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In vitro Antimicrobial Activity of Areca Nut Seed (*Areca catechu* Linn.) Against Pathogenic Bacteria

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***Areca catechu* Linn. possesses medicinal value, in particular, antimicrobial activity. The present study reveals the sensitivity of some pathogenic bacteria to areca nut extracts. In this study the antimicrobial activity of aqueous and methanol extracts of areca nut (seed of *Areca catechu* Linn.) against 7 species of pathogenic bacteria were investigated. Methanol extract inhibited the bacterial growth more effectively than the aqueous extract. Growth of *Klebsiella* sp., *Bacillus* sp., *Salmonella typhi* and *Staphylococcus aureus* were inhibited by the methanol extracts of areca nut. Aqueous extract alone inhibited the growth of *Salmonella paratyphi*. *Proteus* and *E. coli* were resistant to both the extracts.**

Key words: *Areca catechu*, areca nut, antimicrobial, antibacterial, Disc diffusion, methanol extract, aqueous extract.

Plants are potential sources of medicines. The potential of higher plants as source for relief from illness can be traced back to over five millennia to the written documents of early civilization in China, India and the nearest. Since ancient times majority of the population depend on traditional medical practitioners for their medicinal needs, who often employed plant extracts, essential oils or other such natural products for treatment. However the potential of higher plants as source for new drugs is still largely unexplored. Pharmacological screening of compounds of natural origin is highly essential in identifying novel therapeutic agents that aid in the treatment of pathogens, in particular, microorganisms. Oral bacteria have been showing increased resistance to most of the antibiotics that are therapeutically used in the treatment of oral infections. This increase in resistance and adverse effects has led scientists to explore antimicrobial herbal compounds which could be used for effective treatment of oral diseases[1-5].

Areca catechu Linn. (Palmae, Arecaceae) commonly known as Betel Palm or Betel Nut tree is grown in India, Malaysia, Taiwan and many other Asian countries for their economically important seed crop[6]. It is a medium sized tree; stem grows to a height of 20 m and leaves are 1.5-2 m long, pinnate and crowded with leaflets. The seeds of *Areca catechu* Linn., also known as areca nut, are used extensively as masticator either alone or in combination with betel leaves. The chemical composition of areca nut (seed of *Areca catechu* Linn.) is composed of alkaloids belonging to the pyridine group, the most important one being arecoline. Other alkaloids include arecaidine, arecolidine, guvacine, guvacoline, isoguvacine and

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norarecaidine and norarecoline. The active principle responsible for toxicity in *A. actechu* is the alkaloid arecoline[6]. Aqueous extracts of the nuts are used as vasoconstrictor and adrenaline potentitisor in rats[6-9].

The entire parts of the plant have been potentially explored for medicinal properties. Areca nut is used in veterinary medicine as vermifuge for tape worms. Roots of the plant have been used for liver problems; leaves for combating cough and bronchial trouble. Juice of tender leaves, mixed with oil, is applied in lumbago. Young green shoots induce abortion in early pregnancy. Besides its use as de-obstruent in flatulence and dropsy bark of the plant is also employed for choleric infection. The plant has been employed in the treatment of microbial diseases such as rhagades, small pox, venereal sores, syphilis, cholera and dysentery and also for fractured bones[6,7].

Chewing the areca nut with betel leaves is a traditional habit in several of the Asian countries, including India. Areca nut is a mild psychoactive material and also an aphrodisiac[10]. Indians have less dental caries than the Western population, possibly due to the antibacterial activity against gram positive bacteria that are responsible for dental caries. The antibacterial activity of ethanolic extracts of *A. catechu* against *Helicobacter pylori* has been reported by Wang and Huang[11]. Areca nut is also antiviral, with activity against HIV reported by Kusumoto *et al.*[12]. Use of areca nut is associated with incidence of many a type of oral diseases, most importantly oral cancer. Areca nut habits play a crucial role in development of oral cancer has already been correlated to by various researchers[13-17]. Catechin, an ingredient in areca nut, is a chemo-preventive agent against several tumors in animals[18, 19].

In view of the potential of seeds of *Areca catechu* Linn. as a reservoir for exploration of new drugs in the battle of diseases, this study was aimed at investigating the antimicrobial action of extracts of areca nut against some important pathogenic bacteria.

MATERIAL AND METHODS

Collection of Sample

Areca nut was collected in a sterile screw capped bottle (100 ml) from Ettumanoor, Kerala, India. Attempts were made to study the antimicrobial activity of aqueous and methanol extracts of areca nut.

Bacterial Cultures

For antimicrobial assay, seven bacterial strains namely *Klebsiella* sp., *Bacillus* sp., *Salmonella typhi*, *Salmonella paratyphi, Staphylococcus aureus, Escherichia coli,* and *Proteus* sp. were used. Bacterial cultures used in this study were obtained from the culture collections of School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India.

