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The study of volatile fractions of cabbage leaves (*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC.) and determination of its antibacterial and antifungal activity

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ABSTRACT

Background: Along with a widespread application of cabbage as a food and fodder crop, it also possesses therapeutic properties. In traditional medicine, this plant is used in gastritis and gastric ulcer, colitis, diabetes, inflammations of upper airways, musculoskeletal system, disorders of cardiovascular system, and skin. **Materials and Method:** Using the method of gas chromatography, the component composition of cabbage leaves of Yaroslavna and Bilosnizhka cultivars was studied. Testing control strains of microorganisms were used for the determination of the antimicrobial activity of chloroform, ethyl acetate, and 96% ethanol extracts from cabbage cultivars leaves. **Result:** The general amount of volatile compounds in the studied plant material types was almost equal. Allyl isothiocyanate, dihydropseudoionone, and ar-turmerone were chosen as marker compounds for cabbage leaves of Yaroslavna cultivar, while nerolidol and 3,5-di-tret-butyl-4-hydroxybenzaldehyde were chosen for cabbage leaves of Bilosnizhka cultivar. Testing control strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris,* and *Candida albicans* were sensitive to all tested extracts from cabbage leaves of Yaroslavna and Bilosnizhka cultivars. **Conclusion:** The results provide the reason for the development of new antibacterial drugs based on cabbage leaves.

Keywords: Antibacterial and antifungal activity, cabbage, volatile compounds

INTRODUCTION

Nowadays, more and more attention is paid not only to the plants traditionally used with therapeutic purposes but also to the widespread agricultural crops, which are a part of a human daily diet and have a large raw material base. Cabbage – *Brassica oleracea* L. *var. capita*, from the *Brassicaceae* family, is one of such plants. It holds one of the prominent places among vegetable crops by planting acreage and I one of the leading food products in many countries of the world.[1-4] Its plant raw material is used in the treatment of gastritis and gastric ulcer, colitis, gout, inflammations of joints, skin and cardiovascular disorders, diabetes, inflammation of upper airways, as well as for normalizing lipid metabolism.[1-4] Some sources claim that cabbage leaf extracts are effective in hepatitis, liver cirrhosis, and pancreatitis.[5,6] In addition, according to the Korean and Indian scientists, this plant possesses antibacterial, antioxidant, antihistamine, and antitumor activities.[5,7-9] However, the chemical composition of cabbage has not been studied to the full extent.

The name "volatile components" unites structurally diverse compounds, which have in common a property to evaporate

under normal conditions. Along with that, these compounds show a wide spectrum of therapeutic activity: Antioxidant, antibacterial, antifungal, anthelminthic, anti-inflammatory, antitumor, antinociceptive, hypotensive, cardioprotective properties, and other.[10]

Squalene is a triterpenoid compound, which is a structural unit of the skin. Experimental studies of Taiwanese and Austrian scientists show that squalene has antioxidant and anticarcinogenic properties and is a selective singlet oxygen agent. This compound inhibits the growth of lung, skin, and mammary gland tumors, and also prevents the development of colon sarcoma.[11,12] Squalene has anti-inflammatory, hypotensive, cardioprotective properties, lowers the blood cholesterol level. This compound is used for skin moisturizing and its elasticity improvement.[11,12] In addition, in studies in animals, squalene increased the level of testosterone and improved the reproductive function, increased the bioavailability of medicines.[11]

Dihydropseudoionone (synonym: Geranylacetone) and nerolidol (synonym: Peruviol) are natural sesquiterpene compounds with a wide spectrum of biological activity. Both compounds show antioxidant and antibacterial effect to Gram-positive and Gram-negative bacteria, antifungal, antiparasitic, and insecticidal activity.[13-15] Polish scientist discovered the property of dihydropseudoionone to inhibit growth of melanoma and leukemia cells.[13] Taiwanese and Malaysian scientists claim nerolidol to possess antitumor, antinociceptive, and anti-inflammatory activity and its ability to increase the sensitivity of bacteria to the action of antibiotics.[15] Brazilian scientists have determined in experiments *in vivo* that nerolidol at the dose of 500 mg/kg inhibits the formation of gastric ulcer up to 50%.[14,15]

According to the literature sources, the sesquiterpenoid compound ar-turmerone has antibacterial, antifungal, anti-inflammatory, and antitumor activities.[16]

Phytol, from the chemical point of view, is an unsaturated diterpenoid alcohol, which is formed after the chlorophyll decomposition.[17,18] Results received by Brazilian scientists prove that phytol preparations show antibacterial, anti-inflammatory, antioxidant, and antinociceptive properties.[18,19] Spanish and Brazilian scientists detected that at the dose of 75 mg/kg phytol increased the latency of epileptic seizures in animals and decreased their mortality level.[18]

