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RESEARCH ARTICLE

Phytochemical Research and Antimicrobial Properties of Lipophylic Extracts of Some Species of *Salix* L. Genus from Ukraine

Natalia Borodina[1], Ain Raal[2,*], Volodimir Kovalyov[1], Tetiana Osolodchenko[3], Oleh Koshovyi[1], Hoai Thi Nguyen[4] and Andrey Komissarenko[1]

[1]*Department of Pharmacognosy, The National University of Pharmacy, Kharkiv, Ukraine*
[2]*Institute of Pharmacy, Faculty of Medicine, University of Tartu, Tartu, Estonia*
[3]*Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine, Kharkiv, Ukraine*
[4]*Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Hue University, Hue City, Vietnam*

Abstract:

Background:

Willows are indispensable for the creation of anti-erosion plantations, preliminary soil-improving crops in the reclamation of disturbed lands, as well as for energy plantations designed to produce the biomass suitable for biofuels. In the process of care and thinning of these plantations, there are a huge amount of waste remains - young willow branches and leaves, which would be advisable to use in medical and pharmaceutical practice to create new supplements and medicines. They are known to possess antimicrobial, antifungal, and antiviral properties.

Objective:

The aim of the current paper is to determine the chemical composition of lipophylic extracts of some species of *Salix* L. genus and to study their antimicrobial properties.

Methods:

The lipophylic extracts from *Salix cinerea* L., *S. incana* Schrank, *S. caprea* L., *S. sachalinensis* F. Schmidt, *S. acutifolia* L., *S. fragilis* L., *S. caspica* Pall., *S. rosmarinifolia* L. and *S. myrsinifolia* Salisb. fresh shoots were obtained using chloroform in the Soxhlet apparatus. The composition of volatile components and carboxylic acids was determined using quantitative content of chlorophylls and carotenoids was measured spectrophotometrically. The antibacterial activity was studied by the agar diffusion method.

Results:

The methods used allowed identifying 46 volatile compounds in the lipophylic extracts from the shoots of the plants of *Salix* L. genus, where eugenol and squalene dominated; as well as 42 organic acids, among which benzoic, methoxybenzoic, salicylic acids prevailed, and palmitic, linoleic and linolenic acids were found to prevail among fatty acids. All lipophylic extracts studied showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans*.

Conclusion:

The results obtained indicate the possibility of the creation of a novel antimicrobial agent using the lipophylic extracts from the shoots of *Salix* L. genus rich in different biologically active substances.

Keywords: Antibacterial activity, Carotenoids, Clorophylls, Lipophylic extracts, *Salix* L. shoots, Volatile compounds.

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1. INTRODUCTION

One of the prospective groups of plants, which, according to the literature data, are known to possess antimicrobial, antifungal, antiviral properties, are the plants from the *Salicaceae* family [1 - 5]. Willows (genus *Salix* L.) are indispensable for the creation of anti-erosion plantations, preliminary soil-improving crops in the reclamation of disturbed lands, as well as for energy plantations designed to produce the biomass suitable for biofuels [6 - 8].

The leader countries by area of land covered by energy crops in Europe are: Italy - 57 thousand hectares (the largest

* Address correspondence to this author at the Institute of Pharmacy, Faculty of Medicine, University of Tartu, Nooruse 1, 50411 Tartu, Estonia;
Tel: +372 7375288; Fax: +372 7375289; E-mail: ain.raal@ut.ee

area in Europe), Poland - 13 thousand hectares, Sweden - 12 thousand hectares, Germany - 11 thousand hectares, Denmark - 10 thousand hectares, Finland - 8 thousand hectares. According to the estimates of the Bioenergy Association of Ukraine, the potential bioenergy 21 million tons, includes about a third - 7 million tons AD - energy crops on 2 million hectares of land. The total area of land involved in cultivation energy crops in Ukraine - about 4 000 hectares for August 2018. Most of them (approx. 3,000 ha) located on the territory of Volyn, Kyiv and Zhytomyr regions [6, 7, 9]. In the process of care and thinning of these plantations, there are a huge amount of waste remains - young willow branches and leaves, which would be advisable to use in medical and pharmaceutical practice to create new supplements and medicines.

According to the literature data and our own phytochemical research on the willow genus plants, it was determined that they have a diverse chemical composition and contain various classes of natural substances [10 - 14]. We have previously carried out a GC/MS research on the volatile compounds and carboxylic acids of the shoots of *Salix caprea* L., *S. purpurea* L., *S. viminalis* L., *S. triandra* L., *S. cinerea* L., *S. fragilis* L., *S. rosmarinifolia* L., *S. myrsinifolia* Salisb., *S. acutifolia* L., *S. nigricans* Smith., *S. fragilis* L., *S. daphnoides* Vill. of Ukrainian flora [15 - 20].

