

Original Research Article

Antioxidant, hypoglycemic and anti-diabetic activities of *Ziziphus spina-christi* (L) Willd (Rhamnaceae) leaf extract

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Abstract

Purpose: To investigate the antioxidant, hypoglycemic and antidiabetic activities as well as the phenolic composition of *Ziziphus spina-christi* (ZSC)

Methods: Eighty percent methanol extract (ZSC-1), as well as ethyl acetate (ZSC-2), *n*-butanol (ZSC-3) and aqueous (ZSC-4) fractions were administered orally to mice at doses of 250 and 500 mg/kg of body weight. The hypoglycemic and anti-diabetic activities of these fractions in the treated mice were evaluated after 7 and 15 days, using glibenclamide as a standard. Antioxidant activities *in vitro* were determined using stable free radical DPPH and ABTS radical scavenging techniques.

Results: The strongest ($p < 0.001$) anti-diabetic activity (25.59 and 39.48 % after 7 and 15 days, respectively) was found following treatment with 500 mg/kg ZSC-3 fraction. Similarly, the highest ($p > 0.001$) hypoglycemic effect was achieved with 500 mg/kg ZSC-3 treatment (29.07 and 35.56 % after 7 and 15 days, respectively). ZSC-1 possessed the highest content of total flavonoids (36.2 mg/g) and total polyphenol (82.3 mg/g).

Conclusion: Fraction ZSC-3 displayed potential hypoglycemic activity while ZSC-1, ZSC-2 and ZSC-3 possess remarkable DPPH scavenging ability equivalent to 89, 96 and 80.3 % of the activity of the standard drug respectively.

Keywords: *Ziziphus spina*, Rhamnaceae, Hypoglycemic, Anti-diabetic, Flavonoids, Polyphenol

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INTRODUCTION

Diabetes mellitus, a metabolic disorder, causes damage to the heart, blood vessels, nerves, kidney and eyes. The disease may be inherited result from a deficiency of insulin production by pancreases and is also related to ineffective insulin production. Within the next 25 years, diabetes is projected to be one of the major causes of death throughout the world. As the current effective oral therapy for the treatment of diabetes causes side effects, new alternatives are required [1].

In modern world, medicinal plants are used to treat many diseases, every year, a large numbers of novel natural product are isolated with efficacy against various diseases. Therefore medicinal plants are a potential source of therapeutic agents [2]. In this study plants we assessed the potential of *Zizyphus spina-christi* which belong to Genus *Zizyphus* and family Rhamnaceae. *Ziziphus* species are a group of small trees that grow in many regions of the world. They belong to but are native to subtropical and temperate regions.

More than forty *Ziziphus* species are distributed widely throughout the Mediterranean, Africa, Asia, and tropical America [3]. *Ziziphus spina-christi* has grown wild in the southern and western regions of the Kingdom of Saudi Arabia for many years, but its cultivation has been expanded, particularly into the central region of the Kingdom. The Arabic name of the plant is Sidr or *Nabag*, and it is used for various medicinal purposes. Species of this genus are used traditionally for the treatment of various diseases such as fever, insomnia, skin infections, urinary problems, digestives disorders, obesity, liver complaints and diabetes [4].

A clinical study of three species of this genus *Ziziphus spina-christi*, *Zizyphus jojoba*, and *Zizyphus vulgaris* revealed a great effect on insulin secretion. A literature survey revealed that a number of terpenoids, flavonoids, isoquinoline alkaloids, cyclopeptide, and glycosidic terpenes are found in various quantities in almost all *Ziziphus* species. The leaves of the plants in that genus contain ceanothic and betulic acids, different saponins and flavonoids, triterpenoids [5]. Pharmacological screening studies of different crude extracts of *Zizyphus spina Christi* showed antidiarrheal [6], central depressant effect and antinociceptive effect. Phenolic compounds can also act as antioxidants through the chelation of metal ions, the preventing of radical formation and the improving of the antioxidant endogenous system [7].

Different extracts and fractions from different parts of the plants showed bactericidal and antiviral activities against different microorganism [3]. In the current investigation, we aimed to evaluate the composition of the flavonoid and polyphenolic compounds in addition to the antioxidant and the anti-diabetic activities of the extract and various fractions of ZSC.

EXPERIMENTAL

Plant material

The leaves of *Ziziphus spina-christi* were collected from Mecca Road, Jeddah, Saudi Arabia in April 2016. The plant was authenticated by Prof Kadry Abdelkhalik, Umm Al-Qura University, Faculty of Science, Biology Department, Meccah, Saudi Arabia. A voucher specimen (no. Sh-Am-2016) has been kept in the herbarium of phytochemistry Department

Chemicals

Methanol, *n*-hexane, chloroform, butanol and ethyl acetate were analytical or HPLC grade.

