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SHORT COMMUNICATION

Evaluation of Antioxidant and Anti-diabetic Activity of Stem Bark of *Ziziphus jujuba* (L.) Gaertn

S. Shanthi*, S. Latha, G. Karthikeyan, P. Jeeva, A. Janani and K. Karthick

Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai – 600116, Tamil Nadu, India; shanthi.s@sriramachandra.edu.in

Abstract

Diabetes Mellitus (DM), a significant metabolic disorder that increases mortality and morbidity, affects people all over the world. Recently, many individuals are exploring CAM treatment that may have fewer side effects due to the prominent side effects of allopathic medications. *Ziziphus jujuba* (L.) Gaertn. (Rhamnaceae) stem bark has been a vital part of the conventional system of medicine for the management of diabetes, inflammation, wounds, fever, asthma and liver disorders. In spite of its pharmacological significance, *Ziziphus jujuba* stem bark lacks scientific evidence of its antidiabetic and antioxidant potential. Hence, an attempt has been made to evaluate the anti-diabetic effect of the stem bark of *Ziziphus jujuba* using alpha-amylase inhibition assay. In this research work, we further investigated the antioxidant activity of the *Ziziphus jujuba* using DPPH and Nitric oxide radical scavenging assay. The Chloroform (CEZJ), ethyl acetate (EAZJ) and Ethanol (EEZJ) extracts of *Ziziphus jujuba* stem bark were used in the study. In the DPPH radical scavenging assay, EEZJ had an IC50 value of 117.1 µg/ml, however, the value for the nitric oxide scavenging assay was 64.65 µg/ml. The *Ziziphus jujuba* was additionally examined for its *in-vitro* antidiabetic potential using an alpha-amylase inhibition assay, and it was discovered to possess a considerable percentage of alpha-amylase inhibition. The IC50 of EEZJ was found to be 34.68 µg/ml. These results imply that the *Ziziphus jujuba* possesses considerable antioxidant and anti-diabetic properties. To thoroughly establish the anti-diabetic potential of *Ziziphus jujuba*, additional *in vitro* experiments utilizing cell lines and other enzymes may be conducted.

Keywords: α Amylase, Bark, Diabetes, *Ziziphus jujuba*

1. Introduction

A serious metabolic illness called Diabetes Mellitus (DM) causes abnormalities in the metabolism of lipids, carbohydrates, and proteins as well as high plasma glucose levels as a result of insulin resistance, inadequate insulin, or both. The leading metabolic disease responsible for mortality and morbidity worldwide is Diabetes Mellitus (DM). Approximately 2.8% of the world's population currently has it, and by the year 2025, that number is expected to increase to nearly 5.4%. It is one of the most prevalent metabolic disorders[1]. Macrovascular issues, which affect the heart, brain, and extremities, and microvascular complications, which impact the blood vessels of the eye, kidney, and nerves, can be roughly categorized as diabetes-related complications (diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy). Compared to non-diabetics, people with diabetes have a higher risk (around two to four times higher) of having Coronary Heart Disease (CHD) and stroke. In addition, poorly managed diabetes can have an impact on the developing foetus in pregnant women, which can result in birth abnormalities in the newborn[2,3]. Diabetes patients experience cellular and organ damage because of oxidative stress, which is brought on by an imbalance between the production of Reactive Oxygen Species (ROS) and their scavenging by endogenous

*Author for correspondence

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antioxidants[4]. Increased antioxidant supplementation can mitigate these effects[5]. There are several different therapy modalities available today to manage diabetes. Only insulin injections can be used to manage type 1 diabetes. Oral hypoglycemic treatments such as glucosidase inhibitors, sulphonylureas, biguanides, thiazolidinediones, and meglitinide analogues are currently used to treat type 2 diabetes. When diet, exercise, and weight loss are unable to regulate blood glucose levels, these medications can be beneficial. However, the development of complementary and alternative medicines with negligible or nonexistent adverse effects is necessary due to the side effects associated with the use of such pharmaceuticals. The study of extracts to learn about their effectiveness and safety, along with its mechanism of action has sparked an increase in interest in phytomedicine, conventional formulations, and these remedies[6]. People in India use a variety of conventional medical practices to manage diabetes, including Ayurveda, Siddha, Unani, and Homeopathy.

