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STUDY OF THE CHEMICAL COMPONENTS OF CO2 EXTRACTS FROM THE FRUITS OF *SORBUS AUCUPARIA* L.

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The article presents the results of the study of the chemical composition of Sorbus aucuparia L. CO_2 subcritical extract. For the first time in Kazakhstan, 20 grams of brown Sorbus aucuparia L. extract were collected using subcritical carbon dioxide extraction. The current study was directed mainly to the chemical compositions of subcritical Sorbus aucuparia L. CO_2 subcritical extract.

The Sorbus aucuparia L. extract's chemical compositions were determined using gas chromatography/mass spectrophotometry (GC-MS). The extract included the following main compounds: 5-Methyl-2(3H)-furanone (30.18 %), 5-(3-Ethoxy-4,5-dihydro-isoxazol-5-yl)-5-methyl-imidazolidine-2,4-dione (3.20 %), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (2.53 %). Determined fatty acid profile and the moisture content of raw vegetable ingredients of Sorbus substance.

Quantitative determination of fatty acids of ethanol extract was carried out. The results of the analysis for fatty acids in the study showed that linoleic (37.7 %) and oleic (50.5 %) were the most prominent fatty acids.

***The aim** of this study is to determine the component composition by using the GC-MS method and fatty acid to study the Sorbus aucuparia L. extract obtained by CO_2 extraction, which grows in Kazakhstan.*

***Materials and methods.** To determine the possibility of using Sorbus aucuparia L., we carried out the composition and fatty acid of the extract obtained by CO_2 extraction in subcritical conditions of Sorbus aucuparia L. by a certain GC-MS method.*

***Results**. The raw materials of the plant were collected in accordance with GACP requirements. Conducted subcritical CO_2 extraction of plant raw materials showed a 20 g extraction yield. Chemical compounds were discovered, bioactive components were identified, such as 5-Methyl-2(3H)-furanone (30.18 %), 5-(3-Ethoxy-4,5-dihydro-isoxazol-5-yl)-5-methyl-imidazolidine-2,4-dione (3.20 %), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (2.53 %).*

***Conclusions**. The possibility of using the obtained CO_2 extract of Sorbus aucuparia L. in the field of pharmaceutical products as a substance and a drug*

***Keywords:** Sorbus aucuparia L., GC-MS analysis, CO_2 extraction, fatty acid*

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1. Introduction

Kazakhstan accounts for a natural flora of over 6000 plant species [1]. The exact number of medicinal plant species present in Kazakhstan remains uncertain as the list continues to expand annually. More than 150 plant species have been employed in both official and folk medicine for various ailments. This review focuses on a selection of medicinal plants growing in the territory of the Republic of Kazakhstan that have traditionally been used to alleviate skin diseases.

Sorbus aucuparia L. (Fig. 1) is a perennial fruit tree belonging to the family Rosaceae.

In the Republic of Kazakhstan, *Sorbus aucuparia* L grows in Kazakhstan (Fig. 2).

Genus *Sorbus aucuparia* L. is characterized by significant diversity what inflicts taxonomic difficulties. Recent studies provide data on its polyphyletic nature. Interspecific hybridization, apomixis and polyploidy, which have contributed to diversification process, seem to play a crucial role in plant evolution [2, 3].

All simple-leaved species are included in the first four subgroups, whereas the two latter comprise the pinnate-leaved species [4, 5].

However, many forest fruits remain underused, mainly due to their characteristic aroma, which is less pleasant for consumers, although their phytonutrients content is exceptionally high [6, 7]. These forest fruits can supply various raw materials rich in certain compounds of nutritive and health interest [8].

Mountain ash, also known as rowanberry (*Sorbus aucuparia* L.), is one of the underused species, similar to other related species [9].

Presently, it is used in wood production and ornamental horticulture and can be seen in gardens and parks [10, 11]. It is appreciated for its nutrition and for its

medicinal properties [12], especially for its bright red coloured fruits with their functional properties [10].

Therefore, for this purpose, the method of subcritical extraction was used, during which the effect of milder operating conditions (65 bar, 23 °C) was studied. Previously, this trend was not considered as most liquid CO_2 extraction reports were performed under supercritical conditions, which included higher extraction pressures (up to 600 atm) and temperature (up to 60 °C).

Also, CO_2 extraction was chosen because of its clean, cold, and undamaged nature.

