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Research Article

Correlation of Tannins Isolated from Several Medicinal Plants against the Inhibition of Alpha Amylase Activity

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ABSTRACT
Tannins are class of phenolic compounds which exhibited significant therapeutic effect against various diseases. Here the study was carried out to screen the alpha amylase inhibitory activity of tannins enchanced fraction isolated from the fruit rind of *Terminalia chebula, Punica granatum, Embilica officianalis, Aegel marmelos* and seeds of *Mucuna pruiens*. Total tannins content were analysed by UV spectrophotometer. Different concentration was prepared to evaluate the alpha amylase inhibitor activity in each sample and the percentage of amylase inhibition varies with respect to the tannins content. From this study, the tannins fractionated from the *Terminalia chebula* fruit rind showed highest inhibition of 83.70% was obtained at the concentration of 1000µg/ml and IC**50** value of 470.0±1.41 was obtained with respect to the various concentrations (1000,500,250,125,62.5µg/ml). This result indicated that tannins from the *Terminalia chebula* fruit rind act as an effective inhibitor of alpha amylase enzyme and suggested that it may be a significant antidiabetic agent for the management of diabetes.

Key Words: Medicinal plants, Tannins, α-amylase inhibition, diabetes

INTRODUCTION

Diabetes mellitus is a chronic disorder due to lack of insulin secretion and organs are not responding to insulin which is secreted in the pancreas[1]. The international diabetic federation estimates that currently 371 million people living with diabetes and another 200 million peoples are at high risk of developing disease. Half of a billion peoples are expected to be living with diabetes by 2030. In world wide peoples are suffering with type II diabetes [2]. Some synthetic drugs were available for the management of diabetes which is showing side effects also[3]. So the medicinal plants are alternative for the management diabetes due to their negotiable side effects. In India, *Momordica charantia, Gymnema sylvestre, Syzygium cumini* and *Azadirachta indica* were commonly recommended for the management of diabetes [4].

Inhibition of carbohydrate hydrolyzyable enzyme activity such as alpha amylase is one of the approach to control the glucose levels in the blood [5]. Therapeutic drugs, such as acarbose, miglitol and voglibose are currently used as alpha amylase inhibitors. But the side effects of these drugs are abdominal distention, bloating, meteorism, flatulene and diarrhoea[6]. So screening of alpha amylase inhibitors in medicinal plants are necessary with subject to search the more effective and safer antidiabetic agent. Plants are an important source of chemical constituents with potential for inhibition of alpha amylase enzyme. Therefore in this study, fruit rind of *Terminalia chebula* (Combretaceae), *Punica granatum* (Punicaceae), *Embilica officianalis* (Euphorbiaceae), *Aegel marmelos* (Rutaceae) and seeds of *Mucuna pruriens* (Fabaceae) were selected on the basis of their biological efficacy against various diseases. Fruit rind of *Terminalia chebula, Punica granatum, Embilica officianalis* and *Aegel marmelos* were exhibited the pharmacological activity such as antioxidant, antibacterial, anti-inflammatory, digestive, diabetes, antiulcer due to their phytochemical constituents[7, 8, 9]. Seeds of *Mucuna pruriens* exhibited the pharmacological activity such as antidiabetic, male infertility, antioxidant, anti microbial activity and parkinson's disease[10].

Table 1: Tannins content in the plant extracts (Study plants).

S.no.	Plant extracts	Tannins content (%)
1.	***Terminalia chebula***	85.47
2.	***Punica granatum***	79.84
3.	***Embilica officianalis***	55.50
4.	***Aegel marmelos***	28.78
5.	***Mucuna pruriens***	44.55

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Table 2: Alpha amylase inhibitor activity of Tannins fraction of study plants

Sl. No	Test sample	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
		1000	83.70±0.34	
		500	51.65±2.56	
1	*Terminalia chebula*	250	36.98±1.82	470.0±1.41
		125	35.69±1.03	
		62.5	31.46±1.32	
		1000	63.35±0.49	
		500	51.53±0.58	
2	*Punica granatum*	250	44.78±1.47	480.0±0.98
		125	35.96±1.48	
		62.5	32.37±0.87	
		1000	62.40±0.67	
		500	51.36±2.09	
3	*Embilica officianalis*	250	44.47±2.62	410.0±1.65
		125	37.77±1.34	
		62.5	33.73±1.52	
		1000	51.17±0.58	
		500	45.68±2.26	
4	*Aegel marmelos*	250	38.22±1.64	910.0±1.47
		125	32.95±1.32	
		62.5	23.07±1.55	
		1000	56.65±0.95	
		500	49.91±0.65	
5	*Mucuna pruriens*	250	41.45±1.22	560.0±1.20
		125	36.71±1.64	
		62.5	26.52±1.52	

The present study was carried out to screen the alpha amylase inhibitory activity of tannins fraction isolated from the various parts of the represented study plants.