Inhibiting α-glucosidase and α-amylase to reduce glucose absorption in the intestine is one of the therapeutic strategies being used in the therapy of type 2 DM. Alpha-amylase (-1,4-glucan-4-glucanohydrolases) is a well-known salivary and pancreatic secretory product that initiates the intestinal mucosa's conversion of complex carbohydrates into a mixture of oligosaccharides and disaccharides. Alpha-glucosidase further causes the digestion of these sugars into monosaccharides by its action. The present alpha-amylase and glucosidase inhibitors in clinical usage are constrained in their ability to treat diabetes and its complications due to side effects including hypoglycemia, diarrhoea, gas, and intestinal bloating[7]. Therefore, there is a significant need to look for complementary and alternative therapies that have few adverse effects and can be used as a supplement to the treatment of diabetes mellitus.

The antihyperglycemic effects of plants are mostly brought about by their capacity to raise insulin secretion, restrict glucose absorption in the intestine, or promote the action of metabolites in insulin-dependent activities. The literature lists more than 400 kinds of medicinal plants that have hypoglycemic effects. Yet, the quest for novel anti-diabetic agents derived from plants is still alluring since they have compounds that show different and secure effects on diabetes mellitus. Most plants have secondary metabolites such as glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc. that are frequently associated with having antidiabetic effects. The selected plant *Ziziphus jujuba* (L.) Gaertn. (Rhamnaceae) is used traditionally in India for the treatment of diabetes[8-14]. Even though the plant is used for many years, there is a lack of evidence for its therapeutic efficacy against diabetes. Thus, the present research work is aimed to investigate the antidiabetic activity of *Ziziphus jujuba* stem bark by *in-vitro* alpha amylase method besides determining its anti-oxidant activity *in vitro*.

2. Materials and Methods

2.1 Collection of Plant Materials

The stem bark of the plant, *Ziziphus jujuba* (L.) were collected from the herbal garden of Sri Ramachandra Institute of Higher Education and Research (SRIHER), Porur, Chennai, and authenticated by a botanist. Dr. P. Jayaraman, Plant Anatomical Research Centre (PARC), Tambaram, Chennai and the voucher specimen number was PARC/2019/2567.

2.2 Preparation of Extracts[15]

The stem barks of *Ziziphus jujuba* were gathered, dried, and ground into a coarse powder. It was then passed through sieve (mesh no: 80). The coarse powder (500 g) was macerated with petroleum ether (PEZJ), chloroform (CEZJ), ethyl acetate (EAZJ) and ethanol (EEZJ) separately for 72h, 48h and 24h with occasional shaking. In order to completely remove the solvent, the extract was filtered and concentrated using a rotary vaccum evaporator (Superfit, Supervac). The concentrated extract was stored in an airtight container.

2.3 Determination of Physicochemical Parameters

Physicochemical parameters to assess the purity were done according to the standard procedures[16]. Ash value, extractive value and loss on drying were determined according to the standard methods of Indian Pharmacopoeia. Ash value indicates the Inorganic material whereas the extractive value

represents the active constituents. Determination of loss on drying is the measurement of volatile principles and moisture content.

2.4 Preliminary Phytochemical Analysis

The PEZJ, CEZJ, EAZJ and EEZJ extracts were subjected to preliminary phytochemical investigations. The extracts were investigated for the presence of various primary and secondary metabolites according to standard procedures[17].

