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Antimicrobial activity and component composition ethanolic extract of *portulaca oleracea* L.

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Abstract

Medicinal plants possess certain therapeutic properties including synergistic actions. Plant extracts and individual biological active compounds play a crucial role in the treatment and prevention of different diseases. Recently, much attention has been paid to research aimed at finding antimicrobial agents among medicinal plants used in folk medicine. According to the World Health Organization (WHO), the problem of antimicrobial resistance is a global threat to public health and health development. Thus, the need to create new antimicrobial agents of plant origin determines the relevance of the study. In this regard, the purpose of this study is to study the component composition and antimicrobial activity of the ethanolic extract of *Portulaca oleracea* L. The methods of Gas-Chromatography–Mass Spectrometry (GC–MS) and High-performance liquid chromatography (HPLC) were used to determine the component composition. Antimicrobial activity was studied by the method of two-fold serial dilutions using test cultures of microorganisms *Staphylococcus aureus* ATCC 6538-P, *Streptococcus pneumoniae* ATCC BAA-660, *Acinetobacter baumannii* ATCC 1790, *Bacillus subtilis* ATCC 11778, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231, and *Candida albicans* - clinical isolate. Based on the results of GC-MS analysis, 35 compounds were identified in the ethanolic extract, and organic compounds as Catechin, Epicatechin, Naringin, Phloridzin were detected by HPLC analysis. The research results showed that the ethanolic extract of *Portulaca oleracea* L. it has antimicrobial activity (Bactericidal, fungicidal, sporocidal) against all tested museum strains of microorganisms. Thus, the ultrasonic ethanolic extract of *Portulaca oleracea* L. is promising for the pharmaceutical industry, has great potential for antimicrobial activity against pathogenic bacteria.

Keywords: Antimicrobial activity, Component composition, Medicinal plant, *Portulaca oleracea* L., Ultrasonic ethanolic extract.

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1. Introduction

Phytochemistry is the study of chemical or phytochemical compounds found in plants. These compounds are mainly secondary metabolites in plants produced under the influence of biotic and abiotic stress factors. Secondary metabolites are important components in human life with different pharmacological properties [1-3].

Alkaloids have been reported to have antihyperglycemic, anti-inflammatory, anesthetic, analgesic properties; terpenoids perform antimicrobial, antitumor, antidiarrheal functions [4]. Flavonoids have antiallergic, anti-inflammatory, antioxidant, antimicrobial, antidiarrheal, antitumor, wound healing, cardioprotective, neuroprotective activity [2, 4, 5]. Fatty acids have anti-inflammatory, antioxidant, antimicrobial activity [5]. Polyphenols exhibit anti-inflammatory, antiallergic, antimicrobial, antibacterial, cardioprotective, and antioxidant effects [6]. It has been reported to be a rich source of omega-3 fatty acids, which is important for preventing heart attacks and boosting the immune system [4].

In Kazakhstan, there are more than 1400 species of wild herbs, and only 230 species are used in official medicine. More than 1000 species are used in traditional medicine for centuries. The pharmacological properties of many plants are not studied enough. This is due to the incomplete study of species of medicinal plants [7].

Resistance to antimicrobial agents is a current problem. Since, there are no new antimicrobial agents for clinical trials. Multidrug-resistant bacteria (superbacteria) spread rapidly, causing diseases that cannot be treated with conventional antimicrobials. Shortage of antibiotics and antifungal drugs has a significant impact on healthcare systems around the world, regardless of economic growth [8, 9].

Thus, one of the most promising objects for introduction into official medicine is *Portulaca oleracea* L. (Portulacaceae family) widely used in folk and official medicine in many countries [10-13]. The species *Portulaca oleracea* L. is a widespread annual herbaceous medicinal and food plant.

According to literature data, flavonoids, alkaloids, fatty acids, terpenoids, sterols, phenolic compounds, proteins, and minerals are present in ethanolic and aqueous extracts of *Portulaca oleracea* L. [14]. Scientific studies conducted in different years, confirm the antioxidant activity of the methanol extract of *Portulaca oleracea* L. by the content of total phenol, flavonoids, carotenoids [15] and the fraction of phenolic compounds from the crude extract of *Portulaca oleracea* L. [16]. Polysaccharides from this species exert antidiabetic activity [17]. and have a pronounced antitumour effect [18].

