



Table I

Constituents of *C. lancifolius*

Tests	
Alkaloids	Absent
Free anthraquinones	Present
Bound anthraquinones	Present
Saponins	Present
Flavonoids	Present
Tannins	Present

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Antidiabetic potential of *Conocarpus lancifolius*

## Antidiabetic potential of *Conocarpus lancifolius*

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### Abstract

The antidiabetic activity of *Conocarpus lancifolius* extract was investigated *in vitro*, as alpha glucosidase inhibition and *in vivo* as alloxan induced diabetic rabbits with other biochemical parameters (LDL, HDL, SGPT, SGOT, creatinine, urea and triglyceride). Alpha-glucosidase inhibition activity was performed by using acorbose as standred. Methanolic extract show alpha-glucosidase inhibition activity. The dose of 200 mg/kg body weight significantly ( $p < 0.05$ ) decreases the blood glucose level, plasma total cholesterol, triglycerides and LDL in treated rabbits as compared to diabetic rabbits. This dose significantly increased the level of HDL in treated group. The activity of SGOT and SGPT also significantly ( $p < 0.05$ ) decreased in treated diabetic rabbits. Phytochemical studies show the presence of glycosides, tannins, saponins and terpenoids. The antidiabetic potential is may be due to its saponin contents.

### Introduction

World Health Organization estimated that for health care needs, four-fifth of the total populations till confidence on the plant medicine (Farnsworth et al., 1985). Among the plants, *Carica papaya* (Sadeque and Begum, 2010), *Carissa spinarum* (Hegde and Joshi, 2010), *Cestrum nocturnum* (Qadir et al., 2014), *Chenopodium murale* (Saleem et al., 2014), *Cocculus hirsutus* (Thakare et al., 2009), *Convolvulus arvensis* (Ali et al., 2013), *Dodonaea viscosa* (Khan et al., 2013), *Khamira Gaozaban Ambri Jadwar Ood Saleeb Wala* (Akhtar et al., 2013), *Ofipomoea staphylyna* (Bag and Mumtaz, 2013), *Suaeda fruticosa* (Rehman et al., 2013), *Trianthema decandra* (Balamurugan and Muthusamy, 2008) and *Trichodesma sedgwickianum* (Saboo et al., 2013) showed hepato-protective effect.

*Conocarpus lancifolius* Engl., an ornamental tree, is native to coastal and riverine areas of East Africa (Baroon et al., 2012). The leaves are glossy in appearance with relatively fewer trichomes on both surfaces (Redha et al., 2011). The antidiabetic capacities of *C. lancifolius* consumed locally in Pakistan have not been presented. However,

up to now a little phytochemical or pharmacological work was done.

### Materials and Methods

#### Collection and extraction of plant material

The plant material was collected from surroundings of Lahore (Pakistan). The plant was identified as *C. lancifolius* Engl. by Prof. Altaf Ahmad Dasti, Taxonomist Institute of pure and applied Biology, Bahauddin Zakariya University, Multan.

For the purpose of effective extraction, whole plant of the *C. lancifolius* was dried under the shade for 17 days. The plant material was coarse grounded and then grounded to fine powder. This powder was subjected to extraction through the simple maceration method at room temperature occasionally shaking for 24 hours. The weighted amount (1 kg) of plant material was kept in large size well caped containers and added 1200 mL of methanol as solvent, to get the maximum extraction results; the flask having mixture was placed in



ultrasonic bath for about half hour daily. Filtration was done after the addition of solvent. Same process was carried out 3 times to get the maximum extracted volume. The methanol (18.3 g) extract was collected in separate sample bottles and designated with code as CLM.

#### *Phytochemical screening*

The presence of alkaloids, glycosides, tannins, flavonoids, anthraquinones and saponins were screened according to the method of (Trease and Evan, 1998).

**Alkaloids:** About 5 g of powdered drug was boiled in dilute hydrochloric acid. It was filtered and made alkaline by the addition of dilute ammonia solution. This alkalized solution was extracted with 5 mL of chloroform. The chloroform layer was then extracted with 10 mL dilute acetic acid. To the acetic acid extract, few drops of Dragendorff's reagent was added (Orange precipitate or turbidity indicate the presence of alkaloids).

