Antibacterial Effects of Phytofabricated Silver Nanoparticles Against Some Selected Bacteria.

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ABSTRACT

The increase in multidrug resistance microorganisms has initiated the creation of other means of combating the damages caused by these microbes by scientists. This has led to the creation of more affordable, environmental friendly and cost effective antimicrobial agents in which formation of nanoparticle using plant leave extracts fits in rather than chemically synthesized antibiotics which have previously been in used. In this study, the efficacy of phyto-fabricated silver nanoparticle and its antibacterial effect against some bacteria which were collected from a research laboratory located in Awka, Anambra state were identified. To generate the aqueous extract of silver nanoparticle, the various plant leaves were dried for one week at a room temperature of 40^{o}C. The dried leaf were pulverized, dissolved in 100mL deionized water and heated to a boiling temperature for 5 minutes until a nanoparticle is formed through the formation of dark brown colour and a double fitration is carried out using whatman no 1 filter paper. The synthesised silver nanoparticle was centrifuged, dried and used for antibacterial analysis. The antibacterial analysis was carried out using agar well diffusion method. The results from the antibacterial screening revealed that the tested organisms were susceptible to silver nanoparticles with *Proteus mirabilis* showing the highest zone of inhibition for cashew fabricated silver nanoparticle at 28.00±0.45 and concentration of 3.13mg/ml, while the lowest zone of inhibition using *Carica papaya* fabricated silver nanoparticle was observed with *Pseudomonas aeruginosa* at 4.00±0.36 and concentration of 6.25mg/ml. The negative control (DMSO) used did not produce any zone of inhibition on the tested organisms. Statistically, there was significant (P<0.005) inhibition of bacteria pathogenic strains among the means of *A. muricata, C. papaya and A. occidentalis* fabricated silver nanoparticle and ciprofloxacin standard antibiotics. The high antibacterial inhibition of the tested bacterial strains by *A. muricata, C. papaya and A. occidentalis* fabricated silver nanoparticle at minimal inhibitory concentration of 6.25mg/mL could be exploited as biostatic and biocidal agent. This shows that nanoparticle synthesised using biologically materials like plant part, are highly potent, cheaper to synthesized and as well, less toxic.

Key words: Antibacterial activity; Phytofabricated Silver Nanoparticles; Bacteria

INTRODUCTION

Nanotechnology is a promising field for generating new types of nano-materials with biomedical applications (Chen *et al*., 2011). The scientific research and application of nanotechnology have increased rapidly in recent years. The properties of nanoparticles such as durability, high diffusion and versatile chemical and biological activities have increased its importance in technological application (Nabila and Kannabiran, 2018). Metal nanoparticle synthesis researches have been increasing in number due to potential application in nanotechnology (Isaac *et al*., 2013). Nanoparticles are used in many industrial fields, especially in electrical, biomedical, automotive and chemical sectors due to their superior properties (Gurmen *et al*., 2008). Therefore many researchers have studied the synthesis of nanoparticles using various physical, chemical and biological methods (Remya *et al*., 2017).

Generally, nanoparticles are seen as particles having a size of 100nm or less. Due to this property of theirs, they have a large surface area that allows them to be used in new applications, and they tend to react differently from large substance containing the same composition particle (Abou El-Nour *et al*., 2010). However, it is stated that the synthesis of nanoparticle with green chemistry approaches using biological entities such as plants, fungi, bacteria, algae and actinomycetes provides additional advantages over other methods because it is simple, cost effective, reliable, and environmentaly-friendly unlike the synthesis of nanoparticle using conventional physical and chemical methods which has a low yield, quite expensive and produces toxic reducing agents such as citrate, borohydride, or other organic compounds that can negatively impact the environment and it is also difficult to prepare silver nanoparticle with a well-defined size (Malik *et al*., 2010). For this purpose, the use of biological synthetic approaches for many researchers is increasingly important (Kumar and yadav, 2009).The biological methods involve synthesizing silver nanoparticles using plant materials such as leave, seeds, roots etc (Dhaka *et al*., 2023).

