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Antioxidant and Antidiabetic activity of *Opuntia dillenii*

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ABSTRACT

Opuntia dillenii, commonly known as the prickly pear cactus, has been traditionally used in various cultures for its medicinal properties. This study aimed to investigate the potential antidiabetic activity of Opuntia dillenii, focusing on its antioxidant properties. Using in vitro and in vivo models, the antioxidant capacity of Opuntia dillenii extracts was assessed, and its effect on glucose metabolism was evaluated. The results indicated that the extracts exhibited significant antioxidant activity, as evidenced by the scavenging of free radicals and the reduction of oxidative stress markers. Furthermore, in diabetic animal models, treatment with Opuntia dillenii extracts led to a notable decrease in blood glucose levels and improved insulin sensitivity. The findings suggest that the antioxidant components of Opuntia dillenii play a pivotal role in its antidiabetic effects. This study underscores the potential of Opuntia dillenii as a natural therapeutic agent for diabetes management and highlights the importance of antioxidants in modulating glucose metabolism.

Keywords: Opuntia dillenii, antidiabetic activity, antioxidant, glucose metabolism, oxidative stress, natural therapeutic agent, insulin sensitivity.

INTRODUCTION

TYPES OF ANTIOXIDANTS

1. Synthetic antioxidants
2. Natural antioxidants

Synthetic antioxidants contain molecules from the phenolic group, which stop oxidative stress, free radicals, and numerous biological processes linked to the detrimental effects of free radicals. Examples include tertiary butyl hydroquinone (TBHQ), butylated hydroxyl anisole (BHA), and butylated hydroxyl toluene (BHT), as well as nordihydroguaiaretic acid (NDGA), gallic acid esters (propyl gallate), and others. Natural antioxidants act on lipid free radicals and break the chain. e.g.: Mineral antioxidants, Vitamins, Phytochemicals.[16]

Role of antioxidants in diabetes mellitus

Diabetes mellitus is seen as an oxidative stress condition brought on by an imbalance between the body's natural antioxidant defences and the creation of free radicals.

Systemic inflammation, endothelial dysfunction, decreased pancreatic cell output, and reduced glucose uptake by peripheral organs are all caused by oxidative stress. Antioxidant reduce vascular complications and decrease the death rate from cardiovascular disease and other causes in patients with type 2 diabetes mellitus (T2DM) who were at risk.[17]

The alpha glucosidases and alpha amylase are the enzymes that are involved in the digestion of carbohydrates. The activity of these enzymes is responsible for breakdown of oligosaccharides into monosaccharides. It reduces the postprandal increase of blood glucose, so it is an important strategy in management of blood glucose level in Type 2 DM and borderline patients. Natural alpha glycosides and alpha

amylase inhibitors from plant source offers attractive approach for control of hyperglycemia.

Cardiovascular complications such as endothelial dysfunction and accelerated Atherosclerosis are the leading cause of morbidity and mortality associated with DM. For the treatment of oxidative stress in DM, several antioxidants have been developed, including the use of vitamins, supplements and some components of plants and fresh fruits.[18]

Opuntia dillenii

Opuntia dillenii, a well-known member of the Cactaceae family, is used as a medicinal plant with a height of about 1-1.8 metres in various countries and grows in the desert, semi-desert, tropical and sub-tropical areas.[34]

Fig 1: Picture of Opuntia dillenii

Taxonomical classification

a. Kingdom - Plantae (Plants)
b. Subkingdom - Tracheobionta (Vascular plants)
c. Super division - Spermatophyta (Seed plants)
d. Division - Magnoliophyta (Flowering plants)
e. Class - Magnoliopsida (Dicotyledons)
f. Subclass - Caryophyllidae
g. Order – Caryophyllales
h. Family - Cactaceae (Cactus family)
i. Species - Opuntia dillenii (Ker Gawl.) (Erect pricklypear)
j. Genus – Opuntia Mill. (Prickly pear).[35]

Synonyms

a. AndhraPradesh - Nagajemudu, Nagadali
b. Bengal – Nagphana
c. Gujrat - Chorhathalo Nagphan
d. Himachal Pradesh – Chhittarthor
e. Hindi - Hathhathoria, Naghhana
f. Karnataka - Papaskalli, Chappatigalli
g. Kerala – Palakakkali
h. Maharashtra - Nagamullu chapal
i. Orissa – Nagophenia
j. Punjab – Chittarthohar
k. Sanskrit - Mahavriksha, Vajrakantaka
l. Tamilnadu - Nagathali, Sappathikalli .[36]

Geographical source

Native to South-Eastern USA.