2.5 DPPH Free Radical Scavenging Assay[18]

Wickramaratne *et al.,* have states that DPPH was newly dissolved in ethanol to create a DPPH (0.2 mM) solution[18]. Following that, the freshly made solution was set aside (in the dark) until usage. One ml of various extract concentrations (50, 100, 200, 400, 800, and 1000 µg/ml) was added to one ml of DPPH solution. To make the control, ethanol solution was used in place of the extract. The resulting combination was then left to incubate for around 30 minutes at room temperature in the dark. A UV-Visible spectrophotometer (Perkin elmer, LAMBDA 35 set to 517 nm was used to measure the final absorbance after 30 minutes. As a reference, quercetin was used. The results of each determination were made three times. Using the following formula, the extracts' percentage radical scavenging activity was determined.

Percentage DPPH Scavenging activity

$$= \left\{ \frac{ABS_{CONTROL} - ABS_{SAMPLE}}{ABS_{CONTROL}} \right\} \times 100$$

ABS - Absorbance

2.6 Nitric Oxide Radical Scavenging Assay[19]

The Griess assay technique was used to perform the nitric oxide scavenging assay, according to Revathi and Rajeswari[19]. Various extract concentrations (50, 100, 200, 400, 800, and 1000 µg/ml) were combined with 2.0 ml of Sodium nitroprusside (10 mM) in phosphate-buffered solution before being incubated for 4 hours at 37 °C. The aforementioned solution was incorporated into 0.5 ml of Griess reagent (1% Sulphanilamide, 2% Phosphoric acid and 0.1% Naphthyl ethylene diamine dihydrochloride in distilled water) after the incubation period. Without using any samples, the control solution was made. A UV spectrophotometer was used to measure the absorbance at 546 nm. The standard used was vitamin C and each determination was made three times.

2.7 Alpha-amylase Inhibition Assay[20]

According to Shanthi *et al.*, the assay was conducted using the 3,5-Dinitrosalicylic acid (DNSA) reagent[20]. A 500 µl of enzyme solution and 1 ml of extract at various concentrations (50, 100, 200, 400, 800, and 1000 µg/ml) were combined, and the mixture was then incubated at 37 °C for 10 min. After that, each test tube received 500 µl of the starch solution, and they were all incubated at 37°C for 10 min. After 5 minutes of incubation in a bath of boiling water, the reaction was stopped by adding 1 ml of DNSA solution. Following cooling and dilution with 10ml of water, it was detected at 540 nm. 100% enzyme activity is represented by the control. By including a suitable control without enzyme or starch, absorbance resulting from the test sample was eliminated. The standard utilized was acarbose. Three duplicates of each determination were made.

$$\% \text{ INHIBITION} = \frac{EC - (ET - TC)}{EC} \times 100$$

3. Results

Ziziphus jujuba is a little tree or shrub in the family Rhamnaceae. The stem bark has long been used in traditional medicine to treat conditions like diabetes, inflammation, wounds, fever, asthma, and liver diseases. Despite its pharmacological importance, there isn't enough proof to support the antioxidant and anti-diabetic properties of the stem bark of *Ziziphus jujuba*. Hence, an attempt has been made to evaluate the anti-diabetic effect of the stem bark of *Ziziphus jujuba* using alpha-amylase inhibition assay. In this study, we further evaluated the anti-oxidant effect of the plant using DPPH and Nitric oxide radical Scavenging assay. Physico-chemical parameters such as ash values, extractive values and loss on drying were carried out using standard procedure. The results are given in Table 1. The extracts were prepared with petroleum ether, chloroform, ethyl acetate and ethanol separately by maceration process. The Preliminary phytochemical study was carried

out for CEZJ, EAZJ and EEZJ of the stem bark of *Ziziphus jujuba* and the results were tabulated in Table 2. Phytochemical Investigations revealed the presence of alkaloids, quinones, terpenoids, flavonoids, phenols, tannins, saponins, glycosides, carbohydrates, coumarins and steroids.

