

Scientific paper

Investigating the Polyphenolic Profile and the Antioxidant and Antibacterial Activity of Tarragon (*Artemisia Dracunculus* L) Cultivated in Central Romania

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Abstract

The chemical composition, the antioxidant and antibacterial properties of *Artemisia dracunculus* L. leaves were examined through the utilization of four solvents for extraction. These solvents included ultrapure water, ethanol, methanol and acetic acid. The values reached for total polyphenols were between 77.2 mg gallic acid equivalent (GAE)/g for the acetic acid extracts and 192.1 mg GAE/g for the methanolic extracts. The total flavonoids were identified at 46.4 mg quercetin equivalent (QE)/g for the acetic acid extracts and 126.4 mg QE/g for the methanolic extracts. The IC₅₀ antioxidant capacity values determined by the 2,2-diphenyl-2-picrylhydrazyl (DPPH) method were between 14.66 µg/mL (acetic acid extracts) and 20.33 µg/mL (methanolic extracts). 23 phenolic compounds were identified using the High Performance Liquid Chromatography (HPLC) method. The methanolic and the aqueous extracts have on very good antibacterial activity on the *Staphylococcus aureus* 231 and *Enterococcus faecalis* 428 strains. *A. dracunculus* L. leaf extracts are rich in a diverse range of valuable active chemical and biological compounds.

Keywords: polyphenols, antioxidant, antibacterial, phenolic compounds, tarragon.

1. Introduction

Tarragon (*Artemisia dracunculus* L.) is a perennial herbaceous plant that originated from the Nordic hemisphere and has spread across Europe, Central and Eastern Asia, India, Western North America and Southern to Northern Mexico.^{1,2} It belongs to the *Artemisia* genus, which encompasses 500 species, each with distinct aromas and unique biological properties. With a height that can reach up to 150 cm, tarragon has a tall, branching stem adorned with lanceolate leaves that emit a delightful fragrance. The plant also produces whitish flowers. In Europe, tarragon is primarily cultivated and utilized either in its fresh or dried form for its aromatic qualities in culinary applications and traditional or complementary medicine.^{4–6} When grown to maturity and during inflorescence periods, tarragon thrives and can yield significant harvests.

Artemisia species are rich in compounds with therapeutic properties against diseases such as malaria, hepatitis, cancer, diabetes, depression, seizures, inflammation, and fungal, bacterial, or viral infections.^{7–13} The stems and leaves of *A. dracunculus* L. are used in international and traditional cuisine, while the flowers and other parts of the plant are used in alternative medicine.^{14–17} Plant-based derivatives used as alternative medicines are in high demand, as these are considered safe and reliable compared to expensive synthetic drugs that come with secondary effects.

Herbs and spices contain many phytonutrients, viable sources of natural immunity-simulating antioxidants.^{18–21} Of these, phenols are one of the countless general classes of naturally occurring vegetal metabolites; we presently know over 8,000 phenolic forms.^{22–26}

A. dracunculus L. is rich in essential oils rich in flavonoids, phenolic acids, coumarins, sterols, fatty acids, alkalamides and other valuable compounds that make tarra-

gon a useful plant.^{7,27–35} The volatile compounds found in the plant are monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids, derivatives of phenylpropanes, polyacet- ylenes and others elements.^{7,27,31,33,34,36–46} These com- pounds participate to the bioactive qualities of the differ- ent parts of the tarragon.

