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# *In vitro* Inhibitory Activities of α-Amylase and Pancreatic Lipase of Some Fruit Extracts

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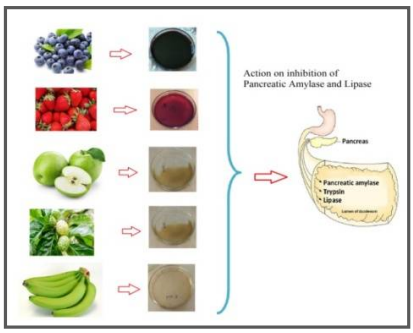
## ABSTRACT

*To explore the alcoholic extracts of some fruits on inhibition of amylase and lipase enzyme systems. Fruits like Vaccinium corymbosum (Blueberry), Fragarria anannasa (Strawberry), Granny smith apple (Green apple), Musa paradisica (Banana) and Morinda citrifolia (Indian Mulberry) were selected for the study. The alcoholic extracts of the fruits were examined for their phenolic, flavanoidal and anthocyanin content. Amylase and lipase inhibitory activity of the extracts were determined according to the reported methods. All the alcoholic extracts exhibited potent inhibitory activities for amylase and lipase at 100 µg $mL^{-1}$. The activity is compared with that of reference standard acarbose and orlistat respectively. Vaccinium corymbosum exhibited a potent activity against amylase with IC50 of 19.7 ± 0.654 and Granny smith apple against Lipase inhibition with IC50 of 22.8 ± 1.985. The other fruit extracts also showed the inhibitory activities with their IC50 values below 60 µg $mL^{-1}$. The presence of polyphenolic compounds resulted in the inhibitory actions of the extracts. Thus, the extracts can be used for reducing the progression of acute pancreatitis if they are used as dietary supplements.*

## Graphical Abstract




**Keywords:** Amylase, Lipase, Pancreatitis, Polyphenolics.

## INTRODUCTION

Pancreatic amylase and lipase are the major enzymes in the digestive system catalyzing the process of hydrolysis of complex food to simple and easily digestible molecules. Amylase has an initial role in the hydrolysis of starch to smaller oligosaccharides like maltose, maltriose and a number of oligoglucans. Whereas lipase, digest dietary triglycerides to triacylglycerols. Both the enzymes have a key role in diagnosis of acute pancreatitis [**1-3**]. Acute pancreatitis is a self limiting disease with inflammation of exocrine part of pancreas, left untreated may lead to chronic conditions involving extra organ manifestations. Amylase and Lipase levels rise eventually and reach its peak within 5- 8 hours of the onset of disease. The rise in the levels of these enzymes contributes to the premature intracellular activation of zymogens, leakage of enzymes leading to activation of self destruction pathway of acinar cells [**4**]. Reactive oxygen species have been reported to have a key role in the disease [**5**]. Although the treatment is restricted to symptomatic relief, many antioxidants like N-acetyl cysteine, Melatonin, Pentoxyfilline have been reported for their beneficial effects in treating the disease [**6**].

Reduction of risk factors of diseases has been associated with high dietary intake of fruits. Fruits are abundant source of bioactive phytochemicals that benefit health by affecting various physiological processes [**7**]. Phenolics are a group of plant metabolites that exert their action through a broad spectrum of mechanisms and their intake is associated with lower mortality rate [**8**]. Fruits comprise of the most copious amounts of dietary antioxidants and differ in their bioavailability depending upon their physical chemical properties [**9**]. The present study has been designed to evaluate the activities of fruits like *Vaccinium corymbosum (Blueberry), Fragarria anannasa (Strawberry), Granny smith apple (Green apple), Musa paradisica (Banana) and Morinda citrifolia (Indian Mulberry)* on inhibition of enzymes which further can be used in the treatment of acute pancreatitis.

## MATERIALS AND METHODS

**Chemicals:** All the chemicals used in the study were of analytical grade and were purchased from Sigma chemicals Pvt. Ltd.

