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Estimation of phenolic content, flavonoid content, antioxidant properties and alpha-amylase inhibitory activities of *Capparis spinosa* L.

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Abstract
The methanolic extract of *Capparis spinosa* was studied to evaluate the phenolic content, and flavonoid content, α-amylase inhibition and antioxidant activities using *in-vitro* methods.
The extraction of the medicinal plant was carried out by maceration in methanol. The findings revealed the presence of elevated amounts of phenols and flavonoids in *C. spinosa*. The phenolic content was 555±0.5mg gallic acid equivalent per gram, while the flavonoid content was 102.59±0.6mg rutin equivalent per gram. The plant displayed a considerable free radical scavenging activity on DPPH with IC_{50} of 0.07±0.02mg/ml in comparison to the standard antioxidant gallic acid (IC_{50} 0.03±0.002mg/ml). The α-amylase inhibitory activity of the extract exhibited an IC_{50} value of 0.34±0.003mg/ml compared to the reference drug acarbose (0.064±0.001mg/ml). The methanolic extracts of the aerial parts of *C. spinosa* have significant antioxidant and α-amylase inhibitory activities. This outcome indicates that caper can be employed in the development of plant-based foods for the treatment of diabetes mellitus.

Keywords: Libya, gallic acid, plant extract, α-amylase, *Capparis spinosa* L

1. Introduction
Defects in the process of insulin secretion, action, or both can result in a group of metabolic disorders characterized by high blood sugar known as diabetes mellitus. Numerous problems are associated with the chronic hyperglycemia of diabetes involving dysfunction and long-term damage to different organs such as the eyes, kidneys, nerves, heart, and blood vessels. These medical conditions have been linked to the excessive production of free radicals leading to oxidative stress, therefore antioxidants have been considered as a significant treatment [1, 2].
The principal cause of fasting hyperglycemia is the elevated rate of glucose production by the liver in the presence of hyperinsulinemia; the impaired suppression of this process by insulin after a meal and the reduction of insulin-dependent glucose transport into skeletal muscles contribute nearly equal to the postprandial hyperglycemia [3].
Recently, several research projects have been focused on the role of oxidative stress in the progression of various complications of diabetes [4]. Free radicals are progressively generated in the body as a result of normal metabolic processes and continuous exposure to environmental stimuli. Oxidative stress arises from the imbalance between radical-producing and radical-scavenging mechanisms, which lead to increased free radical production and reduced activity of antioxidant defenses systems. The main causing factor of the oxidative stress in diabetes is the uncontrolled production of free-radical oxygen species. However, other underlying processes including auto-oxidation of glucose, non-enzymatic protein glycosylation, impaired glutathione metabolism, the formation of lipid peroxides, and alteration in antioxidant enzymes were also involved in the pathogenesis of diabetes mellitus [5, 6, 7, 8].
The management of Type 2 diabetes is still associated with several adverse reactions. Most of the available anti-diabetic drugs such as sulfonylurea, metformin, and meglitinides may cause different side effects involving hypoglycemia, fever, nausea, vomiting, diarrhea, and decreased appetite [9].
Herbal medicine is concerned with the utilization of plant ingredients to treat diseases. Moreover, the field has become an alternative to reduce the side effects caused by conventional treatment. Phytotherapy has shown anti-diabetic activities and curative properties in traditional treatment regimens. Furthermore, herbs are available, easier to process, and used with fewer side effects [10]. For decades, the medicinal plants are used for the management of diseases, as they are rich in therapeutic agents and biologically active ingredients that have

health-promoting effects [11]. Plants commonly contain significant concentrations of antioxidant compounds, tannins, and flavonoids. According to the above fact, the present study suggests that the antioxidant action exerted by medicinal herpes is related to their antihyperglycemic properties on diabetes mellitus [12].

Therefore, medicinal herbs such as *Capparis spinosa* (*C. spinosa*) continue to have a profound role in healthcare systems [13], the plant is one of the most important economical species in the *Capparidaceae* family which has a wide range of diversity (about 40-50 genera and 700-900 species) [14]. *C. spinosa* is native to the Mediterranean Basin and widely distributed in Libya, in which it is considered as an endemic plant in the Al-Jabal Al-Akhder area [15].

In general, *C. spinosa* has an extensive range of bioactive compounds such as alkaloids, flavonoids, steroids, terpenoids, and tocopherols [16]. The herb is a rich source of flavonoids; hence, many studies have identified and quantified these compounds. Rutin and quercetin are detected in *C. spinosa* as the main flavonoid glycosides [17]. Rodrigo *et al*. 1992 reported that fresh capers contain rutin, kaempferol-3 rutinoside, kaempferol-3-glucoside, and kaempferol-3-rhamnorutinoside [18]. Additionally, other flavonoids such as quercetin 3-O-glucoside, quercetin 3-O-glucoside-7-O-rhamnoside, and quercetin 3-O-[6´´´-α-L-rhamnosyl-6´´-β-D-glucosyl]-β-D glucoside were also identified in the aerial parts of *C. spinosa* [19].

