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Effects of operational conditions on biosynthesized silver nanoparticles using *Anogeissus leiocarpus* leaf extract

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ABSTRACT

The yield of synthesized silver nanoparticles from *Anogeissus leiocarpus* leaf (ALNP) was investigated by varying some operational parameters such as time, ratio of extract to $AgNO_3$ and concentration on the production of the nanoparticles for optimization. The effects of the operating parameters on the biosynthesis of ALNP was monitored using the UV-Vis Spectroscopy. The biosynthesized AgNPs was evaluated for activities against some fungi and bacteria pathogens. Dose dependent activities against the pathogens used were observed with zones of inhibition ranging from 16-26 mm. *Pseudomonas aeruginosa* was the most susceptible organism. Optimization of the synthesis of *Anogeissus leiocarpus* silver nanoparticle was obtained at 60 minutes, concentration; 3 mM, ratio 1:2 of $AgNO_3$ and 50 % of ethanol.

KEYWORDS: Nanoparticles, operational variables, *Anogeissus leiocarpus.*

1. INTRODUCTION

Nanotechnology impacts every aspect of human life every day (1-3). Use of nanomaterials has increased significantly in devices such as nanofilms, nanoparticles, nanowires and nanoclusters due to biomedical applications in tissue engineering, drug-gene delivery, and biosensors [4-6].

Nanomaterials from copper, zinc, magnesium, titanium, gold, silver, dielectric and semiconductor been developed [7-11]. Silver and gold nanoparticles showed highest possibilities as they displayed significant antibacterial activities [12-14]. Of all the methods involve in nanoparticle production, the use of plants extract eliminates the conditions of high temperature, pressure, energy and the use of harmful and less expensive reducing agents [15].

Increase in risk of spread of disease, debility, severe illness and mortality due to antimicrobial resistance is a major worldwide health challenge. It was estimated to be associated with close to 5 million deaths across the globe in 2021. This is caused majorly due to drug misuse, and also lack of adequate access to appropriate new and existing vaccines and medicines among locals [16].

Recently efforts are directed on developing new antimicrobial agents that can effectively eliminate bacteria, hence, it is imperative to find novel antimicrobial treatment agents. Nature holds an unlimited pool of agents that could serve as alternatives to combat the growing threat of antimicrobial resistance. In the recent time, there is an increase interest in phytochemicals from medicinal plants which could serve as potential sources of antimicrobial drugs as reliable alternative which have been affirmed from scientific sources.

Anogeissus latifolia is a member of Combretaceae family. It's a medium-sized tree of 20-36 meters in height. Several studies have reported that *Anogeissus leiocarpus* possess potentials for activities against microorganisms, parasitic worms and other parasitic disease agents [17-19]. Lately, the use of plant nanoparticles as antimicrobial agent is a new field of study. Synthesis and antimicrobial properties of some AgNPs with several plant extracts have been reported [20-22]. Our group have reported synthesis, characterization and antimicrobial activity of some AgNPs using different plant extracts [23-25]. In the present study, AgNPs was prepared using a low cost, simple and environmentally friendly method using the leaf extracts of *A. leiocarpus*. The effect of different experimental variables, such as concentration of $AgNO_3$, time, percentage of ethanolic plant extract used and volume ratio of plant extract to $AgNO_3$ solution on the synthesis of the silver nanoparticles were studied. The characteristics (size and morphology) of metal nanoparticles are influenced by the various synthesis parameters which determines the uses and applications of nanoparticles [26]. In this study, AgNPs was prepared using the leaf *A. leiocarpus.* This study aims at examining the best conditions of synthesis of *A. leiocarpus* silver nanoparticle and also to determine their usefulness as potential alternative antimicrobials.

2. MATERIALS AND METHODS

2.1 Preparation of plant extract

Anogeissus leiocarpus leaves were collected and identified at Forest Research Institute of Nigeria. $AgNO_3$ salt used was prepared from Merck Company (Germany). Freshly collected leaves were washed and air-dried at 32^oC for 48 hours. Leaves were ground and boiled for 10 minutes in 40% ethanol in the ratio 1:10.

Figure 1: *Anogeissus leiocarpus* leaves

2.2 Synthesis of silver nanoparticles

Equal amount (10 mL) of extract and aqueous solution of $AgNO_3$ were heated at 70^oC for 60 minutes. A colour changed from light brown to deep brown was noticed which confirms production of nanoparticle. The formation of *A. leiocarpus* silver nanoparticles (ALNP) was also confirmed by UV spectrophotometer.

2.3 Effect of amount of $AgNO_3$

About 5 mL of 1 mM solution of $AgNO_3$ was added to 5 mL extract and heated at 70 ºC for 10 mins. A change in colour was observed which indicates the formation of nanoparticles. The absorbance of the resulting solutions was measured spectrophotometrically. This was repeated with various amount of silver salt solution (2 and 3mM).

