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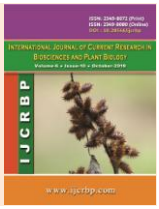

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The comparative antibacterial effect of aqueous extract of *Carica papaya* leaves and *Carica papaya* nanoparticles against selected bacteria

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ABSTRACT

Silver salt and its colloidal formulations have been used since ancient times to treat ulcers, burns chronic wounds and other infections but its use was discontinued due to the interfering effects of salt and the development of new antibiotics, However, almost a decade back, renew interest grew for nanosilver owing to its high surface area to volume ratio and size-dependent unique optical, electrical, and thermal properties. Silver nanoparticles were synthesized using eco-friendly method with extract of *Carica papaya* as reducing and stabilizing agent. The silver nitrate solution was used as precursor. A visible colour change from yellow to dark brown confirmed the formation of the nanoparticles and the UV-Vis spectroscopy showed an absorbance of 0.69 at wavelength 435 nm for the silver nanoparticle. The antimicrobial activity of the synthesized nanoparticles was studied against *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus*. The silver nanoparticles biosynthesized showed antimicrobial activity against the test isolates with zone of inhibition ranging from 10-15mm. Antimicrobial activity of silver nanoparticles was statistically significant as compared to Chloramphenicol at 0.05% level of confidence. Generally, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values for Silver nanoparticle ranged from 0.22 – 0.42 g/ml and 1 – 30 cfu/ml respectively, against the test organisms. *S. aureus* was most sensitive while *E. coli* and *S. typhi* were least sensitive to silver nanoparticles, while the activities of *C. papaya* extract ranged from 0.32 – 0.42 g/ml and 6 – 36 cfu/ml respectively, against the test organisms *E. coli* and *S. aureus* were more sensitive while *S. typhi* was least sensitive to *C. papaya* nanoparticles.

Introduction

Silver has been used since ancient times for its microbicidal properties. Silver salt and its colloidal formulations have been used to treat ulcers, burns and chronic wounds, sepsis, acute epididymitis, tonsillitis, and infections and to prevent eye diseases in infants (Sintubin et al., 2011), but its use was discontinued due to the interfering effects of salt and the development of effective new antibiotics (Edwards-Jones, 2009). However, almost a decade back, nanosilver made a remarkable comeback owing to its high surface area to volume ratio and size-dependent unique optical, electrical, and thermal properties (Schmid, 1992). Silver nanoparticles (AgNPs) are now one of

the most commercialized nanomaterials having applications in over 200 products such as antimicrobial coatings, medical devices, molecular diagnostics and photonic devices, sensors, textiles, home water purifiers, cosmetics, electronics, household appliances, conductive inks, pastes, and fillers (Lin et al., 2011; Sintubin et al., 2011; Wijnhoven et al., 2009). Based on their structure, polymeric nanoparticles can be classified into nanocapsules and nanospheres. Based on the composition, nanoparticles are classified as:i) Organic nanoparticles, ii) Inorganic nanoparticles, iii) Organic –inorganic hybrids, iv) Carbonaceous nanostructure, v) Liposome that can be filled with specific materials, vi) Biological nanoparticles such as proteins and viruses.

Silver is a well-known antimicrobial agent against a wide range of over 650 microorganisms from different classes such as Gram-negative and Gram-positive bacteria, fungi or viruses (Nakkala et al., 2014).Silver has been described as therapeutic agent for many diseases (Veeraputhiran, 2013). In the 18th century, during childbirth it became a common practice to administer drops of aqueous silver nitrate to newborn's eyes to prevent the transmission of *Neisseria gonorrhoea* from infected mothers (Daniel et al., 2014). Silver nanoparticles synthesized using plant extracts (from different sources) have been used for analyzing their antimicrobial activities against different microbes. Nanoparticles have been shown to accumulate inside the membrane and can subsequently penetrate into the cells causing damage to cell wall or cell membranes (Kalishwarlal et al., 2010).

