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In vitro antimicrobial effects of grape seed extract on peri-implantitis microflora in craniofacial implants

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ABSTRACT

Objective: To determine the antimicrobial effects of grape seed on peri-implantitis microflora. **Methods:** The grape seed extract was tested against peri-implantitis microflora most commonly found in craniofacial implants including reference strains of *Staphylococcus aureus (S. aureus)*, *Escherichia coli (E. coli)*, *Candida albicans (C. albicans)* and clinical strains of *S. aureus*, *Klebsiella pneumonia (K. pneumonia)* and *Candida parapsilosis (C. parapsilosis)* by disk diffusion test. Minimum inhibitory concentrations (MIC) and minimum cidal concentrations (MCC) were determined using modified agar dilution millpore method. The extract was further combined with polyethylene glycol and propylene glycol, and was tested for antimicrobial effects. **Results:** Grape seed extract showed positive inhibitory effects with *S. aureus* at MIC of 0.625 mg/mL and MCC of 1.25 mg/mL respectively. However the extracts showed minimal or no reactivity against strains of *E. coli*, *K. pneumonia*, *C. parapsilosis* and *C. albicans*. The use of grape seed extract in combination with polyethylene glycol and propylene glycol also showed dose dependent inhibitory effect on *S. aureus*. **Conclusions:** The results of the study showed that grape seed has potential antimicrobial effects which can be further studied and developed to be used in the treatment of infected skin-abutment interface of craniofacial implants.

1. Introduction

Maxillofacial defects may be a resultant of treatment consequence of neoplasm, trauma and or congenital defects. Previously the rehabilitation of facial defects had been limited or poorly accepted due to inadequacy of materials and retention. With the development of newer materials such as silicone elastomers and medical adhesives the prosthetic rehabilitation of the lost body parts has been considered as an acceptable treatment option for patients with craniofacial defects and may be preferable to complex surgical reconstruction. Furthermore, the use of craniofacial implants offers patients with the state of the art treatment for rehabilitation of facial defects. The greater retention that these implant retained facial prosthesis offer provide a great boost to the patient's psychology and overcomes the limitations of adhesives such as adhesive related skin irritation, breakdown and discoloration of the margins of the prosthesis, lack of retention for large prosthesis, and variable positioning of the prosthesis. In light to these benefits there are related complications to implant retained prosthesis, importantly tissue reactions of the implant skin interface[1].

Histological studies of the peri-implant abutment skin interface have shown that there is a lack of strong physical barrier between the soft tissue and the implants. It has been noted that the seal is more dynamic in nature comprising of inflammatory cells[2,3] and dependent on the load of the implant site from the host response to the implant material, surface, stability, exogenous agents, and mechanical factors. A lack of proper hygiene maintenance along with the compromised skin interface can lead to change in the resident skin microflora. Many microbiological studies have shown *Staphylococcus aureus* to be a common isolate from infected craniofacial implant-abutment interface[4].

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Few isolated strains of *Escherichia coli (E.coli)*, *Klebsiella pneumonia (K. pneumonia)*, *Candida albicans (C. albicans)*, *Candida krusei (C. krusei)* and *Candida parapsilosis (C. parapsilosis)* have also been found from these sites[5].

Recently there have been many research focused towards the antimicrobial properties of herbal products. Grape (*Vitis viniferea*) is one of the most palatably edible fruits, grown all over the world and is considered to have many nutritional and medicinal properties. It has been reported that grape contains a large amount of phenolic compounds, especially the seed which is considered to contain 60%-70% of the total phenolic content of the fruit, comprising of monomeric phenolic compounds such as (+)-cathechins, (-)-epicatechin and (-)-epicatechin-3-o-gallate, and dimeric, trimeric and tetrameric procyanidins.

Grape seed extract obtained from grapes grown in Hasandede, Emir and Kalecik Karasi wine cultivars in Turkey showed concentrations of 2.5%- 5% exhibited the most inhibitory effect against a wide variety of microorganisms including *E. coli*, *K. pneumoniae*, and *S. aureus*[6]. A similar grape seed extract product IH636 was tested against 21 strains of gram positive and gram negative cocci which showed gram positive cocci to be more susceptible, especially *S. aureus*. In presence of 1 mg/mL, 99% inhibition was reported with no further bacterial recovery[7]. Complete inhibition of 43 clinical strains of Methicillin resistant *S. aureus* was noted at concentration of 3 mg/mL crude grape seed proanthrocyanide extract[8].

More researches based on grape seed extract can be beneficial to gain more knowledge towards its potential in medicinal science. This study deals with the antimicrobial effects of "Breko exGrape seed OPC 30" grape seed extract on the most commonly found microflora that exists around infected skin-abutment interface of craniofacial implants and its future prospects.

