



E-ISSN: 2278-4136

P-ISSN: 2349-8234

[www.phytojournal.com](http://www.phytojournal.com)

JPP 2021; 10(1): 961-963

Received: 01-11-2020

Accepted: 07-12-2020

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## Studies on antimicrobial properties of areca nut *Areca catechu*

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**Abstract**

Areca nut is the seed of the Areca palm (*Areca catechu*). This study evaluates the antibacterial, properties of the areca nut in vitro against common bacterial pathogens. Both gram positive and gram negative bacteria were tested against areca nut extract by measuring the growth of the organisms using the spectrophotometric method. It was found that test organisms viz., *Staphylococcus aureus* and *E. coli* were susceptible to the areca nut extract and 85-90% growth inhibition was recorded. Higher inhibition was found for *Staphylococcus aureus*. The concentration needed for maximum inhibition of bacterial growth was found to be 50 µg/3ml for both bacteria. These results indicate that betel nut extract have significant antibacterial properties and may be a potential source of extraction bioactive compounds for use in antimicrobial preparations.

**Keywords:** Areca nut extract, antibacterial activity, herbal extract

**Introduction**

Infection is the major cause of death and to prevent these infectious diseases, different potent antimicrobial agents are used. However, antimicrobial agents that have been widely used today can result in different side effects and changes on intestinal microbiota [Chempakam B. *et al.*, 1993] <sup>[1]</sup>. In addition, bacteria have been reported to show increased resistance towards common antibiotics which have been used therapeutically for the treatment of infectious diseases [Park YB, *et al.*, 2002] <sup>[2]</sup>. The increase in resistance and adverse effects of antimicrobial agents have lead researchers to explore novel anti-infective herbal compounds which could be used for effective treatment of these diseases. Medicinal plants have been used in traditional treatment in various parts of the world, especially in rural areas [Kusumoto IT, *et al.*, 1995] <sup>[4]</sup>. Approximately 80% of the population in developing countries still use traditional medicines for their health care [Van Wyk, *et al.*, 1980] <sup>[5]</sup>. The natural products derived from medicinal plants are known to produce biologically active compounds [Kusumoto IT, *et al.*, 1995 and Vijayan P, *et al.*, 2004] <sup>[4, 6]</sup>. The areca nut is the seed of the areca palm (*Areca catechu*), which grows in much of the tropical Pacific, Asia, and parts of east Africa. Several authors around the world have studied the anti-bacterial, antiviral, and antifungal activities of areca nut extract in isolated micro-organisms [Nisreen J M, 2016, Steven Y. C. *et al.*, 2015, Mobley, 2004, Iwamoto M., *et al.*, 1991 and Keshava Bhat Sarpangala *et al.*, 2017] <sup>[8-12]</sup>. Areca nut has been known to reduce dental caries by possibly inhibiting gram positive microorganisms that are responsible for dental caries [Chang MC, *et al.*, 2001] <sup>[14]</sup>. Chewing the areca nut with betel leaves (betel quid) is a traditional habit in India and in many Asian countries. Betel quid chewing has been reported to improve oral hygiene [Chempakam B. *et al.*, 1993] <sup>[1]</sup> and motility of food and thereby reduce the absorption of food and thus arrest weight gain. It has also been shown to be anti-diabetic and to reduce cholesterol levels [Park YB, *et al.*, 2002] <sup>[2]</sup>. Areca nut is a mild psychoactive material [Kusumoto IT, *et al.*, 1995] <sup>[4]</sup> and has been reported to be an aphrodisiac. One of the ingredients in areca nut, catechin, is believed to be a chemo preventive agent against several tumors in animals [Kusumoto IT, *et al.*, 1995] <sup>[4]</sup>. In this report, we have studied the anti-bacterial activity of areca nut extract in isolated micro-organisms. The main constituents of areca nut are polyphenols, fat polysaccharides, and protein. Besides these, the nuts contain alkaloids arecoline (0.1-0.7%) and others in trace amounts such as arecadine, guvacoline, and guvacine. de Miranda *et al.* found that the hydrolysable tannins in the tannin fraction of aqueous extracts of Areca nut, which include tannic acid, are responsible for the antibacterial properties of the nut and that prolonged intraoral exposure to the nut can suppress bacteria in the mouth. The aim of this study was to evaluate the antibacterial properties of the areca nut extract in vitro using isolated microorganisms.

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## Materials and Methods

### Microorganisms

Both gram positive and gram-negative bacterial species viz., *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8739) procured from department of Veterinary Microbiology, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture & Technology, Pantnagar were used for the study.