Phthalic acid esters are widely used in the production of flexible polymers. Scientists from many countries argue about their toxicity and benefits.[20-24] There is an opinion that natural phthalates show no toxic effect on human body and possess antibacterial, antifungal, anthelminthic, and antibacterial to Gram-positive bacteria effects.[20,23,24]

Sulfur-containing compounds are biologically active secondary plant metabolites, which are mainly accumulated by *Brassicaceae* plants. According to the literature data, sulfur-containing compounds show antioxidant, antiviral, anti-inflammatory, antifungal, antiparasitic, and cytostatic effects.[22,25-27] They are used in the treatment of osteoarthritis, diabetes, and cardiovascular disorders.[22] Italian scientists have determined that sulfur-containing compounds inhibit the production of inflammatory mediators, as well as the growth of *Helicobacter pylori* colonies.[22,26,28] Collaborative researches of Italian, French, and Canadian scientists have proven that natural sulfur-containing compounds are effective in the treatment of neurodegenerative disorders, in particular, disseminated sclerosis and Alzheimer's and Parkinson's diseases.[22,25]

Information on scientific research of volatile fraction and antibacterial activity of leaves of *B. oleracea* L. var. *capita* by other scientists was found and analyzed in the literature. A group of Indian scientists studied the volatile components of dried and frozen leaves of *B. oleracea* L. var. *capita*. During the experiment, 33 volatile substances were identified and were found that about 37.39% of the volatile fraction were glucosinolates (allyl isothiocyanate, 3-isothiocyanato-1-propene, isothiocyanatocyclopropane, 4-isothiocyanato-1-butene, iberin, 1H-indole-3-carboxaldehyde, 5-methoxy-1H-indole-3-carboxaldehyde, methyl-3,7-diaza-4-indol-3'-ylmethyl-7 (N) -methyl-6,8- dioxo2-thia-cis-bicyclo [3.3.0] octyl-exo-4-carboxylate-2 (S)-oxide). In addition, this raw material contained di- (2-ethylhexyl) phthalate and phthalic acid hept-4-ylisobutyl ester.[29] Scientists Jayalakshmi *et al*. in ethanolic extracts of the leaves of this plant identified diethylphthalate, terpene compounds phytol and isophytol. [30] Japanese scientists studied the primary and secondary metabolites of six Japanese varieties of *B. oleracea* L. var. *capita* by gas chromatography. As a result of the experiment, from 40 to 50 compounds were identified, among which fatty and amino acids predominated.[31]

A group of scientists from the Republic of Congo and Zambia found that ethanolic and hydroalcoholic extracts of the leaves of *B. oleracea* L. var. *capita* at a concentration of 50 μg/mL were slow the growth of *Staphylococcus aureus* and *Salmonella enterica* compared to amoxicillin.[32] Studies by Nigerian scientists showed that the growth retardation zones of *S. aureus* (16–22 mm), *Escherichia coli* (16–20 mm), and *Pseudomonas aeruginosa* (8–15 mm) of methanolic and ethanolic extracts of the leaves of *B. oleracea* L. var. *capita* at concentrations above 250 μg/mL were on par with ciprofloxacin.[33]

From the work of Indian researchers, it is known methanolic extracts of the leaves of *B. oleracea* var. *capitata* f. *rubra* at a minimum inhibitory concentration of 0.25–4 mg/mL moderately delayed growth zone of *E. coli* by 12.5 mm, *Candida albicans* by 11.7 mm, and *Bacillus subtilis* by 13.9 mm. The zone of growth retardation of these microorganisms in the study of water extracts did not exceed 8.5 mm. The growth retardation zone of *Proteus vulgaris* and *Aspergillus niger* using water and methanolic extracts was in the range of 6.0–6.7 mm.[34] The 80% of methanolic extracts of the leaves of *B. oleracea* var. *capita* L. at a dose of 50 mg/disk caused a slowing down of *E. coli* by 17 mm, *Salmonella typhi* – by 14 mm, *B. subtilis* – by 12 mm, and *S. aureus* – by 19 mm was found by disc diffusion method.[35] Indian scientists Rameshwari and Ayshwarya studied the antimicrobial activity of ethanolic, methanolic, acetone, chloroformic, and water extracts of the leaves of *B. oleracea* var *capitata rubra* at doses of 100 and 200 mcg r compared to ampicillin by method diffusion into agar. The study found all extracts at a dose of 200 μg showed better antimicrobial activity. The best results were observe for

