The Study of the Composition of Chloroform Fraction of *Anemone nemorosa* L.

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Expanding the range of herbal drugs is among the priorities of the modern pharmacy. The analysis presented in this article is drawn from a broader qualitative study examining the composition of *Anemone nemorosa* L. chloroform fraction. The study revealed 38 compounds, and 32 of them were identified. The investigated lipophilic extract has an antimicrobial activity both in terms of gram-positive and gram-negative microorganisms. The maximal antimicrobial action of the investigated extract was shown to *Escherichia coli*. The significant content of biologically active substances in the lipophilic volatile fraction of *Anemone nemorosa* indicates the prospects for further study.

Introduction

In recent years, there has been observed an increasing interest in medicinal herbs [1]. This indicates a need to understand the various aspects of making and producing new phyto-remedies and introducing them into practical use. *Anemone nemorosa* L. is a herbaceous perennial plant of the buttercup *Ranunculaceae* family [2-4], is widely used in folk medicine as an antitumor, sedative, bactericidal, antimicrobial, antifungal, anti-inflammatory, spasmolytic diaphoretic, expectorant, and diuretic drug [5-11]. The major biologically active substances in *Anemone nemorosa* are: alkaloids, glycosides (proto-anemonin, anemonin, ranunculin, some types of saponins, tannins), vitamin C, resins, organic acids (chelidonic acid), coumarins, flavonoids, and γ-Linolenic acid [12-15].

Evidence suggests that this herb is referred to as a regionally rare plant of administrative territories of Ukraine [16-17]. Taking into account the wide experience of its , a wide spectrum of pharmacological activity, the content of valuable biologically active compounds [7], as well as the results of phytochemical and pharmacological studies, it becomes clear that the further use of *Anemone nemorosa* as a valuable medicinal plant material is an urgent task of pharmacy and

pharmaceutical biotechnology in view of the prospects of scientific research on development and introduction into production of new phyto-remedies.

At present, we carry out a systematic study of lipophilic compounds. The main objective of this study is to investigate and determine the component composition of the chloroform fraction of *Anemone nemorosa* and study of its antimicrobial properties.

Experimental part

Material and methods

The object of the study was herb of *Anemone nemorosa* L. that was collected in 2018 in an ecologically clean region of the Carpathians (Ivano-Frankivsk region, Ukraine), and the lipophilic extract of *Anemone nemorosa* L. The shade-dried aerial parts were powdered and extracted with the chloroform using a Soxhlet extractor, then the solvent was removed in vacuo until dry extract.

Analysis of biologically active substances of the lipophilic fraction was performed using chromatograph Agilent Technology 6890 GC System with mass spectrometry detector HP 5973 Mass Selective Detector. The components were separated in a fused silica column Restek Rtx-5MS being 30 m long and having an internal diameter of 0.32 mm. The stationary liquid phase was 0.25 μm thick and consisted of 95% of polymethylsiloxane and 5% of phenylpolysiloxane. The carrier gas was helium. The velocity of the carrier gas was 2.0 ml/min. The thermostat of the column was heated in such a program: the initial temperature of 60°C was maintained for 10 minutes, and then the temperature was increased with the tempo of 20°C/min to 280°C. This temperature remained constant for 30 min, the temperature of the detector and the evaporator was 280°C. Biologically active substances were identified by their retention time, comparing it with standards and with the library of mass spectra NIST 05 and WILEY 2007, the total number of spectra being more than 470000.

Antibacterial and antifungal bioassay

Four Gram-negative bacteria (reference strains *Pseudomonas aeruginosa* (ATCC 27853 (F-51)), *Escherichia coli* (ATCC 25922) and clinical multi-drug resistance (MDR, according to [19]) strains *Pseudomonas aeruginosa*, *Escherichia coli*), five Gram-positive bacteria (reference strains *Staphylococcus aureus* (ATCC 25923 (F-49)), *Staphylococcus epidermidis* (191), *Bacillus licheniformis* (VKPM-7038) and clinical MDR strains *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA)), and four strain of yeast (reference strains *Candida albicans* (ATCC 885-653), *Candida albicans* (ATCC 668-853) and clinical MDR strain *Candida dubliniensis* and *Candida albicans* resistant to nistatin sensitive to itraconazole and fluconazole) were used for antibacterial and antifungul activity (disc diffusion and serial dilutions assay).

Table 1. Antibacterial and antifungul activity of chloroform fraction of *Anemone nemorosa* L.