2. Material and methods

2.1. Materials

Commercially available grape seed extract "Breko exGrape seed OPC 30" was obtained from East Asiatic Public Company Ltd, Thailand. It is a natural procyanidin extract made from white grape seeds without the inclusion of skins and stems, grown in the south of France. The test organisms used in the study were as follows: *S. aureus* ATCC 6538, *E. coli* ATCC 25922, *C. albicans* ATCC 10231 and clinical strains of *S. aureus*, *K. pneumonia* and *C. parapsilosis*. These strains were obtained from the Dept. of Oral Microbiology, Mahidol University, Thailand.

2.2. Methods

2.2.1. Disc diffusion method

The stock solutions of the grape seed extract was prepared in water. The microorganisms were incubated in enriched blood agar at 37 ℃ for 24-48 hours. Fresh isolates were then transferred to distilled water and a microbial suspension was prepared and the turbidity adjusted to 0.5 McFarland's solution to yield approximately 10^8 CFU/mL. The inoculum was evenly spread in Mueller Hinton Agar (MHA). Two-fold dilution of the stock solution was performed to obtain grape seed extract concentrations ranging from 20 mg/mL to 1.25 mg/mL. 6 mm sterile paper discs were prepared and 20 μL of each of the concentration was transported onto each disc. These discs were placed in MHA and incubated at 37 ℃ for 24-48 hours, following which the inhibition zones were measured.

2.2.2. Measurement of Minimum inhibitory concentration (MIC) and minimum cidal concentration (MCC)

The minimum inhibitory concentrate of grape seed extract was determined by modified agar dilution method[9]. Various concentrations of grape seed extract were mixed along with Muller Hinton agar to obtain extract concentration ranging from 10 mg/mL to 0.078 mg/mL in the agar. 0.45 μm porosity Millipore membranes were placed into the prepared MHA and were inoculated with 20 μL of microbial suspension which approximately contained 10^4 CFU/spot. The agar plates were incubated at 37 ℃ for 24-48 hours and observed for subsequent microbial growth on the Millipore membranes.

After determination of MIC, the membranes were transported from the agar to Difco™ Brain Heart Infusion broth solution in test tubes. The broth media were then incubated at 37 ℃ for 24-48 hours to observe for microbial growth and the MCC was determined.

2.2.3 Preparation of anti-microbial paste

The MCC extracts were mixed with propylene glycol and polyethylene glycol which served as a base for drug delivery. Brain heart infusion agar was prepared and was inoculated with fresh strains of the microorganism, which were prior adjusted to 0.5 McFarland's solution to yield approximately 10^8 CFU/mL before inoculation. A 4 mm diameter copper coil was used to create wells in the agar which were subsequently filled with the antimicrobial paste. After an incubation time of 24-48 hours at 37 ℃, the inhibition zones so formed around the wells were observed and measured to determine the antimicrobial properties of the paste.

2.3. Statistical analysis

All tests were done in duplicates for verification of the results. The values were expressed in mean. To determine the correlation between concentration of extract and inhibition zone, Spearman rank correlation coefficient was used. Statistical significance was set at P-value <0.05. All data analyses were performed using SPSS (Version 17).

3. Results

3.1. Disk diffusion test

Both strains of *S. aurues* showed sensitivity to the effects of grape seed extract with inhibition zones ranging from 12.5 mm to 7 mm. *C. albicans* showed minimum growth inhibitory effects to grape seed concentration with inhibition zones measuring from 8 mm to 7.5 mm. However strains of *E. coli, K. pneumoniae, C. parapsilosis* showed no sensitivity to the effects of the extract, even at a concentration of 20 mg/mL.

Table 1
MIC and MCC results of grape seed extract (mg/mL).

Tested strains	MIC	MCC
S. aureus ATCC 6538	0.625	1.250
S. aurues clinical strain	0.625	1.250
C. albicans ATCC 10231	< 20	–
C. parapsilosis clinical strain	< 20	–

3.2. MIC and MCC results

The MIC and MCC was carried out based on the results of the disk diffusion test. The test results showed potent effects of the grape seed extract with MIC at 0.625 mg/mL and MBC at 1.250 mg/ml for both strains of *S. aureus*. However even at a high concentration of 20 mg/mL extract in agar, MIC for *C. albicans* and *C. parapsilosis* could not be established (Table 1).

3.3. Antimicrobial paste

Overall the grape seed extracts mixed with propylene glycol and polyethylene glycol showed a dose dependent inhibitory effects on *S. aureus* where increasing the concentration of the grape seed extract in the base increased the zone of inhibition (*S. aureus* ATCC 6538 Spearman corr =0.995 and *S. aureus* clinical strain Spearman corr = 0.987 at $P<0.05$; Table 2, Figure 1). The positive control was used as 4% Erythromycin which showed a mean inhibition zone of 43 mm and negative control was 1:1 mixture of propylene glycol and polyethylene glycol which did not exhibit any inhibition zone.

Table 2
Mean inhibition zone (mm) measured with grape seed extract paste.

