



RESEARCH ARTICLE

Comparative analysis of medicinal plants from Kurdistan region-Iraq for chemical constituents and bioactivities

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Abstract

The medicinal plants in Kurdistan-Iraq are used widely for the treatment of many diseases. Therefore, it is very interesting to look after the components of these plants as they are involved directly in the therapy. The collected samples from Dohuk City in the Kurdistan Region of Iraq were *Adiantum capillus-veneris*, *Polygonum avicular*, *Urtica dioica*, *Tribulus terrestris*, *Artemisia splendens* Willd, *Scirpus lacustris*, *Mentha longifolia*, *Thymus kotschyanus*, *Achillea santolina* and *Anthenis des champs*. The elemental contents of these samples were evaluated by inductively coupled plasma mass spectrometry (ICP-MS). The antibacterial and antioxidant activities were identified for the methanolic extracts of these plants. This study aims to identify the components, evaluate elemental content and analyze the biological activities of certain medicinal plants used in the region. The qualitative analysis of the phytochemical by screening out the methanolic extract of the collected samples showed the presence of phenolic compounds, proteins, fatty acids, amino acids, anthraquinones, alkaloids, tannins, flavonoids, saponins and steroids. Furthermore, the extracts have been analyzed to determine the presence of elements. The most abundant elements of the total 29 elements analyzed for the plant samples were iron, manganese, potassium and magnesium. This finding is important for potential drug discovery.

Keywords

medicinal plants; phytochemicals; elemental analysis; antibacterial activity; antioxidant activity; ICP analysis

Introduction

This investigation aims to conduct a comparative analysis of medicinal plants native to the Kurdistan region of Iraq. By comparing their chemical constituents and bioactivities, this study seeks to expand our knowledge of the potential therapeutic applications of these plants.

Plants have been used for therapy since ancient times. The fact that medicinal plants are readily available for use in health care and therapy is the primary reason why 2/3rd of the public trusts these plants (1). Nowadays, herbal medicines are preferred by a lot of the world's population because of how well they work with the human body. More than 80 % of the population in underdeveloped nations receives their main medical treatment from plants used for therapeutic purposes. This is due to limited access to hospi-

tals, basic healthcare facilities and exorbitant pricing (2). The existence of phytochemicals and active components such as tannins, terpenoids, flavonoids and alkaloids in medicinal plants increases the efficiency of treatment and therapy (3). Mineral composition is also important because of its nutritional qualities and health benefits as well as its compliance with dietary standards necessary for a balanced diet and its deficiencies can be the cause of different diseases (4). Essential elements, which include the main elements and some minor elements, serve different purposes, such as electrolytes, enzymes, vitamins and hormones and they are important for many biological processes that are essential to life (5). Several components make their way into plant and human food chains depending on the soil and groundwater's geological origin. Metals like cadmium and lead aren't necessary for human nutrition, yet they're nevertheless big environmental and soil pollutants since they may be harmful even in low amounts (6).

The antioxidant effects of natural sources are commonly linked to the bioactivity of phytochemicals, including phenolic compounds, flavonoids and carotenoids (9). These chemicals can significantly impact human health due to their pharmacological actions. These actions can include reducing inflammation, allergies and infections caused by microbes, viruses and even some cancers. Additionally, they may protect the heart and improve blood flow by relaxing blood vessels (10, 11). To this point, scientists are currently exploring the power of natural antioxidant molecules from medicinal plants. These molecules might hold the key to preventing various diseases, including diabetes, cancer, high blood pressure and even Alzheimer's. Their effectiveness comes from their ability to combat oxidative stress, a cellular imbalance that causes health issues.

The taxonomy of these plants refers to the fact that *Artemisia splendens* Willd, *Achillea santolina* and *Anthemis des champs* belong to the *Asteraceae* family. *Mentha longifolia* and *Thymus kotschyanus* belong to the *Lamiaceae* family, as shown in Table 1.

Table 1. Family taxonomy with English and Kurdish names for the selected plants.

