Comparison of antimicrobial activities of silver nanoparticles synthesized from *Sphaerostephanos asplenioides J. Sm*

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Abstract

Background/Objectives: Green nanotechnology is generating attention of researchers toward ecofriendly biosynthesis of nanoparticles. In this study, the possible role of *Sphaerostephanos asplenioides J. Sm* extract in reducing silver nitrate into silver nanoparticle is highlighted.

Methods/Statistical analysis: A comparative study was made in preparing the silver nanoparticles using the boiling method and microwave irradiation method for the *S. asplenioides*. The synthesis of silver nanoparticles were prepared by adding silver nitrate solution [3mM] and [5mM] to the plant extract. The silver nanoparticles were characterized using the UV-Visible Spectroscopy.

Findings: The antimicrobial assay was carried out using the disc diffusion method which showed promising antibacterial effects against the 15 tested microorganisms. The phytochemical constituent determination shows the positive results expect for the test for terpenoids and test for the reducing sugar.

Improvements/Application: It can be concluded that both methods (Boiling Method and Microwave Irradiation Method) are effective in synthesizing of silver nanoparticles using the leaves extracts of plants.

Keywords: *Sphaerostephanos asplenioides* J. Sm, antimicrobial activity, Silver nanoparticles, Silver nitrate, microwave irradiation and boiling method.

1. Introduction

The recent past has witnessed the advancements of nano drug delivery technologies that can increase efficacy and safety, extend patent lives and provide competitive differentiation for biopharmaceuticals. The large size of most biopharmaceuticals, combined with their other molecular properties, lead to poor physical and chemical stability within the body and limited membrane permeability and severe toxicity when applied systematically [1]. Therefore researchers are developing a range of new delivery technologies and materials to enable these new drugs to be delivered intact to their target sites [2]. The majority of drug products are in solids, so that nanoparticles are expected to have a broad impact on drug product development [3-6].

Bio-inspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is cost effective and no need to use high pressure, energy, temperature and toxic chemicals [7].

Plant source can be the multi-resistant drug for microbes which can be produced by natural, non-toxic biological method of synthesis using silver nanoparticles (Ag NPs) [8].

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 [9].

Sphaerostephanos asplenioides belongs to the family of Ipteridaceae Marsh Fern. It possesses medicinal properties and act as medicine for treating fever and also used for treating fever. The phytochemical constituents present in the leaf extract are Alkaloids, Glycosides, Flavonoids, Tannins, Saponins and phenolic compounds.

In the present study, two methods of nanoparticle synthesis were compared synthesis by employing UV-Visible spectroscopy, for the reducing silver ions present in the aqueous solution of silver nitrate by the help of *S. asplenioides* J. Sm extract and Atomic Absorption spectroscopy for the determination of elemental silver present in the silver nitrate/plant extract. It also focuses on the use of leave extract of medicinally important plant, *S.*

asplenioides J. Sm as a template for silver nanoparticles synthesis and to exploit their medicinal importance in terms of antimicrobial activity.

2. Materials and Methods

2.1. Materials

For the synthesis of silver nanoparticles, *Sphaerostephanos asplenioides J. Sm* was collected from Bulolo District, Morobe Province, Papua New Guinea. The extract was used for reducing and capping agent. Silver nitrate was used at the Applied Sciences Department.

2.2. Preparation of boiled extract

Extract have been prepared by using fresh leaves of *S. asplenioides* weighing 60grams.Washed thoroughly thrice in distilled water, cut into fine pieces, transferred into a 500mL Erlenmeyer flask with 100mL of distilled water and boiled for 10 minutes. It was then filtered to obtain the plant extract.

2.3. Synthesis of nanoparticles from boiling method

3mM and 5mM solution of Silver nitrate ($AgNO_3$) was prepared and mixed 30mL of the plant extract with 80mL of 3mM and 5mM silver nitrate. Observe the formation of colour change and set wavelength (λ) maximum at using the UV-Visible spectrophotometer. Store the solution in room temperature for 24 hours for the complete settlement of nanoparticles. After 24 hours centrifuge the reaction mixture and discard the supernatant.

