International Journal of Mathematics and Physical Sciences Research ISSN 2348-5736 (Online)
Vol. 4, Issue 1, pp: (90-95), Month: April 2016 - September 2016, Available at: www.researchpublish.com

Green Synthesis of Silver Nanoparticles and Their Antibacterial Activities of the Crude Extracts of Brucea Antidysenterica Leaves

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Abstract: **The aim of this study was to synthesize silver nanoparticle from the crude extracts of *Brucea antidysenterica* leaves in order to find possible sources for future novel antibacterial activity in pharmaceutical formulations. Silver nanoparticles were prepared by chemical reduction method and it was confirmed with the help of UV-Vis absorption spectroscopy and compared its antibacterial activity with 80%methanol crude extract. The synthesis of nanoparticles was confirmed by change in colour from yellowish to dark brown. Further, it was confirmed by UV-Vis spectrometer which the biosynthesis of silver nanoparticles. The peak is clearly visible around 438 nm which indicate the reduction of silver ions occurred due to the water-soluble phytochemicals present in the leaf extract. The synthesized silver nanoparticles have strong antibacterial activity against E.coli and S.aureus than standard and 80%methanol extract. A very low concentration of silver nanoparticle (0.008mg/mL) gave antibacterial performance. Applications of Ag nanoparticle based on these finding may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems. Leaf of *Brucea antidysenterica* can be used as source of silver nanoparticle to prevent diseases associated with antibacterial activity.**

Keywords: **antibacterial activity, *Brucea antidysenterica*, nanoparticle, E.coli.**

I. INTRODUCTION

The term “nanoparticles” is used to describe a particle with size in the range of 1nm-100nm, at least in one of the three possible dimensions. In this size range, the physical, chemical and biological properties of the nanoparticles changes in fundamental ways from the properties of both individual atoms/molecules and of the corresponding bulk materials [1].

Silver nanoparticles are of interest because of the unique properties (*e.g.*, size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles. The most popular chemical approaches, including chemical reduction using a variety of organic and inorganic reducing agents, electrochemical techniques, physicochemical reduction, and radiolysis are widely used for the synthesis of silver nanoparticles [2].

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. The main mechanism considered for the process is plant-assisted reduction due to phytochemicals. The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Flavones, organic acids, and quinones are water-soluble phytochemicals that are responsible for the immediate reduction of the ions. Phytochemicals are involved directly in the reduction of the ions and formation of silver nanoparticles [3]. The present work is also a part of this new line of research. In this study, the reducing agent comes from extracts of *Brucea antidysenterica*, which is a plant rich in alkaloids, quassinoids and polyphenols.

The exact mechanism which silver nanoparticles employ to cause antimicrobial effect is not clearly known and is a debated topic. There are however various theories on the action of silver nanoparticles on microbes to cause the

microbicidal effect. Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. There is formation of "pits"on the cell surface, and there is accumulation of the nanoparticles on the cell surface. The formation of free radicals by the silver nanoparticles may be considered to be another mechanism by which the cells die [4].

There have been electron spin resonance spectroscopy studies that suggested that there is formation of free radicals by the silver nanoparticles when in contact with the bacteria, and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death. It has also been proposed that there can be release of silver ions by the nanoparticles, and these ions can interact with the thiol groups of many vital enzymes and inactivate them. The bacterial cells in contact with silver take in silver ions, which inhibit several functions in the cell and damage the cells [5].

Then, there is the generation of reactive oxygen species, which are produced possibly through the inhibition of a respiratory enzyme by silver ions and attack the cell itself. Silver is a soft acid, and there is a natural tendency of an acid to react with a base, in this case, a soft acid to react with a soft base. The cells are majorly made up of sulfur and phosphorus which are soft bases. The action of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to cell death. Another fact is that the DNA has sulfur and phosphorus as its major components; the nanoparticles can act on these soft bases and destroy the DNA which would definitely lead to cell death. The interaction of the silver nanoparticles with the sulfur and phosphorus of the DNA can lead to problems in the DNA replication of the bacteria and thus terminate the microbes [6]. In this study, the results of the synthesized silver nanoparticles and antibacterial activity using extracts of the plant *Brucea antidysenterica* were discussed.

