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Antimicrobial activity of plant extracts of rose by-products from the essential oil industry against saprophytic and pathogenic microorganisms

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In a series of experiments, the inhibitory effect of extracts derived from by-products of rose-oil industry against pathogenic microorganisms, causing food toxicoinfection and intoxication, and saprophytic bacteria, yeasts and fungi, causing food spoilage, were determined. The lowest antimicrobial activity was established for aqueous extracts of the rose waste material while the highest antimicrobial activity was demonstrated by the aqueous-alcoholic (70%) extracts. The latter inhibited the growth of the test-pathogenic bacteria and yeast to varying degrees, with minimum inhibitory concentration (MIC) ranging from 6 ppm to 600 ppm. In determining the effect of the extracts on saprophytic microorganisms, all the extracts were found to inhibit the growth of spore-forming bacteria *Bacillus subtilis*, yeast *Candida utilis* and fungi *Rhizopus arrhizus*. The growth of the remaining fungi in the study was suppressed only by the 70% alcoholic extracts, with a MIC = 600 ppm for all. Gram-positive bacteria were more sensitive to the plant extracts tested (IZ=8-22.5 mm), with a MIC of 60 ppm. The Gram-negative bacteria tested were less sensitive (IZ=8-12.5 mm) with a MIC of 600 ppm. This was due to the difference in the structure and composition of the cell wall of the two bacterial groups. Therefore, alone or in combination with other extracts, they can be used for bio-preservation of foodstuffs.

Keywords: antimicrobial activity; waste valorization; rose species.

INTRODUCTION

There are 4 types of oil-bearing rose species in the world: *Rosa damascena* Mill., *Rosa centifolia* L., *Rosa gallica* L. and *Rosa alba* L. Distillation is the main method for extraction of most of the essential oils [1] along with extraction with various solvents. Hydrodistillation of *R. damascena* produces aromatic products such as rose oil and rose water. The essential oils in the flowers are widely used in pharmacy, perfumery, food industry, etc. A large amount of waste rose flowers remains as a by-product in the production of rose oil and rose water.

Many possibilities for utilization of the waste rose biomass have been investigated: obtaining aromatic products and increasing the essential oil yield [2]; utilization of waste rose biomass as a source of feed or feed additives [3]; use for compost [4]; biogas production; bio-adsorption of heavy metals [5]; isolation of bioactive substances and their application in the food industry or medicine [6]. The extracts from rose by-products were found to be rich in biologically-active substances [7-9]. Hence, the aim of the present study was to investigate the antimicrobial activity of rose by-products against saprophytic and pathogenic test microorganisms.

MATERIALS AND METHODS

Rose by-products investigated

	Designation	Species	Industrial processing	Location
1	RD/SD	*Rosa damascena*	Hydro-distillation	Mirkovo, Sofia
2	RD/CO_2	*Rosa damascena*	Extraction with supercritical CO_2	Mirkovo, Sofia
3	RD/H	*Rosa damascena*	Extraction with n-hexane	Zelenikovo, Brezovo
4	RD/F	*Rosa damascena*	Extraction with freon	Plovdiv
6	RA/SD	*Rosa alba*	Steam distillation	Tarnichane, Kazanlak
7	RA/CO_2	*Rosa alba*	Extraction with super-critical CO_2	Mirkovo, Sofia

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The extracts using water, 30% and 70% ethanol were prepared as described [7]. Briefly, 150 g of dry rose waste was treated with 1000 mL of water or ethanol solution for 1 h at 60°C with constant stirring, and then left for 24 h at room temperature. The mass was filtered, and the insoluble residue was returned for a second extraction with 500 mL at the same conditions. After filtration, the collected extracts were combined.

Determination of the antimicrobial activity against pathogenic and saprophytic micro-organisms.

