DOI: 10.7251/QOL1503045K **UDC:** 665.528.292.94

Original scientific paper

Antimicrobial Activity of Hypericum perforatum Essential Oil

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Abstract: European Centre for Disease Prevention and Control (ECDC) and World Health Organization (WHO) initiate public awareness campaign about antimicrobial substances and their rational uses due to the increasing prevalence of multidrug resistant strains of bacteria. The objective of this study was to evaluate antimicrobial activity of *Hypericum perforatum* essential oil and reference antimicrobial drugs against the growth of certain bacteria Staphylococcus aureus, Salmonella typhimurium i Pseudomonas aeruginosa.

Keywords: antimicrobial substances, antimicrobial activity, Hypericum perforatum, oleum hyperici.

Introduction

The usage of plants as medicine presents a very important phenomenon in the traditional medicine. By the time, almost all the folklore claims on these species has been scientifically proved and their usage in medicine and related fields have been reported. Until of the beginning of the twenty century, about 90% medical drugs were based on natural substances. Since olden times it is known antimicrobial activity of some plants (Borchardt et al 2008). In recent years, it was shown about 78000 aromatic and medical plants (Kovačević 2010). More than 1340 plants are recognised as potential antimicrobial components source, but only small number of plants was investigated (Wilkins 1989).

Hypericum perforatum L. (Hypericaceae) or St. John's Wort is one of the most popular medical plants worldwide. The genus *Hypericum* has about 400 species worldwide (Hickey et al., 1971). *Hypericum perforatum* L. is a perennial plant that bears extensive creeping rhizome with stem up to 1m high. The yellow flowers appear in broad cymes at the ends of the upper branches. *Hypericum perforatum* L. exhibits antibacterial, antiviral, antimycotic, anti-inflammatory and antidepressive activities (Singh Pal, 2006; Couceiro et al., 2006). *Oleum Hyperici* is obtained by maceration of the fresh flowering tops of *Hypericum perforatum* L. Hypericaceae (St. John's Wort) in olive, sunflower or wheat-germ oil exposed to sunlight for 40 days.

Figure 1. Hypericum perforatum L. and Oleum Hyperici

Oleum Hyperici is widely used for its various significant therapeutic activities. It is used internally in official and folk medicine for the treatment of dyspepsia and topically for the treatment of open wounds and blunt injuries, myalgia, first-degree burns, haemorrhoids, as an antiseptic, for liver and stomach complaints, diarrhea, ulcers of the stomach and duodenum, intestinal catarrh, as an antiphlogistic agent in the treatment of inflammation of the bronchi and urogenital tract, treatment of biliary disorders, bladder irritation, etc. Furthermore, it is well know use of *Oleum Hyperici* in massage due to rheumatic diseases (Neuwald 1954, Yesiland *et al.*1999).

Numerous compounds with documented biological activities have been reported from *H. perforatum* e.g. napthodianthrones, hypericin and pseudohypericin, different flavonoids like quercetin, hyperin etc., phloroglucinols, essential oils and xanthones (Upton, 1997; Bystrov, 1975; Gurevich *et al.*, 1971; Holzl *et al.*, 1989; Kitanov *et al.*, 1987; Rocha *et al.*, 1995; Khosa *et al*., 1982; Weyerstahl *et al.*, 1995).

Data concerning *Oleum Hyperici* chemical composition and pharmacological activity are limited. Studies proved the presence of red pigment-hypericin, pseudohypericin, flavonoids (quercetin, kampferol and biapigenin), tetrahydroxyxanthon and volatile oil (0.3%; main components of the volatile oil are aliphatic hydrocarbons, including, among others, 2-methyloctane, undecane, furthermore dodecanol, the prenylated phloroglucine derivative hyperforin and mono- and sesquiterpenes: including, among others, a-pinene, caryophyllene, additionally also 2-methyl-3-buten-2-ol). The *H. perforatum* (hyperforin) extracts effectiveness against methicilin-resistant *Staphylococcus aureus* and penicillin-resistant *Staphylococcus aureus* were previously reported ((Reeichling et al. 2001, Mschempp et al. 1999.). Different phytochemical constituents of *H. perforatum* like xanthones and flavonoid - hyperforin have been shown to be effective antimicrobials, antivirals and antibacterials against gram-positive bacteria and gram-negative bacteria. Clinical results showed that *Oleum Hyperici* have been effective in the treatment of psychogenic disturbances, depressive states and/or nervous excitements, with fewer side effects (Mennini and Gobbi 2004, Bradley 2006, Bradley 2009, Wolf, 1993, Gudžić et al., 2001, Fox et al. 2001).

