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Extraction of *Areca catechu* L. fruits in methanol and water to check its antimicrobial property

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Abstract

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against microbial strains. In this study, methanol and aqueous extracts of *Areca catechu* were tested for their antimicrobial activity against *E. coli*, *B. subtilis*, *Enterobacter* and *P. chrysogenum*. Extraction was done by cold percolation method. Extracts were diluted by serial dilution. Minimum inhibitory concentration (MIC) was measured by using spectrophotometer (OD at 600 nm). Methanol extract was active against *E. coli*, *B. subtilis* and *P. chrysogenum*. Aqueous had antimicrobial potential against *B. subtilis* and *P. chrysogenum*. *Areca catechu* could be a possible source to obtain new and effective herbal medicines to treat infections caused by microorganisms.

Keywords: Antimicrobial, *Areca catechu*, minimum inhibitory concentration (mic)

Introduction

Medicinal plants have been discovered and used in traditional medicine practices since ancient times. Plants synthesise several of chemical compounds that is used for treatment of many diseases. WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants. The use of phytochemicals as natural antimicrobial agents commonly called "biocides" is gaining popularity as alternative to antibiotics. Phytochemicals and pharmacological study of medicinal plants is growing interest in recent studies [1].

Areca catechu L. (Palmae, Arecaceae) commonly known as Betel palm or Betel nut tree is a species of palm. *Areca* palms are growing in India, Malaysia, Taiwan and many other Asian countries for their economically important seed crop. *Areca catechu* L. is an important economical plant in tropical and subtropical areas. Its ripe fruit is widely used in traditional Chinese medicine for treatment of constipation, oedema, beriberi and dyspepsia. Pharmacological research have shown areca nut possesses psychoactive [2], anti-depressant [3], anti-melanogenesis [4], anti-inflammatory [5], Hepatoprotective [6], anti-oxidant [7], anti-tumor [8] and cytotoxic activities [9].

Considering the vast potentiality of plants as sources for natural drug, this study aimed to investigate *in vitro* antimicrobial activity of extracts from *Areca catechu* L. against four microorganisms.

Materials and Method

Plant materials

Plant dried fruit were collected randomly from Surat, Gujarat, India. Plant dried fruit were washed in tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Extraction

Extraction was done by cold percolation method [10, 11]. 10 g of air-dried powder was taken in 100 ml of distilled water and 100 ml methanol in a conical flask. The flask was boiled for 10 min and kept it for 24 h at room temperature in shaking condition. After 24 h, extract solution was taken out in to glass petri plates (put the plates at room temp for air dry and allow it to

completely dry). The dry extract was weighed and extractive yield was calculated by following formula.

Extractive yield = (weight of dry extract/ 10 g) X 100
Extract was preserved in refrigerator until use.

Microbial strains

The standard microorganisms were used for antimicrobial study. The bacterial strains were grown in the nutrient broth and maintained on nutrient agar slants at 4° C. The following bacterial strains were used for this study: *Enterobacter aerogenes* ATCC13048, *E. coli* ATCC25922, *B. subtilis* ATCC6633 and *P. chrysogenum* ATCC10108.

Antimicrobial study

Antimicrobial study was determined by broth dilution method. Microbial culture was inoculated in N broth and incubated for 12h at 37°C. Plant extract was dissolved in dimethyl sulphoxide and prepared it in six different concentrations (5, 2.5, 1.25, 0.625, 0.312, 0.156 mg/ml). Dimethyl sulphoxide was used as control. Total seven tubes were prepared for each microbial strain per one plant extract. Tube 1 to 6 was used as test and tube 7 was used as control. 0.9 ml N broth was added in each test tube and 0.1 ml extract was added at increasing concentration in each tube. All tubes were incubated overnight. Microbial growth was observed by using spectrophotometer (OD at 600 nm).



