STUDIES REGARDING THE ANTIBACTERIAL ACTIVITY (CMI) OF VEGETAL EXTRACTS OBTAINED FROM GRAPE SEEDS AND MEMBRANES

STUDII PRIVIND ACTIVITATEA ANTIBACTERIANĂ (CMI) A EXTRACTELOR VEGETALE OBŢINUTE DIN SEMINŢE ŞI PIELIŢE DE STRUGURI

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Abstract. *At the Research Development Station for Viticulture and Wine Production of Iaşi, the antibacterial activity of four vegetal extracts with a total content of polyphenols between 26.7 and 37.7 g gallic acid/L and four vegetal preponderantly anthocyanin extracts with content between 511.8 and 2804.3 mg/L was studied. The total polyphenolic extracts obtained from grape seeds belonging to the Merlot, Băbească neagră and Fetească neagră varieties were the most active, their antibacterial (CMI) activity being obvious in concentrations of 3 – 4 mg/mL, in comparison to the Gram positive and Gram negative test strains. Nevertheless, the antibacterial (CMI) activity of the preponderantly anthocyanin extracts was different depending on the vine variety and on the used bacterial strain. The preponderantly anthocyanin extract obtained from the membranes of grapes belonging to the Fetească neagră variety stood out by inhibiting the development of test strains in the 3 mg/mL concentration.*

Key words: the vegetal extract, activity antibacterial, *Vitis vinifera*

Rezumat. *În cadrul Staţiunii de Cercetare Dezvoltare pentru Viticultură şi Vinificaţie Iaşi a fost studiată activitatea antibacteriană a patru extracte vegetale cu conţinut în polifenoli totali cuprins între 36,7 şi 37,7 g acid galic/L şi a patru extracte vegetale preponderent antocianice cu un conţinut între 511,8 şi 2804,3 mg/L. Extractele polifenolice totale obţinute din seminţele de struguri aparţinând soiurilor Merlot, Băbească neagră şi Fetească neagră au fost cele mai active, activitatea antibacteriană (CMI) manifestându-se la concentraţii de 3 – 4 mg/mL faţă de tulpinile test Gram pozitive şi Gram negative. Activitatea antibacteriană (CMI) a extractelor preponderent antocianice a fost însă diferită în funcţie de soiul de viţă de vie cât şi de tulpina bacteriană utilizată. S-a remarcat extractul preponderent antocianic obţinut din pieliţele boabelor de struguri aparţinând soiului Fetească neagră care a inhibat dezvoltarea tulpinilor test la concentraţia de 3 mg/mL.*

Cuvinte cheie: extracte vegetale, activitate antibacteriana, *Vitis vinifera*

INTRODUCTION

The use of plants as remedy in the treatment of various disorders dates back to ancient times. However, the discovery of antibiotics and their mass manufacture brought about their privileged use for the cure of diseases caused by microorganisms.

Therefore, in time, Gram-positive and Gram-negative bacterial resistance to a wide range of antibiotics appeared

For that reason, the studies conducted in specialty labs want to detect within plants and to promote new active principles with antibacterial effect.

In literature, there are many scientific works, whose subject was the study of polyphenols. These chemical substances isolated from various medicinal plants showed antibacterial activity in addition to their cytostatic features. We might name the papers of Hisanori A. and collaborators, 2001; Hidetdsh A. and collaborators, 2002; Chunxia W. and collaborators 2006; Mbata T.I. and collaborators, 2007; Doughari S.M. and collaborators, 2007, who obtained extracts from plants rich in hydroxicumarin, derivates of flavonoids, flavonols, flavonons, anthocyans, proanthocyanidin hydroxystilbene etc., with antibacterial activity against pathogenic strains isolated from various infectious processes.

