

## Alpha Amylase Inhibitory Activity of Different Extract of *Terminalia arjuna* Bark

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### Abstract

In India, medicinal plants are used by all sections of people either directly as folk remedies or in different indigenous system of medicine. The present study was based on the use of ethnomedicinal plant bark to evaluate the alpha amylase inhibitory activity. Bark of *Terminalia arjuna* was collected and air dried and soxhlet extracted by using standard methods for flavonoid, alkaloid, steroid and different solvents. These extracts obtained were then tested for the alpha amylase inhibitory activity using the chromogenic DNSA method and starch iodine min triplicates. Data were expressed as mean  $\pm$  SEM (standard error of the mean). Data were analyzed by one-way analysis of variance and *P* values were considered significant at *P*<0.05. Among the tested extracts, methanol, free flavonoids and bound flavonoids extract exhibited significant alpha amylase inhibitory activity. Results of the present study revealed that methanol extracts of *T. arjuna* bark showed great antidiabetic potential and may be exploited for future antidiabetic drugs.

**Key words:** Bark, alpha Amylase, Antidiabetic, methanolic

### Introduction

Diabetes is one of the common diseases found in both developed as well as developing countries and one of the estimation it has been found that 1/3 rd of diabetic person use some form of complementary and alternative medicine (1). Herbal products are very important ingredients which can be used in the development of drug (2).

Now very less modern medicines are available to act as a remedy for various diseases, in such situation many bioactive compounds of medicinal plants are used as anti diabetic, chemotherapeutic, anti inflammatory and anti arthritic agents[3]. Plants produce secondary metabolites rich in flavonoids, phenolic compounds, alkaloids and steroids which help in prevention of various diseases (4).  $\alpha$ -Amylase is a enzyme that catalyses the hydrolysis of internal linkage of  $\alpha$ -1, 4-glycosidic bond in starch to yield products like glucose and maltose (5). Thus, inhibition of the alpha amylase enzyme in the digestive tract of humans is being considered to be effective in controlling diabetes by decreasing the absorption of glucose from starch (6).

*Terminalia arjuna*, traditionally has been used as a medicinal plant for the prevention heart diseases. It has been described as three humours viz., vata, pitta and kapha in Ayurveda (7). The tree is commonly known as Arjuna and belongs to family *Combretaceae*. Bark of *T. arjuna* has been used in the traditional system and repoted to contain different groups of chemical compounds like flavanoids, phenolics, and phyto sterols [8]. Arjunolic acid is an important compound (2, 3, 23-trihydroxyolean-12-en-28-oic acid) used as an antioxidant, antiallergic, and antiasthmatic (9).

The diabetes leads to increased risk of other complications such as cardio vascular disease, peripheral vascular disease complications. There are many agents available to treat diabetes, but

the main disadvantages of these drugs are that they required to be given to patients for their whole life (10). As herbal plant products are used as medicine since ancient time by people of different countries for the treatment in diabetes mellitus. Hence, there is increasing emphasis on the use of plant products for the effective management of the disease. So far there are no reports showing the comparative alpha amylase inhibitory activity of polar, non polar, free flavonoids, bound flavonoids, alkaloid and steroid extract of *T. arjuna* bark. Hence in the present study an attempt was made to compare the alpha amylase inhibitory activity of different extracts of the bark of *T. arjuna*.

### Materials and Methods

**Collection of Plant Material:** Bark of *Terminalia arjuna*, was collected from the eastern region of Rajasthan i.e. Jaipur. Plant was identified by the senior taxonomist of the Department of Botany, University of Rajasthan and Voucher specimen no: RUBL211458 was submitted to the Herbarium, Department of botany, University of Rajasthan.

**Preliminary phytochemical screening:** The phytochemical analysis to test the presence of alkaloids, flavonoids, steroids in the powdered sample was carried out using standard methods (11-12).

### Preparation of Extracts

**Flavonoid extraction:** Bark of *Terminalia arjuna* was collected; shade dried, finely powdered and Subramanian & Nagarjan method was used for extraction of flavonoids (13). 100 grams of sample with 80 % of hot methanol was soxhlet extracted on a water bath for 24 h and filtered. The filtrate was re-extracted using separating funnel successively with petroleum ether in the first fraction and ethyl ether in the second fraction and ethyl acetate in the third fraction. As petroleum ether dissolves fatty substances in it, the fraction of it was discarded, where as fractions of ethyl ether and ethyl acetate were further analyzed for free and bound flavonoids respectively. The ethyl acetate sample was refluxed with 7% H<sub>2</sub>SO<sub>4</sub> for two hours so that hydrolysis removed bounded sugars and again in separating funnel, the filtrate

was refluxed with ethyl acetate. To neutralize the obtained filtrate it was washed with distilled water. Thus, the ethyl ether fraction contain free flavonoids and ethyl acetate fractions contains bound flavonoids were dried in vacuum and weighed. The obtained extracts were stored at 4°C.

