THE STUDY OF ANTIMICROBIAL EFFECT OF GRAPE SEED EXTRACT

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Abstract: In this study, an experiment on the antimicrobial activity of grape seeds from *Vitis vinifera* L. was performed. *Vitis vinifera* L. is known for its health benefits because of its high content of phenolic compounds. Grape seed extract was made from interspecific wine cultivars. Three pathogens (*Candida tropicalis*, *Escherichia coli*, and *Enterococcus faecalis*) were selected for the experiment. The antimicrobial effect was determined using a disc diffusion method. The high efficiency of grape seed extract was observed on *Candida tropicalis* (12.9 mm) and *Enterococcus faecalis* (9.1 mm). Grape seed extract was proven to be effective in inhibiting these pathogens.

Key Words: grape seed extract, antimicrobial activity, disc diffusion method

INTRODUCTION

*Vitis vinifera* L. is one of the most grown fruits in the world, with more than 67 million tons produced annually. There are many proven health benefits from consuming raw grapes, or grape products such as wine or juices (Friedman 2014). Grape seeds contain 60–70% of the grape’s total extractable phenolics (Godevac et al. 2010). Due to their high phenolic compound, grape seeds have the ability to decrease oxidative stress, which makes them beneficial to human health. They also have the ability to promote the growth of beneficial bacteria in the intestinal tract (Brenes et al. 2016). The effect of grape seed extract (GSE) on three common pathogens, and its utilisation in practice, will be evaluated in this study.

MATERIAL AND METHODS

Biological Material

Key material for the preparation of tested extracts was a mixture of seeds of interspecific *Vitis vinifera* L. cultivars (‘Cerason’ (red), ‘Marlen’ (red) and ‘Erlon’ (white)) provided by the Department of Viticulture and Enology at Mendel University in Brno (MENDELU). Seeds were separated from other fractions (impurities, parts of plant), sorted, and dried to 8% moisture. 100 g of fine crushed grape seeds, with 1 litre of 75% methanol, were put into glass jars and stirred regularly. The extraction of substances took place in the dark, at a temperature of 21 °C for 168 hours.

Decanted extract from the jar was centrifuged. Then the evaporation of methanol from the extract was performed at 70 °C in a vacuum evaporator (IKA, Germany) with a constant rotation (70 rpm). 100% pure GSE was tested.

Disc Diffusion Method

Three microorganisms were tested for GSE antimicrobial activity: *Candida tropicalis* (CCM8223), *Escherichia coli* (CCM7229), and *Enterococcus faecalis* (CCM4224). Petri plates with two types of media (Biokar Diagnostics, France) - CHL (for *C. tropicalis*) and PCA (other
microorganisms) - were inoculated (density of inoculum was 0.27 MF). Inoculum was spread evenly on the surface and left to rest for 15 minutes.

The disc diffusion method was used to evaluate the antimicrobial effects of GSE. Paper discs (Fisher Scientific, Czech Republic) 9 mm wide in diameter were saturated by 30 μl of extract and inserted into Petri plates with pathogens. Three Petri plates were used for testing and in each plate there were three discs. Additional Petri plates with each pathogen (but without the discs) were prepared for use as a standard. Also, one plate with discs saturated with distilled water was used for each microorganism as a control.

RESULTS AND DISCUSSION

Petri plates were incubated in suitable conditions of thermostats (Candida tropicalis at 25 °C, Escherichia coli and Enterococcus faecalis at 37 °C). Two measures of inhibition growth zones’ diameters were taken after 24 and 48 hours of incubation. The experiment conducted on 23. 2. 2016 studied the effects of GSE on three model pathogens: Candida tropicalis, Escherichia coli, and Enterococcus faecalis. Results depicted in Figure 1 and Figure 2 are average of the measurements of zones taken in Petri plates. If this average was less than 9 mm (diameter of the paper disc), GSE did not exhibit the expected inhibition. Standard deviation was used in Figures 1 and 2 (marked as error segment in each column). Conclusiveness of inhibitory effects of GSE against microorganisms was evaluated by Wilcoxon signed-rank test. P-value was 0.01 in Candida tropicalis (both 24 hours and 48 hours measurements), in other microorganisms were measurements identical, so test could not be made.

