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***In vitro* antidiabetic activity of methanolic extract of Citrus limon, Punica granatum, Musa acuminata peel**

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Abstract

Diabetes is a clinical syndrome characterized due to absolute or relative deficiency of insulin. The intestinal digestive enzymes play a vital role in the carbohydrate digestion. Recent decades have experienced a sharp increased in the incidence and prevalence of diabetes mellitus. One antidiabetic therapeutic approach is to reduce gastrointestinal glucose production and absorbance through the inhibition of carbohydrate digestive enzyme such as alpha-amylase. Medicinal plants have been reported to play an important role in modulating glycemic responses and have preventive and therapeutic implications. The aim of the current study was to evaluate the methanolic extracts of three kinds of fruit peels (Lemon, pomegranate and Banana) for its *in vitro* antidiabetic activity. Our result suggests that methanol extracts of all three extract exhibit dose-dependent increase in percentage inhibitory activity on alpha amylase enzyme. Acarbose was used as a standard drug. Maximum alpha amylase inhibitory activity from banana peel was found to be 80.87% at 1000µg/ml. The I_{c50} values of alpha amylase inhibitory activity of Lemon, Pomegranate, and banana were found to be 135.354%, 157.928% and 185.384% respectively. The findings indicate banana peel was found to be more potent and posses hypoglycemic effect and hence for the management of diabetes mellitus can be utilized as complement.

Keywords: Lemon peel, Pomegranate peel, Banana peel, alpha amylase

1. Introduction

Fruits peel help to alleviate chronic diseases has served mankind since ages as they are reservoirs of important medicinal components (Nair *et al.*, 2013) [3].

As many diseases like cancer, arthritis and liver diseases find no complete cure in allopathy. Fruits peel plays considerable role in the development of modern herbal medicines (Nair *et al.*, 2013) [3]. Diabetes mellitus is epidemic in India as a result of societal influence and changing lifestyles. Diabetes has been known in India for centuries as 'a disease of rich man' but now spread among all masses. (Narkhede *et al.*, 2011) [4].

Diabetes is a chronic metabolic disorder in which homeostasis of the carbohydrate, protein and lipid metabolism is improperly regulated by the pancreatic hormone, insulin; resulting in an increased blood glucose level i.e. hyperglycemia (Singh & Kumar 2015) [5].

The treatment of diabetes involves the decrease postprandial hyperglycemia by causing retardation in distillation of glucose through inhibition of carbohydrate hydrolyzing enzymes such as α -amylase and α -glucosidase. For management of type 2 diabetes currently a variety of therapeutic drugs are addressable these agents include hypoglycemic agents such as miglitol voglibose and acarbose that competitively and reversibly inhibit α -glucosidase enzyme from intestine as well as pancreas (Singh & Kumar 2015) [5]. However, these drugs are associated with flatulence and diarrhea in the patients, gastrointestinal side effects such as abdominal pain, which might be caused by excessive inhibition of pancreatic α -amylase resulting in fermentation of undigested carbohydrates in the colon by colonic flora (Nair *et al.*, 2013) [3]. Therefore, a good scheme to manage postprandial hyperglycemia with lesser side effects is to assess the natural inhibitors from waste material which has less inhibitory effect against α -amylase. Thus objectives of our study is to investigate *in vitro* antidiabetic activity of methanolic extracts of Lemon, Pomegranate and Banana peel. The fruits peel extract under study can alternative as a therapeutic agents and can be used as potential source of novel bioactive compounds for treating diabetes mellitus type 2.

Material and Methods

Collection of fruits

Fresh Fruits were collected from local market of Akola, Maharashtra

Extraction of fruits peel

The shade dried fruits peel powdered mechanically (Banana, Pomegranate, Lemon) and sieved through sieve no 20 and stored in an air tight container. By using Soxhlet apparatus extraction was carried out by hot percolation method. Methanol was used as a solvent. With 100 ml of methanol about 10 gm of powder was extracted. The extract was preserved in refrigerator for further use.

In vitro methods employed in antidiabetic studies

Inhibition of alpha amylase enzyme

The procedure was done by DNSA method described by Bernfeld P. (Bernfeld 1955) [1].

Calculation of 50% inhibitory concentration (IC₅₀)

The concentration of the plant extracts required to scavenge 50% of the radicals (IC₅₀) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by

$$I \% = (Ac - As) / Ac \times 100$$

Where Ac is the absorbance of the control and As is the absorbance of the sample.