Thanks to this variety of volatile and phenolic com- pounds, tarragon has antioxidant^{30,38,47}, anticancer⁴⁵, hepatoprotective⁴⁷, immunomodulating⁴⁸ and antineo- plastic properties, and can be used to treat gastritis, der- matitis, epilepsy, or various forms of allergies.^{27,42,49–51}

Tarragon shows broad-spectrum antibacterial activ- ity, including against human pathogens, such as *Pseu- domonas aeruginosa*, *E. coli*, *S. aureus*, *Salmonella typhi- murium*, and *S. epidermidis*, as well as *Proteus vulgar- is*.^{29,30,33,38,43,52–58} More than that, *A. dracunculus* L. has shown antifungal activity against certain fungal species, such as *Candida albicans*, *Cryptococcus neoformans*, *As- pergillus niger*, *Microsporum canis*, *Trichophyton rubrum*, *Microsporum gypseum*, and *Fonsecaea pedrosol*.^{59–61}

2. Experimental

2. 1. *A. dracunculus* L. samples

This paper aims at showing the qualities of tarragon cultivated in the sub-mountainous area of the Sibiu de- pression in Central Romania, with a relief characterized by hills, river valleys, and terraces.

A. dracunculus L. plants were collected from the commune of Rășinari, with GPS coordinates of latitude: 45°42'0.00"N and longitude: 24°04'0.01"E at a 573 m alti- tude, the plantation having an area of 1 hectare. The sam- ples were registered with voucher number 366 of the Mi- crobiology Laboratory of the Research Centre in Biotechnology and Food Engineering (CCBIA) within the Faculty of Agricultural Sciences, Food Industry and Envi- ronmental Protection, "Lucian Blaga" University of Sibiu, Romania. The climate in the area is humid and cool, with more rainfall in June, which is favourable to rich, varied vegetation. The average annual temperature is 9.5 °C, with big variations throughout the day, with winds dominantly blowing from the South-Southwest, which makes snow melt faster thus influencing drought periods. Soils here are typical for premontane areas, varying from cambic chernozems to alluvial, clay-sandy soils.

The harvesting area exhibits a naturally low degree of fertility, with alluvial soils in various stages of evolution, slightly acidic. Improvement and fertilization work has been carried out, including the temporary removal of ex- cess water. The predominant climate is characterized by a high frequency of temperate oceanic air coming from the west, especially during the warm season, and a low fre- quency of temperate continental air from the northeast and east. *A. dracunculus* L. is not a very demanding plant, so it develops well in these conditions, generating small,

valuable, useful crops. The tarragon culture was founded with rooted cuttings, planted at 25 cm between them in parallel rows, at the end of April. Fertilization of the land was carried out in autumn with semi-fermented manure (40t/ha), no irrigation operations being necessary. Period- ically the weeds were weeded, and the flower stalks were removed. The whole plants (stem with leaves) were collect- ed on June 10, 2022, in the afternoon, stored in crates in a thin layer and transported to the laboratory on the same day. The leaves were separated from the stem and prepared for drying.

2. 2. Chemicals, Reagents, Bacterial Strains and Culture Media

We have used analytically pure reagents from Sig- ma-Aldrich GmbH, Steinheim, Germany: absolute etha- nol 99.8%, methanol 99.9%, acetic acid 99.8%, Folin-Cio- calteu reagent, sodium carbonate 7.5%, sodium nitrite 5%, aluminum chloride 10%, sodium hydroxide 1M, DPPH/2,2-difenil-2-picrilhidrazil, Trolox (6-hy- droxy-2,5,7,8-tetramethylchroman-2- carboxylic acid), gallic acid (≥99%), quercetin (≥95%), kaempferol (≥97%), luteolin (≥98%), apigenin (≥99%), davidigenin (≥99%), rutoside (≥94%), aridiol (≥98.5%), artemidine (≥98%), artidin (≥95%), coumarin (≥99%), herniarin (≥98%), sco- poletin (≥97%), caffeic acid (≥98%), chicory acid (≥95%), chlorogenic acid (≥95%), p-coumaric acid (≥98%), ferulic acid (≥99%), syringic acid (≥95%), vanillic acid (≥97%), 2-methoxycinnamic acid (≥97%), 4,5-di-o-caffeoylquinic acid (≥90%), sakuranetin (≥95%).

