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Evaluation of Antibacterial Efficacy of Dragon Fruit Peel Extract on Subgingival Anaerobes – An In Vitro Study

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Abstract

Aim & Background: To evaluate the antibacterial efficacy of dragon fruit peel extract on subgingival anaerobic organisms using agar well diffusion method.

Method: 15 subgingival plaque samples were taken from systemically healthy chronic periodontitis patients. The collected samples were transported to the laboratory within 4 hours of sample collection and incubated in the tryptic soy agar medium in an anaerobic chamber. After the colony growth, three wells are created using a sterile cork. 25 µl, 50 µl, 100 µl of dragon fruit peel extract obtained by ethanol maceration- diffused into three wells and incubated. Zone of inhibition is determined after 24 hours.

Result: Zone of inhibition after 24 hours was evaluated and maximum zone of inhibition for dragon fruit peel extract was 15 mm for 100 µl with mean of 14.53 ± 0.516.

Conclusion: The dragon fruit extract has shown to inhibit the growth of subgingival anaerobe and can be used as an adjuvant in treatment of periodontitis.

Clinical Significance: Dragon fruit peel extract can be used as an alternative to chlorhexidine because of chlorhexidine side effects like bacterial resistance.

Keywords: Periodontitis, dragon fruit peel, in vitro, subgingival anaerobes.

Introduction

Human periodontitis is associated with a widely diverse and complex subgingival microbiota encompassing both Gram positive and Gram-negative bacteria, facultative and anaerobic organisms. Dragon fruit contains antibacterial content- betalain pigments and the peel contain flavonoids, alkaloids and terpenoids.

Gingivoperiodontal diseases, including gingivitis and periodontitis, are caused by dental plaque, which is a biofilm. The biofilm present in the gingival crevice, and later in the periodontal pocket, is extremely diverse, with up to 100 culturable species from a single pocket. [1]

More anaerobes increase in their overall proportions on diseased state whereas there is reduction in aerobe and facultative species. [2]

Gram-positive species includes Peptostreptococcus spp. Bifidobacterium species, Eubacterium, Lactobacillus, Propionibacterium, Actinomyces naeslundii. Gram-negative species includes Veillonella parvula, Prevotella oralis, Bacteroides ovatus.

The dragon fruit is enriched with several phytochemical constituents having tremendous pharmacological properties. It is traditionally used as a coloring agent. Phenolic compounds inhibit the growth of bacteria with the ability of damaging the cytoplasmic membrane and proteins & inactivating some bacterial enzymes. [3]

The applications of dragon fruit include its use as an antimicrobial, antidiabetic, antioxidant, anticancer as well as nutraceutical. [Table 1] The flesh, peel, and seeds of the fruit are sours of phytoconstituents and has abundant source of betacyanin, vitamin C, and lycopene. [4] This study is aimed to prove the antibacterial efficacy of dragon fruit peel extract on subgingival anaerobes by agar well diffusion method.

Materials and Methods

Systemically healthy patients, Chronic periodontitis patients with probing depth ≥ 5 mm were included in the study.

Patients requiring immediate care, pregnant and lactating patients, any form of tobacco users, alcohol users, patients using mouth wash or other plaque controlling agents or in any antimicrobial therapy were excluded from the study.

Dragon Fruit Extract Preparation

The extract was prepared by ethanol maceration method. [1] [Figure 1] The extract was prepared by the following method at Alpha omega research foundation, Salem. The extract was refrigerated till use.

- The making of the fruit extracts begins with washing clean the dragon fruit with running water, then separate the peel and pulp of the fruit.
- Dragon fruit peels roasted using the oven in 50 °C for 3 h. The dried peel of dragon fruit mashed using the blender, then sifted so that it becomes simplicial powder and after that weighed.
- The next process is ethanol 96% maceration. Soaking was done by using a ratio of 7.5 times the weight of the powder so the amount of ethanol.

Subgingival Plaque Collection

After supragingival scaling was done, the site from which the sample to be taken was isolated using cotton rolls in order to avoid saliva contamination. Using sterile curette subgingival plaque samples were collected. The samples with blood were discarded.

15 subgingival plaque samples were taken from systemically healthy chronic periodontitis patients. The collected samples were transported to the laboratory within 4 hours of sample collection using thioglycolate broth. The samples were incubated in the tryptic soy agar medium in an anaerobic jar. [Figure 2]

After three days, the colony growth was achieved, [Figure 3] colonies were collected and mixed with blood agar. Four wells were created using sterile cork and 25 µl, 50 µl, 100 µl of dragon fruit peel extract obtained by ethanol maceration was diffused into three wells. In another well chlorhexidine as, positive control was added. Zone of inhibition was determined after 24 hours. [Figure 4]

Results

The zone of inhibition was determined after 24 hours of incubation. Maximum zone of inhibition for dragon fruit peel extract was 15 mm for 100 µl. Statistics was done using SPSS software version 26. Mean for zone of inhibition for 100 µl was 14.53 ± 0.516. [Table 2]

Discussion

The oxidative stress created by free radicals can be prevented by the intake of antioxidants. Dragon fruit peel extract has rich anthocyanins and betacyanins which can inhibit free radicals and better option as an antioxidant supplement.[10]

Total phenolic content (TPC) assay demonstrated that peels of both Hylocereus species contained higher phenolic content than the pulps. The betalains from dragon fruit peels exhibit a comparable activity with quercetin, a proven strong anti-inflammatory compound. This ability may be ascribed to their strong antioxidant capacity in scavenging free radicals which is the cause of provoking inflammatory processes - their capacity in reducing tumour necrosis factor-alpha (TNF-a) expression.[11]

