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Phytochemical Analysis and Antioxidant Activity of *Urtica urens* Leaves from Msallata, Libya

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Abstract: *Urtica urens* is a medicinal plant in Msallata, Libya. This study aimed to evaluate its phytochemical composition, antioxidant activity, and some metal contents. The leaves of the plant were extracted with water, ethanol, chloroform, and ether and analyzed for various active compounds, such as tannins, glycosides, proteins, alkaloids, flavonoids, and coumarins. The total antioxidant assay measured the antioxidant activity of the aqueous and ethanolic extracts. After wet digestion, the calcium, magnesium, iron, copper, and zinc metal levels were determined by Atomic Absorption Spectrophotometry (AAS). The results showed that *Urtica urens* leaves contained a complete set of active compounds in all extracts except chloroform and ether. The ethanolic extract had the highest yield (17.32%) and the highest total phenol content (8.96 mg/g), while the aqueous extract had the most increased total antioxidant activity (5.26 mg/g). The plant was rich in calcium (127,720 mg/kg) and magnesium (4,772 mg/kg), while the other metals were in moderate amounts. The plant's moisture content was 7.58%, the ash content was 5.73%, the total protein content was 28.04%, and the total alkaloid content was 0.22%. The study revealed that *Urtica urens* is a potential source of bioactive compounds with antioxidant properties and mineral nutrients.

Keywords: *Urtica urens*; Antioxidant activity; Bioactive compounds; Mineral composition

1. Introduction

There is an excessive dependency on a limited range of plant species. Introducing greater diversity in production and consumption patterns, particularly by incorporating currently underutilized plant species, can significantly improve nutrition and health outcomes, enhance livelihoods, and promote ecological sustainability [1]. Numerous countries employ medicinal plants to treat various inflammatory conditions, with their use in traditional medicine based on extensive empirical knowledge accumulated over generations by diverse communities [2]. However, many plant species' efficacy and the specific bioactive components responsible for their therapeutic effects remain unknown. In order to establish the active constituents and elucidate these plants' pharmacological properties, further experimental research is necessary [3].

Urtica urens, as shown in Figure 1, a member of the *Urticaceae* family and also known as Dwarf nettle, Bobatsi, common nettle, burning nettle, and small nettle, is utilized for its therapeutic properties. Its applications include treating asthma, heart problems, anemia, diabetes, ulcers, pulmonary tuberculosis, arthritis, bronchitis, and muscle and joint rheumatism [4]. The pharmacological and chemical characteristics of *Urtica urens* support its use as a traditional herbal remedy for various ailments. Recent studies have highlighted this plant's antibacterial, antioxidant, anti-inflammatory, antiviral, and antifungal properties [5,6], warranting further attention.

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Figure 1. *Urtica urens* leaves

Phenolic compounds are a vital component of the human diet and are naturally occurring substances found in various food sources, including herbal beverages, fruits, vegetables, nuts, seeds, and flowers [7]. Numerous studies have demonstrated a strong correlation between the total phenolic content of certain fruits and vegetables and their antioxidant activity [8]. Antioxidants are compounds that can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or progression of oxidative chain reactions [9,10]. The redox characteristics of phenolic compounds are primarily responsible for their antioxidant properties, which aid in the absorption and neutralization of free radicals, the quenching of singlet and triplet oxygen, and the decomposition of peroxides [11]. Phenolic acids, phenolic diterpenes, flavonoids, and lignans are some phenolic components with antioxidant activity [12,13].

Antioxidants are naturally occurring substances that can protect cells from oxidative damage caused by free radicals. Herbal plants are a rich source of antioxidants, with various phenolic compounds, flavonoids, and carotenoids identified as potent antioxidants [14]. These antioxidants can scavenge free radicals, prevent lipid peroxidation, and protect cellular components from oxidative stress. For example, the antioxidant activity of Curcuma longa (turmeric) has been attributed to its phenolic compounds, particularly curcuminoids, which exhibit strong free radical scavenging activity. Similarly, the flavonoids present in Ginkgo biloba have been found to possess potent antioxidant activity, protecting against oxidative damage in various cell types. The carotenoids present in saffron (Crocus sativus) have also been shown to exhibit strong antioxidant activity, which can protect against oxidative damage in the brain and other organs [15,16].

Also, herbal plants are an excellent source of minerals, which play a vital role in maintaining various physiological functions in the human body [17]. Minerals are essential nutrients required in small quantities for numerous metabolic processes such as energy production, enzyme function, and immune system regulation [18]. Several herbal plants, including *Urtica dioica* (stinging nettle), are rich in minerals such as calcium, iron, magnesium, potassium, and zinc. *Urtica dioica* is mainly known for its high calcium content, which can aid in bone health. Additionally, its iron content can help prevent anemia and improve oxygen transport. Magnesium and potassium are also significant in *Urtica dioica*, aiding muscle function, nerve transmission, and blood pressure regulation. Zinc, another essential mineral, is also present in *Urtica dioica*, which can promote immune function and wound healing [19,20].

Urtica urens is a medicinal plant renowned for its diverse applications in treating inflammation, allergies, diabetes, and urinary disorders. However, it is worth noting that the phytochemical composition and antioxidant potential of *U. urens* leaves can vary significantly due to geographical variations and environmental factors that influence plant growth and quality. Consequently, exploring the chemical and biological properties of U. urens leaves from different regions and countries is imperative. In this study, we comprehensively assessed *U. urens* leaves obtained from Msallata, Libya, a specific geographical location not previously documented in the literature. The analysis encompassed a phytochemical examination, determination of metal content, quantification of total phenols and flavonoids, and evaluation of antioxidant activity. By focusing on this unexplored area, we aimed to shed light on the unique characteristics of *U. urens* leaves from Msallata, Libya. To ensure a comprehensive understanding, we compared our research findings with prior studies investigating Urtica species' phytochemical composition and antioxidant potential from diverse regions and countries.

