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RESEARCH PAPER

DETERMINATION OF *IN VITRO* ALPHA AMYLASE INHIBITORY AND ANTIGLYCATION STUDIES OF ROOT AND STEM EXTRACTS OF *URENA SINUATA*

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Medicinal plants form the basic resource for the search of novel medicinal agents since ages. The search for new medicaments continues in preservance of human health due to various disease conditions. The plant *Urena sinuata* has been used in the treatment of diabetes in alternative systems of medicine. Diabetes is a chronic metabolic disorder of carbohydrates leading to many serious consequences. The remedial treatment suffers from lot of draw backs. In view of this, the present study underwent to investigate the anti-diabetic potential of root and stem extracts of the selected plant by *in vitro* methods - inhibition of alpha amylase and antiglycation. The hydroalcoholic extracts of the plant served appreciable role against diabetes forming evidence to its use in traditional systems of medicine. The results indicated that root hydroalcoholic extract showed equivalent activity in comparison to standard drug, acarbose in inhibiting alpha amylase at 500 µg/ml. Moreover, the root hydroalcoholic extract showed more antiglycation activity as compared to standard drug, rutin at 500 µg/ml.

Key words: Alpha amylase, Antiglycation, *Urena sinuata* root extracts, Anti-diabetic potential.

INTRODUCTION

Plants preparations are used in traditional medicine for the treatment of various diseases, such as type-2 diabetes mellitus. Some medicinal plants are capable of controlling the complications of this metabolic disease at different levels, for example, providing antioxidant compounds that act against oxidative stress and protein glycation and others which are capable of inhibiting the catalysis of digestive enzymes and thus contribute to the reduction of hyperglycemia and hyperlipidemia. Type-2 diabetes mellitus (T2DM) is a metabolic disease that affects about 422 million people in the world, being characterized by several factors, including hyperglycemia and hyperlipidemia, as well as increased oxidative stress and protein glycation [1]. Imbalance in cellular metabolism of carbohydrates and lipids is observed in noninsulin dependent diabetes mellitus. Such a condition predisposes to increase in postprandial blood glucose levels [2-3]. Enzyme pancreatic α-amylase is the main enzyme, which plays a key role in starch hydrolysis and

converts in to small oligosaccharides. These are then further catalyzed by α-glucosidase into glucose which after absorption enters in to the blood stream. This process ensures in haste that ultimately predisposes the condition of postprandial hyperglycemia. Thus, impeding the process of digestion of starch could play a vital role in the management of diabetes [4]. Drugs such as acarbose, voglibose, and miglitol are known to inhibit glycolytic enzymes. However, their nonspecific response and adverse effects like abdominal discomfort and diarrhea [5] restrict their frequent use. In spite of the development of pharmacological agents for the treatment of diabetes, the use of medicinal plants is considered a complementary treatment for this disease [6]. Use of herbal remedies seems to be a promising approach in the treatment of diabetes in terms of no or less side effects and being economical [7]. Studies indicate that secondary metabolites, such as polyphenols (flavonoids and phenolic acids) [8-9] and terpenes present in medicinal plants are able to scavenge free radicals and reduce non-enzymatic glycation [10-12] and inhibit α-amylase, α-glucosidase and lipase [13-14].

The plant *Urena sinuata* belonging to family *Malvaceae*, is a shrub used in native medicine for several ailments. It is a wild shrubby plant with some folk medicinal uses in its native areas. Its parts like roots, stems, flowers, fruits are used as natural remedy. The roots of the plant are sweet, slightly cooling, antirheumatic and antipyretic [15]. The root decoction is used in the treatment of enteritis, dysentery, tonsillitis. A poultice prepared from the roots and leaves is used as emollient and is given for snake bites, sprains and bruises. The flowers are used as expectorant in dry and inveterate chronic coughs. An infusion of the flowers is used in gargle and throat bronchitis [16]. The leaves are prescribed in inflammation of the intestines and the bladder. The original distribution of *U. sinuata* is uncertain, but it has been suggested that the native distribution is Asiastic [17]. It is native to India and Srilanka [18]. The studied pharmacological studies are thrombolytic, sedative, anxiolytic, analgesic, anti-helminthic, anti-diarrhoeal, anti-oxidant, anti-inflammatory, anti-pyretic, and anti–rheumatic activities. The review of literature on this plant species finds no reports on the alpha amylase inhibitory and antiglycation activities. Hence, the present study was aimed at determining the *in vitro* antidiabetic potential by alpha amylase inhibitory and antiglycation models for the plant root and stem extracts.

