Antimicrobial activity of synthesized silver nanoparticles and phytochemical screening of the aqueous extract of *Antiaris toxicaria*

S.Gopalakrishnan[1], Philip Kaupa[2], S. Yamini Sudha Lakshmi[3*], Fouzia Banu[4]

[1&2]*Department of Applied Sciences, PNG University of Technology, LAE, Papua New Guinea.*
[3]*Deptartment of Medical Biochemistry, University of Madras,Taramani Campus, Chennai-600113, India*
[4]*Dept. of Biochemistry, JBAS College for Women, Teynampet,Chennai-600018.*
*yasula2000@yahoo.com

Abstract

Background/Objectives: *Antiaris toxicaria* is used as a traditional medicine around many parts of Papua New Guinea. The main aim of this study was to evaluate the compatibility of *A. toxicaria* by carrying out the phytochemical screening, synthesizing silver nanoparticles using silver nitrate and to determine their antimicrobial activity.
Methods/Statistical analysis: Antimicrobial activity was determined by agar paper disk diffusion essay and the zone of inhibition was measured against 14 microorganisms.
Results: All the test microorganisms were highly susceptible to the green synthesis of silver nanoparticles. Phytochemical screening of *A. toxicaria* reveals the presence of alkaloids, glycosides, terpenoids, reducing sugars, saponins and phenolic compounds.
Conclusion/Application: The nanoparticles synthesized from the aqueous plant extract of *A. toxicaria* contains the phytochemicals and it is proved to have the antimicrobial activity.

***Key words*:** *Antiaris toxicaria*, antimicrobial activities, silver nano particles, phytochemical screening.

1. Introduction

Recently, silver nanoparticles synthesis is among the most interesting scientific areas of inquiry in the pharmaceutical and biomedical fields. Silver nanoparticles are synthesized and stabilized by Physical, Chemical and the Biological methods [1-3]. In current trend, biological synthesis of metallic nanoparticles is gaining importance because it is reliable and eco-friendly[4].

During the past decade, lots of work has been done in biological system to address a wide range of field problems utilizing nanomaterials and nano-devices [5]. Synthesis of silver nanoparticles using plant extract is a fast, simple, convenient, eco-friendly and cost effective method for the biological synthesis of silver nanoparticles. Recently, researchers have turned towards the use of biological systems such as bacteria, fungi and plant extracts to synthesis biocompatible silver metals [4-6]. Using plant extract to reduce Ag+ to Ag0 which is incorporated into phytochemical constituents of plant extract to fabricate silver nanoparticles (AgNPs) has been gaining much attention [7]. In this method, plant extract acts as the reducing, capping and the stabilizing agent for the silver nanoparticle formation. The major advantage of using plant extracts for silver nanoparticle synthesis is that they contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [8,9,4,3]. These compounds are synthesized by primary or rather secondary metabolism of living organism. A large number of phytochemicals has been shown to have inhibitory effects on all types of microorganisms. In Papua New Guinea, as a tropical country, there are different species of plants and a good number of them are believed to be medicinal plants. In this study, *A. toxicaria* is a locally identified medicinal plant which was used in the synthesis of silver nanoparticles. In this research, the active phytochemical constituent of *A.toxicaria* was identified and was used with Silver Nitrate to synthesize silver nanoparticles.

2. Materials and Method

2.1. Test microorganisms

Fourteen microorganisms species were used in this study namely *Bacillus cereus (G+), Citrobacter freundii (G-), Enterobacter aerogenes (G-), Escherichia coli (G-), Klebsiella pneumoniae (G-), Micrococcus luteus (G+), Neisseria gonorrhea (G-), Proteus vulgaris (G-), Pseudomonas florescens (G-), Salmonella typhimurium (G-), Staphylococcus aureus (G+), Streptococcus pneumoniae (G-), Trichomonas vaginalis (Protozoa), Candida albicans* (Yeast). All this microorganisms were obtained from Microbiology Laboratory, Applied Science Department, Papua New Guinea University of Technology. The microorganisms were cultured in the Muller-Hitron Broth (MHB) overnight at 25 and 37ºC depending on their inoculation temperature.

2.2. Extraction of Antiaris toxicaria

Fresh healthy leaves of *A. toxicaria* was collected from Bulolo in August 2014 and the preparation started when the leaves are still fresh. The leaves were thoroughly washed with distilled water and then were cut into thin strips. The extracts of the leaves were obtained by the simple boiling method or Decoction method. The leaves were boiled with 1:2 ratio of water to leaves. The crude was extracted by filtering with muslin cloth when it starts to boil and the first bubbles formed.

