ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In-vitro Antibacterial and Antifungal Effect of Areca Nut Extract

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ABSTRACT

Areca nut is the seed of the areca palm (*Areca catechu*). Several author demonstrated the antimicrobial activity of Areca nut aqueous extracts against bacterial pathogens like MRSA, *H. pylori*, and oral microorganisms. The aim of this study was to evaluate the antibacterial and antifungal properties of the areca nut *in vitro* using isolated organisms. A variety of human bacterial isolates, both gram positive and gram negative, were tested against areca nut extract by measuring the growth of the organisms using the Disk diffusion method. Antifungal activity of areca nut extract was also tested against unicellular yeast fungus *Candida albicans* using tube method. It was found that both gram negative and gram positive organisms were susceptible to the areca nut extract. The concentration needed for 100% inhibition of growth was found to be 3.3-7 µg/ml for gram negative organisms and 16 µg/ml for gram positive bacteria. The extract was also found to inhibit the growth of *Candida albicans* at a concentration of 16 µg/ml. It can be concluded from our results that areca nut extract plays an important role as antibacterial and antifungal activity due to the presence of bioactive chemical compounds. Our results suggest that areca nut extract could serve as potential source of bioactive compounds. Further research is needed in which the extract could possibly be exploited for pharmaceutical use.

Keywords: *In-vitro,* Antibacterial, Antifungal, Areca Nut Extract

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ISSN: 0975-8585

INTRODUCTION

Infection is the major cause of death in developing countries. 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 [1]. In addition, bacteria have been reported to show increased resistance towards common antibiotics which have been used therapeutically for the treatment of infectious diseases [2, 3].

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 [4]. Approximately 80% of the population in developing countries still use traditional medicines for their health care [5]. The natural products derived from medicinal plants are known to produce biologically active compounds [4, 6].

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 [7, 8, 9, 10, 11, 12]. Areca nut has been known to reduce dental caries by possibly inhibiting gram positive microorganisms that are responsible for dental caries [13].

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 [9] 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 and antifungal properties of the areca nut extract *in vitro* using isolated microorganisms.

MATERIALS AND METHODS

Bacterial strains

Local bacterial isolates used in this study are listed in **Table 1.** The bacterial strains were activated and cloned three successive times in nutrient agar and stored on nutrient agar slants at 4°C. The identification of the local bacterial isolates was confirmed using conventional biochemical tests [14].

Table 1: Bacterial and fungal isolates used in the study

Bacterial isolate	Source
Escherichia coli Central health lab,	Baghdad
Streptococcus pyogenes	Babylon University/ College of Medicine
Pseudomonas aeruginosa	Central health lab, Baghdad
Staphylococcus aureus	Kufa University/ College of science
Candida albicans	Isolated in this study

Isolation and identification of *Candida albicans*

C. albicans isolates were recovered from women with vaginitis attended to Marjan hospital, Hilla, Iraq. Swabs were taken from patient by using sterile cotton swabs with transport media. The samples were cultured on Sabouraud dextrose agar supplemented with chloramphenicol to prevent bacterial contamination and incubated at 37°C. The fungal culture was examined according to colonies, cellular morphology and germ tube formation [14].

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 1hour. This extract was then concentrated by evaporation. The yield of the extract was found to be 6.4%. The extract was then re-suspended in distilled water and diluted to the desired concentration.

Disk diffusion method

Antimicrobial activity test was determined by using Mueller–Hinton agar plates and carried out according to the protocol described by [15] as follows: The overnight bacterial cultures grown on Mueller-Hinton broth (Oxoid, UK) were adjusted to the density of 0.5 McFarland turbidity standard with a DENSIMAT (Biomérieux). The inocula of the tested bacteria were streaked on to Mueller-Hinton agar (Oxoid, UK) plates using a sterile swab. Sterile filter discs (diameter 6 mm) (Whatman Paper No. 1, England) were impregnated with the areca extract nut in different concentration (62.5, 125, 250, and 500 mg/ml) placed on the appropriate agar medium (Oxoid. UK). Distilled water (20 µl/disc) were used as negative control. After incubation at 37 °C for (18-24) h, the diameter of the inhibition zone was measured [16]. The diameter of the zones of inhibition around each of the discs was taken as measure of the antimicrobial activity. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

Antifungal activity of areca nut extract

The tube method was used against unicellular yeast fungus *Candida albicans*. Areca nut extract inhibited the growth of *C.albicans* and the concentration needed for100% inhibition was found to be 16.67 µg/ml.