MATERIALS AND METHODS

Plant material***:*** The dried fruit rind of *Terminalia chebula, Punica granatum, Embilica officianalis, Aegel marmelos and* seeds of *Mucuna pruriens* were procured from the local market, thanjavur district, tamilnadu. The raw materials

were authenticated by botanist, A.V.V.M.Sri.Pushpam College, poondi, thanjavur district. The plant materials were subjected to coarse powder using a mechanical grinder.

Chemicals: Alpha amylase enzyme was obtained from Hi media lab, Mumbai. All other chemicals used in the experiments were procured from the Central Drug House, Delhi.

Apparatus: UV spectrophotometer-2202, syntronics (India) used for the estimation of tannins.

Extraction method: Each 100g of study plants were extracted with 600ml of demineral water and adjusted the pH to 9 using 10% of potassium hydroxide. Thereafter, it was allowed to stand for one hour at room temperature. Further it was filtered through 100 mesh muslin cloth and adjusted the pH to 7 using 5% of hydrochloric acid, during that period precipitate was obtained. To the same 4 liters of methanol were added with manual stirring and allowed it to stand for 30minutes. Then methanol soluble fraction was filtered through ordinary filter paper and concentrated using water bath at 90^{o}c under fume hood. Thus the obtained extracts were used for the screening of alpha amylase inhibitor activity.

Quantitative estimation of tannins in the study samples by UV spectrophotometer

Sample preparation: Accurately weighed 0.1g of each extract separately into 250ml flat bottomed flask. To that added 100ml of purified water and refluxed at 95^{0}c using water bath for one hour. Then transferred the solution into 500ml of volumetric flak and made the volume up to the mark with purified water. Thereafter, it was filtered through whatman paper and used for analysis.

Standard preparation: Accurately weighed 0.1g of tannic acid in 100ml volumetric flask and made the volume up to the mark with purified water. From that 1 ml of solution pipette out into 100ml volumetric flask and made the volume up to the mark with purified water.

Reagent preparation***:*** Prepared 1% solution of potassium ferric cyanide and ferric chloride in100ml volumetric flask using purified water.

Preparation of reagent blank: Each 1ml of potassium ferric cyanide and ferric chloride were pipette out into 10ml volumetric flask and made the volume up to the mark using purified water.

Preparation of test solution and standard solution: Accurately about 0.2ml of test and 1 ml of standard solution was pipette out separately into 10ml volumetric flask. To the same each 1ml of potassium ferric cyanide and ferric chloride were added and mixed well. Then made the volume up to the mark with purified water. Exactly after 30minutes absorbance was measured[11]at

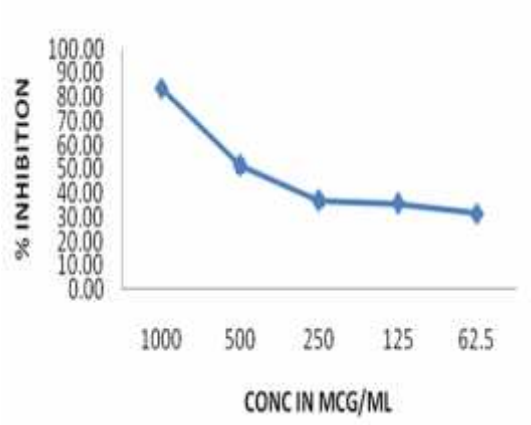

Fig. 1. Alpha amylase inhibitory activity of Tannins fraction of Terminalia chebula fruit rind at the various concentrations

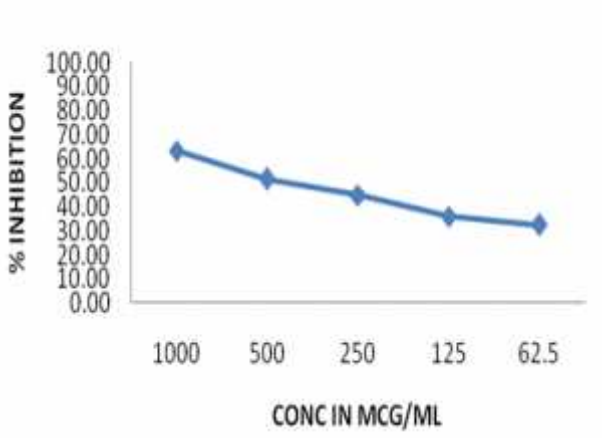

Fig. 2. Alpha amylase inhibitory activity of Tannins fraction of Punica granatum fruit rind at the various concentrations.

