WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 12, 258-266. Research Article ISSN 2277– 7105

ANTIBACTERIAL ACTIVITY OF *ARECA CATECHU L.* FRUIT EXTRACT LOADED SILVER NANOPARTICLES

Foysal Hossen[1], Md. Mamun Or Rashid[2]*, Mohammad Morshad Alam[1], Mir Salma Akter[1], Md. Tanvir Hossain[3], Kazi Nahid Akhter[2]

[1]Department of Microbiology, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.

[2]Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.

[3]Department of Applied Chemistry and Chemical Engineering, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.

Article Received on
05 Oct 2015,

Revised on 27 Oct 2015,
Accepted on 17 Nov 2015,

***Correspondence for Author**
Md. Mamun Or Rashid
Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.

ABSTRACT

Silver nanoparticles are synthesized by $AgNO_3$ as a precursor and $NaBH_4$ was used as reducing agent. Here we tried to investigate the antibacterial activity of polyaniline coated silver-extract nanoparticles using *Areca catechu L.* fruit extract. Prepared nanoparticles were characterized by Visual inspection, Ultraviolet-visible spectroscopy, Scanning Electron Microscopy (SEM) techniques. Antibacterial activities of the synthesized Ag-Extract nanoparticles were tested against *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. UV–Vis spectrum of reaction mixture showed strong absorption peak with centering at 400 nm. SEM results revealed that the particle size of these combined nanoparticles were 78.5 to 100 nm in most cases. The result revealed that *Areca catechu L.* coated Ag-Extract NPs showed strong antibacterial activities against most of the tested species. 34±0.68 mm zone of inhibition was found against *S. aureus*, whereas it was 24±0.40 mm for the standard ciprofloxacin (positive control). Again, maximum zone of inhibition 19±0.30 mm was found for *S. typhimurium*, 35±0.48 mm for *E. coli* and 29±0.35 mm for *P. aeruginosa*. This result suggests that *S. typhimurium* is more resistant than *E. coli* and *P. aeruginosa*. In-vivo

investigation is needed on human model before establishment of antibacterial efficacy of AgNPs-extracts (A. catechu) in drug development program for safe health care services.

KEYWORDS: Silver nanoparticles, *Areca catechu L*., Antibacterial activity, Scan Electronic Microscopy (SEM).

INTRODUCTION

Nanoparticle research is currently the most studied branch of science with the number of uses in various fields - biomedical, optical and electronic fields etc.[1,2] The contribution of nanoparticles to modern medicine is tremendous. Indeed there are some instances where nanoparticles are used to analyze and in therapies that simply cannot be performed otherwise.[3] Nanoparticles have a relatively large surface which has the ability to bind, adsorb and carry other compounds such as drugs, probes and proteins.[4] Nanoparticles of silver which have the size range between 1 nm and 100 nm are known as silver nanoparticle.[5] A common application of silver nanoparticles in antimicrobial coatings is increasing now-a-days. Many textiles, keyboards, wound dressings, and biomedical devices now contain silver nanoparticles that provide protection against bacteria by continuously releasing a low level of silver ions.[6]

Areca nut tree (Areca catechu) is referred to as betel nut tree; which is a species of palm tree that grows in tropical humid regions like Asia Pacific and parts of east Africa.[7] The main chemical constituents of areca nut are polyphenols, fat, polysaccharides, fiber, and protein.[8] Besides these, areca nut contain catechin, tannins (15%), gallic acid fat, gum and alkaloids like arecoline (0.1-0.7%) arecaine (1%) and others in trace amounts such as arecadine, guvacoline, and guvacine.[9] These chemical components have been reported for their antidiabetic, blood pressure regulating activity, antiulceogenic, antioxidant activity, anticonvulsant activity, CNS. stimulant activity, oxytocic activity, antifertility, anthelmintic, antibacterial, antifungal effects and antiviral activity.[10,11]

However, there are some research works done on the antibacterial activities of Areca catechu extract and silver nanoparticles separately, there is no established finding on their combined effect. In our study, we tried to evaluate the antibacterial activity of *Areca catechu L*. fruit extract (water soluble fraction) loaded silver nanoparticles coated by polyaniline.

MATERIALS AND METHODS

Materials

Silver nitrate (MERCK, Germany) used as a silver precursor and sodium borohydride (LOBA CHEMIE) used as reducing agent. All other reagents used in this study were analytical grades and collected from the laboratory of the Dept. of Pharmacy and Dept. of Microbiology, Noakhali Science and Technology University.