Lipophilic extracts of many plants have pronounced antibacterial properties [21]: such as eucalyptus and sage extracts are the main active components of such medicines as Chlorophyllipt and Salvin [22, 23], lipophilic extracts from *Calendula officinalis* [24], *Persicaria bistorta* [25], *Crataegus submollis Sarg* [26], *Genista tinctoria* [27], *Dahlia varieties* [28] and other spices [29, 30]. The main active ingredients of the lipophylic extracts are chlorophylls, carotenoids, the combination of unsaturated fatty acids, volatile compounds, and other biologically active substances that show various types of pharmacological activity [24, 31 - 33]. Thus, for the purpose of complex research, and further rational application of the plant raw material, we continue the research on the lipophylic extracts, obtained from the plant raw material of willow genus plants.

The aim of the study was to determine the chemical composition of lipophylic extracts of some species of *Salix* L. genus, as well as to study their antimicrobial properties.

2. MATERIALS AND METHODS

2.1. Plant Material

The plant material studied was collected from nine Salix genus species in June and July of 2015-2016 in Zakarpattia, Kharkiv and Kyiv regions (Table **1**), the shoots were collected from at least ten different trees of same species, and a united sample was formed. The plant raw material samples were dried at room temperature for 10 days and were stored in well closed bags on the shelves at room temperature. All *Salix* species studied were identified by the taxonomic guide [34, 35] plus using the expert opinion of the botanist Horelov A.M. from the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv). The voucher specimen (No 98-107) is deposited in the herbarium of the Pharmacognosy Department, National University of Pharmacy, Kharkiv, Ukraine.

2.2. Extraction of Lipophylic Compounds

Lipophylic extracts were obtained from the 20.0 g of the crushed dried plant material (1-3 mm) of *Salix cinerea* L., *S. incana* Schrank, *S. caprea* L., *S. sachalinensis* F. Schmidt, *S. acutifolia* L., *S. fragilis* L., *S. caspica* Pall., *S. rosmarinifolia* L. and *S. myrsinifolia* Salisb. by exhausting extraction of the crushed plant raw material under the same conditions using chloroform in the Soxhlet apparatus [26, 36 - 38]. The Soxhlet extractor (product identification number 8730/500ml) of borosilicate thermal glass SIMAX, a round-bottomed flask of 1000 ml capacity, produced in the Czech Republic, a one-place heating mantle LOIP LH-110 (LAB-KN-1000), produced in Russia, were used. The lipophylic extracts obtained were concentrated on the complete extragent removal and were used for further research. The quantitative content of the main biologically active compounds in the lipophylic extracts from the shoots of the plants of *Salix* L. genus was determined spectrophotometrically.

2.3. Determination of Sum of Lipophilic Extracts

The sum of lipophilic extracts was determined gravimetrically, according to the State Pharmacopoeia of Ukraine [36, 38].

Table 1. Studied shoots of different species of *Salix* genus collected in Ukraine.

Sample	Species	Origin	Geographical Coordinates
1	*Salix cinerea* L.	Near the river Tisa, Steblivka village, Khust district, Zakarpattia region	48° 4' 50.081" N 23° 24' 29.376" E
2	*S. incana* Schrank		
3	*S. caprea* L.		
4	*S. sachalinensis* F. Schmidt	Near Shubkiv village, Rivne district, Rivne region	50° 41' 21.656" N 26° 29' 48.314" E
5	*S. acutifolia* L.	Near Shelestove village, Kolomak district, Kharkiv region	49° 51' 54.709" N 35° 12' 16.081" E
6	*S. fragilis* L.	Near the river of Klenove village, Bohodukhiv district, Kharkiv region	50° 8' 56.076" N 35° 41' 49.416" E
7	*S. caspica* Pall.	M.M. Gryshko National Botanical Garden of the NAS of Ukraine (Kyiv)	50° 24' 56.837" N 30° 33' 46.429" E
8	*S. rosmarinifolia* L.		
9	*S. myrsinifolia* Salisb		

2.4. Determination of Sum of Carotenoids and Chlorophylls

The quantitative estimation of the content of the sum of carotenoids (in terms of β-carotene at the wavelength 453 nm), and chlorophylls (in terms of chlorophyll A at the wavelength 670 nm) in the lipophylic extracts studied (in accurately weighed amounts of extracts of 0.05 g) was carried out spectrophotometrically on the Hitachi U3210 spectrophotometer by the generally accepted procedure [24, 39, 40]. The content of the sum of carotenoids (X, mg%) in terms of β-carotene and chlorophylls (X, mg%) in terms of chlorophyll A was calculated using the formula: $X = (10xAxVx100)xE^{1\%}_{1cm}$ m, where: 10 - the content of chlorophyll A or β-carotene in 1 ml of a 1% solution, mg; A - optical density of the solution studied; V - volume of a measuring flask, ml; $E^{1\%}_{1cm}$ - extinction of chlorophyll A in chloroform at the wavelength 670 nm, which comprises 944,5, or $E^{1\%}_{1cm}$ - extinction of β-carotene in chloroform at the wavelength 453 nm, which comprises 2400; m - weight of a lipophylic extract sample, g [36, 39, 40].