Botanical Name	Family	English Name	Kurdish Name
<i>Adiantum capillus-veneris</i>	Pteridaceae	Maidenhair Fern, Venus Hair	Gia Zava
<i>Polygonum aviculare</i>	Polygonaceae	Knotgrass	Qadqadok
<i>Urtica dioica</i>	Utricaceae	Stinging Nettle	Kazink
<i>Tribulus terrestris</i>	Zygophyllaceae	Caltrop, Goat's head	Sey Didang
<i>Artemisia splendens</i> Willd	Asteraceae	Wormwood, Sagewort	Giya Band
<i>Scirpus lacustris</i>	Cyperaceae	Bulrush, Club-rush	Dambiz
<i>Thymus kotschyanus</i>	Lamiaceae	Kotschy's Thyme	Chatra Dark
<i>Mentha longifolia</i>	Lamiaceae	Horse Mint, Wild Mint	Ping
<i>Achillea santolina</i>	Asteraceae	Santolin Yarrow	Beywijan
<i>Anthemis arvensis</i>	Asteraceae	Corn Chamomile	Baybin

To enhance the level of medical care provided to people all over the world, it is essential to conduct more scientific studies on the herbal plant samples that are already available. As the primary and secondary phytoconstituents responsible for the effectiveness of medicinal plants, discovering which essential and trace elements are present in them is critical. Minerals are crucial for living things to reproduce, grow, stay healthy and work properly. In this context, the elemental analysis of plants provides valuable information about their nutritional content, environmental factors and potential medicinal properties.

The chosen plants have been traditionally utilized in therapy due to their valuable concentrations of active chemical elements. Various research have examined the possible antibacterial and antioxidant properties of these plants (7). Metabolic processes have a key role in regulating the concentrations of various elements within living organisms. By recognizing patterns in the levels of these elements regulated by metabolism in commonly consumed foods, it is possible to detect deficiencies or contamination that could eventually lead to specific diseases (8). The selected medicinal plants contain a wide range of elements in different concentrations.

No prior research has been documented that looked at how the plants *Mentha longifolia*, *Achillea santolina* and *Anthemis des champs* grown in Iraq's Kurdistan Region fight free radicals, act as antioxidants or kill bacteria. As a result, this study sought to conduct phytochemical analyses and ascertain the biological functions of these plants for use by researchers in the creation and design of various new drugs.

Materials and Methods

Reagents and Chemicals

Analytical-grade chemicals were used in the entire work. NaOH, Na₂CO₃, H₂SO₄ and DPPH were purchased from Sigma-Aldrich. Gallic acid from Thermo Fisher. Methanol and L-Ascorbic acid were procured from Roche. F.C. reagents were obtained from Ottokemi. Ferric chloride from Hach. Chloroform from Fisher Chemical. Dragendorff's reagent from Alpha Chemika.

Sample Preparation

The selected plants, including *Adiantum veneris*, *Polygonum aviculare*, *Urtica dioica*, *Tribulus terrestris*, *Artemisia*

splendens Willd, *Scirpus lacustris*, *Thymus kotschyanus*, *Mentha longifolia*, *Achillea santolina* and *Anthenis des champs* were collected in June and September 2021 from the region of Dohuk in Benarinke village, with geographical coordinates: 36°53'09.5"N 43°14'34.7"E. This region is surrounded by mountains and the springs of water running in this area. The plants were authenticated by Prof Dr. Saleem Shahbaz at the College of Agriculture, University of Dohuk-Kurdistan Region, Iraq.

The plants were washed with water and air-dried in a ventilated and clean place under the shade for 3 weeks. The dried specimens were pulverized into powder form using an electric grinder and then securely stored in suitable containers.