Microorganisms

To evaluate antibacterial activity, four different ATCC bacterial cultures were used. The ATCC cultures were collected from the department of microbiology, Dhaka Shishu (Children) Hospital and from the department of microbiology, University of Dhaka. The cultures were *Staphylococcus aureus* ATCC 25923; *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028.

Collection and selection of the plant material

Areca catechu L. nuts were collected from the local market of Sonapur, Noakhali, Bangladesh. The collected fruits were washed thoroughly with distilled water several times and peeled cautiously.

Preparation of plant extract

After collection the undesired residues were separated and the nuts were washed with sterile water to remove dust. In hot air oven at 40^0C the nuts were dried out overnight. The dried nuts were cut into small pieces then grounded to powder in a grinder. After that, 200 grams of the powder were taken in beaker and mixed with 1000 ml of distilled water. The nut powder was stirred vigorously to mix up properly. It was then boiled for an hour followed by cooling to room temperature. After that, the suspension was filtered through a piece of sterile cotton fabric and later Whatman filter paper.

Synthesis of Silver Nanoparticles

1M silver nitrate was prepared for the synthesis of silver nanoparticles by reducing aqueous silver nitrate in the presence of sodium borohydride.[12,13] Briefly, a 100-mL aqueous solution of 1.0 X 10^{-3} M silver nitrate was mixed with a 300-mL aqueous solution of 2.0X10^{-3} M sodium borohydride. After adding $AgNO_3$, the solution color changed to yellow from colorless. The reaction mixture turned into dark brown color from brownish-yellow color within 20 minutes of mixing. Color change indicated the formation of silver nanoparticles

(AgNPs). As the concentration of solution is very less, it was concentrated 10 times to get desired concentration. The prepared AgNPs were further characterized.

Preparation of Ag-Extracts nanoparticles

Coating of Extract-AgNPs were done according to the K. Gopalakrishnan, 2012 with slight modification.[14] The prepared silver nanoparticles solution and *Areca catechu L*. fruit extract were mixed together by a magnetic stirrer for continuous stirring. The solution was stirred for 30 minutes at 35^0C and maintain standby for 2 hours. The pH of the solutions was monitored before and after mixing was performed. In second step, 29 ml aniline dissolved in 300 ml distilled water and 40 ml H_2O_2 added in 20 ml distilled water in separate conical flaxes. Two conical flaxes was taken in two hands and mixed with the first step solution for 30 minutes with continuous stirring at room temperature. After 23 hours of stirring on magnetic stirring coating was done. As a result, polyaniline coated Ag-extract NPs were found. These NPs solution then taken for centrifugation at 6000 rpm for 10 min. The precipitate solid residue was taken and dried at room temperature for further experimentation and characterization.

Characterization techniques

Silver nanoparticles were characterized by visual inspection, UV spectroscopy and Scanning Electron Microscopic (SEM) analysis.

Antimicrobial assay

In-vitro antibacterial activity of the samples was determined by deploying the disc diffusion method using Mueller–Hinton Agar (MHA) with determination of inhibition zones in millimeter (mm), which conform recommended standards of the National Committee for Clinical Laboratory Standards according to Rahman et al, 2014.[15] In order to do this, firstly we made Mueller–Hinton Agar plates aseptically. After that, we prepared bacterial lawn on MHA plates using the reference bacterial species, which were growing fresh culture in Luria Bertani broth. Next, previously prepared paper discs of each samples including aqueous extract of Areca catechu, silver nanoparticle, coated silver nanoparticle were placed on each bacterial lawn on MHA plates. The paper discs were prepared by impregnating the sterile paper discs of 4 mm in diameter into the three different test samples each of which was at a concentration of 20 mg/ml. The impregnated paper discs were dried out at 37°C for 24 h in a sterile condition. Standard antibiotic discs (Ciprofloxacin) were used as positive respectively. Finally, the agar plates were incubated at 37°C for 24 hours and the zone of inhibition were observed.

RESULTS AND DISCUSSION

Assessment of AgNPs through visual inspection

The formations of silver nanoparticles were confirmed visually. Within a short period of the reaction, mixer color turned into dark brown from brownish yellow color. This color changing confirmed the synthesis of Ag nanoparticles (Figure 1).

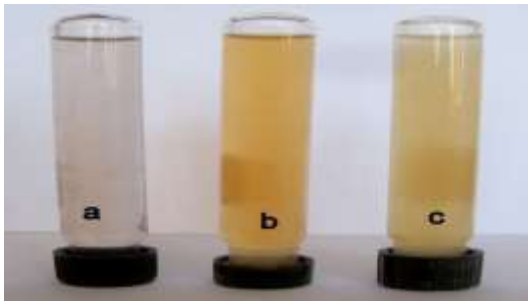


Figure1. Visual assessment of a) Ag NPs b) Extract c) Coated Ag NPs-Extract

Characterization of AgNPs by UV-Vis spectrum

UV–Vis spectrum of reaction mixture at different wavelengths ranging from 300 to 700 nm showed strong absorption peak with centering at approx. 400 nm which indicated the formation of Ag-NPs (Figure 2).

