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SCRENING OF PHYTOCHEMICAL AND INVITRO ANTIDIABETIC ACTIVITY OF METHONALIC FRUIT EXTRACT OF *Abutilon Indicum*

K.Kameswararao*, S.Satish, S. Naga SatyaRamalakshmi, T. Preethi Sai lakshmi, B S Chandra Sekhar

Department of Pharmaceutics, Adarsa College of Pharmacy,G.Kothapalli,Gokavaram Mandal, East Godavari District, Andhra Pradesh - 533285, India.

ABSTRACT

Diabetes is a group of chronic metabolic disorder characterised by high blood sugar levels over a prolonged period of time. One of the approaches is to reduce production and absorption of glucose from gastrointestinal tract through the inhibition of carbohydrate digesting enzymes such as alpha amylase and alpha glucosidase .In the present study the methanoic fruit extracts of *Abutilon indicum* was screened for alpha (α)-amylase inhibition using an *in vitro* model. The plant extracts were also examined for its antioxidant activities, reducing power capacity, estimation of total phenolic content, flavonoid content and flavonol content. The study revealed that the different concentrations of the methanolic fruit extract possessed a very good amount of total phenolics, flavonoid and flavonol and exhibit anti oxidant activity. The methanolicfriut extract exhibited significant α-amylase inhibitory activity with an IC50 value 225.54±3.537µg/ml and well compared with standard acarbose drug 213.27± 2.758. Thus, it could be concluded that due the presence of antioxidant components the plant extract have well prospective for the management of hyperglycemia, diabetes and the related condition of oxidative stress.

Key words: Diabetes, *Abutilon indicum*, *in vitro* antidiabetic, α- amylase.

Corresponding Author: **K. Kameswararao** Email: kameshkancharla@gmail.com

INTRODUCTION

Diabetes mellitus is a worldwide increasing problem entailing enormous financial burden and medical care policy issues. According to International Diabetes Federation (IDF), the number of individuals with diabetes and its complication in 2019 crossed 366 million, with an estimated 4.6 million deaths every year (Lucia k keter.et.al 2012).According to the World Health Organization (WHO), up to 80% of the population in developing countries uses plants and its products as a traditional medicine for primary health care needs.

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The WHO has listed 21,000 plants, which used for medicinal purposes around the world. Among these, 2500 species are in India. There are about 800 plants which have been reported to show antidiabetic potential. Vast collections of plant-derived phytoactive principles representing numerous natural bioactive compounds have established their role for possible use in the treatment of diabetes (Banerji *et al* 1999).

Diabetes is a chronic metabolic disorder of multiple etiologies as a result of defects in insulin secretion or insulin action characterised by hyperglycaemia. It is one of the major public health problems worldwide. CVD, neuropathy, nephropathy and retinopathy are among the major risks that are associated with diabetes.

Abutilon indicum is a small shrub native to tropic and sub tropical regions belongs to Malvaceae family,

fruiting throughout the year. It is used as laxative, demulcent, anti- inflammatory, anthelmentic, diuretic, aphrodisiac, etc.In traditional medicine, *A. indicum* various parts of the plant are used as a demulcent, aphrodisiac, laxative, diuretic, sedative, astringent, expectorant, tonic, anti-inflammatory, anthelmintic, and analgesic and to treat leprosy, ulcers, headaches, gonorrhea, and bladder infection. The whole plant is uprooted, dried and is powdered. In ancient days, maidens were made to consume a spoonful of this powder with a spoonful of honey, once in a day, for 6 months until the day of marriage, for safe and quick pregnancy.
The plant is very much used in Siddha medicines. The root, bark, flowers, leaves and seeds are all used for medicinal purposes by Tamils.The leaves are used as adjunct to medicines used for pile complaints. The flowers are used to increase semen in men (Pandit kumar et al., 2011; Ramachandran, 2008.)

Anti -Diabetic Medication

Drugs used in diabetes treat diabetes mellitus by lowering glucose level in the blood. With the exceptions of insulin exenatide, liraglutide, and pramlintide all are administered orally and are thus called oral hypoglycemic agents or oral anti hyperglycemic agents. These are different classes of anti diabetic drugs and this selection depends on nature of the diabetes, age, and situation of the person as well as other factors.

