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RESEARCH ARTICLE

BETEL LEAF- A PATHOGEN'S ENIGMA

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ABSTRACT

Betel (Piper betle) is the leaf of a vine belonging to the Piperaceae family, which includes pepper and kava. It is mostly consumed in Asia, combined with tobacco, slaked lime and areca nut and is used as a recreational practise and for oral hygiene. Areca nut extract have been popularly known to exert carcinogenic actions on oral cavity. In this study we have attempted to analyse the effects of betel leaf oil on common periodontal pathogens like *Prevotella Intermedia, Porphyromonasgingivalis, Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum.* The minimum inhibitory concentration (MIC) required to inhibit the growth of these pathogens was analysed using the TUBE DILUTION METHOD and it was concluded that betel leaf oil has potent antimicrobial actions.

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INTRODUCTION

A very common recreational practices followed especially by the rural people of Asia is the use of husk of areca nut as 'herbal chewing sticks' instead of plastic bristle brushes to maintain oral health and hygiene. The basis for using plants as an oral hygiene aid is attributed to the inherent chemical properties present in these plants to maintain oral hygiene and suppress the growth and proliferation of the pathogenic species within the bio film present in the oral cavity (Majijose *et al.,* 2012). *Areca catechu* palm is a single trunked palm that grows upto 30 metres. The parts of the plant have reportedly been used as local medicines. *Surendiran et al* reported the antibacterial effects of areca nut leaves extract against *B.cereus*and *B. flurescens.* It was reported that areca nut seed inhibited the growth and propagation of *S.mutans.* Few studies have also reported the antioxidant properties of this plant material.The alcoholic extract of the husk fibres of areca nut showedstrong antifungal activity against *C.albicans* (Majijose *et al.,* 2012).

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There are multiple systemic effects of *Areca Catechu* (betel nut), in addition to giving a feeling of well being secondary to its euphoric effects, it increases salivation and alertness and has anti migraine properties. In addition it is hepatotoxic and predisposes a person to Type II diabetes, hypertriglycedemia and coronary heart disease. It causes infertility in men and aggravates the effects of Vitamin D defeciency. Also, Betel quid chewing is associated with a higher prevalence of bleeding on probing where higher clinical levels of disease existed, and with a likelihood of subgingival infection with *A. actinomycetemcomitans* and *P. gingivalis*. In addition, the arecoline which is a major constituent of betel nut when used in combination with the *Areca nut* has shown to reduce the antibacterial effects and superoxide production of neutrophils. This effect may contribute to a less efficient elimination of bacteria from the periodontal environment. Inhibition of the antimicrobial functions of the neutrophils may alter the microbial ecology of the oral cavity, and this may be one possible mechanism by which area nut compromises the oral health of users of the areca nut products. The leaf on the other hand has significant health benefits. Ethanolic extract of the leaf prevents radiation induced breaks in the DNA in a concentration dependent manner, in addition to the

radioprotection properties, it also has anti mutagenic properties as evident from a study done on two tobacco specific mutagens, N'- nitrosonornicotine (NNN) and 4-(nitrosomethylamino)-l-(3-pyridyl)-l-butanone (NNK). The anti mutagenic effects was caused by hydroxychavicoland has exhibited dose dependent suppression of dimethylbenzathrecene induced mutagenesis in S. *typhimurium* strain with TA98 metabolic activation (Amonkar *et al.,* 1986). It is a common recreational practise to chew betel leaves, especially after consuming a meal and it has shown to have significant reductions in the antimicrobial load especially when combined with cardamom and cloves. The phenolic constituent allylpyrocatechol from the leaves showed activity against obligate oral anaerobes responsible for halitosis (Ramji,Iyer, and Chandrasekaran, 2002). The common periodontal pathogens implicated in the pathogenesis of periodontitis are Aggregatibacter actinomycetemcomitans, Prevotella Intermedia, Porphyromonas Gingivalis and Fusobacterium nucleatum. To estimate the minimum inhibitory concentration of the betel leaf over the periodontal pathogens, the tube dilution method was used, it is a microtechnique for determining antibiotic susceptibilities by the serial dilution method.

MATERIALS AND METHODS

Preparation of the betel leaf oil

The extract of betel leaf is obtained by the steam distillation process. Steam distillation is a special type of distillation for temperature sensitive materials like natural aromatic compounds. Separation by distillation at normal boiling points is not an option so water or steam is introduced into the distillation apparatus. The efficacy of the betel leaf oil was compared with a placebo.

MIC procedure for anaerobes

9 dilutions of each drug have to be done with thioglycolate broth for MIC. In the initial tube 20 microliter of drug was added to 380 microliter of thioglycolate broth. For dilutions 200 microliter of thioglycollate broth was added into the next 9 tubes separately. Then from the initial tube 200 microlitre was transferred to the first tube containing 200 micro litre of thioglycollate broth, this was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200 microliter was transferred to second tube to make 10^{-2} dilution.

