Research Article

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International Journal of Drug Development and Research
1791-809X

2022
Vol. 14 No. 7: 963

Evaluation of *in-vitro* anti-diabetic potential of Achyranthes aspera by α-amylase inhibition method

Muhammad Rashad[1]*, Muhammad Athar Adil[2], Sohaib Peerzadah[2] and Malik Saadullah[1]

Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences Government College University Faisalabad, Pakistan

Corresponding author:
Muhammad RASHAD

rphrashad@gmail.com

Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences Government College University Faisalabad, Pakistan

Citation: Rashad M, Adil MA, Peerzadah S, Saadullah M (2021) Evaluation of *in-vitro* anti-diabetic potential of Achyranthes aspera by α-amylase inhibition method. Int J Drug Dev Res J, Vol.14 No. 7: 963.

Abstract

Many ideas have been drawn to employ plants therapeutically since the time of their ancient healing proclivity towards human health. The mythological profile of Achyranthes aspera reveals that it has been utilized to treat a variety of illnesses. In this study, Achyranthes aspera was standardized using a variety of in vitro analytical methods. Methanol was used to extract the dry powdered plant material, which was then treated with dichloromethane for liquid-liquid extraction. Furthermore, plant extracts (methanol and dichloromethane) were evaluated for antioxidant activity in vitro against the free radical DPPH. As connected with normal ascorbic acid, it was then measured for percent inhibition and inhibitory concentration (IC50). Maximum antioxidant efficacy was recorded with IC50 values 14.25 ± 0.013 µg /ml in methanol and 95.61 ± 0.015 µg/ml in dichloromethane extracts respectively. High performance liquid chromatography results showed 55.965% of quercetin which indicates the presence of flavonoid contents. The effective fractions (methanol and dichloromethane) were further investigated by in vitro α-amylase inhibitory activity for anti-diabetic potential and calculated in terms of percentage inhibition and IC50. Both extracts displayed substantial α-amylase inhibition activity, but the highest inhibition was observed with an IC50 21.58 ± 0.02 µg/ml in crude methanol extract. It is concluded that Achyranthes aspera can be used for isolation of novel compound which will be effective against diabetes mellitus (DM).

Keywords: Achyranthes aspera; Anti-oxidant; Anti-Diabetic; DPPH Assay; α-amylase inhibition.

Received: 19-July-2022, Manuscript No. IJDDR -22-12884; **Editor assigned:** 22-July-2022, Preqc No. PQ-12884; **Reviewed:** 13-Aug-2022, QC No.Q-12884; **Revised:** 18-Aug-2022, Manuscript No. IJDDR-22-12884(R); **Published:** 26-Aug-2022, DOI: 10.36648/1791-809X-16.07-963

Introduction

The traditional history remedies are passed from generation to generation by folk cognition. Furthermore, notwithstanding synthetic chemistry great development, many of these conventional treatments have not yet been replaced [1-2].

The role of both nutraceuticals and responsive essential as herbal remedies is the best applied in the healthcare system by medicinal plants. The use of plant extracts in tumor, diabetes, ulcer treatment, and bacterial infection supports their therapeutic importance. For different reasons, different countries' population has used herbal medicines like cultural requirements, historical uses, ecological and religious ceremonies objectives [3].

The new diagnostic advancement and management of disease approaches have increased the function of herbal drugs. Over the past couple of decades, natural products are used as a substantial starting material source in the pharmaceutical industry. Certain medicinal plant extracts have been used by pharmaceuticals to produce therapeutic formulations such as Atropa belladonna, Digitalis purpurea digoxin, and Rauwolfia serpentine reserpine [4]. They may not be required for metabolism, but may be specifically related to the environmental adaptation role or may be used as an achievable mechanism of protection for organism survival [5].

Metabolic syndrome or diabetes mellitus is an endocrinological confusion that is triggered either by acquired insulin or hereditary

production deficiencies in different ways or by resistance caused by many factors that oppose natural insulin action in the body. Normally the impairment of insulin in human body gives support to hyperglycemic condition characterized by elevated level of blood sugar which in turn can cause several complications including diabetic ketoacidosis such as nephropathies, progressive multiple organ diseases, coronary artery disease, neuropathy and retinopathy. Expressively hyperglycemia is a disorder of the umbrella under which many problems arise [6].