Previously, we obtained a carbon dioxide extract from the raw material of *Portulaca oleracea* L. under subcritical conditions. The constituent composition was determined, triterpenoids, phytosterols, unsaturated and polyunsaturated fatty acids, tocopherols and other biologically active compounds were identified, which significantly contribute to its therapeutic properties, such as antimicrobial and antioxidant activity [13, 19].

The use of ultrasound allows not only to significantly accelerate the production process but also to increase the extraction of the main product compared to other methods [20, 21]. Determination of 8 components of *Portulaca oleracea* herb by HPLC from 20 different locations using methanol extracts obtained by ultrasound and reverse refrigerator methods has been reported [22]. The development of a metabolic database method for *Portulaca oleracea* based on microwave extracts has also been reported [23] optimization of the conditions for ultrasonic and microwave synergistic extraction of *Portulaca oleracea* polysaccharides has a positive effect on the experimental results [24]. There are many literature reports about ultrasonic extraction of *Portulaca oleracea* based on different solvents (ethanol, methanol) [22, 24]. However, some research has focused on the study of the component composition of ultrasonic ethanolic extract of *Portulaca oleracea*, but only a few research have focused on the study of pharmacological activity.

Therefore, this study is aimed at determining the phytochemical composition of the ethanolic extract of *Portulaca oleracea* by GC-MS method, and study the antimicrobial activity.

2. Materials and Methods

2.1. Collecting of Plant Raw Materials

The aboveground parts of *Portulaca oleracea* L. was collected in the flowering phase; in the floodplain of the Talas River (Zhambyl region, South Kazakhstan). Drying of raw materials was carried out in a well-ventilated room at a temperature of +25±5 ºC. Storage of *Portulaca oleracea*'s raw materials was carried out at a temperature of +15°C -+25°C, humidity not more than 65% [25].

2.2. Obtaining an Ultrasonic Ethanolic Extract

An ultrasonic ethanolic extract of *Portulaca oleracea* herb was obtained from the aboveground part; extractant 70% (v/v) ethanol (1:10) [25].

2.3. Identification the Component Composition Method of Analysis

The analysis of the samples was performed using gas chromatography coupled with mass spectrometry (GC-MS) on an Agilent 7890A/5975C system [26]. A sample volume of 2.0 µL was injected at a temperature of 250°C in splitless mode for the analysis of the extracts. Separation was achieved using a DB-35MS capillary column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) with helium as the carrier gas at a constant flow rate of 1 mL/min. The temperature program for chromatography started at 40°C and increased at a rate of 5°C/min until reaching 280°C, where it was held for 15 minutes. The total analysis time was 63 minutes. Detection was performed in SCAN mode with a mass-to-charge (m/z) range of 34–750. Agilent MSD ChemStation software (version 1701EA) was used for system control, data acquisition, and processing. The data analysis included determining retention times, peak areas, and interpreting spectral information obtained from the mass spectrometric detector. The mass spectra were identified using the Wiley 7th edition and NIST'02 libraries, which contain over 550 000 spectra.

2.4. Analyze of Plant Extracts by HPLC

A volume of 10 µL of the extract was analyzed using the high-performance liquid chromatography (HPLC) method on a Shimadzu LC-40 liquid chromatography system. The analysis was conducted with an injection temperature of 40°C. The separation was achieved on a C18 chromatographic column (25 cm length, 4.6 mm inner diameter, and 5 µm film thickness) under isocratic conditions with a water-acetonitrile mobile phase at a constant flow rate of 1 mL/min, using varying ratios of the solvents. The Shimadzu Lab Solutions software was employed for system control, data acquisition, and processing. The data analysis included determining retention times and peak areas for the separated components.

2.5. Determination of Antimicrobial Activity Microbial Strains

Bacterial and fungal species included in the study were represented as the following reference strains: *Staphylococcus aureus* ATCC 6538-P, obtained from the Republican Collection of Microorganisms (RCM), Astana, Kazakhstan; *Streptococcus pneumoniae* ATCC BAA-660, *Acinetobacter baumannii* ATCC 1790, *Bacillus subtilis* ATCC 11778, *Escherichia coli* ATCC 8739, and *Candida albicans* ATCC 10231 obtained from American Type Culture Collection (ATCC), USA.

