RESEARCH ARTICLE

Obtaining of *Geranium sanguineum* phytoextracts and study of their anti-microbial properties

I. L. Benzel[1], O. I. Hordiienko[2], T. A. Hroshovyi[2], L. V. Benzel[1], O. V. Pokryshko[3]

[1]Department of Pharmacognosy and Botany, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine, [2]Department of Pharmacy Management, Economics and Technology, I. Horbachevsky Ternopil State Medical University, Ukraine, [3]Department of Microbiology, Virology and Immunology, I. Horbachevsky Ternopil State Medical University, Ukraine

Abstract

Introduction: Along with the use of the latest pharmaceutical agents, the healing properties of medicinal plants, *Geranium sanguineum* one of them, are more and more widely used in medical research and practice. The presence of tannins in the plant causes its astringent and anti-inflammatory properties. This herb infusion in the form of rinsing proves a positive effect in cases of inflammations of mucous membrane of upper respiratory tract. Decoction of rhizomes and roots was used in traditional medicine as well as in dermatology as an antitumor agent. Therefore, the aim of our research was to develop optimal conditions for obtaining lyophilized phytocomplexes out of *G. sanguineum* herb for further study of their biological properties. **Materials and Methods:** The *G. sanguineum* herb was stored as raw material; the extracts were obtained out of it. By changing the main parameters (degree of shredding, extraction solvent, extraction time, ratio of herbal raw material mass and extraction solvent amount, and multiplicity of extractions), the content of the main groups of active substances was determined. Tannins assay was carried out by permanganametometric titration and the amount of polyphenolic compounds, hydroxycinnamic acids, and flavonoids - by means of spectrophotometric methods. The study of antibacterial and antifungal activity of the extract was performed by double serial dilutions in a liquid nutrient medium. **Results and Discussion:** It was established that the optimal degree of *G. sanguineum* herb grinding was 0.5–1.0 mm, and the best extraction solvents were ethyl alcohol of 50% concentration and purified water. The completeness of biologically active substances obtaining out of the investigated raw materials was most often succeeded by purifying water for 45 min and 50% ethanol - for 30 min. The ratio between raw material and extraction solvent was 1:30 (extraction solvent - 50% ethyl alcohol) and 1:15 (extraction solvent - purified water). The optimal multiplicity of extraction for aqueous extractors is triple, and for aqueous and alcohol extractors, a double extraction was enough. **Conclusion:** The obtained extracts of *G. sanguineum* herb have antibacterial and antifungal properties and can be used for the development of new drugs for prevention and treatment of infectious diseases of different etiologies.

Key words: Antimicrobial action, biologically active substances, extraction, *Geranium sanguineum*, phytosubstance

INTRODUCTION

The use of herbs in therapy is important for the treatment and prevention of many diseases. Hence, the search and development of new herbal drugs as well as rational and integrated use of raw materials of wild and cultivated plants in Ukraine are topical issue of contemporary pharmaceutical research and practice.

Geranium sanguineum L. is one of the herbs that have long been used in traditional medicine; it is a perennial herb of the geranium family (*Geraniaceae*). Above ground and underground organs of *G. sanguineum*

Address for correspondence:
O. I. Hordiienko, Department of Pharmacy Management, Economics and Technology, I. Horbachevsky Ternopil State Medical University, Ternopil, Ruska str, 36, Ukraine. E-mail: hordiienko.oi@gmail.com

Received: 16-05-2018
Revised: 18-06-2018
Accepted: 28-06-2018

contain tannins, flavonoids, vitamins, polysaccharides, hydroxycinnamic acids, and other groups of biologically active substances. Infusions and decoctions of this herb and rhizomes of this plant are recommended to use as astringent, anti-inflammatory, wound healing and antibacterial agent in cases of diarrhea, dysentery, acute and chronic enterocolitis, pulmonary hemorrhage, mouthwash in diseases of its mucous membrane, and for washing purulent wounds. Furthermore, various parts of this plant are part of antitumor infusion. The experimental studies proved antioxidant and antiviral properties of blood-red geranium extracts.[1-4]

Taking into account the pharmacological effects caused by the biologically active substances, which are present in the *G. sanguineum* herb and its sufficient raw material amount on the territory of Ukraine, the aim of our research was to develop optimal conditions for obtaining lyophilized phytocomplexes out of this raw material for further study of their biological properties.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

G. sanguineum herb was harvested in Lviv region, Ukraine, during the flowering period in 2013–2014. The raw material was identified by Ph.D. L.V. Benzel, the Associate Professor of the Department of Pharmacognosy and Botany of Danylo Halytsky Lviv National Medical University, Lviv (Ukraine).