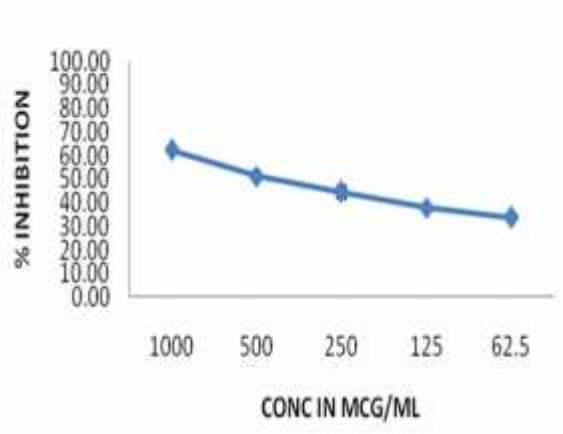

Fig. 3. Alpha amylase inhibitory activity of Tannins fraction of Embilica officianalis fruit rind at the various concentrations.

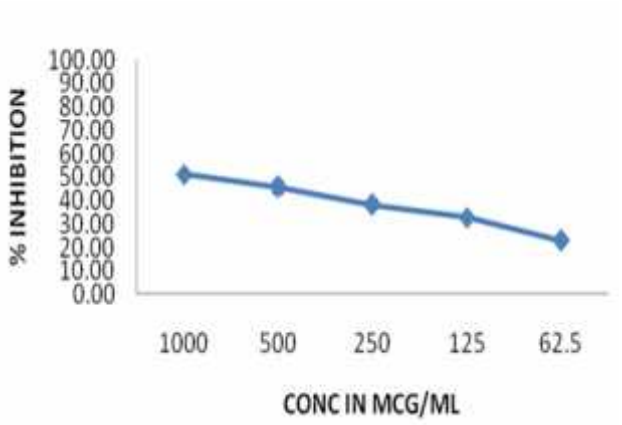

Fig. 4. Alpha amylase inhibitor y activity of Tannins fraction of Agel marmelos fruit rind at the various concentrations.

720nm. Tannins content was calculated from the sample and standard absorbance.

Alpha amylase inhibition assay

Preparation of reagents

1. *Dinitro salicylic acid solution (DNS)*: Accurately weighed about 1g of DNS and 12 g of sodium hydroxide were dissolved in 100ml of distilled water.
2. Starch solution: Accurately weighed about 1g of starch dissolved in 100ml of distilled water.
3. Solution of alpha amylase enzyme: About 2mg of enzyme was dissolved in 1ml of distilled water.

Preparation of test solution: Each 5 mg of study samples were dissolved in 5ml of DMSO separately to obtain the solution of mg/ml concentrations. Each of these solutions were serially diluted separately to obtain lower concentrations.

Experimental protocol: Alpha amylase inhibitory activity was performed by as per the method described by Bernfeld[12]. In brief, 100µl of the each test extract was allowed to react with 200µl of alpha amylase enzyme and 100µl of 2Mm of phosphate buffer (pH-6.9). After 20minutes incubation, 100µl of 1% starch solution was added. The same was performed for the controls where 100µl of the test extract was replaced by buffer. After 5minutes incubation, 500µl of DNS reagent was added to both the control and test. They were kept in boiling water bath for 5 minutes. The absorbance was recorded at 540nm using ultraviolet spectroscopy and the percentage of incubation of alpha amylase enzyme was calculated by the following formula:

$$\text{Inhibition } (\%) = 100\left(\frac{\text{control} - \text{test}}{\text{control}}\right)$$

Suitable reagent blank and inhibitor controls were simultaneously carried out.

RESULTS AND DISCUSSION

The tannins content in the plant extracts were represented in table 1. In this study the inhibitory activity of tannins fraction from the represented medicinal plants against alpha amylase, a key enzyme related to type II diabetes were evaluated. Among the five study plants tested against alpha amylase inhibition (Table 2), tannins enhanced fraction of *Terminalia chebula* fruit rind showed strong inhibiton of 83.7% with an IC**50** value of 470.0±1.41; the significant inhibition was exhibited in the tannins fractions of fruit rind of *Punica granatum*,

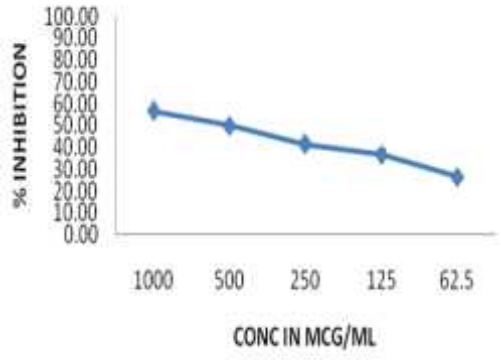

Fig 5. Alpha amylase inhibitor activity of Tannins fraction of Mucuna pruriens seeds at the various concentrations.

