

ANTIMICROBIAL ACTIVITY OF THE *HYPERCUM PERFORATUM* L. PLANT

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The goal of this study was to determine the level of antimicrobial activity of ethanolic extract of St. John's wort (*H. perforatum* L.) toward some phytopathogenic bacteria and fungi. The fungi *Fusarium oxysporum* (Schlecht) and *Penicillium canescens* (Sopp) were isolated from fruit which was in a process of deterioration. The antifungal activity of the ethanolic extract with concentration of 5–45 mg/ml in the period of 72 hours, was determined by the method of spore counting (Nauber's chamber). It was concluded that the sample with 45 mg/ml of ethanolic extract of *H. perforatum* showed most fungistaticity. When $1 \cdot 10^2$ spores of fungi were inoculated, the number of spores was decreased to 5 spores for *F. oxysporum*, and to 15 spores for *P. canescens*. The bacteria *Pseudomonas glycinea* and *Azotobacter chroococcum* showed extreme sensitivity to the presence of 25 mg of the extract of *H. perforatum*. Bacterial growth was inhibited by 53 %. Higher degree of resistance to the extract of *H. perforatum* was demonstrated by the bacteria *Bacillus subtilis*. With 25 mg of extract per disc, an inhibition of 25 % was observed, but with 5 mg extract, an inhibition of 1.8 % was detected. The minimum inhibition concentration of ethanolic extract of *H. perforatum* depends on the phytopathogenic bacteria type (G^+ , G^-), and varies between 1.25–3.5 mg/ml.

Key words: St. John's wort (*H. perforatum*); antibacterial activity; antifungal activity

АНТИМИКРОБНА АКТИВНОСТ НА БИЛКАТА *HYPERCUM PERFORATUM* L.

Целта на ова испитување беше да се определи антимикробната активност на етанолен екстракт од кантарион (*H. perforatum* L.) во однос на некои фитопатогени бактерии и габи. Габите *Fusarium oxysporum* (Schlecht) и *Penicillium canescens* (Sopp) беа изолирани од плодови во распаѓање. Антимикробната активност на етанолниот екстракт со концентрација од 5 до 45 mg/ml беше определувана во текот на 72 часа со методот на броење спори (Nauber-ова комора). Пробата со 45 mg/ml етанолен екстракт од кантарион покажа најголема антифунгицидност. Кога беа изолирани $1 \cdot 10^2$ спори од габите, бројот на спори од *F. oxysporum* се намали на 5, а за *P. canescens* на 15. Бактериите *Pseudomonas glycinea* и *Azotobacter chroococcum* покажаа особена чувствителност и на 25 mg екстракт од кантарионот, при што бактериското растење беше инхибирано за 53 %. Поголема отпорност спрема екстрактот на *H. perforatum* покажа бактеријата *Bacillus subtilis*. 25 mg екстракт на диск предизвика 25 % инхибиција, а 5 mg екстракт доведе до 1.8 % инхибиција. Минималната концентрација на инхибиторот од етанолниот екстракт од *H. perforatum* зависи од фитопатогените бактерии од типот (G^+ , G^-) и варира од 1,25 до 3,5 mg/ml.

Клучни зборови: кантарион (*H. perforatum*); антибактериска активност; антифунгицидна активност

INTRODUCTION

In recent years, the consumption of St. John's wort (*H. perforatum*) – derived products has increased dramatically. Presently, it is one of the

most consumed medicinal plants all around the world [12]. The commercially available *H. perforatum* derived products include phytopharmaceuticals and nutraceuticals, teas, tinctures, juices, and oily macerates [6]. *H. perforatum* has a wide range

of medicinal applications, including skin wounds, eczema, burns, diseases of the alimentary tract, and psychological disorders [2].

Ethanollic extracts of *H. perforatum* contain many phenolic compounds (hypericin, hyperforin and their derivatives, rutin, hyperozide, quercetin, chlorogenic acid, flavonols, and flavones), suggesting that they could have important antioxidant properties [9].

Hypericin was shown to possess antibacterial, antiviral, and anti-inflammatory activity [10]. Hyperforin, a phloroglucin derivate, is the main compound involved in antidepressant activity [3], and was shown to have antimicrobial activity. Hyperforin exhibited good activity against methicillin-resistant strains of *Staphylococcus aureus*, with MIC value of 1.0 µg/ml [8].

H. perforatum is used both for therapeutic purposes and as a flavouring in the preparation of foods and alcoholic beverages. Both the drug and its derivatives (infusion, alcoholic tincture, fluid extract) are used in the flavouring industry to prepare liqueurs, especially digestive and tonic bitters [5].

