus* in Brazil (17). Different parts of this plant, such as seeds, bark, fruit, and leaves have been used in traditional medicine as a remedy for *diabetes mellitus* in many countries. The leaves are also used to strengthen the teeth and gums, to treat leucorrhoea, stomachalgia, fever, gastropathy, strangury, dermopathy, constipation, and to inhibit blood discharges in the faeces. It is considered as a rich source of antioxidative and redical scavenging phytoconstituents. The seeds of this fruit retain extraordinary medicinal properties, which have remained largely undiscovered

Biological activities

- The antimicrobial activity of *Syzygium cumini* leaves extract and the crude hydroalcoholic extract was evaluated against Candida krusei and against multi-resistant strains of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus* by disk diffusion method, micro dilution broth method **Silva M.L.A *et al*., (2007)**

- The oral administration of *Syzygium cumini* seed extracts has shown the amelioration of learning-related memory of old rats by eight-arm radial maze task and the chemical analyses of this extract has revealed substantial amounts of polyphenols and free-radical scavenging activity and hence it was concluded that the Syzygium *cumini* seed extract improves learning-related memory in old rats, probably, by instigating the antioxidant defense of the brain **Rahaman *et al.*,(2013)**

- **Mohamed *et al.,* (2013)** has reported that the methanolic extracts of *Syzygium cumini* leaves has more antioxidant activity in both DPPH and FRAP methods, more anti bacterial activity.

- **Frobes *et al.,*(2009)** has revealed that the raspberry and blackberry fruits has superior levels of anthocyanins and their hexane, ethyl acetate and methanol extracts showed good antioxidant activity.

- The extracts of raspberry and blackberry fruits hasthe greatest potential to inhibit cancer cell growth, inhibiting colon, breast, lung, and gastric human tumour cells by 50, 24, 54 and 37%, respectively **Frobes *et al.,*(2009)**

Phytochemical constituents

- The higher content of both total phenols and flavanoids in methanolic extracts of *Syzygium cumini* leaves and contains essential oils which has the abundant constituents of a-pinene (32.32%), b-pinene(12.44%), trans-caryophyllene (11.19%), 1, 3, 6-octatriene (8.41%), delta-3-carene (5.55%), a-caryophyllene (4.36%), and alimonene (3.42%) **Mohamed *et al.,* (2013)**

Tamarindus indica

The tamarind tree (*Tamarindus indica* L.) of the familyCaesalpinaceae is found in both tropical and subtropical regions of the world. Almost every part of the tree is reported to possess biological activities and uses in industry. The roots have a curative value in chestcomplaints and leprosy.

Biological activities

- It was reported that the methanolic extracts of fruit pulp extracts of *Tamarindus indica* had good antioxidant activity. The extracts of Tamarindus indica were also identified to have antibacterial activity against gram positive and gram negative bacteria **Tril *et al*.,(2014)**

Phytochemical constituents

- It has been reported that among the various solvents like methanol, ethyl acetate and hexane, the most of the antioxidant phenolics were extracted from the methanolic extracts of seeds, veins and skin of *Tamarindus indica* and among that the methanolic leaf extract had highest phenolic content. The HPLC analysis of methanolic leaf extract revealed the presence of catechin, epicatechin, quercetin and isorhamnetin **Razali *et al*.,(2011)**

- **Rao and Gowda (2008)** has identified two proteins in the seeds of *Tamarindus indica* which were Kunitz-type trypsin inhibitor and Class III endochitinase which were purified by a single-step chitin bead affinity chromatography and Class III endochitinase protein was identified accounting for >50% of the total seed protein, is an acidic glycoprotein exhibiting a very low endotype hydrolytic activity toward chitin derivatives and it was concluded that on the basis of its abundance, accumulation without any pathogenesis-related stimulus, temporal regulation, amino acid composition, and very low enzyme activity, that protein designated “tamarinin” physiologically serves as the major storage protein.

- **Tril *et al*.,(2014)** has reported the presence of larger amount of glucose and tartaric acids in the fruit pulp extracts of *Tamarindus indica* and also the DMSO extracts contained the highest phenolic and flavonoids compounds and these extracts exhibited the best metal-ion chelating properties.

- Phytochemical investigation of the root bark of Tamarindus indica afforded n-hexacosane, eicosanoic acid, b-sitosterol, octacosanyl ferulate, 21-oxobehenic acid, (+)-pinitol, apigenin and vitexin. The presence of the bioactive compound (+)-pinitol in this plant is being reported for the first time **Renuka et al., (2006)**

Activity of papain enzyme on protein degradation

Papain and chymopapain are the two main enzymes of papaya. Papain is cysteine protease, also known as papaya proteinase I, from the peptidase C1 family and may be extracted from the plant's latex, fruit, leaves and roots. Papain is simple enzyme which contains 212 amino acid residue chains

- **Minha *et al*.,(2012)** has reported that the protease enzymes like papain, bromelin, actindin and zingibain has an ability to hydrolyse proteins present in topside myofibril extracts

- The antioxidant property of body, foot and viscera of Donax cuneatus were tested after hydrolysing using commercial protease enzyme s like pepsin, trypsin and papain using scavenging ability and reducing power assays **.Nazeer *et al.,*(2012)**

- Researches had shown that the purified sulphated polysaccharide conjugate using protease enzyme like papain has less antioxidant activity than the crude extracts **Bei-Weizhu *et al* .,(2008)**

- The hydrolysis of flax seed proteins using protease enzyme s like thermolysin and pronase which was then mixed with activated charcoal and centrifuged and filtered. The filtrate was found to have high Fischer ratio and thus found to have multiple health benefits **Chibiike. C. Udeniguse and Rotimi.E.aluko (2010)**

- **Lijun You *et al*.,(2012)** has reported that the protein hydrolysates *Misguinus anguillicaudatus* (Loach) was prepared by Papain digestion and the fractions were found to have antioxidant, antihypertensive and antipoliferative activities

- The toxicity of the drugs can be reduced by the degradation of drug using protease enzyme, papain which was determined by spectrometric studies **Hitesh. *et al.,* (2012)**

Isolation of D-Pinitol from plant extracts

Pinitol is an anti diabetic biomarker. Its pharmacological significance is highly remarkable and there are a volly of reports on its use in medicinal formulations.

- **Dewangan *et al*.,(2014)** has isolated the bioactive carbohydrate D-pinitol from the ethanolic extracts of leaves of *Bauhinia variegate*, a plant belonging to the family of *Leguminosea* using column chromatography. Identification of chemical constituents was done by various techniques viz.MP, TLC, IR, NMR & LC-MS techniques.

- **Sharma *et al*.,(2014)** has reported the isolation of Pinitol as a major constituent on the ethanolic extract of *Argyrolobium roseium* on the method based on Proton Nuclear Magnetic Resonance (PNMR) which was used for quantification and identification. Pinitol was isolated by column chromatography followed by crystallization in methanol and the identification of the isolated pinitol was done on the basis of H-NMR,13C-NMR,DEPT(35^{o},90^{o} and 135^{o}) experiments and mass spectral data

- **Indumathi *et al.,* (2013)** has identified the presence of pinitol in 18 chosen plants which mainly belonged to Leguminnosae family and was quantified using HPTLC techniques

- **Poongothai (2012)** has concluded that HPTLC method for identifying and quantifying pinitol and it was regarded as a simple, accurate and cheap method and can also be utilized for quantitative determination of Pinitol in *Pisonia grandis* plant

- **Riberio *et al.,* (2011)** has investigated the levels of inositols (myo-inositol, D-pinitol and ononitol), soluble carbohydrates and proteins in the cotyledons of *Phaseolus vulgaris* and *Vigna unguiculata* sprouts and D-pinitol was detected only in quiescent

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present work was aimed at the identification of the antidiabetic molecule pinitol in chosen antidiabetic medicinal plants and its isolation by a simple method using a protease enzyme, Papain

The methodology comprises of:

- Extraction of plant materials
- Preliminary colour test of extracts
- TLC examination of extracts
- TLC examination of enzyme treated extracts
- UV spectral analysis of isolated compound from enzyme treated extracts
- Identification and quantification of pinitol in the selected extracts by HPLC

3.1 Collection of plant materials

The leaves of *Psidium guajava, Magnifera indica*, the leaves and flower of *Bougainvillea glabra*, the peels of *Citrus sinensis, Punica granatum, Pithecelleoum dulce,* the seeds of *Syzygium cumini* and the flowers of *Musa paradisica* were collected from local areas of Coimbatore. The peels of *Psidium guajava* (both ripened and unripened)and that of *Nephelium lappaceum*, the leaves and flowers of *Mirabilis jalapa* were collected from Gudalur area of The Nilgiris. The peels of *Tamarindus indica* were collected from Karur. The leaves of *Abelmoschus esculentus* and the leaves and seeds of *Cicer arinetium* were collected from Salem.thirteen plant materials were chosen and selected plant parts were taken up for the present work. The plant materials were collected and wereair dried and pulverized. Totally 18 samples were taken.

3.2 Extraction of plant materials

Extraction of the chosen plant samples was done with hydroethanol (80:20) and water as solvents.

The dried plant material (25 g) was extracted wih hydroethanolic(80:20) solvent(100ml) over a boiling water bath for one hour. The extract was filtered after cooling and was concentrated in a rotary evaporator.

The dried plant materials (10 g) were extracted with water by refluxing for one hour and the filtrate was concentrated under vaccum

Fresh *Abelmoschous esculentus* pods were extracted by soaking 25 g of the pods overnight in water (100 ml).

The weight of each extract concentrate was noted.

The percentage of residue was calculated using the formula:

$$\text{Weight percentage} = \frac{\text{Weight of substance extracted}}{\text{Weight of the original material}} \times 100$$

3.3 Phytochemical colour tests of extracts

All the extracts were subjected to phytochemical analysis by standard tests

3.3.1Test for alkaloids (Phytochemical Techniques by (Raaman.N)

3.3.1.1 Mayer's test

A fraction of the plant extract was treated with few ml of Mayer's reagent [1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml of water] and observed for the formation of cream coloured precipitate **(Evans1997**)

3.3.1.2 Wagner's test

A fraction of the plant extract was treated with few ml Wagner's reagent [1.27 g of iodine and 2 g of potassium iodide in 100 ml water] and observed for the formation of reddish brown colour precipitate. **(Evans 1997)**

3.3.1.3 Hager's test

A few ml of the plant extract was treated with Hager's reagent (saturated aqueous solution of picric acid) and observed fot the formation of prominent yellow precipitate **(Evans 1997)**

3.3.2 Test for Flavonoids

3.3.2.1 Test with sodium hydroxide

A small amount of the plant extract was treated with aqueous NaOH and observed for the formation of yellow orange colour

3.3.2.2 Test with concentrated sulphuric acid

A fraction of the plant extract was treated with concentrated H_2SO_4 and observed for the formation of orange colour

3.3.2.3 Shinoda test

Sample was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips were then added to the filtrate followed by few drops of concentrated HCl. A pink, orange or red to purple colouration indicates the presence of flavonoids

3.3.3 Test for tannins

The plant extract was dissolved in water and heated on a water bath for one hour. The filtrate was treated with ferric chloride and observed for the formation of dark green colour.

3.3.4Test for carbohydrates

3.3.4.1 Molisch's test

Few drops of Molisch's reagent was added to each of the sample dissolved in distilled water and 1ml of concentrated H_2SO_4 was added along the side of the test tube. Formation of a red or dull violet colour at the interphase of the two layers was a positive test **(Sofowora, 1993).**

3.3.4.2 Fehling's test for free reducing sugar

Sample was dissolved in distilled water and filtered. The filtrate was heated with 2 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars **(Sofowora, 1993)**.

3.3.5 Test for Quinone

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

3.3.6 Test for terpenoids

3.3.6.1 Salkowski test

The sample was taken in a test tube and few ml of chloroform was added. Then concentrated sulphuric acid was added carefully along the side. Reddish brown coloured solution indicate presence of terpenoids

3.3.6.2 Libermann-Burchard test

The sample was treated with chloroform, acetic anhydride and adds few drops of concentrated H_2SO_4 and observed for the formation of dark green colour

3.3.7 Test for sterols

3.3.7.1 Libermann-Burchard test

The sample was treated with chloroform,acetic anhydride and few drops of concentrated H_2SO_4 was added. Formation of dark pink and red coloured solution indicates the presence of sterols

3.3.7.2 Test with concentrated sulphuric acid

The sample was treated with ethanol and concentrated H_2SO_4 and observed for the formation of violet blue or green colour

3.3.8 Test for phenols

3.3.8.1 Ferric chloride test

The sample was treated with 5% ferric chloride and observed for the formation of deep blue or black colour.

3.3.8.2 Lead acetate test

A small amount of extract was treated with lead acetate and observed for the formation of a white precipitate

3.3.9 Test for proteins

3.3.9.1 Ninhydrin test (aqueous)

The sample was treated with aqueous ninhydrin, when formation of blue colour solution indicates the presence of aminoacid and purple colour indicates the presence of proteins.

3.3.9.2 Ninhydrin (acetone)

The sample was treated with ninhydrin in acetone and observed for the formation of purple colour

3.3.9.3 Biuret test

The sample was heated in distilled water and filtered, the filtrate was treated with 2% copper sulphate solution, to this 95% ethanol and potassium hydroxide were added. The formation of pink ethanolic layer indicates amide group.