MATERIALS AND METHODS

The plant materials, roots and stems of *Urena sinuata* were collected in the month of March in morning hours from the grounds of Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada, Andhra Pradesh, India. Herbarium was prepared and the sample was identified from the plant taxonomist Dr. P. Satya Narayana Raju, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur (**Figure 1**). All the chemicals used were of analytical grade.

Fig. 1. The different parts of plant *Urena sinuata* (*Malvaceae*). a) whole plant b) stems c) leaf d) roots

Extraction

The roots and stems were washed off the debris, and dried in sun for 10 days. Then, they were powdered coarsely and extracted by soxhlation using hydroalcoholic solvent.

Preliminary phytochemical screening

Further, the root and stem hydroalcoholic extracts were subjected to the preliminary phytochemical screening by using the standard methods [19-22].

In vitro antidiabetic activity studies

Alpha amylase inhibition assay

The extract (0.2 ml) was added to 0.2 ml of 0.02 M sodium phosphate buffer at pH 6.9, followed by addition of 0.2 ml enzyme solution. The mixture was incubated at 25°C for 10 min. After incubation, 0.2 ml of 1% starch solution was added and incubated at 25°C for 10 min. Finally, 0.2 ml DNSA (Dinitrosalicylic acid) reagent was added to it, and absorbance was read at 620 nm. Acarbose was used as standard substance [23, 24]

Antiglycation assay

Bovine serum albumin (500 µl) was added to glucose (400 µl), followed by addition of 100 µl each of extract and phosphate buffer saline. The mixture was placed at 60°C for 24 hrs. After that, 10 µl of 100 % *w/v* TCA (Trichloroacetic acid) was added and whole content was placed at 4°C for 10 min and finally, centrifuged for 4 min at 1300 rpm. The precipitate obtained was dissolved in phosphate buffer pH 10. Absorbance was read at 400 nm. Rutin was used as a standard substance [25, 26]. The percentage inhibition was calculated by using the formula:

% inhibition = Control absorbance – Test absorbance / Control absorbance × 100

Statistical analysis

All the values were expressed as ±SD. The statistical significance was calculated using one way ANOVA Tukey's test by Graph Pad Prism 5.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The results of the qualitative phytochemical screening indicated that carbohydrates, gums, reducing sugars, alkaloids, phenols, glycosides, tannins, flavonoids, sterols were present in both hydroalcoholic extracts (**Table 1**).

Table 1. Preliminary phytochemical screening of hydroalcoholic root and stem extracts of *Urena sinuata*

S. No.	Phytochemical	HAERUS*	HAESUS*
1	Carbohydrates	+	+
2	Gums	+	+
3	Alkaloids	+	+
4	Reducing sugars	+	+
5	Phenols	+	+
6	Tannins	+	+
7	Flavonoids	+	+
8	Glycosides	+	+
9	Phytosterols	+	+

*HAERUS - hydroalcoholic extract of root; HAESUS - hydroalcoholic extract of stem; '+' indicates present.

Alpha amylase inhibition assay

The results of alpha amylase inhibition indicate that hydroalcoholic extracts showed significant alpha amylase inhibitory activity. HAERUS (Hydroalcoholic extract of roots of *Urena sinuata*) exhibited inhibition of α-Amylase 49.05, 79.24, 86.79, 90.56, and 92.45% at 100, 200, 300, 400, and 500 µg/ml concentrations. Further, HAESUS (Hydroalcoholic extract of stems of *Urena sinuata*) exhibited inhibition of α-Amylase 86.7, 88.6, 90.5, 92.4. and 94.3% at 100, 200, 300, 400, and 500 µg/ml concentrations. Standard drug Acarbose exhibited % inhibition of α-Amylase 83.0, 86.7, 88.6, 92.4, and 94.3 at 100, 200, 300, 400, and 500 µg/ml concentrations (**Table 2**). In this study, *in vitro* inhibitory effect of hydroalcoholic extracts of *U. sinuata* on amylase and antiglycation activities was evaluated. Antidiabetic activities of *U. lobata* have been reported with its leaves in which showed huge inhibitory effect on both alpha amylase and alpha glucosidase [27].