Comparison of anti diabetic medication (Abate N *et al*., 2007, Gupta R *et al*., 2007)

Sulfonylurea (glyburid,glimipride,glipizide) stimulating insulin release by pancreatic beta cells by inhibiting the K ATP channel. Metformin: acts on liver to cause decrease in insulin resistance.Alphaglucosidase inhiitor (acarbose, miglitol, voglibose): reduces glucose absorbance by acting on small intestineto cause decrease in production of enzymes needed to digest carbohydrates. Thiazolidinedione (pioglitazone, rosiglitazone): Reduce insulin resistance by activating PPAR-GAMA in fat and muscle. Most anti-diabetic agents are contraindicated in pregnancy, in which insulin is preferred. Biguanides: It reduces hepatic glucose output and increase uptake of glucose by the periphery including skeletal muscle. Although it must be used with caution in patient with impaired liver or kidney function. Typical reduction in glycated hemoglobin values for metformin is 1.5 – 2%. Phenformin (DBI) was used from 1960s through 1980s, butwas withdrawn due to lactic acidosis risk.

Objective

The main objective of present work is to determine the anti diabetic potential of methanolic fruit extract of *Abutilon indicum* by using in vitro method (α-amylase inhibitory activity).

METHODOLOGY

Collection of plant material: The fruits of *Abutilon indicum* were available throughout the year. They were collected from a farmer field near by our college G.Kothapalli.

Figure no.1. Friut of *Abutilon indicum*

Extraction of plant material: The fruits are dried and seeds are removed then the fruits are grinded into fine powder. The powder was extracted with methanol by using soxhlet apparatus. Further the extract is concentrated.

Phytochemical analysis: Prepared extract is evaluated for alkaloids, glycosides, tannins, resins, flavanoids and phenolic compounds.

Preliminary Phytochemical Screening of Methanoilc Fruit Extract of the *Abutilon Indicum*

The methanolic fruits extract of *Abutilon indicum* taken 10g in 50ml of methanol and subjected to extraction (Kiritikar KR, 1999)

The filtrate was subjected to Molisch's test. Formation of reddish brown ring indicated the presence of carbohydrates.

1. Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution [brown color indicated the presence of carbohydrate.]
2. Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours. Blue coloration of the spot indicated the presence of phenols.
3. Test for flavonoids: Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added. A pink or red coloration of the solution indicated the presence of flavonoids in the drugs.
4. Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added. Flocculent white precipitate indicated the presence of flavonoids.
5. Test for tannins: Braemer's test: To 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the drug.

6. Test for steroid/terpenoid: Liebermann-Burchard test: To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids.
7. Test for alkaloids: Draggandroff's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggandorf's reagent. Orange coloration of the spot indicated the presence of alkaloids.
Hager's test: The extract was treated with few ml of Hager's reagent. Yellow precipitation indicated the presence of alkaloids.
Wagner's test: The extract was treated with few ml of Wagner's reagent. The reddish brown precipitation indicated the presence of alkaloids.
8. Tests for Glycosides: Legal's test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution. Pink to red color solution indicates the presence of glycosides.
9. Test for Saponins: Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15minutes.1cm layer of foam formation indicates the presence of Saponins
10. Test for Anthraquinones: Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. Pink or red coloration of aqueous layer indicated the presence of Anthraquinones.
11. Test for Amino acids: Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent. Blue color indicated the presence of amino acids.
12. Test for fixed oils and fats: Press small quantity of the petroleum ether extract between two filter paper Oil stains on the paper indicated the presence of fixed oils.

Estimation of Total Phenolic Compounds

Total phenolic content was determined by the Folin Ciocalteu method.(Kumaran A *et al*., 2006) To 0.5ml of 1-5 mg/ml of methanolic fruit extract made up with 0.5ml of distilled water, 0.5 ml of Folin Ciocalteu reagent was added and gently mixed .After 2 minutes 0.5ml of 100mg/ml sodium carbonate was added. The contents were mixed and allowed to stand for 2 hours. The optical density of the blue coloured samples was measured at 765 nm spectrophotometrically (Elico double beam uv visible spectrophotometer). Standard gallic acid of concentration 100-500 microgram/ml was used. The concentration of total phenolics is expressed as milligram of gallic acid) /g of mixture. All determinations were carried out in triplicate.

