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# Biological activities, GC-MS, and HPLC analyses of different parts of *Urtica dioica* L.

*Original Article*

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*Abstract:*
Current research aimed to reveal the antibacterial and antioxidant activities of extracts of leaves and seeds of *Urtica dioica.* Moreover, GC-MS and HPLC analyses of the extracts were performed. The highest activity was observed in the ethyl acetate extract of seeds against *Escherichia coli* (13 mm). The DPPH radical scavenging activity was decreased in the following order at 1000 µg/mL concentration: rutin > BHT > ethyl acetate extract of leaves > ethyl acetate extract of seeds > acetone extract of seeds > acetone extract of leaves. The highest CUPRAC activity was determined in the acetone extract of leaves, and the lowest CUPRAC activity was determined in the ethyl acetate extract of seeds. This research showed that *U. dioica* represents a good source of natural antioxidants and antibacterial agents.

*Key words:*
*Urtica dioica*, reactive oxygen species, bacteria, phytochemical content

*Apstrakt:*
**Biološke aktivnosti, GC-MS i HPLC analiza različitih biljnih delova vrste *Urtica dioica* L.**
Ovo istraživanje imalo je za cilj ispitivanje antibakterijske i antioksidativne aktivnosti ekstrakata listova i semena vrste *Urtica dioica*. Takođe, sprovedene su GC-MS i HPLC analize navedenih ekstrakata. Najizraženija antibakterijska aktivnost zabeležena je kod etil-acetatnog ekstrakta semena, i to u odnosu na vrstu *Escherichia coli* (13 mm). Aktivnost neutralisanja DPPH slobodnih radikala pri koncentraciji od 1000 µg/mL opadala je sledećim redosledom: rutin > BHT > etil-acetatni ekstrakt listova > etil-acetatni ekstrakt semena > acetonski ekstrakt semena > acetonski ekstrakt listova. Najizraženija CUPRAC antioksidativna aktivnost utvrđena je kod acetonskog ekstrakta listova, dok je najslabija zabeležena kod etil-acetatnog ekstrakta semena. Dobijeni rezultati ukazuju na to da vrsta *U. dioica* predstavlja dobar izvor prirodnih antioksidanasa i antibakterijskih jedinjenja.

*Ključne reči:*
*Urtica dioica*, reaktivne vrste kiseonika, bakterije, fitohemijski sastav

## Introduction

Reactive oxygen species are free radicals that can create a chain of damage in cells and tissues. Antioxidants act by preventing the production of radicals that disrupt the balance, minimizing the effects of free radicals that occur and alleviating or repairing oxidative damage (Arıduru, 2022). Nowadays, synthetic compounds with antioxidant properties (butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butyl hydroquinone (TBHQ)) are added to foodstuffs to protect against the harmful effects of reactive oxygen molecules. However, recent studies on synthetic antioxidants have shown that these compounds also have harmful effects and can lead to various diseases when taken in large amounts. For this reason, researchers have recently begun to search for natural plant sources with high antioxidant content (Yıldız et al., 2019).

The number of drugs used in the treatment of infections is increasing day by day. Research is focused on new substances that will have the least side effects on humans and will affect a wide range of microorganisms in small doses. Plants have been both food and drug sources in antimicrobial activity studies for living organisms from the past to present (Arıduru, 2022).

The side effects of artificial substances have increased greatly in recent years, and microorganisms have developed high resistance to artificial drugs such as antibiotics, which has further increased the importance of natural herbal ingredients, and many studies have been conducted. In this way,

the use of plant extracts as antioxidants as well as antimicrobials is being developed and recommended (Arıduru, 2022).

Nettle (*Urtica dioica* L.) is a herbaceous plant from the Urticaceae family that can be annual or perennial. Nettle is consumed as a rich food source due to its high vitamin and mineral content, and is used in many other areas. All parts of the plant have been used in medicine, food, paint, as fertilizer, and cosmetics from past to present days due to its rich variety of components such as saponins, phenolic compounds, phytosterols, tannins, fatty acids, chlorophylls, carotenoids, lignans, flavonolignans, alkaloids, mucilage, vitamins, polysaccharides, proteins, and amino acids. In recent years, an increase in the use of nettle in the cosmetics and food sectors has been observed (Öztürk & Özdemir, 2024).

In this survey, biological activities, GC-MS, and HPLC analyses of the extracts of *Urtica dioica* leaves and seeds were investigated.

