



Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: <http://www.elsevier.com/locate/jtcme>

Antidiabetic potential of *Asparagus racemosus* Willd leaf extracts through inhibition of α -amylase and α -glucosidase

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ARTICLE INFO

Article history:

Received 26 October 2016

Received in revised form

19 September 2017

Accepted 6 October 2017

Available online 30 April 2018

Keywords:

Asparagus racemosus Willd

Diabetes mellitus

α -amylase

α -glucosidase

ABSTRACT

The aim of this work was to evaluate the inhibitory activities of different extracts of *Asparagus racemosus* Willd on α -amylase and α -glucosidase at varying concentrations. Diabetes mellitus is a clinical condition characterized by hyperglycaemia in which an elevated amount of glucose circulates in the blood plasma. α -amylase and α -glucosidase inhibitors are used to achieve greater control over hyperglycemia in type 2 diabetes mellitus. This study is to treat the diabetes using natural resources. We aimed to evaluate of *Asparagus racemosus* Willd by digestive enzymes inhibitory activity. n-hexane, chloroform, ethyl acetate, methanol, and aqueous was used to extract the root of the *Asparagus racemosus* Willd. The different extracts were then used to study its digestive enzymes activity α -amylase and α -glucosidase inhibitory activity. The significant inhibitory effect of α -amylase and α -glucosidase enzyme and exhibited lower inhibitory activity than acarbose was extracted by the ethyl acetate and aqueous extracts of the plant. Flavonoids, Tannins and phenolic, Saponins, Amino acids, Protein are the major phytochemical constituents present. The total flavonoid content plant extracts of ethyl acetate and aqueous showed dose dependent 23.45 ± 1.33 mg rutin equivalent/g and 25.81 ± 0.82 mg rutin equivalent/g respectively. The total triterpenoids content plant extracts of ethyl acetate, aqueous showed dose dependent 109.8 ± 5.6 mg ursolic acid/g and 95.6 ± 7.5 mg ursolic acid/g respectively. The antidiabetic potential and to develop medicinal preparations and nutraceuticals and function foods for diabetes has revealed.

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1. Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to the secreted insulin. Such a deficiency results in increased blood glucose level, which in turn can damage many of the body's systems, including blood vessels and nerves.¹ One of the therapeutic approaches is to decrease the postprandial hyperglycemia, by retarding the absorption of glucose by inhibition of carbohydrate-hydrolyzing enzymes, such as α -amylase and α -glucosidase.² From this point of view, many efforts have been made to search for more effective and safe inhibitors of

α -glucosidase and α -amylase from natural materials to develop physiological functional foods to treat diabetes.³ Many traditional plants have been reported in India for diabetes, but only a small number of these have received scientific and medical evaluation to assess their efficacy. On the basis of ethno medical/tribal information *Asparagus racemosus* Willd has been used to treat and prevent diabetes. *Asparagus racemosus* Willd possess a diverse number of pharmacological activities including antioxidant and free radical scavenging activity,^{4–6} anticholinesterase action^{7,8} and anti-inflammatory property. However, the studies on anti-diabetic effects of *Asparagus racemosus* Willd extracts were not focused on the enzyme inhibitory activity thus, The present study is designed evaluate the in-vitro antidiabetic activity of extract of *Asparagus racemosus* Willd extracts and to understand how the extract acts against α -glucosidase and α -amylase enzymes.

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Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

2. Materials and methods

2.1. Identification of plant materials

The root plant of *Asparagus racemosus* Willd Family *Asparagaceae* was collected from the forests of Doddabetta in Nilgiris. The plant species was identified and authenticated by the Dr.S. Rajan, PhD Field Botanist Survey of Medicinal Plants and Collection Unit. Department of AYUSH Ministry of Health and Family Welfare, Govt of India. The plant was deposited in the herbarium of the Department of Pharmacognosy, JSS College of Pharmacy, Ooty. The leaves of the plant were used in study.

2.2. Chemicals

α -amylase from starch potato soluble, α -glucosidase from one gram of rat-intestinal acetone powder, p-nitrophenyl- α -D-glucopyranoside and dinitrosalicylic acid were purchased from Sigma chemicals. All the chemicals used in the study are of analytical grade.

2.3. Preparation of crude extract

The leaves of the plant were sun dried and ground to a coarse powder and stored in an air tight container. This coarse powder was subjected to successive extraction with n-hexane (68 °C), chloroform (61 °C), ethyl acetate (77 °C), and methanol (64 °C) by continuous soxhlation and aqueous extracts by maceration process. After collection of extracts, it is kept at temperature 37 °C until solvent is completely evaporated. Then finally dried in desiccator.

