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***Original Research Article***

# Biochemical Studies, Antimicrobial Activity and Green Synthesis of Aqueous Extract (AE) of *Citrus aurantium* Leaves

[1]* Kehinde J. Ayantola, [2] Olatunde A. Oseni [3] Margaret O. Oseni

[1]*Department of Science Laboratory Technology, Faculty of Science, Ekiti State University Ado-Ekiti, Nigeria. P.M.B 5363, Ado Ekiti, Nigeria.*
[2]*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Ekiti State University, Ado-Ekiti, Nigeria. P.M.B 5363, Ado Ekiti, Nigeria.*
[3]*Department of Chemistry, Faculty of Science, Federal University, Oye-Ekiti, Nigeria P.M.B 373, Oye Ekiti, Nigeria.*

ABSTRACT

The goal of this study was to examine the biochemical properties and green synthesis characterization of the aqueous extract (AE) of *Citrus aurantium* leaves. Exactly 20 grams of the air-dried leaves were ground into powder and mixed with 100 ml of distilled water and ethanol, which were separately shaken overnight and filtered to obtain aqueous and ethanol extracts that were then used for the analyses, using conventional analytical techniques of phytochemical analyses, in vitro antioxidant studies, green synthesis, and antimicrobial studies. The research findings indicated that the AE displayed a wide range of the phytochemical constituents under investigation when compared the ethanol extract. The total phenolic content concentration rose from 20 mg/mL to 100 mg/mL as well as in flavonoids concentration. The FTIR spectra of the C. aurantium showed different absorption peaks for the AE (3951, 3997) $cm^{-1}$, CuNP (3991, 3967) $cm^{-1}$, AgNP (3945, 3198) $cm^{-1}$, and ZnNP (3950, 3694) $cm^{-1}$, respectively. The green synthesis of synthesized nanoparticles of the *Citrus aurantium* also showed different absorption peaks. *Citrus aurantium* aqueous extract and its silver, zinc, and copper nanoparticles exhibited a significant percentage of mycelia growth inhibition on the two fungi species (Trichophyton verrycosum and Epidermophyton floccosum) under investigation. These compounds demonstrated strong antibacterial activity by inhibiting the growth zones of four bacteria: Ralstonia solanacearum, E. coli, Pseudomonas glycinea, and Staphylococcus aureus. From the study, C. aurantium was observed to possess medicinal properties to justify its use in ethno-medicinal treatment of diseases that can be used for drug development.

**Keywords:** Aqueous Extract; Antioxidant Potential; Antimicrobial Activity; Green Synthesis; Metallic Nanoparticles

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## Introduction

Known by many different names, such as grapefruits, lemons, limes, oranges, mandarins, and citrons, the genus Citrus comprises a large variety of species with varying sizes and shapes within the Rutaceae family.[1, 2] Their yearly yield of approximately 100 million tons places them among the principal horticultural crops with global agricultural significance.[3] The evergreen *Citrus aurantium* L. tree can reach a maximum height of five meters. Other names for it include bigarade, sour orange, bitter orange, and Seville orange. It is believed to have been grown in the US, Spain, and Italy, and to have originated in eastern Africa and Syria. Its fragrant white blossoms are its most famous feature.[4] Several studies on the bioactivity of compounds derived from *C. aurantium* have been carried out. The health benefits of *Citrus aurantium* are derived from its chemical composition. Terpenoids, vitamins, minerals, and phenolic compounds make up the chemical composition.[5] Flavonoids, which are phenolic compounds present in *Citrus aurantium*, are recognized as significant components because of their positive effects on health as well as their physiological and pharmacological actions.[5]. *C. aurantium* leaves are frequently utilized as a source of edible fruit in traditional medicine.

*Corresponding author. Email: kweki.godfrey@delsu.edu.ng

Tel: +2347034331346

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The pharmacological benefits of *C. aurantium* are derived from both traditional and modern sources, particularly in treating fever, diarrhea, and digestive disorders. Since it contains a variety of bioactive substances, such as minerals, essential oils, polyphenols, and flavonoids, *Citrus aurantium L.* is one of the species that has been used medicinally. Since the 19th century, the majority of significant antibiotic classes, including tetracycline, cephalosporin, aminoglycosides, and macrolides, have faced a setback in their viability due to the growth in microbial resistance. Its effects are currently widespread due to treatment failure linked to microorganisms resistant to multiple drugs, and this has raised concerns about global health.[6]. These days, the synthesis of green nanoparticles (NPs) without the use of harmful regular chemicals, high pressure, or high temperatures is affordable and environmentally beneficial when using bio-reducers. One attractive aspect of synthesizing nanoparticles with biomaterials—especially plant extracts—is their biocompatibility. [7, 8] Because nanoparticles are naturally occurring, exposure to bodily fluids did not reveal their significant toxicity. The most promising materials in the field of biomedicine are iron-oxide magnetic nanoparticles. Numerous biomedical applications are made possible by low levels of toxicity, bioavailability, significant magnetic attitudes (superparamagnetism), and adaptable surface functionalization. Therefore, the nature of the phytoconstituents present in the extracts and the intrinsic tolerance of the microorganisms are responsible for the variation in their sensitivity. Therefore, the purpose of this study was to examine the biochemical properties, antimicrobial properties, and environmentally friendly synthesis of metallic nanoparticles derived from the AE of *Citrus aurantium leaves.*

