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ANTIMICROBIAL ACTIVITY OF AN ACTIVE BIOLOGICAL BIOPRODUCT OBTAINED FROM GRAPE SEEDS

ACTIVITATEA ANTIMICROBIANĂ A UNUI BIOPRODUS BIOLOGIC ACTIV OBȚINUT DIN SEMINȚELE DE STRUGURI

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Abstract. *At the Research Station for Viticulture and Enology Iasi, a polymeric condensed proanthocyanidins was obtained from Fetească neagră grape seeds, through a phase preparation, which under determined conditions with hydrogen peroxide, led to the production of a water-soluble bio product with antibacterial, antifungal and antioxidant properties. Evaluation of the antimicrobial activity of the bio product was performed by determining minimum inhibitory concentration (MIC) and minimal bactericidal concentration (CMB) against Staphylococcus aureus and Escherichia coli. From the analysis of the obtained data it was found that the bio product reacted differently from the tested species, being more active against Staphylococcus aureus (G +). The determined MIC was 1.5 mg/mL and the CMB 2.0 mg / mL. In the case of the Escherichia coli (G-) test, the MIC and CMB values were equal but increased to 3.0 mg/mL.*
Key words: seed, proanthocyanidins, inhibitoriy, bactericidal

Rezumat. *La Staţiunea de Cercetare Dezvoltare pentru Viticultură şi Vinificaţie Iaşi, din seminţele de struguri din soiul Fetească neagră, prin extracţii etapizate s-a obţinut un preparat de proantocianidine condensate (PA) polimerice, care tratat cu peroxid de hidrogen, în condiţii determinate, a condus la obţinerea unui bioprodus solubil în apă, cu proprietăţi antibacteriene, antifungice şi antioxidante. Evaluarea activităţii antimicrobiene a bioprodusului s-a efectuat prin determinarea concentraţie minime inhibitorie (CMI) şi a concentraţiei minime bactericide (CMB) faţă de speciile Staphilococcus aureus şi Escherichia coli. Din analiza datelor obţinute s-a constat că bioprodusul a reacţionat diferit faţă de speciile testate, fiind mai activ faţă de Staphilococcus aureus (G+). Valoarea CMI determinată a fost de 1,5 mg/mL, iar valoarea CMB de 2,0 mg/mL. În cazul testului efectuat faţă de specia (G-) Escherichia coli, valorile CMI şi CMB au fost egale, dar au crescut la 3,0 mg/mL.*
Cuvinte cheie: seminţe, proantocianidine, inhibitoriu, bactericid

INTRODUCTION

In the treatment of infectious diseases, the availability of products with antibacterial activity is insufficient, so numerous researches have focused on the investigation of natural products as sources of bioactive molecules (Valgas *et al,*

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2007). This research has grown, as it has been found to increase the resistance of pathogenic bacteria to antibiotics. Among the natural compounds extracted from plants, polyphenols have been shown to have antibacterial functional properties.

Grape seed is the richest source of phenolic extractable compounds whose antibacterial activity has been studied using either total polyphenolic extracts or subclasses of phenolic compounds, namely phenolic acids, quinones, flavanes, flavonols and tannins. The evaluation of antimicrobial activity of phenolic compounds extracted from various natural sources: grape marc, grape seeds, white and red wines has been the subject of numerous studies.

Rodriguez - Vaquero *et al*. (2007) and Papadopoulou *et al.* (2005) have determined the antibacterial activity of polyphenolic extracts obtained from white and red wine, establish the inhibition of the development of the species *Staphylococcus aureus* and *Escherichia coli*. Similar results were obtained by Radovanović *et al.* (2009), the diameter of the inhibition zone of the species *Staphylococcus aureus and Escherichia coli* being 16 - 22 mm and 12 - 20 mm respectively. Jayaprakaska *et al* (2003) determined the antibacterial activity on crude polyphenolic extracts obtained from grape seed extracted into acetone-water-acetic acid (90: 9.5: 0.5) and methanol-water- acetic acid (90: 95: 5) using different species of Gram-positive and Gram-negative bacteria. The minimum inhibitory activity (MIC) against *Staphylococcus aureus* was 1000 ppm / mL, and for *Escherichia coli* at 1250 ppm / mL.