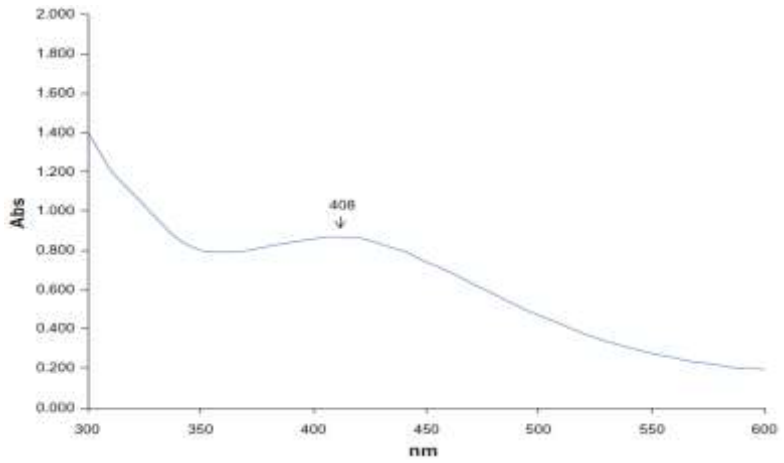


Figure.2. UV–Visible spectrum of AgNPs

Analysis of Ag-NPs by SEM

SEM analysis showed the synthesis AgNPs. It was shown that irregular AgNPs were formed with diameter of 78.5 to 100 nm in most cases; however few particles were larger than 100 nm (Figure 3). The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent. The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements.

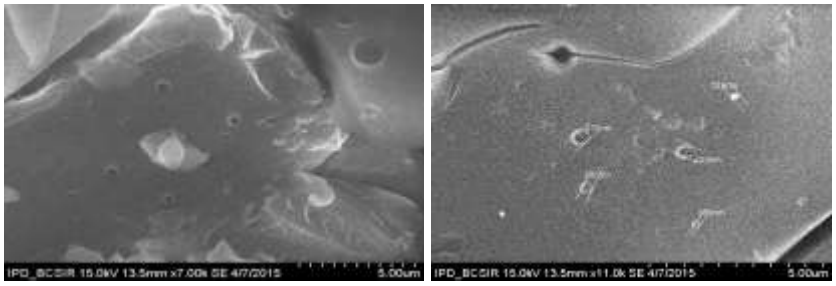
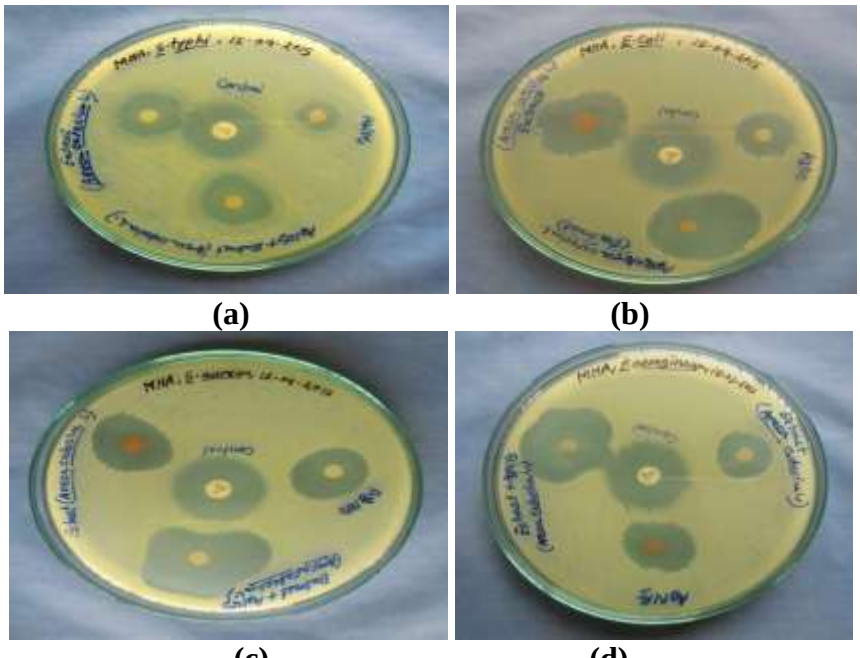


