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Antibacterial effectiveness of Betel leaf (piper betle) extract on red complex bacteria: An invitro study

Sheryl Dolly.A, Gayathri.K, Sunanda Rao, Sindhujaa.R, Mohamed Rashik, P.L.Ravishankar

Department of Periodontics, SRM Kattankulathur Dental College &Hospital, Potheri- 603203,Tamilnadu,India.

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ABSTRACT

INTRODUCTION: The betel leaf plant (piper betle), commonly seen in India has been known traditionally for its antibacterial properties to manage a variety of illnesses including dental and other ailments. The objective of this invitro study is to evaluate the effectiveness of betel leaf extract against the red complex bacteria.

METHODS: This study was conducted using the serial tube dilution technique for betel leaf extract and the positive control amoxicillin at concentrations of1%,2%,5%, and 10% in order to determine the MIC values. The pure drug was dissolved in 10 mg/ml of dimethyl sulfoxide to make the stock solution. A total of 400 µl was prepared by adding 300 µl of BHI broth and 100 µl of stock solution in the initial tube to prepare four serial dilutions in different test tubes containing 200 µl of BHI broth. 200 µl of the previously made bacterial suspension was added to each serially diluted tube. Each tube was then incubated for 24 hours at 37°C in an anaerobic jar to check for turbidity, which signals the growth of the organisms. This serial dilution was repeated for each microorganism. Each tube's turbidity was contrasted with amoxicillin, which serves as a positive control. The drug's minimum inhibitory concentration (MIC) for that specific test organism was determined to be the lowest concentration of the drug in the tube that did not exhibit any turbidity.

RESULTS: The results of the present study shows thatPorphyromonasgingivalis is susceptible to betel leaf extract at 5% concentration and Amoxicillin at 1% concentration. Treponema denticolais susceptible to betel leaf extract at 10% concentration and Amoxicillin at 2% concentration whereas Tannerella forsythia is resistant to the betel leaf extract even at 10% concentration and is susceptible to Amoxicillin at 2% concentration.

CONCLUSION : Betel leaf extract, particularly at 5% concentration, is considerably effective in preventing the development of Porphyromonasgingivalis and significantly effective in inhibiting the growth of Treponema denticola at 10% concentration.

Keywords:betel leaf, red complex bacteria, chronic periodontitis

I. INTRODUCTION

Periodontal disease ranks among the most prevalent oral diseases in today's society, with a high prevalence.It is an inflammatory condition that affects the gums and surrounding tissues of the teeth.[1]. Periodontitis causes the deterioration of alveolar bone, the periodontal pockets to form, and clinical attachment loss. While there are several contributing factors to periodontal disease, the presence of bacteria is the primary cause since it plays a significant role in the pathogenesis. [2],[3]

More than 500 distinct species of bacteria are considered to reside within the oral cavity and have been related to a number of illnesses. Porphyromonasgingivalis, Treponema denticola, and Tannerella forsythia, which are considered the putative periodontal pathogens, constitute to the red complex, which emerges later during biofilm development (previous names Bacteroides forsythus, or Tannerellaforsythensis).[4]As a result, lowering their levels in the oral cavity, is crucial for the management and prevention of periodontal disease. [5]

Scaling and root planing, the nonsurgical mechanical procedures for periodontal disease, are less successful as a result of bacteria's capacity to penetrate periodontal tissues, mandating the administration of antibiotics to halt or decrease bacterial infections. Antibiotics are therefore used in conjunction with scaling and root planing as a therapy. Local administration of the antibacterial agents in the periodontal pockets have a greater impact due to its direct action on the area that is diseased, having greater impact and lowers the risk of adverse drug reactions. [6],[7].

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The rise in drug-resistant pathogenic microorganisms that afflict people and animals, together with some undesirable side effects of antibiotics, has inspired a significant amount of curiosity in the development of new plant-based antimicrobial alternatives. Alkaloids, flavonoids, glycosides, saponins, resins, oleoresins, sesquiterpenes, phenolic compounds, lipids, and oils are a chemical substances found in medicinal plants [8]. The most significant benefit touted for the therapeutic use of medicinal plants in treating a variety of ailments is their safety, in addition to being affordable, efficient, and readily available. [9].

The betel leaf plant (piper betle), commonly seen in India has been known traditionally for its therapeutic properties to manage a variety of illnesses including dental and other ailments. The piper betle hasbeen primarily used to treat a number of ailments, including headache, restricted urination, nerve weakness, sore throats, respiratory diseases, constipation, inflammation, wounds and boils. The antimicrobial , antihistaminic, anti inflammatory, antioxidant, anti mutagenic, anti hemolytic, antiulcer, antibacterial, antifungal, anti – diabetic, antiseptic, local anestheic, anti – nociceptive, as contraceptive activities are also well documented[10].