Baydar *et al*. 2004, studied the antimicrobial activity by the diffusion method against 13 Gram positive and Gram negative bacteria using polyphenolic extracts obtained from grape seeds and found that Gram positive bacteria, *Staphylococcus aureus, Bacillus cereus* and *Bacillus subtilis* were inhibited by concentrations small amounts of phenolic compounds compared to Gram negative species *Escherichia coli* and *Pseudomonas aeruginosa*.

At the Viticulture and Oenology Research and Development Station in Iaşi, from the grape seed of Fetească neagră variety, by stepwise extractions a preparation of polymeric condensed proanthocyanidins (PA) was prepared, which was treated with hydrogen peroxide, under certain conditions, resulted in a water-soluble bioproduct with antibacterial, antifungal and antioxidant properties. The evaluation of the antimicrobial activity of the bioproduct was performed by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) against *Staphylococcus aureus* and *Escherichia coli.*

MATERIAL AND METHOD

The bioproduct was physically and chemically characterized by determining the solubility in distilled water, the amount of total polyphenols - CFT, mg GAE / mg product (method Singleton and Rossi, 1965), polyphenolic index - IP (spectrophotometric at 280 nm) and antioxidant activity - AA (%) / 100 µg bioproduct (Method Brand-Williams *et al*., 1994, with modification of Miliauskas *et.al*.,2004). Determination of the antimicrobial activity of the active bioproduct was performed

qualitatively in the preliminary test by diffusion method on agarified medium with stainless steel cylinders and quantitatively by the dilution method on the liquid culture medium of the test microorganisms.

REZULTS AND DISCUSSIONS

The bioproduct obtained under optimal fractionation conditions is in the form of yellowish brown crusts, is completely soluble in distilled water, has an amount of polyphenolic compounds total of 0.625 mg GAE / mg and an antioxidant activity of 91.37% at concentration of 100 µg / mL (table1).

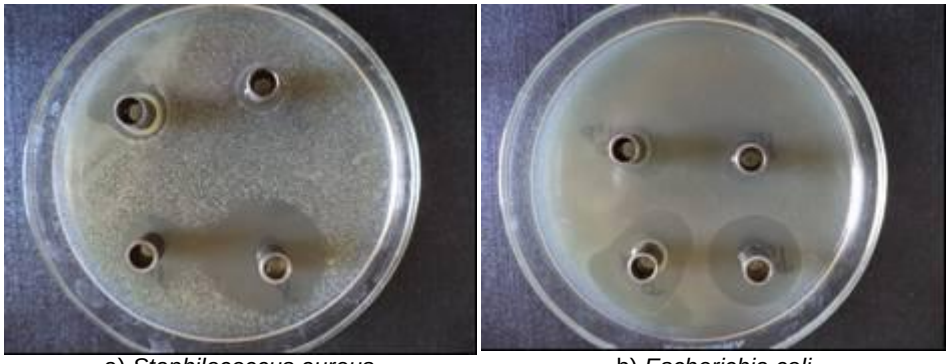
Table 1

Physico-chemical characteristics of the active bioproduct

Activ bioproduct	color		Solubility in distilled water	CFT, mg GAE/mg product	IP 280 nm	A. antioxidant % /100 µg	
	before fractionation	after fractionation				30 min	60 min
	brown crusts	yellow - brown powder	completely	0.625	18.4	91.37	92.44

Determination of antimicrobial activity in the preliminary test.