Furthermore, we discussed plausible factors contributing to the observed variations in our extracts' phytochemical content and antioxidant activity. Factors such as location, climate, soil

conditions, harvesting time, and extraction methods were considered in our analysis. This research endeavor contributes novel insights into the chemical and biological properties of *U. urens* leaves from a distinct geographical area, potentially unraveling new therapeutic applications. By examining the unique attributes of *U. urens* leaves from Msallata, Libya, we expand the existing knowledge base and pave the way for further exploration of this valuable medicinal plant.

2. Experimental

2.1 Chemical reagents

In the present study, the solvents and chemicals mentioned below were utilized, which were of AR grade. Chloroform (99.5%), methanol (99.5%), and ethanol (96.5%) were obtained from Sigma Aldrich, along with gallic acid and DPPH reagent (2,2-diphenyl-1-picrylhydrazyl). Rutin, nitric acid, hydrochloric acid, sodium hydroxide, ferric chloride, and aluminum chloride from Associated Chemicals. Folin-Ciocalteau reagent, ethyl acetate (AR grade, 99.5%), ascorbic acid, and hydrogen peroxide (30%) were purchased from Promark Chemicals. At the same time, copper (II) sulfate, sodium carbonate, mercuric chloride, and potassium iodide were obtained from Prestige Laboratory Supplies.

2.2 Plant collection and preparation

This study collected Urtica urens leaves from Msallata, Libya, a region known for its diverse flora and fauna, between February and May 2020. A plant specialist in the Department of Botany, Faculty of Arts and Sciences at El-Mergib University conducted a morphological identification of the plant samples. Voucher specimens were then deposited in the Laboratory of Plant Science within the Department of Botany at El-Mergib University. Before analysis, the leaves were thoroughly cleaned using distilled water to remove any dirt or impurities and then air-dried for 15 days at room temperature (25±2ºC). The dried leaves were then milled to a flour consistency with a laboratory ultra-centrifugal mill ZM 200 (Retsch GmbH, Haan, Germany) with a particle size of 0.2 mm to obtain 100 g of *Urtica urens* powder, which was stored in glass vials at room temperature. This process ensured that the leaves were prepared correctly for subsequent analysis of their phytochemical and heavy metal composition.

2.3 Preparation of plant extracts

To extract phytochemicals, 10 g of the dried *Urtica urens* leaves plant material was subjected to maceration at room temperature for 72 hours using 100 mL of different solvents, including distilled water, ethanol, chloroform, and petroleum ether. The resulting extracts were filtered through Whatman No. 1 filter paper using a Buchner funnel. The filtrates were subsequently evaporated to a dry state using a rotary vacuum evaporator at 40°C under reduced pressure, as described in previous studies. This method ensured that the phytochemicals present in the *Urtica urens* leaves were effectively extracted and concentrated for subsequent analysis [21-23].

To determine the yield of the extract a dried sample of each extract was weighed, and the weight of the soluble constituents was measured. The dried samples were stored in the dark at 4°C until further analysis. The following equation was used to calculate the yield of the extract being analyzed [24]:

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

In this equation, W_1 denotes the weight of the concentrated *Urtica urens* obtained via evaporation, while W_2 refers to the weight of the dried plant material used for extraction with each solvent.

2.4 Moisture and ash contents

3.00 g of the plant sample powder were weighed in a dry and clean crucible, then placed in a drying oven at 100°C for 3 hours. The crucible was then cooled in a desiccator for 15 minutes and reweighed. This process was repeated several times until a constant weight was obtained. The moisture content can be calculated using the following equation [25]:

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

Where: W_1: weight of the sample before drying, W_2: weight of the sample after drying.

For ash content determination, 3.00 g of the plant sample were weighed in a clean, dry porcelain crucible and placed in a muffle furnace at 550°C for 3 hours. The crucible was then cooled in a desiccator for 15 minutes and reweighed. The ash content can be calculated using the following equation [25]:

$$\%\ \text{Ash} = \frac{W_1}{W_2} \times 100 \quad (3)$$

Where: W_1: ash of weight after burning the sample, W_2: weight of the sample before the burning process.

2.5 Total protein content

The protein content in plant samples was estimated using the Kjeldahl method with some modifications. The plant material is oxidized using concentrated sulfuric acid, which oxidizes all the components except for the nitrogen present in proteins. The nitrogen is then reduced to ammonia, which is determined using back titration in the presence of methyl red indicator [26].

The total protein content in plants can be calculated by converting the nitrogen percentage using the following equation [26]:

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

2.6 Phytochemical screening

Phytochemical screening was performed on aqueous, ethanol, chloroform, and petroleum ether extracts obtained from the leaves of *Urtica urens* using

standard methods described in the literature [27-32]. The extracts were screened for alkaloids, tannins, saponins, steroids, flavonoids, coumarins, phenolics, terpenoids, quinones, glycosides, carbohydrates, and proteins.

2.7 Total alkaloid content

The total alkaloids were estimated using the gravimetric method with some modifications [25]. 5.00 g of the dried plant powder were placed in a 250 mL flask, and 200 mL of 10% acetic acid in ethanol were added. The mixture was left for 4 hours, then filtered and concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise until the precipitation was complete. The mixture was then centrifuged to separate the precipitate, which was collected, washed with diluted ammonium hydroxide, and filtered again. The remaining product obtained from this process is the alkaloids. The residue was dried weighed, and the percentage of alkaloids was calculated.

2.8 Total phenols content

The total phenolic content in the water and ethanol extracts of the studied plant was determined using the Folin-Ciocalteu method with some modifications [31]. 0.2 mL of the extract was mixed with 1 mL of 10% diluted Folin-Ciocalteu reagent and incubated for 4 minutes in the dark. Then, 0.8 mL of 7.5% sodium carbonate solution was added, and the volume was adjusted to 10 mL with solvent. After 30 minutes, the absorbance of the solution was measured at 765 nm. The total phenolic concentrations were calculated using a standard calibration curve prepared with various concentrations of gallic acid (10-60 mg/L). Gallic acid was used as a reference substance, and the results were expressed as gallic acid equivalent.

