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Investigation of the Antimicrobial Activity of Rhaponticum (*Rhaponticum Carthamoides* D.C. Iljin) and Shrubby Cinquefoil (*Potentilla Fruticosa* L.)

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Key words: *antimicrobial activity; rhaponticum; shrubby cinquefoil.*

Summary. *The aim of the study was to determine antimicrobial activity of rhaponticum and shrubby cinquefoil extracts.*

Material and Methods. Ethanol extract from the leaves of rhaponticum (Rhaponticum carthamoides D.C. Iljin) and shrubby cinquefoil (Potentilla fruticosa L.) was produced at the Department of Food Technology, Kaunas University of Technology.

The antimicrobial activity of the viscous extract or rhaponticum and shrubby cinquefoil was evaluated using standard microorganism cultures (bacteria Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 33499, Pseudomonas aeruginosa ATCC 27853, Proteus mirabilis ATCC 12459, Bacillus subtilis ATCC 6633, Bacillus cereus ATCC 8035 and fungi Candida albicans ATCC 60193). The minimum inhibitory concentration (MIC) of the examined preparations was determined.

Results. Both studied preparations – rhaponticum (Rhaponticum carthamoides D.C. Iljin) and shrubby cinquefoil (Potentilla fruticosa L.) – demonstrated similar antimicrobial activity. The highest sensitivity to the studied preparations was observed in microbes with eukaryotic cell structure: Candida albicans, which is a fungus, and a spore-forming prokaryotic bacterium, Bacillus cereus. The highest resistance was observed in Escherichia coli and Klebsiella pneumoniae.

Conclusions. The studied preparations – viscous extracts of rhaponticum and shrubby cinquefoil – are substances with antimicrobial activity against gram-positive (Staphylococcus aureus and Enterococcus faecalis) and gram-negative (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Proteus mirabilis) bacteria, spore-forming bacteria (Bacillus subtilis and Bacillus cereus), and fungi (Candida albicans).

Introduction

Human organism is continuously exposed to a number of stressors that weaken the immune system to a greater or lesser extent and promote immunosuppression. Immunosuppression is a state of the body where humor or cellular immunity is weakened or compromised, resulting in the increased risk of opportunistic infections and complications of various infections. Immunosuppression may be caused either by bacterial or viral infections, or by environmental factors, such as allergens or pesticides. Suppressed immunity may also be secondary to poor nutrition, tumors, physical stress, endogenous immune reactions, or prolonged radiotherapy (1, 2). During the recent years, microorganisms have demonstrated a striking increase in resistance to antibiotics and chemotherapy drugs, while marked immunosuppression resulted in the return of the long-forgotten diseases (such as tuberculosis) and further spread of opportunistic infections secondary to acquired human immunodeficiency. For this reason, stimulation of the immune system is attracting increasing attention. The concept of immunostimulation includes all preventive or therapeutic measures aimed at stimulating the immune system (3, 4). Since specific immunostimulation is applied for the prevention of only some infectious diseases, increasingly more attention is focused on nonspecific immunostimulation. It has been established that nonspecific immunity is stimulated by certain active components, such as polysaccharides, lectins, proteins, and peptides found in plants (5, 6). The

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majority of these substances are not real antigens, but rather antigen mimetics, also called mitogens. This is an antigen-independent stimulation of macrophages, granulocytes, complement and natural killer cells. Since nonspecific antigen-independent stimulants have no effect on immune memory cells, their pharmacological effect rapidly subsides, and thus such medications are administered continuously or at certain intervals.

The comparison of nonspecific vegetal immune system stimulants (including beefsteak plant *Perilla frutescens* L. Britton preparations) with synthetic ones indicates a significant superiority of the former. They are less sensitizing and have milder adverse effects. Differently from synthetic preparations based on certain chemical substances, the majority of natural preparations provide a positive therapeutic effect via synergistic and complementing pharmacological activity of chemical compounds. These compounds affect one or several pathogenic targets related to certain physiological processes (7). According to literature, polysaccharides in purple coneflower activate T-lymphocyte receptors and increase interferon production, while extracts of bigroot geranium (*Geranium macrorrhizum L.*) have antioxidant properties (8, 9).