3.1 DPPH Radical Scavenging Activity

The *invitro* antioxidant activity of CEZJ, EAZJ and EEZJ extracts of the bark were performed using DPPH free radical scavenging assay. The EEZJ exhibited more activity than the CEZJ and EAZJ extracts. At the concentration of 1000 µg/ml, the EEZJ, CEZJ and EAZJ possess the percentage inhibition of 83.09 %, 79.96 % and 77.63 % respectively. The EEZJ showed percentage inhibition in concentration dependent manner and maximum inhibition was observed at 1000 µg/ml. The IC50 values of the extracts are tabulated in Table 3. The IC50 values of CEZJ, EAZJ and EEZJ were found to be 313 µg/ml, 144.1 µg/ml, and 117.1 µg/ml respectively. The IC50 value of the EEZJ shows a quicker response than the other two extracts.

Table 1. Physico-chemical parameters of *Ziziphus jujuba* stem bark powder

S. No.	Parameters	Percentage W/W
I Ash Values		
1	Total Ash	7.3 ± 0.06
2	Acid insoluble Ash	1.15 ± 0.01
3	Water insoluble Ash	4.85 ± 0.02
II Extractive Values		
1	Water soluble extractive	26.4 ± 0.07
2	Ethanol soluble extractive	17.3 ± 0.05
III. Loss on Drying		
		3 ± 0.06

Each value represents mean ± SD (n=3)

3.2 Nitric-Oxide Radical Scavenging Assay

The invitro antioxidant activity of CEZJ, EAZJ and EEZJ extracts of the bark was performed using Nitric oxide radical scavenging assay. The EEZJ exhibited more activity than the CEZJ and EAZJ extracts. At 1000 µg/ml, the EEZJ, CEZJ and EAZJ extracts showed the percentage inhibition of 72.35 %, 53.99 % and 72.45 % respectively. The IC50 values of the extracts are tabulated in Table 4. The IC50 values of CEZJ, EAZJ, and EEZJ were found to be 76.77 µg/ml, 65.85 µg/ml, and 64.65 µg/ml respectively. The potent antioxidant activity was observed with EEZJ extract and it was confirmed by IC50 value.

Table 2. Phytochemical screening of various extracts of *Ziziphus jujuba* stem bark

S. No	Constituents	Chloroform	Ethyl Acetate	Ethanol
1	ALKALOIDS	+	+	+
2	QUINONES	-	+	-
3	TERPENOIDS	+	+	+
4	FLAVONOIDS	-	+	+
5	PHENOL	+	-	-
6	TANNINS	-	+	+
7	SAPONINS	+	-	+
8	GLYCOSIDES	+	+	+
9	CARBOHYDRATE	+	-	-
10	PROTEIN	-	-	-
11	COUMARINS	+	-	-
12	STEROIDS	+	+	+

" + " = Present " – " = Absent

Table 3. Effect of different extracts of *Ziziphus jujuba* in DPPH radical scavenging activity

S. No.	Concentration (µg/ml)	Percentage Scavenging			
		Standard (Quercetin)	CEZJ	EAZJ	EEZJ
1	50	76.29 ± 0.40	60.44 ± 0.85	50.42 ± 0.15	40.67 ± 0.24
2	100	81.45 ± 0.41	60.55 ± 0.13	60.22 ± 0.11	56.84 ± 0.39
3	200	83.61 ± 0.45	64.99 ± 0.27	69.48 ± 0.10	76.83 ± 0.23
4	400	83.77 ± 0.14	74.33 ± 0.55	72.08 ± 0.67	82.45 ± 0.28
5	800	84.62 ± 0.24	75.38 ± 0.47	77.48 ± 0.48	82.77 ± 0.71
6	1000	84.88 ± ss0.41	79.96 ± 0.23	77.63 ± 0.18	83.09 ± 0.35

The value represents Mean ± Standard Deviation of the triplicates
CEZJ - Chloroform extract of *Ziziphus jujuba*; EAZJ-Ethyl acetate extract of *Ziziphus jujuba*; EEZJ- Ethanol extract of *Ziziphus jujuba*.