Several authors reported that essential oils increase bactericidal effects of antimicrobial drugs (Hubner 2003). Excessive use of antimicrobial drugs and food additives in control of human and animal disease and infection was resulted in appearance of resistant bacteria. Due to that, there is a need for development of natural protection for humans and animals from bacterial infections. *Staphylococcus aureus*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* are the most common pathogenic bacteria that cause health and economical problems.

Salmonella spp. is a group of bacteria that can common cause of foodborne illness. In development European countries, Salmonella caused 84.5% of all epidemics and it is second pathogen in world that causes hospitalization adult persons (Linscott, 2011, Bacon, et al. 2003.,Fratamico., et al. 2005.,Sofos 2008.).

Staphylococcus aureus is a bacterium frequently found in the human respiratory tract and skin. About 25% healthy humans and animals are long-term carriers of *S.aureus* in nose. In different condition of temperature and pH some of strains of *Staphylococcus aureus* **cause food poisoning due to secreting termostable enterotoxin**. Stafylococcal toxin fast reacts. The first symptoms of poisoning appear 30 minutes to six hours after ingestion.

Pseudomonas aeruginosa is a Gram-negative opportunistic phatogen, widespread in nature, particularly in wet areas. This bacterium is well adapting to different environments conditions, such as natural and artificial environments, environment with antimicrobial drug, disinfectants or antiseptics. Previously was reported that *P.aeruginosa* thrives on the moist surfaces, and it can survive more than month on the dry floor, on dry filer paper until 150 days, in water more than 300 days (Hurst 1966). Due to all this characteristics and reputation for being resistant to disinfection, this opportunistic nosocomial phatogen can cause a wide range of infections, and is a leading cause of illness in immunocompromised individuals. In particular, it can be a serious pathogen in hospitals.

Pseudomonas aeruginosa is the most common cause of infection of drinking water that is a significant route of transmission in hospitals. It is still not clear if the colonization results from the water in the distribution system, or personnel use within the hospital. It causes urinary tract infections, particularly (Balcht et all. 1994., Iglewski 1996.,Anzai , et all. 2000., NNIS 1996). This illness are treated with antimicrobial drugs, but this treatment is often not effective due to appearance of resistant strains (Burnie et al.2009., Mahboubi and Kazempour 2009., Nostro et al. 2004., Poole K., 2004).

Beside bacterial species with developed resistance to one class of antimicrobial drugs, today it is obvious an increase in number of multiresistant bacteria with resistance on several antimicrobial drugs, different in chemical content and mechanism of acting (Hayouniet all. 2008., Newell et all. 2010., Wright et all. 2009., Hachem et all.2007).

The aim of this work is to investigate the influence of *H. perforatum* essential oil on growth of *Staphylococcus aureus*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* in relation to activity of different antimicrobial drugs. Furthermore, the aim is related to examination whether *H.perforatum* essential oil has bactericidal or bacteriostatic activity on these bacteria.

Material and methods

H.peforatum essential oil

It is used *Oleum Hyperici* obtained by maceration of the fresh flowering tops of *Hypericum perforatum* L. Hypericaceae (St. John's Wort) in olive oil exposed to sunlight for 40 days.

After 40 days, oil is filtered two times through the flax cloth, put into dark bothle, and stored in the dark place. *Oleum Hyperici* is used as a whole, mixed with 96% ethanol (1:1, and 2:1), and mixed with olive oil (1:2, 1:5 and 1: 10).

Test microorganism and medium

For examination of antimicrobial activity of *Oleum Hyperici* it is used referent culture *Staphylococcus aureus WDCM 00013, Salmonella typhimurium WDCM 0031 and Pseudomonas aeruginosa*WDCM 00024 (BCCM™/LMG BACTERIA COLLECTION, Belgium). Strains are cultured in nutrition broth and incubated on 37^0C/18h. Petri dishes with Müeller - Hinton agar (Laboratorios CONDA S.A Spain) are inoculated with 0.1 ml bacterial suspension with concentration 10^8cell/ml.

