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ANTIMICROBIAL ACTIVITY OF EXTRACTS OBTAINED FROM SELECTED FRUIT AND VEGETABLE PROCESSING BYPRODUCTS

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INTRODUCTION

Processing of fruits, vegetables, cereals, and other agricultural raw materials generates significant quantities of byproducts, which are often discarded as waste. In recent years, increased environmental awareness and the growing adoption of circular economy principles have stimulated interest in the valorization of agro-food by-products as sustainable and renewable sources of valuable bioactive compounds, including polyphenols, pigments, dietary fibers, and other functional biomolecules. Their effective utilization contributes not only to waste reduction and environmental protection but also to the development of innovative, value-added bio-based products. Therefore, the present study aimed to evaluate the antimicrobial activity of pigment-rich extracts from selected fruit and vegetable processing by-products against selected Gram-positive and Gram-negative bacteria, and yeasts, specifically sour cherry pomace and the peels of eggplant, red onion, purple sweet potato, beetroot, pomegranate, tangerine, orange, lemon, grapefruit, and lime.

MATERIALS AND METHODS

Extracts preparation: Pigment-rich extracts from selected fruit and vegetable by-products were obtained by the ultrasound-assisted extraction (50 °C, 30 min) (table 1). The extracts was completely dried using a vacuum evaporator and then dissolved in a specific volume of the solvent dimethyl sulfoxide (DMSO) to achieve a final concentration of 50 mg/mL (disc diffusion method) and 100 mg/mL (agar well diffusion method).

Antimicrobial activity: Gram-positive bacteria, Gram-negative bacteria, lactic acid bacteria and yeasts were used to evaluate the antimicrobial properties of the tested extracts. After overnight growth of cultures at 37 °C in Mueller Hinton broth (Gram-positive and Gram-negative bacteria), MRS broth at 32 ° C (lactic acid bacteria), and Mueller Hinton broth with 2 % glucose at 28 ° C (yeast), cell density was adjusted to 10^6 cell/mL. Antimicrobial activity was preliminarily determined using the disc diffusion method. Agar plates were inoculated directly from the suspension with a sterile cotton swab according to the CLSI protocol. Filter paper discs (d=6 mm, Macherey-Nagel, Germany) were then placed on the inoculated medium and 10 µL of extract was applied to the discs. Petri dishes were incubated, and zones of inhibition (mm) were measured with a ruler after 24 hours. Kanamycin (50 µg) and nystatin (100 U) discs (Biolab, Hungary) were used as positive controls and DMSO as negative control. Furthermore, based on the obtained results, antimicrobial activity of selected extracts (red onion, pomegranate and lime peel) was also assessed using the agar well diffusion method (figure 1). Briefly, after agar plate inoculation wells (d=9 mm) were created in agar and filled with 100 µL of extract. Petri dishes were incubated, and zones of inhibition (mm) were measured with a ruler after 24 hours (table 2).

