RESEARCH ARTICLE

Comparison between Total Phenolic and Flavonoid Contents and Antidiabetic Activities of Different Parts of *Capparis spinosa* L. growing in Aleppo, Syria

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ABSTRACT

Caper (*Capparis spinosa* L.) is a xerophytic shrub with remarkable adaptability to harsh environments. This plant species is of great interest for its medicinal/pharmacological properties and its culinary uses. The present study aimed towards the comparison of total phenol and total flavonoids, investigate *in vivo*, *in vitro* antidiabetic activity of aqueous and methanolic (80%) extract of leaves, flowers, stems, and fruits of *C. spinosa* growing in Aleppo, Syria. *In vitro*, the study was completed by evaluating the inhibitory activity of these extracts on α-amylase enzyme using two methods: the first is DNSa method which depended on chemical reactions between plant extracts, α-amylase enzyme, and starch using 3-5 dinitro salicylic acid as the color reagent, whereas the second method was done by using thin-layer chromatography (TLC) method. We used iodine staining to visualize inhibitory activity, where a blue color spot on the TLC plate was taken as a positive α-amylase inhibitory test for the corresponding compound, as well as, evaluation of the antidiabetic activity of aqueous and methanolic leaves extracts of *C. spinosa* on alloxan-induced diabetic rats. The results revealed that leaves extracts possessed significant activity of inhibition of the α-amylase enzyme *in vitro* model compared to other extracts. Bioautography revealed that α-amylase was inhibited by most of the flavonoids separated on the TLC plates. Methanolic leaves extract reduced significantly fasting blood glucose and enhanced regeneration of β cells after the 14th day in diabetic rats treated with a dose of 200 mg/kg body weight, comparable to that of the reference drug, glimepiride (1 mg/kg). The results presented here provide evidence based on the use of *C. spinosa* leaves as hypoglycemic agents in the treatment of type 2 diabetes (T2DM).

Keywords: Alloxan, Alpha-amylase, Antidiabetic activity, Bioautography, *Capparis spinosa*, Glimepiride, Total flavonoids content (TFC), Total phenols content (TPC).

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INTRODUCTION

Diabetes comprises two types, *viz*., type 1 and type 2. Type 1 diabetes is frequently alluded to as a phenomenon of diabetes that is insulin-dependent (IDDM) and known to influence just 5% of the diabetic population. It is a result of cell intermediated immune system destruction of the insulin delivering and secreting β-cells of the pancreas, which brings about a deficiency of insulin for the body.[1] T2DM is a non-communicable disease with leading causes of death worldwide due to associated long term side effects, which include ketoacidosis, hyperosmolar coma accompanied with chronic disorders, retinopathy, renal failure, neuropathy, skin complications, as well as, increasing cardiovascular risks.[2] According to the World Health Organization (WHO) estimates, the number of adults with diabetes in the world will rise from 135 million in 1995–300 million in the year 2025.[3] Current therapeutic measures to treat this disorder include the use of insulin and other agents, such as, amylin analogs, α-glycosidase inhibitors, sulphonylureas, and biguanides. These drugs also have certain adverse effects, such as, causing hypoglycemia at higher doses, liver problems, lactic acidosis, and diarrhea.[4] Management of diabetes without any side effects is still a challenge to the medical community. There is a continuous search for alternative drugs. Therefore, it is prudent to look for options in herbal medicines for diabetes. Thus, due to an increase in demand by patients to use natural products with antidiabetic activity investigations on hypoglycemic agents derived from medicinal plants have gained popularity in

