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A Study on the Phytochemical Analysis, Silver Nanoparticle Synthesis and Antibacterial Activity from Seed Extract of *Areca catechu* L.

P. Rama Bhat[1*], V. H. Savitri[1], P. G. Laxmi[1] and E. P. Jenitta[1]

[1]Department of Biotechnology, Alva's College, Moodbidri – 574 227, Karnataka, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author PRB designed the study, supervised the work, managed the analyses of the study, wrote the first draft of the manuscript and edited the manuscript. Authors VHS and PGL carried out all laboratory work. Author EPJ managed the practical work, wrote the protocol and the literature search. All authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out to screen phytochemicals like carbohydrates, terpenoids, resins, saponins, tannins and alkaloids in the seed extract of *Areca catechu* by standard protocols and antibacterial activities of seed extract as well as silver nanoparticles prepared from seed extracts. The characterization of silver nanoparticles was studied by using FTIR, SEM and UV- Visible spectrophotometer. Antibacterial activity was done with aqueous, methanolic and $AgNO_3$ seed extracts against five bacterial species viz., *Salmonella typhi, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis* by agar well diffusion method. The seed extract showed positive results for carbohydrate, terpenoids, tannins and saponins. The colour of the solution after treating with $AgNO_3$ was changed from light brown to dark brown confirmed the reduction of silver ion in presence of plant extract and formation of silver nanoparticles. Maximum absorption was observed at 400 nm and the size of silver nanoparticles

**Corresponding author: E-mail: bhat_pr@rediffmail.com;*

produced was oval in shape with the diameter of 553-610 nm. *Pseudomonas aeruginosa* was found to be the good organism resistant against areca nut seed extracts while others were showed intermediate effects.

Keywords: Areca nut; silver nanoparticles; antibacterial activity.

1. INTRODUCTION

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent against several disease, minor side effects and economic viability. Several compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic [1,2]. Recently, natural plants have received much attention as sources of biological active substances including antioxidants. Numerous studies have been carried out on some plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids which prevent free radical damage, and reducing risk of chronic diseases [3].

The development of antibiotic resistant bacteria and the toxicity caused by prolonged drug treatment has led to the wide use of medicinal plants by the traditional medical practitioners for curing various diseases in their day to day practice [4]. Indians have long been using many of the herbal products and formulations to counter the microbial activity on open wounds and skin infections well before the inventions of antibiotics.

Now-a-days, nanotechnology has grown to be an important research field in all areas including medicinal chemistry. It is expected to revolutionize both science and society. There is growing need to develop eco-friendly and body benign nanoparticles process without use of toxic chemicals in the synthesis to avoid adverse effects in the biochemical applications [5]. Synthesis and characterization of nanoparticles is an important area of research as selection of size and shape of nanoparticles provide an efficient control over many of the physical and chemical properties.

Nanoparticles are synthesized using plant materials. The various phytochemicals present within the plant result in effective reduction of silver salts to nanoparticles [6,7]. The synthesized silver nanoparticles were used effectively against multidrug resistant bacteria. It has reported that silver nanoparticles are nontoxic to humans and most effective against bacteria, virus and other eukaryotic micro-organisms at low concentrations and without any side effects [8,9].

Research on such plants can provide useful information for their exploitation in treating diseases. Areca nut (*Areca catechu*) is one such plant which has been used in different system of traditional medication for the treatment of diseases and aliment of human being. Areca nut is used for treatment of a mental disorder called schizophrenia and eye disorder called glaucoma; as a mild stimulant and as a digestive aid and also some people used as recreational drug because it spreads central nervous system. Areca nut contains main biochemical compounds like polyphenols, fat, starch and alkaloids [10-12]. It cleanse the mouth, impart a sweet aroma to it, enhance its beauty and cleanse and strengthen the voice, tongue and teeth, the jaws and the sense organs [13]. Areca was touted as a medicine for digestive and dental health and also used to facilitate bowel movements and reduce intestinal worms [14-16,1,17].

