



## Isolation and Identification of $\alpha$ - Amylase activity Inhibiting compounds from *Bryophyllum Pinnatum*

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### Abstract

*Bryophyllum pinnatum* (*B. Pinnatum*) is an herb growing widely and utilized in folkloric medication in tropical Africa, tropical America, India, China, and Australia. Aqueous extract of *B. pinnatum* leaf was tested for alpha amylase inhibition properties which showed significant inhibitory activity against alpha amylase. *B. pinnatum* found to have maximum alpha amylase inhibitory activity at a concentration of 100 $\mu$ g/ml. A compound Digitoxin showed maximum binding energy with alpha amylase comparative to drug Metformin, Metformin at concentration 100  $\mu$ g/ml showed 67.34 $\pm$ 0.002% inhibitory effects on the alpha-amylase activity with an  $IC_{50}$  value 3.25 $\mu$ g/ml. The alpha-amylase inhibitory activity of 79.41 $\pm$ 0.002% with an  $IC_{50}$  value 2.54 $\mu$ g/ml was shown by aqueous leaf extract of *B. pinnatum* at a concentration 100  $\mu$ g/ml. The plant extract showed potential alpha-amylase inhibitory activity in a dose dependent manner.

**Keywords:** *Bryophyllum pinnatum*, Alpha-Amylase, Digitoxin, Metformin, Autodock vina.

### Introduction

Diabetes can be described as a metabolic disorder, characterized by persist hyperglycemic with disturbance of carbohydrate, protein and fat metabolism due to inadequacy in insulin secretion action or both<sup>1</sup>. It results either insufficient emission of hormone insulin, an inadequate reaction of target cells to insulin or a combination of these components. This disease needs medical diagnosis, treatment and changes in life style. The disease is associated with lower quality of life and increased threat for transience and morbidity. As stated by the report of International Diabetes Federation, around 194 millions of people have diabetes and by 2025 there'll be 333 million diabetes sufferers worldwide. There are two main classes of diabetes, Type 1 and Type 2 diabetes.

Type I is insulin dependent diabetes mellitus. Around 10-15% of patients have this polygenic disorder. The pancreas produces insufficient quantity of insulin, outcomes the demand of insulin injections to restrict the blood glucose level. It is characterized by an instantaneous start, usually before the age of 30 years<sup>2</sup>.

Type II is non-insulin dependent diabetes mellitus. It results from a decrease in the amount of insulin produced and decrease in the sensitivity of the cells to insulin. Around 90-95% of patients have type II diabetes. This type II diabetes is treated with exercise and diet, and if elevated glucose levels persist, diet is supplemented with oral hypoglycemic agents. Those individuals who usually control type II diabetes by the action of diet, physical exercise, in their body, may be required insulin injection<sup>2</sup>.

Amylases are a group of enzymes that catalyzes the hydrolysis of the glycosidic linkages in starch and various other oligosaccharides<sup>3</sup>.  $\alpha$ -glucosidase and  $\alpha$ -amylase are the important enzymes involved in the metabolism of carbohydrates and also involved in the breakdown of long chain carbohydrates and alpha glucosidase shatter down starch and disaccharides to glucose. They serve as the leading digestive enzymes and help in intestinal absorption. For the treatment of diabetes, alpha amylase and glucosidase inhibitors are the possible targets in the development of steer compounds<sup>4</sup>. Higher plants, animals and microorganisms are found to produce different protein inhibitors of alpha amylases and alpha glucosidases in order to control the operation of this enzymes<sup>5</sup>. Some of these protein inhibitors acts by directly blocking the active center of the enzyme at varied native sites<sup>6</sup>.

In animals, inhibitors of  $\alpha$ -amylase decrease high glucose levels which can be obtained after a meal by slowing down the speed of breakdown of starch to sugars<sup>7</sup>. Low insulin levels stop the rapid clearing of extracellular glucose from the blood plays important part for diabetic people<sup>8</sup>. In order to keep their glucose levels under control diabetics tend to have low alpha amylase level. Plants also use alpha amylase inhibitors as a defense purpose as a protection from insects. These inhibitors change the digestive activity of alpha amylases and proteinases in the abdomen of insects and inhibit their normal feeding behavior. Therefore alpha amylase inhibitors have possible roles in controlling blood sugar levels and crop protection<sup>9</sup>. For a long time natural products from plants have been used for the treatment of polygenic disorder, commonly in developing countries wherever the facility are limited and affordability and access to modern treatment is a problem<sup>10</sup>.

