**ORIGINAL PAPER**

**Nig. J. Pharm. Res. 2016, 12 (1) pp 59-64**
**ISSN 0189-8434** **Available online at http://www.nigjpharmres.com**

# Phytochemical Composition and Comparative Evaluation of Antimicrobial Activities of the Juice Extract of *Citrus Aurantifolia* and its Silver Nanoparticles

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

## Abstract

**Background**: *Citrus aurantifolia* juice has been useful for the treatment of various infections and green synthesis of silver nanoparticle using lime juice may offer added advantages.
**Objective**: The phytochemical composition and comparative evaluation of antimicrobial activities of the crude juice extract and biosynthesized Silver nanoparticle (SNPs) from *Citrus aurantifolia* juice was investigated.
**Materials and methods**: Phytochemical, antimicrobial evaluation (agar well diffusion) and biosynthesis of SNPs was done using Crude extract of *Citrus aurantifolia.* The SNPs were characterized by colour changes, spectroscopy and Fourier-Transform Infrared (FTIR) Spectroscopy.
**Results**: The juice extract contained bioactive compounds such as flavonoids (710mg/100g), tannins (525mg/100g), phenols (65mg/100g) and terpenes (56mg/100g). Changes in colour, UV-Vis Spectroscopy at 300-550nm ranges and FTIR revealed the functional groups present in the biosynthesized SNPs. The crude extract and SNPs exhibited varying antimicrobial activities against some selected pathogens including *Streptococcus pyogenes* ATCC 19615, *Klebsiella pneumoniae* ATCC 10031, *Bacillus* sp, *Actinobacillus* sp., *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The crude extract has more antibacterial potential against the tested pathogens than the biosynthesized SNPs. The crude extract also had higher antimicrobial activities against *Streptococcus pyogenes* which were resistant to ciprofloxacin. The result revealed that the crude extract was more effective than the SNPs produced and the Minimum Inhibitory concentration (MIC) also showed increasing activities with an increase in the concentration of the juice extract and SNPs.
**Conclusion**: Crude extract of *Citrus aurantifolia* contain bioactive compound with potent antimicrobial potential and the extract was more effective than the biosythesized SNPs.
**Keywords**: *Citrus aurantifolia* juice, Silver nanoparticle, phytochemical, antimicrobial activities, minimum inhibitory concentration.

## INTRODUCTION

The need for new, potent and affordable drugs for the treatment of microbial infections in the developing world is one of the issues facing global health today. Valid drugs for the treatment of these infections are however restricted at a particular level by factors ranging from microbe resistance to safety, compliance and cost. The use of medicinal plants for curative purposes is as old as mankind and can be traced from the beginning of civilization (Awoufack *et al*.,, 2013).
Citrus fruits belong to the *Rutaceae* family and this family is one of the main tree crops grown worldwide. *Citrus aurantifolia*, a medicinal plant and also known as key lime, originated from Malaysia and commonly referred to as Mexican lime or West-Indian lime. Lime is a small shrub-like tree ranging from 3.5 to 9 m in height and 2.5 to 7.5 m in width, the fruit is typically round, green to yellow in color and about 3-6 cm in diameter (Moro and Basile, 2000). *Citrus aurantifolia* juice is consumed as a stomach ache reliever tonic. Mixed with oil, it is used as a vermifuge. The juice is considered antiseptic, astringent, a remedy for intestinal hemorrhage and a disinfectant for any kind of ulcer. Lime is often used as flavor enhancer of other fruits and as a meat tenderizer. Dishes flavoured with lime require a little salt, which makes lime an ally in sodium restriction diets. The lime extract is used in cosmetic products due to its content of sugar and hydroxy acids. Lime has also been used as expectorant and mouthwash (Alexander and Paul, 1995).
Lime juice contains bioactive compounds such as saponins, alkaloids, tannins, phenolics, flavonoids and terpenoids which are antibacterial (Norman, 2015). These bioactive compounds give lime extract properties, which make it useful for any hydrating cosmetic formulation

such as hair care formulations and also in various industries. Based on the affordability and easy availability and other unique properties of *Citrus aurantifolia* juice, it can thereby be used as an alternative medicine for the treatment of some infectious diseases. It can also be introduced into modern technology for the production of silver nanoparticles.

