Research Article

Investigation of CO$_2$ Extract of *Portulaca oleracea* for Antioxidant Activity from Raw Material Cultivated in Kazakhstan

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Medicinal plants remain as an important resource in the fight against many diseases, especially in developing countries. Antioxidants are substances capable of delaying, retarding, and preventing the oxidation of lipids or substances that delay or prevent free radical reactions during lipid oxidation. Natural antioxidants such as ascorbic acid, tocopherol, phenolic compounds, and flavonoids are a safe alternative to chemical antioxidants. In present work, results of antioxidant activity of raw materials from the cultivated plant *Portulaca oleracea* are presented. The extraction time was optimized to 780 minutes; the yield of extractive substances was 1.25% in the production of CO$_2$ extract under subcritical conditions. For the first time, the antioxidant activity of *Portulaca oleracea* CO$_2$ extract was determined by the amperometric method. Gas chromatography-mass spectrometry (GC-MS) chemical analysis of *Portulaca oleracea* CO$_2$ extract dissolved in hexane revealed 37 components, including a complex mixture of aldehydes, alkanes, alkenes, esters, diterpenes, steroids, vitamin E, and carbohydrates. The investigation results showed that the *Portulaca oleracea* CO$_2$ extract was promising for pharmaceutical, cosmetic, and food industries and had great potential for the prevention and treatment of diseases caused by oxidative stress.
1. Introduction

In the fight against diseases, medicinal plants still remain their importance and are a promising source of medicines, especially in developing countries [1]. About 3.4 billion of people use herbal medicines. Natural products were the integral parts of the ancient system of traditional medicine. According to the World Health Organization (WHO), medicinal plant is any plant which contains substances used for therapeutic purposes [2, 3].

Natural antioxidants are the interest for medicine among different biological activities. Antioxidants are important substances produced in the organisms to suppress oxidative stress. They can be divided into enzymatic and nonenzymatic ones. Oxidative stress is associated with a large number of lifestyle-related diseases, such as cardiovascular disease, cancer, diabetes, and aging. Excessive oxidative stress can lead to oxidation of biomolecules, which is accompanied by cell damage and intervenes in the pathogenesis of many human diseases [4].

Antioxidants are able to interact with free radicals to stop the chain reaction without damaging vital molecules [5]. These are compounds capable of delaying, retarding, and preventing the oxidation of lipids or substances that delay or prevent free radical reactions during lipid oxidation. Natural antioxidants such as ascorbic acid, tocopherol, phenolic compounds, and flavonoids are a safe alternative to chemical antioxidants [6, 7].

Currently, there are different methods for evaluating the antioxidant activity of compounds. So, electrochemical and spectrometric methods are widely used, as they are characterized by high sensitivity and the ability to analyze quickly. When using amperometric detection, compounds containing hydroxyl groups are well oxidized, and the determination level of polyphenols and flavonoids is $10^{-9}$–$10^{-12}$ grams. The amperometric method allows measuring the amount of all antioxidants in the sample, which makes this investigation method more accurate [8–12].

It is noted that the therapeutic effect of extractive preparations does not depend on one active substance, but on the complex of all biologically active substances contained in it, enhancing, slowing down, or changing the type of action of basic substances [13].

Previous phytochemical studies [14–17] indicate that the medicinal plant *Portulaca oleracea* L. contains terpenoids, alkaloids, flavonoids, organic acids, minerals, and vitamins. This indicates the potential for antioxidant activity.

In our study, for the first time, the antioxidant activity of *P. oleracea* CO2 extract was determined by the amperometric method.

2. Materials and Methodology

2.1. Plant Material and *Portulaca oleracea* CO2 Extract.

Raw material: the above-ground part of the cultivated plant *P. oleracea* was collected in 2-3 decades of August, 2020, in flowering phase. The place of collecting was N 42°52′07.8″; E 71°20′42.8″ (Zhambyl region, Kazakhstan). The plant samples were identified by specialists of the Institute of Botany and Phytointroduction (Almaty city); typical specimen is stored in the herbarium fund of this institute.