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recent years. Compared to synthetic drugs, herbal preparations are frequently considered to be less toxic with fewer side effects.[5] In recent years, herbal medicines have started to gain importance as a source of hypoglycemic agents. It is estimated that more than a thousand plant species are being used as a folk medicine for diabetes.[6] The medicinal plants have many types of phytochemicals, like phenols, flavonoids, alkaloids, saponins, terpenes, and glucosinolate, which plays a very important role in fighting disease,[7] as an antidiabetic activity through a different mechanism of actions, like insulin-like action or secretion, regeneration of beta cells of the islets of Langerhans, hypoglycemic effect, hepato-pancreatic protective effect, and reduced glucose absorption, favoring peripheral glucose utilization, as well as, glycogenolysis or reducing carbohydrate absorption, inhibition of aldose reductase activity, reduction of lactic dehydrogenase and γ-glutamyl transpeptidase, inhibition glycogen-metabolizing enzymes, increasing glyoxalase 1 activity in liver, increasing the creatine kinase levels in tissues, inhibition of glucose-6-phosphate system besides being antioxidants, and immunomodulators.[8] Syria is known for its wealth of plant species with medicinal properties, which have been used since early times. In fact, more than 3,500 species belonging to 131 families have been found in Syria, hundreds of which may have medicinal and therapeutic significance. From the most common plants is used for the treatment of human ailments in north Syria,[9] *C. spinosa* (CS) is one of the most important wild plants in Syria belonging to the Capparidaceae family and it has been employed traditionally for its beneficial effects on human diseases.[10] Previous chemical studies on *C. spinosa* have shown the presence of alkaloids, indole, flavonoids, lipids, aliphatic glucosinolates, and polyphenols, and additionally, the plant is recognized as a rich source of flavonoids, such as, rutin, kaempferol, quercetin, and its derivatives. These constituents display a significant role in the pharmacological activity of *C. spinosa*, including antioxidant, anti-inflammatory, anti-allergic, antihistaminic, hypolipidemic, anti-mutagenic, anti-proliferative, anti-microbial, anti-helminthic, hepato-protective, and anti-nociceptive effects.[11] In this context fits this present work, as part of a contribution to a better knowledge of medicinal plant in the region of Syria. Thus, we have determined the total phenolic content and concentration of flavonoids of aqueous and methanolic extracts of leaves, flowers, stems, and fruits. Then, the antidiabetic activity of the mentioned plant parts was evaluated *in vitro* by studying the inhibition activity of these extracts on α-amylase enzyme, by using two different methods: the first was DNSa method, which depended on chemical reactions between plant extracts, α-amylase enzyme, and starch, and we used 3-5 dinitro salicylic acid as a color reagent, whereas the second method was done by using TLC method, and we used iodine staining to visualize inhibitory activity, where a blue color spot on TLC plate was taken as a positive α-amylase inhibitory test for the corresponding compound in order to confirm the antidiabetic activity of aqueous and methanolic extracts of leaves of *C. spinosa* on alloxan-induced diabetic rats.

MATERIALS AND METHODS

Chemical Materials

Methanol (Eurolab, UK), alloxan and Folin-Ciocalteu phenol reagent (Sigma-Aldrich, Switzerland and Germany), dimethyl sulfoxide, α-amylase enzyme from malt and dinitro salicylic acid (Himedia Labs), starch, sodium hydroxide, sodium potassium tartrate, phosphoric acid, monosodium phosphate, and disodium phosphate (Merck, Germany), aluminum chloride (Scharalau Chemie, Spain), rutin (Extrasynthese Genay, France), kaempferol, gallic acid (Titan Biotech Ltd., India), glimepiride (Ibn Al-Haytham Pharmaceutical Industries, Aleppo, Syria). All other chemicals, unless and otherwise mentioned, were obtained from research laboratories in the Department of Pharmacognosy, Faculty of Pharmacy, University of Aleppo, Syria.

Plant Materials

Fresh aerial parts (leaves, flowers, stems, and fruits) of *C. spinosa* were collected from different regions of Aleppo in the north of Syria and authenticated by Dr. Ahmad Jaddouh, an expert at the Faculty of Agriculture, University of Aleppo, Syria. The plant materials were washed under running tap water, shade dried, and then, powdered using a mechanical grinder and airtight containers with proper labeling for future use.

Extraction Procedure

The fine powder of each part of the plant (leaves, stems, fruits, and flowers) (50 grams) was extracted with 500 mL of water and methanol (80%), three times for 1-hour in an ultrasonic bath (POWERSONIC 405, Hwashin Technology Co., Korea). The temperature was maintained at 50 and 30°C for water and methanol extracts, respectively. The ratio of plant material and the solvent was 1:10. The extracts were filtered through a paper filter (Whatman No. 1) and evaporated to dryness under reduced pressure by the rotary evaporator. The obtained crude extracts were stored in dark glass bottles and refrigerated at -4°C until use.[7]

In vitro Study

Determination of Total Phenols Content (TPC)

The TPC was determined by using the Folin-Ciocalteu assay. The reaction mixture was prepared by mixing 0.5 mL of the methanolic and aqueous solution of plant extracts, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 mL 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 mL methanol. The samples were thereafter incubated in a thermostat at 45°C for 45 minutes, and the absorbance was determined using a spectrophotometer at λ_{max} = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/mL) from the calibration line. Then, the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).[12,13]

Determination of Total Flavonoids Content (TFC)

The amount of flavonoid content in the extracts was measured by the aluminum chloride colorimetric assay. The sample contained 1-mL of the methanol solution of the extract in the concentration of 1-mg/mL and 1-mL of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using a spectrophotometer at λ_{max} = 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and a dilution series of rutin of concentration 0.01, 0.02, 0.03, 0.04, and 0.05 mg/mL was prepared, and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/mL) on the calibration line.[13,14]

