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Grape Seed Extract Exerts Abhesive Effect Against *Staphylococcus aureus*: *In vitro* Study

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ABSTRACT

Staphylococcus aureus is a pathogen known to cause biomaterial-associated infections of implants and devices. Adherent *Staphylococcus aureus* are highly resistant to the bactericidal activity of phagocytes and they are also resistant to most antimicrobial agents. This study aimed to evaluate the antibacterial activity and the abhesive property of hydro-distillation of *Vitis vinifera* seeds extract against *Staphylococcus aureus*. The Minimum Inhibitory Concentration (MIC) of susceptibility *Staphylococcus aureus*, isolated from patients, to oxacillin and hydro-distilled grape seed extract were determined by microdilution method using Muller-Hinton broth. Oxacillin or grape seed extract was added either before or after bacterial adhesion in the well of micro-titer plate. Five out of 24 isolates collected from infected burns and 7 out of 8 isolates collected from infected wounds were resistant to oxacillin (MIC≥8 μg mL^{-1} and the MICs of grape seed extract were ranged from 1.152 to>150 μg mL^{-1}. The growth of *Staphylococcus* is effectively inhibited by the extract of grape seed when the extract is added either before or after bacterial adhesion (MIC≤150 μg mL^{-1}). It concludes that grape seed extract inhibits the growth of oxacillin-resistant *Staphylococcus aureus* and it exerts abhesive effect against it.

Key words: Grape seed extract, *Staphylococcus aureus*, abhesive effect

INTRODUCTION

Bacterial adherence, a prerequisite in the medical implants infections, initially reversible but later becomes irreversible (Busscher *et al*., 2010; Matl *et al*., 2008). The biomaterial surface is colonized and bacteria develop an environment that protects them from host defenses and antibiotics (Verran and Whitehead, 2005; Pereni *et al*., 2006; Maddikeri *et al*., 2008). Adherent bacteria are highly resistant to the bactericidal activity of phagocytes and they are also resistant to most antimicrobial agents (Zimmerli and Sendi, 2011). Therefore, the best prophylactic approach would be, to prevent bacterial adhesion or to kill bacteria shortly after adhesion, during reversible phase. Vancomycin and/or gentamicin in the irrigating solutions may be useful in reducing *Staphylococcus epidermidis* adhesion to intraocular lens during cataract surgery (Abu el-Asrar *et al*., 2000). Synthetic substances like copper additives in silicon implants,

polytetrafluoroethylene coated with antibiotics, titania nanotubes incorporated with sliver nanoparticles and some poloxamers have been shown to have abhesive (anti-adhesive) activity which makes them useful as detergents (Gosau *et al*., 2010; Matl *et al*., 2008; Zhao *et al*., 2011; Portoles *et al*., 1994).

Natural substances obtained from herbs and plants also have abhesive activity. The essential oil of *Boswellia papyrifera* showed considerable activity against both *Staphylococcus epidermidis* DSM 3269 and *Staphylococcus aureus* ATCC 29213 biofilms i.e., prevents bacterial adhesion at sub-minimum inhibitory concentrations (Schillaci *et al*., 2008). Sub-inhibitory concentrations of the carvacrol and thymol oils attenuated biofilm formation of *Staphylococcus aureus* and *Staphylococcus epidermidis* strains on polystyrene microtitre plates (Nostro *et al*., 2007).

The antibacterial activity of *Vitis vinifera* seed extract against *Staphylococcus aureus* is contradictory. Mayer *et al*. (2008) found that fractionated proanthocyanidins and gallate esters had antibacterial activity towards gram positive and negative microorganisms. At a concentration of 2000 μg mL^{-1}, grape seeds extract significantly reduced the formation of multi-species biofilm grown on saliva-coated hydroxyapatite (Furiga *et al*., 2009). The objective of this study is to evaluate the antibacterial activity and the abhesive property of hydro-distillation of *Vitis vinifera* seeds extract on *Staphylococcus aureus*.

MATERIALS AND METHODS

This study was conducted in Department of Microbiology in cooperation with Department of Pharmacology, College of Medicine, Al-Mustansiriya University in Baghdad, Iraq. Clinical isolates of *Staphylococcus aureus* were collected from infected wounds (n = 8) and burns (n = 24) of patients admitted in Al-Kindey Teaching Hospital.