**Fruit Material:** Fresh fruits of *Vaccinium corymbosum*, *Fragarria anannasa*, *Musa paradisica* and *Granny smith apple* were purchased from the local market. *Morinda citrifolia* fruit was collected from the institutional premises. All the fruits were processed in to puree and subjected to extraction with ethanol in an orbital shaker for 24 h. The resultant extracts were evaporated to dryness and used for evaluating the activities.

**Determination of Anthocyanin Content:** The anthocyanin content is determined by pH differential method. Briefly, 1 mL of the known concentration of the extract is taken in a 10 mL of volumetric flask for preparing two dilutions of the sample. One solution is adjusted with KCl buffer pH 1.0 and the other with Sodium acetate buffer pH 4.5. Both the solutions were incubated for 15 min at room temperature. The absorbance of the mixture was measured at 510 nm and 700 nm against distilled water blank [**10, 11**]. All the measurements should be made between 15 min to 1 h. The anthocyanin content is calculated as follows

$$\text{Total Anthocyanins (mg/100g of extract)} = \frac{A \times MW \times 1000}{\in \times C}$$

Where, A is absorbance ($A_{510}$-$A_{700}$), pH 1.0-($A_{515}$-$A_{700}$), pH 4.5, MW is molecular weight for cyanidin 3-glucoside) 449.2, € is the molar absorptivity of cyanidin 3-glucoside) 26 900 and C is the concentration of the buffer in milligrams per milliliter.

Anthocyanin content was expressed as milligrams of cyanidin 3-glucoside equivalents per 100 g of fresh apple peel for the triplicate extracts.

**Determination of Phenolic Content:** The total phenolic content of the extracts was determined using modified colorimetric Folin Ciocalteu estimation. In brief, 0.5 mL of known concentration of the extract was taken in the test tube and 2.5 mL of Folin Ciocalteu reagent dissolved in water was added. The solution is incubated at room temperature for 5 min. Now add 2.5 mL of 7.5% of sodium bicarbonate solution and the mixture is diluted with 3mL of deionized water. After 45 min, the reaction mixture absorbance is checked at 765 nm using a spectrophotometer. The measurement is compared to a standard curve of Gallic acid and the content of phenolics is expressed as Gallic acid equivalents per 100g [**12**].

**Determination of Flavanoidal Content:** The flavonoidal content was determined by taking 1 mL of known concentration of the extract and 1 mL of 2% aluminium chloride in methanol solution was added. The mixture is diluted further with 3 mL of deionized water and was incubated at room temperature for 1 h. After the incubation the reaction mixture absorbance was checked at 415nm using a spectrophotometer. The measurement is compared to a standard curve of Rutin and the content of flavonoids is expressed as rutin equivalents per 100g [**13**].

**Determination of Amylase inhibitory activity:** Amylase inhibitory activity was measured according to Bernfeld [**14**] with slight modifications. The inhibitory activity of the enzyme was expressed in liberated maltose units. Briefly, 1 mL of the alcoholic extracts were mixed with α-amylase and incubated for 30 min. After incubation, 1 mL of 1% starch solution was added and incubated again at 37°C for 10 min. The resultant reaction was blocked by the addition of 1 mL DNS reagent containing Sodium potassium tetrahydrate in sodium hydroxide and 3,5-Dinitrosalicylic acid. The mixture was heated in water bath for 5 min and the absorbance was measured at 540 nm against Blank where amylase enzyme is replaced with phosphate buffer. The amylase inhibitory activity was expressed as

$$\%\ \text{Amylase inhibition} = 100 - \frac{\text{Maltose(test)}}{\text{Maltose (Control)}} \times 100$$

**Determination of Pancreatic Lipase inhibitory activity:** The alcoholic extracts were subjected to lipase inhibitory activity according to this 0.1 mL of different concentrations of ethanolic extract were mixed with 8 mL of olive oil emulsion and 1mL of porcine pancreatic lipase followed by incubation for 60 minutes. Add 1.5 mL of mixture solution containing 95% ethanol and acetone in the ratio of 1:1, to stop the reaction. The fatty acids thus liberated was determined by titrating the reaction mixture against 0.02M NaOH (standardized with 0.01M Oxalic acid) using the indicator Phenolphthalein [**15**]. The percentage lipase inhibition was calculated as

