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Antimicrobial Ffects of Five Indigenous Spiceextracts Synthesized On Silver Nanoparticles on Selected Microorganisms

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Abstract
Medicinal plants are the mainstay of modern medicine as their usehave been known and used for centuries. Aqueous extract of Piper guineense, Capsicum annuum, Monodora myristica, Allium sativum and Cinnamonum cassiaused as spices were synthesized into silver nanoparticles. Scanning electron micrograph, dispersive X-ray and Fourier Transform Infrared Spectroscopy of each spice-silvernitrate particle were evaluated. The antibacterial properties of each spice-silver nitrate nanoparticle(AgNPs)were evaluated on 4 bacteria namelySalmonella typhi, Escherichia coli, Staphylococcus aureus and Bacillus subtilis at varied concentrations of 80,40, 20 and 10 mg/mlon agar culture media. The SEM Figure/Picture shows that they are well dispersed with no agglomerations. EDX shows that there were $AgNP_S$presence. The FTIR shows the presence of some functional groups, wavelength and absorbance.
A. sativum-AgNPshighest zone of inhibition was recorded against S. typhi, E. coli, and B. subtilis at 80 mg/mL, C. annuum-AgNPsthe plant extract was found to be active against B. subtilis and E. coli at 80mg/mL, P. guineense highest zones of inhibition was recorded at 80 and 40 mg/ml against S. aureus,
C. cassie-AgNPs extract highest zone of inhibitions was againstE. coli, B. subtilis and S. aureus, M. myristica-AgNPs water extracts highest zone of inhibition was recorded against B. subtilisand S. typhiat 80 mg/ml. Therefore, synthesized $AgNP_S$ of spices can be utilized in the development of therapeutic medicine.

Keywords: *Spices, silver nanoparticles, agar diffusion antibacterial activity, bacteria, scanning electron microscopy, Energy dispersive X-ray, Fourier transform infrared*

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I. Introduction

The emergence of multiple drug resistant (MDR) bacterial strains has been on the increase in the last three decades (Islam *et al.,* 2014).This is as a result of mismanagement of antimicrobial products, release of untreated waste from pharmaceutical industries into the environment, self-medication without clinical test to ascertain the causative microbes, incomplete dosage, counterfeit drugs among other factors can lead to bacterial resistance to antimicrobial agents (Yang *et al.,* 2017; Abuga and Gaobotse, 2019). The absence of immediate solution in tackling the incidence of multiple drug resistant bacteria will add to the health burden currently encountered by medical personnel and the public with a resultant loss of finance and time, prolonged stay in the hospital as well as mortality and morbidity (Reda *et al.,* 2019). Sequel to this, more effort is being directed in the search for effective drugs and technologies by researchers all over the globe to help tackle the menace caused by MDR bacteria. Various technology haveexploited, however, nanotechnology has shown great potentials in the combat against MDR bacteria (Abuga and Gaobotse, 2019).

Nanotechnology is an emerging field, which utilizes nanoparticles (NPs) in various applications such as in food packaging, as preservatives, in cosmetics, as carriers of therapeutic agents in nanomedicine (Shalaby *et al*., 2015; Alsammarraie*et al.,* 2018; Khan *et al.,* 2019). NPs are currently being exploited in the treatment of infectious diseases; they are regarded as a bridge between atomic structures and large sizes of materials (Alsammarraie*et al.,* 2018). The structural design of NPs is simple and come in different sizes ranging between 1 - 100 nm. Their peculiar features such as high energy on their surfaces together with a large surface area to

mass ratio makes NPs effective in any given reaction,they are involved in. NPs also exhibit new specific properties in their particle distribution, size and shape (Mahardika *et al*., 2021). Nanoparticles that are inorganic in nature are unique and provide different functions to users (Alsammarraie*et al.*, 2018). Due to high efficiency in catalysis, biosensing and optics has made gold (Au) and silver (Ag) NPs to be exploited in nanomedicine (Bouqellah*et al.*, 2019). However, silver nanoparticles (AgNPs) have exhibited promising potentials when employed in chemical reactions as well as excellent carriers of antioxidants and antimicrobial substances (Alsammarraie*etal*., 2018; Maghimaa and Alharbi, 2020).

