

## Original Research Article

# *In vitro* Studies on Anti-diabetic and Anti-ulcer Potentials of *Jatropha gossypifolia* (Euphorbiaceae)

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### Abstract

**Purpose:** To evaluate  $\alpha$ -glucosidase and  $\alpha$ -chymotrypsin enzyme inhibitory activity of *Jatropha gossypifolia* as a probable remedy for the management of diabetes and ulcer.

**Methods:** Different extracts and fractions of the root, leaf and stem bark of the plant were screened for their  $\alpha$ -glucosidase and  $\alpha$ -chymotrypsin inhibitory activity using standard *in vitro* inhibition assays. Acarbose and chymostatin were used as positive control, respectively.

**Results:** *n*-Butanol and ethyl acetate fraction showed maximum enzyme inhibition for  $\alpha$ -glucosidase with  $67.93 \pm 0.66$  and  $67.67 \pm 0.71$  % and half maximal concentration ( $IC_{50}$ ) of  $218.47 \pm 0.23$  and  $213.45 \pm 0.12$   $\mu$ g/ml, respectively. Dichloromethane and ethyl acetate leaf fractions exhibited maximum  $\alpha$ -chymotrypsin inhibition activity of  $85.08 \pm 0.38$  and  $83.87 \pm 0.70$  %, and  $IC_{50}$  of  $133.1 \pm 0.68$  and  $134.5 \pm 0.12$   $\mu$ g/ml, respectively, Acarbose exhibited enzyme inhibition activity of  $92.14 \pm 0.38$  % with  $IC_{50}$  of  $38.24 \pm 0.1$   $\mu$ g/ml, while chymostatin exhibited  $93.67 \pm 0.38$  % enzyme inhibition and  $IC_{50}$  of  $8.24 \pm 0.11$   $\mu$ g/ml.

**Conclusion:** The presence of bioactive secondary metabolites with enzyme-inhibiting activity lends some support for the traditional use of this plant in the management of diabetes and ulcer. However, further investigation of the plant including identification of its active components is required.

**Keywords:**  $\alpha$ -Chymotrypsin,  $\alpha$ -Glucosidase, Inhibition, *Jatropha gossypifolia*, Anti-diabetic, Anti-ulcer

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## INTRODUCTION

Plants have always played a major role in the treatment of human and animal diseases as a therapeutic source for traditional medicine [1]. Enzymes are usually made up of proteins that are helpful in catalyzing chemical reactions. Enzymes increase the rate of reactions until they acquire the equilibrium, although they do not alter thermodynamic properties of the reaction [2].

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 [3]. Elevated postprandial hyperglycemia (PPHG) is one of the risk factors [4]. PPHG is elevated by the action of  $\alpha$ -glucosidase and  $\alpha$ -amylase. Inhibition of these enzymes plays a major role in managing PPHG in diabetic patients. Inhibition of  $\alpha$ -glucosidase enzyme activity leads to a reduction in disaccharide hydrolysis which has beneficial

effects on glycemic index control in diabetic patients [5,6].

Several  $\alpha$ -glucosidase inhibitors have been isolated from medicinal plants for the development of new drugs with increased potency and lower adverse effects than the existing drugs [7].  $\alpha$ -Chymotrypsin is a serine protease enzyme, which acts to alleviate ulcers and digesting the polypeptide of damaged tissue [8]. Recently, serine protease inhibitors from potato and other plants have also been reported to have inhibitory effects on tumor cell growth [9,10]. Such inhibitors have potential therapeutic use. Herbal medicines become important factors in the treatment of gastro-duodenal diseases, as they prove themselves to be free of side effects and are less expensive than synthetic drugs. Clinical research has confirmed the efficiency of several plants for the treatment of gastroduodenal diseases [11,12]. The medicinal properties of many plants are mainly assigned to the flavonoids present, but they may be influenced also by other compounds such as coumarins, alkaloids, terpenoids, tannins, phenolic acids and antioxidant micronutrients such as Cu, Mn and Zn [13,14].

