ANTIMICROBIAL EFFECT OF GRAPE SEEDS EXTRACT

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ABSTRACT

It is known, that antioxidants in grape seeds have antimicrobial effect. A high content of proanthocyanidins in these seeds is rather important. These compounds are studied and evaluated as one of possibilities how to treat a great number of different diseases.

In our study we focused on monitore of antimicrobial effect of grape seeds extract (GSE) on bacterial culture *Staphylococcus aureus*. The effect of eight concentrations of extract of grape seeds (0, 1.25, 2.5, 5, 10, 15, 25 and 50 $mg \cdot ml^{-1}$) was studied. We were focused on the study of study of antioxidant activity (method Free Radicals). Have also been studied the growth curves and the number of microorganism in this culture. The increased concentration of applied extract of grape seeds led to the increase values of antioxidant capacity.

Keywords: grape seeds extract**,** antimicrobial aktivity, oxidation stress

1. Introduction

Among the foodborne diseases, bacterial infections have been previously thought to be eliminated by the end of last century (Fu & Li, 2014). Keeping in timeline of several past decades, both chronic gastrointestinal infection such as food intoxication are growing public health problem worldwide (Rajkovic et al., 2010). From the environment, microbial contamination may enter the agric-food chain via crops such as fruits or vegetable and cause two types of food poisoning (Park et al., 2012). The aim of the thesis was to study the antimicrobial aktivity of grape seeds extract (GSE).

2. Material and methods

This experimental study was performed with seeds of grapevine (*Vitis vinifera* L.) cultivar „Marlen“. The experimental material originated from the Department of Viticulture and Enology, Faculty of Horticulture in Lednice na Moravě (Czech Republic).

Staphylococcus aureus (NCTC 8511) was obtained from the Czech Collection of Microorganisms, Faculty of Science, Masaryk University, Brno, Czech Republic. Strains were stored as a spore suspension in 20% (*v*/*v*) glycerol at −20 °C. Prior to use in this study, the strains were thawed and the glycerol was removed by washing with distilled water. The bacterial strain was incubated in the presence of cultivation medium (meat peptone 5 $g \cdot L^{-1}$, NaCl 5 $g \cdot L^{-1}$, bovine extract 1.5 $g \cdot L^{-1}$, yeast extract 1.5 $g \cdot L^{-1}$ (HIMEDIA, Mumbai, India)), sterilized MiliQ water with 18 MΩ) at 600 rpm and 37 °C in Incubator Hood TH 15 (Edmund Buhler GmbH, Hechingen, Germany). pH of the cultivation medium was adjusted at 7.4 before sterilization. Sterilization was carried out at 121 °C for 30 min. in sterilizer (BMT, Brno, Czech Republic). Grown bacterial culture was diluted by cultivation medium to OD_{600} = 0.1 prior to use in the following experiments. The prepared medium was pipetted into 25 mL flasks and GSE (0, 31.25, 62.5, 125, 25, 375, 625 and 1 250 $\mu g \cdot ml^{-}$) were added. Thus prepared samples were cultured 24 hours.

Solution containing bacteria, cultivation medium and various concentrations of GSE was mixed and pipetted into plastic tubes (3 mL) (AnalytikJena, Jena, Germany). Subsequently, a SPECORD 210 device (AnalytikJena) was used for measuring of the solution absorbance at a wavelength of 600 nm every 30 min for 24 h. A carousel for eight samples was used. All measurements were done in five replicates. The resulting absorbance were averaged and

recalculated to the control variant, which represented 100%. Cuvette area was thermostated throughout the experiment to 37 °C (F12/ED Julabo, Seelbach, Germany). The SPECORD device was controlled by the WinASPECT Version 2.2.7.0 program package (AnalyticJena).

Determination of antioxidant activity by the Free Radicals method is based on ability of chlorophyllin (the sodium-copper salt of chlorophyl) to accept and donate electrons with a stable with a stable change of maximum absorption. This effect is conditioned by an alkaline environment and the addition of catalyst. A 150 µl volume of reagent is injected into a plastic cuvette with subsequent addition of a 6 µl sample. Absorbance is measured at 450 nm in the second minute of assay and the 10th minute. Difference of the two absorbancies is considered as an outputting value.

3. Results

In this study, we have tested the extracts of GSE for their antimicrobial aktivity. The antimicrobial activity of the extracts of grape seeds were studied in different concentrations (0, 1.25, 2.5, 5, 10, 15, 25 and 50 µg·ml^{-1}) against Gram-positive microorganism *Staphylococcus aureus*.

The increasing of concentration of GSE leads to the increasing of antioxidant activity. The increased antioxidant activity was well evident with the increasing concentration of applied extract of grape seeds. The values of antioxidant activity of *S. aureus* are presented in Fig. 1.

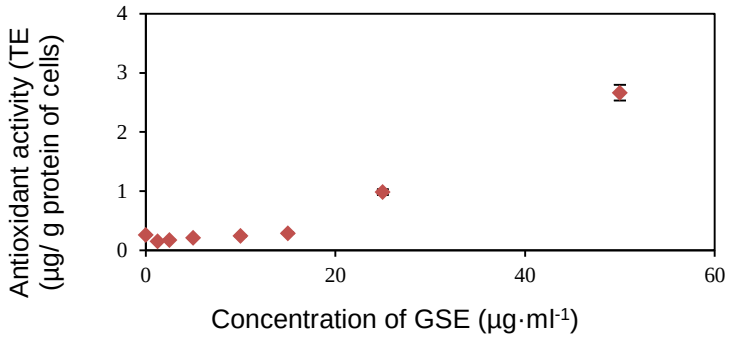

Figure 1. Values of antioxidant activity of *S. aureus.*

Moreover, they were also monitored by inhibition zone. Minimum inhibitory concentration (MIC) shows significant antibacterial properties. Our data show that concentration 5 µg·ml^{-1} was antimicrobial effect.

Concentration of GSE (µg·ml^{-1})	0	1.25	2.5	5	10	15	25	50
Zone of inhibition (mm)	0	0	0	2	4	6	8.5	12.8

Table 1. Zone of inhibition (mm).

4. Conclusions

It is well konwn that grapevine seeds are rich in antioxidants. The results indicate the values of antimicrrobial aktivity and the possibility of the application of above-mentioned analytical techniques in microbiology in determination of oxidative stress in bacterial cultures. Values of Values of antioxidant activity of S*. aureus* ranged from 352 to 1159 µg/ g of equivalent of trolox / 1 g protein of cells.

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REFERENCES

1. Fu L. L. and Li J. R. (2014), Microbial source tracking: A tool for identifying sources of microbial contamination in the food chain. Food Science and Nutrition, **54**, 699-707.
2. Park S., Szonyi B., Gautam R., Nightingale K., Anciso J. and Ivanek R. (2012), Risk factors for microbial contamination in fruits and vegetables at the preharvest level: A systematic review. Journal of Food Protection, **75**, 2055-2081.
3. Rajkovic A., Smigic N. and Devlieghere F. (2010), Contemporary strategies in combating microbial contamination in food chain. International Journal of Food Microbiology, **141**, 29-42.