In terms of accelerated progression in the globally prevalence, it will quite appropriate to the suggestion of (DM) as modern era of an epidemic. Nevertheless, rising diabetes burden has retarded socio-economic development in many countries that the disease has taken on a daunting role for global health. As per the data of global statistics (2015), 5 million females and 2 million males have diabetes mellitus with a prevalence rate of 8.8 percent of disease and as reported by WHO, nearly 90 percent of people with diabetes milletus are non-insulin dependent diabetes mellitus (NIDDM) patients. The common symptoms of any type of diabetes mellitus are fatigue of extreme level, polyphagia, weight loss polydipsia, polyuria and slow wound healing [7].

Materials and Methods

Collection of plant

Fresh plant was harvested in September 2018 from Rehman garden, Lahore, Punjab, Pakistan. It was identified from a botanist in the department of botany, Government College University Lahore, Punjab. Plant was verified by Prof. Dr. Zaheer-ud-Din Khan. After verification of plant, voucher specimen number 3590 was allotted, and specimen was submitted in the herbarium.

Preparation of extract

Plant material was first rinsed with purified water to remove dust, and then dried under shade for fifteen days. After drying, the material was subjected to grind in an electric grinder. The powder obtained was macerated at room temperature for about 5 days using analytical grade methanol and dichloromethane as a solvent and then filtered. To concentrate the extract, rotary evaporator at 40 °C and reduced pressure was used. The same procedure was adapted for the dichloromethane extract.

In vitro antioxidant activity by DPPH free radical scavenging method

Antioxidant activity of different extracts of A. aspera (methanol and dichloromethane) were measured using the procedure described in [8] with minor alterations.

Preparation of sample stock solution: Dissolved 5 mg of each extract in 5 ml of methanol respectively to prepare sample stock solution (methanol and Dichloromethane). For a uniform dissolution of each extract, they were sonicated. From stock solution, dilutions of different concentrations were prepared (62.5, 125, 250, 500 and 1000 µg/ml). Similar procedure was adapted for preparing the standard ascorbic-acid dilutions.

Preparation of DPPH stock solution

A stock solution of DPPH 0.004 mg in 100 ml of methanol was made in a volumetric flask completely covered in aluminum foil and kept at 25 ° C before any further usage.

Procedure

Add 1 ml of each sample and standard solution in test tubes (62.5, 125, 250, 500 and 1000 µg/ml), 2 ml of DPPH solution, and 1 ml methanol to obtain 4 ml mixture in each tube. The mixture was shaken comprehensively and incubated for 30 min in dark at 25 °C. A standard ascorbic-acid solution was employed as a positive control, whereas a negative control was made by dissolving 2 mL of DPPH solution in 1 mL of methanol. Using a blank sample of methanol, measured the absorbance at 517 nm. The whole process was carried out in a triplicate [9].

In terms of inhibition (%) of DPPH, the plant extract's antioxidant capacity was calculated using the equation.

$$\text{Inhibition (\%)} = \frac{[\text{Absorbance of control - Absorbance of sample}]}{[\text{Absorbance of control}]} \times 100$$

Nonetheless, by comparing inhibition rate to extract concentrations, IC_{50} was calculated from graph

High performance liquid chromatography

A 0.45 m Millex-HV PVDF membrane was used to filter the sample (Millipore, New Bedford, MA, USA). HPLC analysis was performed on a Shimadzu chromatographer integrated with a ternary pump (Shimadzu LC-20AT) and DAD analyzer (Diode Assay Detector) (Shimadzu SPD-M20A, Japan) and conducted on an analytical HPLC column (Phenomenex® ODS 100 A 250 mm 4.60 mm, 5 µm) accompanied by a C18 guard column (2.0 cm 4.0 mm; 5 µm) The methodological approach were gradient, with methanol: water as the mobile phase. The solvent was first acetonitrile: water (2: 8 v/v) and subsequently increased to acetonitrile: water (8: 2 v/v) in around thirty min at a 1.0 ml / min. The mobile phase was newly produced and degassed by sonication prior to use. The injection volume utilized was 20 µL, and the temperature was held constant at 25 °C. The UV spectrum was recorded across a wide range of wavelengths, from 450 to 200 nm. The concentration, height or peak area against the concentration of the material was plotted to determine. All peak area or height was specifically proportional to analyte concentration for peaks which were well resolved from each other [10-12]. The concentration of a compound, peak area or height versus the concentration of the material was plotted to determine. All peak area or height was specifically proportional to analyte concentration for peaks which were well resolved from each other.