methanolic extract. When used it, the growth retardation zone of *Lactococcus lactis* was 6 mm, of *P. aeruginosa* and *S. aureus* was 7 mm, of *E. coli*, *Micrococcus* sp., and *P. vulgaris* was 8 mm, and of *Serratia marcescens* and *B. subtilis* was 10 mm. That was significantly higher than the reference drug ampicillin. However, it was found that ethanolic extracts had slightly less activity compared to methanolic extracts. The largest zones of growth retardation were observed for *P. aeruginosa* (8 mm), *Micrococcus* sp. (6 mm), and *E. coli* (5 mm). The size of the growth of retardation zones of *S. marcescens*, *P. vulgaris*, *L. lactis*, *B. subtilis,* and *S. aureus* did not exceed 4 mm, but they were on a par with ampicillin. Chloroformic extracts had little antibacterial activity against *P. aeruginosa*, *E. coli*, *B. subtilis,* and *S. aureus*. The diameter of the growth retardation zone of them did not exceed 4 mm.[2]

A group of Chinese and Korean scientists studied the antibacterial activity against seven strains of bacteria and four strains of fungi of extracts of the leaves of *B. oleracea* L. var. *capitata* f. *rubra*, which were obtained by non-polar (chloroform, dichloromethane, diethyl ether, and toluene) and polar (ethanol, methanol, and distilled water) solvents. It was found that chloroform extracts had the largest zone of growth retardation of *S. enterica* Typhimurium (12 mm), *E. coli* (11–13 mm), *S. aureus* (11–14 mm), *Listeria monocytogenes* (12 mm), *Bacillus cereus* (14 mm), *C. albicans* (15 mm), *Aspergillus fumigatus* (8.5 mm), *Aspergillus*. *flavus* var. *flavus* (10 mm), and *A. niger* (9 mm). Ethanolic extracts showed antibacterial activity against *S. enterica* Typhimurium, *E. coli*, *S. aureus*, and *B. cereus*, the growth retardation zone of which was 10–11 mm, of *Listeria monocytogenes* was 13 mm. Toluene and dichloromethane extracts slowed the growth of strains of Gram-positive and Gram-negative bacteria, methanolic – only Gram negative. Ethyl ether and water extracts did not show antibacterial activity.[36]

Scientists from Iraq and Malaysia have conducted joint studies of the bacteriostatic and bactericidal action of methanolic extracts of the leaves of *B. oleracea* L. var. *capitata* f. *rubra* in comparison with streptomycin. The results showed that the studied extracts at a concentration of 500 mg/ml had a bactericidal effect against strains of *B. subtilis*, *E. coli*, *P. aeruginosa,* and *S. enterica* serovar typhimurium, the growth retardation zone of which was 17–20 mm. It was on a par with the reference drug.[37]

The study of ethanolic extracts of the leaves of *B. oleracea* grown in Bangladesh showed significant antibacterial activity against *A. fumigatus*, *Citrobacter divergens,* and *Klebsiella pneumoniae*, with a growth retardation zone of 13 mm, 8 mm, and 20 mm, respectively.[38]

It is known that the qualitative composition and quantitative content of biological activity compounds, and accordingly their therapeutic effect, depend on many factors, including the place of growth, climatic conditions, and others. Volatile fractions and antibacterial activity of leaves of *B. oleracea* L. *convar. capitata* (L.) *Alef. var. alba DC.* of Ukrainian cultivars Yaroslavna and Bilosnizhka were not studied. Taking into account these facts, the study of the component composition and quantitative content of volatile compounds in cabbage leaves and their antibacterial and antifungal activity were much to the point.

The purpose of the current work was the study of qualitative composition and quantitative content of volatile components in cabbage leaves of Yaroslavna and Bilosnizhka cultivars and study antibacterial and antifungal activity of ethanolic, chloroformic, and ethyl acetate extracts from them.

MATERIALS AND METHODS

Materials

The objects of the research were the cabbage leaves of Yaroslavna and Bilosnizhka cultivars, harvested in the phase of formation of the productive organ (head). The vegetation period of the studied plants was 15–16 weeks. The raw material was cultivated according to the standards of Good Agricultural and Collection Practices at experimental grounds of the Institute of Horticulture and Melon Growing of the National Academy of Agrarian Sciences of Ukraine (Merefa, Kharkiv Region, Ukraine). Samples of cabbage leaves of Yaroslavna and Bilosnizhka cultivars for analysis were harvested in 2016–2018.

Dry extracts from cabbage leaves of Yaroslavna and Bilosnizhka cultivars were prepared for the study of antibacterial activity. Chloroform, ethyl acetate, and 96% ethanol were used as the extractants. These solvents best extracted compounds of terpenic nature and sulfur-containing compounds from herbal raw materials according to the literature.[28] Dry extracts were dissolved in 96% ethanol.