$$\text{Lipase inhibition (\%)} = \frac{(A - B)}{\text{A}} \times 100$$

Where, A = lipase activity; B = activity of lipase when incubated with extract

## RESULTS AND DISCUSSION

In the present study different fruit extracts was evaluated for invitro alpha amylase and pancreatic lipase activity. The phytochemical parameters evaluated were total phenolic, total flavanoids and total anthocyanin content in different alcoholic fruit extracts. Among these different alcoholic fruit extracts of blue berry, *Fragarria anannasa*, Granny Smith apple, *Morinda citrifolia* and *Musa paradisica* was measured.

*Musa paradisica* was found to have higher total phenolic content for blue berry around 9.34 ± 0.23 when compared to other extracts, but for total flavonoid content *Morinda citrifolia* has higher value around 36.78 ± 1.01 when compared to other extracts, whereas total anthocyanin content was found to higher for blue berry around 25.34 ± 1.12 when compared to other extracts and the values are expressed in mean ± SEM and the results are represented in table 1.

**Table 1.** Total Phenolic content, Total Flavonoidal content and total Anthocyanin content in alcoholic extracts of selected fruits

| S.No. | Plant name | Total Phenolic content (mg of Gallic acid equivalent $g^{-1}$) | Total Flavonoidal Content (mg of Rutin equivalent $g^{-1}$) | Total Anthocyanin Content (mg of Cyanidin-3-glucoside |
|---|---|---|---|---|
| 1 | *Vaccinium corymbosum* (Blueberry) | 9.34 ± 0.23 | 36.78 ± 1.01 | 25.34 ± 1.12 |
| 2 | *Fragarria anannasa* (Strawberry) | 2.89 ± 0.19 | 7.23 ± 0.11 | 4.34 ± 0.20 |
| 3 | *Granny Smith apple* (GSA) | 8.56 ± 0.26 | 35.89 ± 0.87 | 0.0 |
| 4 | *Morinda citrifolia* (Indian Mulberry) | 9.01 ± 0.45 | 37.12 ± 0.78 | 16.3 ± 0.45 |
| 5 | *Musa paradisica* (Banana) | 7.92 ± 0.13 | 29.43 ± 1.09 | 17.89 ± 0.26 |

The phytochemical screening of alcoholic fruit extracts showed the presence of phenols, flavanoids and anthocyanains. The amount of phenols was found be highest in *Vaccinium corymbosum* and the amount of flavanoids and anthocyanins was found to be highest in *Vaccinium corymbosum*, whereas the lowest values of total phenols (2.89 ± 0.19), total flavanoids (7.23 ± 0.11) was found to be for *Fragarria anannasa* whereas, the lowest values for anthocyanins (0.0) for granny smith apple. The potent α-amylase inhibitory activity of the alcoholic fruit extracts may be due to the presence of total phenolic and flavonoid content (Table 1).

The enzymatic inhibition of different alcoholic fruit extracts with was measured in µg $mL^{-1}$. The concentration of extracts were chosen was 10, 20, 40, 60, 80,100 µg $mL^{-1}$. Among the different fruit extracts the maximum inhibition of different enzymatic levels was found to be at 100 µg $mL^{-1}$ (Fig. 1).