Various methods have been employed in the synthesis of AgNPs, they include chemical, sonochemical, microwave, ultrasonification, irradiation and green processes (Chung *et al*., 2016). All the aforementioned methods have harmful effects to the environment except green synthesis. They are also expensive and utilizes harmful chemicals in their synthesis (Shalaby *et al*., 2015; El-Deeb *et al*., 2016). As a result of this, green synthesis is always preferable since it is safe, cost effective, efficient, does not utilize high pressure and temperature (Maghimaa and Alharbi, 2020). The use of medicinal plants and spices among varying methods employed in green synthesis of AgNPs have proven to be suitable in the formation of stable AgNPs within a short period of time due to the abundant phytochemicals they possess (Bashir *et al*., 2015)

Medicinal plants are the backbone of modern medicine, their use in medicine have been known and utilized for centuries. Medicinal plants have taken lead roles as sources of important bioactive compounds to tackle infections caused by MDR bacteria (Otunola*et al.*, 2017). However, these herbal drugs could sometimes encounter problems in the delivery system, thus, become inefficient in the treatment of microbial infections. Some of these hindrances include low bioavailability, instability in biological, poor permeability and solubility among many other factors. These hindrances can be overcome when bioactive compounds in herbal drugs are encapsulated or attached to NPs, which enhances significantly the pharmacokinetics and the overall performance of the herbal drug. Medicinal plants are of great importance in green synthesis of NPs. They can control the shape and size of NPs depending on the phytochemicals they possess, which act as a capping layer. Different medicinal plants as well as spices have been employed in the production of AgNPs (Otunola*et al*., 2017).

Spices such as *Piper guineense* (Mgbeahuruike*et al.*, 2018), *Capsicum annuum* (Rajalakshmi and Puviyarasu, 2019), *Monodora myristica* (Akise *et al.*, 2020), *Allium sativum* (Andleeb *et al.*, 2020), and *Cinnamonum cassia* (Kushwaha *et al.*, 2021), are common to most cuisines across the globe. Aside the culinary benefits spice the aforementioned spices have, they also confer medicinal benefits and have been used by consumers as remedies for various diseases. Varying biological functions attributed to these spices include anticancer, hypoglycemic, antidiabetic, antihypertensive, immunomodulatory, hypolipidemic, antioxidant and antimicrobial among many others. The phytochemical investigation of these spices shows that they are rich in tannins, phenols, alkaloids, flavonoids, saponins and carotenoids, which have been demonstrated to exhibit strong antioxidant properties and can be considered as a reducing factor in green synthesis of AgNPs (Dinda *et al*., 2019; Sharma *et al*., 2020).As such, this study was aimed at evaluating the antibacterial efficacy of five spices synthesized with AgNPs against some selected pathogens.

II. Materials and Methods

Collection of spices

Piper guineense, *Capsicum annuum*, *Monodora myristica*, *Allium sativum* and *Cinnamonum cassia*were purchased at Garki market Abuja. These spices were identified by a taxonomist from the Herbarium Unit of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja.

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.

Fig. 1 Picture showing the extract of un-synthesized sample and the dark brown colors showing extract after synthesis of $AgNP_S$.

Test microorganisms

Salmonella typhi, Escherichia coli, Staphylococcus aureus and *Bacillus subtilis* were obtained from NIPRD. These test microorganisms were authenticated using selective media and biochemical tests.

Antimicrobial susceptibility study

Agar diffusion technique was used (NCLS, 2003). One gram each of spice-$AgNP_S$sample was dissolved in 1.0ml of dimethysuphoxide (DMSO) and added to 5.0mL sterile distilled water. A concentration of 80 mg/mL was made and further 1:2 dilutions were done to obtain 40, 20, 10 mg/ml. Muller Hinton agar Petri dishes were prepared and each inoculated with the specific test bacteria and allowed to dry. Five wells were bored on the seeded Muller Hinton agar with 6mm cork borer. The base covered with molten agar to avoid flow at the base. 100µl of the diluted crude 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 with Vernier clapper and recorded accordingly.