2.9 Total flavonoid content

The total flavonoid content in plant extracts was determined using the modified aluminum chloride method [32]. Rutin was used as a reference standard, and the total flavonoid content was expressed as rutin equivalent. A standard calibration curve was prepared with different concentrations of rutin (1-60 mg/L). The method was applied to determine the total flavonoid content in the water and ethanol extracts of the studied plant.

For the assay, 1 mL of the extract (water or ethanol) or rutin solution was mixed with 0.3 mL of sodium nitrite ($NaNO_2$) and 4 mL of distilled water. After 5 minutes, 0.3 mL of aluminum chloride solution was added, and the mixture was left for 6 minutes. Then, 2 mL of 1 M sodium hydroxide solution was added, and the volume was adjusted to 10 mL with distilled water after 10 minutes. The absorbance of the solution was measured at 510 nm.

2.10 DPPH radical scavenging assay

The antioxidant activity of aqueous and ethanolic extracts of *Urtica urens* was evaluated by determining their ability to scavenge DPPH radicals using a modified method based on Kirby and Schmidt (1997) [33]. The scavenging percentage of DPPH radicals was calculated using the following formula:

$$\%\ \text{DPPH scavenging} = \frac{\text{ABS of negative control} - \text{ABS of sample}}{\text{ABS of negative control}} \times 100 \quad (5)$$

$$\%\ \text{DPPH scavenging} = \frac{A_O - A_S}{A_O} \times 100 \quad (5)$$

In order to assess the antioxidant activity of the extract and ascorbic acid, 2 mL of DPPH solution was mixed with 1 mL of the sample solution (water or ethanol extract) or ascorbic acid solution, and the volume was adjusted to 10 mL. The mixture was kept dark for 30 minutes, and the absorbance was measured at 517 nm. The absorbance of the DPPH solution was also measured using the same method in the absence of the sample or ascorbic acid to determine the baseline.

The extract's free radical scavenging activity was evaluated in triplicate and expressed in terms of ascorbic acid, which was used as a reference substance. A standard calibration curve was prepared using varying concentrations of ascorbic acid (4-10 mg/L) to determine the antioxidant potential of the samples [33].

2.11 Minerals content

The mineral elements (Fe, Zn, Mn, Cu, Mg) were determined using an Atomic Absorption Spectrophotometer (VARIAN 220 FS), while Ca and Na were measured using a Flame photometer (PFP7 Jenway). The wet digestion method was utilized to oxidize all organic matter in the plant material using nitric acid and hydrogen peroxide [34-37].

To digest the plant material, 1.00 g of the plant powder was mixed with approximately 10 mL of concentrated nitric acid in a beaker and then heated for 10 minutes until the fumes disappeared. If the brown fumes persist, 10 mL of acid is added and heated. Next, 10 mL of hydrogen peroxide solution was added, and the mixture was boiled until the solution became clear and colorless. After cooling, the solution was filtered using filter paper and transferred to a 100 mL volumetric flask. The volume was adjusted to 100 mL using deionized water.

2.12 Statistical analysis

The results were presented as the mean ± standard error of the mean (SEM) obtained from multiple parallel measurements. Statistical significance was determined at a p-value of less than 0.05. The statistical analysis and database management were carried out using the Excel statistical software package developed by Microsoft Office 2016.

3. Results and Discussion

3.1 Phytochemical analysis

The study included conducting a phytochemical screening on active substances extracted from *Urtica urens* plant using four solvents with different polarities. This type of screening is considered a qualitative analysis that provides preliminary information on the type of active compounds present in the plant. It is important to note that the solvent used can impact the compounds extracted. Nevertheless, phytochemical screening is still helpful for detecting potential active compounds in the plant material [38].

The results of the phytochemical screening were expressed using symbols to indicate the extent of the change observed in the extract. The symbol (+++) was used when a visible change occurred, indicating the presence of the active substance (if present). A moderate change was marked by the symbol (++), while a slight change was suggested by the symbol (+). If no changes occurred that matched the conditions of each test, the result was expressed by the symbol (-). The results of the phytochemical screening are summarized in Table 1.

Table 1. Phytochemical Screening of *Urtica urens*

Phytoconstituent	Solvent			
	Water	Ethanol	Chloroform	Ether
Alkaloids	+	++	-	-
Phenolics	++	+++	+	-
Flavonoids	++	++	+	-
Terpenoids	-	-	-	+
Steroids	+	-	++	+++
Saponins	-	-	-	-
Tannins	+	+	-	-
Coumarins	++	++	+	-
Carbohydrates	+	++	-	-
Glycosides	+	+	-	-
Proteins	++	+	-	-

The qualitative results of the *Urtica urens* plant, as shown in Table 1 of the four extracts, indicate that a complete set of active compounds, including tannins, glycosides, proteins, alkaloids, flavonoids, coumarins, and alkaloids, are present in both aqueous and alcoholic extracts, but absent in chloroform and ether extracts (excluding phenols, flavonoids, and coumarins, which appeared in low concentrations in the chloroform extract). The presence of steroids and terpenes in the ether extract was also noted, which aligns with the polar properties of the solvent and solute.

The study by Kumar et al. [39] demonstrated the presence of glycosides, alkaloids, flavonoids, and reducing sugars in various extracts of the *Urtica urens* plant, which is consistent with our current study findings. However, they reported the absence of polysaccharides and proteins, which contradicts our present study. Similarly, Grara et al. [40] conducted a study focusing on the leaves of another *Urtica* species (U. diocica). They found the presence of flavonoids and tannins, while terpenes and saponins were absent, which aligns with our current study. However, they observed the absence of coumarins and alkaloids, which differs from our recent results. Furthermore, Nigam et al. [41] conducted a study on the same *Urtica* species (U. diocica) and agreed with our current study regarding the absence of saponins and terpenes. However, their findings differ from ours regarding the absence of carbohydrates and glycosides.