Rhaponticum (*Rhaponticum carthamoides* D.C.) is a common medicinal plant. It originates from southern Siberia and is widely cultivated in Central and Eastern Europe. In Lithuania, this plant is grown in botany gardens or plant collections. This plant has been found to have pharmacological properties (10, 11). The principal biologically active components of the plant are ecdysteroids and flavonoids. Examination of Rhaponticum (*Rhaponticum carthamoides* D.C. Iljin) extract showed that it contained flavonoids, compounds that are prevalent in the plant kingdom. Rhaponticum leaves accumulate large amounts of flavonoids (up to 2%). Flavonoids not only have the effect characteristic of vitamin P, but also demonstrate antioxidant activity and strengthen the immune system in the presence of infectious diseases (12–14). The plant also contains significant amounts of phenolic acids (15).

The *Potentilla* genus belongs to the Rosaceae family, and shrubby cinquefoil (*Potentilla fruticosa*) is one of the shrubby plants of this family. This is a perennial plant originating from North America. This plant grows across the world, but is most common in the temperate climate zones. Shrubby cinquefoil does not grow naturally in Lithuania, but is common in public and private green places (16). This is not only a decorative plant, but a medicinal one as well. Shrubby cinquefoil has been found to contain large concentrations of flavonols, phenylcarbonic acids, and yeasts. In addition to that, roots of some *Potentilla* species are edible, and leaves of *P. fruticosa* in some places are used as food supplements and in cosmetics. Extracts of this plant inhibit lipid peroxidation. Photochemoluminescence testing showed that extracts of this plant have a potent antioxidant effect (17). The project "Functional Ingredients and Food Supplements of Vegetal Origin for Safety and Quality" revealed extracts of Rhaponticum and shrubby cinquefoil to have immunostimulating characteristics, but we did not find any data on antimicrobial properties of these extracts, which encouraged us to conduct experimental studies. Therefore, the aim of the study was to determine the antimicrobial activity of Rhaponticum and shrubby cinquefoil extracts.

Material and Methods

Ethanol extract from the leaves of Rhaponticum *(Rhaponticum carthamoides* D.C. Iljin*)* and shrubby cinquefoil (*Potentilla fruticosa* L.) was produced at the Department of Food Technology, Kaunas University of Technology. Two samples (20 grams each) of chopped herb were used for the investigation. A volume of 200 mL of ethanol was added, the resulting mixture was then shaken for 2 hours in a mechanical shaker and was subsequently filtered through the Büchner funnel. Another 200 mL of ethanol was added; the mixture was shaken for 2 hours, then filtered, and concentrated with a rotary evaporator at 40°C.

To evaluate the antimicrobial activity of the viscous extracts of shrubby cinquefoil and rhaponticum, microbiological examination was repeated using five samples of the same preparation. The preparation (0.4 g) was used for each testing of the shrubby cinquefoil and rhaponticum extract.

The preparations of shrubby cinquefoil and rhaponticum were weighed, and 0.4 g of viscous extract was dissolved in 10 mL of 96% ethanol – this was the first main solution of the examined preparation (test tube 1). Subsequently 2 mL of the main solution from test tube 1 were poured into test tube 2 containing 8 mL of solution (4 mL of saline + 4 mL of 96% ethanol), thus obtaining the second main preparation solution. A volume of 4 mL of the second main preparation from test tube 2 was poured into test tube 3 containing 4 mL of solution (3 mL of saline + 1 mL of 96% ethanol), thus obtaining the third main preparation solution.

Viscous extracts of rhaponticum and shrubby cinquefoil dissolve well only in 96% ethanol, while resulting in precipitations in aqueous solutions. For this reason, in order to avoid precipitations, sterile saline was complemented with a specific amount of 96% ethanol. In addition to that, to prevent antimicrobial effect of 96% ethanol, solutions were prepared so that ethanol concentration in Petri dishes with Mueller-Hinton agar would be less than 9%.

On preparation of the main solutions of the tested preparations, working solutions of the preparations were prepared (Tables 1 and 2) in 10 mL of Mueller-Hinton agar (Mueller-Hinton Agar, Becton, Dickinson and Company), where the inhibitory effect of the studied preparations (minimum inhibitory concentration, MIC) on the growth of standard microorganisms was evaluated.

Solutions 1–3 were prepared from the solution of the first main preparation, solutions 4–6 were prepared from the solution of the second main preparation, and solutions 7–10 were prepared from the solution of the third main preparation. Subsequently, a sterile inoculation loop was used to introduce the prepared microorganism suspension into a semisolid growth medium – the Mueller-Hinton agar on a Petri dish containing a certain concentration of the studied preparation. Bacterial culture samples were incubated for 24 hours at the temperature of 37°C.