It is thought that silver atoms bind to thiol groups of enzymes forming stable SAAg bonds with thiol containing compounds and then it causes the deactivation of enzymes in the cell membrane that involve in trans membrane energy generation and ion transport. It was proposed that Ag^+ ions enters the cell and intercalates between the purine and pyrimidine base pairs disrupting the hydrogen bonding between the two anti-parallel strands and denaturing the DNA molecule. Bacterial cell lysis could be one of reason for its antibacterial property (Geetha et al., 2014).

Carica papaya

Carica papaya is an evergreen shrub or small tree that grows best in full sun to light shade. The papaya plant has been described with a large variety of adjectives, which acknowledge the structural and functional complexity of this giant tropical herb. *C. papaya,* with a somatic chromosome number of 18, is the sole species of this genus of the Caricaceae, a family well represented in the Neotropics, which includes six genera with at least 35 species (Fisher, 2008). *C. papaya* as well the leaf is a good source of Vitamin A (Carotene), Vitamin B1 (Thiamine), Vitamin B2 (Riboflavin), Vitamin C (Ascorbic acid), Vitamin E, Niacin, Minerals such as Calcium, Iron, Phosphorous, Potassium, Proteins, Fats, Calories, Carbohydrates, β-carotene, Fibers and Folate that helps to boost the number of platelets present in the blood (Ming et al., 2008). *C. papaya* is used in the treatment of various human diseases and also has a great potential in suppressing various plant pathogenic fungi (Nguegno et al., 2017; Murthy et al., 2019).

This research seeks to synthesize silver nanoparticles from *C. papaya*, with the aim of investigating its antimicrobial properties against selected bacterial strain. More importantly it may serve as a guideline for further investigation.

Materials and methods

Plant sampling and preparation

C. papaya leaves were collected from the compound of Achievers University, Owo and taken to the laboratory. The samples were authenticated by Dr T. Temikotan, a plant Biologists in the Department of Biological Sciences of Achievers University, Owo. The plant samples were washed thoroughly under running tap to remove dirts and debris and subjected to various analyses as described in the following text.

Proximate analysis of plant material

Plant material was subjected to proximate analysis which includes: moisture content, ash and lipid, crude fiber.

Moisture content

A 5 g portion of the papaya leaves was transferred to crucible and placed in the oven at 105°C for 1

hour, then removed from the oven and put in desiccators and allowed to cool. The sample was weighed (the sample + crucible) and recorded. Then it was transferred back to the oven at 105°C for 30 minutes then return back to desiccator to cool and weighed, the procedure was repeated until a constant weight was observed.

The moisture content was established as shown below.

Crucible + papaya leaves – crucible = weight of the leaves moisture content

$$\frac{\text{Weight of fresh sample - Weight of the dry sample}}{\text{Weight of the fresh sample}} \times 100\ \%$$

Ash content

A 5 g portion of the papaya leaves was transferred to crucible and then placed into the muffle furnace at 200°C for 1 hour.

Lipid content

Cleaned and dried crucible was weighed as w_1 and 5g oven dried sample was added and re-weighed as w_2. Round bottom flask with petroleum ether (40-60°C) up to three quarter of the flask, Soxhlet extractor was fixed with reflux condenser and the heat source was adjusted so that the solvent boils gently. The samples were put inside the thimble and inserted into the Soxhlet apparatus and extract under reflux was carried out with petroleum ether (40-60°C) after the barrel of the extractor is emptied, the condenser was removed and the thimble was removed, taken into oven at 100°C for one hour and later cooled in the desiccators and weighed again (w_3) (AOAC, 2015).

$$\% \text{ Fat weight loss sample} = \frac{W_2 - W_1 \times 100}{W_3 - W_1 \times 1}$$

Solvents: pet ether, N- hexane, dichloromethane and chloroform.

