Enhancement of Anti-Diabetic Potential of *Chebulic myrobalan*

Dissertation submitted in partial fulfillment of the requirement for the degree of

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IN

BIOTECHNOLOGY

By

Manisha Sharma (181826)

Under the guidance of

Dr. Garlapati Vijay Kumar

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT

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DECLARATION

I decree hereby that the work presented in this report entitled **"Enhancement of Anti-Diabetic Potential of *Chebulic myrobalan* "** in partial fulfilment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology submitted in the department of Biotechnology, Jaypee University of Information Technology, Waknaghat is a real record of my own work carried out over a period from August 2021 to May 2022 under the supervision of Dr Garlapati Vijay Kumar.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

(Manisha Sharma, 181826)

SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the B. Tech. thesis entitled **"Enhancement of Anti-Diabetic Potential of *Chebulic myrobalan*"**, submitted by Manisha Sharma (181826) at Jaypee University of Information Technology, Waknaghat, India, is a bonafide record of their original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

(**Signature of Supervisor**)

Dr. Garlapati Vijay Kumar

Associate Professor

Department of Biotechnology and Bioinformatics,

JUIT, HP-173234,India.

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(Manisha Sharma, 181826)

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Abstract

The foods we consume have a major impact on our blood glucose levels. We must maintain a nutritious diet on a regular basis in order to manage diabetes. Green leafy vegetables, bitter melon, walnuts, and grains are all anti-diabetic foods. These include omega-3 fatty acids, which help to reduce insulin resistance. These omega-3 fatty acids may also lower the risk of heart attack, stroke, and other cardiovascular diseases. In recent years, Diabetes Mellitus has become more common. It is affecting the majority of the population. Unhealthy eating habits, sleeping habits, lifestyle, thinking all have contributed to serious health problems nowadays. Enhancing anti-diabetic foods is possible approach to decrease or control the increment of disease. Including them in eating routine on a regular basis in a right amount can result in decrease in disease.

Keywords: Anti-diabetic food; Diabetes Mellitus; blood glucose level; *Chebulic myrobalan*

CHAPTER 1
Introduction

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Diabetes mellitus causes high blood sugar level. It occurs because pancreas is not producing enough insulin. Symptoms included are vision is blurry, thirst is increased etc. Diabetes mellitus, usually famous as diabetes, is a collection of metabolic illness from an extreme level of glucose in blood level (hyperglycemia) over an extended duration. Symptoms frequently comprise frequent the act of excreting, raised thirst and raised craving. Diabetes can be fatal if left untreated.There are numerous energy complexities. Diabetic ketoacidosis, hyperosmolar hyperglycemic state, or end of life. Chronic enduring complexities contain heart failure, and other serious long term complications.

Diabetes is caused by either the pancreas producing insufficient insulin or the body's cells reacting incorrectly to the insulin generated. Hormone called insulin that aids in the transport of glucose from the bloodstream into cells, where it is used for energy. Diabetes mellitus is divided into three categories.

- **Type 1** -Syndrome is caused either by destruction of beta cells in the pancreas, which also restricts the pancreas for releasing more blood sugar. Earlier, this disorder was referred to as "insulin-dependent diabetes mellitus" or "juvenile diabetes." An autoimmune reaction triggers the loss of beta cells. There is also no recognised cause for this autoimmune reaction. Even while Type 1 diabetes usually originates through youth or adolescence, it also can appear at any age.
- **Type 2** - begins with insulin resistance, a disease in which cells fail to respond to blood sugar correctly. A shortage of blood sugar can reoccur as the condition proceeds. Earlier, this syndrome was classified as "non-insulin-dependent diabetes mellitus" or "elderly diabetes." Even though the risk of developing type 2 diabetes is lower in most senior adults, a huge increase in the rate of overweight in group youth has resulted in higher levels of type 2 diabetes in teenagers.

- A combination of rising body heaviness and a decreased activity is the most widely recognized condition.
- **Gestational diabetes**-Gestational diabetes would be the tertiary major kind, or it occurs while expectant moms who have never had diabetes develop high blood pressure through pregnancy. After delivering, blood glucose in mothers suffering gestational diabetes usually starts to recover. Girls who had gestational diabetes before birth, on the other hand, had a higher risk of developing type 2 diabetes later in life.