**Alkaloids Extraction:** Alkaloids were extracted from bark of *T. arjuna* by well established method [14]. Hundred grams of sample was extracted in 20ml methanol after shaking of 15 min. Filtrate obtained was kept for drying and then the residual mass was treated with 1% H<sub>2</sub>SO<sub>4</sub> (5ml.) for 2 times. After this, extraction was performed in 10ml. Chloroform using separating funnel. The chloroform layer which was organic by nature was rejected and the other aqueous layer was basified utilizing 30% NH<sub>4</sub>OH of PH=9- 10. Again basified layer was extracted in 10ml chloroform and organic layer of chloroform which was in a lower position was collected in a flask and the step was repeated with fresh chloroform. Extracts was thus obtained was dried in vacuum for further use.

**Steroid Extraction:** Steroids were extracted from bark of *T. arjuna* by well established method (15). The Fine powdered hundred gram of sample of plant bark was extracted in petroleum ether for two to four hours. Then it was filtered and residual mass was treated with 15% ethanolic HCl for four hours. Further it was extracted in ethyl acetate and washed with distilled water to neutralize the extract. To remove the moisture content of the neutral extract it was passed over Sodium sulphate and was dried in vacuum.

**Extraction in different polar and non polar solvents:** Powdered bark of *T. arjuna* (20 g) was taken in three flasks and water, methanol and petroleum ether were used as solvent. The dried material and solvents were taken in a 1:10 ratio. Those were kept at soxhlet unit for complete one day. Obtained extracts were thus filtered and the filtrate was subjected to dry in vacuum to obtain extract. The residual extract that obtained was stored in a refrigerator at 4°C in sterile glass bottles.

### ***In vitro* alpha amylase inhibitory assay**

**Starch iodine assay:** Screening of alpha amylase inhibitors were performed using Xiao et al method in test tubes with slight modifications based on the starch iodine test [16]. The assay mixture was about 120  $\mu$ l of 0.02M sodium phosphate buffer (pH 6.9), 1.5 ml of salivary alpha amylase and bark extracts at a concentration from 0.5-1.5  $\text{mgml}^{-1}$  (w/v) were incubated at 37°C for 10 min. After that, soluble 1% starch was added at each reaction mixture and incubated at 37°C for 15 min. Then 60  $\mu$ l of 1 M HCl was added to the reaction mixture to stop the enzymatic reaction and immediately 300  $\mu$ l of iodine reagent was added. If any change in colour was noted and at 620nm the absorbance was read. The plant extracts was not added to the control reaction showing 100% enzyme activity. Extract control was also included to check if any absorbance produced by bark extract. Thus different colour obtained indicates the presence of starch (dark-blue), absence of starch (yellow) and partially degraded starch (brownish) in the reaction mixture. If inhibitor was present in the extract of plant bark it inhibits the degradation of starch added to the enzyme assay mixture and form a dark-blue colour complex whereas no colour showed the absence of inhibitor.

**3, 5-dinitrosalicylic acid assay:** The inhibition assay of bark extract of *T. arjuna* was performed using the chromogenic DNSA method (17). The assay mixture consists of 500  $\mu$ l of 0.02 M sodium phosphate buffer (pH 6.9), 1ml of salivary alpha amylase and 400  $\mu$ l extracts at concentrations from 0.5-1.5  $\text{mgml}^{-1}$  were incubated at 37°C for 10 min. After pre-incubation, 580  $\mu$ l of 1% starch solution was added at each tube and incubated at 37°C for 15 min. Using 1.0 ml DNSA reagent the reaction was terminated and tubes were placed in boiling water bath for 5 min. After this, cooled to room temperature and at 540nm the absorbances were measured. The control did not contain any bark extract represented 100% enzyme activity. Extract control was also a part to check if any absorbance produced by bark extract except for the enzyme.

Formula for calculation of the percent inhibition of alpha amylase:

% Relative enzyme activity = (enzyme activity of test/enzyme activity of control)  $\times$  100.

Percent Inhibition of the  $\alpha$ -amylase activity = (100% - Relative enzyme activity).

**Statistical analysis:** Experiments were performed in triplicates for three different sets and  $\pm$  standard error of the mean was used for calculation. Graph pad prism5 software was used for ANOVA, linear regression and statistical difference analysis. The  $\text{IC}_{50}$  values were calculated.

### **Results**

Out of the solvent extracts methanol extract exhibited maximum inhibition of alpha amylase activity as compared to free flavonoid, bound flavonoid, alkaloid, steroid and other solvent extract extracts. The maximum activity of methanol extract may be due to presence of potential inhibitory compound in extract. Water, petroleum ether, alkaloid and steroid extracts showed negligible inhibitory activity with insignificant  $\text{IC}_{50}$  value.