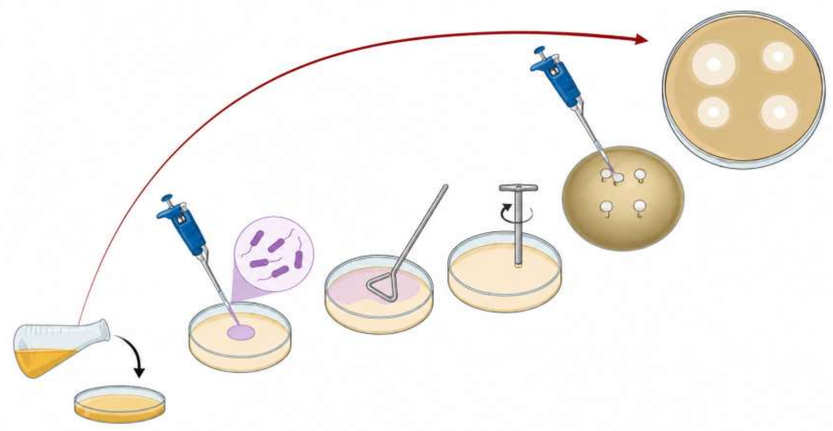

Figure 1. Shematic diagram of agar well diffusion method

RESULTS

Table 1. Overview of selected fruit and vegetable by-product extracts

Sample number	By-product type	Targeted pigments	Solvent	Pigment concertation
1	Sour cherry pomace	anthocyanins	30 % ethanol + citric acid	152.84 mg/100 g dm
2			70 % ethanol + citric acid	111.39 mg/100 g dm
3	Eggplant peel	anthocyanins	30 % ethanol + citric acid	75.92 mg/100 g dm
4			70 % ethanol + citric acid	69.71 mg/100 g dm
5	Red onion peel	anthocyanins	30 % ethanol + citric acid	424.39 mg/100 g dm
6			70 % ethanol + citric acid	322.12 mg/100 g dm
7	Purple sweet potato peel	anthocyanins	30 % ethanol + citric acid	124.49 mg/100 g dm
8			70 % ethanol + citric acid	116.89 mg/100 g dm
9	Pomegranate peel	anthocyanins	30 % ethanol + citric acid	21.59 mg/100 g dm
10			70 % ethanol + citric acid	12.99 mg/100 g dm
11	Beetroot peel	betalains	water	1153.42mg/100 g dm
12			ethanol	926 mg/100 g dm
13	Tangerine peel	carotenoids	96 % ethanol	12.76 mg/100 g dm
14			acetone	20.02 mg/100 g dm
15	Orange peel	carotenoids	96 % ethanol	15.29 mg/100 g dm
16			acetone	21.23 mg/100 g dm
17	Lemon peel	carotenoids	96 % ethanol	0.49 mg/100 g dm
18			acetone	2.23 mg/100 g dm
19	Grapefruit peel	carotenoids	96 % ethanol	5.56 mg/100 g dm
20			acetone	6.88 mg/100 g dm
21	Lime peel	chlorophylls	96 % ethanol	9.11 mg/100 g dm
22			acetone	19.39 mg/100 g dm

Table 2. Antimicrobial activity of pigment-rich extracts against selected test-microorganisms expressed as growth inhibition zones (mm) (agar well diffusion method)

Test-microorganisms		Growth inhibition zone diameter (mm)*									
		Gram-negative bacteria			Gram-positive bacteria			Yeasts			Latic acid bacteria
Sample number		*E. coli*	*Salmonella*	*P. aeruginosa*	*S. aureus*	*L. monocytogenes*	*B. subtilis*	*S. cerevisiae*	*C. utilis*	*Rhodot-horula*	*L. plantarum*
5	Red onion peel	13	14	12	13	24	17	-	15	13	18
6		13	14	14	12	25	17	-	16	12	18
9	Pomegran-ate peel	-	-	-	-	21	-	17	19	26	-
10		-	-	-	-	21	-	16	18	21	-
21	Lime peel	12	13	12	12	15	17	-	23	17	-
22		-	-	-	-	13	11	-	17	16	-
Control**		22	24	10	22	10	19	19	31	20	8

*including 9 mm well diameter; **kanamycin (50 µg) for bacteria, nystatin (100 U) for yeast

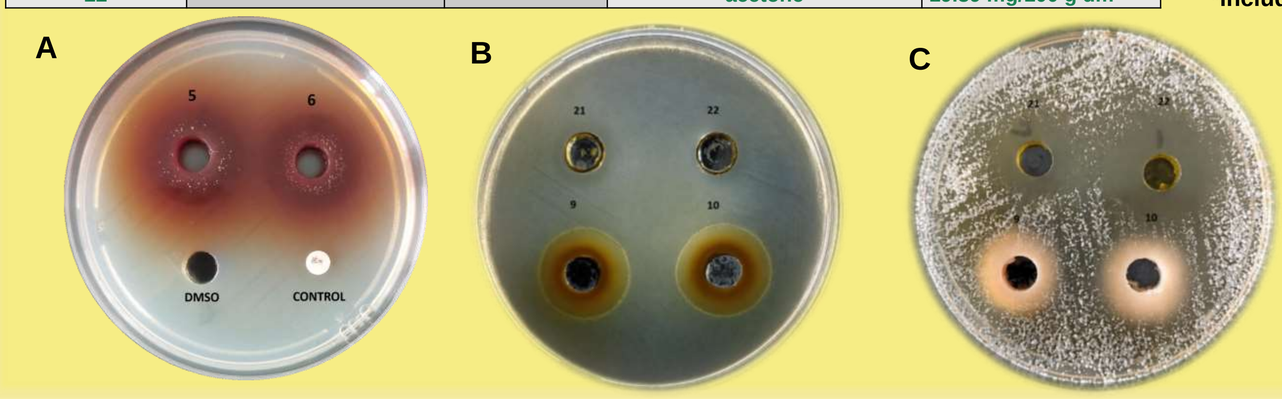

Figure 2. Microorganism growth inhibition zones caused by applied extracts (100 mg/mL) A: red onion peel extracts (5, 6) against *L. monocytogenes*; B: pomegranate (9, 10) and lime (21, 22) peel extracts against *L. monocytogenes;* C: pomegranate (9, 10) and lime (21, 22) peel extracts against *Candida utilis* (DMSO= solvent; CONTROL - kanamycin 50 µg disc).

CONCLUSIONS

Based on the preliminary results obtained using the disc diffusion method, most of the extracts did not show antimicrobial activity against the selected test microorganisms. However, certain extracts showed some antimicrobial potential, such as anthocyanin rich extracts of red onion and pomegranate peel as well as chlorophyl rich extract of lime peel. The results of agar well diffusion method showed that red onion extract had the highest antimicrobial potential among the tested extracts, inhibiting the growth of almost all tested microorganisms at a concentration of 100 mg/mL. Pomegranate and lime extracts also showed antimicrobial potential against selected microorganisms. Gram-positive bacteria *L. monocytogenes* has proven to be the most susceptible to the extracts among the tested microorganisms. Pigment-rich extracts from fruit and vegetable processing by-products could be a promising antimicrobial agents source but additional studies including detailed analyses of their active compounds, concentrations and mechanisms of action are required to fully assess the antimicrobial properties.

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