### Extraction of Areca nut

A hot water extract of the areca nut was prepared by boiling the nuts (100 g) in 500 ml of distilled water for 1 hour. This extract was then air dried. The yield of the extract was recorded to be 6.4%. The extract was then re-suspended in distilled water and diluted to the desired concentration.

### Determination of antibacterial activity

A loopful of bacterial strains were inoculated into a nutrient broth (100 ml) and kept overnight at room temperature for incubation. About 0.1 ml of this overnight culture was incubated in the presence and absence of increasing concentrations of the extract (10-100 µg) for 8 hours in 3 ml of nutrient broth. The absorbance was recorded at 530 nm immediately after inoculation of bacterial cells and continued for 8 hours at intervals of 1 hour. The experiment was set up in triplicates and the average value was taken. Inhibition of bacterial growth was calculated by comparing the optical densities of the control and experimental culture tubes.

### Result and Discussion

The Areca nut extract screened for antibacterial activity against two important and frequent human pathogens *Staphylococcus aureus*, cause of several infections like Boils, impetigo, food poisoning, cellulitis, and toxic shock syndrome (Tong *et al.*, 2015) [9] and *E. coli*, causing serious food poisoning, septic shock, meningitis, or urinary tract infections (Mobley *et al.*, 2004) [10]. The aqueous extract of Areca nut extract exhibited antimicrobial activity against test bacteria in different concentrations (Table-1).

**Table 1:** Anti-bacterial activity of areca nut extract

Strains/Concentration*	Percent inhibition			
	10 µg	20 µg	50 µg	100 µg
<i>E. coli</i>	0	50	85	85
<i>Staphylococcus aureus</i>	0	70	90	90

\*Concentration is expressed per 3 ml of medium.

The results obtained in the present study demonstrate the bioactivity of water-based areca nut extract against test human pathogens. This bioactivity may be attributed to the chemical nature of the active compounds present in the extract. In an investigation of the antibacterial properties of aqueous extracts of the areca nut against selected oral microorganisms, it was found that the hydrolysable tannins in the tannin fraction, which include tannic acid, are responsible for the antibacterial properties of the nut [Steven Y. C. *et al.*, 2015] [9]. Present study showed that areca nut extract was slightly more effective against *E. coli* in comparison to *Staphylococcus aureus*. Further, inhibition is increasing with increasing concentration of the extract to some extent (Table-1). Arecanut extract has earlier shown to be 100% inhibitory for gram negative and an positive organisms, however in our study inhibition ranged from 85-90%, which may be due to different test organisms, extraction method and final concentration of the extract in test solution (Anthikat and

Michael, 2009, Bhat *et al.*, 2017) [7, 12]. The aqueous extract of Areca catechu L. have been found to be effective for inhibiting the growth and propagation of several bacteria (Bhat *et al.*, 2017) [12]. Areca nut was also found to inhibit the growth and propagation of *Streptococcus mutans*, the bacteria that causes dental caries (Iwamoto *et al.*, 1991) [11]. However, the chewing of tobacco along with betel nut may be the major causative agent of oral cancer (Chang *et al.*, 2001) [14]. Areca nut has also been implicated as a cause of submucous fibrosis, which is probably due to decreased collagen metabolism (IARC, 2004, Bhat *et al.*, 2017) [13, 12]. Several investigators have demonstrated the antimicrobial activity of Areca nut aqueous and ethanolic extracts against bacterial pathogens (Boniface *et al.*, 2014, Bhatt *et al.*, 2017, Anthikat and Michael, 2009) [15, 12, 7]. Mycobacterium tuberculosis [Steven Y. C. *et al.*, 2015] [9] and oral microorganisms which are responsible for common bacterial diseases like skin infections, UTIs, peptic ulcer, tuberculosis, and dental caries. Areca nut has been known to reduce dental caries by possibly inhibiting gram positive microorganisms *Streptococcus mutans* that are responsible for dental caries. Findings of the present study and along with other earlier studies indicate possible use of betel nut chewing in skin infection treatment, oral hygiene and possible inhibition of dental caries and other bacterial and fungal infections. Results further suggest that areca nut extract could serve as potential source of bioactive compounds. More investigations are needed to exploit full potential of areca nut for pharmaceutical use.

### Acknowledgement

Financial assistance under All India Coordinated Research Project, CIWA, Bhuvneshwar and Central Agricultural University, Imphal to carry out the research is gratefully acknowledged.

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