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# Pericarp of unripe areca fruit-a potent antimicrobial source

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## ABSTRACT

*Areca catechu* is grown for the important seed crop. The present study highlights the antimicrobial activity of steam distillates of pericarp of areca fruit. The pericarp of unripe areca fruits were collected, cut into small pieces and subjected to steam distillation. The steam distillate obtained was subjected to antibacterial activity against Gram positive and Gram negative bacteria in liquid media. Antifungal activity of steam distillate was carried employing poison food technique. Among bacteria tested, more inhibition was observed in case of *E. coli* followed by other bacteria. All tested fungi were found to be sensitive among which *A. terreus* was least affected. It can be concluded that antimicrobial activity of steam distillate is due to active constituents present in the distillate. The steam distillate could be employed in the treatment of infections caused by enteric bacteria, food poisoning bacteria and opportunistic fungi. Further experiments should be conducted in animal models to validate the activity *in vivo*.



## KEYWORDS

Areca catechu;
Antimicrobial activity;
Steam distillate;
Poison food technique;
Pericarp.

## INTRODUCTION

*Areca catechu* (betel nut) is a slender, single-trunked palm that can grow to 30 m (100 ft). It is cultivated from East Africa and the Arabian Peninsula across tropical Asia and Indonesia to the central Pacific and New Guinea. The nut is chewed as a stimulant masticatory by 5% of the world's population, making it more popular. Use of betel nut is often culturally or socially ritualized, and there are elaborate ceremonies attending its use in various Asian and Pacific cultures. In India and Pakistan, by comparison, betel nut is consumed in quantities greater than local production can supply, and it is imported in large quantities annually. Although not recommended for use due to health risks, the plant nevertheless has a long history of cultural importance in many parts of the world, and this will likely continue[1]. *Areca catechu* is grown for the important seed crop, the Betel nut. The fruit flesh on the seed has psychoactive properties (stimulating effects) and in South-east Asia is used as such by chewing on the fruit. It produces euphoria, heightened alertness, sweating, salivation, a hot sensation in the body and an increased capacity to work[2]. Areca fruit is a fibrous, ovoid drupe, 5-10 × 3-5 cm (2-4 × 1.2-2 in), yellow to orange or red when ripe; pericarp fibrous, ca. 6 mm thick. Seed usually 1, ovoid, globose, or ellipsoidal, 3-4 × 2-4 cm (1.2-1.6 × 0.8-1.6 in), base sometimes flattened[1].

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition[3]. Essential oils (volatile components) can be ex-

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tracted using a variety of methods. Currently, the most popular method for extraction is steam distillation in which water is heated to produce steam that carries the most volatile chemicals of the aromatic material with it. The steam is then chilled (in a condenser) and the resulting distillate is collected. The Essential Oil normally float on top of the Hydrosol (the distilled water component) and may be separated off [4].

The pericarp of any fruit is made up of three distinct layers namely the exocarp, the mesocarp and the endocarp. The pericarp of areca fruit is considered as a waste material and is often used only for burning process. No data is available on the antimicrobial activity of the pericarp of unripe areca fruit. In this study, we have prepared the steam distillate from the unripe areca fruit pericarp and scientifically explored the efficacy of steam distillate to inhibit bacteria and fungi.

## MATERIALS AND METHODS

### Steam distillation of areca pericarp

The distillation apparatus consists of a 1000ml distilling flask (to boil the mixture of plant material and water), a condenser (to condense the steam to obtain the steam distillate), and a receiving vessel. A known weight of (100 grams) small pieces of Areca pericarp was subjected to steam distillation in the assembly. When heated up, the plant cells release their components and some of them are volatilized and carried by the steam. The volatile components were collected into the receiving flask during 3 hours of steam distillation[5,6].

### Antibacterial activity of areca pericarp distillate

Gram positive bacteria namely *Bacillus subtilis, Staphylococcus aureus* and Gram negative bacteria namely *Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* were used as test bacteria. The antibacterial activity of steam distillates was tested in liquid nutrient media with minor modifications[7]. The sterile nutrient broth tubes containing steam distillate of Areca pericarp were inoculated with standardized volumes of 24 hours old broth cultures of test bacteria followed by incubation at 37$^0$C for 24 hours. After incubation, the optical density was measured at a wavelength of 560 nm as a guide to microbial growth. The experiment was performed in three trials and each set included a control broth without distillate.