## Materials and Methods

*Plant identification and authentication*
The leaves of *C. aurantium* were collected from Iworoko-Ekiti (Latitude $7^0 43^1 53^{11}$ N, Longitude $5^0 15^1 48^{11}$ E), Ekiti State, Nigeria, in November 2020. The leaf sample was authenticated and deposited at the herbarium unit of the Department of Plant Science and Biotechnology, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria. It was identified and confirmed by Mr. Omotayo, the chief technologist, with the code number UHAE2020094.

*Preparation of plant leaf extract*
After being cut off from the plant, the *C. aurantium* leaves were cleaned to get rid of any dirt under running water. The leaves were then ground into a powder after being allowed to air dry for a few days. For efficient extraction using a mechanical shaker, 20 grams of powdered leaves were combined with 100 milliliters of distilled water and shaken overnight. The resulting mixture was filtered with the help of muslin cloth, and the filtrate of the leaf sample was taken and used for further phytochemical analyses, in vitro antioxidant analyses, and green synthesis analyses.

*Phytochemical analysis*
Chemical screening for the phytochemical constituents of flavonoids, alkaloids, saponins, phenolic compounds, and steroidal nuclei was done on the AE of *C. aurantium leaves as reported by* [9, 10, and 11].

*In Vitro Antioxidant Activity of the Leaf Sample*

*Determination of 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay of AE of the leaves of C. aurantium:*
The free radical scavenging ability of the AE of the leaves of *C. aurantium* against the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was evaluated using a concentration range of between 20 and 100 mg/ml as described by [12].

*Determination of nitric oxide (NO) radical scavenging assay of AE in the leaves of C. aurantium*
By using modified techniques, the AE of *C. aurantium* leaves was assessed for its capacity to scavenge free radicals from nitric oxide radicals using a concentration range of between 20 and 100 mg/ml.[13]

*Determination of the ferric-reducing antioxidant power of AE in the leaves of aurantium*
Using a modified method of [14], the ferric reducing power of AE in *C. aurantium* leaves was ascertained by comparing the antioxidant power to the reduction of Fe3+ ferricyanide in stoichiometric excess.

*Determination of $Fe^{2+}$ chelation ability of AE in the leaves of C. aurantium*
Using a modified technique, the AE of *C. aurantium* leaves was found to be able to chelate Fe2+. [15, 16]

*Quantification of total phenolic, flavonoid, and ascorbic acid contents of AE of the leaves of the C. aurantium using a concentration range of between 20 and 100 mg/ml*
Estimation of total phenolic content of AE of the leaves of the *aurantium*
The total phenol content of AE of the leaves of the *C. aurantium was* determined based on the method of [17]

*Determination of total flavonoid of AE of the leaves of the C. aurantium*
The total flavonoid content of AE of the leaves of the *C. aurantium was* determined using a colorimetric assay method developed by [18].

*Determination of ascorbic acid content of AE of the leaves of the C. aurantium using 2,4-dinitrophenylydrazine (DNPH) reagent.*
The ascorbic acid content of the AE of the leaves of the *C. aurantium was* analyzed by the modified spectrophotometric Genesys 10-S (USA) method described by 19.

*Green synthesis analyses of AE of the leaves of the C. aurantium*
*Preparation of metal ion solutions*
0.1 M solutions of silver, zinc, and copper were separately prepared in deionized water.

*Synthesis of copper, silver, and zinc nanoparticles*
About 60 g of the sliced leaves was boiled in 600 ml of deionized water on the hot plate to obtain the leaf extract.
After being filtered, the resulting extract was stored in a 250 ml round-bottom flask and refrigerated. Each conical flask contained an equal volume (20 mL) of 0.1 M $AgNO_3$, ZnCl, and $CuSO_4$ solution. Each extract was added separately, and the mixture was stirred for 10 to 45 minutes at 70°C on a hot plate. The color shift that occurred when the corresponding metallic ion was bioreduced into metallic nanoparticles was observed.

