Antimicrobial activity of synthesized silver nanoparticles and phytochemical screening of the aqueous extract of *Similax latifolia*

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Abstract

Background/Objectives *Similax latifolia* 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 *S. latifolia* in synthesizing silver nanoparticles and determine their antimicrobial activity.
Methods/Statistical analysis: Antimicrobial activity was carried out via Agar Paper Disk diffusion essay and measuring the zone of inhibition against 14 microorganisms.
Findings: All the microorganisms used were found to be highly susceptible to the synthesized silver nanoparticles. Phytochemical screening of *S. latifolia* reveals the presence of alkaloids, glycosides, terpenoids, reducing sugars, saponins and phenolic compounds.
Improvements/Application*:* The nanoparticle synthesized from the aqueous plant extract of *S. latifolia* proved to have the antimicrobial activity.

***Key words*:** *Similax latifolia*, antimicrobial activities, agar well diffusion assay, phytochemical screening.

1. Introduction

It has been witnessed that the advancements of nano drug delivery technologies can increase efficacy and safety, extend patent lives and provide competitive differentiation for biopharmaceuticals [1]. The majority of drug products are in solids, so that nanoparticles are expected to have a broad impact on drug product development. The phyto-based silver nanoparticles can be used in hospitals (eg. Surgical apparel, bedclothes, dressings, catheters), food industry (e.g., food containers), cosmetic, textiles (eg., sportswear, towels, carpets), mobile phones, household goods, water disinfection etc [2-6]

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, *Similax latifolia* locally identified medicinal plant which will be used in the synthesis of silver nanoparticles. In this research, the active phytochemical constituent of *S. latifolia* was identified and with the plant's extract was used with Silver Nitrate to synthesize silver nanoparticles. Presently, there is no study carried out on the biological property of this plant. Therefore this study was undertaken to determine the phytochemical constituents of *S. latifolia* and synthesizing silver nanoparticles from the plant extract as well as determining the antimicrobial activity of the synthesized silver nanoparticles.

2. Meterials and method

2.1 Test microorganism

Fourteen microorganism 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 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 *Similax latifolia*

Fresh healthy leaves of *Similax latifolia,* 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 *S. latifolia* using standard methods.

2.4 Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized using silver nitrate and *S. latifolia* 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 *S. latifolia* 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.

3. Results

Table 1 below gives the phytochemical screening results of *S. latifolia.* 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 *S. latifolia* 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 *S. latifolia.* 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).

Table 1. Results for phytochemical constituent's determination

Phytochemicals Test		**Plant extract**
Phytochemicals	***Type of Test***	***Similax latifolia***
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 *Similax latifolia* 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+	13mm	15mm	-	16mm	32mm
Citrobacter freundii	G-	15mm	14mm	-	10mm	0
Enterobacter aerogenes	G-	12mm	12mm	-	0	16mm
Escherischia coli	G-	9mm	10mm	-	12mm	10mm
Klebsiella pneumonia	G-	10mm	10mm	-	0	8mm
Micrococcus luteus	G+	11mm	10mm	-	6mm	10mm
Neisserria gonorrhea	G-	10mm	10mm	-	0	10mm
Proteus vulgaris	G-	14mm	13mm	-	20mm	10mm
Pseudomonas fluorescens	G-	9mm	9mm	-	20mm	18mm
Salmonella typhimurium	G-	12mm	12mm	-	0	0
Staphylococcus aureus	G+	10mm	10mm	-	14mm	8mm
Streptococcus pneumoniae	G-	10mm	10mm	-	2mm	4mm
Trichomonas vaginalis	Protozoa	9mm	9mm	-	6mm	8mm
Candida albicans	Yeast	12mm	12mm	-	20mm	16mm

Ca – Chloramphenicol; Sa – Streptomycin; Extract – *Similax latifolia* extract

4. Discussion

The reduction of silver ions from the silver nitrates to silver metal in colloid with the plant extract of *Similax latifolia* was visible by 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 [7, 8]. 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.

Disease causing microbes that have become resistant to drug therapy are an increasing public health problem. Therefore there is an urgent need to develop new bactericides. Silver nanoparticles take advantages of the oligodynamic effect that silver has on microbes3.Biosynthesis of silver anoparticles has already been reported as

clean, cost effective and non-toxic to environmental routes. Green synthesis offers improvement over synthetic, chemicals and micro-organisms [9]

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 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, all the microorganisms are susceptible to silver nanoparticles but are resistant to the plant extract.

5. Conclusion

The nanoparticle synthesized from the aqueous plant extract of *Similax latifolia proved to have the antimicrobial activity.*

6. References

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