2.3. Phytochemical screening

Tests for phytochemical constituents were carried out for Alkaloids, glycosides, flavonoids, tannins, reducing sugar, saponins, steroids and phenolic compounds from the extract of *A. toxicaria* using standard methods.

2.4. Synthesis of Silver nanoparticles

Silver nanoparticles were synthesized using silver nitrate and *A. toxicaria* extract. Firstly, 3mM and 6mM of $AgNO_3$ were added into separate 100 mL conical flask. Then 20 mL plant extract was added to 80 mL of the different concentrations of $AgNO_3$ solutions (3mM & 6mM) in the two separate 250 mL canonical flask. The solution was incubated in a rotary shaker at room temperature for 20 minutes and periodically was observed for colour change. The solution was heated on a water bath at 25-95 ºC to observe the synthesis rate. Then it went centrifugation at 8000 rpm for 25 minutes and the pellets were obtained. The supernatant was discarded and the pellet was dissolved again in distilled water and undergone centrifugation. The pellet was collected by using alcohol. Characterization/Analysis of silver Nanoparticles was by UV-Visible Spectroscopy. The reduction of the Ag+ ions by the extract of the *A. toxicaria* extract, which leads to the formation of silver nanoparticles were characterized by UV-visible spectroscopy. The bioreduction of silver ions in aqueous solution was monitored by UV-VIS spectra of the solution between 300 nm – 600 nm. Distilled water was used to adjust the baseline. Quantitative determination of the synthesized silver nanoparticle will be carried out using a Varian Cary-50 Bio UV-Visible Spectrometer.

2.5. Antimicrobial test

The antibiotic activity of the synthesized silver nanoparticle was carried out on the 14 microbial species listed in the objective of the current study. Firstly, 33.6 g of Nutrient Agar was dissolve in 1L of distill water. Using a magnetic rod and a magnetic stirrer, the solution was stirred and then undergone sterilization by heating in the autoclave for 2 hours. After sterilization, the media was poured into sterile petri dishes. The media were allowed to solidify for 20 minutes, and then the bacterial species were spread on the media in the petri dishes. Bacterial species were spread on the media plates. Paper disk were placed in petri dishes and 30 mL of concentrated silver nanoparticles solution are placed in the disk and allowed to dry. The paper disk is then placed in the petri dishes that contain the media and the bacterial species and incubated for 24 hours. Then the bacterial growth is determined my measuring the diameter of the zone inhibition.

Table 1. Phytochemical studies

Phytochemicals	*Type of Test*	*Antiaris toxicaria*
Alkaloids	Mayer's Test	+
Glycosides	Liebermann's	+
Flavonoids	Shinoda Test	+
Tannins	Ferric Chloride	-
Reducing Sugars	Fehling's Test	+
Saponins	Foam Test	+
Phenolic Compounds	Ferric Chloride	+
Terpenoids	Acetic anhydride & Chloroform	-
Steroids	Acetic anhydride & Chloroform	-

Table 2.The result for the antibacterial activity of silver nanoparticles synthesized from Antiaris toxicaria and the two different concentrations, 3 mM and 6 mM silver nitrate solution.

Microorganisms	Gram Reaction	3mM $AgNO_3$ & extract	6 mM $AgNO_3$ & extract	Water extract of *A.toxicaria*	Chloramphenol	Streptomycin
Bacillus cereus	G+	14mm	14mm	-	16mm	32mm
Citrobacter freundii	G-	11mm	13mm	-	10mm	0
Enterobacter aerogenes	G-	12mm	11mm	-	0	16mm
Escherischia coli	G-	13mm	12mm	-	12mm	10mm
Klebsiella pneumoniae	G-	10mm	9mm	-	0	8mm
Micrococcus luteus	G+	11mm	10mm	-	6mm	10mm
Neisserria gonorrhoeae	G-	11mm	11mm	-	0	10mm
Proteus vulgaris	G-	13mm	13mm	-	20mm	10mm
Pseudomonas florescens	G-	14mm	10mm	-	20mm	18mm
Salmonella typhimurium	G-	13mm	13mm	-	0	0
Staphylococcus aureus	G+	9mm	9mm	-	14mm	8mm
Streptococcus pneumoniae	G-	9mm	10mm	-	2mm	4mm
Trichomonas vaginalis	Protozoa	10mm	8mm	-	6mm	8mm
Candida albicans	Yeast	13mm	12mm	-	20mm	16mm

3. Results

Table 1 gives the phytochemical screening results of *A. toxicaria*. The extract of *A.toxicaria* was light brown, when adding silver nitrate solution, the colour changes from light brown to immense brown colour when adding the colourless silver nitrate solution. The change of brown colour confirms the continuous synthesis of silver nanoparticles which was read using UV-Visible spectroscopical analysis. The maximum peak absorbance was found to be between 425-454nm respectively for the two different concentrations (3mM & 5mM) of silver nitrate used.