Extensive research has been carried out to screen the bioactivity of these inhibitors because of their remarkable importance in health care and medicine<sup>11</sup>. There is a develop attention in herbaceous treatment because of the side effects related with the oral therapeutic- agent for the cure of diabetes mellitus. So the conventional herbal treatment are mainly used which are collected from plants, play important character in the management of diabetes mellitus<sup>12</sup>.

Scientifically apprise variety of the ethnomedical uses of *B. pinnatum* leaves, the present study was manage to analyze the antidiabetic properties of *B. pinnatum* leaf extract. It is generally known as air plant, love plant, miracle leaf, life plant, panfutti, ghayamari<sup>13</sup>, has been accepted as an herbaceous medication in almost all parts of the world<sup>14</sup> etc. It is a crassulescent herb with opposite, smooth hairy leaves<sup>15</sup> distributed worldwide and growing primarily within the rain forest<sup>16</sup>. It grows widely and used as herbal medication in tropical Africa, India, China, Australia, Asia and Hawaii<sup>17</sup>. It is astringent, bitter in taste, sweet within the post digestive outcome and has potency.

## Materials and Methods

**Sample collection:** *B. pinnatum* fresh leaves were collected from G. B. Pant Engineering College, Pauri and Rudranath, Chamoli region between August, 2015 and June, 2016. Stored at 4°C for further investigation.

**Preparation of extract:** 40gm of fresh grinded leaves were taken and run soxhlet extraction unit with 200ml of distilled water at 100°C. Then the aqueous extract was filtered and concentrates to 50ml of solution. Finally, light brown colour solution was appeared. The extract was further analyzed for phytochemical and alpha amylase enzyme inhibitory assay. The complete assay was performed in triplicate<sup>18</sup>.

**α-amylase enzyme inhibition assay:** 2 mg of starch was dissolved in each of the tubes having 0.2ml of 0.5 M Tris-HCl buffer (pH 6.9) and 0.01 M CaCl<sub>2</sub>. Tubes were boiled for 5 min contained substrate solution and then incubated for 5min at 37°C. 0.2 ml of plant extract taken in each tube of different concentrations of dimethyl sulfoxide. Porcine pancreatic amylase (PPA) was dissolved in Tris-HCl buffer to form (10, 20, 40, 60, 80 and 100µg/ml) concentration of 2units/ml and enzyme solution 0.1ml were added to each of the above tubes. The reaction took place for 10 min at 37°C and was ended by adding 0.5 ml of 50% CH<sub>3</sub>COOH in each tube. Centrifugation of the reaction mixture was done at 3000 rpm for 5 min at 4°C. The optical density of the resulting supernatant was noted at 595 nm using UV-Vis spectrophotometer. Alpha amylase inhibitory activity of plant extract and standard drug was calculated using formula<sup>19</sup>.

**% inhibition** =  $(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test sample}}) / \text{Absorbance}_{\text{control}} \times 100$

Where: Control 1- alpha amylase + starch solution +DMSO, Control 2 –drug +starch solution +DMSO, Test sample 1- Plant

extract + starch solution + amylase + DMSO, Test sample 2- Metformin (drug) + starch solution + amylase + DMSO.

**LC-MS analysis of leaf extract:** An LC-MS result of plant extract was done by CDRI, Lucknow. In this, some highest peaks were observed and compounds were identified by online library based on these resulting peaks, some of the compounds were provided by CDRI in their data base. Furthermore, these compounds were used for docking in Auto dock vina tool. More than 40 compounds were found on highest peak and they are used for further analysis in docking procedure which is an *insilico* procedure<sup>20</sup>.

