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In Vitro Antidiabetic potential of Aegle marmelos

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Abstract

Diabetes Mellitus is a systemic metabolic disease characterized by hyperglycemia, abnormal elevated levels of lipid, and fat in blood and hypoinsulinaemia. This study aims at determining the anidiabetic potential of five traditional medicinal plants such as, *Aegle marmelos*. The selected plant leaves were subjected to solvent extraction and analyzed for their antidiabetic activity using *in vitro* assays such as, á-amylase inhibition assay, glucose difusion assay, glucose uptake by yeast cells, and nonenzymatic glycosylation assay. The extract of *Aegle marmelos* was used at the concentration range of 7.5, 15.5, 31.2, 62.5, 125, 250, 500,and 1000 ug/ml and standard acarbose. The results of the assays suggest that among the selected plants, ethanol extract of the *Aegle marmelos* was significant in inhibiting the activity of á-amylase with IC_{50} value of 1000µg/ml. Further assays proved that the same was efficient in its antidiabetic potential. The phytochemical analysis showed the presence of phenols, flavonoids and alkaloids as major amount. Thus the study suggests that *Aegle marmelos* could be considered as a potential source of natural antidiabetic agents.

Key words: *in vitro* antidiabetic; *Aegle marmelos*,á-glucosidase, á-amylase enzymes

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INTRODUCTION

Diabetes mellitus (DM) is a serious health problem being the third greatest cause of death all over the world, and if not treated, it is responsible for many complications affecting various organs in the body (El-Hilaly *et al*., 2007). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs (Lyra *et al*., 2006). In diabetic rats, the utilization of impaired carbohydrate leads to acceleration of lipolysis, which resulted in hyperlipidemia (Morel and Chisolm, 1989). Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies (Mitra *et al*., 1995). More than 400 plant species have been showed hypoglycemic activity, possess for plants as the source (Oliver-Bever, 1986; Roy *et al*., 2005). Nevertheless search antidiabetic drugs is still attractive because they contain substances which could be used as a viable alternative to chemotherapeutic agents (Esampally *et al*., 2013).

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Traditional medicine (herbal) is used for the treatment of diabetes in developing countries where the cost of conventional medicines is a burden to the population (Saravanan *et al*., 2011). Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes. One of the great advantages of medicinal plants is that these are readily available and have very low side effects. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them (Arumugam *et al*., 2008).

In India different parts of medicinal plants have been used for curing various diseases from ancient times. In this regard, one such plant is *Aegle marmelos*. It belongs to the family Rutaceae and is commonly known as Bael in indigenous system of medicine and has been regarded to possess various medicinal properties (Orwa *et al*., 2009; Chopra *et al*., 1956). *Aegle marmelos* has been widely used in Indian traditional medicine for the remedy of various ailments. All parts of *A. marmelos* such us leaves, fruit pulp, flower, stem bark , root bark, etc. are medicinally useful. A hot poultice of the leaves is applied in opthalmia or severe inflammation of conjunctiva with acute bronchitis and inflammation of the other body parts (Kurian *et al*., 1992).The decoction of root is given with sugar and fried rice for checking diarrhea and gastric irritability in children (Rajadurai *et al*., 2005). Root is one of the

ingredients of 'Dasamoola' a standard Ayurvedic remedy for loss of appetite and puerperal diseases e.g. Inflammation of uterus. Distillation of flowers yielded a drug which is used as tonic for stomach and intestine, anti-dysenteric, antidiabetic, diaphorectic and as local anaesthetic. It is also used in epilepsy and expectorant (Kirtikar *et al*., 1995). Fruits are used in gastric troubles, constipation, laxative, tonic, digestive, stomachic, brain and heart tonic, ulcer, antiviral, intestinal parasites, gonorrhea and epilepsy (Dhankhar *et al*., 2005). Fresh juice of fruit is bitter and pungent, and the fruit extract lower the blood sugar (Kirtikar *et al*., 1995). Decoction of unripe fruit is astringent, useful in diarrhea and chronic dysentery (Robbers *et al*., 2002).

Reported pharmacological activities include antibacterial activity (Rajasekaran *et al*., 2008), antihistamic activity (Nugroho *et al*., 2010), hepatoprotective activity (Singh, *et al*., 2016), insecticidal activity (Kumar *et al*., 2008), hypogiycemic and antioxidant acticity (Upathya *et al*., 2009), immuno modulatory activity (Rajadurai *et al*., 2005), myocardial infarction (Rajadurai *et al*., 2005), testicular activity (Das *et al*., 2006), cardiotonic activity (Dama *et al*., 2010), anxiolytic and antidepressant activity (Kothari *et al*., 2010), wound healing activity (Jaswanth *et al*., 2000), anticonvulsant activity (Sankari *et al*., 2010), anti stress and adaptogenic activity (Duraisami *et al*., 2010), antifertility activity (Joshi *et al*., 2009). Hence the present article deals with the antidiabetic activity of *Aegle marmelos,* determined *by* alpha amylase method, alpha glucosidase method, glucose uptake in yeast cell and non enzymatic glycosidase, and the results are discussed.