**Braemer's test for tannins:** 5 g of drug was extracted with methanol. To methanolic extract, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicates the presence of tannins in the drug.

**Saponins:** 5 g of powdered drug was vigorously shaken with water and noted for production of froth (persistent froth for 20 min indicates the presence of saponins).

**Borntrager's test for free anthraquinones:** 5 g of powdered drug was extracted with hot water and filtered while hot. On cooling, it was extracted with carbon tetrachloride. The carbon tetrachloride was separated and washed with water and shaken with dilute ammonia solution (pink to cherry-red color in ammonia layer indicate the presence of free anthraquinones).

**Modified Borntrager's test for bound anthraquinones:** 5 g of powdered drug was extracted with ferric chloride solution and hydrochloric acid. It was heated on water bath for 10 min and filtered while hot. On cooling, it was extracted with carbon tetrachloride. The carbon tetrachloride was separated and washed with water and shaken with dilute ammonia solution color (intense pink to cherry-red coloration of ammonia layer indicate the presence of anthraquinones glycosides).

**Shinoda test for flavonoids:** 5 g of drug was extracted with methanol. To methanol extract, a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid were added. (Pink red or red coloration of the solution indicate the presence of flavonoids in the drug).

#### *In vitro $\alpha$ -glucosidase inhibition assay*

The  $\alpha$ -glucosidase inhibitory activity was assessed by the standard method (Dong et al., 2012) with slight modifications. Briefly, a volume of 60  $\mu$ L of sample solution and 50  $\mu$ L of 0.1 M phosphate buffer (pH 6.8) containing  $\alpha$ -glucosidase solution (0.2 U/mL) was

incubated in 96 well plates at 37 °C for 20 min. After pre-incubation, 50  $\mu$ L of 5 mM *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37°C for another 20 min. Then the reaction was stopped by adding 160  $\mu$ L of 0.2 M NaCO<sub>3</sub> into each well, and absorbance readings (A) were recorded at 405 nm by micro-plate reader and compared to a control which had 60  $\mu$ L of buffer solution in place of the extract. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. The concentrations of test compounds which inhibited the hydrolysis of substrates (butyrylthiocholine) by 50% (IC<sub>50</sub>) were determined by monitoring the effect of increasing concentrations of these compounds in the assays on the inhibition values (Rehman et al., 2013). The IC<sub>50</sub> values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA).

#### *In vivo antidiabetic study*

##### *Animal selection and treatment*

Thirty male rabbits each of weighing 1-1.5 kg were used and all rabbits were checked for evidence of any infections. All the rabbits were housed in the animal house of the Department of Pharmacy B.Z.U Multan. They were housed in the steel cages under standard laboratory condition (light period 8:00 am to 8:00 pm 21± 2°C, relative humidity 55%, green fodder and water was available as labatum). The protocol was approved by Ethical Committee of Department of Pharmacy Bahauddin Zakariya University Multan. Groups were control (non-diabetic), control group (received vehicle only, diabetic), alloxan induced diabetic rabbits treated with *C. lancifolius* 100 mg/kg, alloxan induced diabetic rabbits treated with *C. lancifolius* 200 mg/kg, and alloxan-induced diabetic rabbits treated with diamicron 80 mg/kg (Hader et al., 1994).

##### *Induction of diabetes*

Animals were divided into five experimental groups (control, diabetic, diabetic treated with 100 mg/kg body weight *C. lancifolius*, diabetic treated with 200 mg/kg body weight, *C. lancifolius* and diabetic treated with diamicron 80 mg/kg) Each group contained six rabbits. Before starting experiment the animals in latter four groups were injected. 150 mg/kg of alloxan (sigma chemical co.) dissolved in 10% isotonic saline to induce diabetes (Issa et al., 2000). The alloxan is selective beta-cells cytotoxic drug. It produced all signs and symptoms of diabetes. i.e. hyperglycemia, glycosuria, polydipsia, polyurea, etc. The control group was injected the same volume of isotonic solution as that of diabetic received. The blood glucose level of surviving rabbits was checked after three days of Alloxan injection and rabbits with blood glucose level more than 250 mg/dL on 12 hours fasting were considered as diabetics (Fridewald et al., 1979).