The results in the previous table reveal significant variations in the outcomes of the phytochemical screening concerning the presence, absence, or concentration of bioactive compounds. These discrepancies can be attributed to several factors, including the bioactive substances' characteristics, the choice of solvent, their solubility, and the precision of the testing methods employed. Notably, the selection of solvents played a crucial role in the extraction process, as certain chemical compounds exhibited a preferential extraction pattern over others. This observation aligns with the findings reported by Amabye et al. [42]. Specifically, the

aqueous and ethanol extracts yielded positive results and higher concentrations when detecting the most active substances. On the other hand, the chloroform and ether extracts demonstrated relatively lower results regarding the availability and concentration of the active compounds, albeit to a certain extent, when compared to water and ethanol.

Table 2. Levels of yield, moisture, ash, protein, and alkaloids determined for *Urtica urens*

%	Water	Ethanol	Chloroform	Ether
Yield	11.15±0.56	17.40±1.04	10.05±0.31	6.40±0.13
Moisture	7.58±0.61			
Ash	5.73±0.49			
Protein	28.04±1.40			
Alkaloids	0.22±0.04			

3.2 Yield, moisture, and ash analysis

The findings presented in Table 2 indicate that the alcoholic extract yielded the highest amount, while the ether extract produced the lowest yield. This observation aligns with the results reported in several prior studies [43-45], further supporting our findings.

Moisture content plays a crucial role in assessing the freshness and quality of plant material during analysis. In this particular study, the moisture content of the *Urtica urens* plant was determined to be 7.58%. A comparative analysis was conducted with existing literature to validate our findings, which indicated consistency between our results and previous studies conducted on Urtica dioica and *Urtica urens* plants. For instance, Dhale et al. (2010) [46] conducted a study on Urtica dioica and reported a moisture content of 7.55%, which aligns closely with our findings. Similarly, Temitope et al. (2015) [47] conducted a study on various herbal plants, and their reported moisture content was comparable to our results.

Ash content is a crucial parameter in plant analysis, providing insights into the mineral composition and purity of the plant material. In our study, the ash content of the *Urtica urens* plant was determined to be 5.73%. The results yielded mixed findings after conducting a comparative analysis with existing literature. For instance, Nigam et al. (2014) [41] conducted a study on Urtica urens and reported an ash content of 6.00%, which aligns with our findings. Similarly, Krishna et al. (2016) [48] conducted a study on various medicinal plant species and reported ash content ranging from 2.66% to 12.22%, comparable to our results. However, Paulauskiene et al. (2021) [49] conducted a study on Urtica dioica L. and reported an ash content ranging from 3.06% to 4.70%, which aligns with our findings.

3.3 Total protein and total alkaloids analysis

The total protein content is an essential parameter to consider in plant analysis, as it reflects the plant's nutritional value and potential therapeutic applications. Proteins are the building blocks of life and are involved in various biological processes, such as enzyme catalysis, signal transduction, cell structure, and defense. In this study, the total protein content of the *Urtica urens* plant was determined and found to be 28.04%, as shown in Table 2. The finding of this study was comparable with the literature, and our results are consistent with previous studies. A study by Arros et al. (2020) [50] reported the total protein content of *Urtica urens* to be 24.0%, which is close to our findings. Similarly, the present study's findings were within the range of 14-28% reported by other authors for nettle species from India and South Africa [51,52].

The total alkaloid content is another critical parameter in plant analysis, as it reflects the potential therapeutic applications of the plant. Alkaloids are nitrogen-containing compounds with various pharmacological effects, such as analgesic, antimalarial, and anticancer. In the present study, the total alkaloid content of the *Urtica urens* plant was assessed and determined to be 0.22%, as demonstrated in Table 2. Our findings and the existing literature were compared, revealing a mixture of results. For instance, a study by Shamsa et al. (2008) [53] on some Iranian medicinal plants reported a total alkaloid content ranging from 0.01% to 1.69%, within the same order of magnitude as our findings. Similarly, a study by Erdenechimeg et al. (2017) [54] on Lider-7-tang, which is a traditional Mongolian herbal medicine containing *Urtica urens* as one of the ingredients, reported a total alkaloid content of 0.20%, which is very close to our results. However, some other studies reported higher or lower values of total alkaloid content for *Urtica urens* or other nettle species, which may be due to different factors such as plant part, extraction method, assay technique, environmental conditions, etc.

To summarize, Figure 2 compares the findings on yield, moisture content, ash content, total protein content, and total alkaloid content in the *Urtica urens* plant extract.

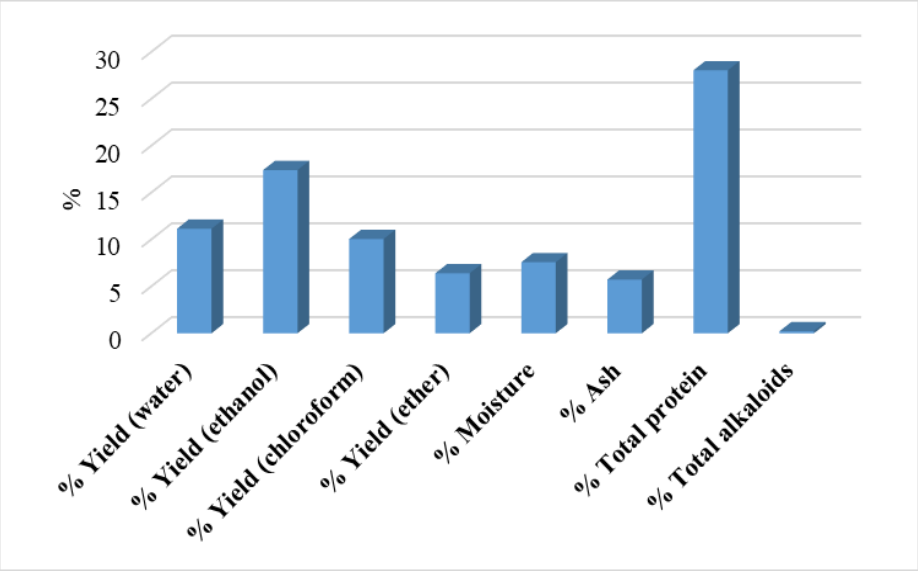

Figure 2. Yield, moisture, ash, total protein, and total alkaloid levels in *Urtica urens*

