Phytotoxic and biomedical activities of synthesized Silver nanoparticles of *Morinda citrifolia* (Noni) leaves.

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Abstract

The synthesis of silver nanoparticles (AgNps) using aqueous extract of noni (*Morinda citrifolia*) seed as the reducing/capping agent was investigated for its anticoagulant, thrombolytic and phytotoxicity activities. The AgNps synthesis was monitored through the colour change 22 minutes at photo activation which turned out to be light brown in colour, then further characterized using UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), EDX and scanning electron microscopy (SEM). The UV-visible spectrum of the AuNPs displayed clear peak at 440.0nm, the prominent peaks of AgNps in FTIR spectrum were 3456.55, 2360.95, 2000.25 and 1637.62 cm^{-1} which show that proteins and phenolic compounds were involved in the forming and capping of the AgNPs. Energy dispersive X-ray (EDX) analysis showed that silver was prominent.

The AgNps showed anticoagulant activities of 49%, at 15µg/ml, and thrombolytic activities of 46%. The biomedical and phytotoxicity properties of the AgNps have established the medical, agricultural and economic importance of the AgNps.

Keyword: *Morinda citrifolia*, Phytotoxic, biomedical, Silver nanoparticles

Introduction

Plants are known for their nutritional and medicinal uses in which *Morinda citrifolia* (Noni) is a part of. Noni has been extensively used in folk medicine for over 2,000 years. It has been reported to have broad therapeutic effects, including cancer activity, in both clinical practice and laboratory animal models [1]. Noni has traditionally been used for colds, flu, diabetes, anxiety, and high blood pressure, as well as for depression and anxiety. The green fruit, leaves, and root/rhizomes were traditionally used in Polynesian cultures to treat menstrual cramps, bowel irregularities, diabetes, liver diseases, and urinary tract infections [2]. The mechanism for these effects remains unknown. The hypothesis that *Morinda citrifolia* possesses an antifungal preventive effect at the initiation stage of carcinogenesis was studied [3]. The advent of green technology into the synthesis of nanoparticles has greatly revolutionized the field of nanotechnology. Firstly, it has opened up the possibility of using biomolecules/substances of diverse origin in its synthesis and secondly, it has widened its applicability in different areas of human endeavours. Utilization of green synthesized nanoparticles transverses medical and biomedical applications to solving environmental problems such as land and water pollution, through material engineering to applications in agriculture. Quite a number of biological macromolecules/substances have been employed as capping and stabilizing agents for the green synthesis of nanoparticles [5]. Hence, in the present investigation, green synthesis of silver nanoparticles using the seed extract of *Morinda citrifolia*, its phytotoxicity and biomedical applications are presented and discussed.

Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. The need for biosynthesis of nanoparticles rose as the physical and chemical processes were costly. Often, chemical synthesis method leads to presence of some of the toxic chemical absorbed on the surface that may have adverse effect in the medical applications [4,6]. This is not an issue when it comes to biosynthesized nanoparticles via green synthesis route. So,

in the search of cheaper pathways for nanoparticles synthesis, scientist used microbial enzymes and plant extracts (phytochemicals). With their antioxidant or reducing properties they are usually responsible for the reduction of metal compounds into their respective nanoparticles. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method, there is no need to use high pressure, energy, temperature and toxic chemical [15, 17, 21]. The present study investigates the green synthesis of silver nanoparticles from *Morinda citrifolia* seed and screen its phytotoxic and biomedical applications.

Plate1: *Morinda citrifolia* Plant

Materials and Methods

Sample collection and identification

Morinda citrifolia fruits were obtained from a garden in Ibadan, Oyo State by plucking and were examined closely to ensure no sign of infections before conveying to the laboratory in sterile polythene bag. It was identified by Professor A. T Ogunkunle, a taxonomist in the Department of Pure and Applied Biology of Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Preparation of sample

The fruits were split open and the seeds were extracted. The seeds were washed with distilled water, drained aseptically, air dried and pulverized after two weeks of drying. 1g of the seed powder was weighed and dissolved in 300 ml of distilled water, and heated in water bath at 60 ^{0}C for 1 hour. The solution was filtered

using Whatman No. 1 filter paper. The filtrate was centrifuged at 4000 rpm for 15minutes, the supernatant was decanted and stored for further use.