EXPERIMENTAL SECTION

Plant material and production of extracts of Hypericum perforatum

H. perforatum plants (flowers, including approx. 7 cm of stem) were collected in the region of Kragujevac, central of Serbia, and the biomass was freeze-dried on the same day. The specimens were identified in the Plant Biology at Faculty of Science, University of Belgrade (voucher 0799HP at Herbarium of the Department).

An ethanollic extract was prepared by extraction of the biomass (72 g) with ethanol:water solution (80:20). The mixture was filtered through a paper filter (Whatman, No. 1) and the resulting total ethanollic extract of *H. perforatum* (20.8 g) was stored in a dark glass bottle for further processing.

Microorganisms

The fungi *Fusarium oxysporum* (Schlecht) and *Penicillium canescens* (Sopp) were isolated from a fruit in the process of deterioration. The fungi were reseeded on potato-glucose agar, on

which they developed for 7 days at room temperature of 20 °C under alternating day-night light conditions. They were then reseeded on a new potato-glucose substrate, where they developed for another 7 days. The reseeded procedure was performed four times, after which the pure cultures needed for the experiments were obtained.

Phytopathogenic bacteria cultures were obtained from the microbial collection of the Faculty of Science, University of Kragujevac.

Antibacterial activity of the ethanollic extract of H. perforatum

The antibacterial activity of 2.5 – 25 mg of ethanollic extract of *H. perforatum* was investigated by the disc-diffusion method on agar [7, 13]. The activity level was established on the basis of the inhibition zone formed after 48 hours of bacteria development and are presented in mm. The disc with ethanol didn't show any zone of inhibition.

The minimal inhibitory concentration of the ethanollic extract was determined by a routine *in vitro* procedure [1]. Briefly, the extract was tested by the macrobroth 2-fold serial technique. The ethanollic extract of *H. perforatum* was dissolved (in a minimum quantity) in phosphate buffer, pH = 8 (10 mg/ml, 7 mg/ml). All tests were performed in Mueller-Hinton broth with addition of certain concentration of extract and 0.1 ml of bacterial spore suspension ($5.4 \cdot 10^6$ CFU/ml) [4, 14]. The results presented were determined after 48 hours.

Antifungal activity of the ethanollic extract of H. perforatum

The antifungal activity of 5 – 45 mg/ml plant extract (in phosphate buffer, pH = 8) was investigated by the method of spore counting (Nauber's chamber) [11]. In each sample of broth 0.2 ml inoculum of the fungi ($1 \cdot 10^2$ CFU/ml spores) was added.

RESULTS AND DISCUSSION

The results of the research of antibacterial activity (via the disc diffusion and microdilution methods) of the ethanollic extract of *H. perforatum* to phytopathogenic bacteria are presented in Table 1.

Table 1
Antibacterial activity of ethanolic extract of *H. perforatum*

Microorganism	Diameter of zones of inhibition (mm) ^a				Minimal inhibitory concentration (mg/ml) ^b
	25	10	5	2.5	
<i>Pseudomonas fluorescens</i> (G-)	8	1	0.5	/	1.25
<i>Pseudomonas phaseolicola</i> (G-)	6	2	0.5	/	1.25
<i>Pseudomonas glycinea</i> (G-)	8.5	1.5	1	/	2.5
<i>Erwinia carotovora</i> (G-)	6	1.5	0.5	/	1.25
<i>Enterobacter cloacae</i> (G-)	7.5	1.5	0.5	/	2.5
<i>Klebsiella pneumoniae</i> (G-)	5	1	0.5	/	3.5
<i>Agrobacterium tumefaciens</i> (G-)	8	2.5	0.5	/	2.5
<i>Azotobacter chroococcum</i> (G-)	8.5	2	1	/	2.5
<i>Bacillus mycoides</i> (G+)	6.5	2	2	/	3.5
<i>Bacillus subtilis</i> (G+)	7	1	0.5	/	3.5

^a Values are the mean of three replicates.

^b Values are the mean of three replicates using $5 \cdot 10^6$ of each culture.

The results indicate that the ethanolic extract of *H. perforatum* has high antibacterial activity towards all ten investigated phytopathogenic bacteria. The highest inhibition is noticed with 25 mg extract, when the inhibition is 30–50 % (inhibition zones are 6–8,5 mm). The 5–10 mg extract show significantly smaller inhibition, in the range of 6.25–15.6 %, with inhibition zones of 1–2.5 mm. The lowest amount of 2.5 mg shows no inhibitory effect.