In order not to lose sulfide derivatives and volatile substances, we chose to dry and extract the plant at low degrees. For effective yield and evaluation of the extract composition, we chose subcritical CO_2 extraction.

In this paper, results of the bioactive compounds of *Sorbus aucuparia* L. obtained by subcritical carbon dioxide extraction are shown.

The results of the study will allow us to improve the development of an effective substance based on a natural antibacterial agent and, in the future, to obtain mild medicine.

The aim of this study is to determine the component composition and fatty acid by GC-MS method of *Sorbus aucuparia* L. extract obtained by CO_2 extraction, which grows in Kazakhstan.

Fig. 1. Picture of *Sorbus aucuparia* L.

Fig. 2. Distribution map of *Sorbus aucuparia* L., in Kazakhstan

2. Planning (method logy) of research

In Table 1 a representation of the research planning process is shown.

Table 1

Planning of the research

Step 1	Obtaining CO_2 extract from the collected underground part of *Sorbus aucuparia* L.
Step 2	GC-MS analysis of CO_2 extract of *Sorbus aucuparia* L.
Step 3	Quantitative determination of fatty acids CO_2 extract of *Sorbus aucuparia* L.
Step 4	Determining the moisture content of raw vegetable ingredients of CO_2 extract of *Sorbus aucuparia* L.

3. Materials and methods

3. 1. Plant Material

To obtain CO_2 extract, *Sorbus aucuparia* L., has been collected in the city Almaty of Kazakhstan province, in the autumn of 2023.

The plant raw materials were collected from the city Almaty of Kazakhstan in compliance with the requirements of the Good Agricultural and Collection Practice (GACP). The plant material was dried at the room temperature of 25±5 °C, equipped with ventilation. After drying, the raw material was ground to 1–3 mm using an IKA M20 laboratory mill.

According to the requirements of Good Manufacturing Practice (GMP), the production process begins with sanitization of the room, equipment, and processed clothing to prevent microbial contamination of production [13]. Based on the principles of GACP, the technology of collection, processing, drying and storage of raw materials *Sorbus aucuparia* L. has been developed in Fig. 3.

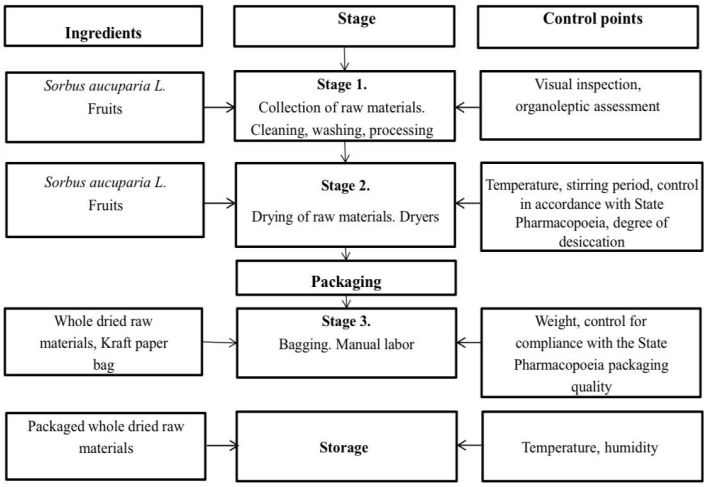

Fig. 3. Technological scheme of raw material procurement *Sorbus aucuparia* L.

3. 2. Extraction procedure

Next, we selected subcritical CO_2 extraction to obtain the optimal form of extraction.

The low efficiency of traditional extraction methods and many disadvantages of the extract, such as contamination of the extract and residues of organic solvents in it, prevented the further use of the resulting natural active ingredient [14]. To eliminate these shortcomings, the technology of subcritical CO_2 extraction (SCCE) is used, which is safe for the environment and satisfies the current desire of the whole world for «green» technology [15]. This method is sensitive to high temperatures and allows the most effective way to obtain an easily oxidizable and decomposable ingredient [16].

The process of extracting *Sorbus aucuparia* L., using carbon dioxide from the roots was carried out according to the following parameters: temperature +18–23 °C and working pressure 57–65 atmospheres, extraction time 8 hours. The percentage of extraction yield was 20 g.

The extraction was obtained using a carbon dioxide extraction plant (installation of carbon dioxide flow-

through extraction-5L) in the Zhanapharm (Pharmaceutical company, Almaty city of Kazakhstan), under subcritical conditions according to the institution national standard 27658-1910 – LLP-02-2011, and liquefied carbon dioxide was used as an extractant (national standard 8050-85).