The genus *Urena* consists of two species *U. lobata* and *U. sinuata*, which are used by native practitioners in Bangladesh, India and other traditional systems of medicine. The results of the present study were compared with α-amylase inhibitory effects obtained in previous studies [28]. The results of the present study showed that both hydroalcoholic extracts of root and stem of the plant showed equivalent activity when compared to standard drug, Acarbose. Hydroalcoholic extract of roots of *U. sinuata* was able to show prominent activity than stem extract at various concentrations (**Table 2, Figure 2**).

Table 2. % Inhibition of α-Amylase by *Urena sinuata* extracts

S. No.	Concentration µg/ml	% Inhibition of α-Amylase		
		HAERUS	HAESUS	STD
1	100	49.05±0.05*	86.7±0.05*	83.0±0.05*
2	200	79.24±0.05*	88.6±0.05	86.7±0.05*
3	300	86.79±0.05	90.5±0.05*	88.6±0.05*
4	400	90.56±0.05*	92.4±0.05	92.4±0.05
5	500	92.45±0. 05	94.3±0.05*	94.3±0.05*

#The values were expressed as mean ± SEM, n= 5, at * p< 0.05 when compared with standard by one-way ANOVA, Tukey test. HAERUS - Hydroalcoholic extract of roots of *Urena sinuata* and HAESUS - Hydroalcoholic extract of stems of *Urena sinuata,* STD – Standard Acarbose, SEM - Standard Error Mean, ANOVA - Analysis of Variance.

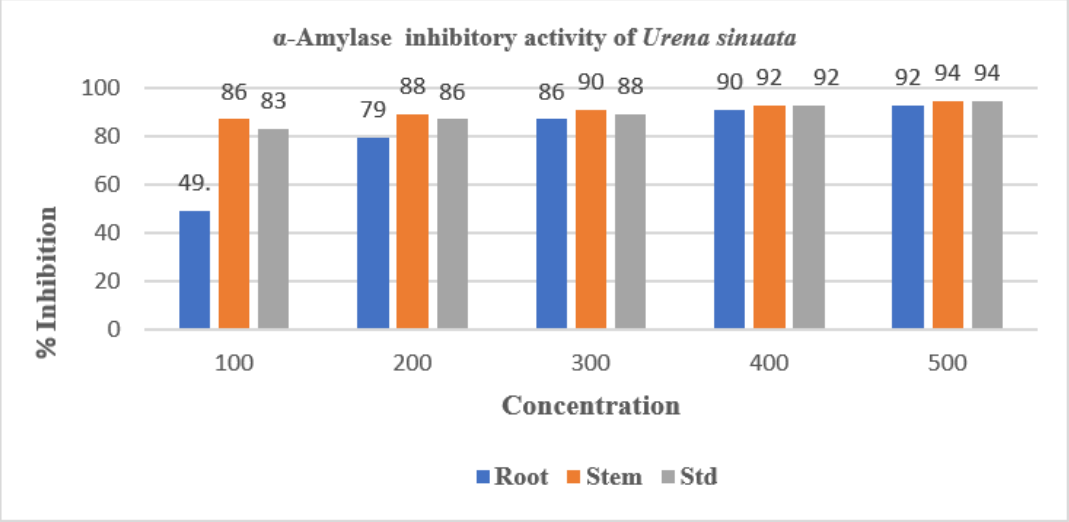

Fig. 2. α-Amylase inhibitory activity of *Urena sinuata* extracts

Antiglycation assay

Antiglycation activity was studied for the hydroalcoholic extracts of the plant *Urena sinuata* roots and stems. The study was conducted by using various concentrations of the extract ranging from 100, 200, 300, 400, and 500 µg/ml. HAERUS showed 67.3, 75.5, 79.5, 83.6, and 97.9% antiglycation activity (**Table 3**).