*Jatropha gossypifolia* belongs to the family, "Euphorbiaceae" and the order, "Geraniale". The common name of *J. gossypifolia* is bellyache bush, pignut or fignut. In Yourba land it is known as "Lapalapa" [15]. *Jatropha gossypifolia* is an erect, monoecious, deciduous, soft wooded and perennial shrub which grows with the average height of 2.5 m but exceeding 4 m in some areas. Its leaves are sticky which are covered with extra-floral nectaries, deeply 3-lobed and bronze when juvenile but green when mature. Flowers are deep red in color with yellow centres. Fruits are triocular, explosively dehiscent capsules having a single seed per locule. Capsules are green in color at maturity which turn pale green before dehiscence and are sparsely hairy. Seeds are spherical, carunculate and greyish-brown with copious starchy endosperm [16]. It is a bushy gregarious shrub which grows mostly throughout India. Its bark contains the alkaloids named jatrophine and a lignin named jatrodien found in the stems [17].

Ethnobotanical uses of *J. gossypifolia* have been reported for cancer, diarrhea, dysentery, skin diseases (leprosy), arthritis, ulcer, gum infections and wound healing [18,19]. This study was aimed at investigating the occurrence of constituents in *Jatropha gossypifolia* plant

extracts with enzyme inhibition activity against  $\alpha$ -glucosidase and  $\alpha$ -chymotrypsin enzymes.

## EXPERIMENTAL

### Chemicals and drugs

Enzymes including  $\alpha$ -Glucosidase (CAS 9000-81-1 Sigma-Aldrich GmbH USA), and  $\alpha$ -Chymotrypsin (CAS 9001-08-5 Sigma-Aldrich GmbH USA), substrates p-nitrophenyl glucopyranoside (CAS1866-15-5 Sigma-Aldrich UK), N-succinyl phenyl-alanine-P-nitroanilide, DTNB 5,5-dithio-bis-nitrobenzoic acid (CAS 69-78-3 Sigma-Aldrich Germany), Acarbose and Chymosatin Sp. (CAS 1933-04-4 Sigma-Aldrich France) were used for enzyme inhibition study. For preparation of buffer, di-potassium hydrogen phosphate ( $K_2HPO_4$ ), potassium di-hydrogen phosphate ( $KH_2PO_4$ ), potassium hydroxide used were of extra pure analytical grade. All the chemicals used in the extraction process were obtained from Merck Group Frankfurter Strabe 25065293 Darmstadt Germany

### Plant material

*J. gossypifolia* was collected from the surroundings of the district of Bhawalpur, Punjab, Pakistan. The plant was identified by Dr Muhammad Arshad (late) of Cholistan Institute of Desert Studies (CIDS) in The Islamia University of Bahawalpur (IUB) and a voucher specimen (no. 3210/CIDS/IUB) was deposited in the CIDS, IUB herbarium for future reference deposited for future reference.

### Extraction

Firstly whole plant specimens were collected and the leaves, stem/bark and root portion of the plants were separated. The stem/bark and the root portions were subjected to shade drying whereas the leaves were first ground and the frozen at  $-20\text{ }^\circ\text{C}$  for three days following which they were freeze dried using lyophilizer.

The dried plant material was ground to fine powder. The powdered root portion (1 kg) was initially extracted with dichloromethane (DCM) successively for 72 h and then with methyl alcohol (MA) again for 72 h at room temperature with periodic agitation. The stem/bark powder (1 kg) was also extracted with MA for 7 days with periodic agitation.

The leaves powder (1 kg) was extracted with MA for 7 days with periodic agitation. All of these extracts were filtered and then concentrated under reduced pressure using a rotary

evaporator at 37 °C and was finally obtained as semisolid these extracts were weighed and stored in a refrigerator in air tight vials.

The concentrated methyl alcohol (MA) crude extracts of the leaves portion was further subjected to fractionation with n-hexane (nH), MC, ethyl acetate (EA) and n-butanol (nB) [thrice with each solvent] respectively. The filtrates of the respective fractions thus obtained were concentrated under reduced pressure by rotary evaporator at 37 °C. The respective concentrates were weighed and stored in labeled airtight vial in a refrigerator and named as JGRD (*Jatropha gossypifolia* roots dichloromethane extract), JGRM (*Jatropha gossypifolia* root methanol extract), JGLCr (*Jatropha gossypifolia* leaves crude extract), JGLH (*Jatropha gossypifolia* leaves hexane extract), JGLD (*Jatropha gossypifolia* leaves dichloromethane extract), JGLE.

