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Urtica dioica: phytochemical and antimicrobial

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Abstract

Urtica dioica, a well-known wild plant with remarkable healing and medicinal properties, has been extensively utilized in both traditional and modern medicine. In this study, the primary objective was to extract diverse compounds from Urtica dioica using the Soxhlet extraction method, resulting in three distinct extracts: hexane, ethyl acetate, and ethanol. Gas chromatography-mass spectrometry (GC-MS) was employed to identify the oily compounds present in the hexane extract, while column chromatography (CC), thin layer chromatography (TLC), and high-performance liquid chromatography (HPLC) were used to investigate the phenolic compounds in the ethyl acetate and ethanol extracts. These extracts yielded phenolic compounds. To evaluate the antimicrobial activity, a disc diffusion assay was conducted on each extract against Gram-positive bacterium *Staphylococcus aureus* and four Gram-negative microorganisms, including *Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Escherichia coli*. The results revealed that the extracts effectively inhibited the growth of *K. pneumoniae* and *P. aeruginosa*. In the second part of the study, it was observed that the growth of *K. pneumoniae* and *P. aeruginosa* was inhibited, while fatty acids showed effectiveness against *S. typhi* and *S. aureus*. These findings suggest that the different phenolic compounds present in the extracts exerted varying effects on the growth of each bacterium. Overall, this study highlights the potential of *Urtica dioica* as a valuable source of phenolic compounds with natural antimicrobial properties, making it a suitable candidate for utilization in pharmaceutical preparations. The investigation demonstrates the significance of exploring natural resources for novel therapeutic agents.

Keywords: *Urtica diocia*, separation, fatty acids, phenolics, antimicrobial

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1. Introduction

Wild plants play a crucial role in maintaining biodiversity in nature and serve as a valuable resource for numerous industries, particularly the pharmaceutical sector. Unlike chemically synthesized medicines, which are relatively easy to produce, wild plants offer distinct advantages. With the advancements in scientific disciplines in recent years, there has been a notable surge in the exploration and utilization of wild plants [1,2]. *Urtica dioica*, a natural plant widely found in the Iraqi environment, represents a diverse and abundant source of medicinal compounds. Its distribution varies across different locations, thriving in cold and temperate regions as thickets while also spreading in gardens and orchards [3,4]. Typically, this plant blossoms and bears fruit in March, characterized by its herbaceous annual nature and classification within the *Urticaceae* family. Covered in fine, hair-like thorns, it features broad leaves with a heart shape and serrated edges. In English, it is commonly referred to as stinging nettle. The term "urtica" is derived from the Greek word "uro," meaning "to burn," which reflects the plant's stinging bristles. Additionally, its English name, Nettle, is connected to the word "needle" and its equivalent, "Noed," which further highlights its prickly nature[5,6,]. Throughout history, fresh *Urtica dioica*, commonly known as stinging nettle, has been employed to stimulate blood flow and provide warmth to arthritic or paralyzed limbs. This practise has been passed down since ancient times, and it involves the deliberate striking of afflicted areas with plant materials [7,8]. Furthermore, this plant is traditionally used in various internal applications, serving as a means to purify the blood, induce menstruation, promote diuresis, alleviate nasal and menstrual bleeding, relieve rheumatism, combat eczema, address anaemia, nephritis, haematuria, jaundice, menorrhagia, and manage diarrhea [9,10]. *Urtica dioica* synthesizes a diverse array of organic compounds with notable medicinal properties. Among these compounds are phytosterols, saponins, flavonoids, tannins, sterols, fatty acids, carotenoids, chlorophylls, proteins, amino acids, and vitamins [11,12]. Various studies have identified specific compounds present in this plant, including beta-sitosterol, trans-ferulic acid, dotriacontane, erucic acid, ursolic acid, scopoletin, rutin, quercetin, and p-hydroxybenzalcohol. These compounds contribute to the plant's therapeutic potential and underline its significance in traditional medicine and natural remedies [13,14]. *Urtica dioica* exhibits a wide range of pharmacological activities, as reported in scientific studies. These activities include antioxidant properties, which help protect against oxidative stress, anti-inflammatory effects to alleviate inflammation, antiulcer and anti-colitis actions to

address gastrointestinal disorders, antiviral properties for combating viral infections, anticancer potential against certain types of cancer [15,16,17].

Antibacterial and antimicrobial actions to inhibit the growth of bacteria and microorganisms, antifungal effects against fungal infections[18]. antiandrogenic properties for hormonal regulation, insecticidal activity against insects, immunomodulatory effects to modulate the immune system, hypocholesterolemic and hypoglycemic actions for managing cholesterol and blood sugar levels respectively, cardiovascular effects to support heart health, analgesic properties for pain relief, natriuretic actions to promote sodium excretion, hypotensive effects to lower blood pressure, hepatoprotective properties to protect the liver, and potential benefits in managing rheumatoid arthritis. These diverse pharmacological activities highlight the extensive therapeutic potential of Urtica dioica[19,20,21]. The present study aims to characterize the chemical compounds found in *U. dioica* by utilizing chromatographic methods. This research contributes to the understanding of the chemical composition of *U. dioica* and explores its potential as a natural source for antibacterial agents.