№	Tipe of species		Species of bacteria and fungi	Diameter of inhibitory zones (mm ± SD)					Minimum Inhibitory (MIC) and Bactericidal /Fungicidal Concentration (MBC/MFC)	
				Extract of *Anemone nemorosa*	DMSO	Water	Ampicilin (10 mcg)	Itraconazole (10 mcg)	MIC (μg/mL)	MBC /MFC (μg/mL)
1	Gram-negative bacteria	reference strains	*Pseudomonas aeruginosa* (ATCC 27853 (F-51))	7.2 ± 0.3	6.8 ± 0.2	NA	NA	NA	NT	NT
2			*Escherichia coli* (ATCC 25922)	14.2 ± 0.4	8.0 ± 0.4	NA	15.2 ± 0.4	NA	250	500
3		clinical strains	*Pseudomonas aeruginosa* (n=4)	NA	NA	NA	NA	NA	NT	NT
4			*Escherichia coli* (n=4)	NA	6.4 ± 0.2	NA	7.4 ± 0.2	NA	NT	NT
5	Gram-positive bacteria	reference strains	*Staphylococcus aureus* (ATCC 25923 (F-49))	10.2 ± 0.8	7.3 ±0 .3	NA	18.2 ± 0.8	NA	NT	NT
6			*Staphylococcus epidermidis* (191)	9.4 ± 0.6	7.4 ± 0.2	NA	19.4 ± 0.5	NA	250	500
7			*Bacillus licheniformis* (*BKIIM-7038*)	11.5 ± 0.3	7.2 ± 0.4	NA	18.8 ± 0.6	NA	NT	NT
8		clinical strains	*Staphylococcus aureus* (n=4)	9.1 ± 0.6	7.2 ± 0.2	NA	12.2 ± 0.4	NA	NT	NT
9			MRSA (n=4)	NA	NA	NA	NA	NA	NT	NT
10	Fungi	reference strains	*Candida. albicans* (ATCC 885-653)	10.3 ± 0.5	8.0 ± 0.2	NA	NA	15.4 ± 0.6	250	500
11			*Candida albicans* (ATCC 668-853)	10.2 ± 0.3	8.0 ± 0.2	NA	NA	16.2 ± 0.2	NT	NT
12		clinical strains	Candida *dubliniensis* (n=4)	6.6 ± 0.2	NA	NA	NA	NA	NT	NT
13			*Candida albicans* (n=4)	8.0 ± 0.7	7.0 ± 0.3	NA	NA	7.6 ± 0.4	500	1000

NA: no activity; NT: Not Tested; diameter of zone of inhibition (mm) including the disc diameter of 6 mm; data are presented as mean ±SD (n = 3).

After adjusting turbidity of each broth culiture of bacteria with saline (0.5 McFarland), inoculation of Mueller Hinton and Sabouraud agar plates was performed by using cotton swabs (6 mm diameter and containing 0.2 μm of extract).

Results and discussion

The first set of analyses examined the chromatogram of the extracts under study (Figure 1).

Figure 1 and Table 2 show the results obtained. In the result of the study, the quantitative content of 38 volatile compounds was established, 32 of which were identified (Figure 1 and Table 2).

Table 2. Identified compounds of *Anemone nemorosa* L. chloroform fraction

№	Retention time	Substance	Content %
1	6.659	Caprylic aldehyde	0.17
2	7.317	Limonene	0.52
3	8.231	4-Ethyldacane	0.41
4	14.562	7-n-Tripropyldecan	0.47
5	14.791	Icosane	0.18
6	15.134	Pentadecane	0.15
7	15,691	2,3,6-Trimethyl-4-octene	0.12
8	18.020	2-Methylpenta-decane	0.22
9	19.249	2-Methyltricosane	0.32
10	20.050	Heneicosane	0.44
11	20.593	2,4-bis(1,1-dibutyl)phenol	0.60
12	23.579	Heptadecane	0.19
13	24,122	Tetracosane	0.21
14	24.894	Heneicosane	0.52
15	27.724	11-(1-Ethylpropyl)-Heneicosane	0.24
16	29,224	Tetracosane	0.36
17	29.967	Pentacosane	0.32
18	30.453	Etyl palmitate	1.23
19	32.582	Ethyl linoleate	0.88
20	32.711	Dibutyl Sebacate	6.11
21	32.954	Stearyl Alcohol	0.77
22	33.411	Fumaric acid 2,2-dicloroethyl tridecyl ester	0.25
23	33.726	Acetyltributyl citrate	31.21
24	34.626	Oleamide methylate	0.55
25	34.726	Oleamide	1.28
26	35.712	Dibutyl Sebacate	0.33
27	36.155	Dioctyl Sebacate	0.83
28	36.612	Heneicosane	0.54
29	38.170	Decyl octyl adipate	0.79
30	38.699	Oleamide	1.26
31	40.542	Docosane	0.39
32	46.030	Pelargonic acid tridecyl ester	1.15
33	46.173	2,4-Difluoro-benzoic acid nonadecyl ester	3.47
34	48.145	Rimuene	2.81
35	48.388	Pyridine-3-carboxamide	2.54
36	48.545	1,5-Dimethyl-benzacridine	0.78
37	48.774	Loxapine	1.92
38	48.916	Metoxyacetic acid heptadecil ester	1.19

