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Harnessing traditional Indian medicine: evaluating the *in vitro* anti-diabetic parameters of *Arundo donax* root extract

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Abstract
This work aims to evaluate the potential for Arundo donax root extract, which is well known as an ingredient of traditional Chinese as well as indian medicine, to prevent diabetes and to compare the results with the commonly-used prescription medicines. In a series of *in vitro* assays the extract has shown pronounced inhibitory activity against α-amylase and α-glucosidase with the calculated IC_{50} values of 10 µg/ml and >9 µg/µl, respectively, which indicates that the investigated extract has the potential for an effective treatment of type 2 diabetes. Moreover, the DPPH assay of the extract yielded a feeble radical scavenging activity of 57.28% at a concentration of 10µg/ml which shows that the extract has good antioxidant potential than that of ascorbic acid.. Altogether, these data indicate the feasibility of Arundo donax root extract in anti-diabetic activity considering the area of traditional Indian medicine. Subsequent studies are required to better understand how it works and to confirm its potential uses in treatment.

Keywords: *Arundo donax,* traditional Indian herbal, α-amylase, α-glucosidase, DPPH

Introduction

Diabetes mellitus is a major public health problem in the world today the current global prevalence being estimated at 537 million and expects to increase dramatically in the next few decades. These are an adult onset type that is caused by a long-standing hyperglycemia arising from defects in insulin secretion or action, which results in complications such as cardio vascular disorders, and neuropathies [1]. Oral hypoglycemic agents like metformin, sulphonylureas and insulin have remained widely used for synthetic antidiabetic drugs whereas long-term efficacy and side effects are known to be shortcoming [2]. This has led to increasing concern for searching natural products from sources such as medicinal plants which provide bioactive products with lesser side effects but with more therapeutic values [3]. Natural medicine for diabetes has benefited from the Traditional Indian Medicine (TIM) more than anything in its growth. Since antiquated, TIM has employed an array of herbs aiming at rebalancing, revitalizing the body and supporting the entire organism [4]. Numerous studies suggest that most herbal drugs involved in this present study; Panax ginseng, Astragalus membranaceus, and Berberis aristata, have possible hypoglycemic effects, improve or Dop-optimize the action of insulin and prevent diabetic complications [5]. Such formulations show efficacy in antidiabetic management due to their antioxidant, anti-inflammatory, and enzyme inhibition properties. The following plant with antidiabetic activity is relatively unknown but has lately gained much attention, Arundo donax, also known as giant reed. Arundo donax is a perennial reed wild plant belonging to Poaceae family, native to the temperate and subtropical regions of Asia, Europe and part of Africa [6]. Specific parts of A. donax have been known to be used in traditional medicine as anti-inflammatory, antimicrobial and diuretic. There is evidence that this plant contain bioactive composite like flavonoids alkaloids and phenolic acids which may explain why it has antidiabetic effect [7]. Consistent with various studies, *Arundo donax* has deemed to possess a potential for antidiabetic activity and modulating the mechanisms pertinent to glucose homeostasis and oxidative stress. Other bioactive constituents of A. donax may pose potential α-amylase and α-glucosidase inhibitory effects, implicated in carbohydrate hydrolysis that indeed reduces post-prandial high blood sugar levels.

Actually, it may decrease oxidative stress that plays a role in the onset of insulin resistance and the development of diabetes in general [8]. Specific objectives of this paper include; establishing the enzyme inhibitory effects of *Arundo donax*, To determine the antioxidant activity of *Arundo donax*. It is hoped that understanding its mode of action may lead to the discovery of additional ways of including this plant in indian medicine and modern phytotherapy for diabetic treatments [9].

Materials and Methods

Collection and Pretreatment of *Arundo donax*

Adult roots of Arundo donax were harvested from a plant of northern plane in the phoolpur pryagraj area of uttar pradesh, India. The roots were sun dried under a clean polythene bag for 6-7 days until dry and was later milled into coarse powder. The dried plant material was kept in airtight containers at room temperature to avoid case of absorption of moisture [10, 11]. The plant identifies were scientifically cross-checked with Botanical Survey of India, central regional center, Allahabad and has the Ref no [2022-23/671].

Extraction of *Arundo donax* Roots

The powdered samples whose particle size was below 0.5mm since more surface area is better were subjected to Soxhlet extraction with ethanol as the solvent [12, 13]. The solvent, ethanol, was heated, vaporized, and the vapors rose into the Soxhlet chamber, and became liquid to drip back into the extraction flask. This process was being repeated until all soluble compounds had been extracted from the solution. The ethanol was subsequently removed from extract using a rotary evaporator at room temperature [14-16].

