

Original Research

FT-IR and GC–MS analyses and antioxidant activities of extracts of the wild type *Saussurea sordida* and *Saussurea alpina* native to Kazakhstan

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Abstract

Objective: Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide un-limited opportunities for new drug leads because of the unmatched availability of chemical diversity. The objects of the study were the medicinal plant raw materials *Saussurea sordida* Kar&Kir and *Saussurea alpina* DC. collected from southern Kazakhstan during the flowering period in the spring-summer months of 2021. experimental and theoretical substantiation of the technology for obtaining phytosubstances *S. sordida*, *S. alpina* and their study of antioxidant activity.

Methods: Identification of functional groups was performed using Fourier transform infrared spectroscopy (FT-IR). The component composition of extracts of two types of medicinal plant raw materials was studied using gas chromatography with mass spectrometric detection.

Results: The present study aimed to analyze the bioactive constituents of the plants *S. sordida* and *S. alpina*. The ethanol extracts were subjected to phytochemical screening and Fourier transform infrared (FTIR) spectroscopy. Phytochemical screening of the extract revealed the presence of phenolic compounds. FT-IR spectroscopic analysis revealed various characteristic bands corresponding to various functional groups, such as amines, alcohols, phenols, alkanes, alkenes, and conjugated acids, in the formulation extract. FT-IR analysis of peak values for various functional compounds, such as alcohols, phenols, carboxylic acids, aldehydes, amides, amino acids, anhydrides, esters, ketones, unsaturated aliphatic, aromatics, unsaturated heterocycles, amines, nitro compounds, alkanes, alkenes and sugars. For the first time, the compositions of petroleum ether extracts from medical plants, such as *Saussurea sordida* and *Saussurea alpina*, were investigated through GC–MS analysis. The antioxidant activity of the medicinal plants showed that the *S. alpina* extract had greater activity than the *S. sordida* extracts according to both methods (the content of antioxidants was measured in terms of quercetin and gallic acid).

Conclusion: According to the results of our study, the biologically active substances of this plant relative have an antioxidant effect due to the presence of phenolic compounds in their composition.

Keywords: *S. sordida*, *S. alpina*, biologically active substances, antioxidant activity

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INTRODUCTION

Medicinal plants, which are used in traditional medicine or traditional medical practices as medicines, are among the most useful natural resources in the world, as they play an important role in health care in many ancient and modern cultures^{1,2}. Therefore, natural products and traditional medicines derived from plant species have incomparable advantages, such as rich clinical experience and unique diversity of chemical structures and biological activities, and can be used in drug development



and discovery³. *Saussurea* is a large, predominantly East Asian genus belonging to the family *Asteraceae*, and includes approximately 465 species found in the Arctic and temperate zones of the Eurasian and North American continents. Many representatives of the genus are also used in pharmaceuticals, cosmetology, and official and traditional medicine^{4,5}. The genus *Saussurea* is well known for its rich chemical composition and wide range of biological activity, therefore, it is necessary to resolve issues related to the conservation of the biological diversity of species of the genus *Saussurea*, which requires a preliminary in-depth study of the composition, morphology and geography of the species⁶. Currently, the scope and boundaries of the genus, as well as the relationships between its constituent substances and pharmacological activities, remain the subject of debate and continue to be revised⁷⁻¹³. There are 41 species of *Saussurea* growing on the territory of Kazakhstan^{14,15}. The objects of this study are *S. sordida*, *S. alpina*, which has a identified as quercetin 5-O-β-D-glucopyranoside. Similarly, flavonoids 6, 7 and 10 were characterized as kaempferol 5-O-glucoside, kaempferol 7-O-glucoside and isorhamnetin 5-O-glucoside. Flavone and flavonol 5-O-glucosides are comparatively rare in the plant kingdom¹⁶.

Medicinal properties of the genus *Saussurea* are gaining popularity in modern traditional medicine systems, including Chinese and Tibetan traditional medicine. According to the literature, the following pharmacological activities of the genus *Saussurea* were determined: Anticancer/antitumor effect; Hepatoprotective effect; Immunostimulant effect; Anti-angiogenesis effect; Antidiabetic effect; Antimicrobial effect; Anti-inflammatory effect; Activity against coronavirus (COVID-19); Antibacterial and antifungal effect; Antioxidant effect; Antihyperlipidemic and antihyperglycemic effect. See Table 1. Taxonomic Insights into *Saussurea* genus within

the *Asteraceae* Family. The genus *Saussurea* belongs to the *Asteraceae* family (Figure 1). In the picture below, a phyllonic tree was created based on the *Saussurea* species that grows in Kazakhstan. As shown in the picture, 39 species grow in Kazakhstan. In our study, we consider two species *S. sordida*, *S. alpina*. Based on the results of these studies, species of this plant family were found to exhibit multiple types of activity in terms of pharmacological action. It becomes clear that in terms of phytochemical composition, the medicinal plants *S. sordida*, *S. alpina* are closer (similar or identical) to other species of the genus *Saussurea*. Thus, the present work was carried out with the aim of studying the phytochemical composition, as well as biochemical and histological studies of these species of medicinal plants (*S. sordida*, *S. alpina*) growing in the South Kazakhstan.

METHODS

Plant Material

Above-ground parts of medicinal plant raw material *S. sordida*, collected in the South Kazakhstan during the flowering period in the spring-summer months of 2021 in Tolebi district, as well as above-ground parts of medicinal plant raw material *S. alpina* collected from the height of 3050 m of Sayram peak in Tolebi district. Drying of herbs was carried out in South Kazakhstan Medical Academy in a drying oven at room temperature 25±2°C.

FT-IR Spectroscopic Analysis

All plant material was collected in South Kazakhstan (*S. sordida*, *S. alpina*) 2021 and was identified by Dr. Issayeva E. specimens are kept in the herbarium of the Laboratory of Pharmacognosy, South Kazakhstan Medical Academy, Kazakhstan. Botanical

Table 1. Pharmacological activity of plants of the genus *Saussurea* according to the results of studies presented in different literature reviews

Authors	Type of plant	Therapeutic effects of <i>Saussurea</i> herbs on the human body
Jehan S Al-Brahim, et al 2023	<i>S. costus</i>	The antimicrobial properties of IONPs were evaluated on nine pathogenic microbes, which showed that the nanoparticles have antimicrobial activity against <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> , <i>Shigella</i> sp., <i>Staphylococcus</i> sp. and <i>Aspergillus niger</i> , with potential applications in therapeutic and biomedical fields.
Hajo Idriss, et al 2023	<i>S. costus</i>	The acetic acid extract of <i>Saussurea costus</i> has been shown to possess phytochemical profile, molecular additivity, anti-candida and antiviral activities. Was found to have active properties indicating inhibition of SARS-CoV-2. The extract showed significant antimicrobial activity (in vitro) against <i>Candida albicans</i> .
Min Liu, et al 2021	<i>S. involucreta</i>	A new auronc glycoside was isolated from the extract of the above-ground parts of <i>Saussurea involucreta</i> . All the identified compounds showed in vitro inhibitory activity against α-glucosidase. Among them, compounds 1 and 6 showed significant inhibitory activity against α-glucosidase with IC50 values of 47.1 and 57.7 μM, respectively.
Yang Wang, et al 2020	<i>S. lappa</i>	Pharmacological studies included extracts, essential oils and monomeric components represented by dehydrocostal lactone. It has antitumor, anti-inflammatory and antibacterial effects on the digestive system.
Gasha S. Ahmed, et al 2023	<i>S. costus</i>	The antibacterial and antifungal activities of essential oil, hexane-chloroform, methanol and aqueous extracts of <i>Saussurea costus</i> root was evaluated against <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus epidermalis</i> , <i>Enterobacter cloacae</i> , <i>Enterococcus faecalis</i> , <i>Klebsiella pneumoniae</i> , <i>Acinetobacter baumannii</i> , <i>Escherichia coli</i> and <i>Candida albicans</i> .
Sana Naseer, et al 2022	<i>S. lappa</i>	Antioxidant potential refers to the ability of a plant to absorb reactive oxygen species (ROS), which increases the therapeutic potential of the plant.
Sana Naseer, et al 2022	<i>S. lappa</i>	<i>S. lappa</i> extracts have antibacterial potential against six ATCC and three multidrug resistant (MLD) strains were evaluated by agar well method. The research showed antibacterial effect.