Nanotechnology is a modern field of research and is one of the rapid growing interdisciplinary areas of science and technology. The production of Silver Nanoparticles (SNPs) has been widely recognized with a large applicability in so many sectors including the health sector, mechanics, drug-gene deliveries, chemical industries, space industries textile industries and also in the pharmaceutical industries for the production of Shampoos, soaps, detergents, toothpastes and other pharmaceutical products (Hungund *et al.*,, 2015). As a result of their small size (1 – 100nm), nanoparticles exhibit unique physicochemical and biological functional properties when compared to macro-sized particles. Among the metallic nanoparticles, the silver nanoparticle has received a unique and greatest attention due to their broad spectrum of antimicrobial activities against many prokaryotic and some eukaryotic microorganisms (Suresshkumar *et al.*,, 2010). SNPs have little chance of drug resistance which provides a solution to multidrug resistance problems (Lim *et al.*,, 2012). SNPs has been widely applied in various fields such as food packaging, catheters, textile and coating, dental, medical therapeutics and diagnosis. Drug resistance has been reported as one of the most serious threatening and widespread problems in all the developing countries (Stevanovic *et al.*,, 2012). Silver and silver-based products have been reported as the most popular and effect antimicrobial agents to kill pathogenic bacteria (Paulraj and Seung, 2013).

Zhu *et al.*,, (2000) reported the limitation in the use of a chemical and physical method for SNPs synthesis. This necessitates the advancement of biological synthesis over the chemical and physical methods of synthesis. This involves the use of bacteria, fungi, algae, carbohydrates, microorganisms and plant extracts or plant biomass for the production of SNPs. The use of microorganisms for SNPs biosynthesis has its own limitations such as time-consuming, processes of maintaining cultures, subsequent extraction and recovery steps, expensive and the gradual loss of the potentials to synthesize SNPs due to mutations (Reddy *et al.*,, 2015).

This has made the use of plants for the synthesis of SNPs advantageous over other methods of synthesis and SNPs biosynthesized using plants have been known to be very potent. Hence, these SNPs can also be used as an alternative to drugs for the treatment of microbial infections. Several researchers have synthesized SNPs and based on their works, these SNPs have been useful in the treatment of various infections. Rajesh *et al.*, (2010) reported that SNPs synthesized from the leaves of *Pongamia pinata* was active against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Staphylococcus aureus*. Hungund *et al.*, (2015) reported that biosynthesis of SNPs using lime juice had activity against *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Salmonella typhimurium*. Bharani Devi *et al.*,, (2015) reported the antibacterial activity of SNPs synthesized from Lactobacillus sp., and some fruit peel extracts against *E. coli* and *Staphylococcus aureus*.

Thus, this study aimed at investigating the phytochemical composition of *Citrus aurantifolia* juice and to biosynthesize SNPs using the crude juice extract of *Citrus aurantifolia*. To characterize the SNPs and to compare the antimicrobial activity of the juice extract and the biosynthesized SNPs against some selected pathogens in vitro.

## Materials and Method

### Preparation of Plant Material and Extraction

Fresh fruits of *Citrus aurantifolia* were obtained from Bodija market in Ibadan, Oyo state Nigeria. The fruits were washed thoroughly, rinsed with distilled water and smashed inside a grinder. The smashed fruits were filtered to remove the debris and the filtrate was centrifuged at 10000 rpm for 10 minutes to obtain the liquid fruit extract (Oikeh *et al.*,, 2013). The supernatant was stored air tight in a refrigerator and kept for further experiment.

### Phytochemical Analysis

Qualitative and quantitative phytochemical screening was carried out on the crude extract to determine the presence of active secondary metabolites present in the fruit. The phytochemical assays for alkaloids, flavonoids, saponins, tannins, cardiac glycosides, anthocyanins, phenols and terpenes were carried out according to established procedures (Trease and Evans, 1983; Harbourne, 1983; Meda *et al.*,, 2005; Singleton *et al.*,, 1999; Van-Burden and Robinson, 1981; Obadoni and Ochuko, 2001; Uruquiaga and Leighton, 2000).

### Testing For the Purity of Extracts

The purity of the extracts was determined by the streaking of the extract on freshly prepared Nutrient and MacConkey agar plates. This was then incubated at 37°C for 24 hours. After incubation, the plates were checked for the growth of contaminant microorganisms.

