Antimicrobial activity of synthesized silver nanoparticles and phytochemical screening of the aqueous extract of *Mussaenda ferruginea*

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Abstract

Background/Objectives: *Mussaenda ferruginea* is used as a traditional medicine around many parts of Papua New Guinea. It is used traditionally in the treatment of malaria and fever. The main aim of this study was to evaluate the compatibility of *M. ferruginea* in synthesizing silver nanoparticles and determine their antimicrobial activity.

Methods/Statistical analysis: 3mM and 6mM concentration of silver nanoparticles were prepared and checked for the antimicrobial activity using agar paper disc diffusion assay and measuring the zone of inhibition against 14 microorganisms. Also the photochemical screening was carried out for the aqueous extract of the plant.

Findings: All the microorganisms used were found to be highly susceptible to the synthesized silver nanoparticles. Phytochemical screening of *M. ferruginea* reveals the presence of alkaloids, glycosides, terpenoids, reducing sugars, saponins and phenolic compounds.

Improvements/Application: All the microorganisms are susceptible to silver nanoparticles but are resistant to the plant extract.

***Key words*:** *Mussaenda ferruginea,* antimicrobial activities, agar well diffusion assay, phytochemical screening.

1. Introduction

Nanotechnology is an important field for modern research dealing with design, synthesis, and manipulation of particle structure ranging from approximately 1-100 nm. Tremendous growth in this emerging technology has introduced novel, fundamental and applied frontiers, including the synthesis of nanoscale materials and exploration or utilization of their exotic properties in physical chemistry and optoelectronics [1]. To date, metallic nanoparticles are mostly prepared from noble metals i.e. Au, Ag and Pt but among them, Ag is the metal of choice in the biological method due to its unique size, shape and distribution by exhibition of improved properties [2,3]. Using plant extract to reduce Ag^{+} to Ag^{0} which is incorporated into phytochemical constituents of plant extract to fabricate silver nanoparticles (AgNPs) has been gaining much attention [4].

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. 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 are easily available, safe, and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions, and are quicker than microbes in the synthesis. Nano particles are being synthesized from a variety of medicinal plants [5,2,6,7].

Medicinal plant metabolites are the active part of constituent of medicinal plants. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs and antimicrobial drugs. Medicinal plants 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. These compounds are synthesized by primary or rather secondary metabolism of living organism. A large number of phytochemicals belonging to several chemical classes has been shown to have inhibitory effects on all types of microorganisms in vitro. The microorganisms removal efficiency has been studied extensively and the results are very encouraging to offer a technology at an affordable price tag to the third world countries, where the water borne diseases are a threat to their everyday life. The outline of the study has been published [8]. In Papua New Guinea as a tropical

country, there are different species of plants and quiet a good number of them are believed to be medicinal plants. In this study, *Mussaenda ferruginea*, *a* locally identified medicinal plant was used in the synthesis of silver nanoparticles. In this research, the active phytochemical constituent of *M. ferruginea* was identified and the antimicrobial study was also carried out with the silver nanoparticles of the plant *M. ferruginea*

2. Materials and Method

2.1 Test Bacteria

Fourteen microorganisms species are used in this study namely namely *Bacillus cereus (G+), Citrobacter freundii (G-), Enterobacter aerogenes (G-), Escherichia coli (G-), Klebsiella pneumoniae (G-), Micrococcus luteus (G+), Neisseria gonorrhoea (G-), Proteus vulgaris (G-), Pseudomonas fluorescens (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 *Mussaenda ferruginea*

Fresh healthy leaves of *Mussaenda ferruginea,* 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

The phytochemical constituents' tests were carried out for the nine important phytochemical's; Alkaloids, glycosides, flavonoids, tannins, reducing sugar, saponins, steroids and phenolic compounds from the extract of *Mussaenda ferruginea* using standard methods.

2.4 Synthesis of silver nanoparticles

Silver nanoparticles were synthesized using silver nitrate and *Mussaenda feerrugenia* 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.

