

In vitro inhibitory effect of *Hypericum perforatum* L. ethanol extract on microbial growth

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Abstract: *Hypericum perforatum* L. is a medicinal plant, traditionally used in few European countries as a sedative, antiseptic, pain-killer and a diuretic. *Hypericum perforatum* L. is a remedy for wounds and a calming agent for inflammatory processes. It has an antimicrobial effect on many viruses, bacteria and fungi. *Candida albicans* is a member of the normal flora of mucous membranes of the respiratory, gastrointestinal and female genital tracts. In such places, they can become dominant and cause pathological conditions, particularly in people who suffer from a compromised immune system. It is believed that the frequent use of broad-spectrum antibiotics and corticosteroids has a significant impact on the pathogenic effect of the fungus, due to its favorable impact on its reproduction. This paper presents the results of microbiological analysis of the effect of extract of St. John's wort collected from locality Tuzla - northeastern Bosnia on 21 strains of *Candida albicans*, isolated from different regions of the human body, and four types of bacteria: *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The results of the research show resistance of *Candida albicans* strains and the tested gram negative bacteria to St. John's wort, and sensibility of the investigated gram-positive bacteria to the extract.

Key words: St. John's wort, antimicrobial activity, *Candida albicans*, gram positive and gram negative bacteria.

Introduction

Hypericum perforatum L. is a perennial herbaceous plant up to 1 meter in height. It has spindle-shaped roots. At the top stem, it has branches with elongated and oval leaves. The flowers are yellow, collected in shieldlike

blossom. There are about 50 stamens grown together in 3 bundles above which a triple dark red pistil rises. When rubbed, flowers emit blood-red juice. The fruit is a capsule with a papillary oil tank (Lakusic 2004). It is widely spread in Europe, Asia and North Africa. *Hypericum perforatum* L. has very complex composition with a large number of bioactive components. These are primarily naftodiatrons, flavanols, flavonoids, floroglucinols and essential oils. A large number of components that have antimicrobial effects have been isolated from *Hypericum perforatum* L. In most of the isolated active components there are floroglucinol derivatives, hiperforin being among the most important. Floroglucinol derivatives identified in lipophilic fraction in few species of the genus *Hypericum* show antifungal and antibacterial activity against microorganisms such as *Staphylococcus aureus*, *Bacillus subtilis*, *Nocardia gardenen* (Trifunović *et al.* 1998, Ishiguro *et al.* 1987), while these derivatives have no activity towards *Candida albicans* (Vajs *et al.* 2003). Other researchers have shown that extracts of St. John's wort obtained by extraction with CO₂ under high pressure showed antifungal activity towards *Candida albicans* (Šmelcerović *et al.* 1998).

The fungus *Candida albicans* lives as a saprophyte on the skin, mouth mucosa, vagina and digestive tract of its host - humans. The entrance routes for the fungus in humans include the mucous membranes, damaged skin, nails, vagina and digestive tracts. It is believed that frequent use of broad-spectrum antibiotics, as well as corticosteroids have a significant impact on the pathogenic effect of the fungus, due to their favorable impact on its reproduction. Candidiadis prophylaxis involves the prevention of reproduction of the existing *Candida* in the body and personal hygiene. It has been shown that the use of small amounts of hiperforin even in strains with reduced sensitivity, there is no synergic resistance when used with clinical antibiotics (Hubner 2003).

Antiviral activity of the plants comes from hypericin which is considered to have also an antidepressant effect. Antiviral activity of hypericin has attracted great attention, especially when it comes to the treatment of AIDS (Glisic *et al.* 2006). Popadic (2000) reports that hypericin shows toxic activity against cancer cells in humans. Essential oils, tinctures, extracts and other galenic preparations are obtained from St. John's wort. St. John's wort oil acts generally on the damaged skin and vaginal mucous membranes as a beneficial lubricant and toning agent. In an external application of St. John's wort oil on open areas of the skin, it is necessary to protect these areas from direct sunlight due to the well-known phototoxic effect it may have if these areas are exposed to sunlight (Schaffner *et al.* 1999).