Extraction: for the first time, CO2 extract from the above-ground part of the wild plants of *Portulaca oleracea* was obtained under precritical conditions, namely, pressure was 45–52 atmospheres, temperature was 19–22°C, extraction time was 540 minutes, and yield was 0.7%, as well as its component composition [18].

The extraction parameters of the cultivated plant *P. oleracea* were compared, which made it possible to recommend a change in the extraction time. Crushed air-dried raw materials (stems, leaves, and flowers) were extracted under pressure of 45–52 atmospheres, temperature of 19–22°C, extraction time of 780 minutes, and yield of 1.25%. The component composition was determined for the new extract.

2.2. The Determination of CO2 Extract Component Composition of *Portulaca oleracea* Cultivated Raw Material. CO2 extract of *Portulaca oleracea* and the different fractions from CO2 extract of *Portulaca oleracea* were injected using the 10 μL Agilent syringe into the sample injection device of a Gas Chromatograph 7890A (Agilent, USA) coupled with a mass spectrometric detector 5975°C (Agilent, USA) in split mode- 10:1, injected sample volume- 1.0 μL and inlet temperature- 250°C. Chromatography was performed using a DB-35MS capillary column with a length of 30 m, an inner diameter of 0.25 mm, and a film thickness of 0.25 μm (Agilent, USA). The carrier gas Helium (>99.995%, Orenburg-Tehgas, Russia) was supplied at a constant rate of 1.0 mL min$^{-1}$.

The temperature of the column thermostat was programmed from 50°C (holding time 1 min) to 270°C (holding time 15 min) with a heating rate of 5°C min$^{-1}$. The analysis time was 60 minutes. The MSD interface temperature was 320°C, the temperature of the quadrupole was 180°C, and the ion source temperature was 230°C. It is detected in the ion-scanning mode in the range of mass numbers m/z 34–750 a.m.u.

Agilent MSD ChemStation software (version 1701EA) was used to control the gas chromatograph system and the system for recording and processing chromatographic data. The data processing included the determination of the retention times of the substance; peak areas as well as the processing of the spectral information obtained using the mass spectrometric detector. Mass spectra were identified applying the Wiley 11th edition and NIST’02 [18].

2.3. The Extraction of Biological Active Substances by Fractionation of *Portulaca oleracea* CO2 Extract. Fractions analysis of *P. oleracea* CO2 extract was carried out on a chromatograph. Sample preparation: fractionation was carried out on a silica gel column. Name of samples: 1st fraction - hexane, 2nd fraction - dichloromethane, 3rd fraction- ethyl acetate, 4th fraction - methanol. Gas chromatography with mass spectrometric detection (Agilent 7890 A/5975°C) was used as a method of analysis.
2.4. Evaluation of Antioxidant Activity of Portulaca oleracea CO₂ Extract. The investigation was carried out on the basis of the technique for measuring the total content of fat-soluble antioxidants in food [19], drinks and food products, dietary supplements, and extracts of medicinal plants [20] by the amperometric method using the "Tsvet Yauza 01-AA" developed by "Khimavtomatika," the scientific and production association (Moscow, Russia). Gallic acid was the standard for fat-soluble antioxidants; the range of determination was 0.00125–25 mg of gallic acid/g (%). Acetone acidified with phosphoric acid was used as an eluent. Eluent preparation and calibration of gallic acid solutions with a mass concentration of 0.1, 0.2, 0.4, 1.0, and 2.0 mg/dm³ were carried out according to the certified method.

Quercetin served as a standard for water-soluble antioxidants; the range of determination was 0.2–4000 mg of quercetin/dm³. 70% ethyl alcohol was used as an eluent. Preparation of eluent and calibration solutions of quercetin with a mass concentration of 0.2, 0.5, 1.0, 2.0, and 4.0 mg/dm³ were carried out according to the certified method.

2.5. Method for Determining Antioxidant Activity by the FRAP Method. 0.25 ml of 0.2 M phosphate buffer (pH = 6.6) and 0.25 ml of 1% solution of potassium hexacyanoferrate (III) were added to 0.1 ml of the test substances in the concentration range of 0.25, 0.5, 0.75, and 1.0 mg/ml [21, 22].