## Materials and Methods

### *Collection of the plant material*

The sample of *U. dioica* used in this study was collected from Dereli district in Giresun in September 2019. The collected above-ground parts were brought to the laboratory. The voucher was preserved at Giresun University in Turkey. Fresh leaves and seeds of *U. dioica* were dried in the laboratory and ground with an electric grinder. The powder was kept in an airtight container until extraction.

### *Extraction*

Leaves and seeds of *U. dioica* were separated and dried. The 20 g of powdered leaves and seeds of *U. dioica* were extracted in a shaker for 48 h utilizing 200 mL of acetone and ethyl acetate, separately. The extracts were filtered and residues were evaporated (40 °C) with a rotary evaporator (Murugan & Parimelazhagan, 2014).

### *Chemical characterization*

### *GC-MS analysis*

The GC-MS analysis of the extracts of *U. dioica* was performed using the Agilent equipment. The constituents were identified after comparison with those available in the computer library (Wiley 9-Nist 11) attached to the GC-MS instrument, and the results obtained have been tabulated.

### *HPLC analysis*

Quantitative analysis of bioactive compounds was carried out by High-Performance Liquid Chromatography (HPLC, Agilent 1260 Infinity). Gallic acid, caffeic acid, hesperidin, and rutin hydrate were used as the phenolic compounds to be tested.

### *Biological activity*

### *Antibacterial activity*

The disc diffusion method was utilized to reveal antibacterial potencies of extracts of *U. dioica* leaves and seeds. Discs were loaded with 20 µL plant extract (dissolved in dimethyl sulfoxide (DMSO) at 30 mg/mL concentration), separately. Gentamycine was used as the positive control. Diameters of zones were measured with a ruler (Murray et al., 1995; Saric et al., 2009).

The minimum inhibitory concentration (MIC) values of the extracts (prepared 30 mg/mL concentration in DMSO) were determined with 96-well plates by the method of Yiğit et al. (2009). Furthermore, 96-well plate was incubated at 37 °C for bacteria overnight (Yiğit et al., 2009).

### *Test bacteria*

Eight bacterial species were used in this work. *Salmonella enterica* serovar Typhimurium ATCC 14028 was obtained from Giresun Province Control Laboratory; *Enterococcus faecalis* ATCC 29212 was obtained from the Department of Molecular Biology at Rize University; *Proteus mirabilis* ATCC 25933, *Pseudomonas aeruginosa* ATCC 27853, *Citrobacter freundii* ATCC 43864, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13385, and *Bacillus subtilis* ATCC 6051 were acquired from the Department of Biology at Giresun University.

### *Antioxidant activity*

Antioxidant activity was evaluated using five tests: Total phenolic content, Total flavonoid content, Total antioxidant capacity, DPPH radical scavenging activity, and CUPRAC activity.

### *Total phenolic content*

Total phenolic compounds were determined by the method of Slinkard and Singleton (Slinkard & Singleton, 1977) with some modifications. The absorbance was recorded at 760 nm, using a spectrophotometer (Slinkard & Singleton, 1977). The amount of total phenolic compounds was calculated as µg of gallic acid equivalents (GAE)/mL. The data were presented as the average of triplicate analyses.

### *Total flavonoid content*

Absorbance was measured at 510 nm. Catechin was used as a standard, and the results were expressed as µg catechin equivalent (CE)/mL. The tests were performed in triplicate (Zhishen et al., 1999).

### *Total antioxidant capacity*

The phosphomolybdenum method was utilized to investigate the total antioxidant capacity of the extracts. Absorbance was read at 695 nm. Ascorbic acid was used as the standard. The total antioxidant capacity was expressed as µg ascorbic acid equivalent (AAE)/mL. The tests were performed in triplicate (Prieto et al., 1999).

### *DPPH radical scavenging activity*

The DPPH radical scavenging activity of the plant extracts was measured according to the procedure described by Brand-Williams et al. (1995). Appropriate dilution series (250-1000 µg/mL) were prepared for each extract. The absorbance was measured spectrophotometrically at 517 nm (Brand-Williams et al., 1995).

The DPPH radical scavenging activity (DPPH RSA) was calculated using the following equation:

$$\text{DPPH RSA } (\%)=(A_0-A_1/A_0)\times 100$$

$A_0$ is the absorbance of the control
$A_1$ is the absorbance of the sample

### *CUPRAC activity*

Dilution series (250-1000 µg/mL) were prepared for each extract. Absorbance was measured at 450 nm. The BHT was utilized as a standard antioxidant substance (Özyürek et al., 2009).