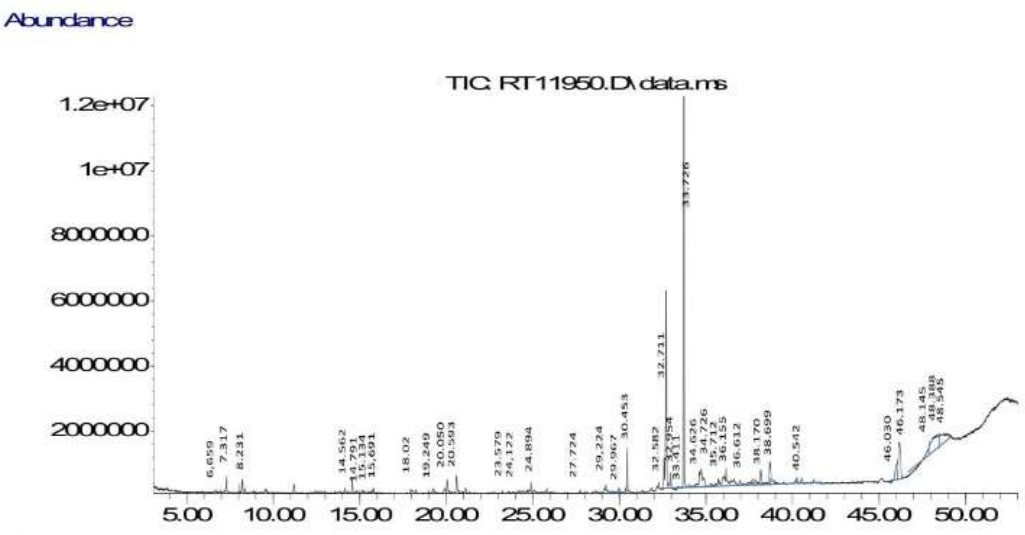

Figure 1. Chromatogram of volatile compounds of the *Anemone nemorosa* L. chloroform fraction

As it can be seen from these data, in the study of the component composition of the *Anemone nemorosa* chloroform fraction, 32 compounds were identified, they being paraffinic hydrocarbons, esters, fatty aldehydes and terpenes.

Further analysis showed that the dominant component in the sample was Acetyltributyl citrate – 31,21%. Among others, there were Oleamide – 3,09%, Loxapine – 1,92%. The identified esters were: Dibutyl sebacate – 6,44%, 2,4-Difluorobenzoic acid nonadecyl ester – 3,47%, Metoxyacetic acid heptadecil ester – 1,19%, Pelargonic acid tridecyl ester – 1,15%. The following alkanes were present: Heneicosane – 1,5%, Tetracosane – 0,53%, Docosane – 0,39%. Terpenes in the sample were presented by Monocyclic monoterpenes limonene – 0,52% and Diterpene rimuene – 2,81%.

The results obtained of the study biological activity of *Anemone nemorosa* chloroform fraction suggest that the lipophilic extract under study has antimicrobial and antifungal activity against referece and clinical MDR strains Gramm-positive bacteria and fungi. The maximal antimicrobial action of the investigated extract was shown in relation to *E. coli.* When applying the method of serial dilutions, it was found that the maximum bactericidal effect of the tested extract was expressed relative to *E. coli* in dilution 1:4.

Conclusions

This study set out to analyse the composition of *Anemone nemorosa* chloroform fraction. The *Anemone nemorosa* lipophilic fraction was obtained using the method of exhaustive extraction with chloroform in the Soxhlet extractor. The yield was 6,93 %.

Using chromatography–mass spectrometry, the component composition of the volatile matter of the *Anemone nemorosa* chloroform fraction was obtained for the first time.

38 substances were discovered, with 32 of them being identified.

This study has shown that the *Anemone nemorosa* lipophilic extraction has an antimicrobial action on reference and clinical MDR strains of both gram-positive, gram-negative microorganisms and *Candida spp.* The maximal antimicrobial action of the investigated extract was shown in relation to *E. coli.*

One of the more important findings to emerge from this study is that the significant content of biologically active substances in the *Anemone nemorosa* lipophilic volatile fraction indicates the prospect of their further study in order to create new medicinal products.

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