We analysed the antibacterial activity of *A. dracun- culus* L. extracts on six bacterial strains isolated in the Mi- crobiology Laboratory (CCBIA/ULBS), of which three Gram-positive: *Clostridium perfringens* 211, *Enterococcus faecalis* 428, *Staphylococcus aureus* 231, and three Gram-negative: *Escherichia coli* 29, *Pseudomonas aerugi- nosa* 323, *Salmonella typhimurium* 14. We used specific growth media: Mueller Hinton agar, Mueller Hinton broth (Sigma-Aldrich GmbH, Steinheim, Germany), Cefoxitin Antimicrobial Susceptibility discs, 30 µg (Thermo Fisher Scientific™ Oxoid™)

2. 3. Methods

2. 3. 1. Extract Preparation

500g of *A. dracunculus* L. leaves are dried for three days in the Memmert incubator at 40 °C (until reaching a constant mass).

After drying, the leaves are ground to a particle size of 100–500 microns, resulting in a fine powder. Each 10g of powder is homogenized with 100 mL of the following solvents: ultrapure water, ethyl alcohol, and distilled water (in a 1:2 ratio), methanol and distilled water (in a 1:2 ra- tio), 9% v/v acetic acid. The extraction takes place in cov- ered recipients, in the dark, at room temperature.

Periodically, the samples are homogenized on a magnetic stirrer, and after 24 hours, they are filtered using Whatman filter paper no. 54. The extraction process is repeated three times, and the resulting extracts are concentrated using the IKA RV 3 rotary evaporator at a rotation speed of 300 rpm, and finally weighed.

The dried powders are stored at 3 °C for analysis. The dry powders were resuspended in distilled water in a 1:1 ratio and methanol in a 1:1 ratio for the determinations.

2. 3. 2. Determining Total Polyphenol Contents (TPC)

To determine the total polyphenol contents, we used a slightly modified Folin-Ciocalteu spectrophotometric method, i.e. homogenized 100 µL aqueous extract (1mg/mL concentration) with 2.5 mL ultrapure water, 100 µL Folin-Ciocalteu reagent, and incubated for 10 minutes at room temperature. Then, we added 250 µL of 20% sodium carbonate, and incubated the samples again for 30 minutes at room temperature in the dark. We used a UV-1900 SHIMADZU spectrophotometer (Shimadzu Corporation, Kyoto, Japan) to read the samples at a wavelength of 760 nm and compared them to the control samples containing the same reagents, while the extract was replaced with distilled water. We used gallic acid for the calibration curve and expressed total polyphenol values in mg equivalent to gallic acid/g of dry extract. All determinations were performed in triplicate.⁶²

2. 3. 3. Antioxidant Activity Assessment

The antioxidant activity involved testing compounds that have the ability to donate hydrogen or eliminate free radicals in the presence of DPPH (2,2-diphenyl-2-picrylhydrazyl) using a slightly modified spectroscopic method.⁶² A stock methanolic solution of DPPH (25:100) is prepared by dissolving 25g of DPPH in 100 mL of methanol and stored at a temperature of –20 °C in the dark. From the stock solution, a working solution is prepared in a ratio of 10:90. The working solution is obtained by homogenizing 10mL of the stock solution with 90mL of methanol. The samples are prepared in a ratio of 1:1 (dry extract:methanol). 20 µL of the sample is weighed and homogenized with 180µL of the working solution.

The mixture is left to react in the dark at a temperature of 20 °C. After 30 minutes, the absorbance is read using a UV-1900 SHIMADZU spectrophotometer at a wavelength of 515 nm. The control sample is obtained following the same procedure, with the extract replaced by methanol. A calibration curve is constructed using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2- carboxylic acid), and the results are expressed in milligrams of Trolox equivalent per gram of dry substance (mg TE/g). The inhibition percentage (I) is calculated according to the equation:

$$\% I = \frac{Ab - Aa}{Ab} \times 100,$$

where Ab is the absorbance of the control, and Aa is the absorbance of the reaction between the sample and the radicals.

