

Arecanut As An Elixir For Dental Caries?

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Abstract: Areca nut is the seed of the fruit of the areca palm (*Areca catechu*) and plays an important role in traditional practice in some regions in south and south-east Asia countries specially India. Areca nut has known to have many beneficial effects according to Ayurvedic literature, though its excessive use causes dangerous adverse effects hence the good effects are masked. Numerous studies have concluded that patients with arecanut chewing habits had low incidence of caries. Through the present study an attempt was made to understand the direct effect of Arecanut on common cariogenic bacteria, *Streptococcus mutans* and *Streptococcus Sobrinus*. The effect of various concentrations of methanol and DMSO extracts of arecanut was tested against *S mutans* and *S sobrinus*. The *in vitro* antimicrobial activity was performed by agar well diffusion method. Based on the results, our study failed to show significant inhibitory effect on cariogenic pathogens like *S. mutans* and *S. sobrinus*.

Keywords: Arecanut, DMSO extract, methanol extract, *S. mutans* and *S. sobrinus*.

I. Introduction

In Ayurveda, the ancient system of Indian medicine literature speaks about chewing of plants and its products since many centuries among which Arecanut (AN) was being used for treatment of many diseases. The habit of chewing the AN for its stimulating qualities is indulged in about one tenth of the world's population which makes it one of the most consumed psycho active substance¹. AN is being consumed alone or as one of the components of betel quid. Along with carbohydrates, fats, proteins, phenols, it contains bioactive components like alkaloids and tannins which have been demonstrated to elicit inhibitory effects on selected microorganisms². Dried form of AN is said to strengthen gums, sweeten the breath, remove bad taste and produce a stimulant and exhilarant effect, and thus it was suggested that it could be used as a beneficial component of a dentifrice. It has also been reported that areca nut exerts a direct antimicrobial effect against oral bacteria including *Streptococcus mutans*, *Streptococcus salivarius*, *Candida albicans* and *Fusiform nucleatum*³.

Dental caries is known to be one of the most common forms of dental diseases. Worldwide, approximately 2.43 billion people (36% of the population) have dental caries in their permanent teeth⁴. Tooth decay is caused by biofilming on the teeth and maturing to become cariogenic (causing decay). Certain bacteria in the biofilm produce acid in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose. The most common bacteria associated with dental cavities are the mutans streptococci, most prominently *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*), and lactobacilli. Caries occur more often in people from the lower end of the socioeconomic scale than people from the upper end of the socioeconomic scale. Regular brushing with tooth brush and cleaning aid, maintaining good oral hygiene reduces the incidence of dental caries⁵.

Numerous studies on AN extract have been conducted to check the antimicrobial effect of oral microflora. Our literature review showed only few *in vitro* studies done using AN in relation to dental caries. According to limited evidence available, patients with AN chewing habits had low incidence of caries. In our present study an attempt was made to understand the direct effect of AN extracts on common bacteria causing dental caries like *S. mutans* and *S. sobrinus*.

II. Materials And Methods

2.1. Preparation of methanolic extract of arecanut

Commercially available processed and roasted form of AN was purchased from the AN market, Shimoga. The AN was pulverized to fine powder using blender and was stored in air tight container till use. The methanolic extract of the same was prepared using Soxhlet apparatus. 250ml of methyl alcohol was taken in the flask of soxhlet apparatus. 85gms of powdered AN was taken in a muslin cloth and put in central tube of apparatus. Tap water was allowed to pass continuously through the condenser of apparatus. The extract was obtained after 4 cycles and was taken in bottle. The bottle was kept in a hot water bath at a temperature of 56°C until the alcohol evaporated leaving behind the powdered form of extract⁶. The powder was transferred to a clean vial and stored at 4°C until use. 17.6gms of lyophilized arecanut powder was obtained from 85gms of

powder (Fig.1).The alcoholic extract obtained after soxhlet extraction was dissolved in five concentrations (2000, 4000, 6000, 8000 and 10000 µg/ml) of 30% methanol (Fig.2) and 30% dimethyl sulfoxide (DMSO) separately (Fig.3).



Figure 1: Methanolic extract of areca nut powder



Figure 2: Areca nut extract dissolved in 30% methanol



Figure 3: Areca nut extract dissolved in 30% DMSO

2.2. Bacterial strains and the test media

Our study was carried out at Nucleobase Life Science Research Lab, HRBR Layout, Bengaluru. Bacterial species like *S.mutans*(MTCC 497) and *S.sobrinus* (ATCC 33478) which are most commonly associated with dental caries were used in this study. The bacterial cultures of *S.mutans* and *S.sobrinus* were swabbed on separate Brain Heart Infusion agar plates in an anaerobic chamber to produce a lawn cultures.

2.3. Agar bacterial inhibition assay

The protocol was followed according to an article published by Astal ZY et al, 2005. To determine Minimum Inhibitory Concentration of bacteria various dilutions of the extracts was taken in a sterile agar plate⁷.

- The bacterial cultures of *S.mutans* and *S.sobrinus* were swabbed using sterile cotton on separate Brain Heart Infusion agar plates to produce a lawn cultures.
- Agar plugs were removed from the plates using sterile cork borer to produce 7 mm diameter well.
- To each well, 100µl of different concentrations of areca nut extract was loaded.
- At the center of respective plate 100µl of 30% DMSO or 30% methanol was loaded as control.
- All the plates were allowed to diffuse at room temperature for 20 minutes.
- The plates were later incubated anaerobically in candle jar at 37°C for 24h and examined for growth inhibition zones of bacteria around the wells bored measured in millimeters.

III. Results

The antimicrobial effect of various concentrations of methanol and DMSO extracts of AN was tested against *S. mutans* and *S.sobrinus*.