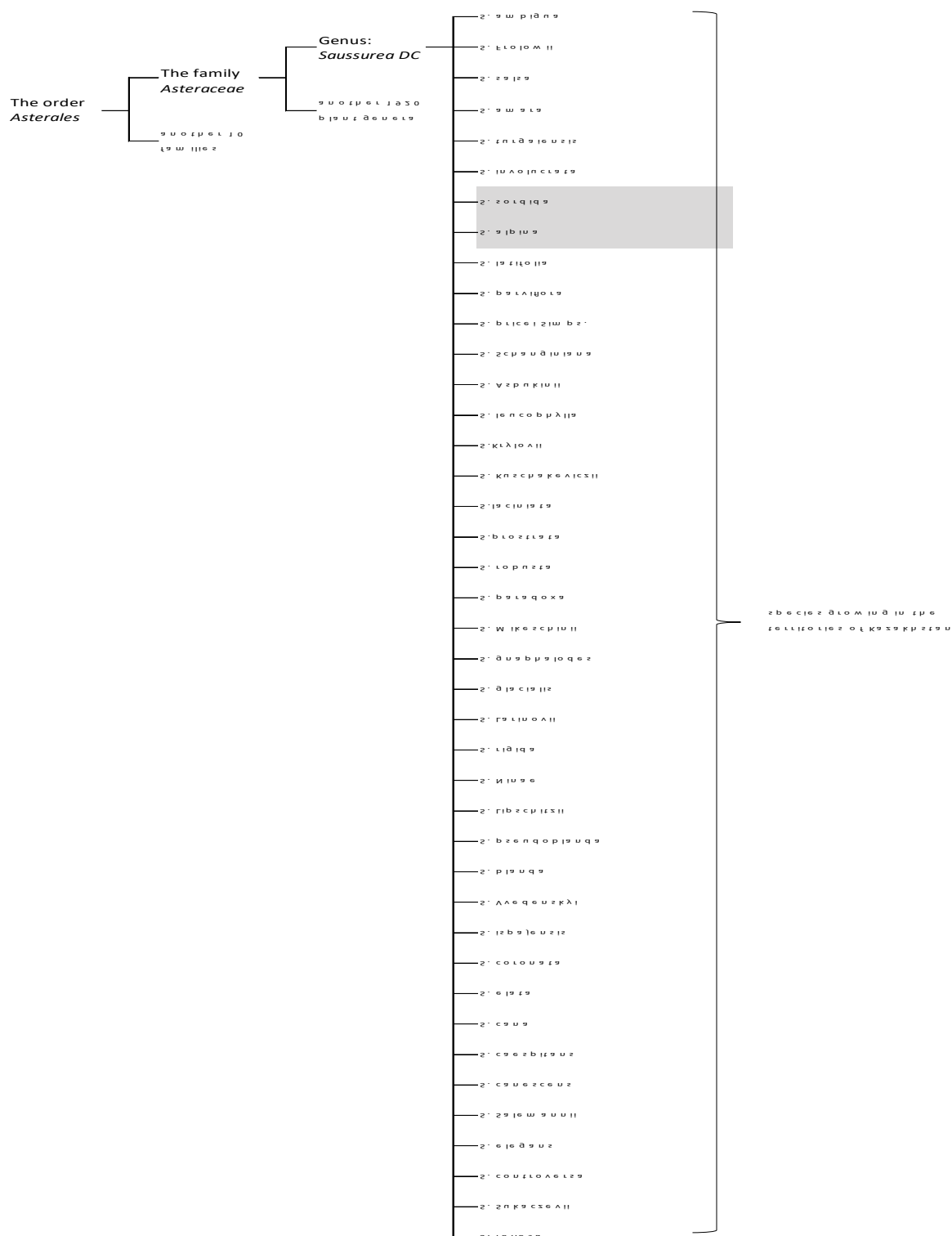


Figure 1. *Saussurea* species growing in Kazakhstan

name, taxonomic authority, plant part, voucher specimen number, place and date of collection of each plant.

Determination of organic compounds in plant extract

A Shimadzu GC-MS chromatograph (Japan) with an ultra inert DB-35 MS capillary column (30 m × 0.25 mm inner diameter × 0.25 µm film thickness; USA) was used for Gas Chromatography-Mass Spectrum Analysis of the metabolites. The oven temperature was set at 40°C for 3 min (isothermal), programmed to 30°C at a rate of 5°C/min, and kept constant at 280°C for 15 min (isothermal); the temperature of the injector was 280°C. Helium was employed as the carrier gas, with a flow rate of 1.40 mL/min. Diluted samples (1% v/v) were injected at a split ratio of 15:1 in a volume of 1 µL. The following were the MS running specifications: 280°C for the interface, 220°C for the ion source, 70 eV for the EI mode, and 35–500 amu for the scan range. Detection was carried out in the SCAN mode m/z 34–750. The Agilent MSD ChemStation software (version 1701EA) was used to control the gas chromatography system, record and process the obtained results and data. Data processing included determination of retention times, peak areas, as well as processing of spectral information obtained with a mass spectrometric detector. To interpret the obtained mass spectra, the Wiley 7th edition and NIST02 libraries were used (the total number of spectra in the libraries is more than 550 thousand).

Antioxidant activity of extracts of *S. alpina* and *S. sordida*

The determination of the total content of antioxidants in extracts from the aerial parts of *S. alpina* and *S. sordida*. The concentration of antioxidants (mg/L) was measured using calibration graphs of the dependence of the output signal (NAC) on the concentration (mg/L) of quercetin and/or gallic acid. The method used allows you to convert the current generated during the oxidation of antioxidants contained in the extract on the surface of the working electrode into a digital signal. In this case, the strength of the electric current depends on several factors: the nature and concentration of the analyzed substances, the type of material of the working electrode and the potential applied to the electrode. The antioxidant activity of the extract was studied using the amperometric method. Analysis of each extraction sample was performed in 6 replicates.

Content of antioxidants in terms of quercetin calculations were made using the equation below:

Content of antioxidants in terms of gallic acid calculations were made using the equation below:

Where X_g is the mass concentration of antioxidants found from the calibration curve, mg/l; V_n - volume of extract of the analyzed sample, ml; m_a - weight of the analyzed substance, g; N is the dilution factor of the sample being analyzed.

RESULTS

Aerial parts of *S. alpina* and *S. sordida* were grinded by an analytical mill and packed in polyethylene bags. The grinded

mass with 50 g of *S. alpina* and *S. sordida* from the aerial parts was extracted with 70% ethanol (Figure 2). The vibrational spectrum of a chemical molecule is considered to be a unique physical property. Absorption spectra of dried herbal extracts obtained in the range 4000–400 cm⁻¹ are shown in figure 3. The main vibrational range of the molecules, called “mid-infrared”, lies between wave numbers from 4000 to 400 cm⁻¹. All the spectra of the studied samples (70% ethanol extracts from *S. sordida* and *S. alpina*) absorption bands reflecting the general chemical composition were detected. In the IR spectra of the studied medicinal plant extracts, characteristic absorption bands of the aromatic part of flavonoids can be identified: The region of 3385 - 2850 cm⁻¹ indicates a symmetric (sym) and asymmetric (asym) stretching of polymeric hydroxyl group (O–H), H-bonded stretching, which is characteristic of phenolic compounds (Table 2), 1680-1615 cm⁻¹ (carbonyl group of γ-pyrone), The stretching of the C–H and C=C–C aromatic bond appears in the region of 1620-1470 cm⁻¹ (skeletal vibrations of aromatic rings), 2950-2880 cm⁻¹. There are absorption bands due to valence vibrations of free groups with phenolic compounds (frequencies 3750-3700 cm⁻¹), intermolecular hydrogen bonds in dimers and polymers (frequencies 3400-3200 cm⁻¹) characteristic peaks are observed from 2000 to 1380 cm⁻¹ [17-18]. The results of the obtained spectra are shown in Figures 3-4.