2.4. Synthesis of nanoparticles from microwave irradiation

3mM and 5mM $AgNO_3$ was prepared and mixed 30mL of aqueous solution of plant extract with 85mL of 3mM and 5mM silver nitrate in a 250mL Erlenmeyer flask. Place the beakers to the microwave irradiation at a frequency of 2.45GHz in a domestic Microwave oven (Sharp), at power output of 100W in a cyclic mode (on 15seconds, off 15seconds) to prevent overheating and the irradiation process was conducted for a minimum of 15 cycles. The colour change was checked periodically. Centrifuge at 10,000rpm for 15 minutes to obtain pellet. Obtain the pellets and add 9mL for the characterization of silver nanoparticles. Colour was change, and then quantitative characterizing was be done by UV-Visible spectrophotometer.

2.5. Analysis of Silver nanoparticles

2.5.1. UV-Vis Spectra Analysis for the boiled extract

The reduction of pure silver ions were observed by measuring the UV-Vis spectrum of the reaction at different time intervals taking 1ml of the samples, compared with 1 ml of distilled water used as blank. UV-Vis spectral analysis has been done by using Varian Cary-50 Bio UV-Vis Spectrophotometer at maximum wavelength of 200nm-800nm.

2.5.2. UV-Vis Spectra analysis for Microwave irradiated extract:

The irradiation process was conducted for a minimum of 5 up to maximum of 15 cycles. The reduction of Ag ions was monitored by sampling an aliquot (2 mL) of the solution after 5, 7, 9, 12 and 15 cycles and measuring the UV-Vis spectra of the solution. Absorption measurements were carried out similar to that of boiled extract. The samples were analysed by using the Varian Cary-50 Bio UV-Vis Spectrophotometer at maximum wavelength of 200nm-800nm.

2.6. Antimicrobial activity assay

The antibiotic sensitivity test was carried out by employing the disc diffusion method using the following tested microorganisms, *Bacillus cereus, Bacillus subtilis, Citrobacter freundii, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Neisseria gonorrhoea, Proteus vulgaris, Pseudomonas fluorescens, Salmonella typhimurium, Staphylococcus aureus, Streptococcus pneumoniae, Trichomonas vaginalis and Candida albicans*.

2.7. Elemental determination of silver by atomic absorption spectroscopy

The elemental determination of silver was determined by employing the Absorption Spectroscopy. Atomic absorption spectroscopy (AAS) was used to analyze the varying concentration of Ag^+ ions in the solution over a period

of time (Varian). The conversion of Ag^+ to Ag^0 can inferred with this measurement. During the course of the reaction at regular intervals, the aliquots of samples were withdrawn and centrifuged at 14,000–15,000 rpm so that the supernatant solution would contain the unreacted silver nitrate (Ag^+ ions) for the reason that Ag^+ ions are much smaller than Ag^0 and the pellets will contain the Ag nanoparticles (Ag^0). The supernatant solution was then analyzed by AAS to detect the amount of Ag^+ ions. The rate of decrease in the concentration of the Ag^+ ions depicts the conversion of Ag^+ to Ag^0. Deionized water was used in this procedure as the precipitation of silver is highly sensitive to the presence of Cl^-.

2.8. Phytochemical Constituents determination

The phytochemical screening determination carried out are; test for alkaloids, test for glycosides, test for flavonoids, test for tannins, test for reducing sugar, test for saponins,test for phenolic compounds and test for terpenoid and steroid.

3. Results and Discussions

3.1. UV-Visible Absorbance Studies

It is generally recognized that UV-visible spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspensions [10]. Here, leaf extract of *S. asplenioides* J. Sm changed the color of silver nitrate solution from transparent to dark yellow brown due to the reduction of Ag^+ ions to Ag^0. These color change arise because of the excitation of surface plasmon vibrations with the silver nanoparticles
[11].The surface plasmon resonance (SPR) peak centered at 440 nm and 445nm for boiling method (Fig 1and 2)and 450nm and 465nm for microwave irradiation method(Fig 3 and 4) affirmed the reduction of Ag^+ to Ag^0.