Pathogenic test-microorganisms at concentrations (in brackets, cfu/mL) were used as follows: *Escherichia coli* ATCC 25922 (1.0×10^{12}), *Salmonella abony* NCTC 6017 (2.0×10^{8}), *Staphylococcus aureus* ATCC 25923 (4.0×10^{8}), *Listeria monocytogenes* ATCC 19111 (4.6×10^{9}), *Proteus vulgaris* ATCC 6380 (5.0×10^{11}), *Enterococcus faecalis* ATCC 19433 (1.2×10^{11}), *Candida albicans* NBIMCC 74 (2.1×10^{11}), *Pseudomonas aeruginosa* NBIMCC 1390, (1.0×10^{12}); saprophytic test-microorganisms at concentrations (cfu/mL in brackets): *Bacillus subtilis* ATCC 19659 (5.0×10^{9}), *Penicillium chrysogenum* ATCC 28089 (2.0×10^{7}), *Fusarium moniliforme* ATCC 38932 (1.0×10^{6}), *Aspergillus niger* ATCC 1015 (1.2×10^{7}), *A. flavus* ATCC 9643 (2.8×10^{7}), *Rhizopus arrhizus* ATCC 11145 (8.0×10^{6}), *Candida utilis* ATCC 42402 (4.6×10^{8}). All strains were deposited in the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria.

Preparation of the suspensions of the test pathogenic or saprophytic microorganisms: The test pathogenic or saprophytic microorganisms were cultured on Luria Bertani medium with glucose (LBG) agar medium (LB Broth, Miller-Novagen, Merck, Germany) at 37 ± 1ºC for 24–48 h for the pathogenic test-microorganisms and at 30 ± 1ºC for 24–48 h for the saprophytic microorganisms. Using a sterile loop biomass of the well-grown pathogenic or saprophytic microorganisms was suspended in sterile saline solution in order to obtain suspensions of the corresponding pathogenic or saprophytic microorganisms. The concentrations of the suspensions of the microorganisms were estimated after counting the single colonies formed after spread plating of the corresponding test-microorganism, followed by incubation of the inoculated Petri dishes at 37 ± 1ºC for 24–48 h for the pathogenic test-microorganisms and at 30 ± 1ºC for 24–48 h for the saprophytic test-microorganisms.

The antimicrobial activity was studied by the disc-diffusion method: sterile melted LBG agar medium (LB Broth, Miller Novagen, Merck, Germany) was poured in Petri dishes and after the hardening of the agar, the dishes were spread-plated with suspensions of the respective pathogenic test-microorganism. Sterile melted LBG agar medium was mixed with the respective saprophytic microorganism suspension and the mixture was poured in empty Petri dishes and was left to solidify. Decimal dilutions of the extracts in saline solution were prepared. Ampicillin paper discs (BB-NCIPD Ltd.) were used as positive control for *E. coli* ATCC 25922, *S. abony* NCTC 6017, *S. aureus* ATCC 25923, *P. aeruginosa* NBIMCC 1390, *B. subtilis* ATCC 6633; nystatin paper discs (BB-NCIPD Ltd.) were used as positive control for *C. albicans* NBIMCC 74*, P. chrysogenum* ATCC 10106, *F. moniliforme* ATCC 38932, *A. niger* ATCC 9029, *A. flavus* ATCC 9643; and actidione paper discs (BB-NCIPD Ltd.) were used as positive control for *S. cerevisiae* ATCC 7754. Saline solution containing 1% (v/v) Tween® 80 was used as negative control. The experiments were conducted with dilutions 10^{0}, 10^{-1}, and 10^{-2} in order to determine the minimum inhibitory concentration (MIC). The used paper discs were 6 mm in diameter. Six μL of the corresponding dilution were pipetted on the corresponding paper discs. The results were recorded as diameters of the clear zones around the paper discs, in millimeters, after 24–48 h of incubation of the Petri dishes at an optimal temperature for the growth of the corresponding test-microorganism − 37 ± 1ºC for 24–48 h for the Petri dishes with the pathogenic microorganisms and at 30 ± 1ºC for 24–48 h for the Petri dishes with the saprophytic microorganisms. The MIC was defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth [10]. The MICs, in ppm, were calculated based on the obtained results [11].