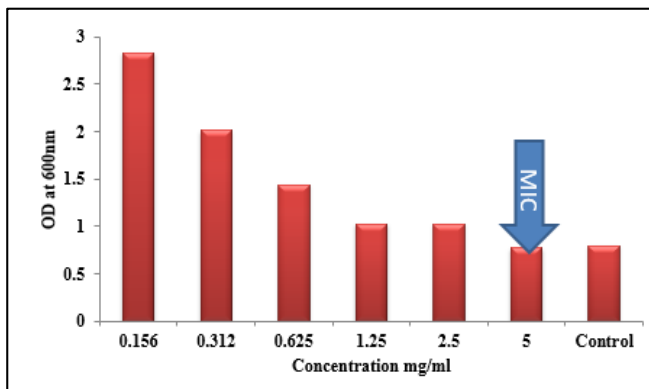
Fig 1: Extraction of *A. catechu* in methanol and water

Results and Discussion

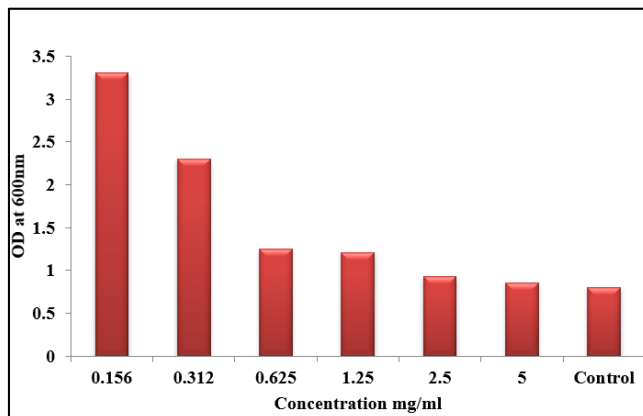
Scientists from different sectors are investigating plants for their antimicrobial usefulness. Studies have found thousands of plants which have inhibitory effects on a range of microorganisms *in vitro*. Surprisingly, only around 10% of all the plants have been investigated in this purpose. In this study, it was observed and compared the antimicrobial activity among methanol and water extract of *A. catechu* nut. Methanol extract gave the higher extractive yield (4.52g/10g powder) than water extract (2.37g/10g powder). Graphs 1-6 summarize the results obtained from this study.

Graph 1 represents antimicrobial study of methanol extract of *Areca catechu* against *E. coli*. Methanol extract showed antibacterial activity against *E. coli* with the minimum

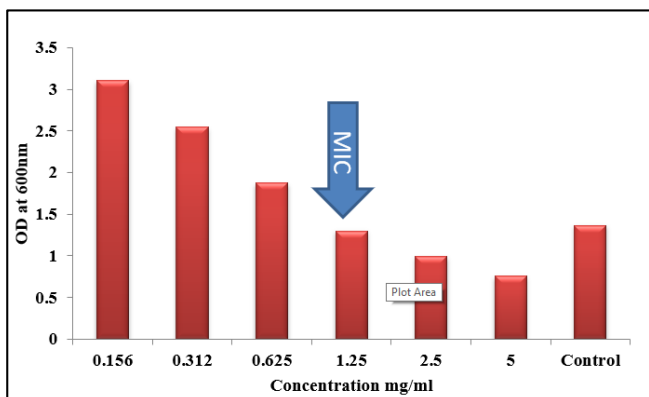
inhibitory concentration (MIC) value 5mg/ml (Graph 1). It also had antibacterial potential against *B. subtilis* with MIC value 1.25mg/ml (Graph 2). It was not effective against *Enterobacter* (Graph 3) but it was effective against fungal strain *P. chrysogenum* with MIC value 5 mg/ml (Graph 4). Aqueous extract of *Areca catechu* was active against *B. subtilis* (Graph 6) and *P. chrysogenum* (Graph 8) with MIC values 5mg/ml and 1.25mg/ml respectively. It was not active against *E. coli* (Graph 5) and *Enterobacter* (Graph 7). Methanol extract was more active against bacterial strains while water extract was more active against fungal strain.



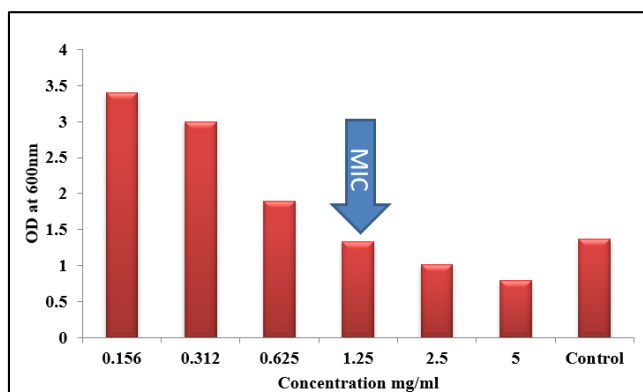
Graph 1: Antimicrobial activity of methanol extract of *Areca catechu* against *E. coli*



