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Antimicrobial and antioxidant potential of wild growing *Silene baccifera* (L.) Roth. (Caryophyllaceae) fruits juice

Tatjana Mihajilov-Krstev[1*], Bojan Zlatković[1], Marija Ilić[2], Vesna Stankov-Jovanović[2], Violeta Mitić[2]

[1]University of Niš, Faculty of Sciences and Mathematics, Department of Biology and Ecology, Višegradska 33, 18000 Niš, Serbia
[2]University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Višegradska 33, 18000 Niš, Serbia

** E-mail: tatjanamk@pmf.ni.ac.rs*

***Abstract*:**

Mihajilov-Krstev, T., Zlatković, B., Ilić, M., Stankov-Jovanović, V., Mitić, V.: ***Antimicrobial and antioxidant potential of wild growing Silene baccifera (L.) Roth (Caryophyllaceae) fruits juice. Biologica Nyssana, 6 (2), December 2015: 55-58.***

Silene baccifera is widespread plant species in Europe, Asia and North Africa, registered in the list of medicinal plants of India. Insufficiently known, biological activities of juice obtained from fresh fruits of this plant were investigated in this study. Antimicrobial activity of juice was tested against pathogenic gastrointestinal microbial strains, using microwell-dilution method, while antioxidant properties were evaluated employing DPPH and total phenolic and flavonoid content assays. To our knowledge, this is the first study of the juice from fruits of this plant species.

Key words: *Silene baccifera*, fruit juice, antimicrobial, antioxidant activity

***Apstrakt*:**

Mihajilov-Krstev, T., Zlatković, B., Ilić, M., Stankov-Jovanović, V., Mitić, V.: ***Antimikrobni i antioksidativni potencijal soka plodova divljerastuće vrste Silene baccifera (L.) Roth (Caryophyllaceae). Biologica Nyssana, 6 (2), December 2015: 55-58.***

Silene baccifera predstavlja rasprostranjenu biljnu vrstu na području Evrope, Azije i Severne Afrike. Registrovana je na listi lekovitih biljnih vrsta Indije. U ovom radu je ispitivana biološka aktivnost soka dobijenog ceđenjem svežih plodova pomenute vrste. Antimikrobna aktivnost je testirana mikrodilucionom metodom protiv patogena gastrointestinalnog trakta. Antioksidantna aktivnost je procenjena DPPH metodom i određivanjem ukupnog sadržaja fenola i flavonoida. Prema našem saznanju, ovo je prva takva studija soka plodova ove biljne vrste.

Key words: *Silene baccifera*, sok od plodova, antimikrobna, antioksidativna aktivnost

Introduction

Silene baccifera (L.) Roth. (syn. *Cucubalus baccifer* L.) is widely spread plant species in the area of Europe, Asia and North Africa, especially in the regions with a temperate climate (G a j i ć , 1970). It is perennial plant with long, prostrate or ascending (50-150 cm), well-branched stems and white or

greenish nodding flowers sorted in lateral brunches of the inflorescence. Comparatively large (up to 15 mm in diameter), subglobose, fleshy, blackish-red berries are distinctly exerted from the calyx during the ripening period. Blossoms appear in June to August, fruits ripen from July to October (Konrad von Weihe, 1972). *Silene baccifera* is common plant species that usually grows along roadsides and weedy places in the human settlements.

This plant species is registered in the List of Indian Medicinal Plants (www.docslide.us) and is used in traditional medicine for treatment of nephritis, hydropsy, bone-fractures, pulmonary tuberculosis and scrofula (Cheng et al., 2001). To the best of the author's knowledge, there is no literature or field data on its use in official either traditional medicine in Serbia or Balkan countries.

The chemical composition of the underground part of the *S. baccifera* has been poorly investigated. Previously isolated constituents from the whole plant were oligosaccharides (Courtois & Ariyoshi, 1960; 1962), tocopherol and tocotrienol (Ivanov & Aitzetmueller, 1998) and phytoecdysterones and cucubalugenin A (Cheng et al., 2001a; 2001b).

To the best of our knowledge, antioxidant and antimicrobial activity of this plant species were not investigated. In this paper, the first results of antioxidant and antimicrobial activity of the juice obtained from fresh fruits of *S. baccifera* are presented.