Media and Reagents: Nutrient agar (Himedia, India); Mueller-Hinton's agar (Himedia, India); Mueller-Hinton's broth (Himedia, India); leucophiltered red blood cell mass (Almaty City Blood Center, Kazakhstan); sodium chloride, ch.p., (Mikhailovsky plant of chemical reagents, Russia); ethanolic, 96% (Talgar Alcohol, Kazakhstan); purified water.

Method of Twofold Serial Dilutions: The test procedure of antimicrobial activity was carried out in a liquid nutrient medium, Mueller-Hinton broth (MHB) [13]. Mueller-Hinton broth with 5% blood was used for *Streptococcus pneumoniae* ATCC BAA-660. The procedure was performed in sterile 96-well polystyrene culture plates (BIOLOGIX, China). A corresponding liquid culture medium was added to the wells of the plates in an amount of 150 µL. 150 µL of *Portulaca oleracea* ultrasonic extract (antimicrobial agent) were added to the first wells of each series (A1-H1), followed by serial dilutions of 150 µL broth mixture and ultrasonic ethanolic extract of *P. oleracea* from wells 1 to wells 2, the resulting 150 µL mixture from well 2 was transferred to well 3. This was repeated until the required number of doubles was reached. From the last well, 150 µL of the mixture was removed. In this manner, serial dilutions from 1:1 to 1:2048 (76.2-0.037 µg/mL of active component) in an antimicrobial agent:broth ratio were obtained in each well row of the plate (wells A-H). The concentration of the active ingredient mixture with the intended antimicrobial effect is 30% in the initial extract solution.After preparation of the working suspension, 20 µL of inoculum was added to all wells containing 150 µL of the mixture. Thus, the final cell concentration/well after inoculation was ~1.5×10^5 CFU/mL for bacterial cells and ~1.5×10^2 CFU/mL for yeast cells. All samples were incubated for 18-24 hours at 37 ± 1°C to determine minimum inhibitory concentrations (MIC). MIC was defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation in a liquid medium. To determine the minimum bactericidal/fungicidal/ sporicidal concentration after the incubation time and MIC readings, 0.01-0.02 mL of each well of the plate was inoculated onto pre-drawn Petri dishes with an agar medium. Each cell on the dish corresponded to the order number of the well with a certain dilution. After inoculation, the Petri dishes were placed in the thermostat for 18-24 hours, and the cultivation was carried out at a temperature of 37 ± 1°C. Minimum bactericidal (MBC), fungicidal (MFC) and sporicidal concentrations were taken as the lowest concentration of the extract that completely suppressed the growth of the test microorganism on a petri dish after seeding on the appropriate medium.

Note! The *Streptococcus pneumoniae* ATCC BAA-660 was cultured in the presence of 5% CO2.

Spore Preparation: The study was conducted based on the methodology for performing spore preparation [27]. *Bacillus subtilis* ATCC 11778 vegetative cells were grown on NA (nutrient agar) at 37 °C for 24 hours. Then, an isolated colony was cultured on NA for 96 h to produce spores. Phase-contrast microscopy (Leica DM2500) was used to confirm the rate of sporulation. When the rate of sporulation reached more than 90 %, the cells were suspended, transferred into a vial and heated in a water bath (HAAKE, «Thermo Electron») at 75 °C for 10 minutes. By microscopy, it is necessary to make sure that there are no vegetative cells. A count was subsequently made to confirm that sufficient cells were obtained for the tests. A spore suspension designed to give a concentration of 1.5×10^9 spores/mL was made for the strain in the present work.

3. Results

3.1. Component Composition Ethanolic Extract of Portulaca Oleracea and Identification of Compounds by GC-MS

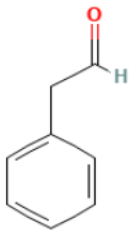
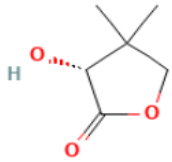
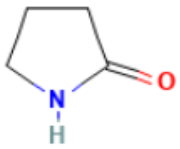
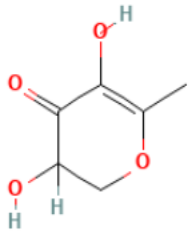
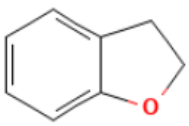
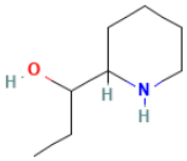
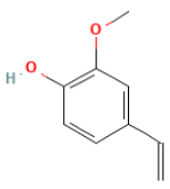
GC-MS is a proven and well-known method for the identification of component composition along with other biologically important compounds like hydrocarbons, esters, and alcohols which are present in medicinal plants [5, 28]. The component composition of ethanolic extract of *Portulaca oleracea* was investigated according to the experimental conditions described in section Material and Methods. Data for chemical compounds, relative content databases presented in Table 1.