The increasing prevalence of microbial resistance has made the management of public health an important issue in the modern world, due to mutation, pollution and changing environmental conditions. This has led to the development of highly evolving superbugs which had rendered a whole generation of antibiotics less effective (Friedman *et al* 2016). Apart from affecting the human health, the research and development for upgrading the existing antibiotics for countering the perils concerning multi-drug resistance in bacteria consumes a large chunk of economy (Founou *et al*., 2017). It is therefore highly desirable to improve the present methodologies with innovative strategies having a broad spectrum mechanism for targeting superbugs using a cheaper means of synthesis such as biological method of synthesizing nanoparticle (Karam *et al*., 2016). The broad spectrum mechanism for targeting approach achieved by synergistic effect of one or more antibiotics administered as a single formation has proved to be quite beneficial but the optimum result are still not achieved (Bush 2017). To circumvent this predicament, scientists are trying to develop drugs for the treatment of such microbial infections of which metallic nanoparticles present a laudable profile (Hoseinzadeh *et al*., 2017). Owing to knowledge of the effect caused by this superbugs, the researcher have been able to synthesized nanoparticle using biological method of synthesis and to check for its ability to inhibit bacteria.

MATERIALS AND METHODS

Study area, sample collection and identification

The study was conducted at the Microbiology Laboratory (Anambra State University, Uli, Nigeria). *Carica papaya Annona muricata* and *Anacardium occidentalis* were sourced from a local environment and identified in the Department of Botany, Nnamdi Azikiwe Univerisity, Awka.

Collection of tested organism and Processing of silver nanoparticle

The tested strains of *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Proteus mirabilis,* were obtained from a research laboratory and a confirmatory test was conducted under aseptic condition. Eosine methylene blue was used to culture *Escherichia coli, and Proteus mirabilis* (non lactose fementer) and mannitol egg yolk polymyxin agar was used for *Bacillus cereus*.

The fresh leave samples were washed with tap water initially and finally washed with distilled water. The leaves were dried for one week at room temperature of about 40^{o}C and then pulverized using industrial blender. 50g each of the pulverized leaves were weighed, dissolved in 100mL deionized water and boiled for 5 minutes until a colour change was observed. The boiled leaf extract was cooled at room temperature and used in the silver nanoparticle synthesis after filtration with whatman no 1 filter paper (Acay *et al*., 2019).

Synthesis and antibacterial assay of silver nanoparticle

The extract and solution was mixed at the rate of 1:5, heated and stirred in a magnetic stirrer at temperature of 70^{o}C for 60minutes. Reduction of the Ag ions resulted in the change of silver trioxonitrate (iv) from white coloured solution to dark brown. The resulting dark colored solution was centrifuged for 5 minutes at 1000rpm to remove the upper liquid phase. It was washed with distilled water until the remaining solid was clear. The

obtained silver Nanoparticles was left to dry in the oven at 65^0c for 48 hours and used for antibacterial assay (Pugazhendhi *et al*., 2018). In the course of antibacterial assay, a stock solution (60mg/mL) of AgNPs was prepared using dimethyl sulphoxide (DMSO) and then different concentrations ranging from 0.47-30.00mg/mL was prepared by two-fold serial dilution in MHB. Susequently, 20 µL of each standardized test bacteria was added into the mixture and vortexed followed by incubation at 37ºC for 24h. The positive and negative controls used are ciprofloxacin and 5% DMSO, respectively. Afterward, the minimum inhibitory concentration (MIC) was taken as the least concentration that inhibited bacterial growth while the minimum bactericidal concentration (MBC) was determined by plating out those broths without visible growth on Mueller Hinton Agar, and the plates were further incubated at 37ºC for 24h.

Safety Considerations

Safety protocols for handling tested bacterial strain and Silver Nanoparticle were strictly adhered to, including the use of personal protective equipment (PPE) and work within a designated laboratory areas.

RESULT

Synthesis of silver nanoparticles

The mixture of pulverized paw-paw, cashew and soursop leaves with colourless $AgNO_3$ solution, showed colour change from light yellow to dark brown which were visually observed when the mixtures were heated at 70 °C in a magnetic stirrer for 60 min.

Antibacterial Activity

The result for antibacterial, minimum inhibitory concentration (MIC) and minimal bacterial concentration (MBC) of the silver nanoparticles are summarized in (Table 1,2,3 and 4).

