GROWTH INHIBITORY ACTIVITY OF *Padina Gymnospora* AGAINST DENTAL PATHOGENS

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Abstract : The study was carried out to evaluate growth inhibitory efficacy of methonolic extract of the marine macro algae plant Padina gymnospora against the dental pathogens, Streptococcus mitis, Streptococcus mutans, Streptococcus salivarius, and Porphyromonas gingivalis.A Disc diffusion assay of the extract used in this experiment to evaluate the bactericidal effect. In vitro inhibitory activity of Padina gymnospora extract shows superior antibacterial properties when compared to standard antibiotics. The extracts indicated the highest zone of inhibition (14mm) in P.gingivalis and moderate inhibition zone (10mm) in S.mutans. P.gymnospora ethanolicic extracts posses a broad spectrum of activity against the bacterial strains responsible for the most common dental diseases. These primary extracts open the possibility of finding new clinically effective antibacterial compounds. Continued further research is necessary to determine the identity of the antibacterial compounds of these marine algal plants.

Key words: Dental pathogens, Disc diffusion, inhibition zone, marine algae.

1. INTRODUCTION

Dental diseases are one of the major public health problems on as they have a considerable impact on individuals and communities by causing pain, suffering, impairment of normal functions and reduced quality of life. Oral microorganisms are known for their pathogenesis in tooth decay, gingivitis, periodontitis and their ability to cause teeth loss [1]. Dental caries and related oral diseases like gingivitis and periodontitis are most common oral diseases in developed as well as developing countries affecting people from all ages of life. The frequency of these oral diseases is continuously increasing as a result of the changing food habit, tobacco use, inadequate exposure to fluorides, and lack of access to dental care [2].

Dental caries (tooth decay) and periodontal disease (gum disease) are the most common oral diseases worldwide. Periodontal diseases and dental caries are two main common dental pathologies affecting humankind [3]. These conditions are caused by plaque forming bacteria and yeast, which reside in the oral cavity. Periodontal diseases have mainly been associated with *Actinomyces*, *Actinobacillus*, *Streptococcus* and *Candida* species [4]. Gram-positive bacteria such as *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus* species and some nonmutans streptococci are closely associated with caries formation [5–9]. Gram-negative bacteria such as *ggregatibacter actinomycetemcomitans*is associated with aggressive deriodontitis [10]. while *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Campylobacter rectus* are associated with chronic periodontitis in adult [11]. Some of these remedies include bisguanide-antiseptics, quaternary ammonium-antiseptics, phenolic-antiseptics and other remedies such as oxygenating agents, and metal ions [12]. Common side effects of some of these may be the staining of the teeth and restorations,taste of food and a burning sensation at the tip of the tongue . Dental plaque is the major cause of dental caries and periodontal disease. Plaque is a habitat for different microorganisms [13]. *Streptococcus mutans is* one of the main opportunistic pathogens of dental caries, which plays a central role in fermentation of carbohydrates resulting in acid production, and leading to the demineralization of the tooth enamel [14]. In addition, other micro flora like *Escherichia coli* and *Candida albicans* are also associated with active caries lesions. *C albicans* is the most common yeast isolated from the oral cavity. It is by far the most commonly isolated fungal species from infected root canals, showing resistance to inter canal medication [15]. Among various causes for oral diseases, poor oral hygiene is one of the majour reason for accumulation of these

microbes and their harmful activities. The oral diseases can be best avoided in most of the cases by the proper maintenance of oral hygiene. This is usually achieved by the regular brushing of teeth, which may be combined with the use of additional oral hygiene products such as mouth rinses and
toothpastes. A recent trend has seen the inclusion of antibacterial agents in many oral hygiene products. A number of chemical antibacterial agents, such as Cetylpyridinium chloride, Chlorhexidine, Triclosan, or antibiotics have been used in the prevention and management of oral diseases. Development of resistance against antibiotics and antiseptics is a growing cause of concern which have limited the preventive measures. Therefore, there is a continuing need to search for new antimicrobial agents [16]. Despite the advances in various field of medicine, oral infections and dental caries are still considered as serious public health problems and inflict a major burden to health care services around the world and especially in developing countries [17]. Development of resistance against antibiotics and antiseptics is a growing cause of concern which have limited the preventive measures. Therefore, there is a continuing need to search for new antimicrobial agents against dental pathogens. The aim of this study was to determine the dental pathogens inhibitory activity of the medicinal marine macro algae plants *Padina gymnospora* against oral microorganisms which are responsible for dental caries.

2. MATERIALS AND METHODOLOGY

2.1 Plant material

The *Padina gymnospora* marine algae plant material was air dried at room temperature without sun rays, and ground in a ordinary powder blender up to fine powder formation. The powdered material (250 g) was extracted with 750ml of ethanol at room temperature for 48h using a mechanical shaker. The resulting slurry was kept for dryness at room temperature. A powdery crude extracts was finally formed. The *Padina gymnospora* crude extracts were then stored at −20◦C for use in further studies of inhibition of oral microbial strains.