Furthermore, the 70% ethyl alcohol extract *S. alpina* and *S. sordida* extracts from South Kazakhstan. The peaks same at 3329, 2927, 1373, 1273, 1045, 879 and 634 cm⁻¹ were identical in two extracts (Figure 3 and Figure 4). These peaks attributed to phenolic and organic compounds. The most prominent signals for the two 70% ethyl alcohol extracts were the major absorption band (Figure 2 and Figure 3) around 3300 cm⁻¹, which can be associated with O–H stretching and C–H stretching vibrations. The peaks between 700 and 1800 cm⁻¹, the fingerprint zone, could be attributed to C=C–C aromatic ring stretching (1580–1615 cm⁻¹, 1450–1510 cm⁻¹) and several aromatic out-of-plane C–H (670–900 cm⁻¹) and in-plane (950–1225 cm⁻¹) bending [14–16]. In addition, peaks for water were observed in the range 1640 cm⁻¹ and 3300 cm⁻¹ on the basis of functional group H and OH (Table 2. Table 3).

S. alpina extract have two different peaks 1772 and 1635 cm⁻¹. This band could be due to stretching vibration of C=C groups, due to aromatic ring deformations, due to flavonoids. Besides the bands previously described, there were other bands encountered. A band at 1772 cm⁻¹, probably related to: stretching vibration of carboxyl groups, stretching of C = O of flavonoids and lipids. A band at 1635 cm⁻¹, related to aromatic ring deformations and to flavonoids and aromatic rings (stretching of aromatic C=C) [19-21]. This study can lead to the production of new herbal medicines from various diseases employing and to creation of new medications.

Study of the chemical composition of medicinal plant raw materials by GC-MS method

In this work, extracts of *S. alpina* and *S. sordida* were studied using gas chromatography with mass spectrometric detection (GC-MS) with petroleum ether. Evaluation of the chemical



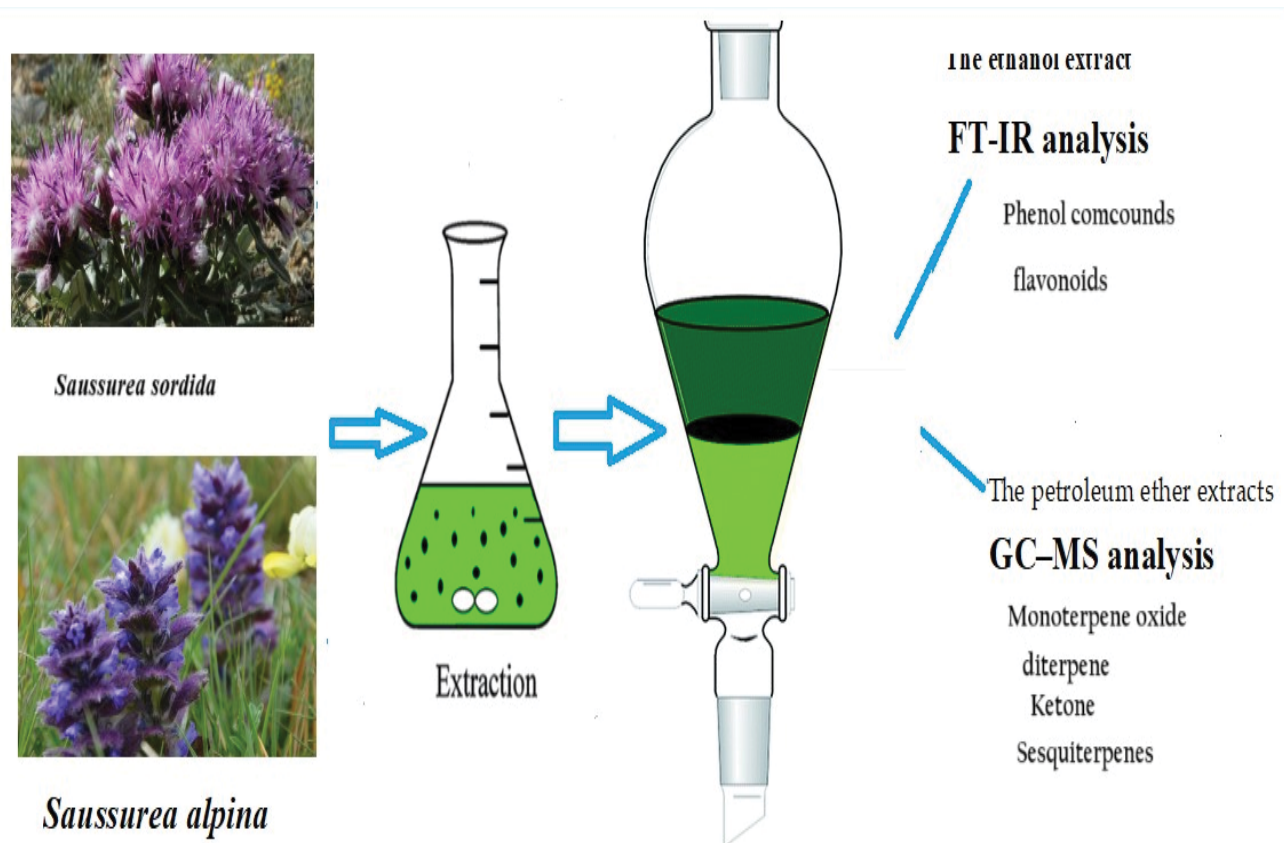


Figure 2. Extraction methods to plants

Table 2. FT-IR spectra analysis of dried medical plant extracts.

Herbs	Absorption spectrum, wave number (cm ⁻¹)							
	3385 - 2850	2950-2880	1785–1640	1680-1615	1600–1300	1450–1050	1080–620	935–710
S. sordida	3329	2927	-	-	1373	1273	1045	879
S.alpina	3329	2927	1772	1635	1373	1271	1045	879

Table 3. Fundamental IR vibrations of phytochemicals present in fractions of *S. Sordida* and *S.alpina*

S. sordida	Functional Group	S.alpina	Functional Group
3329	Imino compounds,NH stretch	3329	Imino compounds,NH stretch
2927	Ac-H carbonyl	2927	Ac-H carbonyl
-		1772	C=C–C aromatic ring stretching
-		1635	Alkenyl C=C stretch
1373	Phenol or tertiary alcohol, OH bend	1373	Phenol or tertiary alcohol, OH bend
1273	Primary or secondary, OH in-plane bend	1271	Primary or secondary, OH in-plane bend
1045	Primary alcohol, C	1045	Primary alcohol, C
879	Alkenyl C=C stretch	879	Alkenyl C=C stretch