Mineral analysis by Inductively Coupled Plasma-Mass Spectroscopy ICP-MS

1 g of plant sample was coldly digested for approximately 8 h in 15 mL of a mixture of nitric acid and perchloric acid (5:1). Then, the mixture was heated gradually up to 115 °C for 2 h. After cooling, samples were diluted with water up to 25 mL and 2–3 drops of HCl were added and then analyzed by ICP-MS (PerkinElmer NexION 5000) (12). The operational parameters for the ICP-MS were as follows: RF power at 1.550 kW; plasma flow rate set at 14 L·min⁻¹; auxiliary gas flow rate maintained at 0.8 L·min⁻¹; nebulizer flow rate adjusted to 0.93 L·min⁻¹; helium collision gas flow rate set to 4.5 mL·min⁻¹; spray chamber temperature set to 2.7 °C; sample depth at 9 mm; sample introduction flow rate maintained at 1 mL·min⁻¹; nebulizer pump set at 0.1 revolutions per second (rps); extract lens 1 voltage set to 1.5 V.

Phytochemical Study

Methanolic Extract Preparation by maceration: 10 g of ground powder samples was mixed with 100 mL of methanol and left at room temperature for 48 h with stirring. After 15 min of centrifugation at 4500 rpm, the mixtures were filtered to separate the supernatants. After that, the solvent was then removed using a rotating evaporator. The dry residue was weighed and kept for further analysis at 4 °C (13).

Phytochemical screening

Qualitative screening of the extracted samples was conducted to detect the presence of phenolic compounds, saponins, flavonoids, terpenoids, steroids, alkaloids, glycosides and tannins (14).

Phenolic compounds Detection

The extract was treated with a ferric chloride solution and the observation of a green, blue or purple color confirmed the existence of phenols (15).

Saponin Detection

The samples were mixed with distilled water in test tubes and the occurrence of prolonged foaming when heated was considered an initial indication of the existence of saponins (16).

Flavonoid Detection

The extract was treated with a solution of sodium hydroxide and the presence of flavonoids was confirmed by the observation of a color change from yellow or orange to colorless upon acidification (17).

Terpenoid Detection

A mixture of concentrated sulfuric acid and chloroform was added to the plant sample, resulting in a reddish-brown coloration if terpenoids were present (18).

Steroid Detection

A combination of chloroform and plant extract was treated with concentrated sulfuric acid, leading to a red upper film and a yellow H₂SO₄ layer with green fluorescence, indicating the presence of steroids (15).

Alkaloid Detection

The extract was treated with Dragendorff's reagent and the observation of an orange-red precipitate confirmed the existence of alkaloids (19).

Glycoside Detection (Keller-Killiani Test)

A mixture of glacial acetic acid, ferric chloride solution, water and extract was prepared and concentrated sulfuric acid was added slowly. The presence of glycosides can be determined by the observation of a brown ring.

Tannin Detection

Samples were mixed with distilled water, filtered and treated with 0.1 % ferric chloride reagent. The presence of tannins was revealed by the blue-green coloring (20).

Total Phenolics Content

The Folin-Ciocalteu method was used to determine the total phenolic content of the extracts. It depends on the hydroxyl groups that exist in plant extracts to assist in the scavenging of free radicals (21). In this method, Phenolates are oxidized with Folin-Ciocalteu reagent to generate a blue-colored complex of molybdenum and tungsten which can be measured at 765 nm with a spectrophotometer. Add 100 µL of extract to 400 µL distilled water and 200 µL of 1 N Folin-Ciocalteu reagent, then add 20 % Na₂CO₃. After 2 h of incubation at room temperature, a UV-visible spectrophotometer (Jenway 7315) measured the absorbance at 765 nm. The phenolic content was quantified as mg of gallic acid equivalents per g of dry weight (mg GAE/g DW) of the sample (22).

Antioxidant Activity

The antioxidant potential of our extracts was assessed using the DPPH (Radical scavenging activity method) (23). Five different concentrations of the sample extracts were prepared. Initially, 1 mL of methanolic DPPH solution (0.004 %) was mixed with 3 mL of the sample extract. Subsequently, the mixture was incubated in darkness for 30 min at 30 °C. Absorbance readings were then obtained at 517 nm using a UV-Vis spectrophotometer. (Jenway 7315).