Table 4. Effect of different extracts of *Ziziphus jujuba* in Nitric oxide radical scavenging assay

S. No	Concentration (µg/ml)	Percentage Scavenging			
		Standard (Ascorbic Acid)	CEZJ	EAZJ	EEZJ
1	10	66.81 ± 0.46	28.30 ± 0.38	60.38 ± 0.41	24.89 ± 0.21
2	50	75.91 ± 0.36	42.78 ± 0.47	62.02 ± 0.22	50.71 ± 0.21
3	100	77.59 ± 0.38	43.63 ± 0.82	66.45 ± 0.20	53.35 ± 0.11
4	200	78.35 ± 0.52	44.68 ± 0.46	69.13 ± 0.41	60.06 ± 0.48
5	400	78.77 ± 0.37	45.69 ± 0.24	70.58 ± 0.16	63.56 ± 0.51
6	800	78.93 ± 0.65	49.13 ± 0.10	70.65 ± 0.16	67.21 ± 0.57
7	1000	79.83 ± 0.53	53.99 ± 0.17	72.45 ± 0.15	72.35 ± 0.01

The value represents Mean ± Standard Deviation of the triplicates
CEZJ - Chloroform extract of *Ziziphus jujuba*; EAZJ-Ethyl acetate extract of *Ziziphus jujuba*; EEZJ- Ethanol extract of *Ziziphus jujuba*.

3.3 Alpha-Amylase Inhibition Assay

The *in vitro* antidiabetic activity of CEZJ, EAZJ, and EEZJ extracts of the bark was performed using alpha-amylase inhibition assay and the results are shown in Table 5. The chloroform extract exhibited maximum inhibition of alpha-amylase than the EEZJ and EAZJ extracts. At the concentration of 1000 µg/ml, the EEZJ, CEZJ and EAZJ extracts possess percentage inhibition of 81.10 %, 92.21 % and 90.70 % respectively. At a dose of 1000 µg/mL, the Acarbose (Standard drug) had the highest percentage of inhibition (93.27 ± 0.63 %). The findings have shown that the CEZJ of *Ziziphus jujuba* efficiently and in a dose-dependent way suppresses the activity of the alpha-amylase enzyme. At concentrations between 10 and 1000 µg/ml for CEZJ, the alpha-amylase enzyme was inhibited to varying degrees (49.70 ± 0.71 to 92.21 ± 0.16 %). The IC50 values of the CEZJ, EAZJ, and EEZJ extracts were found to be 34.68 µg/ml, 44.98 µg/ml and 127.1 µg/ml respectively (Table 6). The findings of the present study supported the traditional use of *Ziziphus jujuba* for diabetes.

4. Discussion

For the treatment of DM, it is crucial to find and create new medications and treatment plans. The number of medicinal plants being tested for their antidiabetic effectiveness has increased, which is encouraging. One of the most cost-effective ways to reduce the disease burden will be to encourage the urban population

Table 5. Effect of different extracts of *Ziziphus jujuba* in *in vitro* alpha-amylase assay

S. No	Concentration (µg/ml)	Percentage Scavenging			
		Standard (Acarbose)	CEZJ	EAZJ	EEZJ
1	10	14.73 ± 0.18	49.70 ± 0.71	27.23 ± 0.51	53.50 ± 0.12
2	50	17.13 ± 0.22	51.86 ± 0.13	67.98 ± 0.14	58.91 ± 0.95
3	100	29.48 ± 0.53	52.25 ± 0.32	74.36 ± 0.29	64.94 ± 0.18
4	200	34.30 ± 0.33	53.55 ± 0.09	78.09 ± 0.21	69.90 ± 0.57
5	400	65.98 ± 0.13	76.95 ± 0.66	79.98 ± 0.95	80.44 ± 0.98
6	800	88.74 ± 0.11	90.79 ± 0.00	87.57 ± 0.56	80.98 ± 0.23
7	1000	93.27 ± 0.63	92.21 ± 0.16	90.70 ± 0.31	81.10 ± 0.11

The value represents Mean ± Standard Deviation of the triplicates
CEZJ - Chloroform extract of *Ziziphus jujuba*; EAZJ-Ethyl acetate extract of *Ziziphus jujuba*; EEZJ- Ethanol extract of *Ziziphus jujuba*.