Folin-Ciocalteu reagent; butylated hydroxyl-anisole (BHA); quercetin; and gallic acid were obtained from Sigma-Aldrich (Germany). Streptozotocin and glibenclamide were purchased from Sigma (USA) and Spimaco (Saudi Arabia) respectively. Other kits used for analysis of lipid analysis were purchased from local suppliers.

Extract preparation

The aqueous methanol extract (ZSC-1) was obtained by extraction of 1 kg of dried leaf powder in 4 L 80 % (v/v) methanol at 25 - 30 °C for four days. The filtrate was collected. The combined filtrates were concentrated at 45 °C under reduced pressure, using a rotary evaporator. To prepare fractions with solvents of different polarities, the concentrated aqueous solution was defatted with hexane and partitioned by solvent extraction using ethyl acetate (ZSC-2), and *n*-butanol (ZSC-3). The organic fractions and the residual aqueous fractions (ZSC-4) were concentrated separately.

Animals

Swiss albino mice male (20-25 g) of approximately the same age were obtained from the Experimental of the Animal Care Center, Faculty of Pharmacy, University of King Saud, Riyadh, and maintained in controlled conditions (temperature, 22 ± 2°C; humidity, 55 %; 12 : 12 h light-dark cycle). The mice were provided with Purina chow and free access to drinking water. Before use, the mice were acclimatized to the experimental conditions for 7 days. The study was approved (clearance no. CBR-4538) by Research Ethics Committee of Experimental Animal Care Society, College of Pharmacy, King Saud University (Riyadh, Saudi Arabia), and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals [8]

Induction of streptozotocin-induced diabetes

Diabetes was induced by injecting 60 mg/kg body weight streptozotocin (STZ) in 0.1M cold citrate buffer (pH = 4.5) intraperitoneally. Diabetes was confirmed by determining fasting blood glucose concentration [9]. The mice were divided into six groups of six mice each. Groups I and II included untreated diabetic control mice and diabetic mice administrated standard (10 mg/kg/day glibenclamide). Group III, IV, V and VI were treated with extracts at the dose level of 250 mg/kg and 500 mg/kg respectively, for 15 days. Finally, on day 15, blood was collected for

hematological and biochemical parameters. Body weight and blood glucose level (with the help of Gluco Check) were measured weekly on overnight-fasted animals. At the end of the experimental period, the animals were fasted overnight, blood (0.2 mL) was collected for various biochemical analyses, and the animals were sacrificed by cervical decapitation.

Evaluation of hypoglycemic and anti-diabetic activities

The drugs were administrated at doses of 250 and 500 mg/kg body weight to each animal. Tween 80 (1 mL) was added as a solvent to each extract/fraction. The experiment was conducted in accordance with a previously reported procedure [10]. Briefly, for hypoglycemic evaluation, six groups of animals were prepared with six mice in each group. Group I was administrated normal saline (the diabetic control), group II was administered 5 mg/kg glibenclamide (standard drug) and groups III, IV, V and VI were administered the test extracts or fractions at 250 and 500 mg/kg body weight.

The extracts, fractions and drugs were orally administrated to animals after fasting for 24 h. Blood samples were collected before administered (zero time) and at 30, 60, 90 and 120 min after the drugs administered and blood glucose level was measured using Reflotron® instrument. For anti-diabetic screening, diabetes was induced in the overnight fasted animals by intraperitoneal injection of 150 mg/kg). The induction of diabetes was completed 72 h after injection; thereafter the experiment proceeded as described for the hypoglycemic experiment.

Glucose tolerance test

All mice were fasted before the experiment. Group 1, the diabetic control, was administrated 1 mL normal saline, group 2 was administered 1 mg/kg glibenclamide, and the other groups were administered ZSC-1, ZSC-2 and ZSC-3 at 250 and 500 mg/kg, respectively. The animals were loaded with glucose (3 g/kg) [11] and blood samples were collected immediately before drug administration and at 30, 60, 90 and 120 min after drug administration.

The serum glucose level was immediately specified by using a glucose estimation kit Refretron (Roche, Germany) to detect the hypoglycemic effects of the tested samples compared to the effects of the test samples compared to the effects of the standard group and control.

