
INVESTIGATION OF ANTIMICROBIAL ACTIVITY OF GEUM URBANUM TINCTURE

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Abstract: The task of modern phytotherapy is to find promising medicinal plants in order to create new herbal medicines. Obviously, herbal medicinal products, to some extent, can be considered as an alternative to antibiotics, while some forms of bacterial infections caused by multiresistant strains of gram positive and gram negative bacteria resistant to other classes of antimicrobial agents and chemicals used to treat a variety of dosage forms. Among the biologically active substances /BAS/ that accumulate in plants and show pharmacological activity, tannins occupy an important place. Such plants include Geum urbanum L. The study of antimicrobial activity of the phytopreparation - tinctures of rhizomes and roots of Geum urbanum L. is an urgent task in terms of prospects for use in medical practice. The goal is to study the effect of tincture of rhizomes and roots of Geum urbanum L. on antimicrobial activity against museum strains of Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica serovar abony. The object of the study was a tincture made from subterranean organs - rhizomes and roots of Geum urbanum L., collected during fruiting in the vicinity of the city of Burgas (Bulgaria). Obtaining tincture was carried out by maceration extraction according to European Pharmacopoeia. To study the antimicrobial activity undiluted tincture and tincture diluted with water in a ratio of tincture-water 1:4, 1:8 and 1:16 were used. Museum strains of microorganisms, as well as standard nutrient media, on which the wells were applied tinctures in certain dilutions. A solvent of 40% ethanol was added to the control wells. The antimicrobial activity of the objects was evaluated by measuring the diameters of the growth retardation zones in Petri dishes. The study of tinctures for antimicrobial activity was performed by diffusion into agar by suppression in the cultivation of bacteria on meat-peptone agar (MPA), followed by incubation in a thermostat at 37° C for 24 hours. Results of obtaining tincture of rhizomes and roots of Geum urbanum L. inhibits the growth of both gram-positive - Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, and gram-negative bacteria - Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica serovar abony. The best results in inhibiting the growth of both gram-positive and gram-negative bacteria were recorded in undiluted tincture obtained from Geum urbanum L.. Tincture of rhizomes and roots of Geum urbanum L. has antimicrobial activity and can be used in medical practice for the treatment of diseases caused by these microorganisms.

Keywords: Geum urbanum, phytotherapy, tannins.

1. INTRODUCTION

The task of modern herbal medicine is to search for promising medicinal plants in order to create new herbal remedies. Among biologically active substances (BAS) contained in plants and possessing pharmacological activity, tannins (tannins) occupy an important place. Given their widespread use in medicine, there is a need to study tannid-containing plants, as well as to obtain new phytopreparations on their basis with their further use in medical practice. These plants include Geum urbanum L.

The purpose of the work is a pharmacognostic study of the roots and rhizomes of Geum urbanum L., including the study of the features of the morphological and anatomical diagnostic signs of medicinal plant raw materials, the phytochemical study of raw materials, as well as the production of a phytopreparation in the form of a tincture, its standardization and the study of antimicrobial activity. To achieve this goal, it was necessary to solve the following tasks:

- to conduct a patent information search of literary sources regarding the chemical composition, pharmacological properties, the use of Geum urbanum L. in folk and official medicine, dosage forms;
- to investigate the features of the morphological and anatomical diagnostic signs of the medicinal product of the Geum urbanum L.;
- to carry out a general phytochemical analysis of the aboveground and underground parts of the Geum urbanum;
- to study medicinal plant raw materials for the content of tannins, depending on the phase of its vegetation, thereby to determine the promising tannid-containing medicinal plant material and the period of its harvesting,
- carry out the standardization of the Geum urbanum L. tincture;
- to study the antimicrobial activity of the Geum urbanum L. tincture in regarding museum strains of Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica serovar abony.

2. MATERIALS AND METHODS

The identification of the main groups of biologically active substances of medicinal plant raw materials using qualitative reactions was carried out by conventional methods of the obtained aqueous, hydroalcoholic and hydrochloric acid extracts. The quantitative determination of tannins in medicinal plant raw materials was carried out by the Leventhal titrimetric method (permanganometry). The method consists in titration with a solution of potassium permanganate of water extraction in the presence of an indicator - indigosulfonic acid.

3. RESULTS

Tincture of rhizomes and roots was obtained from freshly harvested crushed plant materials (raw material-extractant ratio 1:10). A 40% ethyl alcohol solution was used as an extractant. The tincture was prepared by maceration. Crushed medicinal plant raw materials passing through a sieve with holes less than 5 mm was poured with an extractant and infused at room temperature for 7 days, stirred periodically, filtered, the remainder of the meal was washed with a clean extractant and the filtrate was brought to a predetermined volume with a clean extractant.

To study antimicrobial activity, the following objects were used: undiluted and diluted tinctures diluted with water in a tincture-water ratio of 1: 4, 1: 8, and 1:16.

