Sensitivity of Multiresistant Bacteria and Methicillin-Resistant *Staphylococcus aureus* to ethanolic root extract of *Raphanus sativus*

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Raphanus sativus L. (Brassicaceae) is an edible plant, whose root is consumed all over the world. The objective of this study was to test antibacterial potential of _R. sativus_ ethanolic extract on 12 pathogenic bacteria including multiresistant bacterial strains and MRSA. All of the tested bacteria showed sensitivity to the antibacterial effect of _R. sativus_ ethanolic extract with minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) in range of 15 μg/mL – 500 μg/mL. It is interesting to note that _Raphanus_ extract showed the highest activity against multiresistant strain of _Pseudomonas aeruginosa_ with equal inhibitory and bactericidal concentrations of 15 μg/mL, while the most resistant strain to the effect of the extract was _Proteus mirabilis_ (MIC and MBC of the extract were 300 μg/mL and 500 μg/mL, respectively). In disk diffusion assay, zones of inhibitions were measured ranging from 8 mm to 22 mm.

Key words: *Raphanus sativus; ethanolic extract; antibacterial; multiresistant; methicillin-resistant*

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1. INTRODUCTION

Radish, the most common vegetable in the Brassicaceae family, is an edible root cultivated and consumed worldwide (Mithen et al., 2010). The most common radish cultivars are the well-known red varieties, while other varieties vary in size, color and cultivation requirements (Gutierrez and Perez, 2004; Hara et al., 2009). Radish has been used for food since prehistoric times. It was grown for its seed oil in Ancient Egypt, while some radish cultivars are grown for their leaves used as fodder (Al-Shehbaz, 1985; Davidson, 2014; Huxley et al., 1992; Mabberley, 2008). Besides being a food crop, radish also has various medicinal actions. It has been used in Estonian ethnopharmacology for relief of tumor symptoms (Sak et al., 2014), in India for issues like urinary problems and piles (Ahmad and Beg 2001) while in Mexican traditional medicine black radish roots are used for the treatment of cholesterol gallstones, and for decreasing serum lipid levels (Castro-Torres et al., 2012). It has potential for probiotic usage due to the presence of lactic acid bacterial strains such as *Lactobacillus plantarum* and *Lactobacillus fermentum* which could be isolated from fermented radishes (Damodharan et al., 2015). The roots stimulate appetite and digestion, having a tonic and laxative effect upon the intestines and indirectly stimulating the flow of bile (Chevallier 1996). The leaves, seeds and old roots are used in the treatment of asthma and other chest complaints (Duke and Ayensu, 1985). This paper studies growth inhibition of multiresistant bacteria and methicilin-resistant *Staphylococcus aureus* by *Raphanus sativus* root ethanolic extract. Multiresistance is antimicrobial resistance shown by a species of microorganism to multiple antimicrobial drugs. Some of multiresistant bacteria besides mentioned are: *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. *Proteus mirabilis* is a gram-negative, facultative anaerobic, rod-shaped bacterium. It causes 90% of all *Proteus* infections in human. It is widely distributed in soil and water. *Pseudomonas aeruginosa* is a gram-negative rod-shaped bacterium that can cause severe infections, especially in critically ill and immunocompromised patients (Bassetti et al., 2018). *Escherichia coli* is a gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. MRSA is any strain of *Staphylococcus aureus* that has developed multi-resistance to beta-lactam antibiotics, which include the penicillins and the

cephalosporins (Stapleton and Taylor, 2002).

2. MATERIALS AND METHODS

2.1. Sample collection and extract preparation

R. sativus (white variety of radish) was purchased from local supermarket, lyophilized and smashed to a fine powder, prior to extraction with ethanol. The powdered root of *R. sativus* sample (~10 g) was extracted by stirring with 300 mL of ethanol, at room temperature, 150 rpm, for 24 h. The extract was filtered through Whatman No. 4 paper. The residue was then re-extracted twice with additional portions (300 mL) of ethanol. The combined extracts were evaporated at 35 °C (rotary evaporator Büchi R-210, Flawil, Switzerland) to remove ethanol.

2.2. Microorganisms

The Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Staphylococcus aureus* (MRSA strain), *Bacillus cereus* (clinical isolate), Micrococcus flavus (ATCC 10240) and *Listeria monocytogenes* (NCTC 7973); as well as Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 35210), *Proteus mirabilis* and *Enterobacter cloacae* (human isolate), as well as multiresistant strains (MR) of *Escherichia coli* and *Pseudomonas aeruginosa* were used. Isolation and determination of clinical bacteria used in this study was previously described in our study (Kartsev et al., 2018).

2.2.1. Microdilution method

The antibacterial assay was carried out by a microdilution method (CLSI, 2009). The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/mL. Ethanolic extract was dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v) (10 mg/mL) and added in Tryptic Soy broth (TSB) medium (100 µL) with bacterial inoculum (1.0×10^4 CFU per well). The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MICs obtained from the susceptibility testing of various bacteria to tested extracts were determined also by a colorimetric microbial viability assay based on reduction of an INT ((p-iodonitrotetrazolium violet) [2-(4-iodophenyl)-3-(4-nitrphenyl)-5-phenyltetrazolium chloride; Sigma]) color and compared with positive control for each bacterial strain. The MBCs were determined by serial sub-cultivation of 2 µL into microtitre plates containing 100 µL of broth per well and further incubation for 24 h. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate manager 4.0 (Bio-Rad Laboratories) and compared with a blank (broth medium plus diluted extracts) and the positive control. Ampicillin (Panfarma, Belgrade, Serbia) was used as positive control (1 mg/mL in sterile physiological saline). Five percent DMSO was used as a negative control.

