

# Comparison of antimicrobial activities of silver nanoparticles synthesized from *Bridelia minutiflora* Hook. f. through Boiling method and Microwave irradiation method

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## Abstract

**Background/Objectives:** The synthesis of nanoparticles from biological processes is evolving a new era of research interests in nanotechnology. In this study, the possible role of *Bridelia minutiflora* Hook. F. (*B.minutiflora*) extract in reducing silver nitrate into silver nanoparticle is highlighted.

**Methods/Statistical analysis:** 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. The antimicrobial assay was carried out using the disc diffusion method.

**Findings:** A comparative study was made in preparing the silver nanoparticles using the boiling method and microwave irradiation method for the *B.minutiflora*. The results showed promising antibacterial effects against the 15 tested microorganisms. The phytochemical constituent determination shows positive test for terpenoids and reducing sugar.

**Keywords:** *Bridelia Minutiflora* Hook f, antimicrobial activity, Silver nanoparticles, Silver nitrate, microwave irradiation and boiling method.

## I. Introduction

Biological methods of synthesis have paved way for the “greener synthesis” of nanoparticles and these have proven to be better methods due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization [1]. 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 [2].

This has motivated an upsurge in research on the synthesis routes that allow better control of shape and size for various nanotechnological applications. There is an increasing demand for “green nanotechnology” for synthesis of stable nanoparticles. Microorganisms have already been reported as efficient bioreducing agent for metal nanoparticles [3]. Researchers are now focusing on use of plants and plant parts for green synthesis of nanoparticles considering the lower cost of production and easy handling as compared to the chemical routes. Using plant parts also reduces the issues related to elaborate microbial culture handling used in microbe mediated biosynthesis of silver nanoparticles [4].

Leaves are heated over a fire until soft and applied to a sore or ulceration. 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 *Bridelia Minutiflora* Hook. F. 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, *Bridelia Minutiflora* Hook f as a template for silver nanoparticles synthesis and to exploit their medicinal importance in terms of antimicrobial activity.

This 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 [5]

## 2. Materials and methods

### 2.1 Materials

For the synthesis of silver nanoparticles (*B. minutiflora*) 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 *Bridelia Minutiflora Hook f*, 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 ( $\text{AgNO}_3$ ) 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 ( $\lambda$ ) 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  $\text{AgNO}_3$  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 with 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

Atomic absorption spectroscopy (AAS) was used to analyze the varying concentration of  $\text{Ag}^+$  ions in the solution over a period of time (Varian). The conversion of  $\text{Ag}^+$  to  $\text{Ag}^0$  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 ( $\text{Ag}^+$  ions) for the reason that  $\text{Ag}^+$  ions are much smaller than  $\text{Ag}^0$  and the pellets will contain the Ag nanoparticles ( $\text{Ag}^0$ ). The supernatant solution was then analyzed by AAS to detect the amount of  $\text{Ag}^+$  ions. The rate of decrease in the concentration of the  $\text{Ag}^+$  ions depicts the conversion of  $\text{Ag}^+$  to  $\text{Ag}^0$ . Deionized water was used in this procedure as the precipitation of silver is highly sensitive to the presence of  $\text{Cl}^-$ .

## 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 [6]. Here, leaf extract of (*B.minutiflora*) 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 [7].

The surface plasmon resonance (SPR) peak centered at 435 nm and 440nm for boiling method (Figure 1 & 2) and 445nm and 450nm for microwave irradiation method (Figure 3 & 4). This affirmed the reduction of  $Ag^+$  to  $Ag^0$ . Also it can be seen that the absorption increases with increasing silver nitrate concentration (5mM).

Figure 1. UV-Vis Spectrum of 3mM *B. minutiflora* AgNPs (Boiling Method)

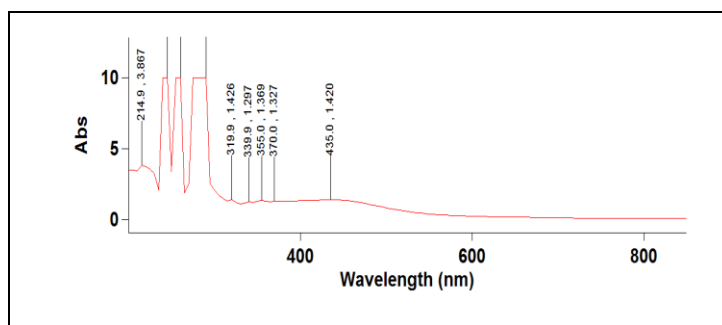


Figure 2. UV-Vis Spectrum of 5mM (*B. minutiflora*) AgNPs (Boiling Method)