Preparation of Extracts

Freshly collected sample of areca nut was cut into small pieces and oven dried at 60 °C for 2 days. The dried samples were ground well into a fine powder with a sterile mortar and pestle. The powder was stored in air sealed containers at room temperature before extraction. The method of Alade and Irobi[20] was adopted for preparation of mace extracts. A fixed weight (5 g) of powdered material was soaked separately in 10 ml of sterile distilled water or methanol for 48 h. Each mixture was stirred at 8 h interval using a sterile glass rod. After 48 h contents were transferred into sterile mortar and ground well using a pestle. Contents were transferred into a sterile container and added water or methanol. The sample was extracted by boiling for 15 min. At the end of the extraction, each extract was passed through Whatman No. 1 filter paper. The filtrate was transferred into a screw capped bottle and stored at 4 °C till use.

Preparation of Bacterial Culture Suspension

Pure isolated colonies of bacterial strains were inoculated into 0.1 % peptone water and inoculated at 37 °C for 48 h. After incubation these culture suspensions were used for lawn culture preparation on Mueller-Hinton Agar (MHA, HiMedia) plates.

Preparation of Discs

The sensitivity disc of 5 mm diameter were punched off from the Whatman No.1 filter paper and sterilized in a water-proof container by autoclaving at 15 lb pressure for 15 min. The sterile discs were soaked in the extract for 10 min and allowed to dry. These discs were used for the antimicrobial assay. Discs coated with methanol and sterile water was used as controls.

Disc Diffusion Assay

The antimicrobial activity was tested

against aqueous and methanol extracts of areca nut. About 15 to 20 ml of Muller-Hinton Agar was poured in the sterilized Petri dishes and allowed to solidify. About 0.2 ml of 48 h old liquid culture of the strains used in this study was spread onto the surface of solidified agar plate using sterile cotton swab. The plates were allowed to dry for 5 min. For agar disc diffusion method, the discs carrying the respective extract were impregnated on the seeded agar plate (2 discs per plate). The plates were incubated at 37 °C for 48 h. The experiment was performed in duplicates.

Zone analysis

The antibacterial activity of the extracts against each bacterial strain was assayed by measuring the diameter of zone of inhibition of growth to the nearest mm. The results were recorded and compared.

RESULTS AND DISCUSSION

Besides its economic importance *Areca catechu* Linn. is also a plant of high medicinal value. Antibacterial and antifungal activity has already been demonstrated in the extracts of areca nut by previous reports[11, 21, 22]. In this study the antibacterial activity of water and methanol extracts of areca nut was assayed against seven species of pathogenic bacteria using disc diffusion technique. Methanol extract was comparatively more effective in inhibiting the growth of the bacterial strains studied (Table 1). *S. typhi* and *Bacillus* sp. exhibited wide zone of inhibition of growth against both the extracts of areca nut. Wide zones of diameters 8 mm and 12 mm, respectively, were produced around *Bacillus* sp. while employing aqueous and methanol extracts. For the same, *S. typhi* exhibited zones with diameters 7 and 13 mm, respectively. Inhibition of growth around discs impregnated with methanol extract alone produced inhibition zones for *S. aureus* and *Klebsiella*. The results of our study are in concordance with the results of Yang and Chou[22] who also observed the antibacterial action of ethanolic extracts of betel nut against *Salmonella*, *S. aureus* and other pathogenic bacteria such as *Neisseria* sp., *Yersinia enterocolitica*, and *Listeria monocytogenes*. Wang and Huang [11] has reported antibacterial activity of areca nut extracts against *H. pylori*.

Table 1. Antimicrobial activity of aqueous and methanol extracts of areca nut (seeds of *Areca catechu*) against pathogenic bacteria

Bacterial strains	Diameter of zone of inhibition (mm)	
	Aqueous Extract	Methanol Extract
E. coli	0	0
S. aureus	0	10
S. paratyphi	8	0
S. typhi	7	13
Klebsiella sp.	0	12
Proteus sp.	0	0
Bacillus sp.	8	12

In this study, *S. paratyphi* responded positively to water extract of areca nut with appreciable zone of growth inhibition of 8 mm diameter. However, no zone of growth inhibition was produced for *S. paratyphi* while using the methanol extract. Neither of the extracts was effective against *E. coli* and *Proteus*. On the contrary Anthikat and Michael [(23)] has reported that aqueous extracts of areca nut inhibited the growth of *E. coli* and *Proteus* along with other bacteria such as *Klebsiella, Salmonella typhimurium, Pseudomonas*, *Streptococcus mutans and Streptococcus viridans* which were also included in their study.

This study has provided more evidence into the potential of areca nut as a source of antimicrobial agents. Majority of the bioactive compounds in areca nut are still unexplored and their chemical nature and pharmacological effects unidentified. More work has to be carried out to determine the efficacy of areca nut to human diseases with respect to other natural products and novel drugs.

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