Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

The Antimicrobial Activity of Grape Seed Extract against Two Important Oral Pathogens

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Article information

Article history:
Received: 5 Mar 2012
Accepted:25 July 2012
Available online: 15 Oct 2012
ZJRMS 2013; 15(1): 43-46

Keywords:
Streptococcus mutans
Aggregatibacter actinomycetemcomitans
Grape seed extract

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Abstract

***Background*:** The antimicrobial properties of plant extracts have shown promise for development of new drugs. This study was conducted to measure the antibacterial activity of grape (*Vitis vinifera*) seed extract against *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans*.

***Materials and Methods*:** In this experimental study the grape seed extract have been prepared with maceration method. The antimicrobial activity of the extract was examined by determining Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) using the macro dilution broth technique.

***Results*:** MIC and MBC for *Aggregatibacter actinomycetemcomitans* was 3.84 mg/mL and 7.68 mg/mL respectively.There were not any inhibitory effects against *Streptococcus mutans*.

***Conclusion*:** The Grape seed extract has inhibitory and bactericidal effects against *Aggregatibacter actinomycetemcomitans*. There were not any bactericidal or bacteriostatic effects against *Streptococcus mutans*.

Introduction

Different plants are used to treat various diseases in different part of the world. There are reports that more than a quarter of medicines administered in industrialized countries are directly or indirectly derived from plants [1]. In-vitro evaluations have shown that an assortment of preparations used in the treatment of non-infectious diseases exhibit some antibacterial activity. These preparations are referred to as "non-antibiotic" antibacterials and might be used as antibiotics or as non-antibiotic antibacterial agents [2]. Despite the fact that the antimicrobial properties of plant extracts have undergone extensive evaluations in the field of clinical microbiology, the effects of these agents need to be further investigated in further clinical trials. Grapes *(Vitis vinifera)* are consumed all over the world and their seeds are rich in phenolic compounds, with an ability to damage microbial cells by exerting an influence on the selective permeability of the plasma membrane, which results in the leakage of vital intracellular substances [3]. GSE (Grape Seed Extract) has exhibited promise as a source for the manufacture of new generations of antibacterial agents for dental use without exerting an influence on the biological equilibrium in the oral cavity [4, 5]. In the present study an attempt was made to evaluate the bacteriostatic and bactericidal effects of GSE against two important bacterial agents involved in dental decay and periodontitis: *Streptococcus mutans* PTCC 1683 and *Aggregatibacter actinomycetemcomitans* ATCC 43718. The null hypothesis tested was "Grape seed extract has no bacteriostatic and bactericidal effects against *Streptococcus mutans* PTCC 1683 and *A. actinomycetemcomitans* ATCC 43718".

Materials and Methods

Preparation of grape (*Vitis vinifera L.)* seed extract: Ground grape seeds (100 gr) were extracted with ethanol: water ratio of 70:30, vol/vol, by maceration method under stirring at 45ºC for 2 h. The extracts were then filtered through whatman No. 1 filter paper under vacuum, and the residue was repeatedly extracted with the same solvent until it was colorless. The resultant filtered extracts were dried in a rotary evaporator at 45ºC.

Antioxidant assay: The GSE was dissolved in MeOH to obtain a concentration of 1×10^{-1} mg/mL. Dilution procedures were carried out to obtain concentrations of 5.00×10^{-2}, 2.5×10^{-2}, 1.25×10^{-2}, 6.25×10^{-3}, 3.13×10^{-3} and 1.56×10^{-3} mg/mL. Diluted solutions (5 mL each)

were mixed with 5 mL of a solution of 2, 2-Diphenyl-1-picrylhydrazyl (0.08 mg/mL) in MeOH and allowed for any reaction to occur for half an hour. The UV absorbance was recorded at 517 nm. The experiment was performed in duplicate and average absorption value was recorded for each concentration. Data were processed using EXCEL and the concentration that yielded a 50% reduction in absorbance (RC_{50}) was calculated. The same procedure was followed for the standard quercetin.

Determination of total phenol content (TPC): The total phenol content of the GSE was determined by the Folin-Ciocalteu method. One mL of the GSE solution in aceton/water (6/4) was transferred to a test tube and then mixed thoroughly with 0.2 mL of Folin-Ciocalteu reagent. After mixing for 3 min, 1 mL of 2% (w/v) sodium carbonate was added. The mixtures were agitated with a vortex mixer and then kept in dark for 30 min, after which they were centrifuged at 12000 g for 5 min. The absorbance of the extracts and a prepared blank were measured at 750 nm using a spectrophotometer.

