Enzyme Assisted Isolation of an Antidiabetic Cyclitol from Extracts of *Pisonia grandis* R.Br.

NANDHINI .M

REG NO: 13PCH011

A Thesis submitted to
Avinashilingam Institute for Home Science and
Higher Education for Women University,
Coimbatore -641 043, Tamil Nadu, India

In partial fulfillment of the requirement for the
Master's Degree in Chemistry
March, 2015

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31.3.15
Signature of the
Supervisor

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Signature of the
Head of Department

Acknowledgement

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LIST OF ABBREVIATION

Rf	Retention factor
UV	Ultraviolet
TLC	Thin Layer Chromatography
HCl	Hydrochloric acid
NaOH	Sodium hydroxide
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid

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Introduction

1. INTRODUCTION

Plants and trees are an immediate source of medicines. The herb plants produce and contain a variety of chemical substances that act upon the body. Herbal medicines, referred to as herbalism or botanical medicine or phytomedicine 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.

Phytochemicals are naturally occurring and have biologically active chemical compounds in plants. Phytochemicals are protective and to prevent from disease. The herbal medicines are non-toxic, have no side effects and are easily available one.

Ayurveda is an Indian traditional medicine system that has been known for nearly 5000 years and still practised widely in India. This system of medicine has cure for numerous diseases. The Siddha medicine originated from South India especially from Tamilnadu, was developed by ancient sages called *Siddhars who* were imbued with divine power.

The Chinese and Japanese people, the people living near the Himalayan Mountains including Himachal Pradesh and Sikkim, as well as the neighbouring states of Tibet, Nepal and Bhutan largely use botanical medicine.

Pisonia grandis is a species of flowering tree in the *Bougainvillea* family*, Nyctaginaceae,* that is distributed throughout the coral cays of the Indian and Pacific Oceans. The species often dominates mature coral cay vegetation, growing in dense stands up to 20 metres tall. *Pisonia* wood is rather weak and soft and decays rapidly when the trees fall. The plant has analgesic, antipyretic, diuretic, wound healing, anti-diabetic, free radical scavenging, anti-inflammatory, anti-arthritic, and antimicrobial, hepatoprotective activity, anxiolytic activity and antiplasmodial properties. It is also commonly seen planted and reared in many households of the local areas of Coimbatore.

Fig.1 *Pisonia grandis* plant

Papaya is a short-lived perennial plant growing to 30 ft (9.14 m) high. Its hollow, herbaceous stem is usually unbranched. The deeply lobed, palmate leaves are borne on long, hollow petioles emerging from the stem apex. Papaya fruits are smooth skinned. They vary widely in size and shape, depending on variety and type of plant. The papaya fruit is about 88.8% water, 9.8 % carbohydrate, 0.8% fiber, 0.6% protein, 0.6% ash and 0.1% fat. A 100g (3.5 oz.) serving of papaya has 39 calories, compared to banana's 92 calories. Papaya also contain 16% more vitamin C than oranges and are a good source of vitamin A (about half of that contained in mango). Consumption of the fruit is reported to aid digestion because of the papain content.

Papain, is also known as **papaya proteinase I**, is a cysteine protease enzyme present in papaya (*Carica papaya*) and mountain papaya (*Vasconcelleacundinamarcensis*).

Fig.2 papaya plant

Papain is used for pain and swelling (inflammation) as well as fluid retention following trauma and surgery. It is used as a digestive aid and for treating parasitic worms, inflammation of the throat and pharynx, shingles (herpes zoster) symptoms, ongoing diarrhea, hay fever, runny nose, and a skin condition called psoriasis.

Papain is also used along with conventional treatments for tumors. Some people apply papain directly to the skin to treat infected wounds, sores, and ulcers.

In the manufacturing sector, papain is used in cosmetics, toothpaste, enzymatic soft contact lens cleaners, meat products and meat tenderizers Papain is also used in some toothpastes and mint sweets as a tooth whitener. It is also used for stabilizing and chill-proofing beer.

1.1 Objectives of the Study

- To isolate the antidiabetic molecule pinitol in extracts of *Pisonia grandis* after treatment with papain enzyme for easy isolation.
- To determine phytochemical constituents of various extracts of *Pisonia grandis* leaves, stems and roots
- To estimate the degradation of constituents in the extracts after enzyme treatment by UV spectrometer.

Review of Literature

2. REVIEW OF LITERATURE

Review of the recent literature pertaining to the present study is presented under the following headings. The review of literature covers the recent period from 2007 till date.

- Production of protease enzyme
- Production, reaction and applications of papain enzyme
- Production and application of bromelain enzyme
- Recent reports on the medicinal plant *Pisonia grandis* R.Br
- Recent reports on the isolation of antidiabetic molecule pinitol
- Quantification, phytochemical aspects and medicinal uses of pinitol from *Pisonia grandis* plant

Enzymes are present in the living organism as cells in minute amount and have a tendency to speed up the metabolic reactions (**Oyeleke and Oduwole, 2009**)

The protease enzymes breakdown proteins and have an important role in metabolic process and are used in the fields of detergents manufacture, leather industry, food industry, pharmaceutical industry, diagnostics, waste management and silver recovery (**Sathiya, 2013**)

2.1 Production of protease enzyme

A protease enzyme was isolated from the microbial organisms *Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus megaterium and Bacillus licheniformis.* The optimum temperature for the isolation of the protease enzyme is 50^0c as evaluated by **Boominathan *et al*., 2009**.

The activity of protease enzymes beyond temperature of 60^0c and at higher temperature the enzymes are denaturated. The optimum condition of pH and temperature needed for production of protease enzyme is reported (**Gitishree *et al*., 2010** and **Oyeleke *et al*., 2011**)

An alkaline protease enzyme was produced from bacteria collected from soil. The activity of enzyme production of bacteria was estimated by spectrophotometric method and the protein degradation activity and the amino acid content of the crude enzyme extract were determined by the Folin-Lowry method (**Pallavi Sinha *et al*., 2013**)

A protease enzyme was produced from *Bacillus subtilis* and it was purified by strain method. The advantage of use of the enzyme is that it eliminates the use of chemicals such as soda, lime and solvents and is eco-friendly (**Sathiya, 2013**)

2.2 Papain enzyme

Papain enzyme is an endolytic plant cyseine protease enzyme extracted from the latex, fruit, leaves and root of *Carica papaya* plant (**Amri and mamboya, 2012; Hitesh *et al*., 2012)**

Papain degrades proteins and cleaves the peptide bonds involving basic amino acids, particularly arginine, lysine and residues of phenylalanine **(Amri and mamboya, 2012; Hitesh *et al*., 2012)**

Papain enzyme can fold in two different size of domains having hydrophobic core. The stability of the enzyme is caused by the strong interaction of three disulfide bridges folded in the molecule (**Braia *et al*., 2013**

Figure (3) below gives the structure of papain enzyme

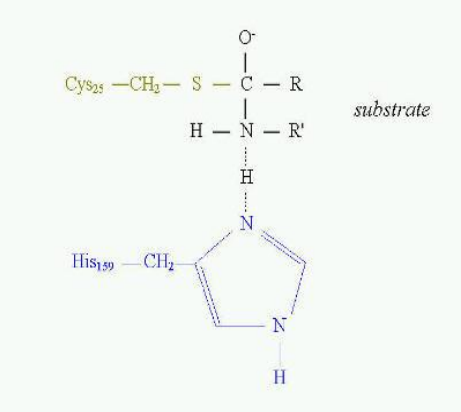


Figure 3 - Papain enzyme

2.2.1 Production of papain enzyme

Production of semisynthetic enzymes by the reduction of benzaldehyde catalysed by papain enzyme is reported. The semisynthetic enzymes are 3-bromoacetyl-N-benzylpyridinium bromide or 3-bromoacetyl-N phenylethylpyridinium bromide which give high yield because of high bonding in the hydrophobic site of papain and have higher catalytic activity in 10% ethanol-phosphate buffer than pure phosphate buffer (**Chen *et al*., 2010**)

The thermal behavior of papain was monitored by differential scanning calorimeter (DSC) (**Lambi *et al*., 2014**)

2.2.2 Reactions of papain enzyme

Silver nanoparticles were deposited on the silica spheres before the papain was coupled to the silica spheres. This improves the proteolytic activity and stability of immobilized papain as characterized by high resolution transmission electron microscopy (HR-TEM), Fournier transform infrared spectroscopy (FT-IR), and UV-visible scanning spectrometer (**Anming *et al*., 2008**)

2.2.3 Applications of papain enzyme

Papain enzyme finds use in the fields of drug design, pharmaceutical preparations and as meat tenderizers. Papain aids the digestive system and it is used in the treatment of arthritis (**Aravind *et al.,* 2103**)

2.3 Bromelain enzyme

Bromelain enzyme contains thiol endopeptidases and components like phosphates, glucosidases, cellulases, peroxidases, glycoproteins, carbohydrates and protease inhibitor. It was accepted as a universal

phytotherapeutical drug and it was effective on oral administration and it has no side effects (**Bhattacharyya, 2008**)

Bromelain enzyme plays a significant role in pharmacological activity and it acts as a anti-inflammatory agent, anti-tumour agent, promotes debridement of burns, inhibits thrombus formation and cures dermatological disorders by (**Tochi *et al*., 2008**)