The serial dilution was repeated upto 10^{-9} dilution for each drug. From the maintained stock cultures of the required organism, 5 microliter was taken and added to 2ml of thioglycollate broth. In each serially diluted tube 200 microliter of the above culture specimen was added. The tubes were incubated for 48-72 hours in anaerobic jar at 37^0C and observed for turbidity.

Media Composition

Thioglycollate medium with Hemin and Vitamin K (for 1 liter)

- Tryptose:- 15gms
- Yeast extract :- 10gms
- Sodium thioglycollate:- 0.50gms
- Sodium chloride:- 2.5gms
- L-cysteine HCL:- 0.5gms
- Sodium bicarbonate:- 0.40gms
- Resazurine :- 0.001 gms
- Hemin :- 0.005gms
- Vit K :- 0.0005gms
- Agar:- 0.75gms

Standard strain of bacteria

- F nucleatum- ATCC no- 25586
- P.gingivalis- ATCC no- 33277
- Aggregatibacter actinomycetemcomitans-ATCC no-25567
- Prevotella intermedia- ATCC no- 25568

The results can be tabulated as follows:

RESULTS

The anaerobic bacteria like fusobacterium nucleatum, porphyromonasgingivalis, prevotella intermedia and aggregatibacteractinimycetemcomitans have all shown sensitivity to betel leaf oil compared to the placebo at 100-25µg/ml. Both P. gingivalis and P. intermedia has been inhibited at a minimum concentration of 0.4µg/ml of betel oil. The action of betel leaf oil on aggregatibacter actinimycetemcomitans is seen at a minimum concentration of 25µg/ml.And fusobacterium nucleatum was inhibited at a minimum concentration of 3.12µg/ml.

MIC (Minimum Inhibitory Concentration)

		100µg/ml	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
Fn	B.oil	S	S	S	S	S	S	R	R	R	R
	Placebo	R	R	R	R	R	R	R	R	R	R
Pg	B.oil	S	S	S	S	S	S	S	S	S	R
	Placebo	S	S	S	S	S	R	R	R	R	R
Pi	B.oil	S	S	S	S	S	S	S	S	S	R
	Placebo	S	S	S	R	R	R	R	R	R	R
Aa	B.oil	S	S	S	R	R	R	R	R	R	R
	Placebo	S	R	R	R	R	R	R	R	R	R

(B.oil- Betel leaf oil ,Fn- Fusobacterium nucleatem, Pg- PorphyromonasGingivalis, Pi- Prevotella Intermedia, Aa- Aggregatibacter actinomycetemcomitans)

DISCUSSION

The fresh leaves of betel vine are popularly known as *Paan*in India, which are consumed by about 15-20 million people in India and Asia. It is cultivated following the traditional methods in India on about 55,000 ha with an annual production worth about Rs 9000 million. A significantly higher prevalence of bleeding on probing was found in betel quid chewers than non-chewers among the subjects with higher plaque level, greater gingival inflammation, deeper probing depth or greater attachment loss. The fresh betel leaves possess antimicrobial, ringworm, antifungal, antiseptic and antihelminthic effects (Sarkar *et al.,* 2000). The leaf has a significant antimicrobial activity against broad spectrum of micro-organisms (Jesonbabu *et al.,* 2012) including *Streptococcuspyrogen*, *Staphylococcus aureus, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa*etc., beside this the leaf extract also poses the bactericidal activity against the urinary tract pathogenic bacteria such as *Enterococococcusfaecalis, Citrobacterkoseri, Citrobacterfruendi, Klebsiellapnemoniae*etc (Chakraborty and Shah, 2011; Agarwal and Singh, 2012). Earlier studies have also evaluated the antimicrobial effect of the ethanolic and methanolic extract of betel leaves on non periodontal pathogens and efficacy have been established. Studies have also failed to demonstrate significant inhibitory effects of the husk of areca nut on the cariogenic and periodontal pathogens and was concluded that the husk could be used as a mechanical cleansing agent rather than antimicrobial activity. Regular chewing of the betel leaves has shown significant changes in the subgingival microflora, especially the levels of *Streptococcus Viridians* has reduced considerably. In addition areca nut extract (ANE) was found to exert different effects in oral cells depending on the supplemented serum level. ANE strongly induced DNA damage, necrotic ballooning, and inflammatory cytokines under lower serum concentration. In a nationwide study conducted in Cambodia, an association was found between the intensity of betel quid use and HIV/AIDS ratio, Tuberculosis and Typhoid.

Conclusion

To conclude areca nut has significant carcinogenic activity and has no antibactericidal effects against periodontal pathogens and its use is limited as a mechanical cleansing agent. In contrast betel leaf has significant bactericidal activity and can significantly inhibit periodontal pathogens at concentrations from 100- 25µg/ml.

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