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Research Article
Studying the Heavy Metals Composition and the Impact of Different Common Solvents on the Extraction Efficiency of Phytochemical Secondary Metabolites from the Leaves of *Ziziphus spina-christi* Grown in Jordan

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Abstract

Objective: The purpose of this study was to investigate the impact of different common solvents on the extraction efficiency of phytochemical secondary metabolites and to determine the heavy metals composition of *Ziziphus spina-christi* (*Z. spina-christi*) medicinal plant available in Jordan. **Methodology:** The dry leaves from Z. *spina-christi* were extracted using different solvents like water, methanol, ethanol, acetone, chloroform, ethyl acetate and n-hexane. Then the extraction yield, the total of flavonoid, tannin and alkaloid contents and heavy metals concentrations of Pb, Cu, Cd, Ni and Co were investigated. **Results:** The highest extract yield was obtained from water extract (46.2%). Ethyl acetate has the highest flavonoids content (145.3±2.3 mg QE g^{-1} DW extract) while methanol extract has the highest concentration of tannins (57.2±5.8 mg GAE g^{-1} DW). The crude powder of *Z. spina-christi* leaves has appreciable amount of alkaloids (10.1±0.02 mg g^{-1}) with permissible heavy metals concentrations. **Conclusion:** The yield and extraction efficiency of secondary bioactive compounds from *Z. spina-christi* leaves were solvent dependent. The leaves extracts of *Z. spina-christi* plant could be a potential source of food products and useful drugs.

Key words: *Ziziphus spina-christi*, phytochemical, secondary metabolites, heavy metals

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plants are rich sources of bioactive compounds which have many health promoting effects like antioxidant, antibacterial, antihypertensive and anti-inflammatory etc[1,2]. Phytochemicals such as, terpenoids, phenolic metabolites and alkaloid are examples of secondary metabolites produced by plants, from which the plants are thought to get their healing properties[3,4]. Among these three groups of phytochemicals, phenolic compounds are the most important for dietary applications. Phenolic compounds include phenolic acids (hydroxybenzoic and hydroxycinnamic acids), polyphenols (hydrolyzable and condensed tannins) and flavonoids. These compounds protect plants, fruits and vegetables from oxidative damage and have been used as antioxidants by humans[5,6].

Flavonoids are generally used as anti-inflammatory, antiviral, anticarcinogenic, antithrombotic, antiallergic and hepatoprotective[7,8]. The tannin-containing plant extracts are utilized as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals[9]. Alkaloids also possess a stimulating effect to act as a topical anesthetic in ophthalmology and to control fever[10]. Rezaie *et al*.[2] have shown that plant materials are known for long times ago by its traditional used for medical purposes.

Extraction processes of these metabolites play a significant and crucial role on the final result and outcome of any medicinal plant[11]. However, extraction yield not only depend on the extraction method but also on the solvent used for extraction. The qualitative and quantitative studies of bioactive compounds from plant materials mostly rely on the selection of proper extraction solvent. The presence of various bioactive compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent. Previous studies have used methanol, petroleum ether, chloroform, ethanol, acetone and water as the solvents for extracting bioactive compounds from different plant species[4,12].

Medicinal plants have been cited as a potential source of heavy metal contamination during cultivation or processing[13]. Heavy metals elements, such as lead (Pb), cadmium (Cd), arsenic (As), etc., have toxic effects on human health. Toxic metals can accumulate persistently in the body over a lifetime. Some heavy metals such as Cu, Fe, Mg, Zn and Co are essential elements for normal growth, development and metabolism of plant. However, high concentrations of these elements lead to physiological disorders in plants[14,15]. Therefore determining the content of the heavy metals accumulation is of high importance. Heavy metals are elements with a specific weight higher than 5 g cm^{-1} [3,16].

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs so it is extremely important to use the most appropriate methods to extract the active components from plant materials[11,17]. Owing to the significance in the above context, such preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy.

In the present study, the *Z. spina-christi* was selected as model food matrices as it is one of the most common medicinal plants available in Jordan[18]. Different parts of this plant is consumed by local population to treat several pathologies such as cough, malaria, wounds, toothache and rheumatic diseases, Alzheimer disease, disease related to reactive oxygen species (ROS) formation, bacterial infection, diabetes and inflammation[1,19].

