ISSN Online: 2349-7785

In vitro Antibacterial Activity of Red Grape Seed Extracts on some Important Human Pathogenic Bacteria

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Received 04 July 2017; accepted in revised form 27 July 2017

Abstract: Red grape seed extracts (RGSE) were found to have antimicrobial properties. This study was conducted to determine the antibacterial potential of grape seed extracts on pathogenic bacteria and compare their properties with the standard antibiotics. RGSEs exhibited variable degrees of antibacterial activities against tested bacteria except for *Enterococcus faecalis*. Marked inhibition was observed with *Pseudomonas aeruginosa* using the methanol extraction. The zones of inhibition using the different aqueous solvents were relatively smaller compared to the zones of inhibition produced by the standard antibiotic discs. HPLC showed the presence of gallic acid in RGSE peaking at 29.670 min. There is a strong potential for RGSE to be used as an antimicrobial agent. It contains gallic acid and acts almost similar to standard antibiotics. However, the effects of these agents need to be further investigated *in vivo*.

Keywords: Red grape seed extracts, antimicrobial activities, gallic acid, pathogenic bacteria.

Introduction

Pathogenic bacteria have always been considered as a major cause of morbidity and mortality in humans. Pharmaceutical companies have produced a number of new antibacterial in the last years; however, resistance to these drugs has increased because of the emergence of bacterial strains with multiple resistances to these antibiotics, and has now become a global concern [1]. Because of this, there has been an increase in demand to develop new and effective antimicrobial agents from other potential natural sources [2,3].

One of the highly potential natural sources of antimicrobials against human pathogens is from plants [4]. Because of this, a multitude of researches has been conducted worldwide searching for new medicine sources, particularly from plants. Several essential drugs are derived from plants such as atropine, codeine, colchicine, digitoxin, morphine and many more [5].

Grapes (*Vitis vinifera L.*) is one among the important fruit crops grown worldwide. Red grape seed extract (RGSE) is well-known to contain many bioactive compounds including catechins, epicatechin, procyanidin, and some dimmers and trimers. Catechins and pyocyanidins are biologically active constituents in green tea and red wine, which are very strong antioxidants that inhibit low density lipoprotein, lower plasma cholesterol and prevent platelet aggregation [6]. These phenolic compounds are also found in cocoa, chocolate products, apple [7,8]. The polyphenols of grape seeds have been recognized for their beneficial role in human health. The grape seed is shown to display bioactivities apart from its antioxidant activity includes anti-inflammatory, anti-bacterial, anti-cancer, antiviral, anti-aging and anti-diabetic [9-14].

The antibacterial activities of grape seed extracts were found to have inhibiting activities against gram-positive bacteria including *Bacillus*

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cereus, *B. coagulans*, *B. subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* at 850-1000 ppm., and also against methicillin resistant *S. aureus* [15,16]. The acetone : water : acetic acid (90 : 9.5 : 0.5) extract was found to be the effective antimicrobial concentration that prevent food deterioration, and some oral pathogens [17,18].

Since more researches based on grape seed extract can be beneficial, this research was conducted to gain more knowledge towards its potential use in medicinal science. The aim of study focused on the determination of the antibacterial potential of RGSE against some of pathogenic bacteria and comparing their bacterial inhibiting properties with the available antibiotics.

Material and Methods

Red grape seeds extract preparation

Red grapes were bought from a local market in Riyadh, Saudi Arabia. The seeds were extracted, collected, and washed with distilled water. The dried red grapes seed were kept to dry at room temperature. Dried seeds were then grounded thoroughly to powder form. Ten grams were extracted and mixed with 100ml of each of the following solvents; ethanol, methanol and water separately. Samples were shaken for three days in a rotator shaker (120 rpm) at 27°C, then filtered with Whatman No.1 filter paper. The resulting filtrate were kept to dry. Each dried crude extract was dissolved in distilled water and sterilized through filtration aseptically with 0.45 µm pore size filter unit (Millipore, USA) and stored in sterile Eppendorf tubes at -4°C for further use.

