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ANTIMICROBIAL ACTIVITY EVALUATION OF LYOPHILIZED JUICE AND WASTE EXTRACT OF RED CURRANT VARIETY REDPOLL

Maja Cvetković[1], Tatjana Mihajilov Krstev[2], Katarina Šavikin[3], Gordana Zdunić[3], Dušanka Kitić[1], Dušica Stojanović[1], Milica Ranđelović[1], Milica Milutinović[1], Dušan Ilić[1], Bojana Miladinović[1]

Berries include a large number of species that are known for their nutritional and health benefits. Several studies have found that red currant has different biological properties, such as antiproliferative, anticancer, antimicrobial, anti-inflammatory, antidiabetic, and antioxidant. The aim of this study was to investigate the antimicrobial activity of lyophilized fruit juice (RPJL) and waste extract (RPWL) obtained from red currant (*Ribes rubrum* L.) variety Redpoll on different Gram-positive (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Enterococcus faecalis) and Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Proteus mirabilis, Enterobacter aerogenes) and one yeast (Candida albicans*). The activity of dominant active compounds (ascorbic acid, quercetin and kaempferol) contained in red currants under the same conditions were also examined to determine their responsibility in the antimicrobial effect. Tested RPJL and RPWL showed moderate antimicrobial activity. The minimum inhibitory (MIC) and microbicidal concentrations (MBC/MFC) of RPJL and RPWL were 100 mg/ml and more than 100 mg/ml. RPJL and RPWL have the same effect on Gram (+) bacteria and the same MIC and MBC value. RPWL showed stronger antimicrobial effects on Gram (-) bacteria while the juice did not inhibit the growth of the Gram (-) bacteria at all. Standard solutions of ascorbic acid and quercetin showed strong inhibitory and microbicidal activity at lower concentrations than tested samples with the MIC/MBC (MFC) =2.5−10 mg/ml. Results showed that red currants could have potential applications as natural antimicrobial agents. *Acta Medica Medianae 2023;62(2): 38-44.*

Key words: *red currants, Redpoll, antimicrobial activity, berries, preservatives.*

[1]University of Niš, Faculty of Medicine, Department of Pharmacy, Niš, Serbia
[2]University of Niš, Faculty of Science and Mathematics, Department of Biology and Ecology, Niš, Serbia
[3]Dr Josif Pančić Institute of Medicinal Research, Belgrade, Serbia

Contact: Bojana Miladinović
81 Zorana Đinđica Blvd., 18000 Niš, Serbia
bojana.miladinovic@medfak.ni.ac.rs
Tel. number: 069/1045097

Introduction

Berries include a large number of species that are known for their nutritional and health benefits. The unique color, fragrance, taste and nutritive content make these fruits valuable food sources. The most commonly investigated berries are strawberry, blueberry, raspberry, black and red currants (1).

The name “currant” was first mentioned in the ancient Greek city of Corinth and was initially used to describe small dried grapes from that region, while the origin of red currant (*Ribes rubrum* L.) comes from Western Europe (2, 3). Red currants belong to the genus Ribes which is classified in the family of Grossulariaceae according to the new taxonomy. It previously belonged to the genus of Saxifragaceae. The genus Ribes includes more than 150 species that are native to northern Europe, Asia, North and South America, and northwestern Africa (4). Currant fruits are berry-shaped, with the seeds enclosed in a fleshy pericarp. The leaves are divided into three to five lobes. Yellow-green flowers appear in April or May, they are arranged on a branch 4−8 cm high, developing three to ten red berries. The berries are born in clusters, which are attached to the branch by short stalks. The fruits nearest to the branch are the largest ones and ripen earlier, while the distal ones are smaller and ripen later. *Ribes rubrum* berries contain a flat pentagonal disk at the top, left over from the sepal. These pentagonal discs are important for the identification of archaeobotanical remains in frozen plant assemblies, as well as in differentiation from black currant (4).

Commercial cultivation of red currant began in the eighties of the XX century on the territory of western Serbia, where a very small part of the plantations are still used today (4).