More recently, studies lead to the development of synergic mixtures from polyphenolic compounds and antibiotics. From this perspective, interesting results were obtained by Zhi Qing H. and collaborators, 2001, 2002; Tiwari R.P., 2005; Esimone C.D., 2006. These synergic mixtures allowed the reduction of antibiotic doses administered for the treatment of diseases triggered by antibiotic-resistant microbial strains.

References related to the antibacterial activity of polyphenolic extracts obtained from grape seeds and skins are rather few and briefly presented. Therefore, our objective was to obtain total and mostly anthocyanic polyphenolic extracts from residue of winemaking process, namely grape seeds and skins, in order to test the antibacterial activity MIC (minimum inhibitory concentration)

MATERIAL AND METHOD

The extractions of total polyphenols from seeds and of anthocyans from the skin of *Vitis vinifera* were performed through Soxhlet continuous method, according to the ratio 1/10 vegetal solid material/ethylic alcohol. The concentrations of total polyphenols (g GAE/L) and of anthocyans (mg/L) were determined by Singleton – Rossi (1965) and Ribereau Gayon – Sonestreet (1965) methods. The amount of dry matter and ash was determined according to OIV methods.

In compliance with the regulations of NCCLS (National Committee for Clinical Laboratory Standards) for the determination of the active biological activity (MIC) of total polyphenolic extracts and of mainly anthocyanic extracts, we used the Mueller – Hinton dilution method in liquid medium in the presence of test bacterial strains: *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15428, *Serratia marcesscen* şi *Staphylococcus aureus* ATCC 29737. Each test strain was inoculated in accordance with the McFarland 0,5 standard (Eucast 2003).

RESULTS AND DISCUSSIONS

The active substances of plants act on microorganisms in various ways. In relation to the polyphenolic compounds, the researchers Zq Hu and collaborators, 2002, Karou D. and collaborators 2005, Esimote and collaborators, 2006 and Akiyama H. and collaborators, 2001 have observed that flavonoids and tannins

modify the permeability of the cell wall, of the cytoplasmatic membrane, of microbial cells by directly fixing on the peptidoglycans. Thus, the substance exchange between the cell and the environment is diminished, ending up with the cell lysis.

Other actions of polyphenolic compounds lead to the disorder of biosynthesis processes of DNA and RNA nucleic acids, to the hindrance of some enzyme functions, interfering in the energy metabolism.

For highlighting the antibacterial activity (MIC) it is used either the diffusometric method (Kirby G.C., 1986; Bauer et. al, 1966) through an agarized medium in Petri dishes, or the dilution method in liquid media, the results being confirmed in an agarized medium (EUCAST, 2003).

The selection of the best method for determining the MIC value of an active principle depends on its physical-chemical features. Extracts rich in active compounds with big molecule should be studied in liquid media through the dilution method as they hardly diffuse in solid media.

Therefore the antibacterial activity (MIC) of total polyphenolic extracts and of anthocyanic ones, obtained from grape seeds, respectively skins belonging to the Cabernet Sauvignon, Merlot, Băbească neagră and Fetească neagră varieties, was studied by means of the Mueller – Hinton dilution method in liquid media.

A compulsory stage before the modeling of the experiment was the concentration of alcoholic vegetal extracts in a rotary evaporator for removing ethylic alcohol.

In table 1 we show the physical-chemical features of concentrated extracts obtained from grape seeds.

Table 1

Physical-chemical features of total polyphenolic extracts from seeds

Parameters	Unit of measure	Concentrated total polyphenolic extracts			
		Cabernet Sauvignon	Merlot	Băbească neagră	Fetească neagră
Total polyphenols	gallic acid g/L	36,7	37,7	36,6	37,5
Dry matter	mg/L	534,6	82,1	234,6	242,8
Ash	g/L	1,3	0,5	1,8	1,0
pH		5,5	5,6	5,5	5,5

By comparing the data shown in table 1 we observed that the values of total polyphenol concentrations of the four polyphenolic extracts are very similar, ranging between 36,6 and 37,7 gallic acid g/L. This similarity did not occur in the case of dry matter and ash, the values being entirely different.