Determination of α-Amylase Activity In vitro by DNSa Method

The starch solution (1% w/v) was obtained by stirring and boiling 1-gram of soluble starch in 100 mL distilled water for 15 minutes. The enzyme solution (1 unit/mL) was prepared by mixing 5 mg of α-amylase in 100 mL of 20 mmol/L sodium phosphate buffer (pH = 6.9) containing 6.7 mmol/L sodium chloride. The extracts were dissolved in Dimethyl sulfoxide (DMSO) to give suitable concentrations (0.2, 0.4, 0.8, 1.2, and 1.6 mg/mL) for the assay. The color reagent was a solution containing (96 mol/L) 3,5-dinitrosalicylic acid (20 mL), (5.31 mol) sodium potassium tartrate in (2 mol/L) sodium hydroxide (8 mL), and deionized water (12 mL). 1 mL of each extract and 1 mL of the enzyme solution were mixed in a test tube and incubated at 25°C for 30 minutes. To 1 mL of this mixture was added 1 mL of the starch solution and the tube was further incubated at 25°C for 30 minutes. Then, 1 mL of the color reagent was added and the stoppered tube was placed into an 85°C water bath. After 15 minutes, the reaction mixture was removed from the water bath and cooled, thereafter, diluted with 9 mL distilled water, and the absorbance value determined at 540 nm. Individual blanks were prepared for correcting the background absorbance. In this case, the color reagent solution was added before the addition of the starch solution, and then, the tube was placed into the water bath. Then, the method was followed as described above. Controls were conducted in an identical manner, replacing extracts with 1 mL DMSO.[15,16] The inhibition percentage of α-amylase was assessed by the following formula:

$$I\alpha\text{ - amylase\%} = (\Delta\text{control} - \Delta\text{sample})/\Delta\text{control} \times 100$$

ΔA control = A test - A blank; ΔA sample = A test - A blank

The concentration of plant extracts required to inhibit 50% of α-amylase activity under the conditions was defined as the IC_{50} value, which is the concentration of the sample (mg/mL) necessary to decrease the absorbance of the α-amylase solution by 50%.

Determination of α-Amylase Activity In vitro by TLC (Bioautography Method)

The TLC was performed on the TLC silica gel 60 F254 plates (Merck, Germany). The extracts were spotted on the plates using a micropipette and allowed to dry. One-dimensional TLC analysis was performed with ethyl acetate:formic acid:glacial acetic acid:methanol, in volume ratio 100:1.1:1.1:2.6, respectively, as mobile phase for flavonoids.[17,18] After the chromatogram was developed, the plates were dried and the spots were visualized sequentially under Ultraviolet (UV) light at 254 and 365 nm, and then sprayed with natural product/polyethylene glycol (NP/PEG). The TLC plates were then incubated in the amylase solution for 30 minutes for the primary reaction between the enzyme and inhibitor. After incubation, the plates were taken out of the amylase solution and incubated in 1% starch buffer of pH 6.9 for 10 to 20 minutes for an enzyme-substrate reaction. The plates were then washed with Gram's iodine solution and observed. These experiments were performed in triplicate to check the reproducibility of the method.[19]

***In vivo* Studies**

In this section, we determined the anti-diabetic activity of leaves of *C. spinosa in vivo* by using alloxan-induced diabetic albino Wistar rats.

Experimental Animals

Fifteen Wistar rats (100–170 grams; 4 months old) were obtained from the Animal House Center of the Faculty of Pharmacy, Aleppo University. The animals were housed in polypropylene cages (three rats/cage) and maintained under controlled room temperature and humidity with 12/12-hour light-dark cycle. The rats had free access to water and food.

Induction of Diabetes

It has been stated that different *Capparis* species indicate effectiveness on diabetes. According to previous studies which were performed, it was administered at a dose range of 100 to 2,000 mg/kg and no toxic effect was observed, so 200 mg/kg body weight (b.w.) dose was selected for the antihyperlipidaemic study.[11,20,21] Diabetes was induced by administration of alloxan monohydrates (150 mg/kg body weight) intraperitoneally. Alloxan was dissolved in normal saline and given to the overnight fasted rats (rats fasted for 12 hours before injection). After 72 hours of alloxan administration, blood samples were drawn from the tail, and glucose levels were determined by using glucometer strips to confirm diabetes induction. Rats having blood glucose levels more than 200 mg/dL were considered as diabetic and taken for study.[20]

Experimental Design

The experimental rats were randomly divided into five groups of three animals each (male alone and female alone). The experiment lasted for 14 days. Group 1 (control) composed of normal rats orally administered with physiological saline. Group 2 (negative control) composed of diabetic rats administered with physiological saline. Group 3 (positive control) composed of diabetic rats administered with glimepiride (reference drug at 0.1 mg/kg b.w.) in physiological saline. Group 4 composed of diabetic rats administered with aqueous extract of leaves (200 mg/kg b.w.).

