IJP (2014), Vol. 1, Issue 12 **(Research Article)**

Received on 29 October 2014; received in revised form, 21 November 2014; accepted, 29 November 2014; published 01 December 2014

IN-VITRO ANTIBACTERIAL ACTIVITY OF *PIPER BETEL* LEAVES EXTRACT AGAINST *STAPHYLOCOCCUS AUREUS* AND *CANDIDA ALBICANS*

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Keywords:

Antibacterial activity, *Piper betel*, Endodontic treatment

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ABSTRACT: Endodontic irrigating solution used in root canal treatment in dental specialty is to eliminate root canal pathogens in necrotic pulp tissues. A microorganism like *Staphylococcus aureus*, *Candida albicans*, *Enterococcus faecalis* were commonly seen pathogens in necrotic pulp tissues. Commonly used root canal irrigating solutions to kill bacteria's in the dental root canal are hydrogen peroxide (H_2O_2), chlorhexidine (CHX) and sodium hypochlorite (NaOCl). These root canal irrigating solutions has detrimental effects on the tissues underlying tooth (periradicular tissues) and affects post-operative healing. NaOCl has tissue toxicity and inhibits phagocytosis. Chlorhexidine inhibits protein synthesis in periodontal ligament cells. CHX and NaOCl exhibit an inflammatory effect in mitochondrial activity in human periodontal cells. Natural products are in great demand for their extensive biological properties and their bioactive molecules. Nontoxic *Piper betel* leaves extract obtained by methanolic extraction method of 500 mg/ml was tested against *Staphylococcus aureus* and *Candida albicans* which are commonly associated with root canal treatment and in failure cases using well agar diffusion method. The result shows piper betel leaves extract has antibacterial activity against these organisms.

INTRODUCTION: Endodontic irrigating solution used in root canal treatment in dental specialty is to eliminate root canal pathogens in necrotic pulp tissues. Micro-organism like *Staphylococcus, Candida albicans, Enterococcus faecalis* was commonly seen pathogens in necrotic pulp tissues. Commonly used root canal irrigating solutions to kill bacteria's in the dental root canal are hydrogen peroxide (H_2O_2), chlorhexidine (CHX), sodium hypochlorite (NaOCl).

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.1(12).792-94

Article can be accessed online on: www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.1(12).792-94

These root canal irrigating solutions has detrimental effects on the tissues underlying tooth (periradicular tissues) and affects post-operative healing. NaOCl has tissue toxicity and inhibits phagocytosis. Chlorhexidine inhibits protein synthesis in periodontal ligament cells. CHX and NaOCl exhibit an inflammatory effect in mitochondrial activity in human periodontal cells. It is time to develop an irrigating solution not associated with the toxic effect.

Natural products are in good demand due to its extensive biological properties and provide a source for developing many types of effective biologically active compounds. There are several reports of plants showing the antibacterial property. *Piper betel* leaves have been long used in India as native medicine. Betel leaves show antibacterial

property against *Streptococcus viridians, S. aureus* and *Streptococcus mutans* present in the oral cavity. Studies show antibacterial action of *Piper betel* against *Staphylococcus aureus, Candida albicans, P.* and *Lactobacillus acidophilus*. The objective of this study is to evaluate the antibacterial property of *Piper betel* leaves extract against *S. aureus* and *Candida albicans* commonly occurring endodontic pathogens which is present in infected necrotic pulp tissues of the affected tooth by agar well diffusion method.

MATERIALS AND METHODS:

Plant Extract: Fresh and healthy *Piper betel* leaves (Vellakodi) collected from Koyambedu market, Chennai, after proper identification. Leaves were washed in tap water and dried, dried leaves were grounded in the grinder, and powdered leaves were used for the preparation of extract. 2g of leaves were soaked in 20 ml of 70% ethanol (1:10) and stored in dark room for 4 days for the secondary metabolites get dissolved 1. It was filtered in Whatman's filter paper no. 1. After filtration, the filtrate was kept in an oven at 50 °C so that ethanol get evaporates. Dried metabolite dissolved in double volume DMSO (dimethyl sulfoxide). Thus the final concentration of extracted metabolite is 500 mg/ml.

Bacterial Strains & Antibacterial Susceptibility Assay: Microbial Strains and Inoculums Preparation: The microorganisms used in this study were clinical isolates of *Staphylococcus aureus* (MTCC 740), *Candida albicans* (MTCC 227) obtained from the Department of Microbiology, Madras Medical College. Bacterial strains stock cultures were maintained at 4 °C on nutrient agar medium. Cultures were prepared by inoculating fresh nutrient broth medium with a loop full of cells from the stock cultures at 37 °C for overnight. To get desirable cell counts for bioassays, overnight grown bacterial cells were sub-cultured in fresh nutrient broth at 37 °C.