The chemical composition of red currants vary depending on the variety, cultivation, location, stage of maturity, harvest and storage conditions (5, 6).

A wide range of nutrients (carbohydrates, vitamins, minerals, organic acids), as well as antioxidant components (polyphenols and vitamin C) make red currant important and useful plant species (7, 8). The primary metabolites of plants are sugars and organic acids, which determine the taste of the fruit (7). Secondary metabolism follows up the primary one and represents the transformation and catabolism of the resulting final products of primary metabolism (9). The dominant group of secondary metabolites in currants is polyphenols and their three most common classes are flavonoids, tannins and phenolic acids. Anthocyanins (delphinidin- and cyanidin-3-*O*-glucoside), flavanols (catechin, epicatechin), flavonols (quercetin, kaempferol, myricetin), condensed tannins (proanthocyanidins), hydrolyzing tannins (ellagitannins and gallotannins) and phenolic acids (hydroxybenzoic acid and hydroxycinnamic acid derivatives) were found in red currants (6, 10-13).

According to the literature, numerous health benefits of red currants come from phenolic components. Several studies have found that they have various biological effects, such as antiproliferative, anticancer, antimicrobial, anti-inflammatory, antidiabetic, and antioxidant (14-17). Berries rich in phenolics also exhibit antimicrobial action against pathogenic bacteria (18). The antimicrobial effect of polyphenolic compounds has been demonstrated in the research of Gatto et al. (2002) and De Pascual-Teresa (2008) (19, 20). These active compounds inhibit the growth of microorganisms, but do not express microbicidal effect (21). *Salmonella, Staphylococcus, Helicobacter,* and *Bacillus* species are the most sensitive bacteria for the berry phenolics (22). In addition, the growth of *Escherichia, Clostridium* and *Campylobacter* species, but not *Lactobacillus* and *Listeria* species, is inhibited by berry phenolics (23). Liegiūtė (2006) showed that sour cherry extracts can suppress the growth of both Gram (+) and Gram (-) bacteria (24). Cranberry juice suppresses the adhesion behavior of *Escherichia coli* and also inhibits the adhesive properties of oral streptococci, thus weakening biofilm formation and mouth colonization (25, 26). The ability of *Helicobacter pylori* to colonize mucous membranes is also inhibited by cranberry extracts (27). While red currant has found its place in the food industry in the preparation of jams, juices, wine, teas and sweets, in medicine and pharmacy could be used to treat and prevent some diseases due to the high content of bioactive components (28-30).

World Health Organization revealed that 1 in 10 people become ill eating contaminated food and 420,000 die every year. Children under 5 are more affected, with 125,000 deaths every year. Food can be contaminated at any point of production and distribution, but also if improperly prepared or mishandled at home. *Salmonella, Campylobacter,* and *Escherichia coli* are among the most common foodborne pathogens that can cause most severe and fatal outcomes on millions people per year. Symptoms usually include fever, headache, nausea, vomiting, abdominal pain and diarrhea. Outbreaks of salmonellosis involve contaminated eggs, poultry and other animal products. Raw milk, raw or undercooked poultry and contaminated drinking water are the main causes of *Campylobacter* foodborne cases. Unpasteurized milk, undercooked meat and fresh fruits and vegetables can be contaminated with enterohaemorrhagic *Escherichia coli* (31).

The aim of this study was to investigate the antimicrobial activity of lyophilized fruit juice (RPJL—Redpoll juice lyophilizate) and waste (RPWL—Redpoll waste lyophilizate) obtained from red currant (*Ribes rubrum* L.) variety Redpoll on different Gram-positive and Gram-negative bacteria and yeast (*Candida albicans*). The second aim was to examine the activity of standard compounds that are found in red currants under the same conditions and to determine their responsibility in the antimicrobial effect. The results could be useful for the food industry where red currants extracts could be used as potential natural preservers.

