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Investigation of Phytochemical and Alpha Amylase Inhibition Activity of *Balanites Aegyptiaca*(L.) Leaves.

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ABSTRACT

In this investigation of *Balanites aegyptiaca* (L.) leaves ethanol extract have been subjected to phytochemical, GC-MS analysis and alpha amylase inhibition activity with a view to assess their antidiabetic potential. Leaves extract of *Balanites aegyptiaca*(L.) were evaluated qualitatively according to standard protocol. The presence of carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids and phenolic compounds were found to be positive. The GC-MS analysis is carried out for the identification of active compounds in the extract sample. The alpha amylase inhibition activity was providing that the ethanol extract exhibited potent inhibitory effect. This investigation brings the data on in vitro alpha amylase inhibition activity of leaves extract and its role in reducing of diabetic problems.

Keywords: *Balanites aegyptiaca*(L.), phytochemical, GC-MS, alpha amylase, antidiabetic.

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INTRODUCTION

Diabetes mellitus is characterized by discontinuity in the metabolism of carbohydrate, protein and fats. It is caused by a relative or absolute deficiency of insulin secretion and different levels of insulin resistance and is also resulting from both genetic predisposition and favouring environmental conditions.

Plants were reporting antidiabetic activity traditionally uses for diabetic therapy plays important role in treatment of diabetes mellitus. The different medicinal plants, mostly traditionally used in common diabetic therapy are able to inhibit α -amylase, which is responsible for the breakdown of oligosaccharides into monosaccharides, which are absorbed [1]. Herbal treatment on diabetes mellitus therapy has supported by World Health Organization (WHO) [2]. Therefore, easy availability of medicinal plants, minimum side effects, low cost and greater acceptance amongst the users is best substitute for the treatment of Type 2 diabetes. The use of *Balanitesaegyptiaca* (L.) leaves ethanol extracts exhibited antidiabetic as well as hypolipidaemic effects on Type 2 diabetic patients [3].

Various parts of *Balanitesaegyptiaca* (L.) plant contain chemical constituents [4] that have high pharmacological importance. Its studies demonstrated as insecticidal, antifungal, antidiabetic, antibacterial, anticancerous, anti helminthic, anti-inflammatory, hepatoprotective and anti parasitic activities of the plant [5-6].

Several phytoconstituents of plant species were reported as α -amylase inhibitors [7] and has different pharmacological activities [8]. In vivo antidiabetic effect of the *Balanitesaegyptiaca* (L.) fruits was studied, it bring forth novel data showing anti hyperglycaemic activity attributed to significant like insulin and partly glipta zone activities in peripheral tissues with increasing muscle basal glucose uptake [9]. The protective effect of *Balanitesaegyptiaca* (L.) may be its high contents of polyphenols and curamins, showed potential antioxidant and antibacterial properties [10]. It can be used as complementary natural antidiabetic agent to prevent diabetic complications [11].

MATERIALS AND METHODS

Plant material

The *Balanitesaegyptiaca* (L.) plant leaves were collected from local area identified and authenticated by colleagues from Department of Botany. The plant leaves are cleaned with distilled water to remove any unwanted particles and dried in shade.

Preparation of Extraction

The *Balanitesaegyptiaca* (L.) air dried leaves were finely crushed and powdered. 10 gm of plant material was mixed in 100 ml of ethanol and kept on a magnetic stirrer for stirring 2 hrs and extracted using a Soxhlet apparatus sequentially in ethanol. The fraction of extract was collected. The solvent was evaporated out to dryness. The extracted material was stored in airtight bottles at 4°C for further investigation studies.

In vitro α -amylase Inhibitory Assay

α -amylase inhibition activity was adopted to screen with the help of a modified 3,5-dinitrosalicylic acid (DNS) method, the reducing sugar (maltose) liberated quantifying under the assay conditions. The enzyme inhibitory activity was noted as a decrease in units of maltose liberated during the assay [12-13].