To prepare the control, 3 mL methanol was mixed with 1 mL DPPH solution. Ascorbic acid was employed as the standard antioxidant positive control. The same conditions of the sample were applied to measure the absorb-

ance of the control. The % inhibition of DPPH was determined using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

Antibacterial Activity

The investigation of antibacterial activity was conducted by the good diffusion method in an agar medium (24). The organic extracts were evaluated for their antibacterial activity against 2 bacterial strains: *Staphylococcus aureus* (NCIMB9518), a gram-positive bacterium and *Escherichia coli* (NCIMB1079), a gram-negative bacterium. Muller Hinton Agar (MHA) plates were sterilized and uniformly swabbed with overnight bacterial strain cultures. Sterilized sticks were used to make wells and 200 μL of the sample extracts at various concentrations (25 mg/mL, 50 mg/mL, 75 mg/mL and 100 mg/mL) were added.

The plates were thereafter placed in an incubator set at a temperature of 37 °C for 24 h. The antibacterial activity was assessed by measuring the diameter of the halo zones of inhibition. Each test was replicated 3 times. Standard antibiotic discs including Amikacin, Ciprofloxacin, Vancomycin and Ampicillin were used as positive controls (25).

Results and Discussion

Phytochemical Screening

The phytochemical qualitative analyses of plant extracts were established to indicate the existence of phenols, flavonoids, terpenoids, saponins, alkaloids, steroids, glycosides and tannins. The detailed methods are described

were found in *A. des Champs* with 33 %, 17 % and 8 % respectively of the total element content. Iron is the most abundant element in *A. capillus-veneris* with 64 %. Some elements have been neglected since their concentration was below the accepted limit as shown in Fig. 1.

The presence of iron, potassium, manganese, calcium, zinc and magnesium in the same plant with detectable levels makes the plant more valuable as in *M. longifolia*, *A. santolina* and *A. des champs* (Table 3). Iron has an important role in the production of hemoglobin and red blood cells (26). Potassium plays an important role in the digestive system and in cardiac and muscle function as well as regulating the functions of body cells and tissues (27). Manganese is involved directly with bone health, metabolism, macronutrients and other biological activities (28).

Bone strength is more related to the accepted level of calcium in the body. Magnesium is necessary for the growth of muscles and energy release. Zinc is required for protein synthesis and many physiological and metabolic functions (29). There are acceptable amounts of Nickel, copper and phosphor, where Cu stimulates the immune system and Nickel for hormone function and lipid metabolism (30, 31)

Antioxidant Activity

The DPPH technique assessed the antioxidant activity of 10 samples by testing them at different amounts, ranging from 0.5 to 15 g/mL. The ascorbic acid was used as a standard radical scavenger. The test showed comparable antioxidant activity of most samples to that of the standard ascorbic acid at different dosages tested (0.5, 3, 5, 10, 15 $\mu\text{g/mL}$). At a concentration of 15 $\mu\text{g/mL}$, the standard ascorbic acid was able to maximally inhibit 92.13 % of the

Table 2. Phytochemical qualitative analysis for methanolic extracts of some medicinal plants.

Plant extract	Phytochemical components							
	Phenolics	Flavonoids	Saponins	Tannins	Glycosides	Alkaloids	Terpenoids	Steroids
<i>Adiantum capillus-veneris</i>	-	+	+	+	+	-	-	+
<i>Polygonum avicular</i>	-	-	+	-	+	+	+	-
<i>Urtica dioica</i>	+	+	-	+	-	+	+	+
<i>Tribulus terrestris</i>	+	+	+	+	-	+	+	+
<i>Artemisia splendens Willd</i>	+	+	+	+	-	-	+	+
<i>Scirpus lacustri</i>	+	-	-	+	+	+	-	-
<i>Thymus kotschyanus</i>	+	+	-	+	-	+	+	+
<i>Mentha longifolia</i>	+	+	+	+	-	+	+	-
<i>Achillea santolina</i>	+	+	+	+	+	+	+	-
<i>Anthenis des champs</i>	+	+	-	+	+	+	+	+

above and the results are given in Table 2. The results showed that terpenoids and phenols were found in most plants.

