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IN VITRO ANTIDIABETIC ACTIVITY OF AEGLE MARMELOS LEAVES

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ABSTRACT
The objective of present study was to evaluate the Invitro Antidibetic activity of Aegle Mormelos leaves extracts. The extract passed significant level of alpha-amylase enzyme inhibition activity. The highest concentration of extract was high effective showing inhibitory activity of alpha-amylase. It also shows the inhibition of glycosylation of haemoglobin in some extract. Hence these effect need to confirmed using further antidiabetic investigation In-vitro and In-vivo models.

KEYWORDS: Aegle Marmelos, Amtodiabetic, α-amylase, Acarbose, Haemoglobin glycosylation.

INTRODUCTION

Aegle marmelos belonging to family Rutaceae, is commonly known as Bael in indigenous systems of medicine and has been regarded to possess various medicinal properties. The bael is one of the scared trees of the Hindus. Leaves are offered in prayers to Shiva and parvathi since ancient times. Its leaves are trifoliate symbolizing the Thrimurthies-Brahma, Vishnu, Shiva, with spear shaped leaflets resembling Thrisoolam the weapon of lord Shiva. In Indian flowering occurs in April and May soon after the new leaves appear and the fruit ripens in 10 to 11 months from bloom March to June.

Herbalism is a traditional medicinal or folk medicine practice based on the use of plant and plant extracts (Arya DS, et.al., 2006). They contain active constituents that are used in treatments of many diseases. Plants are rich source of ecologically developed secondary metabolites, which are potential remedies for different ailments (Nagendra KK, et. al., 2010). Compounds isolate from Aegle marmelos have been proven to be biologically effective against several major diseases including cancer, diabetes and cardiovascular diseases. Thus, objective of the present study was to investigate the α-amylase inhibition activity non-enzymatic glocosylation of haemoglobin different extract of Aegle Marmelos leaves.

MATERIAL AND METHOD

Collection and identification of Plant materials

The leaves of Aegle marmelos were collected and authenticated by Botanist. The leaves were thoroughly washed and dried under oven (40° C-50° C) for 3-4 days. Segregated, pulverized by a mechanical grander to fine powder prior to analysis.

The sample was successively extracted with petroleum ether by hot continuous percolation method in soxhlet apparatus for 24hrs. After all petroleum ether was removed from the filtrate at 40°c using oven and the extract was stored at the refrigerator for futher study.

Preparation of Aegle marmelos leaves stock solution

10mg of Aegle marmelos leaves extract was taken and dissolved in 1ml of Dimethylsulphoxide (DMSO), which is used as stock solution with the concentration of 10,000µg/ml. From this stock solution, different concentration viz, 10, 20, 30mg/ml were prepared using DMSO solution. (Megha G. Chaudhri et. al. ,2013).

Alpha-amylase inhibition assay

Alpha-amylase is an enzyme that hydrolyses alpha linked polysaccharide such as glycogen starch to yield glucose and maltose. Alpha amylase inhibitory activity on based on the starch iodine method that was originally developed by Shekib LA Iraq EI, et al.,1988).

In alpha amylase inhibition method 1ml substrate-potato starch(1%w/v), 1ml of drug solution (Acarbose std drug) of five different concentration such as 20, 40, 60, 80, and 100µg/ml, 1ml of alpha amylase enzyme (1%w/v) and 2ml of acetate buffer. The above mixture was incubated for 1hr. Then 0.1ml iodine-iodine indicator (635 mg Iodine and 1 gm potassium iodide in 250 ml distilled water) was added in the mixture. Absorbance was taken at 565 nm in UV-Visible spectroscopy. % inhibition was calculated

$$\% \text{ inhibition} = \frac{As - Ac}{As} \times 100$$

Ac is Absorbance of Control
As is Absorbance of Sample
All the tests were performed in triplicate.

Non-enzymatic glocosylation of Haemoglobin assay

Antidiabetic activity of leaves of Aegle Marmelos were investigate by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520 nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solution were prepared in phosphate buffer 0.01 M, pH 7.4, 1 ml each of above solution was mixed. 1 ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520 nm. Ascorbic acid was used as a standard drug for assay. Percentage inhibition was calculated as previously published protocol. All the tests were performed in triplicate.

$$\% \text{ inhibition} = \frac{As - Ac}{As} \times 100$$

Ac is Absorbance of Control
As is Absorbance of Sample
All the tests were performed in triplicate.