Phytochemical analysis

The ethanol extract of leaves was investigated qualitatively for the active phytochemical test reporting such as alkaloids, carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids, steroids, terpenoids and phenolic compounds etc. by adopting standard protocol [14-15].

GC-MS analysis

GC-MS analysis was carried out with the help of Shimadzu GC-MS model number QP 2010S. Its column Rxi-5Sil MS, 30 meter length, 0.25 mm ID, 0.25 μm thickness was used. The organic compounds were noted identified by comparing of mass spectra with the inbuilt libraries NIST-11 and WILEY-8.

Statistical analysis

The tests were carried out triplicate in 3 sets and its results expressed in mean ± SD. Values of P < 0.05 were considered as statistically significant.

RESULTS

Ethanol extract of *Balanites aegyptiaca*(L.) leaves exhibited dose dependent porcine pancreatic alpha amylase inhibitory activities. The study is carried out in vitro.

In vitro α-amylase Inhibitory Assay

The activity was investigated through the inhibition of α-amylase, an enzyme that made the digestion of starch and reduction in the glucose absorption. The standard used is acarbose at a concentration of (20-100 μg/ml) much show α-amylase inhibitory activity from 47.17% to 68.81% with an IC₅₀ value 27.90 μg/ml, whereas ethanol extract (20-100 μg/ml) of *Balanites aegyptiaca*(L.) showed potent inhibition activity in a dose dependent manner from 39.96% to 53.02% with an IC₅₀ value of 84.08 μg/ml (Table-1). Compared to standard drug the activity of extract is found to be less but increases with dosage percentage.

Table 1: % Alpha Amylase inhibition activity of standard and leaves extract.

Sr. No.	Concentration In (μg/ml)	% Inhibition of Standard	% Inhibition of extract
1	20	47.17	39.96
2	40	54.38	40.15
3	60	59.45	45.80
4	80	64.32	49.31
5	100	68.81	53.02
IC₅₀ Value (μg/ml)		27.90	84.08

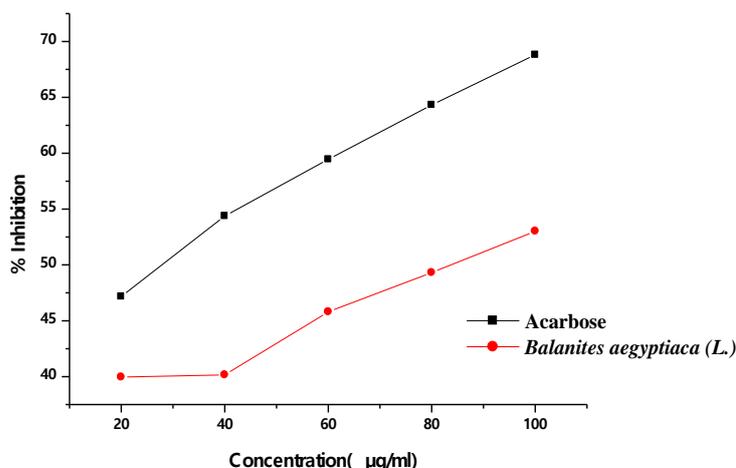


Fig. 1. Alpha Amylase inhibitory activity of *Balanites aegyptiaca* (L.) leaves ethanol extract.

Phytochemical analysis

The qualitative various phytochemical test investigation of *Balanitesaegyptiaca*(L.) leaves ethanol extract were showed the presence of carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids and phenolic compounds as shown in Table-2.

Table 2: Phyto chemical analysis of leaves ethanol extract.

Sr. No.	Phytochemical Tests	Result
1	Alkaloid	-
2	Carbohydrate	+
3	Protein and amino acids	+
4	Glycoside	+
5	Tannin	+
6	Saponin	+
7	Flavonoids	+
8	Steroids	-
9	Triterpenoids	-
10	Phenolic compounds	+

(+) for present and (-) for absent

GC-MS analysis

The GC-MS analysis study reported active phyto compounds of *Balanitesaegyptiaca*(L.) leaves ethanol extract shown in Fig.-2. These contribute to α -amylase inhibitory activity. The chromatogram is run upto 44.0 min. The first peak appears at 16.54 min, overall there are twelve peaks. The probable compounds as peak library search are given in the Table-3. The glycopyrano side is found to be the major constituent of the extract.