Statistical analysis

Data from quadruplicate experiments were processed with MS Office Excel 2013 software, using statistical functions to determine the standard deviation and the maximum estimate error at significance level $p<0.05$.

RESULTS AND DISCUSSION

The results of the experimental studies on the determination of the antimicrobial action of aqueous extracts, extracts (30% and 70% ethanol) are presented in Tables 1, 2 and 3. The type and amount of bioactive substances in the extracts with

antimicrobial effect varied depending on the industrial treatment. Aqueous extracts of rose waste materials contained significant amounts of phenylethyl alcohol, β-citronellol, nerol, geraniol, nonadecane, ferulic acid, gallic acid, 3,4-dihydroxy benzoic acid, neochlorogenic acid, and other substances with inhibitory action [7-9], with the highest content being determined in extract 3 (3RD/H) obtained from *R. damascena* waste, extracted with n-hexane, which also demonstrated the highest antimicrobial activity against the pathogenic microorganisms causing food poisoning and intoxication, included in the study (Table 1).

The MIC for this rose extract varied from 60 ppm to 600 ppm. The results for extracts 1 and 2 of waste raw materials of *R. damascena* were similar. The inhibitory activity of extract 4 of this rose species was lower (Table 1). The aqueous extracts obtained from waste materials of *R. alba* had weak effect on the growth of pathogenic bacteria and yeasts (Table 1).

The data for the experimental studies of water-alcohol extracts (30% ethanol) from rose waste raw materials were similar to the results discussed above (Table 2). The change in the type and concentration of the substances with inhibitory action was weak, therefore the influence of the obtained extracts on the growth of the pathogenic test-microorganisms was similar (Table 2).

The highest antimicrobial activity against the pathogens included in the studies was determined in water-alcohol (70% ethanol) extracts from rose waste materials (Table 3). All aqueous-alcoholic (70% ethanol) extracts from waste raw materials of *R. damascena* inhibited the growth of all pathogenic bacteria and yeast, with MIC values being less than 60 ppm or equal to 600 ppm. The largest inhibition zones (IZ) were determined in the aqueous-alcoholic extracts 3 and 4 of *R. damascena* (Table 3). The inhibitory effect of the aqueous-alcoholic extracts from waste materials of *R. alba* was lower.

Gram-positive bacteria were more sensitive to the plant extracts tested (IZ=8-22.5 mm), with a minimum inhibitory concentration of 60 ppm. The Gram-negative bacteria tested were less sensitive (IZ=8-12.5 mm) with a minimum bactericidal concentration of 600 ppm. This was due to the difference in the structure and composition of the cell wall of the two bacterial groups.

The higher inhibitory activity of the aqueous-alcoholic extracts of roses was a result of the extraction of higher concentrations of geranyl acetate, β-bourbonene, β-cubebene, trans-nerolidol, etc., as well as the increased percentage of phenylethyl alcohol [7]. Their content was high in all rose extracts, regardless of the industrial processing.

It is noteworthy that with increasing the amount of alcohol in the extracts decreased the concentration of organic acids demonstrating inhibitory effect on the growth of the test-microorganisms (Tables 2, 3, 5, 6). This weakly affected the antimicrobial activity and was at the expense of the higher concentrations of the extracted substances with antimicrobial action. The obtained results confirmed the data of studies of other authors for the presence of antimicrobial activity in alcoholic extracts of rose blossoms against *E. coli, Salmonella* sp., *S. aureus, P. vulgaris, P. aeruginosa* [12, 13].