Methods

Gas chromatography

Identification of volatile compounds and determination of their quantitative content were carried out using gas chromatography on the chromatograph Agilent Technologies HP 6890 with mass spectrometric detector 5973 by the following procedure: 0.05 g of the plant raw material was placed into a 20 ml vial where 50 µg of internal standard was added with further calculation of its concentration. Tridecane was used as an internal standard. Ten milliliters of purified water were added to the sample, where the reflux condenser with air cooling was attached, the volatile components were distilled with water vapor during 2 h.[28,39,40]

During the distillation, the volatile components were adsorbed on the inner surface of the reflux condenser. The adsorbed compounds after cooling of the system were washed by slow adding of 3 ml of pure pentane into a dry 10 ml vial. The elution was concentrated by purging (100 ml/min) with highly purified nitrogen to the residual volume of the extract of 10 µl, which was completely taken by a syringe. The further sample concentration was carried out inside the syringe.[39,40]

During the analysis, the following chromatography conditions were adhered to chromatographic column – capillary column DB-5 with inner diameter of 0.25 mm and length 30 m; speed of the gas carrier (helium) – 1.2 ml/min; temperature of the sample injection heater – 250°C, and the thermostat temperature was programmed from 50°C to 320°C with the speed 4°/min.

The mass spectra libraries NIST05 and WILEY 2007 were used for the identification of components, with total number

of spectra over 470,000 combined with the identification software AMDIS and NIST.

The quantitative content of volatile compounds was calculated using the inner standard method. The content of the components (C, mg/kg) was carried out using the following formula:

$C=K_1 \cdot K_2$,

Where, $K_1=P_1/P_2$ (P_1 – the peak area of the studied sample, P_2 – the peak area of the standard);

$K_2=50/M$ (50 – weight of the internal standard (μg), injected into a sample, M – weighed quantity of the sample (g).[39,40]

Study of antibacterial activity

Testing control strains of Gram-positive and Gram-negative bacteria from different taxonomic groups were used for analysis: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *B. subtilis* ATCC6633, and *P. vulgaris ATCC 4636*. Antifungal activity was studied on the reference strain *C. albicans* ATCC 885–653. This set of testing control strains is generally accepted in the primary determination of antimicrobial activity. All test cultures were obtained from the Laboratory of Medical Microbiology with the Museum of Microorganisms of the State Institution Mechnikov Institute of Microbiology and Immunology National Academy of Medical Sciences of Ukraine (Kharkiv, Ukraine). Cultivation media were used according to the type of microorganisms in accordance with existing methodological developments and recommendation.[41,42]

The preparation of microbial suspensions with a determined concentration of microbial cells (optical density) was performed using the turbidity standard (0.5 units on the McFarland scale). We used the apparatus Densi-La-Meter (PLIVA-Lachema, Czech Republic; wavelength 540 nm). The suspension was prepared according to the apparatus instructions and announcement about the Innovations in the Health Care System № 163-2006 "Standardization of preparation of microbial suspensions," Kyiv, Ukraine. The synchronization of the cultures was made using a low temperature (about 4°C).[41,42]

The sensitivity of the microorganism strains to the obtained extracts was determined according to the methodological guidelines "Determination of sensitivity of microorganisms to antibacterial drugs" (Order of the Ministry of Health of Ukraine №167 dated April 5, 2007). The study was conducted using the agar well diffusion method. Mueller-Hinton agar was the medium of choice («HiMedia Laboratories Pvt. Ltd., India). The medium was prepared according to the manufacturer's instructions. The sensitivity of the fungi was determined on the medium Sabouraud dextrose agar. The determination of the sensitivity of the microorganisms to the extracts was carried out on two layers of nutrient medium in Petri dishes. The bottom layer consisted of agar-agar (10 ml). Sterile metal cylinders (8 mm diameter, 10 mm height) were set in the bottom layer of nutrient medium. The top layer was added around the cylinders. Top layer consisted of nutrient agar medium with appropriate standard of daily culture of the microorganism (nutrient medium [14 ml] and microbial solution [1 ml] McFarland 0.5 standard).

Cylinders were removed with sterile tweezers after solidification. Then, the test extract was added into the wells (0.3 ml).

The evaluation of antibacterial activity of the investigated extracts was performed according to the diameter of growth retardation zones:

- 10 mm – resistant the microorganism to the extract
- 10–15 mm – the microorganism is less sensitive to the extract
- 15–25 mm – the microorganism is sensitive to the extract
- 25 mm and more – the microorganism is highly sensitive to the extract[41,42]
- The number of repetitions of each experiment – at least 5.

RESULTS

Significant difference was not detected, according to the analysis of the chemical composition of the volatile components of cabbage leaves of Yaroslavna and Bilosnizhka cultivars harvested in 2016, 2017, and 2018. However, there was a slight fluctuation in the quantitative content of the identified compounds.