Group 5 composed of diabetic rats dosed with hydromethanolic extract of leaves (200 mg/kg b.w.). After overnight fasting, animals of each group were administered orally either physiological saline or extracts or glimepiride via oral metallic cannula. Blood glucose level was measured nearly every other day by means of glucometer strips.[20]

Histological Procedures

Rats were sacrificed and then the sacrificed animals were quickly dissected. The sample of the pancreas was removed and fixed in 10% neutral formalin for 24 hours, followed by washing, dehydration in ascending grades of alcohol, clearing in xylene, and embedding in hard paraffin. The sections were cut by the microtome at 5 µ thick, stuck on clean slides, and allow drying. The sections were deparaffinized in xylene and hydrated to water through descending series of ethyl alcohol. Staining was performed using hematoxylin and eosin stains for nucleus and cytoplasm examination and investigated by light microscope.[22]

Statistical Study

All data were expressed as mean ± standard error of the mean (SEM). The data were analyzed by statistical software package (SPSS version 25). One-way analysis of variance (ANOVA) and Tukey were used to evaluate the significance between means. The data were considered significantly different at $p < 0.01$.

RESULTS AND DISCUSSION

Regulation of glucose level in the blood of the diabetic patient can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species.[23] In the present study, the antidiabetic activity of parts of *C. spinosa* growing in Aleppo city (Syria) was evaluated *in vitro* and *in vivo* using different methods. The results showed that this genus has blood glucose-lowering activity may be the presence of phenols and flavonoids.

Total Phenol Content (TPC)

The chemical constituents extracted from plants, phenolic compounds, can inhibit the absorption of α-amylase in the treatment of carbohydrate absorption, such as, diabetes. There are many fruits and vegetables that contain phenolic compounds, especially, grapes, berries, and tomatoes. Phenolic compounds, for instance, phenolic acids and flavonoids, could promote health benefits by reducing the risk of metabolic syndrome and the related complications of T2DM.[24] As a basis, phenolic content was measured using the Folin-Ciocalteu reagent (FCR). The results were derived from a calibration curve ($Y = 0.116x - 0.04$, $R^2 = 0.993$), and expressed in gallic acid equivalents (GAE) per milligram dry extract weight. In this assay, the FCR phenol detected reagent reacts with phenolic compounds under basic conditions to form chromogens that can be detected at 750 nm. The total phenols were expressed as mg/g gallic acid equivalent, using the standard curve equation, according to the results presented in Table 1. All parts of *C. spinosa* were rich in phenolic compounds, where the TPC varied from 22.4 ± 1.434 to 75.8 ± 8.109 and 26.4 ± 2.007 to 91.4 ± 9.684 mg GAE/g dry extract for aqueous and hydromethanolic extracts, respectively. Among the methanolic extracts, the highest total phenolic value was 91.4 ± 9.684 from leaves extract.

Comparing the works of literature, our results differ from other studies. For instance, TPC ranged from 21.42 to 27.62 mg GAE/g of dry weight in caper leaves methanol extract taken from different sites in India. Caper leaves aqueous extract from Tunisia recorded total phenolics of 33.55 mg GAE/g DW and buds aqueous extracts contained 67.29 mg GAE/g DW, while 427.27 mg GAE/g DW of total phenolics was quantified in the hydroethanolic extract of leaves.[25] This variation in values can be explained by the fact that the phenolic content is influenced by different parameters, such as, time and place of harvest, climate, geographical conditions, method and time of extraction, solubility, and degree maturation of the plan.[26,27]

Total Flavonoid Content (TFC)

The most common groups of plant phenolics are flavonoids, which could be graded, such as, flavones, flavonols, flavanones, flavonols, anthocyanins, and isoflavonoids. It is recognized that flavonoids which display potent antioxidant activity, can prop the regeneration of β-cells.[28,29] As a basis for the quantitative determination, flavonoid contents were determined using aluminum chloride in a colorimetric method. The results were derived from the calibration curve ($Y = 0.043X + 0.004$, $R^2 = 0.998$) and expressed in rutin equivalents (RE) per gram dry extract weight (Figure 1). As shown in Table 2, TFC ranged from 13.8 ± 0.733 to 82.9 ± 4.157 mg RE/g and 14.4 ± 1.473 to 68.7 ± 3.275 mg RE/g, for methanol and aqueous extracts, respectively. The methanolic leaves extracts had the greatest flavonoid content (82.9 ± 4.157 mg RE/g), while the smallest amounts of flavonoids were found in methanolic fruit

