JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

Chemical Composition and Phytochemical Screening of *Juniperus Phoenicea* L.: Evaluation of Antioxidant Activity, Minerals, and Bioactive Compounds

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Abstract: The purpose of the research was to determine *Juniperus phoenicea L.*'s elemental content, antioxidant activity, and phytochemical composition. Phytochemical screening was performed on four plant extracts (water, ethanol, chloroform, and ether). The aqueous and ethanol extracts were also analyzed for their total phenols, total flavonoids, and total antioxidant contents. The levels of macroelements (Na, Mg, Ca) and microelements (Fe, Cu, Zn) in the plant were determined by flame photometry and atomic absorption spectrometry. Moisture, ash, total protein, and total alkaloids were also determined. The results showed that the aqueous and ethanol extracts contained various phytochemicals, such as carbohydrates, proteins, phenols, tannins, flavonoids, alkaloids, coumarins, anthocyanins, saponins, and glycosides. The ethanol extract had higher concentrations of most phytochemicals than the aqueous extract, except for carbohydrates and proteins. The chloroform and ether extracts had lower concentrations of phytochemicals than the aqueous and ethanol extracts. The moisture, ash, total protein, and total alkaloid contents of the plant were 13%, 5.52%, 10.78%, and 1.84%, respectively. The total phenol contents, total flavonoid contents, and total antioxidant activity of the ethanol extract were 49.36±5.24 mg/g, 20.61±2.08 mg/g, and 34.82±2.44 mg/g, respectively. The corresponding values for the aqueous extract were 46.26±2.47 mg/g, 14.80±1.12 mg/g, and 37.32±3.29 mg/g, respectively. The order of abundance for macroelements was Ca (26860±950 mg/kg) > Na (1705.4±85 mg/kg) > Mg (944.4±38 mg/kg), whereas for microelements it was Fe (315.4±18 mg/kg) > Cu (55.52±3 mg/kg) > Zn (35.66±2 mg/kg). These results indicate that *Juniperus phoenicea* L. is a rich source of phytochemicals and elements that may have potential health benefits.

Keywords: *Juniperus phoenicea* L., Phytochemical composition, Antioxidant activity, Elemental content, Medicinal plant, Traditional medicine

Submitted: July 23, 2023. **Accepted**: February 9, 2024.

Cite this: Elsherif KM, Sulaiman MA, Mlitan A. Chemical Composition and Phytochemical Screening of *Juniperus Phoenicea* L.: Evaluation of Antioxidant Activity, Minerals, and Bioactive Compounds. JOTCSA. 2024;11(2):709-22.

DOI: https://doi.org/10.18596/jotcsa.1330273

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1. INTRODUCTION

Herbal medicinal plants are still used in traditional diabetes therapy and have long been a major source of healthcare in developing countries. These plants offer unique benefits because they have a wide range of pharmacological properties, including antioxidant, antiviral, anticancer, antibacterial, antifungal, and antiparasitic properties, and they also contain a large number of medicinal phytochemicals that may help in the creation of novel medications (1). Particularly abundant in these plant extracts are polyphenols, which have the ability to bind with proteins and reduce the activity of enzymes (2). Herbal medicinal plants have the potential to transform healthcare by providing natural alternatives to traditional treatments due to their various bioactive components (3). Furthermore, polyphenols' capacity to selectively interact with certain enzymes makes them appealing candidates for drug development since they may be utilized to

construct focused medicines with few adverse effects (4). As a result, herbal medicinal plants have piqued the interest of academics who are investigating their therapeutic potential and creating novel medications based on their active components. Herbal medicinal plant use in medicine is a fast-emerging sector with bright possibilities for the future of healthcare (5).

Because of its secondary metabolites, which have positive effects and are widely employed in Libyan folk and traditional medicine, *Juniperus phoenicea L.* is regarded as a medicinal plant in Libya (6,7). This plant, which is a member of the *Cupressaceae* family, is also referred to as Ar'ar in Arabic and Phoenician Juniper in English. It is a naturally occurring shrub or small tree that is extensively dispersed throughout Europe, northern Africa, and the Canary Islands. It thrives in Mediterranean coastal regions (8). For centuries, people have used the antiviral, antibacterial, antiseptic, and inflammatory qualities of *Juniperus phoenicea* leaves and fruits. In Libyan traditional medicine, they are also utilized as a natural treatment for digestive, respiratory, and urinary tract issues (9,10). Several studies have reported that *Juniperus phoenicea* L. is rich in a diverse range of compounds, including diterpenoids, flavonoids, lignans, phenylpropanoid glucosides, furanone glucosides, bis-furanone derivatives, norterpenes, and sesquiterpene glucosides (11,12).