The reaction mixture was incubated for 20 minutes at 50°C; the reaction was stopped by adding 0.25 ml of 10% trichloroacetic acid solution. The mixture was centrifuged for 10 minutes (3000 rpm). The upper layer with a volume of 0.5 ml was mixed with 0.5 ml of distilled water and 0.1 ml of 0.1% FeCl₃. The optical density was measured at 700 nm. The antioxidant activity (AOA) of the samples was compared with the AOA of ascorbic acid (AA).

Dilution was made at the rate of 1 mg of substance per 1 ml of solvent. Each sample was tested in three parallel runs. It was carried out at a temperature of 20 ± 2°C, natural light period.

3.3. Antioxidant Activity of Portulaca oleracea CO₂ Extract

3.3.1. Amperometric Method. Determination of the antioxidants sum composition by the amperometric method is based on measuring the electric current caused by oxidation of the antioxidant molecule on the surface of the working electrode at a certain potential, which is converted into a digital signal. Output signals are displayed on the computer screen as peaks. The magnitude of the electric current depends on the nature and concentration of the test substance, the type and material of the working electrode, and the potential applied to the electrode.

The content of antioxidants in the studied samples of the Portulaca oleracea CO₂ extract was calculated in units of the quercetin and gallic acid concentration. The content of water-soluble antioxidants is 35.5386 ± 0.1457 mg/g, and the content of fat-soluble antioxidants is 34.8361 ± 0.0488 mg/g.

The reliability of the correlation coefficient was determined for water-soluble antioxidants r_water = +0.999, p < 0.001 and for fat-soluble antioxidants r_fat = +0.994, p < 0.001. The relationship between concentration and peak area is direct, strong, and reliable, which indicates a high reliability of the approximation.

3.3.2. FRAP Method (Ferric Reducing Antioxidant Power Assay). The FRAP method (ferric reducing antioxidant power assay) is based on the reduction of Fe³⁺ ions to Fe²⁺ by antioxidants. The reduction reaction of K₃[Fe(CN)₆] with antioxidants is used, which is accompanied by the formation of a yellow-colored compound, namely, K₃[Fe(CN)₆]. The measurements are based on the ability of antioxidants to suppress the oxidative effect of reaction particles generated in the reaction mixture. Ascorbic acid was used as a reference drug. Samples were tested at concentrations of 0.25, 0.5, 0.75, and 1 mg/ml (Table 6).

Based on the data analysis, it can be seen that the Portulaca oleracea CO₂ extract at concentrations from 0.25 to 1 mg/ml has a low antioxidant activity compared to the standard solution of ascorbic acid.
Earlier, 41–66 components were found, when studying the component composition of the *Portulaca oleracea* carbon dioxide extract from the raw material of a wild plant. Triterpenoids 6.62%–30.72%, tocopherols 1.46–3.41%, fatty acids 11.31–34.11%, and terpenoids 3.22%–7.07% made up the sum of the main compounds of chromatographic analysis by classes [18]. The component composition of the *Portulaca oleracea* carbon dioxide extract from the raw material of the cultivated plant included 37 components. The most known pharmacologically bioactive compounds of the therapeutic value are given in Tables 7 and 8.

Antioxidant properties of *Portulaca oleracea* are associated with biologically active substances such as gallotannin, omega-3 fatty acids, ascorbic acid, tocopherol, kaempferol, quercetin, and apigenin [29, 30].

Using a simple, fast, and affordable single cell electrophoresis method to measure DNA fiber breakage, the results showed that an aqueous extract of *Portulaca oleracea*, in contrast to ethanol extract, had a high ability to reduce oxidative damage caused by high levels of fat by modulating the activity of antioxidant enzymes [14].