3.4 TLC examination of the extract

Thin layer Chromatographic analysis was performed for all the plant extracts using pre-coated TLC plates (5*10cm).Samples of both hydroethanolic and aqueous extracts were dissolved in their respective solvents one by one separately and were spotted on the TLC plates manually with a capillary tube. The plate was then allowed to develop in chloroform-methanol-water (6:3.5:0.5) solvent system. After development the chromatograph was sprayed with spray reagent, ammonical silver nitrate solution and the spots were allowed to develop by drying the plate in the oven. The R_f values were noted.

3.5 Treatment of plant extracts with papain enzyme

The samples in which the presence of the standard was identified were further treated with protease enzyme, papain for the degradation of proteins present in the extracts. Papain, the protease enzyme was commercially purchased and was stored in a temperature of 5^{o}C.

About 10 mg of the sample was weighed and was solvated in water. The enzyme (5 mg) was added to these sample solution and was allowed to react for an hour. The enzyme treated sample was centrifuged for 5 minutes and the supernatant solution was separated. The filtrates were subjected to TLC analysis for the identification of pinitol in the enzyme treated extracts.

3.6 Isolation of pinitol from the enzyme treated extract samples

The enzyme was found to be insoluble in methanol and hence the filtrate was evaporated to dryness and was dissolved in methanol. The residue settling down was removed by centrifugation. The remaining filtrate was again evaporated to dryness by placing it in the refrigerator overnight. The residue formed was further analysed for the presence of pinitol by UV spectroscopy.

3.7 UV Spectrometric analysis

The pure standard and the pure papain enzyme were dissolved in distilled water and their respective UV spectrum was recorded. The fresh plant extracts of the below samples were also taken up for the UV analysis.

Table 3.The following plant extract samples were taken up for UV analysis

Extract	Sample code
Ethanolic extract of leaves of *Abelmoschous esculentus* treated with enzyme	AELEZ
Ethanolic extract of flowers of *Mirabilis jalapa* treated with enzyme	MJFEZ
Ethanolic extract of leaves *Psidium guajava* treated with enzyme	PULEZ
Ethanolic extract of leaves of *Mirabilis jalapa* treated with enzyme	MJLEZ
Ethanolic extract of peels of *Citrus sinensis* treated with enzyme	CSPEZ
Ethanolic extract of peels of *Punica granatum t*reated with enzyme	PAPEZ
Ethanolic extract of seeds of *Syzygium cumini* treated with enzyme	SCSEZ
Aqueous extract of leaves of *Abelmoschous esculentus* treated with enzyme	AELWZ
Aqueous extract of peels of *Nephelium lappacum* treated with enzyme	NLPWZ
Aqueous extract of peels of *Tamarindus indica* treated with enzyme	TIPWZ
Aqueous extract of peels of *Punica granatum* treated with enzyme	PAPWZ
Aqueous extract of leaves *Psidium guajava* treated with enzyme	PUPWZ

The samples for the UV spectral analysis were taken on the basis of the TLC analysis. The UV spectrometer was first calibrated and wavelength was adjusted between 200nm and 400nm.

Solvent : Distilled water

Range : 200nm-400nm

Instrument: Systronics Brand

Procedure

After calibration the pure solvent was taken in one cuvette and the sample was taken in other cuvette. The scanning was done and the peaks obtained shows the presence of pinitol

3.8 Identification and quantification of pinitol in the extracts by HPLC

High performance chromatography is one of the most powerful tools for the separation, identification and quantification of compounds present in any samples that are dissolved in liquids.

HPLC analysis was done to quantify the pinitol content in the extracts by an optimised method

The following plant extracts were selected for HPLC analysis based on their TLC analysis which revealed the presence of antidiabetic molecule pinitol in the extracts.

Table 4 Ethanolic extracts

Plant extract	**Sample code**
Unripened peel of *Psidium guajava*	PUP_UE
Leaves of *Cicer arientium*	CALE
Peels of *Nephellium lappacum*	NLPE
Flowers of *Bougainvillea glabra*	BGFE
Ripened peels of *Psidium guajava*	PUP_RE
Seeds of *Syzygium cumini*	SCSE
Peels of *Punica granatum*	PAPE
Flowers of *Musa paradisiaca*	MPFE

Table 5 Aqueous extracts

Plant extracts	**Sample code**
Peels of *Punica granatum*	PAPW
Leaves of *Bougainvillea glabra*	BGLW
Unripened peels of *Psidium guajava*	PUP_UW
Seeds of *Syzygium cumini*	SCSW
Peels of *Citrus sinensis*	CSPW
Seeds of *Cicer arientium*	CASW
Leaves of *Psidium guajava*	PULW

3.8.1 Preparation of standard solution

Table.6.

The following standard solutions were prepared for the calibration

Preparation	ppm solutions
40 mg in 1ml of water	40,000 ppm
30 mg in 1ml of water	30,000 ppm
20 mg in 1ml of water	20,000 ppm
10 mg in 1ml of water	10,000 ppm
5 mg in 1ml of water	5,000 ppm
1 mg in 1ml of water	1,000 ppm

3.8.2 Preparation of samples

About 30mg of the selected samples were weighed accurately and was dissolved in 1ml of HPLC grade water.

Solvent: Water-acetonitrile mixture – (30:70)

Column: Amine column

Detector: Refractive Index Detector

3.8.3 Preparation of mobile phase

HPLC grade solvents were used as mobile phase. These solvents were primarily filtered separately one by one by glass filter holder using cellulose filter. The solvents were then placed in the HPLC bottles and were sonicated for 20 minutes for the removal of any air bubbles. After sonication these solvents were used as mobile phase.

3.8.4 HPLC Protocol

The amine column was fixed in the column rack was connected to the instrument. The prepared mobile phase was filled with the mobile phase reservoir and the power was switched on followed by the buttons of the pumps,

RID detector and the column rack. The purge on the pump modules were opened for the activation of pump.

The details required for the system were provided. This includes the setting of the mode, the concentration of the pumps A and B, tigger type, oven temperature, stop time and flow rate. The flow rate was controlled at 1ml/min. Then the instrument was balanced and once it gets stabilized, the injector valve was turned to “load” position and the filtered standards and samples were injected using a Hamilton syringe. After injection the valve was immediately turned to “inject” position.

The sample gets separated into its components inside the column which are identified by the detector and are recorded in the data acquisition system. Those signals that acquire the information such as retention time, peak area and peak height of component are displayed as chromatogram peaks. From these values the quantity of the component in the analyzed fraction can be calculated.

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

The present study was undertaken with the aim of analysing extracts of 18 chosen plant materials from 13 plants for the presence of pharmacologically active sugar alcohol molecule pinitol in them by TLC and High Performance Liquid Chromatography (HPLC) and also for identifying a simple method of its isolation using the protease enzyme, papain.

4.1 Extraction

Each of the 18 plant materials (25 g each) were extracted with hydroethanolic (80:20) solvent for one hour in a boiling water bath. The fresh dried plant materials (10g each) were extracted by reflux method with distilled water for one hour. The extraction with the aqueous solvent by reflux method gave higher yield for most plant materials when compared with hydroethanolic extraction. The percentage yield of residue obtained is given in table 6. Under the above condition seven plant samples gave higher yields in aqueous extraction using reflux method (leaves of *Mirabilis jalapa,* peels of *Citrus sinensis,* peels of *Tamarindus indica,* leaves of *Abelmoschous esculentus,* leaves of *Bougainvillea glabra,* leaves of *Psidium guajava* and leaves of *Mangifera indica*) ranging from 11-13%. However four plant samples showed higher yields in the hydroethanolic solvent using boiling water bath method (peels of *Pthicellobium dulce,* unripened and ripened peel of *Psidium guajava*, flowers of *Bougainvillea glabra*) compared to the aqueous extracts.

Table 7 Yield of residue in plant extracts

		Hydroethanolic extraction		Aqueous extraction	
S.no	**Sample**	**Residue weight (g)**	**Yield (%)**	**Residue weight (g)**	**Yield (%)**
1	AEL	1.8	7.2	1.39	13.9
2	BGL	2.6	10.4	1.28	12.8
3	BGF	2.7	10.8	0.78	7.8
4	CAL	1.9	7.6	1.42	14.2
5	CAS	1.5	6.0	0.78	7.8
6	CSP	2.5	10.0	1.2	12.0
7	MIL	2.16	8.64	1.13	11.3
8	MJL	2.9	11.6	1.42	14.2
9	MJF	2.2	8.8	1.00	10.0
10	MPF	2.07	8.28	0.87	8.7
11	NLP	2.2	8.8	0.53	5.3
12	PAP	1.96	7.84	0.77	7.7
13	PDP	2.6	10.4	0.85	8.5
14	PUL	1.96	7.84	1.45	14.5
15	PUP_U	2.8	11.2	1.09	10.9
16	PUP_R	2.4	9.6	0.88	8.8
17	SCS	2.1	8.4	0.65	6.5
18	TIP	1.7	6.8	1.3	13.0

4.2 Phytochemical screening

The various plant extracts were subjected to preliminary phytochemical screening by standard methods and it revealed that alkaloids, flavanoids, phenols and carbohydrates occur mostly in all plant extracts. The results of the phytochemical screening of various plant extracts are presented in table 8 and 9.

Table 8.Results of colour tests for hydroethanolic extracts

Class of Compound	Test	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Alkaloids	Mayer's test	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
	Wagner's test	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	Shinoda test	+	-	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
	H_2SO_4 test	+	-	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
Tannins	Ferric chloride	+	+	-	+	-	+	+	-	-	+	-	+	+	+	-	-	+	-
Carbohydrate	Molisch's test	+	-	-	+	+	-	+	-	-	+	+	+	+	+	-	+	+	+
	Fehling's test	+	-	-	+	-	-	+	-	-	+	+	+	+	+	-	+	+	+
Phenols	Ferric chloride test	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	+	+	+
Proteins	Ninhydrin test	+	-	+	+	+	-	-	+	-	-	-	-	+	-	-	-	-	+
	Biuret test	+	-	+	+	+	-	-	+	-	-	-	-	+	-	-	-	-	+
Sterols	H_2SO_4 test	-	+	+	+	-	-	+	+	-	+	+	-	+	+	-	-	-	-

Table 9 Results of colour tests for aqueous extracts

Class of Compound	Test	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Alkaloids	Mayer's test	-	+	+	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+
	Wagner's test	+	+	+	-	+	+	+	+	+	-	+	+	-	+	-	+	+	+
Flavonoids	Shinoda test	+	-	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+
	H_2SO_4 test	+	-	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+
Tannins	Ferric chloride	+	+	-	+	-	+	+	-	-	+	-	+	+	+	-	-	+	-
Carbohydrate	Molisch's test	+	-	-	+	+	-	+	-	-	+	+	+	+	+	-	+	+	+
	Fehling's test	+	-	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+	+
Phenols	Ferric chloride test	+	-	+	-	+	-	-	+	-	-	-	-	-	-	+	+	+	+
Proteins	Ninhydrin test	+	-	+	+	+	-	-	+	+	-	-	-	+	-	-	+	+	+
	Biuret test	+	-	+	+	+	+	-	+	-	-	+	-	+	-	+	-	-	+
Sterols	H_2SO_4 test	-	+	+	+	-	-	+	+	-	+	+	-	+	+	-	+	-	-

4.3 TLC Analysis

The concentrated hydroethanolic extracts and the aqueous extracts of all the 18 samples were subjected to TLC examination by an optimized method using chloroform –methanol-water (6:3.5:0.5) solvent system. The samples were compared with standard pinitol of 99% purity. Pinitol in the extracts was identified by the presence of a brown or blackish brown spot developed on spraying with ammonical silver nitrate solution, corresponding to that of the standard pinitol at the same R_f. The R_f values of samples were noted. Sample spots are numbered from left to right.

From the TLC analysis it can be proposed that all the 18 plant extracts of both the extracts may contain pinitol in their extracts. Based on the intensity of the spot 14 samples were chosen for HPLC analysis. Figures 16 to 25 represent the TLC chromatograms of the samples. The R_f value of the sugar alcohol in the chromatograms are tabulated (Tables 10 to 19).

