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Phytochemical Composition of Rosa Gallica and Prospects of Its Use

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Abstract

Natural bioactive compounds have recently become a popular trend in the pharmaceutical, cosmetic, food, and nutraceutical industries due to their beneficial effects on human health. *Rosa* gallica is a rose species widely used in medicine and cosmetics. It exhibits antioxidant, anti-inflammatory and antibacterial activities. The study of *R. gallica* hydrolates – water-soluble, biologically active components that remain in distilled water – appears to be promising. The component composition and properties of the obtained samples of *R. gallica* hydrolate were investigated. Antimicrobial, bifidogenic, and antioxidant activities of *R. gallica* hydrolates were determined. It was found that the samples of *R. gallica* hydrolate contained the highest amount of gallic acid, ellagic acid and the lowest amount of astragalin. It was shown that samples of *R. gallica* hydrolate exhibited enhanced antimicrobial activity against *C. parapsilosis* (inhibition zone was 6.9±2.1 mm) and against *E. coli* (inhibition zone was 7.4±2.2 mm). The results indicate that the obtained samples of *R. gallica* hydrolatum did not have pronounced bifidogenic activity against the test strain *B. adolescentis*; however, they had bifidogenic activity against the test strain *L. casei*. The results revealed that R. gallica hydrolate samples had significant antioxidant activity in terms of DPPH, ABTS, and FRAP (831.67 mg TE g^{-1}, 1825.28 mg TE g^{-1}, and 71.69 mg TE g^{-1}, respectively). It has been demonstrated that the use of hydrolates of plants containing essential oils enables the development of a new line of confectionery products with natural flavors, antioxidants, increased nutritional and biological value, and unique organoleptic properties.

Keywords: *Rosa gallica*; hydrolates; biologically active substances; antimicrobial, bifidogenic, antioxidant activity; rose water

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1. Introduction

The variety of medicinal plants distributed worldwide is remarkable [1]. About 70,000 plant species are reported to have the potential to treat a variety of diseases. According to WHO, 21,000 medicinal plants are used for various medicinal purposes [1-3]. *Rosa gallica* is a species of rose that is commonly used for medicinal, cosmetic, and culinary purposes. *R. gallica* plays an important role as a therapeutic agent for the treatment of certain types of cancer, microbial infections, and diabetes mellitus. It has been reported that *R. gallica* exhibits antioxidant, anti-inflammatory, and antibacterial activities [4]. In the determination of the component composition, the main compounds were compounds such as gallic acid, catechin, quercetin and its derivatives, and rutin [5]. Quercetin and kaempferol glycosides are the most abundant flavonoids in roses among the described compounds [6,7].

The hydrolate (also called hydrosol) is a by-product of the distillation process of plant raw materials in the production of essential oil. During this process, all the fat-soluble and easily digestible plant ingredients form a concentrate in the oil. However, plants also contain water-soluble components that remain in distilled water, forming so-called hydrolates. Compared to decoctions, extractive substances in hydrolates are contained in higher concentration, while hydrolates do not contain salts and colored substances [8]. Although the chemical and biological properties of essential oils have been widely researched, hydrolates are less studied compounds. There is evidence for antibacterial [9], antifungal [10], and antioxidant properties [11,12] of hydrolates of various plants. *R. gallica* hydrolates may also contain small amounts of terpenoids, predominantly included in essential oils [13]. However, in many essential oil producing countries, the use of hydrolates is rejected despite these positive effects.

Data describing mainly pharmacological and botanical properties of *R. gallica* was found in the literature. It should be noted that to date, essential oils have been used primarily for research, while hydrolates have not received much attention [1,12]. In this regard, the study of the methods of producing, the study of the properties and the areas of application of *R. gallica* hydrolates is a relevant task.

This study aimed to investigate the biologically active properties and applications of hydrolate samples from *R. gallica* growing in the Kaliningrad region.

2. Materials and methods

2.1. Objects of research

Hydrolysates from buds of *R. gallica* growing in the Kaliningrad region were used for the study. R. gallica flowers were collected early in the morning (6-8 AM) at the most suitable bud opening phase [14].