2.5 Characterization/Analysis of silver Nanoparticles –UV-Visible spectroscopy

The reduction of the Ag+ ions by the extract of the *M. ferruginea* 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.

2.6 Antimicrobial test

The antibacterial activity of the synthesized silver nanoparticle was carried out on the 14 bacterial and 2 fungal 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. Results for phytochemical constituent's determination

Phytochemicals Test		**Plant extract Sample**
Phytochemicals	***Type of Test***	***Mussaenda ferruginea***
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 Mussaenda ferruginea and the two different concentrations, 3 mM and 6 mM silver nitrate solution.

Bacteria	**Gram Reaction**	**3mM $AgNO_3$ & extract**	**6 mM $AgNO_3$ & extract**	**Extract**	**Ca**	**Sa**
Bacillus cereus	G+	12mm	12mm	-	16mm	32mm
Citrobacter freundii	G-	12mm	-	-	10mm	0
Enterobacter aerogenes	G-	11mm	10mm	-	0	16mm
Escherischia coli	G-	11mm	9mm	-	12mm	10mm
Klebsiella pneumonia	G-	10mm	9mm	-	0	8mm
Micrococcus lutea	G+	11mm	11mm	-	6mm	10mm
Neisserria gonorrhea	G-	10mm	-	-	0	10mm
Proteus vulgaris	G-	14mm	12mm	-	20mm	10mm
Pseudomonas florescens	G-	13mm	9mm	-	20mm	18mm
Salmonella typhimurium	G-	12mm	12mm	-	0	0
Staphylococcus aureus	G+	9mm	9mm	-	14mm	8mm
Streptococcus pneumonia	G-	10mm	10mm	-	2mm	4mm
Trichomonas vaginalis	Protozoa	12mm	9mm	-	6mm	8mm
Candida albicans	Yeast	12mm	11mm	-	20mm	16mm

Ca – Chloramphenicol (Standard)
Sa – Streptomycin (Standard)
Extract – *Mussaenda ferruginea* water extract

3. Results

Table 1 below gives the phytochemical screening results of *Mussaenda ferruginea.* The table gives the name of the test, the phytochemical tested and the result. Results for the synthesis of the silver nanoparticle, the extract of *Mussaenda ferruginea* 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 brow colour confirms the formation of silver nanoparticles because silver nanoparticles exhibit this colour due to its Surface Plasmon Resonance. This was further confirmed with UV-Visible spectroscopy analysis. The UV-Visible spectroscopy analysis of silver colloid solution formed from the plant extract and silver nitrate was taken from the wavelength range of 300 – 700 nm. For 3mM and 6mM Silver nitrate used, the maximum peak was observed around 454nm and 425 nm respectively. Table 2 shows the anti-microbial activity of the synthesized silver nanoparticles. The results showed a little variation in the antimicrobial activity of silver nanoparticles synthesized from *M. ferruginea* . The nanoparticles exhibit the highest antimicrobial activity towards *Bacillus cereus* (15.00 ± 0.10 mm) whilst, the lowest antimicrobial activity (9.00 ±0.10 mm).

4. Discussion and conclusion

The reduction of silver ions from the silver nitrates to silver metal in colloid with the plant extract of *M. ferruginea* was visible through the change of colour of 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 [5, 2]. The formation of silver nanoparticles was also determined by UV-Visible spectroscopy. Measurement of wavelength of silver nanoparticles synthesized from different concentration of silver nitrates (3mM and 6mM) and the plant extract was taken between the wavelength ranges of 300 to 700 nm. The UV-Visible spectroscopy result shows that, the wavelengths of all the samples lie between 415 – 450 nm, which corresponds to the surface plasmon resonance effect of silver. This confirms the formation of silver nanoparticles.

The susceptibility of the test microorganism is related to the inhibition zone size in millimeters via agar well diffusion assay. The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulfhydryl) groups, although other target sites remain a possibility [9]. 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 [10]. Generally the all the microorganisms are susceptible to silver nanoparticles but are resistant to the plant extract.

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