Baghdad Journal of Biochemistry and Applied Biological Sciences

2026, VOL. 7, NO. 2, 155–163, e-ISSN: 2706-9915, p-ISSN: 2706-9907

ORIGINAL RESEARCH ARTICLE

Ruta graveolens L. Leaves as a Potential Source of Natural Antioxidants and Phytochemicals: A Study from Msallata, Libya

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Article Info.

Keywords:
antioxidants,
heavy metals,
phytochemicals,
Ruta graveolens L.,
total phenols

Received: 07.30.2025
Accepted: 09.20.2025
Published online: 04.10.2026
Published: 04.10.2026

Abstract

The phytochemical profile and antioxidant capacity of Ruta graveolens L. leaves, collected from Msallata, Libya, are investigated in this study. Four solvents were used to extract the bioactive compounds: petroleum ether, ethanol, water, and chloroform. Moisture, ash, total proteins, total alkaloids, total phenols, total flavonoids, antioxidant activity, and mineral content in the leaves were measured. Ethanol was the extract with the highest extraction efficiency (16.32%) and the highest concentrations of antioxidant activity (6.47 mg/g), total flavonoids (3.77 mg/g), and total phenols (37.65 mg/g). According to phytochemical screening, all extracts lacked saponins but contained proteins, carbohydrates, phenols, flavonoids, alkaloids, coumarins, glycosides, steroids, and terpenes. Copper was the least common element, according to mineral analysis, whereas calcium, sodium, and magnesium were the most abundant. Iron was the most prevalent heavy metal, followed by zinc, with the overall order being Ca > Na > Mg > Fe > Zn > Cu. These results show the abundance of bioactive chemicals and natural antioxidants in R. graveolens L. leaves, demonstrating their potential uses in the production of functional foods and health promotion.

1. Introduction

Humans have utilized medicinal plants for centuries as traditional therapies and food sources [1–3]. Many bioactive substances, including phenols, flavonoids, terpenes, saponins, quinones, and tannins, have antibacterial, antioxidant, anti-inflammatory, and anticancer qualities, which contribute to their therapeutic potential [4–7]. Modern research has renewed interest in these natural compounds because they show promise as synthetic therapeutic substitutes, especially for chronic and infectious disorders [8,9].

A popular medicinal plant, *Ruta graveolens* (Rue) (Figure 1), received interest because of the presence of several different phytochemicals, such as rutin, coumarins, and essential oils, which support its wide range of biological activities. Its antidiabetic, antibacterial, antifungal, antioxidant, anti-inflammatory, and anticancer effects have all been documented in studies [10–13]. While modern scientific studies support many of these applications and continue to study its pharmacological potential, traditional medicine has used *R. graveolens* for illnesses ranging from inflammation to digestive issues [14,15].

Figure (1): *Ruta graveolens* L.

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How to cite this article: Elsherif, KM., Sulaiman, MA., Mlitan, A. *Ruta graveolens* L. Leaves as a Potential Source of Natural Antioxidants and Phytochemicals: A Study from Msallata, Libya. Baghdad Journal of Biochemistry and Applied Biological Sciences, 2026, Vol. 7, No. 2, 155–163. https://doi.org/10.47419/bjbabs.v7i2.446

Numerous investigations have emphasized the medicinal values of *R. graveolens*. For example, Agidew [16] documented its traditional uses in the treatment of fever, cough, and malaria, whereas Yu et al. [14] showed its anticancer efficacy against human breast carcinoma cell lines. The antibacterial and antifungal properties of its extracts were validated by Ahmed [17], and Diwan [18] discovered a favorable relationship between antioxidant capacity and total phenolic content (TPC). Further evidence of its anti-inflammatory and antioxidant properties was provided by Melnyk [19]. All of these results highlight the potential of *R. graveolens* as a natural source of medicinal compounds.