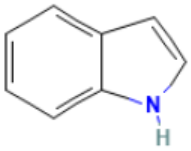
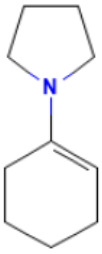
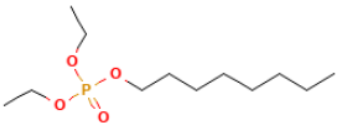
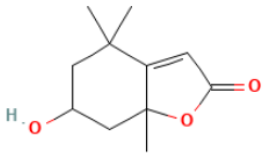
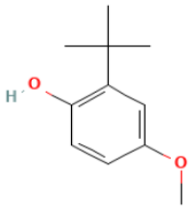
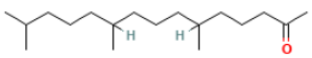
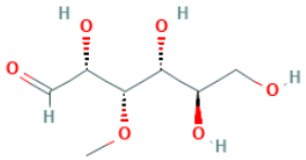
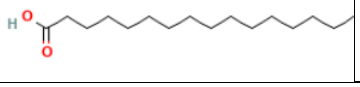
Table 1.
GC/MS analysis ethanolic extract of *Portulaca oleracea.*

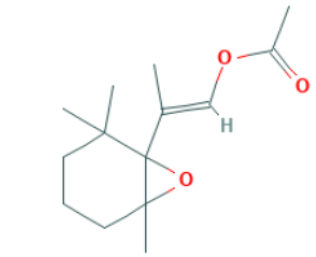
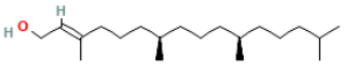
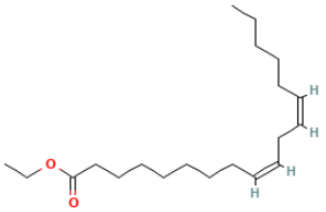
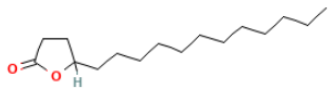
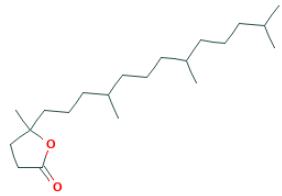
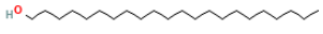
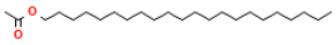
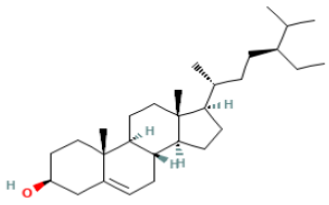
No.	Retention time (In minutes)	Compounds	Molecular formula	Main molecular Ions [m/z]	Peak area [%]
1	11.43	Benzeneacetaldehyde	C_8H_8O	91; 92; 120	5.95
2	12.08	Pantolactone	$C_6H_{10}O_3$	73; 43; 41	1.60
3	13.54	2(1H)-Pyridinone, 6-hydroxy-	$C_5H_5NO_2$	68; 40; 39	1.07
4	14.19	2-Pyrrolidinone	C_4H_7NO	85; 42; 30	1.46
5	14.41	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	43; 44; 144	1.65
6	15.32	N.N'-Bis(2,6-dimethyl-6-nitrosohept-2-en-4-one)	$C_9H_{15}NO_2$	83; 55; 29	2.12
7	15.45	Benzofuran, 2,3-dihydro-	C_8H_8O	120; 91; 119	0.89
8	17.42	Phenol, 2-amino-4-methoxy-	$C_7H_9NO_2$	139; 96; 124	0.58
9	18.63	Conhydrin	$C_8H_{17}NO$	84; 56; 85	2.80
10	19.02	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	135; 150; 107	3.46
11	19.56	Indole	C_8H_7N	117; 90; 89	1.00
12	20.89	Formicacid, 2,6-dimethoxyphenyl ester	$C_9H_{10}O_4$	154; 139; 111	1.04
13	21.49	2-Cyclopenten-1-one, 2-methyl-	C_6H_8O	67; 96; 53	0.69
14	22.13	Pyrrolidine, 1-(1-cyclohexen-1-yl)-	$C_{10}H_{17}N$	136; 165; 137	3.97
15	23.73	Phosphoric acid, diethyl octyl ester	$C_{12}H_{27}O_4P$	155; 99; 127	0.40
16	25.62	4H-Pyran-4-one, 2-methoxy-6-methyl-	$C_7H_8O_3$	69; 140; 97	1.39
17	25.81	2(4H)-Benzofuranone. 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_3$	111; 43; 109	1.07
18	26.00	Ethanediamide, n-dodecyl-N'-(2-thiazolyl)-	$C_{18}H_{20}N_2O_3$	127; 100; 128	0.62
19	26.19	3-tert-Butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$	165; 137; 180	1.05
20	26.52	2-Naphthalenamine	$C_{10}H_9N$	143; 115; 166	0.51
21	26.95	Quinoline, 2-ethyl-	$C_{11}H_{11}N$	156; 157; 129	0.66
22	28.69	1-{2-[3-(2-Acetyloxiran-2-yl)-1,1-dimethylpropyl] cycloprop-2-enyl}ethanone	$C_{14}H_{20}O_3$	43; 193; 123	7.62
23	28.84	2-Pentadecanone,6,10,14-trimethyl-	$C_{18}H_{36}O$	43; 58; 71	2.28
24	29.90	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	73; 74; 87	18.01
25	31.87	Hexadecanoicacid	$C_{16}H_{32}O_2$	43; 73; 60	4.83
26	32.35	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo [4,1,0] hept-1-yl)-propenyl ester	$C_{14}H_{22}O_3$	43; 111; 123	2.15
27	34.15	Phytol	$C_{20}H_{40}O$	71; 43; 57	13.7
28	35.57	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	67; 81; 41	1.45
29	36.19	2(3H)-Furanone, 5-dodecyldihydro-	$C_{16}H_{30}O_2$	85; 43; 55	4.06
30	39.60	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	99; 43; 55	0.80
31	40.76	Behenic alcohol	$C_{22}H_{46}O$	43; 57; 55	5.21
32	42.42	1-Docosanol, acetate	$C_{24}H_{48}O_2$	43; 57; 97	1.68
33	43.07	Phthalic acid, di (2-propylpentyl) ester	$C_{26}H_{26}O_4$	157; 149; 69	1.26
34	45.47	1,4-Benzenedicarboxylic acid,bis (2-ethylhexyl) ester	$C_{24}H_{38}O_4$	161; 70; 112	0.74
35	58.49	γ-Sitosterol	$C_{29}H_{50}O$	43; 55; 41	2.21

