

ANTIMICROBIAL ACTIONS OF ARECANUT, BETEL LEAF AND BETEL QUID
AGAINST CERTAIN ORAL PATHOGENS – A REVIEWSarvangala Keshava Bhat^{1*}, Prashant Kumar Kalladka² and Sukesh Bhat³¹Scientist, Arecanut Research and Development Foundation, Varanashi Towers, Mission Street, Mangaluru, 575001, DK, Karnataka, India.²Director, Himani Dental and Oral Cancer Diagnosis Centre, Kalladka, 574222, DK, Karnataka, India.³Assistant Professor, Kanachur Institute of Medical Sciences, Deralakatte, 575018, DK, Karnataka, India.

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ABSTRACT

Several species of harmful pathogens including *Streptococcus mutans*, *Bacillus subtilis*, *Lactobacillus casei*, *Porphyromonas gingivalis*, *Enterococcus faecalis*, *Candida albicans*, etc. harbour human oral cavity causing various problems including tooth decay, biofilm formation, gingivitis and others. Humans have the habit of chewing arecanut (*Areca catechu*), either alone or in combination with the leaf of *Piper betle*, calcium hydroxide and with or without tobacco in the form of betel quid due to its medicinal properties including improving oral hygiene, since few thousands of years. Both arecanut and betel leaf exhibits several medicinal properties including antibacterial actions. Ample research papers now exist confirming such beneficial activities in these two major chewing substances. The research papers which highlight the antibacterial actions of arecanut, betel leaf and betel quid against certain major harmful oral bacteria and fungus are retrieved and the findings are presented in this article.

KEYWORDS: Arecanut, Betel leaf, Betel quid, Oral pathogens, Antibacterial, Antifungal.

INTRODUCTION

Arecanut is the seed of areca palm, *Areca catechu* L., of Palmae family. This nut is also called betel nut in several parts of the world as it is generally chewed along with the leaf of betel vine, *Piper betle* L. of Piperaceae family. The betel quid (tambula) is generally a mixture of arecanut, betel leaf and slaked lime (Calcium hydroxide). Several other ingredients such as the leaf of tobacco, sweeteners, certain spices like cardamom, saffron, cloves, aniseed, turmeric, mustard, etc., are sometimes added as per local preferences. Chewing of betel quid is a popular habit in most parts of the world, especially in South and South East Asian countries such as India, China, Bangladesh, Indonesia, Myanmar, Thailand, Malaysia, the Philippines, etc.^[1]

Chewing of arecanut and betel quid is not of recent origin, but goes back to few thousands of years. The earliest archaeological evidence of arecanut chewing has been found in the fossils dated back to about 3000 BC in Duyong cave in the Philippines.^[2] Similar evidence has been reported from Vietnam, where the stains of arecanut were reported in the teeth of human fossils of bronze age (1200-3300 BC).^[3] In India, arecanut chewing has been mentioned later on in 650 BC in the work of the famous

Sanskrit poet, Magha in Sisupala Vadha of the epic Mahabharata.^[4] The medicinal properties of this nut were cited as early as 4th century AD by the famous Scholar Vagbhata.^[5]

According to Ayurveda, chewing of betel quid (tambula sevana) after every meal improves digestion, acts as a mouth freshener, relaxes the mind, improves physical and mental stamina and creates a feeling of well being apart from several other health benefits.^[6] Role of betel leaf in oral hygiene was indicated in several ancient texts as well.^[7] Ample research has been done in recent years on the antimicrobial effects of these common chewing products against oral pathogens. Such works are retrieved, presented and discussed in this write up.