The raw material was dried to an air-dry state and grinded. The grinded raw material was of a dark green color and a peculiar, choking odor.

Plants Extraction

Using the dried *G. sanguineum* herb, the extracts were prepared, changing the main parameters (degree of grinding, extraction solvent, extraction time, ratio of raw material weight and extraction solvent amount, and extraction multiplicity), then the content of the main groups of active substances was determined.

Phytochemical Screening

Tannins assay was carried out by permanganametometric titration[5] and the quantity of polyphenolic compounds, hydroxycinnamic acids, and flavonoids - by means of spectrophotometric methods.[6-8] The study of the content of these substances was carried out in a five-fold repetition with subsequent statistical processing of results.[9]

Using the optimal technological conditions for isolation of biologically active substances out of *G. sanguineum* herb, aqueous as well as aqueous and alcohol lyophilized phytosubstances were obtained.

Microbiological and Antifungal Test

Antimicrobial activity of the obtained phytocomplexes was studied *in vitro* according to the State Pharmacopoeia of Ukraine.[9] Dry extract of *G. sanguineum*, obtained of herb, was used for the research.

The study of antibacterial and antifungal effect of the extract on the vital activity of microorganisms was carried out by two-fold serial dilutions in a liquid nutrient medium (meat-peptone broth). A standardized daily suspension of test strains of the following microorganisms: *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, and *Candida albicans* ATCC 885-653, a cell concentration in them was 0.5 per McFarland, were used in the experiments. The research was conducted in a ten-fold repetition.

The evaluation of the results was carried out according to the growth inhibition degree of a test culture of the microorganisms of the test substance in a certain dilution. Statistical analysis of the results was carried out by the method of variation statistics with the assessment of average values and mean error. The statistical significance of differences between the mean values during the analysis was estimated using Student's criterion (t). The difference between the values was statistically significant at probable variance $P \leq 0.05$.

RESULTS

The results of studying the optimal conditions for biologically active substances extraction out of *G. sanguineum* herb by some extraction solvents, depending on various factors, are presented in Tables 1-5.

The information in Table 1 proves that the optimal degree of *G. sanguineum* herb grinding is 0.5–1.0 mm, when the maximum amount of phenolic compounds, hydroxycholic acids, and tannins is extracted; raw material grinded to 1.0–2.0 mm may be also used.

The best extraction solvents for the *G. sanguineum* herb are ethyl alcohol of 50% concentration and purified water, using which the maximum amount of phenolic compounds is isolated [Table 2].

By investigation of the effect of extraction time on the completeness of isolation of biologically active substances [Table 3], it has been established that the completeness of their extraction out of the studied raw material is performed using purified water for 45 min and 50% ethanol - during 30 min. Further increase of extraction time has not caused any significant increase in the content of active substances in the extracts obtained.

Table 1: Study of the dependence of biologically active substances extraction completeness on the degree of grinding

Degree of grinding of raw material, mm	Biologically active substances assay (%), $\bar{x}\pm\Delta\bar{x}$, *n*=5			
	Tannins	Amount of polyphenols	Flavonoids	Amount of hydroxycinnamic acids
>5.0	3.00±0.17	3.32±0.16	0.052±0.02	0.65±0.03
2.5-5.0	7.05±0.11	4.22±0.23	0.12±0.07	1.14±0.08
2.0-2.5	12.24±0.79	17.53±0.85	0.18±0.09	1.70±0.09
1.0-2.0	14.89±0.85	20.12±1.25	0.22±0.11	2.46±0.18
0.5-1.0	16.17±0.96	24.43±1.64	0.27±0.14	2.55±0.23
0.1-0.5	10.50±0.62	13.72±0.59	0.15±0.06	2.07±0.15
<0.1	8.31±0.58	3.42±0.17	0.032±0.01	0.69±0.04

Table 2: Study of the influence of extraction solvent nature on the completeness of biologically active substances extraction

