International Journal of Universal Pharmacy and Bio Sciences 10(3): May-June 2021

INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES

IMPACT FACTOR 4.018*

ICV 6.16*

Pharmaceutical Sciences **Research Article……!!!**

IN VITRO EVALUATION OF ANTIDIABETIC POTENTIAL OF CUMINUM CYMINUM SEED EXTRACT

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KEYWORDS:

Cuminum cyminum, antidiabetic, α-Amylase, α-Glucosidase, ethanolic and aqueous.

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ABSTRACT

Diabetes mellitus a chronic metabolic disorder characterized by rise in blood glucose levels known as " hyperglycemia." which requires a life long treatment. It is of two types, type I(IDDM) accounting for 5% prevalence and type II(NIDDM) for 95% prevalence among diabetics. Alpha Glucosidase inhibitors exhibit their effect by down regulation of the digestion of starch in the small intestine. Long term use of these hypoglycaemic agents results in adverse effects. So research investigations have verified the safety and effectiveness of herbal medicines for the treatment of diabetes. Antihyperglycaemic effect of plants is due to the presence of different phytochemicals or secondary metabolites like flavonoids, terpinoids, alkaloids, glycosides or carotenoids. Acarbose is the most commonly prescribed α-glucosidase inhibitory drug. The inhibition of α-amylase, α-glucosidase activity (IC50 values) is by the tested plant extracts. Our aim of the study is to evaluate the antidiabetic potential of Cuminum cyminum seed extract by using invitro α-amylase and α-Glucosidase inhibitory assay. The present study confirms the traditional claim that Cuminum cyminumexhibits anti-hyperglycemic. The results of α-Amylase inhibition assay and α-Glucosidase Inhibiton assay of Cuminum cyminum seed extracts reflects that ethanolic extract have more anti diabetic potential in compare to aqueous extract. Our study justifies the traditional use of Cuminum cyminumin for diabetes management.

INTRODUCTION:

Diabetes mellitus (DM) is a metabolic disease associated with hyperglycemia. Diabetes is the major cause of morbidity and mortality worldwide1. DM is estimated to affect about 366 million by 2030. Incidence of diabetes is increased dramatically, this increment not only affect the individual health but elevate the cost of health care and has implications for economic and social issues of the society2. Type II diabetes (T2D) has increased during the recent years, its affect 90-95 of diabetic patients. T2D is one of the risk factors for cardiovascular diseases3. Unlimited elevation in blood glucose levels leads to increase oxidative stress, which induce damage cell tissue and causes vital changes in the function and structure of organs1. Exogenous insulin and/oral hypoglycemic drugs can be used to control hyperglycemia in diabetic patients4. Sometimes the side effect due to interaction between medications could induce side effects more serious than the disease itself. So search for natural alternatives to control diabetes and maintain blood sugar levels is very important. These natural alternatives are safer than pharmacological drugs. There has been an increase in the use of herbal products to complement conventional drugs in the treatment of various diseases5.

Figure no.1 Plant picture

Herbal medicines have been proposed as suitable alternatives in the management of diabetes due to their availability and few side effects6. It was reported previously that herbs regulate blood glucose levels through different mechanisms i.e. lowering carbohydrate absorption, enhancing insulin sensitivity and peripheral glucose uptake, stimulating the secretion of insulin and endogenous incretins, preventing cell apoptosis by exerting antioxidant effects and promoting glycogenesis or inhibiting glycogenolysis7. Cumin (*Cuminum cyminum* L.) is herbaceous plant belong to Apiaceae family. Cumin is indigenous to the Mediterranean region and has been popularly used as spice. Previous studies suggested that cumin possess a broad range of pharmacological activities such as antibacterial8, antioxidant9, hypoglycemic10 and anti-carcinogenic11. Cumin plant contain 5% volatile oil, 22% fat, 10% protein and 11% fibers. The major volatile components of cumin plant are cuminaldehyde, cymene and terpenoids12. The aim of the present research was studying the anti-diabetic potential of crude ethanol extract of cumin plant.

MATERIALS AND METHODS

Collection of plants Leaves:

The seeds of the selected medicinal plant were collected from nearest local grocery shop. Identified and authenticate with the help of botanist Dr Shiva kumar, Assistant professor, osmania university and voucher of the same kept in herbarium for further reference.