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Free anthraquinones	Present
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Saponins	Present
Flavonoids	Present
Tannins	Present

Table II

Inhibitory effects of <i>C. lancifolius</i> on $\alpha$ -glucosidase	
Concentration (mg/mL)	% Inhibition
0.5	99.1
0.25	98.7
0.125	99.8
0.0625	98.9
0.03125	98.1
0.0156	99.1
0.0078	99.0
0.0039	98.5

Table III

Effect of <i>C. lancifolius</i> extract on blood glucose level in alloxan induced diabetic rabbits					
No. of days	Glucose level				
	Control	Diabetic	Diabetic with Extract (100 mg/kg)	Diabetic with Extract (200 mg/kg)	Diamicran (80 mg/kg)
0	93.0 (5.1)	270.4 (3.3)	270.0 (5.4)	269.0 (4.7)	272.0 (5.9)
7	91.0 (4.3)	268.2 (4.1)	155.0 (5.1)	144.5 (5.5)	232.1 (3.2)
14	93.0 (5.1)	271.0 (6.3)	146.2 (3.7)	138.2 (5.6)	230.3 (4.3)
21	97.0 (4.3)	271.0 (4.4)	134.7 (4.2)	130.8 (4.4)	222.4 (5.4)
28	92.0 (4.4)	265.0 (5.6)	130.3 (5.1)	124.6 (3.4)	220.3 (4.4)

Number of rabbits in each group = 6; Values are expressed as mean and SD within the parenthesis

The last two groups were given the *C. lancifolius* extract, i.e. Group 1 received 100 mg/kg *C. lancifolius* extract daily for 30 days; Group 2 received 200 mg/kg *C. lancifolius* extract daily for 30 days.

#### Biochemical analysis

The plasma glucose levels were measured, using a glucose oxidase kit (Accu Check Advantage Rosche Chemicals Switzerland). The plasma total cholesterol, triglyceride, HDL, and ALT & AST levels were evaluated by enzymatic test kits (Randox). LDL level was calculated by using following formula:

$$\text{LDL} = \text{Total cholesterol} - \text{HDL} - (\text{triglyceride}/5)$$

Creatinin clearance was determined with the help of Chemistry analyzer Micro Lab 200 (Merck).

#### Statistical analysis

The experimental results were expressed as mean  $\pm$  standard deviation (SD) of three replicates. The data were subjected to one way analysis of variance (ANOVA) Turkey's Test was used to test for differences among Means for which ANOVA indicated a significant.

#### Results and Discussion

The phytochemical screening of arial part of *C. lancifolius* is shown in Table I. *In vitro*  $\alpha$ -glucosidase inhibitory activities of the plant extracts aforementioned are summarized in Table II. Methanol extract possessed excellent  $\alpha$ -glucosidase inhibitory activity even at low concentration. Methanol extract studied showed the most potent inhibitory activity 99.8% with  $\text{IC}_{50}$  found to be 14.9 mg/mL compared to the standard (acorbse) which exhibited 92.2% inhibition with  $\text{IC}_{50}$  found to be 14.9 mg/mL inhibition on  $\alpha$ -glucosidase. Table III shows the antidiabetic effect of *C. lancifolius* treated (100 and 200 mg/kg) as compared to treated

with 80 mg/kg diamicon, control and diabetic. Blood glucose concentration of diabetic rabbits  $269.2 \pm 5.8$  mg/dL (mean  $\pm$  SD) was increased significantly ( $p < 0.05$ ) as compared to the control group. In control group level of blood glucose was  $93.2 \pm 5.4$  mg/dL. Treatment with methanolic extract 200 mg/kg of *C. lancifolius* significantly decreased the blood glucose levels of diabetic treated group with mean value  $136.0 \pm 3.15$  mg/dL ( $p < 0.05$ ) and their glucose levels were within normal range (75-150 mg/dL). Diamicron has no significant effect ( $226.2 \pm 4.1$  mg/dL) as compared with *C. lancifolius* methanol extract ( $136 \pm 3.1$  mg/dL).