Antimicrobial drugs

For examination of bacterial sensitivity on referent antimicrobial drugs, it is used antibiogram method. It is used discus papers for antibiogram with adequate antimicrobial drugs („Liofilchem" s.r.I Italy): amikacin 30μg, azitromicin 15μg, cefotaxim 30μg, cefuroxim 30μg, ciprofloxacin 5μg, chloramphenicol 30 μg, erythromycin15 μg, gentamicin 10 μg ofloxacin 5 μg, penicillinG 10IJ, tetracycline 30 μg,vancomycin 30 μg, neomycin30μg.

Test method

For examination of *Oleum Hyperici* influence on inhibition of growth of *Staphylococcus aureus, Salmonella typhimurium* and *Pseudomonas aeruginosa* it is used difusion method of essential oil with cilinder (diameter 9 mm). Cilinder was put on the surface of the inoculated medium (Müeller- Hinton- agar-MHA). 100μl of essential oil was dropped in cilinder by micropypet, while in control 100μl of 96% ethanol.

Beside essentail oil, discus papers were put on plates as a referent antimicrobial drugs. Plates were incubated for 24h at 37ºC

Statistical analyses

Each trial was repeated three times, and results were presented as a diameter of growth inhibition zones and values were expressed as mean in millimetres.

Type of acting

It is determinated type of acting of essential oil. To examine wheather *Oleum Hyperici* has bactericidal or bacteriostatic properties, small pieces of agar from the zone of inhibition was taken and transferred to the nutrition broth. Incubation was lasted for 24h at 37ºC. Bactericidal properties of *Oleum Hyperici* were defined as a clear nutrition broth after incubation. Bacteriostatic properties of *Oleum Hyperici* were defined as a turbid nutrition broth after incubation.

Results and discussion

Table 1. Growth inhibition zone of Staphylococcus aureus, Salmonella typhimurium, Pseudomonas aeruginosa after treatment with different antimicrobial drugs

Antimicrobial drugs	Zone of inhibition in mm*		
	Staphylococcus aureus	Salmonella typhimurium i	Pseudomonas aeruginosa
Amikacin	16	18,3	30
Azithromycin	25	23,6	23,3
Ciprofloxacin	26,6	27,3	37,6
Cefuroxim	30	20	6
Cefotaxime	25,6	29	27,6
Gentamycin	20,6	17,3	29,3
Erythromycin	28,3	1	2,33
Ofloxacin	26,3	25	24
Penicillin	33,3	3	0,33
Chloramphenicol	25	28,3	0,66
Neomycin	18,3	13,6	20
Tetracycline	30	26,6	23
Vancomycin	14,3	0	0,33

The results obtained after examination of influence of different antimicrobial drugs on the growth of the *Staphylococcus aureus, Salmonella typhimurium, Pseudomonas aeruginosa* were showed in Table 1.

* The showed values are mean values (in mm) of three repetition for inhibition zone

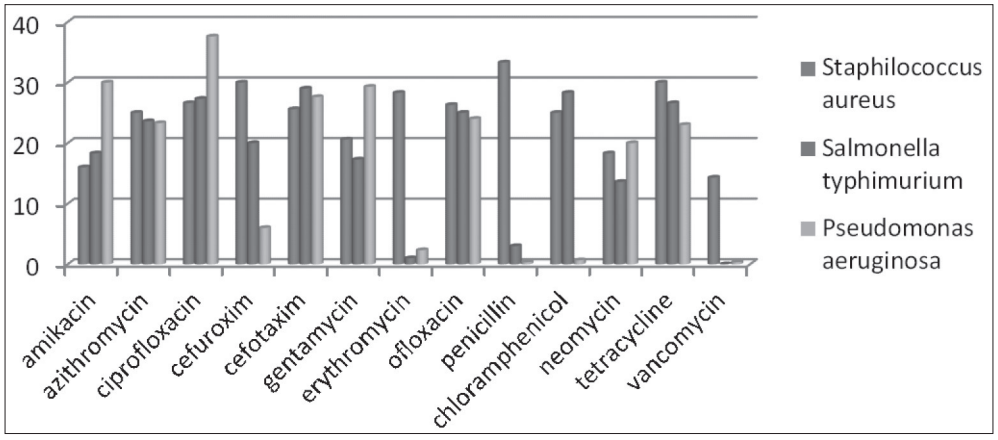

Figure 2. Growth inhibition zone of Staphylococcus aureus, Salmonella typhimurium, Pseudomonas aeruginosa after treatment with antimicrobial drugs

Cyprofloksacin induced the bigest zone of inhibition for the all three bacteria (Table 1). Good inhibitory effect was showed: cefotaksim, ofloksacin, azitromicin and tetraciklin, while amikacin, cefuroksim, gentamicin, eritromicin, penicilin, hloramfenikol, neomicin and vankomicin showed different effect depending on microorganism.