Extraction solvent	Biologically active substances assay (%), $\bar{x}\pm\Delta\bar{x}$, *n*=5			
	Tannins	Amount of polyphenols	Flavonoids	Amount of hydroxycinnamic acids
96% ethyl alcohol	9.47±0.66	5.36±0.34	0.073±0.01	1.13±0.06
70% ethyl alcohol	17.55±0.94	14.05±0.49	0.20±0.15	2.98±0.22
50% ethyl alcohol	18.82±1.11	14.68±0.56	0,27±0,17	3.21±0.31
30% ethyl alcohol	16.74±0.87	13.23±0.38	0.29±0.21	2.92±0.21
Purified water	16.17±0.80	23.95±1.21	0.26±0.16	2.55±0.78

Table 3: Study of the effect of extraction time on the yield of biologically active substances

Extraction time, min	Biologically active substances assay (%), $\bar{x}\pm\Delta\bar{x}$, *n*=5			
	Tannins	Amount of polyphenols	Flavonoids	Amount of hydroxycinnamic acids
50% ethyl alcohol				
15	16.21±0.86	11.28±0.38	0.15±0.04	2.50±0.34
30	19.79±1.12	20.58±1.09	0.22±0.07	2.58±0.42
45	19.22±1.01	17.41±0.92	0.20±0.06	2.15±0.19
60	17.25±0.99	15.74±0.71	0.16±0.05	1.98±0.16
Purified water				
15	12.40±0.63	14.20±0.52	0.15±0.04	1.84±0.12
30	15.64±0.72	15.19±0.67	0.16±0.05	1.95±0.15
45	17.14±0.99	16.48±0.89	0.25±0.09	2.20±0.21
60	15.29±0.69	12.17±0.43	0.22±0.07	1.61±0.09

The information in Table 4 proves that the active substances are better extracted and in greater amount at a ratio between raw material and extraction solvent 1:30 (extraction solvent - 50% ethyl alcohol) and 1:50 (extraction solvent - purified water) because a further increase in the amount of extraction solvent in relation to the weight of raw materials does not allow to significantly increase the yield of active substances in the studied extracts.

According to the results [Table 5], it is established that the optimal extraction multiplicity for aqueous extractors is a 3 time extraction and for aqueous and alcohol extractors, a double extraction is enough.

The summary on the study of optimum conditions for extracts obtaining out of *G. sanguineum* raw material is presented in Table 6.

Taking into account the studied conditions for extraction of active substances out of blood-red geranium herb, a series of aqueous as well as aqueous and alcohol extracts are obtained and their lyophilic drying by means of a

Table 4: Study of the optimal ratio of raw material and extraction solvent

Ratio of raw material/extraction solvent	Biologically active substances assay (%), $\bar{x}\pm\Delta\bar{x}$, *n*=5			
	Tannins	Amount of polyphenols	Flavonoids	Amount of hydroxycinnamic acids
50% ethyl alcohol				
1:10	10.51±0.86	10.43±0.55	0.17±0.01	1.44±0.14
1:15	16.28±1.02	15.40±0.71	0.22±0.02	1.97±0.17
1:20	18.25±1.12	16.80±0.92	0.25±0.03	2.17±0.19
1:30	19.74±1.19	16.95±0.99	0.28±0.02	2.24±0.22
1:50	19.28±1.17	15.56±0.72	0.27±0.03	1.91±0.16
Purified water				
1:10	4.45±0.16	12.11±0.56	0.18±0.01	1.55±0.06
1:15	12.90±0.72	15.24±1.27	0.20±0.01	2.07±0.07
1:20	13.63±0.59	14.94±0.69	0.23±0.01	2.09±0.07
1:30	14.08±0.65	18.17±0.73	0.25±0.02	2.35±0.09
1:50	16.17±0.70	20.33±0.98	0.26±0.02	2.66±1.06
1:75	16.24±0.55	20.25±1.12	0.26±0.01	2.68±0.15

Table 5: Study of the influence of extraction multiplicity on the yield of biologically active substances

