Journal of Dentomaxillofacial Radiology, Pathology and Surgery

Winter2019, Volume8, Number 1

Research Paper: The effect of different Vitis vinifera seed extracts on Streptococcus mutans and sobrinus bacteria

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Citation: Khodadadi E, Moghadamni A, Rajabnia R, Khafri S, Zarei R. The effect of different Vitis vinifera seed extracts on Streptococcus mutans and sobrinus bacteria. Journal of Dentomaxillofacial Radiology, Pathology and Surgery. 2019; 8(1):14-19. http://dx.doi.org/10.32598/3dj.7.4.145

http://3dj.gums.ac.ir

Article info:
Received: 2019/01/17
Accepted: 2019/02/10

Keywords:
Streptococcus sobrinus
Streptococcus mutans
Plant Extracts

ABSTRACT

Introduction: Continued use of chemical agents to reduce the levels of tooth decay bacteria has adverse effects; hence, numerous recent studies have replaced conventional chemicals with plant-derived agents. The aim of this study was to investigate the effect of Vitis vinifera seed extracts on Streptococcus mutans and sobrinus bacteria.

Materials and Methods: In this descriptive experimental study, the Vitis vinifera seeds were dried, the obtaining powder was poured into separate dishes to prepare aqueous, alcoholic and acetone extracts and the desired solvents were added. After being placed in the shaker incubator and passing through the filter paper, the solvents were transferred to the plates and placed in the oven to evaporate.

Results: The minimum inhibitory concentration (MIC) of the aqueous extract was 8 µg/ml for Streptococcus mutans and 2 µg/ml for Streptococcus sobrinus bacteria. Alcoholic and acetone extracts were not able to inhibit bacterial growth at initial concentrations. Therefore, the higher concentrations were evaluated, but none of them was effective.

Conclusion: The aqueous extract of Vitis vinifera seeds was the only one which inhibited bacterial growth, and the alcoholic and acetone extracts had no antibacterial effect.

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Journal of Dentomaxillofacial Radiology, Pathology and Surgery

Introduction

Dental caries is the most common chronic disease worldwide that affects all age groups from children to adults (1).

The prevalence of dental caries in industrialized countries is still high despite the development of fluoride consumption and other preventive methods (2). Acid production by oral acidogenic microorganisms following carbohydrate consumption is a determining factor in the onset of dental caries (3). In fact, it has been proven that microorganisms are the major etiological factors in the formation of dental caries (4).

As Streptococcus mutans is highly acidogenic and results in dissolution of hard tooth tissue via producing short acidic chains; therefore, it is the most important pathogen that causes dental caries. This bacterium produces insoluble extracellular polysaccharides by metabolizing sucrose that increase their adherence to the tooth surface and form biofilms (5).

Various chemical antimicrobial agents such as fluoride, chlorhexidine and so on are used in different forms such as mouthwash, gel, varnish and so on to reduce the Streptococcus mutans levels (6).

Continued use of these chemical antimicrobial agents has adverse effects. For example, long-term use of chlorhexidine can change the tooth color and taste sensation as well as destroy the oral microflora (7).

Therefore, in recent years, many studies have investigated plant-derived antimicrobial compounds as an alternative to commonly used chemicals in preventing caries (8).

The extract obtained from Vitis vinifera seed contains polyphenolic compounds that have a wide range of biological activities including antioxidant, anti-cancer and anti-inflammatory effects (9, 10). The Vitis vinifera is named grapevine in Iran called (vine) Tak, belongs to the family Vitaceae and has different genus (15).

Studies have shown that these polyphenolic compounds are also effective in oral health and have anti-gingivitis and anti-caries properties. According to clinical data, the antioxidant properties of Vitis vinifera seed are more than vitamin C and E due to the high content of polyphenolic compounds, especially proanticyanidins and oligomeric flavan-3-ol units especially, catechin and epicatechin (12).

Wei Zhao et al. (2014) indicated that Vitis vinifera had a dose-dependent effect on the growth inhibition and biofilm formation of Streptococcus mutans and occurrence of enamel caries (13). In 2008, Furgia et al. demonstrated the strong antiplaque effect of the combination of grape seed extract (GSE) and amine fluoride (12).

Since early childhood caries is still recognized as a common public health problem for infants and children (14 and 15), it seems that relying on the antimicrobial properties of GSE can be an axiliary method to prevent the occurrence of these caries. Thus, the aim of this study was to evaluate the effect of different Vitis vinifera seed extracts on Streptococcus mutans and sobrinus bacteria.