Figure3. SEM image of AgNPs

Assessment of antibacterial activity

The aqueous extract and AgNPs-Extract showed strong and moderate antibacterial activity against several test organisms. The aqueous extract of Areca catechu, AgNPs and AgNPs-Extract were subjected to antibacterial assay against both gram positive and gram-negative bacteria namely *Staphylococcus aureus*, *S. typhimurium*, *Escherichia coli* and *Pseudomonas aeruginosa*. This study observed and compared the antimicrobial activity of three different samples on both gram positive and gram negative bacteria (Figure 5). Ciprofloxacin was used as a positive control. The result of antibacterial activity, measured in term of diameter of zone of inhibition in mm is shown in Table 1. All three types of test samples showed inhibitory effect against all of the bacteria used in this study (Figure 4). Among all of the test samples, AgNPs-Extract showed highest inhibitory effect against both gram positive and gram negative bacteria whereas AgNPs showed lowest and aqueous extract of Areca catechu showed moderate activity (Figure 5).



(a) **(b)**

(c) **(d)**

Figure4. Antibacterial activity of Areca catechu formed Ag-NPs on (a) *S. typhi* (b) *E. coli* (c) *S. aureus* (d) *P. aeruginosa*

Table1. Antibacterial activity of Areca catechu coated Ag-NPs

Items	Zone of inhibition (mm)			
	S. aureus,	***S. typhi,***	***E. coli,***	***P. aeruginosa***
Extract	22±0.54	16±0.22	28±0.45	18±0.48
AgNPs	21±0.36	10±0.28	18±0.57	21±0.34
AgNPs-Extract	34±0.68	19±0.30	35±0.48	29±0.35
Ciprofloxacin	25±0.40	23±0.40	23±0.40	25±0.40

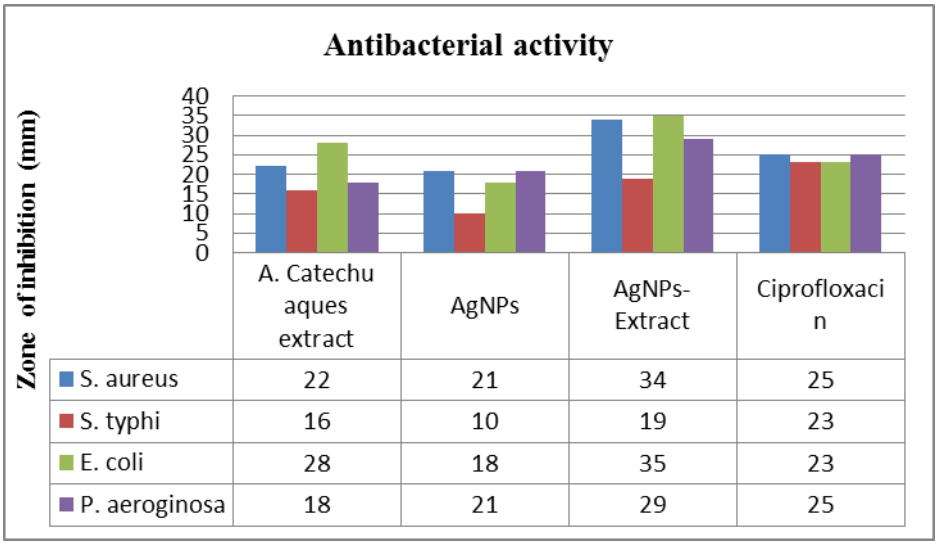


Figure5. Graphical representation of antibacterial activity of Areca catechu coated Ag NPs on *S. aureus, E. coli, S. typhimurium, P. aeruginosa*

There are various reports which have been providing the evidences that silver nanoparticles were used as powerful tool against multidrug-resistant bacteria.[16,17] The mechanism of the inhibitory effects of Ag ions on microorganisms is partially known. It is reported that the positive charge on the silver ion is the reason for antimicrobial activity as it can attract the negatively charged cell membrane of microorganisms through the electrostatic interaction.[18,19] In our experiment, when we compared the antibacterial activity of AgNPs, Areca catechu extract and coated AgNPs-Extract, it was found that AgNPs-Extract have shown more antibacterial activity than either AgNPs or Areca catechu extract alone against these bacterial strains (Table 1), even taken in double in some cases. The antibacterial efficacy of synthesized coated AgNPs enhances because the use of silver and Areca catechu extract, as silver reduced in nano form which increases its surface area, thus make AgNPs more reactive and Areca catechu extract enhances the therapeutic efficacy of AgNPs due to its good antibacterial efficacy. Our experiment value revealed that coated AgNPs-Extract showed excellent antibacterial activity against all types of test bacteria (Figure 5) with highest zone of inhibition was 35±0.48mm, found against *E. coli*.