II. MATERIALS AND METHODS

Plant collection:

The leaf of *Brucea antidysenterica* was collected from local area of kerenwary, Motta Woreda, East Gojjam zone, Amhara region, Ethiopia which is approximately 18 km from Motta and 381Km from Addis Ababa in April, 2014 and was identified by abotanist, Dr. Ali Sied, Biology Department, Science Faculty, Bahir Dar University. A voucher specimen was deposited at Bahir Dar University Herbarium for further reference.

Sample preparation:

Crude extract preparation:

The collected fresh leaf of *Brucea antidysenterica* was cut into small bits to facilitate drying and air dried at room temperature for three weeks under a shed until it became well dried. The dry plant materials, leaf take separately and grind to a uniformly using an electric grinder to obtain a fine powder [7]. The ground plant material in the extracting solvent was placed on ice in a water bath for 1 h.

The powdered leaf (100g) was homogenization with 80% methanol (1000mL) placed on a shaker and soaked for 48 hours. The residue was filtered using a Buchner funnel under vacuum through Whatman No. 1 filter paper. Chlorophyll and water were removed using activated charcoal, and $MgSO_4$ respectively and concentrated using Böchi rotary vacuum evaporator under reduced pressure at 40^{o}C temperature to obtain the crude extract. All extracts were kept at−20 ◦C until antibacterial tests were performed.

For silver nanoparticles:

The dried (20g) *Brucea antidysenterica* leaves were cut into small pieces and boiled in a 250 mL Erlenmeyer flask along with 200 mL of distilled water for 10 min.

After boiling, the color of the aqueous solution changed from colourless to yellow color. The aqueous extract was separated by filtration with Whatman No. 1 filter paper and then centrifuged at 1,200 rpm for 5 min to remove heavy biomaterials.

Brucea antidysenterica leaf extract was stored at room temperature to be used for biosynthesis of silver nanoparticles from silver nitrate.

Procedure for silver nanoparticle determination:

One mM aqueous solution of silver nitrate was prepared for synthesis of silver nanoparticles. 1mL of this solution was added to 5 mL extract of the plant material with stirring magnetically at room temperature. At this stage, the color of the reaction mixture changes from yellow to dark brown and brown black suspended particles are produced, indicating the

formation of silver nanoparticles. The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 6000 rpm for 20 min. Different concentrations of silver nitrate were used to standardize the optimum concentration of silver nitrate for synthesis of silver nanoparticles. The concentrations ranged to 0.5mM, 1mM, 2mM, and 3mM of silver nitrate.

Characterization of silver nanoparticles:

The reduction of metallic Ag^+ ions was monitored by measuring the UV- Vis spectrum after about 48 hours of reaction. To determine the maximum production of silver nanoparticles, a small aliquot with different concentration was drawn from the reaction mixture and a spectrum was taken on a wavelength from 250nm to 600nm on UV-Vis spectrophotometer.

Procedure for antibacterial activity determination:

The comparative antibacterial activities of the 80%ME (80%methanol extract) and (AgNPs) Silver nanoparticles synthesized were effectively accessed against one Gram (+)ve (*staphylococcus aureus*) bacteria and one Gram (-)ve (*Escherichia coli* (E. coli)) bacteria as test microorganisms obtained from Amhara Regional Laboratory.

Disc diffusion method was followed for testing crude extract and their respective Silver nanoparticles containing solution. The ager medium which was cooled at 45 ^oC transferred four (two for each bacterium) Petri dishes equally. The dishes were kept at room temperature in a laminar hood. After the medium was solidified, three cups of ~0.5 cm diameter were made by a cork-borer in each Petri dish at about 1.5 cm away from the disk-wall [8].

