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Evaluation of Antibacterial and Antifungal Effects of Different Extracts of *Vitis vinifera* L. cv. Ghizil Uzumskin and Seeds extracted by Deep Eutectic Solvents and Ultrasonic

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Introduction

The pathogenic microorganisms, especially bacteria and fungi, are the main causes of infectious diseases and food contamination. Due to the resistance of pathogenic bacteria and fungi to common drugs and the tendency to food preservatives of natural origin, researchers are looking for antimicrobial agents of plant and organic origin as alternative compounds. Research results have shown that compounds extracted from grape seeds have antimicrobial and antioxidant properties (Baydar et al., 2004). The method of extraction and the type of solvent is one of the most important steps that can have a great impact on the extraction of antimicrobial compounds and ultimately the ability of the extract to interact with different microorganisms. Meanwhile, in recent years, a new generation of green solvents called deep eutectic solvents have been introduced, which, while being biocompatible, are easily synthesized and inexpensive. One of the techniques to increase extraction efficiency is ultrasonic extraction, which is performed with common laboratory equipment, for example, an ultrasonic bath (Ali et al., 2019). Due to the fact that no research has been done in this field so far on *Vitis vinifera* L. var. Ghizil Uzum grape, therefore, the aim of this study was to investigate the antibacterial and antifungal properties of different extracts of *Vitis vinifera* L. var. Ghizil Uzum grape skin and seeds extracted by different methods.

Material and Methods

Preparation of hydroalcoholic extract of grape skin and seed: After preparing the grapes and its scientific confirmation, the powdered skin and seed samples were used to prepare the extract. The

maceration method and rotary evaporator (Heidolph, Schwabach, Germany) were used. The extracts were stored in a refrigerator at 4 °C until the experiment (Mayeli et al., 2019).

Preparation of extract by deep eutectic solvent method: For this purpose, the modified method of Hsieh et al. (2020) was used. For this purpose, choline chloride and glycerol (Sigma-Aldrich, Germany) were used in a ratio of 1 to 2 and the temperature was 80 °C.

Preparation of extract by deep eutectic solvent method with sonication: For this purpose, an ultrasonic bath with a temperature of 80 °C was used for 30 minutes with a power of 140 watts and a frequency of 37 kHz. The supernatant was dried with a lyophilize device. The extract was stored at 4 °C until use (Hsieh et al., 2020).

Preparation of grape skin and seed extract concentrations: 5% DMSO solution (Sigma-Aldrich, Germany) was used to prepare the dilutions. After vortexing, the contents were passed through a sterile polytetrafluoroethylene (0.45 µm) syringe filter.

Investigating the antibacterial and antifungal effect of the extracts: The *L. monocytogenes* (19115ATCC), *S. typhimurium* (14028ATCC), *S. aureus* (1112PTCC), *E. coli* (25922ATCC) and fungi *A. flavus* (5006PTCC), *P. citronium* (5304PTCC), *Cladosporium* (5202PTCC) and *F. solani* Complex (5284PTCC) were prepared from Iranian Research Organization for Science and Technology. To prepare a fresh microbial culture, a colony of the bacteria was transferred on solid agar medium (Quelab, Canada) and incubated for 24 hours at 37 °C. Also, a colony of activated molds was kept in a Sabouraud dextrose agar culture medium for 7-10 days at a temperature of 27 °C in an incubator.

Broth microdilution method was used to determine the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC). Dilutions of the desired extracts including: 50, 25, 12.5, 6.25, 3.12, 1.56, .78, .39, .19 mg were prepared and evaluated. For well diffusion method, the desired amounts were used to prepare the extract in concentrations of 2, 1 and 3 mg from the skin and seeds extracts separately. Dimethyl sulfoxide (DMSO) 5 % was used for negative control, ampicillin disc 10 µg for positive control in antibacterial tests, and miconazole 10 µg for antifungal tests (Delgado Adámez et al., 2012; Radulescu et al., 2020).

Result and Discussion

The results of agar well diffusion method: The largest zone of inhibition halo for *L. monocytogenes*, *E. coli* and *S. aureus* bacteria in the vicinity of the extracts extracted from the grape seed and skin is related to the extract extracted by the DES-sonication method and the smallest diameter of the growth inhibition halo is related to the extract extracted by the maceration method ($p \leq 0.05$). Grape skin and seed extracts are rich in polyphenols and proanthocyanidins.

Depending on their chemical structure and concentration, polyphenols and their metabolites can act as activators or inhibitors of microbial growth. These metabolites selectively inhibit the growth of foodborne pathogens. Based on another research, it has been shown that the sensitivity of Gram-positive bacteria to grape seed extract is higher than that of Gram-negative bacteria, which is consistent with the results obtained in this study (Rhodes et al., 2006).

The results of the antifungal properties of the extracts by agar well diffusion method: According to the obtained results, there is a significant difference between the growth inhibition by the types of grape skin and seed extracts studied and their different concentrations. In general, with the increase in concentration, the growth inhibition increased. On the other hand, the extraction method also played a role in the performance of the antifungal compounds of the extract ($p \leq 0.05$). Resveratrol is one of the most important natural compounds in grapes, especially the colored ones. Resveratrol is a phytalaxin stilbene that is produced by some plants in response to ultraviolet radiation and growth from fungal pathogens, such as *B. cinerea*, and leads to a decrease in the germination of condyles and the growth of mycelium (Ribes et al., 2018; Favaron et al., 2009). In a study, methanol extract of grape leaves was evaluated against *A. niger*. The MIC results showed that 75.4 µg/ml had an inhibitory effect (Sharifi-Rad et al., 2014).

The results of determining the minimum inhibitory concentration (MIC) and the minimum bactericidal and fungicidal concentration (MBC, MFC): According to the results obtained from the present study, the lowest MIC and the lowest MBC for *S. aureus*, *L. monocytogenes*, *S. typhimurium* and *E. coli* are related to the skin and seed extracts extracted by the combined sonication-DES method. which shows the efficiency of this method in extracting phenolic compounds compared to other conventional methods ($p \leq 0.05$). Also, in this method, *L. monocytogenes* and *S. aureus* have the lowest MIC and MBC levels compared to other studied bacteria, which indicates the high sensitivity of these bacteria to the phenolic compounds found in grape seed and skin extracts ($p \leq 0.05$). Also, the antifungal results showed that the lowest MIC and the lowest MFC for *Cladosporium* fungus are related to the extract of the seed extracted by sonication method ($p \leq 0.05$). Also, in this method, compared to other studied fungi, *Cladosporium* has the lowest MIC and MFC, which indicates the high sensitivity of this fungus to the phenolic compounds found in grape seed and skin extracts ($p \leq 0.05$).

Conclusions

According to the results, it was estimated that all the techniques for extracting the extract including maceration, ultrasonic and deep eutectic solvent, each of them alone have a suitable ability to extraction, with this now, the combination of ultrasonic methods and deep eutectic solvent in the extraction of phenolic compounds has a much higher capability than other methods. The grape seed extract extracted by sonication-DES method has a higher ability in controlling and inhibiting

the growth of pathogenic bacteria and fungi and spoilage microorganisms. Therefore, the researched method can be practically used to extract active compounds with high efficiency.

Keywords: Antimicrobial, Deep eutectic solvents, Ghizil Uzum, Ultrasonic.

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Declaration of conflict of interest

The authors declare that there is no conflict of interest.

Statement on ethics

The current research was conducted in accordance with scientific principles and the results were reported correctly and without distortion.