Evaluation of biochemical parameters

Triglycerides (TG), cholesterol, lipoprotein high density (HDL-C), very low density lipoprotein (VLDL-C) and low density lipoprotein (LDL-C) were calculated by using Refretron diagnostic kit (Roche, Germany).

Determination of total flavonoids

The total content of flavonoids was measured using an $AlCl_3$ colorimetric assay, based on the method of Zhang *et al.* [12], with quercetin as a reference compound. One milliliter of the sample was mixed with methanol extract solution containing 2 % $AlCl_3$. The mixture was maintained at 30 °C for 15 min to allow the formation of a complex between the aluminum chloride and the flavonoids. The complex was measured at 430 nm using an UV-Vis Spectrophotometer (UV-1650 PC, Shimadzu-Germany). Quercetin concentrations from 00-100 mg/L were used to construct a calibration curve. Total flavonoid content (TFC) was expressed as quercetin equivalent (QE) in mg/g dry weight of plant extract.

Assessment of total phenolic content

The concentration of phenolic content was quantified in accordance with the method of Shahat *et al.* [13]. Fifty microliters of the test samples were mixed with 2 mL of 2 % sodium carbonate and the mixture were maintained at 25 - 30 °C for 2 min. After the addition of Folin-Ciocalteu reagent (50 %, 100 μ L), the reaction was allowed to proceed for 30 min at room temperature, after which the absorbance was measured at 720 nm. Gallic acid was used to construct a standard curve and the phenolic content present in the extract was expressed as gallic acid equivalents.

Antioxidant activity

DPPH radical scavenging activity

The effect of the extract, fractions and positive control (BHA) on DPPH scavenging free radical activity were determined based on the method described by Khalifa *et al.* [14] with minor change. The stable free radical DPPH was dissolved in methanol to produce a 100 μ M solution. The DPPH solution (3 mL) was added to different concentration ranging from 20 - 100 μ g/mL of the tested samples. One ml of methanol instead of the plant extract was used for control. After 30 min, the absorbance was measured at 520 nm and the DPPH inhibitory activities (D) was computed as in Eq 1.

$$D (\%) = \frac{A_0 - (A_t/A_0)100}{\dots\dots\dots} (1)$$

where A_t = absorbance of the extract, A_0 = absorbance of control

Determination of ABTS radical scavenging activity

The radical scavenging activity of the total extract and different fractions of ZSC and the positive control, BHA, were measured against the 2, 2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid (ABTS) radical cation by using the method of Smeriglio *et al* and Shahat *et al* [15]. Aqueous solution of 2.45 mmol/L potassium persulfate and 7 mmol/L of ABTS in water were prepared. The two solutions were mixed in a 1:1 v/v ratio and stored in the dark for 6 h at room temperature to allow production of the ABTS radical. The stock solution of ABTS was diluted with methanol. The tested samples were prepared at 5 mg/mL. Serial dilutions of the tested samples were prepared and the volumes were adjusted to 1 mL using methanol. ABTS solution was added to different concentration of the test samples, mixed strongly and incubated at 30 °C for 20 min. The absorbance of the solution was recorded at 700 nm. The affinity of test samples to reaction with the ABTS free radical was calculated as in Eq 2.

$$S (\%) = \frac{A_0 - (A_t/A_0)100}{\dots\dots\dots} (2)$$

where S, A_0 and A_t are radical scavenging activity, absorbance of control and absorbance test sample, respectively.

Statistical analysis

The data are expressed as mean \pm standard deviation (SD) and were statistically analyzed using the one-way analysis of variance (ANOVA) or Student's t-test, followed by Dunnett's multiple comparison test. Statistical significance was set at $p < 0.05$, or $p < 0.01$, or $p < 0.001$

RESULTS

Anti-diabetic activity

The blood glucose level after treatment of fasted mice with 250 and 500 mg/kg body weight of tested fractions after 7 and 15 days are shown in Table 1. The anti-diabetic activities increased with an increase in the dose of all tested fractions. The highest results 25.59 % (7 days) and 39.48 % (15 days) were found after the administration of 500 mg/kg body weight ZSC-3 compared with standard drug. These tested fractions are further investigated for oral glucose

tolerance test and the determination of different biochemical parameters.

Hypoglycemic activity

The effect of the different plant extracts at a dose of 250 and 500 mg/kg body weight on fasting blood sugar level were assessed in normal mice and the results are summarized in Table 2. The highest decrease in glucose level found by ZSC-3 at 500 mg/kg of body weight was 29.07 % (7 days) and 35.56 (15 days) compared with standard drug. As shown in Table 2, a continuous increase can be observed, hypoglycemic activities increased with the increase in the dose of each fraction from 250 to 500 mg/kg of body weight. The second highest decreases of 24.64% (7 days) and 34.85 (15 days) were found after treatment with 500 mg/kg of body weight of the ZSC-2 fraction.