2.2. Laboratory and analytical equipment

The research equipment used for the study was: Shimadzu Prominence high performance liquid chromatography (HPLC) with binary pump and diode matrix detector using Zorbax C18 column (4.6*250mm 5µm). Gradient elution with increasing nonpolar solvent from 5 to 90%: eluent A – trifluoroacetic acid solution in bidistilled water, B – acetonitrile. The flow rate is 1 mL/min and the analytical wavelength was 254 nm. (Shimadzu, Kyoto, Japan), syringe filter Acrodisc PSF 0. 45µm (Khimmnd, Russia), Class 2 microbiological safety cabinet "Laminar-S"-1,2 Neoteric (Amedix-engineering, Russia), thermostat (Laboratornoe oborudovanie, Russia), SmartSpec Plus spectrophotometer (Bio-Rad Laboratories, USA), CLARIOstar microplate reader (BMG Labtech GmbH, Germany), Labconco sublimation unit (Labconco, USA), Eppendorf 5810 centrifuge (Eppendorf, Germany).

2.3. Preparation of R. gallica hydrolates

Samples of *R. gallica* hydrolates were produced using a steam distillation unit. For this purpose, 9 g of raw material (buds) and 180 mL of distilled water were loaded into the extractor. The number of loading cycles was 10. The extracts were further subjected to concentration by lyophilic drying to reduce the volume by half. The composition of R. gallica hydrolates was analyzed using HPLC method. The hydrolates were prepared prior to analysis. Samples were centrifuged and filtered through a syringe filter.

2.4. Determination of antimicrobial activity of R. gallica hydrolates

Disc diffusion method was used to study the antimicrobial activity of R. gallica hydrolate samples. Test strains of microorganisms were purchased from the Research Institute of Genetics and Breeding of Industrial Microorganisms "Kurchatov Institute" (Moscow, Russia). The following pure cultures of yeast *Candida albicans* (Y-3108), *Candida parapsilosis* (Y-3612), *Candida glabrata* (Y-2813); bacteria *Escherichia coli* (B-11419), *Pseudomonas aeruginosa* (B-8243), *Bacillus subtilis* (B-10271); dermatophytes *Penicillium rubrum* (F-601), *Aspergillus niger* (F-1064), *Myceliophthora thermophila* (F-244) were used for testing. Pure yeast cultures were grown on Sabouraud nutrient medium (Microlab, Russia), fungi – on Czapek medium (Biocompass-S, Russia), bacteria – on LB agarized nutrient medium. Fluconazole-Vertex (Vertex, Russia) with a concentration of 2 mg/mL (to study antimicrobial activity against molds and yeasts) and penicillin (Synthesis, Russia) with a concentration of 2.5 µg/10 µL (to study antimicrobial activity against bacteria) were used as positive controls. The pure cultures were handled under sterile conditions in a class 2 microbiological safety cabinet "Laminar-S"-1,2 Neoteric" (Amedix-engineering, Russia). The concentration of microbial suspension during the experiment was at least 1.5×10^8 CFU/mL. The disks were 6 mm in diameter and the thickness of the agar layer was 4.0±0.5 mm. For the experiment, the test strains of microorganisms were evenly distributed with a spatula over the surface of the nutrient medium on Petri dishes. Prepared test discs impregnated with samples of *R. Gallica* or antibiotic solution or antifungal agent were inoculated into Petri dishes and placed in a thermostat (Laboratornoe oborudovanie, Russia), where the optimal temperature was ensured. Yeast cultures were grown in medium at 28 °C for 48 h, bacterial cultures were grown at 37 °C for 24 h, and dermatophyte cultures were grown at 25 °C for 48 h.