A sterile cotton swab is dipped in to a standardized bacterial test suspension and used to evenly inoculate the entire surface of the nutrient agar plate. Using micro-pipette, fifty micro liters of each 80%ME, $AgNO_3$ solutions and AgNPs(250 mg/mL) was aseptically introduced into a respective agar well. *Ciprofloxacin* (25µg/mL) was used as positive controls and the extraction solvents methanol and distilled water were included as negative controls were laid down on the surface of inoculated agar plate. The plates were incubated at 37^{o}c for 24h.

Measurement of zone of inhibition:

After 24 hours of incubation at 37^oC, distinct zone of bacterial growth inhibition the antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the hole in millimeter. The zones of inhibition of the tested microorganism by the 80%ME and AgNPs synthesized were measured using a millimeter scale.

Determination of minimum inhibitory concentration (MIC):

The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial growth [9]. In agar diffusion, the 80%ME solution(500 mg/mL) was diluted to bring 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.63 mg/mL, 7.81 mg/mL, 3.95 mg/mL and 1.95 mg/mL concentrations were used to determine MIC. Using the same way, various concentrations of the synthesized AgNPs stock solutions, 0.500mg/mL, 0.250mg/mL, 0.064mg/mL, 0.032mg/mL, 0.016mg/mL, 0.008mg/mL and 0.004mg/mL were assayed against the test bacteria.

The inhibition zone was measured after 24 h incubation at 37^oC and the minimum concentration that inhibited growth was considered as MIC value of the extract.

III. RESULTS AND DISCUSSION

Silver nanoparticles:

Reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of the color change. For all the $AgNO_3$ concentrations, the samples changed their visual appearance shortly after addition of the plant extract, indicating that a reduction reaction took place. Initially, the reacting mixture was a slightly yellowish liquid; as the reaction proceeded, the solutions became dark brown. This is a strong indication of the formation of silver nanoparticles: the change in color is due to the strong absorption of visible light due to excitation of the nanoparticle surface plasmons [10].

Fig. 1, showed the visual appearance of test tubes containing the *Brucea antidysentrica* extracts and $AgNO_3$ solution after 48 hours. The test tubes correspond to different $AgNO_3$ concentrations: (a) pure extract, (b) 0.5 mM, (c) 1 mM, (d) 2 mM, and (e) 3 mM. The change in color is an indication of the presence of silver nanoparticles.

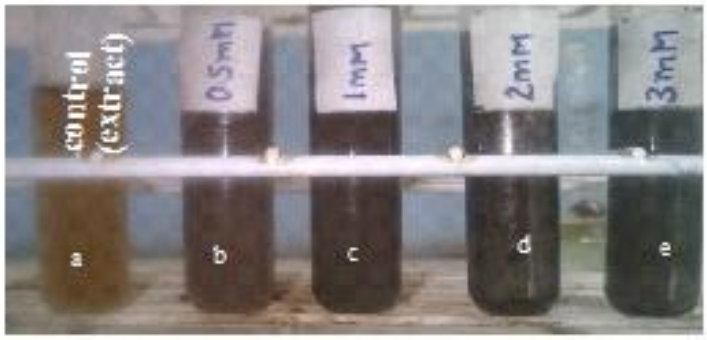

Fig.1: visual appearance of test tubes containing the Brucea anti dysenterica ex tracts and $AgNO_3$ solution after 48 hours

In the reacting samples for different $AgNO_3$ concentrations (0.5mM, 1mM, 2mM, and 3mM,), after the reaction started (6, 12, 24, 48h); the clear time evolution is a signal of the growth of silver nanoparticles. Then after 48h the beginning of the reaction the change in color, and thus the formation of silver nanoparticles, was confirmed by the UV-Vis experiments. The UV-Vis confirms the presence of reductants in the *Brucea antidysntrica* extract. The absorbance of the sample presented a peak in the range of 250 to 300 nm as characteristic of different phytochemicals which serve as reducing agent for the nanoparticle synthesized [11]. The same molecular mechanisms that give antioxidant properties to these molecules must promote the reduction of Ag^+ ions to Ag atoms. The main mechanism is hydrogen abstraction due to the OH groups in the reductants molecules. Silver nanoparticles were prepared using the *Brucea antidysntrica* extracts as reducing agent [12].