Fibre content

A 2g portion *C. papaya* leaves ($\underline{w_1}$) was weighed and put into 250 ml beakers. 50 ml of 1.25% H_2SO_4 acid solution was added and made up to 200 ml with distilled water and stirred. The mixture was heated with continuous stirring for thirty (30) minutes and allowed to cool and settle. Distilled water was added and allowed to settle, then decanted ($\underline{w_2}$). Decantation was repeated for six (6) times consecutively to make the mixture acid free. 50 ml of 1.25% NaOH was added to the mixture and made up to 200 ml with distilled water in a beaker, and heated for thirty minutes with continuous stirring. It was allowed to cool and settle. Distilled water was added and decanted for six (6) times. The mixture was filtered with filter paper and kept for about forty-five minutes (45) for water todrain completely, and weights taken ($\underline{w_3}$).

$$\% \text{ Crude fiber} = \frac{W_2 - W_3}{W_1}$$

Protein content

A 5 g of sample was dissolved with 30ml of concentrated sulphuric acid using 2g of copper sulphate and 16.0g of sodium sulphate salt until a clear green solution was obtained. This was dissolved in distilled water and made up to 100 ml in a volumetric flask; 12.5 ml of the digest was measured into a semi-micro Kjeldahl Markham distillation apparatus and treated with 12.5 ml of 1.25% of sodium hydroxide (NaOH) solution. This was distilled with 10 ml of boric acid and double indicator. The distillate was then titrated with 0.1% HCl solution until a light pink end point was reached. Blank titration was also carried out in similar manner. Distillation was carried out in triplicate and the percentage nitrogen obtained by appropriate calculation.

$$\% \text{ Nitrogen} = \frac{\text{Ml of HCl (blank)} - \text{Ml of HCl (sample)} \times \text{0.1M HCl} \times 14 \times 100 \times 100}{\text{Weight of sample} \times \text{Ml of digest} \times 1000} \times 6.25$$

Phytochemical analysis

The analysis for tannin, saponins, cardiac glycosides and alkaloids were carried out according to standard methods (Sofowara, 1993; AOAC, 1980).

Presence of alkaloids

About 0.5g of the dried sample of the plant each

group was acidified with 1% HCL. About 3ml of each extract was treated with drops of Mayer's reagent another portion with Drangedonff's reagent. Creamy white (Mayer) orange (Drangedonff) precipitates indicate the presence of alkaloids.

Presence of saponins using frothing test

Five ml of the extract was vigorously shaken for two 2 minutes. Formation of stable froth was taken as evidence of the presence of saponin.

Presence of cardiac glycosides using Keller-Killani test

A 5ml of the extracts was treated with 2ml of glacial acetic acid containing a drop of iron iii chlorine $FeCl_3$ solution. This was underlayed with 1ml of conc. H_2SO_4. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just gradually throughout the thin layer.

Presence of tannins

Two drops of 5% ferric chloride $FeCl_3$ were added to 1ml of each extract. A dirty green precipitates indicated the presence of tannins. In the alternative about 1g of each sample was boiled in 20ml of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added. A dirty - green colour or blue black colouration was observed. This indicated the presence of tannins.

Collection and maintenance of microorganisms

Clinical strain of bacteria samples (*Escherichia coli, Salmonella typhi* and *Staphylococcus aureus*) was collected from Federal Medical Centre Owo, Ondo State on agar slant and sample were subjected to biochemical confirmatory test.

Preparation of plant material

After thorough washing, the plant materials were air dried for 10 days under room temperature. The dried parts were ground to coarse powder using thoroughly washed electric home blender machine and kept in clean covered glass containers till needed for extraction.

Extraction procedure

A 25g portion of fine powder of papaya leaves was dissolved in 200 ml water and it was thoroughly shaken to dissolve the powder into the solvent. Then it was heated at 60 °C for 5 – 10 minutes and was shaken intermittently during the process for more interaction between the powdered particles and the solvent and then incubated in water bath for 30 min to facilitate the formation of aqueous extract (Morsy et al., 2014). After the extraction process the plant extracts was filtered with sterilized cotton filter and filter paper. The filtrate was collected in a beaker. This process was repeated three times using cotton and filter paper. Then the filtrate was decanted into a volumetric flask over a 40µl milipore filter and covered with aluminum foil paper and kept in the refrigerator.