The identification of carotenoid pigments in the extracts obtained was carried out by spectrophotometric measurement of extinction values of their 10% solutions in chloroform at the wavelengths 350-500 nm with an interval of 50 nm on the Hitachi U3210 spectrophotometer. The presence of three maxima in the absorption spectra of carotenoid pigments at 420, 450 and 470 nm was detected. According to the literature data, these maxima are characteristic of the absorption spectrum of β-carotene. The peaks in the range of 600-670 nm may be indicative of the presence of chlorophylls in the lipophylic extracts. Thus, the main absorption maxima of the lipophylic substances do not overlap, which allows the simultaneous determination of carotenoids and chlorophyll in the willow shoots extracts spectrophotometrically without preliminary separation [36, 39, 40].

2.5. Determination of Volatile Compounds

The GC/MS analysis of volatile compounds was carried out using the Agilent Technology 6890N chromatograph with the mass-spectrometric detector 5973N, with capillary column HP-5MS (silica, the column length was 30 m, inner diameter - 0,25 mm); carrier gas - helium (flow rate 1 ml/min). The sample volume was 0.1-0.5 µl with the flow split 1/50. The thermostat temperature was 50°C programmed for 4°C/min up to 220°C. The temperature of the detector and the vaporizer was 250°C [15, 16, 41].

2.6. Determination of Organic Acids

Organic acids were also determined by the GC/MS method using the Agilent Technology 6890N chromatograph with the mass-spectrometric detector 5973N. The sample was injected in the column in the splitless mode, the rate of sample injection was 1.2 ml/min within 0.2 min. Detection was held under the following conditions: capillary chromatographic column INNOWAX, the length of which was 30 m, inner diameter - 0.25 mm; carrier gas - helium; carrier gas velocity - 1.2 ml/min; the temperature of the sample injection heater - 250°C, the temperature of the thermostat was programmed for 4°C /min from 50°C up to 250°C; the temperature of the detector - 250°C [16, 17, 42].

Identification of the volatile compounds and organic acids was carried out by comparing the mass-spectra of the compounds with the data of the mass-spectra libraries NIST05 and WILEY 2007 combined with the identification software AMDIS and NIST. The quantitative determination of the compounds was carried out, taking into account the internal standard, and was evaluated in mg/kg of the plant raw material [16, 42].

2.7. Determination of Antibacterial Activity

The antibacterial activity of the extracts was studied by the agar diffusion method in the Mechnikov Institute of Microbiology and Immunology in the Microorganisms and Mediums Biochemistry Laboratory under the supervision of PhD (BiolSc) Osolodchenko T.P [23, 26, 43, 44]. According to the WHO recommendations, the reference strains of *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* 6538 ATCC, *Escherichia coli* ATCC 25922, *Proteus vulgaris* NCTC 4636, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* 9027 ATCC, *Bacillus subtilis* ATCC 6633 and *Candida albicans* 885/653 ATCC were used for the activity of substances assessment. The microbial suspension of microorganisms was prepared using the Densi-La-Meter device (PLIVALachema, Czech Republic; wavelength 540 nm). The suspension was prepared according to the instruction, which is added to the device, and an informational letter on the innovation in the healthcare system of Ukraine [45]. The cultures were synchronized by low temperature (4°C). The microbial burden comprised 107 microbial cells per 1 ml of the medium, and was estimated by the McFarland standard. An 18-24 hours culture of microorganisms was taken for the research. The Mueller-Hinton Agar (Dagestan SIC "Culture Mediums") was used in the experiment. The Sabouraud agar was used for *Candida albicans*. The antibacterial activity of the samples studied was determined using the agar diffusion method, or the "wells" method on two layers of dense culture medium, placed in Petri dishes. The "starvation" media (agar-agar, water, salts) were used for the lower layer. The lower layer represents a pad of 10 mm in height, on which 3-6 thin-walled stainless steel cylinders 8 mm in diameter and 10 mm high are horizontally placed. The upper layer, which consists of an agar culture medium, melted and cooled to 40°C, where a relevant standard of a 24-hour test microorganism culture is placed, is poured around the cylinders. The upper layer was preliminarily mixed until the formation of a uniform mass. The cylinders were pulled out by sterile forceps, and the extract studied, taking into account its volume (0.3 ml), was placed in the slots formed.