Concentration of grape seed (mg/mL)	*S. aureus* ATCC 6538 strain	*S. aureus* clinical strain
1.25	6.5	7.5
2.50	7.0	8.0
5.00	9.0	8.0
10.00	9.75	9.0
20.00	11.5	10.0
31.25	15.0	12.0
62.50	17.5	14.5
125.00	21.5	16.5
250.00	24.0	19.0

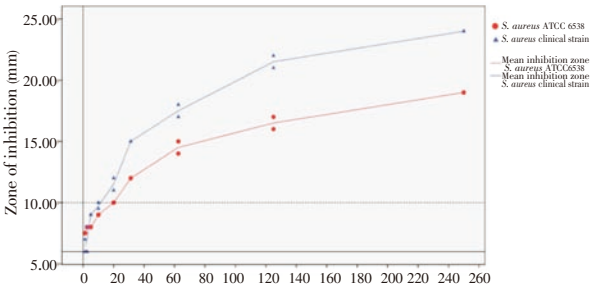

Figure 1. Comparison of the zone of inhibition with the concentration of grape seed extract in the antimicrobial paste.

4. Discussion

The disk agar diffusion method is considered to be a standard screening test to check the antimicrobial activity of a reagent. The disk diffusion test revealed that grape seed extracts showed moderate activity against both strains of *S. aureus* with inhibition zones of 12.5 mm to 7 mm. However the reagent showed minimal or no reactivity against *E. coli, K. pneumonia, C. albicans* and *C. parapsilosis*.

MIC and MCC tests were performed on the micro-organisms which were susceptible to the effects of the grape seed extract, namely *S. aureus* and *C. albicans*. Different quantitative tests, such as broth dilution, agar dilution, and gradient dilution, are available to measure the lowest concentrate of a reagent that inhibits the visible growth of test microbes, ie. MIC. In this study the determination of MIC and MCC was based on the modified Millipore membrane agar dilution method. This method has been used to measure the antimicrobial properties of essential oils extracted from various herbs[9]. The grape seed extract was effective against both strains of *S. aureus* at MIC values of 0.625 mg/mL and MCC at 1.25 mg/mL. However the agar dilution test had limitations and it failed to obtain a homogenous set when the concentration of the extract was increased more than 20 mg/mL in the agar. The test for MIC for *Candida* species which required concentration of more than 20 mg/mL could not be established.

Phenolic contents found in grape seed are partially hydrophobic, and are considered to interact with the bacterial cell wall and lipopolysaccharide interfaces by decreasing membrane stability. The amount of phenolic content in grape seed extract, measured in gallic acid equivalent, has been directly correlated to the antibacterial properties[6, 10]. A study on the bacteriostatic and bactericidal effects of grape seed specially dealt with the pharmacodynamics of gallic acid on *E. coli, S. enteritidis*, and *S. aureus*. Structure-activity correlation assays showed that three hydroxyl groups of the compound are effective against *E. coli* and *S. enteritidis* and all of the substituents

of the benzene ring were effective against *S. aureus*. The study conducted on the effects of muscadine grape seed extract on *H. pylori* suggested that damage to the bacteria occurs on multiple levels including the inhibition of basic energy production and/or virulence factors[11]. Further supportive researches have shown that grape seed extract, resembled THF structurally and interfered with folate mediated one-carbon metabolic pathway of *S. aureus*[7].

Propylene glycol and polyethylene glycol, polymers with dihydric alcohol, are considered to be non-toxic, water-soluble which resists recognition by the immune system. They have been used as solvents and moisturizers in many pharmaceutical preparations. Propylene glycol and polyethylene glycol have also been used as vehicles in the preparation of 3 Mix, which is a mixture of 3 types of antibiotics and has been used to treat pulpally involved primary molars. Propylene glycol and polyethylene glycol serves as good drug delivery system because of its biocompatibility, ease in manipulation, and handling[12]. In the study, propylene glycol and polyethylene glycol combination did not exhibit any microbial inhibitory effects of its own. However the combination of grape seed extract with propylene glycol and polyethyelene glycol showed a dose dependent inhibitory effect on both strains of *S. aureus*.

Infections of the soft tissue surrounding extra-oral implants can be managed by proper treatment planning and patient selection, adequate ventilation of the skin, meticulous maintenance of skin hygiene, administration of antimicrobial agents, and, occasionally, surgical revision and debridement[13]. Although antibiotic preparations may help to decrease the peri-implantitis flora, resistant strains may arise if used for a long duration. Plant extracts can be used as alternatives but like other naturally occurring products the quality control of the batches should be well maintained to obtain uniform results.

The current study showed that grape seed extract "Breko exGrape seed OPC 30" showed moderate effect on *S. aureus* but showed minimal or no inhibitory effects on gram negative and yeast microorganisms. Grape seed extract also exhibited a dose dependent inhibitory effect on *S. aureus* when combined with propylene glycol and polyethylene glycol. These extracts can be further researched and developed to be used as potential anti-microbial agents to treat skin infections around the implant abutment interface of craniofacial implants.

Conflict of interest statement

We declare that we have no conflict of interest.

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