Bacterial strains

Pure cultures of standard bacterial strains tested in this study included Staphylococcus *aureus* (ATCC 25923), Methicillin Resistant *S. aureus* (MRSA) (ATCC 12498), *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* ATCC (25966) and *Salmonella typhymirium* (ATCC 19585). All bacterial strains were obtained from the Microbiology laboratory of King Khaled University Hospital in Riyadh, Saudi Arabia. The cultures were grown on nutrient agar plates at 37Cº for 18 hours and the colonies were suspended in saline (0.85 % NaCl). Its turbidity was adjusted to 0.5 MacFarland standards (10^8 CFU/mL). This suspension preparation was used to inoculate the plates.

Determination of antimicrobial activity

Agar well diffusion assay was performed to examine antibacterial activity of RGSE against pathogenic bacteria. The inoculum of each prepared bacterial suspension were swabbed on Muller Hinton agar (MHA) plates. Wells of 5 mm were cut with a sterile cork borer, and 100 µl of each extract (ethanol, methanol and water) were loaded separately in the wells. Plates were incubated at 37°C for 18-24 hours. Antimicrobial activity was determined by measuring the inhibition zone. Standard antibiotic discs such as ampicillin (AMP 10 µg), Vacomycin (VA 30 µg), Trimethoprim-sulphamethoxazole (SXT 25 µg) and Tetracycline (TE 30 µg) were used as positive controls and for comparison of the size of inhibition zones with the RGSE activities.

High Performance Liquid Chromatography (HPLC) analysis

Chromatographic analysis was carried out on (HPLC, Shimadzu SPD-20A, Japan) to reveal the chemical constituents of RGSE. Gallic acid was separated and collected from the extract using the analytical HPLC. Further to collection, gallic acid was tested in vitro for its individual antibacterial activity again using the agar well diffusion technique, on Muller Hinton plates, where 70 µl of the collected gallic acid were loaded into the well against the tested organisms. Plates were incubated at 37 ÚC for 18-24 hrs. Analysis was compared to a standard gallic acid (GA) using C18 column that was set thermostatically at 25 ÚC. 300 µl of each GA and RGSE were first filtered through 0.45µm syringe filters (Millipore, USA), then the filtrate was inserted and run for 45 minutes. Ultraviolet detection was done at 254 nm. The mobile phase was water : methanol : tetrachloroacetic acid (90 : 1 : 1).

All data results were analyzed statistically using the Predictive Analysis Software (PASW) version 20 (SPSS Inc., IBM, Chicago, Illinois, USA).

Results

The antibacterial activity of the RGSE are shown in Table 1. The results indicated that RGSEs exhibited antibacterial activities at variable degrees against test bacteria. Marked inhibition was observed with *P. aeruginosa* using the methanol extraction of RGSE (21 mm), followed by *S. aureus* with methanol extract (20 mm). The methanol extraction showed zones of inhibition of bacteria between 15 and 21 mm, compared to ethanol extraction (9 - 20 mm) and water extraction (9.5 - 15.5 mm). *E. faecalis* growth was not affected by RGSE using different aqueous solvents. The zones of inhibition using the different solvents (methanol, ethanol and water), were relatively smaller compared to the zones of inhibition produced by the standard antibiotic discs except for the activity against *S. typhimurium* LT2, which has a 2 mm larger zone of inhibition with the methanol extraction than the standard SXT disc. (Table 1 and Fig. 1).

In the HPLC analysis, it was noted that the effect of Gallic acid was slightly negligible or even no effect compared to the crude extract were all phenolic contents are combined. Fig. 2 shows the HPLC profile of the methanol extract of RGSE exhibiting the presence of Gallic acid peaking at 29.670 min.

Discussion

RGSE are known to have antioxidant and antimicrobial activities. This has been reported in several previous studies that investigated on the potential antibacterial activities of the RSGE on some of human pathogenic species (15 - 18). The antibacterial activity of the RGSE is due to the biologically active phenolic flavonoid compounds that act by suppressing bacterial virulence factors including inhibition of biofilm formation, reduction of host ligands adhesion, and neutralization of bacterial toxins [19]. Non-flavonoid compounds particularly Gallic acid have been reported to have antibacterial activity against Gram-positive and Gram-negative bacteria, even better antibacterial activity than gentamicin and streptomycin [20].