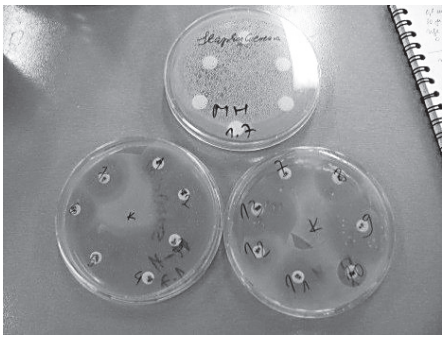

Figure 3. St. aureus

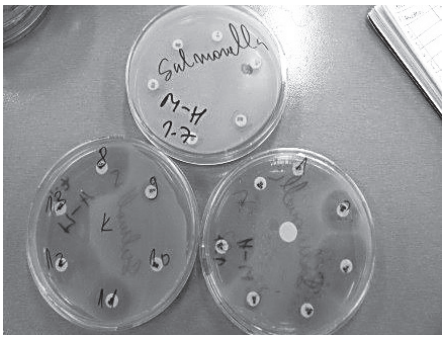

Figure 4. S . typhimurium

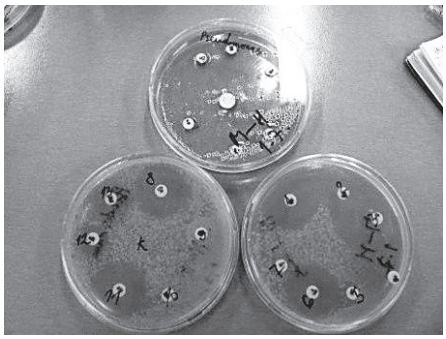

Figure 4. S . typhimurium

The influence of *Oleum Hyperici* on the growth of *Staphylococcus aureus, Salmonella typhimurium, Pseudomonas aeruginosa* were showed in Table 2.

Table 2. Growth inhibition zone of Staphylococcus aureus,Salmonella typhimurium, Pseudomonas aeruginosa after treatment with Oleum Hyperici*

	Zone of inhibition in mm*		
	Staphylococcus aureus	**Salmonella typhimurium**	**Pseudomonas aeruginosa**
Oleum Hyperici	4	0	3.33
Oleum Hyperici:ethanol 1:1	1.66	0	1.66
Oleum Hyperici:ethanol 2:1	2.66	0	6.66
Oleum Hyperici: Olive oil 1:2	4	0	16.66
Oleum Hyperici: Olive oil 1:5	2.33	0	20.00
Oleum Hyperici: Olive oil 1:10	2	0	23.33

* The showed values are mean values (in mm) of three repetition for inhibition zone

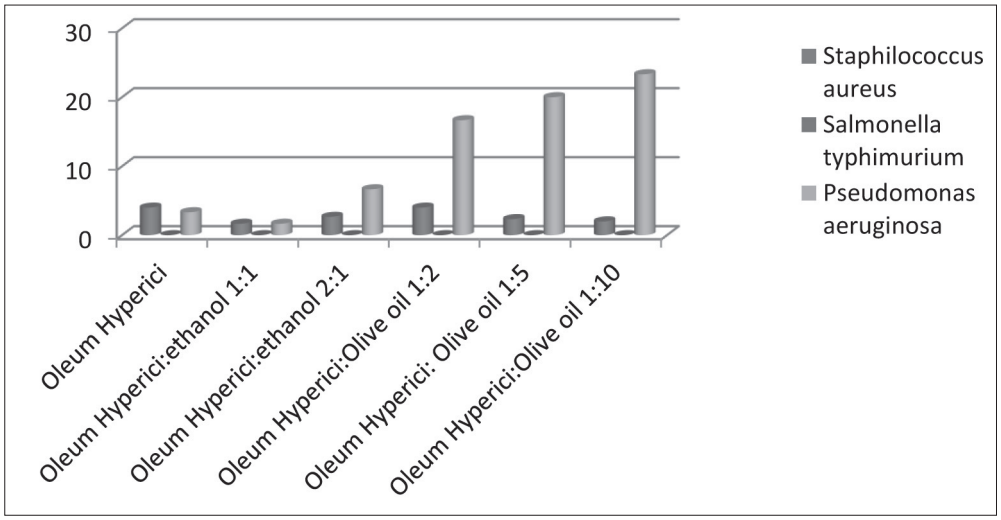

Figure 6. Growth inhibition zone of Staphylococcus aureus,Salmonella typhimurium, Pseudomonas aeruginosa after treatment with Oleum Hyperici