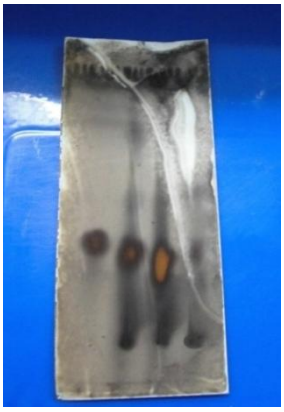

Fig 15. Plate A

1.Standard 2. PULE
3.AELE 4. MJFE

Fig 16 plate B

1.TIPE 2. CALE
3.MILE 4. PDPE

Fig.17 Plate C

1.NLPE 2.PUPUE
3.BGFE 4. PUPRE

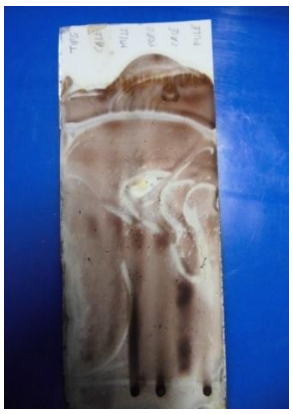

Fig 18. Plate D

1.standard 2. MJLE
3.MPFE 4. CASE

1. SCSE 2.PAPE 3.BGLE 4.CSPE

Fig.19. Plate E

TLC Chromatograms of hydroethanolic extracts

Fig 20. Plate F

1. Standard 2.BGFW 3. PAPW
4. NLPW 5. AEFW

Fig. 21. Plate G

1.PAPW 2.BGFW
3. SCSW 4. MPFW

Fig 22 .plate H

1. Standard 2. CALW
3. PULW 4. PUPUW

Fig. 23. Plate I

1.MILW 2. PUPRW
3.TIPW 4. CSPW

1. BGLW 2. AELW 3.CASW 4.MJFW 5.MJLW

Fig. 24. Plate J

TLC Chromatograms of aqueous extracts

R_f values of pinitol in the chromatograms of hydroethanolic extracts

Table 10

S no	Sample	Major spots	R_f value
1	Standard	Dark brown	0.33
2	PULE	Dark brown	0.33
3	AELE	Brown	0.32
4	MJFE	brown	0.33

Table 11

S no	Sample	Major spot	R_f value
1	TIPE	Brown	0.34
2	CALE	Dark brown	0.33
3	MILE	Brown	0.33
4	PDPE	Brown	0.33

Table 12

S no	Sample	Major spot	R_f value
1	TIPE	Brown	0.32
2	PUP_UE	Brown	0.32
3	BGFE	Brown	0.33
4	PUP_RE	Dark brown	0.33

Table 13

S no	Sample	Major spot	R_f value
1	Standard	Light brown	0.41
2	MJLE	Light brown	0.42
3	MPFE	Light brown	0.41
4	CASE	Light brown	0.41

Tables 14

S no	samples	Major spot	R_f value
1	SCSE	Brown	0.45
2	PAPE	Brown	0.45
3	BGLE	Brown	0.45
4	CSPE	Brown	0.45

R_f values of pinitol in the chromatograms of aqueous extracts

Table 15

S no	samples	Major spot	Rf values
1	Standard	Dark brown	0.35
2	BGFW	Dark brown	0.35
3	PAPW	Brown	0.36
4	NLPW	Brown	0.35
5	AEFW	Dark brown	0.36

Table 16

S no	Samples	Major spot	R_f values
1	PAPW	Brown	0.42
2	BGFW	Brown	0.42
3	SCSW	Dark brown	0.41
4	MPFW	Dark brown	0.42

Table 17

S no	Samples	Major spot	R_f values
1	Standard	Dark brown	0.45
2	CALW	Dark brown	0.46
3	PULW	Dark brown	0.46
4	PUP_UW	Dark brown	0.46

Table 18

S no	Samples	Major spots	R_f values
1	MILW	Brown	0.51
2	PUP_RW	Brown	0.51
3	TIPW	Brown	0.50
4	CSPW	Brown	0.50

Table 19

S no	Samples	Major spots	R_f values
1	BGFW	Brown	0.44
2	AELW	Brown	0.45
3	CASW	Brown	0.45
4	MJLW	Brown	0.44
5	MJFW	Brown	0.44

4.4 Treatment of plant extracts with papain enzyme

The sample solution (10 mg in 1ml) was treated with 5 mg of the enzyme. it was observed that precipitate was formed during the addition of enzyme with the extracts which may be probably due to the degradation of any proteineous matter in the extracts. It was observed that the amount of formation of residue was more in the aqueous extracts compared to that of hydroethanolic extracts indicating that the aqueous extracts contained more proteineous matter. The residue which was separated was observed for the absence of sugar alcohol molecule, hence the filtrate was taken for the further studies.

4.5 TLC analysis for the enzyme treated extracts

The filtrate of the plant extracts treated with enzyme were subjected to TLC examination using chloroform-methanol-water (6:3.5:0.5) solvent system. The enzyme treated extracts were compared with standard pinitol. Pinitol in the enzyme treated extracts was identified by the presence of brown spot on the chromatogram on spraying with ammonical silver nitrate. The developed TLC plates showed two spots, one corresponding to pinitol and the other due to enzyme. It was observed that the spot due to enzyme was below that of the standard. All the plant extracts were found to have both the spots which indicate the presence of pinitol even after the addition of enzyme to the extracts. The developed chromatograms are illustrated in the figure 26 to 30. The R_f are listed in the tables19 to 23. Sample spots are numbered from left to right. Based on the intensity of the spots due to pinitol, 12 samples were chosen for UV analysis to ascertain the nature of the enzyme treated extract concentrate.

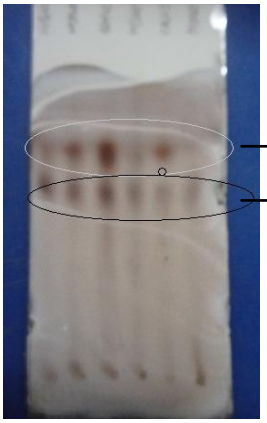

Fig.25 Plate K

1.PUPRE+E 2. MJFE+E 3. SCSE+E
4. MPFE+E 5. PAPE+E

Fig. 26. Plate L

1. NLPW+E 2. CASW+E 3. BGFW+E
4. TIPW+E 5. PAPW+E

Fig. 27 Plate M

1.AELW+E 2. PULW+E 3. MPFW+E
4. BGLW+E 5. MJFW+E 6. SCSW+E

Fig. 28. Plate N

1.CSPW+E 2.CALW+E 3.PUPUW+E
4.CSPE+E 5.MJLE+E 6.BGFE+E

Fig.29 Plate O

1.BG LE+E 2.PULE+E 3.AELE+E 4.CASE+E 5.CALE+E 6.TIPE+E

TLC chromatogram of enzyme treated plant extracts

R_f values of pinitol and enzyme in the Chromatograms of enzyme treated extracts

Table 20

S no	Samples	Major spots	R_f values
1	PUP_RE+E	Brown Brown	0.64 0.52
2	MJFE+E	Brown Brown	0.64 0.52
3	SCSE+E	Brown Brown	0.65 0.52
4	MPFE+E	Brown Brown	0.64 0.53
5	PAPE+E	Brown Brown	0.64 0.52
6	PUP_UE+E	Brown Brown	0.64 0.52

Table 21

S no	Samples	Major spots	R_f values
1	NLPW+E	Dark Brown Brown	0.41 0,27
2	CASW+E	Light Brown Brown	0.42 0.27
3	BGFW+E	Dark Brown Brown	0.43 0.27
4	TIPW+E	Dark Brown Brown	0.43 0.26
5	PAPW+E	Dark Brown Brown	0.41 0.27
6	PUP_RW+E	Brown Brown	0.42 0.26

Table 22

S no	Samples	Major spots	R_f values
1	AELW+E	Brown Brown	0.35 0.20
2	PULW+E	Brown	0.19
3	MPFW+E	Brown	0.20
4	BGLW+E	Brown Brown	0.34 0.20
5	MJFW+E	Brown Brown	0.35 0.20
6	SCSW+E	Brown Brown	0.35 0.19

Table 23

S no	Samples	Major spots	R_f values
1	CSPW+E	Brown Brown	0.43 0.32
2	CALW+E	Brown Brown	0.42 0.33
3	PUP_UW+E	Light Brown Brown	0.43 0.32
4	CSPE+E	Brown Brown	0.42 0.33
5	MJLE+E	Brown Brown	0.43 0.32
6	BGFE+E	Light Brown Brown	0.43 0.32

Table 24

S no	Samples	Major spots	R_f values
1	BGLE+E	Brown Brown	0.72 0.62
2	PULE+E	Brown Brown	0.72 0.63
3	AELE+E	Brown Brown	0.72 0.63
4	CASE+E	Light Brown Light Brown	0.72 0.63
5	CALE+E	Brown Brown	0.72 0.62
6	TIPE+E	Light Brown Brown	0.73 0.63

4.6 UV analysis of the enzyme treated plant extracts

The enzyme treated extract samples were concentrated and macerated with methanol and they were analysed by ultraviolet spectroscopy. The fresh extracts, pinitol and papain were also analysed by UV spectroscopy. The fresh standard treated with enzyme was also analysed. The UV spectrum of the enzyme treated extracts was compared with that of fresh extracts. The UV spectral analysis revealed that the enzyme interacts with the constituent of the plant extracts since the UV spectral contom has been altered for the enzyme treated samples. The spectra of standard and the enzyme confirmed the presence of pinitol in the extracts after enzyme treatment. The figure 31 to 44 illustrates the UV spectra of the samples. The UV spectra shows the flattening of the peaks of the original extracts which indicates that the flattening of the peaks may be due to the interaction of the constituents of the extracts with the enzyme. The degradation of the drug by papain enzyme and its analysis by spectrophotometer was reported in the recent study by Hitesh *et al,.* Hence the interaction between the enzyme and the extracts may have lead to degradation of the constituents of the plant extracts. This nave lead to the bathochromic shift in the UV spectra of enzyme treated extracts. Moreover, the peak values of the enzyme treated extracts confirms the interaction.

UV spectra for the sample

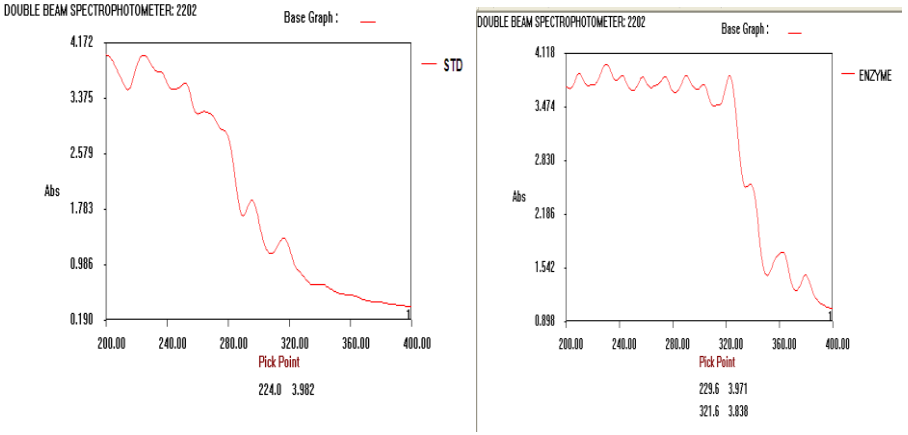

Fig. 30 (a) UV spectra of standard pinitol (b) UV spectra of enzyme

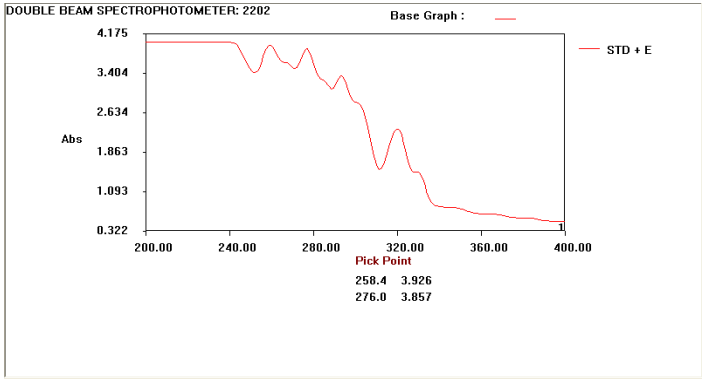

Fig. 31 UV spectra of standard pinitol treated with enzyme

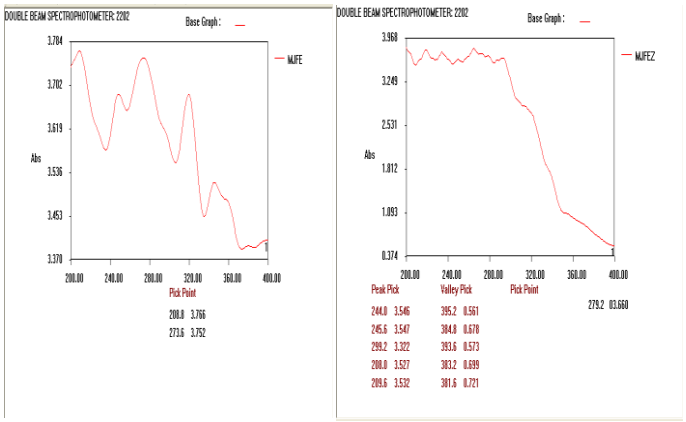

Fig 32 (a) UV spectra of ethanolic extracts flowers of *Mirabilis jalapa* (MJFE) (b) enzyme treated extracts

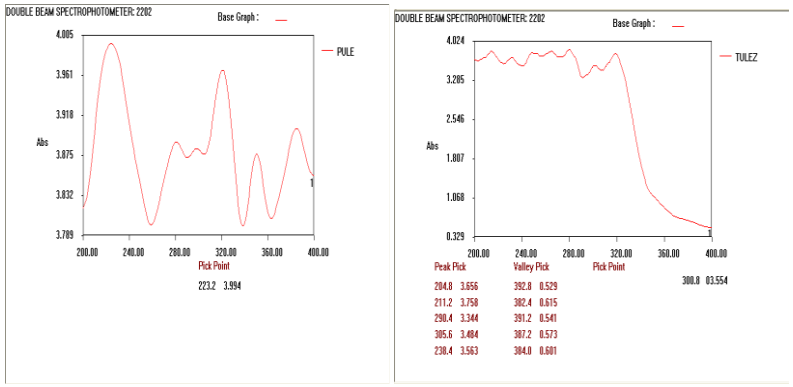

Fig 33 (a)UV spectra of ethanolic extract of leaves of *Psidium guajava* (PULE) b) enzyme treated extracts

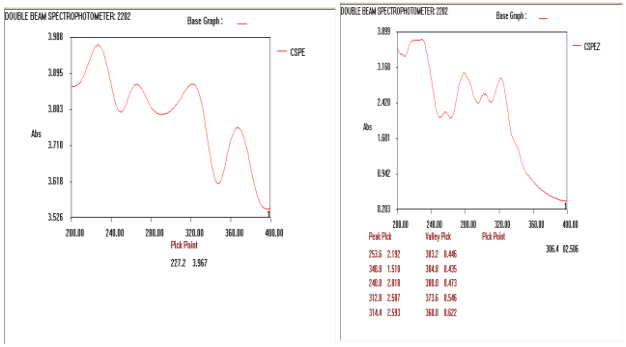

Fig 34 (a) UV spectra of ethanolic extract of peels of *Citrus sinensis* (CSPE) (b) enzyme treated extracts