The microbiological investigation was performed under aseptic conditions. The antimicrobial activity of the viscous extract of rhaponticum and shrubby cinquefoil was evaluated using the standard microorganism cultures (bacteria *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 33499, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 12459, *Bacillus subtilis* ATCC 6633, and *Bacillus cereus* ATCC 8035 and fungus *Candida albicans* ATCC 60193).

The standard microorganism cultures and clinical strains of bacteria were grown on commercially available standardized BBL (Becton Dickinson and Company) growth media – Mueller-Hinton agar. The culture samples were incubated for 24 hours. After this period, the microorganism growth was evaluated at the sites of seeding, where a particular microorganism formed a coating.

During the microbiological testing, the MIC was determined. It is the lowest concentration of an antimicrobial, which still inhibits the growth of bacteria and is calculated using the dilution technique. The evaluation was as follows: G, bacterial growth, i.e., a visible cluster of the studied bacteria grew at the site of seeding; N, no growth, i.e., so signs of bacterial growth (i.e., no clusters of the studied bacteria) at the seeding sites. We performed a visual evaluation of the effect of viscous rhaponticum and shrubby cinquefoil extracts of various concentrations on standard microorganism cultures, compared to the control test findings (the growth of standard microorganism cultures on Mueller-Hinton agar without the tested preparation).

Statistical Analysis. Qualitative data analysis was applied, taking into consideration the clusters of the investigated bacteria and the concentration of the studied preparations.

Results

The findings of the microbiological testing on viscous rhaponticum and shrubby cinquefoil extracts are provided in Tables 1 and 2. All the studied microorganisms were sensitive to certain concentrations of the tested preparations.

The examination showed that *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 3349 were most resistant to the antimicrobial effect of rhaponticum (*Rhaponticum carthamoides* D.C. Iljin) and shrubby cinquefoil extracts (growth inhibition at 30-fold dilution); their MIC was 1330 μg/mL. *Bacillus cereus* ATCC 8035 and *Candida albicans* were the most sensitive microorganisms to the effect of the aforementioned extracts (70-fold dilution); their MIC was 9.4 μg/mL. *Staphylococcus aureus* ATCC 25923 (105-fold dilution; MIC, 15.2 μg/mL), *Enterococcus faecalis* ATCC 29212 (70-fold dilution; MIC, 570 μg/mL), *Pseudomonas aeruginosa* ATCC 27853 (70-fold dilution; MIC, 570 μg/mL), and *Bacillus subtilis* ATCC 6633 (70-fold dilution; MIC, 570 μg/mL) showed a moderate sensitivity to the viscous extracts.

Discussion

The findings of the study showed that both preparations – rhaponticum (*Rhaponticum carthamoides* D.C. Iljin) and shrubby cinquefoil (*Potentilla fruticosa* L.) – demonstrated similar antimicrobial activity. The extracts were effective against gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*) bacteria, as well as against spore-forming bacteria (*Bacillus subtilis*, *Bacillus cereus*) and fungi (*Candida albicans*).

The roots of the studied plant – rhaponticum – were found to contain up to 18% of polysaccharides. Polysaccharides strengthen the immune system and elevate serum levels of IL-2 and IFN-γ (11, 17).

Shrubby cinquefoil contains large amounts of flavonols, phenylcarbonic acids, yeasts, and vitamin C. Shrubby cinquefoil preparations, as antioxidants, stimulate a number of bodily functions, supply the organism with natural vitamins, and inhibit the virulence of flu viruses. Shrubby cinquefoil extracts were also found to have an antimicrobial effect against *Helicobacter pylori* (18).

Studies have shown that the aqueous extract of *Potentilla fruticosa* contains high concentrations of polyphenols, tannins, phenolic acids, and flavonoids (quercetin) and has an antimicrobial effect. The extract suppresses in vitro growth of caries-causing *Streptococcus mutans* (19).

It has been found that the antimicrobial effect of rhaponticum and shrubby cinquefoil extract on clean standard microorganism cultures depends on the certain characteristics of the standard microorganisms.