### Collection of Test microorganisms

Six bacterial isolates (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus* sp. and *Actinobacillus* sp. *Streptococcus pyrogens* ATCC 19615 and *Klebsiella pneumonia* ATCC 10031 were collected from The Department of Medical Microbiology, University College Hospital (UCH), Ibadan and The Federal Institute of Industrial Research Oshodi (FIIRO), Lagos State. The isolates were sub-cultured on plates of Nutrient Agar and incubated for 24 hours at 35-37ºC. The colonies were picked and stored in slants until when needed.

### Biosynthesis of Silver Nanoparticles (SNPs)

The crude juice extracts of *Citrus aurantifolia* was used for the biosynthesis of silver nanoparticles. 100ml of 1mM of the aqueous solution of silver nitrate (AgNO3) was prepared in 250ml Erlenmeyer flasks and 40ml of the juice extract was added into the flasks for the bio-reduction of the AgNO3 into silver (Ag+) ions. This composite was observed for colour changes and then later placed in an incubator for the complete bio-reduction at a temperature of 37 ºC for 24 -72 hours.

**Characterization of Silver nanoparticles**

*Visual observation of the biosynthesized SNPs*

Visual observation was done by observing the gradual colour change of the incubated plant extract and the AgNO3 mixture after incubation for 24 – 72hrs.

**UV–Visible spectroscopy of the extracellular biosynthesized SNPs**

The SNPs were characterized using UV-Visible Spectrophotometer for the verification of the reduction of the AgNO3 to Ag+ by the plant extract. The absorption spectra of the samples were scan and the UV-Vis spectra were recorded at intervals of 24 - 72 hours.

FTIR analysis of the extracellular biosynthesized SNPs

FTIR analysis of the SNPs was done using a potassium bromide (KBr) pellet (FTIR grade) method. The spectrum was recorded using Jasco FT/ IR-6300 Fourier transform infrared spectrometer equipped with JASCO IRT-7000 Intron Infrared Microscope using transmittance mode operating at a resolution of 4 cm−1.

***Antibacterial Evaluation of the Crude Extract and SNPs***

The antibacterial activities of the crude extract and the biosynthesized SNPs produced using the juice extract of *Citrus aurantifolia* against some pathogenic microorganisms were evaluated using Agar-Well Diffusion method. The indicator strains used are *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus* sp. and *Actinobacillus* sp. *Streptococcus pyogenes* ATCC 19615 and *Klebsiella pneumoniae* ATCC 10031. Plates of Nutrient Agar were prepared and the isolates stored in slants were picked and subcultured on the nutrient agar plates and incubated for 24 hours at 37ºC. A colony of each isolate was picked into McCartney bottles containing 10mls of sterile water and 0.5 MacFarland standard was prepared; a sterile swab stick was placed in each suspension and used in spreading each pathogenic isolate on plates of Mueller Hinton Agar (MHA) for sensitivity tests. A sterile 8mm cork borer was used in boring wells on the agar and a micropipette was used in dispensing 100µl of the crude juice extract and 100µl of the silver nanoparticles (SNPs) into the respectively labeled wells. Dimethyl sulfoxide (DMSO), water, $AgNO_3$ and Ciprofloxacin discs were also used as controls. The antimicrobial activities were then determined by measuring the diameter of the zones of inhibition in mm that was produced.

*Minimum Inhibitory Concentration (MIC) Determination*

The MIC of the different crude juice extract and the biosynthesized SNPs was determined using two-fold dilutions method (Russel and Furr, 1977), that is, half strength (10mg/ml) and quarter strength (5mg/ml) of the extract; in addition to the normal strength (20mg/ml) while $AgNO_3$, DMSO and ciprofloxacin discs were used as controls. The sterilized 8mm cork borer was used to bore five wells in the already solidified media inoculated with the microorganisms. Different concentration ratios (100%, 80%, 60%, 40% and 20% v/v) of the extract and SNPs were dispensed into each well. The preparation was left to diffuse for one hour before incubating at 37ºC for 24 hours. The lowest concentration of antimicrobial agent that completely prevented the growth of the bacteria was taken as the MIC of the juice extract and SNPs. The zones of inhibition were then observed and recorded.