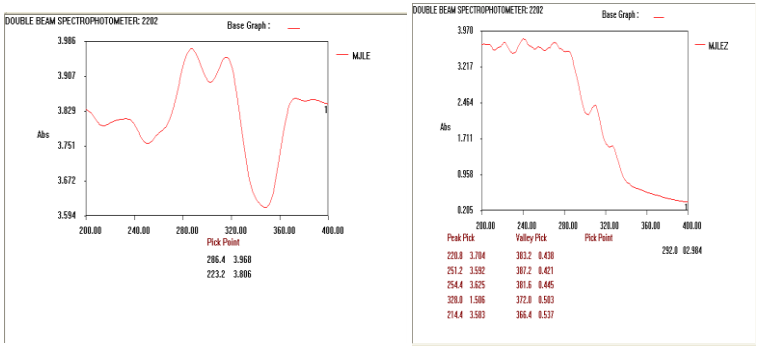

Fig.35 (a) UV spectra of ethanolic extracts of leaves of *Mirabilis jalapa (*MJLE*)* (b) enzyme treated extracts

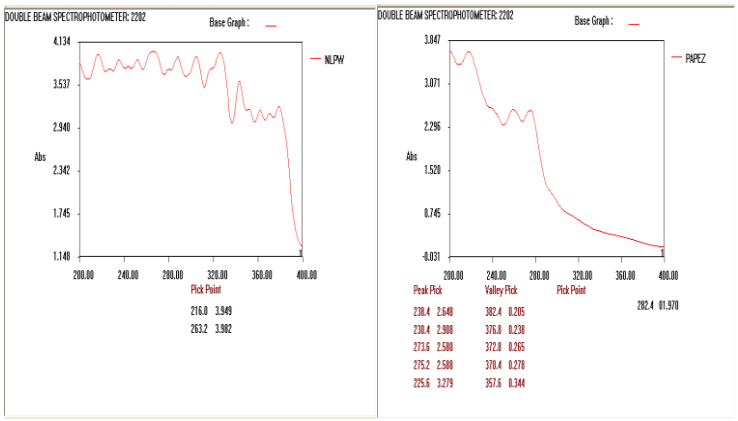

Fig.36 (a) UV spectra of aqueous extract of peels of *Nephellium lappceum* (NPLW) (b) enzyme treated extracts

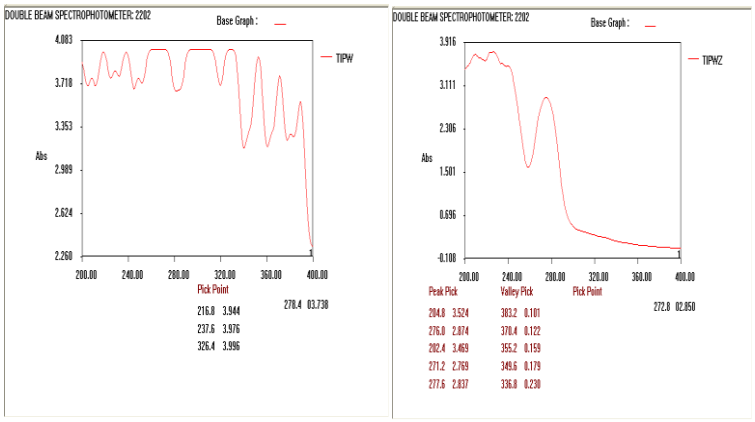

Fig. 37(a) UV spectra of aqueous extract of peels of *Tamarindus indica* (TIPW) (b) enzyme treated extracts

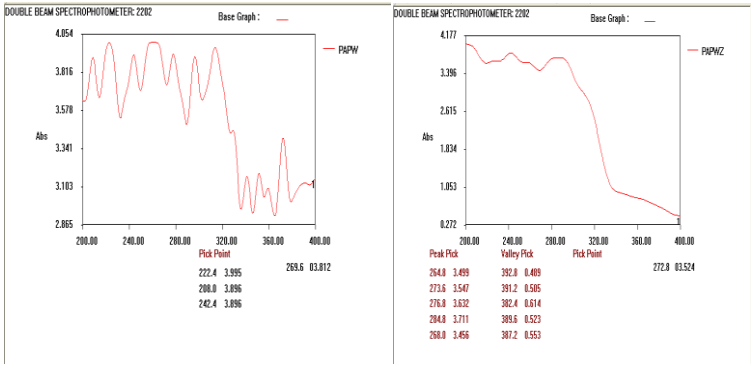

Fig.38 (a) UV spectra of aqueous extract of peels of *Punica granatum* (PAPW) (b) enzyme treated extracts

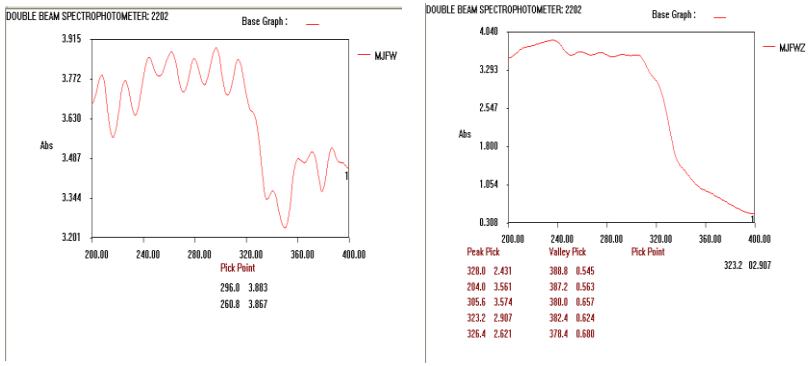

Fig. 39(a) UV spectra of aqueous extract of flowers of *Mirabilis jalapa* (MJFW) (b) enzyme treated extracts

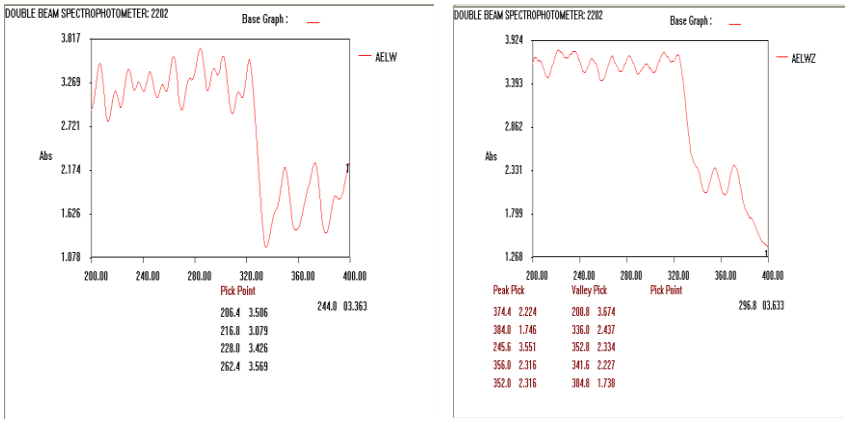

Fig.40 (a) UV spectra of aqueous extract of leaves of *Abelmoschous esculentus* (AELW) (b) enzyme treated extracts

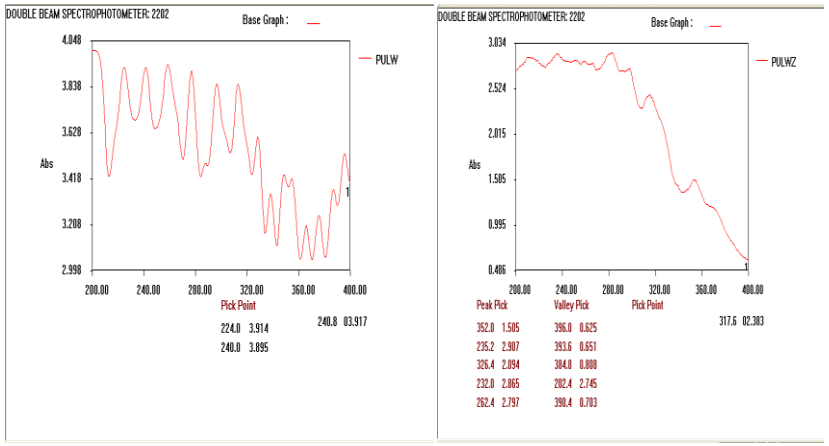

Fig.41 (a) UV spectra of aqueous extract of leaves of *Psidium guajava* (PULW) (b) enzyme treated extracts

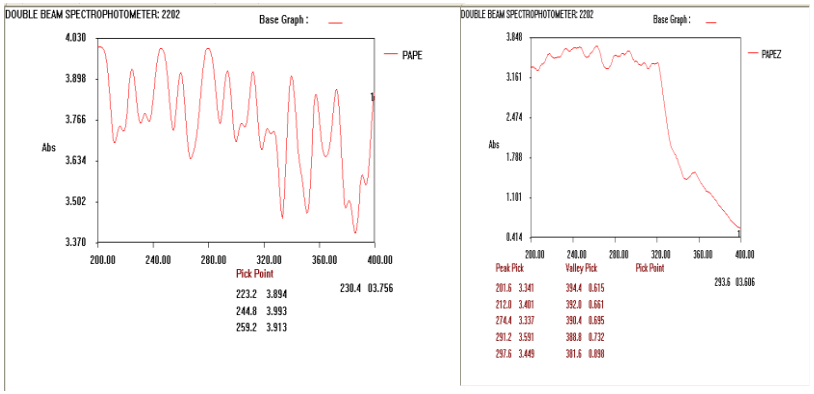

Fig42 (a) UV spectra of ethanolic extract of peels of *Punica granatum* (PAPE) (b) enzyme treated extracts

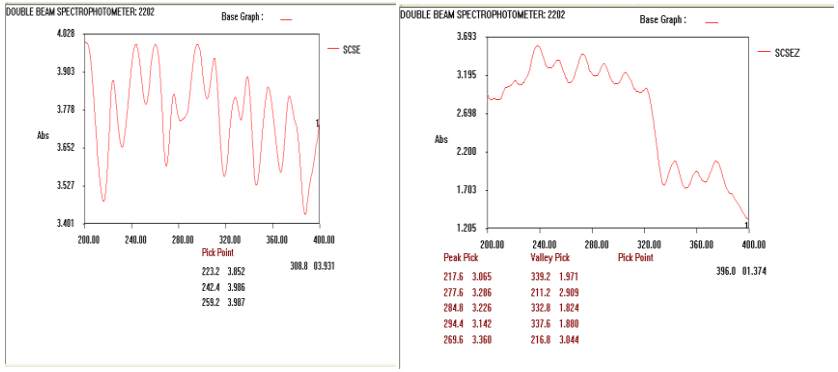

Fig (a) 43 UV spectra of ethanolic extract of seeds of *Syzgium cumini* (SCSE) (b) enzyme treated extracts

4.7 Identification and Quantification of pinitol by HPLC

The amount of pinitol in the extracts was ascertained by HPLC over an amine column using acetonitrile-water mixture as the solvent. Table 24 gives details of sample code, retention time and area obtained from the chromatogram and amount of pinitol quantified. The HPLC chromatograms of the samples are illustrated in figures 46 to 59.