Fourier-transform infrared spectroscopy (FTIR)

The absorbance of the sample as a function of wave number (cm^{-1}) was determined using FTIR (Nicolet iS5, Thermo-scientific Berlin Germany). The FTIR was carried out to identify the functional groups and the types of bonds occurring at the range of 500-4000 (cm^{-1}). FTIR is used as a supportive tool in qualitative bioactive compound screening. Figures 7-11and Table 1-5 below presents the FTIR spectra of AS, CA, PG, Ci, MM.

III. Results

Scanning Electron Microscope / Energy Dispersive X-Ray (Sem/Edx)

Scanning Electron Microscope / Energy Dispersive X-Ray (SEM/EDX) of each spice-silver nanoparticle (spice-AgNP) is shown inFigure 2-6.The scanning electron micrograph of the spice- $AgNP_S$showed that they were crystalline, well dispersed with no agglomerations while EDX further confirmed the presence and formation of $AgNP_s$. FTIR shows some functional groups with different wavelengths

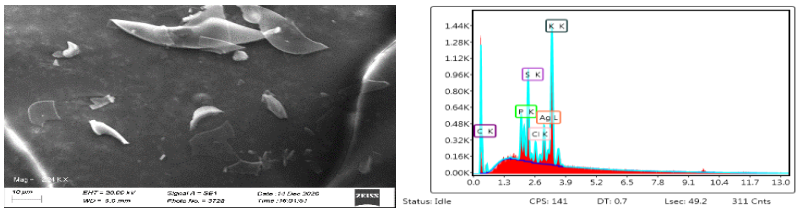

Figure 2: SEM for AS EDX for AS

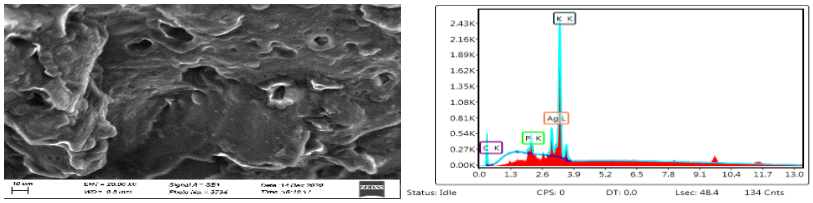

Figure 3: SEM for CA EDX for CA

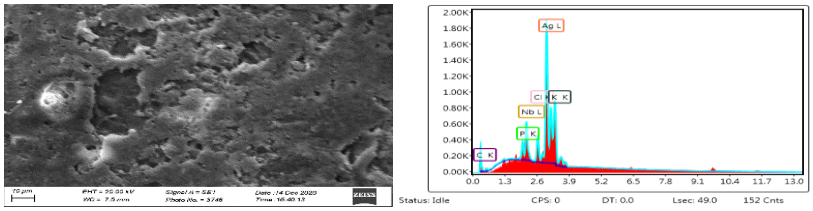

Figure 4: SEMfor PG EDX for PG

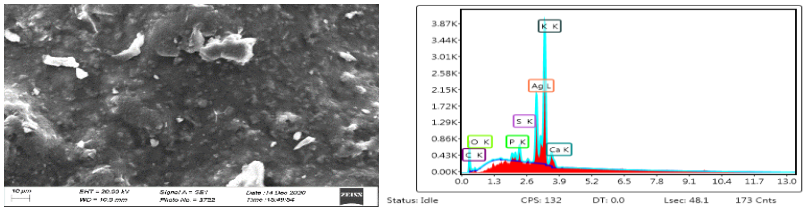

Figure 5: SEM for Ci EDXfor Ci

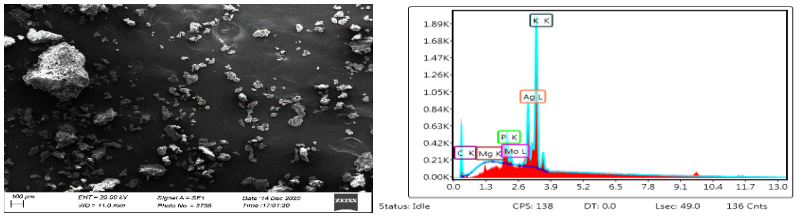

Figure 6: SEMfor MM EDX for MM

Fig. 3 Scanning Electron Microscope / Energy Dispersive X-Ray (SEM/EDX) of each spice-AgNPS: AS (*A. sativum*), CA (*C. annuum*), PG (P. *guineense*)Ci (*Cinnamonum cassia*), MM(*M. myristica*).