Table 1: TPC of plant extracts

	TPC (mg GAE g/g of dry extract)	
Sample	*Aqueous extract*	*Methanolic extract (80%)*
Leaves	71.2 ± 8.521	91.4 ± 9.684
Stems	75.8 ± 8.109	73.8 ± 8.001
Flowers	67.8 ± 7.635	69.8 ± 7.521
Fruits	22.4 ± 1.434	26.4 ± 2.007

TPC: Total phenolic content; GAE: Gallic acid equivalents

Table 2: Total flavonoids content (TFC)

	TFC (mg RE/g of dry extract)	
Sample	*Aqueous extract*	*Methanolic extract (80%)*
Leaves	68.7 ± 3.275	82.9 ± 4.157
Stems	61.9 ± 3.359	68.5 ± 3.125
Flowers	58.1 ± 2.791	34.4 ± 1.578
Fruits	14.4 ± 1.473	13.8 ± 0.733

TFC: Total flavonoids content; RE: Rutin equivalents

extract (13.8 ± 0.733 RE/g). Studies indicated that antioxidant activities of *C. spinosa* are related to the high level of phenolic compounds, as such, the species of this plant have rich alkaloids, lipids, polyphenols, flavonoids, and glucosinolates.[30-33]

Method for *In vitro* Anti-Diabetic Activity

Inhibition Assay for α-Amylase Activity (DNSA)

In an effort for identifying herbal drugs that may become useful in the prevention or alleviation of diabetes,[34] this study is performed. Herbal plants have long been used to treat diabetes, as their principal bioactive components showed good antidiabetic and antioxidant properties.[35] Many herbal plant extracts have been reported for their α-amylase inhibitory activities. These α-amylase inhibitors are also called starch blockers since they prevent or slow down the absorption of starch into the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltotriose, and other simple sugars.[36] Based on our research, it is shown that the potency of α-amylase inhibition is related to the presence of certain compounds, such as, tannins, phenols, flavonoids, and compounds with antioxidant activities.[37,38] So we investigated the presence of the phenolic compounds and flavonoids of the samples. The antioxidant potentials of *C. spinosa* were reported in another study.[39] In our study, the methanolic and aqueous leaf extracts showed the highest inhibition of α-amylase be 90.87 and 86.31% with an IC_{50} value of 0.79 and 0.98 mg/mL, respectively (Tables 3 and 4). This activity could be attributed to the presence of phenols (mg GAE/g sample, Table 1) and flavonoids (mg rutin/g sample, Table 2). Not only are polyphenols capable of reducing oxidative stress, but also they inhibit carbohydrate hydrolyzing enzymes because of their abilities for binding proteins.[40,41] Our results are in accordance with a previous study, in which there was a positive correlation between total polyphenols and flavonoids contents and the ability to inhibit intestinal α-glucosidases and pancreatic α-amylase.[42]

TLC Bioautography

The studies have shown that dietary intake of polyphenols, such as, flavonoids, phenolic, and tannins-rich food influences peripheral glucose uptake in both insulin and noninsulin sensitive tissues. The isolation and identification of secondary metabolites produced by plants are important steps on the way to use these compounds as active principles in medicinal preparations. One of the most effective and inexpensive techniques of plant extracts analysis is bioautography, which is the hybridization of planar chromatographic

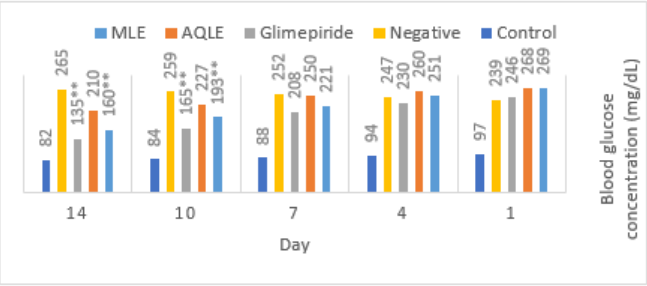


Figure 1: Effect of 14 days of treatment with methanolic and aqueous leaves extracts of *C. spinosa* on serum glucose level after alloxan (150 mg/kg i.p.)-induced diabetes in rats; MLE: Methanolic leaves extract; AQLE: Aqueous leaves extract; Values are presented as the mean ± SEM; n = 3 rats per group; $^{**}p < 0.01$

Table 3: % α-amylase inhibition by methanolic extracts

Sample	*Concentration (mg/mL)*	*Inhibitory activity (%)*	*IC_{50} (mg/mL)*
Leaves	0.2	25.88	
	0.4	38.96	
	0.8	52.41	0.79
	1.2	66.12	
	1.6	75.64	
	2	90.87	
Stem	0.2	19.84	
	0.4	29.74	
	0.8	35.65	1.13
	1.2	48.52	
	1.6	66.21	
	2	80.33	
Flower	0.2	12.98	
	0.4	20.36	
	0.8	28.56	1.46
	1.2	39.36	
	1.6	53.21	
	2	68.91	
Fruits	0.2	11.54	
	0.4	19.32	
	0.8	29.44	1.77
	1.2	38.36	
	1.6	47.88	
	2	53.94	