Synthesis of nanoparticle from *C. Papaya* using silver nitrate ($AgNO_3$)

The modified method of Banala et al. (2015): was used in the synthesis of Silver nitrate from *C. papaya*. A 0.421 g part of silver nitrate salt ($AgNO_3$, Kermel Nigeria), was added to 100 ml of distilled water to dissolve thoroughly. The solution obtained was transferred to an amber coloured bottle to prevent autoxidation of silver. An equal quantity of *C. Papaya* crude extract was also suspended in 100ml of water and mixed thoroughly. A 5ml aliquot of *C. papaya* solution was dispensed into a conical flask and 20 ml of 1 mM $AgNO_3$ was mixed with it (in the ratio 1:4). The solution was then heated in a water bath at 60°C for 30 min until change in colour was observed. A colour change indicates the formation of silver nanoparticles (Baruwati et al., 2009).

Standardization of inocula (preparation of Macfarland's constant)

One percentage (1%) of solution of sulphric acid was prepared and mixed properly. Also, 1% solution of barium chloride was prepared by dissolving 0.5g of dehydrated barium chloride ($BaCl_2$ H_20) in 50ml of distilled water. A 0.5ml of aliquot of barium chloride solution was added to

99.5ml of sulphuric acid solution and it was mixed together. The solution was transferred into a capped tube of the same type used for both control and the test inocula. The solution was kept at room temperature of 27°C. and was used to standardize the inocula by comparing the absorbance using a spectrophotometer (Michael et al., 2010).

Preparation of chloramphenicol (control)

Antibiotic weighing 0.421g (chloramphenicol) was dissolved in 100 ml of distilled water and kept under sterile condition.

Evaluation of antibacterial activity of extracts

Test organisms were suspended in nutrient broth and incubated for 4 hours to obtain a concentration corresponding to McFarlands constant (0.5×10^{-8} cfu/ml). The inoculums were standardized with the prepared barium sulphate solution as described above. Sterile Petri dishes were inoculated by the pour plate method. 1ml of the test inoculums was pipetted aseptically into each Petri dish and about 20ml of sterilized nutrient agar was poured into the inoculated Petri dish. The agar plates were allowed to set. Wells of 6mm diameter were made over the agar plates equidistant from each other using sterile cork borer (bore 4 holes) on the set plate. 0.5ml portion of silver nanoparticles, *C. papaya* extract, silver nitrate and chloramphenicol were introduced into the wells using micropipette. Distilled water was used as negative control. The extracts were allowed to diffuse into agar for about 20 minutes after which the plates were incubated for 24 hours at 37°C. Thereafter the diameter of inhibition zones formed around each well was measured in mm and recorded. The experiments were replicated in triplicates and the values recorded (Cheesebrough, 2006).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in triplicate in test tubes. To 0.5 ml of varying concentrations of the extracts (0.42, 0.32, 0. 22, 0.12, 0.2 g/ml) in test tubes, Nutrient broth (2ml) was added and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standards, was introduced. The procedure was repeated on the test organisms using the aliquots (silver nanoparticle, silver nitrate and pawpaw leaf extract). Tubes containing Nutrient broth only were seeded with the test organisms, as described above, to serve as controls. The culture tubes were then incubated at 37°C for 24 hours. After incubation the tubes were then examined for microbial growth by observing for turbidity.

To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated onto sterile Nutrient agar by streaking. Nutrient agar plates only were also streaked with the respective test organisms to serve as controls. All the plates were then incubated at 37oC for 24 h. After incubation the concentration at which no visible growth was seen was noted as the Minimum Bactericidal Concentration (MBC).

Statistical analysis

Results obtained were subjected to statistical analysis using T- test to separate the means values of the data collected.

Results

Table 1 shows the results of the proximate composition of the *C. papaya*. The moisture content was 84%, ash content was 8%, fibre was 16%, lipid content was 6% crude protein was 5.1% and carbohydrate was 66%.