Material and methods

Plant material and sample preparation

Red currant variety Redpoll was grown and collected from Radmilovac, experimental field of the Faculty of Agriculture, University of Belgrade. Fully ripe berries were harvested from the end of June to the beginning of July of 2020. After picking, the fruits were thawed and pressed in a special press for squeezing fruit, obtaining the juice for further analysis. The residue after straining (waste) was dried on filter paper for one day, after which it was dried in a laboratory dryer at 40^{o}C for 48 hours. The residue left after drying was grinded in a mill. The maceration method was used for extraction of the plant material. In the maceration process, 60% ethanol was used, with a material to extragens ratio of 1:20. The sample was extracted on a laboratory shaker for 60 min at room temperature. After extraction, the ethanol was evaporated using a rotary evaporator.

Antimicrobial activity evaluation

The antimicrobial activity of RPJL and RPWL was assessed using laboratory control strains obtained from the American Type Culture

Collection (ATCC). Gram (+) bacteria used in this assay were *Bacillus cereus* ATCC 10876, *Listeria monocytogenes* ATCC 15313, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 19433. Gram (−) bacteria were represented by *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* ATCC 13076, *Proteus mirabilis* ATCC 12453, and *Enterobacter aerogenes* ATCC 13048. *Candida albicans* ATCC 15313 was used for assessing antifungal activity.

The bacterial and fungal inocula were made from overnight broth cultures. The suspensions with the microorganisms were adjusted to 0.5 McFarland standard turbidity (which corresponds to 107−108 colony forming units (CFU/ml) for bacteria depending on genera and 0.4×105 to 5×105 spore/ml for fungal strains (consensus standard by the National Committee for Clinical Laboratory Standards (NCCLS)) (32). The determination of antimicrobial and antifungal activity (the minimum inhibitory concentration [MIC] and minimum bactericidal/fungicidal concentration [MBC/MFC]) was carried out by microwell dilution method, according to the NCCLS (2003). Tested samples were dissolved in 10% DMSO. The final concentrations of ascorbic acid, quercetin and kaempferol were 20, 10 and 5 mg/ml in 10% DMSO. Serial doubling dilutions of the tested samples and standards were prepared in the 96-well microtiter plates in inoculated nutrient broth. The final volume was 100 µl and the final bacterial concentration was 2×106 CFU/ml in each well and 2×105 of spores for fungal strains. The plates were incubated for 24h at 37oC for bacteria and 48 h at 25ºC for fungi. Microbial growth was determined by adding 20 µl of 0.5% triphenyltetrazolium chloride aqueous solution (33). The minimal concentration where there was no visible growth was defined as the minimal inhibitory concentration (MIC). For MBC/MFC determination, the broth was taken from each well and inoculated into Mueller Hinton agar at 37ºC for 24h for bacteria, or in malt extract agar at 25ºC for 48h for fungal strains. The minimal bactericidal/fungicidal concentration (MBC/MFC) was defined as the lowest concentration of the sample that had killed 99.9% of microorganism cells (32). Doxycycline and nystatin were used as the controls.

Results and Discussion

The antimicrobial activity of tested samples and standards against selected microorganisms is shown in Table 1. Tested RPJL and RPWL showed moderate antimicrobial activity. In this study, red currant RPJL did not inhibit Gram (-) bacteria (*E. coli, P. aeruginosa S. enteritidis, P. mirabilis*, and *E. aerogenes*), while was effective on all Gram (+) bacteria (*B. cereus, S. aureus, E. faecalis*), except *L. monocytogenes*. As other researchers have determined when it comes to the antibacterial effects of herbal extracts, the effect on Gram (-) bacteria is significantly lower compared to Gram (+) ones (34). Red currant RPWL inhibited activity of both Gram (+) and Gram (-) bacteria, except *E. coli*. The minimum inhibitory and microbicidal concentrations, MIC/MBC (MFC) of RPJL and RPWL were 100 mg/ml and more than 100 mg/ml. RPWL shown stronger antimicrobial effects compared to RPJL, especially on Gram (-) bacteria.