Elemental analysis by ICP-MS

The analysis results of the 29 metals showed that the medicinal plants contained a wide range of elements, including potassium, magnesium, calcium, iron and zinc. The most abundant amounts of potassium, magnesium and zinc

DPPH radicals. The IC_{50} value of ascorbic acid was $3.83 \pm 0.30 \mu\text{g/mL}$. IC_{50} value is observed to be significant for different samples as for *A. des champs* $5.89 \pm 0.63 \mu\text{g/mL}$, *Achillea santolina* 4.11 ± 0.17 and *M. longifolias* 5.29 ± 0.31 as shown in Table 4. This indicates that these plants are regarded as a good source for radical scavenging and utilized as antioxidants (Fig. 2). The IC_{50} s of *U. dioica* and *S. lacustri* showed no response as antioxidants (Fig. 3).

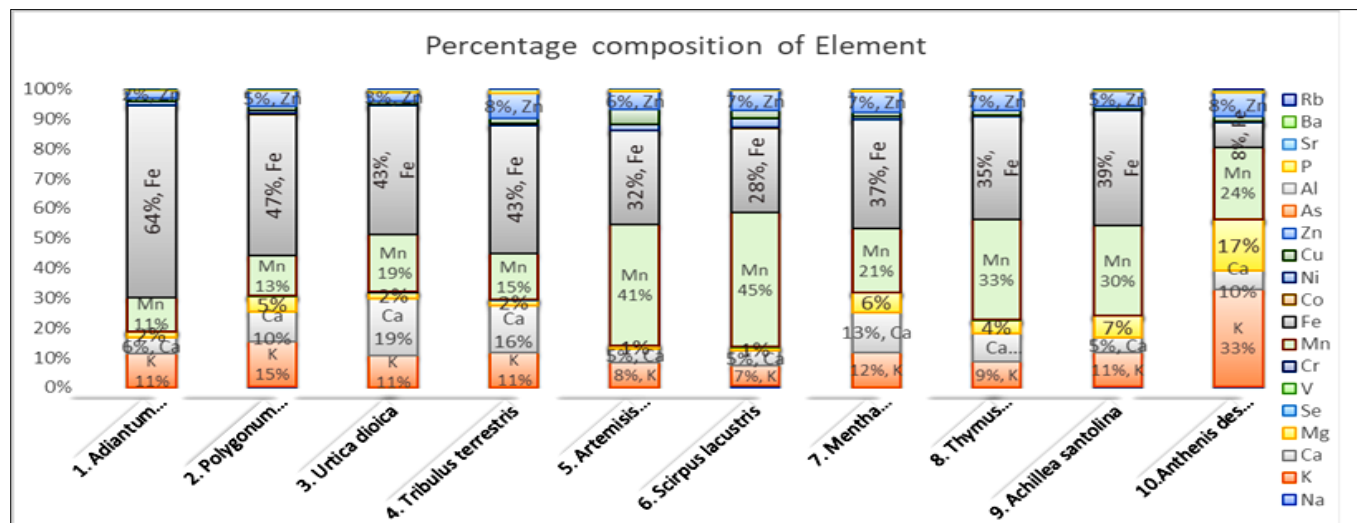


Fig. 1. The % composition of 29 elements in ten medicinal plants by ICP-MS analysis.

Table 3. Trace analysis of elements for three medicinal plants by ICP-MS.