Glucose tolerance

The blood glucose level of mice administered 250 and 500 mg/kg of body weight of all tested fractions was investigated after 30, 60, 90 and 120 min. After the administration of the compounds, it was noted that the glucose level first increased between 30 to 60 min after administration and then decreased between 90 and 120 min as shown in Table 3.

Biochemical profile

The measurement of the other parameters such as cholesterol, triglyceride HDL, LDL and VLDL are presented in Table 4. Here, significant changes can be observed in the lipid profiles with the tested fraction at both dose levels. A very significant change in HDL (96.39 %) was found after ZSC-3 treatment and then after ZSC-2 (71.28 %). Both at 500 mg/kg dose level. The result (96.39 %) was greater than that observed after treatment with the standard drug (95.80 %). Fraction ZSC-3 at dose level 500 mg/kg resulted in highest LDL (63.49 %) followed by ZSC-2 (49.16 %). The same fractions also showed the highest result for VLDL (29.31 and 24.72 %, respectively). At 500 mg/kg bodyweight, the two fractions ZSC-3 and ZSC-2 led to a significant percentage change in cholesterol and triglyceride levels.

Body weight was monitored throughout. After 15 days, significant change of body weight ($p < 0.001$) were evident after the administration of ZSC-2 (500mg/kg) and both doses of ZSC-3 and ZSC-4 (Table 5).

Table 1: Anti-diabetic activity of extracts on fasting blood glucose level in STZ-induced diabetic mice

Group	Dose (mg/kg)	Before treatment	Blood glucose concentration (mg/dL) after treatment			
		Day 0 (Mean ± S.E.)	day 7 (Mean ± S.E.)	% Change	Day 15 th (Mean ± S.E.)	% Change
Group I Diabetic control		307.50±6.41	317.66±4.13	3.30↑	315.50±3.87	2.60↑
Group II Glibenclamide	5	299.16±8.98	158.83±7.80***	46.90↓	153.00±3.19***	48.85↓
Group III (ZSC-1)	250	315.66±2.49	310.16±3.14	1.74↓	302.50±3.56*	4.17↓
	500	326.00±9.70	296.66±4.70*	8.99↓	288.50±6.27*	11.50↓
Group III (ZSC-2)	250	318.66±10.76	287.16±4.19*	9.88↓	277.16±6.06**	13.02↓
	500	305.33±7.68	271.83±3.33**	10.71↓	201.66±3.92***	33.95↓
Group III (ZSC-3)	250	316.83±11.63	276.00±4.28*	12.88↓	226.83±7.18***	28.40↓
	500	315.50±11.13	234.00±5.83***	25.59↓	190.33±2.99***	39.48↓
Group III (ZSC-4)	250	326.00±12.29	299.83±4.58	8.02↓	290.33±11.03	10.94↓
	500	331.66±16.54	295.83±6.02	10.80↓	288.50±3.73*	13.01↓

All values represent mean ± SEM; **p* < 0.05; ***p* < 0.01, ****p* < 0.001; ANOVA, followed by Dunnett's multiple comparison test

Table 2: Hypoglycemic effect of extracts on normal mice treated for two weeks

Group	Dose (mg/kg)	Before treatment	Blood glucose (mg/dl) post-treatment			
		Day 0 (mean±SE)	day 7 (mean±SE)	% Change	Day 15 th (mean±SE)	% Change
Group I Normal saline		109.50±1.70	109.66±2.30	No change	110.83±2.34	No change
Group II Glibenclamide	5	111.66±3.10	62.75±2.50***	43.80↓	56.56±2.81***	49.34↓
Group III (ZSC-1)	250	106.16±1.64	100.06±1.50*	5.74↓	99.93±2.61*	9.16↓
	500	112.33±2.70	99.33±3.74*	11.57↓	93.13±4.13**	17.09↓
Group IV (ZSC-2)	250	109.33±2.26	87.91±2.41***	19.58↓	79.38±2.45***	27.39↓
	500	108.83±2.21	82.01±2.01***	24.64↓	70.90±1.30***	34.85↓
Group V (ZSC-3)	250	102.33±3.26	88.06±1.70**	13.94↓	83.00±1.61***	18.89↓
	500	109.83±1.99	77.90±2.81***	29.07↓	70.76±2.21***	35.56↓
Group VI (ZSC-4)	250	107.00±3.11	108.83±2.92	No change	108.00±1.82	No change
	500	107.83±1.79	101.63±1.71*	5.74↓	98.33±1.89**	8.80↓