## RESULTS AND DISCUSSION

The quantitative analysis of *Citrus aurantifolia* juice extract is shown in Table 1. The bioactive component present in the juice extract includes Flavonoids (710mg/100g), tannins (525mg/100g), terpenoids (65mg/100g) and phenols (56mg/100g). Alkaloids, saponins, anthocyanins and cardiac glycosides were not found in this extract. The results obtained from this study have shown that *Citrus aurantifolia* is a very potent medicinal plant. It has also revealed that the juice contains medicinally important biologically active substances known as phytochemicals which contribute to the unique characteristics of the plants and its antimicrobial potentials. It also acts as a good reducing and capping agents for the stability of SNPs.

**Table 1: Quantitative phytochemical composition of *Citrus aurantifolia* juice extract**

| S/N | PHYTOCHEMICAL PARAMETERS | *Qualitative* | Quantitative (mg/100g) |
|---|---|---|---|
| 1 | Alkaloids | - | _ |
| 2 | Flavonoids | +++ | 710 |
| 3 | Saponins | - | _ |
| 4 | Tannins | +++ | 525 |
| 5 | Phenols | + | 56 |
| 6 | Anthocyanins | - | - |
| 7 | Cardiac Glycosides | - | - |
| 8 | Terpenoids | + | 65 |

The phytochemical screening of *Citrus aurantifolia* has shown the presence of flavonoids, tannins, terpenoids and phenols. The presence of these chemical constituents underscores the importance of *Citrus aurantifolia* juice as a medicinal plant and according to the work of Chakraborty *et al.,* (2004), Flavonoids have an influence on arachidonic acid metabolism and has been known to be a good antimicrobial agent against a wide array of microorganisms. This activity may be due to the ability of flavonoids to form complexes with extracellular and soluble proteins and to complex with bacterial cell walls (Tringali, 2001 and Ayoola, 2008). Tannins are also known to be effective antioxidants, antimicrobial and anti-carcinogenic agents (Lai and Roy, 2004). Terpenoids can be added to proteins to enhance their attachment to the cell membrane in a process known as isoprenylation and they also play a role in traditional herbal remedies and are active against pathogenic microorganisms (Ayoola, 2008) while Phenols are generally germicidal, are usually used in formulating disinfectants and some possess estrogenic or endocrine disrupting activity. They are also the active ingredient in spices that contributes to its flavor, taste and medicinal properties (Walker and Morton, 1993). Hence, it can be said that *Citrus aurantifolia* juice has anti-oxidative, anti-carcinogenic, anti-inflammatory, anti-allergic, anti-thrombotic or vasoprotective and antibacterial effects.


**Figure 1: Production of SNPs from *Citrus aurantifolia* juice A- Before addition of AgNO3, B- After addition of AgNO3, C- Final colour change**

The biosynthesis of SNPs using the juice extract of *Citrus aurantifolia* is shown in Figure 1. As the crude extract of *Citrus aurantifolia* was introduced into the prepared aqueous AgNO3 solution, there was a gradual colour change from a faint light yellow to a slightly yellowish colour and finally to permanent colloidal brown after 24 hours showing the formation of SNPs. It was also observed that the colour intensity increased with the duration of incubation which indicates the formation of more nanoparticles.

This occurred as a result of the vibrations produced by Surface Plasmons (Krishnaraj *et al.*,, 2010) caused by the reduction of silver ion to silver nanoparticles. It was observed that the use of medicinal plant extracts for the synthesis SNPs enhances the reduction and stabilization of silver nitrates in an aqueous medium for the formation of SNPs.

The reduction of silver nitrate (AgNO3) by plant extracts occur through the oxidation of the amino group or some alkaloids or terpenoids present in this plant by the transfer of electrons from the functional group of the bioactive compounds to the Ag+ ions. These plant extract can then passivate the surface of silver nanoparticles and stabilize the nanoparticles produced owing to the coordination of nitrogen atoms or some other groups with Ag atoms at the surface of SNPs. The resulting metallic silver nucleates gradually form SNPs and further stabilizes it electro-statically (Nickel *et al.*, 2000 and Shukla and Makwana, 2014). Borase *et al.*, (2014), also listed the active components of plants such as proteins, flavonoids, terpenoids, polyphenols, phenol hydroxyl and carboxylic groups of arabinose and galactose, phenolic glycosides, reducing sugars, tannins, aliphatic amines, aliphatic alkenes of alkaloids, polysaccharides, aromatic amines, sec-alcohols, water-soluble heterocyclic components and saponins that are also responsible for the bioreduction of silver ions to SNPs.