2. 3. 4. Determining Total Flavonoid Contents (TFC)

To determine total flavonoid contents, we used an adapted colorimetric method⁶³ by homogenizing 1 mL extract (1mg/mL concentration) with 0.5 mL of 5% NaNO₂. The samples were incubated at room temperature for 5 minutes, then we added 0.5 mL of 10% AlCl₃·6H₂O. They were incubated again in the dark for 15 minutes, as we then added 2 mL of 1M NaOH. We used distilled water to dilute the samples to 10 mL, then read them at a wavelength of 510 nm using a UV-1900 SHIMADZU spectrophotometer. We used quercetin for the calibration curve and expressed the results in mg equivalent to quercetin/g of dry extract (QE/g). All determinations were performed in triplicate.

2. 3. 5. Quantifying Phenolic Compounds Through HPLC

We used a slightly modified HPLC method⁶³ to identify valuable phenolic compounds in tarragon extracts with an Agilent 1200 device (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump, automatic injector, and PDS (photo diode system) detector. We set the device to read at the wavelengths: λ = 280 nm, 320 nm, 360 nm. We used a 250 mm × 4.6 mm i.d. and 5.0 µm p.s. C18 Zorbax chromatography column, with a sample injection volume of 20 µL. We used 95/5 v/v water/acetic acid solution as eluent A, and a 100/5/95 v/v/v acetonitrile/water/acetic acid solution as eluent B. The mobile phase was degassed at 22°C for 15 minutes as we established an extraction scheme with the following gradient profile: 0–15 min, 20% B; 15–30 min, 30% B; 30–45 min, 40% B; 40–70 min, 50% B; 70–75 min, 55% B; 75–80 min, 95% B; 80–85 min, 100% B; 85–90 min, 10% B. The resulting values were expressed in µg/g dry extract, as determinations were made on triplicate. To identify and quantify phenolic compounds, we compared the results obtained to the corresponding calibration curves (quercetin, kaempferol, luteolin, apigenin, davidigenin, rutoside, aridiol, artemidine, artidin, coumarin, herniarin, scopoletin, caffeic acid, chicory acid, chlorogenic acid, p-coumaric acid, ferulic acid, gallic acid, syringic acid, vanillic acid, 2-methoxycinnamic acid, 4,5-di-o-caffeoylquinic acid, sakuranetin).

2. 3. 6. Determining Antibacterial Activity (MIC) / Disk Diffusion and Serial Dilution Test

Minimum inhibitory concentration (MIC) is frequently used in microbiology to test whether certain ex-

tracts or compounds can halt the development of microorganisms and to use this information in the field. We activated the bacteria in Mueller Hinton media at 37 °C for 24 hours and brought the inoculum to a density of 0.5 McFarland = 1.5×10^8 Colony Forming Units (CFU)/mL. To do so, we obtained serial dilutions of the extract under analysis, from 2000 µg/mL, 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, to 31.25 µg/mL. Using a pipette, we dropped 10 µL of diluted extract to a disk placed in the Petri dish containing Mueller Hinton solidified culture media covered with the microorganism under analysis (*Clostridium perfringens* 211, *Enterococcus faecalis* 428, *S. aureus* 231, *E. coli* 29, *Pseudomonas aeruginosa* 323, *Salmonella typhimurium* 14). The plates were incubated at 37°C for 24 hours, then we analysed the antibacterial activity of the extracts. The lowest extract concentration that inhibited bacteria growth was considered the minimum inhibitory concentration (MIC). We used cefoxitin disks (30 µg) as a positive control.

2. 3. 7. Statistical Analysis

The results obtained are the mean of three determinations, including the standard deviation of the measurements ($\pm$ standard deviation/SD). We used a one-way analysis of variance (ANOVA), with a statistical significance of $p \leq 0.05$.

3. Results and Discussion

3. 1. Total Phenolic, Antioxidant Activity and Flavonoid Content

The findings presented in Table 1 demonstrate variations in the polyphenol concentrations of tarragon leaf extracts based on the solvent used for extraction. Water, being a polar solvent, has a limited ability to extract compounds with high polarity. Conversely, the inclusion of solvents like methanol or ethanol enhances the extraction process, resulting in a noteworthy increase in the concentration of measurable phenolic compounds.