Figure (4) below gives the structure of bromelain enzyme

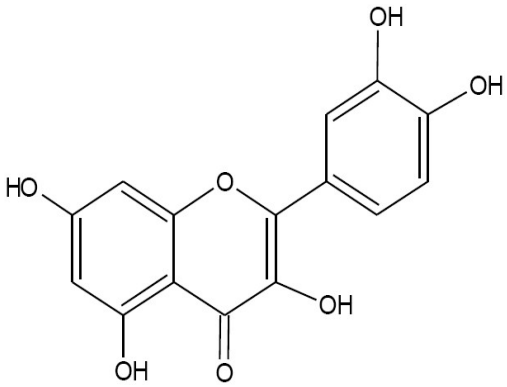


Fig 4 - Bromelain enzyme

2.3.1 Production of bromelain enzyme

Bromelain was extracted from the peel of the pineapple fruit by using of aqueous two phase system. The phase composition of bromelain showed very high stability at pH level neutral and it was suitable for meat tenderization was reported by (**Ketnawa *et al*., 2009**)

Bromelain was extracted from the pineapple of peel, pulp and stem parts using sodium acetate buffer and was separated by ammonium sulphate precipitation, dialysis and ion-exchange chromatography. The better yield of enzyme, enzyme activity and specific activity was shown on the peel part of the pineapple was produced by (**Mohapatra *et al*., 2013**)

2.4 Recent reports on the medicinal plant *Pisonia grandis*

There are recent reports on the isolation of constituents, biological potential and analytical quantification studies on the extracts of *Pisonia grandis.*

2.4.1 Isolation of chemical constituent

The phytochemical investigation of leaves of the *Pisonia grandis* led to the isolation of pinitol and allantoin from *Pisonia grandis* (**Shubashini *et al.,* 2011**)

C-methylated flavonoids have been isolated from the roots of the *Pisonia grandis* plant by an HPLC chromatographic methods (**Sutthivaiyakit *et al*., 2013**)

2.4.2 Biological activity studies

2.4.2.1 Anti-inflammatory activity of *Pisonia grandis*

The polar leaf extract of *Pisonia grandis* reduces inflammation on acute and chronic phases (**Jayakumari *et al*., *2012***), The chloroform extract of leaves also exihibit anti-inflammatory effect (**Vijayalakshmi *et al*., 2014**)

2.4.2.2 Antimicrobial activity on *Pisonia grandis*

The aqueous and ethylacetate extract of leaves of *Pisonia grandis* were analysed by disc diffusion method to determine the antimicrobial activity. The

zone of inhibition of ethylacetate fraction *Pisonia grandis* shows the maximum inhibition (**Jayakumari *et al*., 2014**)

2.4.2.3 Antifungal activity of *Pisonia grandis*

The leaves of ethanol extract of *Pisonia grandis* was analysed for antifungal activity against *Aspergillus niger, Candida albicans, Pencillium citrinum* and *Monascus purpureus* by disc diffusion method. The extracts showed good anti-fungal activity against *Monascus purpureus* (**Shubashini and Poongothai, 2010**)

2.5 Recent reports on antidiabetic molecule pinitol

A review on isolation of pinitol from medicinal plants reveals the significance of this molecule as a highly valuable medicinal product obtained from plants (**Poongothai *et al*., 2013**)

Pinitol was isolated from the leaves of ethanol extract of *Bauhinia variegate* by column chromatography (**Priyanka *et al*., 2014**)

2.5.1 Quantification of pinitol

The leaves, stem and root extracts of *Pisonia grandis* were quantified for pinitol content by HPTLC method (**Shubashini et al., 2011**)

Pinitol was identified and quantified in selected medicinal plants by HPTLC method (**Indumathi *et al*., 2013**)

The petroleum ether extract of stem of *Pisonia grandis* was analysed by GC-MS spectroscopic method (**Poongothai *et al*., 2013**)

2.6 Other activites

Silver nanoparticles synthesis using ethanolic extract of leaves of *Pisonia grandis* was reported by (**Lalitha *et al*., 2012**)

Materials and Methods

3. MATERIALS AND METHODS

The present work on “Enzyme-Assisted Isolation of an Antidiabetic Cyclitol from extracts of *Pisonia grandis* R.Br” is focused on

- Extraction of plant material
- Preliminary examination of extracts
- TLC Examination of the extracts
- TLC examination of enzyme treated extracts
- Isolation of the Cyclitol
- UV Examination of the plant extracts and the enzyme treated extracts.

The methodology pertaining to the above is presented in following below,

3.1 Collection of Plant Materials

The leaves, stem and root of *Pisonia grandis* plant were collected from local areas of Coimbatore. The plant material were air dried and pulverized.

3.2 Extraction

Extract of each of the chosen plant part was prepared by refluxing for six hours and by heating over water bath for one hour Hydro ethanol (80:20) and water were chosen as solvents.

3.21 Method I

The dried plant material (350g) was extracted with 80% ethanol (1000 ml) by refluxing for 6 hours. The extract was filtered and concentrated to give a

residue which was weighed. The same process was done with water as the solvent. Leaves, stem and roots of the plant were extracted by this method.

3.22 Method II

The dried plant material (25g) was extracted with 80% ethanol (100 ml) by heating over a water bath for 1 hour. The extract was filtered and concentrated to give a residue which was weighed. The same process was done with water as the solvent. Leaves, stem and roots of the plant were extracted by this method.

The percentage yield of residue was calculated using the formula:

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

The extracts concentrates were designated as

The leaf ethanol extract (6 hour refluxing) – $PGLE_6$

The leaf ethanol extract (1 hour heating) – $PGLE_1$

The leaf aqueous extract (1 hour refluxing) – $PGLW_1$

The stem ethanol extract (6 hour refluxing) – $PGSE_6$

The stem ethanol extract (1 hour heating) – $PGSE_1$

The stem aqueous extract (1 hour refluxing) – $PGSW_1$

The root ethanol extract (6 hour refluxing) – $PGRE_6$

The root ethanol extract (1 hour heating) – $PGRE_1$

The root aqueous extract (1 hour refluxing) – $PGRW_1$

3.3 Phytochemical colour tests of extracts

All the extracts were subjected to phytochemical analysis by standard tests

3.3.1 Test 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 theplant 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 color

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 color

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 coloration 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.

3.3.4 Test 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 inter phase 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 quinones

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 indicates 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 or 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

The sample was heated in distilled water and filtered, the filtrate 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 the 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 allowed to develop by drying the plate in the oven. The R_f values were noted.

3.4.1 Treatment of plant extracts with papain enzyme

The standard was identified from the samples of extract which were further treated with protease enzyme; Papain was used for the degradation of proteins present in the plant extracts. Papain, the protease enzyme was commercially purchased and was stored in a temperature of 5^oC.

About 100 mg of the sample of the extract was weighed and was solvated in 1 ml of water and it was centrifuged for 5 minutes. The filtrate was treated with 5 mg of the papain enzyme and the contents allowed to stand for

an hour. The enzyme treated sample was centrifuged for 5 minutes and the supernatant solution was collected and it was concentrated. The concentrated extract was subjected to TLC analysis for the identification of pinitol.

3.4.2 Isolation of pinitol from the enzyme treated samples

The enzyme was partially soluble in methanol. The enzyme treated plant extract was evaporated to dryness and was macerated with methanol. The enzyme settles down as a residue. The supernatant solution was collected and concentrated. The concentrate was analysed for the presence of pinitol by TLC method.

3.5 UV spectral analysis

The UV spectrometer was first calibrated and wavelength was set between 200nm and 400nm. Solvent was taken in the two cells for cell matching and then the samples were analyzed.

Solvent : distilled water
Range : 200nm-400nm
Instrument: Systronics Make

The pure standard and the pure papain enzyme were dissolved in distilled water and taken up for UV analysis.

UV spectral analysis was done for

- Raw extracts of the leaves, stems and roots of *Pisonia grandis*
- Plant extract treated with enzyme
- The enzyme treated extract concentrates.