1.1 Anti-diabetic activity of some medicinal plants:-

- Fenugreek – number of insulin receptors in red blood cells may increases fenugreek and improves utilization of glucose in peripheral tissues. It can be obtained from seeds and leaves.
- Turmeric – it increases the regulation of liver function. For diabetic patients intake of omega – 3 fatty acids in food may lower the chance of heart disease , etc.
- Aloe Vera- According to the studies the intake of two tablespoons of the Aloe Vera juice per day can help the blood sugar level to lower down in the patient suffering from diabetes.
- Neem- Intake of Neem leaves in the raw form can also help to lower down the blood sugar level. It can be taken in many ways like boiling in hot water and drinking it as a tea, or having it in the form of powder in limited amount.

1.2 Anti - diabetic foods and vegetables:

- Green Leafy vegetables – nutrients, minerals, and vitamins are present. In this there is presence of high anti-oxidant content. Example – Spinach, cabbage.
- Finger millet – it is also known as ragi, consumption of ragi might help in stabilize their sugar levels in blood.

- Walnuts_– have omega 3 fatty acids in it which is called as ALA (Alpha Lipoic Acid). It reduces the risk of heart attack.
- Citrus fruits – also have anti – diabetic effects. e.g.: lemons, orange , grapes .They give us vitamin C and potassium .
- Whole grain_– It contains more nutrients and high fiber than white grain which means that they have less impact on blood sugar. Glycemic index of whole grain is lower than rice, white bread. Example -: Brown rice, whole grain pasta.
- Almonds and nuts – it makes blood sugar more stable and it is also helpful in digestion.
- Bitter melon – it is also known as karela. it contains lectin that has activity like insulin. It suppressing appetite and lowers blood glucose concentrations by acting on peripheral tissues.
- Garlic- it helps to stabilize cholesterol level to keep the heart healthy and also helps to normalize blood sugar level. It is also resulted a good remedy to lower the blood pressure when intake in regular basis.

CHAPTER 2

Review of Literature

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2.1 What is Anti-diabetic food?

Anti-diabetic foods are huge source of omega-3 fatty acids which have DHA which is docosahexaenoic acid and EPA which means eicosapentaenoic acid, which have main benefit for heart. Intake of these fats is very important for people who have diabetes or bigger risk of heart diseases. A diabetic meal is one that is built specifically for patients or high blood sugar to help them manage the symptoms and risks associated with long-term blood glucose increases (especially: heart failure, kidney defect, overweight). There is no unanimity across guideline assessments, including the American Diabetes Association (ADA) and Diabetes UK, that one diet is superior than others. That's also due to a scarcity of long-term, heavy papers on the subject.

To obese or severely overweight diabetes patients, the most important aspect like any meal is that it causes increased fat reduction. Losing weight has resulted in an increase in glucose level balance and a fall in insulin levels. One of most widely accepted approach is to avoid sweets and starchy foods in the meal while raising highly nutritious texture, particularly dissolve texture. Similarly, diabetic patients can be advised to limit their consumption of foods with a high Sugar content (GI), even when the ADA and Diabetes UK state that further evidence is essential.

2.2 Advantages of Anti-diabetic foods

- These are low in calories.
- It helps in reducing cholesterol.
- It also helps in lower glucose levels.
- These anti-diabetic foods are rich source of vitamins, amino-acids, etc.
- It has anti-diabetic, anti-inflammatory, anti-oxidant properties.

2.3 *Chebulic myrobalan*

Chebulic myrobalan plant possesses medicinal activities such as anti-diabetic, anti-oxidant, anti-microbial etc. Fruit of this tree owns health benefits and can be used as traditional medicine for household remedies. In this, there is presence of many phytochemicals such as alkaloids, flavonoids, polyphenols, terpenes etc.

2.4 Botanical explanation

Tree of *chebulic myrobalan* fruit is about 50-80 feet in height. The bark of the tree is dark brown in color with some cracks. The color of the flower is dull white to yellow. It has unpleasant odor. The flowers appear in tree in the month of May – June. The fruit appears in the month from July– September. When Fruit is unripe it is of green color and when it is ripe it is of yellowish grey in color.

2.5 Uses and Benefits of *Chebulic myrobalan*

Chebulic myrobalan has greatest capacity of healing wounds. It lowers Blood sugar. It reduces cholesterol. It is used for dysentery. It promotes digestion. It manages diabetes. It enhances weight loss. It also maintains heart health. It heals infection. It also improves oral hygiene. These diseases have beneficial effects like diabetes, skin diseases, constipation, ulcers etc.