The antioxidant activity of the *Portulaca oleracea* medicinal plant material was determined at the plant maturation stages. It was determined by the reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH), by reducing properties of iron extracts (FRAP) and by the amount of ascorbic acid.

| Table 1: The results of chromatographic analysis of *Portulaca oleracea* CO₂ extract. |
|---|
| **No.** | **Retention time (min)** | **Compound** | **Identification probability (%)** | **Percentage (%)** | **Groups of biological active compounds** |
| 1 | 11.0 | 4-Cyclopentene-1, 3-dione | 84 | 0.79 | Ketone |
| 2 | 12.6 | 4-Hydroxy-butyric acid | 93 | 0.46 | Butyrolactone |
| 3 | 12.8 | 1-Butene, 4-isothiocyanato- | 85 | 0.32 | Isothiocyanic acid, 3-butenyl ester |
| 4 | 13.5 | 1-(1’-Pyrolidinyl)-2-propanone | 95 | 0.99 | Ketone |
| 5 | 14.6 | 1-Amino-2,6-dimethylpiperidine | 67 | 0.27 | Piperidine derivative |
| 6 | 15.8 | 2,5-Dimethyl-4-hydroxy-3(2H)-furanone | 77 | 0.26 | Ketone |
| 7 | 16.9 | 4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl- | 90 | 2.44 | Pyran derivative |
| 8 | 17.5 | Benzyl nitrile | 93 | 1.21 | Benzene derivatives |
| 9 | 18.5 | Benzofuran, 2,3-dihydro-1- | 80 | 1.06 | Benzofuran derivatives |
| 10 | 20.4 | 2-Methoxy-4-vinylphenol | 93 | 2.07 | Phenol |
| 11 | 20.7 | Pyrazine, 2-ethyl-5-methyl- | 85 | 0.96 | Pyrazine derivative |
| 12 | 22.8 | Pyrrolidine, 1-(1-cyclohexen-1-yl)- | 71 | 0.94 | Pyrrolidine derivative |
| 13 | 23.2 | 4-(2, 6, 6-Trimethylcyclohexa-1, 3-dienyl)but-3-en-2-one | 78 | 0.39 | Steroid |
| 14 | 25.1 | Sucrose | 72 | 10.85 | Carbohydrate |
| 15 | 26.0 | Phosphonofluoridic acid, (1-methylethyl)-, cyclohexyl ester | 70 | 1.43 | Ester |
| 16 | 26.2 | 3’, 5’-Dimethoxyacetophenone | 76 | 1.85 | Ketone |
| 17 | 26.9 | Megastigmatrinone | 75 | 0.21 | Steroid |
| 18 | 27.4 | 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol | 80 | 1.31 | Alcohol |
| 19 | 29.0 | Imidazolo [1,2-a] pyrimidine-2,5(1H,3H)-dione, 3,7-dimethyl-4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol | 65 | 0.36 | Ketone |
| 20 | 30.6 | Benzoic acid, 4-hydroxy-3, 5-dimethoxy-, hydrazi | 91 | 2.21 | Heterocyclic compound |
| 21 | 31.2 | Phthalic acid, isobutyl octadecyl ester | 64 | 3.03 | Ester |
| 22 | 31.5 | Octahydro-2H-quinolinone | 66 | 1.70 | Ketone |
| 23 | 33.8 | Phyto | 76 | 5.80 | Diterpene |
| 24 | 34.1 | Linoleic acid ethyl ester | 72 | 0.69 | Ester |
| 25 | 35.6 | Desulphosinigrin | 70 | 16.96 | Carbohydrate |
| 26 | 36.6 | 5, 10-Diethoxy-2, 3, 7, 8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1’,2’-d]pyrazine | 71 | 0.47 | Pyrazine derivative |
| 27 | 39.0 | Henecicosane | 87 | 0.81 | Alkane |
| 28 | 40.6 | Heptacosane | 80 | 0.68 | Alkane |
| 29 | 41.4 | 2-Methyhexacosane | 84 | 1.02 | Alkane |
| 30 | 42.1 | Hexacosane | 93 | 20.67 | Alkane |
| 31 | 43.6 | Octacosene | 91 | 1.85 | Alkane |
| 32 | 46.4 | Tetratetracontane | 79 | 1.31 | Alkane |
| 33 | 48.7 | Octacosanol | 74 | 2.67 | Alcohol |
| 34 | 50.9 | Vitamin E | 95 | 6.42 | Derivative of tocol |
| 35 | 54.7 | β-Sitosterol | 90 | 3.78 | Steroid |
| 36 | 55.3 | Phytol, acetate | 70 | 0.96 | Ester |
On the basis of the obtained results, it was concluded that the total phenol content and antioxidant activity in mature Portulaca oleracea plants were higher than in immature plant stages [31].