$$\text{Amount of Quercetin} = \frac{\text{Concentration of Sample}}{\text{Concentration of Standard}} \times \frac{\text{weight of Standard}}{\text{weight of Sample}} \times \text{Potency}$$

α-amylase Inhibitory Activity

The colorimetric test was used to assess the α-amylase inhibition activity from each extract with acarbose as a standard component. Heating and agitating the potato starch (0.25 g) in 50 ml distilled water for about 15 minutes yielded the starch solution (0.5 percent w/v). α-amylase (0.001 g) was mixed in 100 ml, 20 M

SPB (pH 6.9) with 6.7 mM NaCl to make the enzyme solution (0.5 unit/mL). Both extracts (methanol and dichloromethane) were diluted in a solvent to produce different concentrations (100, 200, 300, 400, and 500 µg/mL). A solution containing 20 mL 3,5-dinitrosalicylic acid (96 mM), 12 mL distilled water and 8 mL of 5.31 M sodium-potassium tartrate in 2 M NaOH was used as the colour reagent. In ach tube, 1 ml of enzyme solution and 1 ml of each extract was mixed and incubated at 25 °C for 30 minutes. 1 ml of above obtained mixture was mixed with starch solution (1 ml), incubated for 30 minutes at 25 °C. The closed tube was then put in 85 °C water bath with 1 ml of the colour reagent added. After 15 minutes, the mixture was taken from the water bath and cooled, then diluted with 9 ml of distilled water; absorbance was measured at 540 nm. Separate blanks were produced to adjust the absorbance of the backdrop. In this scenario, the colour reagent solution was introduced before the starch solution, and the tube was then put in the water bath. The additional operations were carried out in the same manner as described before. The controls were carried out in the same way, except instead of plant extracts, 1 ml of the corresponding solvents were used. As a positive control, acarbose solution (concentrations of 10, 50, and 100 g/mL) was utilised [13]. The percent inhibition of -amylase was calculated using the formula given below:

$$\text{Inhibition (\%)} = \frac{\text{Abs.of control - Abs.of compound}}{\text{Abs.of control}} \times 100$$

Results

In-vitro antioxidant activity of Acyranthes aspera by DPPH radical scavenging method

At ratios of 62.5, 125, 250, 500, and 1000 µg/ml, the antioxidant properties of various polarity extracts of Acyranthes aspera (Methanol and dichloromethane) was investigated. The % inhibition (**Figure 1**) and IC_{50} values of all extracts were calculated. According to the findings, all of the extracts were less effective than standard ascorbic acid (IC_{50} 7.621 ± 0.011 µg/ml), although crude methanol extract displayed substantial antioxidant activity (IC_{50} 14.25 ± 0.013 µg/ml, respectively) (**Table 1 and Figure 2**). A lower IC_{50}, on the other hand, suggests more inhibitory efficacy.

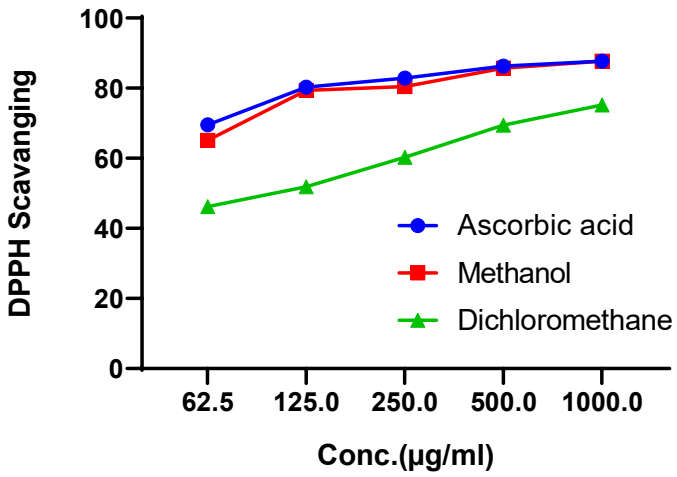

Figure 1 Percentage inhibition of different solvent extracts (Methanol and Dichloromethane) of Achyranthes aspera and Standard ascorbic acid.