The measurements were compared to a standard curve of prepared gallic acid solution and expressed as grams of gallic acid equivalents (GAE) per 100 grams of the extract, which was determined from known concentrations of gallic acid standard prepared similarly.

Bacterial strains and growth conditions: The tested bacterial strains included the gram negative reference strain *A. actinomycetemcomitans* ATCC 43718 and the gram positive strain *S. mutans* PTCC1683. The mentioned bacteria were cultured in Brain Heart Infusion (BHI, Difco, Detroit, MI) broth including 10^{cc} of sheep texture blood, 20 mL of Hemin, 10mL of vitamin K and 1 cc of Fetal bovine serum under aerobic conditions at 37ºC. One loop of stored culture was inoculated in to BHI agar media over night at 37ºC, and the culture was examined for the all bacteria to be assured of their growth.

Antimicrobial activity: The antimicrobial activity of GSE on the two reference strains was examined by determining the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) using the macro dilution broth technique (According to clinical laboratory standards institute (CLSI) 2007, M2A9 and M7A7 USA).

One gram GSE was sterilized by 0.22 µm millipore filter and diluted in 12 tubes. The final concentrations of GSE ranged from 245 mg/mL to 0.12 mg/mL (two fold dilutions). The same 0.5 MacFarland (1.5×10^8 CFU/mL) suspension was diluted with BHI broth and a bacterial inoculums of equivalent 10^5 CFU/mL was inoculated in to the tubes containing test compounds dilutions and incubated anaerobically at 37ºC for 48 hours. The MIC was defined as the lowest concentration of test compound able to caused complete growth inhibition. The MBC was defined as the lowest concentration of test compound that did not permit any visible growth on the appropriate agar plate after incubation period (99.9 killed). Each concentration of extracts was tested in triplicate.

Results

The antioxidant activity of the GSE was determined by the mentioned method and the RC_{50} value was found to be 2.90×10^{-3} mg/mL. The RC_{50} value of the positive control, quercetin, was 2.78×10^{-5} mg/mL, on the other hand the TPC of GSE was 70 g GAE/100 g. The antibacterial activity of grape seed extract against the two bacterial strains is shown in Table 1. In general the MIC and MBC values against *A. actinomycetem*comitans ATCC 43718 was 3.84 mg/mL and 7.68 mg/mL respectively. The GSE has not showed any bactericidal or bacteriostatic effects against SM PTCC 1683.

Discussion

In this study the effect of GSE (*Vitis vinifera*) has been evaluated against two most important bacterial strains in dental pathologies. *A. actinomycetemcomitans* was first identified as a possible periodontal pathogen in 1975 in localized aggressive periodontitis (LAP) and it's highly association with periodontal disease in adolescents has been established [6].

On the other hand *S. mutans* is the primary causal agent for dental caries specially in the initiation and development stages [7], this microbe was first described in 1924 [8]. Grape (*Vitis vinifera*) seeds are considered rich sources of polyphenolic compounds, mainly monomeric catechin and epicatechin, gallic acid and polymeric and oligomeric procyanidins [9]. Grape phenolics are simple molecules, such as hydroquinone, pyrocatechol, caffeic acid, ferulic acid, p-coumaric acid, gallic acid, ellagic acid and resveratrol [10]. Furthermore, GSE is a rich source of diverse bioflavonoids, collectively known as grape seed proanthocyanidins extract [11]. Polyphenols are well documented to have microbicidal activities against a huge number of pathogenic bacteria [3, 12].

The mechanism of polyphenols toxicity against microbes may be related to inhibition of hydrolytic enzymes (proteases and carbohydrolases) or other interactions to inactive microbial adhesions, cell envelope transport proteins and non specific interactions with carbohydrates [3]. It means that phenolic antibacterial activity is due to enzyme inhibition by the oxidized compounds possibly through a reaction with sulfhydryl groups or through more non specific interactions with the proteins [3, 13], further more flavonoids and tannins can bind or form precipitates with various proteins [14].