Because of its antioxidant activity—that is, its capacity to stop or slow down the oxidation of other molecules by free radicals—it has therapeutic benefits. Free radicals are very reactive, unstable chemicals that may harm DNA, lipids, proteins, and organisms. By giving electrons or hydrogen atoms to free radicals, antioxidants can neutralize them and shield the organism against oxidative stress and inflammation (13). Natural products, especially those produced from plants, have long been considered a valuable source of medicinal compounds. Plants' medicinal and aromatic characteristics were found gradually via trial and error, with some successes and disappointments. Vitamin E (tocopherol), vitamin C (ascorbate), and polyphenols are examples of antioxidant compounds having strong antioxidant activity against free radicals. These substances function by scavenging and neutralizing these radicals, converting them into stable molecules or ions (14, 15). The presence of hydroxyl groups in their phenolic structures increases their capacity to efficiently prevent radical processes. Because of their favorable impacts on human health, phenolic compounds, a broad collection of natural chemicals, have been the subject of substantial investigation. They are crucial in preventing and treating conditions including cancer, diabetes, hypertension, and Alzheimer's disease because they counteract oxidative stress (16). The antioxidant capacity of essential oils and extracts of *Juniperus phoenicea L*. derived from various plant parts, including leaves, berries, and seeds, has been documented in a number of investigations. The essential oil of red juniper (*Juniperus phoenicea L*.) leaves, for instance, exhibited strong radical-scavenging action against DPPH (2,2-diphenyl-1-picrylhydrazyl), a stable free radical that is frequently used to gauge a substance's antioxidant capacity, according to Aouadi et al. (17). The high amount of α-pinene (74.14%), a monoterpene molecule with shown antioxidant and anti-inflammatory capabilities, was attributed by the scientists to this action. An additional investigation by Elmhdwi et al. (18) found that the Moroccan *Thymus* satureioïdes leaves, which are related to *Juniperus phoenicea L.*, had a significant antioxidant activity against DPPH and another synthetic free radical called ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)). The authors proposed that the extract's phenolic components, which include tannins and flavonoids, were the cause of this action. Certain trace elements are present in live organisms in incredibly low quantities and have a substantial impact on physiological processes that are common to all living systems. Based on evidence from several processes, including hormone production, enzyme activity, and acid-base balance, their function is thought to be catalytic (19). Given their involvement in the body's synthesis of active chemical components, it is important to comprehend the impact of trace elements in medicinal plants on human health. Furthermore, a crucial area of study is the relationship between the elemental profiles of medicinal plants and the historic uses of those plants for healing (20).

This investigation sought to assess the chemical constituents and possible medicinal qualities of *Juniperus phoenicea L.*, which was harvested from the Libyan Msallata region. Total phenols, total flavonoids, total antioxidant activity, ash, moisture, and mineral content (Na, K, Ca, Mg, Zn, Fe, and Cu) were all measured. Moreover, phytochemical screening was performed on four distinct extracts (aqueous, ethanolic, chloroformic, and ether) of *Juniperus phoenicea L.* to detect the presence of several bioactive components. The findings of this study may help create new therapeutic compounds made from *Juniperus phoenicea L.* and offer significant new insights into the plant's possible medical uses.

2. EXPERIMENTAL SECTION

2.1. Plant sampling and processing

Between February and May 2022, the aerial part of *Juniperus phoenicea* was collected from the Msallata area of Libya. A specialist in plants from the Misurata University Faculty of Science's Botany Department recognized the plant samples based on their morphology. The voucher specimens were retained at the department's Laboratory of Plant Science. The gathered plant samples were washed, then left to air dry on lab tables for 15 days at ambient temperature (24–25°C). An air conditioner in the laboratory area helped to keep the temperature steady as the material dried. After

that, the dehydrated plant material was blended into a uniform powder and kept for later use in an airtight container. About 50.0 g of dried material from the plant was prepared and stored in airtight containers for further analysis. To avoid deterioration and maintain their genetic integrity, the dried plant samples were kept in opaque, airtight containers at -18°C.

2.2. Plant Extraction

A modified version of the process described in the literature (3, 21) was used to manufacture the extracts. In summary, 100 mL of a chosen solvent (water, ethanol, chloroform, or ether) was combined with 10 g of dried powdered plant material, and the mixture was allowed to extract for 72 hours at room temperature while being continuously shaken. The resultant extracts were concentrated at 40°C under vacuum using a rotary evaporator (Buchi R-215, Switzerland) after being filtered using Whatman filter paper (No. 4). For a maximum of one month, the concentrated extracts were kept in the dark at 4°C to avoid deterioration and maintain their efficacy.

2.3. Phytochemical screening

The main classes of phytochemicals found in extracts from *Juniperus phoenicea*, such as alkaloids, flavonoids, saponins, terpenoids, phenols, tannins, glycosides, carbohydrates, proteins, coumarins, and anthocyanins, were qualitatively examined using conventional analytical techniques (22–24).

2.4. Quantitative analysis

2.4.1. Determination of yield

To calculate the yield of each extract, dried samples were weighed, and the weight of the soluble constituents was determined. The yield of the extract under investigation was calculated using the equation provided by (25):

$$\text{Yield (\%)} = \frac{W2}{w1} \text{ x } 100 \quad (1)$$

The weight of the concentrated plant extract obtained by evaporation is denoted by w_1 in the equation above, and the weight of the dried plant material used for extraction with each solvent is indicated by w_2.

2.4.2. Determination of Moisture and ash

After weighing 3.00 g of the fresh plant sample in a clean, dry crucible, it was dried for three hours at 100°C in an oven. The crucible was reweighed after 15 minutes of cooling it in a desiccator. Until a steady weight was achieved, this procedure was repeated. Using equation (26), where w_1 and w_2 represent the sample weights before and after drying, respectively, we were able to calculate the moisture content of fresh samples:

$$\%\, Moisture = \frac{W1-W2}{W1}\, x\, 100 \quad (2)$$

3.0 mg of the plant sample, weighed in a dry, clean porcelain crucible, were heated to 550°C for three hours in a muffle furnace in order to ascertain the amount of ash present. The crucible was reweighed after 15 minutes of cooling it in a desiccator. Equation (27) was used to determine the ash content.

$$\%\, Ash = \frac{weight\ of\ ash}{weight\ of\ sample}\, x\, 100 \quad (3)$$

2.4.3. Determination of total protein

To estimate the protein content in plant samples, the Kjeldahl method was utilized with certain modifications. In this method, the plant material is oxidized with concentrated sulfuric acid, which oxidizes all the components except for the nitrogen present in proteins. The nitrogen is subsequently reduced to ammonia, which is determined through back titration in the presence of a methyl red indicator (28). The total protein content of plants can be calculated by converting the percentage of nitrogen using the equation below:

$$Total\ protein\ \left(\frac{g}{100\ mL}\right) = Nitrogen\ content\ \left(\frac{g}{100\ mL}\right) x\ 6.25 \quad (4)$$