3. 4. Chemical detection of active compounds

After obtaining the extract, we determined the component composition of this extract using the GC-MS method.

The chemical detection of active compounds analysis was carried out at the Department of Analytical, colloidal Chemistry And Technology Of Rare Elements Center, Al-Farabi Kazakh National University by gas chromatography with mass spectrometric detection using an Agilent 7890B/5977A chromatography [17].

Chromatographic analysis conditions: sample volume 1.0 µl, sample entry temperature 240 °C, with a flow division of 1:10. The separation was performed using a WAXetr chromatographic capillary column with a length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.25 microns at a constant carrier gas velocity (helium) of 1 ml/min. The chromatography temperature is programmed from 40 °C (0 min exposure) to 260 °C with a heating rate of 10 °C/min (20 min exposure). Detection is carried out in the SCAN mode m/z 34-850. The Agilent MSD ChemStation software (version 1701EA) was used to control the gas chromatography system and record and process the results and data obtained. Data processing included the determination of retention time and peak areas, as well as the processing of spectral information obtained using a mass spectrometric detector. To decipher the obtained mass spectra, the Wiley 7th edition and NIST'02 libraries were used (the total number of spectra in the libraries is more than 550 thousand).

3. 5. Statistical analysis

Determination of Fatty Acid Composition One volume of sample was extracted by 20 times the volume of chloroform:methanol (2:1) for 5 min. Then, the mixture was filtered through a paper filter to obtain a clear extract that was evaporated in a round bottom flask on a rotary evaporator at a bath temperature of 30–40 °C until dried. Then, 10 mL of methanol and 2–3 drops of acetyl chloride were added, and a methylation reaction was performed at 60–70 °C for 30 min. Then, methanol was evaporated on a rotary evaporator, and the dry residue was dissolved in 5 mL of hexane. An aliquot of the upper hexane layer was directly taken and analyzed using a GC-MS system (Carlo Erba 4200, Cornaredo, Italy) with a capillary column (30 m×0.25 mm, 0.25 µm). Helium is used as a carrier gas. It was operated under the following conditions: oven temperature of 188 °C for 1 h. The injector temperature was set at 188 °C, and the detector temperature was set at 230 °C [18]. The identification of the compounds was based on a comparison of their mass spectra and retention indices with those of the synthetic compounds spectral library of the National Institute of Standards and Technology (NIST11).

Determining the moisture content of raw vegetable ingredients.

The study was conducted by method 2.2.32 SPH RK Volume 1, «Weight loss during drying».

4. Results

Sorbus aucuparia L. The study of the chemical composition of the CO_2 extract revealed 55 chemical compounds. The list and chromatogram of these chemical bonds are shown in Fig. 4 and Table 2