Fourier-transform infrared spectroscopy (FTIR) Figures

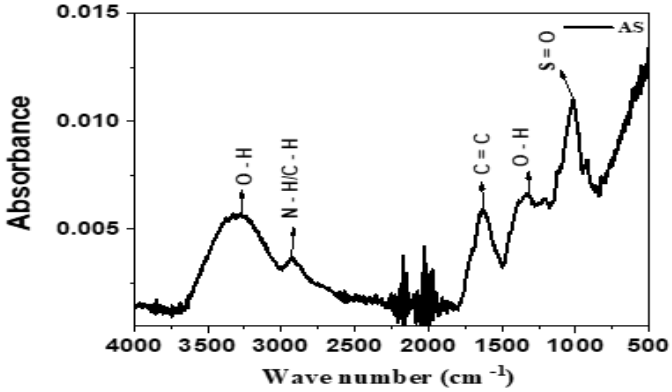

Fig. 7. FT-IR spectra of *Allium sativum*(AS)

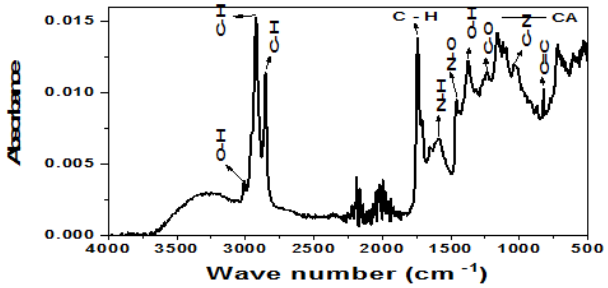

Fig. 8. FT-IR spectra of ***Capsicum annuum***(CA)

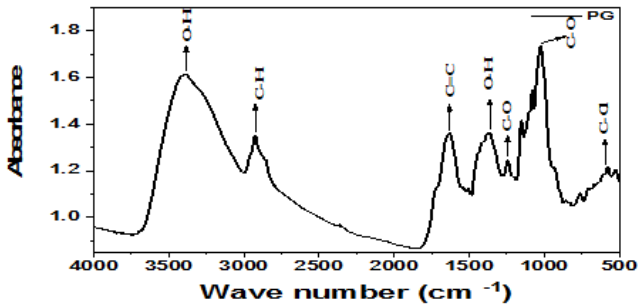

Fig. 9. FT-IR spectra of ***Piper guineense*** (PG)

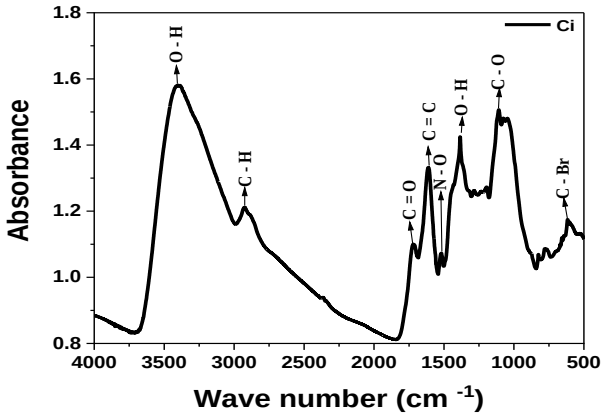

Fig. 10. FT-IR spectra of cinnamonum (Ci)