High performance liquid chromatography

HPLC spectrum was used to calculate the amount of quercetin present in methanol extract of Achyranthes aspera (**Figure 3**). The peak of quercetin in sample spectrum was compared with

Table 1: IC_{50} of Ascorbic acid, Methanol and Dichloromethane

Samples	IC_{50} values (µg/ml)
Ascorbic acid	7.621 ± 0.011
Methanol	14.25 ± 0.013
Dichloromethane	95.61 ± 0.015

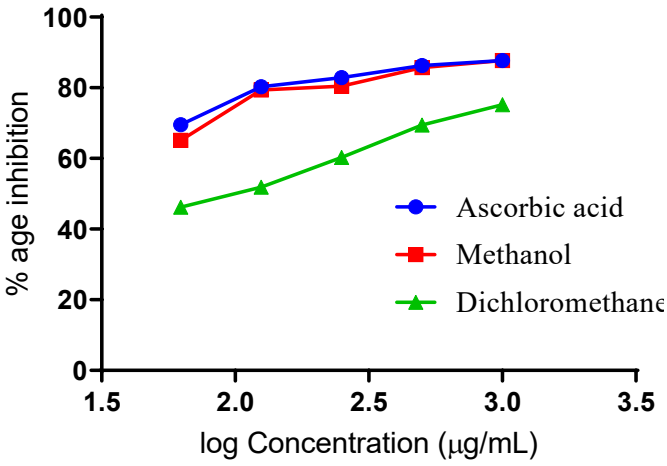

Figure 2 IC_{50} of ascorbic acid, methanol and dichloromethane

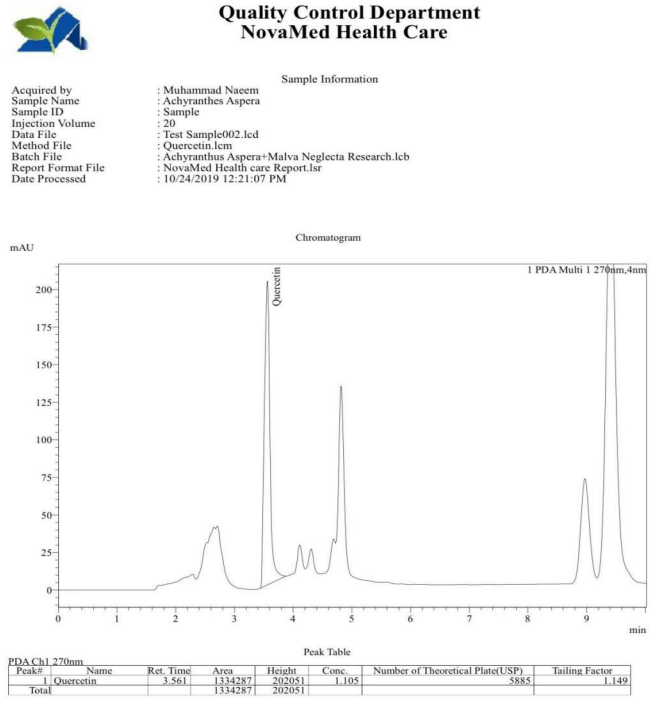

Figure 3 High performance liquid chromatographic spectrum of Achyranthes aspera

standard quercetin peak and amount of quercetin was calculated by following formula

$$\text{Amount of Quercetin} = \frac{1334287}{1175426} \times \frac{24.85}{49.89} \times 98.98$$