Preparation of grape seeds extract: Aqueous extract of *Vitis vinifera* was prepared by simple hydro-distillation. In brief, 1 g of fine powder of grinded grape seeds in 100 mL distilled water (1%) was boiled, the vapor separated and condensed to obtain clear colorless liquid that was more concentrated in volatile polar compounds. UV-Visible spectra of distilled aqueous extract was recorded on a Aquarius (Cecil series with scanning ability, France) from 150 to 900 nm wavelength at room temperature with a 10 mm length quartz cell with a scan rate of 600 nm min^{-1}. Serial dilutions of extract were done with distilled water.

Susceptibility of *Staphylococcus aureus*: Antimicrobial susceptibility of the clinical isolates and the Minimum Inhibitory Concentrations (MICs) of oxacillin and grape seeds extract were determined by microdilution method using Muller-Hinton broth and a standard inoculum of 0.5 McFarland.

A sterile micro-dilution plate of 96 wells (8×12) of 250 μL volume per well was used.

In one series of experiments 15 μL oxacillin (two fold serial dilutions ranged from 0.25 to 32 μg mL^{-1} final concentration) or equivalent volume of grape seed extract (at different concentration) were added into wells. Then after 1 h, 220 μL Muller-Hinton broth and 15 μL bacterial suspensions (≈1.5×10^6 microorganism) in sequences added. The negative control contained antimicrobial and broth. Plates were incubated for 18-24 h at 37°C.

In another series of experiments the wells were inoculated with 220 μL Muller-Hinton broth and 15 μL bacterial suspensions (≈1.5×10^6 microorganism) and then the plates were incubated for 18-24 h at 37°C. On the next day the Muller-Hinton broth was discarded from each well and a layer of bacterial adherence was observed in the well. Wells containing adherent bacteria were used

for testing the effect of oxacillin or the extract of grape seed. To these wells 15 μL oxacillin (two fold serial dilutions ranged from 0.25 to 32 μg mL^{-1} final concentration) or grape seed extract and 220 μL Muller-Hinton broth were added and then the plates were incubated for 18-24 h at 37°C.

The MIC was determined by observing all wells for visible growth. The MIC was defined as the lowest concentration of antimicrobial at which the well appeared clear. Strains were categorized as susceptible to oxacillin at MIC<8 μg mL^{-1}.

RESULTS

UV-Visible spectra of hydro-distilled grape seeds extract (1%) showed two peaks at 192.5 nm (O.D. 0.471) and 277.5 nm (O.D. 0.187). Table 1 showed that 5 out of 24 isolates collected from infected burns and 7 out of 8 isolates collected from infected wounds were resistant to oxacillin (MIC≥8 μg mL^{-1}). The MICs of grape seeds extract were ranged from 1.152 to>150 μg mL^{-1} (Table 2). Nine out of twelve clinical isolates that resistant to oxacillin were inhibited by grape seeds

Table 1: Susceptibility of isolated *Staphylococcus aureus* to oxacillin

	MIC (μg mL^{-1}) of oxacillin		
Isolate	Control	Before adhesion	After adhesion
1	8	2	8
2	0.25	0.25	0.25
3	0.25	16	0.5
4	0.25	1	>32
5	>32	4	32
6	8	8	>32
7	0.25	0.25	0.25
8	0.25	16	>32
9	0.25	8	>32
10	0.25	32	32
11	2	32	>32
12	2	32	>32
13	0.5	>32	16
14	0.5	16	16
15	1	32	16
16	4	32	16
17	8	16	32
18	4	0.25	2
19	4	1	8
20	0.25	>32	>32
21	0.25	16	32
22	1	32	32
23	>32	>32	32
24	0.25	16	32
25	32	32	16
26	32	32	32
27	32	32	32
28	32	32	16
29	>32	16	>32
30	32	1	8
31	8	1	1
32	0.25	0.25	32

Table 2: Susceptibility of isolated *Staphylococcus aureus* to hydrodistillation of *Vitis vinifera* seeds extract

