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Original Article

Antimicrobial activity of *Evernia prunastri* (oakmoss) resinoids

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Abstract:

Infections caused by strains of bacteria that show resistance to a large number of antibiotics represent one of the leading problems today. The aim of this work is to examine new, natural resources with potential antimicrobial effects. The antibacterial activity of lichen resinoids (*Evernia prunastri*) was tested against reference strains *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and clinical isolates of bacteria *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. coli*, by the disc-diffusion method. Each 10 µl of resinoid, of different concentrations, was applied to sterile discs with a diameter of 6 mm. The tested resinoid exhibited the most significant antibacterial activity against *Pseudomonas aeruginosa* ATCC 27853, while it was the weakest against the clinical isolate of *Escherichia coli*. The results indicate that the tested oakmoss resinoid shows moderate antimicrobial activity against the tested strains.

Key words:
antimicrobial activity, resinoid, lichen, bacteria

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Apstrakt:

Antimikrobna aktivnost rezinoida vrste *Evernia prunastri* (hrastova mahovina)

Infekcije izazvane sojevima bakterija koji pokazuju rezistenciju na veći broj antibiotika predstavljaju jedan od vodećih problema današnjice. Cilj ovog rada je ispitivanje novih, prirodnih resursa sa potencijalnim antimikrobnim dejstvom. Antibakterijska aktivnost rezinoida hrastove mahovine (*Evernia prunastri)* ispitivana je na referentne sojeve *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 i kliničke izolate bakterija *S. aureus*, *S. epidermidis*, *P. aeruginosa* i *E. coli*, disk-difuzionom metodom. Po 10 µl rezinoida, različitih koncentracija nanošeno je na sterilne diskove prečnika 6 mm. Ispitivani rezinoid ispoljio je najznačajniju antibakterijsku aktivnost prema *Pseudomonas aeruginosa* ATCC 27853, dok je najslabiji prema kliničkom izolatu *Escherichia coli*. Rezultati ukazuju da ispitivani rezinoid hrastove mahovine pokazuje umerenu antimikrobnu aktivnost na ispitivane sojeve.

Ključne reči:
antimikrobna aktivnost, rezinoid, hrastova mahovina, bakterije

Introduction

The introduction of antimicrobial agents in the treatment of infectious diseases saved millions of lives. Antimicrobial agents have the ability to eliminate microorganisms by acting on various metabolic and structural targets such as interfering with cell wall synthesis, with nucleic acid synthesis or by other ways of inhibiting (Tenover, 2006; Bobbarala, 2012). The production of the first antibiotics represents the greatest achievement in medicine. However, bacterial resistance to certain antibiotics increases significantly with age (Grundmann et al., 2011). Tenover (2006) cites as known cases of resistance, resistance of *Escherichia coli* to third generation cephalosporins, emergence of vancomycin-resistant *Staphylococcus aureus* and multiresistance of *Pseudomonas aeruginosa*, which is why there is a constant need to find new antimicrobial agents. According to data from the World Health Organization (2020), not a single new class of antibiotics has been developed since 1987. Until now, the most clinically important classes of antibiotics are derived from molds, but we can except that basis for the identification of new antimicrobial compounds can be lichen.

Lichens represent a community formed by algae or cyanobacteria and fungi, developing a mutually beneficial relationship or symbiosis (Calcott et al., 2018). In this symbiosis, algae or cyanobacteria represent the photobiont, while fungi represent the mycobiont. Nash (2008) considers lichen *Evernia prunastri* (L.) Ach. „vegetation pioneers" because of their high stress tolerance. Most lichen are slow-growing and long-lived, which indicates that they retain an unchanged morphology for a long period of time (Nash, 2008).