35 components were identified in the ethanolic extract of *Portulaca oleracea*. Compounds with pharmacological activities presented in Table 2 (According to PubMed, Wiley, PubChem, Springer, Taylor, and Elsevier databases).

Table 2.
Pharmacological activity ethanolic extracto f *Portulaca oleracea.*

No.	Compounds	Chemical structure	Activity	PubChem CID
1	Benzeneacetaldehyde		Potential agent targeting breast cancer and CSCs.	998
2	Pantolactone		Quorum sensing inhibitors	439368
3	2-Pyrrolidinone		Antioxidant and anticancer effect	12025
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-		Antioxidant and apoptotic effects, anti-diabetic, antioxidant, antibacterial, antiviral, antifungal activity [29] anticancer activity [30]	119838
5	Benzofuran, 2,3-dihydro-		Anti – infection, Anti-HIV, anti-cancer, antibacterial, antifungal activity [30]	10329
6	Conhydrin		Sedative, anticonvulsant and pain reliever	10314
7	2-Methoxy-4-vinylphenol		Anti-inflammatory effect, antioxidant antibacterial, anti-inflammatory [29] antioxidant, anticancer [29, 30]	332

No.	Compounds	Chemical structure	Activity	PubChem CID
8	Indole		Potential antibacterial activity	798
9	Pyrrolidine, 1-(1-cyclohexen-1-yl)-		Anticholinesterase activity and antioxidant properties	70768
10	Phosphoric acid, diethyl octyl ester		Enhance drug penetration through the skin	209698
11	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-		Benzfuran derivatives have biological activity as antidepressant, antitumour, antiviral, antifungal, antioxidant, antipsychotic [31]	14334
12	3-tert-Butyl-4-hydroxyanisole		Antioxidant and a human xenobiotic metabolite	8456
13	2-Pentadecanone, 6,10,14-trimethyl-		Antibacterial, anti-nociceptive and anti-inflammatory activities, anti-diabetic potential, moderate anticholinesterase activity [32]	10408
14	3-O-Methyl-d-glucose		Metabolic stability	8973
15	Hexadecanoic acid		Anti-inflammatory, antioxidant, hypocholesterolemic [33]	985

