https://doi.org/10.31925/farmacia.2024.5.21 **ORIGINAL ARTICLE**

GC-MS BASED CHARACTERIZATION, ANTIMICROBIAL ACTIVITY OF GARLIC CO_2 SUBCRITICAL EXTRACT *(ALLIUM SATIVUM)*

AIGERIM BAZARALIYEVA [1]*, AKNUR TURGUMBAYEVA [1], ELMIRA KARTBAYEVA [1], ASSEM KALYKOVA [1], SERJAN MOMBEKOV [2], AIMAN AKHELOVA [2], AINASH ATIMTAIKYZY [3], ALIMA BEKBAGANBETOVA [1], AHMET BEYATLI [4], KYMBAT BEKMURATOVA [3], SERIKBAYEVA ELMIRA ASYLBEKOVNA [2], KURALAY BEKESHEVA [5]

[1]*Higher School of Medicine, "al-Farabi Kazakh National University", Almaty, Kazakhstan*
[2]*School of Pharmacy, "Asfendiyarov Kazakh" National Medical University, Almaty, Kazakhstan*
[3]*"Astana" Medical University, MUA, Nur-Sultan, Kazakhstan*
[4]*University of Health Sciences, Department of Medicinal and Aromatic Plants, 34668, Üsküdar, Istanbul, Türkiye*
[5]*JSC Scientific Centre for Anti-infectious Drugs, Head of quality department, Almaty Kazakhstan*

**corresponding author: abazaralieva24@gmail.com*

Manuscript received: June 2024

Abstract

The article presents the results of the study with purpose whose the chemical composition and antimicrobial effect of garlic CO_2 subcritical extract determination.15 grams of brown volatile garlic extract were collected using subcritical carbon dioxide extraction. The current study was directed mainly to the chemical and antimicrobial study of subcritical garlic extract. The garlic extract's composition was determined using gas chromatography/mass spectrophotometry (GC-MS). The extract included the following main compounds: manool, viridifrolol, podocarpa-1,8,11,13-tetraen-3-one, 14-isopropyl-1,13-dimethoxy-5, (+)-2-bornanone, linolic acid ethyl ester, 12-O-methylcarn and β-caryophyllene. Also, it was determined the fatty acid profile and the moisture content of raw vegetable ingredients of garlic 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 the linoleic (64.9%) and the palmitic acid (25.0%) were the most prominent. Antimicrobial activity was determined by the method of serial dilutions. Five different strains were used to evaluate the antimicrobial activity: two Gram-positive coccus strains (*Staphylococcus aureus*, *Enterococcus faecalis*), two Gram-negative bacilli (*Escherichia coli*, *Pseudomonas aeruginosa*) and one yeast (*Candida albicans*). The results of testing the microbiological activity of garlic CO_2 extract identified that it exhibits various effects against both bacteria and fungi. The extract demonstrated significant antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The extract also showed antifungal activity against *Candida albicans*.

Rezumat

Articolul prezintă rezultatele studiului cu scopul a cărui compoziție chimică și efect antimicrobian al determinării extractului subcritic de usturoi CO_2. 15 grame de extract volatil de usturoi brun au fost colectate utilizând extracția subcritică cu dioxid de carbon. Studiul actual a fost îndreptat în principal spre studiul chimic și antimicrobian al extractului subcritic de usturoi. Compoziția extractului de usturoi a fost determinată cu ajutorul cromatografiei de gaze/spectrometriei de masă (GC-MS). Extractul a inclus următorii compuși principali: manool, viridifrolol, podocarpa-1,8,11,13-tetraen-3-one, 14-izopropil-1,13-dimetoxi-5, (+)-2-bornanonă, ester etilic al acidului linolic, 12-O-metilcarn și β-cariofilen. De asemenea, a fost determinat profilul acizilor grași și conținutul de umiditate al ingredientelor vegetale crude din substanța de usturoi. S-a efectuat determinarea cantitativă a acizilor grași din extractul etanolic. Rezultatele analizei acizilor grași din studiu au arătat că linoleicul (64,9%) și acidul palmitic (25,0%) au fost cele mai proeminente. Activitatea antimicrobiană a fost determinată prin metoda diluțiilor seriale. Cinci tulpini diferite au fost utilizate pentru evaluarea activității antimicrobiene: două tulpini de coccus Gram-pozitiv (*Staphylococcus aureus*, *Enterococcus faecalis*), doi bacili Gram-negativi (*Escherichia coli*, *Pseudomonas aeruginosa*) și o drojdie (*Candida albicans*). Rezultatele testării activității microbiologice a extractului de usturoi CO_2 au identificat faptul că acesta prezintă diverse efecte atât împotriva bacteriilor, cât și împotriva ciupercilor. Extractul a demonstrat o activitate antibacteriană semnificativă împotriva *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* și *Pseudomonas aeruginosa*. Extractul a demonstrat, de asemenea, activitate antifungică împotriva *Candida albicans*.

Keywords: garlic, subcritical carbon dioxide extraction, GC-MS, microbiological activity

Introduction

Garlic (*Allium sativum*) has been used in the treatment of various minor and deadly diseases since ancient times. Garlic contains volatile, water-soluble and oil-soluble organosulfur compounds, essential oils, dietary fibre, sugars, flavonoids and pectin [1].

It contains approximately 33 sulphur compounds, 17 amino acids, enzymes, mineral salts, vitamins and valuable essential oils [2-9]. According to Gebreyohannes

G *et al*. (2013), garlic is thought to have over 200 chemical compounds that can shield the human body from a number of ailments [10].

Garlic (*Allium satívum*) is a perennial herbaceous plant of the onion subfamily. Bactericidal, inhibitory, anti-viral and antitumour properties of garlic have been found [11].