Positive results were observed in Petri plates with Candida tropicalis. After 24 hours there were inhibitory zones with a diameter of 12 mm. There was no sharp distinction between the microbial colony and the discs, so we can assume that growth was weakened, but not entirely inhibited. Zones expanded after 48 hours to a diameter 12.9 mm. It is obvious that the inhibitory effect increases with a longer duration of application.

The inhibition of Escherichia coli was barely noticeable. After 24 hours, no negative effect on growth was observed. Yet Escherichia was growing more intensely around the discs. There was no increased growth around the discs visible after 48 hours. Again, there was no sharp distinction between the bacteria and the disc.

Better measurements were taken in the colony of Enterococcus faecalis. After 24 hours there was an average diameter of zones 9 mm, but the antimicrobial activity increased after a longer period of application to 9.1 mm.

Figure 1 Diameter of inhibitory zones in mm (on Y axis) according to microorganism (on X axis) after 24 hours
Figure 1 describes the antimicrobial effect of GSE on *Candida*, *Escherichia* and *Enterococcus*. The most evident inhibition can be seen on the blue field marking *Candida* yeast. There was no particular effect on *Escherichia* or *Enterococcus* (the diameter of zones was 9 mm). 9 samples were measured from every variant of microorganism (n=9).

![Figure 2 Diameter of inhibitory zones in mm (on Y axis) according to microorganism (on X axis) after 48 hours](image)

The progress of GSE activity on tested bacteria is depicted in Figure 2. Antimicrobial effects increased in both *Candida* and *Enterococcus*. There was no inhibition noted in the colony of *Escherichia*. 9 samples were measured from every variant of microorganism (n=9).

Health beneficial compounds can be found in different parts of the *Vitis vinifera* L. plant. Scientists proved the presence of antimicrobial properties in its leaves (Katalinic et al. 2013), anti-carcinogenic effects from its stems (Sahpazidou et al. 2014), and an antifungal effect from its pomace against *Botrytis cinerea* (Mendoza et al. 2013).

Recorded information in this study suggests that GSE has an antimicrobial effect. GSE in our study has a bigger inhibition effect in Gram-positive bacteria and yeast than in Gram-negative bacteria. There are many studies on food pathogens with similar results. GSE used in Spain also had better results on Gram-positive bacteria (Delgado Adámez et al. 2012), *Staphylococcus aureus*, and *Bacillus cereus*, than on *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* (Oliveira et al. 2013). To inhibit Gram-positive bacteria they used 850–1000ppm of GSE (Jayaprakasha et al. 2003).

A study of heated water soluble GSE (made of 'Ison' and 'Carlos' cultivars) showed the high potential of GSE to be a valuable natural preservative in beverages because of its antimicrobial activity against *Escherichia coli* (Kim et al. 2008). The results on *Escherichia coli* vastly differed from measurements in this paper. Such may be caused by different bacteria strains, or by the amount of antibacterial compounds in seeds used for the preparation of extracts. The properties of GSE could be enhanced in the future by using different types of solvents during extract production. Higher phenolic content could be obtained with ethyl acetate-water solvent (Jayaprakasha et al. 2001).

Studies testing GSE activity against pathogens do not end at common food borne organisms. A Kuwaiti study shows that 3 mg/ml of proanthocyanidin GSE (equivalent to 20.7 µg/ml flavonoid content) can be used for the inhibition of methicilin-resistant *Staphylococcus aureus* (Al-Habib et al. 2010). This proves the high utilisation of GSE in medicine.
Grape extract from skins and seeds of cultivar 'Arinto' was also tested against viruses. A Portuguese team of scientists found that this natural extract inhibits the replication of adenovirus type 5 irreversibly (Matias et al. 2010).

CONCLUSION

Extracts made from grape seeds were prepared and tested. Results varied according to each species. Higher antimicrobial activity was visible in colonies of Candida and Enterococcus. Suggested use of GSE against Candida tropicalis is 24 hours; against Enterococcus faecalis is 48 hours. This data shows that GSE can be used as an effective treatment against some pathogens. That is why it is important to utilise waste from the winemaking industry and spread information about this valuable commodity.

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REFERENCES