Results showed that *Oleum Hyperici* (diluted with olive oil) has good antimicrobial effect on the growth of *Pseudomonas aeruginosa*, while weak effect was observed with pure *Oleum Hyperici* and also diluted with ethanol (1:1 and 2:1).

These results agree with the previously reported results about antimicrobial properties of *Hypericum perforatum* (Gibson 2004, Saddige 2010, Gudžić et al 2001., Fox et all 2001, Šmelcerović et al 1998).

Oleum Hyperici had showed very weak inhibitory effect on growth of *Staphylococcus aureus,* but in our investigation, *Oleum Hyperici* in all combination did not show inhibitory effect on growth of *Salmonella typhimurium*.

Many studies showed antimicrobial effect of essential oils on Gram-positive bacteria. Additionally, it is indicated higher sensitivity of Gram positive bacteria on essential oils than Gram negative bacteria, which are *Pseudomonas aeruginosa* and *Salmonella typhimurium* (Juliano et al 2000, Ruberto, et al 2000, Senatore et al 2000.,Canillac and Mourey 2001., Demetzos and Perdetzoglou 2001, Lambert et al 2001., Marino et al 2001., Cimanga, et al 2002., Pintore .,et all.2002, Males et al .2006, Harpaz et al., 2003; Okoh et al., 2009).

There is no clear evidence about antimicrobial activity of essential oil, due to very different antimicrobial activity of essential oil on different testing microorganism, so it is difficult to determine correlation between antibacterial activity of essential oil and its main chemical compounds (Cavanagh, Wilkinson, 2005).

Furthermore, there is a difference between antimicrobial activities of essential oil and separate chemical compounds (Delaquis et al 2002).

Inhibitory activity of essential oil is a result of combined effects of active and nonactive oil compounds, which can induce additive, synergistic or antagonistic influence on antimicrobial activity of oil (Xianfei et al 2007).

Content of active compounds in *Hypericum p.* oil depend on geographical origin, ecological and agronomic condition and way of picking plants. Many compounds are sensitive to light, humidity or heat, so it is very important how preparations of *Hypericum p.* are stored. Phenolic derivatives of *Oleum Hyperici* (particularly thymol and carvacrol) had multiply activity, which is depending on concentration of these compounds in *Hypericum p.* (Panizzi, et al 1993. Svoboda et al 1998., Bakkali et al 2008,. Hyldgaard et al 2012.,Jayasena., Jo, C. 2013, Burt 2004., Lv F., et al 2011.,Bajpai et al 2012).

Based on obtained results, *Oleum Hyperici* showed good inhibitory effect on the growth of the *Pseudomonas aeruginosa,* thus it is suggested for use in pharmaceutics industry for preventing diseases caused by *Pseudomonas aeruginosa*.

Bactericidal effect in all combination of *Oleum Hyperici* was found for *Staphylococcus aureus, Salmonella typhimurium, Pseudomonas aeruginosa,* while bacteriostatic effects for *Pseudomonas aeruginosa,* respectively.

Conclusion

1. Results are showed that *Oleum Hyperici* showed antimicrobial activity on the growth of *Pseudomonas aeruginosa* and *Staphilococcus aureus*. There is a difference between antimicrobial activity of *Hypericum p.* essential oil in depending of type of microorganism and used thinner.
2. *Oleum Hyperici* showed inhibitory effect on the growth of *Salmonella typhimurium*
3. *Oleum Hyperici* showed bactericidal effect on growth of *Staphylococcus aureus* and bacteriostatic on *Pseudomonas aeruginosa*.

The knowledge obtained from this study could be applied for the development of novel formulation for *Oleum Hyperici* and its wider application in food industry, and thus preventing the consumers from poisoning spread by these pathogens.

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Recived: 10.04.2015.
Accepted: 04.12.2015.