The purpose of this study was to assess the antibacterial activity and chemical composition of *R. graveolens* extracts gathered from Libya. The experiment involved determining the phytochemical contents (phenols, flavonoids, tannins, saponins, and alkaloids), assessing the antimicrobial activities against certain bacterial and fungal strains, and evaluating antioxidant activity. The findings support the potential use of *R. graveolens* in natural product-based therapies and offer further information on its pharmacological significance.

2. Materials and Methods

The collection and preparation of *R. graveolens* L. leaves was the first experimental step in this study. Other steps included phytochemical screening, extraction using various solvents, and quantitative measurements of proteins, alkaloids, phenols, flavonoids, antioxidant activity, and mineral content. Figure 2 depicts the general workflow of the experimental techniques.

2.1. Collection and preparation of the plant

R. graveolens L. leaves were obtained from Msallata, Libya, which is noted for its flora and fauna, between February and May 2020. Using morphological analysis, a plant specialist from the Department of Botany, El-Mergib University, identified the plant samples. After that, voucher specimens were stored in the Plant Science Laboratory of the same department. The leaves were carefully cleaned with distilled water to remove any dirt or impurities and were air-dried for 15 days at room temperature (25 ˚C) to ensure purity. After that, 100 g of *Urtica urens* powder was prepared by coarsely crushing the dried leaves in a