Streptococcus Mutans: No zone of inhibition on the culture plate was noted (Fig.4). Areca nut extract did not demonstrate any antimicrobial property for *S.mutans* when dissolved in 30% DMSO and 30% methanol (Table:1)

Streptococcus Sobrinus: No zone of inhibition on the culture plate was noted (Fig.5). Areca nut extract did not demonstrate any antimicrobial property for *S.sobrinus* when dissolved in 30% DMSO and 30% methanol (Table:2).



Figure 4: *Streptococcus mutans* (MTCC 497) culture plates showing no zone of inhibition

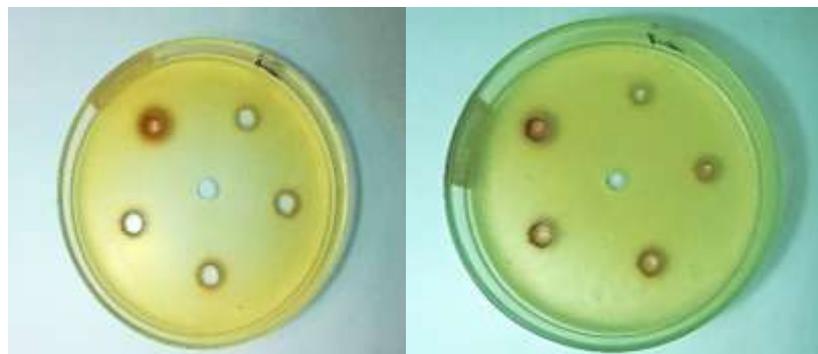


Figure 5: *Streptococcus sobrinus* (ATCC 33478) culture plates showing no zone of inhibition

Table 1. Inhibition of *S.mutans* growth as measured by the agar inhibition assay. Inhibition is expressed as the diameter of the inhibition zone (cm)

	30% DMSO extract	30% Methanol extract
2000 µg/ml	0	0
4000 µg/ml	0	0
6000 µg/ml	0	0
8000 µg/ml	0	0
10000 µg/ml	0	0

Table 2. Inhibition of *S.sobrinus* growth as measured by the agar inhibition assay. Inhibition is expressed as the diameter of the inhibition zone (cm)

	30% DMSO extract	30% Methanol extract
2000 µg/ml	0	0
4000 µg/ml	0	0
6000 µg/ml	0	0
8000 µg/ml	0	0
10000µg/ml	0	0

IV. Discussion

Throughout the world, plants and herbs have had a unique place in human civilizations. According to Ayurvedic literature herbal plants contains several medicinally active substances with therapeutic properties that can be utilized in the treatment of human diseases. Plants have been known to have the ability to synthesize secondary metabolite as defense mechanisms against many microbes which prove that these plants are antibacterial in nature³. The tribal and most of the rural population in India depend largely on these plants for their health care, however their beneficial effects of these plant products are yet to be scientifically proved.

Moller et al in 1977 examined the influence of betel chewing on the prevalence of dental caries which showed a strong inverse relationship between the prevalence of dental caries and the intensity of betel chewing.⁸ Howden in the year 1984 compared the effects of betel nut chewing on all aspects of dental health against an identical population who did not chew. The results of his survey showed, that for betel nut chewers the prevalence of dental caries was 23% whereas for non-chewers it was considerably and statistically significantly greater at 49%.⁹ A survey was carried out by Nigam and Srivastava to find out the effects of betel chewing on dental caries and oral hygiene. He observed low incidence of caries and poor oral hygiene in patients who chew AN¹⁰. Reena and Michael evaluated the antibacterial, antifungal, and antiviral properties of the areca nut *in vitro* using isolated organisms. In their study the aqueous extract of Areca catechu L. was found to be effective for inhibiting the growth and propagation of *S.mutans*, the bacteria that causes dental caries¹¹. Prabhu conducted an *in vivo* study to rule out the antimicrobial effect of chewing betel nut and its combinations from saliva of the subjects. He found that betel leaves +cardamom + clove had better reduction rate of microflora than betel leaves+AN+Clove +Cardamom+Lime. Betel leaves and AN combination did not show significant reduction of micro florain subjects¹².

Keeping these results in consideration and with the limited literature available on both *in vitro/vivo*, an *in vitro* study was carried out to determine the effect of AN on common bacteria causing dental caries. To the best of our knowledge our study was the first *in vitro* study to carry on cariogenic bacteria especially *S. sobrinus*. In contrast to above mentioned studies, our study on AN extracts did not demonstrate any antimicrobial activity against tested organisms. This could be due to many extrinsic and intrinsic factors produced by the plant extract against particular organism. The variable diffusability in agar medium, lack of the solubility of active compounds in aqueous solutions, antibacterial property may not always demonstrate zone of inhibition to commensurate its efficacy. Sometimes the insufficient quantities of active compounds during the crude extract show activity with dose levels, or there could be other constituents exerting antagonist effect of bioactive agents⁶. The cariostatic property of AN could also be due to the betel stain which often coats the surface of the teeth, may act as a varnish or physical barrier against microorganisms which forms biofilms on the teeth¹³.

V. Conclusion

Areca nut chewing has always been a topic of controversy all over the world. It has many beneficial effects when used occasionally whereas its excessive use causes dangerous adverse effects hence the good effects are masked. Our study in contrast to other similar study, failed to show any significant inhibitory effect on the cariogenic pathogens like *S.mutans* and *S.sobrinus*. However, negative results do not imply the lack or absence of bioactive substances nor the plant is inactive. We would like to conclude that the low incidence of dental caries observed in population with dental caries could be due to mechanical cleansing rather than its antimicrobial activity. Further studies on AN and its different forms needs to be explored to probe the effect of oral microflora and dental caries which could be possibly exploited for pharmaceutical use and helpful for prevention and management of caries.

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