## Results and discussion

### *Chemical characterization*

A total of 19 compounds were determined by GC-MS analysis of the acetone extract of seeds of *U. dioica* (**Tab. 1**). The oleic acid (**Fig. 1**) compound, determined at a rate of 63.35% in this study, constitutes the main component of the acetone extract of seeds of *U. dioica*. Other major components contained in the extract are 13.83% butane, 1,1,3-trimethoxy- (**Fig. 2**), 7.84% hexadecanoic acid, and 5.52% phenol, 2,4-bis(1,1-dimethylethyl).

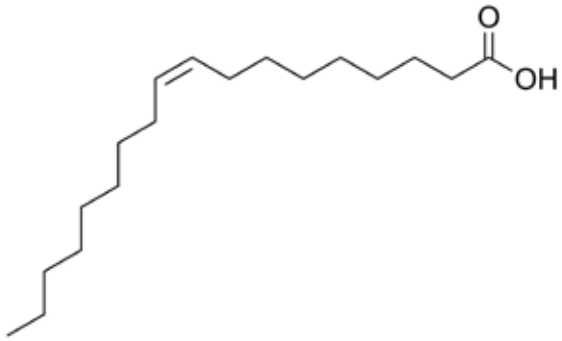


**Fig. 1.** Structure of oleic acid

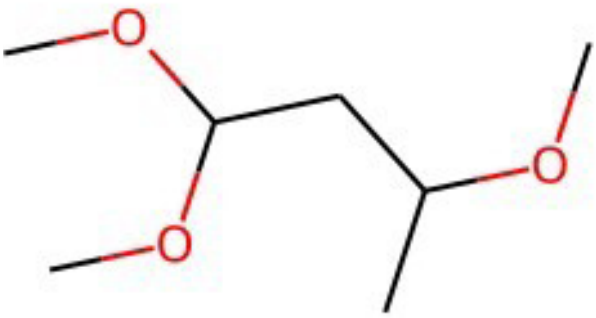


**Fig. 2.** Structure of butane, 1,1,3-trimethoxy

Oleic acid is an unsaturated fatty acid found as glyceryl esters in various vegetable oils such as hazelnut and olive oil. It is used commercially as a pharmaceutical solvent and in the preparation of oleates and lotions. It is also known that oleic acid has the antibacterial combination. One of

**Table 1.** The GC-MS analysis of the acetone extract of seeds of *Urtica dioica*

| Rt (min) | Compound Name | Area (%) |
|---|---|---|
| 5.61 | 1,3-Dimethoxypropene | 0.18 |
| 7.08 | Butane, 1,1,3-trimethoxy- | 13.83 |
| 21.34 | 1,1,3,5-Tetramethoxyhexane | 0.19 |
| 26.80 | Ethanone-2,2,2-D3, 1-Phenyl- | 0.77 |
| 28.65 | 1-Naphthalenamine, 1,2,3,4-tetrahydro- | 0.73 |
| 29.22 | Phenol, 2,4-bis(1,1-dimethylethyl)- | 5.52 |
| 32.00 | Benzophenone | 1.42 |
| 39.46 | Benzoic acid, 2,4,6-trimethyl-, trimethylsilyl ester | 0.39 |
| 40.25 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 0.64 |
| 41.67 | Hexadecanoic acid, methyl ester | 0.43 |
| 42.68 | cis-Vaccenic acid | 0.36 |

| | | |
|---|---|---|
| 43.01 | Hexadecanoic acid | 7.84 |
| 45.46 | 9,12-Octadecadienoic acid, methyl ester | 0.28 |
| 45.59 | 6-Octadecenoic acid, methyl ester | 1.93 |
| 46.84 | Oleic Acid | 63.35 |
| 50.60 | 2-hydroxy-1-(hydroxymethyl)ethyl ester | 1.25 |
| 52.02 | Di-(9-Octadecenoyl)-Glycerol | 0.1 |
| 52.30 | 1-octadecanol | 2.3 |
| 53.31 | Cyclopentadecanone, 2-hydroxy- | 0.91 |