The volume of the medium for the upper layer fluctuated between 14 and 16 ml. The dishes were dried for 30-40 min at room temperature, and then were placed into a thermostat for 18-24 hours. Diameters of the inhibition zones of the microorganisms were measured by a ruler with the measurement error ±0.1 mm.

2.8. Statistical Analysis

The mean and Standard Deviation (SD) of the sample were calculated according to the monograph "Statistical analysis of the results of a chemical experiment" of the State Pharmacopoeia of Ukraine, 2.2. The average sample µ was

calculated as the arithmetic mean of all variants (n=5 of combined samples). At the same time, the spread of options around the average is characterized by the magnitude of the standard deviations. The uncertainty of this estimate is characterized by the value of the confidence interval, in which the true value μ is given with the given two-way probability P_2. Under uncertainty, the confidence interval is understood, usually for the 95% significance level. Limit values of the confidence interval were calculated using Student's criterion. Quantitative data are presented as the mean ± SD [23].

3. RESULTS

3.1. Total Content of Lipophylic Compounds, Chlorophylls and Carotenoids

The lipophylic extracts from the shoots of the plants of *Salix* L. genus were obtained. The lipophylic extracts obtained represent a salvelike mass of a dark-green colour with a characteristic pleasant aromatic odour. The lipophylic fractions are insoluble in water, soluble in chloroform, alcohol, hexane, vegetable oils. The percentage content of the extract in terms of the dried plant raw material comprised the highest yield - from *Salix myrsinifolia* Salisb. shoots - 15.71±0.10%, *Salix acutifolia* L. - 8.12±0.09% (Table **2**).

3.2. Content of Volatile Compounds

Forty-six volatile compounds were identified in the lipophylic extracts from the shoots of willow genus plants by the GC/MS method (Table **3**).

3.3. Contents of Organic Acids

Forty-two organic acids were detected, of which benzoic acid (from 1149.7 mg/kg to 3308.3 mg/kg), methoxybenzoic (from 7514.7 mg/kg to 14056.5 mg/kg), salicylic acid (from 1178.0 mg/kg to 5642.0 mg/kg) prevailed (Table **4**).

3.4. Antimicrobial Activity

The results of the research on the antimicrobial properties of lipophylic extracts by the agar diffusion method are given in (Table **5**).

Table 2. Quantitative content of the main biologically active substances in the lipophylic extracts from the shoots of the *Salix* genus plants.

Lipophylic Extract Sample		Value		
		Content of the Sum of Lipophylic Compounds, %	Content of the Sum of Chlorophylls, mg/%	Content of the Sum of Carotenoids, mg/%
1	*Salix cinerea* L	10.72±0.09	68.3±0.61	27.9±0.09
2	*Salix incana* Schrank.	11.17±0.09	36.5±0.35	13.5±0.17
3	*Salix caprea* L.	11.49±0.13	63.4±0.29	34.6±0.14
4	*Salix sachalinensis* F. Schmidt.	10.92±0.09	66.2±0.30	33.7±0,21
5	*Salix acutifolia* L.	8.12±0.09	58.8±0.36	31.2±0.08
6	*Salix fragilis* L.	10.34±0.11	62.1±0.02	41.5±0.02
7	*Salix caspica* Pall.	9.41±0.10	26.4±0.22	12.6±0.24
8	*Salix rosmarinifolia* L.	9.86±0.29	38.1±0.45	18.6±0.10
9	*Salix myrsinifolia* Salisb.	15.71±0.10	46.2±0.28	17.8±0.29

Table 3. Volatile compounds of the lipophylic extracts from the shoots of the *Salix* L. genus plants.