2.4.4. Determination of total alkaloids

To estimate total alkaloids, the gravimetric technique with certain adjustments (29) was used. First, 200 mL of 10% acetic acid in ethanol was poured into a 250 mL flask containing 5.00 g of the dried plant powder. After letting the combination remain for four hours, it was filtered and concentrated to a fourth of its original volume in a water bath. Gradually adding concentrated ammonium hydroxide, the precipitation was allowed to finish. Following a centrifugation of the mixture, the precipitate was recovered, cleaned with diluted ammonium hydroxide, and filtered once more. The precipitate was dried, weighed, and the proportion of alkaloids was computed. The residual product generated from this operation represents the alkaloids.

2.4.5. Determination of total phenols

The total phenolic content of the plant under study was ascertained by applying specific modifications to the Folin-Ciocalteu technique (30) in order to extract the plant's water and ethanol. In order to use this procedure, 0.2 mL of the extract and 1 mL of 10% diluted Folin-Ciocalteu reagent were combined, and the mixture was then incubated for 4 minutes in the dark. After that, 0.8 mL of a 7.5% sodium carbonate solution was added, and solvent was used to raise the volume to 10 mL. After 30 minutes, the solution's absorbance at 765 nm was determined. A standard calibration curve was created using different amounts of gallic acid (10-60 mg/L) and used to quantify the total phenolic contents. As a benchmark, gallic acid was used, and the outcomes were presented as gallic acid equivalents.

2.4.6. Determination of total flavonoids

The amount of flavonoids present overall in the plant extracts was determined using the modified aluminum chloride technique (31). Rutin equivalent was utilized to express the total flavonoid content, with rutin serving as the standard reference. Several rutin concentrations (ranging from 1 to 60 mg/L) were used to create a standard calibration curve. The whole flavonoid

content of the plant's water and ethanol extracts under study was determined using this approach.

In order to perform the experiment, 0.3 mL of sodium nitrite solution ($NaNO_2$), 4 mL of distilled water, and 1 mL of the extract (ethanol, water, or rutin solution) were mixed. After five minutes, the mixture was allowed for six minutes before 0.3 mL of aluminum chloride solution was added. After that, 2 mL of a 1 M sodium hydroxide solution was added, and after 10 minutes, the volume was increased to 10 mL using distilled water. Next, the solution's absorbance was calculated at 510 nm.

2.4.7. Total antioxidants (DPPH radical scavenging assay)

The free radical scavenging ability of aqueous and ethanolic extracts was measured using the technique reported by Al-Mustafa et al. (32) using ascorbic acid as a reference. Each extract was mixed with 2.0 mL of methanolic DPPH solution to perform the experiment. Following a 30-minute dark incubation period at room temperature, the preparations' absorbance at 517 nm was determined. Using the following formula, the antioxidant activity of the plant extracts was determined:

$$\% I = \frac{A_{Blank} - A_{sample}}{A_{Blank}} x\, 100 \qquad (5)$$

A_{sample} is the absorbance of the tested plant extract, % I is the inhibition in DPPH absorbance, and A_{blank} is the absorbance of the control reaction (containing all reagents except the plant extract). The plot of inhibition (%) against extract concentration was also used to determine the extract concentrations that gave 50% inhibition (I_{C50}). Three copies of each sample were used.

2.4.8. Determination of micro and macro elements

Mineral element concentrations, including those of Fe, Zn, Mn, Cu, and Mg, were measured with an atomic absorption spectrophotometer (VARIAN 220 FS), while Ca and Na were measured with a flame photometer (PFP7 Jenway). To eliminate all organic matter in the plant material, the wet digestion method was employed, which involved the use of nitric acid and hydrogen peroxide (33,34).

1.00 g of plant powder and around 10 mL of concentrated nitric acid were mixed in a beaker, and the mixture was heated for 10 minutes until the vapors disappeared in order to complete the plant material digestion process. An extra 10 mL of acid is added and heated once more if the brown odors continue. After adding 10 mL of hydrogen peroxide solution, the mixture was heated to a boil, at which point the solution turned clear and colorless. Following cooling, the mixture was moved to a 100 mL volumetric flask and filtered using filter paper. Then, deionized water was added to get the amount up to 100 mL.

2.4.9. Statistical analysis

Each measurement was made for three replicates and the mean value was reported. The results were reported as the mean ± standard error of the mean (SEM) attained from several parallel measurements. Excel statistical software package developed by Microsoft Office 2016 was used for statistical analysis and database management.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening

Table 1: Phytochemical screening results

Solvent				Bioactive compounds
Ether	**Chloroform**	**Ethanol**	**Water**	
-	-	++	+++	Carbohydrates
-	-	++	+++	Proteins
-	+	+++	+++	Phenols
-	+	+++	+++	Tannins
-	-	+++	++	Flavonoids
+	-	++	+	Alkaloids
+	+	++	++	Coumarins
-	-	+	+	Anthocyanin
-	-	+++	++	Saponins
-	+	++	+++	Glycosides
+	++	-	-	Steroids
+	+	-	-	Terpenoids

(+++) visible change occurred, (++) moderate change, (+) very slight change, (-) no change occurred

Table 1 displays the phytochemical screening results of four solvent extracts of *Juniperus phoenicea* L. The aqueous and ethanol extracts were found to contain carbohydrates, proteins, phenols, tannins, flavonoids, alkaloids, coumarins, anthocyanins, saponins, and glycosides. These phytochemicals have essential biological functions, such as providing energy, serving as structural components or signaling molecules, and exhibiting antioxidant, anti-inflammatory, antimicrobial, and pharmacological effects on the nervous system. Saponins can lower cholesterol, modulate the immune system, and inhibit tumor growth, while glycosides can have various effects, including cardiac, antidiabetic, and anticancer effects (31,35).