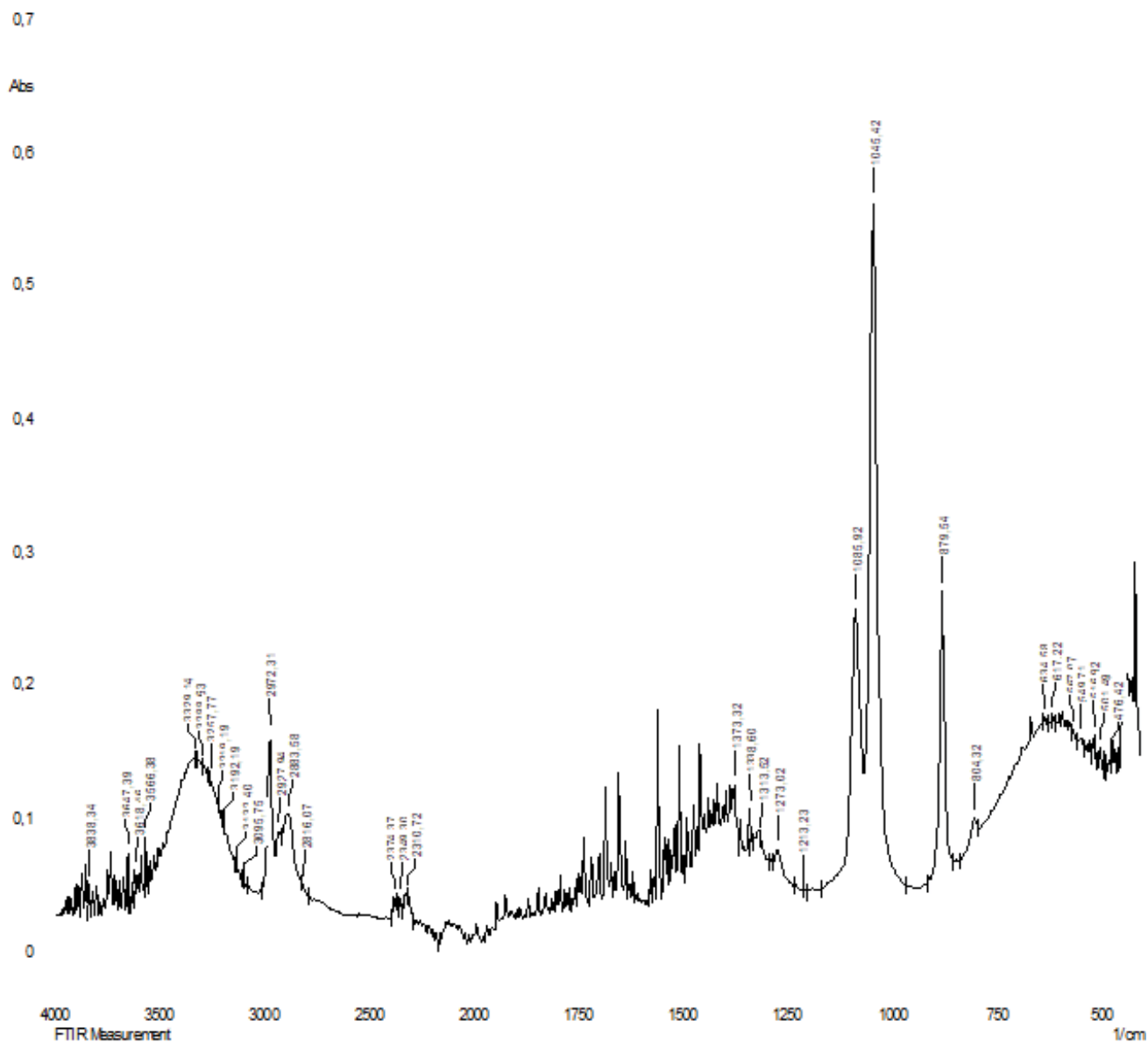


Figure 3. IR spectrum of liquid 70% ethanol extract of *S. sordida*

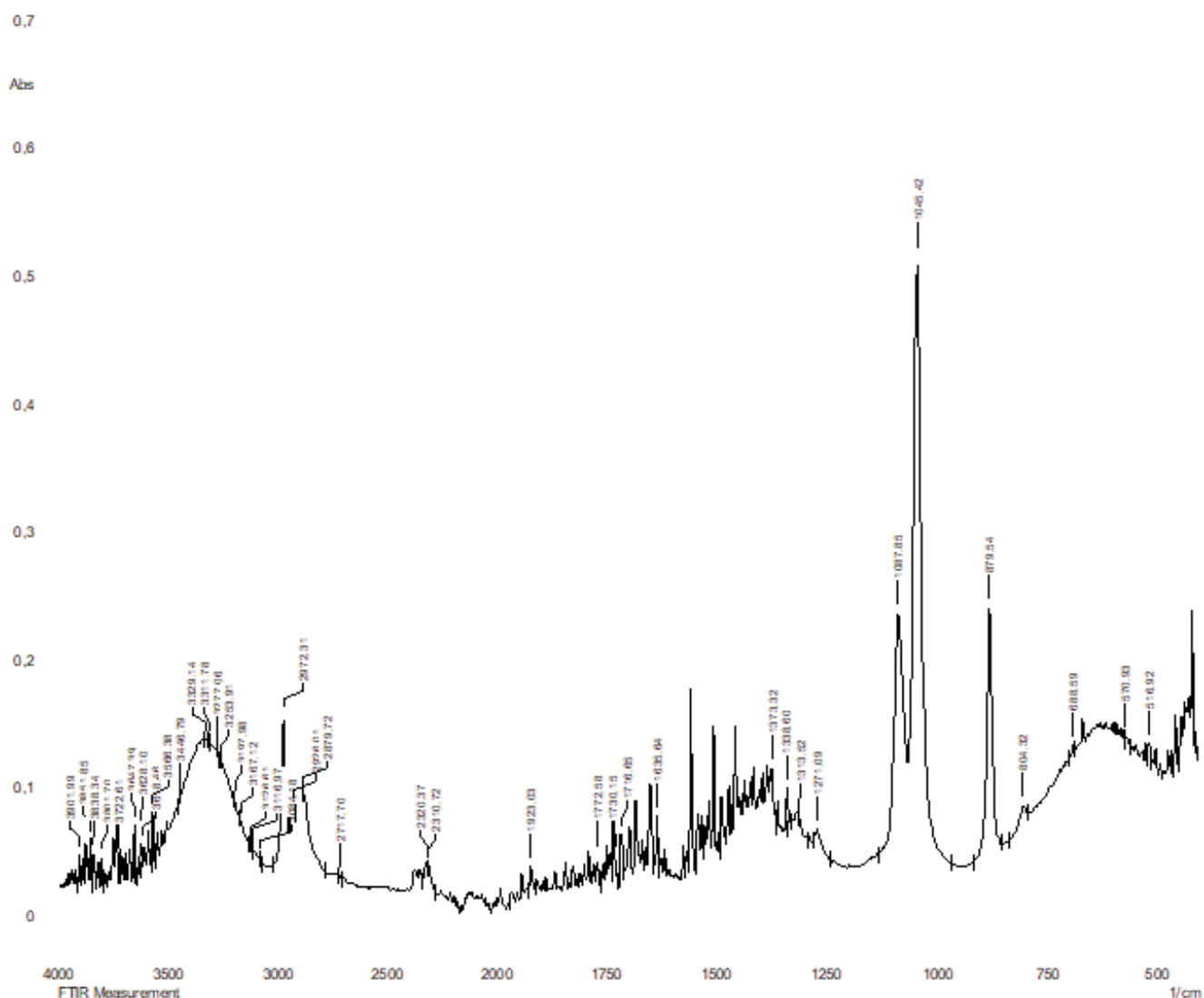


Figure 4. IR spectrum of liquid 70% ethanol extract of *S. alpina*

structure and composition of the extracts can designate various biological potential of different medicinal plant extracts. However, to the best of our knowledge, there is no report on GC–MS based plant metabolic characterization to reveal the presence of various bioactive compounds in petroleum ether extracts. Therefore, the GC–MS analysis was performed in a predetermined study. A total of 37 peaks were observed in all plant parts, each peak designated the bioactive compounds that were recorded by relating their peak retention time, molecular weight, molecular formula to that of the known compounds proposed by the NIST library (Table 4).

Based on the results of the study of *S. sordida*, 37 compounds were identified. These compounds belong to different chemical classes, including hydrocarbons, esters of fatty acids, triterpenes, aldehydes, vitamins. Of these, substances with a high proportion were identified: (+)-2-Bornanone -20,45%, phytol – 11,57%, 2-pentadecanone,6,10,14-trimethyl – 9,14%,

2,4-dimethyl-2,4-pentandiol – 6,87%, eudesm-4(14)-en-11-ol – 5,47%, 5-eocosene,(E)- - 4,09%, hexacosane – 3,29 %. According to the results of the study of *Saussurea alpina*, 29 compounds were identified (Table 5). Of these, substances with high proportion were identified: (+)-2-Bornanone -26,79%, phytol – 14,78%, 2-pentadecanone,6,10,14-trimethyl – 9,58%, 2,4-dimethyl-2,4-pentandiol – 6,68%, eudesm-4(14)-en-11-ol – 7,45%, hexacosane – 3,25% and Pulegone -1,75% *Saussurea alpina* DC extract have Pulegone bioactive compound. According to GC-MS results, *Saussurea alpina* DC contains the biologically active substance Pulegone. Pulegone is used in flavorings, perfumery, aromatherapy (as a component of essential oils), and also to produce menthol. The GC-MS analysis of the petroleum ether extracts of *S. sordida* and *S. alpina*, showed promising biological activities, which has resulted in the identification of 37 and 29 compounds (Figure 5 and Figure 6). These constituents belong to 9 classes of compounds including hydrocarbons, fatty acids, esters, alcohols, aromatics,

Table 4. Identified phytochemicals in petroleum ether extract of *S. sordida* through the GC–MS technique