Volatile Components mg/kg	*S. cinerea* L	*S. incana* Schrank	*S. caprea* L.	*S. sachalinensis* F. Schmidt	*S. acutifolia* L	*S. fragilis* L	*S. caspica* Pall	*S. rosmarinifolia* L.	*S. myrsinifolia* Salisb
Cyclohex-2-en-1-one	215.28	187.70	48.86	319.48	273.67	369.61	141.98	170.09	163.79
2-oxybenzaldehyde	63.86	87.89	20.20	79.49	83.70	62.83	78.77	91.68	50.74
Benzyl alcohol	238.33	467.66	149.20	553.15	228.03	213.73	421.38	561.73	428.99
Cyclohexane-1.2-diol	416.95	807.97	46.56	1641.47	580.53	1058.41	849.50	928.46	295.09
trans-linalool oxide	18.85	56.31	12.06	18.36	20.59	30.78	14.89	25.57	26.59
cis-linalool oxide	20.36	50.26	8.06	51.33	10.14	44.86	24.95	33.18	21.29
Epoxylinalool	10.83	24.86	17.48	18.96	7.88	7.27	59.80	16.10	20.09
Benzoic acid	424.73	1618.45	210.33	5741.79	708.87	861.94	1545.34	1238.24	833.95
Benzene-1.2-diol (pyrocatechol)	592.46	397.65	854.23	1048.51	628.28	2100.44	990.97	632.71	425.89
Dec-2-enal	51.32	85.00	18.94	15.59	17.97	52.70	35.62	94.79	36.67
Phthalic aldehyde	22.18	194.28	18.15	36.17	42.11	57.15	98.26	87.76	84.04
Salicylic alcohol	160.01	120.33	170.53	504.36	367.16	510.07	857.56	296.88	163.53
Nonanoic acid	41.49	98.23	234.25	217.36	46.98	23.87	54.15	185.53	42.76
2-methoxy-4-vinyl phenol	57.75	212.59	27.26	119.20	55.16	85.33	87.34	175.76	20.96
Eugenol	164.22	259.72	140.03	161.41	100.73	123.17	154.25	169.94	98.92
Butylbutyrate	30.96	185.69	39.03	32.71	26.78	19.13	34.28	79.83	45.02

(Table 3) cont.....

Salicylic acid	276.07	168.34	121.76	196.60	212.35	210.02	250.74	260.34	234.90
Capric acid	74.53	66.78	18.00	160.41	18.80	29.90	47.32	58.52	21.98
Tetradecane	56.48	56.14	32.96	60.43	61.25	33.54	50.22	83.89	133.12
4-(2.4.4-trimethyl-cyclohexa-1.5-dienyl)-but-3-en-2-one	20.59	15.10	2.94	4.41	8.78	5.89	55.01	51.86	21.44
β-ionone-5.6-epoxide	36.86	12.20	15.84	46.48	39.30	39.83	41.63	65.65	38.33
2.6.10-trimethyldodecane	13.30	76.54	74.76	60.10	81.60	77.75	38.77	149.09	19.31
4.4.7a-trimethyldodecane-5.6.7.7a-tetrahydro-1-benzofuran-2(4H)-one	91.07	204.89	165.20	168.69	107.75	215.20	208.89	447.05	146.07
2.4-bis(1.1-dimethylethyl)phenol	13.51	73.52	51.47	72.46	11.55	28.42	41.00	74.65	22.21
Lauric acid	220.75	285.42	123.45	192.84	110.10	449.16	510.25	526.12	255.32
Tetradecanal	24.87	68.33	42.49	61.61	61.97	43.80	78.52	90.06	35.29
Myristic acid	268.07	405.37	315.05	275.46	215.71	368.24	308.73	1197.02	278.42
6.10.14-trimethylpentadec-2-one	114.64	204.94	175.69	136.09	165.90	59.89	60.15	161.67	125.03
Pentadecanoic acid	443.63	282.94	233.19	125.96	161.06	77.78	166.52	250.76	82.29
Palmitoleic acid	1290.61	527.26	275.08	580.32	717.47	554.94	1287.37	2643.83	252.60
Palmitic acid	5866.08	4592.24	3342.82	4503.05	6521.40	3810.27	6341.07	19464.72	3994.83
Heptadecanoic acid	173.96	238.17	87..24	181.57	100.62	165.56	170.03	251.47	144.12
Phytol	692.28	599.84	339.79	341.15	180.63	275.99	279.08	482.54	650.11
Linolenic acid	2269.51	2911.96	785.86	1132.94	1443.62	1910.94	2904.31	2000.35	751.21
Linoleic acid	1203.77	2148.99	777.56	1126.81	1350.56	1084.33	1605.23	2500.02	802.25
Oleic acid	969.62	1139.36	172.41	141.85	437.54	807.61	323.58	666.98	264.10
Stearic acid	422.67	882.77	308.52	831.60	361.08	358.36	414.70	792.33	392.48
Tricosane	143.70	181.18	1122.58	270.18	164.55	218.96	232.99	152.29	169.01
Tetracosane	81.26	158.82	168.86	201.34	64.82	89.57	69.98	152.29	95.97
Pentacosane	113.81	367.08	214.93	86.94	106.86	136.39	154.28	168.14	109.50
Hexacosane	678.45	1528.22	1137.42	2053.68	998.76	787.91	1196.76	519.34	2217.24
Heptacosane	510.15	938.71	1172.35	1103.58	529.40	832.90	2615.19	899.30	782.03
Squalene	1775.08	1923.09	495.15	587.30	2153.98	1172.75	1393.84	283.13	569.45
Nonacosane	852.71	2050.17	580.13	589.35	1244.20	856.51	1474.10	303.36	891.84
Phenylethyl alcohol	112.54	544.77	223.90	422.69	41.43	443.87	1182.48	830.70	501.61
Nonanal	39.42	48.91	37.51	53.31	372.01	19.34	94.59	44.72	15.56

Table 4. Organic acids of the lipophylic extracts from the shoots of the *Salix L.* genus plants.