All values represent mean ± SEM. **p* < 0.05; ***p* < 0.01, ****p* < 0.001; ANOVA, followed by Dunnett's Multiple comparison tests

Table 3: Effect of extract fractions on oral glucose tolerance (OGT) in mice

Group	Dose (mg/kg)	Before treatment	Blood glucose level post-treatment			
		(0 h)	30 min	60 min	90 min	120 min
Group I Normal saline		117.33±2.92	298.33±5.18***	347.66±6.18***	249.66±6.40***	224.00±9.54***
Group II Glibenclamide	5	115.50±3.55	195.66±5.55***	169.50±4.92***	151.83±3.27***	131.50±2.76**
Group III (ZSC-1)	250	112.00±2.89	282.50±6.98***	310.50±17.65***	270.33±7.84***	216.83±10.77***
	500	112.00±2.70	298.83±5.24***	323.83±6.72***	234.33±7.33***	208.50±3.12***
Group IV (ZSC-2)	250	115.00±2.23	289.33±6.40***	314.00±7.35***	213.16±5.53***	199.00±5.05***
	500	115.50±2.77	289.50±5.50***	339.83±6.28***	189.16±6.06***	173.33±5.88***
Group V (ZSC-3)	250	117.33±3.92	261.16±9.60***	317.83±5.36***	223.16±6.59***	168.33±6.04***
	500	115.66±1.76	178.16±7.73***	316.83±7.12***	180.66±7.73***	153.00±4.15***
Group VI (ZSC-4)	250	118.83±3.10	279.50±6.11***	320.33±5.22***	283.00±6.56***	216.16±3.29***
	500	112.83±2.13	294.16±4.52***	324.66±5.32***	242.66±7.14***	193.66±6.13***

All values represent mean ± SEM; ***p* < 0.01, ****p* < 0.001; ANOVA, followed by Dunnett's multiple comparison test

Total flavonoids

The total flavonoid content of the extract and fractions were measured by using the AlCl₃ assay; the results are presented in Figure 1. The flavonoid content was highest in 80 % methanol

extract (ZSC-1, 36.2 mg/g), followed by ethyl acetate (ZSC-1, 31.2 mg/g), n-butanol (ZSC-3, 28.2 mg/g), and the aqueous fraction (ZSC-4, 24.6 mg/g). ZSC-1 showed the highest flavonoid content, followed by ZSC-2 and ZSC-3 extracts; ZSC-4 showed the lowest flavonoid content.