Areca catechu L. belongs to the family Arecaceae or Palmae. The areca nut palm is cultivated as cash crop or plantation crop (Plate 1). It is the fourth most commonly used social drug, ranking after nicotine, ethanol and caffeine. It has been mentioned in the Sanskrit manuscripts and used as food, medicine, social and religious purposes. The palm is believed to have originated in the Philippines, but is widespread in cultivation for their seeds and is considered naturalized in southern China, Taiwan, India, Sri Lanka, Cambodia, Laos, Thailand, Vietnam, Malaysia, Indonesia, Bangladesh, Maldives, New Guinea, many of the islands in the Pacific Ocean, and also in the West Indies.

The aim of the present study was to carry out the phytochemical analysis, antimicrobial activity and synthesis of silver nanoparticles from the seeds of *Areca catechu*.

Plate 1. Areca nut plant bearing fruits in bunches and dehusked dried seed – chali

2. MATERIALS AND METHODS

2.1 Preparation of Extract from the Fruit Parts of the Collected Plant

The fruits from selected plant were collected from Hebri, Udupi District, and Karnataka. They were then dried, dehusked, cleaned by washing several times with deionized water and allowed it to shade dry for 7-8 days. Then it was kept in hot air oven at 60℃ for 24- 48 hours until it was dried completely. The dried seeds were powered.

2.2 Preparation of Aqueous and Methanol Extract

Five gram of plant sample was taken in a conical flask with 50 ml of distilled water to get aqueous and 25 ml of distilled water and 25 ml of methanol to get methanol extract of the sample taken separately, mixed and covered with aluminum foil. Then it was kept in water bath at a temperature of 50℃ - 60℃ for 7 -8 hours, the mixture in the flask was allowed to pass through Whatman filter paper no. 1 and filtrate was put in a clean weighed china dish. This was kept in water bath open, till all the filtrate evaporated leaving behind a film of plant residue. The china dish was weighed before and after the evaporation of the filtrate, to know that how much residue was obtained. The extract was stored in screwed tubes which is stored at -20℃ to prevent the loss of bioactive compounds until further use.

2.3 Phytochemical Screening of the Plant Extract

Phytochemical tests for carbohydrates, saponins, tannins, alkaloids, terpenoids and resins were carried out on the aqueous and methanol extracts reconstituted in respective solvents using standard protocols [18,19].

2.4 Synthesis of Silver Nanoparticles

Fruit parts were collected and air dried for 2-3 days and then kept in the hot air oven at 60℃ for 24-48 hours until it is dried completely. The dried sample was grounded to fine powder using mortar and pestle. For the synthesis of silver nanoparticles, 1 Mm aqueous extract of silver nitrate was prepared; 3 g of fruit powder was mixed with 350 ml of silver nitrate solution and centrifuged at 2000 rpm for 30 minutes. The supernatant were collected and heated at 95℃. A change in the colour of the solution was observed after heating the mixture for 10 minutes. The extracts were stored at 4℃ for further use.

2.5 Characterization

The reduction of pure Ag^{+} ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer. Fourier Transform Infrared Spectroscopy (FTIR) was used to recognize the functional groups bound to the silver surface and involved in the formation of silver nanoparticles. The lyophilized powder sample was examined by Infrared (IR) spectrum at the spectral range of 1000-4000 cm^{-1}. Scanning Electron Microscopic (SEM) analysis of silver nanoparticles was done using SEM machine. Thin films of the sample was prepared on a carbon coated copper grid by dropping a very small amount of the sample on

the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 minutes.

2.6 Antimicrobial Screening of the Crude Extracts

The bacterial strains used for present investigation are *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Bacillus subtilis, Staphylococcus aureus* and *Salmonella typhi* were obtained from P.G Department of Biotechnology, Alva's College and Alva's MLT College, Moodbidri. A loop full of culture was inoculated into nutrient broth and incubated at 37℃ for 24 hours to obtain a bacterial culture. This procedure was carried for the selected bacterial cultures to obtain inoculums of particular broth cultures. The well method was employed to assay the plant materials for antimicrobial activity. Petri dishes were plated with Mueller Hinton Agar media and allowed to solidify for 30 mins. The test organisms were then spread on the surface of the media using sterile swab stick. Cork borer was used to bore wells in media. The aqueous, methanolic and silver nanoparticle extract of different concentrations (50 µl, 100 µl and 150 µl) were separately dispensed into the wells of different plates using a micropipette. A negative control of water and a positive control of Amphicillin were kept and the extract was allowed to diffuse for 30 mins at room temperature. The plates were incubated at 37℃ for 24 hours and the zones of inhibition were measured.