**Extract with maximum inhibitory effect on the alpha amylase activity:** Methanolic extracts (at a concentration 0.5-1.5 mg/ml) showed maximum  $\alpha$ -amylase inhibitory activity from 46.93 $\pm$ 0.03% to 48.26 $\pm$ 0.03% with an  $\text{IC}_{50}$  value of 5.1642 mg/ml. At the same concentration free flavonoid extracts also showed good inhibitory activity i.e. 40.97 $\pm$ 0.08% to 43.23 $\pm$ 0.06 % with an  $\text{IC}_{50}$  value of 38.2825 mg/ml (Table-1).

**Extracts with insignificant inhibitory effects on the  $\alpha$ -amylase activity:** Water, petroleum ether, alkaloid and steroid extracts showed minimum inhibitory activity from 25.50 $\pm$ 0.12% to 26.13 $\pm$ 0.03%, 10.13 $\pm$ 0.03% to 12.13 $\pm$ 0.09%, 1.23 $\pm$ 0.03 % to 2.63 $\pm$ 0.13% and 1.70 $\pm$ 0.01% to 2.37 $\pm$ 0.06% respectively with an insignificant  $\text{IC}_{50}$  value (Table-2).

### **Discussion**

Plants produce the secondary metabolites like flavonoids, tannins, alkaloid, and other serve

**Table 1.** Extract with maximum inhibitory effect

Sr. No.	Name of extract	Concentration Mg/ml	% inhibition	Regression Equation	IC <sub>50</sub> value (mg/ml)
1	Methanol	0.5	46.93±0.03	Y=4.928+0.101X	5.1642
		1.0	47.63±0.03		
		1.5	48.26±0.03		
2	Free flavonoids	0.5	40.97±0.08	Y=4.799+0.127X	38.2825
		1.0	42.30±0.01		
		1.5	43.23±0.06		
3	Bound flavonoids	0.5	30.67±0.12	Y=4.53+0.168X	628.0584
		1.0	32.47±0.13		
		1.5	33.16±0.13		

Values are given as mean±SEM (n=3), ANOVA was used which show significant difference with respect to control (P<0.05).

**Table 2.** Extract with insignificant inhibitory effect

Sr No	Name of extract	Concentration Mg/ml	% inhibition	Regression	IC <sub>50</sub> value(mg/ml)
1	Water	0.5	25.50±0.12	Y=4.342+0.056X	56231325190.391
		1.0	25.93±0.05		
		1.5	26.13±0.03		
2	Petroleum ether	0.5	10.13±0.03	Y=3.779+0.205X	90369.737
		1.0	11.03±0.06		
		1.5	12.13±0.09		
3	Alkaloids	0.5	1.23±0.03	Y=2.883+0.624X	2471.7241
		1.0	2.17±0.03		
		1.5	2.63±0.13		
4	Steroids	0.5	1.70±0.01	Y=2.883+0.624X	2471.7241
		1.0	2.06±0.08		
		1.5	2.37±0.06		

Values are given as mean±SEM (n=3), ANOVA was used which show significant difference with respect to control (P<0.05).

as a defense agent against pathogens and various other disorders. Diabetes is part of the chronic diseases that occur either when the level of insulin (a hormone) decreases or when the body is unable to use the insulin produced. In a recent WHO report, it is reported that more than 400 million

people live with diabetes (18). Inhibition of alpha amylase by an agent is being considered to be effective in controlling diabetes. In present investigation bark extracts of *T. arjuna* were tested for their antidibetic activity. In one of the previous study the methanolic extracts of the leaves of

*Terminalia arjuna*, *T. bellerica*, and *T. chebula* were evaluated for hypoglycemic screening and an oral glucose tolerance test (OGTT) in normal rats and showed that *T. chebula* extract had a better hypoglycemic effect in normal and glucose induced hyperglycemic rats (19). In present study it was observed that methanolic extract of bark of showed significant % inhibition of alpha amylase at all tested concentrations with good IC<sub>50</sub> value. All previous study was based on a test of one of the crude extract of *T arjuna* for their hypoglycemic effect and there is not any comparative study of all extract for their effect on inhibition of alpha amylase activity. Hence there is meager literature available to compare the results obtained in the present study. In this study, we compared IC<sub>50</sub> value of  $\alpha$ -amylase inhibitory activity of the crude extract of steroids, free flavonoids, bound flavonoids and alkaloids isolated from the bark of *T. arjuna* with polar and non polar solvent. Further studies are required to determine the mode of action of these plant extracts as alpha amylase enzyme inhibitors and to qualify the action of different constituents in the extract. The results of this study directs further researches to evaluate the therapeutic potentialities of methanolic, free flavonoids and bound flavonoids of bark of *T. arjuna* in the management of diabetes either alone or in a combinatorial therapy.

### Conclusion

The present study showed that the methanolic extract of bark of *T. arjuna* showed maximum inhibition of alpha amylase activity with less IC<sub>50</sub> value. Hence the extract may be useful as better therapeutic agent especially for the treatment of diabetes mellitus.

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