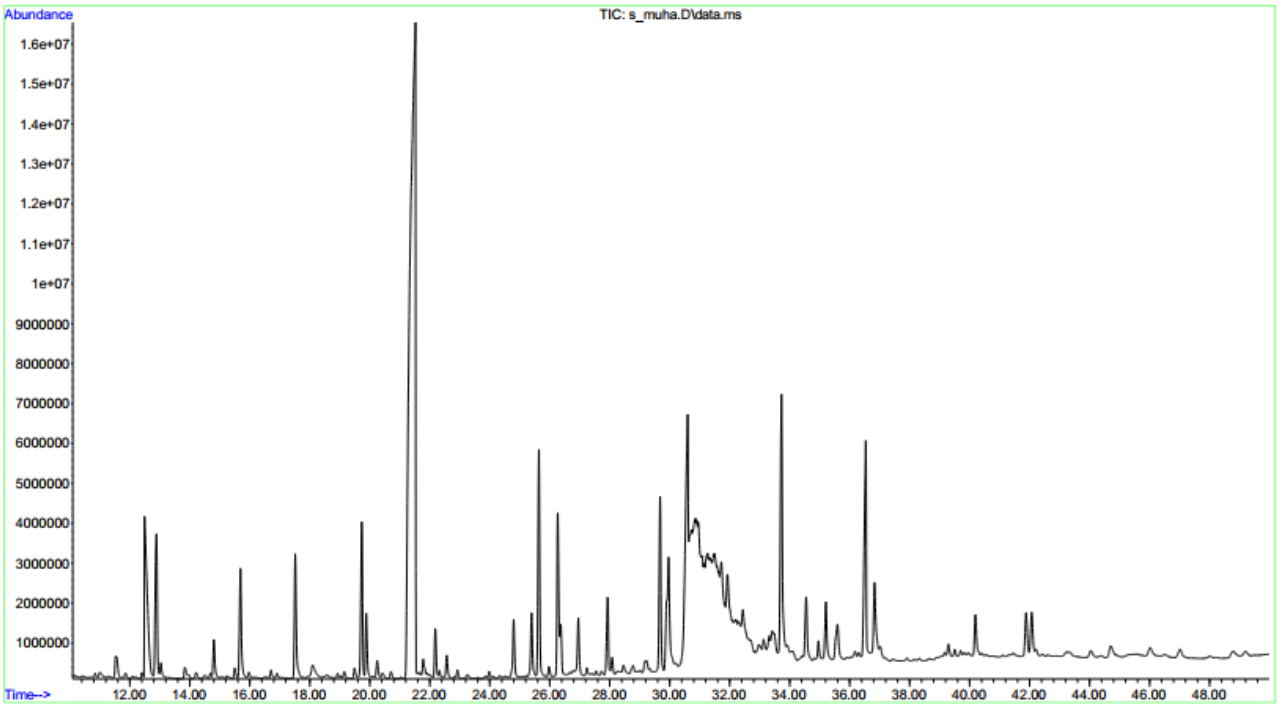

Fig. 4. Chromatogram of *Sorbus aucuparia* L. extract obtained by CO2 extraction

Table 2

Results of GC-MS of subcritical carbonic acid extract of *Sorbus aucuparia* L.

No.	Holding time, min	Component name	Probability of identification %	Percentage content %
1	11.54	3-Isoxazolamine, 5-methyl-	73	0.49
2	12.41	2,4-Hexadienoic acid, ethylester	78	0.09
3	12.51	**Acetic acid**	97	3.92
4	12.88	**Propanoic acid, 2-oxo-, methyl ester**	73	2.44
5	13.04	1,2-Ethanediol, diacetate	84	0.26
6	13.84	Formic acid	73	0.25
7	14.21	Benzaldehyde	85	0.11
8	14.8	2,3-Butanediol	93	0.59
9	15.5	2-Furancarboxaldehyde, 5-methyl-	86	0.13
10	15.69	2,3-Butanediol	86	1.70
11	15.97	1,2-propanediol	83	0.10
12	17.52	Butanoicacid, 4-hydroxy-	68	1.96
13	19.15	2,5-Furandione, 3,4-dimethyl-	75	0.11
14	19.49	2(5H)-Furanone	93	0.18
15	19.73	**2-Butene, 2,3-dimethyl**	68	2.24
16	19.88	1,2-Cyclopentanedione	83	0.96
17	20.25	Urea, 1-methylcyclopropyl-	72	0.32
18	20.71	2-Furanmethanol	68	0.13
19	21.5	**5-Methyl-2(3H)-furanone**	84	30.18
20	21.78	2,4,5-Trihydroxypyrimidine	80	0.55
21	22.19	Benzyl Alcohol	94	0.72
22	22.57	2-Hexenoic acid, 5-hydroxy-	62	0.39
23	22.92	Phenylethyl Alcohol	80	0.15
24	23.98	Maltol	63	0.08
25	24.8	Phenol	95	0.88
26	25.39	1,2-Digydroxyethyl-3,4-hydroxy-3(2H)-furan-5-one	78	1.06
27	25.64	**5-(3-Ethoxy-4,5-dihydro-isoxazol-5-yl)-5-methyl-imidazo-lidine-2,4-dione**	65	3.20
28	25.98	2(3H)-Furanone, 5-acetyldihydro-	84	0.16
29	26.27	SorbicAcid	91	3.15
30	26.95	1,3-Dioxol-2-one,4,5-dimethyl-	75	1.17
31	27.93	2-Hydroxy-gamma-butyrolactone	82	1.18
32	28.08	Phenol, 4-ethyl-	85	0.24
33	28.46	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	77	0.28
34	29.23	Uric acid	60	0.39
35	29.68	**4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl**	90	2.53
36	30.21	Hexanoic acid, 3-hydroxy-, ethyl ester	69	0.19
37	31.72	**1,2,3-Benzenetriol**	62	3.67
38	31.93	**Benzofuran, 2,3-dihydro-**	67	3.68
39	32.44	**Benzoic acid**	64	3.55
40	33.13	2,5-Pyrrolidinedione	82	0.75
41	33.72	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	90	5.55
42	34.55	9,12-Octadecadienoic acid, ethylester	90	1.79
43	34.96	1,2-Ethanediol, 1-(2-furanyl)-	67	0.68
44	35.21	Isosorbide	93	1.28
45	36.18	1,2-Benzenediol, 3-methoxy-	71	0.43
46	36.53	1-Isopropoxy-2,2,3-trimethylaziridine	66	1.08
47	36.83	2-Hexenoic acid, 5-hydroxy-	62	1.88
48	37.02	1,2-Benzenediol	60	0.47
49	39.5	Ethyl 3-hydroxybenzoate	74	0.15
50	41.88	Hydroquinone	81	0.72
51	42.07	**l-ascorbic acid**	90	4.05
52	44.04	α-d-Lyxofuranoside, methyl	73	0.69
53	44.71	Octadec-9-enoic acid	75	0.43
54	46.02	9,12-Octadecadienoic acid	61	0.27
55	47.02	Sucrose	62	0.29