Extraction of plants leaves:

The seeds of the plant were cleaned and air dried under shade, then powdered with the help of grinder. 100 gm of plant powdered material was added in 1000 ml of petroleum ether, ethanol and aqueous solvents respectively and then kept on a magnetic stirrer for 2 hrs. Thereafter, it was extracted one by one using a soxhlet apparatus sequentially with these solvents. The extracts were collected and the solvents were evaporated out to dryness. The obtained materials were stored in airtight bottles, labelled and kept at 4^0C for further studies. The resulting extracts were used to study for their porcine pancreatic α-amylase inhibitory activity, screened for the presence of amylase inhibitors under in vitro conditions by using 3, 5-Dinitrosalicylic acid (DNS) method.

Figure- Extraction process using soxhlet apparatus

Glass wares

All glasswares used were of Borosil make and purchased from authentic supplier

Chemicals:

All chemicals, reagents and solvents used were extra pure, A.R. grade.

Acarbose (Glucobay 50 Tablet-Bayer Pharma, Thane),α-amylase ex-porcine pancrease extrapure (SRL, Mumbai),3,5-Dinitrosalicylic Acid (Loba Chemie, Mumbai),Sodium hydroxide (SDF, Mumbai)Ethanol 99% Absolute,Solvents:Ethanol and distilled water were used for experimental purpose.

Instruments:

pH-meter (Elico LI 120), Boiling Water bath, Heating Mantel (Remi),Thermostat (Metalab), UV-Vis spectrophotometer (Bio Era)

Phytochemical Analysis

For phytochemical analysis Ethanolic and aqueous extract of *Cuminum cyminum* seed part was used. Phytochemical analysis of extracts were performed according to standard methods.

Test for Alkaloids (Mayer's Test)

Presence of alkaloids in extract was confirmed by formation of yellow precipitates due to treatment of extract with Mayer's reagent.

Test for Flavanoids (Ferric Chloride Test)

Appearance of blackish red color indicated presence of flavanoids after treating aqueous extract of polyherbal formulation with few drops of $FeCl_3$ solution.

Test for Tannins:

Few drops of $FeCl_3$ solution (1%) were mixed with 1 ml of polyherbal formulation extract. Absence of tannins was confirmed by Absence of black, blue, green or blue green precipitate

Test for Phenols (Ferric Chloride Test)

In this test, 3 ml of distilled water was added to 1 ml of extract and then extract was treated with few drops of neutral $FeCl_3$ solution (5%). Presence of phenols in sample was indicated by formation of a dark green color.20

Test for Saponins (Foam Test)

Presence of saponins in herbal mixture extract was confirmed by formation of persistent foam due to vigorous shaking of mixture of distilled water (2 ml) and 1 ml of extract.

Test for Proteins (Biuret Test)

Proteins content in extract was determined by mixing 1ml of herbal formulation extract with 1 ml of NaOH solution (10%). The mixture was ignited and presence of proteins in extract was confirmed by appearance of purplish violet color due to treatment of above solution with a drop of $CuSO_4$ solution (0.7%).

Test for Fats and Fixed oils (Stain Test)

Small amount of the extract was compressed between two filter papers. Formation of greasy blot (stain) on the filter paper confirmed the presence of fixed oils in sample.

Test for Carbohydrate

For carbohydrate determination Molisch's test was used. In 2ml of extract added few drop of molisch's solution and sulphuric acid was added. Purple colour indicated carbohydrate presence.

Test for Glycoside

For determination of glycosides, 5ml extract was treated with 2ml of glacial acetic acid and then one drop of $FeCl_3$ solution was added. After that 1ml of concentrated H_2SO_4 was added. At interphase, brown color ring appeared indicating the presence of glycosides.

Test for Terpenoids (Salkowski Test)

To 1 ml of the solvent extract, 2 ml of chloroform was added. Then 3 ml of conc.H_2SO_4 was added carefully to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids.

***In- vitro* antidiabetic activity**

1. *In vitro* α-amylase Inhibitory Assay

A modified 3, 5-dinitrosalicylic acid (DNSA) method was adopted to estimate α-amylase enzyme inhibition activity, by quantification of reducing sugars (maltose) liberated under the assay conditions. The enzyme inhibition activity was determined as a decrease in units of maltose liberated. 1ml solution of the each extracts concentrations ranging from 20-100□g/ml were incubated with 1ml of 1unit/ml porcine pancreatic Î±-amylase enzyme for 30 minutes at 370C. Then 1 mL (1% w/v) potato starch solution was added and the mixture was further incubated at 370C for 10 minutes. The reaction was stopped by adding 1 ml DNSA reagent (12.0 gm of sodium potassium tartrate tetrahydrate in 8 ml of 2M NaOH and 96 mM 3, 5- dinitrosalicylic acid solution) and the contents were heated in a boiling water bath for 5minutes. Then reaction mixture was removed from the water bath and cooled. Thereafter it diluted with distilled water. The absorbance was measured at 540 nm using UV-Spectrophotometer.A blank was prepared without plant extract and another without the α-amylase enzyme, replaced by equal quantities of buffer (20 mM Sodium phosphate buffer with 6.7 mM Sodium chloride, pH 6.9 at 250C). Control representing 100% enzyme activity without plant extract was incubated.