2.6 Proposed Approach: Enhancing Anti-diabetic potential of Chebulic Myrobalan juice using pectinase

Haritaki is a fruit from “Chebulic Myrobalan tree”. Its common name is Harad .Haritaki fruit tastes sweet, sour, bitter depending upon its types. Color of fruit is green .It is found in the forests of Northern western ghats .It has been used as Ayurvedic medicine in India. It has some nutritional values like anti-oxidant, anti-diabetic, anti-bacterial, anti-cancer. Chebulic myrobalan is called as “the king of medicine”. Chebulic myrobalan is commonly used for diabetes, high cholesterol etc. Tannins, flavonoids, steroids are identified in fruit of myrobalan. Tannins are polyphenolic substances. Chebulic myrobalan is known as king of medicines because it has some great power of healing. I have bought some dried fruit of chebulic myrobalan from local market, which is totally dried and very hard and are black in color.

CHAPTER 3
Materials and methods

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3.1 Materials required

- *Chebulic myrobalan* dried fruit – it is easily available in local store; it was bought from a near store from the local market. They are hard in nature as compared to other fruits and highly bitter in taste.
- Mixer Grinder
- Pectinase Enzyme
- Sieve
- Distilled Water
- Beaker
- Glass Rod
- Test Tubes
- Test Tube Stand , Dropper
- pH paper
- Centrifuge tubes
- Cuvette

- Filter paper
- Pipette
- Tips
- Spatula
- Conical flask
- Measuring cylinder
- Wash bottle
- Funnel
- Ice Bucket
- Aluminum Foil
- Tissue paper
- Falcon tubes
- Air Tight plastic Bags

3.2 Chemicals utilized

- ABTs (2,2 – Azinobis -(3-Ethylbenzthiazolin – 6 – Sulfonic Acid))
- Folin Ciocalteau Reagent
- Sodium Carbonate
- Pectinase Enzyme

3.3 Instruments

- Weighing Balance- It is a tool used to measure weight or mass. These are also called as weight balance, weight scales etc. It is an essential tool in laboratories.
- Mixer Grinder – it is used to mix and grind a variety of food items. A grinder food processor is a handy mill that merges joining and crushing operations.
- Incubator – it is used to maintain and grow cell cultures. Incubator maintains optimal temperature, humidity.
- Pipette- it is used to transfer small amount of liquid from one location to another. Without pipette it would be messy and unsafe.
- Water Bath– It is used to incubate samples in water at a stable hotness more a lengthy period of time. Temperature range is from 30 degree – 100 degrees Celsius.
- Uv- visible Spectrophotometer
- Centrifuge
- Ice Machine

3.4 Preparation of *Chebulic Myrobalan* Powder

- After bought from the market fruit was sun dried for 2-7days. Until the fruit gets totally sun dried.
- After sun drying the fruits it was broken into small pieces so as to make the grinding process easy.
- The small pieces were allowed to grind using mixer grinder slowly as due to hardness of the fruit.
- Obtained powder was sieved as it was hard to grind each and every part of the fruit. The powdered material was collected.

- Collected powdered was stored in a clean and moisture free packet. The packet was sealed and stored to avoid any kind of air entering the powder.

3.5 Preparation of *Chebulic Myrobalan* samples (with and without Pectinase)

- Using powder 50% solution of the Chebulic myrobalan was prepared.
- 10-10gm of powder sample was weighed using weighing balance. Avoiding all kinds of error while weighing.
- Three test tubes were placed in the test-tube stand marked as 1, 2 and 3.
- 50ml of distilled water can measured using a measuring cylinder and transferred into beaker.
- As such three different beakers were taken with 50-50ml of distilled water in each.
- Now slowly chebulic powder was added into each beaker and a sterilized glass rod was used to mix it well.
- Test tube marked as 1 was filled up with solution containing 50ml water and 10gm of chebulic powder.
- For test tube marked as 2, the solution was added after adding 0.05gm of pectinase enzyme in powdered form. This makes the pectinase concentration 1% (1gm/l) in 50ml of solution with 10gm of powder. Low concentration solution was prepared.

- For test tube marked as 3, the solution was added after adding 0.25gm of pectinase enzyme which makes its concentration 5% (5gm/l) in 50ml of the solution mixed with 10gm of powder. High concentration of the solution was prepared.
- The test tubes were kept in water bath which was preheated at 90 degrees Celsius. It was kept for 5mins for the activation of Pectinase Enzyme.
- After incubation the test tubes were kept in ice bucket containing ice for the inactivation of the Pectinase Enzyme.
- After cooling down the test tubes was kept at room temperature.
- After reaching the temperature up to room temperature the solution was carefully transferred into falcon tubes which were marked in same sequence.