To current knowledge, there have been no studies done on the impact of solvents on the extraction efficiency of bioactive compounds from the *Z. spina-christi* leaves available locally in Jordan. Therefore, this study was designed to investigate the impact of different common solvents like water, methanol, ethanol, acetone, chloroform, ethyl acetate and n-hexane on the extraction efficiency of phytochemical secondary metabolites and to determine the heavy metals composition of *Z. spina-christ*.

MATERIALS AND METHODS

Plant material and extraction: Fresh leaves of *Z. spina-christi* were collected from different locations of Jordan valley. Prior to the extraction, the leaves were washed with sterile water to remove any associated debris and dried at room temperature. After that, the leaves were powdered with a mechanical grinder and stored in an air tight container. In order to extract the crude bioactives, the powdered leaves were extracted by maceration with seven different common solvents (like water, methanol, ethanol, acetone, chloroform, ethyl acetate and n-hexane) at 20% (w/v) concentration for 2 days at the room temperature with occasional shaking. The mixtures were filtered through Whatman no: 4 and then membrane filter (0.45 um). The filtrates were concentrated in rotary evaporator (Buchi, Switzerland) at 40°C and the crude concentrated extracts were calculated for their extractive value. Then, transferred to brown colored sample vial and stored at -20°C for further analysis[20].

Quantitative phytochemical analysis

Determination of total flavonoid content (TFC): Aluminium chloride colorimetric method was used for flavonoids determination. Different plant extracts in methanol (mg mL^{-1}) were separately mixed with 1.5 mL of ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M sodium acetate and 2.8 mL of distilled water. It was kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with double beam UV spectrophotometer. Quercetin was used as the standard for a calibration curve and the results were expressed as mg of quercetin equivalents per gram of extract dry weight (mg QE g^{-1} DW)[21].

Determination of tannin content: The tannins were determined by Folin-ciocalteu method. About 0.1 mL of each sample of extract was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin-ciocalteuphenol reagent, 1 mL of 35% Na_2CO_3 solution and dilute to 10 mL with distilled water. The mixtures were shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 μg mL^{-1}) were prepared in the same manner as described earlier. Absorbance for tests and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of gallic acid equivalent/g of extract dry weight (mg GAE g^{-1} DW)[22].

Determination total alkaloid content: About 5 g of dry powdered leaves of *Z. spina-christi* was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added, covered and allowed to stand for 48 h. After filtration, the extract was concentrated using a water bath to 1/4th of the original volume. Concentrated ammonium hydroxide was added in drops to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue is the alkaloid, which was dried and weighed[23].

Heavy metals analysis: The concentration of heavy metals including lead (Pb), copper (Cu), cadmium (Cd), nickel (Ni) and cobalt (Co) were measured in the leaves of *Z. spina-christi*. For this purpose, plant sample was dried in oven at 72°C for 48 h. Subsequently, the plant dry matter was powdered and extracted according to the method described by Kovacs *et al.*[24]. Two grams of powdered sample was mixed with 10 mL of 65% HNO_3 and remained overnight at room temperature. Then, the digest was heated at 85°C to evaporate acid. One mL of 30% H_2O_2 was added; consequently, the digest was filtered and diluted with deionized water to 50 mL. Heavy metals concentration in leaves was determined using inductive coupled plasma (ICP-OES) (Optima 2000, PerkinElmer, USA). Appropriate working standard solutions were prepared for each element.

Statistical analysis: The one-way analysis of variance (ANOVA) and Tukey's test at the significance level $p<0.05$ were conducted using SPSS statistical software (version 10). Data were reported as mean±standard deviations. All experiments were performed in three different sets each in triplicates (n = 3).

RESULTS

Yield of extracts: The yield percentage of different *Z. spina-christi* leave extracts are shown in Fig. 1. Yields range from 0.81-46.2%. The highest significant extractive yield ($p<0.05$) was obtained from water extract (46.2%), followed by acetone (24.3%), then chloroform (24.2%), methanol (21.4%) ethanol (20.3%), ethyl acetate (15.0%) and n-hexane (0.81%).