Fatty and Organic Acids mg/kg	***S. cinerea* L**	***S. incana* Schrank**	***S. caprea* L.**	***S. sachalinensis* F. Schmidt**	***S. acutifolia* L**	***S. fragilis* L**	***S. caspica* Pall**	***S. rosmarinifolia* L.**	***S. myrsinifolia* Salisb**
Caproic acid	309.99	283.82	481.92	122.50	217.14	325.79	190.59	259.65	212.20
Hex-3-enoic acid	142.83	23.28	153.37	35.14	73.79	108.61	52.66	59.98	73.16
Hex-2-enoic acid	220.15	94.04	168.25	62.24	130.43	147.21	51.14	62.82	55.28
Caprylic acid	75.53	413.78	200.61	101.87	252.76	225.83	120.76	177.79	432.61
Oxalic acid	45.18	189.97	120.82	36.15	128.00	60.11	183.32	50.57	443.75
Nonanoic acid	45.18	243.37	286.89	106.05	156.40	97.50	143.66	183.02	605.63
Malonic acid	123.25	147.17	217.01	25.19	43.57	42.72	224.88	13509	729.48
Fumaric acid	239.27	48.99	46.79	28.24	26.89	27.80	88.12	53.25	114.93
Levulinic acid	724.07	471.53	419.34	532.11	395.42	244.86	375.01	426.61	564.50
Succinic acid	346.05	96.06	341.18	106.18	206.79	144.16	204.31	96.96	721.49
Capric acid	374.28	176.92	497.39	207.81	114.67	176.69	248.79	281.46	497.94
Benzoic acid	1149.73	1267.62	3308.34	12022.09	2487.17	1781.07	1769.43	1510.94	1150.79
Phenylacetic acid	1228.36	460.13	188.63	145.24	201.61	137.92	202.52	153.74	222.75
Salicylic acid	1485.28	1732.71	1570.53	3326.22	5641.97	4908.12	1741.72	1178.00	1577.21
Lauric acid	3964.28	764.49	1043.03	546.24	218.29	528.31	399.82	700.96	655.84
Malic acid	368.60	153.70	160.95	111.90	55.76	145.08	122.12	41.33	184.82
Myristic acid	71.44	1238.10	3419.50	622.14	1226.43	2404.38	1366.41	839.90	920.53
2-methoxybenzoic acid	1783.52	2304.67	2063.70	12596.52	14056.49	12597.76	8414.86	1415.93	1514.66
Cinnamic acid	382.91	567.11	297.99	435.63	621.30	128.92	1244.41	167.96	181.70
Pentadecanoic acid	2814.81	723.83	591.22	391.91	260.59	464.87	536.04	990.49	281.29
Azelaic acid	419.24	999.01	2727.57	600.81	1031.75	1539.51	967.52	2062.09	986.60
Palmitic acid	732.53	14335.08	18854.48	10542.30	13831.28	11853.84	15147.02	17477.43	13040.34
Palmitoleic acid	3475.33	505.92	1132.65	365.48	528.05	318.63	562.45	1105.55	564.24

(Table 4) cont.....

Heptadecanoic acid	53.88	582.04	674.28	615.27	535.53	892.22	488.49	678.47	400.18
Citric acid	213.41	280.79	637.73	107.32	466.78	572.62	369.41	806.79	1121.22
Stearic acid	285.02	2483.04	2111.21	1916.87	1673.44	1595.92	1873.65	5379.59	1686.41
Oleic acid	1557.95	2460.63	2936.84	1689.92	1822.03	1017.79	1629.86	3809.41	1069.50
Linoleic acid	2487.45	6534.01	9049.08	5040.43	7067.81	8814.67	6765.29	10796.87	3951.01
Linolenic acid	2797.13	6207.82	9338.40	6309.04	7176.23	5957.93	7911.48	6444.38	5708.69
Vanillic acid	47.84	841.68	617.01	565.58	378.40	249.16	499.37	900.17	853.19
2-oxypalmitic acid	475.16	975.35	1144.86	1137.54	880.14	862.78	1247.58	1551.56	734.17
Arachidic acid	138.12	1299.97	864.53	1401.89	257.56	463.41	746.14	883.65	1027.57
Heneicosanoic acid	258.15	176.18	165.35	523.75	69.86	269.54	127.77	110.44	114.86
p-Coumaric acid	-	-	-	-	-	-	3690.79	-	-
Behenic acid	824.53	3104.42	1075.70	4025.91	1253.53	1189.16	1462.78	1694.53	1833.87
Octadicarboxylic acid	82.24	198.28	406.40	79.58	108.47	456.93	231.92	118.82	96.66
p-Oxybenzoic acid	269.85	1567.73	130.88	540.31	203.07	180.11	403.86	92.03	92.02
Tricosanoic acid	181.84	687.87	494.38	414.08	274.94	259.03	497.27	330.99	357.01
Syringic acid	11.23	485.80	181.72	56.01	241.95	134.65	315.91	211.20	148.67
Gentisic acid	64.14	848.76	170.45	89.96	65.29	84.93	197.29	140.11	182.59
Tetracosanoic acid	969.44	7872.52	963.28	5024.78	1631.18	904.59	2819.31	1123.31	1292.13
Ferulic acid	384.48	597.84	875.78	312.81	1502.00	901.19	662.28	489.17	241.94