Habitat

Open woodlands, Range lands, Grass lands, Road sides etc.

Habit

Erect, Succulent plant with 2 meter in height.

Morphology

- Stem – Shrubby, Branched from base, Broad ovate with spine
- Height - 1-2 meter in height
- Leaf – 3.8 mm long, Pale green conical leaf Broadly obovate, Indulate Modified into spines.
- Flower – yellow in color, Complete flower with four sepals and 12 petals
- Fruit – Obovoid, Bright Red purple to pinkish red color Juicy with spines / Spineless.[37]

Microscopic characters

- Obovate cladodes, 1-7 spines per stem opuntia dilleni
- Irregular Epidermis with wavy cells
- Parasitic stomata
- Pantoporate pollen grain.[38]

Chemical constituents

- Stem – Anthocyanin, Tanin, Rutin, Phenol, Lunamarine, Saponin, Sapogenin, Riavalinidine Catechin etc.
- Fruit – Betalins, Ascorbic acid, Total phenol and essential elements.[39]

MATERIALS AND METHODS

MATERIALS

Plants

The whole plant of *Opuntia dilleni* was collected from Cumbum, Theni district, Tamilnadu, India on 15th April 2023 and was authenticated by Dr. TOJI THOMAS, a Botanist from the department of Botany, St.Thomas College, Pala, Kottayam, Kerala. An herbarium specimen of the same was prepared and kept at the department of pharmacology, St. John's College of Pharmaceutical Sciences and Research, Kattappana, Idukki, Kerala.

Methods

Collection

The whole plant *Opuntia dillenii* were collected from Theni district, Tamilnadu, India on 15th April 2023. The

plant was identified and authenticated by Dr. TOJI THOMAS.

Drying

The plant leaves are collected. Shade dried for several weeks, crushed by hands and dried again.Then the crushed leaves were grinded into coarse powder with help of a mechanical grinder. [64]

Successive extraction

50 grams of the powder was packed in a cotton bag wrapped with Whatman filter paper grade number 1 and inserted into Soxhlet apparatus. The apparatus was arranged as below.

Successive extraction was performed with 400 ml each of petroleum ether chloroform and ethanol according to the increasing order of polarity (petroleum ether, chloroform and ethanol).[65]

Procedure

The extraction began with petroleum ether and the temperature was maintained 60°c the process took 4 days to complete; the obtained extract was evaporated in a boiling water bath and then stored in a desiccator. After the completion of extraction with petroleum ether, the marc left was air dried and used for the next extraction process using chloroform (40°c). The extract obtained after 4 days was evaporated in a boiling water bath and then stored in a dessicator.[66]

The powdered whole plant *Opuntia dillenii* was packed into Soxhlet apparatus and extracted with 70% v/v ethanol in water at 75-79°c for 48 hours. The extract obtained was evaporated at 45°c, then stored in an air tight container.[67]

The extract where as follows:

Petroleum ether extract of plant *Opuntia dillenii.*

Chloroform extract of plant *Opuntia dillenii.*

Ethanol extract of plant *Opuntia dillenii.*

Estimation of anti-oxidant activity

Human beings are exposed to hydrogen peroxide indirectly via the environment, nearly 0.28 mg/ kg/ day with intake mostly from leaf crops. Hydrogen peroxide may enter into the human body through inhalation of vapor of mist and through eye or skin contact.

Principle

Hydrogen peroxide is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH -) that can initiate lipid peroxidation and cause DNA damage in the body. This ability of plant extracts to scavenge hydrogen peroxide can be estimated according to the end of Ruche It al (1989).

Reagent preparation

A solution of hydrogen peroxide (40mM) is prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer.