Table 1 indicates that the ethanol extract contains higher concentrations of most phytochemicals compared to the aqueous extract, except for carbohydrates and proteins. This suggests that ethanol is a superior solvent to water for extracting phytochemicals from *Juniperus phoenicea* L. due to its ability to dissolve both polar and non-polar compounds. On the other hand, the ether extract has more alkaloids than the chloroform extract, while the chloroform extract has larger quantities of phenols, tannins, and glycosides. This may suggest that chloroform is more effective than ether for extracting phenols, tannins, and glycosides from *Juniperus phoenicea* L., while ether is more effective than chloroform for extracting alkaloids from the plant material. This is because chloroform can dissolve both polar and non-polar compounds, whereas ether can only dissolve non-polar compounds, making it better suited for extracting alkaloids.

These results are in line with some previous studies, such as the one by Hamzal et al. (2016), (36) which reported the presence of saponins, flavonoids, tannins, and alkaloids in *Juniperus phoenicea* L. Another study by Amalich et al. (2016) (37) detected various phenols, tannins, flavonoids, steroids, and terpenes, but not alkaloids or saponins, in the leaves. However, a study by El-Sawi et al. (2014) (38) found no tannins, coumarins, or alkaloids in *Juniperus phoenicea* L. leaves, which contradicts the current study's results. Nevertheless, both studies agreed on the presence of carbohydrates, glycosides, terpenes, steroids, flavonoids, and saponins. The current study also concurred with a study by Bouassida et al. (2018) (39) that identified alkaloids, terpenes, and tannins in *Juniperus phoenicea* L. leaves but differed in the absence of glycosides.

3.2. Yield, moisture, and ash contents

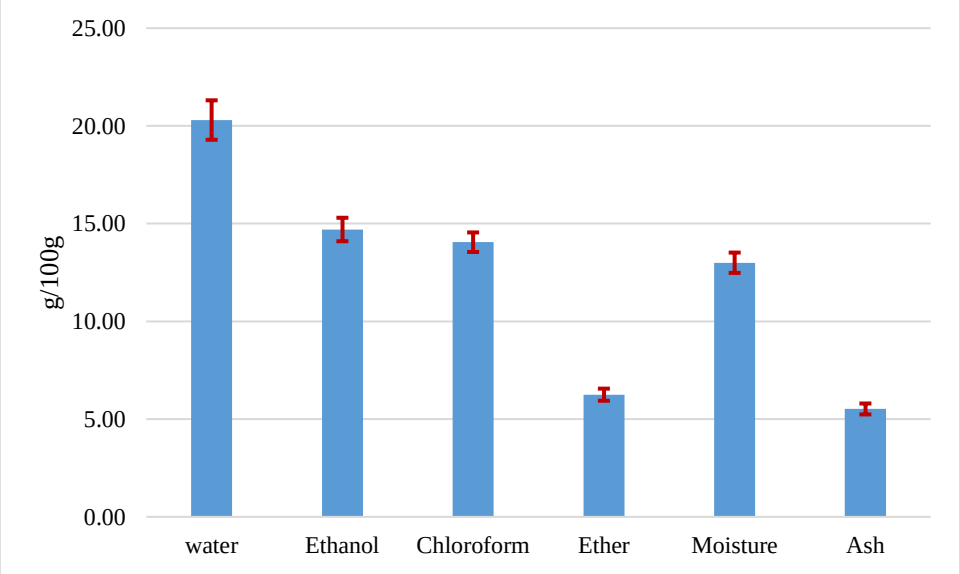

Figure 1: Yield, moisture, and ash contents of *Juniperus phoenicea* leaves

Figure 1 showcases the moisture, ash, and yield values of *Juniperus phoenicea* leaves, obtained through the use of four different solvents: aqueous, ethanol, chloroform, and ether. The results indicate that the ethanol extract exhibited the highest yield, measuring 20.30±0.40% with a 95% confidence interval ranging from 19.98% to 20.62%. Following that, the aqueous extract displayed a yield of 14.70±0.27% with a 95% confidence interval spanning from 14.44% to 14.96%. The chloroform extract showed a yield of 14.05±0.39% with a 95% confidence interval ranging from 13.73% to 14.37%. Lastly, the ether extract demonstrated the lowest yield at 6.25±0.18% with a 95% confidence interval of 6.09% to 6.41%. The leaves' moisture content was found to be 13.00±0.13%, with a 95% confidence range that fell between 12.88% and 13.12%. Furthermore, the results showed that the ash content was 5.52±0.22%, with a 95% confidence interval that included 5.32% and 5.72%.

Previous studies have examined the yield, moisture, and ash content of *Juniperus phoenicea*

leaves or fruits using various solvents and methods. Dane et al. (40) reported a yield of 20.98% for the ethyl acetate extract, which aligns with the findings of the present study. However, Farahat (2020) (41) reported a yield of 9.8% for *Juniperus phoenicea* leaves collected from Egypt using methanol as the solvent. Bouassida et al. (2018) (39) reported a yield of 12.5% for leaves collected from Tunisia using a hydroalcoholic solvent. In a separate study, Amalich et al. (2016) (37) reported a yield of 0.4% for *Juniperus phoenicea* fruits collected from Morocco through hydrodistillation. El-Sawi et al. (2014) (38) documented a moisture content of 7.5% and an ash content of 3.5% for *Juniperus phoenicea* leaves collected from Egypt.