Typically, plant extracts possess intrinsic biological activities, which may further manifest in the biological activities of SNPs as a result of combining the two materials. Therefore, *Citrus aurantifolia* juice extract can potentially be developed into novel nanomaterials with diverse biological activities (Park, 2014). The formation and stability of the colloidal solution of the chemically reduced SNPs were monitored using UV-Vis spectrophotometry analysis at different time intervals and wavelengths. The UV-Vis Spectra of SNPs synthesis using the crude juice extract is shown in Figure 2. The biosynthesized SNPs formed in the reaction media revealed absorption maxima in the range of 300nm - 550nm.

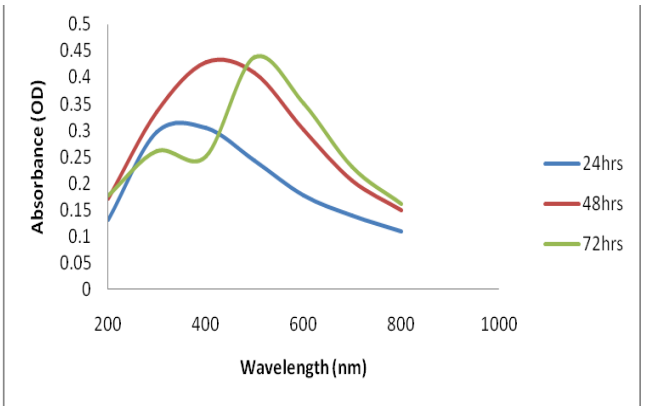


**Figure 2: UV-Vis Spectra of silver nanoparticles synthesis using *Citrus aurantifolia* juice extract**

At 24 hours, the Plasmon band broadened from 300nm - 400nm with an absorbance of 0.15OD while at 48 hours, the broadening of the peak was observed at a wavelength of 350nm - 450nm with an absorbance of 0.2OD. However, at 72 hours, the Plasmon band peaked at 300nm which then further broadened to form the highest peak at a wavelength of 550nm. This was observed at an absorbance of 0.2OD.

The absorbance spectra of the SNPs were analyzed by using a UV-Visible Spectrophotometer at wavelengths ranging between 200 - 800nm at different time intervals. Generally, the Surface Plasmon Resonance (SPR) bands are influenced by the size, shape, morphology, composition, and dielectric environment of the synthesized nanoparticles. The UV-Vis spectrophotometer is mainly used to observe the sizes and shapes of the controlled nanoparticles in the aqueous solutions.

The UV-Vis spectra of the SNPs of *Citrus aurantifolia* centered around wavelengths of 350nm at 24 hours,

400nm at 48 hours and 550nm at 72 hours. This is the characteristic of SNPs and clearly indicates the formation of nanoparticles in the solution.

The Plasmon bands are broad with an absorption tail in the longer wavelengths and an increase in the reading. This could be mainly due to the size distribution of the nanoparticles. The absorption spectra and broadening of the peak of silver nanoparticles formed in the reaction indicated that the particles are polydisperse. Also, it was denoted that all these nanoparticles had an increasing intensity with time (Madhavaraj *et al.*,, 2013) with nanoparticles analyzed at 72hours having the highest intensity. Karthik Raja *et al.*, (2010) reported the plasmon peak of 430nm for synthesized SNPs from Lactobacillus acidophilus 01 strain. Aysha *et al.*,, (2014) obtained absorption peak of 420nm for SNPs biosynthesized using Citrus lemon peel extract.

Balashanmugam *et al.,* (2013) reported good stability and absorption peak of 435nm after 48hours for SNPs produced using *Citrus sinensis* peel extract. Haniff Nisha *et al.*,, (2015) reported that the absorption peak for SNPs from pomegranate was synthesized at 450nm.

