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Comparative Antibacterial Analysis of Green Synthesized Silver Nanoparticle using *Azadirachta indica* (*neem tree*) and *Cassia occidentalis* (*coffee sena*) against *E. Coli* and *Bacillus Subtilis*

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Abstract
Herbal drug delivery can be enhanced with silver nanoparticle (AgNPs) that will enhance their performance and increase their activity. The present study was conducted to determine the antimicrobial activity of Azadirachta indica and Cassia occidentalis Co-synthesized silver nanoparticle against both gram negative and positive bacterial strain including E.coli and B. Subtilis.
AgNPs were synthesized using ethanol and n-Hexane leaf extract of Azadirachta indica and Cassia occidentalis,and were characterized by UV-Vis spectrophotometer and Fourier-transform infrared spectroscopy (FTIR). AiAgNPs and CoAgNPs were used to evaluate their antibacterial activity against gram negative and positive strain as well as their potent inhibitory effects.
The synthesis of silver nanoparticles from leaf extract of Azadirachta indica and Cassia occidentalis were observed by UV-visible spectroscopy. The peaks maxima were observed at 455 nm for silver nanoparticles synthesized from the leaf extracts of Azadirachta indica and Cassia occidentalis. The antibacterial activity of both ethanolic and n-Hexane leaf extracts of Azadirachta indica and Cassia occidentalis against E. Coli and B. Subtilis were determined using disk diffusion method by measuring the diameter for zone of inhibition.
Both the extracts exhibit potential for application that would support the production of AgNPs with broad-spectrum antimicrobial agents to be applied in the fields of medical and pharmaceutical sciences for formulation of new drugs

Keywords: Nanoparticle, *Neem tree, Cassia occidentalis, E. Coli, B. Subtilis*

INTRODUCTION
Numerous studies have reported the use of plant extracts to synthesize AgNPs with significant antimicrobial activities such as: leaf extracts of *Acalypha indica* (Krishnaraj *et al*., 2010), *Solidago altissima* (Kumar *et al*., 2016). Green synthesis is the use of environmentally compatible materials such as bacteria, fungi and plants in the synthesis of nanoparticles (Patra and Baek, 2014). These attractive green strategies are free of the short falls associated with conventional synthetic strategies, i.e. they are eco-friendly (Veerasamy *et al*., 2011). Silver is the most profit-oriented precious metal used in the preparation of NPs and nanomaterials.

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Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process (Morones et al., 2005; Lok et al., 2007). The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burns and open wound. Silver ions (Ag+) and its compounds are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity towards animal cells (Prema, 2011). Rising demand for silver nanomaterials requires the development of eco-friendly synthesis methods. The present study investigate the synthesis of AgNPs *Azadirachta indica* and *Cassia occidentalis* against *E. coli* and *Bacillus subtilis*. The essence of this study was to synthesize silver nanoparticles from two different plants extract (*Azadirachta indica* and *Cassia occidentalis*) and to compare the antibacterial potential of the synthesized silver nanoparticles. Antimicrobial assay (well diffusion assay, minimum inhibitory concentration and minimum bactericidal concentration) was done on both gram positive and gram negative bacterial strain.

Azadirachta indica is a tree in the mahogany family *Meliaceae.* It is one of two species in the genus *Azadirachta*, and is native to some northern part of Nigeria, India, Burma, Bangladesh, Sri Lanka, Malaysia and Pakistan, growing in tropical and semi-tropical regions. It is known as (neem tree) in English, (*Darbejiya, Maina, Charbi*) in Hausa. Neem is a fast-growing tree that can reach a height of 15-20m, rarely to 30-40m.

Cassia occidentalis is an ayurvedic medicinal plant used as a traditional medicine for treatment of various diseases. This plant extracts are known to have antibacterial, antifungal, antimalarial, anti-inflammatory, antioxidant, hepatoprotective and immunosuppression activity. It is grown in all northern part of Nigeria and some western part of Nigeria

MATERIALS AND METHOD

Samples collection and preparation

Fresh leaves of *Azadirachta indica* and *Cassia occidentalis* were collected at federal University Dutse campus during rainy season in an area free from pesticides and other contaminants. The samples were washed thoroughly using tap water and it was allowed to dry under the shade. Authentication was done at botanical survey, Federal University Dutse botanical garden.

