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ANTIBACTERIAL PROPERTIES OF SILVER NANOPARTICLES OF RHIZOMES (*Curcuma longa* and *Zingiber officinale*)

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ABSTRACT
Spices are known to extend shelf life by inhibiting growth or decreasing food borne pathogens. The study into the antibacterial properties on Silver Nanoparticle $AgNP_s$ of *Curcuma longa* and *Zingiber officinale* on *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* bacteria species. The rhizomes were purchased from a market in Abuja, Nigeria and identified in the Herbarium Unit of National Institute for Pharmaceutical Research and Development, Abuja. Aqueous extract of each spice was nanoscaled into silver nitrate nanoparticles. The particles were screened for antibacterial activities against *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* at varied concentrations of 80, 40, 20 and 10 mg/mL in agar culture media. *C. longa*-AgNPs had antimicrobial activities on all the test organisms with varying degrees of zones of inhibition. The highest zones of inhibition (21.5 ± 4.9 mm) were recorded against *B. subtilis* followed by 18.5 ± 9.2 mm against *E. coli* at a concentration of 80 mg/mL. The lowest zones of inhibitions were recorded at 80 mg/mL against all the test isolates except *B. subtilis* where 12.0 ± 0.0 mm zone of inhibition was recorded. *Zingiber officinale*-AgNPs also had antimicrobial activities with varying zones of inhibition for the various test organisms. *Z. officinale* AgNPs with a highest zone of inhibition (17.5 ± 0.7 mm) against *B. subtilis* at 80 mg/mL. while at 40 mg/mL, the plant extract had higher zones of inhibition against the other test organisms. Therefore, the rhizomes can be used for development of effective treatment therapy against bacterial strains.
Keywords: Spices, rhizomes, silver nanoparticles, agar diffusion antibacterial activity, test bacteria, zone of inhibition

INTRODUCTION
The past three decades has experienced the emergence of bacterial strains that are resistant to multiple antibiotics (Islam *et al*., 2014). This resistance has been reported to be as a result of indiscriminate intake and use of broad-spectrum antibiotics, release of untreated pharmaceutical waste into the environment, treatment of infections without proper laboratory diagnosis, incomplete dosage among many other factors contribute to bacterial development of resistance to multiple antibiotics (Yang *et al*., 2017; Abuga and Gaobotse, 2019). This menace is of great public and clinical significance causing loss of time and resources, prolonged hospital stays, which could lead to morbidity and mortality (Reda *et al*., 2019). This however, has raised awareness among researchers in the scientific field to search for novel antimicrobial products and technology that could help curb the spread of bacterial resistance to multiple antibiotics. Among the technology sort out for with promising potentials is nanotechnology (Abuga and Gaobotse, 2019).

Nanotechnology is an emerging field with numerous applications such as in personal care products, packaging materials used in the food industries, systems used in delivering therapeutic agents in the medical sector to improve treatment (i.e. nanomedicine) among many others (Shalaby *et al*., 2015; Alsammarraie *et al*., 2018; Khan *et al*., 2019). Nanotechnology utilizes nanoparticles (NPs), which have been considered as a link between bulk size materials and atomic structures (Alsammarraie *et al*., 2018). NPs have a simple structural design and come in varying sizes ranging from 1 - 100 nm. Unique features of NPs include small in size with high surface energy and a large surface area to mass ratio. There are new specific properties exhibited by NPs by their shape, size and the particles distribution (Mahardika *et al*., 2021). Inorganic NPs are unique in nature as they provide the users with varieties of functions (Alsammarraie *et al*., 2018). Considerable attention has been given to gold (Au) and silver (Ag) NPs owing to their high performance in catalysis, optics, and biosensing. However, silver nanoparticles (AgNPs) have been demonstrated to have great potentials when used as catalyst in chemical reactions, excellent carriers of antioxidant and antimicrobial agent (Alsammarraie *et al*., 2018; Maghimaa and Alharbi, 2020).

There are different methods such as microwave assisted, nonchemical, chemical and green route processes through which AgNPs can be synthesized. With the exception of green synthesis, the aforementioned processes have been demonstrated to have deleterious effect on the environment, they are expensive and use harmful chemicals (Shalaby *et al*., 2015; El-Deeb *et al*., 2016). As such, the synthesis of AgNPs via green synthesis has been utilized owing to its safety, cost effectiveness and efficacy, which does not require high temperature and pressure (Maghimaa and Alharbi, 2020). The use of plants in the synthesis of AgNPs has been reported to be suitable in forming stable AgNPs in a short time. Medicinal plants and spices such as turmeric and

ginger are believed to obtain their therapeutic properties due to the presence of phytochemicals (Bashir *et al*., 2015). These phytochemicals such as flavonoids, alkaloids, phenols among many others are nontoxic, provide a good platform for AgNPs synthesis and act as a natural agent for capping and stabilization of the particles (Dinda *et al*., 2019; Sharma *et al*., 2020).