The anti-microbial activity of the synthesized silver nanoparticles (Table 2) shows a little variation in the antimicrobial activity of silver nanoparticles synthesized from *A. toxicaria.* The nanoparticles exhibit the highest antimicrobial activity towards *Bacillus cereus*

4. Discussion

The reduction of silver ions from the silver nitrates to silver metal in colloid with the plant extract of *A.toxicaria* was visible through the change of colour from the colourless solution of silver nanoparticles and brown solution of plant extract to dark brown or blackish-brown when the two solutions were added [8, 9]. Similarly, the reduction of silver ions and the formation of stable nanoparticles occurred rapidly within 2 h of reaction making it one of the fastest bioreducing methods to produce silver nanoparticles [10, 11].

The susceptibility of the test microorganism is related to the inhibition zone size in millimeters via agar well diffusion essay. Microorganisms are termed susceptible to the nanoparticle when to zone of inhibition is equal to or more than 7 mm in diameter, or resistant with a zone of inhibition less than 7 mm. Generally all the microorganisms are susceptible to silver nanoparticles but are resistant to the plant extract.

5. Conclusion

The silver nanoparticle synthesized from the aqueous plant extract of *Antiaris toxicaria* is proved to have the antimicrobial activity (8).

6. References

1. A. El-Nour, A. Eftaiha, A. Al-Warthan, R.A.A. Ammar. Synthesis and Application of Silver Nanoparticles. *Arabian Journal of Chemistry.* 2010; 3(3), 135-140.
2. A.J. Gavhane, P. Padmanabhan, P.S. Kamble, S.N. Jangle. Synthesis of silver nanoparticles using extract of Neem leaf and triphala and evaluation of their antimicrobial activities. *International Journal of Pharma and Bio Science*. 2012; 3(3), 88-100.
3. A. Saxena, R.M. Tripathi, R.P. Singh. Biological synthesis of silver nanoparticles by using Onion (allium cepa) extract and their antibacterial activity. *Digest Journal of Nanomaterials and Biostructures*. 2010; 5(2), 427-432.
4. S. Yamini Sudha Lakshmi, Fouzia Banu,S. Gopalakrishnan and N.Gajendran. Antimicrobial activity of *Aegle marmelos* (Correa) Linn. Silver nanoparticles. *Indian Journal of Drugs and Diseases.* 2014; 3(1), 294-297.
5. K. Krishna Shyla, N. Natarajan. Customizing zinc oxide, Silver and Titanium dioxide nanoparticles for enhancing groundnut seed quality. *Indian Journal of Science and Technology*, 2014; 7(9), 1376-1381.
6. H. Korbekandi, SiavashIravani. Silver Nanoparticles, The Delivery of Nanoparticles. http://www.intechopen.com/books/the-delivery-of-nanoparticles/silver-nanoparticles. Date accessed: 12/04/2014.
7. Y. Rout, S. Behera, A. Kumar Ojha, P. L. Nayak. Green synthesis of silver nanoparticles using *Ocimum sanctum* (Tulashi) and study of their antibacterial and antifungal activities. *Journal of Microbiology and Antimicrobials*. 2012; 4(6), 103-109.
8. K.N. Thakkar, S.S. Mhatre, R.Y. Parikh. Biological synthesis of metallic nanoparticles. *Nanomedicine: Nanotechnology, Biology, and Medicine*. 2009; 6(2), 257-262.
9. A.M. Awwad, N.M. Salem, A.O. Abdeen. Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. *International Journal of Industrial Chemistry*. 2013; 4(29), 1-6.
10. S. Yamini SudhaLakshmi, F.B. Ezhilarasan, Arumugam, Sahadevan. Green Synthesis of Silver Nanoparticles from *Cleome Viscosa*: Synthesis and Antimicrobial Activity. *International Conference on Bioscience, Biochemistry and Bioinformatics IPCBEE,* IACSIT Press: Singapore, 2011.
11. S. Ponarulselvam, C. Panneerselvam, K. Murugan, N. Aarthi, K. Kalimuthu, S. Thangamani. Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* Linn. G. Don and their antiplasmodial activities. *Asian Pacific Journal of Tropical Biomedicine*. 2012; 2(7), 574-580.

*The Publication fee is defrayed by Indian Society for Education and Environment (*www.iseeadyar.org)

Cite this article as:

S.Gopalakrishnan, Philip Kaupa, S. Yamini Sudha Lakshmi, Fouzia Banu.Antimicrobial activity of synthesized silver nanoparticles and phytochemical screening of the aqueous extract of *Antiaris toxicaria*.*Indian Journal of Drugs and Diseases.* 2015; 4 (1), July.