A. Against cariogenic bacteria

Cariogenic bacteria are usually acidogenic pathogens causing dental caries in the oral cavity. All the three major chewing products such as arecanut, betel leaf and betel quid are reported to be antibacterial against following major cariogenic bacteria:

1. *Streptococcus mutans*

The gram-positive bacteria *Streptococcus mutans* and *S. viridians* are considered to be the primary bacteria

responsible for the initiation of dental decay.^[8] Arecanut is known to suppress the growth and multiplication of these bacteria. It is reported that the aqueous extract of arecanut completely inhibited the growth of *S. mutans* and *S. viridians* at a concentration of 50 µg/ml.^[9] The fatty acids (myristic and oleic acids, etc) of arecanut were reported to be the active principles for such activity.^[10] Two polyphenolic substances (nucliotidase inhibitors), NF-861 and NF-8611 from arecanut were reported to be responsible for the growth inhibition of two strains of *S. mutans*, MT8148(c) and MT6715(g), respectively.^[11] These authors further suggested that such compounds might be used as anti-plaque preventing agents. In another study it was reported that the hydrolysable tannins in the tannin fraction, which include tannic acid, were responsible for the antibacterial properties of this nut and that prolonged intraoral exposure to such nuts can suppress bacteria in the mouth.^[12] The authors also observed that the baked and boiled arecanuts were more potent than the raw nuts. For baking and boiling, tender arecanuts are generally used.^[13] It was earlier reported that tannins are more in tender arecanuts when compared to mature ones.^[14] This might be the main reason for the increased antibacterial actions of baked and boiled nuts when compared to raw nuts. The antibacterial property of tannic acid was also reported in another study.^[15]

Apart from arecanut, the betel leaf, another common ingredient of betel quid, also showed inhibitory effect against *S. mutans*. The methanol extract of betel leaf exhibited larger zones of inhibition compared to its aqueous extract. The zone of inhibition with aqueous extract of betel leaf against this pathogen at 100µg per disk was 9.3mm whereas it was 10.5mm with its methanol extract at the same concentration.^[16] Hydroxychavicol, an important phytochemical of betel leaf, was very effective against *S. mutans* exhibiting a minimum inhibitory concentration (MIC) ranging from 250 to 500µg/ml and a minimum bactericidal concentration (MBC) from 500 to 1000µg/ml.^[17] The mouthwash prepared by Hydrxychavicol oil with 98.5% purity gave an MIC value of 25µg/ml against *S. mutans*.^[18]

Even the betel quid (BQ) containing arecanut, betel leaf, catechu or gambir (*Uncaria gambir*) and slaked lime was also reported to be effective against *S. mutans*. The zone of inhibition obtained with the aqueous extract of BQ containing 3.5g arecanut, 8.0g betel leaf, 2.5g gambir and 2.0g lime against this bacterium was 8.25mm.^[19] All the ingredients of BQ showed significant reduction in microbial population both individually and in different combinations. It was reported that when arecanut alone was chewed, the microbial population in the oral cavity was decreased by 21.08%, but when it was chewed along with betel leaf, lime and gambir the effectiveness was increased by 52.58%.^[20] These authors were of the opinion that the ingredients of BQ, either alone or in combinations could be used as an effective mouth

fresheners which can prevent dental carries and bad breath.

2. *Staphylococcus aureus*

S. aureus is another gram positive bacterium responsible for dental caries. The methanol, ethanol and ethyl acetate extracts of arecanut were reported to be antibacterial against this bacterium. The methanol extract of arecanut at 150µl per well showed an inhibition zone of 21mm against *S. aureus*.^[21] The MIC and MBC against this pathogen were reported to be 5mg/ml and 17mg/ml, respectively for such extract.^[22] In another study, the MIC for the methanol extract of arecanut was reported to be >125µg/ml against this bacterium.^[23] However, the ethanol extract of arecanut was found more potent than its methanol extract against *S. aureus* with MIC and MBC values of 0.377mg/ml and 0.753mg/ml, respectively.^[24] The ethyl acetate extract of arecanut also showed antibacterial property against this bacterium with a zone of inhibition of 9.8mm at 2% concentration.^[25] It was reported that both water and acetone extracts of arecanut effectively inhibited the growth of *S. aureus* at 0.78mg/ml concentration.^[26] The MIC and MBC of the acetone extract of arecanut against *S. aureus* were reported to be 0.625 and 1.25µg/ml, respectively, whereas the figures for the ethanol extract were 2.5 and 5.0 µg/ml, respectively.^[27] The concentration needed for maximum inhibition (90%) of bacterial growth with the aqueous extract of arecanut was found to be 50 µg/3ml for this bacterium.^[28] Certain other studies also confirmed the antibacterial effects of arecanut against *S. aureus*.^[29]