An extract from an underground garlic bulb, especially the compound allicin (diallyl thiosulphinate) contained in it, inhibits the growth of many types of Gram-positive and Gram-negative bacteria [12-14]. Widespread use of antibiotics in the last decades stimulated the emergence of drug-resistant bacteria. This effect has been noticed in both Gram-positive and Gram-negative bacteria. Garlic extract is an excellent alternative to the antibiotics currently used. In addition, essential to study new drugs due to the emergence of bacterial strains resistant to traditional antibiotics.

In 2017, the WHO published a list outlining global priorities for research and development of new antibiotics. Among them, enterobacteria with high resistance to *Escherichia coli* and *Pseudomonas aeruginosa* (resistant to carbapenems) topped the list of critical and urgent threats requiring greater global attention and faster action. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE) were also mentioned by the Organization and classified as high-priority pathogens [15-17]. In addition to multidrug resistance, these bacteria are also common nosocomial pathogens.

The chemical composition of garlic is determined by pre-harvest conditions such as genotype, climate requirements, growing conditions, irrigation, fertilization and harvesting stage. Post-harvest conditions draw attention to processing methods, consumption forms (aged garlic, fresh garlic, cooked garlic, dried garlic and so on), humidity and harvesting time.

Dried garlic contains alliin as well as low levels of sulphur compounds. Oil garlic extract contains allicin and sulphur compounds but not alliin [18-20].

Referring to previous research, the antibacterial properties of CO_2 subcritical extract for the most resistant strains of bacteria were studied.

Subcritical CO_2 extraction requires less pressure and lower temperature (non-supercritical liquid) than supercritical CO_2 extraction. This process is longer, but it retains and protects fragile constituents like essential oils, terpenes and other sensitive chemicals within the plant. Subcritical carbon dioxide extraction refers to the processing of vegetable raw materials with liquid carbon dioxide at pressures of up to 70 atm and temperatures of up to 30°C. Simultaneously, the physiologically active chemicals in plant raw materials are leached through cell membranes and enter the liquid phase. Subcritical extraction makes it possible to obtain universal multicomponent mixtures of substances that are closest to the original composition of the plant. And the physicochemical properties of subcritical CO_2 extracts provide an opportunity for their wide application, both as natural flavouring ingredients and biologically active additives in the production of dietary supplements and pharmaceuticals [21].

Carbon dioxide (CO_2) is frequently used as a supercritical fluid because it has favourable critical characteristics (Tc = 31.1°C, Pc = 7.38 MPa), is non-toxic, inert, chemically stable, inflammable, inexpensive, abundant and environmentally friendly. It is a non-polar solvent that can be used to help polar compounds dissolve more easily since it has strong miscibility with methanol or ethanol. It is chemically located between pentane and toluene. The application of supercritical fluid extraction gives improved extraction yields due to the higher penetration power of supercritical fluid into porous solid materials, higher selectivity, efficiency, stability and adaptability due to the changeable solvation power (temperature, pressure) [22].

Therefore, the purpose of this study is a quantitative assessment of chemical composition and also volatile components of garlic extract, which makes it possible to effectively analyse natural ingredients. The current study was directed mainly to the use of liquid CO_2 as a solvent to extract the volatile oil-rich fraction from garlic seeds with low lipid content. This requires work at much lower pressures. 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). CO_2 extraction is clean, cold and undamaged extraction [22].

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

In this paper results of the bioactive compounds of garlic obtained by subcritical carbon dioxide extraction are shown. Quantitative determination of fatty acids of ethanol extract was carried out. The antimicrobial and antifungal effect of the obtained extract was evaluated.

Materials and Methods

To obtain the CO_2 extract, garlic "Dobrynya" (*Allium sativum*) has been collected in the South Kazakhstan province, during the winter of 2021.

Garlic has been dried in a thermostat at 22°C. The raw materials were peeled and crushed to 10 - 15 mm using cutting tools. Then the crushed raw materials – garlic cloves were sent to the drying cabinet and dried for 14 days until a complete state of dehydration. Statistical analysis used: quantitative determination of fatty acids, moisture content of the raw vegetable ingredients.

The subcritical CO_2 extraction

The extract was obtained from winter garlic "Dobrynya", from dried garlic cloves weighing 1600 g, under subcritical conditions of CO_2 extraction for 8 h at 57 - 65 atm and 18 - 23°C. The subcritical CO_2 extraction was carried out at Zhanafarm LLP, Almaty, Kazakhstan (No. 30, 21.03.2022), on the extraction unit CDFE-5 l (Carbon dioxide flow extraction unit 5^{th} – laboratory). The extractant is liquid carbon dioxide GOST 8050-85.

GC-MS method

To study chemical compounds Gas chromatography – Mass spectrophotometry (GC-MS) methods were used (SPH RK, vol.1, 2.2.28).

Analysis of all prepared samples was performed utilizing an Agilent 7890A gas chromatographic (GC) instrument which was equipped with an Agilent 7693 autosampler (Shimadzu QP-2010S GC-MS). Using GC-MS, mixtures of compounds are separated based on polarity, and the components are analysed by mass spectrometry. The instrument is equipped with an electron ionization (EI) source and can detect the mass range from 10 to 900 *m/z*.

The GC was connected to an Agilent 5975C mass spectrometer (MS). The capillary column (60 m x 0.25 mm *i.d.*) utilised was coated with a 100% dimethylpolysiloxane (Agilent DB-1MS) film (0.25 ηm). Helium at a constant flow rate of 1 mL/min was used as the carrier gas. Each sample was analysed using the following GC oven program: 50°C held for 1 minute, then heated at a rate of 5°C/min to 280°C and held at 280°C for 10 minutes. The inlet was programmed at 280°C in split mode, with a split ratio of 50:1. The transfer tube from the GC to the MS was held at 280°C.