Although extensive research has been done on the benefits of betel leaf as an antibacterial agent, there is still a dearth of scientific sources that demonstrates the herb's efficacy in treating periodontal tissue[11]. Hence, this in vitro study was undertaken to evaluate the Minimal Inhibitory Concentration (MIC) of betel leaf extract against the hypothesised periodontal pathogens, in particular Tannerella forsythia, Treponema denticola, and Porphyromonasgingivalis.

II. MATERIALS AND METHODS

This is a invitro study performed in SRM Kattankulathur Dental College and Hospital, Potheri, Tamil nadu. Institutional Ethics Committee clearance was obtained. Three standardized strains of well-known pathogenic bacteria were obtained from Department of Microbiology, SRM Kattankulathur Medical College and Hospital, Potheri. Porphyromonasgingivalis, Treponema denticola, and Tannerella forsythia were used in this study. The red complex bacteria were chosen as they are the causative organisms of chronic periodontitis according to Socransky's pioneering study[12].

Preparation of betel leaf Extract

Betel leaf powder which is commercially (3V Products, Avadi, Chennai- 62)[Figure 1] available was purchased in order to carry out the study. A clear filtrate was then obtained by using Whatman filter paper for filtering. Moreover, the filtrate was reduced to a low temperature of < 60°C, yielding a solid residue of betel leaf extract. The yield was 6% w/w when 300 gm of betel leaf powder was dissolved in 1 L of ethanol [9], producing 18 gm of residue (extract). Serial dilutions were done accordingly and compared with a positive control amoxicillin.

Microbiological assay

Using the recommendations of Clinical and Laboratory Standards Institute, the present study used the serial tube dilution technique for betel leaf extract and the positive control amoxicillin at concentrations of1%,2%,5%, and 10% in order to determine the MIC values[13].The pure drug was dissolved in 10 mg/ml of dimethyl sulfoxide to make the stock solution. A total of 400 µl was prepared by adding 300 µl of BHI broth and 100 µl of stock solution in the initial tube to prepare four serial dilutions in different test tubes containing 200 µl of BHI broth. 200 µl of the previously made bacterial suspension was added to each serially diluted tube. Each tube was then incubated for 24 hours at 37°C in an anaerobic jar to check for turbidity, which signals the growth of the organisms.This serial dilution was repeated for each microorganism.Each tube's turbidity was contrasted with amoxicillin, which serves as a positive control. The drug's minimum inhibitory concentration (MIC) for that specific test organism was determined to be the lowest concentration of the drug in the tube that did not exhibit any turbidity[Figure 2].

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III. RESULTS

Table 1: MIC values of Betel leaf against Porphyromonasgingivalis, Treponema denticola, and Tannerella forsythia

Betel leaf conc	1%	2%	5%	10%
Porphyromonasgingivalis	R	R	S	S
Treponema denticola	R	R	R	S
Tannerella forsythia	R	R	R	R

Table 2: MIC values of amoxicillin against Porphyromonasgingivalis, Treponema denticola, and Tannerella forsythia

Amoxicillin conc	1%	2%	5%	10%
Porphyromonasgingivalis	S	S	S	S
Treponema denticola	R	S	S	S
Tannerella forsythia	R	S	S	S

Tables 1 and 2 shows the bacterial inhibition of Porphyromonasgingivalis, Treponema denticola, and Tannerellaforsythia exhibited by betel leaf extract (at different concentrations) and control Amoxicillin.

In the present study, Porphyromonasgingivalis is susceptible to betel leaf extract at 5% concentration and Amoxicillin at 1% concentration. Treponema denticolais susceptible to betel leaf extract at 10% concentration and Amoxicillin at 2% concentration whereas Tannerella forsythia is resistant to the betel leaf extract even at 10% concentration and is susceptible to Amoxicillin at 2% concentration.

Figure 1: Commercially available betel leaf powder

Figure 2:Serial dilution of the antibacterial agent with bacterial culture Concentrations in 1%, 2%, 5% and 10% are cultured with bacterial isolate and observed for turbidity

IV. DISCUSSION

Currently, more people around the world are starting to focus more on the usage of natural, herbal medications since they perceive that these medications are fairly beneficial in treating a wide variety of ailments without having too many side effects. Betel leaf (piper betle), used as a traditional medication in India; which has long been recognised as having therapeutic properties to treat a variety of disorders as it is thought to have potent and powerful antibacterial and anti-inflammatory properties[11].