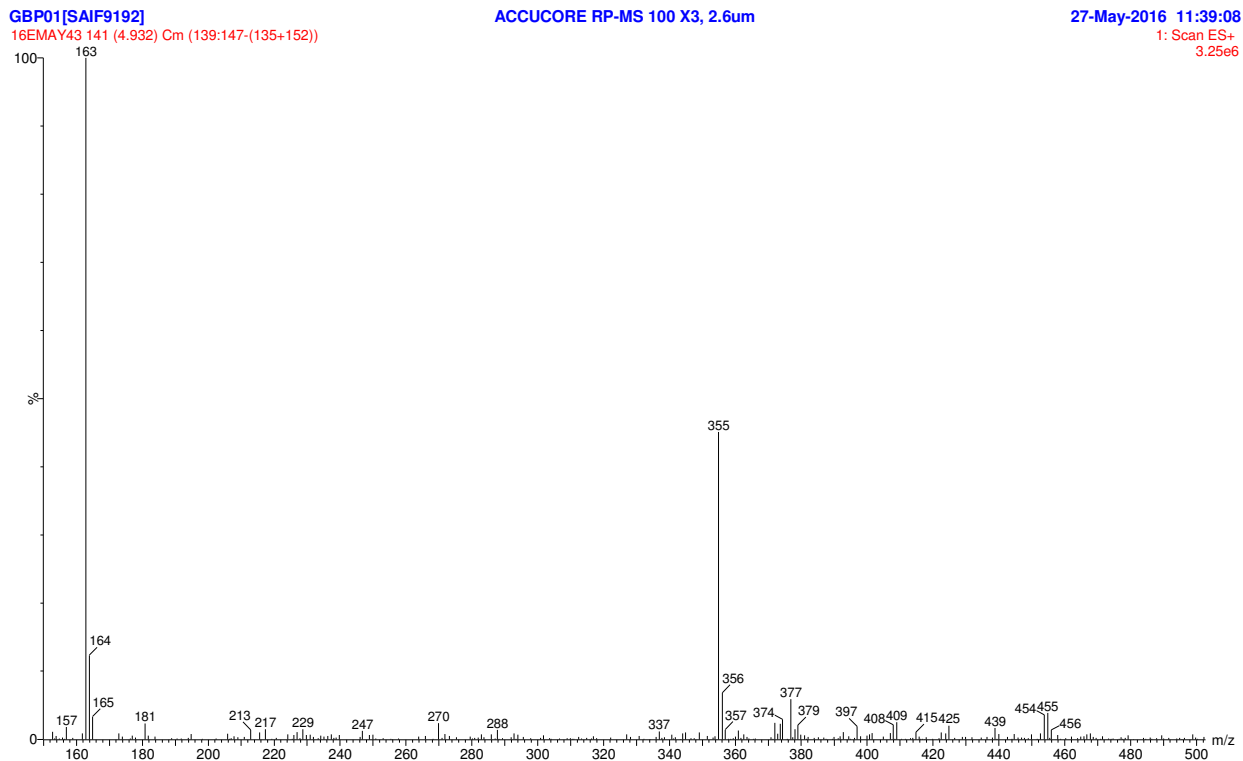
**Docking analysis:** All 40 compounds found to have highest peaks in LC-MS results were docked in Auto dock vina. Grid preparations of all the selected receptors were performed using Auto dock vina. Each compound goes through docking procedure on by one and shows their most finding affinity towards alpha-amylase in config. file. The resulting docking file was read by log file in Auto dock vina tool to analyze the output. The compound which has most binding affinity towards alpha-amylase will be our best result<sup>21</sup>.