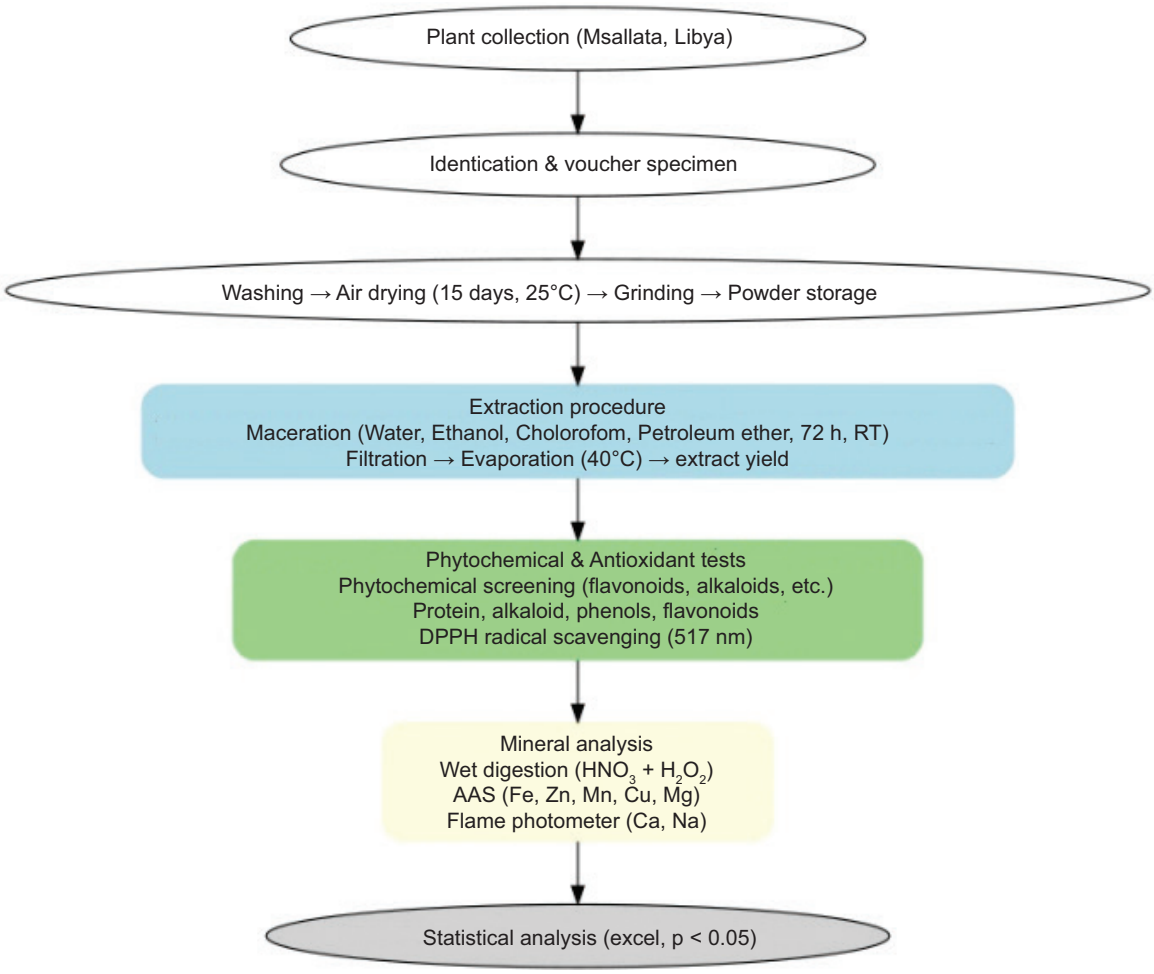

Figure (2): Experimental procedure for an experiment that includes plant collecting, phytochemical and antioxidant analysis, and mineral assay.

laboratory ultra-centrifugal mill ZM 200 (Retsch GmbH, Haan, Germany) to a flour-like consistency [20]. The powder was maintained at room temperature in glass vials, providing the necessary preparation for the later investigation of the phytochemical and heavy metal content of the leaves.

To determine the moisture content in the plant sample, 3.0 g of the powder was weighed in a crucible and dried for 3 h at 100°C. After cooling, the crucible was weighed to determine its final weight. Appropriate formulas were used to calculate the moisture content [21]. The ash content was ascertained by weighing 3.00 g of the sample and heating it at 550 °C for 3 h in a porcelain crucible. After cooling, the crucible was weighed again. The method used to calculate the ash content was documented in the study by Alkherraz et al. [22].

2.2. Extraction procedure

The phytochemicals from the dried *R. graveolens* L. leaves were extracted by maceration. The plant material was soaked in different solvents (water, ethanol, chloroform, and petroleum ether) for 72 h at room temperature. The mixtures were filtered and evaporated at 40 °C under vacuum, following previous studies [23,24]. The extract yield was calculated by weighing the dried samples [25]. This method ensured the effective extraction and concentration of the phytochemicals.

2.3. Phytochemical analysis

R. graveolens L. leaf extracts were screened for phytochemicals using aqueous, ethanol, chloroform, and petroleum ether extracts. The techniques used in this analysis are well established and documented in the literature [26,27]. Using accepted practices, the contents in the extract were examined for the presence of flavonoids, coumarins, phenolics, alkaloids, tannins, saponins, steroids, terpenoids, quinones, glycosides, carbohydrates, and proteins.

2.4. Total protein analysis

The protein content of plant materials was ascertained using the modified Kjeldahl technique [28]. In this process, powerful sulfuric acid oxidises the plant material, destroying all components except nitrogen. After that, the nitrogen is converted into ammonia, and the amount of ammonia is measured via a reverse titration process with an indicator such as methyl red.

2.5. Total alkaloid analysis

The modified gravimetric approach was used to determine the total alkaloids [29]. After being steeped in ethanol and acetic acid, the plant powder was filtered and concentrated. Ammonium hydroxide was used to precipitate the alkaloids, which were then collected, centrifuged, cleaned, and filtered. The alkaloid content in the residue was estimated by drying and weighing it.

2.6. Total phenols analysis

The modified Folin–Ciocalteu technique [29] was used to determine the TPC in the aqueous and ethanolic extracts of the plant. After combining the extract with the Folin–Ciocalteu reagent, the mixture was incubated and diluted using sodium carbonate solution. After 30 min, the absorbance was measured at 765 nm. Gallic acid equivalents were used to calculate and express the phenolic content, and the gallic acid concentrations on the calibration curve ranged from 10 to 60 mg/L.

2.7. Total flavonoid analysis

The modified aluminum chloride approach was used to determine the total flavonoid concentration in plant extracts [3]. Rutin served as the reference standard, and the overall content of flavonoids was expressed as rutin equivalent. To create a standard calibration curve, rutin concentrations ranging from 1 to 60 mg/L were generated.