Table 4: Effect of extract on lipid profile of STZ-induced diabetic mice

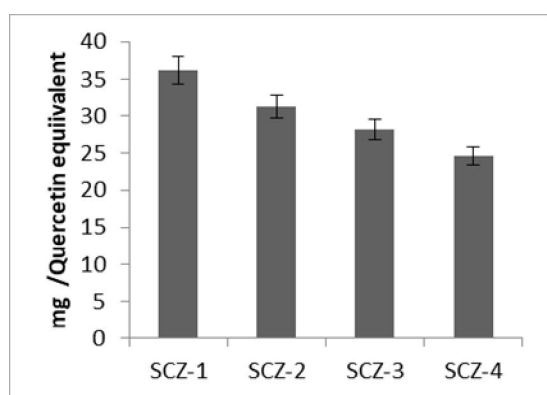
Group	Dose (mg/kg)	Cholesterol (mg/dL)		Triglycerides (mg/dL)		HDL (mg/dL)		LDL (mg/dL)		VLDL (mg/dL)	
		Mean ± SE	% Change	Mean ± SE	% Change	Mean ± SE	% Change	Mean ± SE	% Change	Mean ± SE	% Change
Normal saline		102.02 ± 2.65		100.41 ± 2.79		54.58 ± 2.12		27.36 ± 2.79		20.08 ± 0.55	
Gp I diabetic		209.83 ± 4.41 ^a	105.65	193.33 ± 3.15 ^{***a}	92.53	22.63 ± 0.97 ^{***a}	54.53	148.53 ± 3.9 ^{***a}	442.75	38.66 ± 0.63 ^{***a}	92.53
Gp II glibenclamide	5	120.66 ± 3.56 ^{***b}	42.49	115.66 ± 2.27 ^{***b}	40.17	44.31 ± 2.05 ^{***b}	95.80	53.21 ± 3.87 ^{***b}	64.17	23.13 ± 0.45 ^{***b}	40.17
Gp III	250	201.66 ± 4.52	3.89	183.83 ± 5.15 ^b	4.91	22.46 ± 1.03 ^b		142.43 ± 4.6 ^b	4.10	36.76 ± 1.03 ^b	4.91
(ZSC-1)	500	192.66 ± 3.39 ^{ab}	8.18	179.16 ± 4.54 ^{ab}	7.32	26.91 ± 1.02 ^{ab}	18.92	129.91 ± 3.8 ^{ab}	12.53	35.83 ± 0.98 ^{ab}	7.32
Group IV	250	171.16 ± 4.18 ^{***b}	18.42	171.00 ± 2.19 ^{***b}	11.55	30.36 ± 1.08 ^{***b}	34.16	106.6 ± 4.45 ^{***b}	28.23	34.20 ± 0.43 ^{***b}	11.55
(ZSC-2)	500	143.33 ± 2.34 ^{***b}	31.69	145.33 ± 2.45 ^{***b}	24.82	38.76 ± 1.09 ^{***b}	71.28	75.5 ± 3.02 ^{***b}	49.16	29.06 ± 0.49 ^{***b}	24.72
Gp V	250	157.66 ± 4.36 ^{***b}	24.86	159.00 ± 3.89 ^{***b}	17.75	31.85 ± 0.99 ^{***b}	40.72	94.01 ± 4.81 ^{***b}	36.70	31.80 ± 0.77 ^{***b}	17.75
(ZSC-3)	500	126.00 ± 2.42 ^{***b}	39.95	136.66 ± 3.21 ^{***b}	29.31	44.45 ± 4.65 ^{***b}	96.39	54.21 ± 4.40 ^{***b}	63.49	27.33 ± 0.64 ^{***b}	29.31
Group VI	250	209.33 ± 4.85 ^b	-	195.33 ± 3.95 ^b	-	23.98 ± 0.73 ^b	5.96	146.28 ± 4.01 ^b		39.06 ± 0.79 ^b	
(ZSC-4)	500	193.836 ± 7.08 ^b	7.62	187.50 ± 5.45 ^b	3.10	29.30 ± 0.87 ^{***b}	29.45	127.03 ± 8.2 ^{ab}	14.47	37.50 ± 1.09 ^b	3.01

Gp, Group; All values represent mean ± SEM; * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$; ANOVA, followed by Dunnett's multiple comparison test; ^a compared with normal group; ^b compared with diabetic group

Table 5: Effect of extract on body weight in STZ-induced diabetic mice

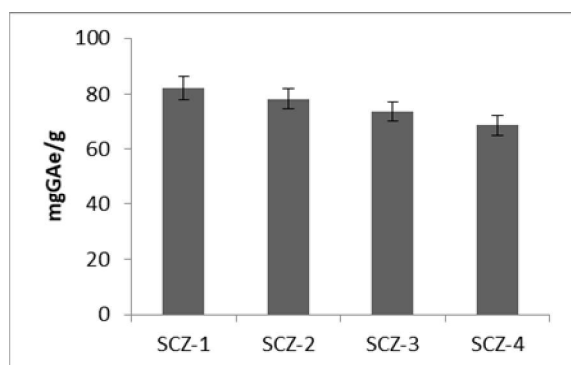
Group	Dose (mg/kg)	day 0 (before treatment) (mean \pm SE)	15 Days (post-treatment) (mean \pm SE)	% Change
Normal (Saline)		20.90 \pm 0.64	24.63 \pm 0.30***	17.86
Group I (Diabetic)		22.36 \pm 0.58	19.96 \pm 0.55***	24.14 \downarrow
Group II (Glibenclamide)	5	22.00 \pm 0.40	26.55 \pm 0.36***	20.68 \uparrow
Group III (ZSC-1)	250	22.40 \pm 0.39	20.45 \pm 0.73*	8.70 \downarrow
	500	22.60 \pm 0.28	20.88 \pm 0.46*	7.59 \downarrow
Group IV (ZSC-2)	250	22.95 \pm 0.73	24.70 \pm 0.25*	7.62 \uparrow
	500	22.05 \pm 0.50	24.61 \pm 0.22***	11.64 \uparrow
Group V (ZSC-3)	250	21.11 \pm 0.43	24.58 \pm 0.28***	16.41 \uparrow
	500	20.93 \pm 0.34	25.13 \pm 0.23***	20.06 \uparrow
Group VI (ZSC-4)	250	20.81 \pm 0.58	19.06 \pm 0.53	8.40 \downarrow
	500	22.50 \pm 0.31	17.66 \pm 0.56***	21.48 \downarrow