**Table 2.** The GC-MS analysis of the ethyl acetate extract of seeds of *Urtica dioica*

| Rt (min) | Compound Name | Area (%) |
|---|---|---|
| 5.61 | 1,3-Dimethoxypropene | 0.19 |
| 7.08 | Butane, 1,1,3-trimethoxy- | 13.37 |
| 17.69 | Heptane, 1,1-dimethoxy- | 0.15 |
| 21.34 | 1,1,3,5-Tetramethoxyhexane | 0.17 |
| 26.80 | Ethanone-2,2,2-D3, 1-Phenyl- | 0.68 |
| 28.65 | 1,2,5-Oxadiazole-3-carboxamide, 4-amino-N-[2-(1H-indol-3-yl)ethyl]-, 2-oxide | 0.76 |
| 29.22 | Phenol, 2,4-bis(1,1-dimethylethyl)- | 5.32 |
| 32.00 | Benzophenone | 1.35 |
| 39.45 | (+-)-1,5-Dihydroxy-6-ethyl-7-methoxy-4-oxo-1,2,3-trihydronaphthalene | 0.37 |
| 40.25 | Phthalic acid, hexyl dodecyl ester | 0.69 |
| 41.67 | Hexadecanoic acid, methyl ester | 0.35 |
| 42.16 | Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester | 0.3 |
| 42.68 | Diethyl Phthalate | 0.37 |
| 43.01 | n-Hexadecanoic acid | 6.81 |
| 43.30 | Octadecanoic acid | 1.72 |
| 45.59 | 8-Octadecenoic acid, methyl ester | 2.01 |
| 46.84 | Oleic Acid | 60.79 |
| 47.19 | cis-Vaccenic acid | 0.63 |
| 50.31 | i-Propyl 9-octadecenoate | 0.11 |
| 50.60 | trans-13-Octadecenoic acid | 1.75 |
| 53.07 | 2-hydroxy-1-(hydroxymethyl)ethyl ester | 0.2 |
| 53.30 | 9,12-Octadecadien-1-ol, (Z,Z)- | 2.7 |

the most characteristic effects of oleic acid is its antioxidant property, since it can directly regulate both the synthesis and the degradation enzymes of antioxidants (Batur et al., 2019; Ramadan et al., 2024).

A total of 22 compounds were determined by GC-MS analysis of the ethyl acetate extract of seeds of *U. dioica* (**Tab. 2**). The oleic acid compound, determined at a rate of 60.79% in this study, constitutes the main component of the ethyl acetate extract of seeds of *U. dioica*. Other major components contained in the extract are 13.37% butane, 1,1,3-trimethoxy-, 6.81% n-hexadecanoic acid (**Fig. 3**), and 5.32% phenol, 2,4-bis(1,1-dimethylethyl) (**Fig. 4**).

A total of 23 compounds were determined by GC-MS analysis of the acetone extract of leaves of *U. dioica* (**Tab. 3**). The oleic acid compound, determined at a rate of 61.77% in this study, constitutes the main component of the acetone extract of leaves of *U. dioica*. Other major components contained in the extract are 14.23% butane, 1,1,3-trimethoxy-, 6.04% hexadecanoic acid, and 5.65% phenol, 2,4-bis(1,1-dimethylethyl).

Hexadecanoic acid, also called palmitic acid, is

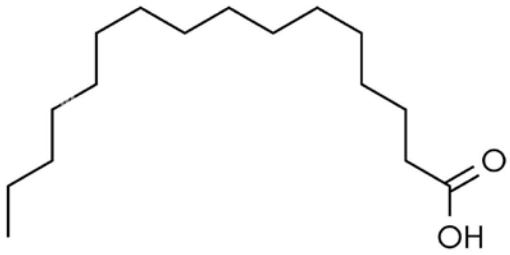


**Fig. 3.** Structure of n-hexadecanoic acid

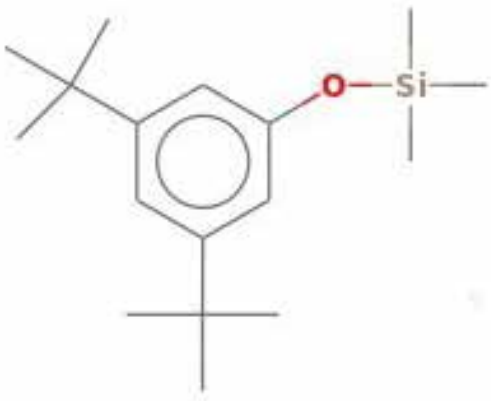


**Fig. 4.** Structure of phenol, 2,4-bis(1,1-dimethylethyl)

a saturated fatty acid with a wide range of uses in industrial, biological, and health areas. It is used to store energy and strengthen cell membranes in the human body. It is also metabolized in the body through various biochemical pathways and plays a role in hormone production and cellular functions. Palmitic acid, obtained from the *Ipomoea eriocarpa* R.Br. plant, has also been reported to inhibit bacteria and exhibit antioxidant properties (Sehim et al., 2023; Ganesan et al., 2024).