Preparation of silver nitrate ($AgNO_3$) solution

The silver nitrate solution was prepared by dissolving 0.17g (1mM) of silver nitrate in 1000ml of de-ionized water. It was stored in an amber bottle and kept in a dark cupboard at room temperature.

Synthesis of silver nanoparticles

Two millilitre (2ml) of the sample (supernatant) was drawn using a sterile hypodermal syringe into a slant bottle and was labelled accordingly. After which 20ml of silver nitrate ($AgNO_3$) was added into the bottle containing 2ml of the supernatant (1:10) and was labelled. The resultant solution was photo-activated by exposure to direct exposure to sunlight until a stable colour change is observed/formed. Which is an evidence of formation of silver nanoparticles. Solution of silver nitrate ($AgNO_3$) alone is used as the control.

Characterization of silver nanoparticles

Fourier Transform Infra-red (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopic analysis was carried out on the powdered form of silver nanoparticles sample using an IR Affinity –IS Spectrometer (LAUTECH, Research laboratory) according to the method of Lateef [7]. 5ml of silver nanoparticles solution was poured into a glass petri dish and was transferred into an oven at a temperature of 40^0C for 4hours in other to change from the liquid to solid state. The solid residue obtained was cooled down at room temperature.

UV-Visible spectrum analysis

Ultraviolet-Visible (UV-Vis) spectroscopy or ultraviolet visible spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles using CCECIL CE model (LAUTECH Research Laboratory). Four milliliters of the synthesized silver nanoparticles were poured into slant bottle and readings were taken on a spectrophotometer at a wavelength of 650nm and bandwidth of 2.0nm. SEM and EDX (plate 4 and figure 3) analyses were

carried out to further characterize the nanoparticles.

Phytotoxicity Screening

The seeds used for phytotoxicity test were healthy beans and healthy maize. In order to show phytotoxic activities of the silver nanoparticle, different concentrations of the silver nanoparticle solutions were prepared (250, 500, 750 µl/ml and absolute) and applied to check germination of both root and shoot of the germinating seeds [13]. Seeds of maize and pea were collected from local market (Wazo market, Ogbomosho Oyo State Nigeria). The assay seeds were sorted for uniformity of size and all damages seeds were discarded. The bioassay seeds were sterilized using NaCl (10% v/v) for 10min, followed by several washes in sterile distilled water. For testing the phytotoxicity, aqueous silver nitrate, the crude seed extracts were used as controls. Bioassay was carried out using petri dishes (90mm diameter) over laid with cotton wool as support.

Sterile distilled water (5ml) was added to the cotton wool in the petri dish and dried completely in a vacuum at 40^0C. Five seeds from each were placed on the cotton wool in a circular pattern and incubated for 7 days at 25^0C in the dark using prepared nanoparticles solution of concentrations 250 µl/ml, 500 µl/ml, 750 µl/ml. The treated seeds of maize and pea were allowed to germinate on a mat of moist cotton wool by addition of 5ml of distilled water daily for 7days. After seven days of incubation the length of roots and shoots of germinated seeds were measured in centimetre (cm). Treated experimental sets were compared with that of control sets where no examined solution was used to treat the seeds. Each experiment was repeated in triplicate [13,14

Anticoagulant and thrombolytic activities of synthesized silver nanoparticles

The anticoagulant activities of the silver nanoparticles were investigated as earlier described by Lateef [9,10]. Exactly 100 µl of the silver nanoparticles prepared as 100 µg/ml were put in sterile Eppendorf tube and 0.5 ml of blood freely provided by a healthy donor was added, while blood collected into EDTA and blood collected in clean plain tube served as positive and negative control respectively. In addition, blood samples were treated with the Noni seed extract and aqueous silver nitrate solution. All the set up were held at room temperature (30 ± 2 °C) for 30 min, and

thereafter examined for anticoagulation. Subsequently, smears prepared from the samples were examined under Olympus microscope to observe the morphology of red blood cells [10, 11].