2.8. DPPH radical scavenging assay

The capacity of ethanolic and aqueous extracts to scavenge DPPH radicals was used to measure their antioxidant activity. A modified method from Kirby and Schmidt (1997) [30] was applied to achieve this goal. A mixture of 2 mL of DPPH solution and 1 mL of the sample solution (water or ethanol extract) or ascorbic acid solution was prepared to measure the antioxidant activity of the extract and ascorbic acid. The entire volume was subsequently lowered to 10 mL. Following that, the mixture was kept in the dark for 30 min, during which the absorbance was measured at 517 nm. The same method was used to measure the absorbance of the DPPH solution without the sample or ascorbic acid to establish a baseline.

2.9. Mineral analysis

The Atomic Absorption Spectrophotometer (VARIAN 220 FS) was employed to analyze the concentrations of essential microelements, namely Fe, Zn, Mn, Cu, and Mg. On the other hand, the Flame photometer (PFP7 Jenway) was utilized for the quantification of essential macroelements, specifically Ca and Na. To eliminate organic matter from the plant material, the wet digestion method was applied, which involved the oxidation process using nitric acid and hydrogen peroxide [31–33].

2.10. Statistical analysis

All experiments were conducted in triplicate, and the mean values and standard deviation (SD) are shown for each result. The reliability of the data was assessed using the standard error of the mean (SEM). The threshold for statistical significance was set at $P < 0.05$. The results were confirmed by the inclusion of suitable controls, which were blank samples devoid of an absorbent. Microsoft Excel 2016 was used to handle the data and conduct statistical analysis.

3. Results and Discussion

3.1. Yield, moisture, and ash contents

Table 1 provides the percentages of yield, moisture, and ash content of the four extracts. The alcoholic extract exhibited the highest yield, while the aqueous and chloroform extracts displayed comparable percentages. Conversely, the ether extract showed the lowest yield. These findings align closely with a previous study conducted by Molnar et al. [34], which investigated the *R. graveolens* L. plant and reported alcoholic extract yields ranging from 9.95 to 14.95%. Another study by Aljaiyash et al. [35] found a relatively higher alcoholic extract yield of 34.18%. A variety of factors, including the freshness of the plant material and the extraction method employed, influence the yield percentage disparity. A comparatively high moisture content of 13.46% was observed in this study. This figure is significantly higher than the measurement given by Dhale et al. (7.55%) [36], but it is close to the data reported by Molnar et al. (12.19%) [34]. The ash content was also found to be relatively high. Nonetheless, this finding is consistent with that of the research project carried out by Nigam and Biswal (6.0%) [37]. It is significant to remember that the amount of ash in a sample can be used as an indicator of the mineral composition of the sample.

3.2. Phytochemical screening results

The presence of proteins, carbohydrates, phenols, flavonoids, alkaloids, coumarins, glycosides, steroids, and terpenes was found in the chemical analysis of various extracts of *R. graveolens* L. leaves, as indicated in Table 2. No saponins were found in any of the extracts. Azalework et al. [38], in their study on *R. graveolens* L. leaves, found a variety of active substances, including saponins, while multiple phenols, carbohydrates, and proteins were absent. The presence of saponins was also reported in other studies, such as those of Perera et al. [39], which differs from the findings of the current research. However, a study by Amabye et al. [12] aligned with the current study in the absence of saponins but differed in the absence of phenols and tannins in this plant. A study conducted by Aljaiyash et al. [35], which investigated both *R. graveolens* L. and Aloe Vera plants, revealed the presence of phenols, carbohydrates, tannins, flavonoids, steroids, and terpenes in alcoholic extracts, while alkaloids and glycosides were not detected in the tested extracts. These variations may be attributed to differences in the extraction methods utilized.