The yield, moisture, and ash of *Juniperus phoenicea* leaves can vary depending on the solvent, method, location, and collection season. Ethanol extract had a higher yield, suggesting better extraction of bioactive compounds than water, chloroform, or ether. Moisture content was higher than in some previous studies, indicating incomplete drying or moisture absorption during storage or transportation. The ash content was also higher, suggesting higher mineral content or impurities. However, these values may vary depending on the location, season, and method of harvesting. For example, the yield and moisture of *Juniperus phoenicea* were higher in the coastal areas than in the inland areas, and lower in the summer than in the winter. The ash content of Juniperus phoenicea was also affected by the soil type, with higher values in clayey soils than in sandy soils.

3.3. Total protein content

Analysis results show that *Juniperus phoenicea* has a considerable amount of total protein, 10.78±0.44%, with a 95% confidence interval between 10.43% and 11.13%. In addition to being an essential macromolecule for plant growth, development, and stress response, protein has nutritional and therapeutic advantages for both people and animals.

Previous research on *Juniperus phoenicea* or related plants from the Cupressaceae family can provide a basis for comparing total protein levels. For instance, El-Gohary et al. (2015) (42) found that the total protein of *Juniperus phoenicea* leaves collected from Egypt was slightly lower at 8.7% compared to the current study. Similarly, Al-Mustafa et al. (2021) (32) reported that the total protein of *Juniperus phoenicea* leaves collected from Jordan was 9.3%, which is also lower than the current result. In contrast, Dzialuk et al. (2011) (43) reported a higher total protein content of 11.9% for *Juniperus communis* needles collected from Poland.

3.4. Total alkaloids content

The current investigation reveals that *Juniperus phoenicea* exhibits a comparatively reduced level of total alkaloids, constituting approximately 1.84±0.08% with a 95% confidence interval ranging from 1.77% to 1.91%. This proportion is notably lower when compared to other plant species. Alkaloids, which encompass nitrogen-containing compounds, possess the potential to impact the nervous system and exhibit various pharmacological characteristics. These properties may encompass analgesic, stimulant, sedative, and hallucinogenic effects. A comparison of the total alkaloid levels of *Juniperus phoenicea* or Cupressaceae plants can be based on previous research on these plants. For instance, Al-Mustafa et al. (2021) (32) found that the total alkaloids of *Juniperus phoenicea* leaves collected from Jordan were lower at 0.9% compared to the current study. Similarly, Bajes et al. (2021) (44) reported that the total alkaloids of *Juniperus phoenicea* leaves collected from Jordan were 0.7%, which is lower than the current result. In addition, Shaboun et al. (2021) (45) reported a lower total alkaloid content of 0.8% for *Juniperus phoenicea* leaves collected from Libya.

The extraction method, the plant part, and the environmental conditions were the main factors that influenced the total alkaloids content (TAC) of *Juniperus phoenicea* in our study. We obtained a higher TAC (1.84%) than previous studies (0.70-0.90%) by using a gravimetric technique, which was more efficient and selective than other methods. We also used the leaves of the plant, which had a higher TAC than other parts.

3.5. Total phenols content

The results obtained from the analysis of aqueous and ethanolic extracts are as follows: The aqueous extract exhibited a value of 46.26±2.47 mg/g, with a 95% confidence interval ranging from 43.67 mg/g to 48.85 mg/g. Conversely, 49.36±5.24 mg/g was the value shown by the ethanolic extract, with a 95% confidence range covering 43.75 mg/g to 54.97 mg/g. Figure 2 shows a visual representation of these numbers. An indication of the amount and diversity of phenolic chemicals found in a plant or plant extract is the total phenol content. The capacity of phenolic compounds to donate electrons or hydrogen atoms to free radicals or reactive oxygen species makes them known for their antioxidant properties. Important biomolecules like proteins, lipids, and DNA are shielded from oxidative damage by this mechanism, which also successfully neutralizes these dangerous substances (46).

Previous studies that used different solvents or methods to assess the total phenol content of *Juniperus phoenicea* leaves or fruits can provide a basis for comparison with the current findings. The total phenolic content of the ethyl extracts of *Juniperus phoenicea L*. came within the same range as found in our investigation, according to Shaboun et al. (45). The authors reported that the total phenols ranged from 21.36 to 84.55 mg/g for different extract concentrations. Also, El-Gohary et al. (2015) (42) found that the total phenol content of *Juniperus phoenicea* leaves collected from Egypt

using methanol as a solvent was much higher at 136.1 mg/g compared to the current study. Al-Mustafa et al. (2021) observed that the total phenol content of *Juniperus phoenicea* leaves collected from Jordan using methanol as a solvent was greater, at 103.6 mg/g. In comparison, *Juniperus phoenicea* fruits gathered from Morocco using hydrodistillation had a substantially lower total phenol concentration (3.8 mg/g) according to Amalich et al. (2016) (37). El-Sawi et al. (2014) (38) found that the total phenol content of *Juniperus phoenicea* leaves collected from Egypt using ethanol as a solvent was lower at 16.1 mg/g compared to the current study.