No.	Compounds	Chemical structure	Activity	PubChem CID
16	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo [4.1.0] hept-1-yl)-propenyl ester		Anti-bacterial agents	5363712
17	Phytol		Anti-infective, antimicrobial, anti-inflammatory,diuretic,anticancer [34, 35]	5280435
18	Linoleic acid ethyl ester		Anti-inflammatory agent	5282184
19	2(3H)-Furanone, 5-dodecyldihydro-		Antimicrobial Potential	97747
20	4,8,12,16-Tetramethylheptadecan-4-olide		Anticancer activities	567149
21	Behenic alcohol		Antiviral activity	12620
22	1-Docosanol, acetate		Antiviral agent	69969
23	γ-Sitosterol		Active ingredient in the product INSADOL. Hypolipidemic agents	457801

3.2. Results of HPLC Analyze

By HLPC analyze ethanolic extract of *Portulaca oleracea* were identified with standard Catechin, Epicatechin, Naringin, Phloridzin (Table 3).

Table 3.
Results of HPLC standard analyze.

No.	Compounds	Retention time (In minutes)	Area	Height of the peak	Concentration, µg/mL
1	Catechin	10.449	9750	957	0.079031
2	Epicatechin	12.489	21760	1992	0.107629
3	Naringin	17.677	47867	3828	0.153994
4	Phloridzin	19.017	14900	1794	0.166396

Ethanolic extract of *Portulaca oleracea* contained such compounds, as Catechin (0.02913 µg/ml), Epicatechin (0.01632 µg/ml), Naringin (0.00880 µg/ml), Phloridzin (0.2257 µg/ml) (Table 4).

Table 4.
Results of analyze ethanolic extract of *Portulaca oleracea*.

No.	Compounds	Retention time (In minutes)	Area	Height of the peak (H)	Concentration, µg/mL
1	Catechin	10.689	1018	95	0.02913
2	Epicatechin	12.455	1033	103	0.01632
3	Naringin	17.661	2729	216	0.00880
4	Phloridzin	18.623	5204	334	0.2257

According to the results of the analysis, the polar substances predominated in the sample compared to non-polar substances. The concentration of substances in the ethanolic extract of *Portulaca oleracea* plant is low, possibly because the total extract requires further separation. Thus, at a wavelength of 254 nm, there are more than 50 organic compounds in the sample (Figure 1).