Curcuma longa commonly known as 'turmeric' and ginger commonly known as 'ginger' are rhizomatous perennial herbaceous plants native to South Asia (Mohammed *et al*., 2019). These plants belong to Zingiberaceae family and have been used as spice in food condiment as well as herbal preparations (Beristain-Bauza *et al*., 2019; Mohammed *et al*., 2019). Curcumene is the most common phytochemical found in turmeric, which possesses lots of medicinal and culinary benefits (Gunes *et al*., 2016; Teow *et al*., 2016). Some of the medicinal properties of turmeric include anticoagulant, anti-inflammatory, anticarcinogenic, antioxidant, antidiabetic, antifungal and antibacterial activities (Mbah-Omeje, 2019). In culinary, turmeric is used as preservative as well as color and flavor to food (Gul and Bakht, 2015; Azhari *et al*., 2018; Teow *et al*., 2019). Onuoha *et al*. (2021) reported methanol extracts of turmeric to have antibacterial activities against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* at all concentrations used.

Ginger (*Zingiber officinale*) has been used since ancient times in traditional medicine in the treatment of various health disorders including but not limited to tumors, inflammation, cardiac disorders, rheumatism, colds, vomiting, nausea and cough among many others (El-Refai *et al*., 2018). Various phytochemicals have been found to be present in ginger and are believed to be responsible for the therapeutic properties it possesses. Some of the phytochemicals include gingerols, zingiberene, flavonoids, alkaloids, among many others (Yang *et al*., 2017). Various studies have reported antibacterial activities of ginger. Onuoha *et al*. (2021) reported methanol extract of ginger to exhibit potent antibacterial activities against *S. aureus* and *E. coli* with zone of inhibition of 21.0 ± 1.4 mm and 14.0 ± 0.0 mm respectively at 80 mg/mL. However, *Salmonella typhi* and *Bacillus subtilis* were all reported to be resistant to the ginger extract (Onuoha *et al*., 2021).

Since *C. longa* and *Z. officinale* contains various phytochemicals and have been demonstrated to have antibacterial potentials, therefore, their use in green synthesis of AgNPs is expected to yield a better antibacterial result. Thus, the aim of this study was to evaluate the antibacterial efficacy of *C. longa*-AgNPs and *Z. officinale*-AgNPs against selected bacterial strains.

MATERIALS AND METHODS

Collection of spices

The spices (*C. longa* and *Z. officinale*), were purchased at Garki market Abuja. These spices were identified in the Herbarium Unit of National Institute for Pharmaceutical Research and Development (NIPRD) by a taxonomist.

Synthesis of Silver Nanoparticles

AgNPs of each extract was synthesized by adopting the method described by Gloria *et al*. (2017). A 20g of each sample was weighed into conical flask of 250ml and 100ml of water was added at 60˚C in a water bath for 10 minutes respectively. Each extract was cooled, filtered using watchman filter paper. Fifteen (15) ml of each extract was added into 45ml aqueous silver nitrate ($AgNO_3$) (0.1M solution) at room temperature and stirred continuously with a magnetic stirrer for 15 minutes so as to get a solution of extract and silver nitrate in the ratio of 1:3. Each conical flask containing the respective extract was wrapped in aluminum foil and kept in the dark to prevent auto-oxidation of silver. After 24 hours, each extract containing silver Nanoparticle ($AgNP_s$) was centrifuged at 3000 rpm for 10 minutes and the resulting pellets were dried in an oven at 100°C for 24 hours. The resultant $AgNP_s$ of each extract was used for antimicrobial assay.

Test Microorganisms

Salmonella typhi, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were obtained from National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. These test microorganisms were authenticated using selective media and biochemical tests.