Polyphenols can be extracted using various methods, and the efficiency of these processes or the solvents used can be correlated with the plant material and the

structure of phenolic compounds. They are abundantly present in the natural environment, comprising different parts of plants, as is the case with samples of *A. dracunculus* L. Polyphenols determined in the methanolic extracts obtained from the leaves of *A. dracunculus* L. showed values of 192.1 ± 5.3 mg GAE/g dry weight (d.w.), which were the most significant in terms of quantity. In the aqueous extracts, 168.3 ± 2.7 mg GAE/g d.w. were identified and quantified, while the alcoholic extracts were, on average, 48% lower. The lowest values were observed in the case of acetic acid extracts, where they did not exceed an average of 77.2 ± 1.4 mg GAE/g d.w. The polyphenol values of tarragon were also found in methanolic extracts (2681 ± 0.12 mg GAE/g d.w.)²⁶ as well in ethanolic extracts (167.20 ± 21.32 µg rutin/mg extract).²⁸ The antioxidant activity characterizes an extract by looking at how its bioactive components can impact various medical.^{30,65} In the case of the extracts in this research, the highest values were identified in methanolic extracts, with a mean DPPH of 76.4 ± 0.4 % and an IC_{50} of 20.3 ± 0.2 µg/ml. The lower the IC_{50} , the higher the extract's antioxidant capacity.

Many studies described the antioxidant potential of *A. dracunculus* L, as they all reached the same conclusion that this is a direct result of the concentration of polyphenols extracted.^{30,65} Other studies found a connection between total polyphenol contents (24.10 mg GAE/g d.w.), total flavonoid contents (20 mg QE/g), and the DPPH test with IC_{50} levels of 65.50 µg/mL³⁸. Significant levels of polyphenols were also found in hydroethanolic extracts (197.22 mg GAE/g d.w.), as their antioxidant activity determined using DPPH and ABTS tests was high. The antioxidant activity of tarragon reached IC_{50} levels of 1.15 mg/mL for DPPH and 0.17 mg/mL for ABTS, while in the case of ascorbic acid, used as a control sample, the values determined were 0.002 mg/mL for DPPH and 0.005 mg/mL for ABTS.^{42,47,66} Flavonoids are a major component found in *A. dracunculus* L. extracts, playing a significant role as the configuration of natural antioxidants in plants.^{65,67,68}

As per Table 1, flavonoid contents found in *A. dracunculus* L. extracts fall between 46.4 ± 0.1 mg QE/g d.w. and 126.4 ± 0.3 mg QE/g d.w. The type of solvent used for extraction influences the level of flavonoids. The methanolic extracts were found to contain the highest amounts

Table 1. Total polyphenol contents, antioxidant activity and flavonoid contents identified in *A. dracunculus* L. leaf extracts.

	Aqueous extract	Ethanolic extract	Methanolic extract	Acetic acid extract
Total phenolic content (mg GAE/g d.w.) $\pm$ SD ^a	168.3 ± 2.7	113.9 ± 1.9	192.1 ± 5.3	77.2 ± 1.4
DPPH scavenging activity (%) $\pm$ SD ^b	51.1 ± 0.6	44.2 ± 0.4	76.4 ± 0.4	41.9 ± 0.3
IC_{50} µg/mL $\pm$ SD ^c	17.4 ± 0.2	16.1 ± 0.3	20.3 ± 0.2	14.6 ± 0.3
TE (mg/g dry extract) $\pm$ SD ^d	683 ± 29	667 ± 31	761 ± 45	567 ± 31
Total flavonoid content (mg QE/g d.w.) $\pm$ SD ^e	92.1 ± 0.2	88.4 ± 0.2	126.4 ± 0.3	46.4 ± 0.1

GAE-gallic acid equivalent; ^bDPPH-2,2-diphenyl-2-picrylhydrazyl; ^c IC_{50} -the concentration at which a substance exerts half of its maximal inhibitory effect; ^dTE-Trolox equivalent; ^eQE -quercetin equivalent, SD-standard deviation, $p \leq 0.05$.

of flavonoids (126.4 ± 0.3 mg QE/g d.w.), followed by water-based extracts (92.1 ± 0.2 mg QE/g d.w.). Ethanolic extracts showed 88.4 ± 0.2 mg QE/g d.w., while acetic acid extracts have significantly lower flavonoid contents (46.4 ± 0.1 mg QE/g d.w.).