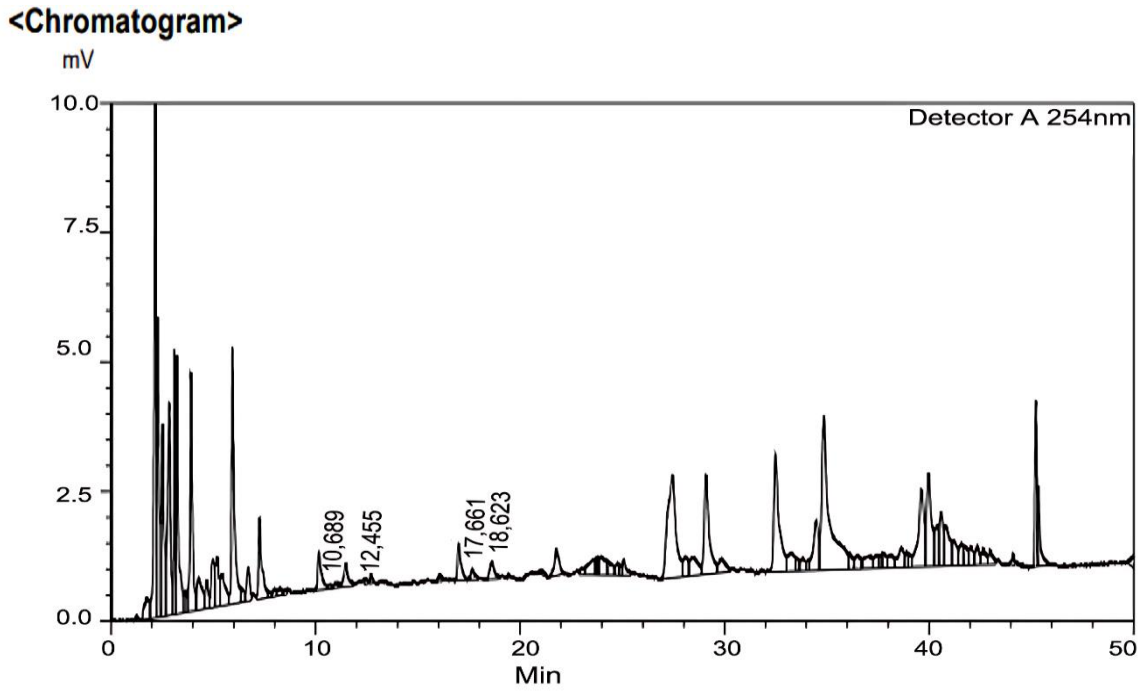


Figure 1.
The common chromatogram ethanolic extract of *Portulaca oleracea.*

Identified compound Naringin got antioxidant, anticancer activity [36] Phloridzin had hypoglycemic action [37] Catechin and Epicatechin had anticancer effect [38].

Determination of Antimicrobial Activity: The minimum inhibitory concentration (MIC), minimum bactericidal (MBC), fungicidal (MFC) and minimum sporicidal concentrations (MSC) of the ultrasonic ethanolic extract of *Portulaca oleracea* L. were determined. The findings are shown in Table 5.

Table 5.
The antimicrobial activity of the ethanolic extract of *Portulaca oleracea*.

№.	Test strain	MIC, µg/mL	MBC / MFC / MSC, µg/mL	MIC of comparison drugs, µg/mL
Non-spore-forming bacteria				
1	*S. aureus* ATCC 6538-P	38.1	76.2	3.9*
2	*S.pneumoniae* ATCC BAA-660	4.75	9.5	15.6*
3	*E.coli* ATCC 8739	19.05	38.1	62.5*
4	*A.baumannii*ATCC 1790	19.05	38.1	1000*
Yeasts				
5	*C.albicans*ATCC 10231	4.75	9.5	2.7**
Spore-forming bacteria				
6	*B.subtilis*ATCC 6633	Not defined	19.0	31.5*

Note: «*» - ampicillin, «**» - fluconazole

The results of these studies showed that all tested reference strains were sensitive to *Portulaca oleracea* ultrasonic ethanolic extract. However, the sensitivity *of these strains* varied (Table 5).

Thus, Gram-positive bacterium *S. pneumoniae* BAA-660 and *C. albicans* ATCC 10231, representing yeast species were the most sensitive to the extract. The MIC that inhibits growth of both microbial species was 4.75 µg/mL. The MBC for *S. pneumoniae* BAA-660 was 9.5 µg/ mL, resulting in MBC/MIC = 2, which indicated bactericidal activity of the extract against this bacterial species. Similarily, MFC for *C. albicans* ATCC 10231 was 9.5 µg/ mL, giving MFC/MBC = 2 pointing at fungicidal activity of the extract against this yeast species. The formal definition of a bactericidal/fungicidal agent means the ratio of MBC/MIC or MFC/MIC is ≤4, while a bacteriostatic/fungistatic agent has an MBC/MIC or MFC/MIC of >4 [39, 40].

S. aureus ATCC 6538-P, representing Gram-positive bacterium, was the most insensitive to the extract compared to other tested Gram-positive and Gram-negative bacterial strains since the MIC value was 38.1 µg/mL. The MBC was determined to be 76.2 µg/mL, which indicated a bactericidal activity of the extract (MBC/MIC = 2) against this bacterial species.

Representatives of Gram-negative bacteria, that is *A. baumannii* ATCC 1790 and *E. coli* ATCC 8739 were also included in these studies. MIC for *E. coli* ATCC 8739 was 19.05 µg/mL, while that for *A.baumannii* ATCC 1790 was found 19.05 µg/mL. MBC values for both bacterial species were at the same - 38.1 µg/mL. The MBC/MIC were in the range 2 for *E. coli* ATCC 8739, which indicated a bactericidal activity of the extract against this bacterial species. In contrast, MBC/MIC for *A.baumannii* ATCC 1790 was 2. This suggested bactericidal activity of the extract.