Silver nanoparticles (AgNPs) of arecanut extract exhibited stronger antibacterial activity against *S. aureus* when compared to its aqueous extract. The MIC and MBC of AgNPs of arecanut extract against *S. aureus* were reported to be 4 and 8 µg/ml, respectively, whereas the figures for arecanut aqueous extract were 512 and 0 µg/ml, respectively.^[30] Similarly, AgNPs extract of arecanut produced a zone of inhibition of 34±0.68mm against *S. aureus* whereas it was only 24±0.40mm with the standard drug, ciprofloxacin.^[31]

The betel leaf was also reported to be antibacterial against *S. aureus*. The methanol extract of betel leaf at a concentration of 400µg/disk showed a zone of inhibition of 6.77±0.25mm^[32], whereas, ethanol, chloroform and petroleum ether extracts of betel leaf, each at 50µl/well, showed a zone of inhibition of 18mm, 10mm and 4mm, respectively.^[33] In another study, a zone of inhibition of 1.2mm was reported with ethyl acetate, 1.3mm with aqueous, 1.45mm with methanol and 1.5mm with petroleum ether extracts of betel leaf, each at 10mg/ml concentration.^[34] However, the ethanol extract of betel leaf showed a zone of inhibition of 13±0.43mm at 1mg/ml concentration and arrived at a MIC of 40µg against this bacterium.^[35] Chloroform and ethyl acetate extract of betel leaves showed the highest zone of inhibition of 17mm and 14mm respectively at a

concentration 30µl against *S. aureus* and ethanol extract showed zone of inhibition of 9mm at a concentration of 30µl.^[36]

3. *Bacillus subtilis*

B. subtilis is the most common gram positive bacterium responsible for the formation of biofilm and dental caries on human tooth.^[37] The ethanol, aqueous and methanol extracts of arecanut were all reported to be effective against this bacterium. At 100% concentration of such extracts the biofilm forming ability of *B. subtilis* was reduced by 40%. The authors suggested that such extracts of arecanut could be used in mouthwash formulations to avoid plaque formation on human teeth. The antibacterial property of arecanut against *B. subtilis* was also reported in another study.^[24] The MIC of the ethanol extract of arecanut, reported by these authors, against *B. subtilis* was 0.188 mg/ml and MBC 0.377 mg/ml. The hydroalcoholic extract of *A. catechu* was also reported to be active against *B. subtilis* at a concentration of 100mg/ml.^[38]

While preparing the 'red type' of arecanut, the immature nuts are boiled before drying and during this process of boiling lots of concentrated liquid called 'chogaru' comes out of the nut. It was reported that such liquid could also be used as antibacterial against *B. subtilis*.^[39] Here also the nanoparticles of arecanut extract exhibited stronger anti-bacterial activity against this pathogen when compared to its plain aqueous extract. The MIC and MBC of silver nanoparticles of arecanut extract against *B. subtilis* were reported to be 16 and 64µg/ml, respectively, whereas such figures for arecanut aqueous extract were 256 and 1024µg/ml, respectively.^[30] Certain other studies also reported similar results.^[40] The zone of inhibition noticed with copper nanoparticles of arecanut was 25 ± 0.59mm at 50 µl of 10% extract per well whereas with plain arecanut extract it was only 11 ± 0.74 mm at that concentration.^[41]