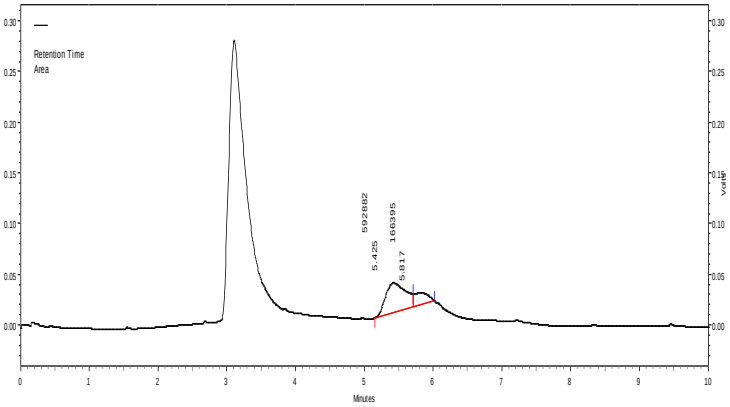

Fig 44 Chromatogram of ethanolic extract of peels of ripened *Psidium guajava*

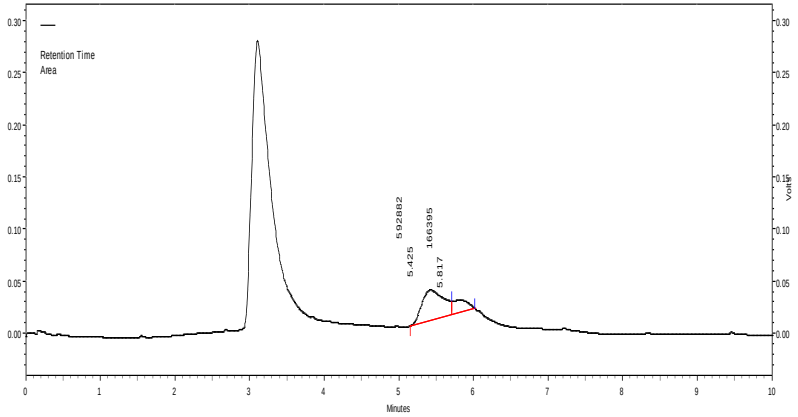

Fig 45 Chromatogram of ethanolic extract of flowers of *Bougainvillea glabra*

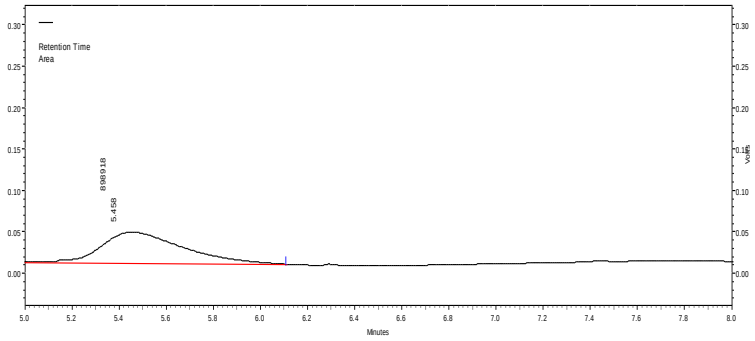

Fig 44a Expanded chromatogram of PUP_RE

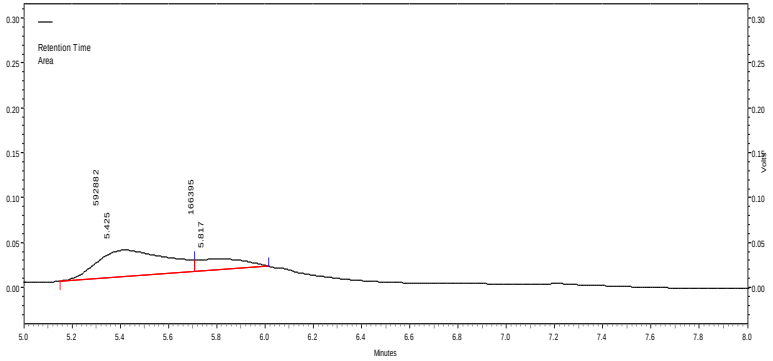

Fig 45a Expanded chromatogram of BGFE

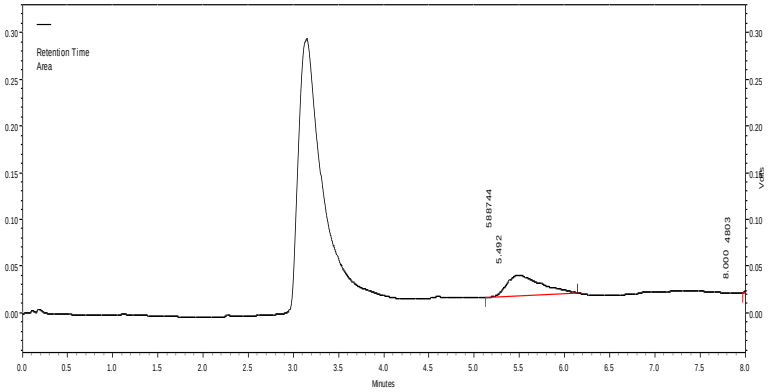

Fig 46 Chromatogram of aqueous extract of leaves of *Abelmoschous esculentus*

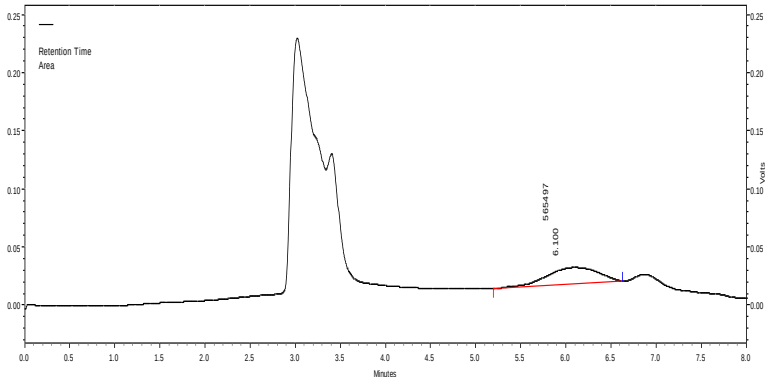

Fig 47 Chromatogram of aqueous extract seeds of *Cicer arientu*m

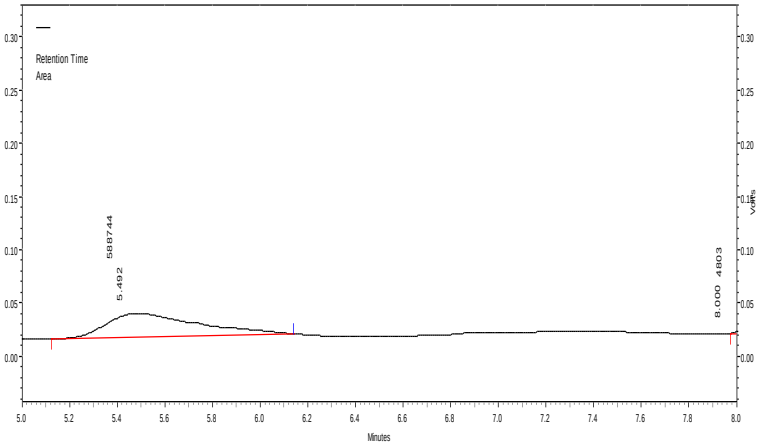

Fig 46a Expanded chromatogram of AELW

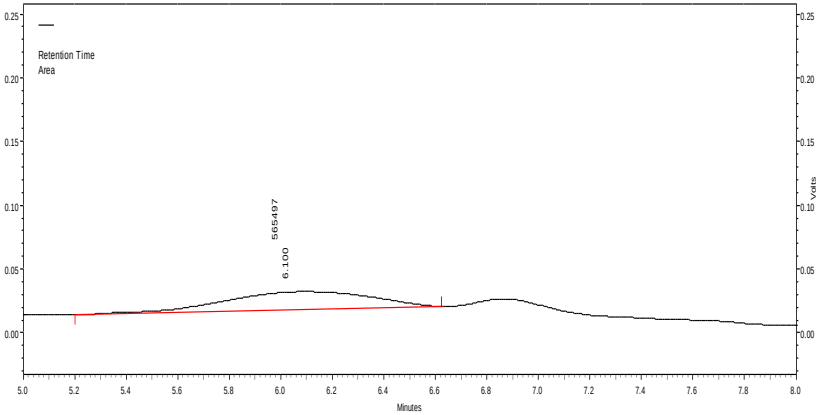

Fig 47a Expanded chromatogram of CASW

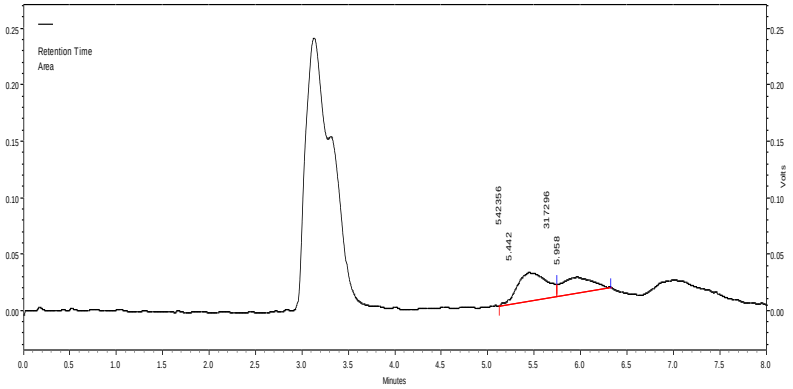

Fig 48 Chromatogram of aqueous extract of peels of *Citrus sinensis*

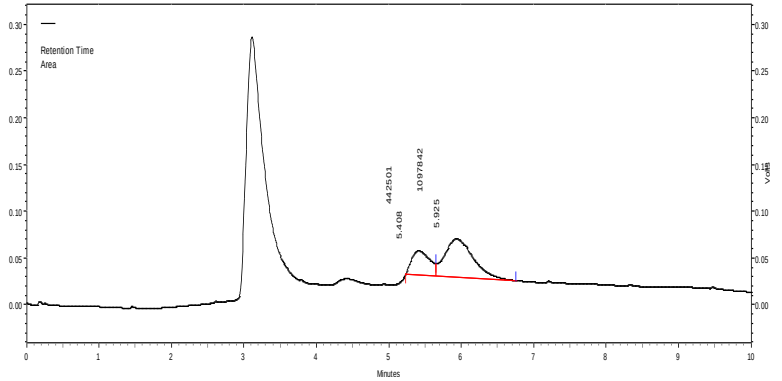

Fig 49 Chromatogram of ethanolic extract of peels of *Punica granatum*

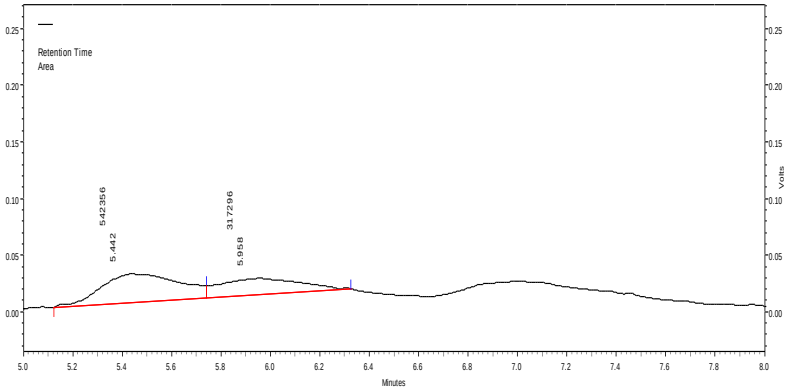

Fig 48a Expanded Chromatogram of CSPW

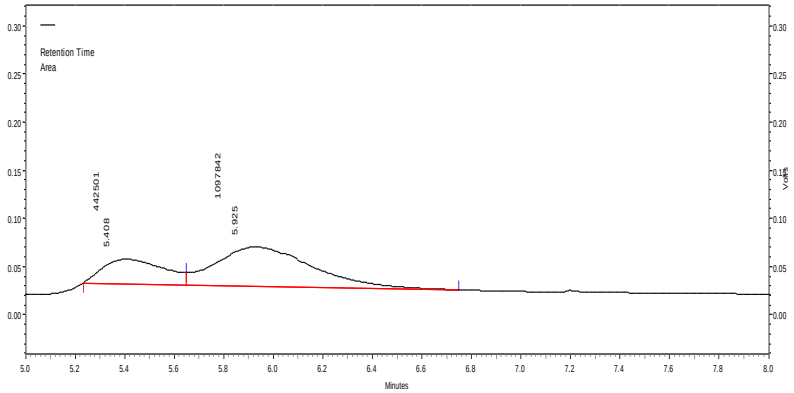

Fig 49a Expanded Chromatogram of PAPE

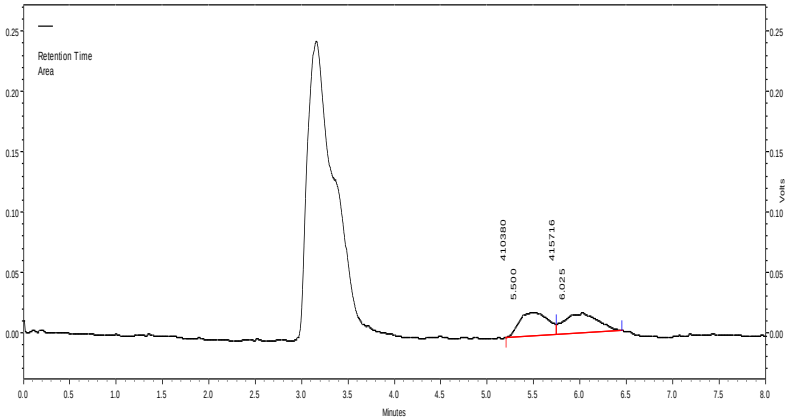

Fig 50 Chromatogram of aqueous extract of seeds of *Syzgium cumini*

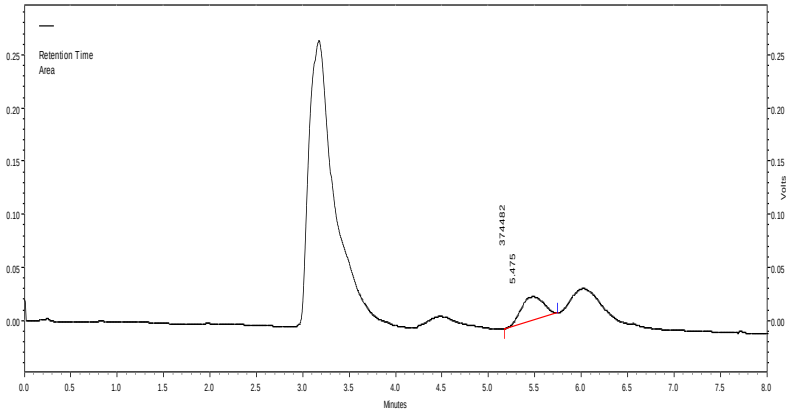

Fig 51 Chromatogram of aqueous extract of peels of *Punica granatum*

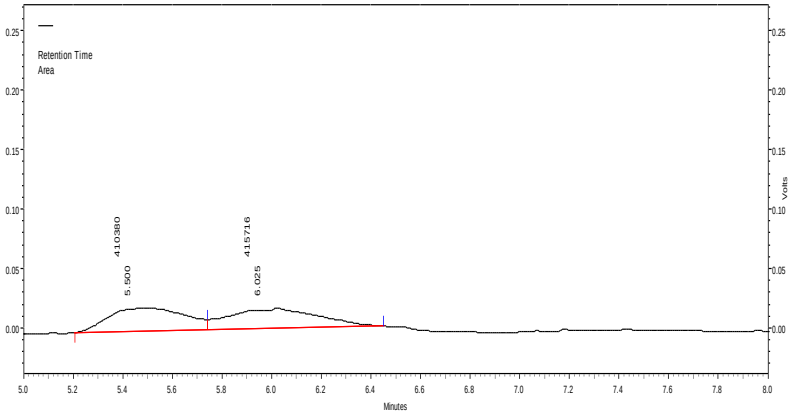

Fig 50a Expanded Chromatogram of SCSW

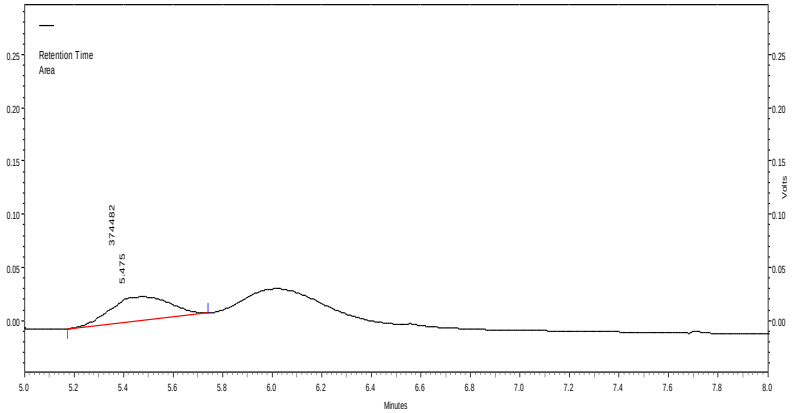