Procedure

Extract (20-60 µg/ml) in distilled water is added to hydrogen peroxide and absorbance 230

nm is determined after 10 min against the blank solution containing phosphate buffer without hydrogen peroxide.[69]

The percentage of hydrogen peroxide scavenging is as follows:

$$\text{Percentage of Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} (\times 100)$$

Estimation of α-amylase inhibition assay

Principle

The principle of the -amylase inhibition assay involves measuring the ability of a test substance to inhibit the enzymatic activity of -amylase.

Procedure

Alpha-amylase inhibition activity was carried out by starch-iodine method. The concentrations 25µg/ml, 50µg/ml, 75µg/ml,100µg/ml of plant extract were prepared by dissolving in distilled water. 500 µl of plant extract and 500 µl 0.02M sodium phosphate buffer (pH 7 with 0.006M NaCl) containing alpha amylase solution (0.5mg/ml) should be taken in each test tube. It is then incubated at 37ºC for 10 min. 500 µl of starch solution (1%) was added and the mixture was incubated for 1 hr. Then 0.1 ml of 1% iodine solution and 5ml distilled water was added to it and the absorbance was taken at 565nm. Sample, substrate and α-amylase blank determination were carried out under the same reaction conditions.

Inhibition of enzyme activity was calculated as:

Percentage inhibition = (A-C) ×100/ (B-C)

Were,

A=Absorbance of the sample,

B= Absorbance of blank (without α-amylase),

C= Absorbance of control (without sample).[70]

Estimation of α-glucosidase inhibition assay

Principle

α-glucosidase will catalyse the conversion of the substrate 4-nitrophenyl--D-glucopyranoside to -D-glucopyranoside and p-nitrophenol under the given conditions (pH=6.8; T = 37 °C), As indicated in the equation below (Equation 1). The latter product's yellow colour is spectrophotometrically evaluated at 405 nm.

[PNPG + α-glucosidase] → α-D-glucopyranoside + PNP (yellow) ⟶ (1)

The acid type of -glucosidase (originating from the prostate) is specifically inhibited since the reaction buffer contains sodium dodecyl sulphate (SDS). This enables accurate measurement of neutral enzyme activity.
Samples were dissolved in dimethyl sulfoxide at various concentrations (10 µL) and treated with p-NPG (250 µL, 3 mM) and phosphate buffer solution (490 µL, 100 mM, p7). The solution was pre-incubated at 37o C for 5 minutes. Then, 250 µL of α-glucosidase enzyme (0.065 U/mL) was added and the reaction continued for 15 minutes. The reaction was stopped by adding 1 mL of 0.2 M Na2CO3. The mixtures were measured at 400 nm using a UV-spectrophotometer.[71,72]

RESULTS AND DISCUSSIONS

Stage 1-outcome of phytochemical analysis

The whole plant of opuntia dillenii were taken for the study of antioxidant activity and phytochemical screening of this plant shows the presence of active constituents like Alkaloids, Flavanoids, Carbohydrates, Terpenoids etc.. The observed phytochemicals and rich phenol content accessed the study and validate its application as folkloric medicine in diabetes mellitus and chemotherapeutic agents for cancer.

Table 1: Phytochemical analysis of different extracts of *Opuntia dillenii*

SL.NO	TEST	SOLVENT(PETROLEUM ETHER)	SOLVENT (CHLOROFORM)	SOLVENT (ETHANOL)
I	ALKALOID TEST			
1.	Dragandroffs test	+	+	-
2.	Hagers test	+	+	-
3.	Mayers test	+	+	-
II	CARBOHYDRATE TEST			
1.	Molisch test	+	+	+
2.	Barfoeds test	-	-	-
III	CARDIAC GLYCOSIDES			
1.	Keller killani test	-	-	-
2.	Bromine water test	-	+	-
IV	PROTEINS & AMINO ACIDS TEST			
1.	Biuret test	-	+	-
2.	Millons test	-	+	+
V	FLAVONOIDS TEST			
1.	Lead acetate test	+	+	+
2.	Ferric chloride test	+	+	+
VI	TANNINS TEST			
1.	Gelatin test	+	+	-
2.	Bromine water test	+	+	-
VII	LIGNINS			
1.	Furfuraldehyde test	+	-	-