Betel leaf also exhibited potent antibacterial activity against *B. subtilis* with the zone of inhibition of 25mm each with ethanol and petroleum ether extracts and 20mm with its chloroform extract at 50µl/well.^[33] An inhibitory zone of >4mm was reported with the water extract of betel leaf (*meeta paan* type) against this pathogen at 10mg/ml concentration.^[42] In another study, inhibition zones of 30mm, 37mm and 38 mm were reported against *Bacillus* sp by the application of ethanol, water and methanol extracts of betel leaf at 50µl concentration, respectively.^[43]

4. *Lactobacillus casei*

While *Streptococcus* spp. are primarily responsible for the initiation of dental decay, other gram-positive bacteria, such as *Lactobacillus* spp. are associated with the progression of such lesion.^[8] It was reported that ethanol extract of arecanut inhibited the growth of *Lactobacillus casei*.^[44] Apart from arecanut, the betel leaf also showed inhibitory effect against this oral

bacterium. The methanol extract of betel leaf exhibited larger zone of inhibition compared to its aqueous extract. The zone of inhibition with aqueous extract of betel leaf against *L. casei* was 11.3mm whereas it was 12.5mm with its methanol extract at 100µg per disk.^[16]

B. Against bacteria causing gingivitis

Gingivitis is a common form of gum disease (periodontal disease) that causes irritation, redness and swelling (inflammation) of gingiva (the part of gum around the base of tooth), eventually leading to tooth loss. *Porphyromonas gingivalis* is a gram-negative oral anaerobic bacterium involved in the pathogenesis of this disease. This disease generally occurs when bacteria and plaque buildup in the mouth and lead to infection. Arecanut has a curative property on this oral disease as well. It was reported that gargling with the ethanol extract of immature arecanut twice a day for 30 seconds for three days completely cured gingivitis in the oral cavity.^[45] These authors further reported that while gargling with chlorhexidine (CHX) at 0.2% concentration the mean gingival index which was 1.20 ± 0.561 on the first day reduced to 0.20 ± 0.414 on the second day and to 0.00 on the third day. Almost similar results were obtained with the gargling of areca extract which was 1.93 ± 0.704 on the first day reduced to 0.40 ± 0.507 on the second day and 0.00 on the third day. Similarly, there was no significant difference in the mean gingival healing time, which was 2.29 ± 0.66 days with CHX and 2.35 ± 0.45 days with arecanut extract.

Apart from arecanut, the betel leaf also showed curative property against this oral disease. The gel prepared from the aqueous extract of betel leaf was reported to inhibit the growth of *P. gingivalis* and *Aggregatibacter actinomycetemcomitans*, the two bacteria often associated with periodontitis, with inhibition zones of 8.95mm and 14.7mm, respectively at 100% concentration.^[46] The mouthwash prepared by using 0.1% betel leaf oil showed effective antibacterial activity against three common periodontal pathogens, viz., *Prevotella intermedia*, *A. actinomycetemcomitans* and *P. gingivalis* with the zone of inhibition ranging from 15 to 25mm.^[47] The mouthwash prepared from the hydroalcoholic extract of betel leaf (50:50 ratio) at 2% concentration was found equally effective with that of 0.2% Chlorhexidine mouthwash.^[48]

C. Against bacteria causing endodontic infections

Enterococcus faecalis is the most common and dominant anaerobic bacterium responsible for human endodontic or root canal infections. The aqueous extract of arecanut was reported to be very effective against this bacterium and found even better than that of CHX, the chemical disinfectant presently used during root canal treatment. With arecanut extract, a zone of inhibition of 8mm was noticed against this pathogen at a concentration of 0.062mg/disk whereas with CHX no inhibition zone was noticed at such a low concentration and the inhibition with CHX commenced only at a concentration of

0.25mg/disc.^[49] In another study, the arecanut aqueous extract treated group showed greater pixels of dead bacteria against *E. faecalis* followed by CHX and normal saline.^[50] These authors suggested that arecanut extract could be considered and used as an alternate herbal disinfectant during root canal treatment.