Antimicrobial susceptibility study

Agar diffusion technique described by NCLS (2003) was used for antimicrobial susceptibility study. One gram each $AgNP_S$ extract was dissolved in 1.0ml dimethysuphloxide (DMSO) and added to 5.0mL sterile distilled water. A concentration of 80 mg/mL was prepared and dilutions 40, 20, 10 mg/ml were obtained by serial dilutions using sterile distilled water. Muller Hinton agar were prepared and dispensed into Petri dishes. Each culture media was inoculated with the specific test bacteria using a pipette and allowed to dry in the biosafety cabinet before boring the wells. Five wells were bored on the seeded Muller Hinton agar with 6 mm Cork borer. The base was covered with a drop of molten agar to avoid flow of extract at the base. A 100µl of the diluted crude extract (i.e. *C. longa*, and *Z. officinale*) was dispensed on the labeled wells. This procedure was repeated for each of the test bacteria. All plates were incubated at 37˚C for 24 hours. The zones of inhibition of each plate were observed and measured using a meter rule and values were recorded accordingly.

Results

The antimicrobial activities of varied concentrations of *C. longa*-AgNPs aqueous extracts are shown in Table 1 while those of *Z. officinale*-AGNPs aqueous extracts are shown in Table 2. All the test isolates were susceptible to *C. longa*-AgNPs aqueous extracts with varying degree of zones of inhibition. The highest zone of inhibition (21.5 ± 4.9 mm) was recorded against *B. subtilis* followed by 18.5 ± 9.2 mm

against *E. coli* all at a concentration of 80 mg/mL. The lowest zones of inhibition were recorded at 10 mg/mL against all the test isolates except *B. subtilis* where 12.0 ± 0.0 mm zone of inhibition was recorded. *B subtilis* was most susceptible to *C. longa*-AgNPs water extract followed by *E. coli*.

Table 1: Antimicrobial Activities of *Curcuma longa* - AgNPs Water Extract

Isolate	Zone of Inhibition (mm)				
	80 (mg/mL)	**40** (mg/mL)	**20** (mg/mL)	**10** (mg/mL)	**C** (50 µg/mL)
S. typhi	$14.5^{c} \pm 0.7$	$13.0^{ab} \pm 2.8$	$12.5^{a} \pm 0.7$	$11.5^{a} \pm 0.7$	$13.0^{ab} \pm 2.8$
E. coli	$18.5^{d} \pm 9.2$	$14.5^{c} \pm 3.5$	$12.5^{b} \pm 0.7$	$11.0^{a} \pm 0.0$	$19.0^{e} \pm 1.4$
S. aureus	$15.0^{d} \pm 2.8$	$13.0^{c} \pm 0.0$	$12.0^{b} \pm 0.0$	$11.0^{a} \pm 0.0$	$21.0^{e} \pm 1.4$
B. subtilis	$21.5^{c} \pm 4.9$	$17.0^{b} \pm 1.4$	$16.5^{b} \pm 4.9$	$12.0^{a} \pm 0.0$	$21.0^{c} \pm 1.4$

Values are means of two determinations. Means with different letter (s) in a row differ significantly at P<0.05

Zingiber officinale-AgNPs water extract was found to possess antimicrobial properties at varying zones of inhibitions (Table 2). All the test isolates were found to be susceptible to the AgNPs aqueous extract of *Z. officinale* at varied concentrations with a highest zone of inhibition of 17.5mm against *B. subtilis* at 80 mg/mL. At 40 mg/ml, the plant extract was found to demonstrate higher zones of inhibition compared to zones of inhibition measured at 80 mg/mL against all the test isolates except *B. subtilis.*

Discussion

Green synthesis of AgNPs have been found to enhance the antimicrobial potentials of medicinal plants and spices. A recent study by Onuoha *et al.* (2021) reported the antimicrobial activities of *C. longa* against four bacterial species viz: *B. subtilis*, *S. aureus*, *S. typhi* and *E. coli* with zones of inhibition at 9.5, 8.5, 11.0, and 10.5mm at 80 mg/ml. These results were similar to that of Gunes *et al.* (2016). However, there was an increase in antibacterial activities of aqueous *C. longa*-AgNPs observed in this study against the aforementioned isolates when compared with the methanol extracts reported by Onuoha *et al*. (2021) who reported an increase in zones of inhibition from 9.5 (methanol extract) to 21.5mm (AgNPs) was observed for *B. subtilis*, 8.5 (methanol extract) to 15.0mm (AgNPs) for *S. aureus*, 11.0 (methanol extract) to 14.5mm (AgNPs) for *S. typhi* and 10.5 (methanol extract) to 18.5mm (AgNPs) for *E. coli* at 80 mg/mL. These increments in zones of inhibition recorded using aqueous extract of *C. longa*-AgNPs is most likely an attributed to the synthesized AgNPs which were capable of penetrating through the membranes of the test microorganisms without much inhibition. Maghimaa and Alharbi (2020) also demonstrated effect of AgNPs on the antibacterial potentials of *C. longa*. In their study, aqueous extract of *C. longa* was found to be active against pathogenic S. *aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *E. coli* and *Candida albicans* with inhibition zones of 14, 15, 15, 14 and 15 mm respectively.