Metals	<i>Mentha longifolia</i>	<i>Achillea santolina</i>	<i>Anthesis des champs</i>
	Mean Concentration \pm STD mg/kg	Mean Concentration \pm STD mg/kg	Mean Concentration \pm STD mg/kg
Na	41.6 \pm 0.6	174.4 \pm 32.4	61.0 \pm 4.3
K	5376.4 \pm 3.5	5841.0 \pm 5.2	12272.0 \pm 21.6
Ca	6146.6 \pm 1.0	2466.0 \pm 6.1	2424.0 \pm 6.0
Mg	2981.2 \pm 1.2	3836.0 \pm 22.5	6382.0 \pm 8.0
Se	18.6 \pm 0.1	19.4 \pm 2.0	23.0 \pm 0.4
V	35.5 \pm 0.1	23.9 \pm 1.5	13.0 \pm 0.2
Cr	56.9 \pm 0.1	17.1 \pm 1.0	33.0 \pm 0.2
Mn	9759.0 \pm 6.1	15362.6 \pm 5.1	9037.0 \pm 7.3
Fe	16883.7 \pm 2.5	19789.3 \pm 5.0	3171.0 \pm 1.3
Co	34.9 \pm 0.0	18.6 \pm 1.0	9.0 \pm 2.8
Ni	453.7 \pm 0.3	156.9 \pm 2.5	106.0 \pm 0.1
Cu	612.8 \pm 1.1	481.5 \pm 5.1	632.0 \pm 1.4
Zn	3233.9 \pm 3.1	2712.3 \pm 4.8	2967.0 \pm 0.3
As	1.3 \pm 0.0	5.9 \pm 1.6	3.0 \pm 1.0
Al	19.8 \pm 0.1	80.0 \pm 1.2	58.0 \pm 2.8
P	326.2 \pm 0.9	275.0 \pm 2.6	377.0 \pm 2.5
Sr	4.2 \pm 0.1	11.1 \pm 1.2	23.0 \pm 1.0
Ba	3.2 \pm 0.0	8.4 \pm 1.0	5.0 \pm 1.0

Table 4. Total phenolic content, IC₅₀ value and % inhibition of standard ascorbic acid and plant extract by DPPH antioxidant activity.

Methanolic extract from plants	IC ₅₀ (μ g/mL)	% Inhibition RSA	TPC (mg GAE/g dry extract wt)
Ascorbic Acid (Standard)	3.83 \pm 0.30	92.13	-
<i>Adiantum capillus-veneris</i>	10.06 \pm 0.73	65.28	49.45 \pm 1.03
<i>Polygonum avicular</i>	7.07 \pm 0.62	85.19	58.95 \pm 0.74
<i>Urtica dioica</i>	-5.50 \pm 0.47	-0.93	5.7 \pm 0.32
<i>Tribulus terrestris</i>	4.49 \pm 0.14	82.87	61.45 \pm 1.25
<i>Artemisia splendens</i> Willd	7.05 \pm 0.40	83.33	58.45 \pm 0.83
<i>Scirpus lacustri</i>	-4.95 \pm 0.38	-41.67	4.2 \pm 0.46
<i>Thymus kotschyanus</i>	7.81 \pm 0.80	75.93	46.7 \pm 0.92
<i>Mentha longifolia</i>	5.29 \pm 0.31	80.09	85.7 \pm 1.50
<i>Achillea santolina</i>	4.11 \pm 0.17	78.24	325.95 \pm 2.26
<i>Anthesis des champs</i>	5.89 \pm 0.63	89.81	60.45 \pm 1.34

Antibacterial activity

The findings from the antibacterial activity assessment of the plant extracts via the well diffusion test indicate the diameter of inhibition zones in millimeters for the various extracts tested against different bacterial strains, namely *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive) bacteria. The antibacterial activity of the methanol extracts wasn't uniform. Some extracts worked better than others. The size of the clear zones (inhibition diameters) caused by the methanol extracts depended on the specific plant used to make the extract. Among the methanol extracts, 4 plants stood out for having the strongest antibacterial effects and they were *A. des champs*, *M. longifolia*, *A. santolina* and *P. avicular*. These 4 extracts produced inhibition zones of 20 mm \pm 0.3 mm, 19 mm \pm 0.9 mm, 18 mm \pm 0.7 mm and 18 mm \pm 0.7 mm re-