The antioxidant activity of methanol extracts of various parts of the Portulaca oleracea and Portulaca grandiflora plants was studied using DPPH. For the first time, it was found that the Portulaca oleracea root had effective antioxidant activity [32].

According to Naciye Erkan, using TBARS analysis, it was shown that the fraction of Portulaca oleracea extract with the highest total quantitative content of phenolic compounds

Table 2: Chromatographic analysis of the hexane fraction of Portulaca oleracea CO₂ extract.

| No. | Retention time (min) | Compound Identification probability (%) | Percentage (%) |
|-----|----------------------|----------------------------------------|----------------|
| 1   | 12.7                 | 2, 4-Heptadienal                         | 89             | 0.96 |
| 2   | 14.4                 | Nonanal                                 | 91             | 0.22 |
| 3   | 15.8                 | 2, 5-Furandione, 3-(1,1-dimethyl)-     | 88             | 0.27 |
| 4   | 16.1                 | Cyclohexanol, 1-methyl-4-(1-methyl)-   | 83             | 0.54 |
| 5   | 16.3                 | Octanoic acid                           | 90             | 0.92 |
| 6   | 16.4                 | Cyclodecene                             | 73             | 0.24 |
| 7   | 18.2                 | Nonanoic acid                           | 87             | 1.45 |
| 8   | 18.7                 | Tetradecane                             | 85             | 0.31 |
| 9   | 19.1                 | 2, 4-Decadienal                         | 87             | 0.67 |
| 10  | 19.4                 | Alfa-copaene                            | 93             | 0.26 |
| 11  | 20.0                 | Decanoic acid                           | 72             | 1.44 |
| 12  | 20.6                 | Caryophyllene                           | 93             | 0.43 |
| 13  | 21.0                 | Undecanoic acid                         | 84             | 0.40 |
| 14  | 21.9                 | 5,9-Undecadien-2-one, 6,10-dimethyl-   | 85             | 0.38 |
| 15  | 22.5                 | Hexadecane                              | 90             | 0.33 |
| 16  | 22.8                 | y-Muurolene                             | 73             | 0.30 |
| 17  | 23.0                 | trans-β-Ionone                           | 90             | 0.28 |
| 18  | 23.2                 | 3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxa- | 74             | 0.24 |
| 19  | 23.2                 | Vanillin                                | 75             | 0.19 |
| 20  | 24.0                 | Nonanoic acid, 9-oxo-, ethyl ester     | 85             | 0.31 |
| 21  | 24.4                 | Heptadecane                             | 82             | 0.36 |
| 22  | 25.6                 | Tridecanoic acid                        | 83             | 0.28 |
| 23  | 26.0                 | 2(4H)-Benzo[1,2-b:5,6-b′]dioxin, 5, 6, 7, 7a-tetrahydro-4,4,7a-trimethyl-(R)- | 74             | 0.82 |
| 24  | 26.1                 | Hexadecane, 2,6,10,14-tetramethyl-     | 69             | 0.15 |
| 25  | 26.3                 | Octadecane                              | 89             | 0.25 |
| 26  | 26.5                 | Hexadecanal                             | 82             | 0.28 |
| 27  | 27.4                 | 3,7,11,15-Tetramethyl-2-hexadecan-1-ol | 84             | 0.45 |
| 28  | 28.3                 | Nonadecane                              | 89             | 0.34 |
| 29  | 28.6                 | 2-Pentadecanone, 6, 10, 14-trimethyl-   | 93             | 2.87 |
| 30  | 29.9                 | Benzoic acid, undecyl ester            | 66             | 0.30 |
| 31  | 30.3                 | Hexadecanoic acid, methyl ester       | 75             | 0.39 |
| 32  | 30.7                 | 1,4-Naphthalenedione, 2, 3, 6-trimethyl-| 71             | 0.50 |
| 33  | 31.1                 | 5, 9, 13-Pentadecatrien-2-one, 6, 10, 14-trimethyl-, (E,E)- | 87             | 0.57 |
| 34  | 31.2                 | Benzoic acid, 4-hydroxy-3, 5-dimethyl-, hydrazide | 86             | 0.35 |
| 35  | 33.9                 | Phytol                                  | 94             | 11.87 |
| 36  | 34.4                 | p-Octylacetophenone                     | 68             | 1.96 |
| 37  | 35.1                 | Ethyl oleate                            | 91             | 5.73 |
| 38  | 38.6                 | Methyl 19-methyl-eicosanoate           | 68             | 2.69 |
| 39  | 39.3                 | 4,8,12,16-Tetramethylheptadecan-4-olide | 86             | 1.53 |
| 40  | 41.8                 | Docosanoic acid, ethyl ester           | 75             | 0.97 |
| 41  | 42.6                 | 13-Methylheptacosane                    | 74             | 1.59 |
| 42  | 49.9                 | γ-Tocopherol                            | 60             | 2.56 |
| 43  | 51.0                 | Vitamin E                               | 93             | 17.62 |
| 44  | 52.0                 | Phytol, acetate                         | 84             | 2.83 |
| 45  | 53.2                 | Campesterol                             | 91             | 4.54 |
| 46  | 53.7                 | Stigmasterol                            | 83             | 2.86 |
| 47  | 54.9                 | y-Stigmasterol                          | 91             | 20.65 |
| 48  | 55.4                 | Phytol, acetate                         | 85             | 4.63 |
had antioxidant activity with the highest rate of lipid peroxidation suppression [33]. The antioxidant activity of aqueous and ethanol extracts of stems and leaves of the Tunisian species of *Portulaca oleracea* was determined using free radical discoloration ABTS, the reducing properties of Fe³⁺ extracts, and phosphomolybdenum analysis [34].