## Results and Discussion

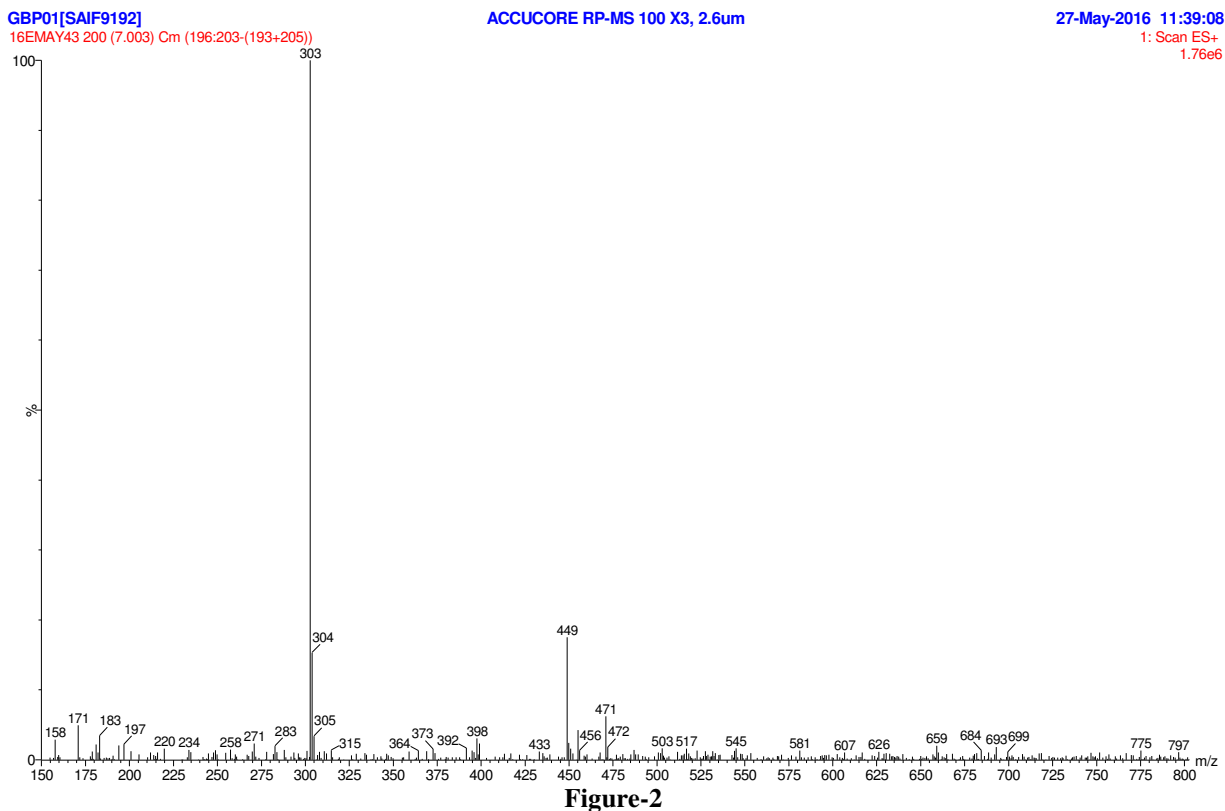
In the early treatment of diabetes, control of post-prandial plasma glucose was difficult. Inhibition of enzymes collaborating within the metabolism method of carbohydrates is one of the therapeutic methods to reduce post-prandial hyperglycemia<sup>22</sup>.

**Phytochemical analysis:** Phytochemical evaluation of the leaf extract was done using different methods<sup>23</sup>. The qualitative assays showed the presence of flavonoid, reducing sugar, tannins, phenol and saponins.

**LC-MS analysis of leaf extract:** An LC-MS result of plant extract was done by CDRI, Lucknow. In this, some highest peaks were observed and compounds were identified by online library based on these resulting peaks. More than 40 compounds found on highest peak and they are used for further analysis in docking procedure which is an *insilico* procedure. These compounds are as follow: 4-hydroxy-3-methoxy-cinnamic acid, 4-hydroxybenzoic acid, 9,12-octadecadienoic acid, ethyl ester, 9-octadecenoic acid, methyl ester, 22-dihydrobrassicasterol, 24-epiclerosterol, astragalol, bersaldegennin-3-acetate, bryophyllin A, bryophyllin B, bryotoxin A, bryotoxin B, caffeic acid, campesterol, clerosterol, clionasterol, codisterol, digitoxin, digoxin, ferulic acid, friedelin, glutinol, hexadecanoic acid, ethyl ester, hexadecanoic acid, methyl ester, isofucosterol, kaempferol, kaempferol-3-o-glucoside, linoleic acid ethyl ester, luteolin, para-coumaric acid, patuletin, peposterol, protocatechuic acid, pseudo taraxasterol, digitoxingenine, quercetin, rutin, stigmasterol, syringic acid, taraxerol. The results of LC/MS is in the form of graph are shown below.