Through the ANOVA test it was established that there are differences in the case of the independent variable, the post hoc test demonstrating that they vary depending on the solvent used.

The scientific literature confirms the fact that *A. dracuncululus* L. contains flavonoids, as these were determined at levels of 50.40 ± 1.60 mg RE/g d.w.⁶⁵, 48.84 ± 2.04 µg rutin/mg extract²⁸, 31.90 ± 0.03 mg/g.³⁰

3. 2. Quantifying Phenolic Compounds Through HPLC Analysis

A. dracuncululus L. leaf extracts contain valuable phenolic compounds in amounts that vary depending on the solvent used. The extracts are rich in luteolin (116.20 ± 5.55 µg/g d.w. – 342.19 ± 7.78 µg/g d.w.). The methanolic extracts presents the highest concentrations. We have also identified and quantified herniarin and chlorogenic acid, at levels between 41.20 ± 0.77 µg/g d.w. and 66.81 ± 0.91 µg/g d.w., as well as 25.21 ± 0.98 µg/g d.w. and 34.14 ± 0.77 µg/g d.w. respectively. Water-based extracts were found to contain the highest amount of chlorogenic acid, followed by ethanolic extracts. Furthermore, Table 2 shows that the

extracts contain significant amounts of quercetin (6.11 ± 0.15 µg/g d.w. – 18.22 ± 0.26 µg/g d.w.), as well as kaempferol, with recorded values between 1.03 ± 0.07 µg/g d.w. and 5.22 ± 0.27 µg/g d.w. Phenolics acids were found at levels over 10 µg/g d.w. in close values for water-based, ethanolic and methanolic extracts to caffeic acid, gallic acid, and vanillic acid. Ferulic acid was determined at a maximum level of 5.12 ± 0.16 µg/g d.w. in methanolic extracts, while syringic acid in water-based extracts (13.20 ± 0.45 µg/g d.w.).

Scopoletin and 2-methoxycinnamic acid were found in all types of extracts, with highest levels in methanolic ones. Subunit values were determined in the case of compounds like apigenin, artemidine, aridiodiol present in water-based, ethanolic, and methanolic extracts.

These compounds were only found in acetic acid extracts. Traces of davidigenin and rutoside were determined in water-based extracts, while artidin and sakuranetin were not found in any of the samples under analysis. In scientific literature, flavonoids and coumarins were found by more studies, while phenolic acids were determined in various amounts, depending on the area where the plant originated, the extraction method, or the equipment used to investigate the compounds.^{65,27–31}

Through the ANOVA test it was established that there are differences in the case of the independent variable, the post hoc test demonstrating that they vary depending on the solvent used.

Table 2. Phenolic compounds identified and quantified in *A. dracuncululus* L. leaf.