2. Materials and Methods

2.1. Plant collection and classification

The plant parts were carefully collected using gloves to maintain their sterility, and they were subsequently placed in a sterile plastic bag and transported to the laboratory. Once the plant's identity was confirmed, it underwent a thorough washing using sterile distilled water to eliminate any surface dust. Subsequently, the plant parts were kept in darkness at room temperature (approximately 25 °C) until they completely dried, as exposure to light can lead to the degradation of phenolics [22]. Following the drying process at room temperature, the plant parts were transferred to a hot air oven (operating at 36°C) and subjected to drying for approximately 48 hours. Eventually, a laboratory mortar and pestle, previously cooled, were employed to crush the dried plant parts rigorously, yielding a fine powder or paste. After fragmentation, the powder was filtered through a sterile cloth and subsequently stored at 20°C until further research could be conducted.

2.2. Extraction of Urtica by Soxhlet Apparatus

The sequential solvent system (hexane, ethyl acetate, and ethanol) was employed in the extraction procedure using the Soxhlet continuous extraction apparatus. The sample, in the form of plant powder, was mixed with 1000 ml of the respective solvent per 100 g of the sample, or at a rate of 100 ml per 10 g. The extraction process lasted for 48–72 hours, or until the solvent's color, derived from the Soxhlet, disappeared, aiming to obtain an extract composed of the plant material and the solvent used. Subsequently, the extracts were concentrated using a rotary vacuum evaporator (RVE) to obtain the crude extract. The resulting crude extract was stored in the refrigerator in 25-ml aliquots and packaged in sealed, opaque glass bottles until ready for use [23].

2.3. *Isolation and Fractions of Urtica Extract by Column*

The separation method described here requires the utilization of a fixed phase (SP), which is exemplified by silica gel, and a moving phase (represented by the solvent). Both the stationary and mobile phases possess distinct attributes, including polarity and adsorption capacity. Alongside the separation column [24], which is a glass column filled with thermally activated silica gel and employed in the separation procedure as depicted in picture (1), multiple solvents with varying polarities were employed in different proportions to achieve multiple components, commonly referred to as fractions, as illustrated in Figure(1-2) (2).

2.4. Thin-layer chromatography (TLC)

In order to select a suitable solvent system for separating the various components present in the crude extract, a thin layer chromatography (TLC) was conducted following a previously outlined method [25]. To detect the presence of phytochemicals, an aluminium-backed TLC apparatus was utilized, and 2 µL of each extract were loaded onto the plate. The TLC plate was developed using a solution comprising chloroform, ethyl acetate, and formic acid in a 10:8:2 v/v ratio. Subsequently, the developed plate was examined under ultraviolet light at 254 nm and 365 nm using a UV-visible spectrophotometer to identify fluorescent compounds. To visualize the different compounds, the TLC plate was then sprayed with vanillin sulphuric acid and subjected to heat. Each detected spot's Rf value was determined and compared with standards to assess their positions. Fractions with identical Rf values were separated, and each fraction was concentrated using a rotary evaporator. After drying, weight measurements were recorded, and the condensed fractions were further analyzed using high-performance liquid chromatography (HPLC) to confirm the presence of phenolic compounds and determine their identities.

2.5. *Analysis of fatty acids by Gas– Chromatography Mass Spectrometry (GC-MS)*

To analyze the contents of the Hexane extract obtained from *U. dioica*, we employed a Shimadzu GC-2010 gas chromatograph coupled with a QP2010 mass spectrometer. The sample was introduced into the GC-MS system using a 30 m glass capillary column coated with a 0.25 m thick film (30 m 0.25 m). Helium served as the carrier gas, flowing steadily at a rate of 1 ml/min. Both the injector and the detector were maintained at a temperature of 250 °C throughout the analysis. The GC was programmed to ramp from 60°C to 280°C at a rate of 15°C per minute. The split ratio was set at 1:3. The total run time for the GC analysis was 35 minutes. The mass spectrometer was operated at an electron energy of 70 eV. The MS scan parameters included a mass range of m/z 40–1000, a scan interval of 0.5 s, a scan speed of 1000 amu s1, and a detector voltage of 1.0 kV. To identify the compounds present, we utilized the NIST08, WILEY8, and FAME Libraries, comparing the mass spectrum of each unknown compound to those of known compounds within the software database. This allowed us to determine the names, molecular weights, and structures of the analyzed materials [26].