The *Pseudomonas glycinea* and *Azotobacter chroococcum* bacteria showed extreme sensitivity to the presence of 25 mg of the extract of *H. perforatum*; the bacterial growth was inhibited by 53 %.

High degree of resistance to the extract of *H. perforatum* was exhibited by the bacteria *Bacillus subtilis*: with 25 mg of the extract, the inhibition zone was 7 mm (25 %) and with 5 mg of the extract, the inhibition zone was 0,5 mm (1.8 %).

The antibacterial activity of the ethanolic extract of *H. perforatum* depends of phytopathogenic bacteria type (G+, G-). The minimal antimicrobial concentration of ethanolic extract of *H. perforatum* is in the range of 1.25–3.5 mg/ml.

The experimental values of inhibition were compared with inhibition zones obtained with sinacillin as standard. The sample with 12.5 mg sinacillin per disc showed an inhibition zone of 16 mm for *P. fluorescens* (G-), and 27.5 mm for *B. mucooides* (G+).

The minimum inhibitor concentration (determined by the dilution method) of sinacillin is 0.675 mg/ml for *P. fluorescens* and 0.0156 mg/ml for *B. mucooides*.

The results of the antifungal activity testing are shown in the Fig. 1.

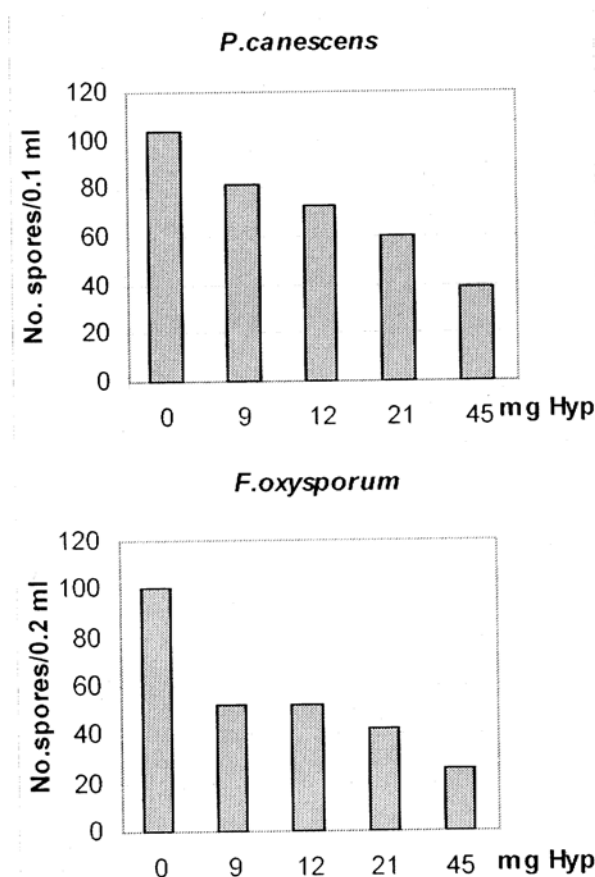


Fig. 1. Antifungal activity of ethanolic extract of *H. perforatum*. The sample was inoculated with $1 \cdot 10^2$ CFU/ml spores. The presented results were determined after 24 hours

By increasing the concentration of ethanolic extract in the sample, a decrease of the spore number for both fungi examined was observed. It was concluded that the sample with the concentration of 45 mg/ml ethanolic extract showed most fungistaticity, decreasing the number of spores to 5 spores for *F. oxysporum* and 15 spores for *P.*

canescens, in the case of inoculation of $1 \cdot 10^2$ spores.

CONCLUSION

It can be concluded that the ethanolic extract of *H. perforatum* shows antibacterial activity for all ten of the investigated bacteria. The antibacterial activity is in the function of the extract concentration. The inhibition zones measured when *P. glycinea* has been tested correspond to an inhibition of 6–53 %. *B. subtilis* shows level of inhibition is 25 % in the presence of 25 mg of ethanolic plant extract.

The lowest mass of extract (2.5 mg per disc) does not exhibit antibacterial activity toward any of the ten investigated bacteria. The minimum inhibitory concentration is in the range of 1.25–2.5 mg/ml for all phytopathogenic bacteria examined.

The antifungal activity of the ethanolic extract of *H. perforatum* against the fungi *P. canescens* and *F. oxysporum*, decreased the number of spores by 62 % for *P. canescens* and by 72 % in samples with *F. oxysporum*.

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