The thrombolytic activity was determined using the methods of Devi [7]. In this case, thrombolytic activity was quantified [10]. Eppendorf tubes containing 0.5 ml of blood were held at 37 °C for 30 min to allow the blood to clot. The weight of clean tube (W1) was subtracted from the weight of tube and blood clot (W2) to obtain the weight of blood clot (W3). Thereafter, 100 µl of the silver nanoparticles, silver nitrate solutions, and noni seed extract were added to each tube containing the blood clot. These were incubated at 37 °C for 90 min, and then inverted to check for clot lysis. The lysed clot was drained, and the weight of the tube with remaining clot was taken (W4) to obtain the weight of clot that was not lysed (W5). The percentage thrombolytic activity was obtained as: The lysed blood clot was also examined microscopically as previously described to observe the morphology of red blood cells [7,10],

Results and Discussion

The sample (whole fruit) was separated into Husk and seed after which the seed was washed with sterilized distilled water, drained aseptically and air dried and blended aseptically after two weeks of drying (Plate 2). The powder was obtained and was stored in screw cap bottle at room temperature. The formation of silver nanoparticles (AgNPs) was visually observed by checking the colour change. The nano particles synthesized from noni seed extract changed from very light orange to deep brown colour after about five minutes when exposed to direct sunlight. After 22 minutes, a stable brown coloration was observed. The control (silver nitrate solution without any sample) remained colourless throughout the photo-activation stage. (Plate 2)

The UV-visible spectrum of the nanoparticles synthesized from Noni seed extract displayed maximum absorbance at the wavelength of 400nm for AgNPs as shown (Figure 1).

The FTIR absorption spectrum of the synthesized silver nanoparticles (AgNPs) showed distinct strong peak at 3456.55, 2941.54, 2360.95, 2000.25 1453.23,1354.94, 1172.76 and prominent peaks at 2978.9,

875.5,788.6, and 1393.4 other minor peaks at 1456.23, and 1364.94 cm $^{-1}$ (Figure 2)