*Characterizations of the synthesized silver, zinc, and copper nanoparticles*
By measuring the UV-visible spectra of the reaction mixture solutions, the biosynthesis of the AgNPs, ZnNPs, and CuNPs in the solutions was tracked. A double beam spectrophotometer (Shimazdu, model UV-1800, Kyoto, Japan) was used to record UV-visible spectra with a resolution of 1 nm between 300 and 800 nm. As a blank, distilled water was utilized. FTIR was used to identify the organic functional groups that were present in the leaf extract, AgNPs, ZNNPs, and CuNPs. These measurements were performed in the diffuse reflectance mode in KBr pellets at a resolution of 4 cm−1 using an instrument from Shimadzu, Kyoto, Japan.

*Antimicrobial analyses of AE and metallic nanoparticles of the leaves of the C. aurantium*
The antibacterial and antifungal analyses were carried out on the AE and synthesized silver, zinc, and copper nanoparticles. The disk diffusion plate method was used for the antifungal and antibacterial analysis. A nutrient agar and potato dextrose agar were prepared and poured into the sterile plate and allowed to gel. Cultures of the organisms (bacteria for nutrient agar and fungi for potato dextrose agar) were taken from stock and inoculated into each agar. Then, an 8 mm well size was made in each of the sterile plates. To ensure that the extract was properly diffusing into the agar, the pre-diffused disk of the synthesized samples was introduced into each well, and the plate was left on the bench. For bacteria, the plates were incubated at 37°C, and for fungi, at 25°C. The plates containing bacteria and fungi were examined a day after they were introduced. It was observed that some extracts had clear zones of inhibition, whereas some had none. The measurement of the zones of inhibition in mm depicts the antimicrobial power of the extract and nanoparticles.

## Results and Discussion

*The phytochemical screening and in vitro antioxidant analyses*
From Table 1.0, it can be deduced that the bitter orange can be said to be a source of saponin, alkaloids, flavonoids, phenols, and steroids depending on the extracting solvent. Phytonutrients are vital in both health promotion and disease prevention. [20] These phytochemicals scavenge the free radicals circulating in the body, thereby reducing the oxidative stress caused by the free radicals.[21] Nevertheless, based on the findings of this investigation, the choice of solvent in extraction is very paramount, depending on the type of phytochemicals we are focusing on. From this point of view, it showed that the ethanol is not good enough for the extraction of this plant, though it extracts a very important steroidal compound. Because saponins have an inclination to repel microorganisms, they are a promising treatment option for yeast and fungal infections. These compounds served as natural antibiotics, helping the body to fight infections and microbial invasion.[22] Some of these phytochemicals, which have been found in *C. aurantium* leaves in this study and are supported by prior research, are associated with the citrus plant's antibacterial ability.[23] Citrus flavonoids have a large spectrum of biological activity, including antibacterial, antifungal, antidiabetic, anticancer, and antiviral activities. Alkaloids are known to have anticancer activities and antibacterial potential as well (Figure 1) . [24]

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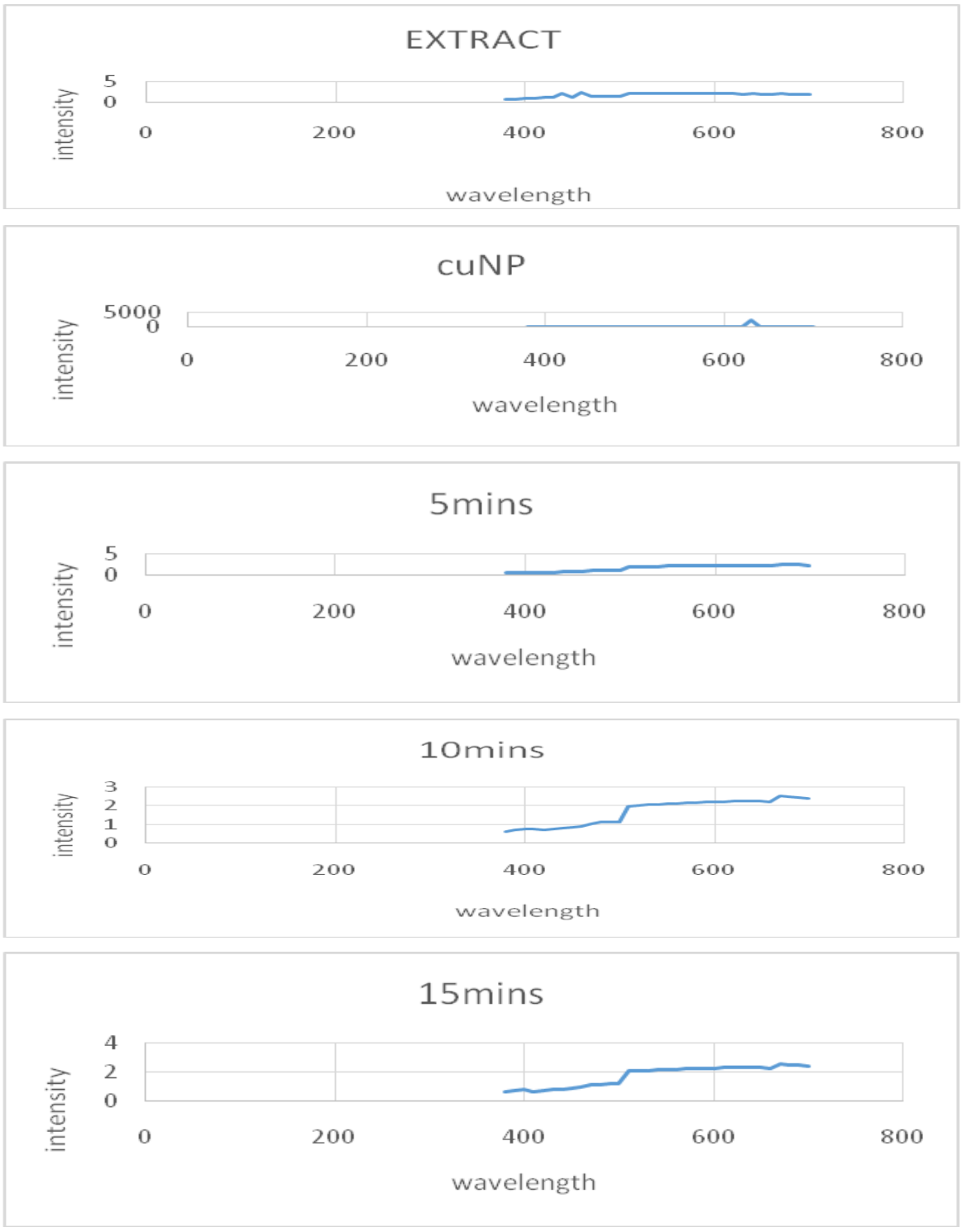