**Figure-1**  
Determination of Digitoxin compound at 163 m/z value whose molecular weight 762.923180 g/mole and molecular formula  $C_{41}H_{62}O_{13}$



**Figure-2**  
Determination of Quercetin at 303 m/z value whose molecular weight 302.04265 g/mol. and molecular formula  $C_{15}H_{10}O_7$

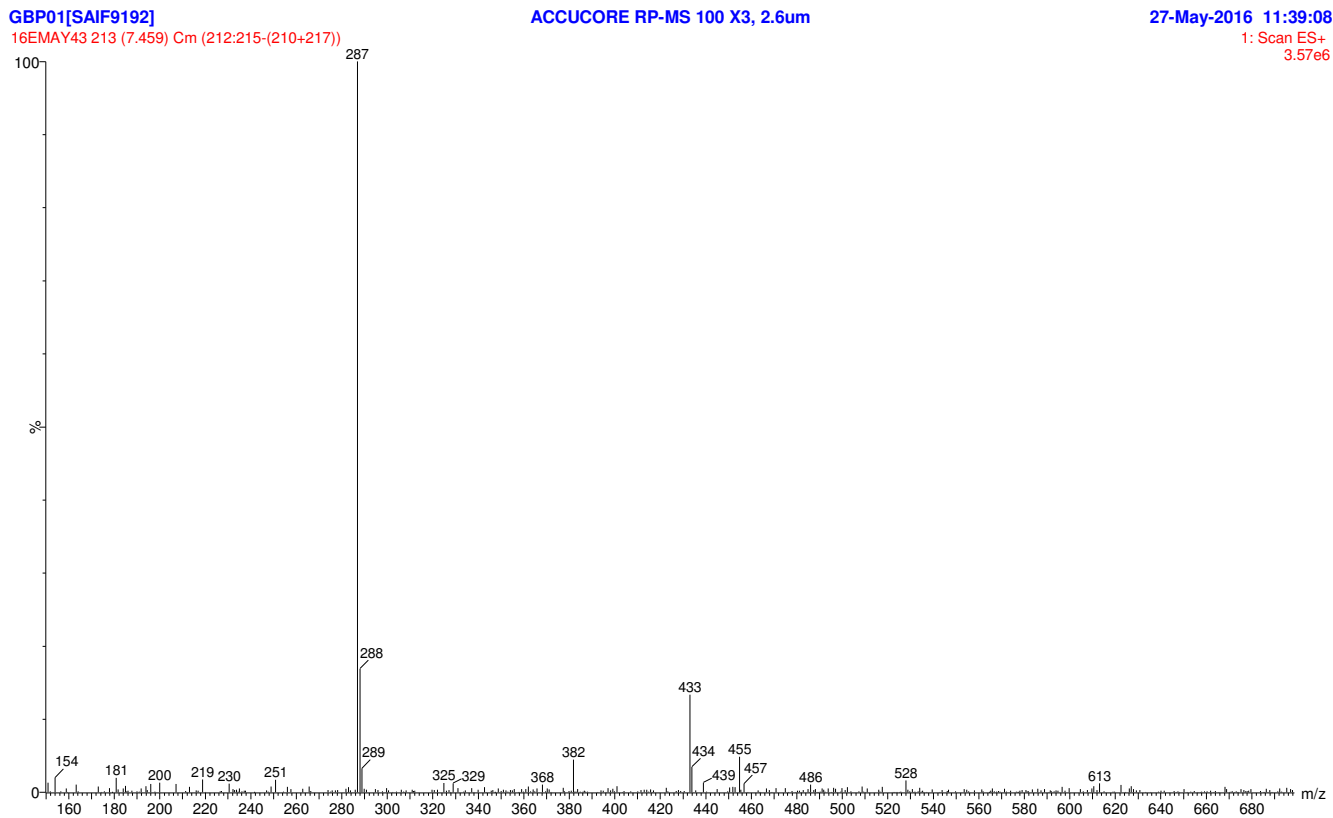


Figure-3

Determination of Kaempferol at 287 m/z value whose molecular weight 286.04774 g/mol. and molecular formula  $C_{15}H_{10}O_6$