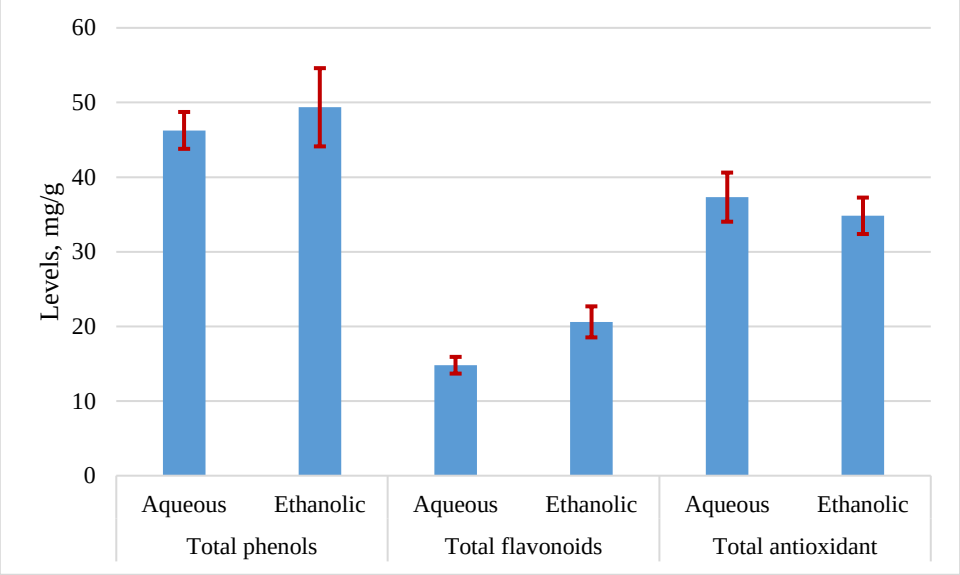

Figure 2: Levels of total phenols, total flavonoids, and total antioxidants in ethanolic and aqueous extracts of *Juniperus phoenicea*

3.6. Total flavonoids content

With a 95% confidence range spanning from 13.77 mg/g to 15.83 mg/g, the total flavonoid concentration in the aqueous extract was found to be 14.80±1.12 mg/g. By contrast, the total flavonoid concentration of the ethanolic extract was greater at 20.61±2.08 mg/g, with a 95% confidence interval that included the range of 18.55 mg/g to 22.67 mg/g. Figure 2 presents these findings. Given that the ethanolic extract had a higher total flavonoid content than the aqueous extract, it appears that ethanol is a better solvent for removing flavonoid components from *Juniperus phoenicea* leaves.

Among the many pharmacological characteristics of flavonoids is their antioxidant activity, which scavenges free radicals and reactive oxygen species to help shield biomolecules like proteins, lipids, and DNA from oxidative damage. Additionally, they have anti-inflammatory properties that can control the release or function of inflammatory mediators such as leukotrienes, prostaglandins, and cytokines. Moreover, flavonoids possess antibacterial properties that can impede the development of bacteria by altering their cell walls, membranes, or enzyme activity. Furthermore, flavonoids have an antidiabetic effect, which may enhance insulin sensitivity or glucose metabolism through changes in signaling, absorption, or transport (47, 48).

Previous studies have reported the total flavonoid contents of *Juniperus phoenicea* leaves or fruits using different solvents or methods, allowing for a comparison with the current findings. For example, Al-Mustafa et al. (2021) (32) discovered that, in comparison to the current investigation, the total flavonoid content of *Juniperus phoenicea* leaves collected from Jordan utilizing methanol as a solvent was much greater at 101.1 mg/g. Comparing the current results with Shaboun et al. (2021) (45), the total flavonoid content of *Juniperus phoenicea* leaves collected from Libya using ethyl acetate as a solvent was found to be lower, at 17.44 mg/g. On the other hand, 0.9 mg/g of total flavonoids were found in *Juniperus phoenicea* fruits that were hydrodistilated and harvested from Morocco, according to Amalich et al. (2016) (37). El-Sawi et al. (2014) (38) found that the total flavonoid content of *Juniperus phoenicea* leaves collected from Egypt using ethanol as a solvent was higher at 27.13 mg/g than in the current study.

3.7. Determination of antioxidant activity: DPPH radical scavenging assay

The aqueous extract, as shown in Figure 2, has an overall antioxidant content of 37.32±3.29 mg/g, with a 95% confidence range that spans from 34 mg/g to 40.64 mg/g. On the other hand, the ethanolic extract had a somewhat reduced total antioxidant content, measuring 34.82±2.44 mg/g with a 95% confidence range that included 31.28 mg/g to 38.36 mg/g. Moreover, for both the ethanolic and aqueous extracts, the concentration needed to inhibit 50% of DPPH radicals (IC_{50}) was found. With a 95% confidence range spanning

from 0.117 mg/mL to 0.131 mg/mL, the aqueous extract's I*C50* value was determined to be 0.124±0.007 mg/mL. Additionally, the I_{C50} value of the ethanolic extract was determined to be 0.133±0.009 mg/mL, with a 95% confidence interval spanning from 0.124 mg/mL to 0.142 mg/mL. Figure 3 provides an illustration of these values. The findings demonstrated the considerable antioxidant activity of both *Juniperus phoenicea* extracts; however, the aqueous extract's activity was marginally greater than that of the ethanolic extract. This result contrasts with other research (32, 49) that found that ethanolic extracts of *Juniperus phoenicea* had higher total phenolic content and antioxidant activity than aqueous extracts. Nonetheless, a few other investigations have also discovered that aqueous extracts of *Juniperus* species exhibit more antioxidant activity than ethanolic extracts (45). This disparity might result from various extraction techniques, solvents, plant components, or sample origins. Non-phenolic substances like alkaloids, terpenoids, anthraquinones, or glycosides may have additive or synergistic effects with phenolic compounds, which might account for the aqueous extract's comparatively larger overall antioxidant concentration. These compounds, along with phenolic compounds, could modulate the redox status of cells or inhibit oxidative enzymes, thereby enhancing the overall antioxidant activity of the extract (32).