№	Retention time, min	Compounds	Molecular formula	MW, g/mol	Identification probability, %	Percentage content, %
1	10,56	Cyclohexanone,5-methyl-2-(1-methylethyl)-,trans-	C ₁₀ H ₁₈ O	154.2	82	0,94
2	10,90	(+)-2-Bornanone	C ₁₀ H ₁₆ O	152.2	98	20,45
3	11,56	Pentadecane	C ₁₅ H ₃₂	212.4	94	0,82
4	12,15	1-Decanol,2-Hexyl	C ₁₆ H ₃₄ O	242.4	79	1,17
5	12,53	Acetic acid, 1,7,7-trimethyl-bicyclo[2,2,1]hept -2-yl ester	C ₁₆ H ₃₄ O	242.4	80	1,58
6	13,51	2,4-Dimethyl-2,4-pentanediol	C ₇ H ₁₆ O ₂	132.2	85	6,87
7	13,86	Hexadecane	C ₁₆ H ₃₄	226.4	92	1,04
8	13,99	Cyclohexanone,5-methyl-2-(1-methylethylidene)-	C ₁₀ H ₁₈ O	154.2	91	1,67
9	14,12	Bicyclo[3,1,1]heptan -3-ol,6,6-dimethyl -2-methylene-	C ₉ H ₁₄ O	138.2	87	0,34
10	15,18	Endo-Borneol	C ₁₀ H ₁₈ O	154.2	89	1,10
11	15,83	7-Oxybiclo [4,1,0] heptan -2-one, 6-methuyl – 3-(1-methylethyl)-	C ₉ H ₁₄ O ₂	154.2	80	2,03
12	16,66	1-Dodecanol, 2-octyl-	C ₂₀ H ₄₂ O	298.5	85	1,13
13	17,31	1-Decanol,2-hexyl-	C ₁₆ H ₃₄ O	242.4	81	1,31
14	18,23	Octadecane	C ₁₈ H ₃₈	254.5	92	0,97
15	18,97	1,4 –Methanobenzocyclodecene, 1,2,3,4,4a,5,8,9,12,12a-decahydro-	C ₁₅ H ₂₂	202.3	70	0,56
16	19,10	5-eicosene, (E)-	C ₂₀ H ₄₀	280.5	83	4,09
17	19,78	Butylated Hydroxytoluene			86	0,78
18	20,67	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296.5	87	2,33
19	20,92	Caryophyllene oxide	C ₁₅ H ₂₄ O	220.3	91	1,53
20	21,24	Phytol	C ₂₀ H ₄₀ O	296.5	78	0,98
21	23,80	Spathulenol	C ₁₅ H ₂₄ O	220.3	86	1,36
22	24,16	Heneicosane	C ₂₁ H ₄₄	296.6	92	2,27
23	24,30	2-pentadecanone, 6,10,14-trimethyl-	C ₁₈ H ₃₆ O	268.5	94	9,14
24	25,60	Eudesm-4(14)-en-11-ol	C ₁₅ H ₂₆ O	222.3	90	5,47
25	27,35	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-,(R)-	C ₁₁ H ₁₆ O ₂	180.2	87	1,61
26	28,33	5,9,13-Pentadecatrien-2-one,6,10,14-trimethyl- (E,E)-	C ₁₈ H ₃₀ O	262.4	81	0,92
27	28,59	1-hexadecanol	C ₁₆ H ₃₄ O	242.4	80	0,96
28	29,66	Hexadecanoic acid, butyl ester	C ₂₀ H ₄₀ O ₂	312.5	81	1,29
29	30,93	1,2- Benzenedicarboxylic acid, bis (2-methylpropyl) ester	C ₁₆ H ₂₂ O ₄	278.3	90	0,54
30	31,06	Hexacosane	C ₂₆ H ₅₄	366.7	92	3,29
31	32,42	Phytol	C ₂₀ H ₄₀ O	296.5	94	11,57
32	33,28	Dibutyl phthalate			94	1,36
33	33,40	9,12,15 – Octadecatrienoic acid, (Z,Z,Z)-	C ₁₉ H ₃₂ O ₂	292.5	83	1,22
34	36,52	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.4	82	2,32
35	37,53	4,8,12,16-Tetramethylheptadecan-4-olide	C ₂₁ H ₄₀ O ₂	324.5	90	2,12
36	38,99	Squalane	C ₃₀ H ₆₂	422,8	93	1,06
37	43,55	9-Octadecenamide,(Z)-	C ₁₈ H ₃₅ NO	281.5	83	1,79

Table 5. Results of chromatographic analysis of the extract <i>S. alpina</i>						
No	Retention time, min	Compounds	Molecular formula	MW, g/mol	Identification probability, %	Percentage content, %
1	10,48	Dodecane,4,6-dimethyl-	C ₁₄ H ₃₀	198.39	77	0,47
2	10,56	Cyclohexanone,5-methyl-2-(1-methylethyl)-,trans-	C ₁₀ H ₁₈ O	154.2	85	0,71
3	10,91	(+)-2-Bornanone	C ₁₀ H ₁₆ O	152.2	98	26,79
4	11,58	Pentadecane	C ₁₅ H ₃₂	212.4	92	0,86
5	12,17	1-Dodecanol,2-octyl-	C ₂₀ H ₄₂ O	298.5	78	1,57
6	12,55	1-Decanol,2-Hexyl	C ₁₆ H ₃₄ O	242.4	74	1,73
7	13,52	2,4-Dimethyl-2,4-pentanediol	C ₇ H ₁₆ O ₂	132.2	89	6,68
8	13,88	Hexadecane	C ₁₆ H ₃₄	226.4	92	0,92
9	14,00	Pulegone	C ₁₀ H ₁₆ O	152.2	90	1,71
10	14,12	Bicyclo[3,1,1]heptan -3-ol,6,6-dimethyl -2-methylene-, [1S-(1α,3α,5α)]-	C ₁₀ H ₁₈	138.2	77	0,35
11	15,19	Endo-Borneol	C ₁₀ H ₁₈ O	154.2	91	1,27
12	15,83	7-Oxybicyclo [4,1,0] heptan -2-one, 6-methuyl – 3-(1-methylethyl)-	C ₉ H ₁₄ O ₂	154.2	85	2,19
13	16,10	Heptadecane	C ₁₇ H ₃₆	240.5	87	1,78
14	17,91	2,4-Dimethyl- 2,4- pentanediol	C ₇ H ₁₆ O ₂	132.2	73	1,72
15	18,24	Octadecane	C ₁₈ H ₃₈	254.5	91	1,01
16	19,57	Heptacosane	C ₂₇ H ₅₆	380.7	78	0,59
17	19,79	Butylated Hydroxytoluene	C ₁₅ H ₂₄ O	220.3	89	1,20
18	20,68	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296.5	89	1,34
19	20,93	Caryophyllene oxide	C ₁₅ H ₂₄ O	220.3	87	1,47
20	21,25	Phytol	C ₂₀ H ₄₀ O	296.5	80	1,02
21	24,30	2-pentadecanone, 6,10,14-trimethyl-	C ₁₈ H ₃₆ O	268.5	94	9,58
22	25,61	Eudesm-4(14)-en-11-ol	C ₁₅ H ₂₆ O	222.3	90	7,45
23	29,43	Tetracosane	C ₂₄ H ₅₀	338.7	81	0,79
24	29,67	Hexadecanoic acid, butyl ester	C ₂₀ H ₄₀ O ₂	312.5	86	1,54
25	31,07	Heneicosane	C ₂₁ H ₄₄	296.6	92	3,25
26	31,33	1-Hexadecyn-3-ol, 3,7,11,15- tetramethyl-	C ₂₀ H ₄₀ O	296.5	77	2,82
27	32,42	Phytol	C ₂₀ H ₄₀ O	296.5	94	14,78
28	33,29	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278.3	94	2,66
29	38,99	Squalane	C ₃₀ H ₆₂	422,8	89	1,75