3.3. Total proteins and total alkaloid contents

Table 1 displays the total protein and total alkaloid content values. There have been no prior investigations on *R. graveolens* L. protein content. However, when compared to the protein content of other plants, it appears to be similar. In a study conducted by Ahmed et al. [17] on the alkaloid content of *R. graveolens* L. and other plants, the alkaloid content ranged from 14–17%, which is significantly higher than the current study result. In a previous study on various medicinal plants in Nigeria [40], including *Ruta bransilensis*, the findings varied from 0.34 to 1.04%, which is comparable to the current study results.

3.4. Total phenolic content

Total phenolic content in extracts was calculated using extrapolation from a calibration curve ($Y = 0.0046X$; $R^2 = 0.9458$) based on gallic acid concentrations. TPC was measured in milligrams of gallic acid equivalence (GAE) per gram. Using a regression equation, the quantification of phenolic components in different quantities of aqueous and ethanolic extracts was achieved, and the findings were reported as gallic acid equivalents (Table 3). There were no significant differences ($P > 0.05$) between the tested concentrations of each extract.

The phenolic content of the aqueous and ethanolic extracts from *R. graveolens* leaves was determined and is presented in Table 3. The results indicated that the plant leaves had a high phenolic content. A comparison with previous studies revealed that the current results were in line with several studies that reported similar values. For instance, Frent et al. [41], Pękal et al. [42], Mokhtar et al. [43], and Diwan et al. [18] reported phenolic contents of 37.98, 39.90, 41.63, and 37.0 mg/g, respectively. However, some studies reported lower or higher values than the current results. For example, Yu et al. [15], Workie and Desta [44], Ahmed et al. [17], and Kaplan [45] reported phenolic contents of 24.96, 142.16, 3.16, 2.90, and 34.12–54.10 mg/g, respectively. These variations could be due to several factors, such as geographical location, extraction method, solvent type, and harvesting time [15]. Because of its possible therapeutic applications, the TPC evaluation is important when analysing plants. According to a recent study by Chen et al. [46], older people who consume phenols

Table (1): Results of yield, moisture, ash, protein, and alkaloids of *Ruta graveolens* +

%	Water	Ethanol	Chloroform	Ether
Yield	13.75 ± 0.47	21.05 ± 0.93	12.60 ± 0.54	9.05 ± 0.21
Moisture	13.46 ± 0.74			
Ash	5.94 ± 0.34			
Protein	11.72 ± 1.12			
Alkaloids	0.28 ± 0.07			

Mean ± SD (for 5 replicates)

Table (2): Phytochemical results for *Ruta graveolens* L.

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

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

Table (3): Amounts of TPC, TFC, TAC, and IC_{50} for *Ruta graveolens* L.

	Water	Ethanol
Total phenols content (mg/g)	35.19 ± 0.74	37.65 ± 0.61
Total flavonoid content (mg/g)	2.91 ± 0.23	3.77 ± 0.09
Total antioxidant content (mg/g)	6.51 ± 0.31	6.47 ± 0.22
IC_{50} (mg/mL)	0.89 ± 0.09	0.85 ± 0.04

Mean ± SD.

through food are less likely to experience cognitive deterioration. Similarly, studies by Liu et al. [47] suggested that phenols might protect against dysbiosis of microbiota and gastrointestinal inflammation. The results of this study demonstrate the possible significance of phenols in maintaining general health and improving well-being in general.

3.5. Total flavonoid content

R. graveolens leaves were used to determine the total flavonoid content (TFC) by using a linear regression equation, Y = 0.0036x, which was obtained from a rutin calibration curve. Strong correlation was indicated by the linear regression coefficient (R^2) of 0.963 that was obtained from this research. The results are displayed in Table 3 and are expressed as mg of rutin equivalent per gram of the dry material.

Compared to values reported in earlier research studies, including Yu et al. [15], (11.90 mg/g), Workie and Desta [44], (118.25 mg/g), Mokhtar et al. [43], (13.97 mg/g), and Kaplan [45], (18.94–54.10 mg/g), the TFC in both the aqueous and ethanolic extracts in this study was generally lower. However, other investigations, including the ones by Diwan et al. [18] (0.8–17.0 mg/g) and Arruda et al. [48] (5.88 mg/g), have findings that are in line with the current study.