2.5. Determination of bifidogenic properties of R. gallica hydrolates

The bifidogenic properties were studied using a method based on the measurement of optical density when the test strains were cultured in liquid nutrient medium. Test strains of microorganisms were purchased from the Research Institute of Genetics and Breeding of Industrial Microorganisms "Kurchatov Institute" (Moscow, Russia). Pure cultures of *Bifidobacterium adolescentis* (AC-1245), *Lactobacillus casei* (B-2873) grown on nutrient media Bifidum-Sreda (State Research Center for Applied Microbiology, Russia) and LB (AppliChem, Germany) were used for testing. Additionally, samples of the studied hydrolate were added to the nutrient media in amounts of 0.0%, 12.5%, and 25.0% of the total water content. Nutrient medium without the addition of hydrolates was used as a positive control for comparative evaluation of the bifidogenic properties of the hydrolates. The test strains were handled under sterile conditions in a class 2 microbiological safety cabinet "Laminar-S"-1.2 Neoteric" (Amedix-engineering, Russia). For this purpose, test strains in the amount of 500 µL were placed in glass tubes with liquid nutrient medium, closed with sterile cotton-gauze plug and cultured in a thermostat (Laboratornoe oborudovanie, Russia) at 37 °C for 24 and 48 hours.

2.6. Study of the concentration of test strains

The concentration of test strains of *B. adolescentis* (AC-1245) and *L. casei* (B-2873) was determined by spectrophotometric analysis on a SmartSpec Plus spectrophotometer (Bio-Rad, USA), provided that if the concentration of test strains in the samples was higher than in the nutrient medium without addition of hydrolates, it indicated the presence of bifidogenic activity of the tested substances.

2.7. Determination of antioxidant activity

The antioxidant activity of *R. gallica* hydrolates was determined by DPPH, FRAP and ABTS methods using a CLARIOstar microplate reader (BMG Labtech GmbH, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) solutions of known concentrations were used as standard solution. Assay results are expressed in milligrams of trolox equivalents per gram of dry weight (mg TE g^{-1}). To determine the antioxidant activity of R. gallica hydrolatum by the DPPH method, 20 µL of extract was mixed with 300 µL of freshly prepared 0.1 mM solution of 2.2-diphenyl-1-picrylhydrazyl. The decrease in optical density compared to the control was recorded at 515 nm. When determining the antioxidant activity using the ABTS method, 300 µL of the prepared ABTS-+ cation-radical solution was added to 20 µL of the extract. ABTS radical was prepared by

mixing aliquots of 7.0 mM ABTS solution and 2.45 mM potassium persulfate solution. The solution was incubated in the dark at room temperature for 16 hours. The optical density was measured at 734 nm. The FRAP method was used to determine the reducing power of the extracts by mixing 300 µL of FRAP reagent and 20 µL of the tested hydrolate. The reaction mixture was incubated for 10 minutes at 37°C in the dark. Optical density was measured at 593 nm. FRAP reagent consisted of 0.3 M acetate buffer (pH 3.6), 10 mM solution of 2,4,6-tripyridyl-s-triazine in 40 mM HCl and 20 mM $FeCl_3 \times 6H_2O$ solution in a 10:1:1 ratio.

2.8. Statistical analysis

All experiments and calculations were performed three times. Results were presented as mean ± standard deviation. Standard statistical methods were used to process the obtained data. Data were subjected to analysis of variance (ANOVA) using Statistica 10.0 package (StatSoft Inc., 2007, USA).