Table 5. Results of the antimicrobial activity study of the lipophylic extracts from the shoots of the willow species.

1% Alcohol Solution of a Lipophylic Extract	Inhibition Zones Diameter. mm. n=6. P=0.95					
	Staphylococcus aureus ATCC 25923	***Escherichia coli ATCC 25922***	***Proteus Vulgaris ATCC 4636***	***Pseudomonas aeruginosa ATCC 27853***	***Bacillus subtilis ATCC 6633***	***Candida Albicans ATCC 653/885***
1. *Salix cinerea* L	30.0 ±0.6	29.8±0.5	22.7±0.3	25.0 ±0.5	30.9 ±0.3	27.2 ±0.5
2. *Salix incana* Schrank	30.0 ±0.5	30.0 ±0.3	23.2±0.5	25.0 ±0.5	31.0±0.3	26.0 ±0.3
3. *Salix caprea* L	31.3±0.5	29.0 ±0.5	24.5 ±0.3	26.2±0.5	31.5±0.5	27.0±0.5
4. *Salix sachalinensis* F. Schmidt.	31.5±0.5	21.4±0.3	20.1±0.3	21.0 ±0.5	31.3±0.5	19.2 ±0.5
5. *Salix acutifolia* L.	29.2±0.3	29.5 ±0.5	19.8±0.5	21.5 ±0.2	26.5±0.6	25.3±0.5
6. *Salix fragilis* L.	28.9±0.5	30.2±0.3	19.0±0.2	22. ±0.5	30.0±0.5	23.2±0.5
7. *Salix. caspica* Pall.	29.2±0.3	28.2±0.5	17.8±0.5	22.2±0.5	26.5±0.5	22.3±0.2
8. *Salix rosmarinifolia* L.	30.2±0.5	27.8±0.5	20.2±0.4	23.0±0.5	31.1±0.4	22.3±0.5
9. *Salix myrsinifolia* Salisb.	24.5±0.3	28.0 ±0.5	21.0±0.5	20.0±0.3	34.3±0.5	24.0 ±0.5

The following criteria were used in the evaluation of the antibacterial activity: 1) absence of the microorganisms growth inhibition zone around the slots, as well as the inhibition zone up to 10 mm, show the absence of sensitivity of the microorganism towards the extract placed in the slot; 2) the growth inhibition zones of 10-15 mm in diameter indicate the low sensitivity of the culture towards the extract studied; 3) the growth inhibition zones of 15-25 mm in diameter are considered as the indicator of the sensitivity of the microorganism towards the extract studied; 4) the growth inhibition zones, the diameter of which exceeds 25 mm, indicate high sensitivity of the microorganism towards the extract studied.

The results of the experimental research show expressed antimicrobial activity of the lipophylic extracts towards the strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli*, as well as the activity towards the yeast fungus *Candida albicans*.

4. DISCUSSION

4.1. Total Content of Lipophylic Compounds, Chlorophylls and Carotenoids

The quantitative content of lipophylic compounds in willow shoots differs depending on the chosen type of the plant raw material, but still is on the quite high level.

According to our data (Table **2**), the content of the sum of

chlorophylls in the shoots of the willow genus plants varies from 26.4 to 68.3 mg/%. The quantitative content of carotenoids in the lipophylic extracts from the shoots of the plants of *Salix* L. genus was also determined spectrophotometrically. The content of carotenoids ranges from 12.6 to 41.5 mg/%. Thus, the sum of chlorophylls in the willow species shoots during the vegetation period is higher compared to that of carotenoids, which confirms the high level of metabolism and anabolism in the plants.

Chlorophylls possess bactericidal and antioxidant activities, improve the state of blood vessels [46, 47]. Carotenoids show a proven anticarcinogenic, immune modulating, antioxidant activity, suppress the photosensibilization processes and lower the risk of cardiovascular disorders, which contribute to the prospects of further research on the properties, obtained during the study of lipophylic extracts [48 - 50].