Table IV shows the antihyperlipidemic effect of *C. lancifolius* extract in alloxan induced diabetic rabbits. Hypercholestrolemia and hypertriglyceridemia have

Table IV

Effect of *C. lancifolius* extract on total cholesterol and triglycerides level in alloxan-induced diabetic rabbits

No of days	Total cholesterol (mg/dL)					Triglyceride (mg/dL)				
	Control	Diabetic	Diabetic with			Control	Diabetic	Diabetic with		
			Extract (100 mg/kg)	Extract (200 mg/kg)	Diamicran (80 mg/kg)			Extract (100 mg/kg)	Extract (200 mg/kg)	Diamicran (80 mg/kg)
0	41.2 (3.5)	64 (4.7)	64.3 (2.9)	66.1 (3.1)	66.9 (3.9)	57.2 (8.3)	139 (9.1)	140.4 (11.5)	135.1 (13.3)	137.9 (9.3)
7	45.3 (2.6)	66 (5.1)	54.5 (3.6)	52.1 (2.7)	58.3 (4.3)	58.1 (8.5)	135.4 (8.5)	104 (9.3)	99.3 (12.2)	104 (9.5)
14	43.1 (2.3)	65.5 (5.5)	53.4 (3.1)	52 (3.1)	55 (3.7)	57.5 (9.1)	136.1 (9.8)	96.5 (10.2)	99.5 (10.3)	100 (12.2)
21	42 (3.4)	64.2 (5.2)	52 (2.3)	51 (3.5)	55.1 (3.8)	57.4 (12)	136 (7.5)	74 (8.5)	80 (8.7)	93 (13.1)
28	43.7 (3.1)	65 (5.5)	52.1 (2.3)	51.3 (3.5)	54.2 (3.6)	59 (10.1)	139 (11)	74 (9.3)	74.2 (8.5)	81.1 (11.5)

Number of rabbits in each group = 6; Values are expressed as mean and SD within the parenthesis

Table V

Effect of *C. lancifolius* extract on HDL and LDL in alloxan-induced diabetic rabbits

No of days	HDL (mg/dL)					LDL (mg/dL)		
	Control	Diabetic	Diabetic with			Diabetic with		
			Extract (100 mg/kg)	Extract (200 mg/kg)	Diamicran (80 mg/kg)	Extract (100 mg/kg)	Extract (200 mg/kg)	Diamicran (80 mg/kg)
0	28.1 (3.5)	34.2 (2.0)	35.3 (2.5)	36.1 (3.1)	35.1 (2.5)	8.9 (2.5)	9.7 (3.1)	8.9 (3.5)
7	28 (3.1)	35.4 (2.7)	36 (3.1)	34.2 (2.7)	34.5 (3.0)	7.3 (3.1)	7 (3.1)	7.7 (3.2)
14	29.4 (3.5)	34 (2.3)	34.5 (3.1)	34 (2.5)	36.2 (3.2)	7.1 (2.7)	7.1 (2.4)	7.5 (2.6)
21	28.2 (2.5)	34.5 (3.1)	34.7 (3.0)	33.7 (2.2)	34.5 (3.1)	6.9 (3.5)	6.7 (3.1)	7.9 (3.1)
28	27.5 (3.1)	35 (2.8)	36.2 (3.7)	36.9 (2.9)	33.5 (2.7)	7.2 (3.2)	7.3 (3.1)	7.2 (4.1)

Number of rabbits in each group = 6; Values are expressed as mean and SD within the parenthesis

Table VI

Effect of *C. lancifolius* extract on serum SGPT and SGOT (IU/L) in alloxan induced diabetic rabbits

No. of days	Serum SGPT (IU/L)					Serum SGOT (IU/L)		
	Control	Diabetic	Diabetic with			Diabetic with		
			Extract (100 mg/kg)	Extract (200 mg/kg)	Diamicran (80 mg/kg)	Extract (100 mg/kg)	Extract (200 mg/kg)	Diamicran (80 mg/kg)
0	202 (51.1)	206 (15.5)	206 (11.5)	203.5 (12.1)	204 (13.5)	197 (13.5)	201 (5.1)	203 (16.4)
7	203 (12.5)	203.5 (12.1)	188.3 (25.3)	183.3 (11.5)	189.3 (15.3)	172 (5.3)	176 (36.3)	180.3 (9.5)
14	200 (13.5)	204 (9.8)	186 (13.5)	185.3 (15.1)	185.3 (5.1)	160 (3.3)	172 (12.5)	178.5 (3.5)
21	204 (15.1)	202 (13.3)	178 (11.1)	180 (13.3)	186 (13.5)	170 (12.5)	168 (13.2)	176.3 (12.1)
28	201 (13.5)	205 (25.1)	178.9 (15.1)	180.5 (23.3)	188.3 (3.5)	171 (3.2)	165 (16.1)	176 (13.5)