2.4. Qualitative phytochemical analysis

The plant extracts of *Asparagus racemosus* Willd in n-hexane, chloroform, ethyl acetate, methanol and aqueous was analyzed for the presence of amino acids, steroids, cardiac glycosides, phenols, tannins, terpenoids, alkaloids, flavonoids, saponins, carbohydrates, reducing sugar.

2.5. Evaluation of bioactive constituents

2.5.1. Total flavonoid content

The total flavonoid content of the n-hexane, chloroform, ethylacetate, methanol and aqueous extracts of *Asparagus racemosus* Willd was determined by aluminium chloride colorimetric method. In brief 50 μ L of the n-hexane, chloroform, ethylacetate, methanol and aqueous extracts of *Asparagus racemosus* Willd (1mg/ml ethanol) were made up to 1 ml with methanol mixed with 4 ml of distilled water and then 0.3 ml of 5% sodium nitrite solution.⁹ 0.3 ml of 10% aluminium chloride solution was added after 5min of incubation and the mixture was allowed to stand for 6min. Then 2 ml of 1 mol/L sodium hydroxide solution were added and the final volume of the mixture was brought to 10 ml with double-distilled water. The mixture was allowed to stand for 15min and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve. The result was expressed as mg rutin equivalent per g dry weight.

2.5.2. Total triterpenoid content

The content of n-hexane, chloroform, ethylacetate, methanol and aqueous extracts of *Asparagus racemosus* Willd obtained by the aforementioned method was determined according to Lu et al with a slight modification and then expressed as milligram ursolic acid equivalent/gram dry weight. Briefly after a 200- μ L n-hexane, chloroform, ethylacetate, methanol and aqueous extracts of *Asparagus*

racemosus Willd solution in a 10 ml volumetric flask was heated to evaporation in a water-bath 1 ml new mixed 5%w/v vanillin acetic solution and 1.8 ml sulfuric acid were added mixed and incubated at 70 °C for 30min.¹⁰ Then the mixed solution was cooled and diluted to 10 ml with acetic acid. The absorbance was measured at 573 nm against blank using a spectrophotometer. The blank consisted of all reagents and solvents without sample solution. The content was determined using the standard ursolic acid calibration curve.

2.6. α -amylase inhibition activity

The α -amylase solution (Prepare 25 ml in Reagent 7.3.1 using Starch Potato Soluble. Facilitate solubilization by heating the starch solution in a glass beaker directly on a heating/stir plate using constant stirring. Bring to boil and maintain the solution at this temperature for 15 min). Inhibitory activity of the n-hexane, chloroform, ethyl acetate, methanol and aqueous extracts of *Asparagus racemosus* Willd were determined.¹¹ Dry residue was dissolved in 1 ml of tris buffer solution (pH 9.1) 2 ml of 0.1 mol/L an ammonia solution and 2 ml n-hexane. A total of 250 μ L of sample and 125 μ L of 0.02 M sodium phosphate buffer (pH 6.9 with 6 mM sodium chloride) containing α -amylase solution (0.5 mg/mL) was incubated at 25 °C for 10 min. After preincubation, 250 μ L of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 6 mM sodium chloride) was added to each tube at timed intervals. The reaction mixtures were then incubated at 25 °C for 10 min. The reaction was stopped with 0.5 mL of dinitrosalicylic acid color reagent. The test tube was then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 5 mL of distilled water and absorbance was measured at 540 nm. Acarbose was used as the positive control. The α -amylase inhibitory activity was calculated as follow;

$$\text{Inhibition (\%)} = (1 - A_s / A_c) \times 100$$

where A_s and A_c are the absorbance of the sample and the control respectively.