Table 1

List of plant extract samples taken up for UV analysis

Extract	Sample code
Ethanolic extract of leaves of *Pisonia grandis* (6 hour)	$PGLE_6$
Ethanolic extract of leaves of *Pisonia grandis* (1 hour)	$PGLE_1$
Aqueous extract of leaves of *Pisonia grandis* (1 hour)	$PGLW_1$
Ethanolic extract of stem of *Pisonia grandis* (6 hour)	$PGSE_6$
Ethanolic extract of stem of *Pisonia grandis* (1 hour)	$PGSE_1$
Aqueous extract of stem of *Pisonia grandis* (1hour)	$PGSW_1$
Ethanolic extract of root of *Pisonia grandis* (6 hour)	$PGRE_6$
Ethanolic extract of root of *Pisonia grandis* (1 hour)	$PGRE_1$
Aqueous extract of root of *Pisonia grandis* (1 hour)	$PGRW_1$
Ethanolic extract of leaves of *Pisonia grandis* treated with enzyme	$PGLE_6$(10mg)+E
Ethanolic extract of leaves of *Pisonia grandis* treated with enzyme	$PGLE_1$(10mg)+E
Aqueous extract of leaves of *Pisonia grandis* treated with enzyme	$PGLW_1$(10mg)+E
Ethanolic extract of stem of *Pisonia grandis* treated with enzyme	$PGSE_6$(10mg)+E
Ethanolic extract of stem of *Pisonia grandis* treated with enzyme	$PGSE_1$(10mg)+E
Aqueous extract of stem of *Pisonia grandis* treated with enzyme	PGSW1(10mg)+E
Ethanolic extract of root of *Pisonia grandis* treated with enzyme	$PGRE_6$(10mg)+E

Ethanolic extract of root of *Pisonia grandis* treated with enzyme	$PGRE_1$(10mg)+E
Aqueous extract of root of *Pisonia grandis* treated with enzyme	$PGRW_1$(10mg)+E
Ethanolic extract of leaves of *Pisonia grandis* treated with enzyme	$PGLE_6$(100mg)+ E
Ethanolic extract of leaves of *Pisonia grandis* treated with enzyme	$PGLE_1$(100mg)+ E
Aqueous extract of leaves of *Pisonia grandis* treated with enzyme	$PGLW_1$(100mg)+E
Ethanolic extract of stem of *Pisonia grandis* treated with enzyme	$PGSE_6$(100mg)+ E
Ethanolic extract of stem of *Pisonia grandis* treated with enzyme	$PGSE_1$(100mg)+ E
Aqueous extract of stem of *Pisonia grandis* treated with enzyme	$PGSW_1$(100mg)+E
Ethanolic extract of root of *Pisonia grandis* treated with enzyme	$PGRE_6$(100mg)+E
Ethanolic extract of root of *Pisonia grandis* treated with enzyme	$PGRE_1$(100mg)+E
Aqueous extract of root of *Pisonia grandis* treated with enzyme	$PGRW_1$(100mg)+E
Concentrate of ethanolic extract (6 hour extract)of leaves of *Pisonia grandis* after enzyme treatment	C-$PGLE_6$(10mg)+ E
Concentrate of ethanolic extract (1 hour extract)of leaves of *Pisonia grandis* after enzyme treatment	C-$PGLE_1$(10mg)+ E
Concentrate of aqueous extract (1 hour extract)of leaves of *Pisonia grandis* after enzyme treatment	C-$PGLW_1$(10mg)+E
Concentrate of ethanolic extract (6 hour extract)of stem of *Pisonia grandis* after enzyme treatment	C-$PGSE_6$(10mg)+E
Concentrate of ethanolic extract (1 hour extract)of stem of *Pisonia grandis* after enzyme treatment	C-$PGSE_1$(10mg)+E

Concentrate of aqueous extract (1 hour extract)of stem of *Pisonia grandis* after enzyme treatment	C-PGSW_1(10mg)+E
Concentrate of ethanolic extract (6 hour extract)of root of *Pisonia grandis* after enzyme treatment	C-PGRE_6(10mg)+E
Concentrate of ethanolic extract (1 hour extract) of root of *Pisonia grandis* after enzyme treatment	C-PGRE_1(10mg)+E
Concentrate of aqueous extract (1 hour extract) of root of *Pisonia grandis* after enzyme treatment	C-PGRW_1(10mg)+E
Concentrate of ethanolic extract (6 hour extract)of leaves of *Pisonia grandis* after enzyme treatment	C-PGLE_6(100mg)+E
Concentrate of ethanolic extract (1 hour extract)of leaves of *Pisonia grandis* after enzyme treatment	C-PGLE_1(100mg)+E
Concentrate of aqueous extract (1 hour extract) of leaves of *Pisonia grandis* after enzyme treatment	C-PGLW_1(100mg)+E
Concentrate of ethanolic extract (6 hour extract)of stems of *Pisonia grandis* after enzyme treatment	C-PGSE_6(100mg)+E
Concentrate of ethanolic extract (1 hour extract)of stems of *Pisonia grandis* after enzyme treatment	C-PGSE_1(100mg)+E
Concentrate of aqueous extract (1 hour extract)of stems of *Pisonia grandis* after enzyme treatment	C-PGSW_1(100mg)+E
Concentrate of ethanolic extract (6 hour extract)of root of *Pisonia grandis* after enzyme treatment	C-PGRE_6(100mg)+E
Concentrate of ethanolic extract (1 hour extract)of root of *Pisonia grandis* after enzyme treatment	C-PGRE_1(100mg)+E
Concentrated of aqueous extract (1 hour extract)of root of *Pisonia grandis* after enzyme treatment	C-PGRW_1(100mg)+E
$PGLE_6$ of extract was analysed on TLC Examination, Isolation of pinitol	TLC - $PGLE_6$
Pinitol was analysed on TLC Examination	TLC – STD

Result and Discussion

4. RESULTS AND DISCUSSION

The present study was undertaken with the main aim of the isolation of an antidiabetic cyclitol from extracts of the medicinal plant *Pisonia grandis* by an enzyme assisted method.

The results are presented below

4.1 Extraction of plant material

Extract of each of the chosen plant part was prepared by refluxing for six hour and by heating over a water bath for one hour. Hydroethanol and water were chosen as solvents. The percentage yield of residue obtained is given in table 2

Table 2 – Percentage yield of residue from the plant extracts

S.No	Sample	Yield (%) in ethanol extraction and aqueous extraction
1	$PGLE_6$	14.75
2	$PGLE_1$	16.59
3	$PGLW_1$	19.21
4	$PGSE_6$	5.62
5	$PGSE_1$	12.58
6	$PGSW_1$	11.36
7	$PGRE_6$	7.36
8	$PGRE_1$	16.07
9	$PGRW_1$	9.49

The yield of extract was high for extraction with water. In the ethanol extraction, it was found that the method of heating over an electrical water bath for one hour duration gave a higher yield of extract compared to the refluxing method.

Table-3 Results of color tests

Compound	**Test**	**$PGLE_6$**	**$PGLE_1$**	**$PGLW_1$**	**$PGSE_6$**	**$PGSE_1$**	**$PGSW_1$**	**$PGRE_6$**	**$PGRE_1$**	**$PGRW_1$**
Protein	Biuret	+	+	+	+	+	+	+	+	+
Flavonoids	Ferric Chloride	+	+	+	+	+	+	+	+	+
Phenols	Ferric Chloride	-	+	+	+	+	+	+	+	+
Carbohydrate	Molisch's	+	+	+	+	+	+	+	+	+
	Fehling's	+	+	+	+	+	+	+	+	+
Terpenoids	Salkowski	+	+	+	-	-	+	+	+	-
Sterols	H_2SO_4	+	+	+	+	-	-	-	-	-

4.2 Treatment of plant extracts with papain enzyme

The samples of the plant extracts were treated with protease enzyme. The concentrated enzyme treated extract was subjected to TLC analysis for the identification of the cyclitol, pinitol. The various trials carried out for the enzyme treatment are listed below in the table 4.

Table 4 List of variations in the amount of enzyme added to the plant extract.

S. No.	Sample code	Weight of the extract (mg)	Weight of the enzyme (mg)
1	$PGLE_6$	100	600
2	$PGLE_6$	100	20
3	$PGLE_6$	100	10
4	$PGLE_6$	100	5
5	$PGSE_6$	10	5
6	$PGSE_1$	10	5
7	$PGRE_1$	10	5
8	$PGRE_6$	10	5
9	$PGLW_1$	10	5
10	$PGLW_1$	100	5
11	$PGLW_I$	100	10

TLC analysis of the enzyme treated plant extracts for the various trials gave the following observations (table 5).

Table 5 Details of experimental trials carried out for enzyme treatment

S.No.	Name of plant extract	Weight of the extract (mg)	Weight of the enzyme (mg)	Inference for experimental trials
1	$PGLE_6$	100	600	Excess of enzyme is present and the pinitol was not isolated
2	$PGLE_6$	100	20	Excess of enzyme is present and the pinitol was not isolated
3	$PGLE_6$	100	10	Excess of enzyme is present and the pinitol was not isolated
4	$PGLE_6$	100	5	Detection of pinitol on chromatogram
5	$PGSE_6$	10	5	Precipitation process does not occurs.
6	$PGSE_1$	10	5	Precipitation process does not occurs.
7	$PGRE_1$	10	5	Precipitation process does not occurs.
8	$PGRE_6$	10	5	Precipitation process does not occurs.
9	$PGLW_1$	10	5	Precipitation process does not occurs.
10	$PGLW_1$	100	5	Precipitation process does not occurred due to minimum amount of enzyme
11	$PGLW_1$	100	10	Detection of pinitol on chromatogram.

The enzyme treated plant extract was evaporated to dryness and was macerated with methanol. The papain enzyme settled down as residue. The supernatant solution was collected and concentrated. The concentrate was analysed for the presence of pinitol by TLC.

Table 6 Yield of residue from the enzyme treated plant extracts

S.No	Sample code	Weight of the extract (mg)	Weight of the enzyme (mg)	Yield of residue after enzyme treatment (mg)
1	$PGLE_6$	100	5	63
2	$PGLE_1$	100	5	69
3	$PGLW_1$	100	10	78
4	$PGSE_6$	100	5	67
5	$PGSE_1$	100	5	87
6	$PGSW_1$	100	10	88
7	$PGRE_6$	100	5	88
8	$PGRE_1$	100	5	90
9	$PGRW_1$	100	10	92

The yields of residues from enzyme treated extract are for 100mg of each plant extract. The ethanol extracts were treated with 5mg of enzyme. The aqueous extracts were treated with 10 mg of enzyme. The residue obtained after concentrating the enzyme treated plant extract was macerated with methanol to isolate the cyclitol molecule which is proposed to be present in the methanol fraction as shown by TLC analysis (Fig 12 to 15).

