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Research Article

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Synthesis and Antibacterial Activity of Silver Nanoparticles using *Citrus aurantifolia* Leaf Extract

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Abstract This studdy investigates synthsis and antimicrobial activity of silver nanoparticles using *citrus aurantifolia* leaf extract to achieve, the fresh leaf of *citrus aurantifolia* was collected, authenticated, prepared, extracted with de-ionzed water and concentrated using standard procedures. Four different concentrations of plant extract were prepared in the ratio of (1:1, 2:1, 3:1, 4:1) by increasing the concentration of plant extract in the solution mixture. 0.17% of 1mM $AgNO_3$ metal ion was added to the prepared plant extract. Then the bio-reduced aqueous component solution turned colloidal and different in colour, which suggested the formation of *Citrus auratifolia* leaf extract silver nanoparticles however, a control solution was made by 10 mL of *citrus auratifolia* leaf extract (solution) without silver nitrate solution. *Citrus auratifolia* leaf extract silver nanoparticles so obtained were scraped, coded (As, Bs, Cs, Ds) thereafter, inhibitory effects were sreened against *S. typhi*, *S. aureus* and *K. pneumonia* using disc diffusion method at three different concentrations of 100µg/ml, 200µg/ml 300µg/ml, 400µg/ml respectively. The nano particle coded As has demostrated inhibitory effects of 4mm and 3mm against *S. typhi* and *S. aureus* at the concetration of 200µg/ml. However, nano particle coded Bs shows inhibitory effects of 4mm and 3mm against *S. typhi* and *S. aureus* at the concetration of 200µg/ml while Cs shows inhibitory effects of 4mm and 2mm against *S. typhi* and *S. aureus* at the concentration of 200µg/ml furthermore, nano particle coded Ds also shows inhibitory effects of 5mm and 3mm against *S. typhi* and *S. aureus* respectively at the concentration of 200µg/ml on the other hand, 3mm, 2mm, 2mm and 1mm inhibitory effects were observed against *K. pneumonia* at the concentration of 300µg/ml for all the sample tested. The activity of the standard drug (amoxicillin) was found to be higher compared to all the samples tested except against *K. pneumonia* in which no inhibition was observe for the amoxicillin at all the concentrations tested. The inhibitory effects observed for the *Citrus aurantifolia* leaf extract silver nanoparticles (As, Bs, Cs, and Ds) were comparable to that of the standard drug (amoxicillin) this could probably be considered as an indication for the antimicrobial property of the *Citrus aurantifolia* leaf extract silver nanoparticles.Reports suggest that bactericidal effect of silver nano particles by destroying the enzymes that transport the cell nutient and weakening the cell membrane or cell wall, leading to increase cell peamiability and cell death.The remarkable antimicrobial effects observed may be due to the formation of nano particles by the leaf extract of *Citrus aurantifolia* since, no inhibition was observed for the *Citrus aurantifolia* leaf extract solution (E) alone against all the microorganisms tested.

Keywords: *Citrus aurantifolia,* silver nanoparticles, antimicrobial

Introduction

Nano is a metric measure of one billionth of a meter and covers a width of 10 atoms. In terms of comparison with real objects, an example that hair is 150,000 nanometers may be given. The rapidly developing nanotechnology is the inter-disciplinary research and development field of biology, chemistry, physics, food, medicine, electronics, aerospace, medicine, etc., which examines the design, manufacture, assembly, characterization of materials that are smaller than 100 nanometers in scale, as well as the application of miniature functional systems derived from these materials. It represents the whole of development activities. As far the nanobiotechnology, on the other hand, it is the result of a combination of biotechnology and nanotechnology branches with a common combined functioning [1]. The reason for the intense interest of scientists nowadays in nanoparticles is that nanoparticles can exhibit different properties and functions than normal bulk materials. The most important factor that enables production of nanostructures in desired size, shape and properties and provides their usage in various fields is that the effects of classical physics are reduced and the quantum physics becomes active. Other reasons for the different behavior of nanoparticles in physical, chemical, optical, electrical and magnetic behavior include the limitations of load carriers, size dependent electronic structures, increased surface / volume ratio, and other factors incurred by the unique properties of atoms [2].

The process of removing toxic and waste metals in the environment includes microorganisms, plants and other biological structures; achieved by means of oxidation, reduction or catalysis of metals with metallic nanoparticles. Metallic nanoparticles produced by biological methods; are used in the biomedical field for purposes such as protection from harmful microorganisms, bio-imaging, drug transport, cancer treatment, medical diagnosis and sensor construction because of their unique properties such as being insulator, optics, antimicrobial, antioxidant, anti-metastasis, biocompatibility, stability and manipulability [3-4].