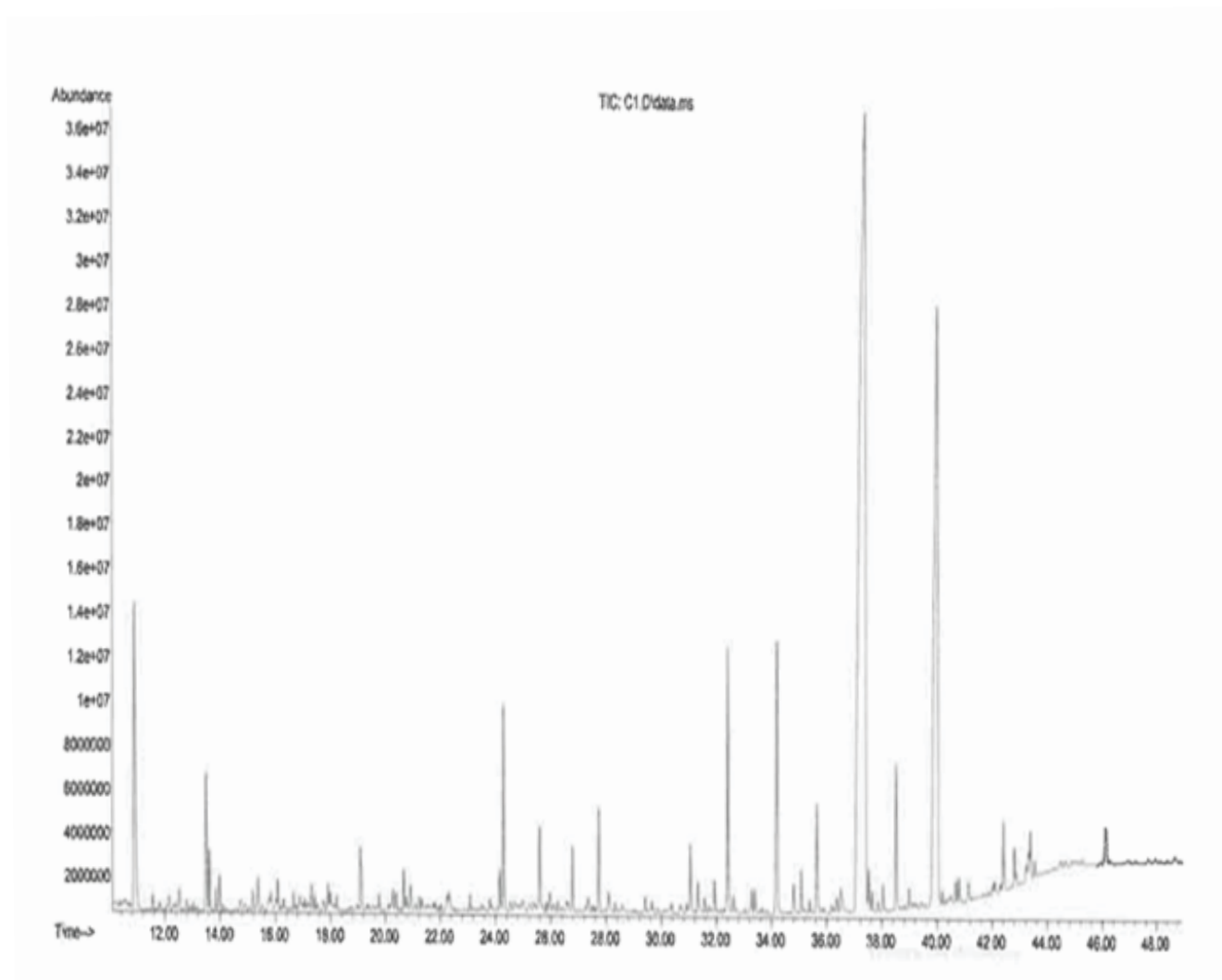


Figure 5. Chromatogram of the extract *S. sordida*

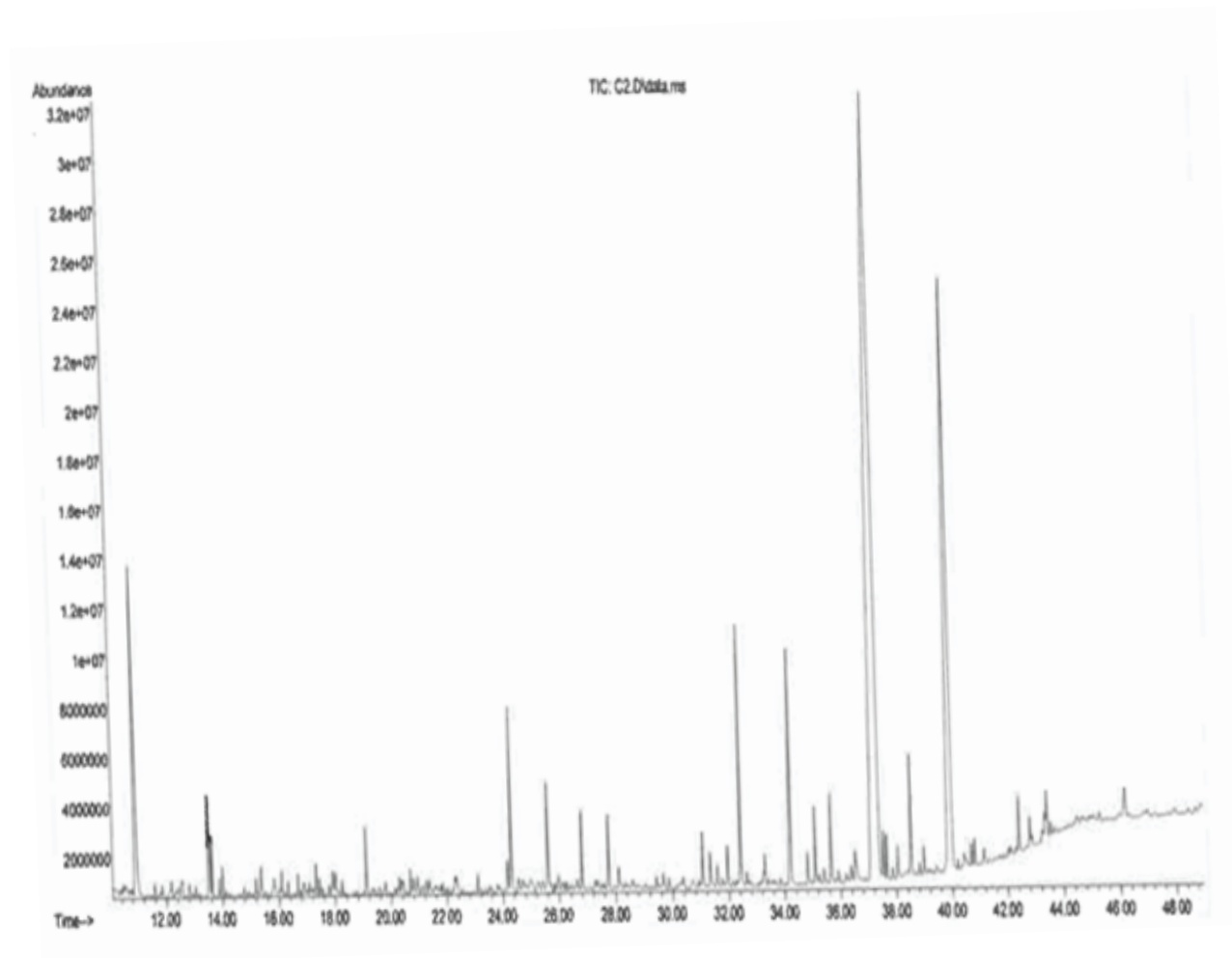


Figure 6. Chromatogram of the extract *S. alpina*

alkamides, steroids, unsaturated alkenamides and alkyne.

Antioxidant activity of extracts of *S. alpina* and *S. Sordida*

Since preliminary studies showed that the largest amount of phenolic compounds from the aerial parts of *S. alpina* and *S. sordida* was extracted with 70% ethyl alcohol, these extracts were used to study the antioxidant activity. Extracts were obtained using the following method: 1 g of dried and crushed raw material (exactly weighed) - the above-ground part was placed in a 100 ml conical flask, 30 ml of 70% ethyl alcohol was added, a reflux condenser was connected and heated in a boiling water bath for 30 minutes. Then the extract was separated from the meal using a paper filter. The procedure was repeated thrice. The resulting total extraction was transferred to a 100 ml volumetric flask and adjusted to the mark with 70% ethyl alcohol. The presence of antioxidants was determined using an in vitro calculation method. Their total content in the analyzed extracts was determined using the peak areas of differential curves, which reflected the dependence of the output signal on the concentration of the sum of phenolic compounds in terms of quercetin or gallic acid.

The figures show graphs of the output signals of quercetin Figure 7 and gallic acid in NAc versus concentration in mg/ml, Figure 8. Data on the total concentration of antioxidants in the studied extracts from the aerial parts of *S. alpina* and *S. sordida* are presented in Table 6.