3. RESULTS

The phytochemical screening of the extract of the *Areca catechu* seed extract showed positive result for carbohydrates, terpenoids, tannin and saponins while negative result for resin and alkaloids as showed in Table 1.

3.1 Synthesis of Silver Nanoparticles

Formation of silver nanoparticles by the reduction of silver ions during the exposure to *Areca catechu* seed extract was recorded by change in colour of the reaction mixture from light brown to dark brown (Fig. 1) after 10 minutes of incubation which indicated the formation of silver nanoparticles.

3.2 Ultraviolet- Visible (UV- Vis) Spectra Analysis

The silver nanoparticles formed in the areca nut seed extract solution exhibited an absorption spectrum ranging from 300-800 nm with a peak at 435 nm (Fig. 2).

Table 1. Phytochemical analysis of methanol and aqueous extract of the areca nut seed

Constituents	Aqueous extract	Methanol extract
Carbohydrates	+	+
Tannins	+	+
Alkaloids	-	-
Terpenoids	+	+
Resins	-	-
Saponins	+	+

+ present, - absent

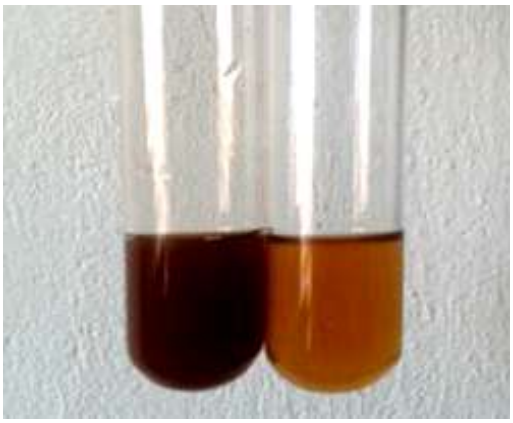

Fig. 1. Synthesis of silver nanoparticles confirmed by color change

3.3 Fourier Transform Infrared Spectroscopy (FTIR)

The spectra of nanoparticles obtained from *Areca catechu* seed extract showed absorption peaks at 3827.07 cm^{-1}, 3871.13 cm^{-1}, 1809.41 cm^{-1}, 1751.05 cm^{-1}, 2337.72 cm^{-1}, 1608.63 cm^{-1}, 1624.84 cm^{-1}, 819.76 $cm^{-1,}$ and 867.57 cm^{-1} for different groups (Fig. 3).

3.4 Scanning Electron Microscopic (SEM) Study

The synthesized silver nanoparticles from areca nut seed extracts were oval in shape with the diameter ranged from 553 to 610 nm under 10,000X magnification (Fig. 4).