The standard α-amylase inhibitor drug Acarbose was used as positive control. The antidiabetic activity was determined through the percentage of inhibition of α-amylase enzyme and calculated by the following equation as

$$\% \text{ Inhibition Activity} = (A\text{Control} - A\text{Test} / A\text{Control}) \times 100$$

IC_{50} values were calculated by using graphical method.

2. α-Glucosidase Inhibitory Activity

A reaction mixture of 2.9 mM 4-Nitrophenyl-β-D-glucopyranoside (pNPG), 0.25 ml of each sample and 6U/ml α-Glucosidase was prepared in sodium phosphate buffer (pH 6.9). This reaction mixture was incubated for 5 min at 250C. The same procedure was repeated for blank. After 5 min, absorbance was calculated at 405 nm. Sample from each extract of concentration 10, 30, 50, 70, 90µg/ml were prepared. These samples were studied for three parameters using UV3000 UV/VIS spectrophotometer. calculated by the following equation as

$$\% \text{ Inhibition Activity} = (A_{Control} - A_{Test} / A_{Control}) \times 100$$

Results:

Extractive values of *Cuminum cyminum* seed extract from solvents of different polarity such as Petroleum ether, ethanol and water was calculated. The highest extractive values of *Cuminum cyminum* seed extract were obtained in water (91.6%), ethanol (73.4%) and Petroleum ether (53.5%).

Phytochemical Analysis

Ethanolic and aqueous extracts of *Cuminum cyminum* seed extract were subjected to qualitative phytochemical analysis. The results of various chemical tests for the detection and identification of phyto constituents present in are summarized in table no.1

Qualitative analysis of *Cuminum cyminum* seed extract

Phytochemical	Ethanolic extracts	Aqueous extracts
Phenolics	+	+
Alkaloids	+	+
Flavanoids	+	+
Saponins	+	+
Glycosides	+	+
Fixed oils	+	+
Tannins	-	-
Carbohydrates	+	+
Terpenoids	+	+

(+)= Present, (-)=Absent)

Estimation of in vitro α-amylase inhibition activity by DNSA method

α-Amylase is key enzymes involve in carbohydrates breakdown and its absorption in intestine. A modified DNS method was adopted to estimate in vitro α-amylase inhibition activity, by quantifying the reducing sugar (maltose) liberated under the assay, using starch as a substrate and acarbose as a standard reference compound at 20 µg/ml, 40 µg/ml, 60 µg/ml 80 µg/ml and 100 µg/ml concentrations of each extracts.

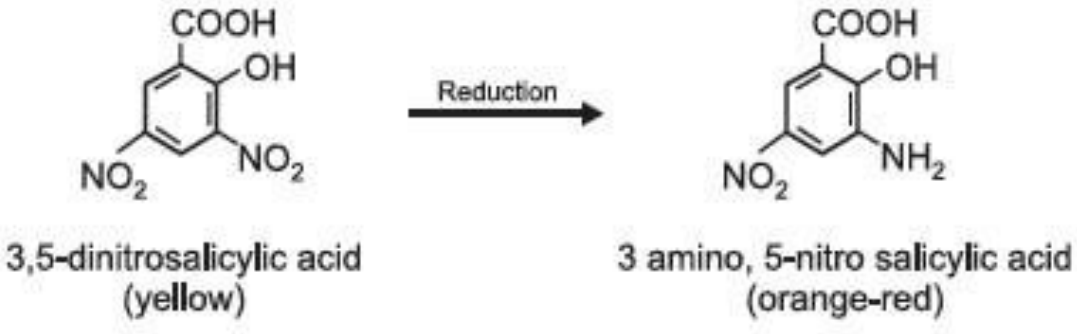

Table-2 α-Amylase inhibition activity of *Cuminum cyminum* seed extracts and Acarbose (Standard α-Amylase inhibitor)