The physical-chemical features of mainly anthocyanic extracts are shown in table 2. In the extracts studied, the concentration of anthocyans varied depending on the variety of *Vitis vinifera*, the highest concentration being found for Cabernet Sauvignon, namely 2804,3 mg/L, followed by the Merlot variety with 2000,8 mg/L.

Concentrated vegetal extracts comprise a wide range of polyphenolic

compounds, anthocyans but we determined only total polyphenols and anthocyans, therefore, we believe that an accurate assessment of MIC activity should take into account the total parameter represented by dry matter (mg/mL).

Table 2

Physical-chemical features of mainly anthocyanic extracts from grape skins

Parameters	Unit of measure	Concentrated mainly anthocyanic extracts			
		Cabernet Sauvignon	Merlot	Băbească neagră	Fetească neagră
Anthocyans	gallic acid g /L	2804,3	2000,8	511,8	1524,3
Dry matter	mg/L	119,3	145,6	71,9	53,4
Ash	g/L	0,56	0,58	0,50	0,78
pH		5,7	5,6	5,6	5,6

After repeating for three times the preliminary test to determine the concentration field, we saw that the antibacterial activity (MIC) could range between 1 – 20 mg/mL.

The results of tests for the antibacterial activity (MIC) of total polyphenolic extracts are shown in figure 1.

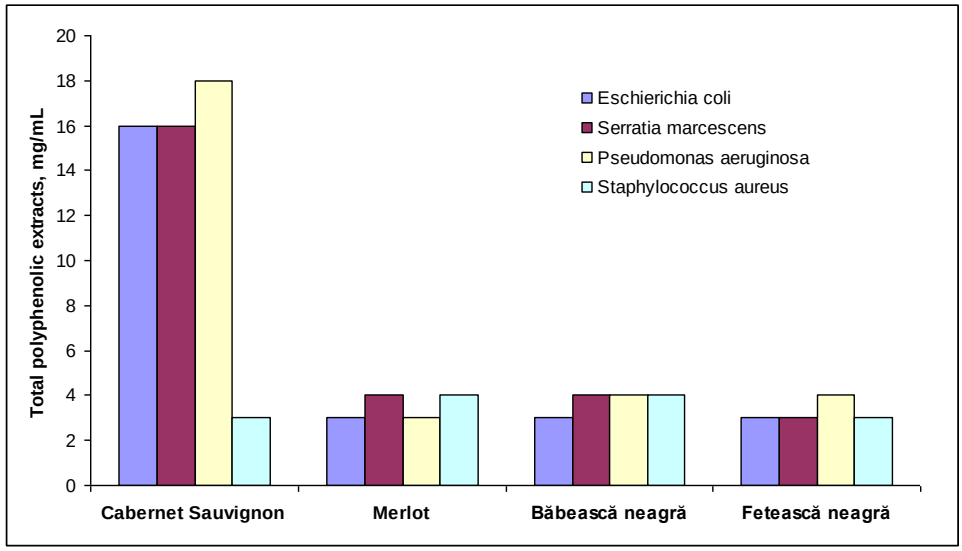

Fig. 1. Determining the biologically active activity (MIC) of total polyphenolic extracts from grape seeds

The graphical representation of data showed that the polyphenolic extract from grape seed of Cabernet Sauvignon variety inhibited the *Staphylococcus aureus* strain when the concentration was of 3 mg/mL. In contrast, in the presence of *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcesscen* test strains, the antibacterial activity is reduced and the values of MIC concentrations range between 16 – 18 mg/mL.

Total polyphenolic extracts obtained from seeds of Merlot, Băbească neagră and Fetească neagră varieties proved much more active. The antibacterial activity (MIC) against Gram-positive and Gram-negative strains occurred for concentrations of 3 and 4 mg/mL.