Statistical analysis

Values are expressed as the mean ± SD. Two-tailed unpaired Student's *t*-test, and ANOVA-One way were used to compare means. Bonferroni test was used for statistical analysis to show if there is any significant differences at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$.

RESULTS

Antibacterial activity of areca nut extract

The Areca nut extract screened for antibacterial activity against Gram's positive bacteria *Staphylococcus aureus*, *and Streptococcus pyogenes*. The Areca nut extract showed antimicrobial activity against these bacteria in different concentrations (Figure-1). The same results demonstrated against Gram negative bacteria *E. coli*. However, the extract showed no antimicrobial activity against *P. areuginosa* (Figure-2).

Antifungal activity of areca nut extract

Results of this study showed that areca nut extract inhibited the growth of *C.albicans* and the concentration needed for100% inhibition was found to be 16.67 µg/ml.

DISCUSSION

Antibacterial effects of aqueous extracts of areca nut were examined against representative bacterial isolates of Gram positive and negative bacteria (*S. aureus* and *E. coli*). The results demonstrated that the bioactivity of water-based extracts against *S. aureus* and *E. coli* may be related to the chemical structure of the active substances. In an investigation of the antibacterial properties of aqueous extracts of the areca nut against selected oral microorganisms found that the hydrolysable tannins in the tannin fraction, which include tannic acid, are responsible for the antibacterial properties of the nut [9].

Several author demonstrated the antimicrobial activity of Areca nut aqueous and ethanolic extracts against bacterial pathogens like MRSA [7], *H. pylori* [8], *Mycobacterium tuberculosis* [11], and oral

ISSN: 0975-8585

microorganisms [9, 17]which are responsible for common bacterial diseases like skin infections, UTIs, peptic ulcer, tuberculosis, and dental caries. areca nut has been knownto reduce dental caries by possibly inhibiting gram positivemicroorganisms *Streptococcus mutans* that are responsible for dental caries. [17].

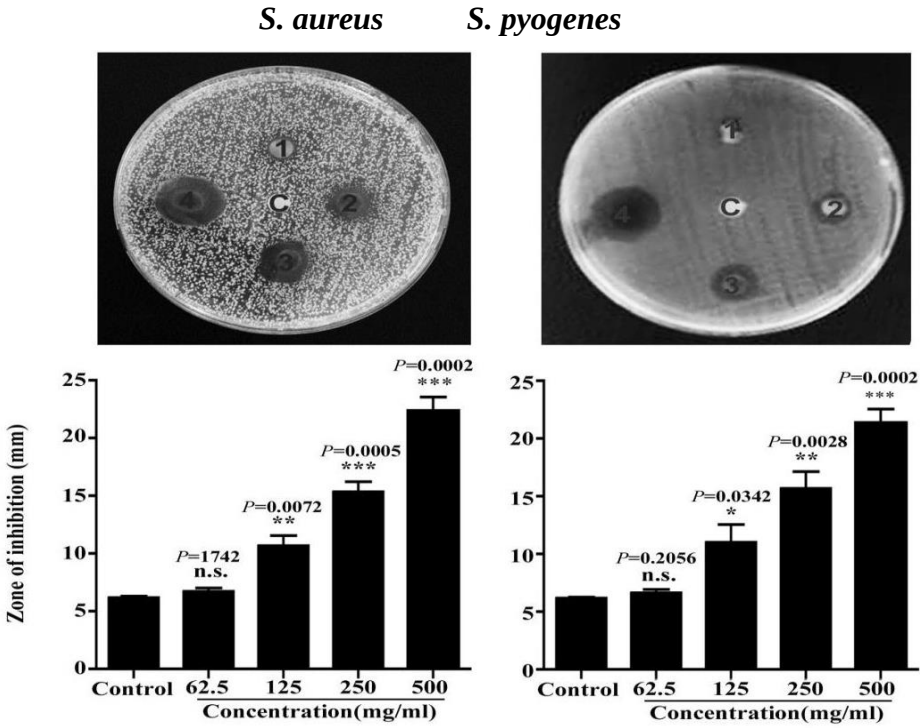

Figure 1: Antimicrobial activity of Areca nut extract against Gram positive bacteria. C= Control (D.W.), 1= 62.5mg/ml, 2=125mg/ml, 3=250mg/ml, 4=500mg/ml of nut extract. Asterisks indicate statistically different from control (D.W). Columns are mean of triplicate determination; error bar are SEM. * *P* <0.05, ** P<0.01, *** P<0.001.