2.6 Analysis of Phenolic compounds by HPLC

HPLC was utilized to validate the identity of the extracted phenolic components. Due to its exceptional sensitivity and speed, HPLC stands as one of the most effective analytical systems for examining plant substances,

including phenols. The analytical HPLC system used in this study consisted of a detector (SPD-10A UV-VIS), a VP pump (LC-10AT), an auto injector (SIL-10AF), and a system controller (SCL-10A VP), all from Agilent Technologies in Santa Clara, CA, USA. The Chiralcel ® OD-RH column (150 mm length, approximately 4.6 mm diameter, 5 mm particle size, Chiral Technologies Inc., Exton, PA, USA) served as the analytical column. The mobile phase employed was a mixture of acetonitrile, water, and phosphoric acid (30:70:0.08, v/v/v) under isocratic conditions at ambient temperature (25 ± 1 ◦C), with a flow rate of 0.4 mL/min. Each run lasted 8 minutes, followed by a 15-minute clean-up period. The separated compounds were detected by the built-in SPD-10A UV-Vis detector at a wavelength of 288 nm. The method described by Skendi et al. [27]. was employed for the identification of phenolic compounds. The confirmation of individual compounds was achieved by comparing the retention time of respective standards (listed in Table 1) with those of the peaks present in the extract.

2.7. Origin and Selection of Microorganism Strains

The efficacy of extracts derived from *U. dioica* was assessed against a range of highly dangerous bacteria. To determine their in-vitro antibacterial properties, five pathogenic bacteria affecting humans were selected for examination, utilizing strains that were readily available from our laboratory's stock culture. Specifically, the investigation focused on the prevalence of two significant food-borne pathogens: Gram-positive strains such as Staphylococcus aureus (ATCC 6538) and Gram-negative strains like Escherichia coli (ATCC 8739), Pseudomonas aeruginosa (ATCC 27853), Klebsiella pneumoniae, and Salmonella typhi. All microorganisms were meticulously stored in bead vials under controlled conditions at a temperature of (-18°C).

2.8. Preparation of the Inoculums

The bacterial strains were cultivated on nutrient agar (NA) based on Mueller-Hinton broth and incubated for 24 hours at 37 degrees Celsius. Following incubation, the strains were sub-cultured before undergoing any antimicrobial testing. To create the inoculums, the bacteria were suspended in a sterile saline solution containing 0.85 percent NaCl. The suspensions' optical density (OD) was carefully adjusted or maintained at a range of 0.4 to 0.6 at a wavelength of 405 nm, which corresponds to a cell density of approximately 0.5 McFarland and ensures an inoculum expected to contain 106 to 108 colony-forming units per milliliter (CFU/ml) [28].

2.9. Method of Disk Diffusion on Agar

The antibiotic susceptibility test was conducted using the standard disc diffusion method with some modifications as described by Zazharskyi et al. [29]. Mueller-Hinton agar (MHA) plates were prepared and marked with the previously prepared inoculums using a sterile swab. Sterilized paper discs (6 mm, Whatman paper N5) were impregnated with 5 µL of each ethyl acetate (I) and ethanol (II) fractions using a solvent composed of 10% v/v dimethyl sulfoxide and 1% v/v tween 80 in deionized water. As controls, positive and negative discs were prepared by diluting the antibiotic solutions Amikacin and Gentamycin (5 g/mL) and the same solvent, respectively, under identical conditions as the extract fractions. The plates were kept at room temperature (RT) and subsequently incubated at 37°C for 24 hours. The antibacterial activity was evaluated by measuring the diameter of the closest inhibition zone in millimeters, encompassing the area around and including the discs' diameter. Each experiment was performed in triplicate, providing three replicates for analysis.

3. Results and Discussion

3.1. Composition of fatty acids in U. dioica

The results obtained from the GC-MS analysis revealed the presence of five saturated fatty acids in the leaves of *Urtica dioica* L. The specific fatty acid composition of the nettle leaves can be found in Table (2). Among the identified fatty acids, Palmitic acid (16:0) stood out as the major component, accounting for 1.96% of the total composition in the leaf parts of the plant. Behenic acid (22:0) was identified as the second major fatty acid component, albeit with a relatively lower concentration of 0.71%. Additionally, significant concentrations of Stearic acid (18:0) and Pentacosylic acid (15:0) were detected, measuring 0.38% and 0.11% respectively. Finally, Myristic acid (14:0) constituted a minor portion, representing 0.08% of the composition, as indicated by figures (3–7). Fatty acids play a crucial role in maintaining overall health by serving as a vital energy source and acting as carriers for fat-soluble vitamins such as vitamin A, D, and E. While the human body requires fatty acids, they can only be obtained through dietary intake [30,31].