3. Results and Discussions

At present, there is no general report on significant rose species with respect to the presence of BASs, their properties, and food applications [14]. In this study, R. gallica hydrolate samples were produced using steam distillation. HPLC analysis detected a number of BASs in each of the hydrolate samples. Table 1 summarizes the identified compounds. The compounds such as gallic acid, ellagic acid, and astragalin, which have not been previously detected in *R. gallica*, were found in our study. The highest amount (Table 1) of gallic acid (4211.0 µg/mL) was contained in the *R. gallica* hydrolate sample. The three derivatives of gallic acid accounted for 340.0 µg/mL, 182.0 µg/mL, and 187.0 µg/mL, respectively. The next most abundant compound found in *R. gallica* hydrolate samples was ellagic acid (3428.0 µg/mL). The 6 ellagic acid derivatives accounted for 161.0 µg/mL, 387.0 µg/mL, 118.0 µg/mL, 21.3 µg/mL, 32.0 µg/mL, and 1025.0 µg/mL, respectively. Astragaline (458.0 µg/mL) with its derivatives (11.0 µg/mL, 56.5 µg/mL, and 10.1 µg/mL) were the least presented in *R. gallica* hydrolate samples. Phytochemical analysis of 10 samples of rose water from Shiraz, Iran, allowed Mahboubi [15] to detect the presence of compounds such as phenylethyl alcohol, geraniol and β-citronellol. A group of researchers [16] found the following compounds in Damask rose hydrolate: cinnamic acid, gallic acid, kaftaric acid, ferulic acid, elagic acid, and 3-o-methyl gallic acid. The main components of the Gallic rose were gallic acid, elagic acid, astragalin, and their derivatives, as in our samples of *R. gallica* hydrolate. It is known that depending on the composition of biologically active substances, plants have a number of biological properties [14]. Therefore, the antimicrobial, bifidogenic and antioxidant properties of the hydrolate samples were investigated in this study. The results of antimicrobial activity of *R. gallica* hydrolates are presented in Table 2. *R. gallica* hydrolate samples (Table 2) showed enhanced antimicrobial activity against yeast *C. parapsilosis* (Y-3612) with the zone of inhibition of 6.9±0.5 mm and against bacteria *E. coli* (B-11419) with the zone of inhibition of 7.4±0.5 mm. The lowest antimicrobial activity (3.7±0.5 mm, 3.4±0.5 mm and 3.8±0.5 mm for *P. rubrum* (F-601), *A. niger* (F-1064), and *M. thermophila* (F-244), respectively) was found against dermatophytes.

In the study [17], no antimicrobial activity was found in the example of rose hybrid hydrolate, which can be explained by the dependence on the season of collection and the place of plant growth, as well as on the extractant and HPLC modes used to obtain the hydrolate. A study [18] investigated the antimicrobial activity of *R. gallica* hydrolates prepared using different extractants. It was found that R. gallica hydrolates had antimicrobial activity against *Listeria monocytogenes*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enteritidis*, in contrast to our study. This fact can be explained by the different content of the main BAS and the peculiarities of the place of growth of *R. gallica*. In addition, this *R. gallica* extract contained six active phenolic compounds: gallic acid, catechin, chlorogenic acid, epicatechin, quercetin-3-O-α-d-(glucopyranoside), and quercetin, which accounted for the higher antioxidant activity of the studied *R. gallica* hydrolates. This study presents an alternative option for the utilization of *R. gallica* hydrolates as readily available sources of natural antioxidants and antimicrobials in food and pharmaceutical industries. *R. gallica* as readily available sources of natural antioxidants and antimicrobial agents in the food and pharmaceutical industries [18]. *R. gallica* hydrolates have been used to treat skin and mucous membrane lesions, digestive problems, eye problems, chronic insomnia, skin and hormonal problems, and neurological disorders [14,19,20]. These products also demonstrated antimicrobial action [21], anti-inflammatory activity [22], and antimutagenic effects on various cells [23], in genotoxic screening [14]. The results of the bifidogenic properties of the hydrolate samples are presented in Tables 3-5. The data in Table 3 show an increased residual cell content of the test strain *B. adolescentis* (AC-1245) without the addition of *R. gallica* hydrolate at 24 h and at 48 h of culturing (8.76×10^7 cells/mL and 9.03×10^7 cells/mL, respectively). When *R. gallica* hydrolate samples were added at 12.5% and 25.0% of the water volume at 24 h and at 48 h of cultivation, the concentration of the test strain *B. adolescentis* (AC-1245) remained essentially unchanged (Table 4). Analyzing the empirical data (Table 5), it was found that when R. gallica hydrolate samples were added at 12.5% of water volume, the concentration of test strain *L. casei* (B-2873) decreased compared to the control, to values of 6.48×10^7 cells/mL at 24 h of cultivation and 6.94×10^7 cells/mL at 48 h of cultivation. When *R. gallica* hydrolate samples were added at 25.0% of water volume, the concentration of the test strain *L. casei* (B-2873) decreased compared to the control, to values of 6.31×10^7 cells/mL at 24 h of cultivation and 6.96×10^7 cells/mL at 48 h of cultivation. The analysis of the table data (Tables 3-5) showed that the obtained samples of R. gallica hydrolatum did not possess pronounced bifidogenic activity against the test strain B. adolescentis (AC-1245), but possessed bifidogenic activity against the test strain L. casei (B-2873). The antioxidant properties of plant extracts can be confirmed using various *in vitro* assays such as radical scavenging 2,2-diphenyl-1-picrylhydrazyl) (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, and FRAP method based on Fe(III) reduction by polyphenols [1]. The values of antioxidant activity are presented in Table 6.