The Agilent 5975C mass spectrometer was operated with an electron energy of 70 eV.

The source, quadrupole and transfer line temperatures were 230°C, 150°C and 280°C, respectively during the experiment. All mass spectra data were recorded from 40 to 500 *m/z* after a 7 min solvent delay, The NIST (National Institute of Standards and Technology) database (version 2.3) was utilised for tentative compound identification.

Quantitative determination of fatty acids

Quantitative determination of fatty acids was carried out by the method of Gas chromatography 2.4.22, Method A, (European Pharmacopoeia 6.0). 1 sample volume is extracted by 20 times the volume of a mixture of $CHCl_3$ and CH_3OH (2:1) for 5 minutes. Then the contents are filtered through a paper filter until a pure extract is obtained, which is evaporated in a round-bottomed flask on a rotary evaporator (EYELA 1300 ROTARY) at a bath temperature of 30 - 40°C to dry. After that, 10 mL of methanol and 2 - 3 drops of acetyl chloride are added to the flask and methylated at a temperature of 60 - 70°C in a special system for 30 minutes. Then the methanol is evaporated on a rotary evaporator, and the sample is extracted from a 5 mL hexane cone and injected into a gas chromatograph.

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".

Antimicrobial Activity Determination

Microbiological analysis by method of serial dilutions.

Antimicrobial activity Determination 1 was carried out in Kazakhstan, Almaty, at the Laboratory of Microbiology "Scientific Centre for Anti-infectious Drugs".

Antimicrobial activity was tested on the following test strains of microorganisms: (1) *S. aureus* ATCC 6538-P is a reference strain for the determination of antimicrobial activity obtained from the Republican Collection of Microorganisms (RCM), Astana, RK; (2) *E. coli* ATCC 8739 is a reference strain for the determination of antimicrobial activity obtained from the American Collection of Type Cultures (ATCC), USA.

Nutrient media and reagents. Muller-Hinton agar (Himedia, India); Muller-Hinton broth (Himedia, India); sodium chloride, H.ch., (Mikhailovsky Chemical Reagents Plant, Russia); ethanol, 96% (Talgar Alcohol, Kazakhstan); purified water.

The studied sample was *Allium sativum* garlic extract: a thick yellow-orange substance with a characteristic smell.

Preparation of a suspension of microorganisms. An aliquot of a daily cultured test strain was selected with a bacteriological loop, transferred to a test tube with a sterile isotonic sodium chloride solution, and thoroughly homogenised until a homogeneous suspension was obtained (ELMI, Latvia), after which the optical density of all prepared stock suspensions of cultures was measured densitometrically (DEN-1, Latvia). The suspension density of each studied strain was 0.5 units according to McFarland, which corresponded to a cell concentration equal to ~ 1.5 × 108 CFU/mL. To prepare working suspensions of bacteria, the stock inoculum was diluted with an isotonic solution 100 times to a concentration of ~ 1.5 × 106 CFU/mL.

The procedure for testing antimicrobial activity was carried out by the method of double serial dilutions in a liquid nutrient medium − Muller-Hinton broth. The procedure was carried out in sterile 96-well culture plates made of polystyrene (BIOLOGIX, China). Previously, the appropriate liquid nutrient medium in the amount of 150 μL was introduced into the required number of wells of the tablet. In all the first wells of the rows (A1-H1), 150 mL of the antimicrobial agent base solution was added, after which a series of serial double dilutions were made: a carefully piped mixture of broth and solution from well No. 1 in the amount of 150 mL was transferred to well No. 2, the resulting mixture from well No. 2 in the amount of 150 mL was transferred to the well No. 3. The action was repeated until the required number of double dilutions was reached. 150 mL of the mixture was

removed from the last hole. Thus, serial dilutions from 1:1 to 1:2048 in the ratio antimicrobial agent: broth were obtained in each row of the tablet (wells A-H) (Table I).

Table I

Dilutions used for antimicrobial activity testing

No. wells	1	2	3	4	5	6	7	8	9	10	11	12
The resulting dilution (antimicrobial agent: broth)	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048

Inoculation and incubation conditions. 20 mL of the working inoculum solution was added to each well containing 150 mL of the combination after the working suspension had been made. After seeding, the number of cells *per* well reached a final concentration of 1.5 105 CFU/mL. The tablets were incubated for 18 to 24 hours in Binder, Germany, at a temperature of 37°C. After the incubation period, 0.01 - 0.02 mL were seeded from each well onto pre-lined Petri dishes with a sterile loop in order to ascertain the bactericidal dilution. Each of the cup's cells represented a well's ordinal number with a specific level of dilution. Petri plates were seeded, then kept in a thermostat for 18 to 24 hours while cultivation took place at 37°C.

Microbiological analysis by method of broth dilution

At the same time, antimicrobial properties were tested on the following strains at the pharmaceutical faculty of the Medical University in Istanbul (Türkiye).

Five different strains were used to evaluate the anti-microbial activity; two Gram-positive coccus strains (*S. aureus* ATCC 25923, *E. faecalis* ATCC 51299) two Gram-negative bacilli (*E. coli* ATCC 25922, *P. aeruginosa* ATT 27853) and one yeast; (*C. albicans* ATCC 90028). All reference strains were obtained from the Department of Medical Microbiology, Medical Faculty of Istanbul University, Türkiye.