Results and Discussion

Regeneration of glucose level in the blood can prevent the various complications associated with the disease. Under a variety of dietary conditions the maintenance of plasma glucose concentration for a long term is the most important and closely regulated processes observed in the mammalian species (Nair et.al 2013) [3]. Management of Diabetes without side effect is still challenge to the medical community. *In vitro* methods play an important role for the preclinical studies for any activity, which make support to the *in vivo* studies. The purpose of the *in vitro* testing is to demonstrate the hypoglycemic activity of fruits peel of Lemon, Pomegranate, Banana the results were tabulated. At the concentration of 100µg/ml, inhibition level was 17.39%, 27.95% and 54.65% for Lemon, Pomegranate and Banana Respectively (Fig 1). The Percentage inhibition at 100 to 1000µg/ml concentrations of all the fruits peel showed a dose dependent increase in percentage inhibition. The highest inhibitory activity of alpha amylase was found to be in Banana peel (80.87%) as compared to Lemon (50.42%)

and pomegranate (72.41%) peel at 1000 µg/ml concentration.

Table 1

Sample	Concentration (µg/ml)	%Inhibition	IC ₅₀ µg/ml
Lemon	100	17.39	135.354
	200	23.26	
	400	33.94	
	800	45.71	
	1000	50.42	

Table 2

Sample	Concentration (µg/ml)	%Inhibition	IC ₅₀ µg/ml
Pomegranate	100	27.95	157.928
	200	39.10	
	400	50.92	
	800	65.23	
	1000	72.41	

Table 3

Sample	Concentration	%Inhibition	IC ₅₀ µg/ml
Banana	100	54.65	185.384
	200	62.37	
	400	69.37	
	800	77.14	
	1000	80.87	

Table 4.

Sample	Concentration	%Inhibition	IC ₅₀ µg/ml
Standard	100	62.11	188.98
	200	66.33	
	400	71.95	
	800	80.23	
	1000	84.43	

The IC₅₀ value for Lemon, Pomegranate and Banana were 135.354%, 157.928% and 185.384% respectively. It was proposed that inhibition of the activity of alpha amylase would delay the degradation of carbohydrate, which would intern cause a decrease in the absorption of glucose, as result the reduction of postprandial blood glucose level inhibition (Singh & Kumar 2015) [5]. The reaction mechanisms involved in inhibition of alpha amylase enzymes by plant protein inhibitors are not clearly understood. But there are some suggestions that the plant protein (Flavonols) might cause conformational changes in structural. In this present study we evaluated *in vitro* alpha amylase inhibitory activity of Lemon, Pomegranate and Banana peel. It was suggested that further studies are required to elucidated the mechanism of antidiabetic potential.

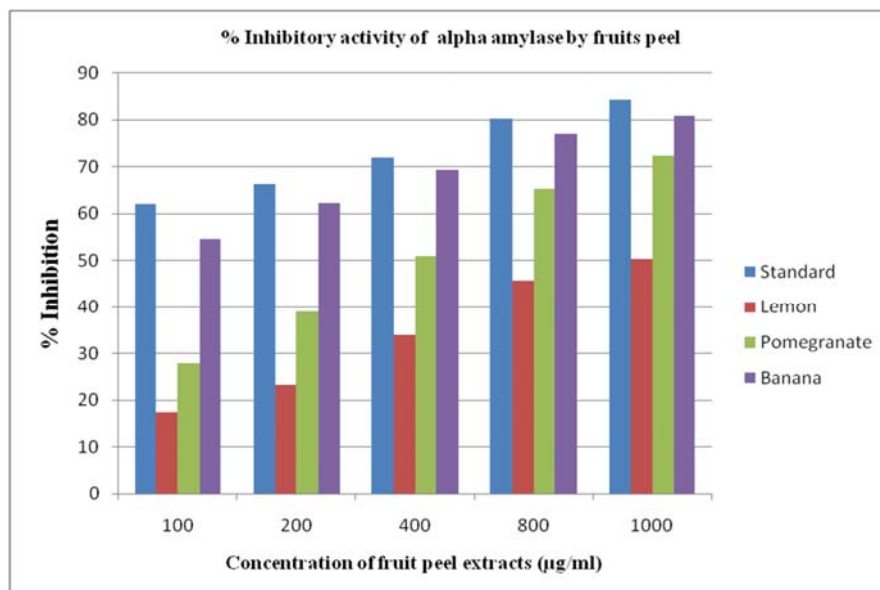


Fig 1: % Inhibitory activity of alpha amylase by standard, Banana peel, Pomegranate peel and Lemon peel.

Conclusion

In conclusions, our findings showed Banana peel has the potential to be explored further to identify the antidiabetic compounds in Banana peel. From this study we can conclude that the presence of these phytochemicals in this peel might be the reason for these inhibition. Further *in vivo* investigation should be done for conforming the antidiabetic activity of these peel.

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