The present work demonstrates the antimicrobial value of RGSE by using various solvents against different species of gram positive and gram negative bacteria. The well diffusion test revealed that RGSEs showed best activity against *P. aeroginosa*, *S. aureus* and *S. typhimutrium*,

Table 1. Average inhibition zones (in mm.) of solvent and aqueous extraction of RGSE using Agar well diffusion method against test organisms

Test organisms	Average inhibition zones (in mm.)			Zone of inhibition (antibiotic discs)
	Methanol extract	Ethanol extract	Water extract	
S. aureus	20.5	20.0	9.5	Amp (10) = 29 mm
MRSA	16.5	19.0	15.5	Va (30) = 25 mm
E. faecalis	-	-	-	SXT(25) = 27 mm
B. subtilis	15.0	9.0	11.0	TE (30) = 25 mm
P. aeruginosa	21.0	14.5	-	TE(30) = 27 mm
E. coli	-	-	12.0	SXT(25) = 27mm
S. typhymirium LT2	20.0	10.0	13.0	SXT(25) = 18 mm

Diameter of zone of inhibition in mm Data represented as mean of three reading
Disc diameter = 6mm
Hole diameter = 6mm
Amp = ampicillin 10 mg/ug/disc
SXT = trimethoprim-sulphamethoxazole 25 mg/ug/disc
Va = vancomicin 30 mg/ug/disc
Te = tetracyclin 30 mg/ug/disc