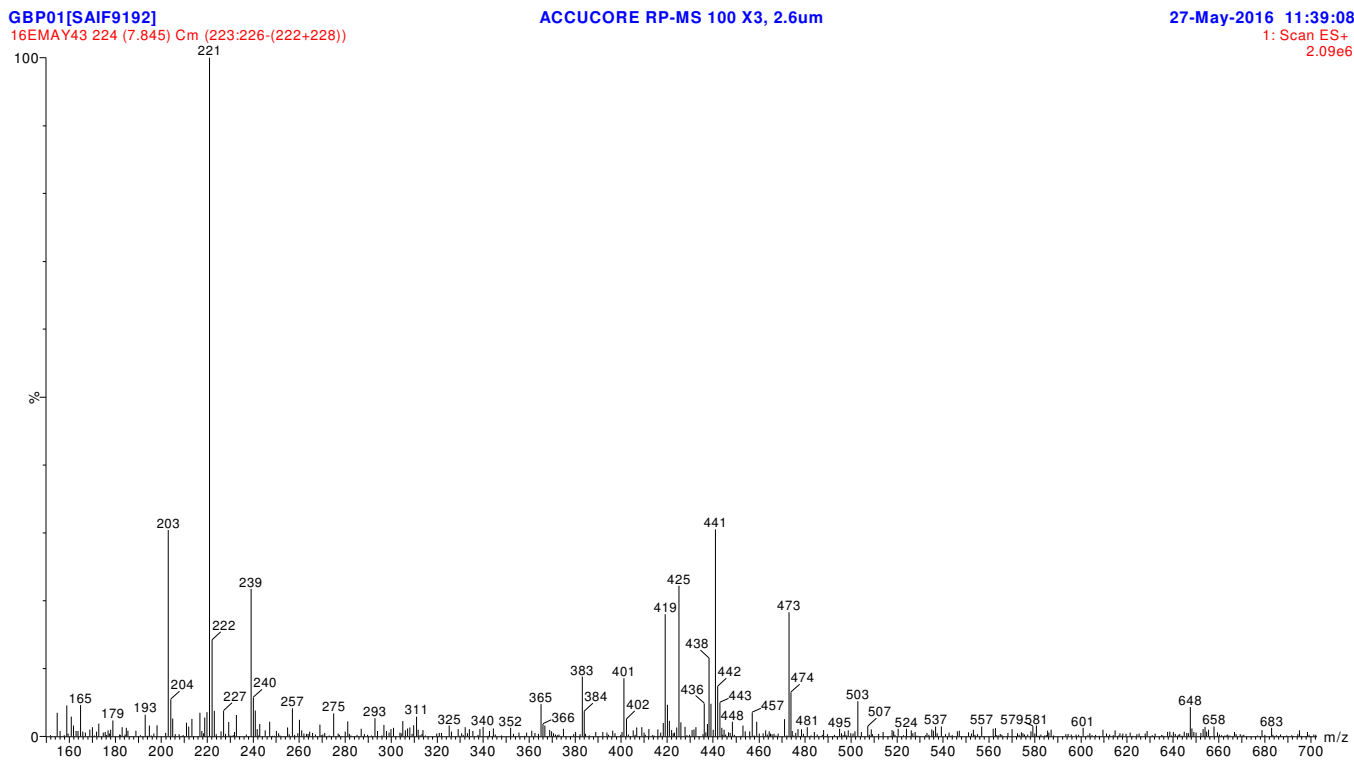
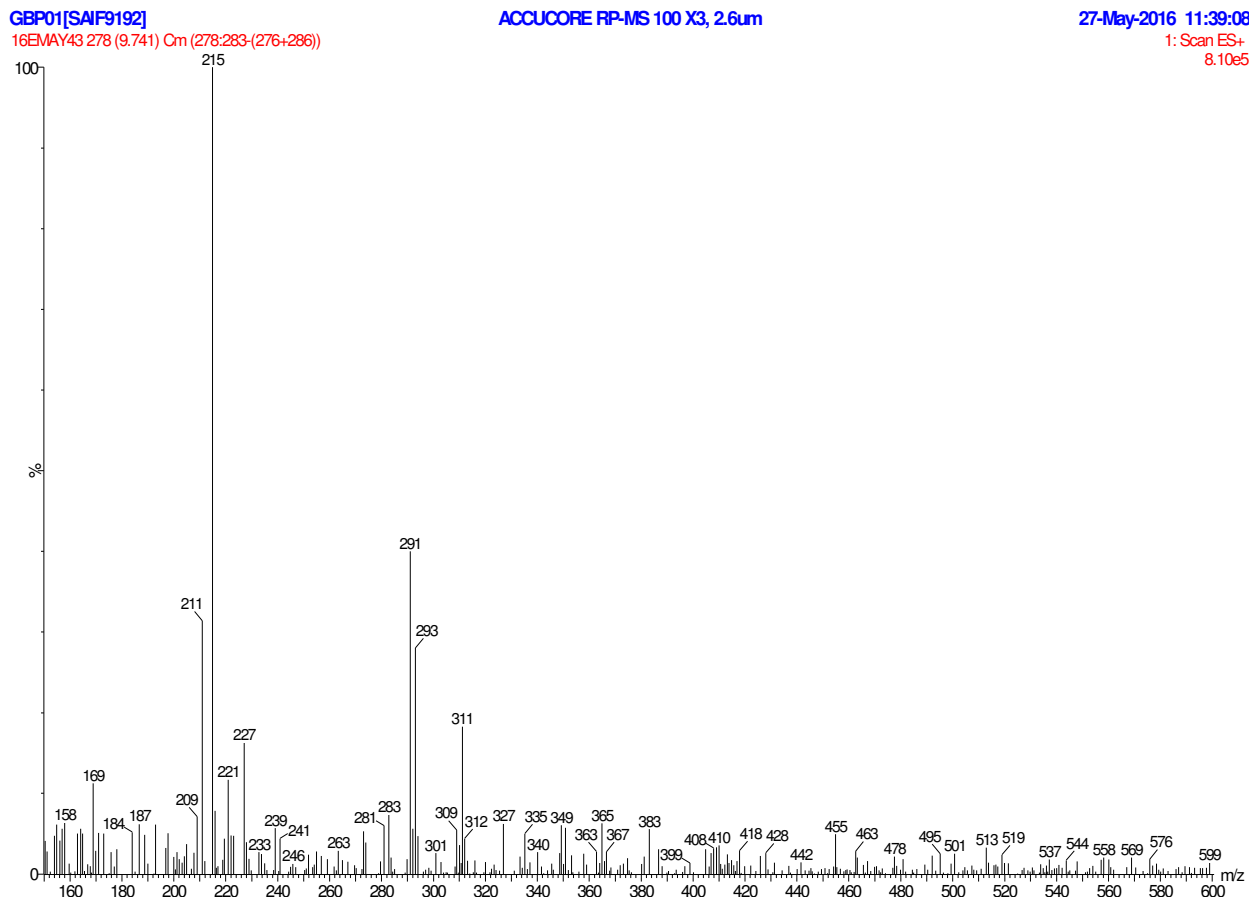