	MIC (μg mL^{-1}) of *Vitis vinifera* seed extract (1%)		
Isolate	Control	Before adhesion	After adhesion
1	1.156	75	9.25
2	37.5	150	75
3	18.5	150	75
4	37.5	75	37.5
5	>150	9.25	1.156
6	37.5	9.25	>150
7	75.0	1.156	18.5
8	>150	9.25	2.312
9	>150	9.25	2.312
10	75.0	150	75
11	>150	2.312	2.312
12	75.0	>150	150
13	>150	1.156	9.25
14	>150	4.625	9.25
15	>150	18.5	9.25
16	>150	37.5	4.625
17	18.5	9.25	75
18	1.156	150	1.156
19	150	>150	150
20	37.5	>150	>150
21	9.25	150	1.156
22	150	37.5	1.156
23	>150	150	>150
24	1.152	150	18.5
25	75	>150	75
26	9.25	150	75
27	150	150	75
28	150	>150	1.156
29	150	>150	>150
30	>150	>150	75
31	37.5	>150	4.625
32	1.152	150	18.5

extract of 1.156-≤150 μg mL^{-1}. Table 2 showed that oxacillin failed to inhibit the growth of *Staphylococcus aureus* in 22 isolates when it added before the adherent microorganisms and in 27 isolates of adherent microorganisms. The growth of *Staphylococcus* is effectively inhibited by the grape seed extract when the extract is added before the microorganisms adhered to wells of microdilution plate in 24 isolates (MIC<150 μg mL^{-1}) and in 28 after adhered microorganisms (MIC≤150 μg mL^{-1}). Fourteen isolates that were susceptible to oxacillin became resistant when oxacillin is added prior to adherent microorganisms and four resistant isolates became susceptible. On the other hand sixteen susceptible clinical isolates became resistant when cloxacillin is added after the microorganisms adhered to the wells while only one resistant isolate became susceptible.

DISCUSSION

The present study clearly showed that hydro-distilled grape extract (1%) inhibits the growth of *Staphylococcus aureus* clinical isolates. The hydro-distilled grape extract which is the polar

fractions of extract showed the antibacterial activity against *Staphylococcus aureus*. This finding is in agreement with others who found the polar fractions of *Vitis rotundifolia* which contained malic and tannic acids, inhibited the growth of *E. sakazakii* while the polyphenol fraction which contained gallic acid catechin, epicatechin, ellagic acid and pigments slightly inhibited the growth of *E. sakazakii* (Kim *et al*., 2009). Moreover, whey protein isolate coating incorporated with grape extract is found to be a promising mean of controlling the growth and recontamination of *L. monocytogenes*, *S. typhimurium* and *E. coli* 0157 in ready to eat poultry products (Gadang *et al*., 2008). Grape seed extract combined with amphotericin B strikingly retarded the growth of yeast (Han, 2007). The main determinant of the antimicrobial activity of grape seed extract is the constituents of proanthocyanidins which consisted from catechin and epicatechin (Mayer *et al*., 2008). Recent study reported that proanthocyanidins extracted from cranberry reduced the formation of biofilms by *S. mutans in vitro* and dental caries development *in vivo* due to the presence of specific bioactive A-type dimers and oligomers (Koo *et al*., 2010). Moreover, in one cross-over clinical study Lavigne *et al*. (2011) found that administration of proanthocyanidine plus propolis once daily offered some protection against *E. coli* adhesion, multiplication and virulence in the urinary tract.

This study indicates that the anti-adhesive effects are possibly due to interactions of the polyphenols constituents of grape seed extract (detected by UV scan at 192.5 nm and 277.5 nm) with binding factors on the bacterial surface (Janecki *et al*., 2011). One of the limitations of this study is the identification of the active ingredient in the grape seed extract that exerts the abhesive effect. It concludes that grape seed extract inhibits the growth of oxacillin-resistant *Staphylococcus aureus* and it exerts abhesive effect against it.

REFERENCES

Abu el-Asrar, A.M., A.A. Kadry, A.M. Shibl, S.A. al-Kharashi and A.A. al-Mosallam, 2000. Antibiotics in the irrigating solutions reduce *Staphylococcus epidermidis* adherence to intraocular lenses. Eye, 14: 225-230.

Busscher, H.J., M. Rinastiti, W. Siswomihardjo and H.C. van der Mei, 2010. Biofilm formation on dental restorative and implant materials. J. Dent. Res., 89: 657-665.