Table 1. Result of proximate composition of the *Carica papaya* leaves.

Sl. No.	Parameters	Percentage (%)
1	Moisture content	84.0
2	Ash content	8.0
3	Fibre content	16.0
4	Lipid content	6.0
5	Crude protein	5.1
6	Carbohydrate content	66.0

Phytochemical screening of papaya leaves

The phytochemical analysis of the leaves (Table 2) showed that the leaves contained saponins, cardiac

glycosides, and alkaloids. Tannin was absent in the leaves.

Table 2. Phytochemical screening of *Carica papaya* leaves.

Constituents	Result
Saponins	+
Tannins	–
Cardiac glycoside	+
Alkaloid	+

+ Represent; – No activity;

UV–Vis spectral analysis (characterization of synthesized nanoparticles)

The eco-friendly synthesis of AgNP was performed under a reflux environment using *C. papaya* L. extract. The Ag+ ion reduction was confirmed by the apparent transformation in the colour of the reaction mixture from yellow to dark brown. Indeed, no color transformation was noticed in the absence of plant extract, under a similar set of circumstances. The formation of AgNP was further confirmed by UV–Vis spectral study, which is an authentic technique to monitor the progress of the reaction during the reduction of Ag+ ions. For this purpose, UV–Vis spectra of green synthesized AgNP prepared at 60 ℃ were measured as displayed in Fig. 1 below.

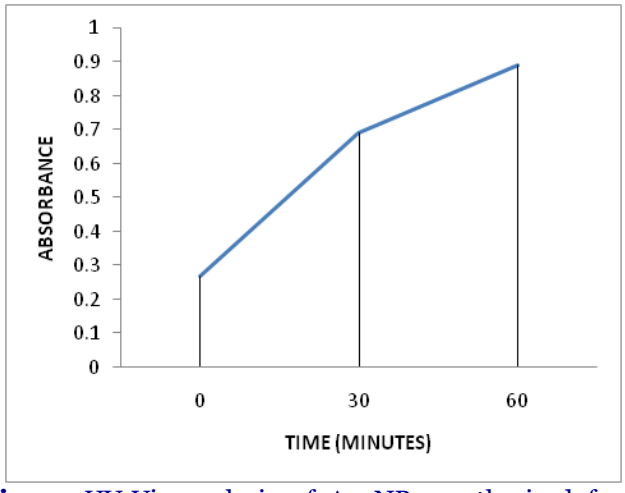

Fig. 1: UV-Vis analysis of Ag NPs synthesized from *Carica papaya*.

The kinetics of the reaction monitored by UV–Vis analysis revealed that the reaction was slow in the beginning; indeed, up to 30 minutes from the start of the reaction, no indication of the formation of NPs was observed (no Ag peak). After 30 minutes, the nucleation was initiated very rapidly and the formation of AgNP occurred very fast until around 30 minutes had passed. This is clearly reflected by the sudden appearance of the characteristic band of AgNP at ~430 nm after 60 minutes of reaction time (blue line in Fig. 1). The reaction was allowed to continue further and no considerable change was observed in the intensity of the Ag peak, which points towards the completion of the reaction in 60 minutes.