Materials and methods

In this experimental study, the effect of different vitis vinifera seed extracts was evaluated on Streptococcus mutans and sobrinus bacteria with Ethics Committee code of MUBABOL. REC1395156. Vitis vinifera grapes were purchased from Qazvin vineyard. The seeds of grapes were separated (6 kg) and dried at room temperature away from sunlight. The dried seeds were powdered by electric grinder, the resulting powder was collected in a glass container, 150 g of this powder was poured into separate calibrated containers to prepare aqueous, alcoholic (70% ethanol) and acetone extracts, and then each of the solvents was added to a volume of 200 ml.

The solutions were transferred to the shaker incubator system and after 72 hours, the containers were removed, the solutions were filtered using a filter paper and poured into the plates placed in the oven to evaporate the solvents and finally, the extract-containing plates were sterilized in a furnace to eliminate any contamination.

Lyophilized powders of Streptococcus mutans (ATCC 35668) and sobrinus (ATCC 27607) were purchased from Iran Scientific and Industrial Research Organization. At

Khodadadi E, et al. The effect of different Vitis vinifera seed extracts on Streptococcus mutans and sobrinus bacteria. 2019; 8(1):14-19.http://dx.doi.org/

first, these lyophilized bacteria were active. They were then transferred to Muller Hinton agar-containing plates using a sterilized swap, and the cultures were incubated at 37 ° C for 24 hours to allow the bacteria to grow fully and to obtain a single colony. Four to six colonies of each bacterial culture were inoculated with 5 ml of Muller-Hinton medium using a sterilized swap and incubated at 37 ° C until the bacteria reached exponential growth phase after 4-6 hours. The turbidity of each tube was compared with that of a 0.5McFarland standard.

Minimal inhibitory concentration (MIC) is the lowest concentration of a drug that can inhibit bacterial growth (12). In this study, determination of MIC of extracts was done using macrodilution method based on CLSI standard 2013(16). Each of the aqueous, alcoholic and acetone extracts was poured into separate tubes at concentrations of 2, 4, 8, 16, 32, 64 and 128 µg/ml (13). For each bacterium, one tube with no extract was considered as control.

After the macrodilution test, re-culture was performed on blood agar medium from the last tube in which the bacterial growth was negative (no turbidity tube). For the extracts that had no effect on the target bacteria (the culture medium was turbid), the re-culture was done on blood agar medium from tube containing 16 µg/ml to the last concentration (128 µg/ml) and the bacterial count was calculated in ml.

The plates were transferred to the incubator and the colonies grown after 48-72 hours were counted and their counts were calculated in one cc. Examination of plates revealed that the aqueous extract inhibited the growth of Streptococcus sobrinus and mutans at concentrations of 2 and 8 µg/ml, respectively. Alcoholic and acetone extracts had no inhibitory effect at any of these concentrations. The experiments were duplicated to ensure the accuracy of the results.

Results

In the measurement using quantitative macrodilution technique, the MIC of aqueous extract was 8 and 2 µg/ml for Streptococcus mutans and sobrinus, respectively. Nevertheless, two alcoholic and acetone extracts failed to obtain the zero bacterial growth at all concentrations (2, 4, 8, 16, 32, 64, 128).

The number of grown colonies of Streptococcus mutans counted in alcoholic and acetone extracts with a concentration of 128 µg/ml was CFU/ML10824000 and CFU/ML16232000, respectively. The number of grown colonies of Streptococcus sobrinus counted in alcoholic and acetone extracts with a concentrations of 128 µg/ml was CFU/ML12544000 and CFU/ML20577000, respectively. The results of the duplicate experiment were similar to those of the initial one.

Fig1. Grown colonies of streptococcus mutans in alcoholic extracts with a concentration of 128 µg/ml

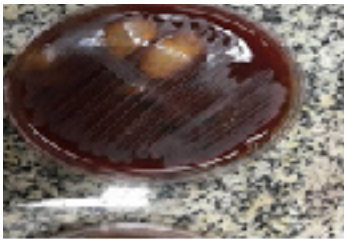

Fig 2. Grown colonies of streptococcus mutans in acetone extracts with a concentration of 128 µg/ml

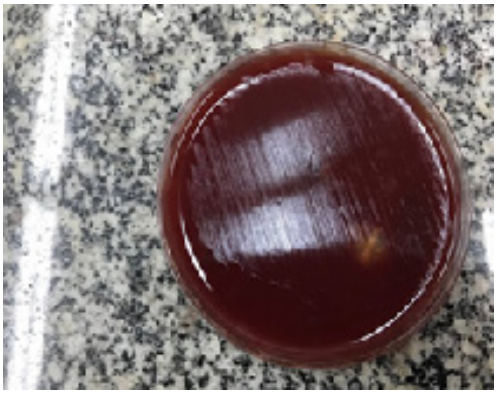

Fig 3. Grown colonies of streptococcus sobrinus in alcoholic extracts with a concentration of 128 µg/ml