HPTLC Analysis

Preparation of Standard Solutions (Gallic Acid)

For standard marker preparation, 10 milligrams of Gallic acid in each were dissolved using 10 milliliters of methanol [17-18]. From the working stock solutions 0.1mg/mL concentrations were prepared and aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mL were pipetted into 10 mL volumetric flasks to yield concentrations of 1, 2, 3, 4, 5 and 6 µg/mL [19].

Solvent System

Toluene: ethyl acetate (7:3 v/v) [20].

TLC Plate

Silica gel coated aluminum plate F254 60 (10x20 cm with 0.2 mm layer Merck, Germany.

Method

Test samples were eluted on pre-coated silica gel aluminum plate with a Camag Thin Layer chromatography Sample applicable with 100µL Hamilton syringe [21-23]. Strips of 6 mm thickness were observed at 15 mm distance from the bottom of the plate and 1.0 to 6.0 µL of Betasitosterol and Gallic acid standards were applied likewise [24-26]. The mobile phase used was toluene: Ethyl acetate and acetic acid (7:3 v/v) were used as the mobile phase and the linear ascending development was done in a glass chamber containing the mobile phase was allowed to equilibrate at room temperature for 30 minutes. After development, plates were dried and the bands were scanned using a Camag Scanner 4. A calibration plot of peak areas against the concentrations of standard markers was made [27-29].

In-vitro Activity

α-Amylase Activity

A 500 µL solution of 10 µg/mL test extract was mixed with 500 µL of 0.02 M sodium phosphate buffer (pH 6.9, 0.006 M NaCl) containing Hog pancreatic α-amylase (0.5 mg/mL) and incubated at 25 °C for 10 minutes. A 500 µL solution of 1% starch was added, and the mixture was incubated at 25 °C for another 10 minutes [30]. The reaction was terminated by adding 1 mL of DNSA, followed by boiling for 5 minutes. After cooling, 10 mL of distilled water was added, and the absorbance was measured at 540 nm using a plate reader. The percentage of α-amylase inhibition was calculated as [16]:

% Inhibition = [(Abs Untreated – Abs Samples) / Abs Untreated] × 100 [31, 34].

α-Glucosidase Activity

A 50 µL solution containing test extract at concentration of 10 µg/mL was incubated with 100 µL α–glucosidase solution at 25 °C for 10 minutes. Then, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside in 0.1M phosphate buffer, pH 6.9 was added and the reaction mixture was allowed to react for 5 minutes. Optical density was determined at 405 nm wavelength. Percentage of α-glucosidase inhibition was determined in the same manner [32, 34].

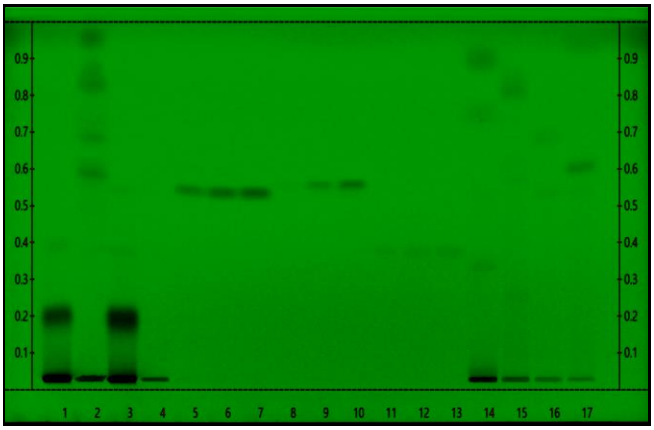
Antioxidant Activity

DPPH Assay

Extract of Arundo donax was evaluated for the DPPH radical scavenging activity by adding 100 µg/mL of extract to 3 mL DPPH solution of 6 × 10^{-5} M in methanol prepared freshly [33]. The mixture was allowed to stand in the dark for 15 minutes, after which absorbance was taken at 517 nm. The lower absorbance pointed to the scavenging activity, and BMG Mars software was used for analysis [33, 34].

Result & Discussion

HPTLC



Abbreviation- Track-2: Sample R1; Track-4: Sample R1; Track-5: Gallic acid standard; Track-6: Gallic acid standard; Track-7: Gallic acid standard; Track-8: Gallic acid standard; Track-9: Gallic acid standard; Track-10: Gallic acid standard; Track-14: Sample R1.

Fig 1: Graph of HPTC analysis of *Arundo donax* root Extract

The HPTLC study of Arundo donax yielded a significant result, with 1.78% gallic acid detected in the test sample (Table 1). The bioactive chemical gallic acid has antioxidant

and anti-diabetic effects. The extract of Arundo donax has a high gallic acid content, which means it might be used therapeutically to manage oxidative stress and diabetes.
The values of Rf at 254 nm, before derivative scaling:
The following Rf values were detected in the HPTLC fingerprint study, which was conducted at 254 nm (Table 2):

Sample (R1): A black spot, or clearly visible band, was seen at an Rf value of 0.58 in the test sample.
An Rf value of 0.58 (black spot) was also seen in the gallic acid standard marker.