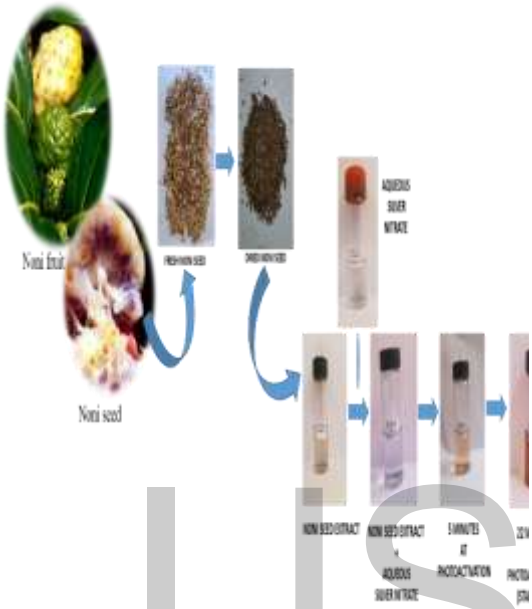

Plate 2: Synthesis of silver nano particles of Noni seed

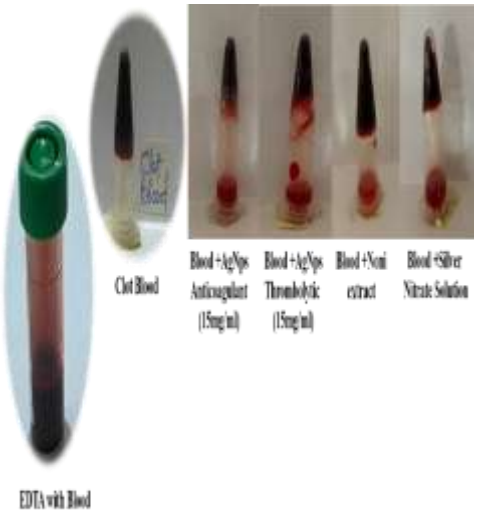

Plate 3: Anticoagulant and thrombolytic activity of Ns AgNPs

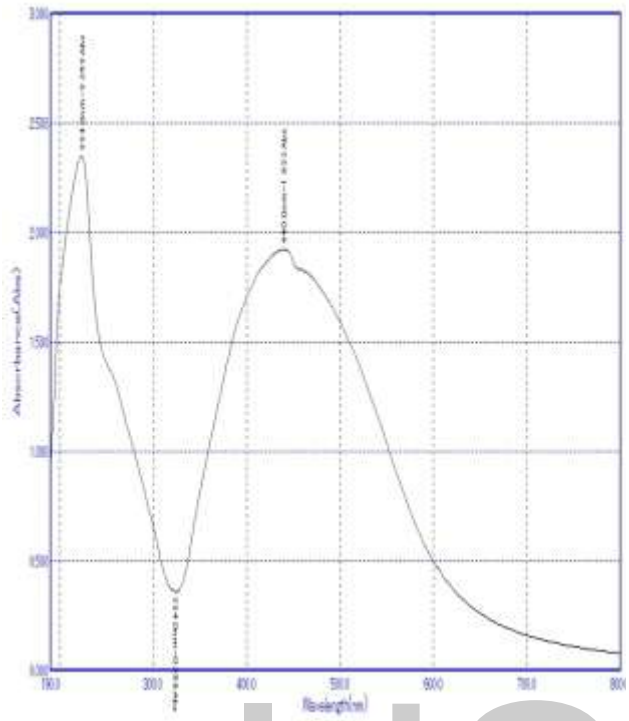

Figure 1: UV-Visible spectrum data of NS AgNPs

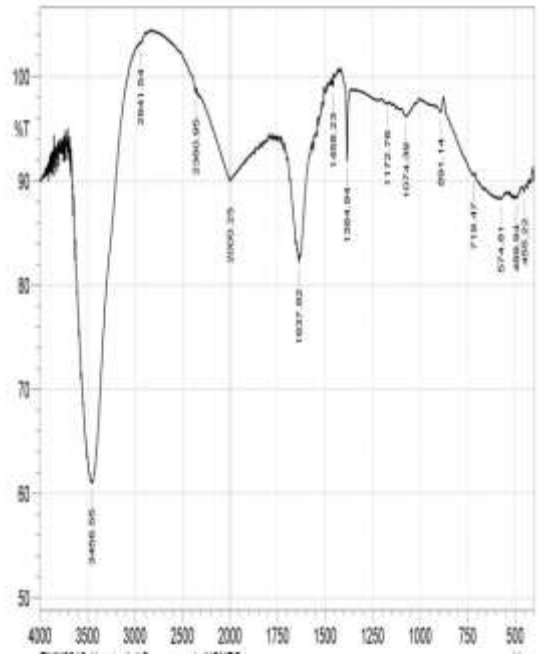

Figure 2: FTIR spectrum data of NS AgNPs

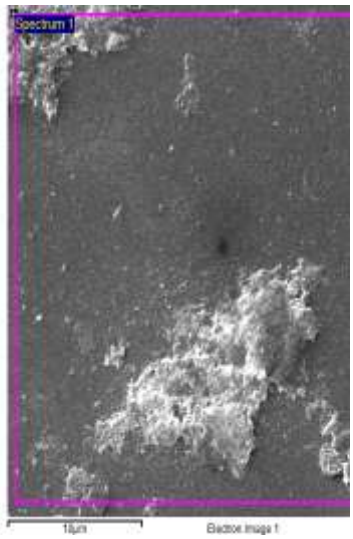

Plate 4: The SEM Micrograph of the synthesized silver nanoparticles

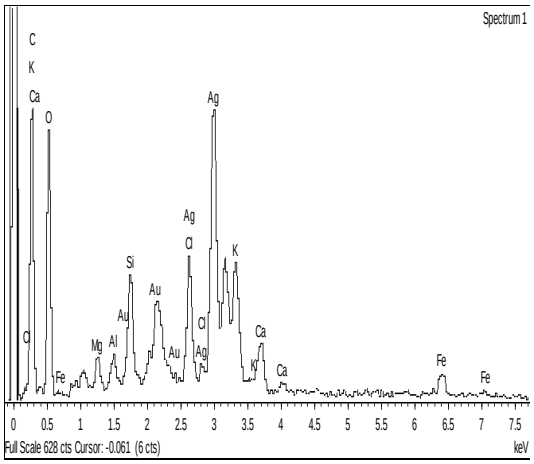

Figure 3: The EDX Micrograph of the synthesized silver nanoparticles

Formation of clots was inhibited by the addition of the biosynthesized nanoparticles to fresh blood of healthy human donor as observed in the positive control (EDTA) whereas coagulation was noticed in the negative control. The silver prevents formation of coagulation (blood clot) when used as anticoagulant when compared to the positive control using EDTA (Plate 3). In the table 1, the phytotoxicity of the AgNps synthesized was evaluated in four concentrations; 250, 500, 750 and 1000 µg/ml, the control used was the crude seed extract and aqueous silver nitrate ($AgNO_3$).

The biosynthesis of AgNPs was mediated by the extract of Noni seed within 22 minutes under exposure to direct sunlight. The deep ruby red colour of biosynthesized AgNPs has been extensively reported by [9] The appearance of deep ruby red colour shows the formation of Ag nanoparticles [17] ,[20] and [19] reported variation in the colour of AgNPs such as ruby brown, red pinkish, yellowish and purple due to the composition of bioactive molecules responsible for the synthesis of the nanoparticles which lend credence to the coloration i.e. brown colouration of the one synthesized in this study.

Table1: Phytotoxicity assay of NS AgNPs

Extract (µl/ml)	Maize		Bean	