Table 1. The antimicrobial activities of grape seed extract determined by macro dilution broth method

Tubes	1	2	3	4	5	6	7	8	9	10	11	12
Concentrations (mg/mL)	245	122.88	61.44	30.72	15.36	7.68	3.84	1.92	0.96	0.48	0.24	0.12
A. actinomicetecomytans	-	-	-	-	-	-	-	+	+	+	+	+
S. mutans	+	+	+	+	+	+	+	+	+	+	+	+

In present study the GSE showed inhibitory effects against *A. actinomycetemcomitans* with MIC =3.84 mg/Land MBC= 7.68 mg/L using broth dilution method. However Song etal [13] reported 0.5mg/L MIC and 2mg/L MBC values for *A. actinomycetemcomitans* by using Polygonum Cuspidatum plant which contains a large number of flavonoids. In this study the GSE had no inhibitory effect on S.mutans, this finding is in opposite with Furiga etal [15] study which Red grape extract with 20% polyphenols and 3% anthocyanin and Red wine extract with 95% polyphenols (mainly catechin) showed approximately 4000µg/mL and 8000 µg/mL MBC for *S.mutans*, also Pascale etal [16] indicated that in the presence of GSE SM showed a decrease of colony forming units(CFU) and under SEM evaluation they found that tannis induced a great disparity in the bacterial size and in the bacterial forms (decrease of the cocoide forms). There are other studies which have point out that the plants with polyphenol compounds have inhibitory effects on *S. mutans* [4, 17].

Bayder etal [18] reported that gram positive bacteria were more susceptible to GSE than gram negative bacteria, considering the differences between gram positive and gram negative bacteria it's obvious that gram positive bacteria have a relatively thick continuous cell wall which is composed largely of peptidoglycan also known as mucopeptide or murein which is a polymer with a backbone of 1,4-linked N-acetylglucosamine and N-acetylmuramate and the lactyl group of which is cross linked through tetrapeptides, also gram positive bacteria sometimes have other cell wall polymers such as the teichoic acid, polysaccharides and peptidoglycolipids which covalently attached to the peptidoglycan, in contrast the peptidoglycan layer in gram negative bacteria is thin [19]. As it has been mentioned before the flavonoids can bind with various proteins and in the gram positive bacteria the proteins content may be stronger than gram negatives.

Although the results of the present study is in contravene with the upper justifications, it should be point out that the kind of reference strains may have an influence on the final results and the conflicting results could be due to intrinsic differences between different strains of the same species. Consequently the findings of our study could be account for SM PTCC 1683 and the GSE (*Vitis vinifera*) with the mentioned extraction process, also it should be point out that there are a number of factors such as climate, geographical area and growing conditions are known to affect the contents of grapes, so the species and harvested parts may be the most important factors affecting the differences [20].

There are different methods for evaluating the antimicrobial activities, Yim et al. [17] used disk diffusion assay for assessing antimicrobial activities of Vitis amurensis against *S. mutans* and *S. sanguis*.

In present study we have used the macro dilution broth method to evaluating the antimicrobial activity according to CLSI guidelines. Peng etal [20] indicated that disc diffusion method reveals the lower antimicrobial activities in comparison with micro dilution method.

This difference between methods may have an important role for making variation in results.

At all with consideration of inhibitory effect of GSE on *A. actinomycetemcomitans*, it may have potential for further development as natural agent in prevention of periodontal disease. Further studies with different species of *S.mutans* and different concentration of GSE are recommended.

Grape seed extract as a natural antimicrobial compound derived from *Vitis vinifera* has inhibition effect against *A. actinomycetemcomitans* ATCC 43718. There was not any bactericidal or bacteriostatic effect observed against *S. mutans* PTCC 1683.

Acknowledgements

This work was supported by Zahedan University of Medical Sciences Research Center and was based on a thesis in partial fulfillment of requirement for degree of doctor of dental surgery (registration code: 90-960).

The authors thank Dr. Mohammad Rahbar for providing the bacterial strains and Dr. Abbas Delazar for providing Grape seed extract

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Tarbiat Modares University.

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Please cite this article as: Mirkarimi M, Amin-Marashi SM, Bargrizan M, Abtahi A, Imani Fooladi A. The antimicrobial activity of grape seed extract against two important oral pathogens. Zahedan J Res Med Sci (ZJRMS) 2013; 15(1): 43-46.