In the preliminary test carried out, the diffusometric method found that the bioproduct had antibacterial activity against both species of tested microorganisms. In the case of the species *Staphylococcus aureus* antibacterial activity not expressed at a concentration of 0.5 mg/mL, but was shown as a concentration of 1 mg/mL. The diameter of the inhibition zone of the test microorganism increased with the increase of the bioproduct concentration so that at the concentration of 2 mg / mL the largest diameter was 26 mm (fig. 1).



a) *Staphilococcus aureus* b) *Escherichia coli*

Fig. 1 Antimicrobial activity of the bioproduct against species *Staphilococcus aureus (a) and Escherichia coli (b)*

In the case of *Escherichia coli*, the antimicrobial activity was evidently starting with the bioproduct concentration of 1.5 mg/mL and 2 mg/mL, the diameters of the inhibition zones of the tested microorganism being close to 18 mm and 21 mm, respectively.

Also, from the data obtained in the preliminary test, the *Staphylococcus aureus* Gram positive species is more sensitive to the complex of phenolic compounds in the obtained bioproduct, compared to the Gram negative species *Escherichia coli.*

Determination of antimicrobial activity by dilution method on liquid medium

The preliminary test revealed that the bioproduct obtained has antibacterial activity starting from the concentration of 1 mg/mL and 1.5 mg/mL, respectively, relative to the species of microorganisms studied. Thus, in the experiment to evaluate the antimicrobial activity on the liquid medium, 7 concentrations/mL were used, conditions which ensure direct contact of the test microorganism cells with the phenolic compound complex from the bioproduct obtained by the physico-chemical treatment of the proanthocyanidinic preparation. The antimicrobial activity of the bioproduct was assessed by the MIC and MBC values. Table 2 and figures 2 and 3 show the results obtained in the determination of antimicrobial activity by the quantitative method on the liquid medium.

Table 2

Antibacterial activity MIC and MBC of the bioproduct obtained against *Staphylococcus aureus* and *Escherichia coli*

Microorganism test	Bioproduct mg/mL						
	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Staphilococcus aureus	+[x]	+[xx]	-[xxx]	-	-	-	-
Escherichia coli	+	+	+	+	-	-	-

[x] development of microorganisms test
[xx] inhibition of visible development of test microorganisms
[xxx] 99% inhibition of test microorganism development

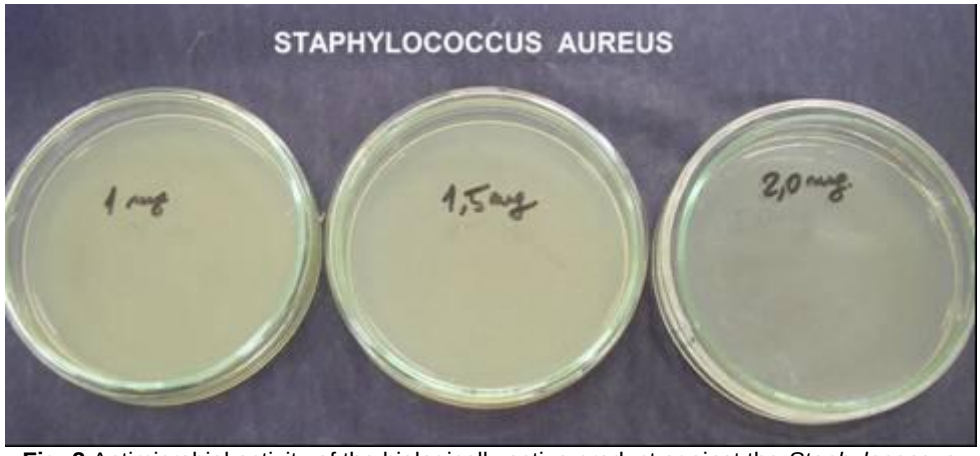


Fig. 2 Antimicrobial activity of the biologically active product against the *Staphylococcus aureus* test microorganism