3.4 Total phenols analysis

The Folin–Ciocalteu colorimetric method was used to determine the total phenolic content of both the alcohol and water extracts. This method reduces a phosphomolybdic-phosphotungstic acid complex by phenolic compounds in an alkaline medium, resulting in a blue color that can be measured spectrophotometrically. The total phenolic concentrations were calculated using a standard calibration curve prepared with various concentrations of gallic acid (10-60 mg/L). Gallic acid was used as a reference substance, and the results were expressed as gallic acid equivalent (GAE). The calibration curve's correlation coefficient (R2) was 0.9458, indicating an excellent linear relationship between the absorbance and the concentration of gallic acid. The calibration curve equation was y = 0.0046x, where y is the absorbance at 765 nm and x is the concentration of gallic acid in mg/L. The calibration curve is shown in Figure 3. Table 3 presents the results obtained for each extract.

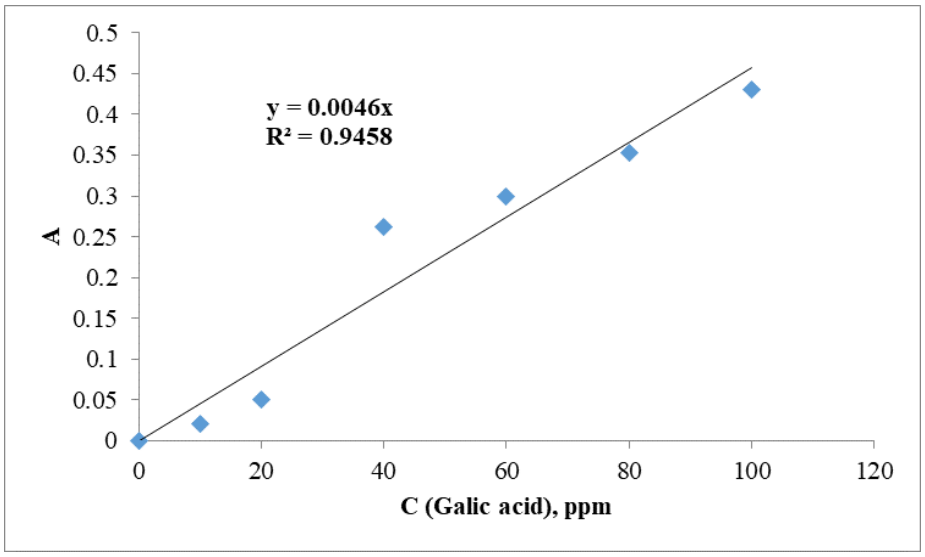

Figure 3. Calibration curve of gallic acid

The total phenol content is an essential parameter in plant analysis, as it reflects the potential therapeutic applications of the plant. In the present investigation, the total phenol content in the aqueous and ethanolic extracts of the *Urtica urens* plant was found to be 14.41 and 8.96 mg/g, respectively. A recent study by Caruso et al. (2021) [55] found that dietary intake of phenols was associated with a reduced risk of cognitive impairment in older adults. Another study by Godos et al. (2021) [56] demonstrated that phenols may protect against gut inflammation and microbial dysbiosis. These findings suggest that phenols may be crucial in promoting overall health and well-being. Upon comparing our findings with previously reported values, we have observed consistency between our results and previous studies conducted

on *Urtica urens*. For instance, Paulauskiene et al. (2021) [49] conducted a study on *Urtica dioica* and reported a total phenol content ranging from 3.28 mg/g to 19.07 mg/g, similar to our findings. Likewise, Paul and Pillai (2021) [57] studied various *Urtica urens* extracts. They reported a total phenol content ranging from 18.72 mg/g to 131.67 mg/g, which aligns with our results for both aqueous and ethanolic extracts of *Urtica urens*. However, it is worth noting that Maaroufi et al. (2017) [58] conducted a study specifically on *Urtica urens* and reported higher total phenol content values. Their findings indicated a total phenol content of 88.75 mg/g for the methanolic extract, an average of 56.50 mg/g for the acetone extract, and the lowest value of 43.92 mg/g for the aqueous extract. These values are significantly higher than our results. Overall, our findings suggest that both the aqueous and ethanolic extracts of *Urtica urens* contain a significant amount of total phenols, which could contribute to their potential therapeutic applications.

Table 3. Levels of Phenols, flavonoids, and total antioxidants determined for *Urtica urens*

	Water	**Ethanol**
Total phenols (mg/g)	14.41±0.80	8.96±0.63
Total flavonoids (mg/g)	2.57±0.23	2.08±0.09
Total antioxidant (mg/g)	4.77±0.29	5.26±0.37
IC_{50} (mg/mL)	0.64±0.07	0.86±0.06