#### **α-Glucosidase assay**

The α-glucosidase inhibition activity was performed according to the standard method with slightly modification [20]. Total volume of the reaction mixture of 100 µl contained 70 µl 50 mM phosphate buffer saline, pH 6.8, 10 µl (0.5 mM) test compound, followed by the addition of 10 µl (0.057 units) enzyme. The contents were mixed, preincubated for 10 min at 37 °C and pre-read at 400 nm. The reaction was initiated by the addition of 10 µl of 0.5 mM substrate (p-nitrophenyl glucopyranoside). Acarbose was used as positive control. After 30 min of incubation at 37 °C, absorbance was measured at 400 nm using Synergy HT microplate reader. All experiments were carried out in duplicates. Inhibition was calculated as in Eq 1.

$$\text{Inhibition (\%)} = \{(\text{Control} - \text{Test})/\text{Control}\} 100 \dots\dots (1)$$

where control = total enzyme activity without inhibitor, and test = activity in the presence of test compound.

IC<sub>50</sub> values (concentration at which there is 50 % enzyme catalyzed reaction) were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

#### **α-Chymotrypsin assay**

Chymotrypsin inhibition activity was performed according to slightly modified method of Rehman et al. A total volume of 100 µl assay mixture contained 60 µl Tris-HCl buffer (50 mM pH 7.6), 10 µl test compound and 15 µl (0.9 units) purified chymotrypsin enzyme (Sigma, USA). The

contents were mixed and incubated for 20 min at 37 °C and pre-read at 410 nm. The reaction was initiated by the addition of 15 µl (1.3 mM) substrate (N-succinyl phenyl-alanine-P-nitroanilide). The change in absorbance was observed after 30 min at 410 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiment. All reactions were performed in triplicates. The positive and negative controls were included in the assay. Chymostatin (0.5 mM well) was used as a positive control. Percentage Inhibition was calculated as in Eq 1.

#### **Statistical analysis**

Mean ± SEM of the data were calculated and the extract concentration providing 50 % inhibition (IC<sub>50</sub>) was calculated from the plot of percent inhibition versus extract concentrations in solution, using Microsoft Excel program. *P* < 0.05 was considered statistically significant. Graph Pad Prism, version 5.01, was used for data analysis

## **RESULTS**

Different extracts and fractions of roots, stem/bark and leaves of *Jatropha gossypifolia* were tested against the α-glucosidase and α-chymotrypsin enzyme inhibitory activity (Table 1) and IC<sub>50</sub> values were also calculated (Table 2).

n-Butanol and ethyl acetate fractions showed maximum % enzyme inhibition for α-glucosidase with 67.93 ± 0.66 and 67.67 ± 0.71 and IC<sub>50</sub> of 218.47 ± 0.23 and 213.45 ± 0.12 % enzyme inhibition, respectively. While maximum α-chymotrypsin % inhibition activity was observed with DCM leaves 85.08 ± 0.38, ethyl acetate leaves 83.87 ± 0.71, DCM roots 81.45 ± 0.69, root methanol 75.00 ± 0.99, crude leaves methanol 71.77 ± 0.57 with IC<sub>50</sub> values of 133.1 ± 0.68, 34.5 ± 0.12, 129.5 ± 0.17, 118.3 ± 0.29, 121.7 ± 0.19, respectively.

## **DISCUSSION**

The use of medicinal plants is mainly based on local tradition and not on scientific knowledge [21]. The chemical constituents of most of these plants are unknown and may have dangerous effects on human health. On the other hand, some plants, which are not reported to be used in herbal medicine, might also possess potential activity. In traditional practices, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases and different neuropharmacological disorders.