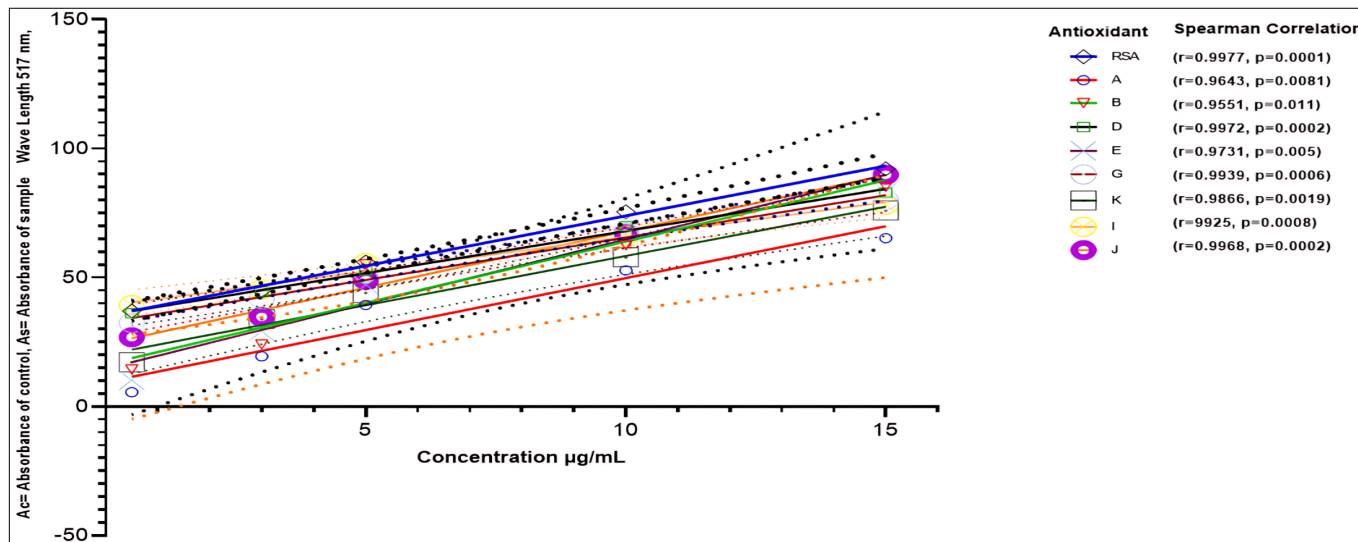


Fig. 2. Antioxidant activity of medicinal plants compared with ascorbic acid as a standard which shows significant response (p < 0.05).

