



Evaluation of Total Phenolic Content and Antioxidant Activity of Three Leaf Extracts of *Ziziphus spina-christi* (Sedr) Grown in Jordan

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Authors' contributions

This work was carried out in collaboration between all authors. Author SMJK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author ASJ contributed to the sample preparation, extraction procedure for antioxidants and preparation of the paper. Author MSYH participated in designing the study, collecting plant material, reading and revising the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The primary aims of this study were to evaluate the total phenolic content and *in vitro* antioxidant activity of three different leaves extracts (methanolic, ethanolic and aqueous) of *Ziziphus spina-christi* grown in Jordan.

Methods: Total phenolic content of the methanolic, ethanolic and aqueous extracts of the leaves of *Ziziphus spina-christi* was determined spectrometrically according to the Folin-Ciocalteu procedure. The antioxidant activities of the leaves extracts of this plant at different concentrations were determined by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging activity and reducing power methods. All the analyzes was made with the use of UV-Visible spectrophotometer, and ascorbic acid was used as a standard antioxidant.

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Jordan. Department of Nutrition and Food Technology, University of Jordan, Amman, Jordan, from April 2015 to December 2015.

Results: The total phenolic content was ranged between 11.8 to 52.5 mg/g expressed in terms of Gallic acid equivalent (mg of GAE/g extract). *In vitro* antioxidant activity of the plant extracts revealed that all the extracts showed good antioxidant power with IC₅₀ values of 21.4, 24.2 and 54.3 µg/mL for methanolic, aqueous and ethanolic extracts, respectively. The reducing power of the extracts was found to be concentration dependent. The results of this study revealed that, the methanolic extract of leaves showed the highest phenolic concentration and largest antioxidant activity.

Conclusion: From this study it may be concluded that *Z. spina-christi* leaves could have potential source of antioxidants for pharmaceutical drug preparations.

Keywords: *Ziziphus spina-christi*; total phenolic content; DPPH; reducing power.

1. INTRODUCTION

Medicinal plants are important for human health, due to their contents of antioxidants and phenols [1,2]. Antioxidants act as radical scavengers, inhibit lipid peroxidation and other free radical-mediated processes, and therefore they protect the human body from several diseases attributed to the reactions of radicals [3,4]. Natural products, particularly those present in medicinal plants, have gained more interest as food ingredients because of their safety, accessibility, and positive impact on health [5].

Ziziphus spina-christi is a deciduous shrub belong to the family *Rhamnaceae* and grow throughout Middle Eastern region including Jordan. It is commonly called as "Sedr" and also known as 'Nabak' [6]. Since ancient times, it has been among the key plants of the Jordanian traditional medicine. *Ziziphus* species have been also used for traditional eastern and western herbalism for long times.

Among *Ziziphus* species multiple pharmacological actions, anti-inflammatory activity has been extensively studied [7,8]. The medicinal properties of this plant depend on the part of the plant and the extract used. For instance, fruits are applied on cuts and ulcers. They are also used to treat pulmonary ailments and fevers and to promote the healing of fresh wounds, for dysentery [9]. The leaves are applied as poultices and are helpful in liver troubles, asthma and fever [10]. The seeds of *Zizyphus* were found to be effective in the improvement of blood glucose and lipid levels in serum of dietary hyperlipidemic rats [11]. Interestingly, the flowers are important source for honey bee. The winter honey (i.e Napak honey) collected from the flowers of the sedr is in high demand by citizens

for its medicinal qualities in addition to its excellent taste and fragrant smell [12].

Recently, many researches were conducted the plant extracts of *Ziziphus* species to be exhibited anti-diabetic effects [13], as well as anti-bacterial [14], antifungal [15], anticancer [16], and antioxidant [8]. The anti-oxidative capacities of ethanol and petroleum ether extracts of *Z. spina-christi* leaves were evaluated by hydroxyl radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, lipid peroxidation and superoxide radical standardization methods [17]. A study done by Yossef et al. [18] proved that *Z. spina-christi* has protective effect against carbon tetrachloride (CCl₄) induced oxidative stress and hepatotoxicity in Albino Wistar rats. And the hepatoprotective effect might be correlated with its antioxidant and free radical scavenger effects.