Gallic acid is present in the Arundo donax extract since the Rf value of 0.58 is the same in the standard and the sample. Gallic acid, which absorbs heavily at 254 nm, is characterized by the black spot seen at this wavelength; this fact further confirms the compound's identification.

Table 1: HPTLC analysis of *Arundo donax* root Extract

Sr. No.	Test Parameter	Units	Results	Method of Testing
2.	Gallic Acid	%	1.78	By HPTLC

Table 2: Rf values of HPTLC fingerprints profile of test samples at 254nm before derivatization

Rf values →	Rf1	Rf2
Sample R1	-	0.58 (black)
Gallic Acid Standard Marker	-	0.58 (black)

Effect of samples R1 on α amylase
At 10 µg/ml, the Arundo donax sample R1 inhibited α-amylase the most effectively, with a 53% inhibition rate compared to 77.42% for the gold standard medication acarbose. This effect was dosage dependent. At 0.001 µg/ml, the inhibitory impact was low at 2.82%, and it reduced as the concentration of R1 dropped. In comparison to acarbose, which had a lower IC_{50} of 1 µg/ml, R1 needed a greater dosage of 10 µg/ml to achieve 50% inhibition (Table 3) (fig 2). Compared to acarbose, R1's potency in blocking α-amylase is lower. Based on these findings, it seems that more research and improvement are necessary to maximize the anti-diabetic effects of Arundo donax and make it more successful than current therapies.

Table 3: Effect of samples R1 on α amylase

Concentration µg/ml	R1(%inhibition)	Acarbose (% inhibition)
10	53	77.42
1	33.59	52.51
0.1	27.43	41.35
0.01	6.59	25.22
0.001	2.82	16.50
IC_{50} VALUE µg/ml	10	1

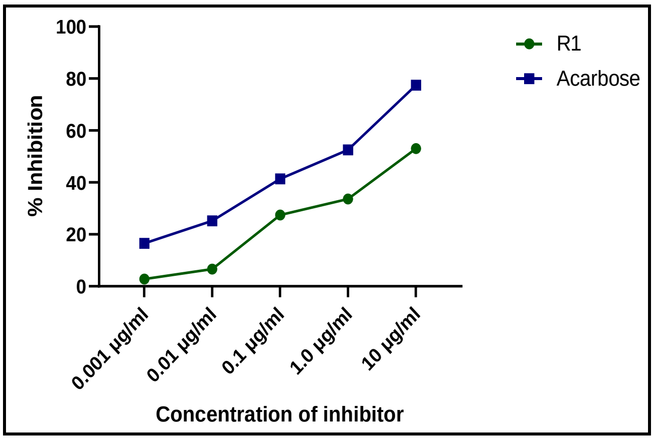


Fig 2: A graph for Comparison of α-Amylase Inhibitory Activity of R1 Extracts of *Arundo donax* Roots

Effect of sample R1 on α Glucosidase:
Similar to the conventional medication acarbose, the Arundo donax sample R1 showed a concentration-dependent inhibitory impact on α-glucosidase. R1 exhibited 56.45% inhibition at 10 µg/ml, a lower level than acarbose's 72.2% inhibition. The inhibitory activity of R1 reduced as the concentration dropped, reaching a minimum of 3.51% inhibition at a concentration of 0.001 µg/ml. Similarly, at a concentration of 0.001 µg/ml, acarbose exhibited an 8.4% suppression of inhibitory action. Although R1 does inhibit α-glucosidase to a lesser extent than acarbose, its inhibitory impact is still noticeable due to the fact that both IC_{50} values were more than 9 µg/ml and >2 µg/ml, respectively. This indicates that Arundo donax might be useful as an inhibitor of α-glucosidase, however it takes greater doses than common anti-diabetic medications like acarbose to provide noticeable suppression.

Table 4: Effect of samples R1 with Acarbose as standard on α-Glucosidase

Concentration µg/ml	R1(%inhibition)	Acarbose (% inhibition)
10	56.45	72.2
1	34.15	41.50
0.1	25.67	16.66
0.01	5.87	11.6
0.001	3.51	8.4
IC_{50} VALUE µg/ml	**> 9**	**> 2**