Table 4: % α-amylase inhibition by aqueous extracts

Sample	*Concentration (mg/mL)*	*Inhibitory activity (%)*	*IC_{50} (mg/mL)*
Leaves	0.2	20.84	
	0.4	28.32	
	0.8	40.87	0.98
	1.2	59.63	
	1.6	74.77	
	2	86.31	
Stem	0.2	15.38	
	0.4	25.78	
	0.8	38.69	1.34
	1.2	49.31	
	1.6	55.52	
	2	66.02	
Flower	0.2	16.45	
	0.4	26.32	
	0.8	36.74	1.43
	1.2	48.21	
	1.6	58.32	
	2	64.97	
Fruits	0.2	12.21	
	0.4	20.54	
	0.8	27.44	1.79
	1.2	34.58	
	1.6	46.91	
	2	54.65	

analysis with the biological detection method.[18] According to the literature, the separation of polyphenols is mostly performed using silica gel and ethyl acetate:acetic acid:formic acid:water (100:11:11:26 v/v) as a mobile phase.[43] In the present study, TLC chromatogram of the methanol, aqueous leaves extracts and standard compounds (quercetin, rutin, kaempferol, and gallic acid), is presented in spots were characterized by R_f values and color under UV light with colors with NP/PEG reagent (UV-NP/PEG, Table 5 and Figure 1). The results of one of the TLC analysis showed that a number of flavonoids and phenolic acid, especially, rutin, quercetin, and gallic acid, were found in two extracts. It is observed from Figures 2C and F that the leaves extracts and standards are positive for α-amylase inhibitors, showed blue stains upon iodine staining at the positions of separated spots on the TLC plate, indicating the separated spot was responsible for the inhibitory activity in the corresponding extract. This reaction we attribute to the starch-iodine complex formation resulting from starch, which was not hydrolyzed by the enzyme due to its inhibition by the compound and hence, got stained blue by iodine on that position. This means that leaves contain rutin, quercetin, and other flavonoid compounds, which may be responsible for inhibitory activity. Previous studies indicate that *C. spinosa* is rich in flavonoids, such as, rutin and quercetin,[30] which have been reported to possess antidiabetic activity.[31]

Estimation of Hypoglycemic Activity of Leaves of *C. spinosa*

Alloxan destroys pancreatic β-cells to induce hyperglycemia. It is notified that a range of 140 to 180 mg/kg dose range of alloxan may be efficient for this purpose in mice.[44] Therefore, the dose of alloxan was selected 150 mg/kg (i.p.).

Table 6 and Figure 1 depicted that the hypoglycemic effect of *C. spinosa* aqueous and methanol extracts on the

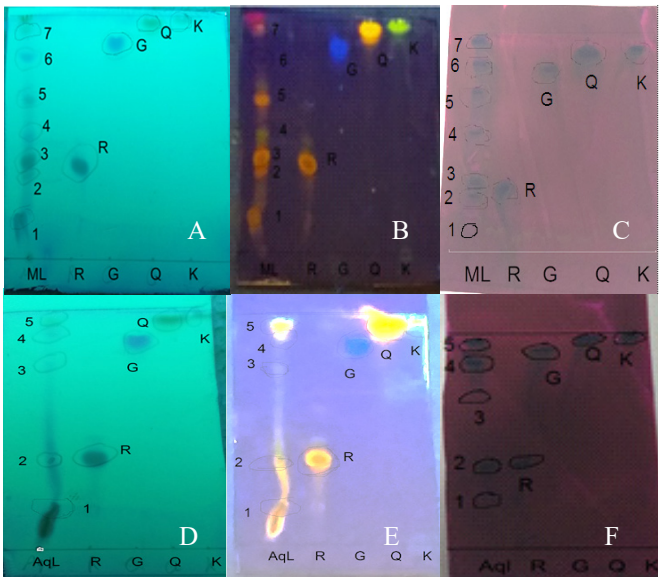


Figure 2: Detection of flavonoid aglycones: **(A)** TLC chromatogram of methanolic leaves extract; **(B)** Detection with NP/PEG reagent under 366 nm; **(C)** TLC-α-amylase bioautogram of methanolic leaves extract; **(D)** TLC chromatogram of aqueous leaves extract; **(E)** Detection with NP/PEG reagent under 366 nm; **(D)** TLC-α-amylase bioautogram of aqueous leaves extract; ME: Methanolic extract; Aql: Aqueous leaves extract; R: Rutin; G: Gallic acid; Q: Quercetin; K: Kaempferol