A survey of the literature revealed that a number of cyclopeptide and isoquinoline alkaloids, flavonoids, terpenoids and their glycosides have been found to occur in various amount in most *Ziziphus* species, and to exhibit *In vitro* antioxidant properties [19]. The leaves of these plants contain betulinic and ceanothic acids, various flavonoids, saponins, tannins and triterpenes [12,20].

The use of natural extracts as alternatives to the chemically synthesized formulations may prove a successful tool in drug technology for treatment of different diseases. To our knowledge no research has been done on the antioxidant activity of *Z. spina-christi* extract grown in Jordan. Therefore, this study aimed to assess the *In vitro* antioxidant activity property of different leaf extracts of *Z. spina-christi* grown in Jordan, and to quantify total phenolic content in different polarity solvents. This study will open

new areas of application of extracts of this plant as antioxidant agents in medical industry.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh Leaves of *Z. spina-christi* were collected from different locations of Jordan valley in spring 2015. The collected plant material was rinsed with distilled water to remove any dust and particulate matter and dried at room temperature for several days.

2.2 Preparation of *Zizyphus spina-christi* Leaf Extracts

Dried leaves (500 g) were ground and extracted in distilled water, ethanol and methanol at 20% (w/v) concentration. The mixtures were mixed on rotary shaker for two hours and then for 15 min in ultrasonic bath. The mixtures were filtered through whatman no: 4 and then membrane filter (0.45 μ m). The excess solvents from the filtrate were evaporated under vacuum using a rotary evaporator (Buchi, Switzerland) at 40°C. The crude concentrated extract was transferred to brown colored sample vial and stored in a refrigerator until used [21].

2.3 Determination of Total Phenolics

The concentration of phenolics in the extracts was determined by the method of Singleton *et al.* [22]. Samples (0.5 mL) were mixed with 2.5 mL of 0.2 N Folin-Ciocalteu reagent (Sigma-Aldrich) for 5 min, then 2 mL of 7.5% sodium carbonate was added. After standing for 2 h at room temperature, the Absorbance was measured at 760 nm using UV/visible spectrophotometer (SL-150 UV/Vis spectrophotometer, India). The content of phenolics was expressed as mg gallic acid equivalents (GAEs) per g of extract. A standard calibration curve for gallic acid was prepared by running a series of standard solutions.

2.4 Antioxidant Activity

2.4.1 DPPH radical scavenging activity

Scavenging activity of the three extracts on DPPH was assessed according to the method reported by Hsu *et al.* [23]. About 0.1 mL of various concentrations of the extract were mixed with 1.9 mL of 0.1 mM DPPH methanolic solution. The mixture was shaken vigorously and left to stand for 30 min at room temperature. The

absorbance was measured at 517 nm against a blank. Ascorbic acid was used as positive control. The DPPH radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_0 - A_1) / A_0 \times 100]$, where A_0 is the absorbance of control (without extract), and A_1 is the absorbance of sample.

The IC_{50} , which is the concentration required to scavenge 50% of DPPH free radical was calculated using dose inhibition curve by plotting the sample concentration versus the corresponding DPPH scavenging activity [24].

2.4.2 Determination of reducing power

The reducing power of the three extracts was determined according to the method of Oyaizu [25]. A 0.25 mL aliquot of various concentrations of the three extracts were mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide and the mixture was heated at 50°C for 20 min. After 2.5 mL of 10% trichloroacetic acid (w/v) was added, the mixture was centrifuged at 650g for 10 min. A 5 mL aliquot of the upper layer was mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride. The absorbance of this solution was measured at 700 nm using a spectrophotometer. Ascorbic acid at different concentrations was used as standard, and phosphate buffer was used as blank solution.

2.5 Statistical Analysis

All experiments were performed in triplicate ($n = 3$), ANOVA test of SPSS statistical software was used to compare the mean values of each treatment. Significant differences between the means of parameters were determined using the LSD and Dunnett tests ($P \leq 0.05$). The results were represented as means \pm standard deviation (SD) of three replicated determinations.

3. RESULTS

3.1 Total Phenolic Contents

The result obtained in this study showed that the amounts of total phenolic content differ significantly among the various extracts of the *Z. spina-christi*. The total phenolic contents in the examined *Zizyphus* extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent. The standard curve equation is: $y = 0.103 x + 0.15$, $R^2 = 0.96$. The

values obtained for the concentration of total phenols are expressed as mg of GAE/ g of extract (Fig. 1). The highest total phenolic content was observed in methanol extract 52.5 ± 0.46 (mg GAE/g extract), followed by the ethanol extract 34.0 ± 0.23 (mg GAE/g extract), while water extract contained the least 11.8 ± 0.51 (mg GAE/ g extract).