DISCUSSION

Some frequencies are absorbed when infrared radiation passes through a sample of an organic compound; however, some frequencies are transmitted through the sample without any absorption. Absorption of infrared radiation is associated with vibrational changes occurring within the molecule caused by infrared radiation. Therefore, infrared spectroscopy can be characterized as vibrational spectroscopy. Different bonds (C–C, C=C, C–C, C–O, C=O, O–H, and N–H) have different vibrational frequencies. If such bonds are present in an organic molecule, they can be detected by determining the characteristic frequency absorption band in the infrared spectrum²². Fourier transform infrared spectroscopy (FTIR) is a high-resolution analytical tool for detecting chemical components and

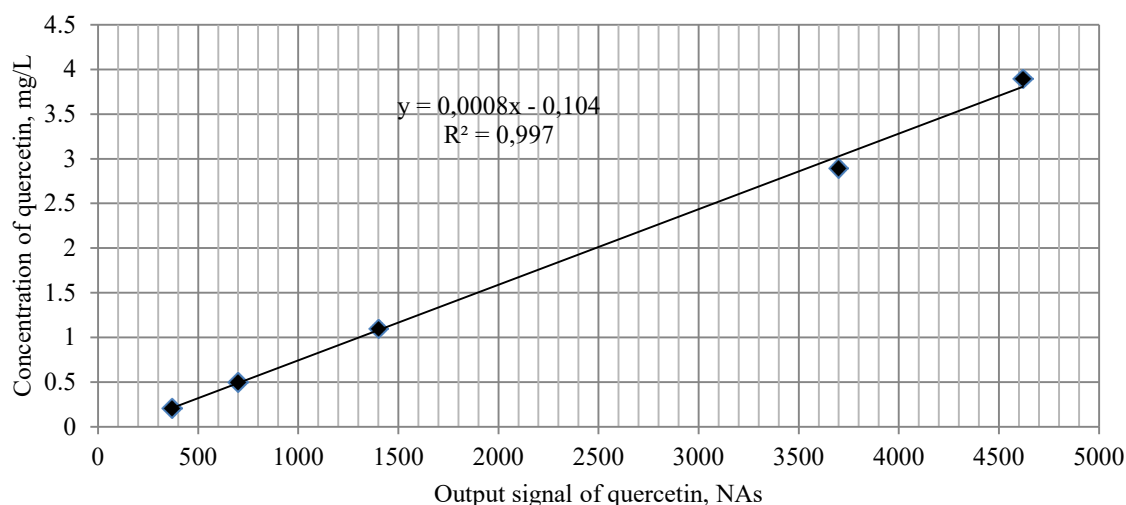


Figure 7. Dependence of quercetin output signal on concentration

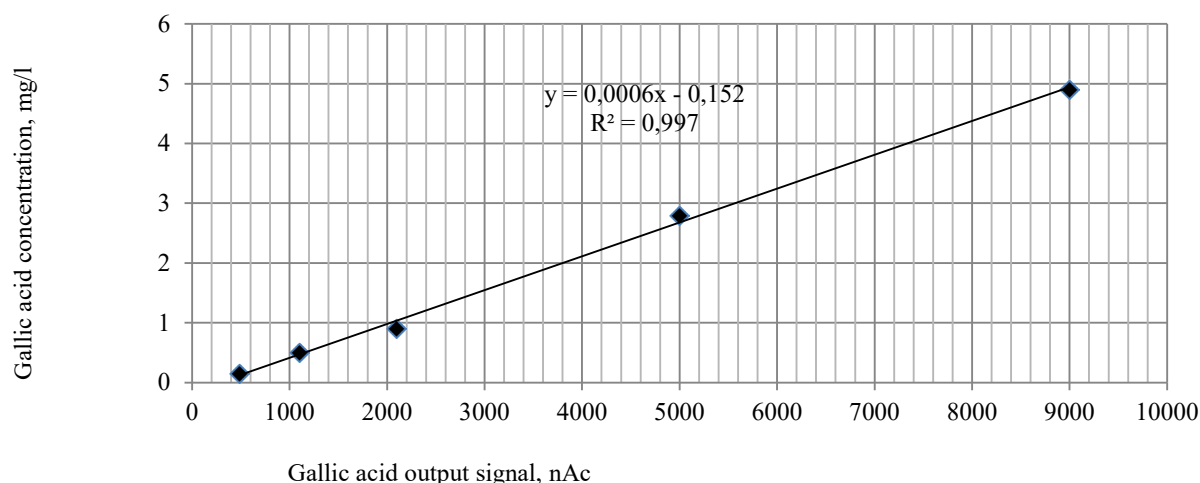


Figure 8. Dependence of the output signal of Gallic acid on concentration

Table 6. Total content of antioxidants in 70% ethanol extracts of <i>C. sordida</i> and <i>S. alpina</i>		
Subject of research	Antioxidant content, calculated as quercetin (mg/g)	Antioxidant content, calculated as gallic acid (mg/g)
70% ethanol extract of <i>S. alpina</i>	0,125 ± 0,005	0,088 ± 0,006
70% ethanol extract of <i>C. sordida</i>	0,056 ± 0,007	0,043 ± 0,008

Table 7 Identified bioactive compounds from <i>S. sordida</i> and <i>S. alpina</i> aerial part through the GC–MS technique			
Name of the Compound	Sature of the Compound	Activity	Reference
(+)-2-Bornanone	Monoterpene oxide	Antitumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide, Anticancer.	[24]
phytol	diterpene	Antimicrobial, Antibacterial, Anticancer, Anti-inflammatory, antioxidant,	[25]
2-pentadecanone	Ketone	Anti-Inflammatory, Antioxidant And Antiulcer Activities	[26]
2,4-dimethyl-2,4-pentanediol	chiral diol	Antimicrobial,	[27]
eudesm-4(14)-en-11	Sesquiterpenes	Antioxidant, Antimicrobial, and Cytotoxic	[28]
5-eocosene,(E)-	Alken	Enzyme inhibition antioxidant	[29]
hexacosane	Alkane	Antimicrobial	[30]

detecting structural compounds. FTIR offers rapid and non-destructive testing of herbal extracts or powders²³⁻²⁴.

The ethanol extracts from *S. sordida* and *S. alpina* was shown to have several bioactive components that were confirmed by FTIR spectroscopy, including alcohol, carboxylic acid, phenolic acids, and aromatic compounds. the functional groups identified from the ethanoic extract are shown in Table 2, 3 and Figure 3, 4. The powerful instance peak is located at peak number 3329, which assigns the NH stretch. The biologically active substances and their derivatives important constituents of plants and are commonly known to possess various medicinal properties. Especially they are known to have antimicrobial and antibacterial activities and the next generation of medications that can be used to treat different illnesses caused by pathogens could be replaced with terpens and their derivatives.

In the present study, (+)-2-Bornanone, phytol, 2-pentadecanone, 6,10,14-trimethyl, 2,4-dimethyl-2,4-pentandiol, eudesm-4(14)-en-11-ol, hexacosane are the lead compounds showing among the subjected bioactive compounds. The biologically active substances of this extracts, have antibacterial, antimicrobial, antioxidant and anti-inflammatory activity (Table 7). Thus, we want to draw attention to the fact that this circumstance indicates that this plants speciese is a potential raw material for drug development.

Thus, the proposed schemes for determination of the above organic substances in various objects by GC-MS methods provide high accuracy and reliability of determination of the determined substances and can be used for determination of some products of plant origin. The possibility of practical application of the identified marker compounds for authentication and quality control of dietary supplements is shown. On the basis of the obtained data it is established, one hundred of two plant species (*S. sordida*, *S. alpina*) contain a number of specific markers that allow their unambiguous identification.

In recent years, a connection has been established between the growth of free radicals (superoxide anion radical, hydroperoxide radical, hydrogen peroxide, hydroxyl radical, etc.) in human biological fluids with the occurrence of dangerous diseases, in particular cardiovascular, cancer, diabetes, etc. Premature aging is also associated with an increase in the number of free radicals. To reduce the level of free radicals in human biological fluids, it is recommended to consume daily foods, medications, dietary supplements and drinks containing natural antioxidants, in particular fruits, berries, vegetables, vegetable oils, tea, honey, etc. Regular preventive consumption of the appropriate level of antioxidants will restore the body's antioxidant status to normal and maintain health and a full life expectancy.

