



ANTIMICROBIAL PROPERTIES OF *ARECA CATECHU* (ARECA NUT) HUSK EXTRACTS AGAINST COMMON ORAL PATHOGENS

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Received on: 09/12/2011 Revised on: 20/01/2012 Accepted on: 09/02/2012

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ABSTRACT

The husk fibres of *Areca catechu* (areca nut) are reported to be used by the people of rural areas of Dakshina Kannada, Karnataka for cleaning their teeth. The beneficial effects of these plant materials are not scientifically proven, so the study was carried out to estimate the antimicrobial properties of *A.catechu* against common oral pathogens. These antimicrobial properties in addition to mechanical cleansing property of the fibers of areca nut could improve the oral health. Alcoholic and aqueous extracts of husk of *Areca catechu* were prepared and antimicrobial properties against common oral pathogens like *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, *Lactobacillus acidophilus*, *Candida albicans*, *Prevotella intermedia* were performed by agar well diffusion method. Alcoholic extract of *Areca catechu* husk fibers showed dose dependent positive inhibitory effect against *Candida albicans* with zone of inhibition of 5-9 mm. Alcoholic and aqueous extracts did not show significant antimicrobial activity against other tested organisms. As our study failed to demonstrate significant inhibitory effect on cariogenic organisms and a periodontal pathogen, we conclude that areca husk when used for routine oral care, would improve the oral health primarily through mechanical cleansing rather than antimicrobial activity. However, the results indicate that areca husk contains chemical components that has antifungal effect. Therefore this plant material can be a potential source for developing natural antifungal agents against *C. albicans* which is a common oral pathogen.

Keywords: *Areca catechu*, Antimicrobial activity, Oral pathogens

INTRODUCTION

The use of plants and plant products as medicines can be traced as far as the beginning of human civilization. Medicinal plants contain large varieties of chemical substances with important therapeutic properties that can be utilized in the treatment of human diseases. The tribal and rural populations of India largely depend on medicinal plants for their health care. The studies on medicinal plants used as folklore remedies have attracted immense attention in scientific world in an attempt to find possible solutions to the problems of multiple drug resistance due to the existing conventional antibiotics¹.

Oral flora comprises of a diverse group of organisms and many of which are pathogenic causing various oral diseases such as dental caries, gingivitis, periodontitis as well as oral candidiasis. Dental caries affects people of all races, countries and economic strata and can occur at any age and in either sex. The bacteria primarily responsible for tooth decay are acidogenic and aciduric Gram-positive bacteria such as *S. mutans*, *S.salivarius*, *S.mitis* and *L.acidophilus*. These bacteria are known to colonize on surfaces of teeth and cause dental caries by production of acids through fermentation of dietary carbohydrates². Similarly periodontal diseases are also caused by various microbial pathogens of great importance such as *P. intermedia* and *P. gingivalis*³. Candidal group of organisms are also a part of oral flora which can cause various forms of oral candidiasis particularly in denture wearers and immune-compromised individuals⁴. Despite extensive researches, oral diseases like dental caries and periodontal diseases continue to be most important global

oral health problems. Frequency of dental diseases is directly proportional to the bacterial load in the oral cavity. Therefore daily oral hygiene practices like tooth brushing are of high importance as they help to remove the accumulated microbes from the teeth through mechanical cleansing action of bristles. The effect will be enhanced if combined with chemical agents having antimicrobial effects.

Areca catechu palm is a slender, single-trunked palm that can grow up to 30 meters. Betel nut palm yields diverse products that are used throughout its range. The husks, shoots, buds, leaves, and roots also have local medicinal uses⁵. Surendiran *et al* reported the antibacterial effect of areca nut leaves extract against *B.cerus* and *P.fluorescens*⁶. It was reported that areca nut seed inhibited the growth and propagation of *S. mutans*⁷. Few studies also reported the antioxidant properties of this plant material⁸.

One of the common traditional practices followed by the people of rural areas of Dakshina Kannada district of Karnataka is the use of husk of areca nut as herbal 'chewing sticks' instead of plastic bristle brushes to maintain oral health and hygiene⁹. As plants have the ability to synthesize secondary metabolites as defense mechanisms against predation by microorganisms, insects, it can be predicted that these plant materials have chemical constituents which are antimicrobial in nature. Therefore we hypothesize that, when husk of areca nut is used for routine oral care, it may be contributing to reduce the microbial load of oral cavity through mechanical cleansing property of fibers, supplemented by

antimicrobial properties. As there is no scientifically proven evidence that these husks contain active components, it would be relevant to conduct a systematic study on their valuable effects which may be accountable for improving the oral health, in addition to the mechanical cleansing.