**Figure 1.0:** Wave scanning showing effect of time result for the extract of *C. aurantium*, Copper nanoparticle at 5mins, 10mins and 15mins.

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**Table 1.0:** Phytochemical screening of aqueous of *C. aurantium leaves*

| Parameter | Aqueous extracts |
|---|---|
| Saponin | + |
| Alkanoids | + |
| Flavonoids | + |
| Phenolic compound | + |
| Steroid | - |

- = Absent +=Present

The amounts of flavonoids and phenolic compounds in the *C. aurantium* leaf extract were displayed in Table 2. Comparing with the standard gallic acid, the extract possessed a good quantity of the two phytochemicals needed by a plant to carry out the biological responsibility. It is observed that at higher concentrations, the leaf extract possessed higher contents than the standard gallic acid used. According to a study, [25] flavonoids have been demonstrated to have the ability to scavenge free radicals and act as antioxidants; this ability is related to the hydroxyl groups in their phenol rings. The flavonoids quercetin, myricetin, rutin, tangeritin, naringin, and hesperidin are frequently found in citrus fruits.[26] Additionally, quercetin exhibits remarkable antitumor properties. Quercetin may have positive effects in combating or helping to prevent cancer, prostatitis, heart diseases, cataracts, allergies/inflammations, and respiratory diseases like bronchitis and asthma.[27] Citrus fruits are rich in the flavonoid glycoside hesperidin. Hesperidin has anti-inflammatory properties in addition to lowering cholesterol.[28]

**Table 2.0:** Total phenolic compounds and total flavonoids contents of AE of *C. aurantium leaves*

| Concentration in mg/mL | 20mg/mL | 40mg/mL | 80mg/mL | 100mg/mL |
|---|---|---|---|---|
| Total phenolic content | | | | |
| Galic acid equivalent | 0.513 ± 0.002 | 0.533 ± 0.002 | 0.606 ± 0.002 | 0.671 ± 0.002 |
| *C. aurantium* | 0.520 ± 0.002 | 0.643 ± 0.002 | 0.697 ± 0.002 | 0.719 ± 0.002 |
| Total flavonoids contents | | | | |
| Gallic acid equivalent | 0.423 ± 0.002 | 0.458 ± 0.002 | 0.505 ± 0.002 | 0.534 ± 0.002 |
| *C. aurantium* | 0.399 ± 0.002 | 0.527 ± 0.002 | 0.563 ± 0.002 | 0.842 ± 0.002 |

Values are mean ± standard deviations of duplicates
Standard curve equation, GAE: Y = 0.005x +0.464 ($R^2$=0.961)
mg of GAE/mg of dry extract