Figure 1.UV-Vis Spectrum of 3mM Sphaerostephanos asplenioides J. Sm AgNPs (Boiling Method)

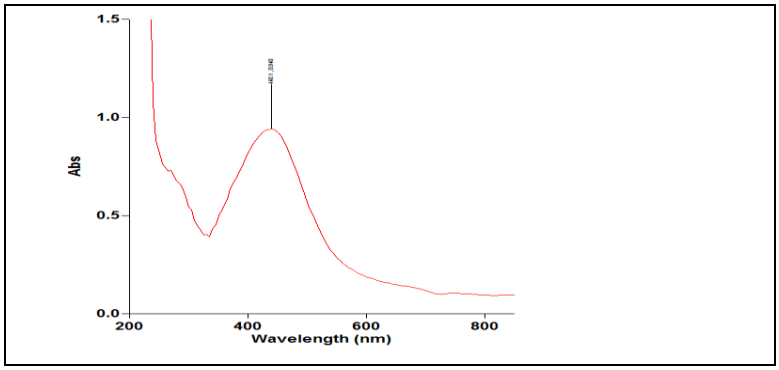


Figure 2.UV-Vis Spectrum of 5mM Sphaerostephanos asplenioides AgNPs (Boiling Method)

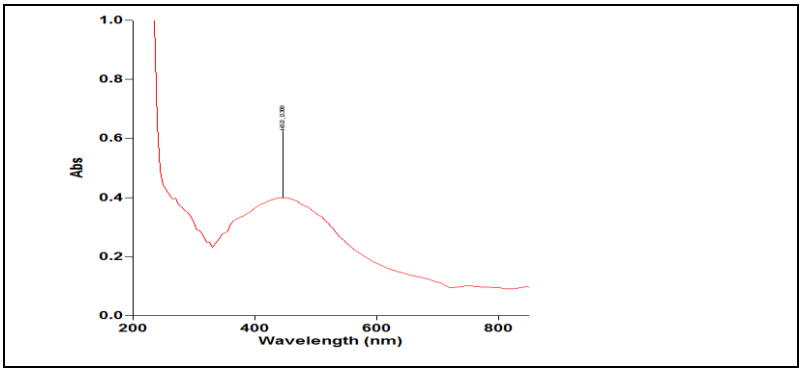


Figure 3.UV-Vis Spectrum of 3mM Sphaerostephanos asplenioides AgNPs (Microwave Irradiation Method)

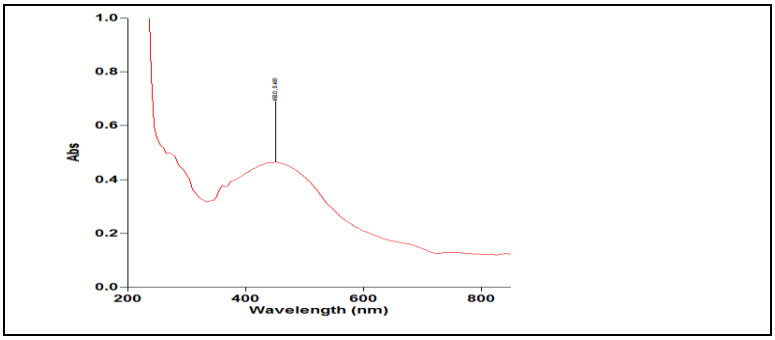
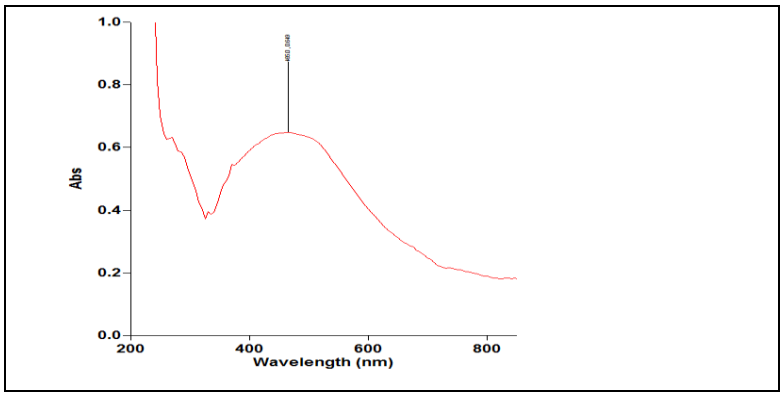