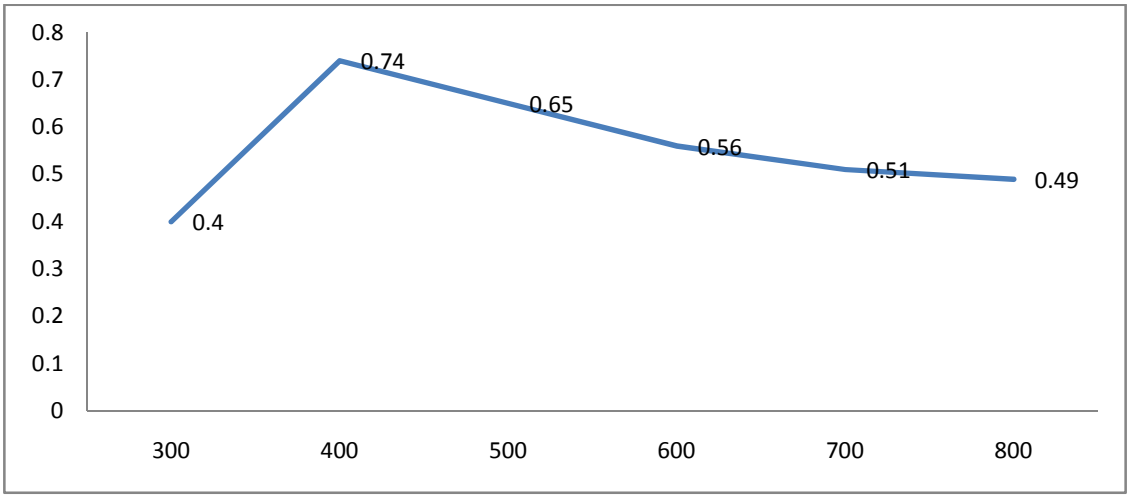

Fig. 2. UV- visible spectroscopy of areca nut silver nanoparticle extract

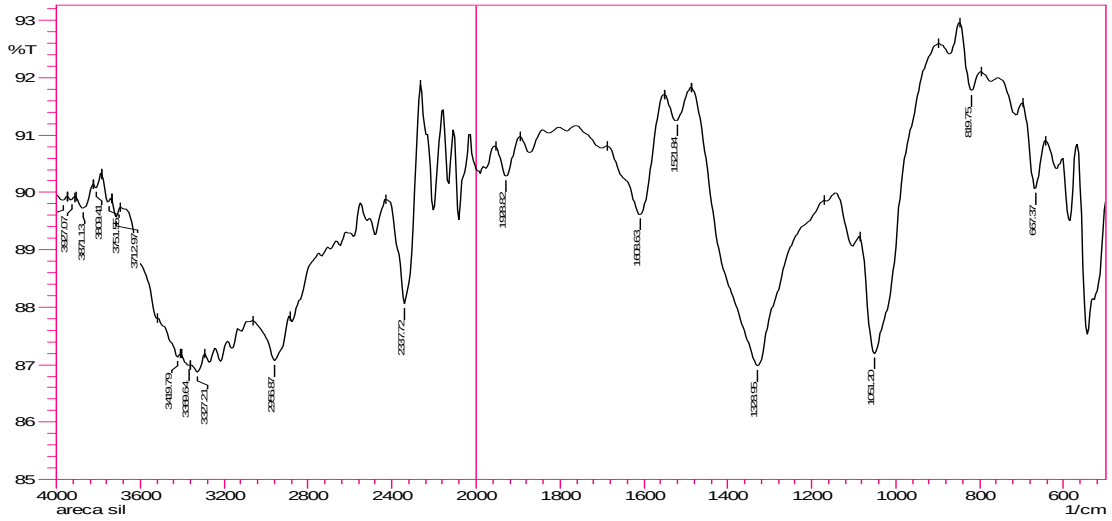

Fig. 3. FTIR analysis showing functional group spectrums

3.5 Antibacterial Activity for Different Concentrations of the Plant Extract

The antibacterial activity of aqueous, methanolic and silver nanoparticles of areca seed showed varied susceptibility and resistance. The different extract *Psuedomonas aeruginosa* showed high sensitivity for the aqueous extract of the plant i.e., the zone of inhibition for this organism was more when compared to other organisms for the aqueous extract of the plant. The *Bacillus subtilis* was less sensitive for the aqueous extract was less (Table 2). *Pseudomonas aeruginosa* was high sensitive for the methanol extract of the seed and *Bacillus subtilis* and *Klebsiella pneumoniae* showed less sensitivity compared to other organisms for the methanol extract (Table 3). *Pseudomonas aeruginosa* showed high sensitivity for the extract of silver nanoparticle synthesized from *Areca catechu. Bacillus subtilis* showed less sensitivity while *Klebsiella pneumoniae* did not exhibit any inhibition (Table 4).

4. DISCUSSION

Medicinal plants are the most productive source of new compounds and drugs of natural origin. Most of the natural products isolated from medicinal plants are the secondary metabolites, which include alkaloids, tannins, flavonoids, steroids, terpenoids, phenylpropanoids and

anthraquinones Some of the products have nutritive value and antifungal and antibacterial activities. Ayurveda has related research efforts which have led to generation of enormous amount of scientific information concerning plants, crude plant extracts, and various substances from plants as medicinal agents during last 30 to 40 years. In the present investigation, the phytochemical analysis of *Areca catechu* seed extract showed presence of carbohydrates, terpenoids, tannin and saponins, and absence of resin and alkaloids by qualitative tests. In one of the earlier study the presence of phenolic constituents and antioxidant activities of areca seeds were reported [12].