Estimation of flavonoids

The method used with slight modifications was followed for estimation of flavonoids.(Kumaran A *et al*., 2006) 0.5ml of concentration 100-500 µg/ml of methanolic fruit extract was mixed with 1ml aluminium trichloride in ethanol (20g/l) and diluted with ethanol to 25 ml. The absorbance at 415 nm was read after 40 minutes at 37 ˚C. Rutin of concentration 0.5mg/ml, 1.0mg/ml, 1.5mg/ml, 2.0mg/ml and 2.5mg/ml was used as a reference compound and absorbance was measured under the same conditions. All determinations were carried in triplicate. The amount of flavonoids in fruit extarct was calculated as milligram of rutin/g of mixture.

Estimation of total flavonols

Total flavonols in the fruit extract was estimated using the method of Kumaran and Karunakaran, 2006.(Kumaran A *et al*., 2006). To 1.0 ml of methanolic fruit extract 1.0 ml of 2% $AlCl_3$ ethanol and 3.0 ml (50 g/L) sodium acetate solutions were added. The absorption at 440 nm (UV-visible spectrophotometer Shimadzu UV 1800) was read after 2.5 h at 20°C for the estimation of total flavonol content in the fruit extract.

Measurement of reducing power

The reducing power of the fruit extract was determined according to the method of Oyaizu, 1986. (Oyaizu *et al*., 1986). The methanolic friut extract (100 µl) was mixed with phosphate buffer (2.5 ml, 0.2 M, *p*H 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 min. Aliquots of 10% trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm and reducing power is determined.

***In vitro* antidiabetic activity**

Inhibition of alpha amylase enzyme: (Hamdam and Afifi 2004, Thalapaneni *et al*., 2008, Heidari R *et al*., 2005).

A total of 500µl of methanolic fruit extract samples and standard drug (100-1000µg/ml) were added to 500µl of 0.20 mM phosphate buffer (PH6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25℃ for 10 minutes. After these, 500µl of a 1% starch solution in 0.02 Msodium phosphate buffer (pH6.9) was added to each test tube. The reaction mixture was then incubated at 25℃ for10 minutes. The reaction was stopped with 1ml of 3, 5-dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 minutes, cooled to room temperature. The reaction mixture was then diluted after adding 10ml distilled water and absorbance was measured at 540nm by

using Elico double beam UV-visible spectrophotometer. Control represents 100% enzyme activity.
Percentage inhibition (I %) was calculated by

I % = (Ac-As)/Ac X 100

Where, Ac is the absorbance of the control, As is the absorbance of the sample

Calculation of 50% Inhibitory Concentration (IC50)

The concentration of the methanolic fruit extract required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the fruit extract.

RESULTS AND DISCUSSION

The dried fruit of *Abutilon indicum* was extracted with methanol. The percentage yield of methanolic fruit extract was found to be 9.5. Preliminary phytochemical screening of the methanolic fruit extract of *A.indicum* revealed that presence of carbohydrates, flavonoids, tannins, triterpenoids, glycosides and phenolic compounds and results are shown in the table No.1.

Evaluation of *in vitro* α-amylase inhibitory activity using methanolic fruit extarct of *Abutilon indicum*:

There was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. At a concentration 100µg/ml of methanolic fruit extract showed a percentage inhibition 29.73 ±0.2454 and for 1000 µg/ml it was 71.65 ± 0.3729. The methanolic fruit extract gave an IC 50 value of 225.54±3.537 results are shown in the Figure no.2 and Table.no.3.

Lack of insulin affects the metabolism of carbohydrates, proteins, fat and causes significance disturbance of water and electrolyte homeostasis. Recent advances in understanding the activity of intestinal enzymes (α-amylase and α-glucosidase both are important in carbohydrate digestion and glucose absorption) have lead the development of newer pharmacological agents. A high postprandial blood glucose to response is associated with micro- and macro-vascular complications in diabetes and is more strongly associated with the risk for cardiovascular diseases than are fasting blood glucose. Hence one of the therapeutic approaches for reducing postprandial (PP) blood glucose levels in patient with diabetes mellitus is to prevent absorption of carbohydrate after food intake. Inhibition of this enzyme (α-amylase) reduced the high postprandial (PP) blood glucose peaks in diabetes. The α-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 effect such as abdominal pain, diarrhoea and soft faeces in the colon.