Table 1: Antibacterial activity of the green silver nanoparticles against tested microbial pathogens

Agent	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas Aeruginosa*	*Proteus mirabilis*
A. muricata	10.17±0.24	15.50±0.59	12.00±0.00	26.00±0.59
C. papaya	12.67±0.50	14.50±0.67	4.00±0.36	22.50±0.58
A. occidentalis	11.00±0.26	20.33±0.86	8.50±0.52	28.00±0.45
Ciprofloxacin	8.17±0.21	0.00±0.00	0.00±0.00	12.83±0.81
DMSO	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 1 revealed the antibacterial activity of the biosynthesized silver nanoparticle against tested bacterial pathogens. *Proteus mirabilis* had the highest inhibition activity against *A. occidentalis* synthesized nanoparticle with the rate of 28.00 ±0.45, and 26.00±0.59. For *A. muricata* synthesized nanoparticle; *E. coli* had an inhibition rate of 20.33±0.86 and for A. *occidentalis* and 0.00±0.00 for ciprofloxacin. *Proteus mirabilis* had the greatest inhibition which showed that *A. muricata* displayed a strong efficacy against bacterial multiplication.

Table 2: Minimium inhibitory bactericidal concentration of the *C. papaya* leave hot water extract mediated silver nanoparticle

S/N	Nanoagent	Dilution	Concentration (Mg/ml)	*Bacillus sp*	*E. coli*	*P. aeruginosa*	*Proteus sp*
1		Neat	50	–	–	–	–

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2	*C. papaya* Ag NPs	1:2	25	_	_	_	_
3		1:4	12.50	_	_	+	+
4		1:8	6.25	+	_	++	++
5		1:16	3.13	++	_	++	++
6		1:32	1.56	++	_	++	++
7		1:64	0.78	++	_	++	++
8		1: 128	0.39	++	+	++	++
9		1:256	0.20	++	++	++	++
MIC(Mg/ml)			6.25	0.39		12.50	12.50
MBC(Mg/ml)			12.50	0.78		25.00	25.00

Key: S/N= Serial number

AgNPs= Silver nanoparticle

Mg/ml= Milligram per milliliter

%= Percent

DMSO= Dimethylsulphonide

++= Dense growth

MIC= + Slight growth

MBC= - No growth

Table 2 showed that the minimal inhibition of the bacterial pathogen increased as the concentration decreases while the minimal bactericidal concentration increased as the rate of concentration tends to increase. Also, *E. coli* showed a greater susceptibility at 3.13mg/ml compared to other bacterial pathogens. Therefore, the rate of susceptibility increased with an increase in concentration of antibacterial agent and also, inhibition could be due the structure of the organism present.

Table 3: Minimium inhibitory bactericidal concentration of the *A. occidentalis* hot water extract mediated silver nanoparticle

S/N	Nanoagent	Dilution	Concentration (Mg/ml)	*Bacillus sp*	*E. coli*	*P. aeruginosa*	*Proteus sp*
1		Neat	50	_	_	_	_
2		1:2	25	_	_	_	_
3		1:4	12.50	_	_	_	_
4		1:8	6.25	_	_	_	_

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5	*A. occidentalis* Ag NPs	1:16	3.13	+	_	_	+
6		1:32	1.56	++	_	_	++
7		1:64	0.78	++	_	_	++
8		1: 128	0.39	++	+	+	++
9		1:256	0.20	++	++	++	++
10			5% DMSO	++	++	++	++
MIC(Mg/ml)			3.13	0.39	0.39		3.13
MBC(Mg/ml)			6.25	0.78	0.78		6.25

Key: S/N= Serial number

AgNPs= Silver nanoparticle

Mg/ml= Milligram per milliliter

%= Percent

DMSO= Dimethylsulphonide

++= Dense growth

MIC= + Slight growth; MBC= - No growth

Table 3, showed that the rate of minimal inhibition of the bacterial pathogen increased as the concentration decreases while the rate of minimal bactericidal concentration increased as the rate of concentration tends to increase. MBC tends to be higher at 6.25mg/ml. The rate of inhibition is more pronounce in *E.coli* and *Pseudomonas aeruginosa*, which shows that silver nanoparticle synthesized using *A. occidentalis* is a highly potent antimicrobial agent.