The antioxidant activity of the *Juniperus phoenicea* aqueous extract in this investigation was found to be lower than that of the same species' methanolic extract, as reported by Al-Mustafa et al. (32). El Jemli et al. observed that it showed more antioxidant activity than other *Juniperus* species' aqueous extracts (49). However, the ethanolic extract of *Juniperus phoenicea* in our investigation showed lesser antioxidant activity than the methanolic extract of *Juniperus phoenicea* published by Al-Mustafa et al. (32) but equal antioxidant activity to the aqueous extract of *Juniperus oxycedrus* reported by El Jemli et al. (49). Additionally, compared to the ethanolic extract of the same species published by Menaceur et al. (50), who used a different extraction technique and a different plant component, the ethanolic extract of *Juniperus phoenicea* in our investigation showed reduced antioxidant activity. It is significant to note that the I_{C50} values in our study were lower than those reported by Fadel et al. (51), who found that the extracts of J. Oxycedrus and J. *Phoenicea* had I_{C50} values of 0.404 and 0.481 mg/ml, respectively. Rahhal et al. (52) observed a similar I_{C50} level of 0.14 mg/mL for the same species; however, Chelouati et al. (13) reported an I_{C50} level of 0.26 mg/mL for J. *phoenicea*.

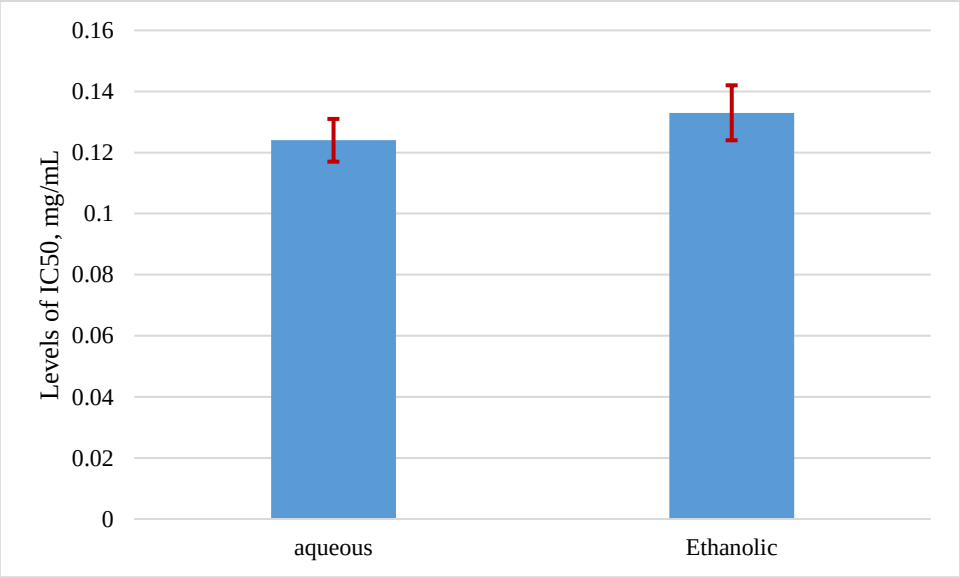

Figure 3: Levels of I_{C50} in ethanolic and aqueous extracts of *Juniperus phoenicea*

3.8. Micro and macro elements contents

The following findings were obtained from measuring the amounts of macroelements (Na, Mg, Ca) and microelements (Fe, Cu, Zn) in *Juniperus phoenicea*: Ca (26860±950 mg/kg) > Na (1705.4±85 mg/kg) > Mg (944.4±38 mg/kg) was the order of abundance for macroelements, whereas the order for microelements was Fe (315.4±18 mg/kg) > Cu (55.52±3 mg/kg) > Zn (35.66±2 mg/kg). The highest level among all the elements analyzed was Ca, which was found to be much higher than Na and Mg. Similarly, Fe had the highest level among the microelements, followed by Cu and Zn, respectively. The levels of these elements are depicted in Figures 4 & 5.