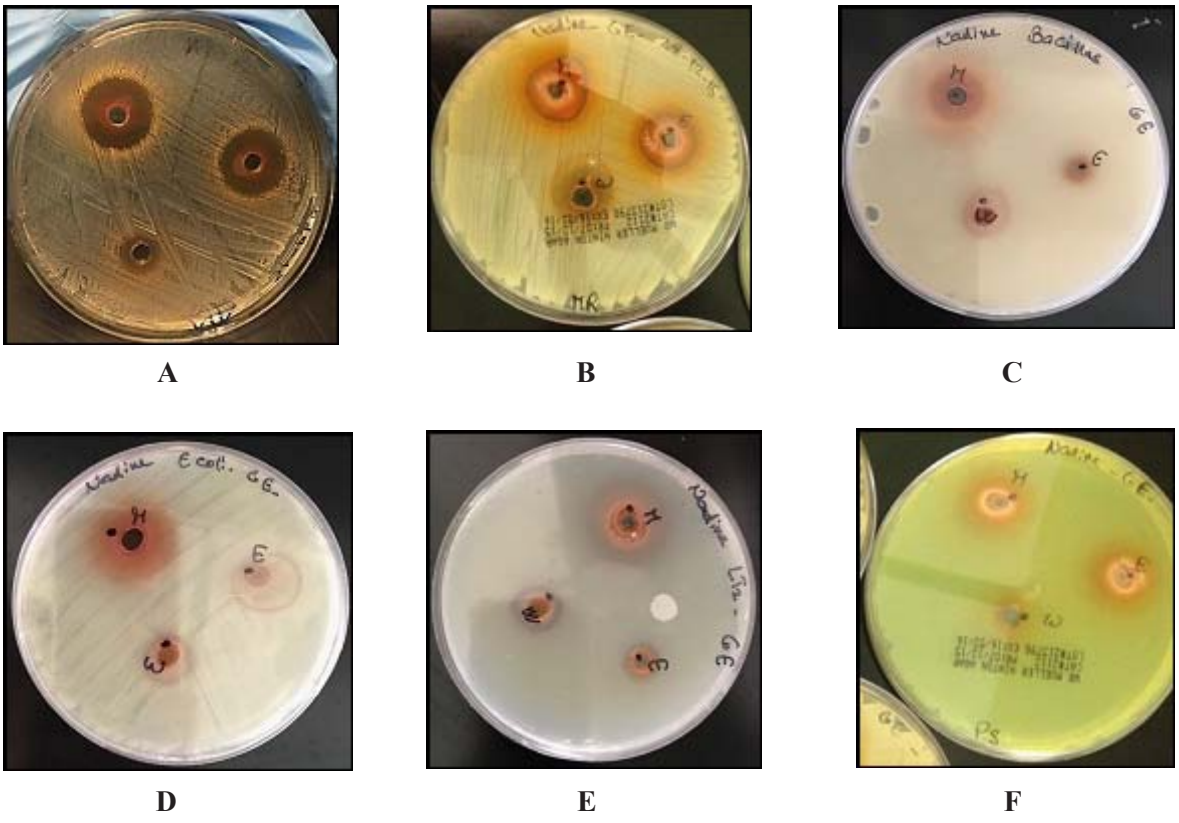

Fig. 1. Photographs showing zone of Antibacterial Inhibition of RGSEs (in mm) by Solvent and Aqueous Extracts against *S. aureus* (A), MRSA (B), *B. subtilis* (C), *E. coli* (D), *S. typhimurium* (E) and *P. aeruginosa* (F)

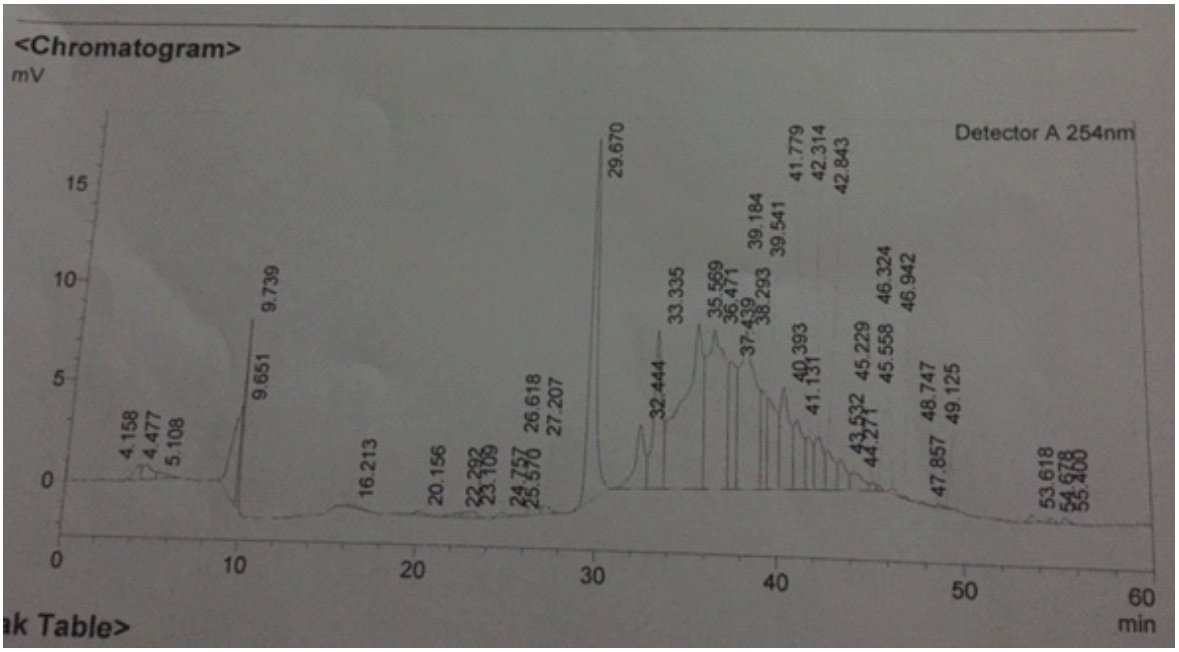

Fig. 2. HPLC profile of the RGSE methanol extract showing the peaking of Gallic acid at 29.670 min

followed by MRSA and *B. subtilis*. However, the extracts showed minimal or no activity against *E. coli* and *E. faecalis*. Table 1 and Fig. 1 clearly shows the antibacterial activity of RGSE against the test organisms. Despite the smaller zones of inhibition displayed RSGE on the test organisms as compared to the standard antibiotic discs, the potential use of RGSE for antibacterial use is clearly manifested. One probable reason why these RGSE did not produced marked inhibition is that most plant extracts generally contain flavonoids in the glycosidic form [21].