As a result of the experiment, 18 volatile compounds were identified in cabbage leaves of Yaroslavna cultivar, which amount to 1531.47 ± 38.29 mg/kg in total. Fifteen volatile compounds accumulated in cabbage leaves of Bilosnizhka cultivar. However, there was no significant difference in the content of the compounds studied in the cabbage leaves of Yaroslavna and Bilosnizhka (1386.67 ± 34.67 mg/kg) cultivars. The chromatograms of volatile compounds of cabbage leaves of Yaroslavna and Bilosnizhka cultivars, obtained by the gas chromatography method, are given in Figures 1 and 2, respectively.

It was found that sulfur-containing compounds prevailed by quantity in cabbage leaves of Yaroslavna cultivar, the content of which comprised 908.08 ± 22.70 mg/kg. Cabbage leaves of Bilosnizhka cultivar mainly accumulated terpenoid compounds (752.91 ± 18.82 mg/kg). Terpenoid compounds represented by four compounds in cabbage leaves of Yaroslavna cultivar and five compounds in cabbage leaves of prevailed Bilosnizhka cultivar, by qualitative composition in both studied plant material types. The results of the experiments carried out are given in Table 1.

After analyzing the component composition of the volatile fractions of cabbage leaves of the both studied cultivars, nine compounds and six compounds were found, which were present only in cabbage leaves of Yaroslavna cultivar and Bilosnizhka cultivar, respectively.

Sulfur-containing compounds, such as phenylethyl isothiocyanate (589.02 ± 14.73 mg/kg), allyl isothiocyanate (147.26 ± 3.68 mg/kg), and iberverin (142.35 ± 3.56 mg/kg), were present in the highest quantity in cabbage leaves of Yaroslavna cultivar. Among terpenoid compounds in this plant raw material type squalene and ar-turmerone prevailed, the content of which comprised 73.63 ± 1.84 mg/kg and 54.98 ± 1.37 mg/kg, respectively. In addition, the total amount of terpenoid compounds in cabbage leaves of Yaroslavna cultivar was 162.48 ± 4.06 mg/kg, which was almost 4.6 times less comparing to their content in cabbage leaves of Bilosnizhka

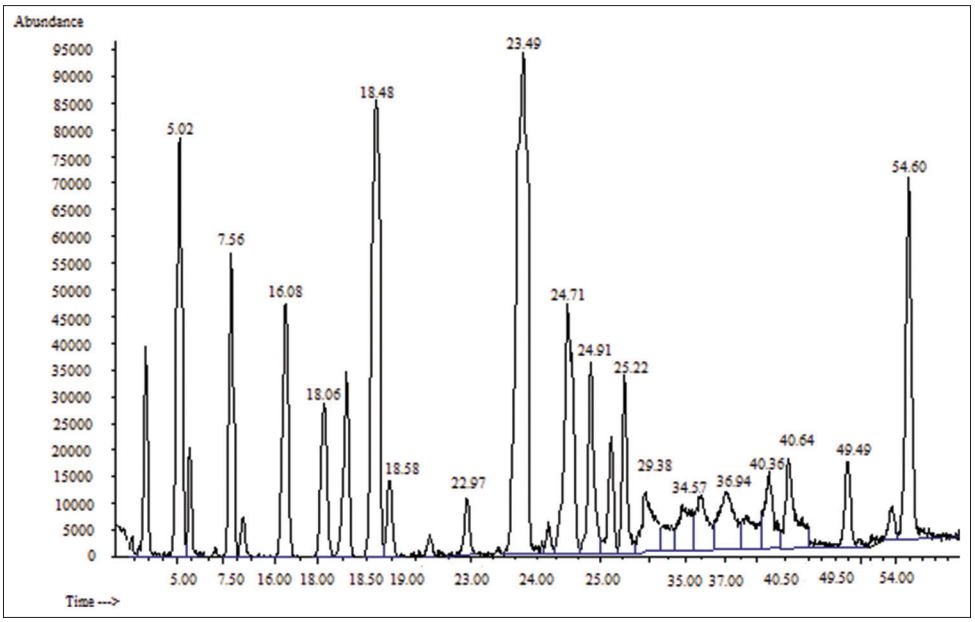

Figure 1: Gas chromatogram of volatile components of cabbage leaves of Yaroslavna cultivar