2.2 Strains of oral pathogens

Four bacterial strains of dental pathogens, *Streptococcus mitis, Streptococcus mutans,* S*treptococcus salivarius, and Porphyromonas gingivalis* were selected for this study. The strains were identified and collected from Rajah Muthia Dental College and Hospital, Annamalai University, Chidambaram, Tamilnadu, India. All the broth with bacterial growth were incubated at 37◦C then they were included in the present experiment.

Statistical Analysis

Statistical analysis was done using one way analysis of Variance - ANOVA test.

2.3 Growth inhibitory activity

Disc diffusion assay

The microbial growth inhibitory potential of the extract was determined using the agar disk diffusion method [18]. 100 µl inocula of all tested microorganisms were inoculated on Casein peptone Soy Agar medium. Sterile Whatmann filter discs (6 mm diameter) were made in agar plate using sterile cork borer. Then 50 µl each of Padina gymnaspora and ethanol solvent extracts were placed in the discs made in inoculated plates. One hundred microlitres of positive drug control (chlorhexidine 5%) and 10% DMSO (solvent control) soaked on filter papers severed as positive and negative control, respectively. All tests were performed in triplicate and zones of inhibition were measured (CLSI, 2009). mg/mland chlorhexidine 0.2% mouthwash. The plates were then incubated at 37 °C for 48 h. zone of inhibition if any around the wells were measured in mm (millimeter). and zones of inhibition were measured from the edge of each disc after the incubation period.

3. RESULTS

In vitro dental bacterial inhibitory activity of Padina gymnospora algal plant extract and their potency were quantitatively and qualitatively assessed by determining the inhibition zone diameter of Disc

diffusion assay. Screening results and inhibitory activity of extract against 4 dental pathogenic bacteria are shown in Table1 and Fig 2. The test shows positive inhibitory activity against the tested oral pathogens.

Dental pathogens	Zone of inhibition in (mm)		
	Negative control (DSMO)	Positive control Chlorhexidine	Experimental *Ethanolic* extract
S.mitis	Nil	22mm	11mm
S.mutan	Nil	24mm	10mm
S.salivarius	Nil	21mm	12mm
P.gingivalis	Nil	23mm	14mm

Table 1

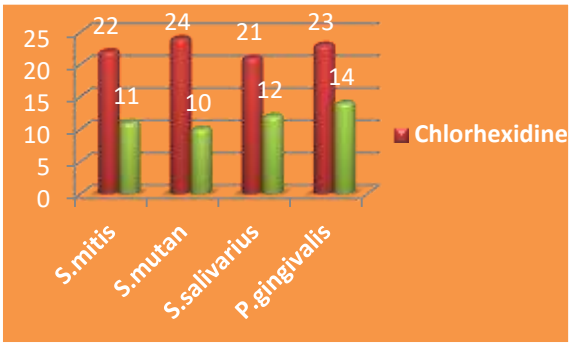

Fig.1
Growth inhibitory activity of *P. gymnosphora*

No strain in this study showed resistance to this extract. The inhibitory zone significantly increased in a dose dependent manner. The extract indicated an inhibition zone of 11mm in S.mitis, 10 mm in S.mutan, 12mm in S.salivarius, and 14 mm in P.gingivalis. The extract showed the highest zone of inhibition (14mm) in P.gingivalis. However, S.mutans shows moderate inhibition zone (10mm). Chlorhexidine was used as the positive control.

4. DISCUSSION

The results of this study provided an insight into the oral bacterial inhibitory properties of the Padina gymnospora extracts.The present study supports the view that P.gymnospora extract might be useful as antibacterial agents against dental pathogens.The findings of this study propose that P.gymnosphora can inhibit the growth of Streptococcus mitis, Streptococcus mutans, Streptococcus salivarius, and Porphyromonas gingivalis. It has also been reported earlier that the crude methanolic extract of dried twigs of Diospyros lycioides demonstrated preferential growth inhibitory activity against the oral pathogens Streptococcus mutans and Porphyromonus gingivalis at 1.25 mg/ml [19]. Dentists reported the activity of ten methanolic plant extracts including Euclea natalensis which showed minimum inhibitory concentration ranging from 0.63 mg/ml to 5 mg/ml against Actinomyces viscosus, Streptococcus mutans and Candida albicans. A study confirmed the MIC of the bark of Euclea natalensis on oral Candida albicans and Streptococcus mutans, the recorded MIC is 5.0 mg/ml while in this study Streptococcus mutans was found to be susceptible at the MIC of 12.5 mg/ml [20]. The activity of Euclea natalensis against Streptococcus mutans was also reported [21,22]. Our study proved that the marine macro algae are rich in a wide variety of secondary metabolites, such as tannins,terpenoids, alkaloids, and flavonoids, which have been found in-vitro to have antimicrobial properties against dental pathogens.

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