All values represent mean \pm SEM; * $p < 0.05$; *** $p < 0.001$; ANOVA, followed by Dunnett's multiple comparison test

**Figure 1:** Total flavonoid content of the total extract and different fractions of *Zizyphus spina-christi*

Total polyphenolic content

The total phenolic contents of *Z. spina-christi* were measured by the Folin-Ciocalteu assay of Singleton *et al* [16] and Shamhat *et al* [13] and reported as mgGallic Acid equivalents (GAE) per g of sample. As shown in Figure 2, the total extract, ZSC-1, had the highest phenolic content (82.3 mg/g), followed by ZSC-2, ZSC-3, and ZSC-4 at 76.3, 73.6, and 68.3 mg/g respectively. ZSC-4 had the lowest total phenol content.

**Figure 2:** Polyphenolic content of total extract and different fractions of *Zizyphus spina-christi*

Antioxidant activity

Antioxidant has been used widely in food industry to prolong the shelf life. Antioxidant term refers to a compound that can be inhibited or delay the oxidation of biomolecules. A numerous number of plant extracts have been investigated for their antioxidant activities [13,17]. The secondary metabolite compounds of the higher plants have been verified in *in vitro* experiments to defend against oxidative damage through reduction or inhibition of the free radicals and oxygen reactive species.

DPPH radical scavenging activity

Five concentrations of the tested samples were used with the positive control; the activities were increased as the concentration increases. The total extract (ZSC-1) and the fractions ZSC-2, ZSC-3, and ZSC-4 exhibited strong antiradical activity by reducing the radical stable DPPH to yellow colored (Figure 3) in comparison with the positive control (BHA) which is a known antioxidant. The Ethyl acetate fraction (ZSC-2) exhibited higher DPPH scavenging activity (96 %) compared with the standard, ZSC-3 and ZSC-4. ZSC-4 fraction showed lowest activity at 100 μ g/mL. A concomitant increase in scavenging activity with an increase in concentration. The hydrophobic DPPH free radical change in scavenging capacity of the extract and different fraction of *Zizyphus spina-christi* is shown in Figure 3.

Total antioxidant capacity

The blue and green ABTS radical cation was generated before the addition of the antioxidant and the degree of radical cation decolorization can be used to measure the relative antioxidant ability of extract compared to that of BHA, The

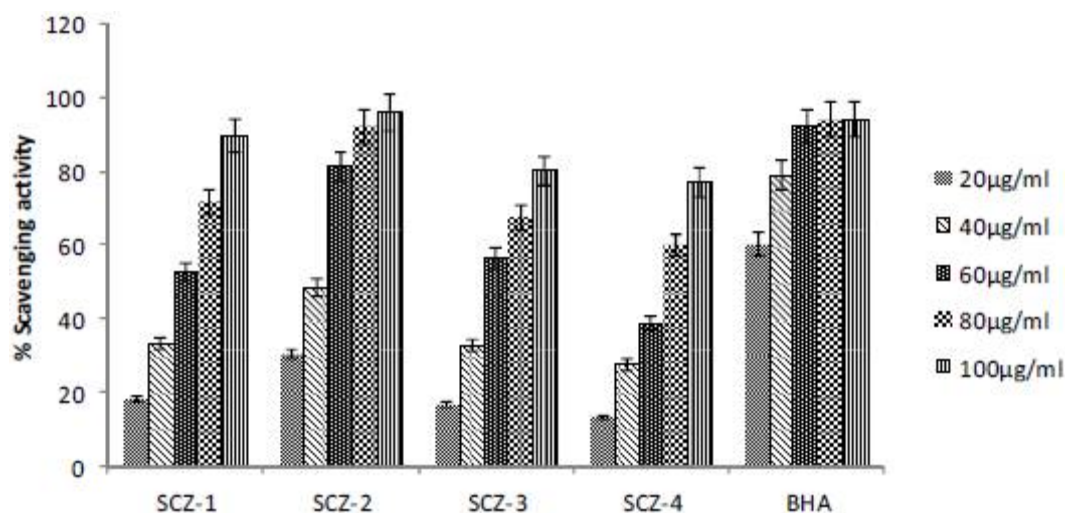


Figure 3: Free radical scavenging activity (DPPH) of ZSC-1 and the different fractions (ZSC-2, ZSC-3, and ZSC-4) of *Ziziphus spina-christi* at different concentrations