Extraction multiplicity	Biologically active substances assay (%), $\bar{x}\pm\Delta\bar{x}$, *n*=5			
	Tannins	Amount of polyphenols	Flavonoids	Amount of hydroxycinnamic acids
50% ethyl alcohol				
1	23.67±1.22	17.39±1.15	0.15±0.04	2.24±0.34
2	5.83±0.38	4.94±0.32	0.04±0.01	0.67±0.42
3	1.00±0.03	1.05±0.04	0.02±0.008	0.17±0.19
4	0.42±0.01	0.42±0.01	0.008±0.001	0.11±0.16
Purified water				
1	19.17±1.13	16.53±0.72	0.19±0.04	3.35±0.12
2	4.54±0.32	4.56±0.34	0.02±0.009	0.64±0.05
3	3.04±0.09	2.05±0.09	0.005±0.004	0.27±0.004
4	0.50±0.01	0.64±0.02	0.002±0.001	0.13±0.001

Table 6: Optimal conditions for extracts obtaining out of *G. sanguineum* raw material

Raw material	Extraction solvent	Degree of grinding of raw material, mm	Extraction time, min	Ratio of raw material/ extraction solvent	Extraction multiplicity
Herb	Purified water	1.0–0.5	45	1:50	3
	50% ethyl alcohol	1.0–0.5	30	1:30	2

G. sanguineum: Geranium sanguineum

sublimation apparatus KS-30 (Czech Republic) is carried out. The obtained lyophilizates are standardized according to the content of the main active substances; they are crumbly, amorphous, from light yellow to light brown color mass with a peculiar odor and astringent taste. The yield of the final product is 25.70–34.50% [Table 7].

The next stage of the research is to study the antimicrobial and antifungal properties of dry extracts of *G. sanguineum* herb, extracted with 50% ethanol, since the largest number of biologically active substances is found. For this purpose, the minimum inhibitory concentration and the minimum bactericidal concentration are determined by means of serial dilutions. The results of the experiment are presented in mg/ml [Table 8].

DISCUSSION

The process of extraction of raw material is of a complex physical and chemical nature associated with surface phenomena by the

Table 7: Characteristic features of lyophilized extracts obtained out of blood-red geranium herb by means of different extraction solvents

Extraction solvent	Yield of phytosubstance, %	Biologically active substances assay, %, $\bar{x}\pm\Delta\bar{x}$, *n*=5			
		Tannins	Flavonoids	Hydroxycinnamic acids	Amount of polyphenols
Purified water	25.70	26.39±1.01	4.96±0.23	5.46±0.33	31.84±1.42
50% ethyl alcohol	34.50	32.64±1.45	6.32±0.21	7.02±0.40	39.77±1.89

Table 8: Study of antimicrobial and antifungal effects of ***G. sanguineum*** phytoextract

Phytoextract	Testing cultures of microorganisms									
	S. aureus ATCC 6538		*E. coli* ATCC 25922		*B. subtilis* ATCC 6633		*P. aeruginosa* ATCC 9027		*C. albicans* ATCC 885-653	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
G. sanguineum	12.5	25	6.25	12.5	6.25	12.5	12.5	25	50	100

G. sanguineum: Geranium sanguineum, C. albicans: Candida albicans, P. aeruginosa: Pseudomonas aeruginosa, B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

interaction of molecules of extraction solvent with molecules of cellular structures of raw materials. Therefore, its detailed study is very important, to obtain a phytosubstance.

The degree of grinding of *G. sanguineum* herb raw material affected the completeness of the obtaining of biologically active substances out of it. Thus, out of raw materials, 0.5–1.0 mm in size, the biologically active substances were maximally obtained: 16.17 ± 0.96% of tannins, 0.27 ± 0.14% - of flavonoids, 24.43 ± 1.64% - amount of polyphenols, and 2.55 ± 0.23% - amount of hydroxycinnamic acids.

In the study of the nature of the extraction solvent, it was established that the highest number of tannins (18.82 ± 1.11%) and the amount of hydroxycinnamic acids (3.21 ± 0.31%) were obtained by extraction with 50% ethyl alcohol. To obtain an extract with a maximum content of polyphenols (23.95 ± 1.21%), it was necessary to use purified water as an extraction solvent. 0.29 ± 0.21% of flavonoids was obtained by extraction with 30% ethanol.