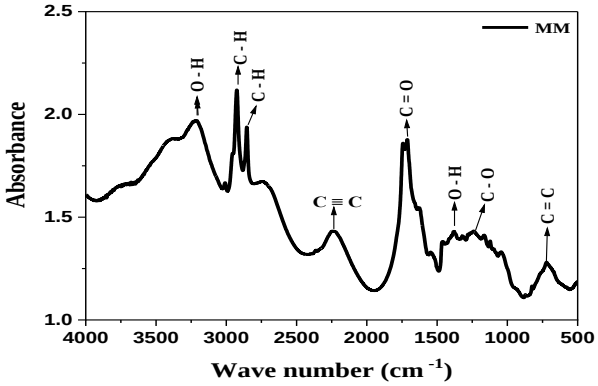

Fig. 11. FT-IR spectra of *Monodora myristica*(MM)

Fourier-transform infrared spectroscopy (FTIR) Tables

Table 1: Vibrational frequencies and wave number of AS

Wave number (cm^{-1})	bond	functional group
3324.03	O-H stretching	alcohol (strong)
2931.11	C-H/N-H stretching	alkane (medium)
1634.40	C=C stretching	alkane (medium)
1330.69	O-H bending	phenol (medium)
1017.83	S=O stretching	sulfoxide (strong)

Table 2: Vibrational frequencies and wave number of CA

Wave number (cm^{-1})	Bond	functional group
3007.92	O-H stretching	alcohol (weak)
2922.94	C-H stretching	alkane (medium)
2852.69	C-H stretching	alkane (medium)
1742.97	C-H bending	aromatic compound (weak)
1587.83	N- H / C=C stretching	cyclic alkane (medium)
1457.42	N-O stretching	nitro compound (strong)
1376.82	O-H bending	alcohol (medium)
1234.56	C-O stretching	alkyl aryl ether (strong)
1039.53	C-N stretching	amine (medium)
824.04	C=C bending	alkene (medium)

Table 3: Vibrational frequencies and wave number of PG

Wave number (cm^{-1})	Bond	functional group
3385.73	O-H stretching	alcohol (strong)
2925.11	C-H stretching	alkene (medium)
1629.02	C=C stretching	α,β-unsaturated ketone (strong)
1365.69	O-H bending	alcohol (medium)
1243.06	C-O stretching	alkyne aryl ether (strong)
1023.13	C-O stretching	vinyl ether (strong)
576.26	C-Cl stretching	halo compound (strong)

Table 4: Vibrational frequencies and wave number of Ci

Wave number (cm^{-1})	Bond	functional group
3404.76	O-H stretching	alcohol (strong)
2925.42	C-H stretching	alkene (medium)
1719.76	C=O stretching	conjugated acid (strong)
1612.32	C=C stretching	α,β-unsaturated ketone (strong)
1518.74	N-O stretching	nitro compound (strong)
1384.58	O-H stretching	carboxylic acid (strong)
1108.47	C-O stretching	aliphatic ether (strong)
616.27	C-Br stretching	halo compound (strong)

Organism	Zone of Inhibition (mm)				
	80 (mg/mL)	**40** (mg/mL)	**20** (mg/mL)	**10** (mg/mL)	**Control** (50 µg/mL)
S. typhi	-	-	-	-	$12.0^{a} \pm 0.0$
E. coli	$12.0^{a} \pm 0.0$				$18.0^{b} \pm 0.0$
S. aureus	-	-	-	-	$28.0^{a} \pm 0.0$
B. subtilis	$12.0^{a} \pm 0.0$	$12.0^{a} \pm 0.0$	$12.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$25.0^{b} \pm 0.0$

Table 5: Vibrational frequencies and wave number of MM

Wave number (cm^{-1})	Bond	functional group
3209.67	O-H stretching	alcohol (strong)
2925.16	C-H stretching	alkene (medium)
2854.12	C-H stretching	alkene (medium)
2241.42	C≡C stretching	alkyne (weak)
1712.34	C=O stretching	carboxylic acid (strong)
1379.90	O-H stretching	carboxylic acid (strong)
1241.04	C-O stretching	alkyl aryl ether (strong)
721.17	C=C bending	alkene (strong)

Antimicrobial Susceptibility Test

The antimicrobial activities of different spice $AgNP^{S}$were ascertained and the zones of inhibitions

Table 6. Antimicrobial activities of *Allium sativum* –$AgNP_s$ water extract

Values are means of two determinations. Means with dissimilar letter (s) in a row differ significantly according to the Duncan's Multiple Range Test (DMRT).