There were carried out the study of the antioxidant activity of methanol, ethanol, and aqueous extracts of *Portulaca oleracea* using such methods as FRAP [31, 34], DPPH [31, 32], phosphomolybdenum [34], TBARS [33], and single cell electrophoresis [14].

The study of the antioxidant activity of extracts from plant materials is of interest to scientists. The authors Syeda A.M. and Riazunnisa K. report on the determination by gas chromatography-mass spectrometry (GC-MS) of the component composition of aqueous and methanolic extracts of Madagascar periwinkle (*Catharanthus roseus*) and drumstick tree (*Moringa oleifera*) and found their antioxidant activity [35]. On the basis of plant extracts, a technology of multifunctional film has been developed [36, 37], which has the prospect of application in the pharmaceutical and food industries.

**Figure 1**: The ratio of the groups of biologically active substances of the hexane fraction of the *Portulaca oleracea* CO₂ extract.

**Table 3**: Chromatographic analysis of the dichloromethane fraction of the *Portulaca oleracea* CO₂ extract.

| No. | Retention time (min) | Compound | Identification probability (%) | Percentage (%) |
|-----|----------------------|----------|--------------------------------|----------------|
| 1   | 26.0                 | 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- | 78          | 1.00            |
| 2   | 27.4                 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 81          | 0.44            |
| 3   | 27.7                 | Tetradecanoic acid, ethyl ester | 89          | 0.99            |
| 4   | 28.5                 | 3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)- | 73          | 0.77            |
| 5   | 28.6                 | 2-Pentadecanone, 6,10,14-trimethyl- | 92          | 2.33            |
| 6   | 33.8                 | Phytol | 94          | 15.32           |
| 7   | 34.3                 | p-Octylacetophenone | 67          | 2.52            |
| 8   | 35.0                 | Ethyl oleate | 88          | 7.15            |
| 9   | 38.1                 | Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester | 65          | 0.62            |
| 10  | 38.6                 | Methyl 19-methyl-eicosanoate | 68          | 1.36            |
| 11  | 39.2                 | 4,8,12,16-Tetramethylheptadecan-4-olide | 90          | 1.97            |
| 12  | 43.2                 | Hexacosane, 9-octyl- | 80          | 4.18            |
| 13  | 45.2                 | Squalene | 86          | 5.73            |
| 14  | 49.9                 | γ-Tocopherol | 66          | 3.28            |
| 15  | 50.9                 | Vitamin E | 95          | 21.22           |
| 16  | 53.2                 | Campesterol | 84          | 4.51            |
| 17  | 54.7                 | γ-Sitosterol | 92          | 22.75           |
| 18  | 55.3                 | Phytol, acetate | 75          | 3.86            |
Table 4: Chromatographic analysis of the ethyl acetate fraction of the *Portulaca oleracea* CO₂ extract.