Table 1. Antimicrobial Activity of the Preparation of Viscous Rhaponticum Extract

Dilution of the Preparation of Viscous Rhaponticum Extract	Standard Microorganism Culture, No.								
	1	2	3	4	5	6	7	8	9
	Staphylococcus aureus ATCC 25923	*Enterococcus faecalis* ATCC 29212	*Escherichia coli* ATCC 25922	*Klebsiella pneumoniae* ATCC 33499	*Pseudomonas aeruginosa* ATCC 27853	*Proteus mirabilis* ATCC 12459	*Bacillus subtilis* ATCC 6623	*Bacillus cereus* ATCC 8035	*Candida albicans* ATCC 60193
Dilution 0 (main, 5-fold) 0.008 g/mL	N	N	N	N	N	N	N	N	N
Dilution 1 (30-fold) 1330 μg/mL	N	N	MIC	MIC	N	N	N	N	N
Dilution 2 (55-fold) 730 μg/mL	N	N	G	G	N	MIC	N	N	N
Dilution 3 (70-fold) 570 μg/mL	N	MIC	G	G	MIC	G	MIC	N	N
Dilution 4 (105-fold) 15.2 μg/mL	MIC	G	G	G	G	G	G	N	N
Dilution 5 (130-fold) 12.3 μg/mL	G	G	G	G	G	G	G	N	N
Dilution 6 (170-fold) 9.4 μg/mL	G	G	G	G	G	G	G	MIC	MIC
Dilution 7 (255-fold) 3.1 μg/mL	G	G	G	G	G	G	G	G	G

G, growth; N, no growth; MIC, minimum inhibitory concentration.

Table 2. Antimicrobial Activity of the Preparation of Viscous Shrubby Cinquefoil Extract

Dilution of the Preparation of Viscous Shrubby Cinquefoil Extract	Standard Microorganism Culture, No.								
	1	2	3	4	5	6	7	8	9
	Staphylococcus aureus ATCC 25923	*Enterococcus faecalis* ATCC 29212	*Escherichia coli* ATCC 25922	*Klebsiella pneumoniae* ATCC 33499	*Pseudomonas aeruginosa* ATCC 27853	*Proteus mirabilis* ATCC 12459	*Bacillus subtilis* ATCC 6623	*Bacillus cereus* ATCC 8035	*Candida albicans* ATCC 60193
Dilution 0 (main, 5-fold) 0.008 g/mL	N	N	N	N	N	N	N	N	N
Dilution 1 (30-fold) 1330 μg/mL	N	N	MIC	MIC	N	N	N	N	N
Dilution 2 (55-fold) 730 μg/mL	N	N	G	G	N	MIC	N	N	N
Dilution 3 (70-fold) 570 μg/mL	N	MIC	G	G	MIC	G	MIC	N	N
Dilution 4 (105-fold) 15.2 μg/mL	MIC	G	G	G	G	G	G	N	N
Dilution 5 (130-fold) 12.3 μg/mL	G	G	G	G	G	G	G	N	N
Dilution 6 (170-fold) 9.4 μg/mL	G	G	G	G	G	G	G	MIC	MIC
Dilution 7 (255-fold) 3.1 μg/mL	G	G	G	G	G	G	G	G	G

G, growth; N, no growth; MIC, minimum inhibitory concentration.

The highest sensitivity to the studied preparations was observed in microbes with eukaryotic cell structure: *Candida albicans*, which is a fungus, and a spore-forming prokaryotic bacterium, *Bacillus cereus*.

The highest resistance was observed in *Escherichia coli* and *Klebsiella pneumoniae*. *Klebsiella pneumoniae* forms a capsule that most probably prevents antimicrobial substances of the preparation from entering the cell.

According to the literature data, standard rha-

ponticum root extract exhibits antimicrobial activity against *Bacillus cereus*, *Bacillus subtilis*, *Bacteroides fragilis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* (20). The results of the studies are similar, although our tested extracts were produced from plants grown in the Kaunas Botany Garden, Vytautas Magnus University, and thus differed in their biological properties and production technique.