To compare the antimicrobial activity of the tincture, an antimicrobial agent from the group of monofluoroquinolones, ciprofloxacin, was used as a reference drug, since it occupies a leading position in terms of the breadth of application in clinical practice and is characterized by a high clinical effect. Ciprofloxacin was added to the wells at a dose of 25 µg. The study of the antistaphylococcal activity of undiluted and diluted tinctures was carried out using the museum gram-positive strain *Staphylococcus aureus*. The study of the antimicrobial activity of diluted and undiluted tinctures was also carried out using some museum gram-positive strains - *Bacillus cereus*, *Bacillus subtilis* and gram-negative strains - *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* serovar abony. A screening study of the antimicrobial activity of tinctures was carried out by diffusion into agar by inhibition during the cultivation of bacteria on meat-peptone agar (MPA) followed by incubation in a thermostat for a day. Wells with a diameter of 8 mm were made in nutrient agar in Petri dishes. After inoculating the agar surface with test cultures of microorganisms and drying the Petri dishes in a thermostat, the calculated number of the objects under study was introduced into the wells. After incubation in a thermostat at a temperature of 37 ° C for 24 hours, using a magnifying glass with an eyepiece micrometer, the diameters of the zones of growth inhibition of microorganisms in Petri dishes around the wells were estimated.

The bactericidal concentrations of the tinctures were studied by the method of serial dilutions in agar. Strains of the studied microorganisms were inoculated on each plate using a replicator stamp. For seeding, we used suspensions of daily test cultures standardized by optical density (from 6 x 10⁸ CFU to 12 x 10⁸ CFU / ml according to the Mc Farland turbidity standard).

Taking into account the results of our research, according to which the largest number of tannins was found during the fruiting period in underground organs - rhizomes and roots, we prepared in laboratory conditions a tincture of rhizomes and roots of *Geum urbanum* L. The tincture was obtained by maceration according to Pharmacopoeia. The ratio of raw materials: extractant (40% ethanol) - 1: 10. Dried raw materials collected during the fruiting period - the roots and rhizomes were crushed, sifted through a sieve with a diameter of 5 mm. The calculated weighed portion of the crushed and sifted raw material was placed into a conical flask with a ground stopper, the calculated volume of 40% ethanol was added, the flask was closed and left in a dark place at room temperature for 7 days with periodic stirring of the mixture during infusion. After 7 days, the resulting tincture was filtered through a paper filter, the remainder of the raw material was washed with a small amount of extractant, re-filtered, both portions of the extraction were combined and, if necessary, brought to the specified volume with 40% ethanol. The standardization of the tincture was carried out according to the following indicators: description, smell, relative density, dry residue, identification and quantification of tannins.

Quality indicators of tincture of rhizomes and roots of *Geum urbanum* L. are presented in Table 1.

Indicator name	Indicators
Description	Transparent solution of dark red color
Odor	Specific aromatic odor
Dry residue,%	1,200
Relative density, g / ml	0,963

Identification (tannins): <ul style="list-style-type: none"> - with 1% gelatin solution - with 1% alcoholic solution of quinine chloride - with 1% solution of iron ammonium alum - with a 10% solution of lead acetate in an acetate acid 	<p>Turbidity appears, disappearing when an excess of 1% gelatin solution is added</p> <p>Amorphous precipitate</p> <p>Dark blue staining (hydrolyzed tannins)</p> <p>White turbidity is formed (a small amount of condensed tannins)</p>
Quantitation: <ul style="list-style-type: none"> - the content of tannins in the tincture, in% - the content of tannins in the tincture in terms of dry residue, in% 	<p>0,309</p> <p>25,72</p>

The results of the screening study of the antimicrobial activity of the *Geum urbanum* L. tinctures performed using gram-positive museum strains: *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus subtilis* are presented in Table 2.

Named bacteria strains	Diameter of growth retardation zones, mm					
	<i>Dilution of the tincture (applied amount in terms of dry residue)</i>				<i>40% ethanol</i>	<i>Ciprofloxacin dose</i>
	<i>Undiluted (1200 mcg)</i>	<i>1:4 (300 mcg)</i>	<i>1:8 (150 mcg)</i>	<i>1:16 (75 mcg)</i>	<i>100 mcg</i>	<i>25 mcg</i>
<i>Staphylococcus aureus</i>	13,5	11,3	9,8	8,3	8,1	25,0
<i>Bacillus cereus</i>	14,2	11,8	9,8	8,2	8,1	22,0
<i>Bacillus subtilis</i>	12,8	10,9	9,7	8,4	8,2	-

The results of a screening study of anti-staphylococcal activity showed that undiluted tincture applied in an amount of 1200 µg (in terms of dry residue): showed the greatest efficiency - the diameter of the growth retardation zone was 13.5 mm.