2.4 Effect of time

Mixture of equal volume of plant extracts and 1 mM $AgNO_3$ was incubated. Sample of the mixtures was taken at time intervals of 0, 15, 30, 45 and 60 minutes of the reaction and the absorbance taken.

2.5 Effect of ratio of volume of plant extract to $AgNO_3$ *solution*

An equal volume of extract ratios (1:1, 1:2 and 1:3) and the silver salt was heated at 70 ºC and colour changed from light brown to dark brown. The UV absorbance of the mixture was taken.

2.6 Effect of Concentration of ethanol solvent used in preparing the extract

Equal volume of different concentrations (10%, 20%, 30%, 40% and 50%) of ethanol solvent was reacted with the same volume of leaf extract at 70 ℃ for about an hour. A change in the colour of the reaction was used to confirm the formation of nanoparticles.

2.7 Antimicrobial assay

The agar diffusion plate method was used for the antibacterial assay while surface plate method was employed for the antifungal assay. Human pathogenic organisms consisting of four bacteria; 2 Gram positive (*Bacillus subtilis* and *Staphyloccocus aureus*) and 2 Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were used for the antibacterial assay. For antifungal assay, one yeast (*Candida albicans*) and three molds (*Aspergillus niger, Penicillium notatum Rhizobium stolonifer*) were used. The microorganisms were obtained from the Faculty of Pharmacy, University of Ibadan. Nutrient agar, nutrient broth, sabourand dextrose agar (SDA), tryptone soya broth agar from Oxoid Laboratories, U.K were used in the study. Methanol (40%) was in used to dissolve the drugs and also served as negative control in the assay. The agar cup well (diameter =8 mm) diffusion and agar broth tube dilution procedures were in the study. Gentamicin (0.5 mg\ml) and Tioconazole (70 µg/mL) served as reference antibacterial and antifungal drug.

2.8 Statistical analysis

Results were analyzed with SPSS software, while the analysis of variance was used to determine significant differences between the values under the experimental conditions examined.

3. RESULTS AND DISCUSSION

3.1 The effect of various operational variables

The rate of formation of AgNs increased with increase in reaction time (from 0-60 minutes) with slight shift in the absorption maxima indicative of variation in the particle size. (Figure 3a). Optimum concentation for *A. leiocarpus* was10^{-3} M (Figure 3b).Excess silver ion is required for optimum synthesis of ALNP (Figure 3c).Change in the concentration of ethanol solvent used during formation of AgNPs is negligible Figures 3d.

3.2 Antimicrobial Result

Anogeissus leiocarpus NP showed dose dependent inhibitory activity against the whole organism in the antimicrobial assay. *P. aeruginosa* was the most susceptible organism while *Rhizobium stolonifera* showed least activity. Other members of the family Combretaceae such as *Anogeisuss acuminata* and *Anogeissus latifolia* palladium nanoparticle have demonstrated similar antimicrobial activity [27, 28]. In all the assays, the synthesized AgNPs demonstrated lower activities than the reference drugs Table 1.

3.3. Discussion

Addition of leaf extract of *A. leocarpus* reduced Ag^+ to Ag metal nanoparticles which caused the observed colour change from faint brown to dark brown ascribed to the surface plasmon resonance phenomenon (SPR) [29]. The synthesized nanoparticles showed a spectrum range around 420 to 460 nm which agree to literature reports that gave AgNPs spectrophotometric absorption measurements in the wavelength ranges between 400-450 nm [30]. The aggregation of the silver nanoparticles in the solution is shown by the presence of broad resonance (Figure 2). The green reduction of the Ag^+ occurs speedily with over 90% of reduction of Ag^+ ions accomplished in less than 1 h after its addition to the leaf extract. Higher SPR peaks intensities were observed at the higher time intervals which indicate the increase in the production of silver nanoparticles (Figure 2a). After addition of the leaf extract to silver nitrate solution at different ratios, the curve shows increased absorbance at higher concentration (3mM) (Figure 2b) and high volume ratio.

The antimicrobial activity of silver nanoparticles synthesized by natural plants extract was investigated against various pathogenic organisms. The synthesized silver nanoparticles showed efficient antimicrobial property due to their extremely large surface area, which provides better contact with microorganisms. The antibacterial activities of metal nanoparticles from *A. leocarpus* leaf extract showed similar and comparable results to other silver nanoparticles from other plant [31,32].