MIC (minimum inhibitory concentrations) was determined by the broth dilution method according to the definition of the Clinical and Laboratory Standards Institute (CLSI 2018). All bacterial strains were cultured on tryptic soy agar (OXOID, Türkiye) medium, while fungus cells were cultured on Sabouraud dextrose agar (OXOID, Türkiye) and incubated aerobically at 35°C for 24 - 48 hours. Bacterial cultures were then suspended in sterile saline (0.85% NaCl) and adjusted to 0.5 McFarland turbidity (10^8 CFU/mL). Negative controls (medium containing only plant extract), positive controls (medium containing bacteria only) and plant extracts (5000 - 90 μg/mL) were placed in U-bottom, 96-well microplates at a final inoculum concentration of 1 x 10^5 CFU/mL. Mueller Hinton Broth (OXOID, Türkiye) and RPMI 1640 (Thermo Fischer Scientific, Türkiye) were used to prepare serial dilutions of the plant extract for bacterial and fungus cells, respectively. All inoculated plates were incubated at 35°C for 24 - 48 hours and MIC values were determined.

Results and Discussion

During the preparation of the winter garlic extract "Dobrynya" 1600 g of the main raw materials are weighed, and the pre-dried herb is crushed and filtered. Then the plant's raw materials are placed in an extractor, where a liquid solvent is added from an adjustable container and pumped into the extractor. Carbon dioxide is neutralised, and the extract and carbon dioxide are separated in the distiller chamber, and the extract is collected in the lower part of the distiller and periodically sampled. Finally, the extract is collected in distillers and poured into the collector for the extractor. As a result, a dark brown extract weighing 15 g was obtained.

Chemical Compositions

Chemical components were discovered in considerable concentrations in the makeup of the garlic extract, according to the study's findings: manool (39.56%), viridifrolol (7%), podocarpa-1,8,11,13-tetraen-3-one,14-isopropyl-1,13-dimethoxy-(5,15%), (+)-2-bornanone (4.29%), thujone (3.49%), linolic acid ethyl ester (3.41%), 12-O-methylcarn (2.56%) and β-caryophyllene (2.17%). The chemical composition of the plant extract was studied at the Pharmaceutical Faculty of Mississippi University, USA. All chemical compounds and their areas in extract, match factors are shown in Table II.

Table II

Results of GC-MS of subcritical carbonic acid extract of *Allium sativum*

No.	**Sample RT**	**Component name**	**Sample match factor**	**Component area**	**Area %**
1	12.0592	Bicyclo[3.1.1]-hept-2-ene, 3,6,6-trimethyl-	95.9	102127.4	0.33
2	12.4722	Camphene	97.6	108284.4	0.35
3	13.1235	Bicyclo[3.1.0]-hex-2-ene, 4-methyl-1-(1-methylethyl)-	95.2	21907.7	0.07
4	13.293	Cyclohexene, 4-methylene-1-(1-methylethyl)-	95.6	42683.6	0.14
5	14.4473	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	89.5	41491.3	0.13
6	14.5268	1,3,8-p-Menthatriene	81.3	20765.1	0.07
7	14.8021	Eucalyptol	98.4	683813.2	2.18
8	15.4429	1,5-Heptadien-4-one, 3,3,6-trimethyl-	94.7	90407.8	0.29
9	15.6811	γ-Terpinene	88.7	31179.1	0.10
10	16.6978	Linalool	68.9	27415.7	0.09

No.	Sample RT	Component name	Sample match factor	Component area	Area %
11	16.8567	Thujone	98.9	1091321	3.49
12	17.1903	Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1.α.,4. B.,5.α.)]	98.6	505038.9	1.61
13	17.9422	(+)-2-Bornanone	98.6	1343652.2	4.29
14	18.7842	endo-Borneol	96.5	206642.9	0.66
15	19.1337	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	80	28843.8	0.09
16	19.4196	1,2,2,3-Tetramethylcyclopent-3-enol	75.5	93945.9	0.30
17	22.2367	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	96.9	190889.2	0.61
18	23.0628	Bicyclo[3.2.0]heptan-2-one, 5-formylmethyl-6-hydroxy-3,3-dimethyl-6-vinyl-	77.1	131567	0.42
19	23.174	Bicyclo[3.2.0]heptan-2-one, 5-formylmethyl-6-hydroxy-3,3-dimethyl-6-vinyl-	77.1	239579	0.77
20	25.721	Bicyclo[2.2.1]heptan-2-one, 5-(acetyloxy)-4,7,7-trimethyl-, endo-	70.1	62984.1	0.20
21	26.3512	Caryophyllene	98.5	680697.2	2.17
22	27.1825	1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	97.9	529581.8	1.69
23	27.9715	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha	95.9	141371.4	0.45
24	28.1834	Selina-3,7(11)-diene	79.1	22761.4	0.07
25	30.1373	Caryophyllene oxide	94	163980.5	0.52
26	30.4127	Viridifrolol	98.7	2190664.6	7
27	30.7092	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo(9.1.0jdodeca-3,7-diene	95.1	2190664.6	0.70
28	34.4212	β-Clovene	80.2	218704.5	0.09
29	35.5915	2-Pentadecanone, 6,10,14-trimethyl-	80.7	27125.9	0.14
30	35.7186	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	82.4	42441.8	0.11
31	37.2807	Cyclohexene, 4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl-	75.7	35183.9	0.07
32	37.5507 I	Isopimara-9(11),15-diene	86.3	21802.5	0.28
33	37.7679	n-Hexadecanoic acid	80.3	86863.9	0.60
34	38.4827	Hexadecanoic acid, ethyl ester	96.6	188899.1	1.67
35	39.5524	Arteannuin b	75.5	522631.5	0.12
36	39.9336	Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)-	74.2	36009.6	0.17
37	40.0395	Manool	97.9	12381022,5	39.56
38	40.6273	1,4-Dimethyl-8-isopropylidene-tricyclo[5.3.0.0(4,10)]-decane	88.1	12381022,5	39.56
39	40.9556	9,12-Octadecadienoic acid-(Z,Z)-	92.1	629790,6	2.01
40	41.0774	cis-Vaccenic acid	86.4	294716.6	0.94
41	41.5169	Linoleic acid ethyl ester	97.3	461184.2	1.47
42	41.5911	9,12-15 – Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	93.7	1065862.5	3.41
43	41.6599	€-9-Octadecanoic acid, ethyl ester	92.6	407659.2	1.30
44	42.1418	Octadecanoic acid, ethyl ester	80	496552.8	1.59
45	43.7039	4,5,6,7-Tetrahydroxy-1,8,8,9-tetramethyl-8,9-dihydrophenaleno[1,2-b]-fufan-3-one	78.4	73042.5	0.23
46	43.3446	Podocarpa-1,8,11,13-tetraen-3-one,14-isopropyl-1,13-dimethoxy	47	396695.3	1.27
47	44.4823	Podocarpa-1,8,11,13-tetraen-3-one,14-isopropyl-1,13-dimethoxy	73.1	1610705.1	5.15
48	45.1707	12-O-Methylcarn	82.9	800726,3	2.56
49	45.642	Kolavenol acetate	79.8	120608.8	0.39
50	45.9756	N-Methyllaurotetanine	74.3	451027.2	1.44
51	48.6391	Phenanthrene 1,2,3,4,4a,9,10,10a-octahydro-6-methoxy-1,1,4a-trimethyl-7-(1methylethyl)-	77.8	665715.5	2.13
52	50.376	(Z)-3-(Heptadec-10-en-1-yl)-phenol	73.1	268113,2	0.86
53	51.8745	12-O-Methylcarnosol	82.8	109453	0.35
54	53.479	Squalene	89.8	423122.4	1.35