When diluted with water in a tincture-water ratio of 1: 4, i.e. in the amount of 300 µg (in terms of dry residue): the diameter of the growth inhibition zone decreased to 11.3 mm, and when diluted with water in a tincture-water ratio - 1: 8, i.e. in the amount of 150 µg (in terms of dry residue): the diameter of the growth inhibition zone continued to decrease and amounted to 9.8 mm.

With further dilution of the tincture in the tincture-water ratio of 1:16, i.e. in the amount of 75 µg (in terms of dry residue): there is practically no anti-staphylococcal activity, as in the case of the extractant - 40% ethyl alcohol: the diameters of the growth inhibition zones for the diluted tincture and extractant are 8.3 mm and 8.1 mm, respectively. In the reference preparation, ciprofloxacin, applied at a dose of 25 µg, the diameter of the growth retardation zone was 25.0 mm, which indicates a moderate anti-staphylococcal activity of the tincture.

The data of the screening study of the antimicrobial activity of the *Geum urbanum* L. tincture performed using other gram-positive museum strains: *Bacillus cereus* and *Bacillus subtilis*, presented indicate that undiluted tincture of rhizomes and roots of *Geum urbanum* L. exhibits the most pronounced antimicrobial effect on *Bacillus cereus* and *Bacillus subtilis* in an amount of 1200 µg (in terms of dry residue): the diameters of growth inhibition zones are, respectively, 14.2 mm and 12, 8mm.

When studying the antimicrobial activity of the tincture against the bacterium *Bacillus cereus* in the reference preparation - ciprofloxacin, applied at a dose of 25 µg, the diameter of the growth inhibition zone was 22.0 mm, which indicates a moderate antimicrobial activity of the tincture relative to the museum strain of *Bacillus cereus*.

The results of studying the antimicrobial activity of the Geum urbanum L. tincture relative to gram-negative museum strains: Escherichia coli, Pseudomonas aeruginosa and Salmonella enterica serovar abony are presented in Table 3.

Named bacteria strains	Diameter of growth retardation zones, mm					
	Dilution of the tincture (applied amount in terms of dry residue)				40% ethanol	Ciprofloxacin dose
	Undiluted (1200 mcg)	1:4 (300 mcg)	1:8 (150 mcg)	1:16 (75 mcg)	100 mcg	25 mcg
Escherichia coli	13,7	12,3	9,3	8,3	8,2	24,0
Pseudomonas aeruginosa	12,9	10,8	9,2	8,2	8,1	-
Salmonella enterica serovar abony	13,4	11,2	9,4	8,4	8,2	22,0

Screening data of antimicrobial activity against gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica serovar abony showed that undiluted tincture of rhizomes and roots of Geum urbanum L. applied in an amount of 1200 µg (in terms of dry residue) also exhibits moderate antimicrobial activity: diameters of growth inhibition zones are 13.7 mm, 12.9 mm and 13.4 mm, respectively. In the study of the antimicrobial activity of the tincture against the bacterium Escherichia coli in the reference preparation - ciprofloxacin, applied at a dose of 25 µg, the diameter of the growth inhibition zone was 24.0 mm, which indicates a moderate antimicrobial activity of the tincture relative to the museum strain of Escherichia coli.

When studying the antimicrobial activity of the tincture against the bacterium Salmonella enterica serovar abony in the reference preparation - ciprofloxacin, applied at a dose of 25 µg, the diameter of the growth inhibition zone was 22.0 mm, which also indicates a moderate antimicrobial activity of the tincture relative to the above-mentioned museum strain.

4. DISCUSSIONS

It is emphasized that the minimum antimicrobial activity of tincture of rhizomes and roots of Geum urbanum L. relative to both gram-positive and gram-negative investigated museum strains of bacteria is observed when diluted with water in a tincture-water ratio of 1: 8, i.e. in the amount of 150 µg (in terms of dry residue): the diameters of growth inhibition zones are in the range from 9.2 mm to 9.8 mm. With a further dilution of 1:16, antimicrobial activity is practically absent, as in the case of the extractant - 40% ethyl alcohol.

5. CONCLUSIONS

The most promising and valuable tannid-containing raw material of Geum urbanum L. for harvesting are rhizomes, then roots and leaves collected during the fruiting period. In order to standardize the tincture of rhizomes and roots of Geum urbanum L., a chemical analysis was carried out according to the following indicators: description, odor, relative density, dry residue, identification and quantitative determination. Moderate antimicrobial activity of rhizomes and roots of geum urbanum L. was established, both against gram-positive and gram-negative microorganisms. The results of a screening study of anti-staphylococcal activity showed that undiluted tincture applied in an amount of 1200 µg (in terms of dry residue): showed the greatest efficiency - the diameter of the growth retardation zone was 13.5 mm. In the reference preparation, ciprofloxacin, applied at a dose of 25 µg, the diameter of the growth inhibition zone was 25.0 mm,

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