In the case of *U. dioica*, commonly known as stinging nettle, its leaves contain a diverse range of compounds, including essential minerals, amino acids, phytosterols, lignans, carotenoids, phenolics, and abundant fatty acids. Among these fatty acids, palmitic acid and stearic acid are two saturated fatty acids found throughout the entire plant. Notably, palmitic acid is particularly abundant in all parts of the plant. Additionally, *U. dioica* exhibits smaller quantities of monounsaturated fatty acids. The main monounsaturated fatty acids present in *U. dioica* are palmitoleic acid, oleic acid, gadoleic acid, and erucic acid. Furthermore, the polyunsaturated fatty acids linoleic acid and a-linolenic acid are also found in U. dioica. These findings have been previously reported by various researchers [32,33,34]. *Urtica dioica* has garnered significant attention due to its diverse pharmacological properties. It plays a crucial role in immune system modulation, cholesterol metabolism, membrane structure regulation, and brain function. Furthermore, fatty acids have demonstrated their efficacy in managing low-density lipoprotein levels and combating various diseases, including skin diseases, arthritis, asthma, lupus erythematosus, cardiovascular diseases, cancer, inflammatory and autoimmune diseases, coronary heart disease, and high blood pressure [35,36]. To thoroughly investigate nettle leaves' chemical composition, a comprehensive chemical analysis was conducted. This involved employing a range of analytical methods and sample preparations. The samples were subjected to extraction using petroleum ether, followed by gas chromatography (GC), flame ionization detection (FID), and mass spectrometry (MS) analyses. The fatty acid profile was determined using GC-FID after extraction with formaldehyde and methanol. Unsaturated fatty acids were found to be more prevalent than saturated fatty acids. Among the unsaturated fatty acids, polyunsaturated fatty acids such as linolenic acid, palmitic acid, and cis-9,12-linoleic acetate were the most abundant [37,38,39].

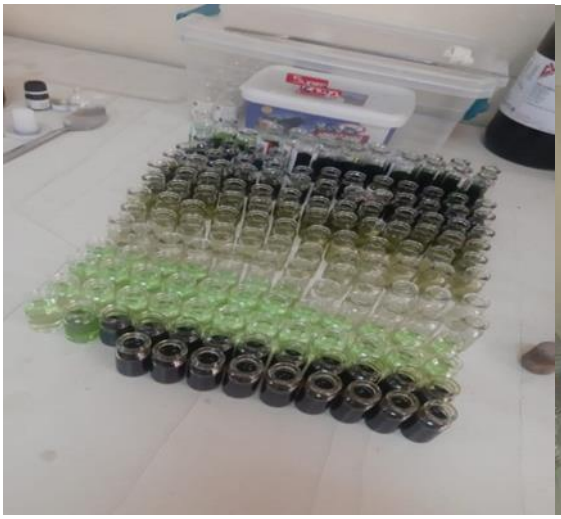


Figure 1. Fractions

Figure 2. Column Chromatography

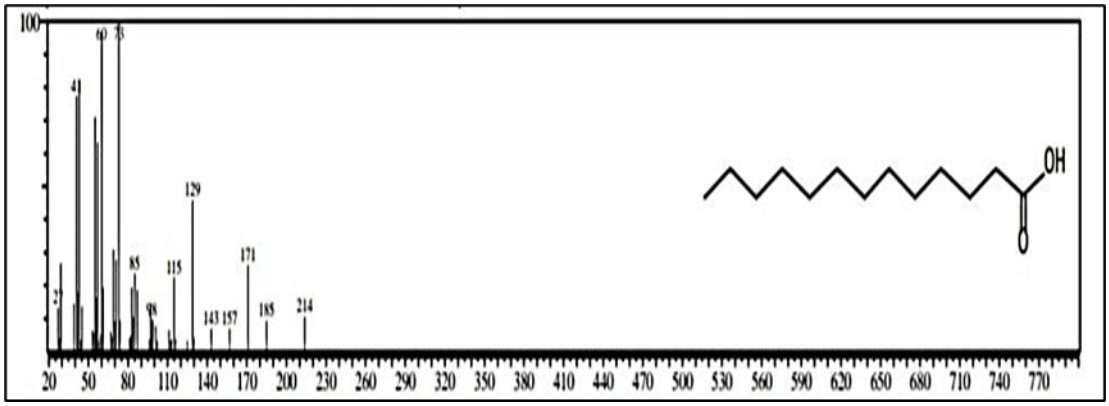


Figure 3. The curve and the structural formula of the compound Myristic acid

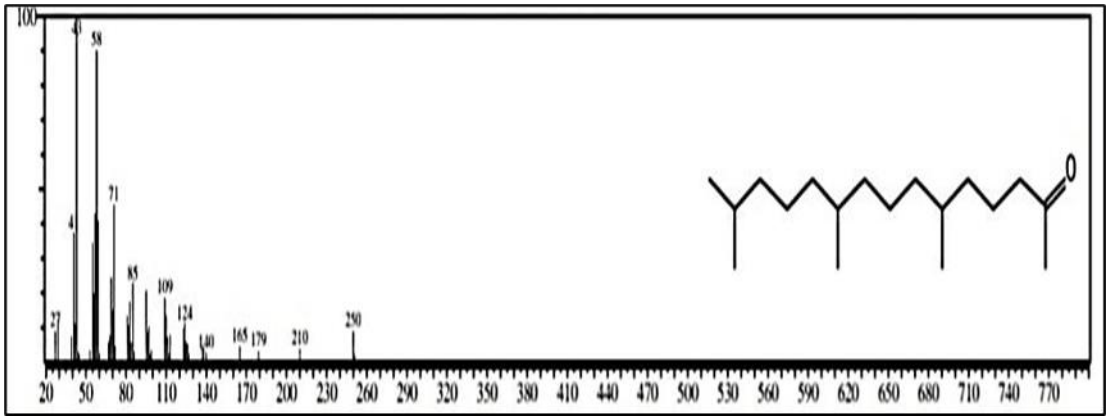


Figure 4. The curve and the structural formula of the compound Pentadecanoic acid