Previous literature confirmed the antihyperglycemic characteristics of *C. spinosa*. Jalali *et al*. 2016 investigated the anti-diabetic effects of aqueous extracts of *C. spinosa* fruit in streptozotocin-induced diabetic rats [20]. Eddouks *et al.* 2004 recorded a significant hypoglycemic effect after a single dose of the plant extract [21].

The methanol extracts of *C. spinosa* were confirmed to have antioxidant free-radical scavenging action in various models [22, 23] and has been suggested as a treatment for pathological conditions associated with oxidative stress [24].

2. Material and Methods

Plant Material

The aerial parts of *C. spinosa* plant were collected during August from Botraba area, about 100 km east of Benghazi, Libya. Samples of the plant were sent to the Department of Pharmacognosy (Faculty of Pharmacy, University of Benghazi) for identification. The aerial parts were left to dry in the open air. The dried plant materials were milled into a fine powder to be used for extraction.

Extraction Preparation

The methanol (70%) extract was obtained through exhaustive cold maceration of the dried plant powder. The solvent was evaporated under reduced pressure (at 40C°). The residual kept for further chemical or biological examination.

Assessment of the Total Phenolic Content (TPC)

The content of total phenolic compounds in the methanolic extract was determined by Folin-Ciaculteau's method [25]. The intensity of the blue color was measured at λ_{max} 725nm against a blank (distilled water). Different concentrations of gallic acid were used to plot the standard calibration curve [26]. Determinations were carried out in triplicates and expressed as mg gallic acid equivalents per gram of dry weight of extract (mg GAE/g) [27, 28]. The following equation was used for the calculation:

C=cV/m

Where, C= TPC, c=concentration of gallic acid obtained from the calibration curve in mg/ml, V=Volume of extract in ml, m=Weight.

Assessment of the Total Flavonoid Content (TFC)

The aluminum chloride colorimetric method was applied. The technique is based on measuring the intensity of the color resulted when flavonoids are complexed with aluminum chloride ($AlCl_3$) [28, 29]. The intensity of the developed yellow color is measured at λ_{max} 420nm in a UV/V spectrophotometer against a blank prepared using one milliliter of methanol instead of the standard solution [30]. The average absorbance values obtained at different concentrations of rutin were used to plot the calibration curve. The TFC in the extract was expressed as milligrams rutin equivalent per gram of dry weight of the sample (mg QE/g). The following equation was used for the calculation:

C=cV/m

Where, C=TFC in mg QE/g, c=Concentration of rutin estimated from the calibration curve in mg/ml, V=Volume of extract in ml, m=Weight.

DPPH Radical Scavenging Assay

The free radical neutralizing ability of the extracts was tested by DPPH radical scavenging assay as described by Bouaziz *et al*. 2010 [31]. The discoloration of the purple-colored methanol solution of DPPH (Sigma-Aldrich, Saint Quentin Fallavier, France) indicates the scavenging capacity of the corresponding extracts. Briefly, to 5mL of a 0.004% methanol solution of DPPH, Aliquots of different concentrations of the test compound in methanol were added. After thirty minutes of incubation at 25Cº, the absorbance was measured against the blank (all reagents except the test compound) at 517nm. Percent inhibition of free radical DPPH (I %) was determined as following:

I% = [(A blank-A sample)/A blank] x 100,

Where A sample refers to the absorbance of the test compound, while the absorbance of the control reaction is A blank. Test compound concentration providing 50% inhibition (IC_{50}, expressed in g/mL) was calculated from the graph of inhibition percentage against extract concentration. Butylated hydroxytoluene (Sigma-Aldrich) was used as positive control standard antioxidant. All tests were performed in triplicates.

α-Amylase Inhibitory Activity

The antihyperglycemic activity of plant extract was calculated by applying the α-amylase inhibition assay proposed by Kusano *et al*. 2011 [32]. The technique is based on the change in the intensity of the blue color developed as a result of iodine binding to starch polymers, the difference in reading between the control (starch and iodine) and test sample (starch, Alpha-amylase, the sample, and iodine) was measured spectrophotometrically at 630nm. α-Amylase (3µg/ml) was dissolved in 20mM phosphate buffer (pH 6.9) to prepare the enzyme solution. Then 200µl of varying concentrations (640µg/ml, 320 µg/ml, 160µg/ml, 80µg/ml, and 40µg/ml) of plant extracts and acarbose were separately pre-incubated with 400µl of the substrate for 15min at 37 °C, then 200µl of α- amylase solution was added to each of them and incubated again for 15min at 37Cº. After the incubation, 800µl of HCl (0.1M) was added to reduce the enzymatic reaction. Followed by the addition of 1000µl of iodine reagent to measure the absorbance at 630nm. The assay was performed in triplicates.