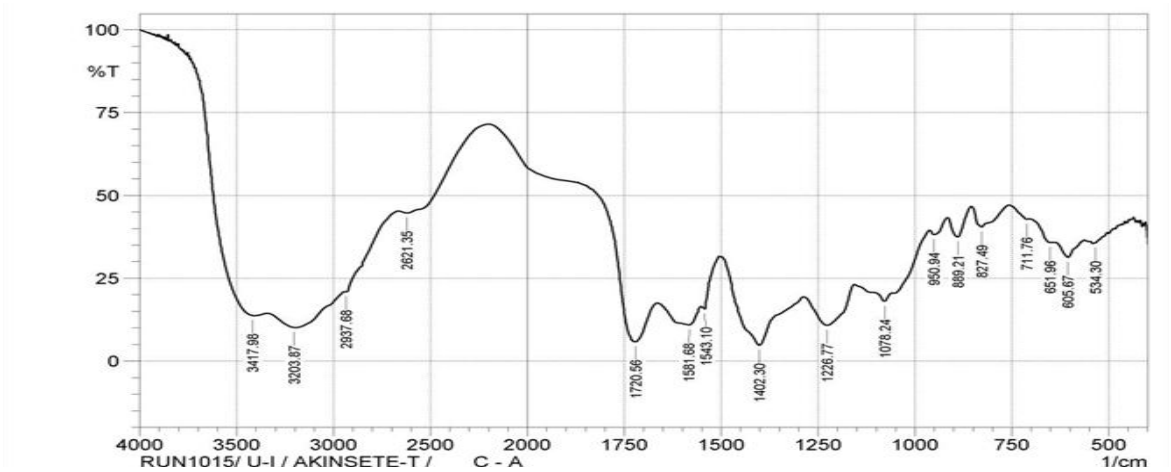


**Figure 3: FTIR analysis of silver nanoparticles synthesized from *Citrus aurantifolia* juice extract**

The FTIR result of the biosynthesized SNPs is shown in Figure 3. The FTIR spectrum of biosynthesized SNPs from *Citrus aurantifolia* juice extract revealed multiple peaks observed between the ranges of 534.30cm-1 and 3417.98cm-1. The peaks observed at 3417.98cm-1 and 3203.87cm-1 reveals strong absorption bands of the N-H secondary amides and the O-H stretching of the hydroxyl groups. The C-H stretching of bands occurs at 2967.68cm-1 and 2621.35cm-1 while the peaks observed at1720.56cm-1, 1581.68cm-1 and 1543.10cm-1 denotes the presence of the C=O anhydride group of amine that is commonly found in protein. Thus indicating the presence of protein as the capping agent and also the presence of C=C aromatic conjugates were observed around the peaks of 950.94cm-1 down to 711.76cm-1 while the stretching of the C-O carbonyl group was revealed by the presence of broad peaks around 1078.24cm-1, 651.96cm-1, 605.67cm-1 and 534.30cm-1 which corresponds to the silver metal.

The FTIR of SNPs from *Citrus aurantifolia* juice had several peaks ranging from 3417.98cm-1 to 534.30cm-1 and consists of various functional groups which includes the secondary amides, the carboxyl groups, the hydroxyl group, the carbonyl groups and anhydride groups commonly found in protein which serves as the capping agent denoting functional groups like the aromatic conjugates, secondary amides, amine groups, carboxyl and carbonyl groups that indicates the presence of silver ions.

The antibacterial activity of crude juice extract and the biosynthesized SNPs from *Citrus aurantifolia* juice extract against the test pathogens is shown in Table 2. A Clear zone of growth inhibition was observed in the test pathogens which confirm the antibacterial potential of the crude extract and the biosynthesized SNPs. The crude extract of *Citrus aurantifolia* had activities against 5 of the test pathogens while the SNPs had activity against 4 of the test pathogens. The zones of inhibition by the crude extract of *Citrus aurantifolia* juice against the pathogenic bacteria were higher than those produced by their biosynthesized SNPs. The crude juice extract of *Citrus aurantifolia* was highly effective against *Streptococcus pyrogens* ATCC 19615 (15mm), *Bacillus* sp. (10mm) and *Staphylococcus aureus* (24mm). *Pseudomonas aeruginosa* was high resistance to the crude juice extract.

The biosynthesized SNPs had antimicrobial activity against *Streptococcus pyrogens* ATCC 19615 (11.0mm), Bacillus sp. (2.0mm), *Actinobacillus* sp. (10.0mm) and *Staphylococcus aureus* (14.0mm). *Klebsiella pneumonia* ATCC 10031 and *Pseudomonas aeruginosa* was high resistance to the biosynthesized SNPs.