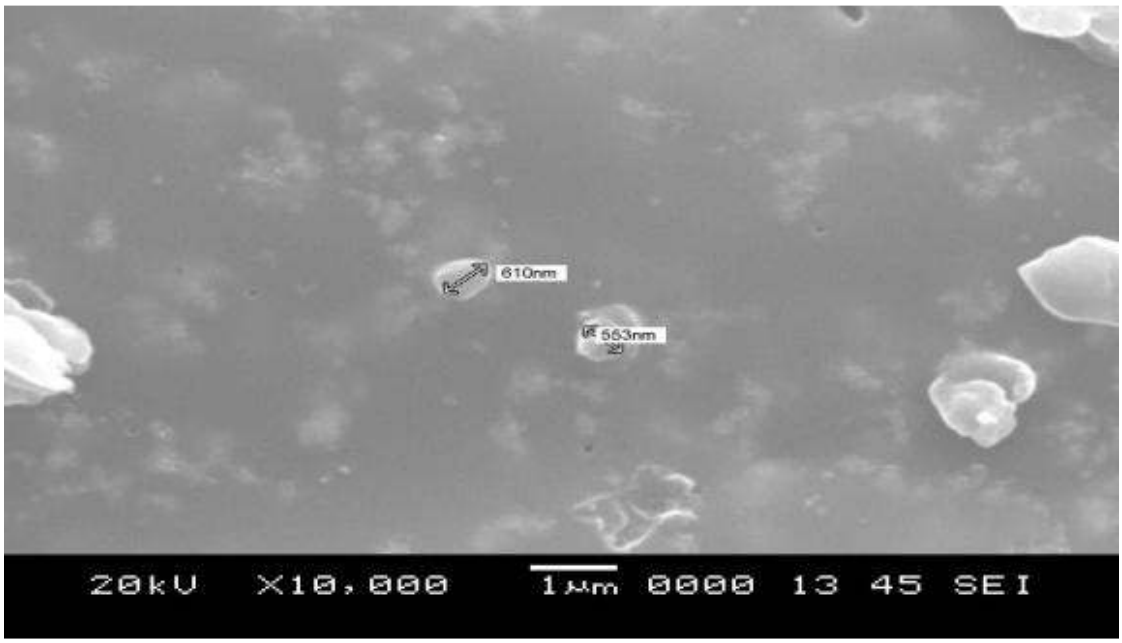

Fig. 4. Silver nanoparticles obtained from *A. catechu* seed extract under SEM

Table 2. Antibacterial activity for the aqueous extract of *A. catechu* seed

Test organisms	Zone of inhibition (mm) for different concentrations of the methanol extract		
	50 µl	100 µl	150 µl
Pseudomonas aeruginosa	13	16	17
Bacillus subtilis	10	15	15
Staphylococcus aureus	10	12	12
Klebsiella pneumoniae	09	13	13
Salmonella typhi	09	12	11

Table 3. Antibacterial activity for the methanol extracts of *A. catechu* seed

Test organisms	Zone of inhibition (mm) for different concentrations of the methanol extract		
	50 µl	100 µl	150 µl
Pseudomonas aeruginosa	5	7	8
Bacillus subtilis	3	3	5
Staphylococcus aureus	4	5	7
Klebsiella pneumoniae	2	4	5
Salmonella typhi	7	9	10

Table 4. Antibacterial activity of silver nanoparticles synthesized from *A. catechu* seed extract

Test organisms	Zone of inhibition (mm) for different concentrations of silver nanoparticles		
	50 µl	100 µl	150 µl
Pseudomonas aeruginosa	15	16	16
Bacillus subtilis	13	15	15
Staphylococcus aureus	14	15	16
Klebsiella pneumoniae	-	-	-
Salmonella typhi	12	13	14

Silver nanoparticles bear characteristic dark brown colour due to the excitation. The change in colour of the reaction mixture after 10 minutes was observed in the present study. Similarly, on other studies with the leaves of *Svensonia hyderobadensis* and stem bark of *Boswellia* [9,20] revealed the synthesis of silver nanoparticles and noted the reduction of silver ion in silver nitrate solution exposed to plant extract followed by colour. Ankanna and Savithramma [5] also reported that the stem bark of *Boswellia ovalifoliata* solution of the silver ion complex started to change the colour from yellow to dark brown colour in aqueous solution. There are few other recent works supporting the present investigation [21,22].