Preparation of the leaf extracts

About 50g of each sample was measured and added into 400ml of ethanol and n-Hexane in order to form the various extracts. It was shaken and agitated to achieve homogeneity. After 5 days, the samples were filtered using funnel and clean white hand-kerchief. The filtered samples were placed in a container for about three weeks for the solvent to evaporate.

Synthesis of the silver nanoparticle (AgNps) from the plant extracts

Silver nanoparticles were prepared from ethanolic and n-Hexane leaf extract of *Azadirachta indica* and *Cassia occidentalis*. To 10 ml of the ethanolic and n-Hexane leaf extract, 90 ml of 1mM silver nitrate solution was added (Dutta *et al.,* 2001). The extentof nanoparticles synthesis was monitored by visual observation of color change from yellow to brown and by measuring the absorbance at 400-600nm.

Antibacterial activity of the nanoparticles, silver nitrate and leaves extracts

The bacterial strains used were *E. coli* and *B. subtilis.* The strains were obtained from microbiology Department of Rasheed Shekoni Teaching Hospital, Federal university Dutse. Stock cultures were maintained at 4°C on nutrient agar slant. The cultures were incubated for 24 hours at room temperature. Agar well diffusion method (Nwokem *et al*., 2010) for bactericidal susceptibility was carried out according to standard method to assess the

presence of antibacterial activity of the synthesized silver nanoparticle. The concentration of the nanoparticle used in the experiment was 1500, 1000, 500 and 250 µg/ml. Well of about 6 mm diameter were made aseptically using sterilized cork borer. Each of the plates was swabbed with *E. coli and B. subtilis* respectively, then the plates were incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the diameter of the zone of inhibition around the well.

RESULT AND DISCUSSION

Table 1: Percentage yield, color and texture of the leaf extract obtained

S/N	Extract	%yield (g)	Texture	Color
1	*A. indica* leaves ethanol extract	4.7	Viscous	Dark green
2	*A. indica* leaves n-Hexane extract	5.1	Oily	Light green
3	*C. occidentalis* leaves ethanol extract	3.7	Viscous	Army green
4	*C. occidentalis* leaves n-Hexane extract	4.5	Oily	Green

Percentage yield of various leaf extract determined with n-Hexane leaf extract of A. indica having the highest yield.

ANTIBACTERIAL STUDIES.

The inhibition zone of antibacterial activity of *Azadirachta indica* synthesized Silver nanoparticle (AiAgNPs) and Cassia occidentalis synthesized nanoparticle CoAgNPswere assessed by measuring the clear zone of inhibition around the wells. Both the AgNPs synthesised from leaf samples types displayed inhibition of *E.coli and B. subtilis*. The highest zone of inhibition (17.5 mm) was recorded for *E. coli*, then by B. *subtilis* (14.8 mm). The growth of both *E. coli* and *B. subtilis* reached exponential phase rapidly in the absence of AiAgNPs and CoAgNPs. However, it was found that the growth of the *E. coli*, then by *B. subtilis* cells were significantly reduced when exposed toAiAgNPs and CoAgNPs. The result has shown that AiAgNPs was found to have higher zone of inhibition against *E.coli* than the standard antimicrobial disc in both the nanoparticles synthesized from ethanolic and n-Hexane extracts (Table 2).

Table 2: Inhibition zone of antibacterial activity of *A. indica* ethanolic and n-Hexane leaf extract on *E. coli* and *Bacillus subtilis*.