Journal of Dentomaxillofacial Radiology, Pathology and Surgery

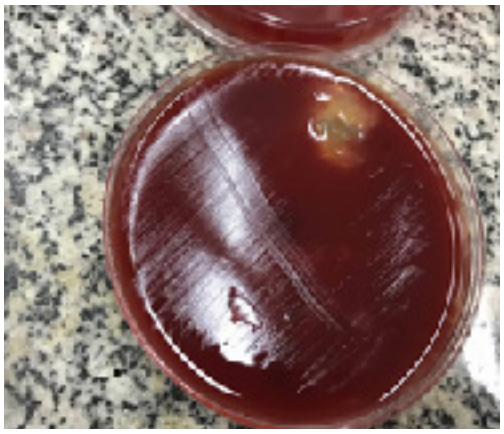

Fig 4. Grown colonies of streptococcus sobrinus in acetone extracts with a concentration of 128

Discussion

In the present study, the antibacterial effect of the aqueous, alcoholic and acetone extracts of Vitis vinifera seed extract was investigated using macrodilution method. Based on the results of this study ,Aqueous extract inhibited bacterial growth at certain concentrations, but alcoholic and acetone extracts had no inhibitory effect on bacterial growth at any concentrations. Given the prominent role of Streptococcus mutans in the onset of caries (17, 18), some methods should be found to reduce the rate of this bacterium in the oral cavity. Existing chemical antimicrobial agents are associated with limitations and complications. Since the plants contain effective and useful compounds for medical use, it seems that they can also be applied in dentistry to promote oral hygiene along with brushing(19). Researches have shown that Vitis vinifera seed extract has inhibitory effects on Streptococcus mutans growth, Wei Zhao et al. in 2014 observed that 4-µg/ml aqueous extract of Vitis vinifera inhibited the growth and biofilm formation of Streptococcus mutans (13). In the present study, in addition to the concentration of 4 µg/ml, concentrations of 2 and 8 µg/ml were used to investigate the concentration effect on the efficacy of the Vitis vinifera seed extract. Moreover, the effect of different extracts (aqueous, alcoholic and acetone) of Vitis vinifera seed was evaluated on both Streptococcus mutans and Sobrinus. Swadas et al. in 2016 compared the anti-Streptococcus mutans activity of Vitis vinifera seed extract with chlorhexidine. They concluded that the alcoholic extract of grape seed had anti-Streptococcus mutans effect, and chlorhexidine gluconate had more antibacterial property compared to all GSE concentrations (20),this result is different from that of the present study, to explaining this difference,it is important to note that several factors such as plant breeding conditions affect the phenolic compounds of grapes , in addition the genetics of bacteria tested effect on their tolerance against extract. Despite the greater efficacy of chlorhexidine in the inhibition of Streptococcus mutans, the use of herbal antimicrobial agents such as Vitis vinifera seed extract may be preferred due to the side effects and limitations of the use of chemical agents (chlorhexidine) such as staining bacterial tolerance (13، 20). Hence, we decided to determine whether the extract affects sobrinus bacteria, which is the effective microorganism at the onset of decay, or not (21). Furthermore, the current study investigated whether different solvents (aqueous, alcoholic and acetone) could play a role in inhibition rate of Vitis vinifera seed extract, and the rate of this efficacy was compared because the extraction of chemical compounds in plants depends on various factors including the used solvent and extraction method (22). Though some studies have proven that the methanol and ethyl acetate solvents are more effective in extracting phenolic compounds from grape seed (23, 24), the aqueous and ethanol solvents were selected due to their lower toxicity. The acetone extract was also compared for a more detailed examination. The result indicated that the aqueous extract was able to inhibit the growth of Streptococcus mutans and sobrinus bacteria, whereas alcoholic and acetone extracts were vice versa, which is contrary to the result of Ana Boucic et al. (2008) who have concluded that 50% ethanol is more effective than water (25). HUA LI et al. (2008) compared the efficacy of different solvent systems in the extraction of phenolic compounds and observed that the highest efficacy was related to acetone:

Khodadadi E, et al. The effect of different Vitis vinifera seed extracts on Streptococcus mutans and sobrinus bacteria. 2019; 8(1):14-19.http://dx.doi.org/

aqueous solvent (70:30) and the lowest one was for aqueous solvent (26) This difference in results could be due to the in vitro differences from beginning to end stages, also the geographical area and weather conditions are among factors that affect the constituents in grape seed(polyphenolic compounds). Based on the present study, it can be concluded that Vitis vinifera seed extract is a good compound for preventing caries, but more studies are needed to select the best solvent. As well as, the grape seed essence may have more antibacterial properties than its extract, which requires further research in this area.

Conclusion

According to the results of the present study, the aqueous extract of Vitis vinifera seed can inhibit the growth of Streptococcus mutans and sobrinus so it can be hoped that this extract can be used as an axiliary method to prevent tooth decay.

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