4.4 TLC analysis

The concentrated hydroethanol and ethanol extract of the 9 plant samples and enzyme treated plant extracts of various concentration 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. Sample spots are numbered from left to right. The extreme left corresponds to standard pinitol.

Figures 5 to 7 represent the TLC chromatograms of plant extracts, **figures 8 to 12** represent the TLC chromatograms of enzyme treated plant extract and **figures 13 to 16** represent the TLC chromatograms of methanol macerated of enzyme treated plant extract.

TLC Chromatogram of raw plant extracts

Fig 5

1. **STD**
2. **$PGLE_6$**
3. **$PGLE_1$**
4. **$PGLW_1$**

Fig 6

1. **STD**
2. **$PGSE_6$**
3. **$PGSE_1$**
4. **$PGSW_1$**

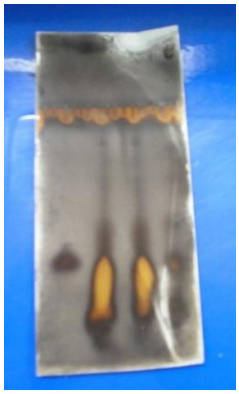


Fig 7

1. **STD**
2. **$PGRE_6$**
3. **$PGRE_1$**
4. **$PGRW_1$**

TLC Chromatogram of enzyme-treated plant extract

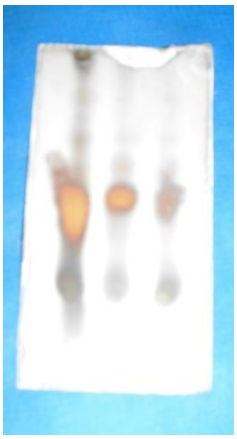


Fig 8

1. **STD**
2. **PGLE$_6$+E**
3. **PGLE$_1$+E**

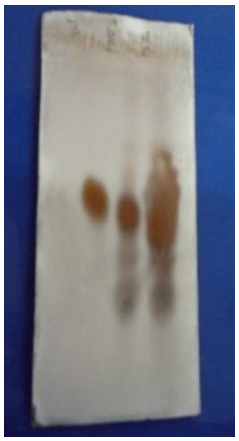


Fig 9

1. **STD**
2. **PGSE$_6$+E**
3. **PGSE$_1$+E**

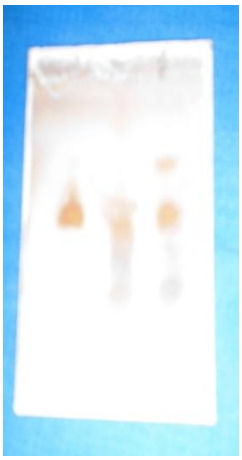


Fig 10

1. **STD**
2. **PGRE$_6$+E**
3. **PGRE$_1$+E**

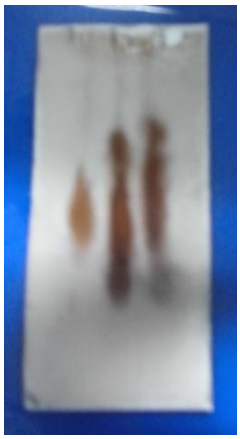


Fig 11

1. **PGLW$_1$+E**
2. **PGSW$_1$+E**
3. **PGRW$_1$+E**

TLC Chromatogram of enzyme-treated plant extracts macerated with methanol

Fig 12

1. **STD**
2. **methanol soluble portion of $PGLE_6$+E**
3. **methanol soluble portion of $PGLE_1$+E**

Fig 13

1. **STD**
2. **methanol soluble portion of $PGSE_6$+E**
3. **methanol soluble portion of $PGSE_1$+E**

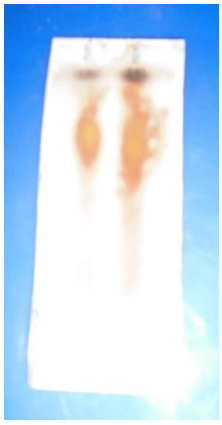


Fig 14

1. **methanol soluble portion of $PGRE_6$+E**
2. **methanol soluble portion of $PGRE_1$+E**

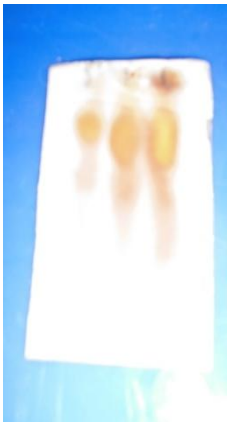


Fig 15

1. **methanol soluble portion of $PGLW_1$+E**
2. **methanol soluble portion of $PGSW_1$+E**
3. **methanol soluble portion of $PGRW_1$+E**

R_f values of TLC chromatograms

Table 7

S.NO	Sample	Major spots	R_f value
1	$PGLE_6$	Dark Brown	0.41
2	$PGLE_1$	Dark Brown	0.43
3	$PGLW_1$	Brown	0.44

Standard R_f : 0.43

Table 8

S.NO	Sample	Major spots	R_f value
1	$PGSE_6$	Brown	0.43
2	$PGSE_1$	Dark Brown	0.41
3	$PGSW_1$	Dark Brown	0.42

Standard R_f : 0.42

Table 9

S.NO	Sample	Major spots	R_f value
1	$PGRE_6$	Brown	0.44
2	$PGRE_1$	Dark Brown	0.45
3	$PGRW_1$	Brown	0.44

Standard R_f : 0.44

Table 10

S.NO	Sample	Major spots	R_f value
1	$PGLE_6$+E	Dark Brown	0.43
2	$PGLE_1$+E	Brown	0.44

Standard R_f : 0.43

Table 11

S.NO	Sample	Major spots	R_f value
1	$PGSE_6$+E	Brown	0.45
2	$PGSE_1$+E	Dark Brown	0.46

Standard R_f : 0.46

Table 12

S.NO	Sample	Major spots	R_f value
1	$PGRE_6$+E	Dark Brown	0.46
2	$PGRE_1$+E	Dark Brown	0.47

Standard R_f : 0.46

Table 13

S.NO	Sample	Major spots	R_f value
1	PGLW1+E	Dark Brown	0.42
2	PGSW1+E	Brown	0.41
3	PGRW1+E	Brown	0.42

Table 14

S.NO	Sample	Major spots	R_f value
1	methanol soluble portion of $PGLE_6$+E	Dark yellow	0.43
2	methanol soluble portion of $PGLE_1$+E	Yellow	0.42

Standard R_f : 0.43

Table 15

S.NO	Sample	Major spots	R_f value
1	methanol soluble portion of $PGSE_6$+E	Yellow	0.45
2	methanol soluble portion of $PGSE_1$+E	Brown	0.46

Standard R_f : 0.46

Table 16

S.NO	Sample	Major spots	R_f value
1	methanol soluble portion of $PGRE_6$+E	Dark Brown	0.45
2	methanol soluble portion of $PGRE_1$+E	Orange	0.44

Table 17

S.NO	Sample	Major spots	R_f value
1	methanol soluble portion of $PGLW_1$+E	Dark Orange	0.43
2	methanol soluble portion of $PGSW_1$+E	Orange	0.44
3	methanol soluble portion of $PGRW_1$+E	Brown	0.44

UV spectral analysis

The pure standard and the pure papain enzyme were dissolved in distilled water and was taken for analysis.

UV spectral analysis was done for

- Raw extracts of the leaves, stems and roots of *Pisonia grandis*
- Plant extract treated with enzyme
- The concentrate of enzyme treated extracts

Chart 1 – UV Spectra of plant extracts, enzyme treated plant extracts and enzyme treated plant extract concentrates

Standard	Raw plant extracts	Enzyme treated extracts	Concentrate of enzyme treated extracts	Enzyme

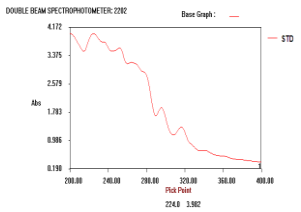
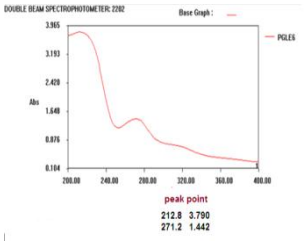
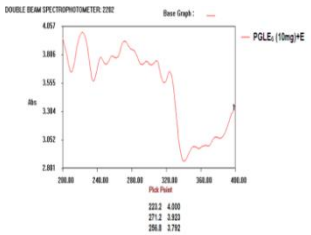
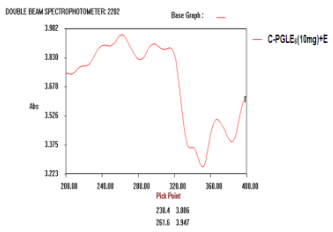
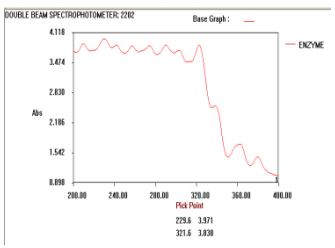

				