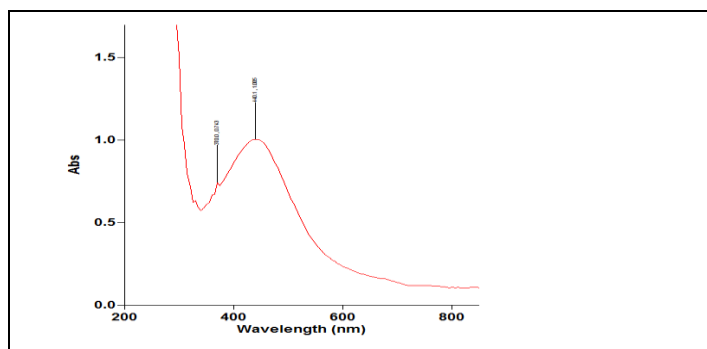


Figure 3. UV-Vis Spectrum of 3mM (*B. minutiflora*) AgNPs (Microwave Irradiation Method)

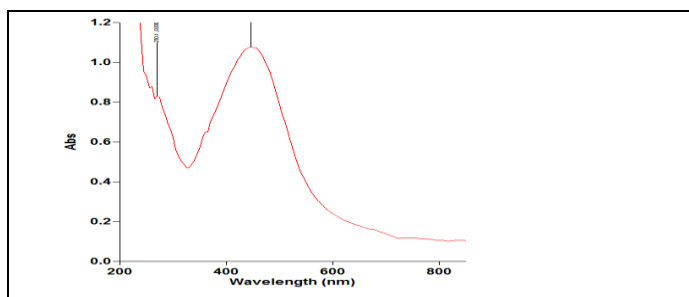
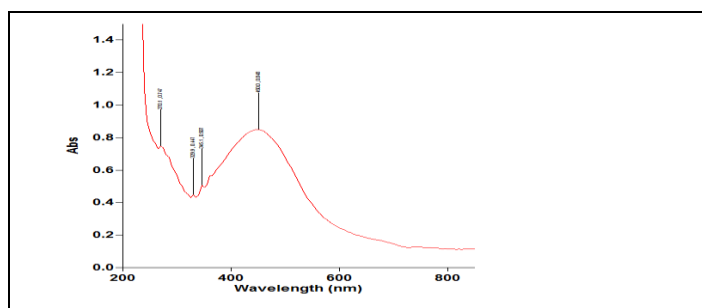


Figure 4. UV-Vis Spectrum of 5mM (*B.minutiflora*)AgNPs (Microwave Irradiation Method)



The microwave irradiation method exposes 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 435nm and 440nm (boiling method) for 3mM and 5mM and 445nm and 450nm (microwave irradiation method) for 3mM and 5mM.

### 3.2 Antimicrobial Activities Analysis

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 microbes. Biosynthesis of silver nanoparticles has already been reported as clean, cost effective and non-toxic to environmental routes. Green synthesis offers improvement over synthetic, chemicals and micro-organisms [8]. 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 (*B.minutiflora*). Results showed that these silver nanoparticles reveal a strong dose-dependent antimicrobial activity against both gram negative, gram positive microorganisms, protozoa and yeast.

The Table 1 & 2 show the inhibition zone (mm) of microorganisms tested using boiling method and irradiation method respectively.

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

Microorganisms	3mM Inhibition zone	5mM inhibition zone
<i>B.cereus</i>	13	12
<i>B.subtilis</i>	12	10
<i>E.aerogenes</i>	14	9
<i>E.coli</i>	11	9
<i>K.pneumoniae</i>	10	10
<i>M.luteus</i>	14	9
<i>P.vulgaris</i>	19	15
<i>P.fluorescens</i>	13	13
<i>S.typhimurium</i>	13	10
<i>S.aureus</i>	11	9
<i>S.pneumoniae</i>	10	11
<i>C.albicans</i>	12	13

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

Microorganism	3mM Inhibition zone	5mM Inhibition zone
<i>B.cereus</i>	42	26
<i>B.subtilis</i>	9	10
<i>E.coli</i>	10	11
<i>P.fluorescens</i>	10	12
<i>C.albicans</i>	15	13

### 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 were 19.6mg/L and 23.4mg/L respectively. Thus 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 are presented in table 3. 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 ( <i>B. minutiflora</i> )
Test for alkaloids	+
Test for glycosides	-
Test for flavonoids	+
Test for tannins	+ (green black colour form)
Test for reducing sugar	-
Test for saponins	+
Test for phenolic compounds	+
Test for Terpenoid and steroid -	-

## 4. Conclusion

The extract of *B. minutiflora* are capable of producing silver nanoparticles extracellular. The rapid microwave heating also provides uniform nucleation and growth conditions, leading to homogeneous nanomaterials with smaller sizes. It provides uniform temperature for the heating of the solution apart from being faster.

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