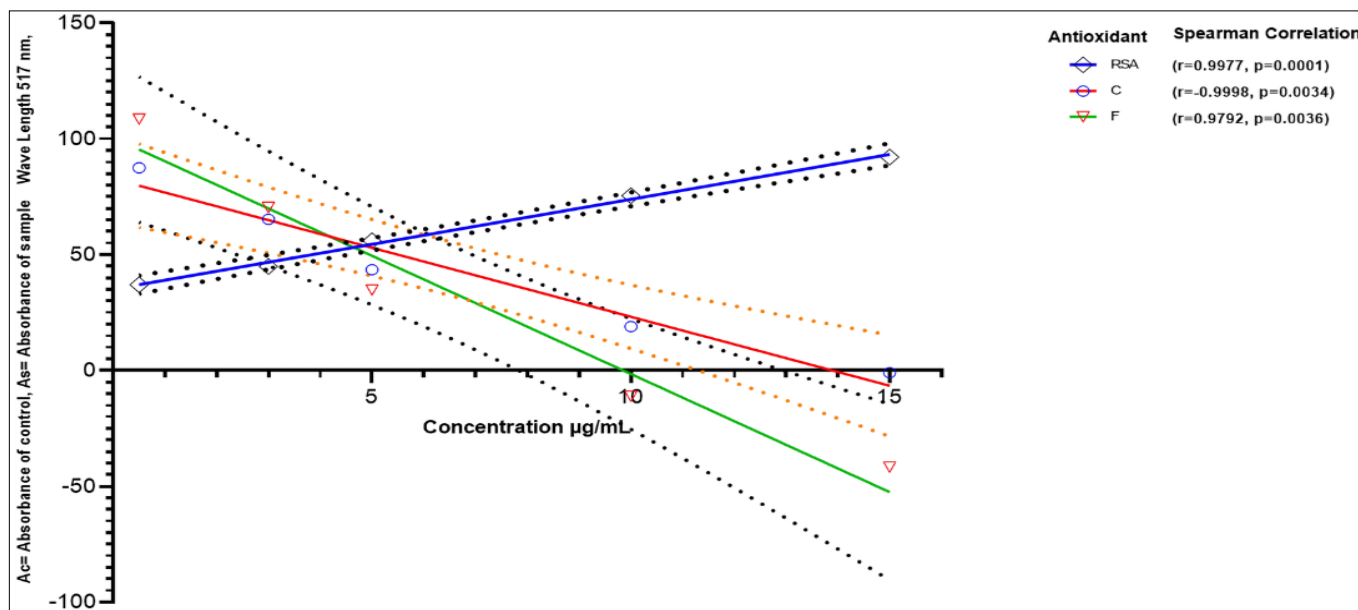


Fig. 3. Antioxidant activity of *Urtica dioica* and *Scirpus lacustris* compared with ascorbic acid as standard which shows no response (p < 0.05).

Table 5. Antibacterial activity of methanolic extracts and standard antibiotics.

Methanolic Extract	Zone of inhibition measured in mm (Mean ± SD)	
	<i>E. coli</i>	<i>S. aureus</i>
<i>Adiantum capillus-veneris</i>	16 ± 0.7	13 ± 0.4
<i>Polygonum avicular</i>	18 ± 0.7	13 ± 0.7
<i>Urtica dioica</i>	NR	NR
<i>Tribulus terrestris</i>	14 ± 0.3	14 ± 0.9
<i>Artemisis splendens Willd</i>	13 ± 1	12 ± 0.7
<i>Scirpus lacustris</i>	NR	NR
<i>Thymus kotschyanus</i>	13 ± 0.7	14 ± 0.4
<i>Mentha longifolia</i>	19 ± 0.9	15 ± 0.7
<i>Achillea santolina</i>	18 ± 0.7	17 ± 0.7
<i>Anthesis des champs</i>	20 ± 0.3	20 ± 0.7
Ciprofloxacin (Cip)	46.5 ± 0.7	44.75 ± 0.3
Amikacin (AK)	12.25 ± 0.3	16.5 ± 0.7
Vancomycin (Va)	20.75 ± 1	25 ± 1.4
Ampicillin (AM)	28.5 ± 0.7	25.5 ± 0.7

spectively. The standard antibiotics used were Ciprofloxacin, Amikacin, Vancomycin and Ampicillin (Table 5).

Total Phenolic components

Phenolic compounds in medicinal plants contribute directly to the antioxidant activity and are regarded as powerful chain-breaking antioxidants for the redox properties of their components (22). The total phenolic content in the extract was assessed using the Folin-Ciocalteu reagent. It was found that *A. des champs*, *M. longifolia* and *A. santolina* had the highest phenolic contents, measuring (325.95 ± 2.26, 85.7 ± 1.50 and 60.45 ± 1.34 mg GAE/g respectively). These values were determined based on a calibration curve (y = 0.004x + 0.0182) with an R² value of 0.9967, using gallic acid concentrations ranging from 15 to 500 µg/mL. The results were expressed in terms of gallic acid equivalents (GAE) per g of dry extract weight (Table 4).

Conclusion

The present research provides important insight into the composition and bioactivities of ten selected medicinal

plants from the Kurdistan region of Iraq. A wide variety of bioactive compounds and essential minerals were identified in the selected plant samples using qualitative phytochemical screening and elemental analysis. Methanolic extracts showed that they possessed considerable antioxidant and antibacterial activities; via free radicals scavenging and inhibited the growth of tested bacteria. At the species level, *Mentha longifolia*, *Achillea santolina* and *Anthenis des champs* were identified as promising candidates having remarkable bioactivities that correlated with high phenolic content.

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Authors' contributions

ANM carried out the practical session of plant collection, the extraction steps until the identification of the chemicals in the plants, and the first draft of the manuscript. LAJ participated in analyzing the analytical techniques of the experiments and reviewing the first draft of the manuscript and some additions. FSHH Phytochemical screening of the plants and their bioactivity, as well as the final draft of the manuscript. All authors read and approved the final manuscript

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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