Our results also suggests that alcohol is the best solvent for the extraction of active compounds as compared to distilled water in most of the test bacteria except for *E. coli*, where water extract showed a wider zone of inhibition compared to methanolic and ethanolic extracts. It is known that the yields of polyphenols decreases with water extraction compared to methanol and ethanol extraction in which the yield of extracts is increased with the time of extraction [22].

The HPLC analysis confirmed the presence of Gallic acid which peaked at 29.670 minutes (Fig. 2). Gallic acid is known to exhibit a wide range of antibacterial effects which are able to change the membranes of some pathogenic bacteria irreversibly [23,24]. Fruits are well known to have high phenolic content; however, each compound has a different antioxidant capacity, depending on its structure, number of aromatic and hydroxyl groups and their distribution in the structure [25,26]. The composition of bioactive phenolic is complex and it is assumed that all account to the overall antioxidant capacity, where their interactions could be additive, synergistic or even antagonistic [27]. In order to gain more insights about the antibacterial activity of grape seeds methanol extract mainly attributed to the phenolic compound, particularly related to the presence of Gallic acid. It was noted that the effect of Gallic acid was slightly negligible or even no effect compared to the crude extract were all phenolic contents are combined. Hence we can assume that the potent antibacterial activity of grape seed extract is mainly due to the synergistic interaction between the secondary metabolites mainly phenolic compound rather due to the individual effect of one phenolic acid namely Gallic acid.

Conclusion

RGSE contain Gallic acid and has the potential to be used as an antimicrobial agent. These results are encouraging and provided a scientific insight to further determine the antimicrobial properties of RGSE. Development of more extraction methods to enhance the yield of RGSE and Gallic acid from red grapes are potential areas for further research. However, the effects of these agents need to be further investigated in vivo.

Acknowledgements

This research project was supported by a grant from the "Research Center of the Female Scientific and Medical Colleges," Deanship of Scientific Research, King Saud University.

References

1. **Levy, S.B. and Marshall, B. (2004).** Antibacterial resistance worldwide: causes, challenges and responses. Nature Med. 10: 122-129.
2. **Kim, S. and Fung, D.Y.C. (2004).** Antibacterial effect of crude water-soluble arrowroot (*Puerariae radix*) tea extracts on food-borne pathogens in liquid medium. Letters in Applied Microbiology. 39(4): 319-325.
3. **Adwan, G. and Mhanna. M. (2008).** In Vitro Antibacterial, Antifungal and Other Medical Properties of Endangered Medicinal Plant Seeds. Journal of Scientific Research. 3: 134-139.
4. **Mahesh, B. and Satish, S. (2008).** Antimicrobial Activity of Some Important Medicinal Plant against Plant and Human Pathogens. World. J. Agri. Sciences. 4: 839-843.
5. **Akerele, O. (2015).** In: Medicinal Plants: Their role in Health and Biodiversity (Chapter 2 pages 11-17). Tomlinson, T.R. and Akerele, O (Eds). University of Pennsylvania Press.
6. **Jeong, W.S. and Kong, A.T. (2004).** Biological properties of monomeric and polymeric catechins: Green tea catechins and procyanidins. Pharm. Biology**.** 42: 84-93.