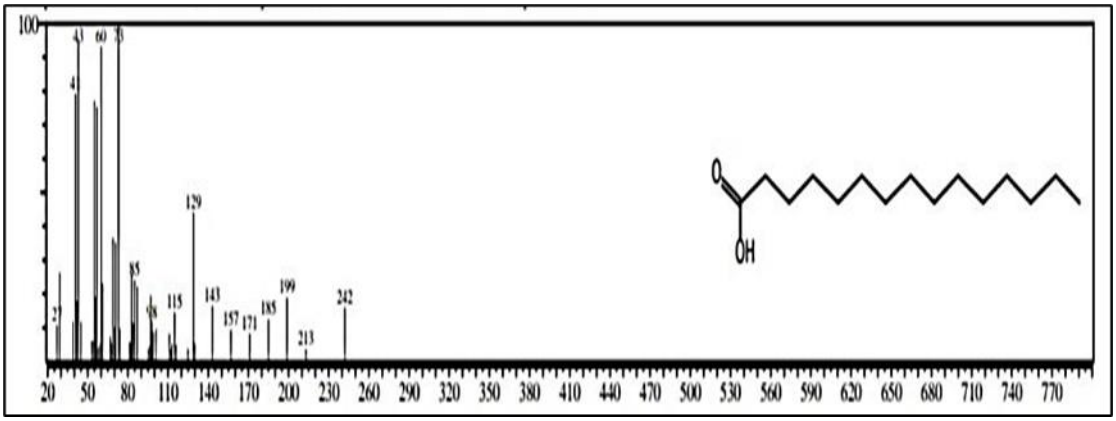


Figure 5. The curve and the structural formula of the compound Palmitic acid

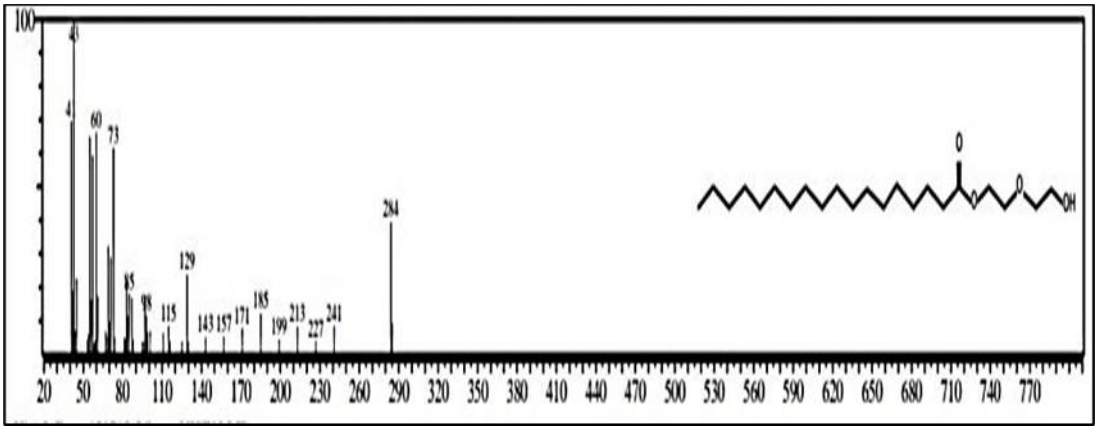


Figure 6. The curve and the structural formula of the compound Stearic acid

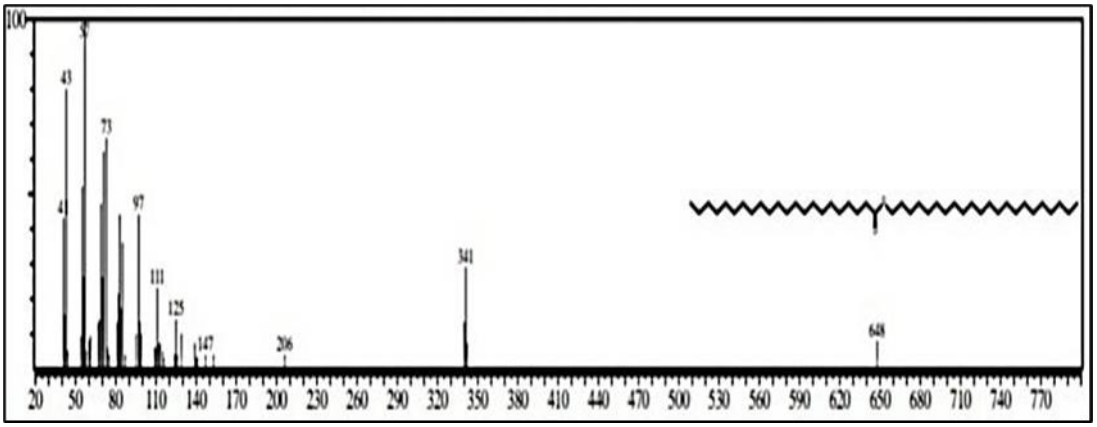
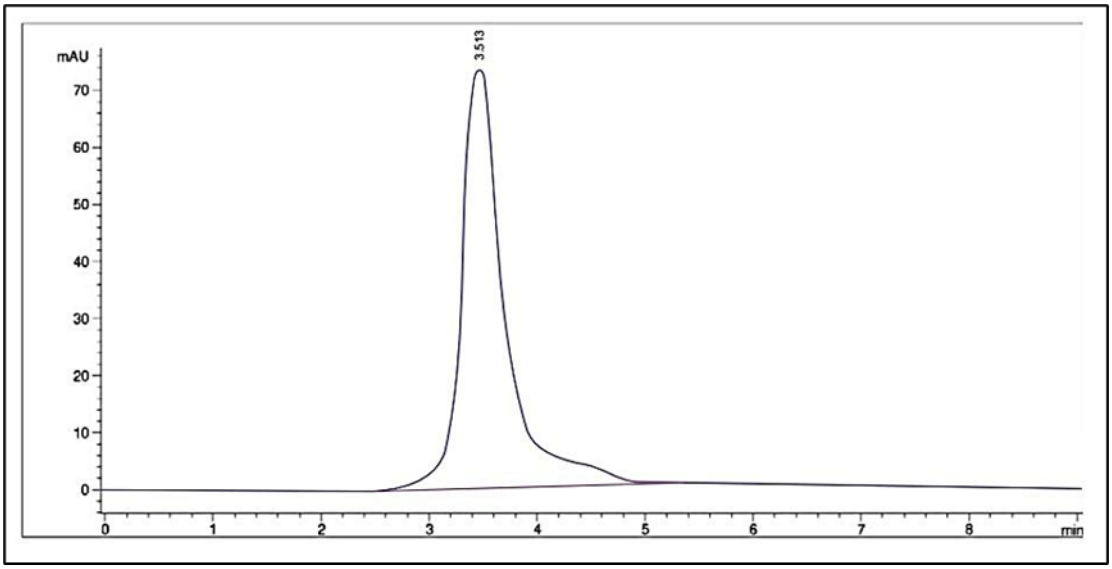
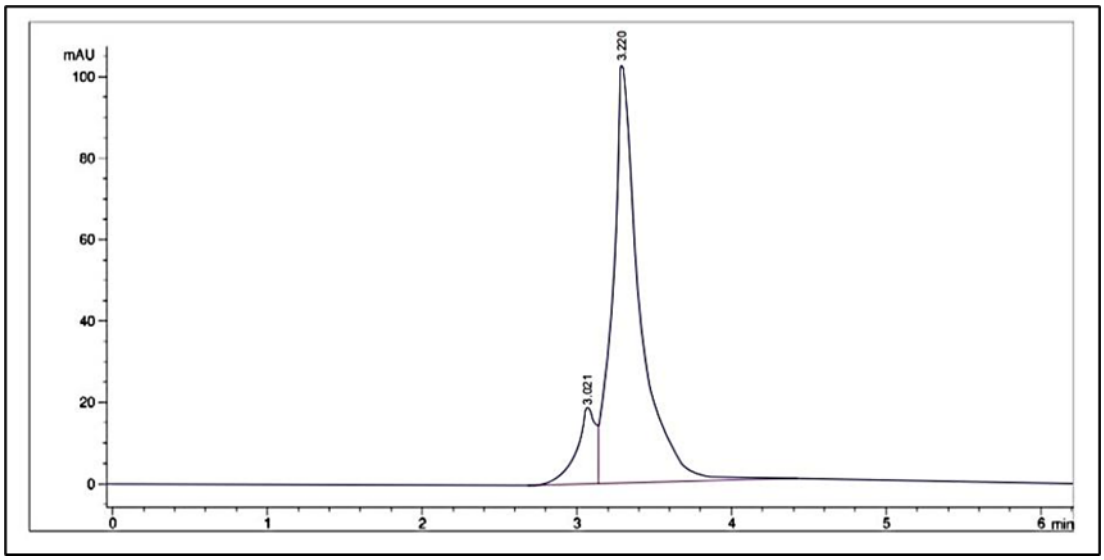


Figure 7. The curve and the structural formula of the compound Behenic acid



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.513	VB	0.3917	797.81285	76.35118	100.0000
Totals :				797.81285	76.35118	



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.021	VB	0.1258	872.37685	18.70055	40.5380
2	3.220	VV	0.2079	1279.62153	103.10608	59.4620
Totals :				2151.99838	121.80663	

Figure 8. HPLC chromatogram of Fraction I-II

Table 1. Standards of phenolic compounds and their retention time

Standards	Retention Time (min)	Concentration (ppm)	Area [1]
p-Coumaric acid	3.523	25	1161,583,252 (0.01)
vanillic acid	3.031	25	445,452,319 (0.01)
Quercetin	3.261	25	12,367,103 (0.01)