Amount of Quercetin = 55.965%

In-vitro antidiabetic activity of Achyranthes aspera by α-amylase inhibition

The α-amylase inhibition action of A. aspera's polar (methanol) and non-polar (dichloro-methane) extracts was investigated at different concentrations (100, 200, 300, 400, and 500 µg/ml), with acarbose serving as a control. The absorption of the extract and standard was determined at 540 nm by using UV/Vis spectroscopy to calculate the percent inhibition. According to the findings, the methanol extract of this plant inhibited α-amylase more effectively than the dichloromethane extract. Both extracts, however, were less effective against α-amylase than regular acarbose. **Tables 2 and 3** provide the % inhibition and IC_{50} values of extracts/standards while (**Figures 4 and 5**) provide a comparison of α-amylase inhibition and IC_{50} values of standard acarbose, dichloromethane and methanol extract.

Table 2: α-amylase inhibition by *Achyranthes aspera* extracts and Acarbose at different concentrations

Concentration (µg/ml)	Acarbose	Methanol	Dichloromethane
100	71.44 ± 0.001	64.545 ± 0.001	53.066 ± 0.010
200	73.05 ± 0.001	69.078 ± 0.010	57.126 ± 0.003
300	77.41 ± 0.001	71.208 ± 0.011	61.237 ± 0.008
400	81.29 ± 0.002	74.101 ± 0.008	65.176 ± 0.015
500	83.55 ± 0.002	78.071 ± 0.001	69.337 ± 0.013

Table 3: IC_{50} of Dichloromethane and methanolic extract of *Achyranthes aspera* and acarbose at various concentrations

Samples	IC_{50} values (µg/ml)
Acarbose	13.10 ± 0.017
Methanol	21.58 ± 0.020
Dichloromethane	84.73 ± 0.012

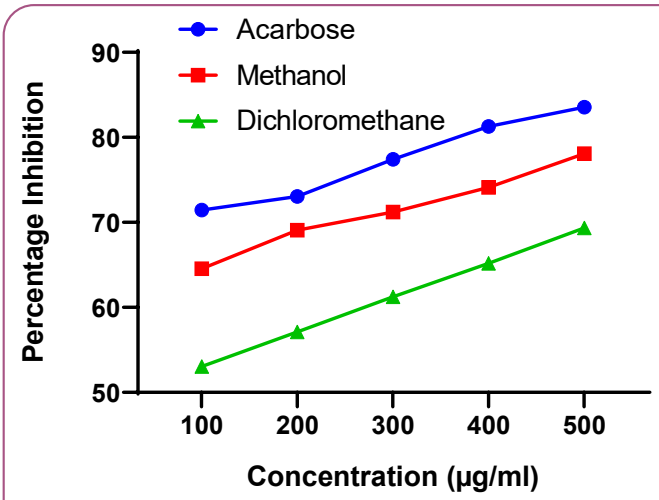

Figure 4 α-amylase inhibition properties of acarbose, dichloromethane and methanol extract of Achyranthes aspera at different concentrations.

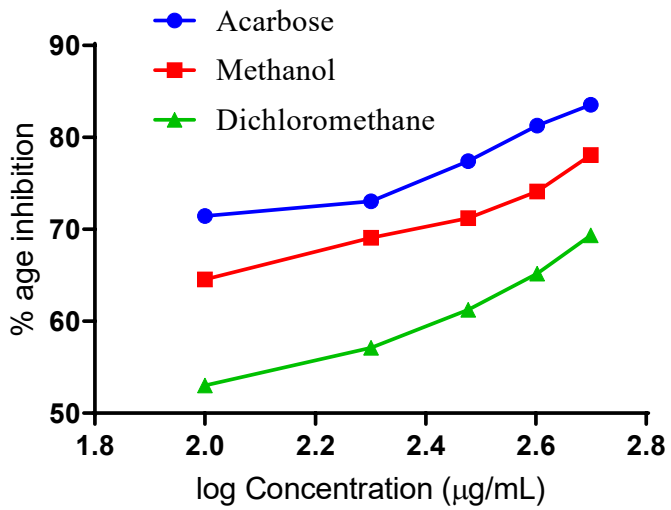

Figure 5 IC_{50} of acarbose, dichloromethane and methanol extract of Achyranthes aspera at different concentrations