Citrus aurantifolia (christm) swingle (lime) belong to the family rutaceae. It is a dense and irregularly branched tree. The stem is spreading and woody, brown in color, with short stiff spines on twigs. The leaves are acute, entire elliptic, oblong-ovate, dark green above, pale-green blow, altanate with narrowly winged leaf petioles. Flowers are white and stand from lea corners. The fruit is round, greenish-yellow with thin skin, juicy, fragrant and very acidic. The seeds are small, avoid and pale [5].

Citrus aurantifolia in its natural state is widely used in West Africa, particularly in Nigeria where it is employed in herbal medicine to treat several illnesses. It forms an essential ingredient in the preparation of most herbal concoctions [6].

This research work was designed to carry out synthesis and antimicrobial activities of silver nano particles using citrus aurantifolia leaf extract will help provide scientific baseline information on plant based silver nanoparticles for use as an antimicrobial agent.

Materials and Methods

Sample collection and Extraction

Citrus aurantifolia L. (Lime) leaf: Fresh leaf of *Citrus aurantifolia* (CA) L. was collected within the premises of Ramat Polytechnic Maiduguri. The plant part was authenticated by a botanist in the Department of Biological Sciences, University of Maiduguri, Borno state and washed several times with water to remove the dust particles and then air dried to remove the residual moisture and pulverized into powder. Then plant extract was prepared by mixing 1% of plant extract with deionized water in a 250ml of conical flask. Then the solution was incubated for 30 min. and subsequently subjected to centrifuge for 30 min. at room temperature with 5000 rpm. The supernatant was separated and filtered with (mm filter paper pore size) filter paper with the help of vaccume filter. Then the solution was used for the reduction of silver ions Ag^{+}) to silver nanoparticles (Ag^{o}).

Synthesis of Silver Nanoparticles

Four concentrations of plant extract were prepared (20, 40, 60, 80 mL) in 20 mL of 1 mM silver nitrate in the ratio of (1:1, 2:1, 3:1, 4:1) by increasing the concentration of plant extract in the solution mixture. 0.17% of 1mM $AgNO_3$ metal ion was added to the prepared plant extract. Then the bio-reduced aqueous component solution turned colloidal and different in colour, which suggested the formation of *Citrus auratifolia* leaf extract silver nanoparticles however, a control solution was made by 10 mL of *citrus* leaf extract (solution) without silver nitrate solution.

After incubation, the solution was centrifuged at 12000 rpm for 4 minutes, and the obtained precipitate mass i.e., *Citrus auratifolia* leaf extract silver nanoparticles was washed three times with distilled water and centrifuged at 12,000 rpm for 3min.The mass was collected and oven dried at 42°C. Thereafter *Citrus auratifolia* leaf extract silver nanoparticles so obtained were scraped, coded (As, Bs, Cs, Ds) and kept for further analysis.

Determination of Antibacterial Activity

Antibacterial Susceptibility of silver nanoparticles

The standard drug Amoxicillin, *Citrus auratifolia* leaf extract solution and *Citrus auratifolia* leaf extract silver nanoparticles (As, Bs, Cs, Ds) were screened against different strains of pathogenic organisms such as Gram positive (*Staphylococcus aureus*) and Gram negative (*Salmonella typhi* and *Klebsiella pneum*onia) using a modified filter paper disc agar diffusion method described [7-8]. A disc of blotting paper was impregnated with 5ml of the standard drug Amoxicillin, *citrus auratifolia* leaf extract solution and (As, Bs, Cs, Ds) correspondingly. These were placed on plate of sensitivity testing agar uniformly inoculated and thereafter incubated at 37ºC for 24hrs. The inhibition zones were observed and then recorded in millimeters using a transparent plastic meter rule [9].

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentrations were determined by disc diffusion method using dilution method. The stock samples were diluted to have 100µg/ml, 200µg/ml, 300µg/ml, 400mg/ml respectively hence, the lowest concentrations that show activity were taken as the MIC [9].