Fig 51a Expanded chromatogram of PAPW

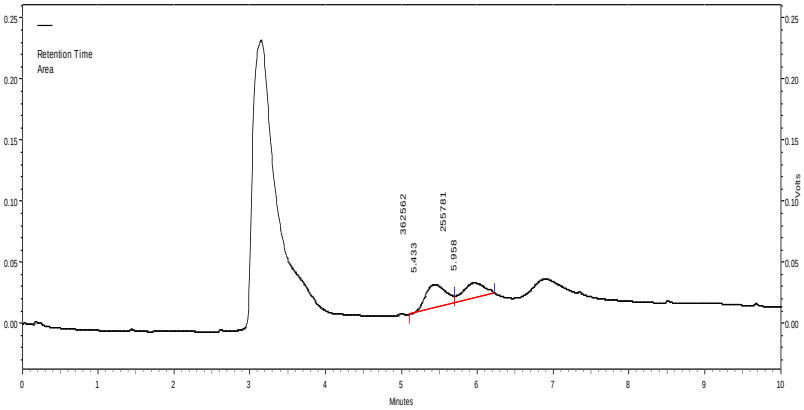

Fig 52 Chromatogram of ethanolic extract of seeds of *Syzygium cumini*

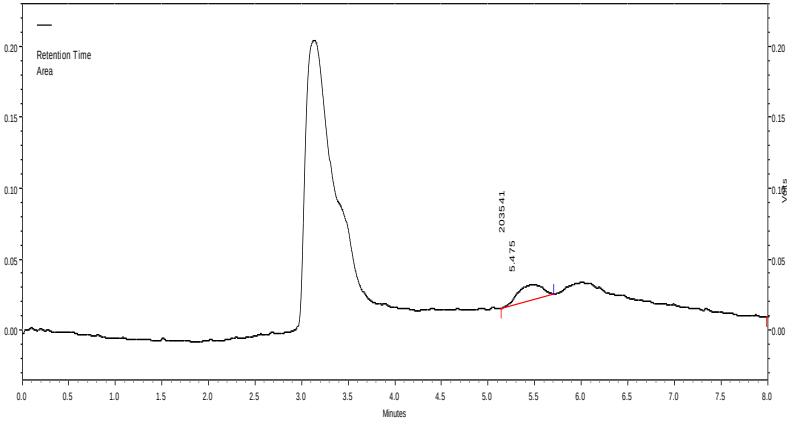

Fig 53 Chromatogram of aqueous extract of peels of unripened *Psidium guaja*

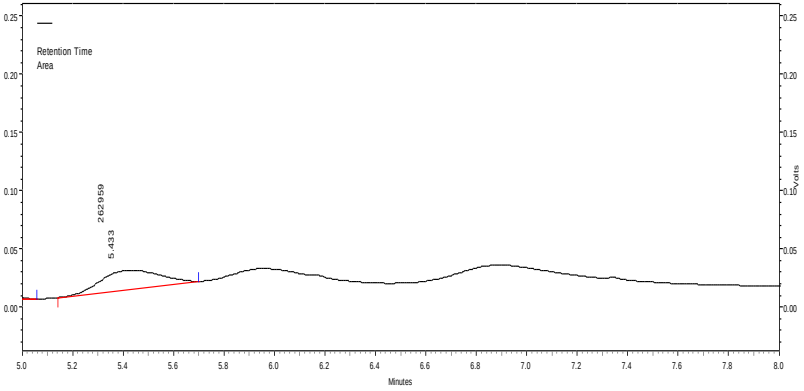

Fig 52a Expanded Chromatogram of SCSE

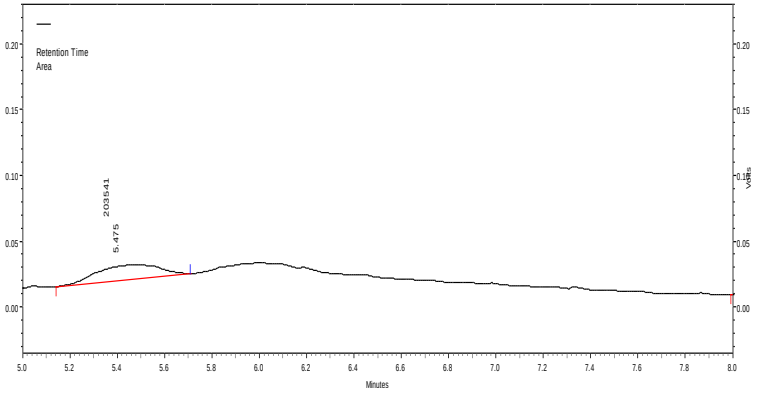

Fig 53a Expanded Chromatogram of PUP_UW

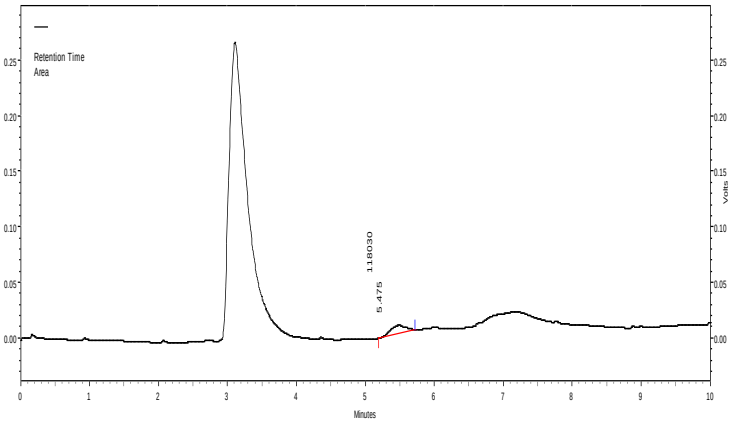

Fig 54 Chromatogram of ethanolic extract of leaves of *Cicer arientum*

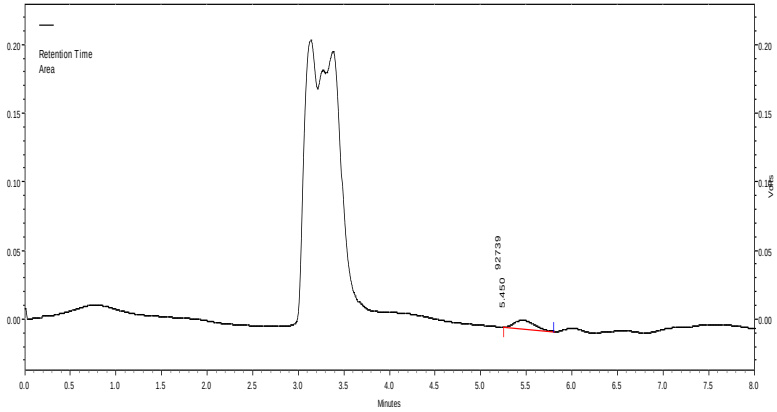

Fig 55 Chromatogram of aqueous extract of leaves of *Psidium guajava*

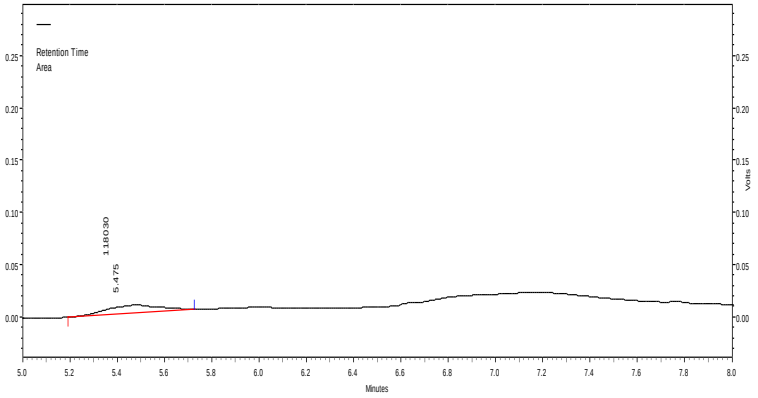

Fig 54a Expanded Chromatogram of CALE

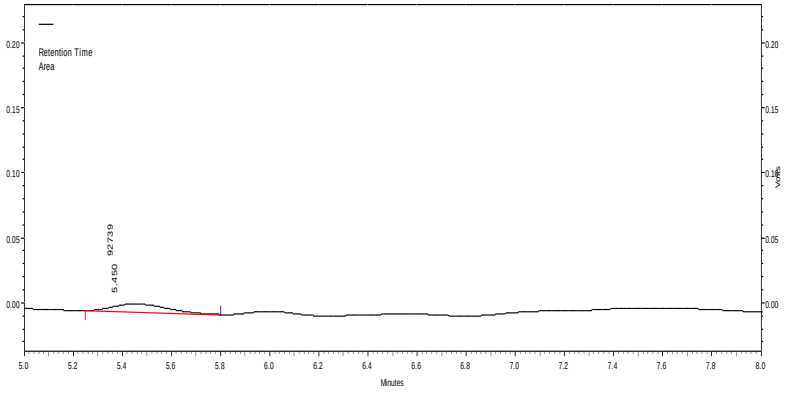

Fig 55a Expanded Chromatogram of PULW

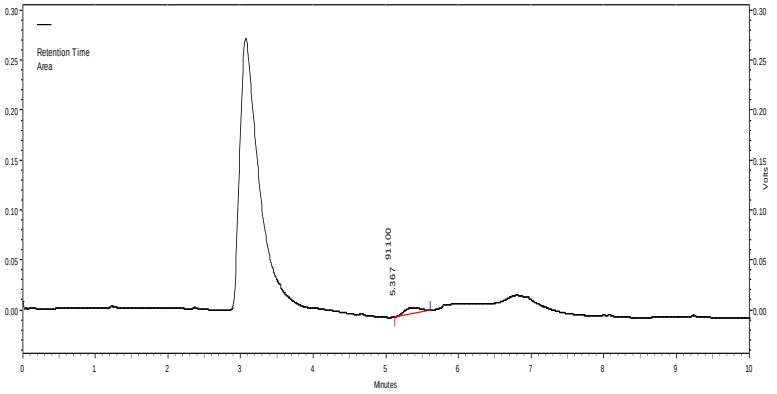

Fig 56 Chromatogram of ethanolic extract of peels of unripened *Psidium guajava*

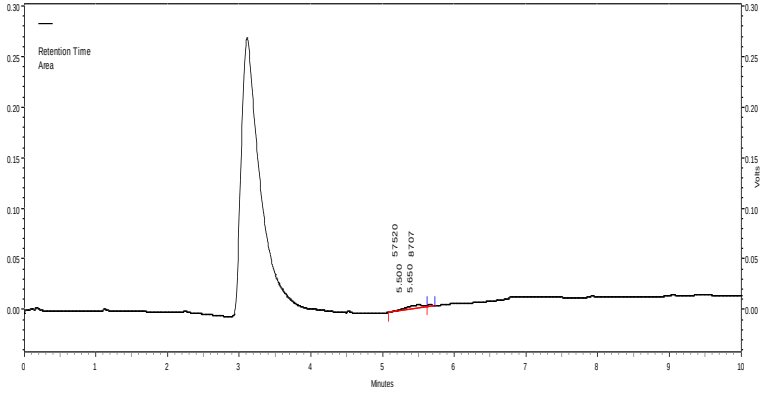

Fig 57 Chromatogram of ethanolic extract of peels of *Nephellium lappaceum*

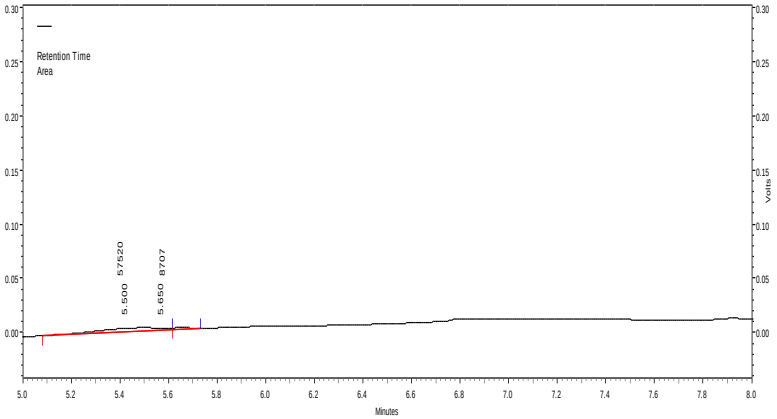

Fig 56a Expanded Chromatogram of PUP_UE

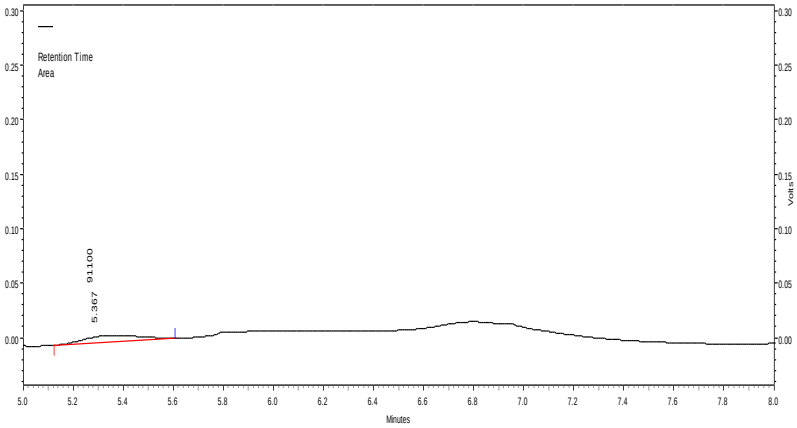

Fig 57a Expanded Chromatogram of NLPE

The HPLC chromatograms of sample extracts were compared with that of standard at various concentrations. Most of the samples were found to possess concentration within the range 1000ppm to 10,000ppm of the active molecule pinitol. The merged chromatograms are shown in the figures 59 to 73