Furiga, A., A. Lonvaud-funel and C. Badet, 2009. *In vitro* study of antioxidant capacity and antibacterial activity on oral anaerobes of a grape seed extract. Food. Chem., 113: 1037-1040.

Gadang, V.P, N.S. Hettiarachchy, M.G. Johnson and C. Owens, 2008. Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a Turkey frankfurter system. J. Food. Sci., 73: M389-M394.

Gosau, M., L. Prantl, M. Feldmann, A. Kokott, S. Hahnel and R. Burgers, 2010. The effects of copper additives on the quantity and cell viability of adherent *Staphylococcus epidermidis* in silicone implants. Biofouling, 26: 359-365.

Han, Y., 2007. Synergic effect of grape seed extract with amphotericin B against disseminated candidiasis due to *Candida albicans*. Phytomedicine, 14: 733-738.

Janecki, A., A. Conrad, I. Engels, U. Frank and H. Kolodziej, 2011. Evaluation of an aqueous-ethanolic extract from *Pelargonium sidoides* (EPs® 7630) for its activity against group A-streptococci adhesion to human HEp-2 epithelial cells. J. Ethnopharmacol., 133: 147-152.

Kim, T.J., J.L. Silva and Y.S. Jung, 2009. Antibacterial activity of fresh and processed red muscadine juice and the role of their polar compounds on *Escherichia coli* O157: H7. J. Applied Microbiol., 107: 533-539.

Koo, H., S. Duarte, R.M. Murata, K. Scott-Anne and S. Gregoire *et al.*, 2010. Influence of cranberry proanthocyanidins on formation of biofilms by *Streptococcus mutans* on saliva-coated apatitic surface and on dental caries development *in vivo*. Caries. Res., 44: 116-126.

Lavigne, J.P., X. Vitrac, L. Bernard, F. Bruyere and A. Sotto, 2011. Propolis can potentialise the anti-adhesion activity of proanthocyanidins on uropathogenic *Escherichia coli* in the prevention of recurrent urinary tract infections. BMC. Res. Notes, Vol 4. 10.1186/1756-0500-4-522.

Maddikeri, R.R., S. Tosatti, M. Schuler, S. Chessari, M. Textor, R.G. Richards and L.G. Harris, 2008. Reduced medical infection related bacterial strains adhesion on bioactive RGD modified titanium surfaces: A first step toward cell selective surfaces. J. Biomed. Mater. Res. A, 84: 425-435.

Matl, F.D., A. Obermeier, S. Repmann, W. Friess, A. Stemberger and K.D. Kuehn, 2008. New anti-infective coatings of medical implants. Antimicrob. Agents Chemother., 52: 1957-1963.

Mayer, R., G. Stecher, R. Wuerzner, R.C. Silva and T. Sultana *et al.*, 2008. Proanthocyanidins: target compounds as antibacterial agents. J. Agric. Food. Chem., 56: 6959-6966.

Nostro, A., A.S. Roccaro, G. Bisignano, A. Marino and M.A. Cannatelli *et al.*, 2007. Effects of oregano, carvacrol and thymol on *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. J. Med. Microbiol., 56: 519-523.

Pereni, C.I., Q. Zhao, Y. Liu and E. Abel, 2006. Surface free energy effect on bacterial retention. Colloids Surf. B Biointerfaces, 48: 143-147.

Portoles, M., M.F. Refojo and F.L. Leong, 1994. Poloxamer 407 as a bacterial abhesive for hydrogel contact lenses. J. Biomed. Mater. Res., 28: 303-309.

Schillaci, D., V. Arizza, T. Dayton, L. Camarda and V. Di-Stefano, 2008. *In vitro* anti-biofilm activity of *Boswellia* spp. oleogum resin essential oils. Lett. Applied Microbiol., 47: 433-438.

Verran, J. and K. Whitehead, 2005. Factors affecting microbial adhesion to stainless steel and other materials used in medical devices. Int. J. Artif. Organs, 28: 1138-1145.

Zhao, L., H. Wang, K. Huo, L. Cui and W. Zhang *et al.*, 2011. Antibacterial nano-structured titania coating incorporated with silver nanoparticles. Biomaterials, 32: 5706-5716.

Zimmerli, W. and P. Sendi, 2011. Pathogenesis of implant-associated infection: the role of the host. Semin. Immunopathol., 33: 295-306.