Number of rabbits in each group=6; Values are expressed as mean and SD within the parenthesis



been reported to occur in alloxan-induced diabetic rabbits. Total cholesterol, triglyceride and LDL mg/dL values increased significantly ( $p \leq 0.05$ ) in Diabetic rabbits. Whereas HDL mg/dL values decreased significantly ( $p \leq 0.05$ ) in diabetic rabbits. *C. lancifolius* extract treatment decreased the cholesterol, triglyceride and LDL values and increased HDL values significantly ( $p \leq 0.05$ ) in diabetic treated rabbits. So, *C. lancifolius* not only decreases the blood glucose level but also eliminate the side effects associated with diabetes in rabbits.

Table V shows the liver function effects of *C. lancifolius* extract in diabetic rabbits. Because diabetes mellitus is the commonest cause of liver failure and hepatomegaly (Chatila and West, 1996) which itself re-presents a huge and rapidly increasing problem. Liver and kidney glutathione levels have been reported to decrease in experimental diabetic rats (Loven et al., 1986).

SGOT and SGPT Values increased significantly ( $p \leq 0.05$ ) in diabetic rabbits. *C. lancifolius* extract treatment decreased ( $p \leq 0.05$ ) SGOT and SGPT values in diabetic rabbits (Table VI).

The effect of *C. lancifolius* extract in alloxan-induced diabetic rabbits on creatinine clearance. Because chronic diabetes mellitus causes renal failure (Sacks, 1997).

Creatinine clearance mg/dL values were not significantly ( $p \leq 0.05$ ) changed in diabetic rabbits.

Blood urea level of diabetic rabbits is not significantly increased and treatment with *C. lancifolius* has not significant effect on blood urea and creatinine concentration.

Diabetes mellitus is a chronic disorder of metabolism caused by an absolute or relative lack of insulin. It is characterized by hyperglycemia in postprandial and fasting state, and its severe form is accompanied by ketosis and protein wasting (Athanasakis et al., 2010).

Diabetes is one of the world's greatest health problems, affecting about 171 million people and most of these will be dominated by those suffering from type II diabetes (Moller, 2001).

In diabetes mellitus there is heterogeneity, so need for appropriate therapy increased. There is a use of traditional medicinal plants to overcome the complications of diabetes (Alamgeer et al., 2013). Our studies offer a natural key to unlock the complications in future.  $\alpha$ -glucosidase inhibitor inhibits the disaccharide digestion and impedes the postprandial glucose excursion to enable overall smooth glucose profile (Casirola and Ferraris, 2006). Thus, natural products of great structural diversity are still a good source for searching for such inhibitors, thereby motivating to explore biologically active compounds from the highly diverse plants.

The aim of this study was to asses to antihyperglycemic

effect of *C. lancifolius* in transiently hyperglycemic rabbits. The dose expressed in mg/kg administered of *C. lancifolius* is different because the traditional way for preparations and volume/kg of administration was used. Studies shows that diabetes causes the abnormalities in lipids, carbohydrate and protein metabolism.

Differences in social structure, psychic stress, obesity, hormonal imbalance and heredity are optimizing the growth of pandemic. At present, the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of biguanides, thiazolidinediones, sulphonylureas, D-phenylalanine derivatives, meglitinides and  $\alpha$ -glucosidase inhibitors in addition to insulin. However, due to unwanted side effects the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes (Oubre et al., 1997). Hence, plants have been suggested as a rich, as yet unexplored source of potentially useful antidiabetic drugs. However, only a few have been subjected to detailed scientific investigation due to a lack of mechanism-based available *in vitro* assays (Bell, 1991). These efforts may provide treatment for all and justify the role of novel traditional medicinal plants having anti-diabetic potentials. The methanolic extract also prevents body weight loss in diabetic rabbits.

The present study provides the evidence that the methanolic extract of *C. lancifolius* have prominent antidiabetic potential due to gluconeogenesis suppression in alloxan-induced diabetic rabbits may be due to saponins contents of *C. lancifolius*.

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