RESULTS AND DICSUSSION

The Aegle marmelos petroleum ether extract revealed a significant inhibitory action on alpha amylase enzyme. At a concentration of 20µg/ml of plant extract showed a percentage inhibition 90.3% and for 100µg/ml plant extract showed inhibition of 94.5%.Results showed in following table.

1. Invitro study using α-amylase inhibition assay

Table 1: Alpha Amylase inhibition.

Blank	Conc. µg/ml	STD		PE		Ethanol	
		Abs	% inh	Abs	% inh	Abs	% inh
0.01	20	0.019	15.7	0.165	90.3	0.48	96.6
	40	0.027	40.7	0.224	92.8	0.50	96.8
	60	0.039	58.9	0.225	92.8	0.56	97.1
	80	0.058	72.4	0.267	94.0	0.58	97.2
	100	0.215	92.5	0.296	94.5	0.60	97.3

The alpha-amylase hydrolysed the α-bond of large polysaccharide like glocogen and starch to yield glucose. Hence by inhibiting the α -amylase may helpful for reducing the post prandial glucose level in blood.

The petroleum ether extract and ethanol extract of Aegle Marmelos revealed a significant inhibitory action of α–amylase enzyme. As a concentration of 20ug/ml of Aegle Marmelos petroleum ether and ethanol extracts showed a % inhibition 90.3% and 96.6% and for 100 ug/ml extract showed inhibition 94.5% and 99.3% as per table No. 1.

Hence P.E. Extract and ethanol extract of Aegle Marmelos leaves showed significant activity as compare to Acarbose standard drug.

2. Invitro non-enzymatic glycosylation of Haemoglobin assay

Table 2: In-vitro non-enzymatic glocosylation of Haemoglobin method.

Blank	Conc. µg/ml	STD		PE		Ethanol	
		Abs	% inh	Abs	% inh	Abs	% inh
0.07	20	0.075	6.66	0.077	9.09	0.076	8.00
	40	0.085	17.64	0.090	22.22	0.085	17.64
	60	0.175	60.00	0.110	36.36	0.086	18.60
	80	0.185	62.16	0.180	61.11	0.095	26.31
	100	0.240	70.83	0.185	82.16	0.015	83.33

Increase in the concentration of globulin blood leads to its binding to haemoglobin which result in formation of reactive oxygen species.[7]

The P.E. and Ethanol extract of Aegle Marmelos leaves shows higher inhibition of glycosylation for the concentration of 20 ug/ml and 40 ug/ml as compared to STD drug.

But in further concentration the inhibition of haemoglobin glycosylation the extracts do not shows the much effect.

Hence the lower concentration of plant extract may helpful for decreasing the formation of the glucose haemoblogin complex and thus increase the amount of free haemoglobin.

The intestinal digestive enzymes alpha-amylase plays a Vitol role in the carbohydrate digestion. Diabetes mellitus is metabolic disorder with increasing incidence throughout the world. Lack of insulin affects carbohydrate, fat and protein metabolism (Rajiv Gandhi and Sasi Kumar, 2012). It was prepared that inhibition of the activity alpha-amylase would delay the degradation of carbohydrate which would in turn cause a decreased in the absorption of glucose as result the reduction of pastprandial blood glucose level elevation (Rhabaso-Lharet and Chiass on 2004).

In present finding reveals that Aegle Marmelos as efficiently inhibits alpha-amylase in vitro in a dose dependent manner. The result infers that plant extract may have significant effect in maintainer postprandial glucose concentration.

CONCLUSION

Thus, present finding, reveals that Aegle Mamerlos leaves shows effective inhibition activity of α-amylase which may helpful for maintaining the past-prandial glucose. It also shows some effect on glycosylation of haemoglobin.

Hence, further investigation of Aegle Mamelose for antidiabetic activity should be done in-vitro and on phyto constituents base.

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