MATERIALS AND METHODS

Plant Collection of plant materials

The plant species namely *Aegle marmelos L.* plant was collected by in and around Poondi, Thanjavur District, Tamil Nadu, India. The plant was identified with the help of the Flora of Presidency of Madras (Gamble,1997) and authenticated by Dr. S. John Britto, RAPINAT Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli (Voucher number of the specimen, RK001).

Preparation of plant powder

The plant was air dried under shade for 10-15 days. Then the dried materials were grinded to fine powder using an electric grinder and stored in air tight bottles. The powder was used for further analysis.

Preparation of the aqueous extract

The leaves were shade dried and coarsely powdered. Then it was boiled until it was reduced to one third and filtered. The filtrate was evaporated to dryness. The extract was made into paste and was subjected to preclinical screening.

Preparation of the Ethanol extract

Ethanolic extract was prepared according to the methodology of Indian pharmacopoeia (Anonymous, 1996). The coarse powder material was subjected to Soxhlet extraction separately and successively with 210ml ethanol and 90ml distilled water. These extract was concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (40æ%C – 50æ%C). The paste form of the extract was put in air tight container and stored in refrigerator.

In vitro antidiabetic activity

Determination of alpha amylase inhibitory activity (Adisa *et al*., 2004), alpha glucosidase inhibitory activity (Adisa *et al* ., 2004), glucose uptake by Yeast Cells and evaluation of the effect of plant fractions on haemoglobin glycosylation (Cirillo, 1962) was made.

RESULTS AND DISCUSSION

Antidiabetic activity

In vitro á -Amylase inhibition Assay

á-amylase is an enzyme that converts starch to glucose in its presence. When á- amylase, glucose and plant extract are taken together as a solution, the plant extract causes the inhibition of enzyme activity (Suhashini *et al*., 2014). The percentage inhibition of á amylase increases from 55% to 92% with increasing concentration of plant extract (7.5 and 1000 µl). The standard drug of Acarbose exhibited the rate of glucose inhibition at maximum level 90% and minimum level 54% (Table 1). The IC_{50} value was 45µg / ml, 49µg / ml for ethanol and aqueous extract respectively, where as it was 42.5µg / ml with the standared.

Inhibition of these enzymes (á-amylase and á-glucosidases) reduced the high Postprandial (PP) blood glucose peaks in diabetes (Sankari *et al*., 2009). Acarbose is the competitive inhibitor of á glucosidases and reduces absorption of starch and disaccharides (Duraipandiyan *et al*., 2006). The amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates. Acarbose is complex oligosaccharides that delay the digestion of carbohydrates. It inhibits the action of pancreatic amylase in breakdown of starch. Synthetic inhibitor causes side effects such as abdominal pain, diarrhoea and soft faeces in the colon. The reaction mechanisms involved in inhibition of á- amylase enzymes by plant protein inhibitors are not clearly understood. But there are some suggestions that the plant protein might cause conformational changes in the structure (Gabbay *et al*., 1976).

Alpha-glucosidase inhibition assay

In this assay, it shows a marginal dose dependent increase in inhibitory activity of á- amylase by different concentrations of the extracts. The á-glucosidase inhibition is a potent action in the treatement of diabetes. The plant extracts source maximum inhibition of 1000 µg / ml than compared to acarbose. The IC_{50} value is 45.5 µg / ml whereas in standared drug it was 47.5µg / ml. From the presence study, it comes to know that *A. marmelos* shows a potent inhibition in á- amylase, á-glucosidase activity. (Table 2).

Table.1. Amylase inhibitory activity of ethanolic and aqueous extract of *Aegle marmelos*

S. No.	Concentration (µg/ml)	Ethanol Extract			Aqueous Extract		
		Absorbance	% of Inhibition	IC_{50}	Absorbance	% of Inhibition	IC_{50}
1	7.5	0.81	55	28	1.08	54	27
2	15.5	0.85	63		1.14	62	
3	31.2	0.87	67		1.15	64	
4	62.5	0.92	76		1.25	75	
5	125	0.93	78		1.23	78	
6	250	0.97	86		1.27	81	
7	500	0.98	88		1.29	84	
8	1000	0.99	90	45	1.32	88	44
9	Standard 100 (Acarbose)	0.96	85	43	0.96	82	41

Table.2. α–glucosidase inhibitory activity of ethanolic and aqueous extract of *Aegle marmelos*

S. No.	Concentration (µg/ml)	Ethanol Extract		Aqueous Extract	
		Absorbance 410nm	IC_{50} (µg/ml)	Absorbance 410nm	IC_{50} (µg/ml)
1	7.5	58	29	50	25
2	15.5	66		63	
3	31.2	70		68	
4	62.5	73		72	
5	125	77		76	
6	250	82		78	
7	500	91		83	
8	1000	95	45.5	93	41.5
9	Acarbose	92	47.5	81	46.5

The present study reveals that *A. marmelos* efficiently inhibited á-amylase enzyme *in vitro*. The reaction mechanisms involved in inhibition of á-amylase enzymes by plant protein inhibitors are not clearly understood. The results suggest that ethanol extract the leaves of *A. marmelos* effectively inhibited á-glucosidase enzymes *in vitro* . The antidiabetic action of *A. marmelos* could also be attributed to the intestinal á-glucosidases inhibitory activity.