As shown in Table 2, Acetic acid **(**3.92 %**),** Propanoic acid, 2-oxo-, methyl ester (2.44 %), 2-Butene, 2,3-dimethyl (2.24 %), 5-Methyl-2(3H)-furanone (30.18 %), 5-(3-Ethoxy-4,5-dihydro-isoxazol-5-yl)-5-methyl-imidazolidine-2,4-dione (3.20 %), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (2.53 %), 1,2,3-Benzenetriol (3.67 %), Benzofuran, 2,3-dihydro- (3.68 %), Benzoic acid (3.55 %), l-ascorbic acid (4.05 %) were found from the CO_2 extract.

Fatty acid profile of the Sorbus aucuparia L.

The fatty acid composition of oil is a major factor determining its best commercial uses, such as nutritional, industrial, or pharmaceutical, and it is influenced by the variety, climate, and areas of production**.** The results of the analysis for fatty acids in the study showed that linoleic (37.3 %) and oleic (50.5 %) were the most prominent fatty acids of *Sorbus aucuparia* L., extract, followed by pentadecane (1.1 %), palmitic (6.3 %), stearic (3.4 %). The lesser content of myristic, pentadecane, and linolenic acid did not exceed 0.50 % of the total fatty acid (Table 3).

Table 3

Fatty acid profile of *Sorbus aucuparia* L.

No.	Parameter	C number: number of double bonds	Class of compound	Content, %
1	Myristic acid	14:0	Saturated	0,6
2	Pentadecanoic acid	15:0	Saturated	1,1
3	Palmitic acid	16:0	Saturated	6,3
4	Palmitoleic acid	16:1	Monounsaturated	0,4
5	Stearic acid	18:0	Saturated	3,4
6	Oleic acid	18:1	Monounsaturated	50,5
7	Linoleic acid	18:2	Polyunsaturated	37.3
8	Linolenic acid	18:3	Polyunsaturated	0.4

Determining the moisture content of raw vegetable ingredients.

Around 1 g of the raw material's mass is dried, brought to a constant mass, and then placed in a drying cabinet for 30 minutes at a temperature of 100–150 °C. The weighing glass is then chilled, weighed, and dried a second time to a constant mass (the difference between the last two weightings is not more than 0.1 g). The following formula can be used to determine the percentage of moisture in raw materials (X): if M is the original mass of the raw materials in grams and $M1$ is the mass of the raw materials brought to a constant weight in grams:

$$X=((M–M\ 1))/M*100=((1–0.948))/1*100=4.8\ \%.$$

5. Discussion

The content of chemical compounds in *Sorbus aucuparia* L. extract may vary depending on the climatic conditions in the regions where the plant grows and the time of harvest and processing.