Compound	Aqueous extract µg/g d.w. ^a	Ethanolic extract µg/g d.w. ^a	Methanolic extract µg/g d.w. ^a	Acetic acid extract µg/g d.w. ^a
apigenin	0.15 ± 0.01	0.21 ± 0.03	0.25 ± 0.02	n.d.
artemidine	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	n.d.
davidigenin	tr	0.04 ± 0.01	0.05 ± 0.01	n.d.
kaempferol	2.28 ± 0.16	5.14 ± 0.21	5.22 ± 0.27	1.03 ± 0.07
luteolin	278.11 ± 6.24	302.20 ± 7.21	342.19 ± 7.78	116.20 ± 5.55
rutoside	tr	0.02 ± 0.01	0.02 ± 0.01	n.d.
quercetin	18.22 ± 0.26	12.33 ± 0.51	15.81 ± 0.66	6.11 ± 0.15
artidin	n.d.	n.d.	n.d.	n.d.
aridiodiol	0.04 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	n.d.
coumarin	24.23 ± 0.34	29.00 ± 0.61	32.77 ± 0.65	20.23 ± 0.34
herniarin	45.45 ± 1.01	59.37 ± 0.87	66.81 ± 0.91	41.20 ± 0.77
scopoletin	3.05 ± 0.44	1.21 ± 0.43	3.44 ± 0.56	1.40 ± 0.22
caffeic acid	19.23 ± 1.12	19.21 ± 1.05	18.99 ± 1.02	10.21 ± 1.01
2-methoxycinnamic acid	1.29 ± 0.14	1.98 ± 0.28	2.99 ± 0.26	0.79 ± 0.06
4,5-di-o-caffeoylquinic acid	0.01 ± 0.12	0.17 ± 0.01	0.27 ± 0.01	n.d.
chicory acid	1.62 ± 0.08	0.98 ± 0.12	2.99 ± 0.15	n.d.
chlorogenic acid	34.14 ± 0.77	31.44 ± 0.75	27.26 ± 0.78	25.21 ± 0.98
p-coumaric acid	4.88 ± 0.25	3.56 ± 0.21	4.78 ± 0.28	3.84 ± 0.32
gallic acid	14.11 ± 0.32	12.00 ± 0.24	12.04 ± 0.16	10.07 ± 0.25
ferulic acid	4.99 ± 0.26	4.72 ± 0.22	5.12 ± 0.16	3.45 ± 0.29
syringic acid	13.20 ± 0.45	13.11 ± 0.54	11.21 ± 0.47	7.77 ± 0.23
sakuranetin	n.d.	n.d.	n.d.	n.d.
vanillic acid	14.22 ± 0.46	15.98 ± 0.65	16.12 ± 0.31	10.56 ± 0.11

^a data expressed as mean $\pm$ standard deviation of triplicate, tr- trace, n.d.-not detected, $p \leq 0.05$

3. 3. Determining Antibacterial Activity (MIC)

The antimicrobial activity of *A. dracunculus* L. extracts differ depending on how it is extracted, and the type of strain tested. As per Table 3, Gram-positive bacteria are more sensitive to the active biological compounds in the extracts, showing lower MIC values than Gram-negative strains. In the case of the *Clostridium perfringens* 211 strains, MIC values fell between 62.5 µg/mL for methanolic extracts, which are richer in phenolic compounds, and 250 µg/mL in the case of acetic acid extracts. *Enterococcus faecalis* 428 reacts with a MIC value of 31.25 µg/mL to methanolic extracts, 62.5 µg/mL to aqueous extracts and with a MIC value of 125 µg/mL to ethanolic and acetic acid extracts. The *S. aureus* 231 strains is the most sensitive to the antibacterial action of *A. dracunculus* L. extracts, with MIC values of 31.25 µg/mL for water-based and methanolic extracts, and 62.5 µg/mL to ethanolic ones. Gram-negative bacteria are more resistant to the antibacterial action of these extracts, recording values between ≥1000 µg/mL and ≥2000 µg/mL in the case of *E. coli* 29 and *Pseudomonas aeruginosa* 323, and 250 µg/mL and ≥1000 µg/mL in the case of *Salmonella typhimurium* 14. We benchmarked these strains with cefoxitin, which showed MIC values between 31.25 µg/mL and 125 µg/mL.

anolic extracts exhibited higher levels of polyphenols and flavonoids, despite water-based extracts being more commonly employed in traditional medicine. On the other hand, acetic acid extracts yielded the lowest results, making it an unfavorable solvent choice. These compounds contribute to the extracts' notable antioxidant activity, suggesting that tarragon could serve as a valuable source of metabolites.