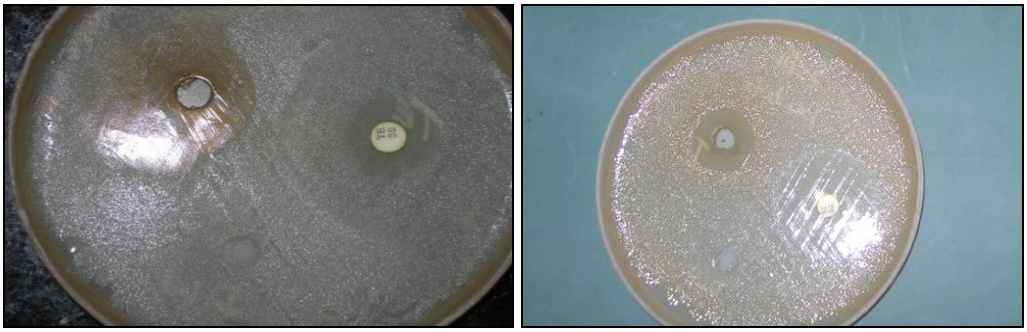
Agar Diffusion Method: Antibacterial activity of the extract was evaluated by the agar well diffusion method of Kirby Bauer. Sterile NA plates were prepared and spread with 60 µl of *Staphylococcus aureus* and *Candida albicans* in triplicates. Three well of 8 mm diameter were bored with sterile borer and first well loaded with 75 µl of standard antibiotic tetracycline (50 µg/ml), second well loaded with betel leave extract and third well with autoclaved distilled water. All the plates were incubated at 37 °C overnight.

RESULTS: Antibacterial activity of *Piper betel* ethanolic extract against *Staphylococcus aureus* and *Candida albicans* was expressed in terms of the mean of the diameter of the zone of inhibition in mm produced by extract at the end of incubation period. The results were given in the table and the figure.

Zone of Inhibition (ZOI):

TABLE: 1 ZONE OF INHIBITION

Microorganism	Tetracycline	*Piper betel*
Staphylococcus aureus	18	16
Candida albicans	22	18



ZOI *S. aureus* and tetracyclin **ZOI *C. albicans* and tetracyclin**

FIG. 1: ZONE OF INHIBITION

DISCUSSION: Endodontic irrigating solution used in root canal treatment in dental specialty is to eliminate root canal pathogens in necrotic pulp tissues. Micro-organism like *Staphylococcus, Candida albicans, Enterococcus faecalis* was commonly seen pathogens in necrotic pulp tissues. Commonly used root canal irrigating solutions to kill bacteria's in the dental root canal are hydrogen

peroxide (H_2O_2), chlorhexidine (CHX), sodium hypochlorite (NaoCl). These root canal irrigating solutions has detrimental effects on the tissues underlying tooth (periradicular tissues) and affects postoperative healing [2]. NaOCl has tissue toxicity and inhibits phagocytosis. Chlorhexidine inhibits protein synthesis in periodontal ligament cells. CHX and NaOCl exhibit an inflammatory effect in mitochondrial activity in human periodontal cells. It is time to develop an irrigating solution not associated with the toxic effect.

Natural products are in good demand due to its extensive biological properties and provide a source for developing many types of effective biological compounds. There are several reports of plants showing the bacterial property. *Piper betel* leaves have been long used in India as native medicine [3, 4] against *Streptococcus viridians, Staphylococcus aureus* and *Streptococcus mutans* present in oral cavity [5]. It has anti hemolytic, antioxidant and anti-inflammatory property [6]. Sugumaran M *et al*., in 2011 7 shows antibacterial action of *Piper betel* against *S. aures* [8], *C. albicans*, *Pseudomonas* and *Lactobacillus acidophilus*. Ethanol extract is used this study and the antibacterial assay was done with well agar diffusion method [9]. Results show the presence of an antibacterial effect in betel leaves on par with Tetracycline. Antibacterial property is due to the presence of 5-(2-propenyl benzodioxole) in betel leaves. Sterol molecules present in betel leaves have surface interaction with bacterial cell wall and membrane leading to pore formation and degradation of bacterial cell components [10]. M. Rama *et al*., in 2013 [11] revealed the presence of antioxidant and antimicrobial property of betel leaves is due to the presence of eugenol.

CONCLUSION: As per this study and other various studies the antibacterial property of Piper betel leaves shows significant antibacterial properties. Extracted oil obtained from *Piper betel* can be replaced with toxic chemical agents used in dental treatment procedures. However, *in-vitro* studies and *in-vivo* clinical studies need to establish the efficient antibacterial activity of *Piper betel* leaves on more pathogens and purification of the biomolecule components present.

ACKNOWLEDGEMENT: My sincere thanks to the Department of Microbiology, Madras Medical College, Chennai for providing microbial resources.

CONFLICT OF INTEREST: Nil

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How to cite this article:
Vinodh G, Kavitha M and Pradeep: *In-vitro* antibacterial activity of piper betel leaves extract against *Staphylococcus aureus* and *Candida albicans*. Int J Pharmacognosy 2014; 1(12): 792-94. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.1(12).792-94.