Fig 1. Prepared solution before centrifugation

- Centrifuged for 10mins at 4000rpm at 22 degrees Celsius temperature.

Fig 2. After Centrifugation at 4000 rpm)

- Clear supernatant solution was obtained after centrifugation which was carefully transferred into fresh and clean falcon tubes.
- pH of each sample was also measured using pH paper.

Fig3. pH of the sample

- The falcon tubes were well marked and stored in cold storage (-20^{o}C) for further testing.

3.6 Preparation of ABTS reagent

- First prepare solution A which consists of 13.2mg of Potassium per sulfate in 10ml of distilled water.
- Then for solution B 32mg of ABTS was weighed and dissolved in 4ml of Distilled water.
- Combine 4ml of solution A and B stored in dark after mixing well for 12-16hours of duration.

3.7 Performing Total Anti-Oxidant Capacity (TAC) By ABTS Assay

- Take clean test tube and marked as 1, in this test tube add 200 micro liters of sample from the first falcon tube and after adding this add 1800 micro liters ABTS reagent.
- Take another test tube, and marked as 2, in this test tube also add 200 micro liters of sample from 2nd falcon tube and then 1800 micro liters.
- ABTS reagent.
- In third test tube and marked it as 3, add 200 micro liter sample from 3rd falcon tube and 1800 micro liters of ABTS reagent.
- Keep the test tubes in test tube stand in a sequence.
- After that incubate test tubes it at room temperature in dark as ABTS is light sensitive 30 minutes.After this, absorbance (y) is measured at 734 nm.

3.8 Performing Total Phenolic Content (TPC) by Folin - Ciocalteu Reagent Assay

- Take a clean and dry beaker, add 20ml of distilled water and then add 2ml of FC reagent using a clean measuring cylinder.
- Incubate it for 5mins in dark area.
- Take 3 clean test tubes add 300 micro liters of juice samples from 3 different Falcon tubes using pipette. Change tips for each juice sample.
- In 4^{th} test tube add 300 micro liter distilled water for standard.
- In each test tube add 1950 micro liter Folin-Ciocalteu reagent using pipette and keep test tubes in test tube stand.
- After incubation 1950 micro liter sodium carbonate anhydrous pure was added in each test tube.
- Test tubes were stand for 90 minutes in dark.
- After that absorbance (y) was measured at 750nm.

CHAPTER 4

Results and Discussion

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4.1 pH – The pH of each juice sample was measured same i.e., 2.

4.2 TAC estimation studies

Table1. Standard Curve utilized for TAC estimation.

Conc. Of Gallic Acid (mg/ml)	Absorbance at 734nm
0.625	0.04
1.25	0.09
2.5	0.2
5	0.4
10	0.8

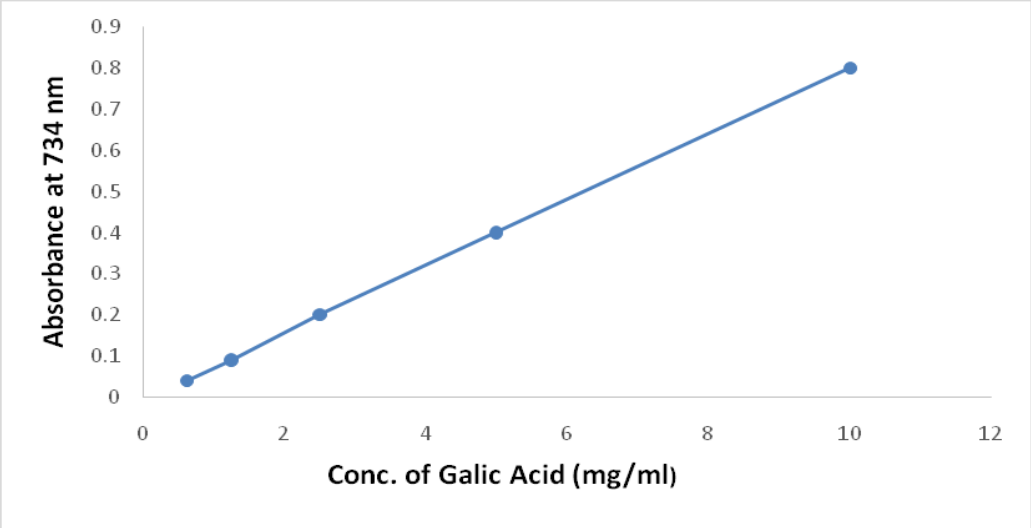

Fig 4. Standard Curve utilized for TAC estimation

Table 2. Samples incubated with and without pectinase and their respective Total Antioxidant Capacity (TAC).