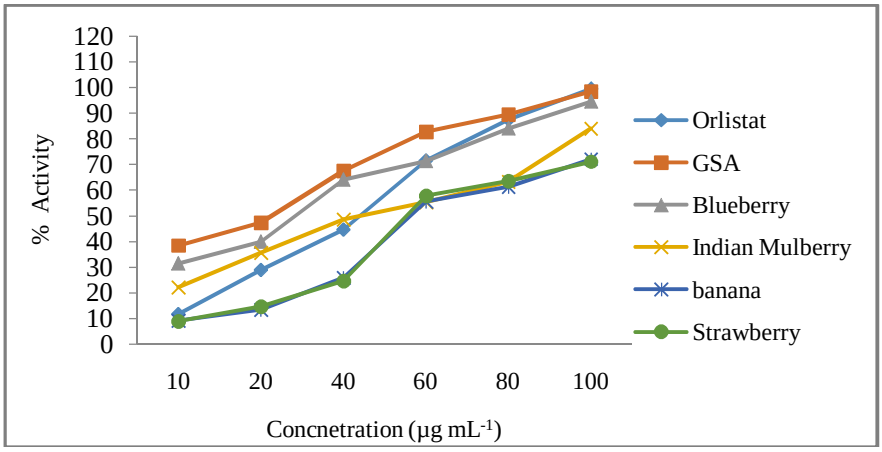



**Figure 1.** Lipase inhibitory activities (%) of alcoholic extracts of selected fruits.

The different percent enzymatic inhibition levels estimated was lipase and amylase levels and was compared with that of standard orlistat and acarbose. The percentage inhibition of lipase activity among different extracts was found to be maximum for granny smith apple which was found be around 98.4 ± 1.98 when compared to standard drug which was found to be around 99.46 ± 0.546

(orlistat), similarly the percentage inhibition of α amylase activity was found to be maximum for *Vaccinium corymbosum* was found to be around 94.56 ± 0.654 when compared to standard drug which was found to be around 90.76 ± 0.764(acarbose).The alcoholic extracts showed almost the similar activity close to that of the standard drug. The $IC_{50}$ values were found to be more potent for granysmith apple and blue berry and the results are represented in Table 2 (Fig. 2).

**Table 2.** IC50 values of the fruit extracts and reference standard Orlistat against Lipase inhibition

| S.No. | Plant name | IC 50 Values in µg $mL^{-1}$ |
|---|---|---|
| 1 | *Vaccinium corymbosum* (Blueberry) | 28.6 ± 1.345 |
| 2 | *Fragarria anannasa* (Strawberry) | 56 ± 0.986 |
| 3 | *Granny Smith apple (GSA)* | 27.6 ± 1.345 |
| 4 | *Morinda citrifolia* (Indian Mulberry) | 33.2 ± 1.976 |
| 5 | *Musa paradisica* (Banana) | 55 ± 0.457 |
| 6 | *Orlistat* (reference standard) | 26.6 ± 0.546 |