Outcome of hydrogen peroxide scavenging assay

The scavenging ability of different extracts of Opuntia dillenii with hydrogen peroxide is shown below. The highest hydroxyl radical scavenging was found in the ethanolic extract of opuntia dillenii followed by chloroform extract and petroleum ether extract. Ascorbic acid was used as the standard

Table 2: Absorbance, Percentage inhibition and IC_{50} values of different extracts of Ethanol, Cloroform and Petroleum ether of *opuntia dillenii.*

CONCENTRATION	STANDARD		ETHANOL		CHLOROFORM		PETROLEUM ETHER	
(µg/ml)	ABS	% INH	ABS	% INH	ABS	% INH	ABS	% INH
20	0.760	22.44	0.69	29.59	0.79	19.38	0.76	22.44
40	0.613	37.44	0.57	41.83	0.65	33.67	0.74	24.48
60	0.406	58.57	0.42	57.14	0.54	44.89	0.64	34.69
80	0.293	70.10	0.30	69.38	0.40	57.18	0.52	46.93
100	0.185	79.12	0.20	79.53	0.33	63.32	0.51	47.95
IC_{50}	58.203		86.604		90.286		96.202	

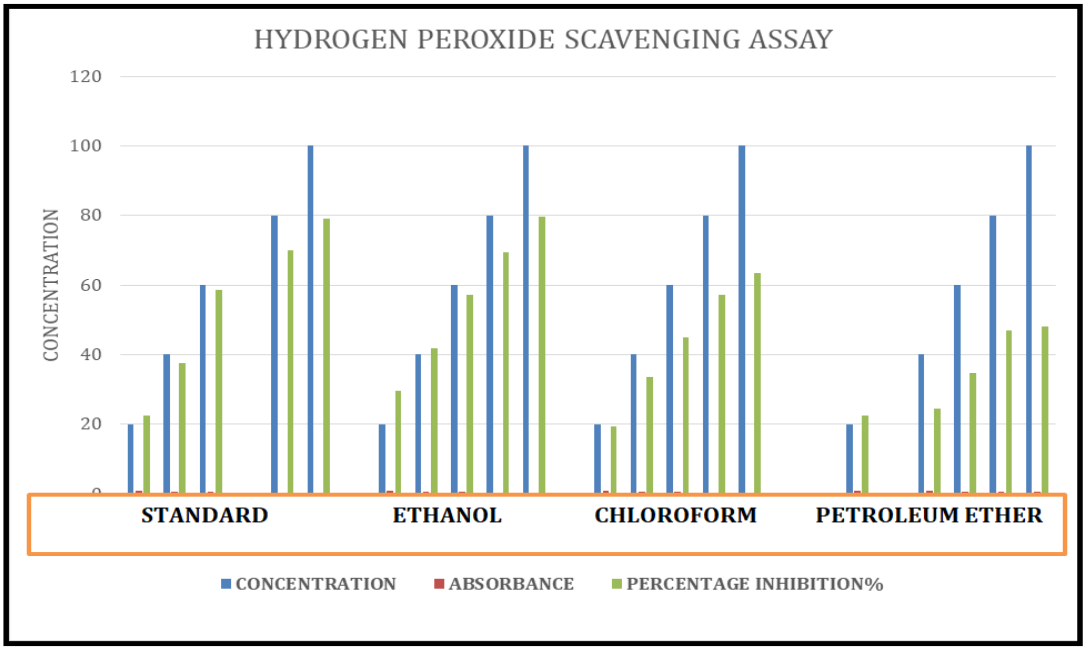

Fig 2: Plot of Hydrogen peroxide scavenging activity of different extracts of Ethanol, Chloroform and Petroleum ether extract of *Opuntia dillenii*.

The antioxidant activity of *Opuntia dilleni* in different extract ethanol, chloroform, petroleum ether was compared with standard. The ethanolic extract shows the better percentage inhibition as compared to other extracts. The IC_{50} value of Ascorbic acid was found to be 58.203mcg/ml and the IC_{50} value of Ethanolic extract was 86.604mcg/ml.

Antioxidants are the molecule that fight free radicals in our body. The free radicals can cause harm to the body. It is estimated from the graph that the antioxidant activity is high in the ethanolic extract when compared to standard.