Absorbance of the sample at 540nm		*Absorbance of Control = 0.513*			
Sr. No.	**Extracts**	**Concentration (µg/ml)**	**Absorbance**	**% of Inhibition**	**IC50 Value (µg/ml)**
1	**Ethanol**	20	0.303	40.94	61.27
		40	0.265	48.34	
		60	0.257	49.60	
		80	0.243	52.63	
		100	0.220	57.12	
2	**Aqueous**	20	0.341	33.53	83.25
		40	0.337	34.31	
		60	0.335	34.70	
		80	0.310	39.57	
		100	0.247	51.85	
3	**Acarbose**	20	0.271	47.17	27.98
		40	0.234	54.38	
		60	0.208	59.45	
		80	0.183	64.32	
		100	0.160	68.81	

 International Standard Serial Number (ISSN): 2319-8141

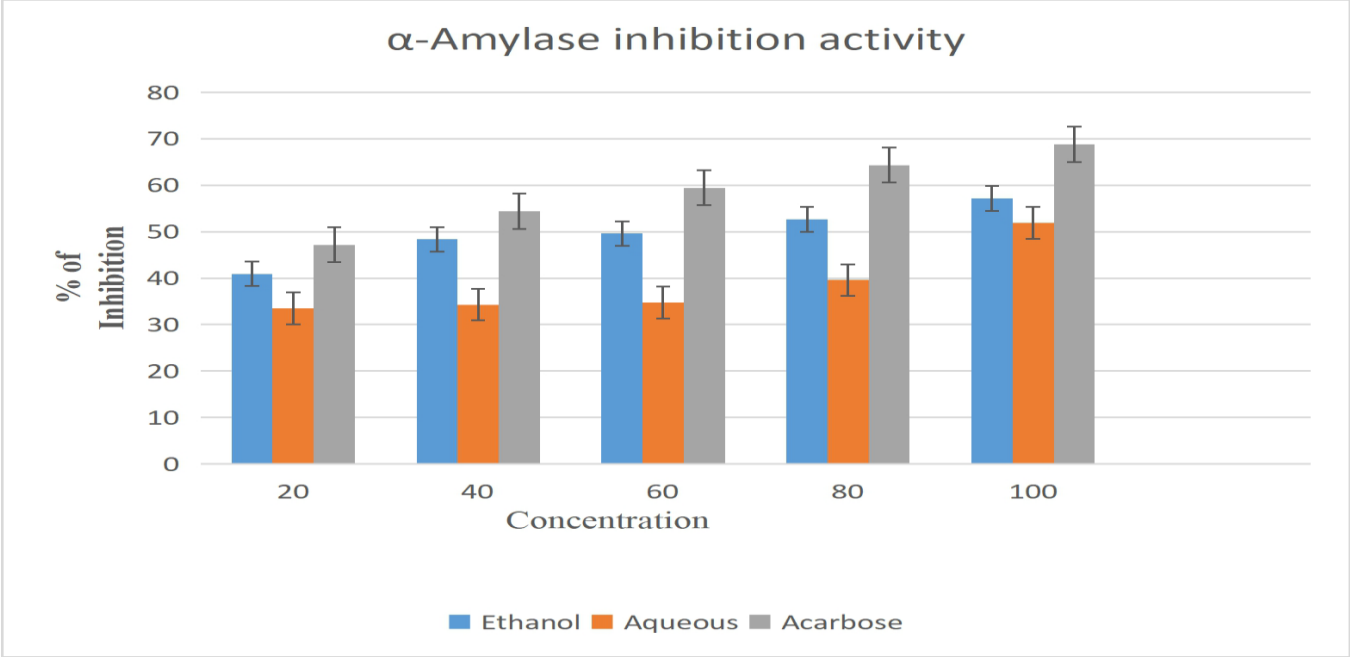

Figure no.2 Comparison of α-Amylase inhibition activity of various extracts

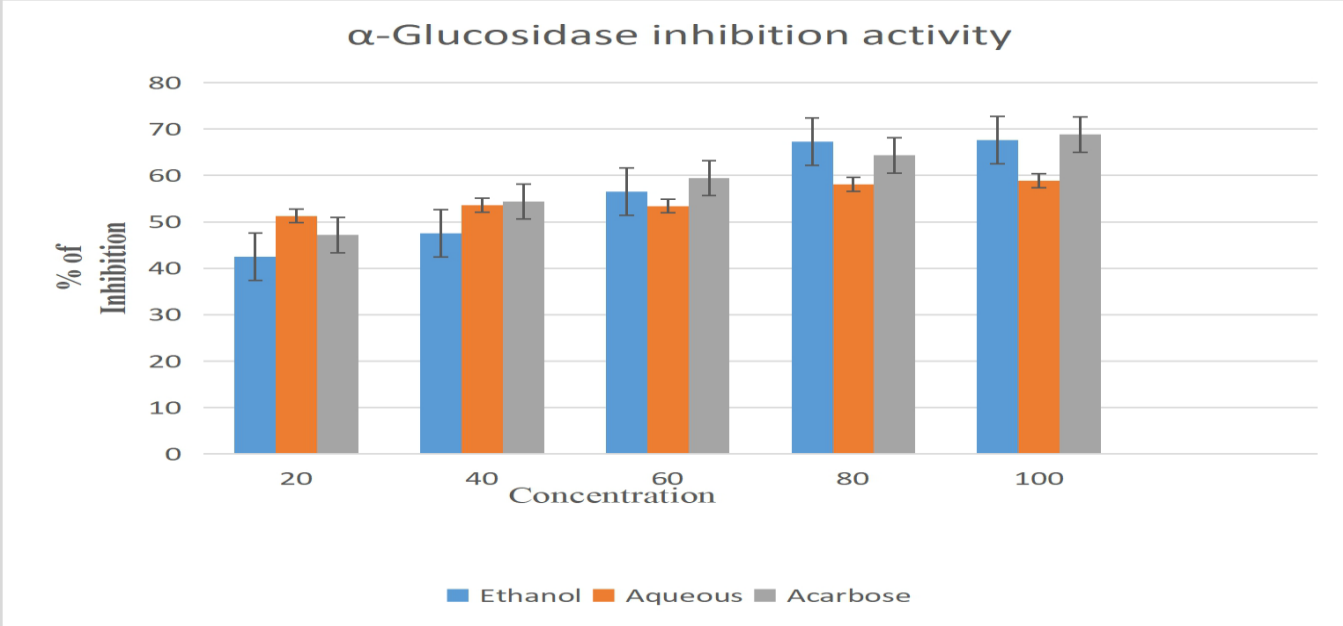

Figure no.3 Comparison of α-Glucosidase inhibition activity of various extracts

Table-3 α-Glucosidase Inhibitory Activity of *Cuminum cyminum* seed extracts and Acarbose (Standard α-Amylase inhibitor)

Absorbance of the sample at 405nm		*Absorbance of Control = 0.513*			
Sr. No.	**Extracts**	**Concentration (µg/ml)**	**Absorbance**	**% of Inhibition**	**IC_{50} Value (µg/ml)**
1	Ethanol	20	0.295	42.49	45.75
		40	0.269	47.56	
		60	0.223	56.53	
		80	0.168	67.25	
		100	0.166	67.64	
2	Aqueous	20	0.250	51.26	97.11
		40	0.238	53.60	
		60	0.239	53.41	
		80	0.215	58.08	
		100	0.211	58.86	
3	Acarbose	20	0.271	47.17	27.98
		40	0.234	54.38	
		60	0.208	59.45	
		80	0.183	64.32	
		100	0.160	68.81	

DISCUSSION:

Qualitative phytochemical screening of plant extract was performed by using aqueous extract. Results have indicated the presence of alkaloids, flavanoids, phenols, glycosides and saponins in herbal extract. These phyto constituents are potent hypoglycemic agents. Alkaloids are reported to produce anti-hyperglycemic effect through potentiating insulin secretion from the