Table 6. Effect of *Ziziphus jujuba*in *in vitro* antioxidant and antidiabetic assay (IC50 value)

S. No	Extracts	IC50 (µg/ml)		
		DPPH radical scavenging assay	Nitric oxide radical scavenging assay	Alpha-amylase inhibition assay
1	EEZJ	117.1	64.65	127.1
2	EAZJ	144.1	65.85	44.98
3	CEZJ	313.0	76.77	34.68

CEZJ - Chloroform extract of *Ziziphus jujuba*; EAZJ-Ethyl acetate extract of *Ziziphus jujuba*; EEZJ- Ethanol extract of *Ziziphus jujuba*.

to lead a healthy lifestyle and use herbal remedies with antidiabetic properties, keeping in mind the recommendations made by the WHO (which include regular exercise and consumption of healthy food) for the effective management of type II DM[21].

It has been shown that DM complications are linked to free radical-induced oxidative stress brought on by glucose oxidation and glycosylated protein degradation[22]. To prevent these issues, anti-diabetic drugs are typically advised in addition to antioxidants. In the current study, DPPH and nitric oxide scavenging assays were used to assess the antioxidant capacity of *Ziziphus jujuba*. The EEZJ extract has shown greater efficacy than other extracts, according to a DPPH scavenging assay. The IC50 values of CEZJ, EAZJ and EEZJ were found to be 313 µg/ml, 144.1 µg/ml, and 117.1 µg/ml respectively. Additionally, *Ziziphus jujuba's* activity was on par with that of regular quercetin.

By using a Nitric-oxide radical scavenging assay, the antioxidant effectiveness of *Ziziphus jujuba* was further confirmed. According to the study, the EEZJ of *Ziziphus jujuba* was more active than the CEZJ and EAZJ extract. The IC50 values of CEZJ, EAZJ, EEZJ extracts were found to be 76.77 µg/ml, 65.85 µg/ml, and 64.65 µg/ml respectively. The potent antioxidant activity was observed with EEZJ extract and it was confirmed by IC50 value.

The alpha-amylase inhibition assay was used to determine the anti-diabetic in vitro efficacies of the CEZJ, EAZJ, and EEZJ. The research found that the *Ziziphus jujuba* CEZJ extract was more active than the other extracts under investigation. The IC50 values of the CEZJ, EAZJ and EEZJ were found to be 34.68 µg/ml, 44.98 µg/ml and 127.1 µg/ml respectively. Alpha-amylase inhibition by *Ziziphus jujuba* was comparable to that of the medication acarbose. These "α - amylase inhibitors" work by preventing the conversion of starch

(1, 4-glycosidic bonds) and other oligosaccharides' to simple sugars like maltose and triose[23]. The findings of the present study supported the traditional use of *Ziziphus jujuba* for diabetes.

In the current study, DPPH and Nitric-oxide radical scavenging assays were used to assess the antioxidant capacity of *Ziziphus jujuba*. The findings showed that *Ziziphus jujuba* has significant antioxidant capacity. By using an alpha-amylase inhibition assay, the *Ziziphus jujuba* was also examined for its potential as an *in-vitro* diabetes treatment, and it was discovered to have a significant percentage alpha-amylase inhibition. The results of the study point to strong antioxidant and anti-diabetic action in *Ziziphus jujuba*. This might be because the phytoconstituents of *Ziziphus jujuba* work together synergistically. Therefore, the findings imply that *Ziziphus jujuba* may be useful in lowering starch absorption and may therefore be useful in the control of diabetes.

5. Conclusion

The current study provides scientific substantiation for the stem bark of *Ziziphus jujuba* possessing antioxidant and alpha-amylase inhibition activity *in vitro*. Therefore, it assists in lowering Post-Prandial Hyperglycemia (PPHG), which is extremely beneficial in diabetes. To thoroughly establish the anti-diabetic potential of *Ziziphus jujuba*, additional in vitro experiments utilizing cell lines and other enzymes may be conducted in future.

6. References

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