The percentage of enzyme inhibition was calculated using the following formula:

% Inhibition = 1-[Abs2–Abs1/Abs4–Abs3] ×100
Where, Abs1=Absorbance of the incubated mixture containing plant extract, starch, and amylase, Abs2=Absorbance of the incubated mixture containing the samples and starch, Abs3=Absorbance of the incubated mixture containing starch and α-amylase, Abs4=Absorbance of incubated solution containing starch only.
The standard graph was plotted by taking the concentration on the X-axis and percentage inhibition on the Y-axis.

3. Results and Discussion
The result of phenolic and flavonoid contents is displayed in Table 1. The methanolic extract of *Capparis spinosa* (MEC) showed high phenolic and flavonoid contents (555mg GA/g and 102mg Rut/g respectively).

Table 1: Total phenols and flavonoid contents of MEC

Sample	Flavonoids mg Rut/ g**	Phenolic mg GA/g***
(MEC)	102.59±0.6	555 ± 0.5

*Values are presented as mean ± SE of 3-test sample observations.
Rutin (Rut) equivalent, * Gallic acid (GA) equivalent

Simple phenols, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives, and flavonoids are different classes of phenolic compounds, which are considered of the most important secondary metabolites generated by plants. Several studies have linked herbs that are rich in such compounds with their capacity to reduce the risk of numerous chronic diseases [33, 34]. The result of this research supports the reports published by Tlili *et al*. 2010 and Mansour *et al*. 2016 concerning the content of phenolic compounds in leaves and buds of *C. spinosa* [17, 35]. The nutritional and medicinal values of *C. spinosa* were confirmed, as it was rich in these compounds. Therefore, the plant can be used as a potential source of phenols and flavonoids.

DPPH Radical Scavenging
The antioxidant activity outcomes are presented in Table (2) in terms of IC_{50}, which is the concentration in mg/ml causing 50% inhibition of the free radical.

Table 2: The antioxidant activity of MEC against gallic acid in terms of IC_{50}±SD

Sample	DPPH % Inhibition*
(MEC)	0.07±0.02
Gallic acid	0.03±0.002

*Values are presented as mean ± SE of 3-test sample observations.

The MEC revealed a significant scavenging effect on the DPPH radical, indicating a strong antioxidant activity compared to gallic acid.
The free radical scavenging capacity observed is related to the high content of phenolic compounds in the extract. The finding of this study agrees with those reported by Germano` *et al*. 2002 [23]. Several investigations have shown a profound relation between the total phenolic contents and the anti-oxidative activity of plants [31, 37, 38].

α-Amylase Inhibitory Activity
All concentrations of MEC exhibited inhibitory effects on the α-amylase enzyme as shown in Table (3).

Table 3: Result of the *in vitro* α-amylase inhibitory assay of (MEC)

Sample	IC_{50} (mg/ml)
(MEC)	0.34± 0.003
Acarbose	0.064±0.001

Controlling blood glucose levels is an essential approach to prevent or limit the complications of diabetes. Inhibition of carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) is a helpful strategy for the regulation of hyperglycemia in patients with type-2 diabetes mellitus [39, 40, 41]. α-Amylase inhibitors decrease the digestion of carbohydrates, resulting in reduced postprandial plasma glucose rise [42]. Acarbose is a well-known drug that exerts its effect through this mechanism and widely used for clinical treatment of diabetes mellitus. It is a competitive inhibitor of α-amylase; the pathway of the inhibition seems to be due to the unsaturated cyclohexene ring and the glycosidic nitrogen linkage that mimics the transition state for the cleavage of glycosidic linkages of the carbohydrate by the enzyme [43, 44]. Lo Piparo *et al*. 2008 investigated the interactions between flavonoids and human α-amylase; they approved that the potency of inhibition of these compounds is correlated with the number of hydroxyl groups on the flavonoid skeleton [45]. These results follow the mechanism of action proposed for acarbose [46]. *C. spinosa* leaves and seeds have been reported to have anti-diabetic properties as their extracts significantly lowered triglycerides and blood glucose levels [47]. Rahmani *et al*. 2003 and Huseini *at el*. 2013 indicated that the fruit extract of *C. spinosa* (400mg three times/day for two months) could reduce the fasting blood glucose, glycosylated hemoglobin, and triglyceride in diabetic patients [36, 48]. This work investigated the inhibitory effect of *C. spinosa* methanolic extract on α-amylase activity.
MEC exhibited a considerable α-amylase inhibitory effect with IC_{50} of 0.34±0.003mg/ml in comparison to the positive control Acarbose (0.064±0.001). This outcome may be due to the presence of different chemical constituents such as phenolic compounds, terpenes, flavonoids, and alkaloids.

4. Conclusion
The present study provides some scientific support for the traditional use of *C. spinosa* as a treatment for some medical conditions such as diabetes. The phytochemical investigation indicated that the plant extract could be a good source for the isolation of bioactive secondary metabolites such as phenols and flavonoids. The selected herb revealed marked antioxidant and anti-diabetic properties and may have the potential to be utilized for the production of food supplements for limiting postprandial hyperglycemia.

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