The main objectives of this study was to test the effects of *Hypericum perforatum* L. ethanol extract on growth of *Candida albicans* and gram positive (*Staphylococcus aureus* and *Streptococcus faecalis*) and gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria.

Materials and methods

Microbiological investigations were carried out at the Microbiological Laboratory, Faculty of Natural Sciences and Mathematics, Tuzla. Twenty one strains of *Candida albicans* isolated from sputum samples (5 strains), tongue swabs (3 strains), vaginal and cervical swabs (4 strains) and throat swab (9 strains) were tested.

Gram positive bacteria *Staphylococcus aureus* and *Streptococcus faecalis*, and Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* were analyzed as well.

St. John's wort extract was prepared from dried flowers collected from the site of Tuzla. The extraction was performed using a Soxlet machine (Banjanin 1998). Plant material was extracted with ethanol. A total of 20 g of dried flowers were wrapped in filter paper and placed in the machine connected to flasks containing the solvent. A flask of ethanol was heated in a water bath. Ethanol evaporated, steam entered the cooler, condensing the dried St. John's wort flowers, from which the red substance was extracted. The extraction process was repeated four times. The obtained extract was heated with steam in the water bath at a temperature of 90° C until dry and then diluted with sterile distilled water at a ratio of 1:2, 1:5, and 1:10. The obtained extract solutions were used to test the sensitivity of *Candida albicans* and bacterial strains. The sensitivity of both bacteria and fungi was tested using diffusion method. The method involves diffusion of the test substance through a solid nutrient medium previously inoculated with a certain microbial culture whose sensitivity is examined (Kavanagh 1972, NCCLS, 1990).

Muller-Hinton agar (MHA) containing 7% sheep blood and Sabourad Maltose agar (SMA) were used for fungi, and Mueller - Hinton agar for bacteria (NCCLS, 1990). The inoculum was prepared with fresh microbial day-old culture. One colony per culture was selected and suspended in sterile physiological solution until turbidity corresponded to 0.5 Mc-Farland scale. The plates were inoculated with the prepared suspension to obtain a homogeneous smear. Finally, holes 9 mm in diameter were made in the agar. A total of 100 µl of ethanol extract solutions were poured into the holes. The tested concentration for fungi was 1:2 of extract and that for bacteria - 1:2 (1ml of extract and 1ml of distilled water), 1:5 (1ml of extract and 4ml of distilled water) and 1:10 (1ml of extract and 9ml of distilled water).

Incubation was performed at 28 - 30° C for fungi and at 37°C for bacteria. After 18-24 hours, the results were determined by measuring the diameter of the inhibition zone in mm.

Results and discussion

The results on the effect of 21 samples of *Hypericum perforatum* L. collected from the Tuzla localities show resistance of *Candida albicans*. Literature data on the antifungal activity of St. John's wort are diverse. Our results are in agreement with those of a group of researchers from Serbia (Vajs *et al.* 2003). However, another group of authors (Šmelcerović *et al.* 1998) confirmed the sensitivity of the reference strain *Candida albicans* ATCC 24493. This should be associated with the methodology of extract preparation.

The results on the microbiological analysis of the effects of St. John's wort extract or *Hypericum perforatum* L. collected from the Tuzla site on *Candida albicans* strains are shown in Table 1 and Figure 1.

Tab. 1. Effect of *Hypericum perforatum* L. extract (solution 1:2) on *Candida albicans* samples

Ref. no.	Origin	Diameter of inhibition zone (mm) SMA	Blood agar
1.	sputum	0	0
2.	sputum	0	0
3.	sputum	0	0
4.	sputum	0	0
5.	sputum	0	0
6.	tongue	0	0
7.	tongue	0	0
8.	tongue	0	0
9.	vaginal and cervical swab	0	0
10.	vaginal and cervical swab	0	0
11.	vaginal and cervical swab	0	0
12.	vaginal and cervical swab	0	0
13.	throat swab	0	0
14.	throat swab	0	0
15.	throat swab	0	0
16.	throat swab	0	0
17.	throat swab	0	0
18.	throat swab	0	0
19.	throat swab	0	0
20.	throat swab	0	0
21.	throat swab	0	0

Fig. 1. Effect of St. John's wort extract (solution 1:2) on *Candida albicans*.