The significance of the bacteriostatic activity of RPJL and RPWL against *B. cereus* is that they can stop this causative agent of alimentary infections accompanied by nausea and vomiting (35). Inhibitory activity of RPWL against *L. monocytogenes* may be helpful in preventing listeriosis, which is also associated with food contamination (36). RPJL didn’t have any effect on the *L. monocytogenes*. In this study, neither RPWL nor RPJL showed activity against *E. coli*, which is responsible for urogenital tract infections, meningitis, pneumonia and sepsis (37).

In this research, standard solutions of ascorbic acid and quercetin showed strong inhibitory and microbicidal activity at lower concentrations than tested samples. The MIC/MBC (MFC) of standards were 2.5−>10 mg/ml. Kaempferol showed no activity, except on *S. aureus*, where MIC/MBC were 2.5/>2.5. There are literature data showing results of kaempferol esters antimicrobial activity on four bacteria (*E. faecalis, S. aureus, E. coli, P. aeruginosa*). MIC values varied from 23−250 µg/ml (38). The difference between the results can be attributed to a different type of sample as well as the different methoxylation patterns. Kaempferol with free -OH groups (without any methoxyl substitution) was the most active.

Values of MIC/MBC for ascorbic acid in our study were 2.5−5/>10.0 mg/ml. One study reported that application of low concentration of vitamin C (0.15 mg/mL) inhibited the growth of *S. aureus* and *E. faecalis* (39). *C. albicans* was more sensitive to the ascorbic acid (10/>10 mg/ml) then to RPJL and RPWL. Quercetin and kaempferol did not inhibit the growth of *C. albicans*.

The literature data on the antibacterial activity of lyophilized juices and waste are scarce, while the results on the effect of extracts of red currants are limited. Data on antimicrobial and antifungal activities of red currants are different. There is a study that examined the inhibition of the growth of Gram (+) and Gram (-) bacteria by the action of juice of different fruits. They showed that red currant juice had antibacterial effect on most of the oral bacterial species tested (*S. gordonii, S. sobrinus, F. nucleatum, A. actinomycetemcomitans, Pseudomonas gingivalis, E. faecalis*), with none, or only very limited cytotoxic effects on human gingival fibroblasts (40). Also, another study shown that extracts with low or medium total phenolics and low anthocyanins were the strongest suppressors of *S. aureus* and *Lactococcus lactis* subsp. *Lactis* (41).

Table 1. Antimicrobial activity of lyophilisates of Redpoll juice (RPJL) and waste (RPWL) and standard substances (ascorbic acid, quercetin and kaempferol) against pathogenic microorganisms (MIC/MBC in mg/mL)

EXTRACTS		RPJL	RPWL	Ascorbic acid	Quercetin	Kaempferol	Doxycycline	Nystatin
Bacterial strains	**Source**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (μg /ml)**	
Gram (+)	**ATCC**							
Staphylococcus aureus	6538	100.0/>100.0	100.0/>100.0	2.5/>10.0	2.5/>5.0	2.5/>2.5	7.81/15.61	
Enterococcus faecalis	19433	100.0/>100.0	100.0/>100.0	2.5/>10.0	2.5/>5.0	nd	0.90/1.90	
Bacillus cereus	10876	100.0/>100.0	100.0/>100.0	2.5/>10.0	5.0/>5.0	nd	0.90/15.61	
Listeria monocytogenes	15313	nd	100.0/>100.0	2.5/>10.0	nd	nd	7.81/15.61	
Gram (-)	**ATCC**							
Pseudomonas aeruginosa	27853	nd	100.0/>100.0	2.5/>10.0	5.0/>5.0	nd	15.61/15.61	
Escherichia coli	25922	nd	nd	5.0/>10.0	5.0/>5.0	nd	15.61/15.61	
Enterobacter aerogenes	13048	nd	100.0/>100.0	5.0/>10.0	5.0/>5.0	nd	7.81/15.61	
Salmonella enteritidis	13076	nd	100.0/>100.0	2.5/>10.0	5.0/>5.0	nd	0.90/1.90	
Proteus mirabilis	12453	nd	100.0/>100.0	5.0/>10.0	5.0/>5.0	nd	7.81/15.61	
Fungal strain	**Source**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (μg/ml)**
Yeast	**ATCC**							
Candida abicans	15313	100.0/>100.0	100.0/>100.0	10.0/>10.0	nd	nd	nd	16.0/16.0

Conclusion

We can conclude that RPJL and RPWL have the same effect on Gram (+) bacteria and the same MIC and MBC values. The difference between these two forms is that the juice does not inhibit the activity of Gram (-) bacteria. Standard solutions of ascorbic acid and quercetin showed strong inhibitory and microbicidal activity.