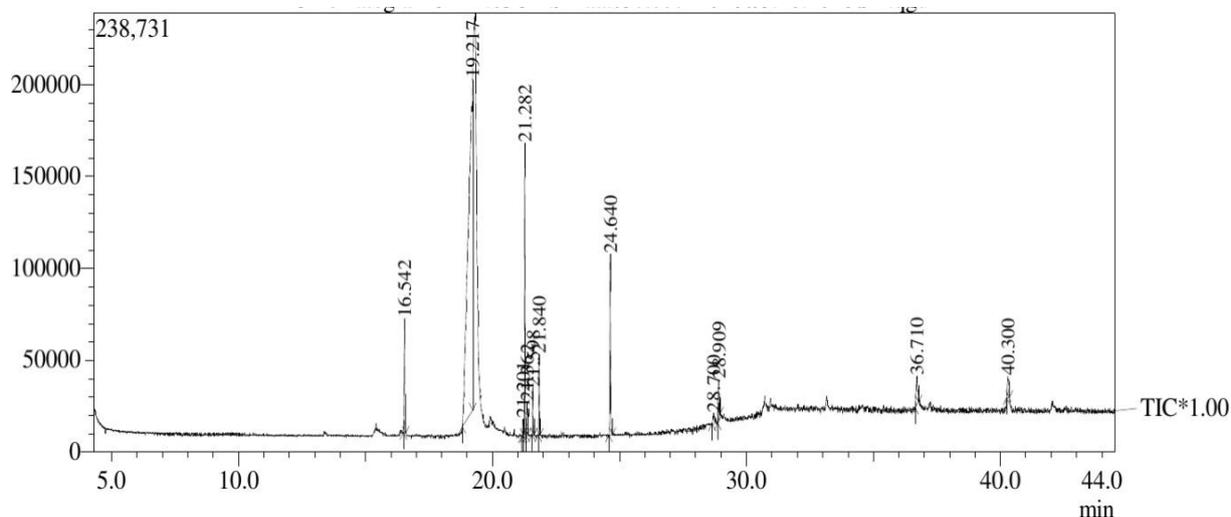


Fig 2: GC-MS Chromatogram of Balanitesaegyptiaca(L.) leaves.

Table 3: Phyto chemicals detected in GC-MS analysis of Balanitesaegyptiaca(L.)extract.

Peak #	R. Time	Area	Area%	Height	Height %	Name	Base m/z
1	16.542	111526	3.42	62288	9.57	Phenol, 2,4-Bis(1,1-Dimethylethyl)-	191.05
2	19.217	2289027	70.11	180437	27.73	Alpha.-D-Glucopyranoside, methyl	53.90
3	21.201	15358	0.47	8944	1.37	1-HexenE, 3,5,5-Trimethyl-	70.05
4	21.282	309071	9.47	159044	24.44	Neophytadiene	68.00

5	21.362	36110	1.11	17667	2.72	1-Hexanol, 4-methyl-	70.05
6	21.598	39860	1.22	25286	3.89	6-Octen-1-ol, 3,7-Dimethyl-, Propanoate	82.05
7	21.840	73787	2.26	43954	6.75	16-Heptadecenal	82.05
8	24.640	210308	6.44	98294	15.11	2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]- (T-Phytol)	71.00
9	28.700	23023	0.71	5812	0.89	1H-Indene, 1-hexadecyl-2,3-dihydro-	117.00
10	28.909	49645	1.52	21750	3.34	1-Tridecanol	57.00
11	36.710	66109	2.02	15622	2.40	Carbonic acid, neopentylcyclohexylmethyl ester	97.05
12	40.300	41068	1.26	11611	1.78	Cyclopentanemethanol, .alpha.-cyclohexyl-2-nitro-	95.05
		3264892	100.00	650709	100.00		

DISCUSSION

The investigation of inhibitory activity of *Balanitesaegyptiaca*(L.) leaves was carried out by using modified 3,5-dinitrosalicylic acid (DNS) method for detecting in vitro anti diabetic activity. Its ethanol extract reported potent alpha amylase inhibition activity at IC₅₀ value 84.08µg/ml as compared to standard IC₅₀ value 27.90µg/ml (Fig.-1).