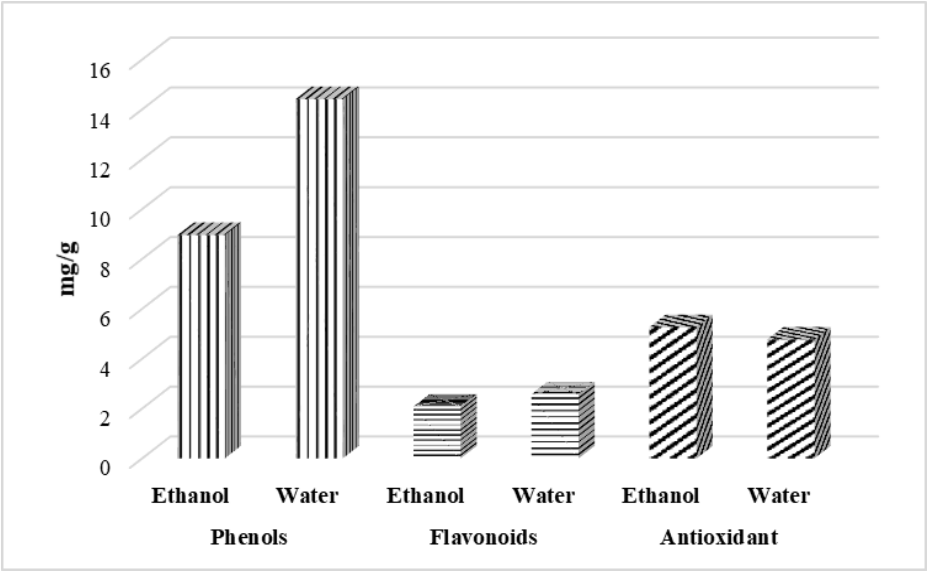

Figure 4. Phenols, flavonoids, and total antioxidant levels in *Urtica urens*

3.5 Total flavonoids analysis

The aluminum chloride colorimetric method was utilized to determine the total flavonoid content of both aqueous and ethanolic extracts. This method is based on forming a complex between flavonoids and aluminum ions, resulting in a yellow color that can be measured spectrophotometrically. The total flavonoid concentrations were calculated using a standard calibration curve prepared with various concentrations of rutin (1-60 mg/L). Rutin was used as a reference substance, and the results were expressed as rutin equivalent. The calibration curve's correlation coefficient (R2) was 0.963, indicating an excellent linear relationship between the absorbance and the concentration of rutin. The calibration curve equation was y = 0.0036x, where y is the absorbance at 510 nm and x is the concentration of rutin in mg/L. The calibration curve is shown in Figure 5. Table 3 shows the flavonoid amount for the studied extracts, while Figure 4 illustrates their obtained concentrations.

In this study, the flavonoid contents of the ethanolic and aqueous extracts were 2.08 mg/g and 2.57 mg/g, respectively. These values are lower than some previous studies on *Urtica urens* and related herbal plants. For example, Paul and Pillai (2021) [57] reported a 6.02 mg/g range to 31.57 mg/g for total flavonoids in *Urtica urens* species. Mzid et al. (2016) [44] reported 31.41 and 29.56 mg/g for ethanolic and aqueous extracts of *Urtica urens* L. leaves, respectively.

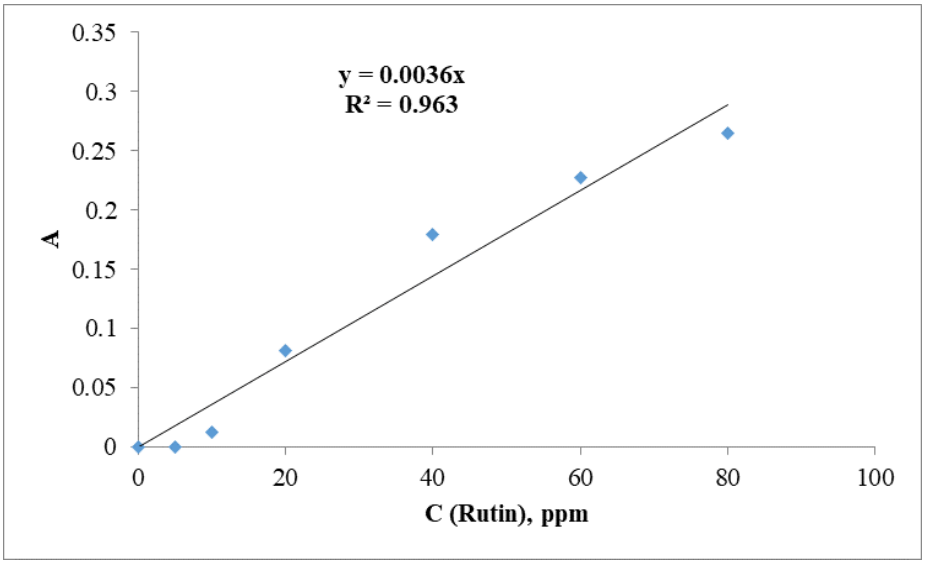

Figure 5. Calibration curve of rutin

However, these values are higher than other studies on *Urtica urens* and related herbal plants. For instance, Maaroufi et al. (2017) [58] reported 0.68, 0.66, and 0.47 mg/g for methanolic, acetone, and aqueous extracts of *Urtica urens*, respectively. Nencu et al. (2015) [59] reported 1.93 and 2.13 mg/g for *Urtica Urens* and *Urtica Dioica*, respectively. Therefore, this study's results agree with some of the previous findings on *Urtica urens* and related herbal plants. The variation in flavonoid content among different extracts of the same plant or related plants may be due to differences in extraction methods, plant parts used, and environmental factors.

3.6 Total antioxidant analysis

The DPPH radical scavenging method was used to assess the total antioxidant activity of *Urtica urens* plant extracts in aqueous and ethanolic extracts. The extract concentrations required to capture 50% of the stable free radical DPPH (I_{C50}) in mg/mL were also determined. The antioxidant content of the plant material was expressed as mg of ascorbic acid equivalent per gram of plant material. However, the DPPH radical scavenging percentage (%I) was calculated using equation (5) and plotted against ascorbic acid concentration, which served as the reference standard (Figure 6). Table 3 and Figure 4 show the antioxidant levels in the aqueous and alcoholic extracts of the studied plant. Table 3 also includes I_{C50} values.