2.7. α -glucosidase inhibitory activity

The rat intestinal α -glucosidase assay was based on the method of Kwon et al.,¹² with slight modifications. One gram of rat-intestinal acetone powder was suspended in 3 mL of 0.9 % saline. The suspension was sonicated 12 times for 30 s at 4 °C. After centrifugation (10,000 g, 30 min, 4 °C), the resulting supernatant was used for the assay.¹³ Fifty microliters of sample solution were pre-incubated with 100 μ L of rat intestinal α -glucosidase solution at 37 °C for 15 min. The α -glucosidase inhibitory activity of the n-hexane, chloroform, ethylacetate, methanol and aqueous extracts of *Asparagus racemosus* Willd were determined. Dry residue was redissolved in 1 ml of tris buffer solution (pH 9.1) 2 ml of 0.1 mol/L an ammonia solution and 2 ml n-hexane. A mixture of 50 μ L of sample and 100 μ L of 0.1 M phosphate buffer (pH 6.9) containing α -glucosidase solution (1 U/mL) was incubated in 96 well plates at 25 °C for 10 min. After preincubation, 50 μ L of 5 mM pNPG solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixture was incubated at 25 °C for 5 min. Before and after incubation, absorbance was recorded at 405 nm by micro plate reader. Acarbose was used the positive control. The α -glucosidase inhibitory activity was expressed as percentage inhibition percent and was calculated as follows;

$$\text{Inhibition (\%)} = (1 - A_s / A_c) \times 100$$

where A_s and A_c are the absorbance of the sample and the control respectively.

3. Results and discussion

3.1. Phytochemical analysis

The n-hexane, chloroform, ethyl acetate, methanol, and aqueous extracts of *Asparagus racemosus* Willd showed the presence of major phytochemical constituents such as Flavonoids, Tannins and phenolic, Saponins, Amino acids, Protein (Table 1).

3.2. Total flavonoid and triterpenoids content

The total flavonoid of n-hexane, chloroform, ethyl acetate, methanol, and aqueous extracts of *Asparagus racemosus* Willd are given in Table 2. The plant extracts (ethyl acetate, aqueous) showed dose dependent 23.45 ± 1.33 mg/g and 25.81 ± 0.82 mg/g respectively. The total triterpenoids of n-hexane, chloroform, ethyl acetate, methanol, and aqueous extracts of *Asparagus racemosus* Willd are given in Table 2 and Fig. 1. The plant extracts (ethyl acetate, aqueous) showed dose dependent 109.8 ± 5.6 mg/g and 95.6 ± 7.5 mg/g respectively.

3.3. Inhibition of α -amylase and α -glucosidase

The α -amylase inhibition of n-hexane, chloroform, ethyl acetate, methanol, and aqueous extracts of *Asparagus racemosus* Willd are given in Table 3. The plant extracts (ethyl acetate, aqueous) showed dose dependent α -amylase inhibition with IC_{50} value 65.85 ± 1.19 and 63.28 ± 0.38 respectively. The IC_{50} value of Acarbose was found to be 11.84 ± 0.19 . The α -glucosidase inhibition of n-hexane, chloroform, ethyl acetate, methanol, and aqueous extracts of *Asparagus racemosus* Willd are given in Table 3. The plant extracts (ethyl acetate, aqueous) showed dose dependent α -glucosidase inhibition with IC_{50} value 68.32 ± 1.02 and 62.65 ± 2.61 respectively. The IC_{50} value of Acarbose was found to be 13.39 ± 0.11 .

To maintain glycemic level in control, in both the fasting and post-prandial states is the treatment goal of diabetes patients. The suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine have been investigated using natural resources.¹⁴

Pancreatic α -amylase is a key enzyme in the digestive system which catalyzes the initial step in the hydrolysis of starch, which is a principal source of glucose in the diet. α -glucosidase, a key enzyme for carbohydrate digestion, has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality to occur in type 2

Table 2

Total flavonoid and triterpenoids content.

S.No	Extracts	Total Flavonoid Content (mg rutin equivalent/g) ^a	Total Triterpenoids Content (mg ursolic acid/g) ^b
1	n – hexane	11.20 ± 2.32	45.7 ± 1.4
2	Chloroform	19.87 ± 0.67	61.3 ± 3.8
3	Ethyl acetate	23.45 ± 1.33	109.8 ± 5.6
4	Methanol	22.67 ± 2.72	75.8 ± 6.5
5	Aqueous	25.81 ± 0.82	95.6 ± 7.5

^{a,b}Average of three determinations, mean $\pm$ SEM.

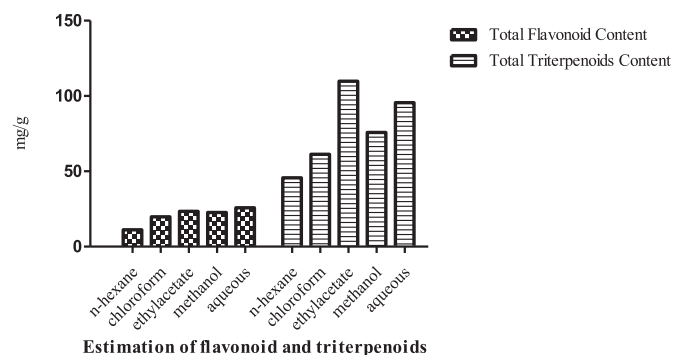


Fig. 1. Estimation of flavonoid and triterpenoids.