Total flavonoid content: The concentration of flavonoids in various plant extracts of *Z. spina-christi* leaves was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of quercetin equivalent, the standard curve Eq.:

$$y = 0.015x+0.005$$

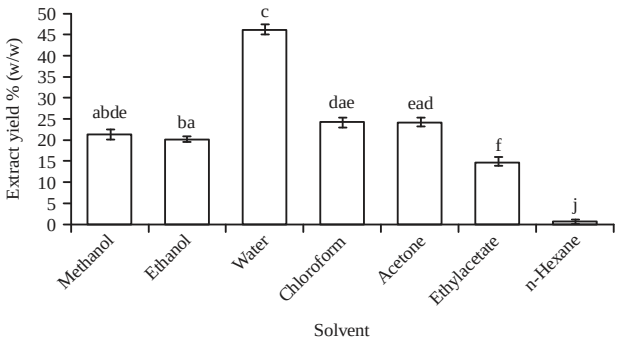

Fig. 1: Extract yield percentage of different extracts of *Z. spina christi*

Values are the mean average of three replications for each solvent±standard deviation. Columns not sharing the same superscript letter are significantly different $p<0.05$

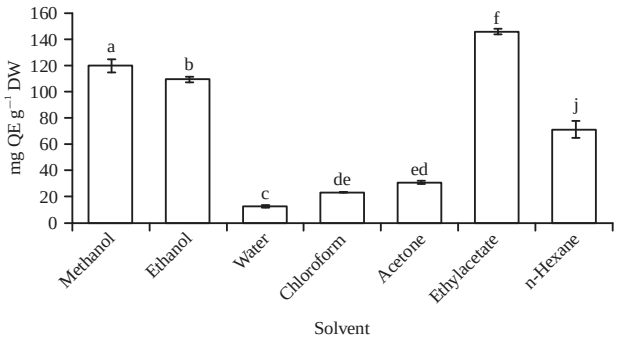

Fig. 2: Total flavonoid content of different extracts of *Z. spina christi* expressed as mg of quercetin equivalent/g of dry extract (mg QE g^{-1} DW)

Values are the mean average of three replications for each solvent±standard deviation. Columns not sharing the same superscript letter are significantly different p<0.05

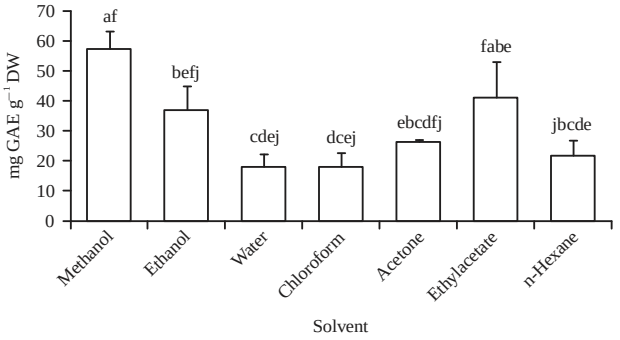

Fig. 3: Total tannin content of different extracts of *Z. spina christi* expressed as mg of gallic acid equivalent/g of dry extract

Values are the mean average of three replications for each solvent±standard deviation. Columns not sharing the same superscript letter are significantly different p<0.05

Table 1: Total concentration of alkaloid in dry leaves of *Z. spina christi*

	Concentration (mg g^{-1} DW)
Alkaloid	10.1±0.02

Value is the mean average of three replications for each solvent±standard deviation

Table 2: Concentration of heavy metals in dry leaves of *Z. spina christi*

Elements	Concentration (ppt)
Nickel (Ni)	0.425
Cobalt (Co)	0.075
Lead (Pb)	1.175
Cadmium (Cd)	0.200
Copper (Cu)	8.525

(R^2 = 0.999) (Fig. 2). Ethyl acetate extract has significantly the highest total flavonoid content (145.3±2.3 mg QE g^{-1} DW of extract) (p<0.05), followed by methanol (120.0±4.9), then ethanol (109.0±2.0), n-hexane (71.3±6.4), acetone (31.3±1.2), chloroform (23.9±0.05) and water (13.0±2.0). The flavonoid contents of the rest of the solvents are also significantly different (p<0.05) except for chloroform and acetone extracts.

Determination of tannin contents: The tannin contents in the examined extracts of *Z. spina-christi* leaves using the Folin-ciocalteu's reagent is expressed in terms of gallic acid equivalent, the standard curve Eq.:

$$y = 0.0.13x+0.23$$

(R^2 = 0.981). The values obtained for the concentration of tannin are expressed as mg of GAE g^{-1} DW of extract (Fig. 3). The tannin contents in the examined extracts ranging from 17.9±4.4 mg GAE g^{-1} DW for water extract to 57.2±5.8 mg GAE g^{-1} DW for methanol extract. The highest concentration of tannin was measured in methanol and ethyl acetate extracts. Water and chloroform extract had the least tannin contents.