Purple coneflower (*Echinacea purpurea*) and beefsteak plant (*Perilla frutescens*) are studied and used as immunostimulants. Purple coneflower contains biologically active compounds that have an antibacterial effect. Studies on bacteriostatic properties of purple coneflower extracts conducted by Jurkštienė et al. (1998) and Gorchen (2003) showed that the best effect was achieved against S*taphylococcus aureus* and *Escherichia coli*. Antibacterial properties of coneflower are related to echinacoside whose effect equals to that of penicillin. Schar (1999) indicated that derivatives of caffeic acid also inhibited *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Tannins and flavonoids – the components of bigroot geranium (*Geranium macrorhizum*) – in aqueous and alcoholic extracts in vitro inhibit the growth of some gram-negative microorganisms, such as *Klebsiella pneumonia*, *Proteus vulgaris*, *Escherichia coli*, and *Pseudomonas aeruginosa*, gram-positive microorganisms, such as *Staphylococcus aureus*, and fungi, such as *Candida albicans*. Geranium extracts increased survival in mice with pneumonia caused by *K. pneumoniae* (9).

Literature data as well as experimental and clinical studies have shown that beefsteak plant extracts have not only immunostimulant, but also a direct antimicrobial effect. An aqueous infusion of beefsteak plant leaves has detoxifying and disinfecting properties, i.e., it exhibits a direct bacteriostatic effect. Later it has been specified that this infusion is effective against *Staphylococcus aureus*, but ineffective against gram-negative bacteria. It has been found that the volatile fraction of beefsteak plant leaf extracts has a strong inhibitory effect on the growth of skin fungi (*Trichophyton*, *Microsporum*, *Epidermophyton*), thus stimulating the immune system, but is ineffective against *Saccharomyces* or *Candida* genera (21).

Conclusions

The studied preparations – viscous extracts of rhaponticum and shrubby cinquefoil – are substances with quite strong wide-spectrum antimicrobial activity against gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*) bacteria, spore-forming bacteria (*Bacillus subtilis* and *Bacillus cereus*), and fungi (*Candida albicans*). Microorganisms with eukaryotic cell structure – fungi *Candida albicans* – were most sensitive to the studied preparations, compared to bacteria with prokaryotic cell structure.

Statement of Conflict of Interest

The authors state no conflict of interest.

Paprastojo rapontiko (*Rhaponticum carthamoides* D.C. Iljin) ir krūminės sidabražolės (*Potentilla fruticosa* L.) antimikrobinio aktyvumo tyrimas

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Raktažodžiai: paprastasis rapontikas, krūminė sidabražolė, antimikrobinis aktyvumas.

Santrauka. *Tyrimo tikslas.* Įvertinti paprastojo rapontiko ir krūminės sidabražolės ekstraktų antimikrobinį aktyvumą.

Tyrimo medžiaga ir metodai. Paprastojo rapontiko (*Rhaponticum carthamoides* D.C. Iljin) ir krūminės sidabražolės (*Potentilla fruticosa* L.)) lapų etanolinis ekstraktas pagamintas Kauno technologijos universiteto Maisto technologijos katedroje.

Nustatytas tirštųjų ekstraktų: paprastojo rapontiko ir krūminės sidabražolės antimikrobinis aktyvumas su etaloninėmis bakterijų: *Staphylococcus aureus ATCC25923, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Klebsiella pneumoniaeATCC 33499, Pseudomonas aeruginosa ATCC 27853, Proteus mirabilis ATCC 12459, Bacillus subtilis ATCC 6633, Bacillus cereus ATCC 8035 ir grybų – Candida albicans ATCC 60193* kultūromis. Nustatyta tirtųjų preparatų MSK (minimali slopinamoji koncentracija).

Rezultatai. Paprastasis rapontikas *(Rhaponticum carthamoides* D.C. Iljin) ir krūminė sidabražolė (*Potentilla*

fruticosa L.) yra antimikrobiškai panašaus aktyvumo. Tirtiems preparatams jautriausi yra eukariotinę ląstelės struktūrą turintys mikrobai – *Candida albicans*, kuri priklauso grybams, taip pat sporinė prokariotinė bakterija *Bacillus cereus*. Atspariausia yra *Escherichia coli* ir *Klebsiella pneumoniae*.

Išvados. Tirti preparatai tirštasis paprastojo rapontiko ekstraktas ir tirštasis krūminės sidabražolės ekstraktas veikia gramteigiamas (*Staphylococcus aureus, Enterococcus faecalis*) ir gramneigiamas (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Proteus mirabilis*) bakterijas, sporines bakterijas (*Bacillus subtilis*, *Bacillus cereus*) ir grybus (*Candida albicans*).

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