D. Against fungus causing oral candidiasis

Oral candidiasis is an infection of the oral cavity by the fungus *Candida albicans*. The arecanut extract is reported to be very effective against this pathogen.^[9] The aqueous extract of arecanut showed a zone of inhibition of 18mm against *C. albicans* at 16.67 µg/ml concentration by plate method.^[51] The hydroalcoholic extract of arecanut was reported to be moderately active against *C. albicans* at a concentration of 100mg/ml and very active at 200mg/ml concentration with the zone of inhibition of 21mm and 24mm, respectively.^[38]

Not only the arecanut seed but also its husk (pericarp) and root were reported to be antifungal. The antifungal effect of the ethanol extract of arecanut husk against *C. albicans* was reported and the authors suggested that this plant material could be a potential source for developing natural antifungal agent against this common oral pathogen.^[52] The ethanol extract of the roots of *A. catechu* also showed effective antifungal activity against *C. albicans* with zone of inhibition ranging from 16.7 to 24.0mm at concentrations between 100 to 500µg.^[53]

Apart from arecanut, the betel leaf was also reported to be effective against *C. albicans*. Hydroxychavicol, the active ingredient isolated from the chloroform fraction of the aqueous extract of betel leaf showed MICs ranging from 125 to 500 µg/ml for 25 different strains of *C. albicans*.^[54] However, the MIC value of the ethanol extract of betel leaf against *C. albicans* was reported to be 1.6mg/mL whereas the figure for the standard drug Fluconazole was only 0.128 mg/mL.^[55] Further, these authors reported that the extracts of young leaves of *P. betle* showed significantly higher ($p < 0.05$) anticandidal activity than that of mature leaves. Among the ethyl acetate and ethanol extracts of betel leaf, the ethyl acetate extract showed better inhibitory effect than that of the ethanol extract against *C. albicans*.^[56] However, in their study, the MIC value of betel leaf extract (125 µg/ml) was found higher when compared to that of the standard drug fluconazole (62.5 µg/ml). Using 40% ethanol extract of betel leaf a zone of inhibition of 9.12mm was reported against *C. albicans* and the figure was found almost similar to that of the 2% concentration of the standard drug Ketoconazole (10.35mm).^[57] Among the three solvent extractions of betel leaf the methanol extract showed better anticandidal activity than the ethanol and aqueous extracts. At a concentration of 40µl of methanol extract of betel leaf a zone of inhibition of 17mm was noticed against *C. albicans*, whereas at the same concentration of ethanol and aqueous extracts the inhibition zones were only 7mm and 2mm, respectively.^[58]

E. Population study

It was reported that arecanut chewing confers a degree of protection against dental caries.^[59-61] In a population study carried out on arecanut chewers and non chewers, it was reported that the prevalence of dental caries in arecanut chewers was 23% whereas in non-chewers it was significantly more at 49%.^[62] The authors further reported that the amount of dental caries as measured by DMFT was also significantly more in non-chewers at 1.162 compared to 0.364 for chewers. The stain caused due to arecanut chewing may act as protective varnish on tooth surface. It was also reported that arecanut chewing significantly lowered the microbial diversity in the oral cavity.^[63] In another study it was confirmed that the more the chewing frequency of arecanut the lesser is the dental caries incidence.^[45] In an observational trial conducted on school children it was noticed that there was a significant reduction in caries status in those chewing arecanut for more than 5 times per day compared to those who chewed 3-5 times per day.^[64] The study carried out in Thailand reported that even in chewers of betel quid containing arecanut and tobacco the mean number of decayed teeth was lower than in non-chewers group.^[65] Similar observations were reported from India where it was noticed that the tooth problems were significantly less in chewers of betel quid with or without tobacco when compared to non chewers.^[66,67]

CONCLUSION

This review highlights that all the major chewing products such as arecanut, betel leaf and betel quid are effective anti microbial in their actions. It is also true against several oral pathogens including bacteria and fungus. The study conducted on chewers of betel quid also supports this. It is high time to work further in these lines.

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