Table 5: R_f values of flavonoid compounds identified and their colors with NP/PEG reagent on TLC chromatogram under UV light

Mobile phase	*Extract*	*Spots*	*UV/ NP/PEG*	*R_f value*
Ethyl acetate:formic acid:glacial acetic acid:methanol (100:11:11:26)	Methanolic (80%)	1	Orange	0.2
		2	Yellowish orange	0.31
		3	Orange	0.39
		4	Light yellow	0.49
		5	Yellowish orange	0.64
		6	Light violet	0.82
		7	Yellowish orange	0.95
		Rutin	Orange	0.38
		Gallic acid	Blue	0.87
		Quercetin	Orange	0.96
		Kaempferol	Yellow-green	0.98
	Aqueous	1	Orange	0.16
		2	Orange	0.34
		3	Light blue	0.72
		4	Yellow	0.91
		5	Yellow	0.93
		Rutin	Orange	0.35
		Gallic acid	Dark blue	0.83
		Quercetin	Yellowish orange	0.91
		Kaempferol	Yellowish green	0.94

blood glucose in normal and diabetic mice. The administration of the glibenclamide (0.1 mg/kg) significantly decreased blood glucose at 14 days, where the level of blood glucose decreased 111 mg/dL ($^{**}p < 0.01$), when compared with the diabetic control group in diabetic rats. The administration of the methanolic leave extract (MLE) (200 mg/kg b.w.) group at 14 days significantly ($^{**}p < 0.01$) decreased blood glucose (approximately 58 mg/dL) when compared with the diabetic control group in diabetic rats. However, no significant difference in blood glucose levels was observed with the aqueous extract (AQLE) (200 mg/kg) group. Several studies have shown the antihyperglycemic and hypolipidemic activities of *C. spinosa*. The putative mechanisms involved in the antihyperglycemic effects of *C. spinosa* include reducing carbohydrate absorption from the small intestine, inhibiting gluconeogenesis in the liver, enhancing glucose uptake by tissues, and β-cell protection/ regeneration. This plant also ameliorates cardiovascular disorders, liver damage, and nephropathy in animal models of diabetes, which are attributed to its antioxidant phytochemicals, such as, phenolic compounds, flavonoids, carotenoids, tocopherols, and terpenes. Antihyperglycemic and hypolipidemic activities of *C. spinosa*, along with its beneficial effects on diabetic complications, make it a good candidate for the management of diabetes.[45]

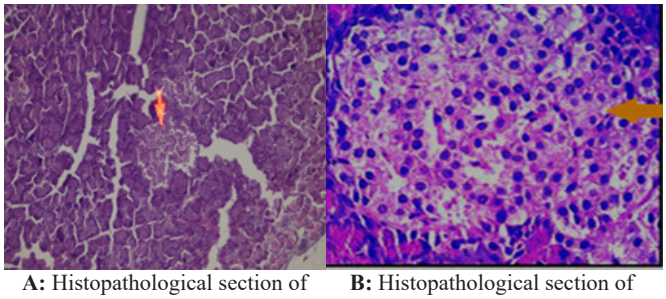
Pathological Observations

The light microscopic examination of pancreatic sections showed them divided into lobules by connective tissue septa. Lobules were composed largely of grape-like clusters of exocrine cells called acini. Islets of Langerhans were embedded forming the endocrine component of the pancreas. They appeared as pale stained oval or rounded areas inside the pancreatic lobules. Pancreatic sections from the negative group which is treated with alloxan showed disorganization of the structure of the endocrine and exocrine cells illustrated in less of Langerhans cells with damaged and necrotic pancreatic acini, as illustrated in Figure 3A. The pancreatic section from group 2 showed normal pancreatic structure (normal islets of Langerhans and normal acini tissues), as illustrated in Figure 3B. On the other hand, group 3 which is treated with aqueous extract of leaves (200 mg/kg) showed moderate improvement in pancreatic islets size, increased number of β-cells, and relatively restored islets covering connective tissue sheet (Figure 3C). Group 4 which is treated with methanolic extract of leaves (200 mg/kg) showed marked improvement with a restored size of islets of Langerhans, also showing regular islets cells with increased number and abundant eosinophilic cytoplasm and central small nuclei; most of these cells restored their rounded shape while few of them still with an elongated shape. The islets covering connective tissue are also relatively stored and regain their normal texture, as illustrated in Figure 3D. Positive control rats treated with glibenclamide (Figure 3E) also manifested complete restoration of the islet of Langerhans, has a normal arrangement of islet cells and the cellularity is likened to that of the normal control. The results of this study were consistent with one of the other previous reports that phenolic extracts from leaves of *C. spinosa* exhibited a hypoglycemic effect in diabetic rats.[31] Recently, reported that *Capparis* species leaves are rich in phenolics were effective in reducing blood glucose of diabetic rats and have high antioxidant activity and at the same time exhibited strong hypoglycemic activity.[46] On the other hand, it is commonly known that alloxan potentially damages the pancreatic β-cells because it led to the antioxidant competence of the β-cells being much lower than cells in other tissues did.[44] Therefore, an extract prepared with 80% ethanol from *C. spinosa* leaves, is abundant in phenolics with superior antioxidant activity.[39] Its hypoglycemic activity might due to its antioxidant activity to enhance pancreatic β-cells viability of alloxan-induced diabetic rats in this study. Furthermore, a decline of increased postprandial hyperglycemia through inhibition of enzymes is used in the management of T2DM, with the main enzymes being α-glucosidase and α-amylase.[47-49]