Figure-4

Determination of Linoic acid at 221 m/z value whose molecular weight 280.24023 g/mol. and molecular formula  $C_{18}H_{32}O$



**Figure-5**  
 Determination of Digitoxigenine at 215 m/z value whose molecular weight 387.12370 g/mol. and molecular formula  $C_{21}H_{22}ClNO_4$

**Table-1**  
 Inhibition of pancreatic alpha amylase by aqueous extract of *B.pinnatum* and standard drug at varying concentration

S. No.	Concentration (µg/ml)	% inhibition by aqueous extract of <i>B.pinnatum</i>	IC <sub>50</sub> value of aqueous extract of <i>B.pinnatum</i>	% inhibition by metformin	IC <sub>50</sub> value of Metformin
1	10	39.51 ±0.002	2.54µg/ml	37.19 ±0.012	3.25µg/ml
2	20	43.24 ±0.001		41.86 ±0.001	
3	40	53.54 ±0.002		51.06 ±0.002	
4	60	59.22 ±0.001		54.17 ±0.001	
5	80	73.86 ±0.001		56.81 ±0.002	
6	100	79.41 ±0.002		67.34 ±0.002	

**In vitro alpha amylase inhibitory assay:** The percentage inhibitory activity exhibited by extract and drug is shown in Table-1 and Figure-6. Metformin at concentration 100 µg/ml showed 67.34±0.002% inhibitory effects on the alpha-amylase activity with an IC<sub>50</sub> value 3.25µg/ml. The aqueous leaf extract

of *B.pinnatum* at a concentration 100 µg/ml exhibited 79.41±0.002% of alpha-amylase inhibitory activity with an IC<sub>50</sub> value 2.54µg/ml. The plant extract showed potent alpha-amylase inhibitory activity in a dose dependent manner.