Total alkaloid content: The result of total alkaloid content in the leaves of *Z. spina-christi* is shown in Table 1. The alkaloid content was found to be 10.1±0.02 mg g^{-1}.

Heavy metals analysis: The concentrations of various heavy metals in dry powder leaves are summarized in Table 2.

DISCUSSION

The use of bioactive compounds in different commercial sectors such as pharmaceutical, food and chemical industries signifies the need of the most appropriate and standard method to extract these active components from plant materials[25]. However, extraction efficiency not only depend on the extraction method but also on the solvent used for extraction[12,26]. In this study, Several leaves extracts of *Ziziphus spina-christi* were obtained by using several solvents like water, methanol, ethanol, acetone, chloroform, ethyl acetate and, n-hexane in order to identify the most appropriate solvent for further extraction and isolation of bioactive compounds such as phytochemical secondary metabolites, from this medicinal plant grown in Jordan.

Results of the present study showed that among all the solvent extracts used, the extract obtained by water showed

the highest yield percentage (46.2%), while the n-hexane extract exhibited the lowest percentage (0.81%) (Fig. 1). Findings of the current study are in agreement with previous investigation of Do *et al.*[26], who reported that maximum extract yield from *Limnophila aromatica* was obtained with water extract. This may be attributable to the higher solubility of proteins and carbohydrates in water. Also, Zielinski and Kozowska[27] obtained the highest extract yield with water. However, higher extraction yield does not necessarily imply that it will also have high concentration of the bioactive compounds[28].

The total content of flavonoid compounds in different extracts was performed using the aluminium chloride method and expressed as milligrams of quercetin equivalents. In the current study, the TFC values of the extracts are ranging from 13.0±2.0 mg QE g^{-1} DW for water extract to 145.3±2.3 mg QE g^{-1} DW for ethyl acetate extract. Methanol and ethanol extracts also offered high values of TFC (Fig. 2). Results of the current study are in accordance with a previous investigation on *Hibiscus tiliaceus* wood, wherein, maximum amount of flavonoid was observed in ethyl acetate extract followed by methanol and other extracts[22]. While Do *et al.*[26] reported that flavonoid content in the extracts decreased with increasing water content in the aqueous solvent.

The total content of tannins in leaves of *Z. spina-christi* was determined using total phenol method using Folin-Ciocalteu's reagent and standard gallic acid. The highest concentrations of tannin were measured in methanol and ethyl acetate extracts, while water and chloroform extracts had the least tannin contents (Table 2). Tambe and Bhambar[22] observed similar trends as in the present study regarding extraction efficacy of solvents towards recovering of tannins from *Hibiscus cannabinus* L. wood.

The total alkaloid content was measured in the powdered leaves of *Z. spina-christi* and was found to be 10.1±0.02 mg g^{-1}. Almost similar quantities of alkaloids were obtained from other medicinal plants such as *Pteris* species using the same extraction method[29].

From another aspect, the determination of the heavy metals concentration in plants can provide useful information about the environmental health and safety. The amount of excess metal ions in food chain results in damage to ecosystems and threatens the human health, if these plants be used as food or pharmaceutical products[14]. Hence, the World Health Organization (WHO) has stressed that the plants with medicinal and curative function, should be assayed for the presence of heavy metals. Analysis of heavy metal content is necessary for final production and processing of herbal products. According to WHO, the specific limit of heavy metals such as Cu, Pb, Ni, Cd and Co in medicinal plants and food are 10,10, 1.5, 1 and 0.2 ppm, respectively[30,31]. In the present study, it is observed that crude powder of *Z. spina-christi* leaves contains the aforementioned metals in lower concentrations than the permissibility (Table 2).

Previously, several studies revealed that the extraction solvents play an important role in the extraction of important secondary bioactive compounds from the medicinal plants. The yield of extraction depends on the solvent with varying polarity, pH, temperature, extraction time and composition of the sample. Under the same extraction time and temperature, solvent and composition of sample are known as the most important parameters[26,32,33]. Since biologically active compounds occur naturally in very small concentrations, the choice of an extraction method and the corresponding suitable solvent is an important step in the drug discovery process[11,12].

CONCLUSION

The solvent effects identified in this study, revealed that the most efficient extraction medium for flavonoids was ethyl acetate. Whereas methanol extraction showed the highest tannins content. The leaves extract has contained appreciable amount of alkaloids, in addition to low concentrations of the heavy metals. Furthermore studies are required to isolate and characterize the bioactive components from *Z. spina-christi*.

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