pancreatic β cells, by modulation of antioxidant enzymes and dropping oxidative damage. An important flavanoid (quecrcetin) has also been reported to decrease the blood sugar level, hepatic gluconeogenesis, glycogenolysis and increased glucose uptake resulting in anti-hyperglycemic potential.

The medicinal plants contain a variety of ingredients that are thought to act on various ailments in a variety of targets by various modes and mechanisms. Plant derivatives with purported hypoglycemic properties have been used in folk medicine and traditional healing systems around the world, as the plant extract and its secondary metabolites have been experimentally proved and widely used as more effective agents against hyperglycemia and oxidative stress. The present study was aims to check anti-diabetic activity of Cuminum cyminum seeds extracts through α-Amylase inhibition assay and α-Glucosidase Inhibiton assay.

CONCLUSION:

Three different extracts of *Cuminum cyminum* were prepared using petroleum ether, ethanol and aqueous extract and undergone with various phytochemical tests to check the presence of phyto constituent in plants extracts (Result mention in table no.1) The present study confirms the traditional claim that *Cuminum cyminum* exhibits anti- hyperglycemic. The results of Î±-Amylase inhibition assay and Î±-Glucosidase Inhibiton assay of *Cuminum cyminum* seed extracts reflects that ethanolic extract have more anti diabetic potential in compare to aqueous extract (Result mention in table no.2 and 3)Further studies are warranted in the isolation and characterization of the active principle(s) and its molecular mechanism of action. Our study justifies the traditional use of *Cuminum cyminum* in diabetes management.

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