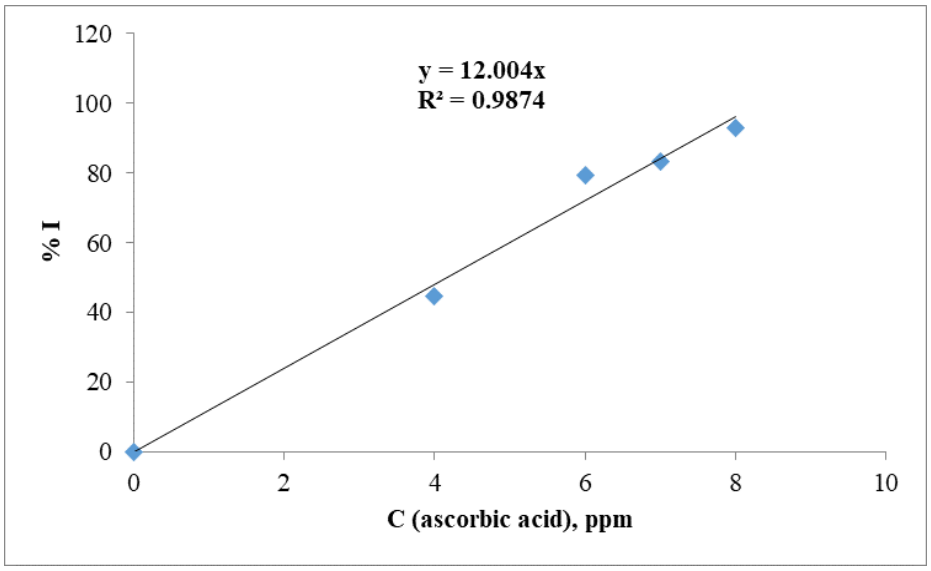

Figure 6. Calibration curve of ascorbic acid

In our study, the *Urtica urens* plant extracts (water and ethanol) exhibited significant antioxidant activity, with total antioxidant values of 4.77 mg/g and 5.26 mg/g, respectively. The IC50 values were also determined to be 0.64 mg/mL and 0.86 mg/mL for the water and ethanol extracts, respectively. These results suggest that *Urtica urens* could be a potential source of natural antioxidants for various food, pharmaceutical, and cosmetic applications.

The observed antioxidant activities of *Urtica urens* plant extracts, both in aqueous and ethanolic forms, can be attributed to bioactive compounds and their inherent mechanisms of action. *Urtica urens* contains a diverse range of phytochemicals, including phenolic compounds, flavonoids, and other secondary metabolites, recognized for their antioxidant properties [9]. The mechanism underlying the antioxidant activity of *Urtica urens* extracts can be attributed to bioactive compounds such as phenolic compounds and flavonoids. These compounds possess hydrogen-donating abilities, effectively scavenging free radicals and inhibiting oxidative stress. The phenolic compounds in *Urtica urens*, including phenolic acids and flavonoids, act as electron donors, neutralizing free radicals and preventing the propagation of oxidative reactions.

Additionally, other secondary metabolites, such as terpenoids and alkaloids, may contribute to the overall antioxidant activity of *Urtica urens* extracts [5,8]. Considering the potent antioxidant activity demonstrated by *Urtica urens*, these plant extracts hold promising potential for various applications in the food, pharmaceutical, and cosmetic industries. Incorporating *Urtica urens* extracts as natural antioxidants in these sectors can contribute to developing healthier and more sustainable products, offering protection against oxidative damage and promoting overall well-being.

The findings show that the ethanolic extract of the plant material possesses a higher overall antioxidant content than the aqueous extract, as determined by the DPPH assay. This indicates that the ethanolic extract contains more compounds capable of scavenging free radicals, highlighting its antioxidant potential. Additionally, the results reveal that the aqueous extract exhibits a lower I_{C50} value than the ethanolic extract in the DPPH assay. This implies that the aqueous extract requires a lower concentration to neutralize 50% DPPH free radicals, indicating its strong antioxidant activity. These two outcomes align as they reflect each extract's distinct types and quantities of antioxidants. While the ethanolic extract may contain a higher total antioxidant content, its efficacy in neutralizing free radicals may be comparatively lower. On the other hand, the aqueous extract may possess a lower total antioxidant content, but its antioxidants could be more potent and reactive in quenching free radicals. Consequently, the results suggest that the solvent's polarity and solubility impact the extraction process and the antioxidant activity of the plant material [60].

The antioxidant activity of *Urtica urens* and related plant species has been studied by other researchers, who have obtained different results from ours. For instance, Paulauskiene et al. (2021) [49] found much higher total antioxidant values in Urtica dioica, a closely related plant, ranging from 52.92 to 95.17 mg/g. Similarly, Maaroufi et al. (2017) [58] found higher total antioxidant values in *Urtica urens* in three extracts (methanol, acetone, water) ranging from 54 to 153 mg/g. Moreover, Mzid et al. (2017) [44] found higher total antioxidant values in *Urtica urens* L. leaves in ethanolic and aqueous extracts with 128.75 and 76.64 mg/g values, respectively. Furthermore, Chahardehi et al. (2009) [61] found higher total antioxidant values in *Urtica dioica* in four extracts (methanol, butanol, chloroform, ethyl acetate, and ether) with a range of 17.20 to 36.40 mg/g. These variations in results may be due to differences in extraction methods, plant parts used, and environmental factors.

3.7 Metal contents

The current study investigated the concentrations of Na, Mg, Ca, Fe, Cu, Zn, and Mn in *Urtica urens* plants, as shown in Figures 7 and 8. Ca and Mg were found to have the highest concentrations, with values of 127,720 and 4,772 mg/kg, respectively. These minerals are necessary for plant growth, development, and human health [62]. Calcium is essential for bone health, whereas magnesium is involved in various physiological processes, including muscle and nerve function [62]. Iron, copper, and zinc concentrations were moderate, with 134.94, 27.72, and 87.34 mg/kg values, respectively. These minerals are necessary for various metabolic processes, including immune function and the formation of red blood cells [63]. Sodium and manganese concentrations were relatively low, with values of 3,658 mg/kg and under detection limits, respectively. Although sodium is an essential nutrient, it is found in many foods, and excessive consumption can cause health problems. Manganese is another vital nutrient, but too much can cause neurological problems [64].