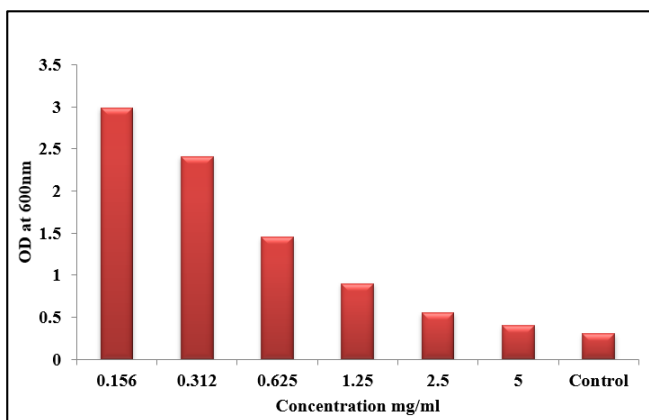
Graph 5: Antimicrobial activity of aqueous extract of *Areca catechu* against *E. coli*



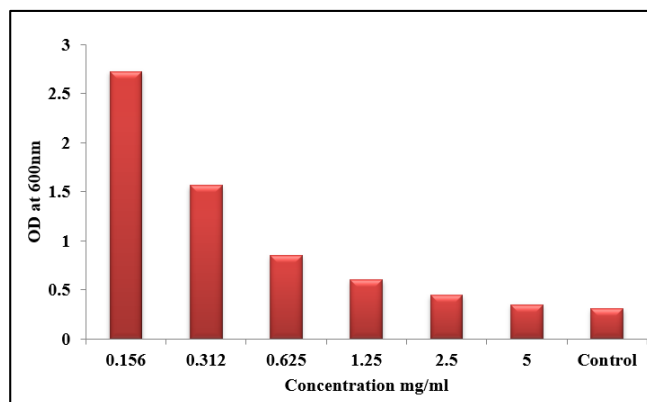
Graph 2: Antimicrobial activity of methanol extract of *Areca catechu* against *B. subtilis*



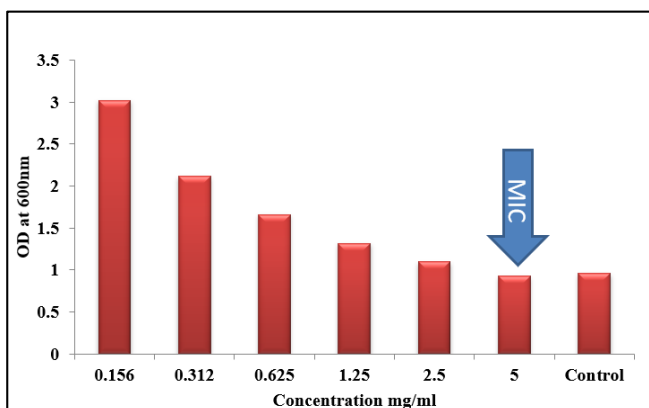
Graph 6: Antimicrobial activity of aqueous extract of *Areca catechu* against *B. subtilis*



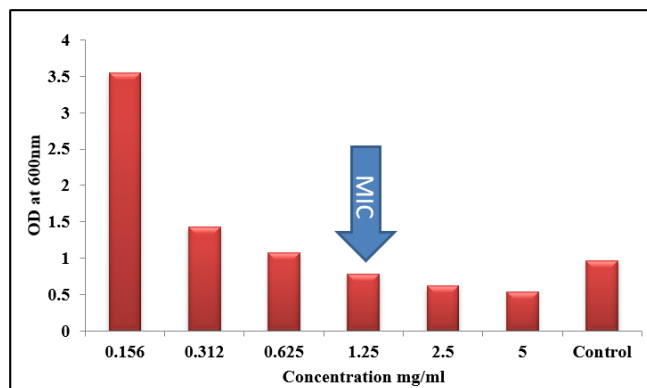
Graph 3: Antimicrobial activity of methanol extract of *Areca catechu* against *Enterobacter*



Graph 7: Antimicrobial activity of aqueous extract of *Areca catechu* against *Enterobacter*



Graph 4: Antimicrobial activity of methanol extract of *Areca catechu* against *P. chrysogenum*



Graph 8: Antimicrobial activity of aqueous extract of *Areca catechu* against *P. chrysogenum*

Conclusion

Areca catechu nut extract showed good activity against microbes. To determine the highest efficacy and optimum concentration of *A. catechu* nut extract as antibacterial drug, more investigation is needed using purified components with different other solvents in various doses.

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