The fatty-acid profile of the *Sorbus aucuparia* L., plant is presented in Table 3 and is expressed as a percentage (%) of total fatty acids (TFA). The profiling of fatty acids showed the presence of eight compounds; of these, four were saturated fatty acids (SFAs), two were monounsaturated fatty acids (MUFAs), and two were polyunsaturated fatty acids (PUFA). Fatty acids containing one or more covalent double bonds between carbon-carbon at various positions on the carbon chain are called unsaturated fatty acids. In general, a high percentage of unsaturated fatty acids (88.2 %) was found. In addition, percentages of MUFAs (50.5 %) were higher than those of PUFAs (37.7 %). The major unsaturated fatty acids were oleic (50.5 %), followed by linoleic (37.3 %), linolenic (0.4 %) and palmitoleic (0.4 %) acids. Oleic acid is considered beneficial, as it has been shown to lower cholesterol levels in low-density lipoproteins [19]. Polyunsaturated fatty acids (PUFAs) are basic components involved in the architecture and function of cellular membranes and play key roles in several biological processes. Linoleic acid is a major constituent of human tissues, and it is considered to be an essential fatty acid [20]. Fatty acids that consist of a single covalent bond between carbon-carbon atoms and are generally solid at room temperature are called saturated fatty acids. Palmitic acid (6.3 %), stearic acid (3.4 %), myristic acid (0.6 %) and pentadecanoic acid (1.1 %) found in vegetable oils are the most important saturated fatty acids. Saturated fatty acids can be synthesized in the human body, and even if no fat is consumed, these types of fatty acids can be synthesized from molecules formed by carbohydrate metabolism. In response to this, unsaturated fats are essential fatty acids that the body needs. They are liquid at room temperature, and most of them are of vegetable origin [21, 22].

The results of this study can provide valuable information about the nutritional value and health benefits of the sample. For example, the high content of linoleic acid indicates that the sample may be a good source of essential fatty acids, which have health benefits, including reducing the risk of heart disease and improving brain function. A complex of fatty acids and the strongest natural antioxidants, are necessary for all people for daily intake, strengthening the body, and increasing its energy and vitality [23].

Particularly, omega fat acid groups, which the body can only acquire externally, are crucial for the functioning of the cardiovascular system. The essential role of omega-3 fatty acids includes promoting heart health in people at risk for or already suffering from cardiovascular disease, slowing the development of vein hardening, lowering blood triglyceride levels, lowering bad cholesterol in heart disease while raising good cholesterol, and lowering the risk of stroke, subsequent heart attacks, and heart attack-related death.

Practical relevance. Extraction of an extract from the studied medicinal plant raw materials of *Sorbus aucuparia*. The practical significance of determining biologically active substances and the chemical composition of fatty acids from the resulting extract of *Sorbus aucuparia.*

The scientific novelty of the study is confirmed by the patent of the Ministry of Justice of the Republic of Kazakhstan, National Institute of Intellectual Property No. 7898, 24.03.2023, issued to the utility model «Method of obtaining carbon dioxide extract from the aboveground part of rowan fruits *Sorbus aucuparia* L.».

Research limitations. A limitation of the study could be considered that during the GC-MS study of the composition of CO_2 extract of the *Sorbus aucuparia* L., some chemical compounds were not identified due to the absence of their characteristics in the automatic library of the NIST02 and Wiley 7th edition database.

Prospects for further research. In further studies, it is advisable to analyze the component composition and fatty acid of CO_2 extract of *Sorbus aucuparia* L., depending on their stages of germination, climatic conditions and place of growth. Also, phytochemical and pharmacological studies of *Sorbus aucuparia* L. can show the prospect of creating new pharmaceutical preparations.

6. Conclusions

This research work was described on the basis of obtaining CO2 extract from medicinal plant raw materials of *Sorbus aucuparia* L.

For the first time, 20 grams of brown volatile extract were collected using subcritical carbon dioxide extraction. For the first time, 55 different types of chemical compounds were discovered, bioactive components were identified, such as 5-Methyl-2(3H)-furanone (30.18 %), 5-(3-Ethoxy-4,5-dihydro-isoxazol-5-yl)-5-methyl-imidazolidine-2,4-dione (3.20 %), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (2.53 %), with a large distribution area in the extract, and with an Furanone-containing compounds cover numerous therapeutic categories viz. Analgesic and anti-inflammatory, anticancer, anticonvulsant, antibacterial and antifungal, antioxidant, antiulcer and anti-TB, etc.

The moisture content of raw vegetable ingredients is 4.8%.

The results of the analysis of fatty acids in the study showed that Linoleic (37.3 %), and Oleic (50.5 %) were the most prominent fatty acids of *Sorbus aucuparia* L., extract.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data Availability

The data will be made available at a reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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