Table 3. Antiglycation activity studies of *Urena sinuata* extracts

Sr. No.	Concentration µg/ml	% Antiglycation		
		HAERUS	HAESUS	STD
1	100	67.3±0.05	42.8±0.05*	57.1±0.05*
2	200	75.5±0.05*	44.8±0.05	73.4±0.05*
3	300	79.5±0.05*	46.9±0.05*	77.5±0.05
4	400	83.6±0.05*	48.9±0.05	79.5±0.05
5	500	97.9±0.05	51.0±0.05*	83.6±0.05*

#The values were expressed as mean±SEM, n= 5, at *p<0.05 when compared with standard by one-way ANOVA, Tukey test. HAERUS - Hydroalcoholic extract of roots of *Urena sinuata* and HAESUS - Hydroalcoholic extract of stems of *Urena sinuata*, STD–Standard Acarbose, SEM - Standard Error Mean, ANOVA - Analysis of Variance.

HAESUS showed 42.8, 44.8, 46.9, 48.9, and 51.0% antiglycation activity. The standard drug, Rutin showed 57.1, 73.4, 77.5, 79.5, and 83.6% antiglycation ability (**Table 3**, **Figure 3**). The results of antiglycation activity study of *Urena sinuata* root and stem hydroalcoholic extracts indicated that root extract exhibited antiglycation activity more than standard drug, Rutin. The activity shown by root extract may be due to the presence of phytochemicals such as phenols, tannins and glycosides. The genus *Urena* has been studied for antiglycation activity. The results of the present study were compared with previous studies [29]. The present investigation was aimed at studying the antidiabetic potential of roots and stems of ancient medicinal plant *Urena sinuata* by two *in vitro* models.

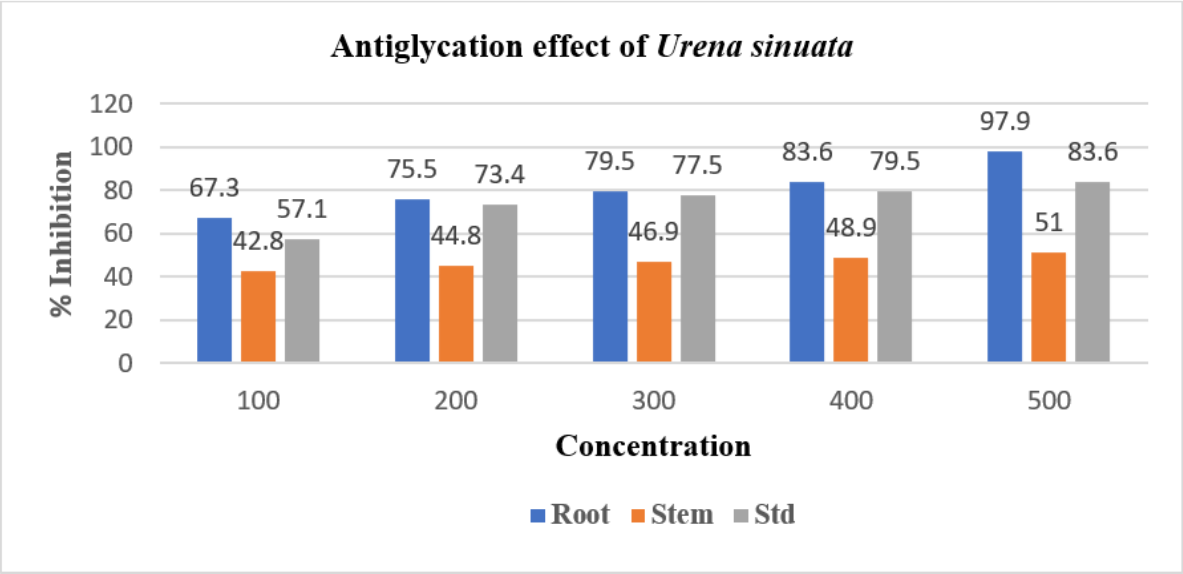

Fig. 3. Antiglycation activity effects of *Urena sinuata* extracts

The results showed that the hydroalcoholic extract of the roots of the plant showed meaningful alpha amylase inhibitory activity as compared to standard drug Acarbose. Further, the root extract of plant showed more antiglycation activity as compared to that of standard drug, Rutin. The genus *Urena* has been used in the treatment of diabetes in ancient system of medicine throughout the World. The current investigation on antidiabetic activity further confirms the usage of the plant for treating diabetes as a treatment option.