In figure 2 we present the results of tests on the antimicrobial activity (MIC) of mainly anthocyanic extracts.

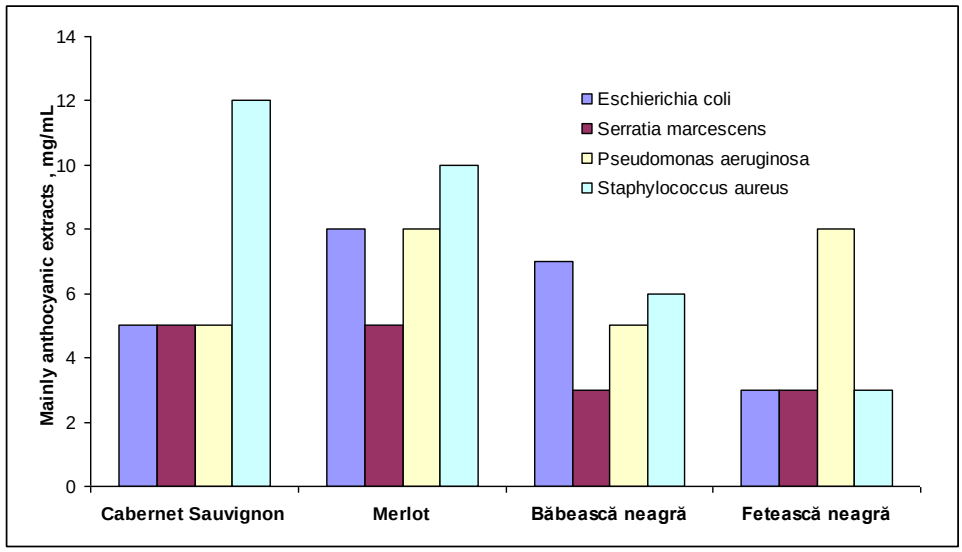

Fig. 2. Determining the biologically active activity (MIC) of mainly anthocyanic extracts from grape skins

The antibacterial activity (MIC) of anthocyanic extracts obtained from grape skins of Merlot and Băbească neagră varieties had an identical behavior in relation to Gram-positive and Gram-negative bacterial strains, small differences occurring in the case of concentrations (MIC). In the graphical representation of data, we saw that the mainly anthocyanic extract from grape skins of Fetească neagră variety inhibits the development of *Escherichia coli, Serratia marcesscen, Pseudomonas aeruginosa* bacterial strains when the concentration is 3 mg/mL and the *Staphylococcus aureus* strain for a concentration of 8 mg/mL.

A different behavior of the antibacterial activity (MIC) was noticed in the case of mainly anthocyanic extract from Cabernet Sauvignon variety. In figure 2 we see that the bacterial activity (MIC) against Gram-negative strains manifested when the concentration was of 5 mg/mL and against Gram-positive *Staphylococcus aureus* strain when it was of 12 mg/mL.

An overview of test data points out that polyphenolic extracts are more active in comparison with mainly anthocyanic ones. The demonstration of the antibacterial activity (MIC) of vegetal extracts of *Vitis vinifera* against Gram-positive and Gram-negative bacteria recommends this source for the preparation of new drugs with wide spectrum of actions.

CONCLUSIONS

1. The antibacterial activity (MIC) was tested „in vitro” using total polyphenolic extracts and mainly anthocyanic extracts;

2. The total polyphenolic extracts from seeds of Merlot, Băbească neagră and Fetească neagră varieties are the most active; the inhibition of test strains development occurred for concentrations of 3 and 4 mg/mL.

3. The best antibacterial activity (MIC) against *Escherichia coli, Serratia marcesscen, Pseudomonas aeruginosa* test strains was achieved by the mainly anthocyanic extracts from Fetească neagră grape skins with a concentration of 3 mg/mL and of 8 mg/mL against *Staphylococcus aureus* strain.

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