In this investigation, we were interested in discovering more concerning betel leaf extract's antibacterial effectiveness, specifically against three key periodontal pathogens, Porphyromonasgingivalis, Treponema denticola,

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and Tannerella forsythia; due to the fact that these microorganisms play a role in the development and spread of several oral diseases, particularly chronic periodontitis. Observations from this in vitro study revealed that the betel leaf extract is effective against Porphyromonasgingivalis at 5% concentration and Treponema denticolaat 10% concentration when compared with Amoxicillin which is effective against Porphyromonasgingivalis at 1% concentration itself ; Treponema denticola, and Tannerella forsythia at 2% concentration. From this we can infer that thePorphyromonasgingivalis was showed higher sensitivity to betel leaf extract when compared to Treponema denticolaand Tannerella forsythia where Tannerella forsythia was resistant even at 10% concentration .The concentration of amoxicillin towards the Porphyromonasgingivalis, Treponema denticola,Tannerella forsythia, was still more than the betel leaf extract, though. Yet, employing the anti- infective therapy can sometimes lead to undesirable side effects that raise newer concerns [14].

Piper betle leaf, a perennial dioecious creeper as described empirically isanplant with aphrodisiac, stimulo-carminative, and fragrant properties. The leaves are reported to show wound healing properties. It is also known that betel leaves can treat a number of ailments, such as halitosis, boils and abscesses, conjunctivitis, constipation, migraines, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, gum swelling, rheumatism, scrapes, and bruises. The antibacterial, antifungal, antiseptic, and antihelminthic effects are also possessed by the fresh betel leaves. The leaf has high antibacterial activity against a variety of pathogens, including Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Streptococcus pyrogen, and Escherichia coli. Moreover, Enterocococcus faecalis, Citrobacter koseri, Citrobacter fruendi, Klebsiella pnemoniae, etcand other bacteria that cause urinary tract infections are all susceptible to the leaf extract's bactericidal effects[15].

The extract from betel leaves contains alkaloids, flavonoids, polyphenols, tannins, monoterpenoids, and sesquiterpenoids, according to the qualitative phytochemical analysis. The presence of phenolic group substantiates the antibacterial efficacy of the betel leaf. Protein denaturation and increased permeability are caused by phenols binding to the bacterial cell walls. The balance of the protein molecules will alter, changing the protein's structure and causing coagulation. Proteins that have become denaturated and coagulated lose their physiological action and become dysfunctional. As a result of changes to the bacterial cell wall's protein structures, cells become more permeable, which inhibits and damages cell growth[16].

The flavonoids in the Piper betle form complex chemicals that combat extracellular proteins and damage bacterial cell membrane integrity. Alkaloids additionally possess an antibacterial effect by disrupting the peptidoglycan portion of bacterial cells, which results in incomplete formation of the cell wall layers and cell death. Also tannin has antibacterial properties by reducing the permeability of the cell membrane or cell wall, which interferes with the cell's capacity to carry out its normal functions and prevents the growth of bacteria[14][17].

The majority of gram-negative bacteria contain a tough-to-penetrate polysaccharide complex on their cell walls. Endotoxin is the antigen that activates a particular immunity, along with the O-specific polysaccharide in its lipopolysaccharide structure. Also, the peptidoglycan layer of gram-negative bacteria is thinner which results in a quicker recovery period when this layer is harmed by antimicrobial activity and alters the sensitivity of the bacterial cell [16][18].The red complex bacteria has a big impact on the pathogenesis of periodontal disease, which results in tissue destruction.

There is concrete evidence that using plant products as an efficient therapy for periodontal diseases, despite the fact that earlier studies, such as those by Pratiwi et al[19], Limsuwan et al. [20], and Heliawati L et al. [21], all demonstrated the antimicrobial properties of betel leaf extract against various organisms.[9]This present study is one of the initial studies to evaluate the antibacterial potency of betel leaf extract against the red complex bacteria, particularly those related to chronic periodontitis.

Given the variety of the species tested against betel leaf's antibacterial activity, comparisons with prior investigations are not warranted in this instance. The current study encourages investigators to carry out future tests assessing betel leaf toxic effects, longevity, and other evaluations followed by clinical trials to provide clarity in the activity of betel leaf against periodontal pathogens and establish significant implication of betel leaf in periodontal disease because there is minimal existing literature that might illustrate the effectiveness of betel leaf specifically against periodontal micro organisms.

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Given the limitations of the current study, it could be stated that betel leaf, as an effective adjunct, if it is determined to be harmless and effective on further investigation, would be considered as a potentialadjunct in addition to conventional treatment in the management of periodontitis to mitigate the adverse effects of synthetic drugs, particularly in the modern age of rapidly advancing clinical dentistry.

V. CONCLUSION

Betel leaf extract, particularly at 5% concentration, is considerably effective in preventing the development of Porphyromonasgingivalis and significantly effective in inhibiting the growth of Treponema denticola at 10% concentration.

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