Material and methods

Plant material

Ripe fruits of wild growing *Silene baccifera* were collected on the territory of Serbia (Kamenica village, Stara planina, E Serbia) and identified by Dr Bojan Zlatković. A voucher specimens, under the acquisition number 6859, were deposited at the herbarium collection of Department of Biology and Ecology, Faculty of Science and Mathematics in Niš (HMN).

Preparation of juice

Cleaned, ripe fruits were tap washed, followed by washing with distilled water and subsequent drying. The fruits were separated from the seeds, cut in small pieces, arranged in clean plastic containers and frozen. Melted at room temperature, the softened material was grounded in mechanical blender to a fine mash prior to filtration and the juice extraction.

Antimicrobial assay

Microbial strains. *In vitro* antimicrobial activity of the tested samples have been investigated against the commonest human gastrointestinal pathogenic microbial strains: *Salmonella enteritidis* (ATCC 13076), *Shigella sonnei* (ATCC 25931), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Listeria monocytogenes* (ATCC 7644), *Bacillus cereus* (ATCC 10786), *Staphylococcus aureus* (ATCC 6538) and *Candida albicans* (ATCC 10031) using microwell-dilution method.

Microwell dilution method. Determination of the minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) was carried out according to the method described by CLSI (2005) with some modifications. An overnight culture of tested strains were used for the preparation of 0.5 McFarland standard turbidity suspension (corresponding 10^8 CFU/mL). A serial doubling dilutions of tested juice were prepared in the range 500.00-0.25 μL/mL, in a 96/well microtiter plate with inoculated Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for yeast. The final volume was 100 μL and the final concentration of bacterial cells was 10^6 CFU/mL in each well. The plates were incubated for 24 h at 37 °C. Chloramphenicol at concentrations ranging from 10.00-0.002 μg/mL was used as positive control. All determinations were performed in triplicate. Microbial growth was determined by adding 20 μL of 0.5% triphenyl tetrazolium chloride (TTC) aqueous solution. MIC was defined as the lowest concentration of juice at which microorganisms showed no visible growth. In order to determine MMC, broth was taken from each well and inoculated on Mueller-Hinton agar (MHA) for bacteria and Sabouraud dextrose agar (SDA) for yeast. The plates were incubated for 24 h at 37°C. The MMC is defined as the lowest concentration of the juice at which 99.9% of inoculated microorganisms were killed.

Antioxidant assay

The antioxidant properties of the tested juice were evaluated employing DPPH and total phenolic and flavonoid content assays. All the assays were carried out in triplicate and average value was considered.

1,1-diphenyl-2-picrylhydrasyl (DPPH) radical scavenging assay. Relatively stable organic radical DPPH has been widely used in the determination of antioxidant activity of single compounds as well as the different plant extracts (Kulišić, 2004). The DPPH˙-assay was performed as described (Stojanović, 2010). Samples of juice methanol solution (10 μL, 232.4, 232.5 and 232.6 mg/mL respectively) were mixed with 90 μmol/L DPPH˙in methanol (1.0 mL), and these

solutions were diluted up to 4.0 mL. After shaking mixtures vigorously, they were stored in darkness for 60 min at room temperature and the absorbances were measured at 515 nm (Perkin-Elmer Lambda 15 UV-VIS spectrophotometer).

Radical scavenging activity of the samples was calculated applying following equation:

DPPH RSC (%) = 100 (A_0−A_1/A_0)

Where: A_0-absorbance of the blank; A_1-absorbance of the sample.

Total phenolic content determination. The total phenolic concentration was determined spectrophotometrically according to modified Folin-Ciocalteu method (Di Majo, 2008). To the samples of juice (100 µL) portions of 1 mL of Folin-Ciocalteu reagent (purchased from "Mol" Belgrade, Serbia) and 4 mL of sodium carbonate (20% v/v) were added and diluted with distilled water up to 20 mL. The mixture was allowed to stand at room temperature in dark place for 30 min and the absorbance of the solution at 750 nm was measured with a Perkin Elmer Lambda 15 UV/VIS spectrophotometer. The total phenolic concentrations were calculated from a calibration curve using gallic acid as a standard. Gallic acid was provided by Sigma Aldrich (Darmstadt, Germany). Data were expressed as gallic acid equivalents per 1 mg of juice. The levels of total infusions in juice determined according to the Folin–Ciocalteu method are not absolute measurements of the amounts of phenolic materials, but are in fact based on their chemical reducing capacity relative to an equivalent reducing capacity of gallic acid.