Significant at $p \leq 0.01$

Antimicrobial activities of *A. sativum* - AgNPs is shown in Table 6. The plant extract was reported to be active against all the test isolates except *S. aureus* at the varied concentrations. The highest zone of inhibition was recorded against *S. typhi, E. coli*, and *B. subtilis* at 80 mg/mL respectively.

Organism	Zone of Inhibition (mm)				
	80 (mg/mL)	**40** (mg/mL)	**20** (mg/mL)	**10** (mg/mL)	**Control** (50 µg/mL)
S. typhi	$12.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	-	$10.0^{a} \pm 0.0$	$15.0^{a} \pm 0.0$
E. coli	$12.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$10.0^{a} \pm 0.0$	$12.0^{a} \pm 0.0$
S. aureus	-	-	-	-	$25.0^{a} \pm 0.0$
B. subtilis	$12.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$20.0^{a} \pm 0.0$

Table 7. Antimicrobial activities of *Capsicum annuum*–$AgNP_s$ water extract

Values are means of two determinations. Means with dissimilar letter (s) in a row differ significantly according to the Duncan's Multiple Range Test (DMRT).

Significant at $p \leq 0.01$

The antimicrobial activities of AgNPs aqueous extract of *C. annuum* is presented in Table 7. The plant extract was found to be active against *B. subtilis* and *E. coli* whereas *S. typhi* and *S. aureus* were found to be resistant to the plant extract at all concentrations. The highest zone of inhibition exhibited by *C. annuum*-AgNPs water extract was at concentration of 80 mg/mL against *E. coli* and *B. subtilis* and the lowest zone of inhibition was recorded against *B. subtilis* at 10 mg/ml.

Table 8. Antimicrobial activities of *Piper guineense* – $AgNP_s$ water extract

Organism	Zone of Inhibition (mm)				
	80 (mg/mL)	**40** (mg/mL)	**20** (mg/mL)	**10** (mg/mL)	**Control** (50 µg/mL)
S. typhi	$15.0^{d} \pm 0.0$	$14.5^{c} \pm 0.7$	$13.5^{b} \pm 0.7$	$12.5^{a} \pm 1.5$	$15.0^{d} \pm 0.0$
E. coli	$16.5^{d} \pm 0.7$	$13.5^{c} \pm 2.1$	$12.0^{b} \pm 0.0$	$11.0^{a} \pm 0.0$	$20.5^{e} \pm 0.7$
S. aureus	$18.5^{d} \pm 2.1$	$18.0^{c} \pm 1.4$	$15.5^{b} \pm 0.7$	$15.0^{a} \pm 0.0$	$27.5^{e} \pm 2.5$
B. subtilis	$17.5^{d} \pm 0.7$	$16.0^{c} \pm 1.0$	$15.5^{b} \pm 0.7$	$15.0^{a} \pm 0.7$	$20.5^{e} \pm 0.7$

Values are means of two determinations. Means with dissimilar letter (s) in a row differ significantly according to the Duncan's Multiple Range Test (DMRT).

Significant at $p \leq 0.01$

The antimicrobial activities of AgNPs aqueous extract of *P. guineense* was reported to be active against all the test isolates as shown in Table 8. The highest zones of inhibition were recorded against *S. aureus* at 80 and 40 mg/mL respectively while the lowest zone of inhibition was recorded against *E. coli* at 10 mg/mL. The antimicrobial activities exhibited by *P. guineense*-AgNPs water extract was demonstrated to be concentration dependent.

Table 9. Antimicrobial activities of *Cinnamonum cassie*–$AgNP_s$ water extract

Organism	Zone of Inhibition (mm)				
	80 (mg/mL)	**40** (mg/mL)	**20** (mg/mL)	**10** (mg/mL)	**Control** (50 µg/mL)
S. typhi	$12.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$10.0^{a} \pm 0.0$	$12.0^{a} \pm 0.0$
E. coli	$12.0^{a} \pm 0.0$	$12.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$17.0^{c} \pm 0.0$
S. aureus	$11.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$10.0^{a} \pm 0.0$	$10.0^{a} \pm 0.0$	$19.0^{d} \pm 0.0$
B. subtilis	$12.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$19.0^{d} \pm 0.0$

Values are means of two determinations. Means with dissimilar letter (s) in a row differ significantly according to the Duncan's Multiple Range Test (DMRT).