2.2.2. Disc-diffusion assay

Antibacterial activity of the extract was also determined using filter paper disc diffusion assay (Sokovic et al., 2008). Inoculums of the test bacteria were prepared equivalent to McFarland Standard 0.5. Uniform bacterial lawns were made using 100 µL inoculums on a nutrient agar plate. Filter paper (Whatman) discs (5.0 mm) soaked with test extract were placed over seeded plates. The plates were incubated at 37 °C for 24 h. Activity was measured in terms of zone of inhibition (mm). The net zone of inhibition was determined by subtracting the disc diameter (i.e. 5.0 mm) from the total zone of inhibition shown by the test disc in terms of clear zone around the disc. Ampicillin (Panfarma, Belgrade, Serbia) was used as positive control (1 mg/mL in sterile physiological saline). Five percent DMSO was used as a negative control.

Table 1. Minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of *Raphanus sativus* root ethanolic extract in mg/mL

Bacteria		*R. sativus* extract	Ampicillin
S. aureus	MIC	0.15	0.74
	MBC	0.15	1.24
S. aureus (MRSA)	MIC	0.1	>1.50
	MBC	0.15	>1.50
B. cereus	MIC	0.05	0.25
	MBC	0.05	0.37
M. flavus	MIC	0.05	0.49
	MBC	0.075	0.74
L. monocytogenes	MIC	0.1	0.37
	MBC	0.15	0.49
P. aeruginosa	MIC	0.2	0.25
	MBC	0.2	0.37
S. typhimurium	MIC	0.25	0.25
	MBC	0.3	0.37
E. coli	MIC	0.25	0.37
	MBC	0.3	0.37
E. cloacae	MIC	0.3	0.49
	MBC	0.4	0.74
P. mirabilis	MIC	0.3	0.74
	MBC	0.5	0.74
MR *E. coli*	MIC	0.05	>1.50
	MBC	0.05	>1.50
MR *P. aeruginosa*	MIC	0.015	>1.50
	MBC	0.015	>1.50

3. RESULTS AND DISCUSSION

The creation of drug-resistant pathogens has induced decline in the efficacy of traditional antimicrobial therapy. The resistance growth has been forced by prevalent use, and in some cases misuse, of antibacterial agents in treating a variety of infections (Spaulding et al., 2018). Previous studies reported good antimicrobial activity for *Raphanus* root (Gutierrez and Perez, 2004). Namely, juice obtained from root showed activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, while ethanolic and aqueous extracts showed activity against *Streptococcus mutans* and *Candida albicans*. In our study, all of the tested bacteria showed sensitivity to the *R. sativus* ethanolic extract with minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) ranging from 15 µg/mL to 500 µg/mL, tested by microdilution method (Table 1). Our results are better compared to previous results obtained by Ngoc et al. (2017) indicating higher activity. They showed that MIC values for *Salmonela typhi* and *Pseudomonas aeruginosa* were 40 mg/mL, while for *Bacillus cereus* and *Staphylococcus aureus* were 20 mg/mL. It is interesting to note that *Raphanus* extract showed the highest activity against multiresistant strain of *Pseudomonas aeruginosa* with equal inhibitory and bactericidal concentrations of 15 µg/mL, while the most resistant strain to the effect of the extract was *Proteus mirabilis* (MIC and MBC of the extract were 300 µg/mL and 500 µg/mL, respectively). The activity of the extract was slightly better

towards Gram negative bacteria compared to Gram positive bacteria. These differences in the activity might be attributed to different composition of cell walls of Gram positive and Gram negative bacteria investigated in this study. The activity of commercial drug ampicillin was more or less uniform towards tested bacterial strains. In most of the cases it was lower compared to the activity of *R. sativus* root ethanolic extract (Table 1). In disk diffusion assay, zones of inhibitions were measured and were in the range of 8 mm – 22 mm (Table 2). Again multiresistant *P. aeruginosa* was the most susceptible bacterium with inhibition zone of 22.0 mm, followed by *Staphylococcus aureus* (MRSA strain) with the inhibition zone of 21.4 mm. The most resistant was *Proteus mirabilis* with inhibition zone of 8.0 mm. Antibacterial properties that are proven for different parts of radish, with the root as the most active, are positively correlated with level of isothyocyanate compounds (Beevi et al., 2009). Component found widely in radishes, 4-(methylthio)-3-butenyl isothiocyanate, has been proven to exhibit strong antimicrobial and antimutagenic activities (Nakamura et al., 2001). Also, plant contains raphanin, which can inhibit growth of *Staphylococcus aureus*, *Escherichia coli*, *streptococci*, *pneumococci* etc. (Yeung, 1985).

Table 2. Diameters of inhibition zones in disc diffusion assay

Bacteria	*R. sativus* extract [mm]	Ampicillin [mm]
S. aureus	18.2	18.1
S. aureus (MRSA)	21.4	17.2
B. cereus	19.6	18.5
M. flavus	15.8	16.4
L. monocytogenes	15.2	18.1
P. aeruginosa	14.4	16.3
S. typhimurium	12.2	14.6
E. coli	10.4	16.7
E. cloacae	14.9	18.6
P. mirabilis	8.0	10.3
MR *E. coli*	9.2	8.1
MR *P. aeruginosa*	22.0	8.3

CONCLUSION

In summary the present study demonstrates good antibacterial activity of *R. sativus* ethanolic extract and is of importance for showing the effect towards strains of bacteria resistant to common therapeutic drugs, especially to *Pseudomonas aeruginosa*. It gives a baseline for researching *Raphanus* compounds involved in the exhibited antibacterial effect, identifying mechanisms of these compounds and also for further development of novel natural antibiotics.

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