Balanitesaegyptiaca(L.) are described as high potential anti diabetic plant with a moderate inhibitory effect on α-amylase activity [16]. Medicinal plants extracts has been reported valuable antidiabetic agents and it involve one or more active components responsible for blood glucose reduction [17].

Phytochemicals or Plant extracts having active role in diabetes management and treatments are involved in decreasing or increasing or stimulating different mechanisms in reducing diabetes. The different parts of the plants researchers have been uses for antidiabetic activities [18]. Phytochemical analysis with different reagents *Balanitesaegyptiaca*(L.) leaves ethanol extract showed the presence of Carbohydrate, protein, amino acids, glycoside, tannins, Saponin, flavonoids and phenolic compounds, which could be help in the development of a suitable monograph for the plant[19]. Investigation of phyto chemicals in Stem bark and kernel extracts were reported different active phyto chemicals with significant pharmacological activities [20-21].

The effective bioactive compounds in *Balanitesaegyptiaca*(L.) leaves are responsible for inhibition were studied by GC-MS analysis technique (Fig.-2). These were reported as Phenol, 2,4-Bis(1,1-Dimethylethyl)-, alpha.-D-Glucopyranoside, methyl, 1-Hexene, 3,5,5-Trimethyl, Neophytadiene, 1-Hexanol, 4-methyl-, 6-Octen-1-Ol, 3,7-Dimethyl-, Propanoate, 16-Heptadecenal, 2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]- (T-Phytol), 1H-Indene, 1-hexadecyl-2,3-dihydro-, 1-Tridecanol, Carbonic acid, neopentylcyclo hexyl methyl ester and Cyclopentane methanol, alpha.-cyclohexyl-2-nitro-(Table-3).

The before reported as an enzyme inhibitor is Neophytadiene [22]. 3,7,11,15-Tetramethyl, [R-[R*,R*-(E)]]- (T-Phytol) have antidiabetic, α-glucosidase inhibition activity responsible for the management of insulin resistance and metabolic disorders [23-24].

Balanitesaegyptiaca(L.) kernel cake has been shown an anti hyperglycaemic and anti hyper lipidemic activity with Alloxan induced diabetes mellitus in rats, which confirmed the use in traditional medicine for the management of diabetes mellitus[25]. The dose dependent hypoglycaemic effect of fruits indicated as reduction in the levels of fasting blood glucose and glycated haemoglobin in diabetic rats[26].

The potential antidiabetic and antioxidant impacts of *Balanitesaegyptiaca*(L.) on streptozotocin induced diabetic and normal rats study provides a scientific evidence for the traditional use in type 1 diabetes mellitus [27].

The Methanolic extract of *Balanitesaegyptiaca*(L.) and the furostanolsaponin exerted in vivo antidiabetic effects through increasing the biosynthesis of insulin with its anti hypercholesterolemic effects [28].

These above findings of *Balanitesaegyptiaca*(L.) leaves revealed scientific evidences in alpha amylase inhibition for the therapeutic benefits in the management of diabetes and its acquired complications.

CONCLUSION

These investigations suggest that *Balanitesaegyptiaca*(L.) leaves ethanol extract has significant role in management of diabetes and its acquired complications. The bioactive phytochemicals were present in the extract reveals α -amylase inhibition activity. The leaves of *Balanitesaegyptiaca*(L.) plant could become a source of natural antidiabetic agents responsible for prevention and management of type 2 diabetes. Therefore, this investigation concluded that *Balanitesaegyptiaca*(L.) leaves ethanol extract shows in vitro alpha amylase inhibition activity in reducing role of diabetic problems.

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