Table 1: Antimicrobial activity of AgNP

Plant/Concentration (mg/mL)	S.a	E.c	B. sub	Ps.a	C.a	A.u	P.n	R.n
A. Leiocarpus								
100	20	24	24	26	20	18	18	16
50	18	20	22	20	18	16	16	14
25	14	18	18	18	16	14	14	12
12.5	12	14	16	14	14	12	12	10
6.25	10	12	12	12	12	10	10	_
3.125	_	10	10	10	10	_	_	_
-ve (40% Methanol)	–	–	–	–	–	–	–	–
+ve	40	38	38	40	28	26	28	28

Key: +ve = Gentamicin 10 µg/mL (Bacteria), Tioconazole 70 µg/mL (Fungi), *Staphylococcus aureus* (S. a), *Esherichia coli* (E.c), *Bacillus subtilis* (B.sub), *Pseudomonas aeruginosa* (Ps.a), *Candida albicans* (C.a), *Aspergillusniger* (A.u), *Penicilliumnotatum* (P.n), *Rhizobium stolonifer* (R.s).

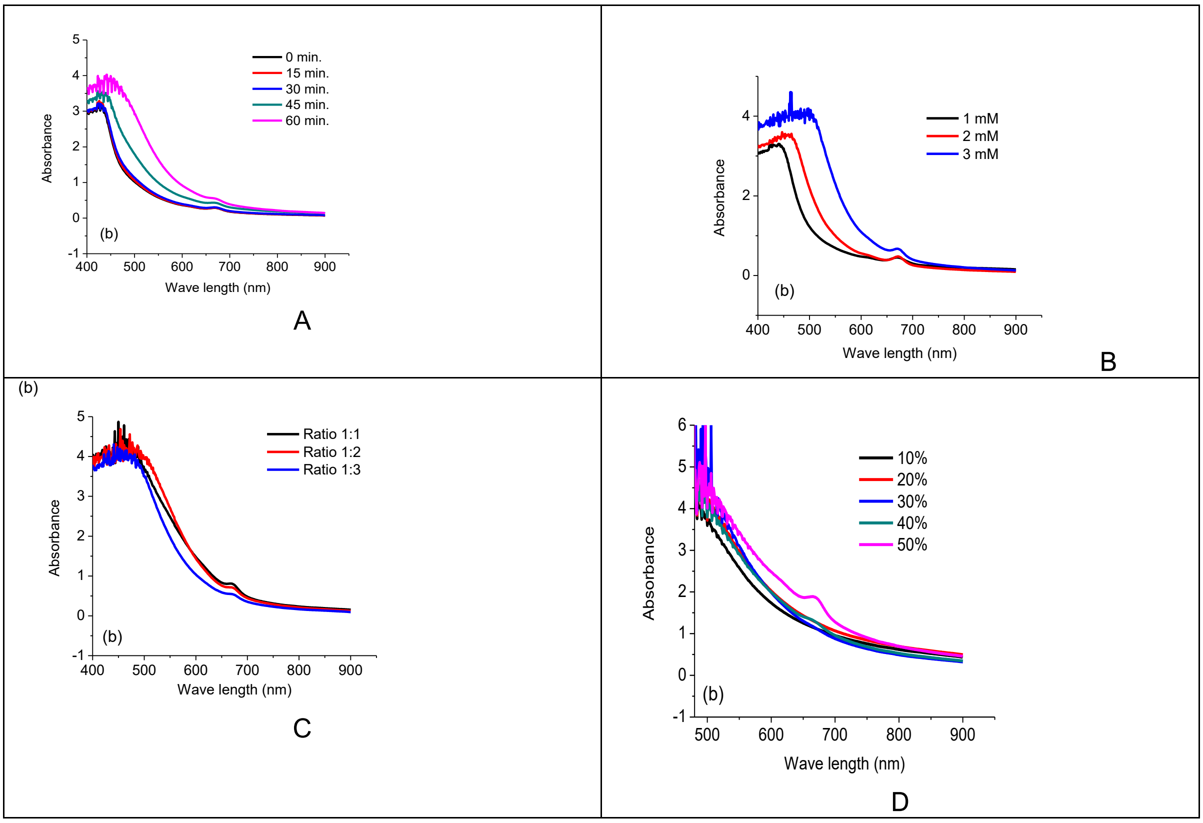

Figure 2a: Effects of time on AgNPs formation *A. leiocarpus*, 2b: Effects of concentration of $AgNO_3$ on AgNPs formation *A. leiocarpus*
2c: Results of change in ratio of $AgNO_3$ solution to plant extracts on AgNPs formation *A. leiocarpus*,
2d: Effects of concentration of ethanolic solvents on AgNPs formation *A. leiocarpus*

4. CONCLUSION

Anogeissus leiocarpus leaf extracts demonstrated reducing ability for the synthesis of the Ag nanoparticles. The concentration of the extract was the most effective parameter for optimization. The synthesized AgNPs showed effective antifungal and antibacterial activities.

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