Fig 16 **STANDARD**	Fig 18 **$PGLE_6$**	Fig 19 **PGLE6(10mg)+E**	Fig 20 **C-PGLE6(10mg)+E**	Fig 17 **ENZYME**

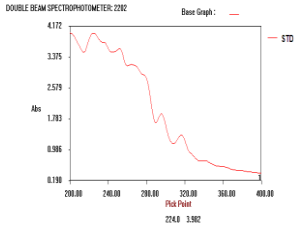
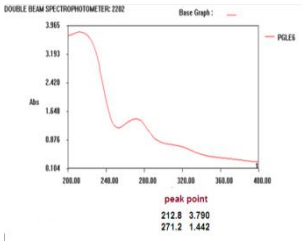
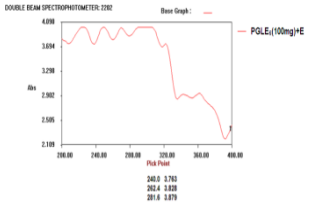
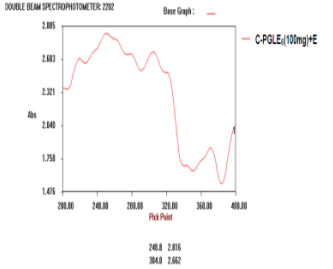
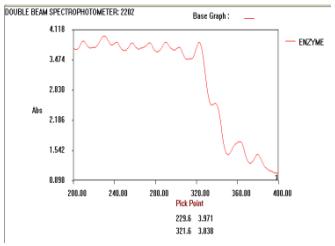

				

Fig 16 **STANDARD**	Fig 18 **$PGLE_6$**	Fig 21 **PGLE6(100mg)+E**	Fig 22 **C-PGLE6(100mg)+E**	Fig 17 **ENZYME**

Standard	Raw plant extracts	Enzyme treated extracts	Concentrate of enzyme treated extracts	Enzyme

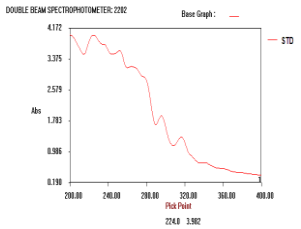
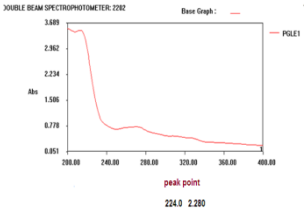
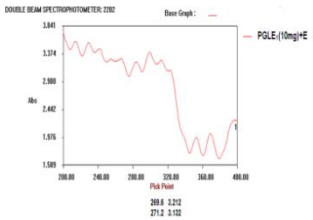
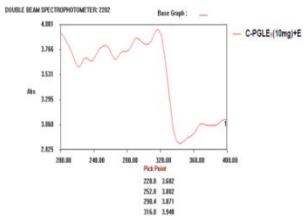
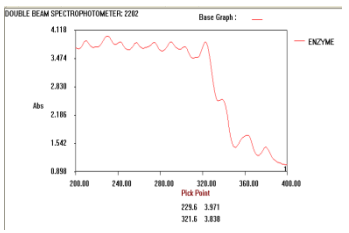

				

Fig 16 **STANDARD**	Fig 23 **$PGLE_1$**	Fig 24 **$PGLE_1$(10mg)+E**	Fig 25 **C-$PGLE_1$(10mg)+E**	Fig 17 **ENZYME**

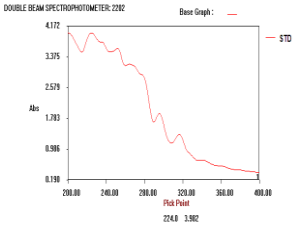
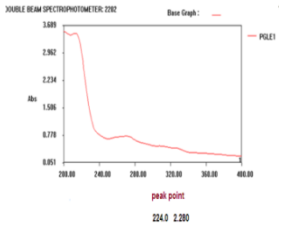
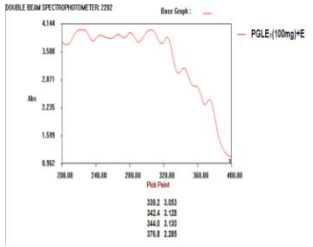
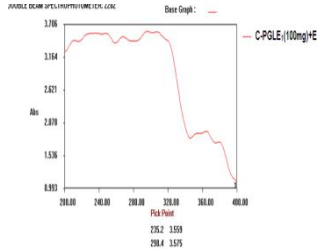
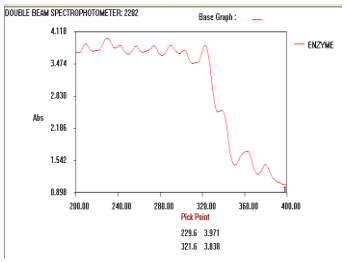
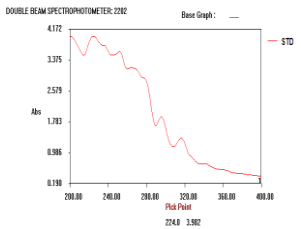
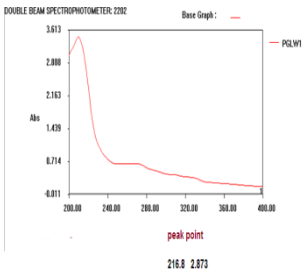
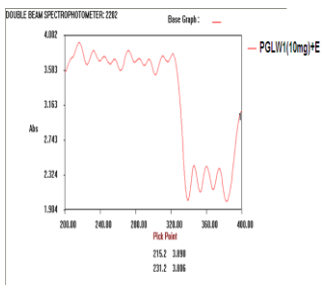
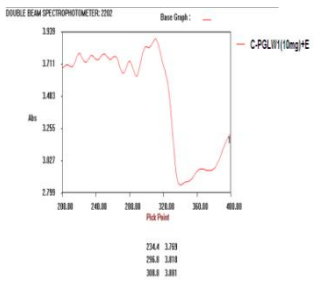
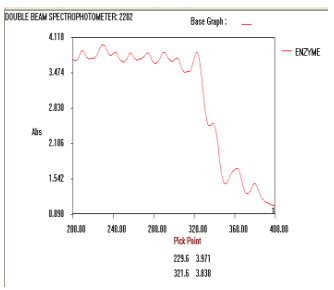
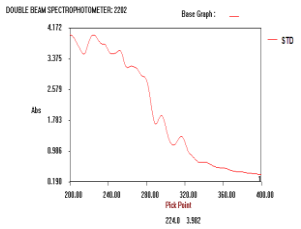
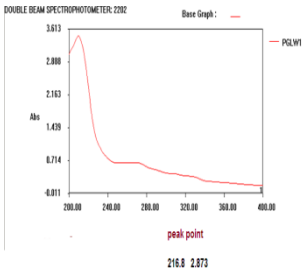
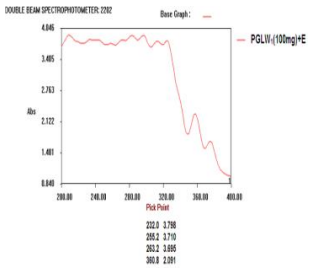
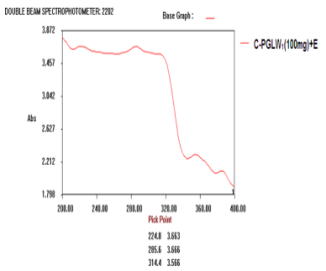
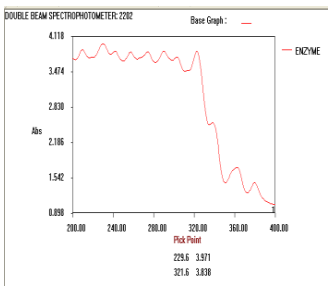

				

Fig 16 **STANDARD**	Fig 23 **$PGLE_1$**	Fig 26 **$PGLE_1$(100mg)+E**	Fig 27 **$PGLE_1$(100mg)+E**	Fig 17 **ENZYME**

Standard	Raw plant extracts	Enzyme treated extracts	Concentrate of enzyme treated extracts	Enzyme
 Fig 16 **STANDARD**	 Fig 28 **$PGLW_1$**	 Fig 29 **$PGLW_1$(10mg)+E**	 Fig 30 **C-$PGLW_1$(10mg)+E**	 Fig 17 **ENZYME**
 Fig 16 **STANDARD**	 Fig 28 **$PGLW_1$**	 Fig 37 **$PGLW_1$(100mg)+E**	 Fig 31 **C-$PGLW_1$(100mg)+E**	 Fig 17 **ENZYME**

Standard	Raw plant extracts	Enzyme treated extracts	Concentrate of enzyme treated extracts	Enzyme