The inhibitory effect of aqueous and aqueous-alcoholic extracts obtained from rose waste raw materials against saprophytic bacteria, yeasts and fungi causing microbial spoilage of food and beverages, was determined in a series of experiments (Tables 4-6). The obtained results confirmed the data obtained in the examination of the antimicrobial activity of the extracts against the pathogenic test-microorganisms. The aqueous extracts from rose waste materials showed a weak antimicrobial effect against spore-forming bacteria, yeasts and fungi (Table 4). 30% aqueous-alcoholic extracts followed in terms of activity (Table 5) and the highest inhibitory effect was determined in 70% aqueous-alcoholic extracts (Table 6). The aqueous-alcoholic extracts of *R. damascena* (1) had the highest antimicrobial action. It should be noted that all 70% aqueous-alcoholic extracts inhibited the growth of spore-forming bacteria of *B. subtilis* ATCC 19659, and the MIC ranged from 60 ppm to less than 600 ppm (Table 6). In the saprophytic yeast *C. utilis* ATCC 42402 the MIC was 60 ppm to 600 ppm. Differences were observed within the fungal test-microorganisms. The effect of the extracts on the spores of the *Aspergillus* species was weaker (Tables 5 and 6). The obtained results confirmed the data from the studies of other authors for the antimicrobial activity of aqueous and aqueous-alcoholic rose extracts against *B. subtilis, Aspergillus* sp., *P. chrysogenum* [13].

The determined high antimicrobial activity of the studied extracts from rose waste materials is a prerequisite for the application of the obtained extracts in food and beverage production as potential bio-preservatives.

Table 1. Antimicrobial activity and MIC of different water plant extracts against pathogenic microorganisms (MO). Inhibition zones (IZ) in mm. d_{disc} =6 mm.

Test-MO / Sample	*E. .coli* ATCC 25922		*S. abony* NCTC 6017		*S. aureus* ATCC 25923		*L. monocytogenes* ATCC 19111		*P.vulgaris* ATCC 6380		*E. faecalis* ATCC 19433		*P. aeruginosa* NBIMCC 1390		*C. albicans* NBIMCC 74	
	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm
1/H_2O 2018	-	-	-	-	9.17 ± 0.47^{a}	600	9.17 ± 0.47^{a}	600	10.17 ± 0.24^{a}	600	10.17 ± 0.24^{b}	600	10.17 ± 0.24^{c}	600	9.17 ± 0.47^{c}	600
2/H_2O 2018	-	-	9.67 ± 0.47^{a}	600	9.17 ± 0.47^{a}	600	9.17 ± 0.47^{a}	600	11.17 ± 0.47^{a}	>60	9.17 ± 0.47^{c}	600	11.17 ± 0.47^{b}	>60	10.17 ± 0.47^{b}	600
3//H_2O 2018	9.17 ± 0.24^{a}	600	14.67 ± 0.47^{b}	60	-	-	12.17 ± 0.24^{b}	60	10.17 ± 0.24^{a}	600	12.17 ± 0.47^{a}	60	13.17 ± 0.47^{a}	60	12.67 ± 0.47^{a}	60
4/H_2O 2018	-	-	9.17 ± 0.24^{a}	600	-	-	15.17 ± 0.47^{c}	60	10.17 ± 0.47^{a}	600	-	-	12.67 ± 0.47^{a}	60	11.67 ± 0.47^{a}	>60
6/H_2O 2018	-	-	-	-	-	-	-	-	12.17 ± 0.47^{b}	60	-	-	9.17 ± 0.47^{d}	600	-	-
7/H_2O 2018	-	-	9.17 ± 0.47^{a}	600	-	-	-	-	10.17 ± 0.47^{a}	600	-	-	-	-	-	-

$^{a, b, c, d}$ – the different letters show statistically different results in a column (one-way ANOVA (Tukey's criterion; $p < 0.05$).

Table 2. Antimicrobial activity and MIC of different plant extracts (in 30% ethanol) against pathogenic microorganisms (MO). Inhibition zones (IZ) in mm. d_{disc} =6 mm.