		Ethanol Leaf Extract		**n-Hexane Leaf Extract**	
S/N	Antibacterial agent	*E. coli (mm)*	*Bacillus subtilis (mm)*	*E. coli(mm)*	*Bacillus subtilis(mm)*
1	Plant extract	5.5	4.2	5.2	3.8
2	Silver nitrate	10.2	9.5	10.4	9.8
3	AiAgNPsNanoparticle	17.5	13.8	17.2	13.5
4	Standard antibacterial disc	16.4	14.3	16.1	14.2

Both ethanolic and n-Hexane leaf extract of CoAgNPs synthesized nanoparticle was found to have shown higher zone of inhibition against *E.coli* thant he standard antibacterial disc (Table 3).

Table 3: Inhibition zone of antibacterial activity of *C. occidentalis* Ethanol and n-Hexane leaf extract and nanoparticle on *E. coli* and *Bacillus subtilis*.

		Ethanol Leaf Extract		**n-Hexane Leaf Extract**	
S/N	Antibacterial agent	*E. coli (mm)*	*Bacillus subtilis (mm)*	*E. coli(mm)*	*Bacillus subtilis(mm)*
1	Plant extract	3.8	3.3	4.40	3.60
2	Silver nitrate	13.3	13.0	13.2	10.0
3	CoAgNPsNanoparticle	16.8	14.8	15.0	14.7
4	Standard antibacterial disc	14.7	13.7	14.8	14.4

Both synthesized nanoparticle extracts were found to inhibit the growth of *E. coli* and *B.subtilis* higher than the non-synthesized nanoparticle. This is not surprising as silver nanoparticles have been reported to show better wound healing capacity, better cosmetic appearance and scar less healing when tested using an animal model (Tian et al., 2006). Silver impregnated medical devices like surgical masks and implantable devices have been shown to significantly enhance antimicrobial efficacy (Furno et al., 2004).

The results of the present study showed that AgNPs synthesized from the extracts of *A. indica and C. occidentalis* in this study are not only more effective but have also shown a higher activity than the plant extract alone. In general, according to the result of the present study, nanoparticle produced using *Azadirchta indica* extract shows highest activity against all the tested bacterial strain (*E. coli* and *Bacillus subtilis*) than *Cassia occidentalis* nanoparticle. This could be due to the presence of major chemical constituents in the extract identified as nimbin and quercetin (Shankar et al., 2004; Tripathy et al., 2009). Previous studies have documented antibacterial properties of silver since 1000 B.C., when silver vessels were used to preserve water (Richard et al., 2002; Castellano et al., 2007). The higher antibacterial activity of the *A. indica and C. occidentalis* synthesized silver nanoparticle than leaf extract and nanoparticle alone observed in the present study could be due to enhancement of the activity by the silver itself which has been reported to have innate antibacterial activity.

According to the present study, nanoparticle produced using *Azadirchta indica* extract shows highest activity against all the tested bacterial strain (*E. coli* and *Bacillus subtilis*) than *Cassia occidentalis* nanoparticle. *Azadirachta indica* nanoparticle for all extract (ethanol and n-Hexane) produces zone of inhibition of 17.5 and 17.2mm against *E. coli* and 13.5 and 13.8mm against *Bacillus subtilis* as seen from (Table 2) for the ethanol and n-Hexane respectively while *Cassia occidentalis* nanoparticle produces zone of inhibition of 16.8 and 15.0mm against *E. coli* and 14.8 and 14.7mm against *Bacillus subtilis* as seen from (Table 3) above respectively. Based on the zone of inhibition produced, synthesized silver nanoparticles prove to exhibit good antibacterial activity against E. coli and *Bacillus subtilis*.

CONCLUSION

The present study demonstrated that the green synthesized silver nanoparticles exhibit high antibacterial activity than extarcts alone. Both the ethanolic and n-Hexane leaf extracts of *Azadirachta indica* and *Cassia occidentalis* showed strong antibacterial activity against *E. coli* and *Bacillus subtilis* and exhibit potential for application the production of AgNPs with broad-spectrum antimicrobial activity to be applied in the fields of medical and pharmaceuticals formulation of new drugs.

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