The amperometric method is based on measuring the electric

current that arises during the oxidation of the substance under study (or a mixture of substances) on the surface of the working electrode, which is under a certain potential. It is known that the amperometric method of analysis has a number of advantages: a low detection limit, high selectivity (only compounds whose molecules can be oxidized are determined; other compounds present even in high concentrations are not determined), small cell volume (0.1-5 μ l) , simplicity of maintenance. The presence of antioxidants was determined using an in vitro calculation method. Their total content in the analyzed extracts was determined using the peak areas of differential curves, which reflected the dependence of the output signal on the concentration of the sum of phenolic compounds in terms of quercetin or gallic acid.

The plant objects studied in this work contain significant amounts of biologically active substances, including phenolic compounds, which have antioxidant properties. As can be seen from Table 6, the antioxidant activity results of medical plants showed that *S. alpina* extract has more activity than *S. sordida* extracts in results of both methods (content of antioxidants in terms of quercetin and gallic acid). The 70% ethanol extract of *S. alpina*: quercetin - $0,125 \pm 0,005$ mg/g; gallic acid - $0,088 \pm 0,006$ mg/g.

CONCLUSION

The main range of vibrations of molecules, called “mid-infrared”, lies between wave numbers from 4000 to 400 cm^{-1} . Absorption bands reflecting the general chemical composition were found in all spectra of the studied samples. Through the use of FT-IR and GC–MS techniques in this investigation, it was shown that the observed biological activities came at the expense of the plant's rich phytochemical content. Further studies are necessary to isolate pure biologically active compounds responsible for the noticed biological potentials. The antioxidant activity results of medical plants showed that *S. alpina* extract has more activity than *S. sordida* extracts in results of both methods (content of antioxidants in terms of quercetin and gallic acid).

AUTHOR CONTRIBUTIONS

Conceptualization, Zh.K.; writing—original draft preparation, A.T.; methodology, A.K., K.R.; Formal analysis K.O., S.O., investigation Zh.S., F.K., supervision, R.O.; project administration, R.K, S.O.; funding acquisition, A.K., validation S.O. K.R. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no competing interests.



References

1. Ameenah GF. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Mol Aspects Med.* 2006 Feb;27(1):1-93.
2. Alves RR, Rosa IM. Biodiversity, traditional medicine and public health: where do they meet? *J Ethnobiol Ethnomed.* 2007 Mar;21:3:14.
3. Yuan H, Ma Q, Ye L, Piao G. The Traditional Medicine and Modern Medicine from Natural Products. *Molecules.* 2016 Apr 29;21(5):559.
4. Fan CQ, Yue JM. Biologically active phenols from *Saussurea medusa*. *Bioorg Med Chem.* 2003 Mar 6;11(5):703-8.
5. Chik WI, Zhu L, Fan LL, Yi T, Zhu GY, Gou XJ, et al. *Saussurea involucrata*: A review of the botany, phytochemistry and ethnopharmacology of a rare traditional herbal medicine. *J Ethnopharmacol.* 2015 Aug 22;172:44-60.
6. Kreuzer M, Tribsch A, Nyffeler R. Ecological and genetic differentiation of two subspecies of *Saussurea alpina* in the Western Alps. *Alp Botany.* 2014 Apr;124(1):49-58.
7. Fan WX, Yang WP, Liu HS. Research progress on culture technologies, chemical components and pharmacological activities of *Saussurea involucrata* cells. *China Journal of Chinese Materia Medica.* 2021 Jul;46(14):3522-3528.
8. Zhang Q, He L, Jiang Q, Zhu H, Kong D, Zhang H, Cheng Z, Deng H, Zheng Y, Ying X. Systems Pharmacology-Based Dissection of Anti-Cancer Mechanism of Traditional Chinese Herb *Saussurea involucrata*. *Front Pharmacol.* 2021 Jun 25;12:678203.
9. Dongdong Zh, Zhaoye W, Jin L, Jianbo Zh. Exploring the possible molecular targeting mechanism of *Saussurea involucrata* in the treatment of COVID-19 based on bio-informatics and network pharmacology. *Comput Biol Med.* 2022 Jul;146:105549.
10. Ahmed GS, Coskun US. Investigation of antibacterial and antifungal activity of *Saussurea costus* root extracts. *An Acad Bras Cienc.* 2023 Jul 24;95(suppl 1):e20230059.
11. Ebraheem A, Amal Z, Omar MS, Giovanni C, Essam AS. *Dolomiaea costus*: an untapped mine of sesquiterpene lactones with wide magnificent biological activities. *Nat Prod Res.* 2023 Nov-Dec;37(23):4069-4079.
12. Mammate N, Oumari FEE, Imtara H, Belchkar S, Lahrichi A, Alqahtani AS, Noman OM, Tarayrah M, Houssaini TS. Antioxidant and Anti-Urolithiatic Activity of Aqueous and Ethanolic Extracts from *Saussurea costus* (Falc) Lispich Using Scanning Electron Microscopy. *Life (Basel).* 2022 Jul 11;12(7):1026.
13. Arshad N, Ishtiaq S, Kamran SH, Rehman MS, Akbar S, Rehman S, Rehman S, Hareeri RH, Fadil SA, Youssef FS, Elhady SS. Evaluation of the Antihyperglycemic and Antihyperlipidemic Activity of *Saussurea hypoleuca* Root in Alloxan-Induced Diabetes in Rat Model and Correlation to Its Major Secondary Metabolites. *Life (Basel).* 2022 Sep 19;12(9):1451.
14. Vasileva AN, Gamaunova AP, Goloskokov VP, Zaiceva LG, Karmysheva NH, Orazova A. *Flora of Kazakhstan*; editor N.V. Pavlov. KazSSR Academy of Sciences: Alma-Ata, Kazakhstan, 1966; Vol. 9; ISBN 978-5-4458-5991-8. (In Russian).
15. Tsukasa I, Sergey S, Oyunchimeg D, Katsuhiko K. *Saussurea* Species from the Altai Mountains and Adjacent Area, and Their Flavonoid Diversity. *Bull. Natl. Mus. Nat. Sci., Ser.* 2010;36:141-154.
16. Katsumi K, Tsukasa I, Junichi K, Tamaki M. Flavonoid Diversity of *Saussurea* and *Serratula* Species in Tien Shan Mountains. *Nat Prod Com.* 2007;2(11):1121-1128.
17. Coates J. Interpretation of Infrared Spectra, A Practical Approach. *Encyclopedia of Analytical Chemistry.* 2000:10815-10837.
18. Park YS, Myeng HI, Ham KS, Kanget SG, et al. Quantitative assessment of the main antioxidant compounds, anti-oxidant activities and FTIR spectra from commonly consumed fruits, compared to standard kiwi fruit. *LWT-Food Science and Technology.* 2015 Sep 63: 346-352.
19. Barud HdS, Júnior AM, Saska S, Mestieri LB, Campos JA, et al. Antimicrobial Brazilian Propolis (EPP-AF) Containing Biocellulose Membranes as Promising Biomaterial for Skin Wound Healing. *Evid Based Complement Alternat Med.* 2013: 703024.
20. Franca J, De Luca M, Ribeiro T, et al. Propolis - based chitosan varnish: drug de-livery, controlled release and antimicrobial activity against oral pathogen bacteria. *BMC Complement Altern Med.* 2014 Dec 12;14:478.
21. Mot A, Silaghi-Dumitrescu R, Sârbu C. Rapid and effective evaluation of the antioxidant capacity of propolis extracts using DPPH bleaching kinetic profiles, FT-IR and UV-vis spectroscopic data. *Journal of Food Composition and Analysis.* 2011;24: 516-522.
22. Urbano M, LdCMD, Pérez PM, García-Olmo J, Gómez-Nieto MA. Ultraviolet–visible spectroscopy and pattern recognition methods for differentiation and classification of wines. *Food Chem.* 2006;97:166–175.
23. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phyto-chemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants (Basel).* 2017 Sep;22:6(4):42.
24. Shegebayev Zh, Turgumbayeva A, Datkhayev U, Zhakipbekov K, Kalykova A, Kartbayeva E, Beyatli A, Tastambek K, Altynbayeva G, Dilbarkhanov B, Akhelova A, Anarbayeva R, Orynassarova K. Pharmacological Properties of Four Plant Species of the Genus *Anabasis*, *Amaranthaceae*. *Molecules.* 2023 May;31:28(11):4454.