Test-MO / Sample	*E. coli* ATCC 25922		*S. abony* NCTC 6017		*S. aureus* ATCC 25923		*L. monocytogenes* ATCC 19111		*P. vulgaris* ATCC 6380		*E. faecalis* ATCC 19433		*P. aeruginosa* NBIMCC 1390		*C. albicans* NBIMCC 74	
	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm
1/30% 2018	10.17 ± 0.47^{a}	600	12.17 ± 0.47^{a}	>60	9.17 ± 0.47^{a}	600	9.17 ± 0.47^{a}	600	11.17 ± 0.47^{a}	>60	9.17 ± 0.47^{b}	600	9.17 ± 0.47^{b}	600	10.17 ± 0.47^{d}	600
2/30% 2018	10.17 ± 0.24^{a}	600	$10.17\pm0.47^{b,d}$	600	9.17 ± 0.47^{a}	600	9.17 ± 0.47^{a}	600	17.67 ± 0.47^{b}	60	10.17 ± 0.47^{a}	600	$9.67\pm0.47^{a,b}$	600	15.17 ± 0.47^{a}	60
3/30% 2018	10.17 ± 0.47^{a}	600	16.17 ± 0.47^{c}	<60	-	-	17.67 ± 0.47^{b}	<60	17.67 ± 0.47^{b}	<60	-	-	10.17 ± 0.47^{a}	600	12.17 ± 0.47^{c}	60
4/30% 2018	9.17 ± 0.47^{a}	600	15.17 ± 0.47^{c}	<60	-	-	15.17 ± 0.47^{c}	<60	16.67 ± 0.47^{b}	<60	-	-	9.17±0.47b	600	14.17 ± 0.47^{b}	<60
6/30% 2018	-	-	-	-	-	-	9.17 ± 0.47^{a}	600	-	-	-	-	-	-	-	-
7/30% 2018	-	-	9.17 ± 0.47^{b}	600	-	-	10.17 ± 0.24^{d}	600	-	-	-	-	-	-	-	-

$^{a, b, c, d}$ – the different letters show statistically different results in a column (one-way ANOVA (Tukey's criterion; $p < 0.05$).

Table 3. Antimicrobial activity and MIC of different plant extracts (in 70% ethanol) against pathogenic microorganisms (MO). Inhibition zones (IZ) in mm. d_{disc} =6 mm.

Test-MO / Sample	*E. coli* ATCC 25922		*S. abony* NCTC 6017		*S. aureus* ATCC 25923		*L. monocytogenes* ATCC 19111		*P. vulgaris* ATCC 6380		*E. faecalis* ATCC 19433		*P. aeruginosa* NBIMCC 1390		*C. albicans* NBIMCC 74	
	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm
1/70% 2018	12.67±0.47^{a}	60	11.67±0.47^{a}	>60	10.67±0.24^{a}	600	18.17±0.47^{a}	60	12.17±0.47^{a}	60	9.67±0.47^{a}	600	10.17±0.24^{a}	600	12.67±0.47^{d}	60
2/70% 2018	12.67±0.47^{a}	60	11.67±0.47^{a}	>60	10.17±0.24^{a}	600	9.17±0.47^{b}	600	15.17±0.47^{b}	60	9.67±0.47^{a}	600	11.67±0.47^{a}	>60	13.67±0.47^{c}	60
3/70% 2018	10.17±0.47^{b}	600	15.17±0.47^{b}	<60	10.17±0.47^{a}	600	17.17±0.47^{a}	<600	9.67±0.47^{c}	600	10.17±0.47^{a}	600	10.17±0.47^{a}	600	15.17±0.47^{b}	<60
4/70% 2018	10.17±0.47^{b}	600	13.67±0.47^{c}	60	10.17±0.47^{a}	600	15.17±0.47^{c}	<600	14.17±0.47^{b}	<60	10.17±0.47^{a}	600	10.17±0.47^{a}	600	16.67±0.47^{a}	<60
6/70% 2018	10.17±0.47^{b}	600	10.17±0.47^{a}	600	10.17±0.47^{a}	600	9.17±0.47^{b}	600	9.17±0.47^{c}	600	-	-	10.17±0.47^{a}	600	9.17±0.47^{f}	600
7/70% 2018	9.67±0.47^{b}	600	10.17±0.24^{a}	600	10.17±0.24^{a}	600	9.67±0.47^{b}	600	9.67±0.47^{c}	600	10.17±0.24^{a}	600	10.17±0.24^{a}	600	10.17±0.24^{e}	600

a, b, c, d, e, f – the different letters show statistically different results in a column (one-way ANOVA (Tukey's criterion; $p <0.05$).