A total of 21 compounds were determined by GC-MS analysis of the ethyl acetate extract of leaves of *U. dioica* (**Tab. 4**). The oleic acid compound, determined at a rate of 60.46% in this study, constitutes the main component of the ethyl acetate extract of leaves of *U. dioica*. Other major components contained in the extract are 12.16% methyl 2-hydroxy-15-tetracosenoate, 7.25% butyraldehyde (**Fig. 5**), 7.85% n-Hexadecanoic acid, and 6.60% 2-hydroxy-1-(hydroxymethyl)ethyl ester.

### *HPLC analysis*

The HPLC analysis of the extracts is given in **Tab. 5**. Gallic acid, caffeic acid, hesperidin, and rutin hydrate standards were used in HPLC analyses of the

**Table 3.** The GC-MS analysis of the acetone extract of leaves of *Urtica dioica*

| Rt (min) | Compound Name | Area (%) |
|---|---|---|
| 5.62 | 1,3-Dimethoxypropene | 0.28 |
| 7.09 | Butane, 1,1,3-trimethoxy | 14.23 |
| 17.70 | Dodecane, 1,1-dimethoxy | 0.17 |
| 21.34 | 1,1,3,5-Tetramethoxyhexane | 0.18 |
| 21.43 | Octadecane, 1,1-dimethoxy- | 0.34 |
| 26.80 | Ethanone-2,2,2-D3, 1-Phenyl- | 0.9 |
| 28.65 | 1-Naphthalenamine, 1,2,3,4-tetrahydro- | 0.89 |
| 29.23 | Phenol, 2,4-bis(1,1-dimethylethyl)- | 5.65 |
| 32.01 | Methanone, diphenyl- | 1.41 |
| 40.25 | Phthalic acid, nonyl pentadecyl ester | 0.74 |
| 41.67 | Hexadecanoic acid, methyl ester | 0.4 |
| 42.16 | Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester | 0.32 |
| 42.68 | Benzothiazole, 2-methyl- | 0.2 |
| 43.02 | Hexadecanoic acid | 6.04 |
| 45.59 | Oleic acid, methyl ester | 2.23 |
| 46.85 | Oleic acid | 61.77 |
| 47.19 | cis-Vaccenic acid | 0.88 |
| 49.34 | Di-(9-Octadecenoyl)-Glycerol | 0.11 |
| 50.62 | 2-hydroxy-1-(hydroxymethyl)ethyl ester | 1.2 |
| 51.22 | n-Propyl 11-octadecenoate | 0.15 |
| 52.30 | Cyclopentadecanone, 2-hydroxy- | 0.69 |

| | | |
|---|---|---|
| 53.32 | 6-Octadecenoic acid | 0.89 |
| 54.27 | n-Propyl 9-octadecenoate | 0.24 |

**Table 4.** The GC-MS analysis of the ethyl acetate extract of leaves of *Urtica dioica*

| Rt (min) | Compound Name | Area (%) |
|---|---|---|
| 5.61 | 1,3-Dimethoxypropene | 0.34 |
| 7.09 | Butyraldehyde | 7.25 |
| 21.42 | 1,1,3,5-Tetramethoxyhexane | 0.17 |
| 26.80 | Benzoic acid, 2-hydroxyethyl ester | 0.66 |
| 28.65 | Gramine | 0.4 |
| 29.22 | Phenol, 2,4-bis(1,1-dimethylethyl)- | 2.87 |
| 32.01 | Methanone, diphenyl- | 0.7 |
| 39.46 | Benzyl (dideuterated )methyl ether | 0.3 |
| 40.26 | Phthalic acid, cyclohexylmethyl butyl ester | 0.34 |
| 41.67 | 1-Nonadecene | 0.23 |
| 42.17 | cis-13-Octadecenoic acid | 0.17 |
| 42.68 | 13-Octadecenal | 0.25 |
| 43.04 | n-Hexadecanoic acid | 7.85 |
| 45.59 | trans-13-Octadecenoic acid, methyl ester | 1.03 |
| 46.95 | Oleic Acid | 60.46 |
| 47.90 | trans-13-Octadecenoic acid | 0.33 |
| 48.48 | Heptadecanal | 0.66 |
| 51.58 | Cyclopentadecanone, 2-hydroxy- | 0.24 |
| 52.31 | 2-hydroxy-1-(hydroxymethyl)ethyl ester | 6.6 |
| 53.47 | 9-Octadecenoic acid | 0.43 |
| 55.33 | Methyl 2-hydroxy-15-tetracosenoate | 12.16 |