| No. | Retention time (min) | Compound                                    | Identification probability (%) | Percentage (%) |
|-----|----------------------|---------------------------------------------|-------------------------------|----------------|
| 1   | 28.61                | 2-Pentadecanone, 6,10,14-trimethyl-         | 89                            | 2.71           |
| 2   | 29.90                | Benzoic acid, hept-2-yl ester              | 64                            | 1.90           |
| 3   | 30.36                | Benzoic acid, pentadecyl ester             | 78                            | 4.05           |
| 4   | 31.43                | Hexadecanoic acid                           | 85                            | 9.46           |
| 5   | 31.85                | Methyl 8,11,14-heptadecatrienoate          | 82                            | 2.15           |
| 6   | 33.76                | Phytol                                      | 67                            | 10.85          |
| 7   | 34.99                | Ethyl oleate                                | 88                            | 7.14           |
| 8   | 50.92                | DL-α-Tocopherol                             | 86                            | 29.69          |
| 9   | 53.17                | Campesterol                                 | 74                            | 5.32           |
| 10  | 54.68                | γ-Sitosterol                                 | 88                            | 26.73          |

Figure 2: The ratio of the main groups of biologically active substances of the *Portulaca oleracea* dichloromethane fraction.

Figure 3: The ratio of the main groups of biologically active compounds of the *Portulaca oleracea* ethyl acetate fraction.
Figure 4: The ratio of the main groups of biologically active compounds of the *Portulaca oleracea* methanol fraction.

### Table 5: Chromatographic analysis of the methanol fraction of the *Portulaca oleracea* CO₂ extract.

| No. | Retention time (min) | Compound | Identification probability (%) | Percentage (%) |
|-----|----------------------|----------|--------------------------------|----------------|
| 1   | 33.8                 | Phytol   | 85                             | 3.6            |
| 2   | 35.0                 | Ethyl oleate | 71                             | 2.7            |
| 3   | 39.0                 | Hexacosane | 84                             | 4.1            |
| 4   | 41.4                 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | 65                             | 3.3            |
| 5   | 45.0                 | Octacosane | 92                             | 53.4           |
| 6   | 48.7                 | Hentriacontane | 80                             | 7.3            |
| 7   | 50.9                 | Vitamin E  | 89                             | 13.8           |
| 8   | 54.7                 | γ-Sitosterol | 80                             | 11.8           |

### Table 6: Change in the optical density of solutions depending on the working solutions concentration.

| No. | Samples                  | Optical density value at concentration (mg/ml) |
|-----|--------------------------|-----------------------------------------------|
|     |                          | 0.25  | 0.5   | 0.75  | 1.0   |
| 1   | Ascorbic acid (AA)       | 1.5539| 1.5928| 1.6775| 1.7738|
| 2   | CO₂ extract of *Portulaca oleracea* (CO₂ P. oleracea) | 0.1314| 0.2659| 0.3878| 0.7519|

### Table 7: Pharmacologically active compounds of the therapeutic value from the *Portulaca oleracea* CO₂ extract.

| No. | Compound                  | Percentage (%) | Pharmacologic effect                                                                 |
|-----|---------------------------|----------------|--------------------------------------------------------------------------------------|
| 1   | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 2.44           | Antidiabetic, antioxidant, antibacterial, anti-inflammatory, antifungal activity [23], and anticancer activities [24] |
| 2   | Benzofuran, 2,3-dihydro-  | 1.06           | Anti-HIV, anticancer, antibacterial, and antifungal activities [24]                    |
| 3   | 2-Methoxy-4-vinylphenol   | 2.07           | Antibacterial, anti-inflammatory [23], antioxidant, and anticancer activities [23, 24] |
| 4   | Desulphosinigrin          | 16.96          | Antioxidant activity [24]                                                             |
| 5   | Vitamin E                 | 6.42           | Antioxidant and anti-inflammatory activities [24]                                    |
Table 8: Pharmacologically active compounds of therapeutic value (hexane, dichloromethane, ethyl acetate, and methanol fraction) of *Portulaca oleracea* CO₂ extract.