Table 3 showed that the lower the result values of the *C. aurantium* extract and the standard vitamin C, the higher the radical scavenging ability, while the higher the values, the lower the radical scavenging ability. Additionally, it demonstrated that when the standard vitamin C and *C. aurantium* extract concentrations rise, the radical scavenging ability also increases. Therefore, the results showed that both the *C. aurantium* extract and the standard vitamin C possessed viable antioxidant thiobabituric acid radical scavenging power as demonstrated in a concentration-dependent manner. [28]
From Table 4, it was indicated that the values of sample and standard increase as the concentration increases. This means that as the value increases, the antioxidant power increases when ferric reducing, and as the value decreases, the FRAP also decreases. According to the results in table 4, *C. aurantium* has the strongest antioxidant power for reducing ferric iron. The ability of *C. aurantium* to function as a reducing agent in the presence of ferric ions is indicated by the FRAP of the AE. A popular test for determining a substance's antioxidant potential is FRAP. Ferric ions can be efficiently converted to ferrous ions by antioxidant compounds, indicating their ability to combat oxidative stress and eliminate free radicals.[28]

**Table 3.0:** Thiobabituric acid reactive species (TBARS) of *C. aurantium leaves*

| Concentration in mg/mL | 20mg/mL | 40mg/mL | 80mg/mL | 100mg/mL |
|---|---|---|---|---|
| Vitamin C | 0.901 ±0.002 | 0.768± 0.002 | 0.622 ±0.002 | 0.604 ±0.002 |
| *C. aurantium* extract | 1.105±0.002 | 0.893± 0.002 | 0.733± 0.002 | 0.707± 0.002 |

Values are mean ± standard deviations of two numbers

**Table 4.0** Ferric reducing antioxidant power (FRAP) of AE of *C. aurantium leaves*

| Concentration in mg/mL | 20mg/mL | 40mg/mL | 80mg/mL | 100mg/mL |
|---|---|---|---|---|
| Vitamin C | 0.025 ± 0.002 | 0.174± 0.002 | 0.210± 0.02 | 0.246± 0.002 |
| *C. aurantium* | 0.404± 0.002 | 0.420± 0.02 | 0.676± 0.002 | 0.743± 0.002 |

Values are mean ± standard deviations of two numbers

Table 5 displays the extract of *C. aurantium* leaves' ability to scavenge nitric oxide radicals. It was observed to show in a concentration-dependent manner because the scavenging power increases as the sample concentration increases. Therefore, *C. aurantium* leaves show nitric oxide radical scavenging power. Nitric oxide is a signaling molecule involved in various physiological processes, including vasodilation, immune response, and neurotransmission. Through the donation of electrons or reaction with reactive nitrogen species, the AE of *Citrus aurantium* may scavenge nitric oxide radicals, preventing potential oxidative stress and preserving a balance in nitric oxide levels. According to the results (Table 5), *C. aurantium* has the strongest antioxidant power for reducing ferric iron. The ability of *C. aurantium* to function as a reducing agent in the presence of ferric ions is indicated by the FRAP of the AE. A popular test for determining a substance's antioxidant potential is FRAP. Ferric ions can be efficiently converted to ferrous ions by antioxidant compounds, indicating their ability to combat oxidative stress and eliminate free radicals.[28] However, specific mechanisms and effectiveness can vary (Figure 2).

**Table 5.0** Nitric oxide radical scavenging ability of AE of *C. aurantium leaves*

| Concentrations (mg/mL) | 20mg/mL | 40mg/mL | 80mg/mL | 100mg/mL |
|---|---|---|---|---|
| Buthylated hydroxyl acetone (BHA) | 0.452 ±0.002 | 0.562± 0.002 | 0.578 ±0.002 | 0.608± 0.002 |
| *C. aurantium* | 0.511 ±0.002 | 0.523± 0.02 | 0.534 ±0.002 | 0.549± 0.002 |