Discussion

DM is the most prevalent insulin resistance condition, influencing 2.8 percent of the global population and expected to rise to 5.4 percent by 2025. It is expected that enhanced oxidative stress, caused by inefficiencies of protein-glycation and oxidation of glucose, will strength the etiology of DM. Many investigations have also suggested the intake of antioxidants in conjunction with others anti-diabetic medications [14]. Plants have a wide range of compounds and are known to be a major source of anti-hyperglycemic drugs. Thus attentive attention was drawn to the increasing need for safer medication standardization of plant-derived drugs [15]. Our analysis is aimed at analyzing the therapeutic potential of A.aspera from the point of view of the above procedural features and establishes criteria to prove its validity.

DPPH activity is considered to be the main and simplest tool for the evaluation of antioxidant activity. Prior phytochemical findings have also shown that plant extracts from Achyranthes aspera includes many phenolic compounds which are powerful antioxidants including quercetin, phenolic acids such as ellagic and gallic acid [16- 17]. The antioxidant ability of Achyranthes aspera extracts resulted the presence of phenolic compounds. Based on the results obtained, it was inferred that methanol extract Achyranthes aspera plant showed maximum antioxidant efficiency in comparison with other extracts also showed that methanol extract possesses the highest total phenol count and important anti-radical activity. So, in this sense it is likely that phenolic contents embedded in methanol fraction have appropriate structures that are used to scavenge the free radicals. Furthermore, it is important to notice that dichloromethane fraction also contained radical scavenging property which is not documented to date, this may mean that dichloromethane fraction of Achyranthes aspera leaves contains not only deserted

the less polar molecules but also these molecules act as scavenging agent against unpaired radicals of DPPH [18-19].

HPLC data also shows that methanol extract of Achyranthes aspera contains maximum quercetin level i.e. 55.965 %, which further strengthen the antioxidant properties of this plant. Hence, it is suggested the use of antioxidants in conjunction with anti-diabetic medications which weaken the symptoms of DM [14].

α-amylase is known to be an effective pancreatic and salivary enzyme for transforming large insoluble starch molecules into easily absorbable form. By comparison, α-glucosidase, which is primarily in the small intestine, catalyzes the conversion of broad carbohydrate molecules into readily absorbable monosaccharide glucose. α-glucosidase and α-amylase inhibitors are often referred to starch blockers as they are meant to slow the rate at which starch absorption occurs in the body from this point of view, they provide an efficient means of reducing postprandial glucose levels [20-21]. The α-amylase inhibitory activity is recognized a significant therapeutic strategy for maintaining postprandial sugar levels in blood, especially in type-2 diabetic patients. Synthetic inhibitors of α-amylase such as acarbose, the commonly clinically used miglitol has a variety of adverse effects including hypoglycemia, extreme gastrointestinal unpleasant sensations such as flatulence and diarrhea [21].

Previous anti-diabetic research on this plant showed that methanol extract of Acyranthes aspera had a major anti-hyperglycemic effect in diabetic rats (STZ-NIC), but the action mechanism for anti-hyperglycemic behavior remains unclear [22]. In this opinion, the current research indicates that the possible mechanism to exhibit the hypoglycemic effect of this plant may be inhibition or reduction of pancreatic enzymes (amylase) of test animals inducted in earlier analysis. In addition, α-amylase inhibition activity of Achyranthes aspera extracts such as methanol and dichloromethane) may be possible due to occurrence of various phytochemicals, including various phenolic contents (flavonoids), terpenoids and tannins [20-23].

Conclusion

The current study might be a beneficial addition to the plant's medicinal application. Antioxidant properties and α-amylase suppression were observed in several extracts of this plant (methanol and dichloromethane). HPLC examination of A. aspera methanolic extract indicated the presence of phenolic compounds (quercetin). This study indicates that the plant might be used to treat a variety of ailments such as diabetes mellitus. However, more attention is required at the molecular level for the extraction and purification of active constituents, as well as the confirmation of their mode of action.

Acknowledgments

We are grateful to Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, and the University of Lahore for providing research facilities and supports. Without their support this research could not be possible.

Conflicts of Interest

The authors declare no conflict of interest.

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