Significant at $p \leq 0.01$

Cinnamonum cassie -AgNPs aqueous extract was observed to be active against all the test organisms with zones of inhibition ranging between against the test organisms as indicated in Table 9. Differences in the zones of inhibition from a particular concentration gradient is ≤1 mm across all the test isolates

Table 10. Antimicrobial activities of *Monodora myristica*–$AgNP_s$ water extract

Organism	Zone of Inhibition (mm)				
	80 (mg/mL)	**40** (mg/mL)	**20** (mg/mL)	**10** (mg/mL)	**Control** (50 µg/mL)
S. typhi	$15.5^{c} \pm 3.5$	$11.5^{b} \pm 0.7$	$11.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$18.0^{d} \pm 0.0$
E. coli	$12.0^{b} \pm 0.0$	$12.0^{b} \pm 0.0$	$11.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$19.0^{c} \pm 0.0$
S. aureus	$11.5^{b} \pm 0.7$	$11.5^{b} \pm 0.7$	$11.0^{a} \pm 0.0$	$11.0^{a} \pm 0.0$	$12.0^{c} \pm 0.0$
B. subtilis	$14.5^{d} \pm 1.4$	$14.5^{d} \pm 3.5$	$11.5^{b} \pm 0.7$	$11.0^{a} \pm 0.0$	$12.0^{c} \pm 0.0$

Values are means of two determinations. Means with dissimilar letter (s) in a row differ significantly according to the Duncan's Multiple Range Test (DMRT).

Significant at $p \leq 0.01$

M. myristica-AgNPs extracts were active against all the test isolates with varying degrees of zones of inhibitions. The highest zone of inhibition was recorded against *B. subtilis* at 80 mg/ml followed by against *S. typhi* at highest concentration of 80 mg/mL. The lowest zone of inhibition was recorded against all the test isolates at the lowest concentration (10 mg/ml) tested. *S. aureus* was found to be least susceptible (with 11.5 ± 0.7 mm at 80 mg/mL) to the antimicrobial activities of *M. myristica*-AgNP3 water extract.

IV. Discussion

Silver nitrate is a chemical reducing agent widely used for synthesis ofsilvernano particles$AgNP_S$ (Hyllested *et al.,* 2015). SEM analysis of the $AgNP_s$ revealed the size, shape, morphology and organization of the $AgNP_S$. It's ability to agglomerate resulted to high surface tension and high surface energy in the extreme fine particles of $AgNP_S$ (Theivasanthi and Alagar, 2012). The EDX measured the distribution of X-ray signals generated by an electron beam on the specimen which was confirmed by the $AgNP_S$ synthesis of the extracts (Song and Kim, 2009). The results showed weak peaks from the EDX due to biomolecules being bound to the surface.

FTIR is used to reveal the $AgNP_S$ capping and reduces thefunctional group from the biomolecule for identification and also played role for the identification and characterization of functional groups (Sasidharan *et al*, 2011).The polarity of the$AgNP_S$contributed to the major role in extracting more specific bioactive compoundsof the carboxylic acid compounds, alcohols, alkenes, alkynes, aliphatic ether, vinyl ether, α β-unsaturated ketone and phenol.

In this study, the antibacterial properties of aqueous extracts of spice-AgNPs exhibited varying degrees of activities evident by zones of inhibition measured from agar well diffusion method of antimicrobial

susceptibility test used. *M. myristica* - AgNPs was found to be active against all the test organism in a concentration dependent manner. *B. subtilis* however was found to be highly susceptible to *M. myristica* - AgNPs with highest zone of inhibition.This result differed from what was reported by Onuoha *et al.* (2021) who stated that methanol extract of *M. myristica* was inactive against all the test organism except *B. subtilis.* with a zone of inhibition of 11.5 while a value of 21mm was observed for *M. myristica*-AgNP as shown in this study at 80 mg/mL. The antibacterial activities exhibited by *M. myristica*could be attributedto the AgNPs which served as a carrier of bioactive agents from *M. myristica*through the cell wall of the microorganisms.