Values are mean ± standard deviations of two numbers

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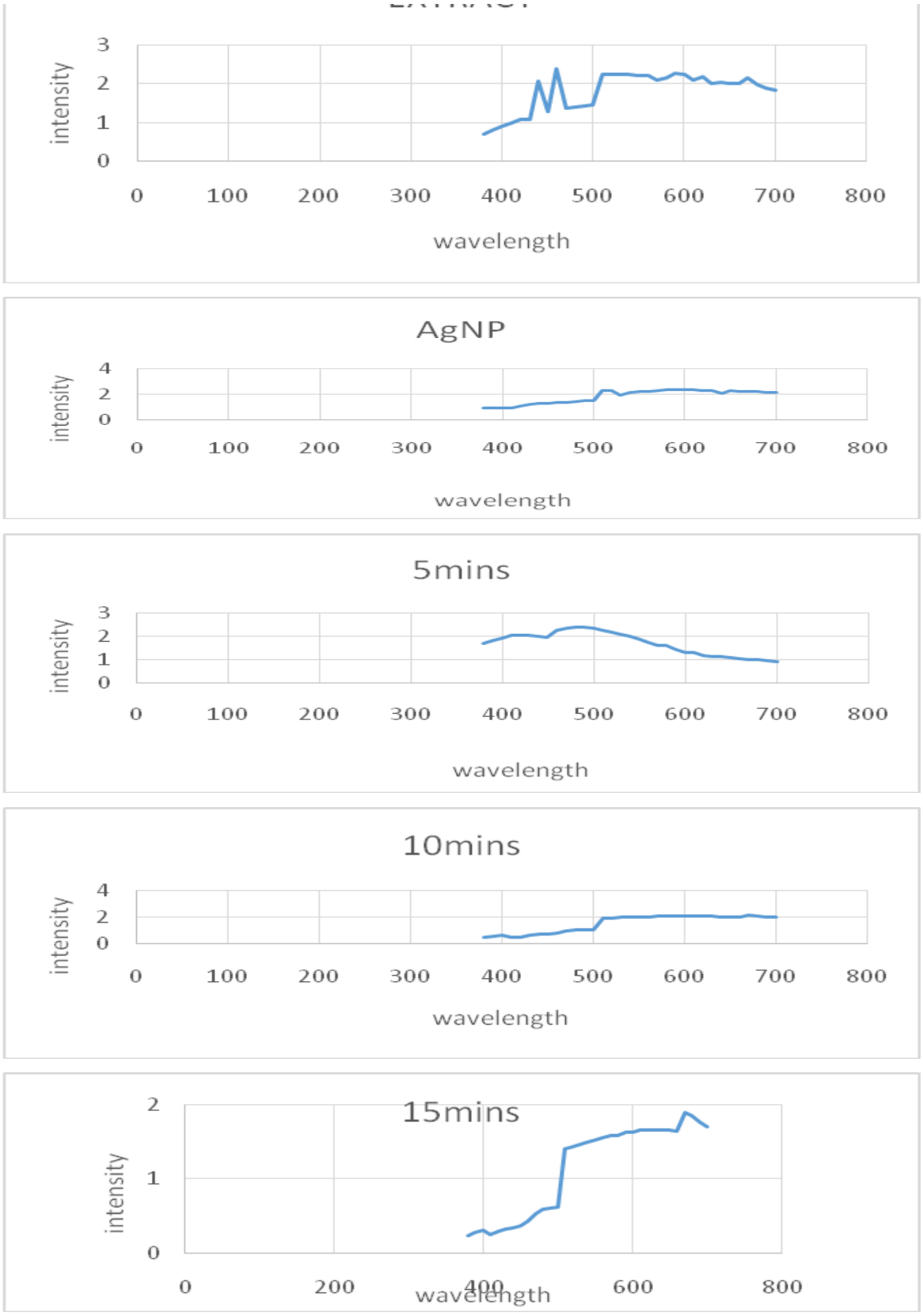


**Figure 2.0:** Wave scanning showing effect of time result for the extract of *C. aurantium* , Silver nanoparticle at 5mins, 10mins and 15mins

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Table 6 shows that the scavenging ability of extract on DPPH radical increases in order with the concentration increment. When compared to regular vitamin C, it was found that the *C. aurantium* leaf extract had a good 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging power. The term "DPPH radical scavenging ability" refers to the *C. aurantium* leaf AE's capacity to neutralize free radicals, especially DPPH radicals. Several bioactive substances, including flavonoids and polyphenols, which are recognized for their antioxidant qualities, are present in *C. aurantium* leaves. The degree of discoloration in the DPPH solution is indicative of the extract's ability to counteract oxidative stress. Greater

reduction of DPPH radicals and a more noticeable change in color from purple to yellow are the outcomes of a stronger antioxidant capacity.

**Table 6.0:** 2, 2-Diphenyl-2Picrylhydrazyl (DPPH) Radical scavenging ability of AE of *C. aurantium leaves*

| **Concentration (mg/mL)** | **20mg/ mL** | **40mg/ mL** | **80mg/ mL** | **100mg/ mL** |
|---|---|---|---|---|
| Buthylated hydroxyl acetone (BHA) | 0.604± 0.002 | 0.622 ±0.00 2 | 0.768 ±0.00 2 | 0.901± 0.002 |
| *C. aurantium* | 0.336± 0.002 | 0.451 ±0.00 2 | 0.520 ±0.00 2 | 0.527± 0.002 |

Values: mean ± standard deviations of two numbers

*Copper, silver, and zinc are synthesized using an AE of* aurantium (effect *of wave canning at different time iintervals)*