7. **Gu, L., House, S.E., Wu, X., Ou, B. and Prior, R.L. (2006*).*** Procyanidin and catechin contents and antioxidant capacity of cocoa and chocolate products. J. Agric. Food. Chem. 54: 4057-4061.
8. **Shoji, T., Masumoto, S., Moriichi, N., Kanda, T. & Ohtake, Y. (2006).** Apple (*Malus pumila*) procyanidins fractionated according to the degree of polymerization using normal-phase chromatography and characterized by HPLC-ESI/MS and MALDI-TOF/MS. J. Chromatogr. A. 1102: 206-213.
9. **Baydar, N.G., Özkan, G. and Çetin, E.S. (2007).** Characterization of grape seed and pomace oil extracts. Grasas Y Aceites. 58(1): 29-33.
10. **Li, H., Wang, X., Li, P., Li, Y. and Wang, H. (2008).** Comparative study of antioxidant activity of grape (*Vitis vinifera*) seed powder assessed by different methodsJournal of Food and Drug Analysis. 16(6): 67-73.
11. **Suwannaphet, W., Meeprom, A., Yibchok-Anun, S. and Adisakwattana, S. (2010).** Preventive effect of grape seed extract against high-fructose diet-induced insulin resistance and oxidative stress in rats. Food and Chemical Toxicology. 48: 1853-1857.
12. **Canbay, H.S. and Bardakc, B. (2011).** Determination of Fatty Acid, C, H, N and Trace Element Composition in Grape Seed by GC/MS, FTIR, Elemental Analyzer and ICP/OESSDU. Journal of Science (E-Journal). 6(2): 140-148.
13. **Su, X. and D'Souza, D.H. (2011).** Grape Seed Extract for Control of Human Enteric Viruses. Applied and Environmental Microbiology. 77(12): 3982-3987.
14. **Mendes, J.A.S., Prozil, S.O., Evtuguin, D.V. and Lopes, L.P.C. (2013).** Towards comprehensive utilization of winemaking residues: characterization of grape skins from red grape pomaces of variety touriga nacional. Industrial Crops and Products. 43: 25-32.
15. **Jayaprakasha, G.K., Selvi, T. and Sakariah, K.K. (2003).** Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. Food Res Int. 36: 117-122.
16. **Al-Habib, A., Al-Saleh, E., Safer, A. and Afzal, M. (2010).** Bactericidal effect of grape seed extract on methicillin-resistant Staphylococcus aureus (MRSA). J Toxicol Sci. 35: 357-364.
17. **Baydar, N.G., Ozkan, G. and Sagdic, O. (2004).** Total Phenolic Contents and Antibacterial Activities of Grape (*Vitis vinifera* L.) Extracts. Food Control. 15: 335-339.
18. **Furiga, A., Lonvaud-Funel, A. and Badet, C. (2009).** In Vitro Study of Antioxidant Capacity and Antibacterial Activity on Oral Anaerobes of a Grape Seed Extract. Food Chemistry. 113: 1037-1040.
19. **Daglia, M. (2012).** Polyphenols as antimicrobial agents. Current Opinion in Biotechnology. 23: 174-181.
20. **Saavedra, M.J., Borges, A., Dias, C., Aires, A., Bennett, R.N., Rosa, E.S. and Simoes, M. (2010).** Antimicrobial activity of phenolics and glucosinolate hydrolysis products and their synergy with streptomycin against pathogenic bacteria. Med. Chem. 6: 174-183.
21. **Rauha, J.P., Remes, S., Heinonen, M., Hopia, A., Kahkonen, M., Kujala, T., Pihlaja, K., Vuorela, H. and Vuorela, P. (2000).** Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Microbiol. 56: 3-12.
22. **Lapornik, B., Prosek, M. and Wondra, A.G. (2005).** Comparison of extracts prepared from plant by-products using different solvents and extraction time. J Food Engineering. 71: 214-222.
23. **Jayaraman, P., Sakharkar, M.K., Lim, C.S., Tang, T.H. and Sakharkar, K.R. (2010).** Activity and interactions of antibiotic and phytochemical combinations against Pseudomonas aeruginosa in vitro. Int. J. Biol. Sci. 6: 556-568.
24. **Borges, A., Ferreira, C., Saavedra, M.J. and Simo, M. (2013).** Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. Microbial Drug Resistance. 19(4): 256-265.

25. **Balasundram, N., Sundram, K. and Samman, S. (2006).** Phenolic compounds and agri-industrial by products: Antioxidant activity, occurrence, and potential uses. Food Chem. 99: 191-203.
26. **Heo, H., Kim, Y., Chung, D. and Kim, D. (2007).** Antioxidant capacities of individual and combined phenolics in a model system. Food Chem. 104: 87-92.
27. **Corral-Aguayo, R.D., Yahia, E.M., Carrillo-Lopez, A. and Gonzalez-Aguilar, G., (2008).** Correlation between some nutritional components and the total antioxidant capacity measured with six different assays in eight horticultural crops. J. Agric. Food Chem. 56: 10498-10504.