The biologically active substances of this extract have antibacterial, antimicrobial, antioxidant and anti-inflammatory activity (Table II). Thus, we want to draw attention to the fact that this circumstance indicates that this plants species 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.

The garlic extract fatty acid profile

The fatty acid composition of the oil major factor determining its best commercial uses such as nutritional, industrial, or pharmaceutical, and it is influenced by the variety, climate and the areas of production**.** The results of the analysis for fatty acids in the study showed that linoleic (64.9%), palmitic (25.0%) acids were the most prominent acid s encountered in the *Garlic (Allium sativum)* extract, followed by linolenic (5.7%), oleic (3.2%), stearic (0.8%) acids. The lesser content of myristic, pentadecane and palmitoleic acids did not exceed 0.50% of the total fatty acid (Table III).

Table III

Relative percent composition of fatty acid in *Garlic (Allium sativum)*

Chain Length	Acid Name	X (%)
$C_{14:0}$	Myristic	0.1
$C_{15:0}$	Pentadecane	0.2
$C_{16:0}$	Palmitic	25.0
$C_{16:1}$	Palmitoleic	0.1
$C_{18:0}$	Stearic	0.8
$C_{18:1}$	Oleic	3.2
$C_{18:2}$	Linoleic	64.9
$C_{18:3}$	Linolenic	5.7

As seen in the Table III, the saturated fatty acids of garlic studied are found in large quantities: palmitic (25%) and Linoleic (64.9%), these fatty acids do not increase blood cholesterol levels in people with cholesterol levels within normal limits.

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):

$$X = ((M-M\ 1))/M*100 = ((1-0{,}948))/1*100 = 5.2\%.$$

where, 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.

Antimicrobial Activity

Previous study results showed that extracts from various plants were less active against fungal species than bacterial species. The results showed that the extracts were more active against the Gram-positive *staphylococci (S. aureus* and methicillin-resistant *S. aureus),* the Gram-negative bacteria *(E. coli* and *P. aeruginosa),* the acid-fast bacilli *(Mycobacterium intracellular).* The results are in agreement with previous studies which indicated that plant extracts were more active against Gram-positive bacteria than those that are Gram-negative [24, 27]. These differences may be attributed to the fact that the cell wall in Gram-positive bacteria consists of a single layer, whereas the Gram-negative cell wall is a multi-layered structure, bounded by an outer cell membrane and quite complex [30]. The different percentages of microbial growth inhibition can be attributed to the different chemical compositions and modes of action of these plant extracts [31-33]. *C. albicans* is an important human opportunistic fungal pathogen that is frequently found as part of the normal human microbiota. It is well-accepted that the fungus interacts with other components of the resident microbiota and that this impacts the commensal or pathogenic outcome of *C. albicans* colonization [34].

Activity testing results of microbiological analysis by using the method of serial dilutions

The antimicrobial activity of *Allium sativum* (garlic) extract has been tested against two different strains of microorganisms: *Staphylococcus aureus* and *E coli*. The results showed that the extract has a different bactericidal effect on each of the strains, which is reflected in the selection in which the bactericidal effect was observed. The results of the study are presented in Table IV.

Table IV

Results of testing the antimicrobial activity of *Allium sativum* garlic extract

Test strain	Breeding in which the bactericidal effect is noted
***S. aureus* ATCC 6538-P**	1:64
***E. coli* ATCC 8739**	1:1

The data obtained indicate the presence of a different bactericidal effect of the extract for different test strains of microorganisms. Thus, about the strain of *S. aureus* ATCC 6538-P, the bactericidal activity was determined at a dilution of 1:64 (Figure 1). The culture of *Escherichia coli* ATCC 8739 in the *in vitro* experiment turned out to be less susceptible, here the antimicrobial effect was observed at a 1:1 dilution (Figure 2).