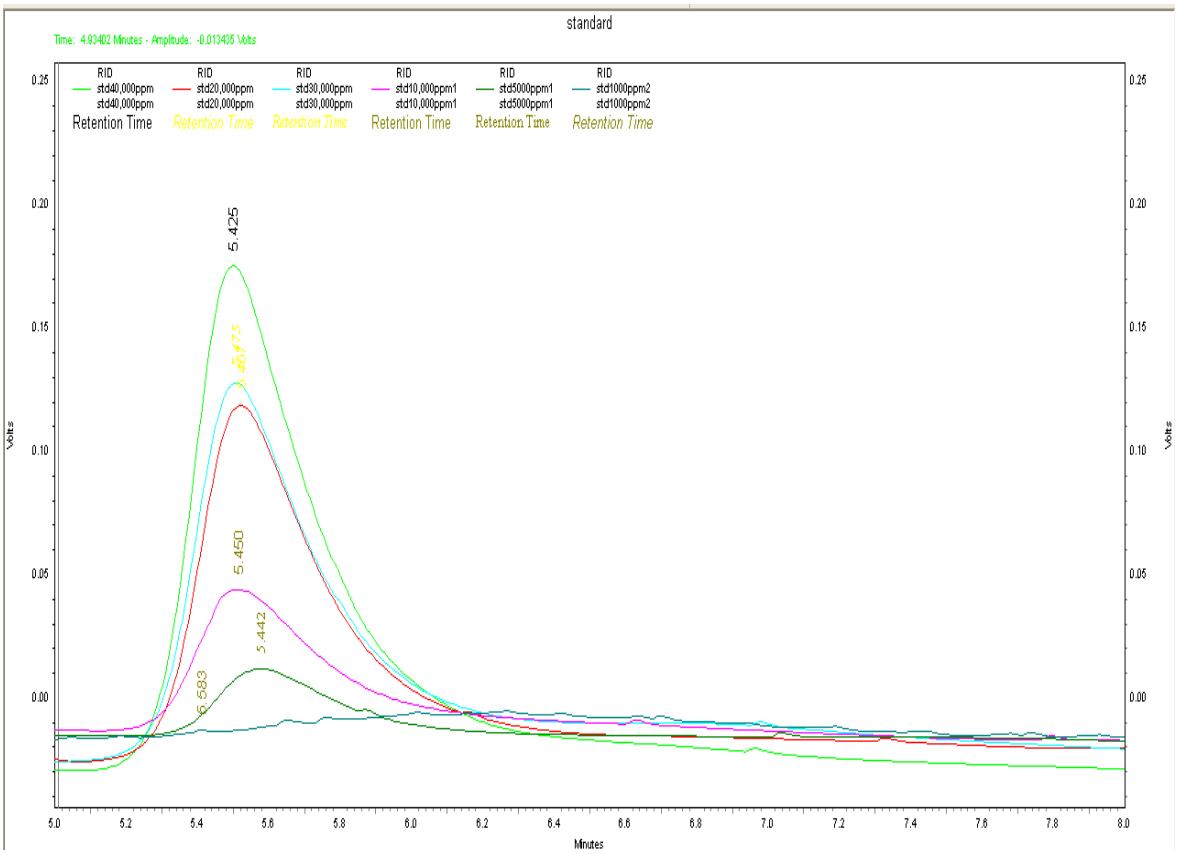

Fig 58 Merged Chromatogram of standards of varying concentration

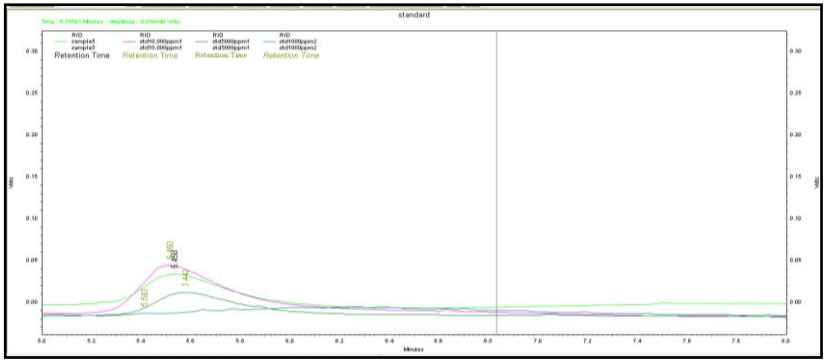

Fig 59 Comparison of HPLC chromatogram of Ethanolic extract of peels of ripened *Psidium guajava* (PUP_RE) with standard

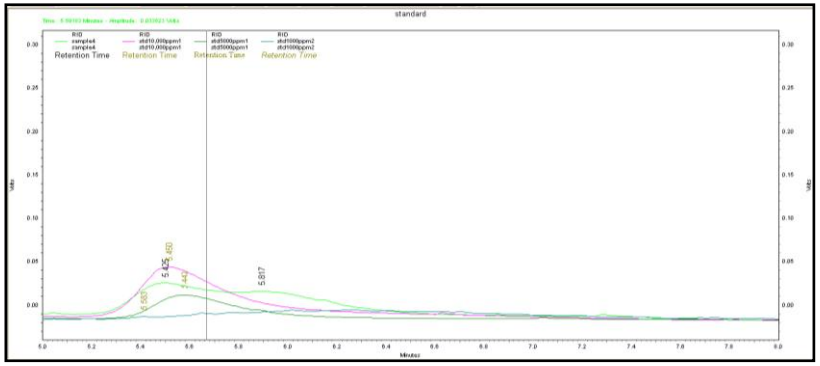

Fig 61. Comparison of HPLC chromatogram of Aqueous extract of Leaves of *Abelmoschus esculentus* (AELW) with standard

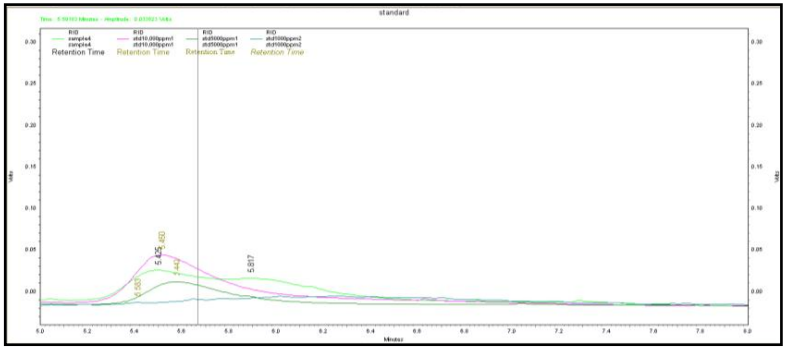

Fig 60. Comparison of HPLC chromatogram of Ethanolic extract of Flowers of *Bougainvillea glabra* (BGFE) with Standard

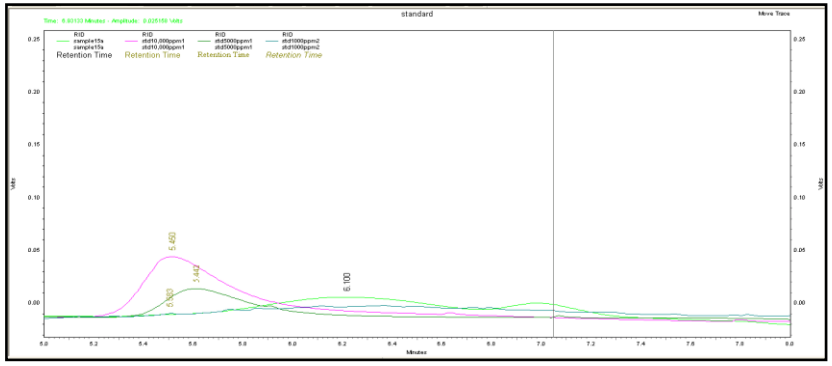

Fig 62. Comparison of HPLC chromatogram of Aqueous extract of Seeds of *Cicer arientum* (CASW) with Standard

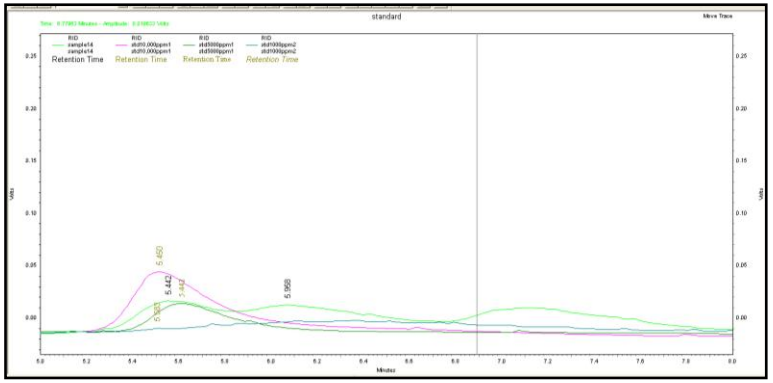

Fig 63. Comparison of HPLC chromatogram of Aqueous extract of Peels of *Citrus sinensis* (CSPW) with Standard

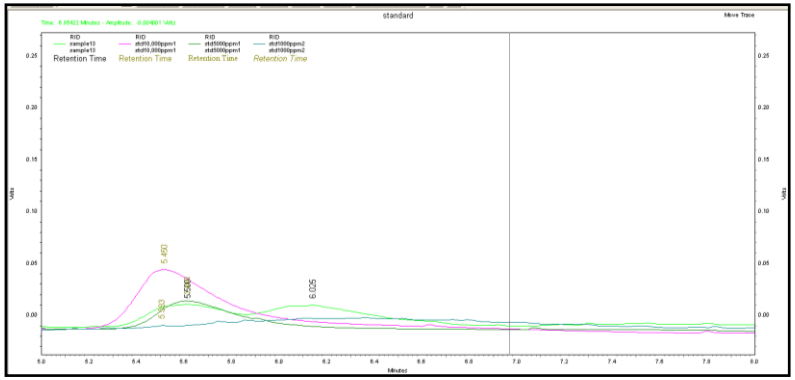

Fig 65. Comparison of HPLC chromatogram of Aqueous extract of Seeds of *Syzygium cumin*i (SCSW) with Standard

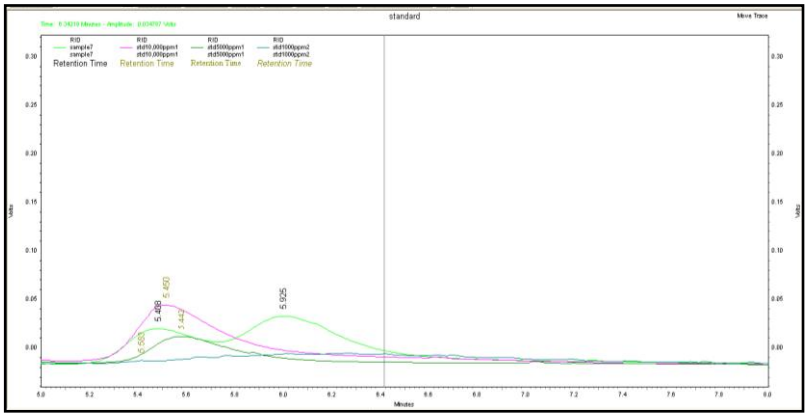

Fig.64 Comparison of HPLC chromatogram of Ehanolic extracts of Peels of *Punica granatum* (PAPE) with Standard