Based on these results, red currants could have important applications as natural antimicrobial agents. Lyophilized juice and waste of red currant (*Ribes rubrum* L.) variety Redpoll might be used as a potential preservative in the food industry.

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Procena antimikrobne aktivnosti liofilizata soka i ekstrakta ostatka ploda crvene ribizle sorte *Redpoll*

Maja Cvetković[1], Tatjana Mihajilov Krstev[2], Katarina Šavikin[3], Gordana Zdunić[3], Dušanka Kitić[1], Dušica Stojanović[1], Milica Ranđelović[1], Milica Milutinović[1], Dušan Ilić[1], Bojana Miladinović[1]

[1] Univerzitet u Nišu, Medicinski fakultet, Katedra za farmaciju, Niš, Srbija
[2] Univerzitet u Nišu, Prirodno-matematički fakultet, Katedra za biologiju i ekologiju, Niš, Srbija
[3] Institut za proučavanje medicinskog bilja „Dr Josif Pančić", Beograd, Srbija

Kontakt: Bojana Miladinović
Bulevar dr Zorana Đinđica 81, 18 000 Niš, Srbija
e-mail: bojana.miladinovic@medfak.ni.ac.rs
Telefon: 069/1045097

Bobičasto voće sadrži veliki broj vrsta poznatih po svojim nutritnivnim i zdravstvenim benefitima. Crvena ribizla pokazuje različite biološke aktivnosti: antiproliferativnu, antikancerogenu, antimikrobnu, antiinflamatornu, antiokisativnu i antidijabetičku akitvnost. Cilj ovog rada bio je da se ispita antimikrobna aktivnost liofiliziranog soka crvene ribizle vrste *Redpoll* (RPJL) i liofilizovanog ekstrakta ostatka (RPWL) na različite Gram (+) bakterije (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Enterococcus feacalis*), Gram (-) bakterije *(Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Proteus mirabilis, Enterobacter aerogenes*) i jednu gljivicu (*Candida albicans*). Drugi cilj bio je ispitati aktivnost dominantnih bioaktivnih jedinjenja (askorbinska kiselina, kvercetin i kempferol), koja se nalaze u crvenoj ribizli, pod istim uslovima, kako bi se procenio njihov efekat u antimikrobnoj aktivnosti. Testirani RPJL i RPWL pokazali su umerenu antimikrobnu aktivnost. Minimalne inhibitorne (MIC) i mikrobicidne koncentracije (MBC/MFC) RPJL i RPWL bile su 100 mg/ml i više od 100 mg/ml. RPJL I RPWL pokazali su isti efekat na Gram (+) bakterije i istu MIC i MBC vrednost. RPWL je pokazao jači antimikrobni efekat na Gram (-) bakterije, dok sok uopšte ne inhibira rast Gram (-) bakterija. Standarni rastvori askorbinske kiseline i kvercetina pokazali su jaku inhibitornu i mikrobicidnu aktivnost pri nižim koncentracijama od ispitivanih uzoraka. MIC/MBC (MFC) standarda bili su od 2,5 mg/ml do 10 mg/ml. Rezultati su pokazali da bi ekstrakti i sokovi crvene ribizle mogli biti upotrebljeni kao potencijalni prirodni konzervansi. *Acta Medica Medianae 2023;62(2):38-44.*

Ključne reči: *crvena ribizla, Redpoll, antimikrobna aktivnost, bobičasto voće, konzervansi*