Moreover, the minimum sporocidal concentration inhibiting the re-growth of *B. subtilis* ATCC 6633 spores was found to be at a dilution 19.0 µg/mL (Table 5).

4. Discussion

Chromatographic methods allow identification, separation, and description of the component composition of biologically active compounds in the field of natural products and plant raw materials [2]. GC-MS analysis showed the presence of 35 components in the ultrasonic ethanolicextract of *Portulaca oleracea*, terpenoids, alcohols, polyunsaturated fatty acids and their derivatives were identified.

In a study conducted by Tamara Fukalova et al. the claim, that the analysis of volatile compounds of the methanol extract of *Portulaca oleracea* was carried out using gas chromatography and mass spectrometry (GC-MS). Fatty alcohols, benzoids, medium chain aldehydes, sesquiterpenoids, pyrazines have been found. The profile of volatile substances showed that fatty alcohols are characteristic of *Portulaca oleracea*. Aldehydes and terpenes may contribute to the antioxidant properties. Sesquiterpenoids have the ability to prevent cardiovascular and oncological diseases [12].

HPLC results showed that the ethanolic ultrasonic extract of *Portulaca oleracea* contains different types of flavonoid compounds. The HPLC analysis was performed under specific conditions and at a specific wavelength of 254 nm. Thus, not all bioactive compounds in the ethanolic ultrasonic extract of *Portulaca oleracea* were detected. Only those compounds that were separated under HPLC conditions and had optimal optical density at this wavelength were the measured compounds.

HPLC is used for the separation, identification and quantification of compounds present in extracts [41]. The range of biological active compounds values is affected by different environmental conditions in different countries such as temperature, rainfall, sunlight, soil characteristics, altitude [42] differences in harvest season, plant genetics, leaf maturity, drying and extraction methods [43].

According to research, in contrast to purified fractions, crude extracts are a complex mixture of bioactive compounds that can act singly or synergistically [44]. The therapeutic effect of extractive preparations depends not on any of the active substances but on the complex of all biologically active substances contained in it, enhancing, slowing down or changing the type of action of the main substances [45, 46].

The flavonoid apigenin isolated from ethanolic extract of *Portulaca oleracea* was reported to have antibacterial activity against *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Enterobacter aerogenes* [47]. Ethanolic crude extract of *Portulaca oleracea* showed high antimicrobial activity against strains of *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Neurospora crassa*, chloroform extract of *Portulaca oleracea* showed a moderate effect on strains of *Klebsiella pneumoniae*, *Aspergillus niger* and *Neurospora crassa* [48].

Sultan, et al. [49] reported a high inhibitory effect extract of *Portulaca oleracea* against *Staphylococcus aureus*. The authors claim that the antimicrobial activity may be due to the presence of fatty acids, which can inhibit oxygen consumption and subsequent cell death.

The results of the present study are in good agreement with the results of the above-listed authors. Moreover, the findings revealed the presence of bactericidal and fungicidal activity of the ethanolic extract of *Portulaca oleracea* against tested reference microbial strains, representing bacterial species: *Staphylococcus aureus* ATCC 6538-P, *Staphylococcus pneumoniae* ATCC BAA-660, *Escherichia coli* ATCC 8739, *Acinetobacterbaumannii* ATCC 1790 and yeast species *Candida albicans* ATCC 10231. The sporicidal activity of this extract was also shown using spores of *Bacillus subtilis* ATCC 6633.

Thus, results of investigation showed that *Portulaca oleracea* ultrasonic ethanolic extract has a pronounced antimicrobial activity.

5. Conclusion

The *in vitro* antimicrobial activity testing determined that the ultrasonic ethanolic extract of *Portulaca oleracea* had bactericidal, fungicidal and sporocidal effects, being a potential source of biocidal compounds. It is able to be concluded that the ultrasonic ethanolic extract of *Portulaca oleracea* may be a potential source for the development of new herbal medicines with antimicrobial activity. Therefore, the current direction of modern pharmacy is the development of new effective antimicrobial and antifungal drugs that will potentially have greater safety and breadth of therapeutic effect. These preliminary data show that the ultrasonic ethanolic extract of *Portulaca oleracea* is a promising source of new natural antimicrobials, as well as a possible source of new medicines. Thus, the production potential can stimulate the domestic economy ensure environmental safety, since the medicinal plant *Portulaca oleracea* it grows in a wide variety of habitats.

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