Furthermore, the study demonstrated that extracts of *A. dracunculus* L. possess potent antimicrobial properties, particularly towards Gram-positive bacteria. However, their efficacy against Gram-negative bacteria was comparatively weaker. This disparity can be attributed to the structural differences in the cellular composition of these bacteria. The presence of a lipopolysaccharide layer, varying in proportions between Gram-positive and Gram-negative bacteria, likely affects the absorption of vital elements, thereby influencing the antimicrobial activity observed. LPS is the major component of the outer membrane of Gram-negative bacteria, contributing greatly to the structural integrity of the bacteria and protecting the membrane from certain types of chemical attack. Showing the above characteristics tarragon plants can be useful as a food/condiment or as an ingredient in natural supplements.

Table 3. Antimicrobial activity of four different *A. dracunculus* L. extracts (MIC, µg/mL).

Strains	Aqueous extract	Ethanolic extract	Methanolic extract	Acetic acid extract	Cefoxitin
<i>Clostridium perfringens</i> 211	125	125	62.5	250	31.25
<i>Enterococcus faecalis</i> 428	62.5	125	31.25	125	31.25
<i>Staphylococcus aureus</i> 231	31.25	62.5	31.25	125	31.25
<i>Escherichia coli</i> 29	≥2000	≥2000	≥1000	≥2000	62.5
<i>Pseudomonas aeruginosa</i> 323	≥1000	≥1000	≥1000	≥2000	125
<i>Salmonella typhimurium</i> 14	500	250	250	≥1000	62.5

The antibacterial and antifungal action of *A. dracunculus* L. extracts were analysed in more studies. These looked at the plant's activity in both solvent-based extracts and essential oils, and their results confirmed the antibacterial properties of tarragon.^{25,33,43,54} Not all bacterial strains showed sensitivity to the compounds of *A. dracunculus* L., for some studies proved lack of reactivity in the case of *E. coli* or *Yersinia enterocolitica*. MIC values were determined from 1 mg/mL to 32 mg/mL for *S. pyogenes*, *S. aureus*, *B. subtilis*, *B. cereus*, *E. coli* *P. vulgaris*, *P. aeruginosa*, getting the best results in the case of *S. pyogenes*, *S. aureus*, *B. subtilis*, (1 mg/mL, 2 mg/mL) and lowest values for *P. aeruginosa* (32 mg/mL).³⁸

4. Conclusions

The findings of this study reveal that tarragon extracts contain beneficial phenolic compounds; however, it is important to consider the extraction solvent used. Meth-

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Povzetek

Kemijsko sestavo, antioksidativne in antibakterijske lastnosti listov *Artemisia dracunculus* L. so v raziskavi proučili z uporabo štirih topil za ekstrakcijo. Ta topila so vključevala ultra čisto vodo, etanol, metanol in očetno kislino. Dosežene vrednosti skupnih polifenolov so bile med 77,2 mg ekvivalenta galne kisline (GAE)/g za ekstrakte z očetno kislino in 192,1 mg GAE/g za metanolne ekstrakte. Skupni flavonoidi so znašali 46,4 mg ekvivalenta kvercetina (QE)/g za ekstrakte očetne kisline in 126,4 mg QE/g za metanolne ekstrakte. Vrednosti IC₅₀ antioksidativne zmogljivosti, določene z metodo 2,2-difenil-2-pikrilhidrazil (DPPH), so bile med 14,66 µg/ml (izvlečki očetne kisline) in 20,33 µg/ml (metanolni izvlečki). Z metodo tekočinske kromatografije visoke ločljivosti (HPLC) je bilo identificiranih 23 fenolnih spojin. Metanolni in vodni izvleček sta imela zelo dobro antibakterijsko delovanje na seva *Staphylococcus aureus* 231 in *Enterococcus faecalis* 428. Ekstrakti listov *A. dracunculus* L. so bogati z raznolikim naborom dragocenih aktivnih kemičnih in bioloških spojin.



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