Fig. 2, showed UV-Vis absorbance for sample with different values of $AgNO_3$ concentrations. (a) Pure extract, (b) 0.5 mM, (c) 1 mM, (d) 2 mM, and (e) 3 mM, the peak around 438 nm corresponds to the absorbance due to surface plasmons in the silver nanoparticles. Note that peak intensity increases with the $AgNO_3$ concentration and that the absorption due to the reducing agent from the extract is observed around 278 nm. The spectra are showed for a reaction time of 48h (Fig. 2). The curves display a pronounced peak around 438 nm, as expected from the plasmon resonance of silver nanoparticles. The UV-Vis peak is more pronounced for higher $AgNO_3$ concentrations, indicating that more nanoparticles per unit volume are formed when this concentration increases. Note that in all the spectra displayed in fig. 2, the peak (observed in the *B. antidysentrica* extract) is also clearly visible around 438 nm which indicate the reduction of silver ions occurred due to the water-soluble phytochemicals present in the leaf extract including quassinoids, alkaloids, triterpenoids, flavonoids and others. The UV-Vis spectra (not observed) of the $AgNO_3$ solution has a peak around 217 nm, as expected for Ag^+ ions. Therefore, the leaf of *B. antidysentrica* can be used for the synthesis of nanoparticle.

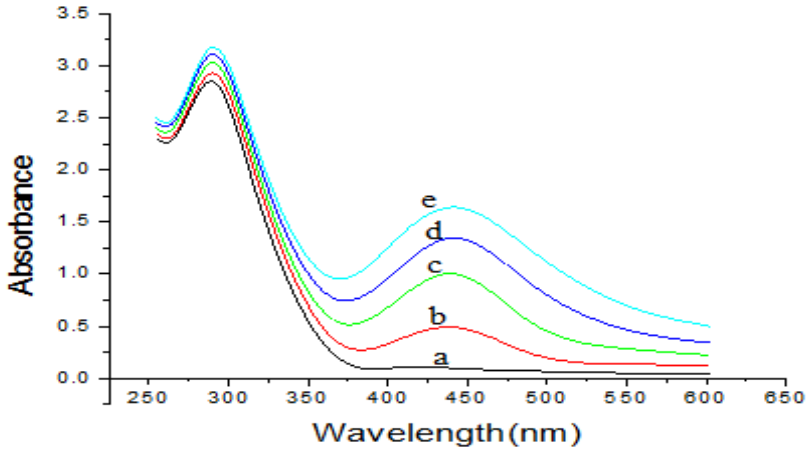

Fig.2: UV-Vis absorbance for sample with different values of $AgNO_3$ concentrations.

Antibacterial activity:

The antibacterial activity of 80%methanol extract and AgNPs were determined by agar well diffusion method after applying the samples solution into well of the inoculums and incubating for 24h at 35^0C, the formation of inhibition zones(clear transparent regions) around the well was observed. The diameter sizes in mm of the zone of inhibition are shown in the Table 1.

TABLE.1: Mean bacterial growth inhibition zones in agar well diffusion method treated with 50 µl of samples (mm).

Samples	Concentration(µl)	inhibition zone(mm)	
		Escherichia coli(mm)	*Staphylococcus auerus*(mm)
80%ME	50	9	7
AgNPs	50	22	17
$AgNO_3$	50	≤ 6	≤ 6
Standard (*Ciprofloxacin*)	50	13	9

Values were expressed as Mean. 80%ME = 80% methanol extract, AgNPs= silver nanoparticles.

The diameter of inhibition zones (mm) around each well with silver nanoparticle solutions and 80%methanol extract are represented in Table 1. The highest antimicrobial activity was registered in silver nanoparticles synthesized against *E. coli*(22 mm) compared to the standard than 80%methanol extract. The lesser antimicrobial activity of the sample was found in 80% methanol extract against *S. aureus* (7 mm) and E. coli (9 mm) but it showed good activity.