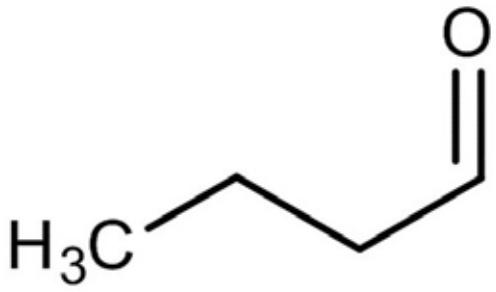


**Fig. 5.** Structure of butyraldehyde

extracts. Caffeic acid wasn't found in any analyzed extracts. Gallic acid, hesperidin, and rutin hydrate were found in the extracts at varying amounts.

***Biological activity***

***Antibacterial activity***

Results of antibacterial activity of ethyl acetate and acetone extracts of *U. dioica* leaves and seeds against test bacteria were presented in **Tab. 6.** While the highest activity was observed in the ethyl acetate extract of *U. dioica* seeds against *E. coli* (13 mm), the lowest activity was observed in the acetone extracts of *U. dioica* leaves, ethyl acetate and acetone extracts of *U. dioica* seeds against *P. mirabilis* and *K. pneumoniae* (6 mm). Generally, the leaf extract of *U. dioica* had better activity than the seed extract of *U. dioica.* The DMSO had no activity. Gentamycine, which was used as a standard antimicrobial agent, had higher antibacterial activity when compared with the plant extracts against bacteria, except for *P. aeruginosa.*

In the study, MIC values of the ethyl acetate and acetone extracts of seeds and leaves of *U. dioica* were determined on strains (inhibition zones of 10 mm and above formed) for which activity was determined by the disc diffusion method. The MIC values of the ethyl acetate and acetone extracts of seeds and leaves of *U. dioica* are demonstrated in **Tab. 7**. The MIC values of ethyl acetate and acetone

**Table 5.** The HPLC analysis of the extracts

| Extract | Phenolic compound | Rt (min) | Area | Amount (0,002 g/2 mL extract) |
|---|---|---|---|---|
| Ethyl acetate extract of leaves of *U. dioica* | Gallic acid | 2.022 | 117.4075 | 0.0065 |
| | Caffeic Acid | - | - | - |
| | Hesperidine | 6.183 | 11.69461 | 0.0096 |
| | Rutin hydrate | 6.537 | 24.78324 | 0.001 |
| Acetone extract of leaves of *U. dioica* | Gallic acid | 2.02 | 104.8308 | 0.0058 |
| | Caffeic Acid | - | - | - |
| | Hesperidine | 6.183 | 11.38039 | 0.0093 |
| | Rutin hydrate | 6.534 | 23.58227 | 0.0095 |
| Acetone extract of seeds of *U. dioica* | Gallic acid | 2.02 | 120.6895 | 0.0067 |
| | Caffeic Acid | - | - | - |
| | Hesperidine | 6.188 | 10.77397 | 0.0089 |
| | Rutin hydrate | 6.542 | 25.08587 | 0.001 |
| Ethyl acetate extract of seeds of *U. dioica* | Gallic acid | 1.998 | 158.5627 | 0.0088 |
| | Caffeic Acid | - | - | - |
| | Hesperidine | 6.182 | 14.42254 | 0.0011 |
| | Rutin hydrate | 6.532 | 29.70686 | 0.0012 |

**Table 6.** Inhibition zones created by the extracts, DMSO, and gentamycine (mm)

| Bacteria | Ethyl acetate extract of *U. dioica* leaves | Acetone extract of *U. dioica* leaves | Ethyl acetate extract of *U. dioica* seeds | Acetone extract of *U. dioica* seeds | Genta | DMSO |
|---|---|---|---|---|---|---|
| *B. subtilis* | 10 | 9 | 9 | - | 32 | - |
| *P. mirabilis* | - | 6 | - | - | 15 | - |
| *C. freundii* | 12 | 11 | 10 | - | 20 | - |
| *E. coli* | 9 | 8 | 13 | 8 | 21 | - |
| *E. faecalis* | 10 | 10 | 8 | 8 | 18 | - |
| *K. pneumoniae* | 10 | 9 | 6 | 6 | 20 | - |
| *S. typhi* | 7 | 9 | 8 | - | 13 | - |
| *P. aeruginosa* | 10 | 10 | 10 | 8 | 8 | - |

extracts of seeds and leaves of *U. dioica* ranged from 3.75 mg/mL to 0.23 mg/mL.