Outcome of α-amylase inhibitory assay using ethanolic extract

The alpha amylase inhibitory assay of Ethanolic extract of *Opuntia dillenii* with standard (Acarbose) is given below.

Table 3: Percentage inhibition and IC_{50} values of different concentrations

CONCENTRATION mg/ml	STANDARD		TEST	
	Absorbance	% Inhibition	Absorbance	% Inhibition
25	25.49	24.59618209	24.93	25.71113561
50	35.46	77.04006713	35.51	80.83792723
70	51.43	129.4839522	47.60	135.9647189
100	59.89	181.9278372	58.69	191.0915105
IC_{50}	101.235		106.027	

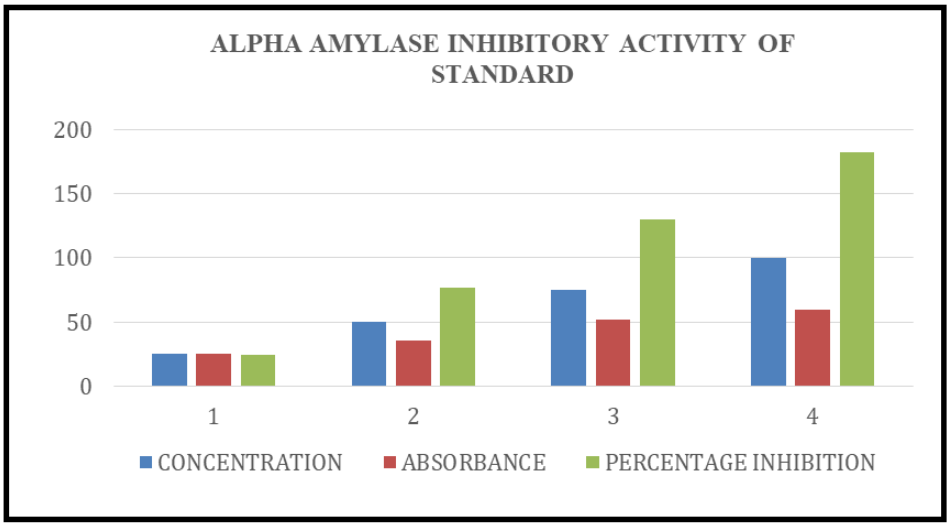

Fig 3: Absorbance, Percentage inhibition values of standard

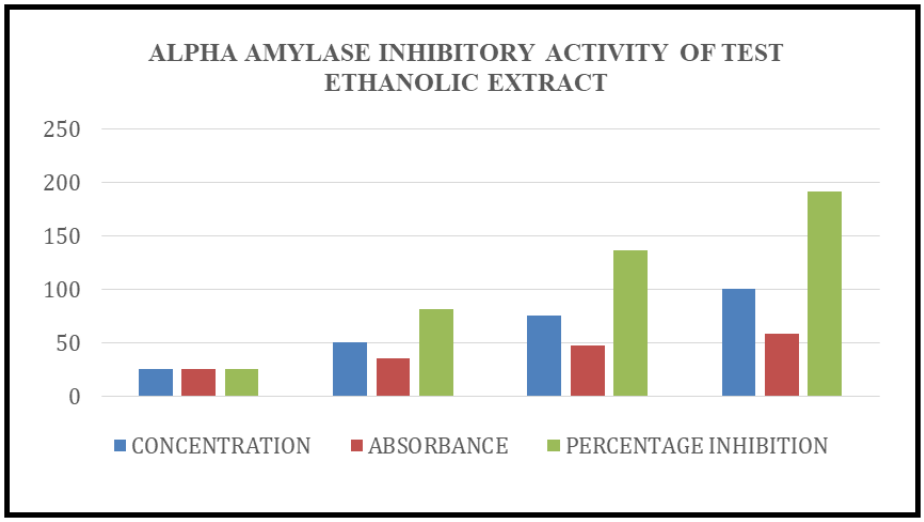

Fig 4: Absorbance, Percentage inhibition values of Ethanolic extract

The Alpha amylase inhibitory activity of *Opuntia dillenii* ethanol concentrate was compared to that of the widely used alpha amylase inhibitor acarbose with IC_{50} values. The IC_{50} estimations of ethanol extract of *Opuntia dillenii* was 106.027µg/ml which was the better on contrasted and standard acarbose 101.235µg/ml.