Previous investigations into the mineral composition of *Urtica urens* and related plants have also yielded fresh insights into these discoveries. Ugulu et al. (2019) [65] conducted a study on wild *Urtica urens* available in the open markets of Izmir, which revealed the following concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn for unwashed plants: 0.03 mg/kg to 0.65 mg/kg, 0.26 mg/kg to 1.60 mg/kg, 10.62 mg/kg to 96.40 mg/kg, 120.50 mg/kg to 721.50 mg/kg, 22.27 mg/kg to 75.41 mg/kg, 0.26 mg/kg to 2.05 mg/kg, 0.16 mg/kg to 6.75 mg/kg, and 25.61 mg/kg to 74.77 mg/kg, respectively. The present research yielded similar results for Fe, Zn, and Cu. Another investigation by Paulauskiene et al. (2021) [49] focused on *Urtica dioica* and determined the concentrations of nine mineral elements (P, K, Ca, Mg, Fe, Cu, Mn, Zn, B) in nettle leaves. The study recorded the highest levels of potassium (average 3.18%) and calcium (average 3.04%). Phosphorus (average 0.82%) was approximately four times lower, while magnesium content (average 0.61%) was six times lower. In terms of microelements, the study reported the highest values for iron (average 224.78 mg/kg), followed by boron (average 49.25 mg/kg) and

manganese (average 44.65 mg/kg). The lowest concentrations were found for zinc (average 18.86 mg/kg) and copper (average 14.23 mg/kg). The present study yielded similar results for copper and calcium but lower values for iron, copper, and zinc. Mzid et al. [44], in their investigation of *Urtica urens* L. leaves, found lower levels of Ca, Mg, Fe, Zn, and Cu compared to the present study. These variances could be attributed to soil composition disparities, different plant parts utilization, and environmental factors.

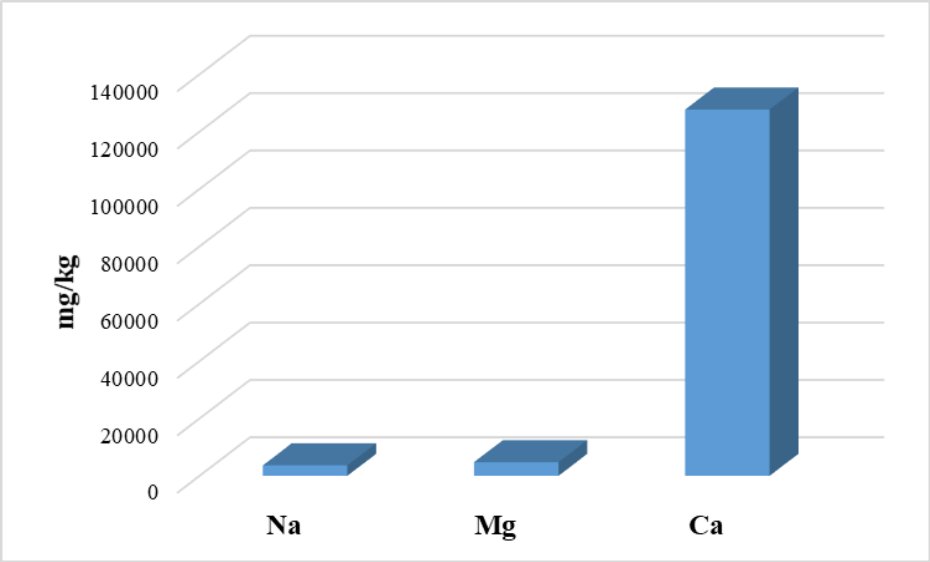

Figure 7. Na, Mg, and Ca levels in *Urtica urens*

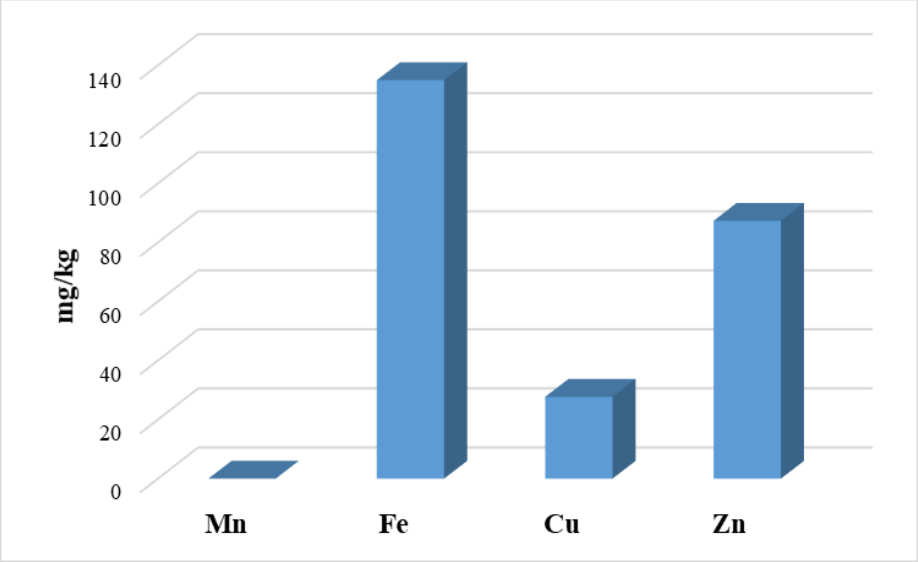

Figure 8. Mn, Fe, Cu, and Zn levels in *Urtica urens*

4. Conclusion

The present study provides comprehensive information on the phytochemical composition, antioxidant activity, and heavy metal content of *Urtica urens* leaves collected from Msallata, Libya. The results indicate that *Urtica urens* leaves are a rich source of bioactive compounds, including phenolics, alkaloids, proteins, and flavonoids, contributing to the plant's antioxidant properties. The aqueous and ethanolic extracts of *Urtica urens* exhibited significant antioxidant activity, with total antioxidant values of 4.77 mg/g and 5.26 mg/g, respectively. The plant was rich in calcium and magnesium, essential minerals required for various physiological functions in the human body. The study provides new insights into the potential health benefits of *Urtica urens* and highlights its potential as a source of bioactive compounds with antioxidant properties. The findings of this study may have important implications for developing new therapeutic agents or functional foods.

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