However, when green synthesis of AgNPs of *C. longa* was used, the zones of inhibition increased for *S. aureus* (from 14 to 18 mm), *S. pyogenes* (from 15 to 17 mm), *P. aeruginosa* (from 15 to 19 mm), *E. coli* (from 14 to 17) and *C. albicans* (from 15 to 17 mm) Maghimaa and Alharbi (2020). Antibacterial sensitivity to *C. longa*-AgNPs was observed to be highest against *S. aureus* and *P. aeruginosa* with +4 mm zones of inhibition from results of the aqueous extract. *P. aeruginosa* have been reported in the past to evade effects of antibiotics through the action of its biofilm. However, the use of *C. longa*-AgNPs was able to negate to a great extent the protective action biofilm of *P. aeruginosa*. These AgNPs were able to function as drug carriers across the biofilm of *P. aeruginosa*, which is in agreement with the reports of Reda *et al.* (2019).

Zinger officinale have been used for centuries in treating bacterial infections (Rahmani *et al.*, 2014). Their phytochemical constituents have made them a good candidate for green synthesis of AgNPs. In this

Table 2: Antimicrobial Activities of *Zingiber officinale* - AgNPs Water Extract

Isolate	Zone of Inhibition (mm)				
	80 (mg/mL)		**80** (mg/mL)		**80** (mg/mL)
S. typhi	$13.5^{b} \pm 2.1$	*S. typhi*	$13.5^{b} \pm 2.1$	*S. typhi*	$13.5^{b} \pm 2.1$
E. coli	$11.0^{a} \pm 0.0$	*E. coli*	$11.0^{a} \pm 0.0$	*E. coli*	$11.0^{a} \pm 0.0$
S. aureus	$12.0^{a} \pm 0.0$	*S. aureus*	$12.0^{a} \pm 0.0$	*S. aureus*	$12.0^{a} \pm 0.0$
B. subtilis	$17.5^{d} \pm 0.7$	*B. subtilis*	$17.5^{d} \pm 0.7$	*B. subtilis*	$17.5^{d} \pm 0.7$

Values are means of two determinations. Means with different letter (s) in a row differ significantly at P<0.05

study, *Z. officinale*-AgNPs was found to be active against all the test microorganisms. Onuoha *et al*. (2021) reported the antibacterial potentials of *Z. officinale* methanol extract against *S. pyogenes*, *E. coli*, *S. aureus*, and *B. subtilis.* However, the methanol extract was only active against *S. aureus* and *E. coli* at all concentration, while *S. pyogenes* and *B. subtilis* were both resistant. However, *Z. officinale*-AgNPs inhibited all the test microorganisms with varying zones of

inhibitions and at different concentrations. The highest zone (18.0mm) of inhibition was recorded against *S. aureus* at 40 mg/mL while it was 17.5mm for *B. subtilis* at 80mg/ml. Dinda *et al*. (2019) reported that an increase in concentration led to a proportional increase in the inhibitory properties of AgNPs plant extract. Shalaby *et al*. (2015) proposed a mechanism by which AgNPs could have exhibited inhibitory activities which include that the positively charged AgNPs attach themselves to the negatively charged cell wall of bacteria causing rupture followed by protein denaturation and subsequently cell death. Usually, there is accumulation of precursors of envelop protein, which leads to proton motive force dissipation when nanoparticles (NPs) or Ag ions attach themselves to bacterial cell wall. There is also outer membrane destabilization or plasma membrane rupture, which causes intracellular ATP depletion whenever AgNPs attaches themselves to bacterial cell wall. These could be some of the reasons why all the test isolates in this study were sensitive to *Z. officinale*-AgNPs. There are the possibility of *Z. officinale*-AgNPs penetrating cell walls of the test microorganism, allowing the particles to interfere with DNA function. Since synthesis of AgNPs reduces the particles into nano-sizes as well as increasing their surface area of interaction to mass surface ratio.

Conclusion

The synthesized AgNPs of *C. longa* and *Z. officinale* had antibacterial activities. Therefore, silver nanoparticles of the plants could be harnessed in developing effective treatment therapy against these bacterial strains.

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