Sample	**Incubation Time with Pectinase Enzyme**	**Temperature (^{o}C)**	**Absorbance at 734mn**	**Pectinase concentration**	**Total Antioxidant Capacity**
Sample 1	5min (without Pectinase)	90	0.211	0%	2.630
Sample 2	5mins	90	0.121	1% (Low)	1.650
Sample 3	5mins	90	0.051	5% (High)	0.745

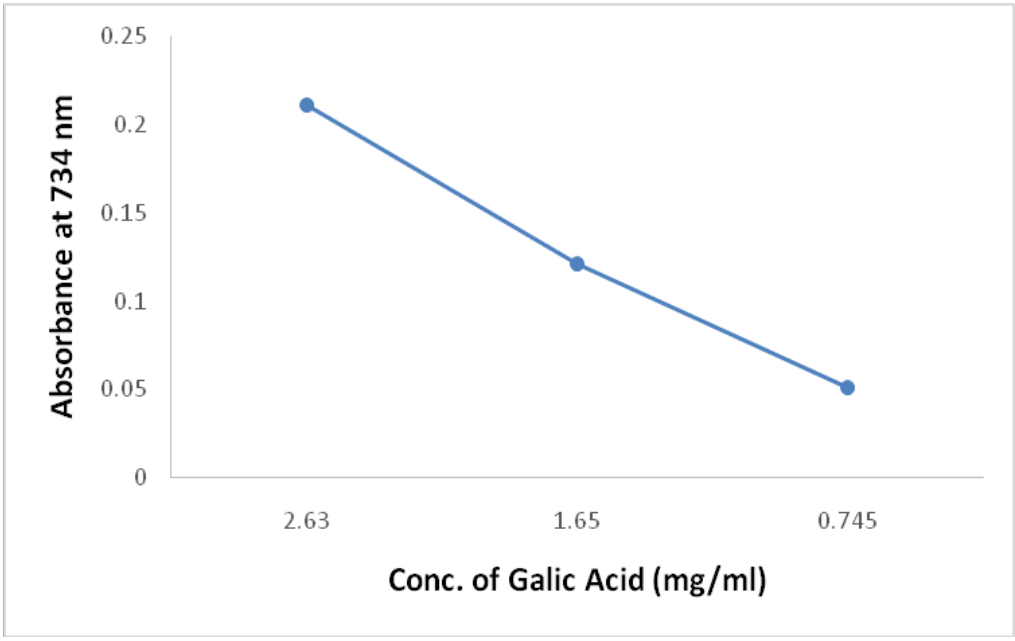

Fig. 5 . Decrease of TAC upon pectinase treatment

4.3 TPC studies

Table 3. Standard Curve utilized for TPC estimation

Conc. Of Gallic Acid (µg/ml)	Absorbance at 750 nm
50	4.268
100	3.466
150	2.267
200	0.172