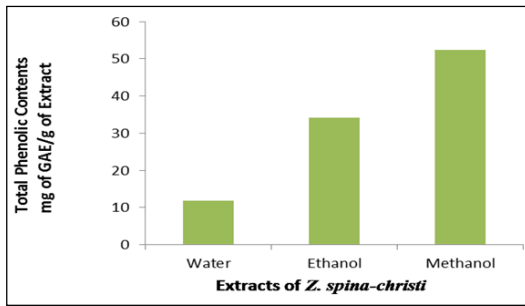


Fig. 1. Total phenolic contents of *Z. spina-christi* leaves extracts (methanol, ethanol and water)

- Values are Mean \pm SD of triplicate determinations.
- Total phenolic contents are expressed as milligram gallic acid equivalent per gram of plant extract (mg GAE/g extract)

3.2 DPPH Scavenging Activity

The scavenging effect of different leaves extract of *Z. spina-christi* on the DPPH free radical was compared with standard antioxidant, ascorbic acid. The results are expressed as % inhibition and are shown in Table 1. Free radical scavenging capacity increased with increasing extracts concentration. *Z. spina-christi* methanolic and aqueous extracts exhibited good radical scavenging activity, with IC_{50} (the extract concentration providing 50% of inhibition) values of 21.4 and 24.2 μ g/mL, respectively. While that of ethanolic extract was 54.3 μ g/mL. When compared with the standard antioxidant ascorbic acid (IC_{50} =7.1 μ g/mL), all extracts offered significantly ($p < 0.05$) lower antioxidant activity.

Table 1. DPPH radical scavenging activity of *Z. spina-christi* leaves extracts

	% inhibition \pm SD at different concentrations of the extracts				
	5 μ g/mL	10 μ g/mL	25 μ g/mL	50 μ g/mL	IC_{50} (μ g/mL)
Methanol	13 \pm 0.02 ^{ac}	27 \pm 0.01 ^{ac}	75 \pm 0.02 ^a	93 \pm 0.04 ^{acd}	21.4
Ethanol	6 \pm 0.03 ^b	9 \pm 0.02 ^b	24 \pm 0.01 ^b	46 \pm 0.03 ^b	54.3
Water	12 \pm 0.01 ^{ac}	25 \pm 0.05 ^{ac}	58 \pm 0.04 ^c	93 \pm 0.02 ^{acd}	24.2
Ascorbic acid	41 \pm 0.04 ^d	49 \pm 0.03 ^d	92 \pm 0.02 ^d	97 \pm 0.03 ^{acd}	7.1

- Values are the average of triplicate experiments and represented as mean \pm standard deviation
- Superscript a, b, c and d showed that means \pm standard deviation in the same column with the different superscript are significantly different at ($p < 0.05$)

However, there were no significant differences between methanolic and aqueous extracts comparing with ascorbic acid at a concentration of 50 μ g/mL.

3.3 Reducing Power

Fig. 2 showed the reductive capabilities of different leaves extract of *Z. spina-christi* compared with ascorbic acid. The reducing power of different leaves extracts increased with the increasing amount of extract. At 50 μ g/mL concentration, the reducing power was found to be a maximum value. The leaves extracts of *Ziziphus* showed absorbance of 0.379, 0.315, 0.133 for methanolic, aqueous and ethanolic extracts, respectively (Fig. 2). Thus, the plant leaves extracts exhibited a lower reducing ability than the standard. Also, the reducing ability was found to be the concentration dependent. With increasing concentration, the reducing ability of all the leaves extracts was found to increase. The results obtained from this study showed that ethanol extract of *Z. spina-christi* leaves significantly has the lowest reductive capability in comparison with the other extracts (methanol and water).