[1] Area represented as mean (n = 5) with coefficient of variation in brackets

Table 2. Fatty acids separated from nettle leaves using GC-MS

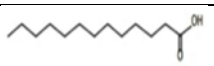
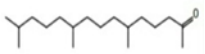
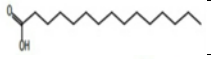
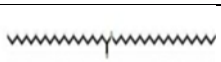
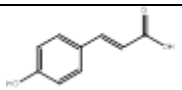
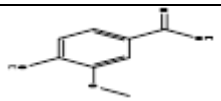
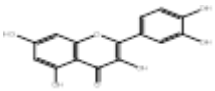
No.	Common name of fatty acid	Chemical nomen-clature	R.t (min)	The area under curve%	The area under curve	structure formula
1	Myristic acid (tetradecanoic)	C 14:0	8.926	0.08	3127428	
2	Pentacosylic (pentacosanoic)	C 15:0	9.580	0.11	4221321	
3	Palmitic acid (hexadecanoic)	C 16:0	11.423	1.96	76351495	
4	Stearic acid (octadecanoic)	C 18:0	13.632	0.38	14782841	
5	Behenic acid (docosanoic)	C 22:0	25.980	0.71	27763072	

Table 3. Phenolic compounds in tow fractions and their retention time.

Fractions	No. of Peak	R .t (min)	Conc. (ppm) [c]	Identifed Compounds	structure formula
I[a]	1	3.513	17.0 ± 0.6	p-Coumaric acid	
II [b]	1	3.021	2.5 ± 0.3	vanillic acid	
	2	3.220	2.4 ± 0.2	Quercetin	

[a] Fractions identified from ethyl acetate extraction; [b] Fractions identified from ethanol extraction, [c] Values represent mean and standard deviation (n = 3)

Table 4. Antimicrobial activity of fraction I– II and fatty acids.

	Concentration	Zone of Inhibition (mm)				
	µg/mL	***K.pneumoniae***	***P.aeruginosa***	***S.typhi***	***S.aureus***	***E.coli***
Fraction I	1.25	4	7	0	0	0
	2.5	7	9	0	0	0
	5	9	11	9	8	8
	10	14	17	11	16	13
	20	19	21	16	19	16
Fraction II	1.25	9	8	0	0	11
	2.5	15	112	0	0	16
	5	17	16	8	6	18
	10	18	19	17	18	19
	20	23	22	19	20	21
Fatty acids	1.25	0	0	5	4	0
	2.5	0	6	8	7	0
	5	0	8	11	9	6
	10	6	10	13	10	8
	20	9	15	19	14	11
Control	Amikacin	22	23	22	21	20
	Gentamycin	20	21	22	24	22

3.2. Composition of Phenolic Compounds in U. dioica Fractions

Two fractions were obtained from the extraction process, each revealing distinct bioactive components of Urtica dioica. Phenolic compounds are known to be abundantly present in *U. dioica*, making them a prominent target for analysis. The first fraction (Fraction I) was obtained through ethyl acetate extraction, while the second fraction (Fraction II) was obtained through ethanol extraction. The composition and content of phenolic compounds in these fractions were analyzed using HPLC at a wavelength of 280 nm, and the results are presented in Table 1. Identification of the phenolic compounds was accomplished by comparing their retention times and peak areas with appropriate standards. In Fraction I, a single major peak was observed for p-coumaric acid, corresponding to a specific standard. In Fraction II, two major peaks were detected and further identified as vanillic acid and quercetin, as illustrated in Figure 8.

Plants are known to possess significant quantities of polyphenols and flavonoids, which exhibit remarkable antioxidant activity and contribute to their defensive and disease-fighting properties. Phenolic compounds, classified as secondary metabolites in plants, are particularly noteworthy due to the presence of hydroxyl groups on their aromatic rings. Importantly, these phenolic compounds do not pose any harm to human health, leading to an upsurge in the utilization of plants with high phenolic content in the food industry, with the aim of enhancing food quality. Notably, the leaves of stinging nettle are rich in natural phenolic compounds, including flavonoids, phenolic acids, anthocyanins, and other phenols, as supported by previous studies [40,41]. Rutin has been identified as the predominant phenolic compound in stinging nettle leaves. Furthermore, investigations into the phytochemical composition of nettle leaves have consistently revealed their richness in phenolic compounds [42].

In this study, we focused on the characterization and pharmacological activities of p-coumaric acid (p-CA) and vanillic acid (VA), two important compounds found in various natural sources. p-Coumaric acid is synthesized by introducing a single hydroxyl group to the phenyl group of cinnamic acid, with the p-CA isomer being the most prevalent form. It is abundantly present in a wide range of fruits, vegetables, and cereals. Alongside cinnamic acid, p-coumaric acid also exists as o-coumaric and m-coumaric isomers, but the p-CA isomer is the most prevalent in nature [43,44]. Notably, research literature has highlighted the diverse pharmacological activities exhibited by vanillic acid and its metabolite vanillyl. Specifically, vanillic acid has been found to inhibit 5'-nucleotidase activity in a specific and targeted manner. Moreover, vanillin, vanillic acid, and vanillyl alcohol have well-established anti-inflammatory properties and exert antioxidant effects by combating oxidative stress. Vanillic acid has been employed in the treatment of neurological disorders, as it effectively attenuates the production of β-amyloid, a key contributor to Alzheimer's disease, in an oxidative stress model [45,46].