4.2. Content of Volatile Compounds

The dominating compounds among all the components are eugenol (4-allyl-2-methoxyphenol, the content of which comprised from 98.92 mg/kg to 259.72 mg/kg), and squalene (from 495.15 mg/kg up to 2153.98 mg/kg). Squalene should be noted as one of the dominating compounds in the lipophylic extracts, which belongs to the compounds of carotenoid nature. The results of experimental and clinical research show squalene has a positive impact on lipid metabolism. In medicine, squalene is added as a component of agents for the external therapy of inflammatory skin diseases. In this case, the property of squalene to deeply penetrate the skin, being able to carry elements of other compounds, is used; as a result, potent medicines can be applied on the skin pointwise, not being troubled of their adverse effects [31].

Aromatic components, such as benzyl alcohol, salicylic alcohol, pyrocathechol, *etc*., are also found in quite a large amount, which indicates the presence of antiseptic properties in the extracts. A large amount of aldehydes and ketones, which comprise over 10% of the total compounds, is also found.

Previously, *cis*-3-hexenyl acetate, *cis*-3-hexenol, and benzaldehyde were found from leaves of six willow varieties [51]. Karimi *et al*. [52] showed that the main compound of essential oil of *S. aegyptiana* was 1,4-dimethoxybensene, also phenylethyl alcohol, carvone and citronellol are present among principal compounds. The phenylethyl alcohol was also found in the *Salix species* studied (41-1182 mg/kg).

4.3. Contents of Organic Acids

A considerable part of the extracts is represented by fatty acids - essential components of plant cells, which take part in lipid biosynthesis and almost all physiological processes, as well as biochemical reactions, which proceed in human and animal cells. Fatty acids show F-vitamin activity, reparative and many others, in addition, they provide pharmacological effects of a range of medicines. The biological value of lipophylic extracts depends on the composition of fatty acids. Linoleic (from 2488.0 mg/kg to 10796.9 mg/kg) and linolenic (from 2487.45 mg/kg to 10796.87 mg/kg) acids are found in quite large quantity in the extracts. Palmitic acid (from 10542.3 mg/kg to 18854.5 mg/kg) dominates among the saturated fatty acids. The high content of hydroxycinnamic acid derivatives and salicylic acid in the lipophylic extracts of willow shoots is of much interest. This might explain their high pharmacological activity to some extent. In our study [53], it was shown that *Salix* spp. branches contain 3.0-10.1 mg/kg of copper. According to Rohnert *et al*. [54], the oxidation of low-density lipoprotein by copper ions is strongly inhibited by different aqueous extracts of *Salix* spp. but linolenic acid has no significant effect on it.

The study of the chemical composition of lipophylic extracts from the shoots of willow genus plants indicates that they must possess a wide spectrum of antimicrobial activity. Thus, the experiment carried out significantly broadens the data on the chemical composition of the plant raw material of *Salix* L. genus plants and extracts on their basis.

4.4. Antimicrobial Activity

The results of the experimental research show expressed antimicrobial activity of the lipophylic extracts towards the strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli*, as well as the activity towards the yeast fungus *Candida albicans*. As it was found by Gonzalez-Alamilla *et al*. [33], the hydroalcoholic extract also showed an antibacterial effect against *S. aureus* (MIC 0.39 mg/ml) and less to *E. coli*. The water infusion of *S. balylonica* leaves showed just weak activity to *E. coli* and no activity against *C. albicans* [55].

Thus, it can be concluded that the lipophylic extracts are a prospective substance for the creation of a novel antimicrobial agent from willow shoots, and further require more detailed research on their antimicrobial activity.

CONCLUSION

1. A comparative study of the chemical composition of lipophylic extracts from the shoots of 9 willow species of Ukrainian flora - *Salix cinerea* L., *S. incana* Schrank, *S. caprea* L., *S. sachalinensis* F. Schmidt, *S. acutifolia* L., *S. fragilis* L., *S. caspica* Pall., *S. rosmarinifolia* L. and *S. myrsinifolia* Salisb - was carried out. 46 volatile compounds and 42 carboxylic acids were detected and identified. The quantitative content of lipophylic compounds in the willow species plant raw material and chlorophylls and carotenoids in the willow shoots extracts was determined. The balanced natural complex of these biologically active compounds is responsible for the pharmacological effect of the obtained lipophylic extracts from the plant raw material studied.

2. For all the studied lipophylic extracts from willow shoots, the antimicrobial activity towards *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans* was established.

3. The results obtained indicate the possibility of the creation of a novel antimicrobial agent using the lipophylic extracts from willow shoots, which will allow the more effective application of the *Salix* L. genus plants raw material, as well as broadening the nomenclature of Ukrainian medicinal products.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No human or animals were used in this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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