Alpha amylase is an enzyme accountable for digestion of carbohydrates to give diverse products of glucose units which may be responsible for hyperglycaemia and evolution of type 2 diabetes mellitus. The ethanol extract of *Opuntia dilleni* was inhibits the alpha amylase activity and decreases the raised blood glucose levels. The ethanol extract of *Opuntia dilleni* was more potent on contrasted with that of standard acarbose. The plant-based α-amylase inhibitor offers a forthcoming helpful methodology for the management of diabetes.

Outcome of α-glucosidase inhibitory assay using ethanolic extract

Table 4: Percentage inhibition and IC_{50} values of different concentrations

CONCENTRATION mg/ml	STANDARD		TEST	
	Absorbance	% Inhibition	Absorbance	% Inhibition
20	12.05	45.52390999	11.43	47.59196185
40	19.14	115.847398	18.17	115.7118529
60	24.64	186.1708861	23.75	183.8317439
80	29.37	256.4943741	29.96	251.9516349
100	35.37	326.8178622	34.89	320.0715259
IC_{50}	182.168		192.105	

The alpha amylase inhibitory assay of Ethanolic extract of Opuntia dillenii with standard (Acarbose) is given below.

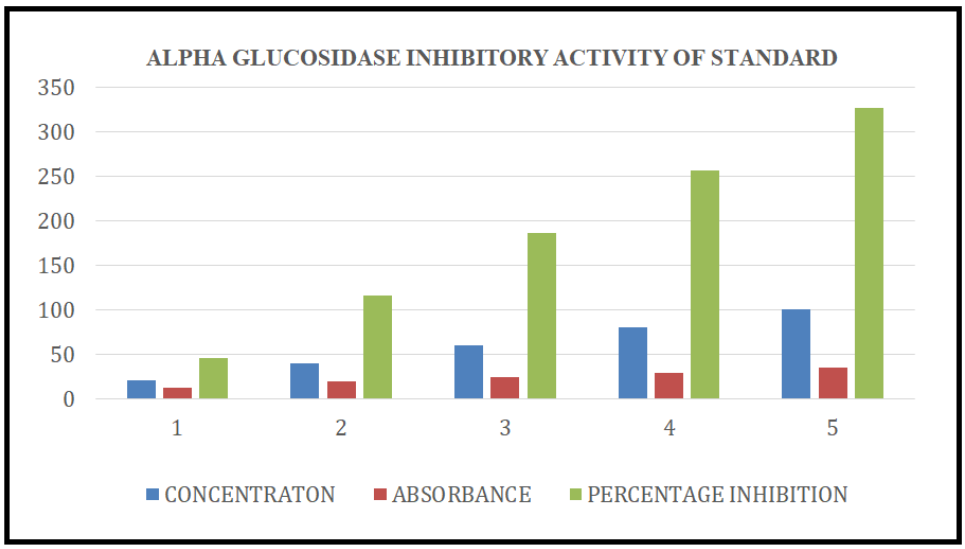

Fig 5: Absorbance, Percentage inhibition values of standard

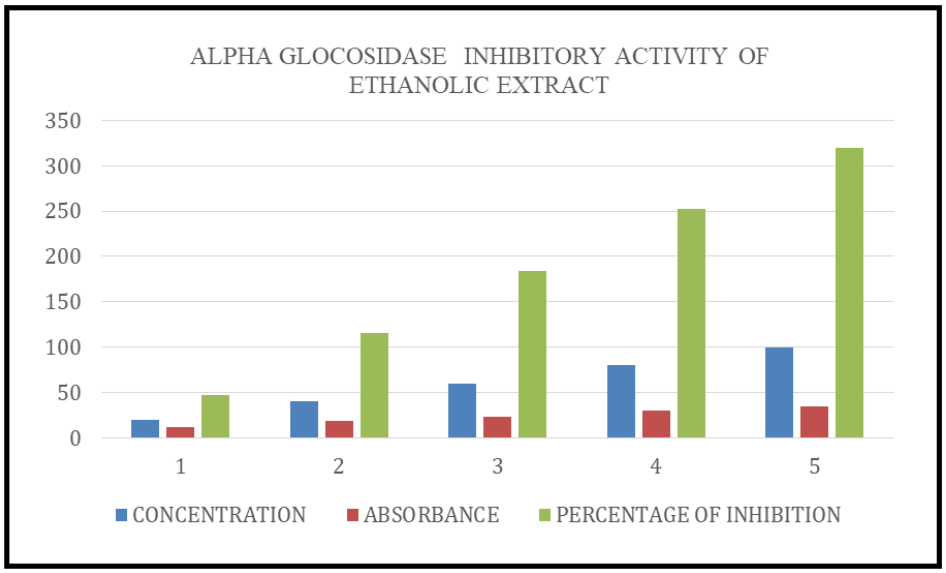

Fig 6: Absorbance, Percentage inhibition values of Ethanolic extract

With respect to IC_{50} values, the alpha glucosidase inhibitory activity of the ethanol extract of Opuntia dilleni was compared to that of the widely used alpha glucosidase inhibitor acarbose. The ethanol extract of Opuntia dilleni had an estimated IC_{50} of 192.105 µg/ml, which was superior to the more common acarbose at 182.168µg/ml.

The alpha glucosidase enzyme works same as that of alpha amylase. The alpha glucosidase inhibitory activity of ethanol extract of Opuntia dilleni was contrasted and basic standard alpha glucosidase inhibitor acarbose with IC_{50} esteems, among them the ethanol extract of Opuntia dilleni shows the more potent activity.

SUMMARY

Diabetic is the one of the most non-communicable disease in the world. It is mainly classified into 3 major types:

Type 1 – auto immune disease

Type 2 – metabolic disorder

Type 3 – gestational diabetic – hormonal disorder

Diabetic scenario in the world

The diabetic around the world in 2021, 537 million adults (2279 years) or living with diabetic - 1 in 10. This number is predicted to rise to 643 million by 2030 and 783 million by 2045. Over 3 in 4 adults with diabetes live in low- and middle- income countries.

Diabetic scenario in India