The impact of time for copper nanoparticles (CuNP) changes with time in contrast to the plant extract. CuNP has a high reducing ability compared to the plant extract. It was observed that CuNP has the highest reducing ability at 15 mins due to its highest peak compared to that of CuNP at 5 mins and 10 mins. The peak increases as the time also increases, as demonstrated in Figure 4. AgNP was revealed to have the highest peak at 5 mins while it reduces as the time increases; therefore, the AgNP at 5 mins has a higher reducing ability than the extract at different time intervals, as depicted in Figure 5. Additionally, it was shown that the ZnNP has its reduction power increasing as the time increases, as displayed in Figure 6. CuNP at 15 mins has the highest reduction power in contrast to other nanoparticles and extracts.
*Citrus aurantium* leaves were utilized to create green synthesis of Cu, Ag, and Zn nanoparticles. The environmentally friendly nanoparticles (NPs). These days, using bio-reducers to create green nanoparticles (NPs) without the use of heat, pressure, or hazardous chemicals is an economical and environmentally friendly process. Due to their biocompatibility, plant extracts are especially desirable for the synthesis of nanoparticles.[29] Since nanoparticles are naturally occurring, there wasn't any discernible toxicity when exposed to bodily fluids or the body itself. Its strong magnetic properties, bioavailability, adaptable surface functionalization, and low toxicity make it suitable for a wide range of biomedical applications. These consist of magnetic resonance imaging contrast agents for diagnostic purposes.[30, 31]. The release of controlled substances and gene therapy.[32, 33] Recently, the field of nanodrugs has seen the release of intelligent nanomaterials that provide synergistic effects of nanomedicines. These materials are referred to as "theragnostic" due to their combination of therapeutic and diagnostic functions. [33] A change in color occurs when taking the UV-Vis spectra of the different synthesized nanoparticles, which are AgNP, CuNP, and ZnNP, respectively. The nanoparticles' absorption spectra were recorded in Figs. 1.0 to 3.0. For every nanoparticle, the result demonstrates a strong surface plasmon band (SPB), with high peaks falling within the wide range of 500–4000 $cm^{-1}$. The surface plasmon resonance of the nanoparticles is dependent on their size, which is very small when absorption dominates the plasmon resonance extinction spectrum. [34] In the case of larger nanoparticles, in order for the result to disclose the bio-reduction of Cu, Ag, and Zn ions to their nanoparticles, respectively. [35] The transformed peak in the artificially produced nanoparticles indicates that their starting size was small, but it increases after some time and is stabilized at a particular wavelength.

*Green Synthesis of Copper, Silver, and Zinc with the AE of C. aurantium (FTIR Spectra Peaks of C. aurantium Extract, Copper, Silver, and Zinc Nanoparticles)*

Below are the spectra peaks of the synthetic nanoparticles along with the AE of *C. aurantium* in copper, silver, and zinc. Extract (395, 1399), CuNP (3991, 3967), AgNP (3945, 3198), ZnNP (3950, 3694)
The extract's spectra peaks, as seen in Figure 4.0, revealed numerous absorption peaks at 3951 $cm^{-1}$ and 3997 $cm^{-1}$. These peaks are caused by the carboxylic acid's C=O or O-H group, which denotes phenol and alcohol groups.[35] As in the CuNP, the band revealed at 3991 $cm^{-1}$ and 3967 $cm^{-1}$ was due to C=O or O-H groups of carboxylic acids, and it indicates alcohol and phenol groups. In the AgNP, peaks of absorption at 3945 $cm^{-1}$ and 3198 $cm^{-1}$ are due to C=O or O-H groups of carboxylic acids, and they indicate alcohol and phenol groups.[36] A similar absorption was also obtained for ZnNP. Since the carboxylic (C-O or O-H) group is present in both the extracted and the synthesized nanoparticles, it was concluded that the synthesis of the nanoparticles has no effect on it.

*Green Synthesis of Copper, Silver, and Zinc with the AE of C. aurantium* (Antimicrobial *Qualities of the Extract from C. aurantium, Copper, Silver, and Zinc Nanoparticles)*

According to the antimicrobial assay, the *C. aurantium* leaf AE demonstrated in vitro antibacterial activity (Table 7) against both Gram-positive and Gram-negative bacteria. The important activity was observed. The activities of the plant extract and its nanoparticles were compared, and the findings showed that the *C. aurantium* plant extract has antibacterial activity. Specifically, it inhibits the growth of four bacteria: *R. solanaceanum, E. coli, P. glycinea*, and *S. aureus*. The outcomes of this study were comparable to those of [37], who evaluated the antimicrobial activity of *Citrus aurantium* against a range of gram-positive and gram-negative bacterial strains. According to Table 7.0, it was discovered that plant extract and CuNP have the highest antimicrobial activity because they each inhibit four different species of bacteria.