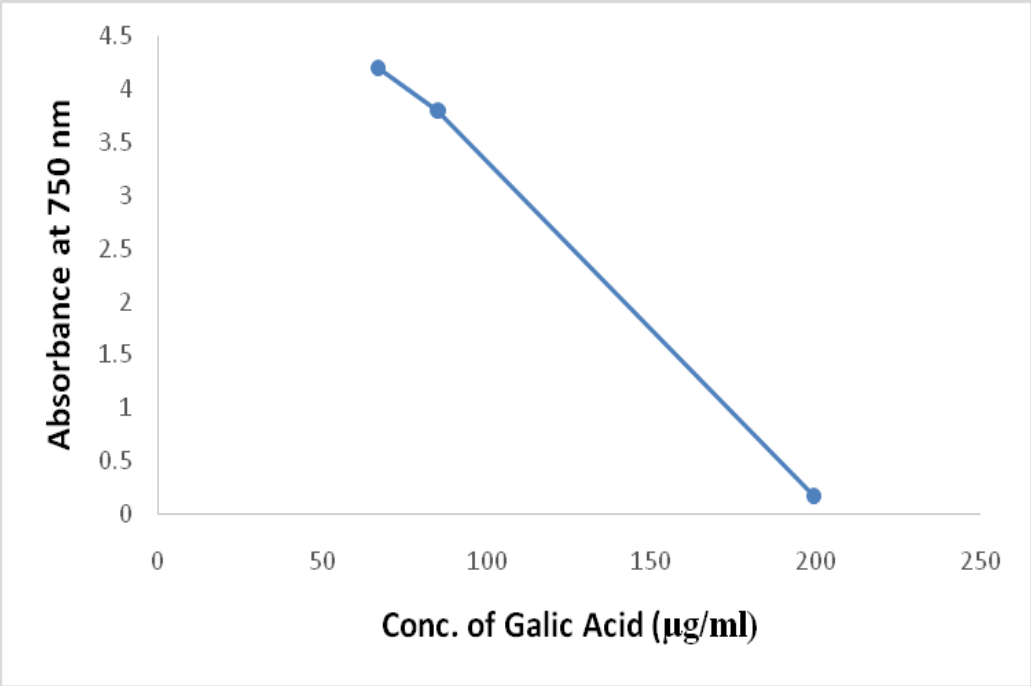

Fig.6. Standard Curve utilized for TPC estimation.

Table 4. Samples incubated with and without pectinase and their respective Total Phenolic Content (TPC).

Sample	Incubation time with Pectinase Enzyme	Temperature (^{o}C)	Absorbance at 750nm	Pectinase Concentration	Total phenolic content
Sample 1	5mins (Without Pectinase)	90	4.2	0%	67.001
Sample 2	5mins	90	3.8	1% (low)	85.035
Sample 3	5mins	90	0.181	5%(low)	199.09

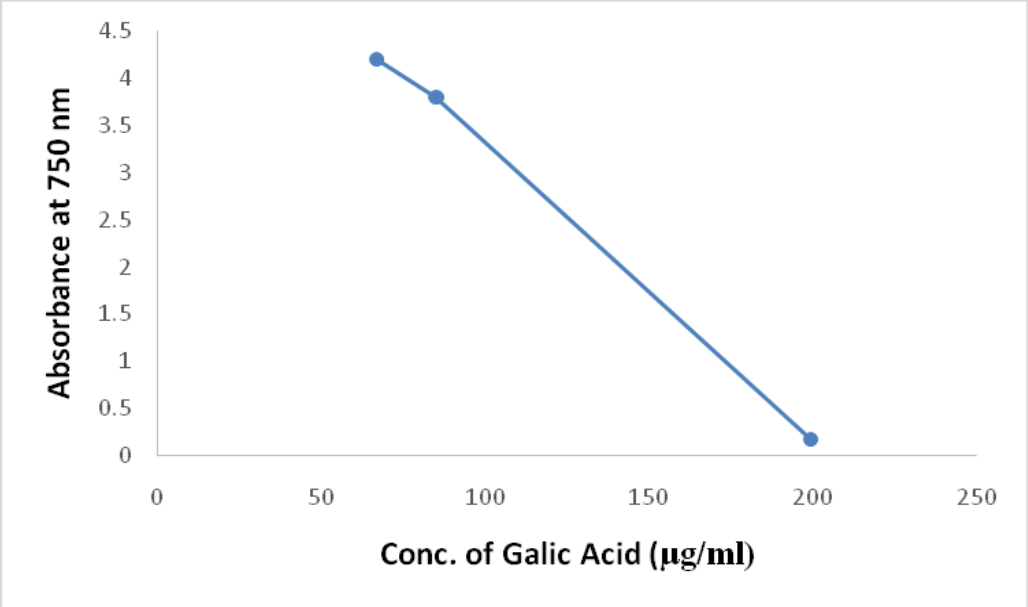

Fig.7. Decrease of TPC upon pectinase treatment.

CHAPTER 5
Conclusions

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- A brown color fine powder was obtained after grinding the sundried fruit of Chebulic Myrobalan that was brought from the local market. After, the sample was prepared and centrifuged with or without pectinase concentration a brown color solution was obtained in supernatant form.
- From Total Phenolic Content Test and Total Antioxidant Capacity Test it was concluded that the addition of Pectinase Enzyme decreases the release of bioactive compound as it increased in Bitter Guard Juice according to research.
- The high (5%) and low concentration (1%) of the Pectinase Enzyme was used in case of Chebulic myrobalan where the activation of the enzyme was done for 5mins at 90 degrees Celsius and in Result of that the decrease in bioactive compounds.

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