Figure 1.

Results of testing the antimicrobial activity of *Allium sativum* garlic extract against *S. aureus* ATCC 6538-P

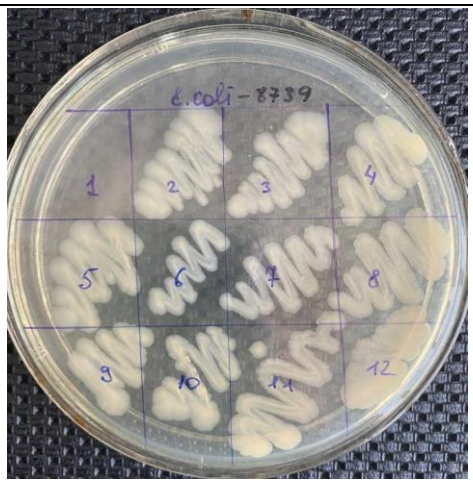

Figure 2.
Results of testing the antimicrobial activity of *Allium sativum* garlic extract against *E. coli* ATCC 8739

At a dilution of 1:64, the extract displayed bactericidal activity against the strain of *S. aureus* ATCC 6538-P. This shows that the extract has considerable antibacterial action against this strain of bacteria even at low concentrations because it was able to kill the germs. The antibacterial action of the extract was only noticed when the extract was diluted 1:1 in the *E. coli* ATCC 8739 culture. This shows that even while the extract still demonstrated some antibacterial action, a greater concentration was required to produce a bactericidal effect against this strain. Overall, these findings imply that *Allium sativum* CO_2 extract may be effective against specific strains of microbes as a natural antimicrobial agent.

Activity testing results of the microbiological analysis by using the method of broth dilutions

The results of the microbiological activity testing of an extract from *Allium sativum* CO_2 showed that it exhibited various effects against both bacteria and fungi. The MIC of the extract was determined against several microbial strains, as displayed in Table V.

Table V
MICs of the extract against various microbial strain

Microorganisms	***A. sativum* CO_2 (MIC µg/mL)**
Bacteria	
S. aureus	500
E. faecalis	475
E. coli	650
P. aeruginosa	650
Fungi	
C. albicans	1000

With MIC values ranging from 475 to 650 g/mL, the extract showed strong antibacterial activity against *S. aureus, E. faecalis, E. coli* and *P. aeruginosa*. This shows that even at relatively low concentrations, the extract was able to prevent the growth of these bacterial strains. With a MIC value of 1000 g/mL, the extract also demonstrated antifungal activity against *C. albicans.* The extract may have potential as a natural antifungal agent, even if the antifungal activity was not as effective as the antibacterial activity.

The fatty acid composition of oil is a critical determinant of its suitability for various commercial applications, including nutritional, industrial and pharmaceutical purposes. This composition is significantly influenced by the cultivar, climatic conditions and geographic regions where the oil is produced. The results of fatty acid analysis illustrate that there are eight different fatty acids in the sample, with palmitic acid and linoleic acid being the most common. Consequently, study outcomes can provide valuable information about the nutritional value and health benefits such as high content of linoleic acid in garlic extract. It indicates a good source of essential fatty acids, which have health benefits, including reducing the risk of heart disease and improving brain function.

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 [35-38].

The tested garlic extract *Allium sativum* demonstrates a bactericidal effect against the studied strains of microorganisms. The test sample showed the greatest antimicrobial activity against the *S. aureus* strain ATCC 6538-P (64-fold dilution). Bactericidal dilution against *E. coli* strain ATCC 8739 was noted a 1:1 dilution.

The second study revealed that *E. faecalis* showed greater susceptibility to garlic extract compared to other microorganisms, while *E. coli* and *P. aeruginosa* were less susceptible. The fungus *C. albicans* showed even less susceptibility to garlic extract.

Allium sativum CO_2 extract demonstrates a variety of actions against both bacteria and fungi, according to tests on its microbiological activity. With MIC values between 475 and 650 mcg/mL, the extract showed substantial antibacterial activity against *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa*. This shows that even at relatively low concentrations, the extract was able to prevent the growth of these bacterial strains.

With a MIC value of 1000 mcg/mL, the extract also demonstrated antifungal activity against *Candida albicans*. The extract may have potential as a natural antifungal agent, even if the antifungal activity was not as effective as the antibacterial activity.

The results of the antimicrobial study are in good agreement with the conclusions of previous studies and show strong antibacterial activity against Gram-positive strains and slightly less strongly on Gram-negative ones [39, 43]. Study outcomes prove that

garlic extract generally exhibit greater antibacterial activity against certain bacterial species compared to their antifungal activity. Specifically, this extract is more effective against Gram-positive bacteria such as staphylococci (*S. aureus* and methicillin-resistant *S. aureus*), Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and acid-fast bacilli (*Mycobacterium* intracellular). This suggests that the structural and biochemical properties of bacterial cells play a significant role in determining their susceptibility to plant-derived bioactive compounds. Overall, findings imply that *Allium sativum* CO_2 extract may have the ability to operate as a natural antibacterial agent, notably against diverse bacterial strains. Its promise as an alternate treatment for various infections brought on by these microorganisms has to be further investigated.

Conclusions

15 grams of brown volatile extract were collected using subcritical carbon dioxide extraction. 54 different types of chemical compounds were discovered, bioactive components were identified, such as manool (39.56%), viridifrolol (7%), linolic acid ethyl ester (3.41%) and β-caryophyllene (2,17%) with a large distribution area in the extract, and with an antibacterial, anti-inflammatory effect. The moisture content of raw vegetable ingredients was set at 5.2%. The study outcomes illustrate that linoleic (64.9%) and palmitic (25.0%) were the most prominent acids encountered in the Garlic extract, followed by linolenic (5.7%), oleic (3.2%), stearic (0.8%) acid.