Determination of total flavonoid content. The amount of total flavonoids was determined with aluminium chloride ($AlCl_3$) colorimetric assay according to a known method (Rice-Evans et al., 1996). Briefly, 0.5 mL of juice was made up to a final volume of 1 mL with reaction medium ($MeO/H_2O/CH_3COOH$=14:5:1). Prepared solution was mixed with $AlCl_3$ reagent (4 mL, 133 mg of $AlCl_3x6H_2O$ and 400 mg of CH_3COONa dissolved in 100 mL H_2O). After 5 min, the absorbance level was measured versus prepared reagent blank (containing the same chemicals, except for the sample) at 430 nm (Perkin-Elmer Lambda 15 UV-VIS spectrophotometer). Total flavonoid content was calculated on the basis of the calibration curve of rutin and expressed by mg rutin/g dry extract. The total flavonoid assay was measured in triplicate.

Results and discussion

Freshly drained juice from the fruits have shown antimicrobial activity on all tested strains of patogenic microbes (**Tab. 1**).

The obtained minimal inhibitory concentrations were in the range 31.2-500.0 µL/mL, and minimal microbicidal concentrations ranged from 125.0-500.0 µL/mL. The tested juice exhibited the highest activity against *P. aeruginosa* and *S. enteritidis* (MIC/MMC=31.2/125 and 62.5/125 µL/mL, respectively). Also, good effect was observed against *E. coli* (MIC=MMC=125 µL/mL). The juice was the least active against *S. aureus* and *C. albicans* (MIC=MMC=500 µL/mL).

Table 1. Antimicrobial activity of juice from *S. baccifera* fruits and reference antibiotics (Tested strains: SB - *S. baccifera* fruits juice, MIC/MMC in µL/mL; A - Antibiotic, MIC/MMC in µg/mL; bacterial strains: *Se – Salmonella enteritidis; Ss - Shigella sonnei, Ec - Escherichia coli, Pa - Pseudomonas aeruginosa, Lm - Listeria monocytogenes, Bc - Bacillus cereus, Sa - Staphylococcus aureus; Ca – Candida albicans*)

Tested strains	*Se*	*Ss*	*Ec*	*Pa*	*Lm*	*Bc*	*Sa*	*Ca*
SB	62.5/125	250/250	125/125	31.2/125	250/500	125/500	500/500	500/500
A	0.035*	0.035*	0.035*	0.078*	0.035*	0.035*	0.035*	0.035**

*Chloramphenicol, **Nystatin

Table 2. The antioxidant activity of juice from *S. baccifera* fruits

Tested samples	**RSA (EC 50 u µg/mL)****	**PPC (gallic acid equivalents, µg/mg of dry matter)**	**PC (rutin equivalents, µg/mg of dry matter)**
*Silene baccifera**	0.65	6.00	15.18

*juice was prepared by dissolving fruit in water;
** EC 50 for BHT as a standard is 0.63 µg/mL

The obtained results of antioxidant activity of juices are summarized and presented in **Tab. 2**.

Comparative study of antimicrobial and antioxidant activities of the fresh juices obtained from the fruits of plant species: *Viburnum lantana, Viburnum opulus, Sambucus nigra, Cornus sanguinea* and *Paliurus spina-christi*, wild growing on the territory of Serbia, showed that all tested juices had inhibitory concentrations in the range from 15.6-500.0 μL/mL and microbicidal concentrations from MBC/MFC=62.5-500.0 μL/mL (Mihajilov-Krstev et al., 2011). Generally, better activity than *S. baccifera* juice had only the juices from *V. opulus* and *C. sanguinea*. Also, the juice of this species demonstrated the best antioxidant activity in comparison to the mentioned juices.

Conclusion

The obtained results showed that *S. baccifera* fruit juice presents a natural source of antimicrobial and antioxidant components that can be applied in prevention and treatment of gastrointestinal diseases and oxidative stress in humans. In future studies, the chemical composition and biological activity of different extracts of certain parts of the plant should be investigated, as well as their cytotoxicity and acute toxicity.

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