ACKNOWLEDGEMENT

We would like to express my gratitude to everyone who has supported, encouraged and helped us during our research work. We are grateful to the Dept. of Pharmacy and Dept. of Microbiology for their support and lab facilities to conduct this research work.

CONFLICT OF INTEREST

The authors claim no conflict of interest for this research work.

CONCLUSION

In our study we found that gram negative bacteria are more susceptible on Ag-Extract NPs rather than Gram positive bacteria. The synthesized combination may enhance the therapeutic efficacy and strengthen the medicinal values. Hence, our results are promising and prove to be an important step in this direction as it decreases the burden of multidrug resistance of patients. Further investigation is needed to establish the antibacterial efficacy of AgNPs-extracts (A. catechu) in drug development program for safe health care services.

REFERENCES

1. Taylor R, Coulombe S, Otanicar T, Phelan P, Gunawan A, Wei L, Rosengarten G, Prasher R, Tyagi H. Small particles, big impacts: A review of the diverse applications of nanofluids. J Appl Phys, 2013; 113: 011301.
2. Hewakuruppu YL, Dombrovsky LA, Chen C, Timchenko V, Jiang X, Baek S, Taylor RA. Plasmonic "pump–probe" method to study semi-transparent nanofluids. Appl Opt, 2013; 52(24): 6041-6050.
3. Murthy SK. Nanoparticles in modern medicine: State of the art and future challenges. Int J Nanomed, 2007; 2(2): 129-141.
4. Wim H De Jong, Paul JA Borm. Drug delivery and nanoparticles: Applications and hazards. Int J Nanomed, 2008; 3(2): 133-149.
5. Graf C, Vossen DLJ, Imhof A, van Blaaderen A. A general method to coat colloidal particles with silica. Langmuir, 2003; 19(17): 6693-6700.
6. http://www.sigmaaldrich.com/materials-science/nanomaterials/silver-nanoparticles.html
7. http://en.wikipedia.org/wiki/Areca_nut (Accessed March 2015)
8. Reena R, Authikat N, Michael A. Study on the Areca nut for its antimicrobial properties. Pharmacognosy, 2009; 1(1): 42-45.

9. Joshi M, Gaonkar K, Mangoankar S, Satarkar S. Pharmacological investigation of Areca catechu extracts for evaluation of learning, memory and behavior in rats. Int Curr Pharma J, 2012; 1(6): 128-132.
10. Peng W, Liu YJ, Wu N, Sun T, He XY, Gao YX, Wu CJ. *Areca catechu L*. (Arecaceae): A review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. J Ethnopharmacol, 2015; 164: 340-356.
11. Patil PR, Rakesh SU, Dhabale PN, Burade KB. Pharmacological activities of *Areca catechu L*. J Pharm Res. 2009; 2(4): 683- 687.
12. Creighton JA, Blatchford CG, Albrecht MG. Plasma resonance enhancement of Raman scattering by pyridine adsorbed on silver or gold sol particles of size comparable to the excitation wavelength. J Chem Soc, 1979; 75: 790-8.
13. Suh JS, DiLella DP, Moskovits M. Surface-enhanced Raman spectroscopy of colloidal metal systems: a two - dimensional phase equilibrium in p-aminobenzoic acid adsorbed on silver. J Phys Chem, 1983; 87: 1540-4.
14. Gopalakrishnan K, Ramesh C, Ragunathan V, Thamilselvan M. Antibacterial activity of Cu_2O nanoparticles on *E. coli* synthesized from Tridax Procumbens leaf extract and surface coating with polyaniline. Dig J Nanomater Bios, 2012; 7: 833-839.
15. Rahman MA, Sultana P, Islam MS, Mahmud MT, Rashid MMO, Hossen F. Comparative Antimicrobial Activity of Areca catechu Nut Extracts using Different Extracting Solvents. Bangladesh J Microbiol, 2014; 31 (1,2): 19-23.
16. Lara HH, Ayala-Nunez NV, Turrent LCI, Padilla CR. Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World J Microb Biot, 2010; 26: 615-621.
17. Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol, 2012; 112: 841-852.
18. Dibrov P, Dzoiba J, Gosink KK, Häse CC. Chemiosmotic mechanism of antimicrobial activity of Ag(+) in vibrio cholera. Antimicrob Agents Chemother, 2002; 46: 2668-2770.
19. Hamouda T, Myc A, Donovan B, Shih A, Reuter JD, Baker RJ. A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial against bacteria, enveloped viruses and fungi. Microbial Res, 2000; 156: 1.