	Shoot	**Root**	**Shoot**	**Root**
250 NPs	13.80 ± 0.92^{ab}	18.73 ± 1.83^{c}	22.15 ± 0.55^{b}	21.80 ± 0.20^{b}
500 NPs	12.43 ± 1.59^{a}	15.93 ± 0.48^{bc}	7.45 ± 4.35^{a}	7.45 ± 4.35^{a}
750 NPs	10.23 ± 1.11^{a}	16.23 ± 1.34^{bc}	19.10 ± 0.10^{b}	19.10 ± 0.10^{b}
1000 NPs	17.10 ± 1.10^{bc}	16.15 ± 1.13^{bc}	19.55 ± 0.35^{b}	19.55 ± 0.15^{b}
NSE	19.63 ± 0.58^{c}	12.23 ± 1.12^{b}	19.00 ± 1.00^{b}	22.50 ± 0.50^{b}
SN	17.90 ± 0.10^{bc}	4.90 ± 0.10^{a}	21.00 ± 1.00^{b}	3.25 ± 0.25^{a}

Values were expressed as mean±SEM and considered significant at $p<0.05$. Values with different superscript along the same column are significantly different

NSE: Noni seed extracts of each category

SN: Silver nitrate

Characterization was carried out to monitor the formation of the silver nanoparticles through UV-Visible spectroscopy analysis and Fourier Transformed Infrared Spectroscopy (FTIR). UV-Vis spectroscopy or ultraviolet-visible spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticle using Spectrophotometer. This means it uses light in the visible and adjacent near-UV and near-infrared (NIR) ranges. The absorption or reflectance in the visible range directly affects the perceived colour of the chemicals involved. The result of UV-Visible provides an insight into the size, distribution, surface properties and optical properties of nanoparticles; as the wavelength of peak absorption depends upon several factors such as particle size, di-electric constant of surrounding media and the inter-particle distance [9]. The UV-visible spectrum of the synthesized silver nanoparticles (AgNPs) displayed maximum absorbance at the wavelength of 420 and 415nm respectively. The values obtained are within the range (390-470nm) established for AgNPs [10]. Also, the values obtained for AgNPs is within the range (400-525nm) reported by [9,10], [18].