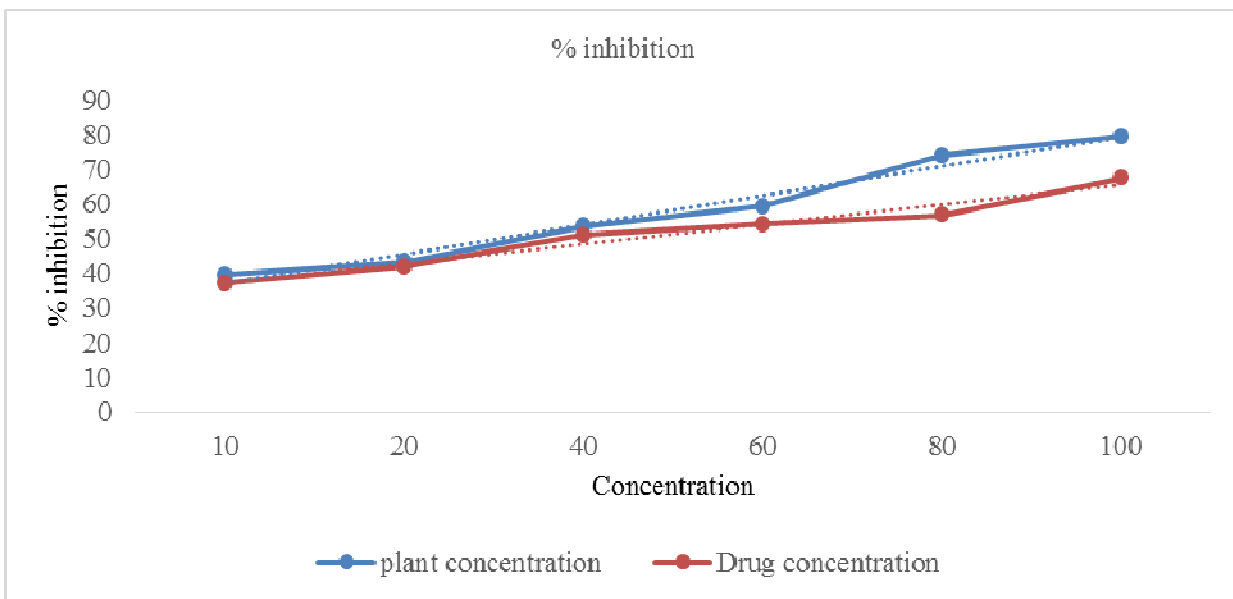


Figure-6

Inhibition percentage of pancreatic alpha amylase by aqueous extract of *B. pinnatum* and drug Metformin at varying concentration

**Docking Analysis:** In autodock vina, all the compounds were docked with pancreatic alpha amylase. A compound Digitoxin from *B. pinnatum* showed more binding affinity towards pancreatic alpha-amylase as compared to standard drug Metformin (Table-2).

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Table-2

In this Digitoxin showed highest binding affinity with pancreatic alpha amylase as compared to drug

Compound name	Binding affinity (kcal/mol)	Drug name	Binding affinity (kcal/mol)
Digitoxin	-11.1	Metformin	-5.0

**Statistical analysis:** The statistical analysis was performed by using ANOVA. Results are expressed as mean ±SD and n =3<sup>24</sup>.

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**Conclusion**

The results of the present study indicate that aqueous extract of *B. pinnatum* showed the maximum alpha amylase inhibitory activity. The plants may essentially contain herbal bioactive compounds inhibiting enzyme activity.

The present study was restricted to the preliminary screening of enzyme inhibitory activities of the selected plant extract. In conclusion, Digitoxin a compound of plant extract has more inhibitory property against alpha amylase than a standard drug named Metformin. This compound uses as drug against diabetes.

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