**TABLE 1: Antibacterial activity of Areca pericarp distillate**

| Treatment | Optical density at 560nm | | | | |
|---|---|---|---|---|---|
| | *E.coli* | *B.subtilis* | *S.aureus* | *K.pneumoniae* | *P.aeruginosa* |
| Control | 0.606 | 0.208 | 0.688 | 0.525 | 0.489 |
| Areca pericarp distillate | 0.313 (48.34) | 0.149 (28.36) | 0.558 (18.89) | 0.433 (17.52) | 0.410 (16.15) |

**Values within parentheses- % inhibition of test bacteria**

**TABLE 2: Antifungal activity of areca pericarp distillate**

| Treatment | Average colony diameter in cm | | | |
|---|---|---|---|---|
| | *A.niger* | *A.oryzae* | *A.nidulans* | *A.terreus* |
| Control | 4.3 | 3.8 | 3.7 | 2.6 |
| Areca pericarp distillate | 3.1 (27.90) | 2.7 (28.94) | 2.7 (27.02) | 2.1 (19.23) |

**Values within parentheses- % inhibition of test fungi**

### Antifungal activity of Areca pericarp distillate

Four species of the genus *Aspergillus* namely *A.niger*, *A.oryzae*, *A.nidulans* and *A.terreus* were selected as target fungi, some of which are known to cause opportunistic mycotic infections in susceptible individuals. The suspension of spores of the test fungi was prepared in a test tube containing 0.85% sterile normal saline containing 0.01% Tween 80 detergent[8]. The antifungal activity was assessed using Poison food technique[9]. The test fungi were inoculated on Sabouraud's dextrose agar plates poisoned with Areca pericarp distillate (10% concentration) by point inoculation. The effect of the distillate on fungal growth was determined by measuring the diameter of the colony obtained on poisoned plate and comparing with control.

## RESULTS AND DISCUSSION

Results of antibacterial activity of steam distillate of areca pericarp revealed the susceptibility of all bacteria tested (TABLE 1). Among bacteria tested, *E.coli* was found to be more inhibited (48.34%) followed by *B.subtilis* (28.36%), *S.aureus* (18.89%), *K.pneumoniae* (17.52%) and *P.aeruginosa* (16.15%). Among bacteria, Gram positive bacteria were found to be more susceptible than Gram negative bacteria, except in case of *E.coli*. TABLE 2 shows antifungal activity of areca pericarp distillate. A reduction in colony diameter of test fungi was observed in plates poisoned with steam distillate. All the species of *Aspergillus* were found to

be susceptible to the distillate. Among fungi tested, *A.oryzae* was found to be more inhibited followed by others. Least inhibition was observed in case of *A.terreus*.

Information about antimicrobial activity of areca is quite lacking. No report is available on the antimicrobial activity of areca pericarp distillate. Thus, the present study becomes relevant and the results are suggestive that the distillate contains active constituents of pericarp which can be exploited against pathogenic microbes. Only limited literatures are avaialbe on antimicrobial activity of betel nut. Tannic acid, found in the areca nut extract, at higher concentrations inhibited the growth of *E. corrodens*, *P.gingivalis*, *C.rectus* and *F.nucleatum*[10]. It was found that the hydrolysable tannins in the tannin fraction, 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[11]. The phenolics in *A. catechu* Linn. were mainly distributed in root followed by fresh unripe fruit, leaf, spike, and vein, while the contents of alkaloids were in the order of root, fresh unripe fruit, spike, leaf, and vein. Tender shoot, the upper young stem of the tree contained a small amount of total phenolics, condensed tannin, and total alkaloids[12]. The phenolics in whole fresh areca fruit involved condensed tannins (92 mg/g of dry wt), hydrolyzable tannins (69 mg/g of dry wt), non-tannin flavans (84 mg/g of dry wt) and simple phenolics (56 mg/g of dry wt). The crude phenolic extract of areca fruit and its two separated fractions exhibited marked antioxidative activity and an antimutagenic effect on 2-amino-3-methylimidazo[4,5-*f*]quinoline toward *Salmonella typhimurium* TA98 and TA100, did not induce chromosomal aberration, and increased the frequency of sister chromatid exchange (SCE) in CHO-K1 cells[13].

## CONCLUSION

The results of the study revealed the potential of steam distillate of areca pericarp being considered as waste generated during harvesting and processing of areca nuts. The distillate could be used against bacteria causing food poisoning as *E.coli* and *S.aureus*, the causative agents of food poisoning, were found to be inhibited. As the study made use of enteric bacteria, the distillate may also be employed to treat infections caused by enteric bacteria. As inhibition of *Aspergillus* species was observed, the distillate could also be used against opportunistic mycotic infections. Further experiments are needed to recover active constituents from steam distillate and validate antimicrobial activity of purified constituents in animal models i.e. *in vivo*.

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