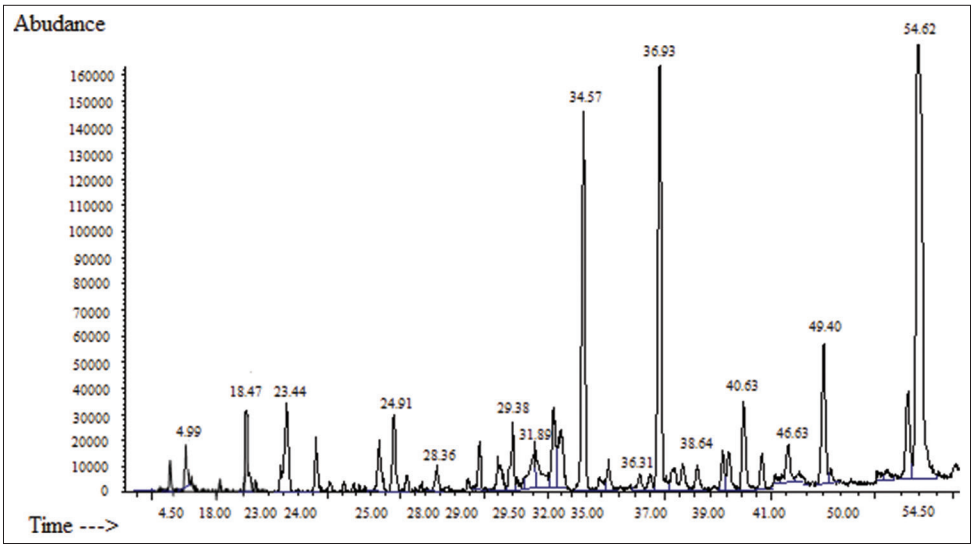

Figure 2: Gas chromatogram of volatile components of cabbage leaves of Bilosnizhka cultivar

cultivar. Besides that, empirical data testify that the acyclic monoterpene dihydropseudoionone (9.33 ± 0.23 mg/kg) was accumulated only in cabbage leaves of Yaroslavna cultivar.

The component composition of phthalates in cabbage leaves of Yaroslavna cultivar is represented by three compounds, the total amount of which comprised 238.06 ± 5.95 mg/kg. Their content was almost twice lower in this plant raw material type than in cabbage leaves of Bilosnizhka cultivar. The dominating compound of this class in cabbage leaves of Yaroslavna cultivar was dibutylphthalate – 134.49 ± 3.36 mg/kg.

The total amount of the four compounds, which were classified as phenolic aldehydes, alcohols, and ketones, detected in cabbage leaves of Yaroslavna cultivar, was 136.46 ± 3.41 mg/kg, which was twice as high as the total amount of these compounds in cabbage leaves of Bilosnizhka cultivar. Dibunol was found in significant amount in cabbage leaves of the indicated cultivar, which amounted to 66.26 ± 1.66 mg/kg.

In addition, the linoleic acid methyl ester (26.02 ± 0.65 mg/kg), phenylethyl cyanide (35.83 ± 0.90 mg/kg), and the heterocyclic aromatic compound indole (24.54 ± 0.61 mg/kg) were also found in this type of the plant raw material.

Sulfur-containing compounds iberverin and phenylethyl isothiocyanate accumulated in cabbage leaves of Bilosnizhka cultivar in total amount of 76.85 ± 1.92 mg/kg, which was 11 times less than the amount of sulfur-containing compounds in cabbage leaves of Yaroslavna cultivar.

Among terpenoid compounds, squalene (651.56 ± 16.29 mg/kg) and phytol (46.22 ± 1.56 mg/kg) prevailed in

Table1: Component composition of volatile fractions of cabbage leaves of Yaroslavna and Bilosnizhka cultivars (X±ΔX)