The results on the antibacterial activity of the test extract against gram-positive bacteria *Staphylococcus aureus* and *Streptococcus faecalis* are presented in Table 2 and Figure 2.

Tab. 2. Effect of *Hypericum perforatum* L. extract on gram positive bacteria

Bacteria	Solution		
	1:2	1:5	1:10
<i>Staphylococcus aureus</i>	34*	25	24
<i>Streptococcus faecalis</i>	30	27	26

* Diameter of inhibition zone (mm)



Fig. 2. Effect of *Hypericum perforatum* L extract on *Staphylococcus aureus*

The results obtained in this paper also confirm the evidence of other researchers (Trifunovic *et al.* 1998, Kavanagh 1972) on the inhibitory effect of St. John's wort extract on the gram positive bacteria tested.

St. John's wort extract has an inhibitory effect on growth of *Staphylococcus aureus* and *Streptococcus faecalis* at ratios of 1:2, 1:5 and 1:10. A low concentration (1:10 of extract) also induces high growth inhibition of these bacteria, forming zones of inhibition of 24 mm for *Streptococcus aureus* and 26 mm for *Streptococcus faecalis*. The results on the antibacterial activity of the tested extract against gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* are presented in Table 3 and Figure 3.

Tab. 3. Effect of *Hypericum perforatum* L. extract on gram negative bacteria

Bacteria	Solution		
	1:2	1:5	1:10
<i>Escherichia coli</i>	0*	0	0
<i>Pseudomonas aeruginosa</i>	0	0	0

* Diameter of inhibition zone (mm)



Fig. 3. Effect of St. John's wort extract on *Pseudomonas aeruginosa*

The present results as well as those of the group of researchers from Serbia (Vajs *et al.* 2003) show resistance of the test gram-negative bacteria to St. John's wort extract. The resistance is exhibited in all test solutions of *Hypericum perforatum* L.

Conclusion

Hypericum perforatum L. collected at the Tuzla locality has diverse antimicrobial effects.

Microbiological analysis revealed that St. John's wort extract collected from the Tuzla site shows no antifungal effect on *Candida albicans* strains isolated from different regions of the human body.

The gram-positive bacteria *Staphylococcus aureus* and *Streptococcus faecalis* showed high sensitivity to this extract, depending on the concentration.

Resistance of gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) on St. John's wort extract was also observed.

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DJELOVANJE EKSTRAKTA KANTARIONA (*Hypericum perforatum* L.) NA MIKROORGANIZME

- originalni naučni rad -

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Rezime

Hypericum perforatum L. je ljekovita biljka koja se tradicionalno koristi u nekoliko evropskih zemalja, kao sedativ, antiseptik, analgetik i diuretik. Ulje od kantariona je lek za rane, smiruje upalne procese, ima antimikrobno dejstvo na mnoge viruse, bakterije, gljivice. *Candida albicans* je pripadnik normalne flore sluzokože respiratornog, gastrointestinalnog, a kod žena i genitalnog trakta. Na takvim mjestima, one mogu da postanu dominantne i da izazovu patološka stanja, naročito kod imunokompromitovanih osoba. Smatra se da česta primjena antibiotika širokog spektra, kao i kortikosteroida ima značajan uticaj na patogeni efekat gljivice, jer utiče povoljno na njeno razmnožavanje. U ovom radu prezentirani su rezultati mikrobiološke analize uticaja ekstrakta kantariona sa lokaliteta Tuzla – sjeveroistočna Bosna, na kvasnicu *Candida albicans*, gram pozitivne i gram negativne bakterije. Ispitivanje je izvršeno na 21 soj *Candida albicans*, izolovanih iz različitih regija ljudskog tijela, i četiri vrste bakterija: *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli* i *Pseudomonas aeruginosa*. Rezultati istraživanja pokazuju rezistentnost sojeva *Candida albicans* na ekstrakt kantariona, kao i ispitivanih gram negativnih bakterija, dok su ispitivane gram pozitivne bakterije senzibilne na ovaj ekstrakt.