Table 6: Effect of 14 days of treatment with methanolic and aqueous leaves extracts of *C. spinosa* on serum glucose level after alloxan (150 mg/kg i.p.)-induced diabetes in rats; MLE: Methanolic leaves extract; AQLE: Aqueous leaves extract; Values are presented as the mean ± SEM; n = 3 rats per group; $^{**}p < 0.01$

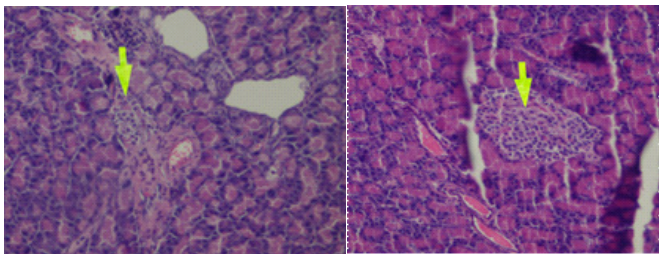
Day	*AQLE*	*MLE*	*Glimepiride*	*Negative*	*Control*
1	268 ± 2.646	269 ± 10.148	246 ± 9.539	239 ± 14.422	97 ± 7.937
4	260 ± 9.539	251 ± 17.776	230 ± 19.974	247 ± 12.124	94 ± 8.888
7	250 ± 18.357	221 ± 8.718	208 ± 10.44	252 ± 7.81	88 ± 7.55
10	227 ± 7.211	193 ± 11.358**	165 ± 11.718**	259 ± 7.55	84 ± 5.568
14	210 ± 13.228	160 ± 18.521**	135 ± 16.093**	265 ± 20.421	82 ± 2
Statistical significance					
		Difference in averages between groups		*Std. Error*	*p*
AQLE		MLE	-51**	9.60324	0.004
Glimepiride		-53**	9.60324	0.004	
MEL		AQLE	51**	9.60324	0.004
Glimepiride		-2	9.60324	0.976	
Glimepiride		AQLE	53**	9.60324	0.004
MEL		2	9.60324	0.976	

**indicates a statistically significant difference between the groups studied at the value of $p < 0.01$



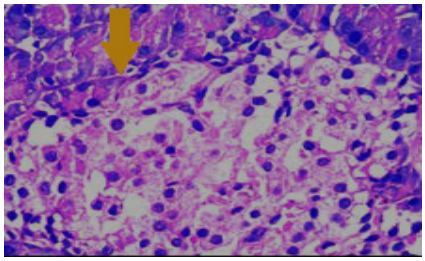
A: Histopathological section of pancreas of alloxnized rats; the arrow points to necrotic ß-cells

B: Histopathological section of pancreas of normal rats; the arrow points to normal ß-cells



C: Histopathological section of pancreas of rats treated with aqueous extract; the arrow points to regenerated ß-cells (less than methanolic extract)

D: Histopathological section of pancreas of rats treated with methanolic extract; the arrow points to regenerated ß-cells



E: Histopathological section of pancreas of rats treated with glimepiride; the arrow points to regenerated ß-cells

Figure 3: Photomicrographs of pancreatic tissues stained with Hand E, X200, A2

Other previous studies showed that *C. spinosa* exhibited inhibitory activities against α-amylase,[50] indicating that *C. spinosa* could also contribute to the treatment of T2DM. All the aforementioned results demonstrated that *C. spinosa*, being underutilized by products, held the huge potential to be developed into a novel dietary phytonutrient for the management of T2DM.

CONCLUSION

In conclusion, our findings revealed that the methanolic leaves extract have high phenolic and flavonoid contents, as well as, antidiabetic activity. Therefore, it appears to be used as a natural source of antidiabetic. Also, it can be speculated that the findings of our work could make the background for further investigation of this species, especially, research concerning individual phenolic compounds.

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