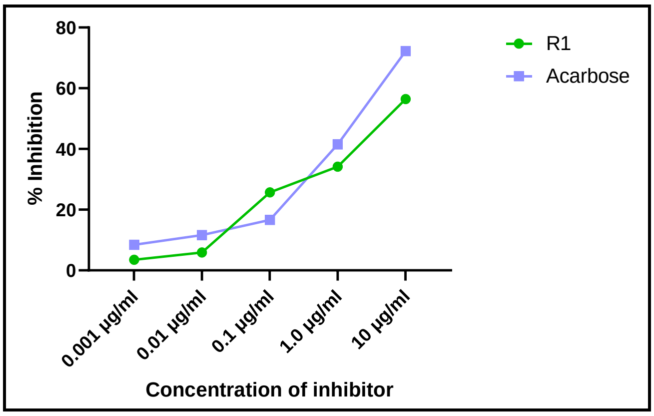


Fig 3: A graph for Comparison of α-Glucosidase Inhibitory Activity of Root extract, R1 Extracts of *Arundo donax* Roots

DPPH Assay:
Possible antioxidant capabilities were shown by the modest DPPH radical scavenging activity of the ethanolic root extract (R1) of Arundo donax. Compared to ascorbic acid, which exhibited a 68.26% suppression of DPPH radicals at its maximum concentration of 10 µg/ml, R1 exhibited a lesser inhibition of 57.28%. With a minimum inhibition of 1.75% at a concentration of 0.001 µg/ml, the antioxidant

activity of R1 declined as the concentration dropped. Throughout all doses, ascorbic acid showed more potent scavenging action, with an IC_{50} value of 2 µg/ml, compared to R1, which had an IC_{50} value of >7 µg/ml. These results indicate that while Arundo donax does have antioxidant characteristics, it is not as effective as ascorbic acid, especially at lower doses.

Table 5: DPPH Radical Scavenging Activity of Ethanolic (R1) Extracts of *Arundo donax* root Compared to Ascorbic Acid

Concentration µg/ml	R1(% inhibition)	Ascorbic Acid (%inhibition)
10	57.28	68.26
1	36.67	42.38
0.1	18.54	26.48
0.01	6.35	14.59
0.001	1.75	3.61
IC_{50} VALUE µg/ml	**>7**	**2**

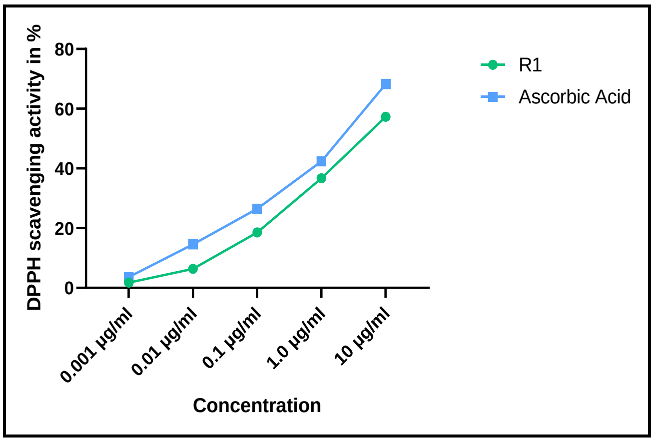


Fig 5: DPPH Scavenging Activity

Discussion

The present investigation on the phytochemical and therapeutic investigation of Arundo donax root extract (R1) revealed novel pharmacological aspects towards diabetes and related metabolic disorders. The extract shown moderate to strong anti α-amylase and α-glucosidase activity indicating its potential for modulating postprandial hyperglycemia. This action may also partially hinder carbohydrate digestion and absorption and therefore contribute to lower blood glucose levels as observed at a concentration of 10 µg/ml R1 inhibited 53% α-amylase and 56.45% α-glucosidase with IC50 values of 10 and >9 µg/ml respectively.

Besides the enzyme inhibition, the antioxidant activity of the R1 ethanol extract was determined using DPPH assay that had shown a concentration dependent radical scavenging effect. At a concentration of 10 µg/ml the extract inhibited the enzymes by 57.28% while the most potent control ascorbic acid which is an antioxidant inhibited the enzyme by 68.26%. Specifically, using the *in vitro* antioxidant activity quantitative model, it was observed that R1 exhibits an IC50 value >7 µg/ml, which shows that R1 has a strong antioxidant effect that may also help in reducing oxidative stress, which is characteristic of diabetes patients.

Conclusion

The overall results of the present study suggest that Arundo donax root extract has the potential for the development of antidiabetic agents due to similar levels of inhibition of carbohydrate-hydrolyzing enzymes, considerable antioxidant activity. The versatile properties of the extract as suggested by this study's findings such as antioxidant, α-amylase and α-glucosidase activity. These findings challenge existing knowledge regarding Arundo donax and should stimulate subsequent studies aimed at identifying the individual constituents in the plant and their functioning in the body, factors that can spur the creation of new classes of phytotherapeutic agents for diabetes mellitus. Therefore, Arundo donax can be viewed as a promising adjuvant to diabetes and excellent solution to control glycemia and enhance the patients' metabolic profile.

Conflict of Interest

None

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