Table 1. HPLC results of *R. gallica* hydrolates

BAS	Output time, min	Concentration, μg/mL
Gallic acid	3.8	4211.0
Gallic acid derivative	5.2	340.0
Gallic acid derivative	9.8	182.0
Gallic acid derivative	10.3	187.0
3,4-dihydroxybenzoic acid (protocatechinic acid)	5.7	16.0
Ellagic acid	18.0	3428.0
Ellagic acid derivative	7.7	161.0
Ellagic acid derivative	12.6	387.0
Ellagic acid derivative	17.0	118.0
Ellagic acid derivative	18.8	21.3
Ellagic acid derivative	23.0	32.0
Ellagic acid derivative	28.3	1025.0
Astragalin	24.7	458.0
Astragalin derivative	23.9	11.0
Astragalin derivative	31.0	56.5
Astragalin derivative	41.0	10.1

Table 2. Antimicrobial activity of *R. gallica* hydrolates

No.	Test strain	Antimicrobial activity, mm
Yeast		
1	*C. albicans* (Y-3108)	3.1±0.5
2	*C. parapsilosis* (Y-3612)	6.9±0.5
3	*C. glabrata* (Y-2813)	2.5±0.5
Bacteria		
4	*E. coli* (B-11419)	7.4±0.5
5	*P. aeruginosa* (B-8243)	2.2±0.5
6	*B. subtilis* (B-10271)	3.2±0.5
Dermatophytes		
7	*P. rubrum* (F-601)	3.7±1.1
8	*A. niger* (F-1064)	3.4±1.0
9	*M. thermophila* (F-244)	3.8±1.1

Table 3. Cell concentration of test strains when grown under standard conditions without the addition of hydrolates

Duration, h	Concentration of cells/mL	
	***B. adolescentis* (AC-1245)**	***L. casei* (B-2873)**
24	8.76×10^7	6.64×10^7
48	9.03×10^7	7.32×10^7

Table 4. Evaluation of bifidogenic properties of hydrolates against pure culture of *B. adolescentis* (AC-1245)

No.	Cultivation duration, h	Concentration of cells/mL
12.5% of hydrolate by volume of water		
1	24	8.82×10^7
2	48	8.97×10^7
25.0% of hydrolate by volume of water		
3	24	8.78×10^7
4	48	8.92×10^7

Table 5. Evaluation of bifidogenic properties of hydrolates against pure culture of *L. casei* (B-2873)

No.	Cultivation duration, h	Concentration of cells/mL
12.5% of hydrolate by volume of water		
1	24	6.48×10^7
2	48	6.94×10^7
25.0% of hydrolate by volume of water		
3	24	6.31×10^7
4	48	6.96×10^7

Table 6. Antioxidant activity of *R. gallica* hydrolate samples

Antioxidant activity, mg TE g^{-1}		
DPPH	**ABTS**	**FRAP**
831.67±24.95	1825.28±57.76	71.69±2.15