TABLE.1: Minimum inhibition concentrations (MIC) of the tested silver particles samples and crude extract.

Sample	Minimum inhibition concentration	
	Bacteria	
	E. coli	*S.aureus*
80%ME	17.81mg/Ml	31.25mg/mL
AgNPs	0.008mg/Ml	0.016 mg/mL

80%ME = 80% methanol extract, AgNPs= silver nanoparticles.

The minimum inhibitory concentration (MIC) was read after 24 h of incubation at 37°C. The MIC was determined as the lowest concentration that inhibited the visible growth of the used bacterium. MIC values were obtained for the synthesized nanoparticles and 80% methanols extract tested against *E. coli* (Gram negative bacteria) and *S. aureus* (Gram positive bacteria). From Table 2, it was found that the MIC (mg/mL) for silver nanoparticles is 0.008mg/mL, and for 80%methanol extracts17.81mg/mL against *E. coli*. The MIC (mg/mL) for silver nanoparticles is 0.016mg/mL, and for 80%methanol extracts 31.25mg/mL against *S. aureus*. Silver nanoparticles showed the better antibacterial activity against *E. coli* and *S. aureus* compared to 80%methanol extract, since the MIC value of silver nanoparticle lower than 80%methanol extract against *E. coli* and *S. aureus*. The MIC of all samples is lower when tested against *E. coli* than when tested against *S. aureus*.

These results can be explained on the basis of the differences on the cellular wall of each strain; the cellular wall for gram-positive strains is wider than the cellular wall for gram-negative strains [13].

The AgNPs were synthesized successfully from this plant which exhibited good antibacterial activity against both *E. coli*, and *S. auerus*. Comparing with crude extract, AgNPs exhibited higher antimicrobial activity than the use of crude extract alone. The exact mechanism for strong antimicrobial activity of the AgNPs is still in debate. But several hypotheses mention that AgNPs may attach to the surface of the cell membrane leading to disturbance of permeability and respiration functions of the cell. The strong antimicrobial activity depends on the large surface area of the nanoparticles which give more surface area for interaction with the organisms than available with those for large particles. Moreover, it is possible that AgNPs not only interact with the surface of membrane, but also can penetrate inside the bacteria. Other reported mechanisms are: uptake of free silver ions followed by disruption of adenosine triphosphate production and DNA replication, formation of reactive oxygen species and direct damage to cell membranes [14].

The antimicrobial effect of hydro-alcoholic extract against these organisms may be due to the ability of the methanol to extract some of the active properties of the genus *Brucea* plants like nigakilactones, alkaloids, triterpenoids, flavonoids and other secondary metabolites which are reported to be antimicrobial [15]. The selected plant species has been used in traditional medicine, so for this the leaf of plant has not been tested to antimicrobial activity.

Therefore, this work supports the medicinal values of *Brucea antidysntrica*s and also revealed that a simple, rapid and economical route to synthesis of silver nanoparticles; and its capability of rendering the antimicrobial efficacy. Moreover the synthesized AgNPs enhance the therapeutic efficacy and strengthen the medicinal values of this plant.

IV. CONCLUSION

The bio-reduction of silver ions from the extract promotes the formation of nanoparticles at room temperature with a fast kinetics and with no harmful chemicals. The method is easy to perform in a single step. UV/Vis spectra show the characteristic plasmon absorption peak for the silver nanoparticles ranging from 400 to 450 nm. The synthesized silver nanoparticle has good antibacterial activity against *E.coli* and *S.aureus* than standard and 80%methanol extract. It is confirmed that silver nanoparticles are capable of rendering antibacterial diseases. In the finding of this study support that *Brucea antidysntrica*s plant is a promising source of synthesis of nanoparticle for utilization in pharmaceutical fields as reducing agent. Applications of Ag nanoparticle based on these finding may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems. From the technological point of view these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

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