No	Component	Molar mass (amu)	Cabbage leaves			
			"Yaroslavna" cultivar		"Bilosnizhka" cultivar	
			Retention time, min	Quantitative content, mg/kg	Retention time, min	Quantitative content, mg/kg
1	Allyl isothiocyanate	99.15	5,02	147.26±3.68	-	-
2	Pentanoic acid (Valeric acid)	102.13	-	-	4.99	13.37±0.33
3	1-Butene, 4-isothiocyanato-	113.18	7.56	29.45±0.74	-	-
4	Benzenepropanenitrile (Phenylethyl cyanide)	131.18	16.08	35.83±0.90	-	-
5	Indole	117.15	18.06	24.54±0.61	-	-
6	Propane, 1-isothiocyanato-3-(methylthio)- (Iberverin)	147.25	18.48	142.35±3.56	18.47	34.53±0.86
7	2-Methoxy-4-vinylphenol (*p*-vinylguaiacol)	150.78	18.58	8.84±0.22	-	-
8	5,9-Undecadien-2-one, 6,10-dimethyl- (Dihydropseudoionone)	194.32	22.97	9.33±0.23	-	-
9	Benzene, (2-isothiocyanatoethyl)- (Phylethyl isothiocyanate)	195.30	23.49	589.02±14.73	23.44	42.32±1.06
10	Butylated Hydroxytoluene (Dibunol)	220.35	24.71	66.26±1.66	-	-
11	Phenol, 2,4-bis (1,1-dimethylethyl)-	206.32	24.91	34.36±0.86	24.91	29.52±0.74
12	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)- (Myristicin)	192.21	25.22	27.00±0.68	-	-
13	Benzophenone (Diphenylketone)	182.22	-	-	28.36	6.13±0.15
14	Ar-tumerone	216.32	29.38	54.98±1.37	29.38	27.84±0.70
15	3,5-di-tret-Butyl-4-hydroxybenzaldehyde	234.34	-	-	31.89	26.73±0.67
16	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester (Diisobutylphthalate)	278.35	34.57	102.10±2.55	34.57	172.64±4.32
17	Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydroxy-, methyl ester	292.42	-	-	36.31	23.39±0.58
18	Dibutyl phthalate	278.35	36.94	134.49±3.36	36.93	217.19±5,43
19	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]- (Nerolidol)	222.37	-	-	38.64	13.92±0.35
20	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	292.46	40.36	26.02±0.65	-	-
21	Phytol	296.54	40.64	24.54±0.61	40.63	46.22±1.16
22	Hexanedioic acid, bis (2-ethylhexyl) ester	370.57	-	-	46.63	20.05±0.50
23	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (Monoethylhexylphthalate)	278.35	49.49	1.47±0.04	49.40	61.26±1.53
24	Squalene	410.73	54.60	73.63±1.84	54.62	651.56±16.29
Total content of volatile compounds			1531.47±38.29		1386.67±34.67	

this type of the plant raw material. In addition, ar-turmerone, valeric acid, and nerolidol accumulated in significant amount, which comprised 27.84 ± 0.70 mg/kg, 13.37 ± 0.33 mg/kg, and 13.92 ± 0.35 mg/kg, respectively.

Compounds of the group of phenolic aldehydes, alcohols, and ketones in cabbage leaves of Bilosnizhka cultivar were found in the amount of 62.38 mg/kg. 2,4-Bis(1,1-dimethylethyl)-phenol and 3,5-di-tret-butyl-4-hydroxybenzaldehyde dominated among the abovementioned compounds, the content of which was insignificantly different and comprised 29.52 ± 0.74 mg/kg and 26.73 ± 0.67 mg/kg, respectively.

The total amount of the esters of benzopropanoic and adipic acids in cabbage leaves of Bilosnizhka cultivar was 43.50 ± 1.09 mg/kg.

The analysis of the component composition of volatile fractions of cabbage leaves of Yaroslavna and Bilosnizhka cultivars showed that among the sulfur-containing compounds, phenylethyl isothiocyanate accumulated in maximum quantity, and among phenolic aldehydes, alcohols, and ketones – dibunol in cabbage leaves of Yaroslavna cultivar. In turn, among phthalates, dibutylphthalate was found in the highest quantity, and among terpenoid compounds – squalene in cabbage leaves of Bilosnizhka cultivar.

Based on the results of the chromatographic study, allyl isothiocyanate, dihydropseudoionone, dibunol, myristicin, and ar-turmerone can be considered as marker compounds for cabbage leaves of Yaroslavna cultivar. Distinctive compounds of cabbage leaves of Bilosnizhka cultivar were valeric acid, diphenylketone, nerolidol, and 3,5-di-tret-butyl-4-hydroxybenzaldehyde.

Table 2: Antimicrobial activity of chloroform, ethyl acetate, and 96% ethanol extracts of cabbage leaves of Yaroslavna and Bilosnizhka cultivars compared to the antimicrobial activity of 96% ethanol

No	Microorganism	Growth retardation zones of microorganism, cm						
		Cabbage leaves extracts						96% ethanol
		Yaroslavna cultivar			Bilosnizhka cultivar			
		Chloroform	Ethyl acetate	96% ethanol	Chloroform	Ethyl acetate	96% ethanol	
1	*Staphylococcus aureus ATCC 25923*	23.00±1.10	25.33±1.19	27.67±1.33	23.33±1.14	25.00±0.25	27.67±1.33	16.33±0.41
2	*Escherichia coli ATCC 25922*	23.00±0.25	23.33±1.10	24.67±1.21	22.67±0.57	22.67±1.09	24.33±1.14	14.67±0.37
3	*Proteus vulgaris ATCC 4636*	21.00±0.25	21.67±1.04	22.67±1.07	20.67±1.01	21.00±1.01	22.33±1.09	14.33±0.36
4	*Pseudomonas aeruginosa ATCC 27853*	21.33±1.00	22.00±0.51	24.33±1.17	21.33±1.05	21.00±0.55	23.33±1.10	14.00±0.55
5	*Bacillus subtilis ATCC 6633*	24.33±1.19	26.67±1.31	27.67±1.35	24.00±0.25	26.33±1.26	28.33±1.33	14.67±0.29
6	*Candida albicans ATCC 653/885*	18.33±0.90	20.33±0.96	21.67±1.06	19.33±0.93	20.00±0.25	21.00±0.025	13.67±0.34

Testing control strains of *E. coli*, *P. aeruginosa*, *P. vulgaris,* and *C. albicans* were sensitive to all tested extracts from cabbage leaves of Yaroslavna and Bilosnizhka cultivars. *E. coli* and *B. subtilis* were sensitive to chloroform extracts and highly sensitive to ethyl acetate and 96% ethanol extracts from cabbage leaves of both cultivars.