Garlic extract demonstrates a bactericidal effect against the studied strains of microorganisms. The study revealed that *E. faecalis* showed greater susceptibility to garlic extract compared to other microorganisms, while *E. coli* and *P. aeruginosa* were less susceptible. The fungus *C. albicans* showed even less susceptibility to garlic extract. Antibiotics have been widely used in both medicine and agriculture around the world. One of the largest hazards to people is drug-resistant bacteria, which have emerged as a result of the increasing use of antibiotics. Both Gram-positive and Gram-negative bacteria have shown this behaviour. In addition to the human body's weakened immunity, this is a severe social issue. Manool - the main antibacterial agent that can be used in the production of new resistant preparations to strains. As shown by the *in vitro* data obtained in this study, *Allium sativum* extract suppressed the growth of a wide range of bacteria, including multidrug resistant (MDR) strains with bactericidal or bacteriostatic action. It promises as an alternate treatment for various infections brought on by these microorganisms has to be further investigated.

Acknowledgement

The authors deeply appreciated Doctor, Professor Samir Anis Ross for the help with GC-MS analysis at the University of Mississippi (Professor of Pharmacognosy, School of Pharmacy, The University of Mississippi). We are grateful to the members of the analytical group in the laboratory of microbiology "Scientific Centre for Anti-infectious Drugs" (Kazakhstan, Almaty).

Conflict of interest

The authors declare no conflict of interest.

References

1. Swiderski F, Dabrowska M, Rusaczonek A, Waszkiewicz-Robak B, Bioactive substances of garlic and their role in dietoprophylaxis and dietotherapy. *Rocz Panstw Zakl Hig.*, 2007; 58(1): 41-46.
2. Block E, The chemistry of garlic and onions. *Sci Am.*, 1985; 252: 114-119.
3. Block E, Garlic and other alliums. *RSC publishing Cambridge: London, UK.*, 2010; 152-166.
4. Morioka N, Sze LL, Morton DL, Irie RF, A protein fraction from aged garlic extract enhances cytotoxicity and proliferation of human lymphocytes mediated by interleukin-2 and concanavalin A. *Cancer Immunol Immun.*, 1993; 37: 316-322.
5. Lawson LD, Garlic: a review of its medicinal effects and indicated active compounds. In: Lawson LS, Bauer R, Editors, Phytomedicines of Europe: chemistry and biological activity, ACS Symposium Series 691. *Am Chem Soc Washington.*,1998; 176-209.
6. Josling P, Preventing the common cold with a garlic supplement: a double-blind, placebo-controlled survey. *Adv Ther.*, 2001; 18: 189-193.
7. Ariga T, Seki T, Antithrombotic and anticancer effects of garlic-derived sulfur compounds. *BioFactors*, 2006; 26: 93-103.
8. Najda A, Błaszczyk L, Winiarczyk K, Dyduch J, Tchórzewska D, Comparative studies of nutritional and health-enhancing properties in the "garlic-like" plant *Allium ampeloprasum* var. *ampeloprasum* (GHG-L) and *A. sativum*. *Sci Hortic.*, 2016; 201: 247-255.
9. Tchórzewska D, Bocianowski J, Najda A, Dąbrowska A, Winiarczyk K, Effect of environment fluctuations on biomass and allicin level in *Allium sativum* (cv. Harnas, Arkus) and *Allium ampeloprasum* var. *ampeloprasum* (GHG-L). *J Appl Bot Food Qual.*, 2017; 90: 106-116.
10. Gebreyohannes G, Gebreyohannes M, Medicinal values of garlic: a review. *Int J Med Med Sci.*, 2013; 5(9): 401-408.
11. Thuy BTP, My TTA, Hai NTT, Hieu LT, Hoa TT, Thi Phuong Loan H, Triet NT, Anh TTV, Quy PT, Tat PV, Hue NV, Quang DT, Trung NT, Tung VT, Huynh LK, Nhung NTA, Investigation into SARS-CoV-2 Resistance of Compounds in Garlic Essential Oil. *ACS Omega*, 2020; 5(14): 8312-8320.
12. Yoshida H, Katsuzaki H, Ohta R, Ishikawa K, Fukuda H, Fujino T, Suzuki A, Antimicrobial activity of the thiosulfinates isolate from oil-macerated garlic extract. *Biosci Biotech Bioch.*, 1999; 63(3): 591-594.
13. Tsao SM, Yin MC, *In vitro* antimicrobial activity of four diallyl sulphides occurring naturally in garlic and Chinese leek oil. *J Med Microbiol.*, 2001; 50: 646-649.