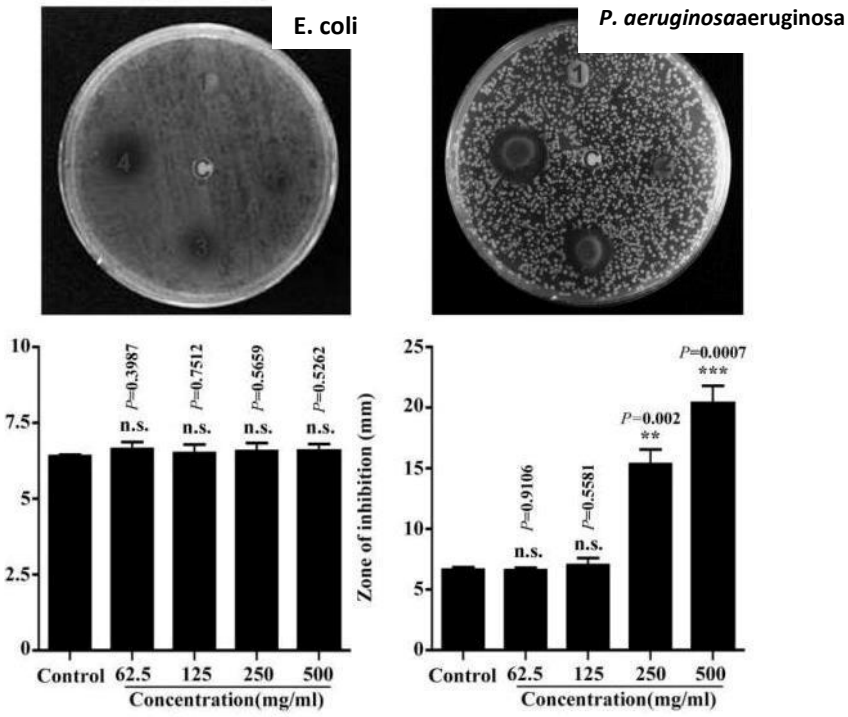

Figure 2: Antimicrobial activity of Areca nut extract against Gram negative bacteria. C= Control (D.W.), 1= 62.5mg/ml, 2=125mg/ml, 3=250mg/ml, 4=500mg/ml of nut extract. Asterisks indicate statistically different from control (D.W). Columns are mean of triplicate determination; error bar are SEM. * *P* <0.05, ** P<0.01, *** P<0.001.

The inability of Areca nut extract to show antimicrobial activity against *P. areuginosa* was expected since the majority of *P. Aeruginosa* strains are resistant to most of antibacterial agents, and this bacterial pathogen considered as one of the major problems in many hospitals. Its high rates for developing resistance against most of the antimicrobials agents initiate a great need for finding other alternative medicine towered it [18].

Results of this study showed that areca nut extract inhibited the growth of *C.albicans* and the concentration needed for100% inhibition was found to be 16.67 µg/ml. Similar findings were reported by other workers [10, 19].The areca nut extract was also found to inhibit the growth of *Candida albicans* at a concentration of 16 µg/ml and inhibited aflatoxin production by *Aspergillus flavus* [10].Therapeutic agents with high killing propensity for fungi are few. The reason why yeast is more susceptible to the extracts than bacteria is unclear but it may be that at any given time, these oils may break up the structural integrity of *C. albicans* faster than they dissociate bacteria.

CONCLUSIONS

These data suggest that Areca nut extract could inhibit the growth of *S. aureus*, *and St. pyogenes*, and *E. coliin vitro*and this activity may contribute to its chemopreventive effect.It also can be concluded from our results that areca nut extract plays an important role as antibacterial and antifungal activity due to the presence of bioactive chemical compounds. Our results suggest that areca nut extract could serve as potential source of bioactive compounds. Further research is needed in which the extract could possibly be exploited for pharmaceutical use.

ACKNOWLEDGEMENTS

The author is thankful to Department of Pharmacology, College of Medicine, Babylon University, Iraq, for the facilities provided in the completion of the work. The author also thankful to Prof. Alaa Al-Charrakh/Babylon University, for critical review and checking of the manuscript.

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