MATERIALS AND METHODS

Preparation of extracts

The fibrous pericarp of areca nut was collected from the local areca nut growers. The husk fibers were washed with distilled water to remove dirt, cut into smaller pieces and air dried for 5 days. The dried husk fiber was then blended using household electric blender. The alcoholic extract was prepared using Soxhlet apparatus. For the preparation of water extract measured quantity of the plant powder was soaked in measured volume of distilled water. After complete extraction (72 hours in shaker), the filtrate was concentrated. Different concentrations of the plant extracts 100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml were prepared by dissolving alcoholic extract in dimethyl sulfoxide and aqueous extract in distilled water.

Collection of test organisms

Caries and plaque samples were obtained and cultured on appropriate media and the isolates were identified by Gram staining, colony morphology and biochemical reactions. These isolates were used as test organisms. The following organisms were studied *S. mutans*, *S. salivarius*, *S. mitis*, *L. acidophilus* and *P.intermedia*.

Assay for antibacterial activity

The antibacterial screening was carried out using agar diffusion method described by Lino and Deogracious with slight modifications. Three or four isolated colonies were inoculated in the 2 ml nutrient broth and incubated till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by World Health Organization. The freshly prepared inocula were swabbed all over the surface of the Muller Hinton agar plate using sterile cotton swab. Five wells of 6 mm diameter were bored in the medium with the help of sterile cork-borer and were labeled properly and 50µl of different concentrations of plant extract and the same volume of positive and negative controls were filled in the wells

with the help of micropipette. Chlorhexidine was used as a positive control. Negative control was distilled water for aqueous extract and dimethyl sulfoxide (DMSO) for alcoholic extract. Plates were left for some time till the extract diffused in the medium with the lid closed and then incubated at 37°C for 24 hours. The zone of inhibition was measured using a scale (Ajaiyeoba 2003)¹⁰. The mean and standard deviation of triplicates of various concentrations of plant extract was calculated and compared with chlorhexidine.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Various dilutions of the extracts which had antibacterial activity in the previous assay were taken in sterile test tubes to determine MIC. Freshly prepared nutrient broth was used as a diluent. Crude extract was diluted by two fold serial dilution method. A tube containing nutrient broth was taken as control. 50µl of the standard culture inocula were added to each test tube except the control tube. All tubes were incubated at 37°C for 24 hours and then examined for growth by observing turbidity. 1ml of bacterial culture was pipetted from the mixture obtained in the determination of MIC tubes which did not show any growth and sub cultured onto Muller Hinton agar and incubated at 37°C for 24 hrs. After incubation the concentration which did not show single colony of bacteria was taken as MBC (Lino A, Deogracios 2006)¹¹.

RESULTS

The effect of various concentrations of alcoholic and aqueous extracts of *Areca catechu* were tested against *S.mutans*, *S.salivarius*, *S. mitis*, *L. acidophilus*, *C.albicans* and *P.intermedia*. The results obtained are tabulated in Table 1.

The values obtained were then subjected to statistical analysis to specify the statistical correlation between the groups. Kruskal-Wallis test, Mann Whitney U test were used to compare and correlate different parameters in subgroups. In this study the MIC and MBC values for *C.albicans* were found to be 75mg/ml and 100mg/ml respectively.

Table 1: Zone of inhibition observed against oral pathogens (Mean ± SD)

Test bacteria	Areca nut alcoholic extract in different concentrations(mg/ml)				Areca nut aqueous extract in different concentrations(mg/ml)				DMSO/DW	CHX
	100	75	50	25	100	75	50	25		
<i>C.albicans</i>	9	7	5	0	0	0	0	0	0	16.6±0.57
<i>S. mutans</i>	0	0	0	0	0	0	0	0	0	18.6±1.15
<i>S.salivarius</i>	0	0	0	0	0	0	0	0	0	19.3± 0.57
<i>S. mitis</i>	0	0	0	0	0	0	0	0	0	18.6±1.15
<i>L.acidophilus</i>	0	0	0	0	0	0	0	0	0	18.0±1.73
<i>P.intermedia</i>	0	0	0	0	0	0	0	0	0	13.5±0.57