Table 4: Minimium inhibitory bactericidal concentration of the *Annona muricata* leave hot water extract mediated silver nanoparticle

S/N	Nanoagent	Dilution	Concentration (Mg/ml)	*Bacillus sp*	*E. coli*	*P. aeruginosa*	*Proteus sp*
1	*A. muricata* AgNPs	Neat	50	_	_	_	_
2		1:2	25	_	_	_	_
3		1:4	12.50	_	_	+	_
4		1:8	6.25	_	_	++	_
5		1:16	3.13	_	_	++	_
6		1:32	1.56	_	_	++	+
7		1:64	0.78	+	_	++	++

8		1: 128	0.39	++	+	++	++
9		1:256	0.20	++	++	++	++
10			5% DMSO	++	++	++	++
MIC(Mg/ml)			0.78	0.39		12.50	1.56
MBC(Mg/ml)			1.56	0.78		25.00	3.13

Key: S/N= Serial number

++= Dense growth

MIC= + Slight growth

AgNPs= Silver nanoparticle

Mg/ml= Milligram per milliliter

%= Percent

DMSO= Dimethylsulphonide

MBC= - No growth.

Table 4, showed that the rate of minimal inhibition of the bacterial pathogen increased as the concentration decreases while the rate of minimal bactericidal concentration increased as the rate of concentration tends to increase. MBC tends to be higher at concentration of 3.13mg/ml. This shows that biosynthesis of silver nanoparticle using *Annona muricata* is also a potent antibacterial agent.

DISCUSSION

Table 1 displays the percentage zone of inhibition and show inhibitory effect at all concentrations against the tested organism using biosynthesized silver nanoparticle are shown in Table 1. The highest zone of inhibition 28.00±0.45 as seen in table 1 was recorded when silver nanoparticle was synthesised using *Anacardium occidentale* inhibited the growth of *Proteus mirabilis*; while the lowest zone 0.00±0.00 was observed when ciprofloxacin was used to inhibit the growth of *Escherichia coli* and *pseudomonas*. Similar observation was recorded by work done by (Isiaka *et al*., 2020; Lateef *et al*., 2018). This showed that, the biosynthesised silver nanoparticle is more potent in the inhibition of microbes compared to drugs as reported by Rai *et al*., 2014.

In the study, table 2, 3 and 4 showed that the green synthesized AgNPs inhibited the microbial growth as the concentration increases. The antimicrobial mechanism may be due to cell wall and membrane damage (Arokiyaraj *et al*., 2017), damage of ribosome and mitochondria, inhibition of thiol group in microbial cell, and the ability of the cell to seize functioning which may have been the reasons for the inhibition (Velusamy *et al*., 2016). Another possible reason for these significant inhibitions by *A. muricata, C. papaya* and *A. occidentalis* AgNPs could be attributed to the adhension of their larger surface area to the tested bacterial strains leading to inhibition of numerous physiological and biochemical processes such as disturbing cell-wall permeability and cellular respiration in the cell (Kim *et al*., 2012). These observations are consistent with previous reports that have demonstrated the potential of AgNPs as antimicrobial agent (Xi-feng and Zhi-Guo, 2016). The Antimicrobial property of silver nanoparticle depends on the size of nanoparticle synthesised (Richard *et a*l., 2012). Smaller size of nanoparticle effectively penetrates cell due to its large surface availability for interaction and interfering with metabolism of cell. The presence of clear zone around the synthesized silver nanoparticle suggest that the silver nanoparticle possess antimicrobial activity which is able to inhibit the growth of the Gram negative and Gram positive bacteria. Statistically, there was significant ($P<0.05$) inhibition among the mean of

A. muricata, *C. papaya* and *A. occidentalis* AgNPs and ciprofloxacin treatment doses on the tested bacteria. From the research work, Gram negative bacteria are more inhibited than Gram positive bacteria which could be due to the nature of the organism present and their physiological features like thick peptidioglycan present in Gram positive cell (Mirzajan *et al*. 2011).

CONCLUSION

Biologically synthesized silver nanoparticle, are highly potent, economical and less toxic when used to inhibit or suppress the growth of microbial cell. They tend to produce a positive result when properly synthesized; with the knowledge of nanoparticle synthesis, its production and utilization, there will be reduction in the hazardous effect of bacterial, especially the superbugs which has been a global issue. In this study, the antimicrobial of phyto-fabricated silver nanoparticle was investigated using leaf extracts of *A. occidentalis*, *Carica papaya* and *A. muricata*. It was discovered that the synthesised silver nanoparticle exhibited antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Proteus mirabilis.*

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