Growth retardation zones of all microorganisms were higher for 96% ethanol extracts than for chloroform and ethyl acetate that were typical for both studied varieties.

The maximum growth retardation zone of *S. aureus* with diameter 27.67±1.33 cm was equal for 96% ethanolic extracts of both cultivars.

The largest diameter of growth retardation zone of *E. coli* (24.67 ± 1.21 cm), *P. aeruginosa* (24.33 ± 1.17 cm), and *P. vulgaris* (22.67 ± 1.21 cm) was found for 96% ethanol extracts from cabbage leaves of Yaroslavna cultivar.

The maximum of growth retardation zones of *B. subtilis* were 27.67 ± 1.35 cm and 28.33 ± 1.33 cm for 96% ethanol extracts from cabbage leaves of Yaroslavna and Bilosnizhka cultivars, respectively.

The 96% ethanol extracts from cabbage leaves of Yaroslavna cultivar showed the greatest extent of suppression of *C. albicans* growth. The results of the determination of antibacterial and antifungal activity of chloroform, ethyl acetate, and 96% ethanol extracts from cabbage leaves of the studied cultivars are given in Table 2 in comparison with the antimicrobial activity of 96% ethanol.

DISCUSSION

By comparing results of the research with the results found in the literature,[29-31] it was found that in the leaf of *B. oleracea* L. convar. capitata (L.) Alef. var. alba DC. of Ukrainian varieties, the qualitative composition of sulfur-containing compounds is almost identical from previously researched.[29] However, it was found that Ukrainian varieties of *B. oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC. accumulate terpene compounds ar-tumerone, nerolidol, myristicin and squalene, phthalates diisobutyl phthalate, dibutyl phthalate, and phenolic compounds p-vinylguaiacol and dibunol.[29,30] In addition, the quantitative content of all identified compounds was determined. This information has not been reported in the publications of other authors.[29-31]

The results of the study of antibacterial activity showed that the chloroform and ethanol extracts of *B. oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC. leaves of Ukrainian varieties increased the diameter of the growth retardation zone of *S. aureus, E. coli, P. aeruginosa, B. subtilis,* and *P. vulgaris* almost twice. As well, they slowed the growth of *C. albicans* by almost the same level as data of literature.[2,32,33,36,37] In addition, the antibacterial activity of ethyl acetate fractions from *B. oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC. leaves of Ukrainian cultivars Yaroslavna and Bilosnizhka was studied for the 1st time.

CONCLUSION

The study of volatile compounds of cabbage leaves of Yaroslavna and Bilosnizhka cultivars by gas chromatography showed slight difference of the quantitative content of volatile compounds between cultivars. However, some difference in the qualitative composition was observed. This indicates the variability of the chemical composition of the volatile substances of cabbage leaves depending on the cultivar.

Sulfur-containing substances prevailed in cabbage leaves of Yaroslavna cultivar (908.08 mg/kg) with phenylethyl isothiocyanate being the dominating compound (589.02 mg/kg). Compounds of terpene nature prevailed in cabbage leaves of Bilosnizhka cultivar in total amount of 752.91 mg/kg. The terpenoid squalene prevailed among those compounds in this type of plant material (651.56 mg/kg).

The marker compounds for cabbage leaves of Yaroslavna cultivar are allyl isothiocyanate, dihydropseudoionone, dibunol, myristicin, and ar-turmerone, while valeric acid, diphenylketone, nerolidol, and 3,5-di-tret-butyl-4-hydroxybenzaldehyde are those for cabbage leaves of Bilosnizhka cultivar.

The study of antimicrobial activity showed sensitivity of testing control strains of *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis, P. vulgaris* and *C. albicans* to chloroform, ethyl acetate, and 96% ethanol extracts from cabbage leaves of both cultivars.

The results of the analysis are consistent with the data of the literature and contribute to the expansion of knowledge about the chemical composition of *B. oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC. The positive results confirmed the literature data of the use of cabbage extracts as an antibacterial agent the possible role of unique composition of the volatile components of this plant as antimicrobial compounds. The results provide the reason for the development of new antibacterial drugs based on cabbage leaves after further research.

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