Evernia prunastri (L.) Ach. is widely distributed in the northern hemisphere. It belongs to the family Parmeliaceae*,* genus *Evernia*, and is popularly known as oak or plum moss. According to the type of structure, it belongs to foliose lichen. They have a dichotomously branched thallus, with more or less branches that are attached at one point. It reaches a width between 0.5 and 3 mm. The upper surface of the lichenis greenish-gray to pale green-yellow in color. The lower surface of the thallus in healthy lichen is the same or a shade lighter in color. If the lichen is damaged, its color becomes grayish and less intense. *E. prunastri* grows on the bark of deciduous trees, most often oak, while occasionally it can be found on conifers in areas of high humidity (Shcherbakova et al., 2021). Therefore, it is not surprising that this species is widely distributed on the territory of Serbia. It likes moist, sunny areas at lower altitudes, and is very rarely found in areas higher than 1,675 m above sea level (Nash, 2001). The species *E. prunastri*is acidophilic and sensitive to the presence of nitrogen, especially ammonia from the air.

E. prunastri is characterized by usnic acid, atranorin and chloratranorin in the upper cortex, while evernic acid is found in the medulla. About 170 compounds were identified in the extract of this species, of which 47 are depsides, 25 are triterpenes and steroids (Joulain & Tabacchi, 2008). Usnic acid is known as an acid with a broad spectrum of antimicrobial activity against Gram-positive bacteria, such as *Streptococcus* spp., *Bacillus* spp., *Staphylococcus aureus* (including methicillin-resistant strains) and Gram-negative bacteria, such as *Proteus vulgaris* (Shcherbakov et al., 2021). Evernic acid, although studied to a lesser extent, has shown antimicrobial activity against *Bacillus mycoides* and *B. subtilis, Escherichia coli* and *Pseudomonas aeruginosa* (Shcherbakova et al., 2021). Atranorin also shows some antimicrobial activity (Studzinska-Sroka et al., 2017).

According to the literature, the resinoid of *E. prunastri* contains monoterpenes, sesquiterpenes, diterpenes and various terpenoids. Also, α-pinene, camphene, β-pinene, limonene, γ-terpinene, p-cymene, trans-pinocarveol, α-copene and α-muurolene are compounds characteristic of both the resinoids and the essential oil of *E. prunastri* (Kahriman et al., 2011).

The ancient Greeks were still familiar with the healing properties of *E. prunastri*, from which they made salves for urogenital diseases. More recently, it has been used for diseases of the digestive tract and respiratory tract (Crawford, 2015). Medical use today is justified by the fact that lichens contain unique biologically active metabolites that have a wide variety of biological effects (antibacterial, analgesic, antiproliferative and cytotoxic) (Kosanic et al., 2013).

It is often used in research because it is easy to collect, transport and prepare for analysis.

Materials and Methods

Samples of lichen *E. prunastri* (**Fig. 1**) were collected in October 2021 in the village of Prekopuce, at the foot of the Jastrebac mountain. The thallus was dried in the air, in a shady place, at room temperature for 10 days.

Fig. 1. Photograph of *Evernia prunastri* (L.) Ach.

Extraction

Dried lichen (10 g) poured with 100 cm^3 of 70% aqueous ethanol solution and left at a temperature of 25 °C. After a certain time, the extract was separated from the dried material by vacuum filtration. The solvent was evaporated in vacuo until a semi-solid residue was obtained. The resulting semi-solid residue was dried at a temperature of 60 °C to a constant mass. The resulting dry residue is the resinoid, that is, the total alcoholic extract.

Disc-diffusion method

To determine the antimicrobial activity of lichen resinoids, laboratory reference cultures from the American Type Culture Collection (ATCC) *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and clinical isolates of bacteria *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. coli*, by the disc-diffusion method. Clinical isolates were isolated from swabs of patients' skin wounds. A turbidity suspension of 0.5 McFarland containing 1.5×10^8 CFU/ml was made from overnight cultures of tested strains of microorganisms grown on nutrient agar (NCCLS - National Committee for Clinical Laboratory Standards, 2003). On sterile Müller-Hinton agar (Torlak) substrates, 0.1 ml of all prepared microorganism suspensions were inoculated. Sterile cellulose discs with a diameter of 6 mm (Himedia, Milano, Italy) were placed on the inoculated surface of the agar plate. The disks were impregnated with 10 µl of different solutions (1, 1/2 and 1/5 v/v) of resinoid in ethanol. A disc with gentamicin (10 µg/ml) was used as a positive control. Petri dishes were incubated at 37 °C for 24 hours. After that, the zone of inhibition was read in mm. All growth inhibition assays were performed in triplicate.