Figure4.UV-Vis Spectrum of 5mM Sphaerostephanos asplenioides AgNPs (Microwave Irradiation Method)



The microwave irradiation method expose more synthesized silver nanoparticles than the boiling method.The advantage of using microwave radiation is that it provides uniform heating around the nanoparticles and can assist the digestive ripening of such particles without aggregation. The microwave radiation heats up a material through its dielectric loss, which converts the radiation energy into thermal energy it can be observed that the silver surface plasmon band occurs at 450 and 465nm for 3mM and 5mM respectively.

3.2. Antimicrobial Activities Analysis

Antimicrobial activity of biosynthesized silver nanoparticles was analyzed against both gram-negative, gram-positive bacteria, protozoa(*T.vaginalis*) and yeast (*C.albicans*) at different concentrations (3mM and 5mM). Both the test microorganisms were found to be resistant for the aqueous extract of *S. asplenioides*. Results showed that these silver nanoparticles reveal a strong dose-dependent antimicrobial activity against both gram negative, gram positive microorganisms, protozoa and yeast. Table 1 & 2 below shows the inhibition zone (mm) of microorganisms tested. It was observed that the microbial growth decreases with the increase in concentration of biosynthesized silver nanoparticles.

Table 1.Maximum inhibitory zone (mm) for the boiling method

Microorganisms	3mM $AgNO_3$ Inhibition Zone	5mM $AgNO_3$ Inhibition Zone
C.freundii	10	25
P.vulgaris	12	13
P.florescens	15	15
S.typhimurium	10	12
C.albicans	10	12

Table 2.Maximum inhibitory zone (mm) for the microwave irradiation method

Microorganisms	3mM $AgNO_3$ Inhibition Zone	5mM $AgNO_3$ Inhibition Zone
B.cereus	16	17
P.florescens	11	12
S.typhimurium	13	12
C.albicans	11	12

3.3. Atomic Absorption Spectroscopy

The concentration (mg/L) of elemental silver present in silver nitrate/plant extract aqueous mixture for boiling method and microwave irradiation method are 24.3mg/L and 21.8mg/L respectively. Thus the presence of Silver in the synthesized Silver Nanoparticles was confirmed through characterization by the Atomic Absorption Spectrometer (AAS).

3.4. Phytochemical Screening Tests

The chemical constituents in the plants or crude extracts are known to be biologically active ingredients. Some chemical constituents are considered as secondary metabolites components. They are directly responsible for different activity such as antioxidant, antimicrobial, antifungal and anticancer.
The phytochemical constituents present in the plant extract(Table3) is indicated as under:
The plus sign (+) represents the phytochemical present while the minus sign (-) represents the absent of phytochemicals.

Table 3. Phytochemical Tests

Type of Screening Test	Plant aqueous extract (*S. asplenioides* J. Sm)
Test for alkaloids	+
Test for glycosides	+
Test for flavonoids	+
Test for tannins	+ (green black colour form)
Test for reducing sugar	-
Detection test for saponins	+
Test for phenolic compounds	+
Test for Terpenoid and steroid	-

4. Conclusion

The major advantage noticed is the time of reaction rate when irradiated with microwave. The extract of *Sphaerosphanos sp.* are capable of producing silver nanoparticles extracellular. Achievements of such a rapid time scales for the synthesis of nanoparticles by various methods of analysis increases the efficiency of synthetic procedures using environmentally benign natural resources as an alternative to chemical synthesis protocols at low cost. Also it was confirmed that the two methods proved to be similar in determining their characteristics. But the advantage of using microwave radiation is that it provides uniform heating around the nanoparticles and can assist the digestive ripening of such particles without aggregation. This rapid microwave heating also provides uniform nucleation and growth conditions, leading to homogeneous nanomaterials with smaller sizes.

5. References

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