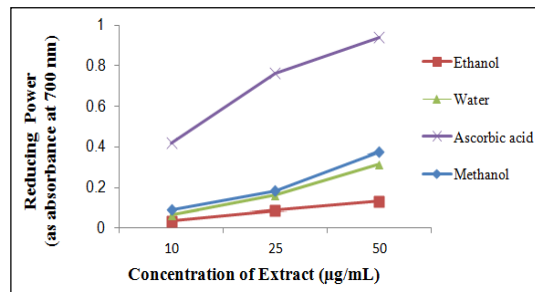


Fig. 2. Reducing power of different leaves extracts of *Z. spina-christi* compared with ascorbic acid

- Values are the average of triplicate experiments and represented as mean \pm standard deviation

4. DISCUSSION

In this study, three different leaf extracts (methanolic, ethanolic and aqueous) were prepared to evaluate the total phenolic content and antioxidant activity of the *Zizyphus spina-christi* grown in Jordan. The results showed that the amount of total phenolic content differ significantly among the various extracts of the *Z. spina-christi*. Methanolic extract showed a highest phenolic contents, followed by ethanolic and aqueous extracts. Our results are in accordance with a previous investigation [26] on *Z. spina-christi* fruit, where in, maximum amount of phenolic was examined in methanol extract followed by acetone and water extracts. Another research group [27], also observed similar trends as in the present study regarding extraction efficacy of solvents towards recovering phenolic compounds from *Tamarix leaves*.

The difference in the amounts of phenolics in different extracts may be attributed to nature of extracting solvent as well as the chemical nature and availability of the compounds extracted [28]. Some studies showed that methanol and ethanol were better extraction solvents for phenolics from plant materials than less polar solvents including acetone and hexane [29]. Plant materials may contain phenolics varying from simple (e.g., phenolic acids, anthocyanins) to highly polymerized substances (e.g., tannins) in different quantities. Moreover, phenolics may also be associated with other plant components such as carbohydrates and proteins. Therefore, there is no universal extraction procedure suitable for extraction of all plant phenolics [30]. In particular, methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols while the higher molecular weight flavanols are better extracted with aqueous acetone. Ethanol is another good solvent for polyphenol extraction and is safe for human consumption [31].

Results in Table 1 showed all extracts presented a good scavenging activity. Lower value of IC_{50} indicates higher antioxidant activity [32]. In addition, as shown in Fig. 2 the reductive ability of the extracts reflected the reducing power of the *Z. spina-christi* as a potential source of antioxidants. The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom. The presence of reductants in

extract causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm [33,34].

All extracts prepared in this study showed good scavenging and reducing power activities. Methanolic and aqueous extracts showed a powerful antioxidant activity, whereas water extracts recorded the lowest content of phenols. This can be explained by the fact that phenolic compounds existing in aqueous extract possess an ideal structure for the scavenging of free radicals since they contain a number of hydroxyl groups acting as hydrogen donors which makes them an important and very powerful antioxidant agents [35].

On the other hand, the obtained results showed that ethanol extract of *Z. spina-christi* leaves significantly has the lowest antioxidant activity in comparison with other extracts. This report is in agreement with the study done by Boeing et al. [36] on berries fruits, where, methanol was the most efficient solvent for extraction of antioxidant compounds, followed by water and ethanol. The present study, therefore, suggests that, ethanol was less efficient in the extraction of antioxidant compounds than methanol, even if their polarities were similar. This may be due to the low solvent properties provided by ethanol, probably because of the presence of the ethyl radical that is longer than the methyl radical present in methanol, resulting in a lower salvation of antioxidant molecules.

It is well known that plant phenolics in general are highly effective free radical scavengers and hence are antioxidants [37]. Thus the *In vitro* antioxidant properties of *Z. spina-christi* leaves may be possibility attributed to the phytochemicals present. According to different reports in the literature, whereas some authors found correlation between the total phenolic content and the antioxidant activity [38], others found no such relationship [39]. Antioxidant activity of extracts is strongly dependent on the solvent due to the different antioxidant potentials of compounds with different polarity [40]. However, the finding from this study regarding Jordanian *Z. spina-christi* antioxidant activity agrees with reported data of previous investigations which revealed a strong influence of extracting solvents on the antioxidant activity of the plant extracts [41].

5. CONCLUSION

Antioxidant properties of plant extracts have become of great interest due to their possible uses as natural additives to replace synthetic ones. To our knowledge, this is the first study dealing with the *In vitro* antioxidant activity of *Z. spina-christi* grown in Jordan. This study revealed that tested plant extracts have moderate to significant phenolic contents and presented a good DPPH radical scavenging and reducing power activities. *Z. spina-christi* leaves could have potential source of antioxidants for pharmaceutical drug preparations. Further studies on this plant species should be directed at a detailed qualitative analysis of all its parts and carried out *in vivo* evaluation of its antioxidant properties.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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