Results and discussion

E. prunastri resinoid inhibited the growth of all tested microorganisms; however, their antimicrobial potential was lower than the applied positive control, gentamicin.

The results of the disc-diffusion method showed antimicrobial activity against reference strains *P. aeruginosa* ATCC 27853 (depending on the resinoid concentration, the zone of inhibition was from 18 mm to 10 mm), while a significantly weaker antimicrobial activity was shown against *S. epidermidis* ATCC 12228 (depending on the resinoid concentration, the zone of inhibition was from 15 mm to 7 mm). Inhibition zone *S. aureus* ATCC 25923 ranged from 14 mm to 9 mm. The weakest antimicrobial effect was shown by *E. coli* ATCC 25922 with an inhibition zone ranging from 10 mm to 8 mm. It is necessary to emphasize that 1/5 v/v ethanol solution of resinoid according to *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 did not show antimicrobial activity (**Tab. 1**).

The resinoid showed the most significant antimicrobial activity against the clinical isolate of *P. aeruginosa*, whose zone of inhibition ranged from 17 mm to 13 mm. It showed moderate antimicrobial activity according to *S. epidermidis* and *S. aureus*, whose inhibition zones ranged from 13 mm to 8 mm, that is, from 10 mm to 7 mm. The weakest antimicrobial activity was against *E. coli*, whose zone of inhibition was from 9 mm to 7 mm (**Tab.**

Table 1. Antimicrobial activity of *E. prunastri* resinoids

Bacterial strains	**Gentamicin**	**Resinoid *E. prunastri* 10 µl**		
		1	1/2	1/5
Staphylococcus aureus ATCC 25923	22	14	9	0
Staphylococcus epidermidis ATCC 12228	20	15	9	7
Pseudomonas aeruginosa ATCC 27853	28	18	16	10
Escherichia coli ATCC 25922	12	10	8	0
Staphylococcus aureus isolate from the wound	19	13	8	0
Pseudomonas aeruginosa isolate from the wound	26	17	13	0
Escherichia coli isolate from the wound	10	9	7	0

1). It is important to note that the 1/5 v/v ethanol solution of resinoid did not show antimicrobial activity against any of the tested clinical isolate, but reference strain *Staphylococcus epidermidis* ATCC 12228 had inhibition zone of 7 mm and inhibition zone of *Pseudomonas aeruginosa* ATCC 27853 was 10 mm. By comparing reference and clinical strains, it is concluded that the highest antimicrobial activity of resinoid in all concentrations is against *P. aeruginosa* ATCC 27853, while it is the lowest against the clinical isolate of *E. coli.*

In this study, the tested *E. prunastri* resinoid showed relatively low antimicrobial activity. The obtained results are in accordance with the results of testing the essential oil of *E. prunastri* from Algeria, which showed a small effect against *E.coli* and a moderate antibacterial effect against *P. aeruginosa* (Chahra et al., 2016). However, the obtained results are in contrast with the results of testing the antimicrobial activity of the essential oil of *E. prunastri* from Turkey. In this study, the essential oil of *E. prunastri* did not show antimicrobial activity against *P. aeruginosa*, *S. aureus, S. epidermidis* (Aslan et al., 2006). In a study conducted by Kahriman et al. (2011), *E. prunastri* essential oil did not show antimicrobial activity against any tested bacteria. Stojanović et al. (2013) showed the antimicrobial effect of *E. prunastri* against *P. aeruginosa* and *E. coli*.

Conclusions

The results of the *E. prunastri* resinoid test indicate a relatively low antimicrobial potential. The tested microorganisms were not equally sensitive to the presence of lichen resinoids, which shows that the tested lichen possesses compounds with antimicrobial properties, and this requires further research to determine antimicrobial agents that could be used in new drugs for the treatment of infectious diseases in humans and animals, as well as diseases plants.

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