FTIR spectroscopy is a highly diverse molecular spectroscopy technique for polymer testing and pharmaceutical analysis, the application of the technique is virtually limitless offering both qualitative and quantitative analysis of a wide range of organic and inorganic samples. FTIR is able which one could distinguish the small absorption and of functionally active residue from the large background absorptions of the entire protein [10]. FTIR spectroscopy is frequently used to find out whether biomolecules are involved in the synthesis of nanoparticles, which is more pronounced in academics and industrial research [15]

Fourier transform infrared (FTIR) spectroscopic analysis was carried out on the powdered form of AgNps sample using an IR Affinity –IS Spectrometer according to the method of [9]. For AgNps, the broad peaks are 3456.55, 2941.54, 2360.95, 2000.25 and 1453.23, 1354.94 and 1172.76 this relate to N-H bond of amines, C=C stretch of alkenes or C=O stretch of amides (Shankar *et al*., 2014). Also, the broadness of the band of the band may be as a result of overlap of both O-H and N-H bond stretching of 1^{o} and 2^{o} amines. The prominent peaks at 2978.9, 875.5,788.6, and 1393.4 and other minor peaks at 1456.23, and 1364.94 cm^{-1} were ascribed to the 3^{o} O-H vibration of alcohols, aromatic nitrogen compounds, N-H bend of 1^{o} amine and C=C stretch of alkynes. It was apparent from these bands that biomolecules rich in amines (N-H) and hydroxyl (O-H) from the seed extract were accountable for the reduction in Ag^{+}, as well as capping and stabilization of AgNPs [23]

The synthesized silver nanoparticles showed anticoagulation properties as the formation of clot was inhibited by their addition to fresh blood. The results obtained are in conformity with anticoagulant potentials of nanoparticles synthesized from

and chemical analysis method using SHIMADSU: IR Affinity-IS-USA. FTIR is frequently used to provide accuracy, reproducibility and also a favourable signal-to-noise ratio. By using FTIR spectroscopy, it become possible to detect small absorbance changes on the order of 10^{-3}, which helps to perform spectroscopy,

diverse biomolecules as previously reported by [8-10]. The AgNPs showed thrombolytic activities of 53.3% respectively. Therefore, the thrombolytic activities of the synthesized nano nanoparticles showed fair thrombolytic properties. Furthermore, AgNPs prevented coagulation of blood and also dissolved blood clot indicating the biomedical potential of AgNPs in the management of blood coagulation disorders [10]. Though blood clotting is required to prevent bleeding, but its dissolution is equally important in preventing thrombolysis and maintenance of haemostasis, of which nanoparticles play important roles in rendering efficient lysis of blood clots [18]. The anticoagulant and thrombolytic potentials of metallic nanoparticles have been recently reported [18].

Maize shoot demonstrated significant difference in the growth rate when compared across the groups. Significant decrease ($p<0.05$) was observed in groups 500 and 750 when compared across the groups. The highest growth value was observed in the NSE group while no significant ($p<0.05$) difference was observed between 1000 and SN. The maize root on the other hand had the least growth value in the SN group with significant decrease ($p<0.05$) when compared across the groups. The highest value was observed in 250 group. Both the bean shoot and root demonstrated significant decrease ($p<0.05$) in the growth of 500 group when compared across the groups. The least value was observed in the SN group for the bean root while the highest value was observed in the NSE followed by 250, for the bean shoot, the highest value was observed in 250 group followed by SN group.

Conclusion

This study has therefore established the relevance of noni plant seed in nano biotechnological applications, particularly in green synthesis of low cost, eco-friendly, safe, reliable and stable Ag nanoparticles. The biosynthesized silver nanoparticles (AgNPs) showed significant potential management of blood coagulation disorders was established as AgNPs efficiently dissolved blood clots and also functioned as excellent thrombolytic agent. Therefore, this study has comprehensively established the potential vast applications of silver nanoparticles synthesized from noni seed extract. The silver nanoparticles synthesized from noni plant leaves can be used as treatment of thrombolysis and other related complications. The phytotoxic activity of

silver nanoparticle can be employed in weed control as alternative to synthetic herbicides currently used for weed control and management.

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