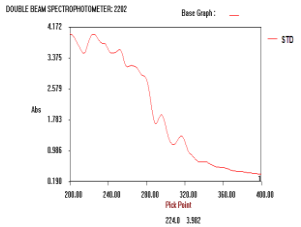
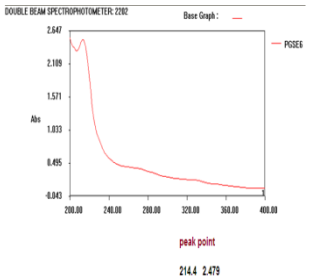
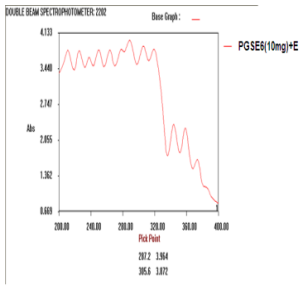
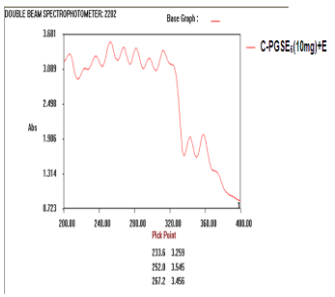
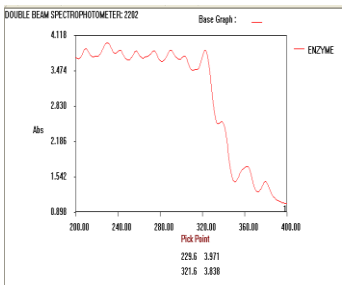

				

Fig 16 **STANDARD**	Fig 32 **$PGSE_6$**	Fig 33 **$PGSE_6$(10mg)+E**	Fig 34 **C-$PGSE_6$(10mg)+E**	Fig 17 **ENZYME**

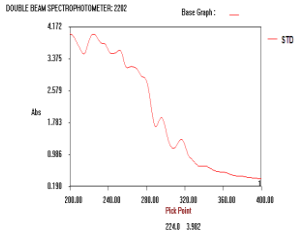
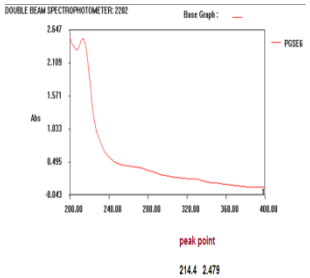
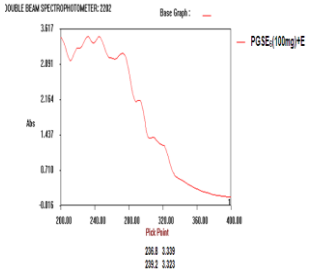
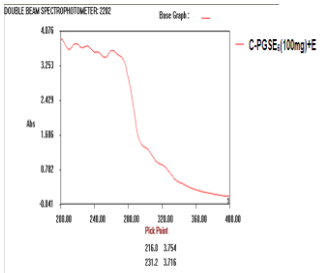
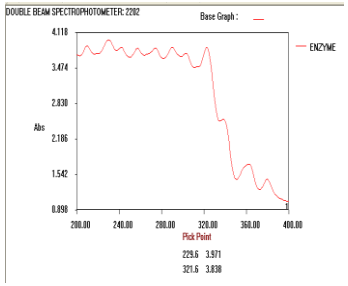

				

Fig 16 **STANDARD**	Fig 32 **$PGSE_6$**	Fig 35 **$PGSE_6$(100mg)+E**	Fig 36 **C-$PGSE_6$(100mg)+E**	Fig 17 **ENZYME**

Standard	Raw plant extracts	Enzyme treated extracts	Concentrate of enzyme treated extracts	Enzyme

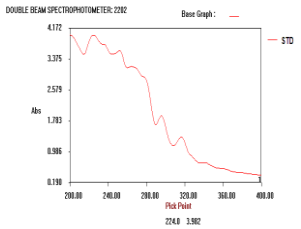
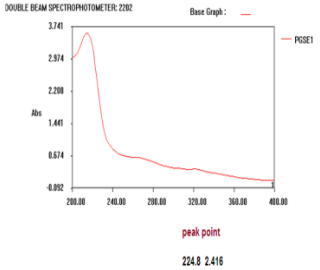
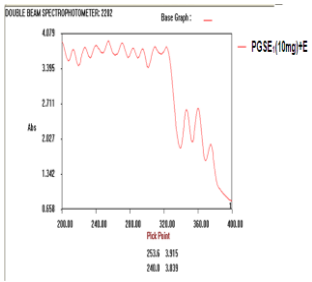
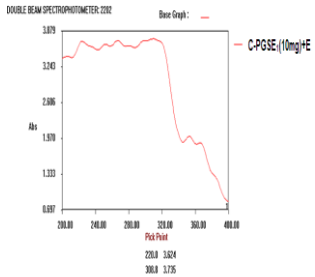
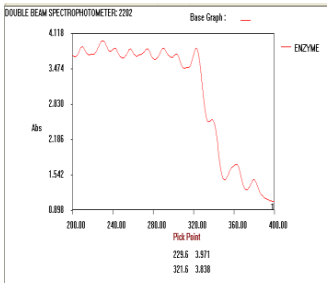

				

Fig 16 **STANDARD**	Fig 37 **$PGSE_1$**	Fig 38 **$PGSE_1$(10mg)+E**	Fig 39 **C-PGSE1(10mg)+E**	Fig 17 **ENZYME**