**Table 1:**  $\alpha$ -Glucosidase and  $\alpha$ -chymotrypsin inhibitory activity (mean  $\pm$  SEM, n = 3) of different extracts of *Jatropha gossypifolia*

Sample code	Glucosidase inhibition (%)	Chymotrypsin inhibition (%)
JGRD	42.57 $\pm$ 0.69	81.45 $\pm$ 0.69
JGRM	52.26 $\pm$ 0.99	75.00 $\pm$ 0.99
JGLC <sub>r</sub>	51.99 $\pm$ 0.57	71.77 $\pm$ 0.57
JGLH	46.47 $\pm$ 0.23	42.47 $\pm$ 0.23
JGLD	55.62 $\pm$ 0.38	85.08 $\pm$ 0.38
JGLE	67.67 $\pm$ 0.71	83.87 $\pm$ 0.71
JGLB	67.93 $\pm$ 0.66	44.49 $\pm$ 0.66
JGAC <sub>r</sub>	43.52 $\pm$ 0.77	64.52 $\pm$ 0.77
Control	92.14 $\pm$ 0.38	93.67 $\pm$ 0.38

\*Control = Acarbose for glucosidase and chymostatin for chymotrypsin; \*JGRD = *Jatropha gossypifolia* roots dichloromethane extract; \*JGRM = *Jatropha gossypifolia* root methanol extract; \*JGLC<sub>r</sub> = *Jatropha gossypifolia* leaves crude extract; \*JGLH = *Jatropha gossypifolia* leaves n-hexane extract; \*JGLD = *Jatropha gossypifolia* leaves dichloromethane extract; \*JGLE = *Jatropha gossypifolia* leaves ethyl acetate extract; \*JGLB = *Jatropha gossypifolia* leaves n-butanol extract

**Table 2:** IC<sub>50</sub> values of  $\alpha$ -glucosidase and  $\alpha$ -Chymotrypsin (mean  $\pm$  SEM, n=3) of different extracts of *Jatropha gossypifolia*

Sample code	Glucosidase IC <sub>50</sub> ( $\mu$ g/ml)	Chymotrypsin IC <sub>50</sub> ( $\mu$ g/ml)
JGRD	NIL	129.5 $\pm$ 0.17
JGRM	<400	118.3 $\pm$ 0.29
JGLC <sub>r</sub>	<400	121.7 $\pm$ 0.19
JGLH	NIL	NIL
JGLD	392.41 $\pm$ 0.68	133.1 $\pm$ 0.68
JGLE	213.45 $\pm$ 0.12	134.5 $\pm$ 0.12
JGLB	218.47 $\pm$ 0.23	NIL
JGAC <sub>r</sub>	NIL	116.9 $\pm$ 0.16
Control	38.24 $\pm$ 0.11( $\mu$ M)	8.24 $\pm$ 0.11

\*Control = acarbose for glucosidase, and chymostatin for chymotrypsin

The history of drug discovery has shown that plants contain active compounds that have become new sources to investigate for the pharmaceutical industry [22].

The extracts from *Jatropha gossypifolia* plants showed significant  $\alpha$ -glucosidase activity.  $\alpha$ -glucosidases comprises a family of enzymes hydrolase, located in the brush-border surface membrane of small intestinal cells and it is the key enzyme by which the final step of digestion is catalyzed, so glucosidase inhibitors can stop the liberation of D-glucose from complex dietary carbohydrates and can delay the glucose absorption which in turn reduce plasma glucose level and decrease hyperglycemia [23]. Several synthetic glucosidase inhibitors such as acarbose, miglitol, voglibose are used as treatment for diabetes but their prices are high and side effects occur [24]. Due to these issues, there has been a search for some other safe and effective glucosidase inhibitors of natural origin in order to develop a physiological functional food and lead compound for diabetic treatment [25,26].

The chymotrypsin enzyme inhibitory activity was performed by using chymostatin as a standard. The relative percent enzyme inhibition along with IC<sub>50</sub> of all the extracts and standard were analyzed. Epidemiological studies demonstrating a decrease in occurrence of breast, colon and prostatic cancers in vegetarian populations has suggested the role of plant proteinase inhibitors in preventing these cancers. A soybean-derived Bowman-Birk protease inhibitor (BBI) either inhibits or prevents development of experimentally induced colon, oral, lung, liver and esophageal cancers. This anti carcinogenic effect of the Bowman-Birk protease inhibitor is thought to stem from its ability to inhibit chymotrypsin enzyme activity [27].

## CONCLUSION

In the light of these findings, we can conclude that the plant extracts screened herein showed inhibitory activity against both of the enzymes and they could be considered for further studies in the treatment of diabetes and ulcer as natural remedy. Further works related to the isolation of

the active constituents through bioassay-directed fractionation are in progress in our laboratory.

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