Table 4. Antimicrobial activity and minimum inhibitory concentration (MIC) of different water plant extracts against saprophytic microorganisms (MO). Inhibition zones (IZ) in mm. d_{disc} =6 mm.

Test-MO / Sample	*B. subtilis* ATCC 19659		*C. utilis* ATCC 42402		*A. niger* ATCC 1015		*P. chrysogenum* ATCC 28089		*F. moniliforme* ATCC 38932		*R. arrhizus* ATCC 11145		*A. flavus* ATCC 9643	
	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm
1/H_2O 2018	-	-	9.17±0.24^{a}	600	-	-	-	-	11.00±0.00^{c}	60	9.17±0.24^{b}	600	-	-
2/H_2O 2018	9.17±0.47^{a}	600	9.17±0.24^{a}	600	9.17±0.24	600	-	-	9.17±0.70^{d}	600	9.17±0.47^{b}	600	-	-
3//H_2O 2018	9.17±0.47^{a}	600	13.17±0.47^{b}	60	-	-	-	-	15.17±0.47^{a}	60	10.17±0.47^{a}	600	-	-
4/H_2O 2018	-	-	14.17±0.47^{b}	<600	-	-	-	-	-	-	9.67±0.47^{a,b}	600	15.17±0.24^{a}	60
5/H_2O 2018	-	-	-	-	-	-	-	-	10.17±0.47^{c}	600	10.17±0.24^{a}	600	-	-
6/H_2O 2018	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7/H_2O 2018	-	-	9.17±0.24^{a}	600	-	-	-	-	13.67±0.47^{b}	60	-	-	10.17±0.24^{b}	600

a, b, c, d – the different letters show statistically different results in a column (one-way ANOVA (Tukey's criterion; $p <0.05$).

Table 5. Antimicrobial activity and MIC of different plant extracts (in 30% ethanol) against saprophytic microorganisms (MO). Inhibition zones (IZ) in mm. d_{disc} =6 mm.

Test-MO / Sample	*B. subtilis* ATCC 19659		*C. utilis* ATCC 42402		*A. niger* ATCC 1015		*P. chrysogenum* ATCC 28089		*F. moniliforme* ATCC 38932		*R. arrhizus* ATCC 11145		*A. flavus* ATCC 9643	
	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm
1/30% 2018	9.17 ± 0.24^{a}	600	13.17 ± 0.24^{a}	<600	15.00 ± 0.10^{a}	>60	-	-	9.17 ± 0.24^{b}	600	$9.67 \pm 0.47^{c,d}$	600	-	-
2/30% 2018	10.17 ± 0.47^{b}	600	9.17 ± 0.24^{b}	600	14.17 ± 0.24^{b}	60	-	-	9.17 ± 0.24^{b}	600	9.17 ± 0.47^{d}	600	9.33 ± 0.47	600
3/30% 2018	10.17 ± 0.24^{b}	>60	16.17 ± 0.47^{c}	60	-	-	-	-	-	-	11.17 ± 0.47^{a}	<600	-	-
4/30% 2018	11.17 ± 0.47^{b}	60	14.17 ± 0.47^{d}	60	-	-	-	-	-	-	$10.17 \pm 0.47^{b,c}$	600	-	-
5/30% 2018	-	-	-	-	-	-	9.17 ± 0.47	600	16.17 ± 0.47^{a}	60	-	-	-	-
6/30% 2018	9.17 ± 0.47^{a}	600	-	-	-	-	-	-	-	-	-	-	-	-
7/30% 2018	9.17 ± 0.24^{a}	600	13.17 ± 0.24^{a}	<600	15.00 ± 0.10^{a}	>60	-	-	9.67 ± 0.47^{b}	600	$10.67 \pm 0.47^{a,b}$	600	-	-

a, b, c, d – the different letters show statistically different results in a column (one-way ANOVA (Tukey's criterion; $p < 0.05$).