Diabetic in India at a glance currently 22.2 million adults, which is estimated to increase 35.7 million in the year 2045. India has estimated 77 million people (1 in 11 Indians) formally diagnosed with the diabetics which makes it the second most affected in the world.

Diabetic scenario in Kerala

In Kerala around 90.5 % it was in the 17th position in 2006. In Trivandrum 16.3 % in 10th position in India. That's why we are taken into special consideration for the management of the disease.

Diabetic is a group of clinical syndromes with glucose metabolic disorder as the main manifestation caused by genetics and environmental factors. There is certain differences etiology, morbidity, symptoms and disease characteristic management.

Insulin resistance and pancreatic β cells function defects (insufficient insulin secretion) are the basic characteristics of Type 2 diabetics. If the blood sugar level more than 200 + we will find variety of adverse outcomes including kidney failure, blindness, neurological damage and increase the risk of cardio vascular diseases including heart attack and stroke.

These tests can be used for diagnosis of diabetics

1. HbA1C – measured
2. FPG – Fasting blood glucose
3. OGTT – oral glucose tolerance test
4. RPG – random blood glucose test

The main adverse effects produced by all allopathy drugs may be worse than the disease. But the value of the herbal drugs comparatively less adverse and toxicological effect. That is the main reason, we are taken into special consideration extract of *Opuntia dilleni* shows the maximum inhibitory activity in Alpha amylase, Alpha glucosidase, these are enzymes accountable for digestion carbohydrates to give diverse products of glucose units which may be responsible for hyperglycaemia.

The conclusion of summery indicates the ethanol extract of *Opuntia dilleni* produce remarkable finding that are comparable to those of the relevant standard. That's why in future we are also making our effort for the distinguish, dynamic de-toxification process for the beneficial effect of human being for the easy and safety administration of drug.

CONCLUSION

According to the findings, *Opuntia dillenii* ethanol extract has strong antidiabetic properties as demonstrated by an in vitro experiment. The ethanol extract of *Opuntia dillenii* produces findings that are comparable to those of the relevant standards. Currently, efforts are being made to distinguish the dynamic components from the various plant extracts and explain the component of activity.

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