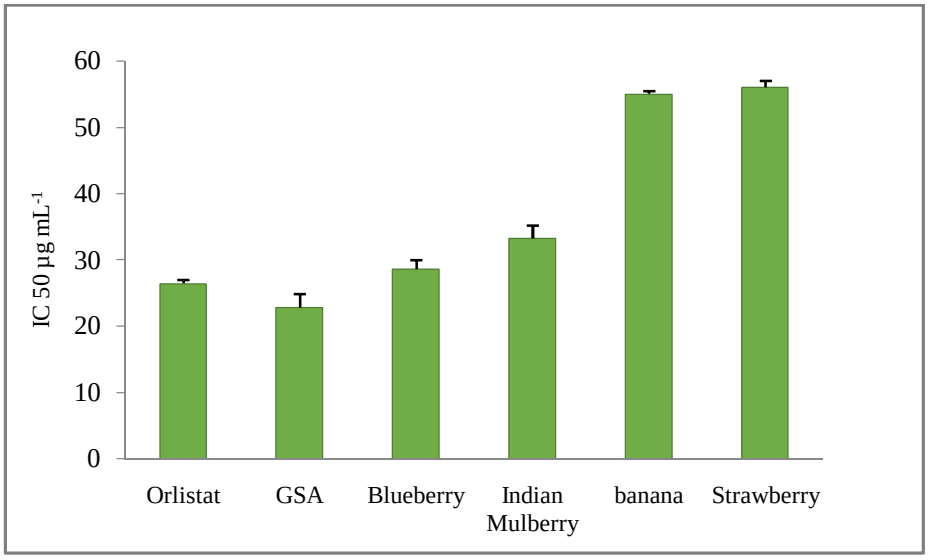



**Figure 2.** IC50 values of the extracts in comparison with reference standard orlistat.

Amylase and lipase two significant enzymes in the prognosis and diagnosis of acute pancreatitis, inhibition of these by plant extracts may provide an alternative therapy for treatment of the disease. A glycerol ester hydrolase (lipase) catalyzes the hydrolysis reaction of different di, tri monoacylglcerols that are absorbed by the human body, if there is an inhibition of the enzyme function, obesity may be prevented and the dietary fat is not directly absorbed from the intestine unless it has been subjected to the action of pancreatic lipase [**16**]. In this process triglycerides must emulsify into fat droplets to generate a substantial substrate for pancreatic lipase activity and the smaller droplets with larger surface would allow a higher catalytic activity. On the other side, Lipase levels are high in disease conditions like acute pancreatitis which is inflammation of exocrine pancreatic tissue and regarded as a significant reliable marker than the other parameters [**17**]. Orlistat an antiobesity agent is a potent inhibitor of pancreatic lipase activity reported to reduce 30% dietary fat absorption [**18**]. In the study the different alcoholic fruit extracts showed inhibitory potential for lipase activity. As expected the different fruits extracts showed activity in a dose dependant manner i.e. the concentration of the drug is directly proportional to the biological activity (Fig. 3). Further, polyphenolic extracts from different plants have been reported to have inhibitory mechanism on pancreatic lipase enzyme systems [**19, 20**]. The IC50 of the extracts was found to be (28.6 ± 1.345 µg $mL^{-1}$) for *Vaccinium corymbosum*, (27.6 ± 1.345 µg $mL^{-1}$) for *Granny Smith Apple*, (55 ± 0.457 µg $mL^{-1}$) for *Musa paradisica*, (33.2 ±

1.976 µg $mL^{-1}$) for *Morinda citrifolia*, and (56 ± 0.986 µg $mL^{-1}$) for *Fragarria anannasa* respectively when compared to orlistat which had and IC50 of (26.6 ± 0.546 µg $mL^{-1}$). All the extracts showed a potent activity at a concentration of 100 µg $mL^{-1}$ (Table 3, Fig.4) Based on the results, inhibition of enzymes is important in the management of progression of acute pancreatitis as well as in breakdown of fats. Therefore, these extracts may prove to be an option of treatment for obesity and acute pancreatitis.

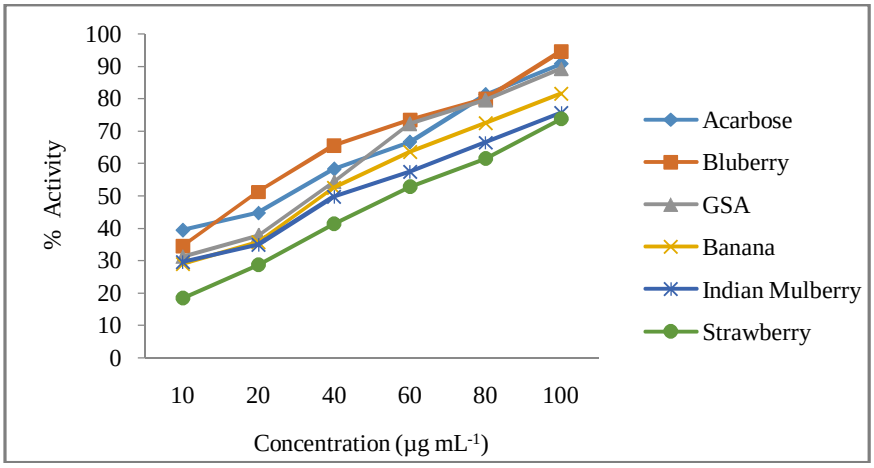



**Figure 3.** α- amylase inhibitory activities (%) of alcoholic extracts of selected fruits.

**Table 3.** IC50 values of the fruit extracts and reference standard acarbose against amylase inhibition

| S.No. | Plant name | IC 50 Values in µg $mL^{-1}$ |
|---|---|---|
| 1 | *Vaccinium corymbosum (Blueberry)* | 19.17 ± 0.654 |
| 2 | *Fragarria anannasa (Strawberry)* | 54.9 ± 0.678 |
| 3 | *Granny Smith apple* (*GSA*) | 34.4 ±1.237 |
| 4 | *Morinda citrifolia (Indian Mulberry)* | 40 ± 1.008 |
| 5 | *Musa paradisica (Banana)* | 38.3 ± 1.124 |
| 6 | *Acarbose (reference standard)* | 26.6 ± 0.764 |