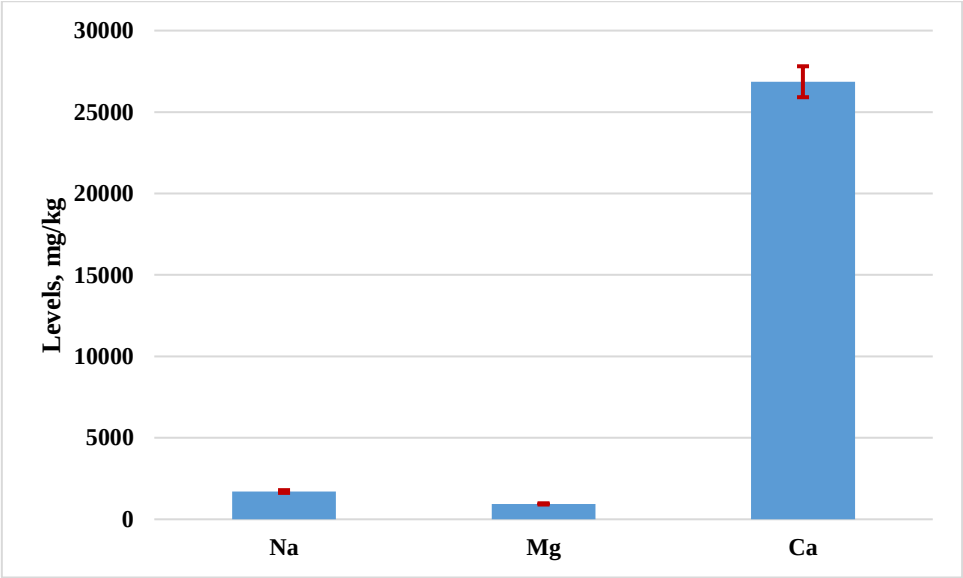

Figure 4: Levels of macroelements in *Juniperus Phoenicea*

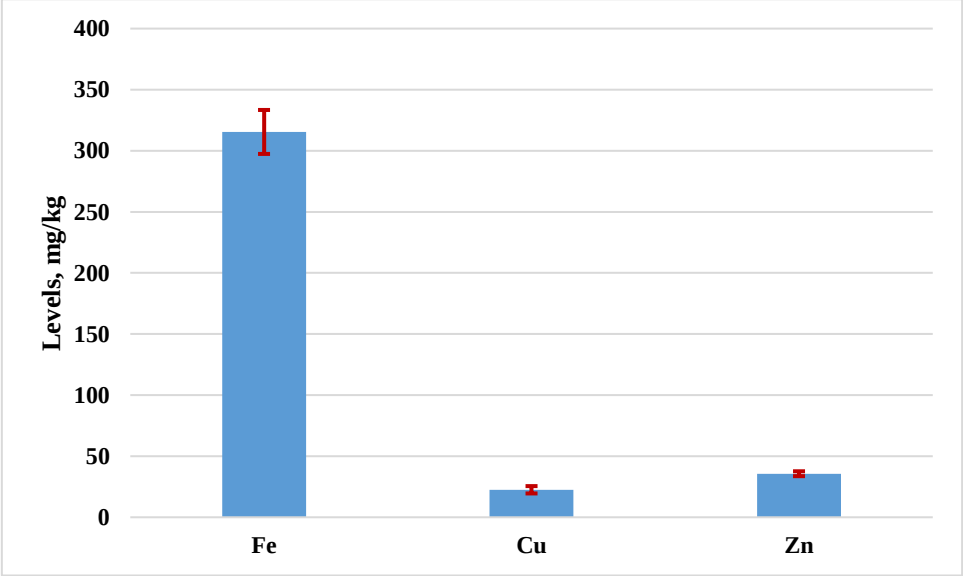

Figure 5: Levels of microelements in *Juniperus phoenicea*

Each element's concentration in *Juniperus phoenicea* shows both its potential influence on human health and its significance for the growth and development of plants. Calcium is a vital macronutrient for plant growth, and it is also essential for human health, especially for bone and tooth formation. Sodium and magnesium are also needed for plant growth, and they have important roles in various physiological processes in humans, such as nerve and muscle function. Iron is a crucial micronutrient for plant growth, and it is required for the synthesis of chlorophyll. In humans, iron is important for the formation of hemoglobin, which transports oxygen in the blood. Copper and zinc are also critical micronutrients for plant growth, and they have important roles in enzyme function in humans. Overall, the amount of these elements in *Juniperus phoenicea* indicates that it may have potential health benefits for humans, particularly for bone and tooth formation, nerve and muscle function, and enzyme activity (53, 54).

The current study's findings show differences in the identified metals' concentrations. Environmental variables such as soil type, temperature, and altitude might be blamed for these variances (55, 56). Table 2 (57–60) compares the amounts of macroelements and microelements found in this study with those documented in the literature for other *Juniperus phoenicea* in order to give more context.

Table 2: Comparing the levels of macro and micro elements in *Juniperus phoenicea* with previous research studies

Na (mg/kg)*	Mg (mg/kg)*	Ca (mg/kg)*	Fe (mg/kg)*	Cu (mg/kg)*	Zn (mg/kg)*	Ref.
1705.4±85	944.4±38	26860±950	315.4±18	55.52±3	35.66±2	This study
1607.6-1803.2**	900.5-988.3**	25763.8-27956.2**	294.6-336.2**	52.06-58.98**	33.35-37.97**	
52.13±4.76	-	1600±0.10	430±.20	-	15.60±0.70	57
6.50±0.87	-	51.61±1.16	0.43±0.06	3.00±0.27	-	58
-	-	-	168.07±0.03 190.11±0.04	31.51±0.03 71.86±0.10	40.71±0.03 13.72±0.11	59
602	370	828	13	9	7	60
681	479	972	22	7	8	
630	1107	1036	16	15	8	
638	652	946	17	11	11	

**Mean ± SD, **95% Confidence intervals*

Through a comparison of mineral concentrations in *Juniperus phoenicea* with the recommended daily intakes (RDIs) for adults, specifically for men and women aged between 19-50, it was found that consuming 100 g of *Juniperus phoenicea* would fulfill and surpass the RDIs for calcium, iron, and copper. However, it would not meet the RDIs for sodium, magnesium, and zinc (RDIs: 750 mg/day for Ca, 2000 mg/day for Na, 300-350 mg/day for Mg, 6-7 mg/day for Fe, 1.3-1.6 mg/day for Cu, 6.2-12.7 mg/day for Zn) (61, 62).

4. CONCLUSION

In summary, this investigation uncovered the elemental content, antioxidant capacity, and phytochemical makeup of the traditional medicine plant *Juniperus phoenicea*. Numerous phytochemicals found in the plant, including glycosides, alkaloids, flavonoids, and phenols, have shown strong antioxidant activity. In addition, the plant had moderate amounts of salt and magnesium and high amounts of calcium and iron—all of which are vital for human health. These findings suggest that *Juniperus phoenicea* may have potential health benefits and therapeutic applications. To validate the biological actions of the plant extracts and their separated components in vivo, more research is required.

5. CONFLICT OF INTEREST

The authors disclose that the research was carried out without any financial or commercial relationships that may be seen as having a conflict of interest. The writers do not belong to or are associated with any organization or institution that has a financial or non-financial interest in the topics or sources covered in this paper. There are no academic, professional, or personal ties between the writers and their work that may slant or affect it. The writers have disclosed no conflicting interests.

6. ACKNOWLEDGMENTS

For providing the resources and encouragement for this study, the authors would like to sincerely thank the Chemistry Department of Misurata University's Faculty of Science.

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