14. Bakri IM, Douglas CWI, Inhibitory effect of garlic extract on oral bacteria. *Arch Oral Biol.*, 2005; 50: 645-651.
15. Brunel AS, Guery B, Multidrug-resistant (or anti-microbial-resistant) pathogens-alternatives to new antibiotics. *SwissMed Wkly.*, 2017; 147: 1-15.
16. Asokan GV, Ramadhan T, Ahmed E, Sanad H, WHO global priority pathogens list: a bibliometric analysis of Medline-PubMed for knowledge mobilization to infection prevention and control practices in Bahrain. *Oman Med J.*, 2019; 34(3): 184-193.
17. Jyoti T, Shrayanee D, Zeeshan F, Saif H, Multidrug resistance: an emerging crisis. *Interdiscip Perspect Infect Dis.*, 2014; 2014: 1-7.
18. Bazaraliyeva A, Moldashov D, Turgumbayeva A, Kartbayeva E, Kalykova A, Sarsenova L, Issayeva R, Chemical and biological properties of bio-active compounds from garlic (*Allium sativum*). *Pharmacia*, 2022; 69(4): 955-964.
19. Vertiz, Angel. Local antinociceptive effect of "snow mountain garlic" in an experimental formalin model. *Farmacia*, 2023; 71(1): 11-17.
20. Kunti Setiowati, Frida, Elicitation of organosulfur bioactive compounds with Fe^{3+} and Zn^{2+} in cell suspension culture of single garlic (*Allium sativum* L.). *Farmacia*, 2022; 70(1): 81-89.
21. Amr E, Subcritical CO2 extraction of a volatile oil-rich fraction from the seeds of *Nigella sativa* for potential pharmaceutical and nutraceutical applications. *J Ess Oil Res.*, 2017; 30: 1-30.
22. Cˇižmek L, BavconKralj M, Cˇož-Rakovac R, Mazur D, Ul'yanovskii N, Likon M, Trebše P, Supercritical Carbon Dioxide Extraction of Four Medicinal Mediterranean Plants: Investigation of Chemical Composition and Antioxidant Activity. *Molecules*, 2021; 26(18): 1-18.
23. Souza AB, Souza MGMd, Moreira MA, Moreira MR, Furtado NAJC, Martins CHG, Bastos JK, Santos RAd, Heleno VCG, Ambrosio SR, Veneziani CSR, Antimicrobial Evaluation of Diterpenes from *Copaifera langsdorffii* Oleoresin Against Periodontal Anaerobic Bacteria. *Molecules*, 2011; 16(11): 9611-9619.
24. Kot B, Wierzchowska K, Piechota M, Czerniewicz P, Chrzanowski G, Antimicrobial activity of five essential oils from *lamiaceae* against multidrug-resistant *Staphylococcus aureus*. *Nat Prod Res.*, 2019; 33(24): 3587-3591.
25. Trevizan LNF, Nascimento KFD, Santos JA, Kassuya CAL, Cardoso CAL, Vieira MDC, Moreira FMF, Croda J, Formagio ASN, Anti-inflammatory, antioxidant and anti-*Mycobacterium tuberculosis* activity of viridiflorol: The major constituent of *Allophylus edulis*. *Radlk J Ethnopharmacol.*, 2016; 192: 510-515.
26. Kaya A, Doğu S, Demirci B. Geographical impact on essential oil composition of endemic *Salvia absconditiflora* collected from different parts of Turkey. *Eur J Life Sci.*, 2022; 1: 55-62.
27. Fusani P, Aiello N, Biazzi E, Tava A, Variability in the Essential Oil Composition of *Achillea erba-rotta* subsp. *moschata* (Wulfen) I. Richardson Growing in the Eastern Italian Alps. *Chem Biod.*, 2022; 19(11): 1-11.
28. Pelkonen O, Abass K, Wiesner J, Thujone and thujone-containing herbal medicinal and botanical products: toxicological assessment. *Regul Toxicol Pharmacol.*, 2013; 65: 100-107.
29. Roslan JB, Characterization of Essential Oil from Malaysian Curry Leaves. *Faculty of Chemical and Natural Resources Engineering, University Malaysia Pahang, Kuantan*. 2014; 1: 1-114.
30. Dolan LC, Matulka R, Burdock G, Naturally occurring food toxins. *Toxins (Basel)*, 2010; 2(9): 2289-2332.
31. Park S, Seetharaman R, Ko M, Kim D, Kim T, Yoon M, Kwak JH, Lee S, Bae Y, Choi YW, Ethyl linoleate from garlic attenuates lipopolysaccharide-induced pro-inflammatory cytokine production by inducing heme oxygenase-1 in RAW264.7 cells. *Int Immunopharmacol.*, 2014; 19: 10.
32. Machado KDC, Islam MT, Ali ES, Rouf R, Uddin SJ, Dev S, Shilpi JA, Shill MC, Reza HM, Das AK, Shaw S, Mubarak MS, Mishra SK, Melo-Cavalcante AAC, A systematic review on the neuroprotective perspectives of beta-caryophyllene. *Phytother Res.*, 2018; 32(12): 2376-2388.
33. Dunkić V, Bezić N, Vuko E, Antiphytoviral activity of essential oil from endemic species *Teucrium arduini*. *Nat Prod Commun.*, 2011; 6(9): 1385-1388.
34. Tsiaganis M, Laskari K, Melissari E, Fatty acid composition of *Allium* species lipids. *J Food Compos Anal.*, 2006; 19: 620-627.
35. Şehitoğlu H, Yarali Karakan F, Kizilkaya B, Öztopuz R, Gülçin I, Investigation of antioxidant properties and bioactive composition of *Allium tuncelianum* ((Kollman) Özhatay, Matthew and Şiraneci) and *Allium sativum*. *J Inst Sci Technol.*, 2018; 8(4): 213-221.
36. Abuova Z, Turgumbayeva A, Jumagaziyeva A, Rakhimov K, Jussupkaliyeva A, Study of component composition and antimicrobial activity of the ophthalmic emulsion based on the safflower flowers (*Carthamus tinctorius L.*). *Int J Microbiol.*, 2022; 2022: 3181270.
37. Parekh J, Chanda S, Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *Afr J Biomed.*, 2007; 10: 175-181.
38. Elisha IL, Botha FS, McGaw LJ, Eloff JN, The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. *BMC Complement Altern Med.*, 2017; 17(1): 133.
39. Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, Hatab SR, Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Front Microbiol.*, 2018; 9: 1639.
40. Othman L, Sleiman A, Abdel-Massih RM, Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. *Front Microbiol.*, 2019; 10: 911.