In our study, we investigated the potential therapeutic properties of quercetin, a flavonoid abundantly present in several edible and medicinal plants. Quercetin has garnered significant attention due to its diverse pharmacological activities, including its role as an antioxidant, anti-inflammatory, anti-microbial, and anti-allergic agent. Moreover, this phytochemical has been associated with chemopreventive, anti-genotoxic, and anti-tumor effects. These findings are in line with previous research [47,48].

3.3. Antimicrobial Activities of U. dioica Extracts

The present study aimed to investigate the inhibitory effect of fatty acids and phenolic compounds extracted from nettles on pathogenic bacteria. The Dig-diffusion method was employed to compare the efficacy of these extracts with antibiotics. The results, summarized in Table 4, revealed that the inhibitory effect varied based on the active compounds present, the bacterial strain tested, and the concentration gradient. Among the three extracts, two were phenolic fractions (designated as Fraction I and II) while the other was fatty acids. Specifically, *K.pneumoniae* exhibited inhibition at concentrations of 1.25 µL/mL for Fraction I and II, and 10 µL/mL for fatty acids. *P.aeruginosa* displayed inhibition at concentrations of 1.25 µL/mL for Fraction I and II, and 2.5 µL/mL for fatty acids. *S.typhi* was inhibited at concentrations of 5 µL/mL for Fraction I and 1.25 µL/mL for fatty acids. Similarly, *S.aureus* exhibited inhibition at concentrations of 5 µL/mL for Fraction I and II, and 1.25 µL/mL for fatty acids.

Finally, *E.coli* was inhibited at concentrations of 1.25 µL/mL for Fraction I, 5 µL/mL for Fraction II, and fatty acids. It was observed that the extracts predominantly inhibited the growth of *K.pneumoniae* and *P.aeruginosa*, while displaying relatively weaker inhibition effects on S.typhi, *S.aureus*, and *E.coli*. This aligns with the presence of p-Coumaric acid alone as the major compound in Fraction I, which efficiently inhibits the growth of *K.pneumoniae* and *P.aeruginosa*. Fraction II showed inhibitory effects on *K.pneumoniae*, *P.aeruginosa*, and *E.coli*, with relatively weaker effects on *S.typhi* and *S.aureus*. Furthermore, the fatty acids were found to inhibit the growth of *S.typhi* and *S.aureus*, but exhibited lower inhibition effects on *K.pneumoniae*, *P.aeruginosa*, and *E.coli*. The broad antimicrobial effect of Fraction II, inhibiting almost all the tested strains except *S.typhi* and *S.aureus*, can be attributed to the presence of vanillic acid and quercetin.

Nowadays, the diverse shapes of secondary metabolites make plants an excellent source for discovering natural antimicrobial agents [49]. The growing popularity of traditional ethnopharmacology and the increasing interest of the healthcare system in utilizing natural bioactive compounds further emphasize the importance of exploring unexplored species to alleviate the strain on the limited natural resources of known medicinal plants [50,51]. Regarding *U. dioica*, the extract derived from this plant exhibits significant antimicrobial activity, as demonstrated by the observed zone of inhibition against *Escherichia coli* (8 mm), *Staphylococcus aureus* (8 mm), *Streptococcus pneumoniae* (9 mm), and other bacteria. Notably, previous studies have also documented the antibacterial properties of *U. dioica* leaves. Additionally, the tested extract exhibited a notable abundance of fatty acids, which have been recognized for their bactericidal effects [52,53].

4. Conclusions

The results obtained in this study revealed the composition and antimicrobial properties of different extracts derived from *U. dioica*. Three distinct phenolic compounds were successfully identified. Notably, this study introduced the use of the Soxhlet apparatus for extracting leaves of *U.*

dioica, resulting in significantly higher yields of phenolic compounds such as p-Coumaric acid, vanillic acid, and quercetin compared to previous reports. The fractions obtained from the *U. dioica* extracts exhibited varying degrees of antimicrobial activity against the bacteria tested in this study. Fraction I demonstrated efficient inhibition of growth in *K. pneumoniae* and *P. aeruginosa*, while Fraction II exhibited the strongest inhibitory effect against *K. pneumoniae*, *P. aeruginosa*, and *E. coli*. Fatty acids showed notable activity against *S. typhi* and *S. aureus* when tested. These differences in antimicrobial activity were attributed to the specific phenolic compounds present in each fraction. However, further in vivo studies and clinical trials are necessary to validate the safety and potential applications of these *U. dioica* extracts as antimicrobial agents. The findings of this study offer valuable insights for the development of an effective method to extract phenolic compounds from *U. dioica*, which can be utilized in a wide range of nutraceutical and pharmaceutical applications.

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Conflicts of Interest

The authors assert the absence of any conflicts of interest and confirm that ethical approval has been obtained.

Ethical approval

We have adhered to all ethical protocols, ensuring the due observance of ethical procedures, and our research has received validation from a provincial ethics commission.

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