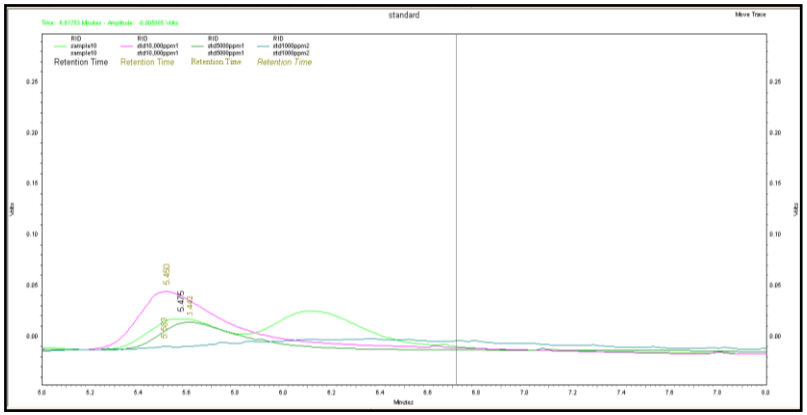

Fig 66. Comparison of HPLC chromatogram of aqueous extract of Peels of *Punica granatum* (PAPW) with Standard

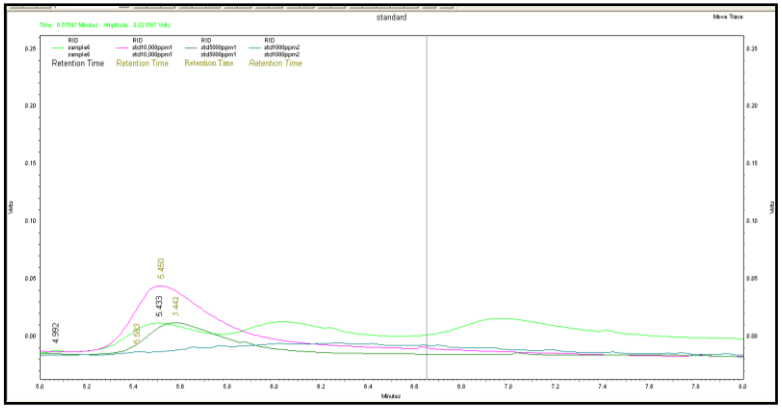

Fig 67 Comparison of HPLC chromatogram of Ethanolic extracts of Seeds of *Syzygium cumini* (SCSE) with Standard

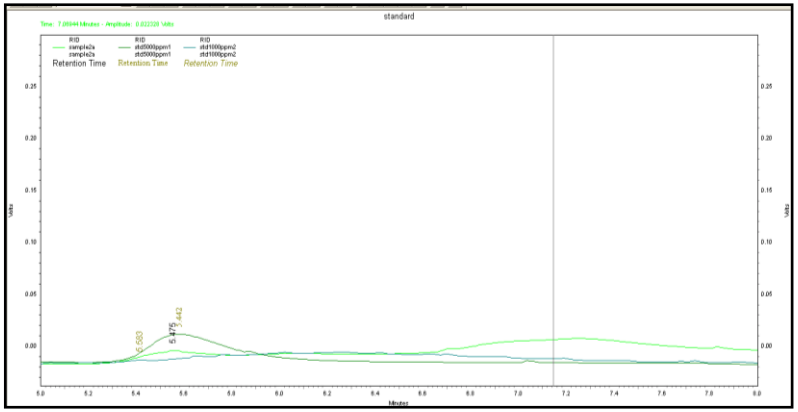

Fig 69. Comparison of HPLC chromatogram of Ethanolic extracts of Leaves of *Cicer arientum (*CALE) with Standard

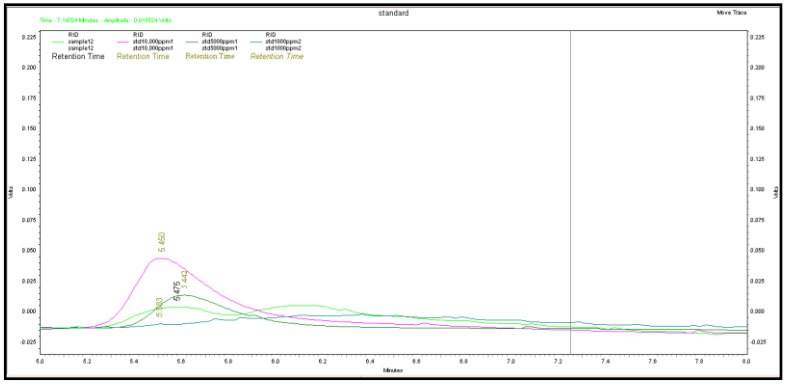

Fig 68 Comparison of HPLC chromatogram of Aqueous Extract of of peels of unripened *Psidium guajava (*PUP_UE*)* with Standard

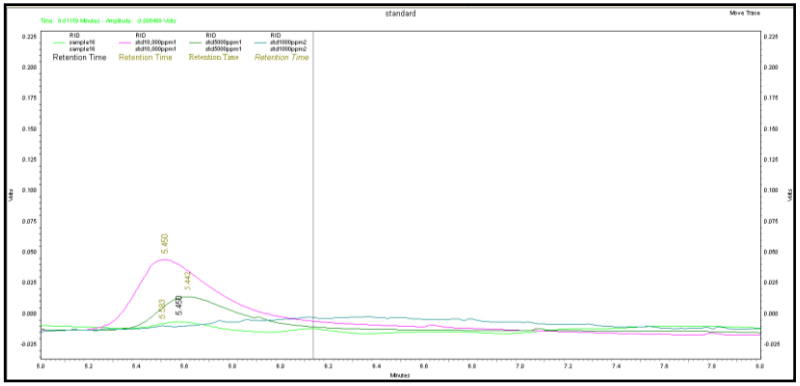

Fig 70.Comparison of HPLC chromatogram of Aqueous Extract of Leaves of *Psidium guajava (*PULW*)* with Standard

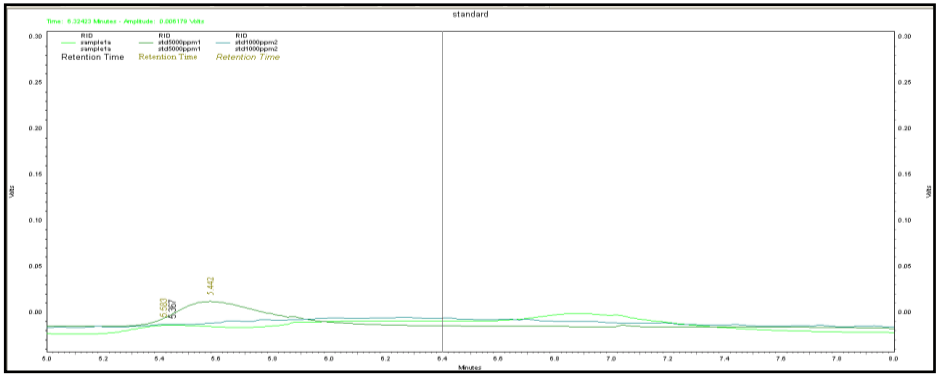

Fig 71 Comparison of HPLC chromatogram of Ethanolic extracts of peels of unripened *Psidium guajava (*PUP_UE) with Standard

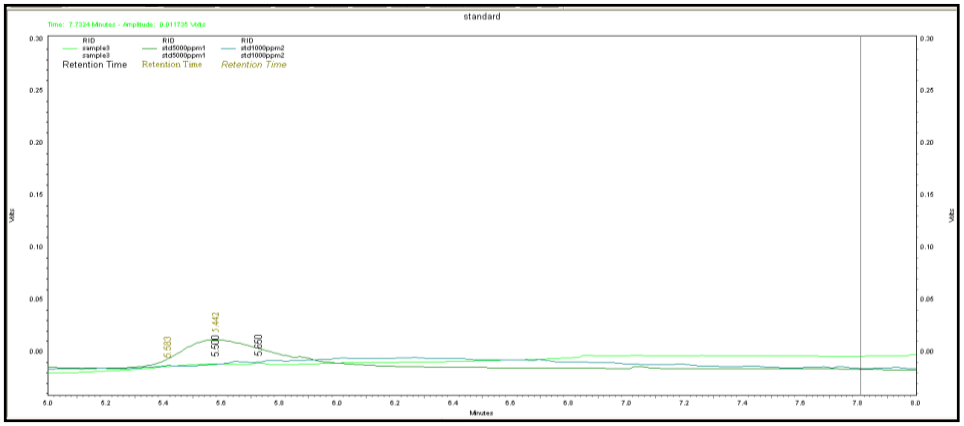

Fig .72 Comparison of HPLC chromatogram of Ethanolic extract of peels of *Nephellium lappaceum* (NLPE) with Standard

The quantity of pinitol in the selected plant extracts was calculated from the calibration curve (Fig 74) drawn for the standard concentrations taken and using the slope and the intercept values and area for each samples, the quantity of pinitol in 20µl of the sample was calculated. From this value the concentration of pinitol per milligram was calculated.

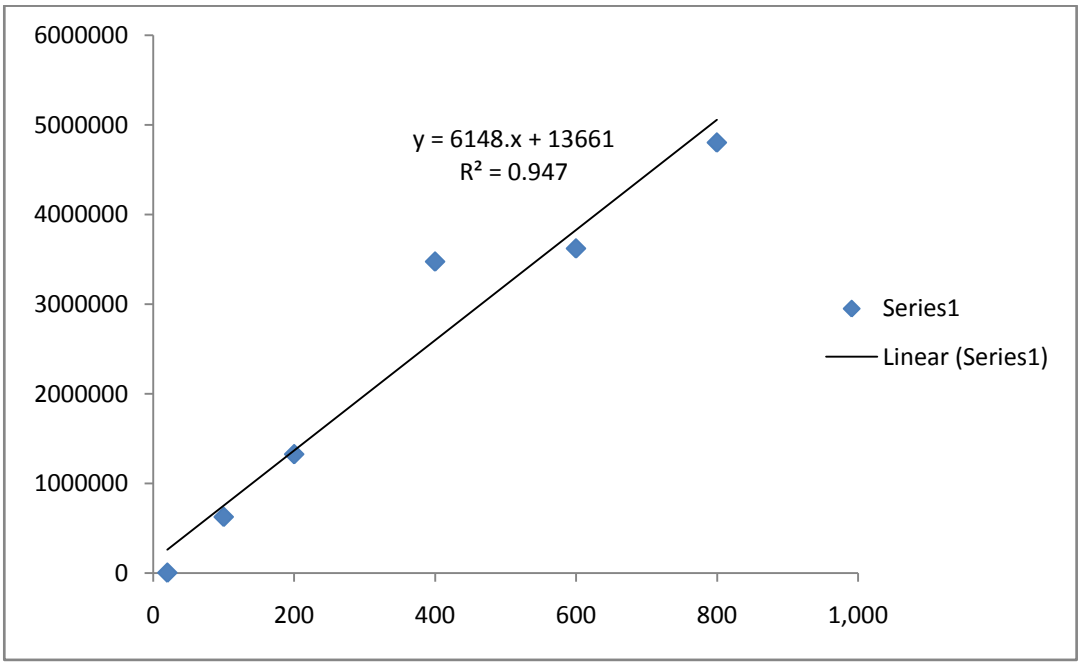

Fig 73 Calibration Graph

Table 25 Quantity of pinitol in the extracts

S No.	Sample code	Retention time(min)	Area(mAU)	Quantity of Pinitol(µg) in 20µl of extract	Quantity of Pinitol (µg) per mg of extract
1	PUP_RE	5.458	898918	143.99	239.98
2	BGFE	5.425	592882	94.21	157.02
3	AELW	5.492	588744	93.53	155.90
4	CASW	6.100	565497	89.75	149.60
5	CSPW	5.442	542356	85.99	143.32
6	PAPE	5.408	442501	69.75	116.25
7	SCSW	5.500	410380	64.53	107.55
8	PAPW	5.475	374482	58.69	97.81
9	SCSE	5.433	362562	56.75	94.58
10	PUP_UW	5.475	203541	30.88	51.47
11	CALE	5.475	118030	16.98	28.29
12	PULW	5.450	92739	12.86	21.44
13	PUP_UE	5.367	91100	12.59	20.99
14	NLPE	5.500	57520	7.13	11.89

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

The present study titled **"Identification and HPLC Quantification of an Antidiabetic Cyclitol Molecule in Selected Plant Extracts and its Isolation by an Enzyme Mediated Method"** was undertaken to identify the antidiabetic molecule pinitol, an antidiabetic cyclitol molecule in the of 13 chosen plants extracts and to identify a simple method of its isolation using the protease enzyme, papain.

The first chapter is a brief introduction to the study. Review of literature is presented in the second chapter. It covers review of recent reports in literature on the following aspects:

- ❖ Medicinal potential of chosen plants
- ❖ Protein degradation by papain enzyme
- ❖ Isolation of pinitol from plant extracts

The third chapter deals with the methodology adopted for the study. The results are presented in the fourth chapter.

The study exposed the following revelations

- ✓ Almost all plant extracts contain pinitol and the molecule was present in higher yield in 14 extracts as identified by TLC analysis and this was ascertained by HPLC method.
- ✓ Among the chosen plant extracts the ethanolic extract of peels of ripened Guava (*Psidium guajava*) was found to contain the higher quantity of the cyclitol molecule
- ✓ The presence of pinitol was ascertained after the degradation of proteins in ethanolic and aqueous extracts using protease enzyme, papain and it was found that pinitol can be isolated by this simple procedure.

This being a preliminary study to identify a method of isolation of an antidiabetic molecule from plant extracts using papain enzyme, further optimization studies are needed to establish this enzyme assisted method. This study also provides evidence of the presence of a medicinally valuable molecule in the chosen plant extracts as ascertained by their HPLC.

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