Glucose uptake in yeast cells

This assay is based on the movement of glucose across the membrane of yeast cells, with the help of the plant extract. The yeast cells were suspended in plant extract and various concentrations of glucose (7.5µg / ml to 1000µg / ml). The plant extract enhanced the to take of glucoseby the yeast cells. The amount of glucose remaining in the solution after incubation was observed. This determines the glucose uptake by the yeast cells (Suhashini *et al*., 2014). The assay was carried out to study the uptake of glucose by the yeastcells.This study is similar for that of how our body intake glucose and what are all modification it undergoes in the cell.The glucose uptake of the yeast cells showed the max antidiabetic action at the concentration of 10005µg / ml than compared to the standared drug Acarbose. The IC_{50} value of plant extract is 42, 40.5 in ethanol & aquous extracts where as in case of acarbose it was found to be 41.5& 36.5 respectively.

Table.3. Effect of ethanol and aqueous extract of *Aegle marmelos*on this Glucose uptake of yeast cell

S. No.	Concentration (µg/ml)	Ethanol Extract		Aqueous Extract	
		% of Inhibition	IC_{50} (µg/ml)	% of Inhibition	IC_{50} (µg/ml)
1	7.5	63	31.5	55	27.5
2	15.5	68		59	
3	31.2	70		67	
4	62.5	75		70	
5	125	77		72	
6	250	80		75	
7	500	82		78	
8	1000	84	42	81	40.5
9	Acarbose	83	41.5	73	36.5

Non enzymatic glucosylation of haemoglobin method

The non enzymatic activity of haemoglobin showed that maximum glycosylation at 1000µg / ml concentration of the extract where as minimum was observed at 7.5 µg / ml concentration of the extract. Ethanolic extract showed the maximum action of 90% inhibition and the aqueous extract showed the minimum activity 62% inhibition (Table 3). The IC_{50}

value was found to be 31µg/ml and 46µg/ml for ethanolic and aqueous extract, where as the standard showed that it was observed to be 45µg/ml.

Nonenzymatic glycosylation of hemoglobin involves the condensation of two abundant reactants within the erythrocyte: glucose and hemoglobin. The reaction takes place slowly and continuously throughout the cell's 120-days lifespan (Evans *et al*., 2008). Initially, this type of post-translational modification was considered to be the NH2- terminal amino group of the a chain as well as several lysine residues on both the a- and the p- chains (f3-Lys 66, a-Lys 61,-Lys 17, and a-Lys 40).

The non enzymatic glycosylation of hemoglobin involves the condensation of 2 reactants within the erythrocyte Heamoglobin & glucose. This reaction occurs throughout the RBC lifespan. Tn DM, higher amount og glycated Hb, indicating poorer control of blood glucose levels. Which leads to CV disease, nephropathy etc. The maximum action 1000µg/ml than compared to standared drug. The IC_{50} value of plant extract is ethanol & aqueous extracts was 46,45.5, whereas in case of Acarbose it was 45& 43.5 respectively (Govindappa *et al*., 2011)..

Table.4. Non enzymatic glycosylation activity of ethanol and aqueous extracts of *Aegle marmelos*

S. No.	Concentration (µg/ml)	Ethanol Extract		Aqueous Extract	
		Absorbance at 443 nm	IC_{50} (µg/ml)	Absorbance at 443 nm	IC_{50} (µg/ml)
1	7.5	62	31	60	30
2	15.5	67		63	
3	31.2	72		67	
4	62.5	75		70	
5	125	80		75	
6	250	84		79	
7	500	87		85	
8	1000	92	46	89	44.5
9	Acarbose	90	45	87	43.5

The antidiabetic properties of plants can be evaluated *in vitro* by several methods such as study of glucose uptake, effect on glycosylation of the haemoglobin and inhibition of alpha glucosidase and alpha amylase enzymes. The results of the work indicate that the selected plants possessed considerable *in vitro* anti diabetic activity at a concentration of 1000µl/ml when compared to that of standard.

On the basis of the results obtained in the present study, it is concluded that the ethanol extract of *Aegle marmelos* has potential antidiabetic activity. It could be attributed to the presence of phytochemicals. Further investigation on this area of research could reveal the active principles are the chemical trials could help in the drug development.

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