Table 1. Preliminary phytochemical screening of the methanolic fruit extract of *Abutilon indicum*

Class of Compound	Tests Performed	Results
Carbohydrates	Molisch's test	Absent
	Fehlings test	
Phenols	Phosphomolybdic acid test	Present
Flavanoids	Shinoda test	Present
	lead acetate test	Absent
Tannins	Braemer's test	Absent
Sterols	Salkowski's test	Absent
Alkaloids	Draggendrof's test	Absent
Glycosides	Legals test	Absent
Saponins	Foam test	Absent
Anthraquinones	Borntragers test	Absent
Amino acid test	Ninhydrin test	Absent
Fixed oils and fats		Absent

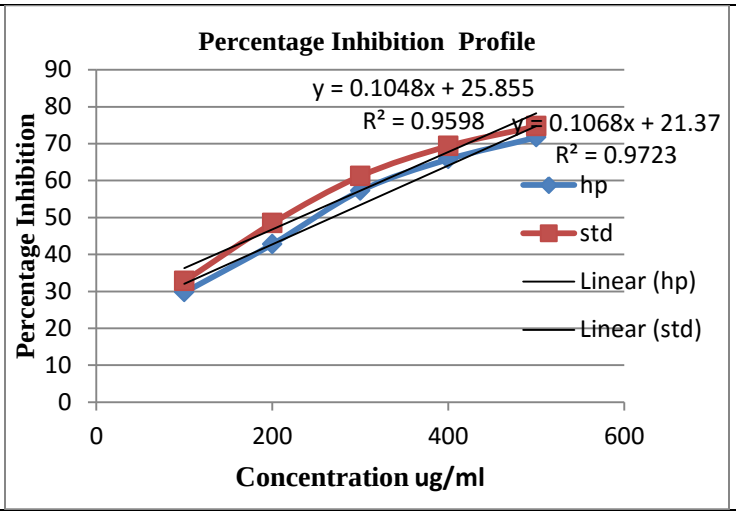
Table 2.Total phenolic, flavaonoid, flavanol and reducing power of methanolic fruit extract of *Abutilon indicum*

Name of the plant	Total phenolic content	Total flavonoid content	Total flavonol content	Reducing power
Methanolic fruit Extract of *Abutilon indicum*	21.34±0.25	0.35±0.01	0.31±0.01	0.32±0.01

Table 3.Results for percentage inhibition of alpha amylase activity for methanolic fruit extract of *Abutilon indicum* and Acarbose(standard).

Sample	Concentration (µg/ml)	%Inhibition	IC50
Methanolic fruit extract of *Abutilon indicum*fruit	100	29.73± 0.2454	225.54± 3.537
	200	42.25 ± 0.1400	
	400	57.23± 0.2454	
	800	65.75± 0.3704	
	1000	71.65 ± 0.3729	
Standard Drug (Acarbose)	100	32.75 ± 0.2425	213.27± 2.758
	200	48.44 ± 0.3704	
	400	61.22 ± 0.2833	
	800	69.33 ± 0.1433	
	1000	74.69 ± 0.3736	

Figure 2.Comparitive inhibitions of alpha amylase of methanolic fruit extract *Abutilon indicum* and standard drug Acarbose



Our finding reveals that fruit extarct of *Abutilon indicum* efficiently inhibits α-amylase enzyme *in vitro*. The reaction mechanisms involved in inhibition of α-amylase enzymes by plant protein inhibitors are not clearly understood. But there are some suggestions that the plant protein (flavanols) might cause conformational changes in structure. Chemical investigations of the plant have showcaffenic acid, P-hydroxy benzoic acid, gallic acid and eallagic acid.

The results suggest that methanol fruit extract of *Abutilon indicum* efficiently inhibits α-amylase enzyme in vitro.

CONCLUSION

The results suggest that methanolic fruit extract of *Abutilon indicum* efficiently inhibits α-amylase enzyme *in vitro* using Acarbose as standard.

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