Antimicrobial activity

The antimicrobial activity of silver nanoparticles was investigated against one Gram positive and two Gram negative bacteria. The synthesized silver nanoparticles exhibited good antibacterial activity against both Gram positive and Gram negative bacteria. The AgNP showed an inhibition of 10 ± 1.5mm against *S. typhi,* which was not statistically lower than an inhibition of 12 ± 2.5 mm of the standard antibiotic, but chloramphenicol was significantly higher than the inhibition of 3 ± 0.5 mm and 6 ± 1.5 mm of silver nitrate and pawpaw extract respectively. Its inhibition of 12 ± 1.5 mm against *E. coli,* was statistically lower than that of 14 ± 2.5 mm than that of chloramphenicol but highly significant than those of silver nitrate and papaya extract with the inhibition values of 2. ± 0.5 mm and 9 ± 1.5 mm, respectively. The inhibition by AgNP against *S. aureus* was 15 ± 1.5mm. This showed statistically significant value that was higher than those of chloramphenicol with inhibition value of 12 ± 0.5 mm and silver nitrate with inhibition of 5 ± 0.5 mm but not significantly higher than that of papaya extract with inhibition of 8 ± 1.5 mm.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts as presented in Fig. 2 shows Ag NP inhibited the selected organisms at 0.32g/ml, 0.22g/ml and 0.42g/ml against *S. typhi, S. aureus* and *E. coli* respectively. Silver nitrate inhibited the selected organisms: *S. typhi, S. aureus* and *E. coli* at 0.42g/ml, 0.32g/ml and 0.42g/ml respectively. The MIC of *C. papaya* leaf extract against the selected organisms: for *S. typhi* 0.32g/ml, while for *S. aureus* 0.42g/ml and for *E. coli* 0.32g/ml. The MIC of chloramphenicol against *S. typhi* was 0.22g/ml, while for *S. aureus* 0.32g/ml and 0.32g/ml for *E. coli.*

Table 3. Antibacterial activity of each sample against selected organisms.

Test organisms	Chl / AgNP	Chl / $AgNO_3$	Chl / PE
Salmonella typhi	12 ± 2.5 mm	12 ± 2.5 mm	12 ± 2.5 mm
	10 ± 1.5 mm	3 ± 0.5 mm	6 ± 1.5 mm
	Ns	Sig	Sig
Escherichia coli	14 ± 2.5 mm	14 ± 2.5 mm	14 ± 2.5 mm
	12 ± 1.5 mm	2 ± 0.5 mm	9 ± 1.5 mm
	Sig	Sig	Sig
Staphylococcus aureus	12 ± 0.5 mm	12 ± 0.5 mm	12 ± 0.5 mm
	15 ± 1.5 mm	5 ± 0.5 mm	8 ± 1.5
	Sig	Sig	Ns

NOTE: Upper values are for the standard antibiotic, chloramphenicol, while lower values in each cell belong to the various reagents as symbolized in the legend.
LEGEND: Chl = chloramphenicol; AgNP = Silver Nanoparticle; $AgNO_3$= Silver nitrate and PE = Papaya leaf extract; ns = no significant difference; sig = significant difference.

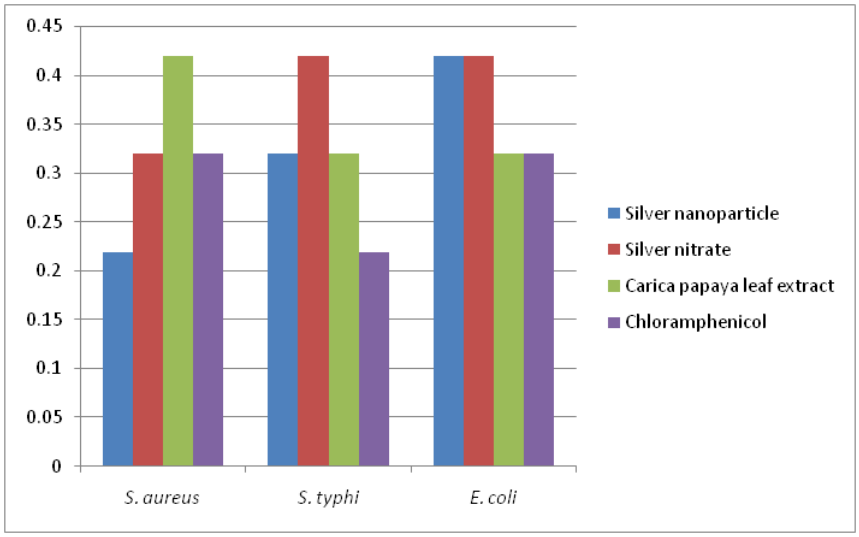

Fig. 2: Minimum Inhibitory Concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC)