Table 3

Inhibitory activity of different extracts of *Asparagus racemosus* Willd.

Groups	Treatment	α -amylase (IC_{50}) mg/mL	α -glucosidase (IC_{50}) mg/mL
I	n – hexane	35.52 ± 0.66	37.92 ± 1.73
II	Chloroform	45.13 ± 0.67	49.16 ± 1.10
III	Ethyl acetate	65.85 ± 1.19	68.32 ± 1.02
IV	Methanol	55.52 ± 1.21	56.88 ± 0.68
V	Aqueous	63.28 ± 0.38	62.65 ± 2.61
VI	Acarbose	11.84 ± 0.19	13.39 ± 0.11

Value are expressed as mean $\pm$ SEM, n = 5.

Diabetes mellitus. α -amylase catalyzes the hydrolysis of α -1, 4-glucosidic linkages of starch, glycogen and various oligosaccharides and α -glucosidase further breaks down the disaccharides into simpler sugars, readily available for the intestinal absorption. The inhibition of their activity, in the digestive tract of humans, is considered to be effective to control diabetes by diminishing the absorption of glucose decomposed from starch by these enzymes.¹⁵ Rat-derived α -glucosidase in the initial screening step of this study, because this stage is the most important for ensuring that the

Table 1
Phytochemical analysis.

S.NO	Phytoconstituents	Extracts of <i>Asparagus racemosus</i> Willd				
		n – hexane	Chloroform	Ethyl acetate	methanol	aqueous
1	Alkaloids	–	+	–	+	–
2	Protein	–	–	+	+	–
3	Flavonoids	+	+	–	+	–
4	Glycosides	–	–	–	+	–
5	Tannins and phenolic	–	+	+	+	–
6	Saponins	–	+	+	+	+
7	Steroids	–	+	–	–	–
8	Amino acids	–	+	+	+	–
9	Carbohydrate	–	–	–	+	+
10	Fat and oils	+	–	–	–	+

+: Present -: Absent.

medicinal plants we chose had inhibitory activities against rat α -glucosidase (which is similar to human α -glucosidase) and could thus be used in the treatment of type 2 diabetes.^{16–18} Therefore, effective and nontoxic inhibitors of α -amylase and α -glucosidase have long been sought.

We have investigated that the *Asparagus racemosus* Willd which is used in traditional ayurvedic medicine for the treatment of several diseases, has been serving as anti-diabetic potential. Previously, this beneficial and priceless herb was not been investigated for its in vitro anti-diabetic activity. This herb has clearly recognized the potential of anti-diabetic activity and come out with the active principles responsible may be total flavonoids and terpenes content compound.

Flavonoids, like anti-oxidants, may prevent the progressive impairment of pancreatic beta-cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes. Although, in the present study, the enzyme inhibitory activity of these extract and fractions were assayed in-vitro, the results from this work should be relevant to the human body. In addition to α -amylase and α -glucosidase inhibitory activities, these phytoconstituents are also reported to have several other biological activities including anti-bacterial, anti-oxidative, anti-cancer etc.¹⁹ This supportive evidence further increases the medicinal importance of this *Asparagus racemosus* Willd indicating that this herb is not only beneficial for diabetes but also may be useful to a number of other human health complications.

4. Conclusion

The plant extract of the *Asparagus racemosus* Willd, has been used for food and medicinal purposes. Our result suggest the inhibitory effects on α -amylase and α -glucosidase and contains a high amount of phytochemical constituents (i.e., total flavonoids and triterpenoids content) Thus, the plant extract of the *Asparagus racemosus* Willd may be used in the management of type 2 diabetes mellitus with few or no side effects. However, Further studies of the plant extract of the *Asparagus racemosus* Willd using in vitro and in vivo models need to be performed to elucidate its insulin mimetic activity and reduction of insulin resistance and also to develop medicinal preparations, nutraceuticals or functional foods for diabetes and related symptoms.

Acknowledgements

The authors are grateful to the principal and management, JSS College of Pharmacy, (Jagadguru Sri Shivarathreeshwara University, Mysuru) Udhagamandalam, Nilgiris, Tamilnadu, – 643001, India for

providing the necessary infrastructure to carry out this research work in successful manner.

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