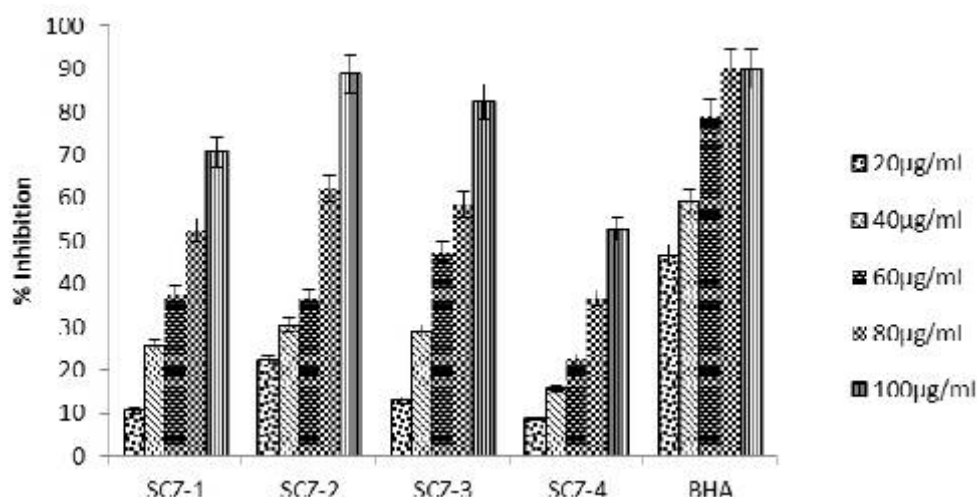


Figure 4: Radical-scavenging activity of extract and fractions from *Ziziphus spina-christi* at different concentrations

total antioxidant capacity of total extract and different fractions of *Zizyphus spina christi* at different concentration, measured by using the ABTS, is shown in Figure 4. A concentration-dependent increase in scavenging was observed. ZSC-4 showed lowest ABTS scavenging activity. The total antioxidant activities of ZSC-1, ZSC-2, ZSC-3 and ZSC-4 result in 70.5 to 91.2 % inhibition in the ABTS.

DISCUSSION

Streptozotocin-induced hyperglycemia has been described as a useful model to study the activity of hypoglycemic agents. Streptozotocin selectively destroys the pancreatic β cells, reduce their activity, and cause diabetes [18]. In the present study, significant weight loss was observed in the diabetic group in addition to an improvement in the weight of rats in the diabetic group treated with fraction ZSC-3. This may be a

result of the ability of the extracts to reduce hyperglycemia in STZ induced diabetes, which is characterized by severe body weight loss, owing to the loss or degradation of structural protein [19].

In the present study, blood glucose levels were four times higher than the initial level after the induction of diabetes. However, treatment with ZSC-3 fraction reduced blood sugar level compared with levels of the control. The ZSC-3 fraction was more effective in controlling the blood glucose level than the other tested samples. This activity may be attributable to the presence of saponins glycosides [5]. Many studies have shown the blood glucose-lowering effect of plant extracts such as *Boehmeria rugulosa* [20], and have sought to evaluate the hypoglycemic effect in STZ- and alloxan-induced diabetes mellitus. Hyperlipidemia is a recognized complication of diabetes mellitus, characterized

by elevated levels of cholesterol, triglycerides and changes in lipoprotein composition.

In the present study, significant hyperlipidemia was observed in STZ-induced rats compared to the normal groups. However, administration of the fractions reduced the hyperlipidemia. ZSC-3 was found to be the most effective, followed by ZSC-2 which indicated that these fractions had the potential to improve insulin secretion. Similarly, many authors have also noticed hypercholesterol and hypertriglycerides condition [21], which supported the data in the present study. The total flavonoids content and polyphenol contents of ZSC-1, ZSC-2, ZSC-3 and ZSC-4 were 36.2, 31.2, 28.2 and 24.6 mg/g and 76.3 mg/1g, 73.6 mg/1g and 68.3 mg/1g respectively. The ethyl acetate (SZC-2) fraction showed DPPH scavenging activity greater than the standard compound (96 %); the activities of ZSC-1, ZSC-3 and ZSC-4 were 89, 80.3 and 77.3 %, respectively. The total antioxidant activities of ZSC-1, ZSC-2, ZSC-3 and ZSC-4 result were between 70.5 and 91.2 % inhibition in ABTS test.

CONCLUSION

Fraction ZSC-3 showed potential hypoglycemic activity in diabetic mice. ZSC is rich in flavonoids and polyphenols, which may be responsible for its strong antioxidant effect. Further studies are required to determine the mechanisms of these effects.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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