The MCB as presented in Table 4 shows that all the extracts had little or no bactericidal activity against the selected organisms. Colony forming unit for silver nanoparticle ranged from 1 – 21 cfu/ml in *S. aureus*, 2 – 30 cfu/ml in *E. coli,* and 6-50 cfu/ml in *S. typhi*. Colony forming unit for Silver nitrate ranged from 11 – 29 cfu/ml in *S. aureus,* 9 – 65 cfu/ml in *E. coli* and 7 – 36 cfu/ml in *S. typhi*. For *C. papaya,* colony forming unit ranged from 6-36 cfu/ml all the selected organisms. The colony forming unit for chloramphenicol ranged from 2-30 cfu/ml in the selected microorganisms (*E. coli, S. typhi*, and *S. aureus*), at all concentrations (0.42 g/ml, 0.32 g/ml, 0.22 g/ml and 0.12 g/ml) (Table 4).

Table 4. Minimum Bactericidal Concentration (MIC).

Conc. (g/ml)	Ag NP	Ag NO_3	P.E	CHL	Ag NP	Ag NO_3	P.E	CHL	Ag NP	Ag NO_3	P.E	CHL
0.42	3	11	6	3	2	9	6	2	1	6	7	4
0.32	7	16	14	10	9	13	15	8	7	14	17	11
0.22	16	21	21	15	22	31	23	17	13	24	25	16
0.12	19	24	27	19	25	53	28	19	15	39	30	23
0.02	24	29	34	27	30	65	33	24	21	50	36	30

Key: Ag NO→ Silver nitrate; Ag NP→ Silver nanoparticles; CHL → Chloramphenicol; P.E → *Carica papaya* leaf extract.

Optical density

The optical density (OD) for all the extract (Silver nanoparticle, Silver nitrate, *C. papaya,* chloramphenicol) was determined at wavelength 430nm and silver nanoparticle had the highest absorbance of 0.69 and silver had the lowest absorbance of 0.27 as shown in Table 5.

Table 5. Optical density for each extract at 430 nm.

Extract	Optical density
Silver Nanoparticles	0.69
Silver Nitrate	0.27
Carica papaya	0.43
Chloramphenicol	0.47

Discussion

The phytochemicals present in the leaves of *C. papaya* could be responsible for the high medicinal properties. The presence of saponins supports the fact that pawpaw leaf has cytotoxic effects such as permealization of the intestine as saponins are cytotoxic (Okwu and Okwu, 2004). It also gives the leaves the bitter taste. Alkaloids are the most efficient therapeutically significant plant substance. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties (Stray, 1998). The presence of alkaloids in the leaves shows that these plants can be an effective anti-malarial agents, since alkaloids consist of quinine, which is an anti-malaria. The cardiac glycosides therapeutically have the ability to increase the force and power of the heart-beat without increasing the amount of oxygen needed by the heart muscle (Okwu and Okwu, 2004).

Results obtained in this study suggest that silver nanoparticles synthesized with *C. papaya* leaf extract could be used as an effective antibacterial material against *S. typhi, E. coli,* and *S. aureus*, these infectious bacterial causes a number of diseases, including typhoid, bacteraemia, chronic wound infection, septic arthritis, skin and soft tissue infections, and respiratory infection. Staphylococcal infections are becomingly increasingly difficult to treat (Leid et al., 2012). In particular, silver ions have long been known to exert strong inhibitory and bactericidal effects as well as to possess a broad spectrum of antimicrobial activities (Soo-Hwan et al., 2011).

Shahverdi et al. (2007), Shirley et al. (2010), Mohsen et al. (2011), and Soo-Hwan et al. (2011) showed that the AgNPs have potent antibacterial activities against *S. aureus and E. coli*. Nada and Saravanan (2010) showed that silver bio-nanoparticles from bacteria have inhibitory and bactericidal effect against Methicillin-Resistant *Staphylococcus aureus* (MRSA). The mechanism of inhibitory action of silver ions on microorganisms is partially known. It is believed that DNA loses its replication ability and cellular proteins become inactivated on Ag+ treatment. In addition, it was also shown that Ag+ binds to functional groups of proteins, resulting in protein denaturation (Nada and Saravanan, 2010).