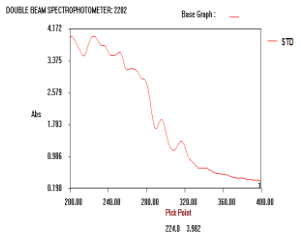
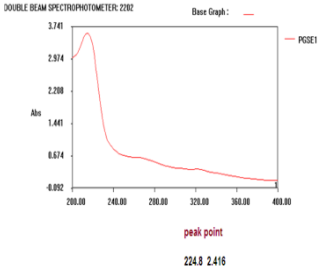
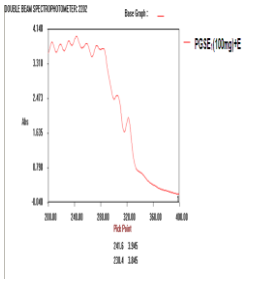
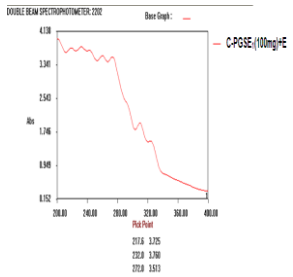
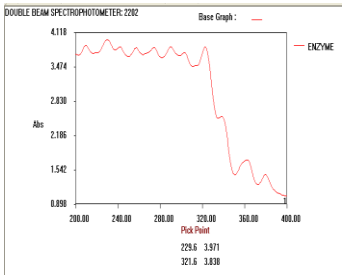
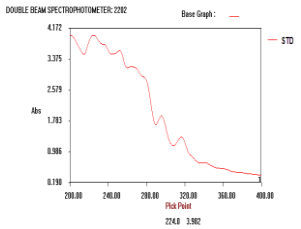
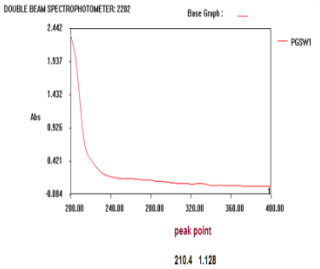
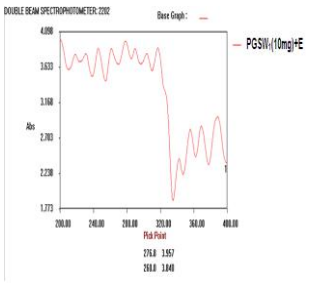
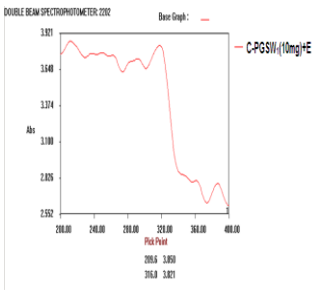
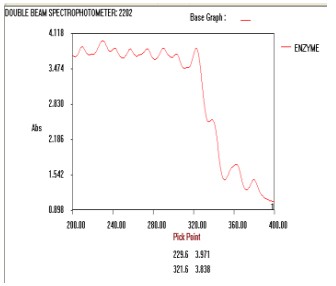
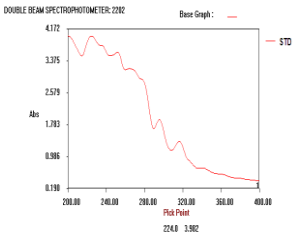
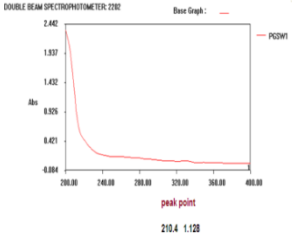
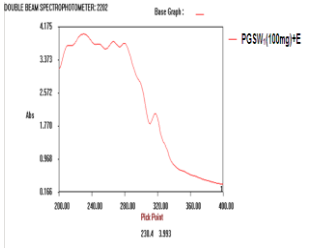
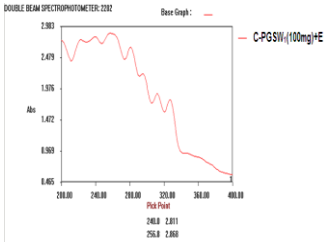
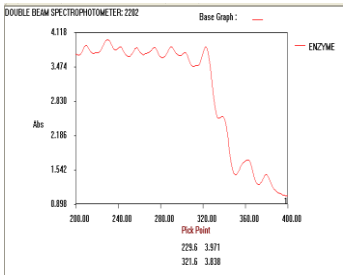
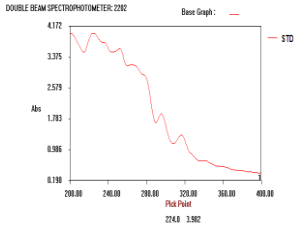
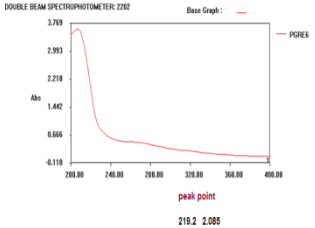
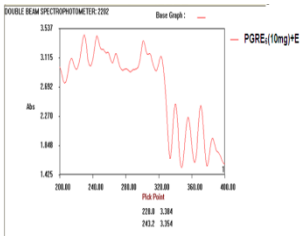
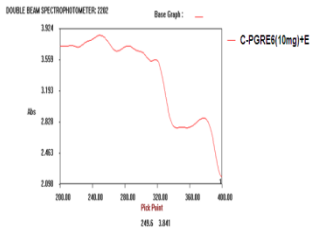
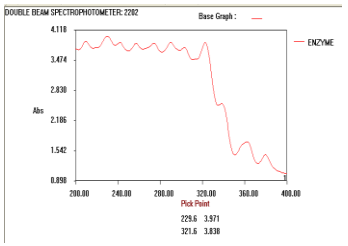
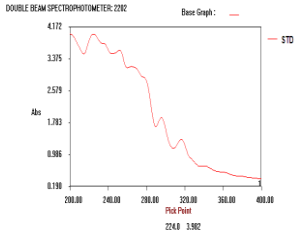
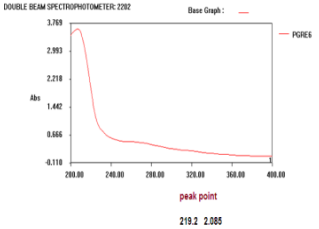
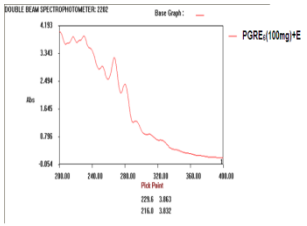
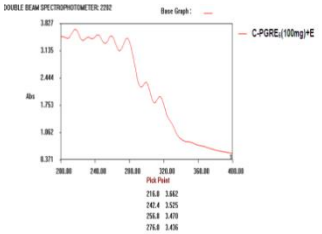
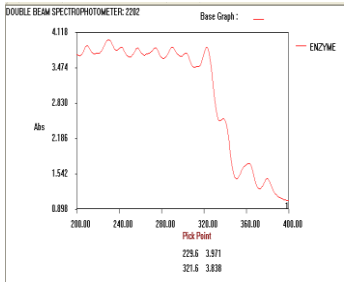
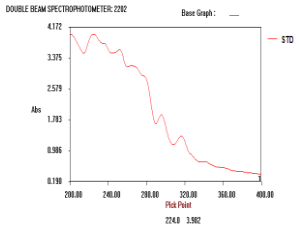
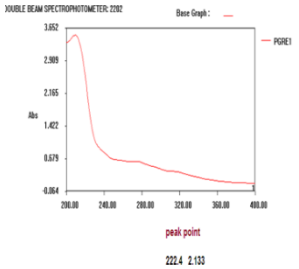
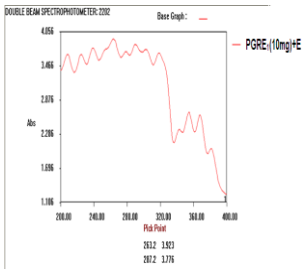
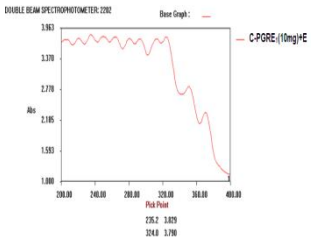
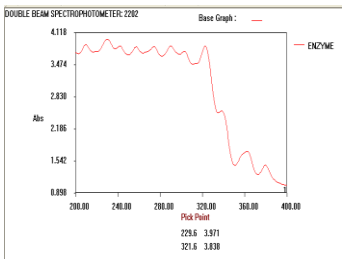
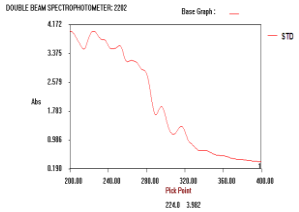
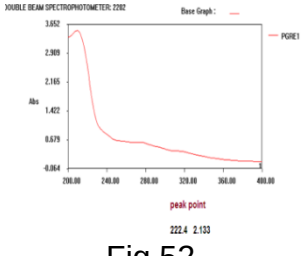
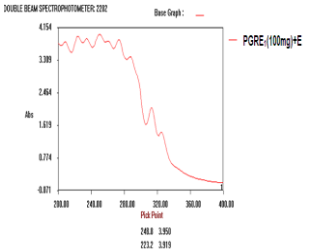
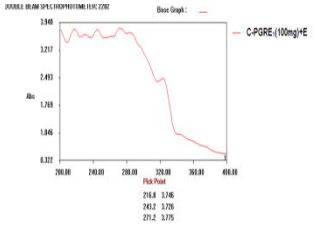
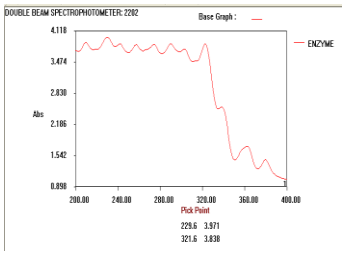

				

Fig 16 **STANDARD**	Fig 37 **$PGSE_1$**	Fig 40 **$PGSE_1$(100mg)+E**	Fig 41 **C-PGSE1(100mg)+E**	Fig 17 **ENZYME**

Standard	Raw plant extracts	Enzyme treated extracts	Concentrate of enzyme treated extracts	Enzyme
 Fig 16 **STANDARD**	 Fig 42 **$PGSW_1$**	 Fig 43 **$PGSW_1$(10mg)+E**	 Fig 44 **C-$PGSW_1$(10mg)+E**	 Fig 17 **ENZYME**
 Fig 16 **STANDARD**	 Fig 42 **$PGSW_1$**	 Fig 45 **$PGSW_1$(100mg)+E**	 Fig 46 **C-$PGSW_1$(100mg)+E**	 Fig 17 **ENZYME**

Standard	Raw plant extracts	Enzyme treated extracts	Concentrate of enzyme treated extracts	Enzyme
 Fig 16 **STANDARD**	 Fig 47 **$PGRE_6$**	 Fig 48 **$PGRE_6$(10mg)+E**	 Fig 49 **C-$PGRE_6$(10mg)+E**	 Fig 17 **ENZYME**
 Fig 16 **STANDARD**	 Fig 47 **$PGRE_6$**	 Fig 50 **$PGRE_6$(100mg)+E**	 Fig 51 **C-$PGRE_6$(100mg)+E**	 Fig 17 **ENZYME**

Standard	Raw plant extracts	Enzyme treated extracts	Concentrate of enzyme treated extracts	Enzyme
 Fig 16 **STANDARD**	 Fig 52 **$PGRE_1$**	 Fig 53 **$PGRE_1$(10mg)+E**	 Fig 54 **C-$PGRE_1$(10mg)+E**	 Fig 17 **ENZYME**
 Fig 16 **STANDARD**	 Fig 52 **$PGRE_1$**	 Fig 55 **$PGRE_1$(100mg)+E**	 Fig 56 **C-$PGRE_1$(100mg)+E**	 Fig 17 **ENZYME**

Standard	Raw plant extracts	Enzyme treated extracts	Concentrate of enzyme treated extracts	Enzyme

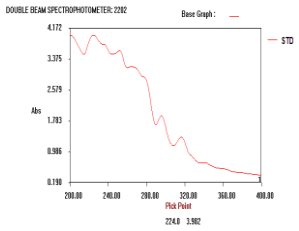
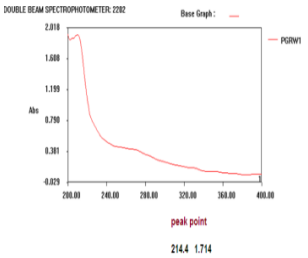
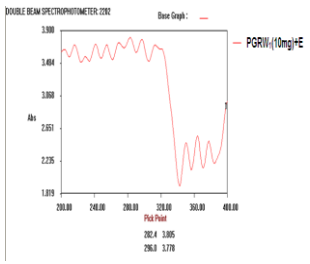
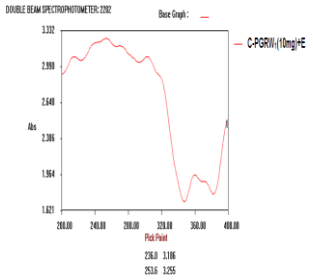
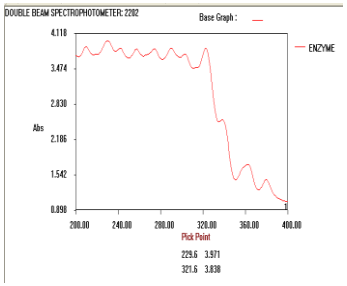

				

Fig 16 **STANDARD**	Fig 57 **$PGRW_1$**	Fig 58 **$PGRW_1$(10mg)+E**	Fig 59 **C-$PGRW_1$(10mg)+E**	Fig 17 **ENZYME**