In the literature, there are many studies about the antibacterial activity of extracts of *U. dioica*. For example, Yılmaz et al. (2022) investigated the antibacterial activity of *U. dioica* extract collected from Muğla, Turkey. It was found that the extract was active against *Staphylococcus aureus* (7.97 mm), but it wasn't active against *Bacillus cereus* and *E. coli* (Yılmaz et al., 2022). In our study, both leave and seed extracts of *U. dioca* had antibacterial activity against *E. coli*. This situation may arise from the use of plants located in different cities.

Saklani and Chandra (2012) investigated the antibacterial activity of petroleum ether, chloroform, ethyl acetate, acetone, methanol, ethanol, and water extracts of *U. dioca* collected from India. Both acetone and ethyl acetate extracts inhibited the growth of *E. coli* and *K. pneumoniae* (Saklani & Chandra, 2012). In our assays, acetone and ethyl acetate extracts of *U. dioca* also had antibacterial activity against *E. coli* and *K. pneumoniae*.

Mirtaghi et al. (2014) stated that the ethanol extract of *U. dioica* from Iran had activity against

*E. coli*, *S. aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus* (Mirtaghi et al., 2014).

**Table 7.** The MIC values of the extracts (mg/mL)

| Bacteria | Ethyl acetate extract of *U. dioica* leaves | Acetone extract of *U. dioica* leaves | Ethyl acetate extract of *U. dioica* seeds |
|---|---|---|---|
| *B. subtilis* | 0.234 | - | - |
| *C. freundii* | 1.875 | 3.75 | 3.75 |
| *E. coli* | - | - | 3.75 |
| *E. faecalis* | 3.75 | 1.875 | - |
| *K. pneumoniae* | 1.875 | - | - |
| *P. aeruginosa* | 1.875 | 3.75 | 3.75 |

### *Antioxidant activity*

### *Total phenolic content*

Phenolics, which exist naturally in an estimated number of 8000, share the identical prevalent structure composed of an aromatic hydroxyl nucleus (Hatami et al., 2014). The total phenolic content of the samples showed large variations, between 110.11±0,006 and 180.88±0,003 µg GAE/mL extract (**Tab. 8**). Based on the results, total phenolic contents of the extracts are decreasing in the following order: Acetone extract of *U. dioica* leaves > Ethyl acetate extract of *U. dioica* leaves > Acetone extract of *U. dioica* seeds > Ethyl acetate extract of *U. dioica* seeds.

### *Total flavonoid content*

Measurement of total flavonoids in the acetone and ethyl acetate extracts of *U. dioica* leaves and seeds was performed by utilizing catechin standards. Total flavonoid contents of the extracts can be seen in **Tab. 9**. Total flavonoid content from the highest to the lowest is acetone extract of *U. dioica* leaves, ethyl acetate extract of *U. dioica* leaves, acetone extract of *U. dioica* seeds, and ethyl acetate extract of *U. dioica* seeds.

**Table 8.** Total phenolic contents of the extracts (µg GAE/mL)

| Plant Extract | Total Phenolic Content (µg GAE/mL) |
|---|---|
| Acetone extract of *U. dioica* leaves | 180.88±0.003 |
| Ethyl acetate extract of *U. dioica* leaves | 160.38±0.0005 |
| Acetone extract of *U. dioica* seeds | 160.11±0.005 |
| Ethyl acetate extract of *U. dioica* seeds | 110.11±0.006 |

Values are expressed as means of three replicates ± SD (n=3)

**Table 9.** Total flavonoid contents of the extracts (µg QE/mL)

| Plant Extract | Total Flavonoid Content (µg QE/mL) |
|---|---|
| Acetone extract of *U. dioica* leaves | 680.37±0.005 |
| Ethyl acetate extract of *U. dioica* leaves | 322.45±0.040 |
| Acetone extract of *U. dioica* seeds | 297.03±0.024 |
| Ethyl acetate extract of *U. dioica* seeds | 174.94±0.017 |

Values are expressed as means of three replicates ± SD (n=3)

### *Total antioxidant capacity*

The total antioxidant capacity of the extracts was shown in **Tab. 10**. The highest and the lowest total antioxidant capacity were determined in the acetone extract of *U. dioica* leaves and the ethyl acetate extract of *U. dioica* seeds, respectively.