CONCLUSION

The present study was conducted to evaluate the phytochemical and *in vitro* antidiabetic activity of *Urena sinuata* L. root and stem hydroalcoholic extracts by α-amylase inhibition and anti-glycation models. The medicinal plant selected was used in traditional system of medicine as antidiabetic. The results of the study proved that the root and stem hydroalcoholic extracts exhibited appreciable alpha amylase inhibitory and antiglycation activity in comparison to standard drugs, Acarbose and Rutin.

The hydroalcoholic extract of roots showed prominent alpha amylase inhibitory activity than stem extracts compared to standard drug Acarbose. Moreover, root extracts showed more prominent antiglycation activity than standard drug, Rutin. The results of the study validate the traditional use of the plant extracts in treating diabetes. In future, *in vivo* studies can be conducted to validate the mechanism of action of the study as well as its use as promising antidiabetic agent.

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REFERENCES

1. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res.* 2010;107(9):1058-70. doi:10.1161/ CIRCRESAHA.110.223545
2. Henriksen EJ, Diamond-Stanic MK, Marchionne EM. Oxidative stress and the etiology of insulin resistance and type 2 diabetes. *Free Radic Biol Med*. 2011;51(5): 993-9. doi:10.1016/j.freeradbiomed.2010.12.005
3. Gruenwald J, Freder J, Armbruester N. Cinnamon and health. *Crit Rev Food Sci Nutr*. 2010;50(9):822-34. doi:10.10 80/10408390902773052
4. Eichler HG, Korn A, Gasic S, Pirson W, Businger J. The effect of a new specific alpha-amylase inhibitor on post-prandial glucose and insulin excursions in normal subjects and Type 2 (non-insulin-dependent) diabetic patients. *Diabetologia*. 1984;26:278-81.
5. Cheng AYY, Fantus IG. Oral antihyperglycemic therapy for type 2 diabetes mellitus. *Can Med Assoc J*. 2005;172(2):213-26. doi:10.1503/cmaj.1031414
6. Ota A, Ulrih NP. An overview of herbal products and secondary metabolites used for management of type two diabetes. *Front Pharmacol*. 2017;8,436. doi:10.33 89/fphar.2017.00436
7. Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. *J Ethnopharmacol*. 2006;106(1):1-28. doi:10. 1016/j.jep.20 06.03.021
8. Choi SY, Jung SH, Lee HS, Park KW, Yun BS, Lee KW. Glycation inhibitory activity and the identification of an active compound in Plantago asiatica extract. *Phytother Res*. 2008;22(3):323-9. doi:10.1002/ptr.2316