The results (Table 6) revealed that *R. gallica* hydrolate samples had significant antioxidant activity in terms of DPPH, ABTS, and FRAP (831.67 mg TE g^{-1}, 1825.28 mg TE g^{-1}, 71.69 mg TE g^{-1}, respectively). A study conducted in Morocco [24] showed that the antioxidant capacity of *R. damascena* hydrolate was 47.07 mg TE g^{-1}, and 100.71 mg TE g^{-1} according to the FRAP test. In addition, *R. damascena* and *R. alba* hydrolates from Bulgaria had antioxidant activity values significantly lower than the activity of *R. gallica* hydrolate samples in our study (32.52 mg TE g^{-1} and 72.72 mg TE g^{-1}, respectively) [25]. Therefore, it can be concluded that the antioxidant activity of rose may vary from species to species. Georgieva et al. (2019) evaluated the phytochemical profile and antioxidant capacity of hydrolates produced by water-steam distillation of *Rosa alba* L. and *Rosa damascena* Mill. The hydrosol compounds were identified using GC-MS system [25]. The results showed that the hydrolates of *R. alba* L. and *R. damascena* Mill were rich in geraniol (42.55 % for *R. alba* L. and 27.25 % for *R. damascena* Mill.) and citronellol (28.70 % in both hydrolates). The antioxidant activity in terms of Fe^{2+} /ascorbic acid inhibition capacity for *R. alba* L. hydrolate at all concentrations tested was higher than that of *R. damascena* Mill. The results suggest that the hydrolates of *R. alba* L. and *R. damascena* Mill. had well-defined antioxidant properties, which can be used as antioxidant dietary supplements to prevent oxidative stress [25]. Puladze et al. (2022) aimed to evaluate the chemical composition and pharmacological properties of *R. gallica* hydrolates grown in Georgia for use in medicine, cosmetology and food industry. Secondary metabolites of R. *gallica* hydrolate were analyzed using HPLC and their antioxidant activity was evaluated *in vitro* and *in vivo* [26].

It was found that the identified BASs (glycyrretinoic acid, hyperoside, caffeic acid, quercetin, and ferulic acid) exhibited dose-dependent antioxidant radical scavenging effects in both ABTS (IC 50 20.0 μg/mL) and DPPH (IC 50 39.0 μg/mL) assays. Antimicrobial activity was observed against *Plasmodium falciparum* strain 3D7 (IC 50 47.2 μg/mL). In *in vivo* experiments, *R. gallica* hydrolates showed marked analgesic (124%) and anti-inflammatory effects (67.58%), and moderate gastroprotective efficacy (28.9%) [27]. At present, however, further research is needed in this direction, focusing on the preliminary isolation of BAS from *R. gallica* hydrolysates with a high degree of purification by anion-exchange chromatography [1], followed by evaluation of their biological activity and areas of application. *R. gallica* hydrolates are the most common ingredients to produce high-

quality rose water, which has potential for use in food, confectionery, culinary, functional and specialized foods, dietary supplements, and as promising antioxidant nutraceuticals [27]. The trend of healthy eating and the desire of consumers to see natural products in the composition of products is very popular nowadays. Manufacturers of food products, including confectionery products, are increasingly giving preference to vegetable raw materials with a higher content of biologically active substances. The use of hydrolates from plants containing essential oils, including R. gallica, makes it possible to create a new range of confectionery products with natural flavors, antioxidants, increased nutritional and biological value and original organoleptic properties [8].

4. Conclusions

As a result of this research, samples of *R. gallica* hydrolate were obtained and their component composition and properties were studied. Antimicrobial, bifidogenic, and antioxidant activities of *R. gallica* hydrolate samples were determined. It was found that high content of gallic acid (4211.0 µg/mL) was detected in *R. gallica* hydrolate samples. The three gallic acid derivatives accounted for 340.0 µg/mL, 182.0 µg/mL, and 187.0 µg/mL. The content of ellagic acid was found to be 3428.0 µg/mL. The lowest amount of astragaline (458.0 µg/mL) was found in *R. gallica* hydrolate samples. *R. gallica* hydrolate samples were shown to have enhanced antimicrobial activity against the yeast *C. parapsilosis* (Y-3612) and the bacteria *E. coli* (B-11419). The results indicate that the obtained samples *of R. gallica* hydrolates did not have pronounced bifidogenic activity against the test strain *B. adolescentis* (AC-1245), but had bifidogenic activity against the test strain *L. casei* (B-2873). The results revealed that *R. gallica* hydrolate samples had significant antioxidant activity according to DPPH, ABTS, and FRAP (831.67 mg TE g^{-1}, 1825.28 mg TE g^{-1}, and 71.69 mg TE g^{-1}, respectively).

Based on the analysis of literature sources, it has been demonstrated that the use of *R. gallica* hydrolates makes it possible to create a new range of food products with natural flavors, antioxidants, with increased nutritional and biological value and original organoleptic properties.

Conflict of interest

The authors declare no conflict of interest.

Author's contribution

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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