| No. | Compound                                      | Hexane fraction | Dichloromethane fraction | Ethyl acetate fraction | Methanol fraction | Pharmacologic effect                                                                 |
|-----|-----------------------------------------------|-----------------|--------------------------|------------------------|------------------|-------------------------------------------------------------------------------------|
|     |                                               | Percentage (%)  | The presence or absence of an ingredient | Percentage (%)        | The presence or absence of an ingredient | Percentage (%)        | The presence or absence of an ingredient | Pharmacologic effect                                                                 |
| 1   | Vitamin E                                     | 17.62           | +                        | 21.22                  | +                | -                                     | -                                     | 13.8                  | +                                        | Antioxidant and anti-inflammatory activities [24]. |
| 2   | Decanoic acid                                 | 1.44            | +                        | -                      | -                | -                                     | -                                     | -                     | -                                        | Triglyceride. Antibacterial, anti-inflammatory, anticancer, and antioxidant activities [23]. |
| 3   | 2-Pentadecanone, 6,10,14-trimethyl             | 2.87            | +                        | 5.33                   | +                | 2.71                                  | +                                     | -                     | -                                        | Antidiabetic potential and moderate anticholine esterase activities [25]. |
| 4   | γ-Tocopherol                                   | 2.56            | +                        | 3.28                   | +                | -                                     | -                                     | -                     | -                                        | Anti-inflammatory property [24]. |
| 5   | Campesterol                                    | 4.54            | +                        | 4.51                   | +                | 5.32                                  | +                                     | -                     | -                                        | Antimicrobial and antioxidant activities [24]. |
| 6   | Stigmasterol                                   | 2.86            | +                        | -                      | -                | -                                     | -                                     | -                     | -                                        | Phytosterols. Sterols have the ability to lower cholesterol levels. It is also effective in cancer prevention [26]. |
| 7   | γ-Sitosterol                                   | 20.65           | +                        | 22.75                  | +                | 26.73                                 | +                                     | 11.8                  | +                                        | Antimicrobial and antioxidant activities [24]. Clinosterol is a class of phytosterols, a triterpenoid. Sterols have the ability to lower cholesterol levels. It is also effective in preventing cancer [26]. |
| 8   | 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- | -               | -                        | 1.00                   | +                | -                                     | -                                     | -                     | -                                        | Benzofuran derivatives have biological activity as an antidepressant, antitumor, antiviral, antifungal, antioxidant, antipsychotic agent [27]. |
| 9   | Squalene                                       | -               | -                        | 5.73                   | +                | -                                     | -                                     | -                     | -                                        | Triterpenoid. Antioxidant, hypolipidemic, and antitoxic effects [28]. |
| 10  | Hexadecanoic acid 9,46                        | -               | -                        | -                     | -                | 9.46                                  | +                                     | -                     | -                                        | Triglyceride. Antitumor and antihistaminic properties [23]. |
| 11  | DL-α-Tocopherol                                | -               | -                        | -                     | -                | 29.69                                 | +                                     | -                     | -                                        | Antioxidant and anti-inflammatory activities [24]. |

-, absence of ingredient; +, presence of ingredient.
4. Conclusions

For the first time, there has been revealed the antioxidant activity of Portulaca oleracea CO₂ extract from raw materials cultivated in Kazakhstan. The results of the study included determination of the composition of the sum of antioxidants in the Portulaca oleracea CO₂ extract by the amperometric method. The composition concentration of the combination of fat-soluble and water-soluble antioxidants was established. The investigation results of antioxidant activity by the FRAP method allow us to conclude that the Portulaca oleracea CO₂ extract in concentrations of 0.25–1 mg/ml has an antioxidant activity, which turned out to be lower than that of ascorbic acid, but promising for the pharmaceutical, cosmetic, and food industries.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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