When extracting *G. sanguineum* herb with 50% ethanol, the most complete extraction of biologically active substances was during a 30 min time. That is, the tannins were obtained in the amount of 19.79 ± 1.12%, polyphenols - 20.58 ± 1.09%, flavonoids - 0.22 ± 0.07%, and hydroxycinnamic acids - 2.58 ± 0.42%. When purified water was used as an extraction solvent, the extraction time was increased up to 45 min. The amount of tannins was 17.14 ± 0.99%, of polyphenols - 16.48 ± 0.89%, flavonoids - 0.25 ± 0.09%, and hydroxycinnamic compounds - 2.20 ± 0.21%. These indices were lower compared to the amounts of biologically active substances obtained by extraction with 50% ethanol.

The study of the ratio between the raw material and the extraction solvent proved that the maximum amount of tannins (19.74 ± 1.19%), flavonoids (2.24 ± 0.22%), polyphenols (16.95 ± 0.99%), and hydroxycinnamic acids (19.74 ± 1.19%) was obtained using *G. sanguineum* herb and 50% ethanol at the ratio of 1:30. Consecutively, when using the purified water as an extraction solvent, the optimal ratio was 1:15 - the maximum amount was obtained; tannins - 17.20 ± 0.72%, flavonoids - 3.74 ± 0.15%, polyphenols - 24.33±1.27%, and the amount of hydroxycinnamic acids was 17.20 ± 0.72%.

The obtained dried aqueous as well as aqueous and alcohol extracts were standardized according to the main groups of biologically active substances. A more promising aqueous and alcohol extract was studied for antimicrobial and antifungal effects. It was established that *G. sanguineum* herb extract had antibacterial effect regarding *S. aureus*, *E. coli*, *P. aeruginosa*, and *B. subtilis*. Antifungal effect was less significant.

CONCLUSIONS

Thus, in the study, the optimal conditions for extraction of biologically active substances out of *G. sanguineum* herb have been established. The obtained phytoextracts have been standardized according to the content of main active substances: Tannins, flavonoids, hydroxycinnamic acids, and the amount of polyphenol compounds. Using a serial dilution method, it has been proved that dry *G. sanguineum* extract has an antimicrobial and antifungal effect that is why the development of a medicinal product is promising.

REFERENCES

1. Kozlovskyi MM, Benzel IL, Lozynskyi IM, Benzel LV, Drul OS, Fedoruk VI. Interferoninducing Properties

of Phytosubstance Plants of the Genus Geranium. Medicine-man. Modern Problems of the Creation, Research and Testing of Medicinal Products: Materials of the XXVII Scientific-practical Conference with International Participation. Kharkiv: NFaU; 2009. p. 213-6.
2. Leucuta S, Vlase L, Gocan S, Radu L, Fodorea C. Determination of phenolic compounds from *Geranium sanguineum* by HPLC. J Liq Chromatogr Relat Technol 2005;28:3109-17.
3. Hammami I, Triki MA, Rebai A. Chemical compositions, antibacterial and antioxidant activities of essential oil and various extracts of *Geranium sanguineum* L. Flowers. Arch Appl Sci Res 2011;3:135-44.
4. Penkov D, Kassarova M, Andonova V, Kostadinov I, Delev D, Georgieva M, *et al*. Study on anti-inflammatory and analgesic effects of total extract of *Geranium sanguineum*, *Astragalus glycyphyllos, Erodium cicutarium* and *Vincetoxinum officinalis*. Sci Technol 2014;4:50-4.
5. Khasnabis J, Rai C, Roy A. Determination of tannin content by titrimetric method from different types of tea. J Chem Pharm Res 2015;7:238-41.
6. Borodina NV, Kovalov VM. Quantitative determination of phenolic compounds *Populus tremula* L. Pharmacom 2001;1:1-4.
7. Korniievska VH, Fursa MS, Yu IK. Study of the content of hydroxycinnamic acids of *Valeriana stolonifera* during the day. Pharm Bull 2001;2:19-21.
8. Lomboeva SS, Tanhaeva LM, Olenikov DN. Method of quantitative determination of the total content of flavonoids in the above-ground part of the orthilia of one-sided (*Orthilia secunda* L.) House). Chem Plant Raw Mater 2008;2:65-8.
9. State Pharmacopoeia of Ukraine/SE. Scientific Expert Pharmacopeial Center. 1st ed. Kiev: SE Scientific Expert PharmacopeialCenter; 2004. p. 492.

Source of Support: Nil. **Conflict of Interest:** None declared.