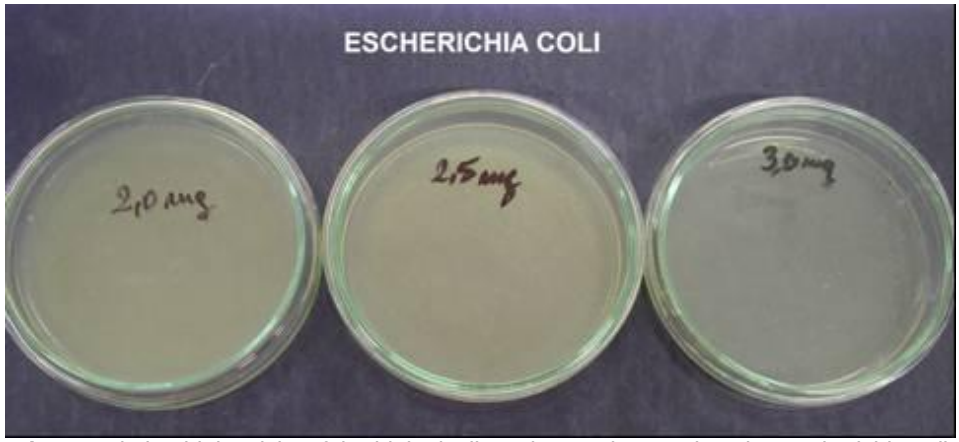


Fig. 3 Antimicrobial activity of the biologically active product against the *Escherichia coli* test microorganism

From the analysis of the data presented in table 2 and figures 2 - 3, the following aspects were found:

- ✓ the minimum inhibitory concentration (MIC) of the bioproduct for the species *Staphylococcus aureus* was 1.5 mg/mL and 3.0 mg/mL for the species *Escherichia coli*;
- ✓ the minimal bactericidal concentration (MBC) of the bioproduct for the species *Staphylococcus aureus* was 2.0 mg/mL and 3.0 mg/mL for the species *Escherichia coli*;
- ✓ In the case of *Escherichia coli*, the antibiotic activity of the CMI and CMB of the bioproduct was at the same concentration of 3.0 mg/mL.

The bioproduct show a more pronounced antibacterial reactivity to gram positive and temperate Gram negative species, as other authors have found when testing total polyphenolic extracts or phenolic compounds isolated from polyphenolic extracts from grape seeds.

The CMB / CMI ratio mark the bactericidal effect to values less than 4 and the bacteriostatic effect to values greater than 4 (O'neil *et al.*, 2004). In the case of the bioproduct tested, the antibacterial activity evaluated according to the CMB / CMI ratio (1.0 and 1.33) defines in particular the bactericidal potential of the test bacterial species.

Regarding the mechanism of action of phenolic compounds on microorganisms Xia *et al.* (2010), suggests that polyphenols by conjugating to the cell wall proteins of microorganisms and especially to key enzymes may be the main way to inhibit the development of microorganisms. Other authors (Cowan *et al,* 1999) have shown that the hydrophobic partial nature of phenolic compounds is responsible for antimicrobial activity. Also, the accumulation and attachment of phenolic compounds to the cytoplasmic membrane may eventually result in the death of microbial cells.

CONCLUSIONS

1. The bioproduct reacted differently to the test species, being more active against *Staphilococcus aureu*s (G+). The determined minimum inhibitory concentration (MIC) was 1.5 mg / mL and the minimal bactericidal concentration (MBC) of 2.0 mg/mL. In the case of the *Escherichia coli* (G-) test, the MIC and MBC values were equal but increased to 3.0 mg/mL.

2. Demonstration of antimicrobial activity against Gram positive and Gram negative microorganisms of phenolic compounds from the obtained bioproduct, is a way to capitalize on it chemically modified proanthocyanidins for solubilization in water, also representing a potential source with a wide utility spectrum in human and animal infectious therapy.

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