**Table 10.** Total antioxidant capacity of the extracts (µg AAE/mL)

| Plant Extract | Total Antioxidant Capacity (µg AAE/mL) |
|---|---|
| Acetone extract of *U. dioica* leaves | 206.42±0.039 |
| Ethyl acetate extract of *U. dioica* leaves | 138.65±0.044 |
| Acetone extract of *U. dioica* seeds | 118.96±0.010 |
| Ethyl acetate extract of *U. dioica* seeds | 104.28±0.039 |

Values are expressed as means of three replicates ± SD (n=3)

### *DPPH radical scavenging activity and CUPRAC activity*

The DPPH scavenging and CUPRAC activities of the extracts are illustrated in **Tab. 11.** The DPPH activity of the extracts ranges between 5.42% to 29.06% at a 1000 µg/mL concentration. The DPPH activity of the extracts and standards increases with increasing concentrations. The activity was decreasing in the following order at 1000 µg/mL concentration: rutin > BHT > ethyl acetate extract of *U. dioica* leaves > ethyl acetate extract of *U. dioica* seeds > acetone extract of *U. dioica* seeds > acetone extract of *U. dioica* leaves.

The highest CUPRAC activity was determined in the acetone extract of *U. dioica* leaves, and the lowest CUPRAC activity was determined in the ethyl acetate extract of *U. dioica* seeds. Both DPPH radical scavenging activity and CUPRAC activity assays standard antioxidants exhibited higher activity than the extracts.

The antioxidant activity of extracts of *U. dioica* was also studied by other researchers. Yıldırım et al. (2013) declared that the methanol extract of *U. dioica* collected from Tunceli, Turkey had DPPH radical scavenging activity (Yıldırım et al., 2013). In our assays, acetone and ethyl acetate extracts of *U. dioica* also had DPPH radical scavenging activity.

Kukrić et al. (2012) investigated the antioxidant activity of the ethanol extract of *U. dioica* collected from Bosnia and Herzegovina. According to the results, it was concluded that the ethanolic extract of *U. dioica* had DPPH radical scavenging activity, ABTS radical scavenging activity, and FRAP activity (Kukrić et al., 2012).

**Table 11.** The DPPH radical scavenging and CUPRAC activities of the extracts and standards

| Plant Extract | Concentration (µg/mL) | CUPRAC Activity (Absorbance (nm)) | DPPH Radical Scavenging Activity (% inhibition) |
|---|---|---|---|
| Ethyl acetate extract of *U. dioica* leaves | 250 | 0.3710±0.029 | 18.91±0.003 |
| | 500 | 0.8866±0.009 | 24.91±0.006 |
| | 750 | 1.1320±0.017 | 26.41±0.003 |
| | 1000 | 1.6396±0.036 | 29.06±0.004 |
| Acetone extract of *U. dioica* leaves | 250 | 0.4716±0.052 | No activity |
| | 500 | 0.9660±0.032 | No activity |
| | 750 | 1.4370±0.059 | 5.07±0.004 |
| | 1000 | 1.7773±0.013 | 5.42±0.003 |
| Acetone extract of *U. dioica* seeds | 250 | 0.3553±0.045 | 4.49±0.002 |
| | 500 | 0.7263±0.012 | 8.76±0.001 |
| | 750 | 1.0490±0.031 | 22.60±0.003 |
| | 1000 | 1.3540±0.022 | 28.83±0.003 |
| Ethyl acetate extract of *U. dioica* seeds | 250 | 0.2813±0.005 | 12.11±0.028 |
| | 500 | 0.5446±0.020 | 22.66±0.006 |
| | 750 | 0.9333±0.019 | 22.83±0.013 |
| | 1000 | 1.1343±0.028 | 29.02±0.002 |
| BHT | 250 | 2.1503±0.050 | 89.15±0.013 |
| | 500 | 2.5356±0.071 | 90.55±0.003 |
| | 750 | 3.1870±0.010 | 91.25±0.010 |
| | 1000 | 3.2833±0.008 | 92.45±0.005 |
| Rutin | 250 | - | 89.10±0.005 |
| | 500 | - | 91.90±0.002 |
| | 750 | - | 92.63±0.012 |
| | 1000 | - | 93.12±0.010 |

Values are expressed as means of three replicates ± SD (n=3)

## Conclusion

The current investigation evaluated the biological activities and chemical composition of ethyl acetate and acetone extracts of seeds and leaves of *U. dioica*. Both *U. dioica* seeds and leaves can be considered as an alternative to synthetic antioxidants and antibacterial substances as natural antioxidant and antibacterial substance sources. The GC-MS analysis revealed the presence of bioactive metabolites with various biological functions, which are fatty acids and their derivatives. According to the results obtained from the assays, *U. dioica* can be used as a natural alternative agent for biomedical applications.

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