Ciprofloxacin also had activity against four of the test pathogens with the highest zone of inhibition recorded for *Actinobacillus* sp. Higher zones were observed in the well loaded with Ciprofloxacin against *Klebsiella pneumonia* ATCC 10031 (21.0mm), *Actinobacillus* sp. (26.0mm), *Pseudomonas aeruginosa* (12.0mm) and *Staphylococcus*

*aureus* (24.0mm). *Streptococcus pyrogens* ATCC 19615 and *Bacillus* sp. was high resistance to ciprofloxacin.

The aqueous solution of $AgNO_3$ used as a control had activity on 3 of the test pathogens with the highest zone of inhibition observed on *Streptococcus pyogenes* ATCC 19615, *Klebsiella pneumoniae* ATCC 10031 and *Actinobacillus* sp. was less susceptible to $AgNO_3$. *Bacillus* sp. and *Pseudomonas aeruginosa* were resistant to the $AgNO_3$. DMSO had no effect on any of the test pathogens; they were all resistant to DMSO.

**Table 2: Antibacterial activity of the crude extract and biosynthesized SNPs from *Citrus aurantifolia* juice against the test pathogens**

| Pathogens | *Citrus aurantifolia* | | Control | | |
|---|---|---|---|---|---|
| | Crude juice extract (mm) | SNPs(mm) | AgNO3 | DMSO | Ciprofloxacin |
| *Streptococcus pyogenes* ATCC 19615 | 15 | 11 | 6 | - | - |
| *Klebsiella pneumonia* ATCC 10031 | 14 | - | 3 | - | 21 |
| *Bacillus* sp. | 10 | 2 | - | - | - |
| *Actinobacillus* sp. | 18 | 10 | 4 | - | 26 |
| *Pseudomonas aeruginosa* | - | - | - | - | 12 |
| *Staphylococcus aureus* | 20 | 14 | - | - | 24 |

All the test pathogens use during this experiment were more susceptibility to the crude juice extract than the biosynthesized SNPs. *Staphylococcus aureus* shows more susceptibility to the crude extract and the biosynthesized SNPs with a zone of growth inhibition 20mm and 14mm respectively.

One of the most remarkable and peculiar properties of *Citrus aurantifolia* juice is their antibacterial activities and currently, the intensified use of its silver nanoparticles in medicine is closely associated with its antimicrobial potentials. The juice extract of *Citrus aurantifolia* and the SNPs exhibited varying antimicrobial activities against the test organisms.

The crude extract of *Citrus aurantifolia* juice and the synthesized SNPs were also subjected to antibacterial activities to check for their Minimum Inhibitory Concentration (MIC). The Minimum Inhibitory Concentration of the crude juice extract of *Citrus aurantifolia* and their biosynthesized SNPs is shown in Table 3.

The MIC of both the crude extract and SNPs against *Streptococcus pyrogens* were both at 20% (20µl/ml). For *Klebsiella pneumonia*, the MIC of the crude extract was also at 20% concentration but the MIC of the synthesized SNPs was not determined since *Klebsiella pneumonia* was resistant to the SNPs.

The MIC of the crude extract against *Bacillus* sp. was at 20% while at 60% concentration of the synthesized SNPs, the growth of *Bacillus* sp. was completely eradicated which indicate that the MIC of the SNPs of *Citrus aurantifolia* against *Bacillus* sp. was at 80% (80µl/ml). For *Actinobacillus* sp., the minimum growth was observed at 20% concentration of the crude extract while there was no growth observed at 20% of the SNPs making the MIC of the

SNPs against the bacteria at a concentration of 40% (40µl/ml).

*Staphylococcus aureus* had its minimal growth for both the crude extract and the SNPs produced at 20% while the MIC of *Pseudomonas aeruginosa* was not determined since it was resistant to both the crude and SNPs produced from *Citrus aurantifolia*.

The extract had activities on five of the six pathogenic organisms which ranged from 8 to 20mm in diameter while the synthesized SNPs had activity on four pathogenic organisms with zones ranging from 2 to 14mm. the extract had the highest activity against *Staphylococcus aureus* (20mm) and the SNPs also had the highest activity against the same pathogen but the activity here was reduced (14mm) while the least activity was observed against *Bacillus* sp. (10mm) and a reduced activity was also observed for the SNPs against *Bacillus* sp. (2mm).