The synthesized nanoparticles of areca nut seed extract exhibited a maximum absorbance peak at 400 nm. Similarly, Geetha et al*.* [23] reported a narrow absorption band at 450 nm and Maribel et al. [24] reported that the absorption peak of silver nanoparticles by chemical reduction method was 412 nm. FTIR analysis was used for the characterization of the extract and the resulting nanoparticles. The biomolecules which are involved in the reduction of silver ions can be identified by absorption peaks in the spectral range of 1000-4000 cm^{-1}. Saifuddin et al. [25] observed the FTIR measurements to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized in leaf broth. The synthesized silver nanoparticle of areca nut seed extract was oval in shape with a diameter ranged from 553-610 nm. Christensen et al. [26] and Charusheela et al. [27] reported spherical shaped nanoparticles of 10-25 nm and Hemanth et al. [28] obtained the nanoparticles of 52-104 nm. In other studies, hexagonal shaped nanoparticles of 80-120 nm and 90-120 nm were reported [21,22]. According to Mie theory, only a single surface Plasmon resonance band is expected in the absorption spectra of spherical nanoparticles, whereas the number of peaks increases as anisotropy increases [29].

In this study on the antibacterial activity of methanol and aqueous extracts from seeds of *Areca catechu* resulted in highest inhibitory zone with *Pseudomonas aeruginosa* and minimum zone of inhibition by *Klebsiella pneumoniae*. Chin and Fernandez [16] examined the antimicrobial performance of methanolic extract of *Areca catechu* seeds against mixed oral flora from tooth scum and Gram negative laboratory isolates. Varying concentrations of *A. catechu* ethanol extract was tested for antimicrobial activity against 0.5 Mc Farl and of mixed oral flora and eight Gram negative clinical isolates by agar well diffusion method. All concentration showed to inhibit oral flora models with zone of inhibition about 7-18 mm. In the present study of silver nanoparticles synthesized using areca nut seed extract exerted a significant antibacterial activity compared with positive control and other bacterial strains which symbolized that the extract have antibacterial activity. By increasing the dose of the extract higher inhibitory zone was found. The antibacterial activity can also be compared with other silver nanoparticles synthesized from leaf extracts of *Svensonia*, which also showed activity against *E. coli, Bacillus subtilis, Staphylococcus aureus* and *Klebsiella pneumoniae* [9]. Maria et al*.* [15] also reported the antimicrobial effect of areca nut extract on oral pathogens which are of other bacterial strains but *Candida albicans* showed positive inhibitory effect. On the other hand, Marium and Tuslima [17] obtained inhibitory effect against Gram negative bacteria when carbon tetrachloride extract was used compared with methanol extract of areca seeds. Similarly, Kabbashi [30] tested ethanolic and methanolic fruit extract of *Balanites aegyptiaca* against two Gram positive, two Gram negative and two fungal species, among which the methanolic extract exhibited high activity only against *Aspergillus niger*, *Bacillus subtilis* and *Staphylococcus aureus*, whereas it was an intermediately active against, *E. coli, Candida albicans* and, *Pseudomonas aeruginosa*. He

concluded that the ethanolic extract proved to be had high activity on all bacteria and fungi.

Senthil et al. [31] and Kumar et al. [1] reported an overview of many phytochemical characteristic of areca nut seed and therapeutic effect on various disease conditions on human beings. Areca nut seed biochemical compounds have been recently recognized as functionally active molecules, possessing antioxidant, antidiabetic, antiallergic and other useful properties, as well as exert protective effects against cardiovascular and other diseases.

5. CONCLUSION

Being an important cash and commercial crop, the areca nut has got multifacinating medicinal properties. The present study confirmed the silver nanoparticle formation using the areca nut seed extract and its antimicrobial properties against selected bacterial species, some of which are human pathogens. Further study required to use these nanoparticles for the treatment of disease using animal models.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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