Antimicrobial activity of the refrigerated dragon fruit analyzed by the broth microdilution assay was better (MIC values: 3,130–6,250 µg/ ml) compared to freshly harvested fruits (MIC: 50,000–>50,000 µg/ ml) against 10 pathogenic gram-positive and 6 gram-negative bacteria. This proved the effectiveness of refrigeration improving the betacyanin content and overall antimicrobial activity of dragon fruit.[8]

Temak et al in 2019[12] used dragon fruit extract with chloramphenicol on the growth of P. aeruginosa which showed synergistic inhibitory activity on burn injured mice. This is in concordance with the present study where inhibition of anaerobic organism was seen in vitro.

Rahayu et al. in 2019[3] used red dragon fruit extract on Streptococcus mutans isolates and measured the zone of inhibition by well diffusion method. Different extract concentration of 100%, 50%, 0.2% chlorhexidine and aquadest sterile in each of the wells, then incubated for 24 h and was measured using a digital caliper. The antibacterial activities were assessed by the presence of inhibition zones after incubating the plates at 37 °C for 24 hours. The results showed that higher concentration on the peel and pulp of the fruit shows the bigger inhibition zone diameter.

Hendrik Setia Budi et al in 2019[13] studied effect of red dragon fruit peel extract on decrease malondialdehyde level of gingival tissue in chronic periodontitis rats. K- is control group of healthy rats, K+ is control group of periodontitis rats given Sodium Carboxymethyl Cellulose (CMC-Na) and P is treatment group of periodontitis rats given gel of red dragon fruit extract for 3 days and 7 days with concentration P1 (1 mg/mL/day), P2 (2 mg/mL/day), and P3 (4 mg/mL/day). Gingival tissues malondialdehyde level of each group was measured by thiobarbituric acid (MDA-TBA) method. The study showed significant decrease on MDA level by 4 mg/mL concentration at 3 days ($p<0.05$).

Diah Setiani in 2020[14] tested antibacterial activity testing of red dragon fruit peel on pseudomonas species on diabetic foot ulcers with red dragon fruit peel with concentrations of 15%, 25%, 50%, 75%, and 95%. The positive control is amoxicillin and the negative control is sterile aquadest. The decoction of red dragon fruit peel with a concentration of 95% has the highest inhibitory zone on the growth of Pseudomonas Sp. bacteria that cause diabetic ulcers compared to the dragon fruit peel with other concentrations which is concordance with the present study where 100 µl showed greater zone of inhibition. Wisnu Setyari Juliastuti in 2020[15] studied

effect of dragon fruit (hylocereus polyrhizus) peel extract on collagen fiber density of rat socket healing with dragon fruit peel extract gel 15%, 30%, 60% was given into the socket. Wistar rats was sacrificed on the fourth and seventh days after tooth extraction and prepared for histopathological examination with Masson's Trichrome staining. There are significant differences in the density of collagen in the treatment group concentration 30%. The result showed that the use of dragon fruit peel extract gel affects the density of collagen fibers at 4 and 7 days after tooth extraction. The finding provides dragon fruit peel extract could promote the healing process through the formation of collagen fibers density

Hendrik Setia Budi 2020[16] studied the potential of red dragon fruit (Hylocereus polyrhizus) peel extract accelerates wound healing process post tooth extraction and Immunohistochemical and histopathological analysis with light microscope showed the number of fibroblast cell and new blood vessel show the increase on the groups that are treated with dragon fruit peel extract gel. Winda Khosasi et al in 2021[17] compared antibacterial efficacy of red beetroots to red dragon fruit peels extract on Streptococcus mutans. Peel extract from both plants with 25%, 50%, 75%, and 100% concentrations were used in well diffusion method and zone of inhibition was seen. Red beetroots peel extract had a significantly higher inhibition zone against S.mutans compared to red dragon fruit peel extract in all concentrations

Limitations

- Minimum sample size
- Isolation of specific organism from the colonies were not determined.

Conclusion

To conclude dragon fruit extract has wide range of antimicrobial and anti-inflammatory properties, thus helps in reducing disease progression by decreasing the both aerobic and anaerobic bacteria. So, dragon fruit peel extract can be used as an adjuvant for scaling and root planing as many bacterial strains shows resistance to chlorhexidine.

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Legend Tables & Figures:

Table 1: Pharmacological Activity of Dragon Fruit

Pharmacological activity	**Active phytoconstituent**
Antioxidant	Betacyanin [5] Lycopene, β-carotene [6] Gallic acid, Vanillic acid, Syringic acid, Protocatechuic acid, p-hydroxybenzoic acid, p-coumaric acid [7]
Anticancer	β-amyrin, β-sitosterol, Stigmast−4-en−3-one[7]
Antimicrobial	Betacyanin[8]
Coloring agent	Betacyanin [9]

Table 2: Descriptive Statistics

Extract Used	**N**	**Minimum**	**Maximum**	**Mean**	**Standard Deviation**
25 µl	15	12	13	12.40	.507
50 µl	15	13	14	13.73	.458
100 µl	15	14	15	14.53	.516

Figure 1: Dragon fruit extract preparation

Figure 2: Agar plates incubated in anaerobic jar

Figure 3: Subgingival colonies on tryptic soy agar

Figure 4: Zone of inhibition