9. Sun C, McIntyre K, Saleem A, Haddad PS, Arnason JT. The relationship between antiglycation activity and procyanidin and phenolic content in commercial grape seed products. *Can J Physiol Pharmacol*. 2012;90(2): 167-74. doi:10.1139/ y11-121
10. Lei F, Zhang XN, Wang W, Xing DM, Xie WD, Su H, et al. Evidence of anti-obesity effects of the pomegranate leaf extract in high-fat diet induced obese mice. *Int J Obes (Lond)*. 2007;31(6):1023-9. doi:10.1038/sj.ijo.0803502
11. Hanamura T, Mayama C, Aoki H, Hirayama Y, Shimizu M. Antihyperglycemic effect of polyphenols from Acerola (Malpighia emarginata DC.) fruit. *Biosci Biotechnol Biochem*. 2006;70(8):1813-20. doi:10.1271/bbb.50592
12. Yajima H, Noguchi T, Ikeshima E, Shiraki M, Kanaya T, Tsuboyama-Kasaoka N, et al. Prevention of diet-induced obesity by dietary isomerized hop extract containing isohumulones, in rodents. *Int J Obes (Lond)*. 2005;29(8): 991-7. doi:10.1038/sj.ijo.0802965
13. Buchholz T, Melzig MF. Polyphenolic compounds as pancreatic lipase inhibitors. *Planta Med*. 2015;81(10): 771-83. doi:10.1055/s-0035-1546173
14. Tan Y, Chang SKC, Zhang Y. Comparison of α-amylase, α-glucosidase and lipase inhibitory activity of the phenolic substances in two black legumes of different genera. *Food Chem*. 2017;214:259-68. doi:10.1016/j.foodchem. 2016.06. 100
15. Abdullah M, Kumar Pk A, Saleh DKMA, Khan AR, Islam N. Insecticidal and repellent activities of the chloroform extracts of Urena sinuata L. against Tribolium castaneum (Herbst) adults. *Univ J Zool, Rajshahi Univ*. 2011;30:25-8. doi:10.3329/ujzru.v30i0.10740
16. Kirtikar KR, Basu BD. Indian Medicinal Plants, 2nd edition, Basu LM: Allahabad, 1935.
17. Austin DF. Caesar's weed (Urena lobata): An invasive exotic or a Florida native? *Wildland Weeds*. 1999;3(1): 13-16.
18. Rondon JB. The subfamily Malvoideae (Malvaceae sl) in the west of Sucre state, Venezuela. UDO *Agrícola Sci J*. 2009;9(3):599-621.
19. Evans WC. Trease and Evans' Pharmacognosy, 14th edition, WB Saunders Co.: London, 1996; 545-6.
20. Wallis TE. Textbook of Pharmacognosy, 5th edition, CBS Publishers: New Delhi, 2005.
21. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments, Fourth edition, Nirali Prakashan: Pune, 1998.
22. Kokate CK, Purohit AP, Gokhale SB. Text book of Pharmacognosy, Nirali Prakashan: Pune, 2007.
23. Sachan AK, Rao CV, Sachan NK. In vitro studies on the inhibition of α-amylase and α-glucosidase by hydro-ethanolic extract of Pluchea lanceolata, Alhagi pseudalhagi, Caesalpinia bonduc. *Phcog Res*. 2019; 11(3):310-4. doi:10.41 03/pr.pr_31_19
24. Kim KT, Rioux LE, Turgeon SL. Alpha-amylase and alpha-glucosidase inhibition is differentially modulated by fucoidan obtained from Fucus vesiculosus and Ascophyllum nodosum. *Phytochemistry*. 2014;98:27-33. doi:10.1016/j. phytochem.2013.12.003
25. Muniz A, Garcia E, Gonzalez D, Zuñiga L. Antioxidant activity and in vitro antiglycation of the fruit of Spondias purpurea. *Evid Based Complementary Altern Med*. 2018:5613704. doi:10.1155/2018/5613704
26. Jovanović JA, Mihailović M, Uskoković AS, Grdović N, Dinić S, Poznanović G, et al. Evaluation of the antioxidant and antiglycation effects of Lactarius deterrimus and Castanea sativa extracts on hepatorenal injury in Streptozotocin-induced diabetic rats. *Front Pharmacol*. 2017;8:793. doi:10.3389/fphar.2017.00793
27. Purnomo Y, Makdasari J, Fatahillah FI. Inhibitory activity of Urena lobata leaf extract on alpha-amylase and alpha-glucosidase: In vitro and in silico approach. *J Basic Clin Physiol Pharmacol*. 2021;32(4):889-94. doi:10.1515/ jbcpp-2020-0430
28. Tamil IG, Dineshkumar B, Nandhakumar M, Senthilkumar M, Mitra A. In vitro study on α-amylase inhibitory activity of an Indian medicinal plant, Phyllanthus amarus. *Indian J Pharmacol*. 2010;42(5): 280-2. doi:10.41 03/0253-7613.70107
29. Perera PRD, Sagarika Ekanayaka S, Ranaweera KKDS. In vitro antiglycation activity of some medicinal plants used in Diabetes mellitus. *Med Aromat Plants*. 2013;2(6):143. doi:10.4172/2167-0412.1000143

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