Capsicum annuum - AgNPs was also found to have antibacterial properties only against *E. coli* and *B. subtilis* at 80 mg/mL with inhibition zones ofrespectively. This was in contrast to the findings reported by Onuoha *et al*. (2021) where only methanol extract was used. Rjalakshmi and Puviyarasu (2019) also reported the antibacterial potency of *C. annuum* when used alone and when synthesized with AgNPs against *Klebsiella pneumoniae* and *S. aureus*. *C. annuum synthesized into* AgNPswas found to exhibit higher antibacterial activities than the former. Kushwaha *et al.* (2021) proved that green synthesis of AgNPs is important in improving the antibacterial efficacy of herbal drugs. In their study, *Cinnamonum cassie*-AgNPs was reported to have higher antibacterial activities against the tested organism (i.e., *E. coli* and *B. subtilis*) compared to extracts without NPs. Their result is quite similar to the result obtained in this study where all the isolates were found to exhibit some degree of susceptibility for all the concentrations used. Although, earlier study by Onuoha *et al.* (2021) reported *C. cassie* methanol extract had a better antibacterial activity when compared with the AgNPs synthesized *C. cassie* water extract. However, the methanol extract was reported to have antibacterial activities against *S. aureus* only at concentration of 80 mg/mL, which is contrary to the result obtained in this study. This can be an attribute of the solvents used, since bioactive compounds vary in solubility in different solvents.Mgbeahuruike*et al.* (2018) made emphasis on type of extracting solvent as contributing factor to the antimicrobial properties of *Piper guineense.* In their investigation, all the solvents (i.e ethanol, methanol, hexane and chloroform) had alkaloid-rich piperamide with the exception of aqueous extract and was attributed for better antibacterial activities compared to the water extract. The methanol extract of *P. guineense* was reported by Onuoha *et al*. (2021) to exhibit antibacterial properties. However, this study reported an improved antibacterial activity of *P. guineense* - AgNPs against all the test organism across all concentration.

Allium sativum have been used for centuries as food additives as well as in treating infectious diseases. Their therapeutic properties are often attributed to allyl cysteine and allicin (Reda *et al.,* 2019). *A. sativum* have been demonstrated to possess antibacterial properties against both gram positive and negative bacteria. However, the study of Onuoha *et al.* (2021) reported methanol extract of *A. sativum* to be active only against gram positive bacteria, which was contrary to the result of Lekshmi *et al.* (2015). *A. sativum* - AgNPs were found to exhibit varying degree of antibacterial activities against all the test organism except *S. aureus*. The difference in solvent used could be the reason for this dissimilar result.Some mechanisms have been proposed by Shalaby *et al.* (2015) on the mechanisms through which AgNPs synthesized plant extracts exhibit bactericidal or bacteriostatic effects. These include the attachment of positively charged AgNPs to the negatively charged cell wall of bacteria thereby causing rupture of cell membrane. This is followed by protein denaturation which often result in cell death. Another mechanism involves the accumulation of protein envelop precursors, which leads to dissipation of proton motive force when Ag or NPs ions attaches themselves onto the cell wall of bacteria. Another possible mechanism is plasma membrane rupture or destabilization of outer membrane when intracellular ATP gets depleted as a result of actions of AgNPs whenever they bind to cell wall of bacteria. These mechanisms among many others could be the reason why AgNPs synthesized spices had improved antibacterial activities. Silvernano particles (AgNPs) have unique properties of small sizes with large surface area which makes it possible for them to penetrate the bacteria cell wall thereby allowing the NPs to interfere with proper functions of DNA (Shalaby *et al.,*2015).

V. Conclusion

The synthesized AgNPs of five spices used in this study has antibacterial activities and could be utilized in the development of drugs that can be used for effective treatment therapy against bacteria species threatening human health.

Conflict of interest: The authors declare there was no conflicts of interests.

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