Key-DMSO-Dimethyl sulfoxide,DW-Distilled water,CHX-Chlorhexidine

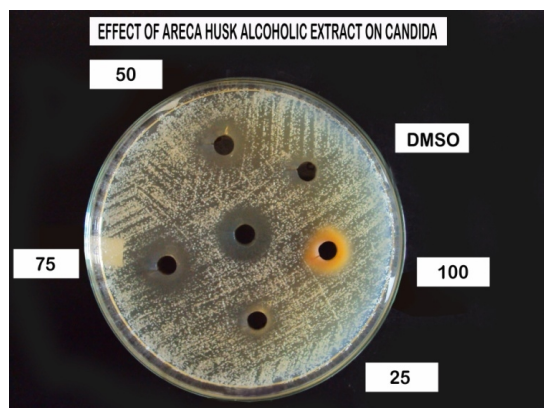


Figure 1: Effect of *Areca catechu* alcoholic husk extract *Candida albicans*

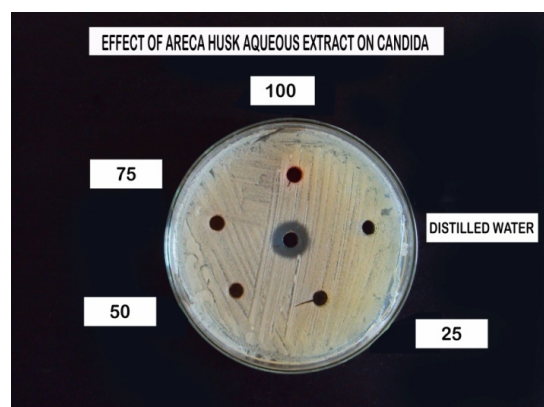


Figure 2: Effect of *Areca catechu* aqueous husk extract on *Candida albicans*

DISCUSSION

The present study was aimed to assess the antimicrobial activity of husk fibers of areca nut against dental caries causing bacteria like *S. mutans*, *S. salivarius*, *S. mitis*, *L. acidophilus*. In addition to these periodontal pathogens such as *P. intermedia* and *C. albicans* were also tested. Alcoholic as well as aqueous extracts of the husk fiber did not show strong antibacterial activity against the test organism except *C. albicans*. The zone of inhibition shown by alcoholic extract of areca husk fibre against *C. albicans* ranged from (9-5 mm), while the aqueous extract did not show any effect against the same. The differences in the observed activities of alcoholic and water extracts may be due to their varying degrees of solubility of the active constituents¹². It was found that the antibacterial activity reduced with decreasing concentrations of the plant extract. The inhibition produced by the plant extracts against particular organism depends upon various extrinsic and intrinsic parameters. Due to variable diffusability in agar medium, the antibacterial property may not demonstrate zone of inhibition to commensurate its efficacy¹³. Therefore MIC and MBC value has also been computed in this study. MIC is the lowest concentration of antibacterial substance required to produce a sterile culture. In this study the MIC and MBC values for *C. albicans* were found to be 75mg/ml and 100mg/ml respectively.

Due to incomplete investigations of these plant materials against oral pathogens, no data is available in the literature concerning the antimicrobial effects for

comparative analysis of our results. Only study reported so far is by Reena *et al* who evaluated the antimicrobial properties of areca nut seed material against *S. mutans* and found 100% inhibition at 100µg⁷. Although we could not demonstrate antimicrobial effects of these plant materials on other species except candida, these results do not prove inactivity of plant nor lack of bioactive constituents. Phytochemical constituents of areca nut husk fibers reported earlier includes phenolic compounds such as flavanoids and alkaloids¹⁴. These chemical constituents confer antimicrobial properties to the plant materials through various mechanisms which include substrate deprivation, membrane disruption, intercalation into cell wall or DNA and enzyme inhibition¹⁵. As areca husk are rich in these phytochemical constituents, in spite of negative results obtained in our study, antimicrobial property of these plant materials cannot be completely ruled out. The reasons for this may be insufficient quantities of active compounds in the crude extracts to show activity with the dose levels employed. Alternatively, even if the active constituent is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents¹³.

Further systematic studies using purified components in variable doses are required to verify the results obtained in the present study. Possibility of antimicrobial effect of these plant materials against other bacterial species which were not tested also may be considered.

CONCLUSION

Although husk fiber of *Areca catechu* did not show significant antimicrobial effect on oral pathogens, we propose that these plant materials are also contributing to oral health through efficient mechanical cleaning. Our results indicate that areca husk contains chemical components that have antifungal effect therefore this plant material can be a potential source for developing natural antifungal agents against *Candida albicans* and for exploitation of bio-active compounds of plant origin for eco-friendly disease management.

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Source of support: Nil, Conflict of interest: None Declared