The silver atoms bind to thiol groups (-SH) in enzymes and subsequently cause the deactivation of enzymes. Silver forms stable S-Ag bonds with thiol-containing compounds in the cell membrane that are involved in trans-membrane energy generation and ion transport (Klueh et al., 2018). It is also believed that silver can take part in catalytic oxidation reactions that result in the formation of disulfide bonds (R-S-S-R).

Silver does this by catalyzing the reaction between oxygen molecules in the cell and hydrogen atoms of thiol groups: water is released as a product and two thiol groups become covalently bonded to one another through a disulfide bond (Davies et al., 2007). Another one of the suggested mechanisms of the antimicrobial activity of silver was proposed that Ag+ enters the cell and intercalates between the purine and pyrimidine base pairs disrupting the hydrogen bonding between the two anti-parallel strands and denaturing the DNA molecule (Ranganath et al., 2012).

Literature suggests that there are several mechanisms by which silver nanoparticles could be killing the microorganisms: (i) destructuring the cell wall and ceasing the cell permeability, (ii) formation of free radicals, (iii) inactivating important enzymes by interacting with thiols, (iv) interaction of AgNPs with DNA and interruption of DNA replication and translation and by dephosphorylating the tyrosine residues on peptides inhibiting the signal transduction and growth in bacteria (Kim et al., 2007 and 2011).

MIC for each extract on each isolate was determined and it was observed that silver nanoparticle had the lowest MIC of 0.22g/ml against *S. aureus* as compared to silver nitrate, chloramphenicol and *C. papaya* leaf extract which had an MIC of 0.32 g/ml, 0.32 g/ml and 0.42 g/ml respectively. This could be attributed to the cellular makeup of *S. aureus* it has a thick peptidoglycan layer which makes *C. papaya* leaf extract require a higher concentration to inhibit it growth (Adams and Moss, 2009). While for *S. typhi,* chloramphenicol had the lowest MIC of 0.22 g/ml as compared to silver nanoparticle and *C. papaya* leaf extract which had same MIC of 0.32 g/ml, silver nitrate had the highest MIC of 0.42g/ml. Silver nitrate formed a shiny silver coat on the agar which inhibited the growth of the organism on the surface of the agar, this could be linked a unique feature of silver as suggested by Prathna et al. (2014).

For *E. coli,* chloramphenicol and *C. papaya* leaf extract had the lowest MIC of 0.32 g/ml as compared to silver nitrate and AgNPs which had a higher MIC of 0.42 g/ml each. The antimicrobial activity of *C. papaya* leaf extract was different from that of Romasi et al. (2012) possible due fact that he used a male papaya tree for his research or environment factors and the extraction procedure used. The MBC for each extract as presented in Table 5. It was observed that silver nanoparticle, chloramphenicol, *C. papaya,* and silver nitrate all had bacteristatic effects and not bactericidal effect thus explaining why there was growth at all concentration. Possibly if the extract were left in contact with the organisms for longer period of time, complete bactericidal activity could be achieved, also the concentration were too low to have bactericidal effect on them.

The optical density as presented in Table 6 shows that silver nanoparticle had an absorbance of 0.69 as compared to silver nitrate and *C. papaya* which had an absorbance of 0.27 and 0.43 respectively. Therefore confirms that nanoparticle has been synthesized because the OD of silver nanoparticle was higher than those of silver nitrate and *C. papaya* extract.

Conclusion

Silver has already proved itself as a powerful inorganic compound, and can be used correctly, prevent infection. As a board-spectrum agent, it kills a wide range of microorganisms. Currently, the usage has increased dramatically in the developed world across a huge range of applications, but the impact has not reached the areas of developing world), where the greatest impact could be seen through incorporation into application associated with the production of uncontaminated food and water. The result of this study confirms that the nanoparticles synthesized have satisfactory inhibitions against the test microorganisms.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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