Table 6. Antimicrobial activity and MIC of different plant extracts (prepared with 70% ethanol) against saprophytic microorganisms (MO). Inhibition zones (IZ) in mm. d_{disc} = 6 mm.

Test-MO / Sample	*B. subtilis* ATCC 19659		*C. utilis* ATCC 42402		*A. niger* ATCC 1015		*P. chrysogenum* ATCC 28089		*F. moniliforme* ATCC 38932		*R. arrhizus* ATCC 11145		*A. flavus* ATCC 9643	
	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm
1/70% 2018	13.17 ± 0.47^{a}	<600	14.17 ± 1.24^{a}	60	9.17 ± 0.47^{a}	600	9.17 ± 0.47^{a}	600	13.33 ± 0.47^{a}	60	13.33 ± 0.47^{a}	60	$9.00 \pm 0{,}00$	600
2/70% 2018	14.17 ± 0.47^{a}	<600	10.17 ± 0.47^{b}	600	14.17 ± 0.47^{b}	60	12.17 ± 0.24^{b}	600	10.17 ± 0.47^{b}	600	10.17 ± 0.47^{d}	600	-	-
3/70% 2018	11.17 ± 0.24^{b}	60	15.67 ± 0.47^{a}	60	9.17 ± 0.47^{a}	600	$10.17 \pm 0.47^{a,c}$	600	10.17 ± 0.47^{b}	600	9.67 ± 0.47^{d}	600	-	-
4/70% 2018	10.17 ± 0.47^{c}	600	17.17 ± 0.47^{c}	60	-	-	10.67 ± 0.47^{c}	600	13.17 ± 0.24^{a}	60	11.17 ± 0.47^{c}	<600	-	-
5/70% 2018	9.67 ± 0.47^{c}	600	9.17 ± 0.47^{b}	600	9.17 ± 0.47^{a}	600	9.67 ± 0.47^{a}	600	-	-	9.67 ± 0.47^{d}	600	-	-
6/70% 2018	10.67 ± 0.47^{c}	600	9.67 ± 0.47^{b}	600	9.17 ± 0.47^{a}	600	9.17 ± 0.47^{a}	600	9.17 ± 0.47^{c}	600	9.67 ± 0.47^{d}	600	-	-
7/70% 2018	13.17 ± 0.47^{a}	<600	14.17 ± 1.24^{a}	60	9.17 ± 0.47^{a}	600	9.17 ± 0.47^{a}	600	-	-	12.17 ± 0.47^{b}	>60	-	-

a, b, c, d – the different letters show statistically different results in a column (one-way ANOVA (Tukey's criterion; $p < 0.05$).

CONCLUSION

The inhibitory effect of plant extracts derived from waste raw materials from the rose essential oil industry against pathogenic microorganisms, causing food toxicoinfection and intoxication, and saprophytic bacteria, yeasts and fungi, causing food spoilage, were determined. The lowest antimicrobial activity was established for aqueous extracts of the rose waste material while the highest antimicrobial activity was demonstrated by the aqueous-alcoholic (70%) extracts. The latter inhibited the growth of the test-pathogenic bacteria and yeast to varying degrees, with MICs ranging from 6 ppm to 600 ppm. In determining the effect of the extracts on saprophytic microorganisms, all the extracts were found to inhibit the growth of spore-forming bacteria *B. subtilis*, yeast *C. utilis* and fungi *R. arrhizus*. The growth of the remaining fungi in the study was suppressed only by the 70% alcoholic extracts, with a MIC = 600 ppm for all. Gram-positive bacteria were more sensitive to the plant extracts tested (IZ=8-22.5 mm), with a minimum inhibitory concentration of 60 ppm. The Gram-negative bacteria tested were less sensitive (IZ=8-12.5 mm) with a minimum bactericidal concentration of 600 ppm. This was due to the difference in the structure and composition of the cell wall of the two bacterial groups. Therefore, alone or in combination with other extracts, they can be used for the biopreservation of food and beverages.

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