Embilica officianlis were 63.35±0.49, 62.40±0.67 with an IC**50** value of 480.0±0.98, 410.0±1.65 respectively; also the tannins fraction from the fruit rind of *Aegel marmelos* and *Mucuna pruriens* seeds showed the appreciable inhibition of 51.17±0.58, 56.65±0.95 with an IC**50** value of 910±1.47, 560.0±1.20 respectively. Figure 1 to 5 showed the different concentration depended alpha amylase inhibitory activity of tannins isolated from the represented medicinal plants.

Polyphenols rich in medicinal herbs significantly reduces the alpha amylase activity which has been already reported[13]. However, the present study also revealed that the tannins (Polyphenols) are effective against to inhibit the alpha amylase activity. It has been reported that, the human alpha amylase also correlated with the free OH (Hydroxy) groups in the tannins[14]. According to that, the possible mechanism of action probably the free OH groups of tannins correlated with alpha amylase enzyme which results the formation of hydrogen bonding and its assist to inhibit the enzyme activity.

CONCLUSION

From the compilation of study results, it was concluded that the tannins enhanced fraction of fruit rind of *Terminalia chebula* significantly inhibited the alpha amylase activity and it may be an effective, safer antidiabetic agent for the management of diabetes. Also the major innovation from this research work was the percentage of inhibition of alpha amylase increased with respect to the percentage of tannins content.

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Hajra Subhadip, Mehta Archana, Pandey Pinkee, Jhon Jinu, Mehta Pradeep, Vyas Suresh Prasad. Free Radical Scavenging and A-Amylase Inhibitory Activity of *Swietenia Mahagoni* Seeds Oil. International Journal of Pharmacognosy and Phytochemical Research 2013; 5(1): 51-56.
2. Peyman, Behvar Asghari, Mohammad Ali Esmaeili, Hossein Dehghan, Iraj Ghazi. α-Glucosidase and α-amylase inhibitory effect and antioxidant activity of ten plants extracts traditionally used in Iran for diabetes. Journal of Medicinal Plants Research 2013; 7(6): 257-266.
3. Iniyan GT, Dineshkumar B, Nandhakumar M, Senthilkumar M, Mitra A. Invitro study on α-amylase inhibitory activity of Indian medicinal plants, *Phyllanthus amarus*. Indian J Pharmacol. 2010; 42: 280-282.
4. Mitra A. Effects of Composite of Tulsi Leaves, Amla, Bitter Gourd, Gurmur Leaves, Jamun Fruit and seed in type II Diabetic patients. J Clin Diagn Res. 2007; 6: 511-20.
5. Prashanth D, Padmaja R, Samiulla DS. Effects of certain plant extracts on alpha amylase activity. Fitoterapia 2001; 72: 179-81.
6. Anwesh Bag, Subir Kumar Bhattacharya, Rabi Ranjan Chattopadhyay. The development of *Terminala chebula* Retz. (Combretacea) in clinical research. Asian Pacific Journal of tropical biomedicine 2013; 3: 244-252.
7. Saleem Banihani, Samer Swedan, Ziyad Alguraan. Pomegranate and type 2 diabetes. Nutrition Research 2013; 33: 341-348.
8. Prasan R Bhandari, Mohammad, Ameeruddin Kamdod. Embilica officianalis (Amla): A review of potential therapeutic applications. Int J Green Pharma 2012; 6: 257-69.
9. Gupta D, John, Kumar Pankaj, Kaushik, Yadav R. Review of *AEGEL MARMELOS* CORR. FRUITS. International Journal of Pharmaceutical Sciences and Research 2012; 2: 2031-2036.
10. Sharma Brijesh Kumar, Ahmed Shamin, Sing Rahul, Verma Rajesh kumar and Kumar Nilesh. A review on Mucuna pruriens: Its phytoconstituents and therapeutic uses. Novel Science International Journal of Pharmaceutical Science 2012; 1: 308-312.
11. Diaz AM, Ageger A. Analytical methods of tannins and their applications to *Myrtus Communis* seed extracts. Fitoterpia 1986; 58: 395-407.
12. Bernfeld P. Amylase, *α* and *β*. *Methods in Enzymology*. Academic Press, New York, 1955, 149–158.
13. Paloma Michelle de Sales, Paula Monterio de Souza, Luiz Alberto Simeoni, Perola de Oliveira Magalhaes, Damaris Silveira. *α*- Amylase Inhibitors: A Review of Raw Material and Isolated compounds from the Plant Source. J Pharm Pharmacut Sci 2012; 15: 141-183.
14. Kandra L, Gyemant G, Zajacz A, Battab G. Inhibitory effects of tannins on human salvary a-amylase. Biochem Biophys Res Commun 2004; 319: 1265-1271.