The activity of the SNPSs aligned with the work of Hungund *et al.,* (2015) where SNPs synthesized using *Citrus aurantifolia* juice had the least activity against *Staphylococcus aureus*. Also, the juice extract had activity against *Klebsiella pneumonia* (14mm) which was resistant to the SNPs produced from this extract.

Therefore, it can also be said that the juice extract is more potent against pathogenic microorganisms than the SNPs. This may due to the antibacterial activity of lime juice which resulted in changes in cell morphology in the form of bacterial cell wall broken and hollow to be able to inhibit the growth of deadly bacteria according to the findings of Norman (2015).

However, some of these test pathogens which were susceptible to ciprofloxacin which is a broad spectrum antibiotic were highly susceptible to the extracts. For instance, *Streptococcus pyrogens* was resistant to ciprofloxacin, so it can be said to be an antibiotic resistant organism but it was highly susceptible to *Citrus aurantifolia* juice and its SNPs with zones of 15mm and 11mm respectively while Bacillus sp. which was also resistant to Ciprofloxacin was found to be susceptible to *Citrus aurantifolia* and its SNPs with zones of 10mm and 2mm respectively. This has revealed that medicinal plant extracts and their SNPs can also exhibit broad spectrum

activities. Gordana *et al.,* (2012) reported the effectiveness of Citrus lemon extract (20µl) against Listeria sp. Madhuri *et al.,* (2014) reported the high susceptible to *K. pneumoniae* (14mm) and low susceptibility of B. cereus (12mm) to *Citrus sinensis* peel extract.

**Table 3: MIC of *Citrus aurantifolia* juice extract and their SNPs on the test pathogens**

| | Zones of Inhibition (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | (100%) | | (80%) | | (60%) | | (40%) | | (20%) | |
| Pathogens | Crude | SNPs | Crude | SNPs | Crude | SNPs | Crude | SNPs | Crude | SNPs |
| *Streptococcus pyogenes* ATCC 19615 | 15 | 11 | 12 | 8 | 8 | 5 | 2 | 5 | 2 | 2 |
| *Klebsiella pneumonia* ATCC 10031 | 14 | - | 9 | - | 5 | - | 3 | - | 1 | - |
| *Bacillus* sp. | 11 | 3 | 7 | 1 | 5 | - | 3 | - | 1 | - |
| *Actinobacillus* sp. | 16 | 9 | 12 | 6 | 7 | 4 | 4 | 3 | 1 | - |
| *Pseudomonas aeruginosa* | - | - | - | - | - | - | - | - | - | - |
| *Staphylococcus aureus* | 19 | 12 | 14 | 10 | 9 | 7 | 1 | 6 | 2 | 1 |

The MIC illustrates a decreasing inhibitory effect of the juice extracts and the SNPs as the concentration decreases as there was a progressive decrease in the thickness of the layer of colonies on the streak but also a decrease in the number of colonies from low concentrations to high concentrations thus reflecting a dose-dependent sensitivity. This implies that antimicrobial activity of a substance is concentration dependent, which is in concordance with the report of Dubey *et al.,* (2010) and Oboh (1997), that antimicrobial activity is a function of the active ingredient reaching an organism.

**CONCLUSION**

In conclusion, the crude extract of *Citrus aurantifolia* contains medicinally important bioactive phytochemicals with good antimicrobial potentials against the tested pathogens used in this study. The crude juice extract is a good reducing and capping agents for the stability of SNPs. The SNPs contain various functional groups such as secondary amides, the carboxyl groups, the hydroxyl group, the carbonyl groups and anhydride groups commonly found in protein which serves as the capping agent denoting functional groups like the aromatic conjugates, secondary amides, amine groups, carboxyl and carbonyl groups that indicates the presence of silver ions. The crude extract has more antibacterial potential against the tested pathogen than the biosynthesized SNPs from the crude juice extract. The crude extract exhibits more potent antimicrobial activities against *Streptococcus pyrogens* which were resistance to ciprofloxacin.

Although the plant extract showed greater antimicrobial activities against microorganisms, the biologically biosynthesized SNPs of *Citrus aurantifolia* could still be of immense use as effective growth inhibitors in microorganisms, making them applicable to medical devices and antimicrobial control systems.

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Conflict of Interest: None declared

Received: 23 June, 2016

Accepted: 29 July, 2016