Studies on *R. graveolens* have revealed varying levels of flavonoid concentration, which can be attributed to many factors, such as the study location, the extraction method utilized, and the solvent employed. Numerous studies have attempted to pinpoint the precise kinds of flavonoids that are present. For example, *R. graveolens* has a high concentration of phenolic compounds, with a TPC of 41.63 mg/g and a flavonoid content of 13.97 mg/g, according to the investigation by Mokhtar et al. [43] into the phenolic profile of the plant. Nine phenolic compounds were identified in this experiment, comprising three phenolic acids and six flavonoids. With 464.95 g/g, rutin was found to be the most prevalent phenolic component in *R. graveolens*. Syringic acid came in second with 179.74 g/g, while naringenin came in third with 109.78 g/g. In another investigation by Elansary et al. [49], conducted in Northern Saudi Arabia, various polyphenols such as quercetin, isochlorogenic acid, chlorogenic acid, and p-coumaric acid were detected in *R. graveolens*. However, specific quantification of these polyphenols was not provided in the study.

3.6. Total antioxidant activity

Ascorbic acid (AA) was used as the reference ingredient to create a calibration curve for determining the DPPH free radical scavenging ability of the aqueous and ethanolic extracts of *R. graveolens* leaves. With a linear regression coefficient of R^2 = 0.9874 and a linear regression equation of Y = 12.004x, the calibration curve was a straight line. The DPPH radical scavenging capacity of each extract was determined using this curve. The mean values of the DPPH free radical scavenging activity at different dosages of both leaf extracts are shown in Table 3. The I_{C50} values (i.e., the concentration of *R. graveolens* extract or control sample that inhibited 50%) are also included in Table 3. These values were determined by plotting the inhibition percentage against the concentration of the plant extract or control sample and analyzing the resulting graph.

The antioxidant activity of *R. graveolens* plant extracts has been investigated by several previous studies. These studies used different solvents for extraction and various

methods for antioxidant evaluation. Alotaibi et al. [24] reported values of 0.94 mg/g and I_{C50} = 56.6 µg/mL, which were consistent with the current study results. Al-Ghamdi et al. [50] also reported similar results, ranging from 3.48 to 8.81 mg/g, using different solvents. Kaplan's study [45] showed a similar range of results, from 2.5 to 7.36 mg/g, with the ethanolic extract having the highest value. Ahmed et al. [17] also reported a similar range of results, from 5.02 to 7.47 mg/g.

Based on the results of this study as well as earlier research, it has been determined that the *R. graveolens* plant has significant antioxidant qualities. These qualities are vital for protecting the body from oxidative stress and the harmful effects of free radicals. This high concentration of flavonoids and phenolic substances in this plant greatly enhances its antioxidant capacity. It is significant to remember that environmental elements such as pH and temperature might have an impact on the antioxidant activity of *R. graveolens*. The antioxidant and antibacterial qualities of agitated cultures of different Rue species, including *R. graveolens*, were assessed in a study by Szewczyk et al. [11]. *R. graveolens* has a wide variety of actions, including antioxidant, anti-inflammatory, spasmolytic, sedative, antibacterial, antifungal, and antiviral characteristics, according to the findings. Jianu et al. [51] revealed that the essential oil produced from *R. graveolens* has antioxidant activity, as proven by its capacity to decrease peroxide and thiobarbituric acid levels and scavenge DPPH radicals.

3.7. Mineral contents

The concentrations of certain essential elements (Na, Ca, Mg, Mn) and heavy metals (Fe, Cu, Zn) in *R. graveolens* were determined using flame emission spectroscopy and atomic absorption spectroscopy. The results are presented in Figures 3 and 4.

The elemental concentrations in the examined plant samples are displayed in the figures. Every mineral element was found in every sample. The results showed that the concentration of calcium was highest. Magnesium and sodium were likewise highly concentrated, and iron had the highest concentration of all the heavy metals. The other elements had varying concentrations, with copper having the lowest. The element concentrations were in the sequence Ca>Na>Mg>Fe>Zn>Cu.