Figure 1: Effect of (As, Bs, Cs, Ds) against bacterial strain S. typhi

Figure 2: Effect of (As, Bs, Cs, Ds) against bacterial strain S. aureus

Figure 3: Effect of (As, Bs, Cs, Ds) against bacterial strain K. pneumonia

Table 1: The Result of Synthesis of Silver Nanoparticles Using Citrus Leaf Extract

S/No	Sample	Ratio(v/v)	Colour after 24 hrs	*Citrus a.* leaf extract silver nanoparticle
1	A	1:1	Tan	As
2	B	2:1	Latte	Bs
3	C	3:1	Linen	Cs
4	D	4:1	Off white	Ds
5	E	-	Yellow	-

Key: A, B, C, D = *Citrus aurantifoloia* leaf extract solution + $AgNO_3$

E= *Citrus aurantifoloia* leaf extract solution

(As, Bs, Cs, Ds) = *Citrus a.* leaf extract silver nanoparticles

Table 2: The Result of Antibacterial Susceptibility of silver nanoparticles

S/No	Organism	Zone of Inhibition (mm)					
		As	Bs	Cs	Ds	E	F
1	*Salmonella typhi*	4	3	4	5	R	8
2	*Staphylococcus aureus*	3	2	2	3	R	10
3	*Klebsiella pneumonia*	3	2	2	3	R	R

Key:

(As, Bs, Cs, Ds) = *Citrus aurantifolia* leaf extracts silver nanoparticles

E= *Citrus aurantifoloia* leaf extract solution

F= Amoxicillin (standard drug)

R= Resistant

Table 3: The Result of Minimum Inhibitory Concentration of silver nanoparticles

S/No	Organism	Zone of Inhibition (mm)					MIC (µg/ml)
		A	B	C	D	F	
1	*Salmonella typhi*	4	3	4	5	8	200
2	*Staphylococcus aureus*	3	2	2	3	10	200
3	*Klebsiella pneumonia*	3	2	2	3	-	300

Results and Discussion

The synthesis of the silver nanoparticles was confirmed by the characteristic colour change from greenish to tan, latte, linen and off white respectively, for the ratios of 1:1, 1:2, 1:3 1:4 of *Citrus a.* leaf extract solution together with AgNO3 as shown in the (Table 1). The nano products formed were coded (As, Bs, Cs, Ds). This result is in consonance with the findings of Sivakumar and Vidyasagar (2014) reported that during the synthesis of silver nanoparticles using Annona reticulata the color of the reaction mixture after 20 min, at room temperature, changed to dark brown, indicating the formation of AgNPs.

Silver has displayed antibacterial, antiviral and antifungal properties when used in the nanoscale. AgNPs are widely used for coating of medical devices, disinfection of medical devices, home appliances and water purification [10].

The inhibitory effects of *Citrus a*. leaf extract silver nanoparticles As, Bs, Cs, and Ds were sreened against *S. typhi, S. aureus* and *K. pneumonia* using disc diffusion method at three different concentrations of 100µg/ml, 200µg/ml 300µg/ml, 400µg/ml respectively. The nano particle coded As has demostrated inhibitory effects of 4mm and 3mm against *S. typhi* and *S. aureus* at the concetration of 200µg/ml. However, The nano particle coded Bs shows inhibitory effects of 4mm and 3mm against *S. typhi* and *S. aureus* at the concetration of 200µg/ml . The nano particle coded Cs shows inhibitory effects of 4mm and 2mm against *S. typhi* and *S. aureus* at the concetration of 200µg/ml. The nano particle coded Ds also shows inhibitory effects of 5mm and 3mm against *S. typhi* and *S. aureus* respectively at the concetration of 200µg/ml on the other hand, 3mm, 2mm, 2mm and 1mm inhibitory effects were observed against *K. pneumonia* at the concentration of 300µg/ml for all the sample tested. The activity of the standard drug (amoxicillin) was found to be higher compared to all the samples tested except against *K. pneumonia* in which no inhibition was observe for the amoxicillin at all the concentrations tested. However, no inhibition was observed for the *Citrus aurantifoloia* leaf extract solution (E) against all microorganisms tested.

The inhibitory effects observed for the *Citrus aurantifolia* leaf extract silver nanoparticles (As, Bs, Cs, and Ds) were comparable to that of the standard drug (amoxicillin) this might be considered as an indication of antimicrobial property of the *Citrus aurantifolia* leaf extract silver nanoparticles.Reports suggest that bactericidal effect of silver nano particles by destroying the enzymes that transport the cell nutient and weakening the cell membrane or cell wall, leading to increase cell peamiability and cell death [11]. The remarkable antimicrobial effects observed may be due to the formation of nano particles by the leaf extract of *Citrus aurantifolia* since, no inhibition was observe for the *Citrus aurantifoloia* leaf extract solution (E) alone against all the microorganisms tested.

Conclusion

In conclusion, this study reveals the antimicrobial activity of *Citrus aurantifolia* leaf extract silver nanoparticles against selected microorganisms. The result of this study shows that *Citrus aurantifolia* leaf extract silver nanoparticles are effective in inhibiting the growth of microorganisms such as *S. typhi, S. aureus and K. pneumonia* respectively.

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