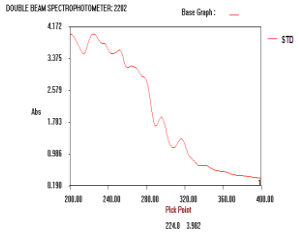
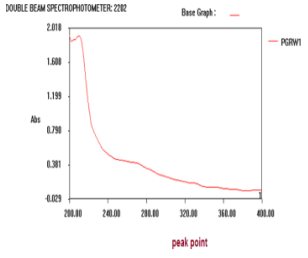
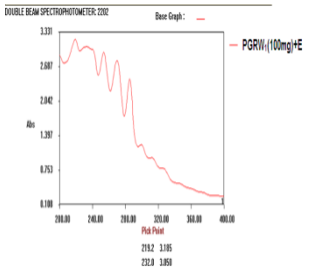
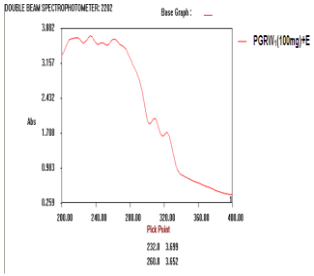
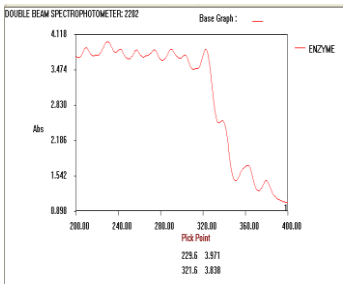

				

Fig 16 **STANDARD**	Fig 57 **$PGRW_1$**	Fig 60 **$PGRW_1$(100mg)+E**	Fig 61 **C-$PGRW_1$(100mg)+E**	Fig 17 **ENZYME**

Standard	Standard isolate from TLC	Raw plant extract	Standard + Enzyme	Enzyme

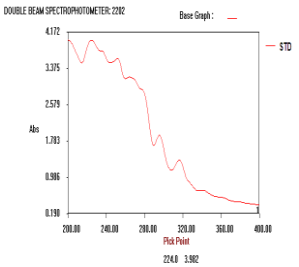
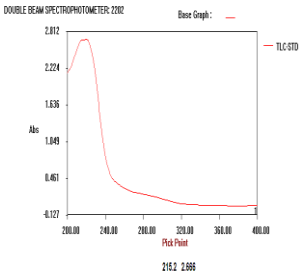
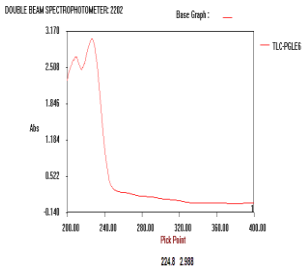
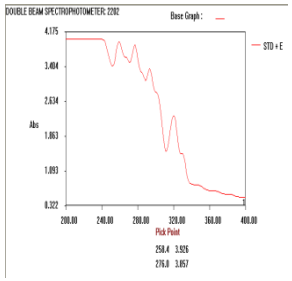
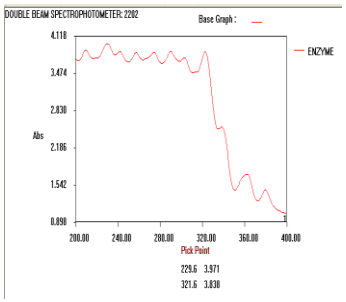

				

Fig 16 **STANDARD**	Fig 62 **TLC-STD**	Fig 63 **TLC-PGLE$_6$**	Fig 64 **STANDARD+ENZYME**	Fig 17 **ENZYME**

Figure 16 and **17** represents the UV spectra of standard pinitol and enzyme.

Figure 18-**31** represents the UV spectra of the leaf extracts and their enzyme treated extracts.

Figure 32-**46** represents the UV spectra of the stem extracts and their enzyme treated extracts.

Figure 47-61 represents the UV spectra of the root extracts and their enzyme treated extracts.

Figure 62 and **63** represents the UV spectra of the isolate the standard and the isolate the standard from the $PGLE_{6.}$

Figure 64 represents the UV spectra of the combination of enzyme and standard.

The wavelength of maximum absorption of raw plant extract, enzyme treated plant extract and the concentrates of enzyme treated plant extracts is tabulated in table 18

From the UV analysis, it was observed that pinitol was present in the plant extracts. It was also identified that in the spectra of the enzyme treated extracts interaction of enzyme.

The wavelength of maximum absorption pertains to the peaks of highest intensity in the UV spectra.

Table 18 Wavelength of absorption of plant extract sample (in nm)

STANDARD	ENZYME	STD+ENZYME	$PGLE_6$	$PGLE_6$(10mg)+E	C-$PGLE_6$(10mg)+E	$PGLE_6$(100mg)+E	C-$PGLE_6$(100mg)+E
224	229	258	212	223	238 261	281	248

STANDARD	ENZYME	STD+ENZYME	$PGLE_1$	$PGLE_1$(10mg)+E	C-$PGLE_1$(10mg)+E	$PGLE_1$(100mg)+E	C-$PGLE_1$(100mg)+E
224	229	258	224	269	290 316	344	235 298

STANDARD	ENZYME	STD+ENZYME	$PGLW_1$	$PGLW_1$(10mg)+E	C-$PGLW_1$(10mg)+E	$PGLW_1$(100mg)+E	C-$PGLW_1$(100mg)+E
224	229	258	216	215 231	308	232	224 285

STANDARD	ENZYME	STD+ENZYME	$PGSE_6$	$PGSE_6$(10mg)+E	C-$PGSE_6$(10mg)+E	$PGSE_6$(100mg)+E	C-$PGSE_6$(100mg)+E
224	229	258	214	287	252	236	216

STANDARD	ENZYME	STD+ENZYME	$PGSE_1$	$PGSE_1$(10mg)+E	C-$PGSE_1$(10mg)+E	$PGSE_1$(100mg)+E	C-$PGSE_1$(100mg)+E
224	229	259	224	253	308	230, 241	232

STANDARD	ENZYME	STD+ENZYME	$PGSW_1$	$PGSW_1$(10mg)+E	C-$PGSW_1$(10mg)+E	$PGSW_1$(100mg)+E	C-$PGSW_1$(100mg)+E
224	229	259	210	276	209	230	256

STANDARD	ENZYME	STD+ENZYME	$PGRE_6$	$PGRE_6$(10mg)+E	C-$PGRE_6$(10mg)+E	$PGRE_6$(100mg)+E	C-$PGRE_6$(100mg)+E
224	229	258	219	228	249	229	216

STANDARD	ENZYME	STD+ENZYME	$PGRE_1$	$PGRE_1$(10mg)+E	C-$PGRE_1$(10mg)+E	$PGRE_1$(100mg)+E	C-$PGRE_1$(100mg)+E
224	229	258	222	263	235	223 248	271

STANDARD	ENZYME	STD+ENZYME	$PGRW_1$	$PGRW_1$(10mg)+E	C-$PGRW_1$(10mg)+E	$PGRW_1$(100mg)+E	C-$PGRW_1$(100mg)+E
224	229	258	214	282	253	219 232	232

STANDARD	ENZYME	STD+ENZYME	TLC-STD	TLC-$PGLE_6$
224	229	258	215	224

The UV spectral analysis revealed that the enzyme treated extracts showed UV absorbance corresponding to both the standard and the enzyme. Not much interaction between the active molecule of the extract and the enzyme was revealed.

Summary and Conclusion

5. SUMMARY AND CONCLUSION

The present study titled “Enzyme Assisted Isolation of an Antidiabetic Cyclitol from Extracts of *Pisonia grandis* R.Br” was undertaken to identify the antidiabetic molecule pinitol in the extracts of *Pisonia grandis* plant.

The first chapter is a brief introduction of the study. Review of literature is presented in the second chapter under the following headings

- Production of protease enzyme.
- Production, reaction and applications of papain enzyme.
- Production and application of bromelain enzyme.
- Recent reports on the medicinal plant *Pisonia grandis* R.Br.
- Recent reports on isolation of antidiabetic molecule pinitol.
- Quantification, phytochemical aspects and medicinal uses of pinitol from *Pisonia grandis* plant.

The third chapter deals with the methodology adopted for the study.

The results are presented in the fourth chapter. The study facilitates the following revelations.

- The aqueous extraction gave higher yield of extract concentrate
- Heating the plant material with ethanol for one hour over an electrical bath can yield and higher yield of residue compared to refluxing for six hours. This observation is a significant one.
- The hydroethanol and aqueous extracts of *Pisonia grandis* plant contained proteins, carbohydrates, sterols, flavonoids, phenols and terpenoids as shown by positive phyochemical color tests.
- The extracts of *Pisonia grandis* plant were found to possess pinitol in their hydroethanol and aqueous extracts by as shown by the TLC analysis of these samples.

- The concentrates of ethanolic and aqueous extracts of leaves, stem and roots of *Pisonia grandis* after enzyme treatment were found to contain pinitol as revealed by TLC analysis.

- The UV spectral analysis revealed that the enzyme treated extracts showed UV absorbance corresponding to both the standard and the enzyme. Not much interaction between the active molecule of the extract and the enzyme was revealed.

- This method can be furthur optimized for the isolation of pinitol from extracts of *Pisonia grandis* without resorting to tedious column chromatography.

  Future studies will be concentrated on optimizing this method.

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