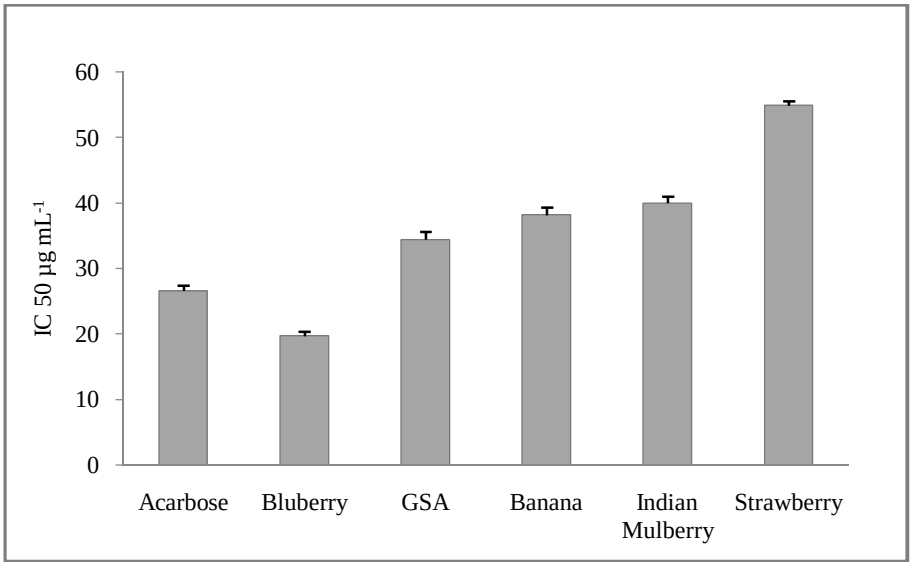



**Figure 2.** IC50 values of the extracts in comparison with reference standard acarbose.

Many natural products inhibit α-amylase enzyme, which is responsible for breakdown of starch to maltose then to glucose results in reduction of postprandial hyperglycemia [21]. In the contrary, Pathological conditions like acute pancreatitis have increased levels of amylase as a result of abnormal digestion of the acinar cells, leakage of the enzyme in the circulation [4]. The present study revealed the potent inhibitory activities of the plant extracts with IC50 values of (19.17 ± 0.654 µg $mL^{-1}$) for *Vaccinium corymbosum*, (34.4 ±1.237 µg $mL^{-1}$) for *Granny smith apple*, (38.3 ± 1.124 µg $mL^{-1}$) for *Musa paradisica*, (40 ± 1.008 µg $mL^{-1}$) for *Morinda citrifolia*, and (54.9 ± 0.678 µg $mL^{-1}$) for *Fragarria anannasa* when compared to Acarbose with (26.6 ± 0.764 µg $mL^{-1}$), a drug used in reducing postprandial hyperglycemia but reported to have untoward effects [22]. Plant products with potential antidiabetic activity consist of various phenolic and flavanoidal compounds and inhibition of α-amylase could be the strategy for its activity [23]. Thus, the ability of the extracts to inhibit the enzyme was discovered in the study.

## APPLICATION

The rationale behind the study include application of such fruits in the form of formulations or as nutraceuticals in disease conditions like acute or chronic pancreatitis, which do not have any specific therapy and the treatment majorly focuses on symptomatic treatment. Nutrition is a major drawback in such disease conditions. By intake of fruits directly or indirectly in the form of nutraceutical formulations like syrups, can help in reduction of progression of the disease as well as improving the overall condition of the patient.

## CONCLUSION

In summary the alcoholic fruit extracts showed potent inhibitors of digestive enzymes lipase and α-amylase. Based on the results there is a possibility of development of newer preparation from edible fruits which are common in the dietary supplements for antidiabetic, antiobesity and in pathological conditions like pancreatitis. Further biological screening has to be investigated to identify and isolate the bioactive phytochemical responsible for the enzymatic inhibition.

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