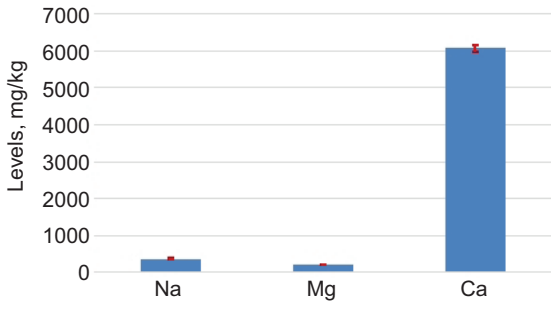

Figure (3): Na, Mg, and Ca concentrations in *R. graveolens.*

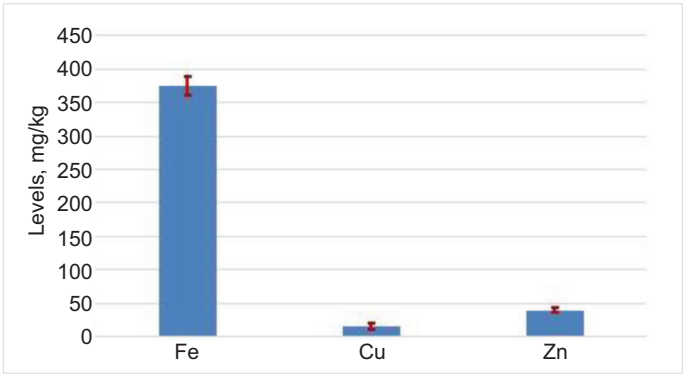

Figure (4): Mn, Fe, Cu, and Zn concentrations in *R. graveolens.*

The present study findings are consistent with the investigations of Ozyigit et al. [52], which revealed calcium and iron concentrations of 6,839.97 mg/kg and 279.569 mg/kg, respectively. Their average values for the concentrations of copper, sodium, zinc, and magnesium, however, were less than the findings of the present investigation. Moreover, the manganese level of 78.279 mg/kg was reported in the same research, which countered the results of the current investigation. Similar to the current findings, another study conducted by Khalid et al. [53] discovered iron, zinc, and copper in the leaves of *R. graveolens*.

4. Conclusions

This study assessed the phytochemical composition, mineral content, and antioxidant capabilities of *R. graveolens* L. leaves collected from Msallata, Libya, using extracts of ethanol, water, chloroform, and petroleum ether. Ethanol produced the greatest levels of flavonoids, phenolics, and antioxidant activity among the extracts. According to mineral analysis, zinc and copper were comparatively low, whereas calcium, sodium, magnesium, and iron were the most prevalent elements. Except for saponins, phytochemical screening revealed the existence of a number of beneficial chemicals. According to these findings, the leaves of *R. graveolens* are a rich natural source of antioxidant chemicals, especially flavonoids and phenolics, which are known to have a variety of biological functions. To establish their biological significance and possible uses, more research is required, including the isolation of individual compounds and *in vivo* assessments, as the current findings are restricted to *in vitro* experiments and compositional analysis.

Acknowledgments

The authors would like to express their sincere gratitude to the Chemistry Department, Faculty of Science, Misurata University, for providing the facilities and support for this research.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Compliance with Ethical Standards

This article does not contain any studies involving human or animal subjects.

Authors' Contributions (CRediT Taxonomy)

Contributor Role	Degree of Contribution		
	Lead	Equal	Supporting
Conceptualization	KME		
Data curation	MAS		
Formal analysis	KME	MAS	
Funding acquisition		MAS	AM
Investigation		MAS	AM
Methodology	MAS		
Project administration	KME		AM
Resources	MAS		AM
Software	KME	MAS	
Supervision	KME		AM
Validation	MAS		
Visualization	MAS		AM
Writing-original draft	MAS		
Writing-review & editing	KMW		AM

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