**Table 7.0:** The antibacterial characteristics of copper, silver, zinc, and *C. aurantium* extract nanoparticles

| | *R. solanacearum* | *E. coli* | *P. glycine* | *S. aureus* | *S. faecalis* | *X. phasaoli* | *E. aerogenes* | *S. typhi* |
|---|---|---|---|---|---|---|---|---|
| *C. aurantium* extract | 7.0 | 5.0 | 1.0 | 7.0 | - | - | - | - |
| ZnNP | 5.0 | - | - | - | - | - | - | - |
| AgNP | 10.0 | - | - | - | - | - | - | - |
| CuNP | 1.0 | - | - | 2.0 | 1.0 | 2.5 | - | - |
| CIPRO | 23 | 18 | 28 | 30 | 32 | 28 | 26 | 20 |

CIPRO = CIPROFLACIN (500mg)/20ml of Distiled water ($H_2O$)

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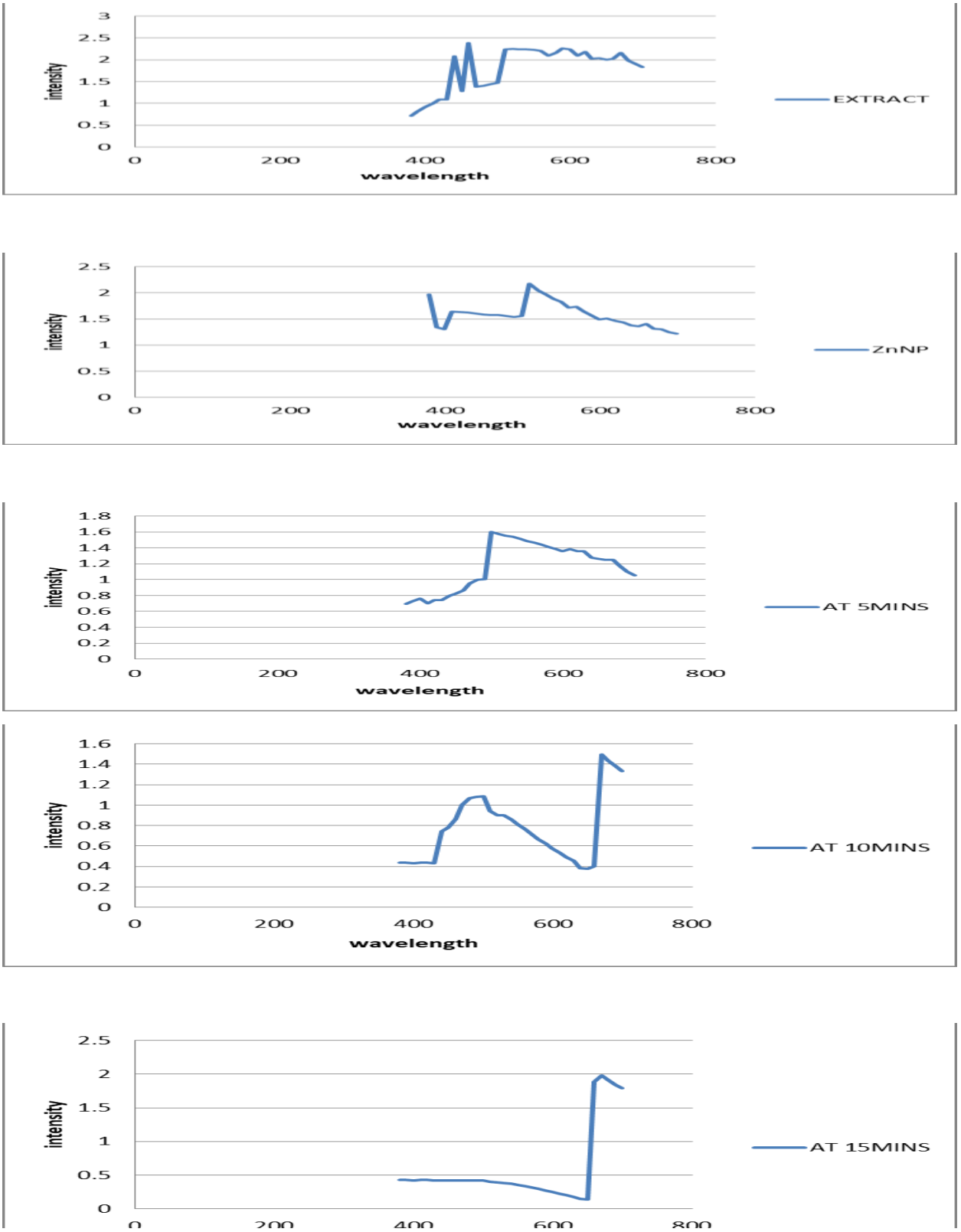


**Figure 3.0:** Wave scanning showing effect of time result for the extract of *C. aurantium* , zinc nanoparticle at 5mins, 10mins and 15mins.

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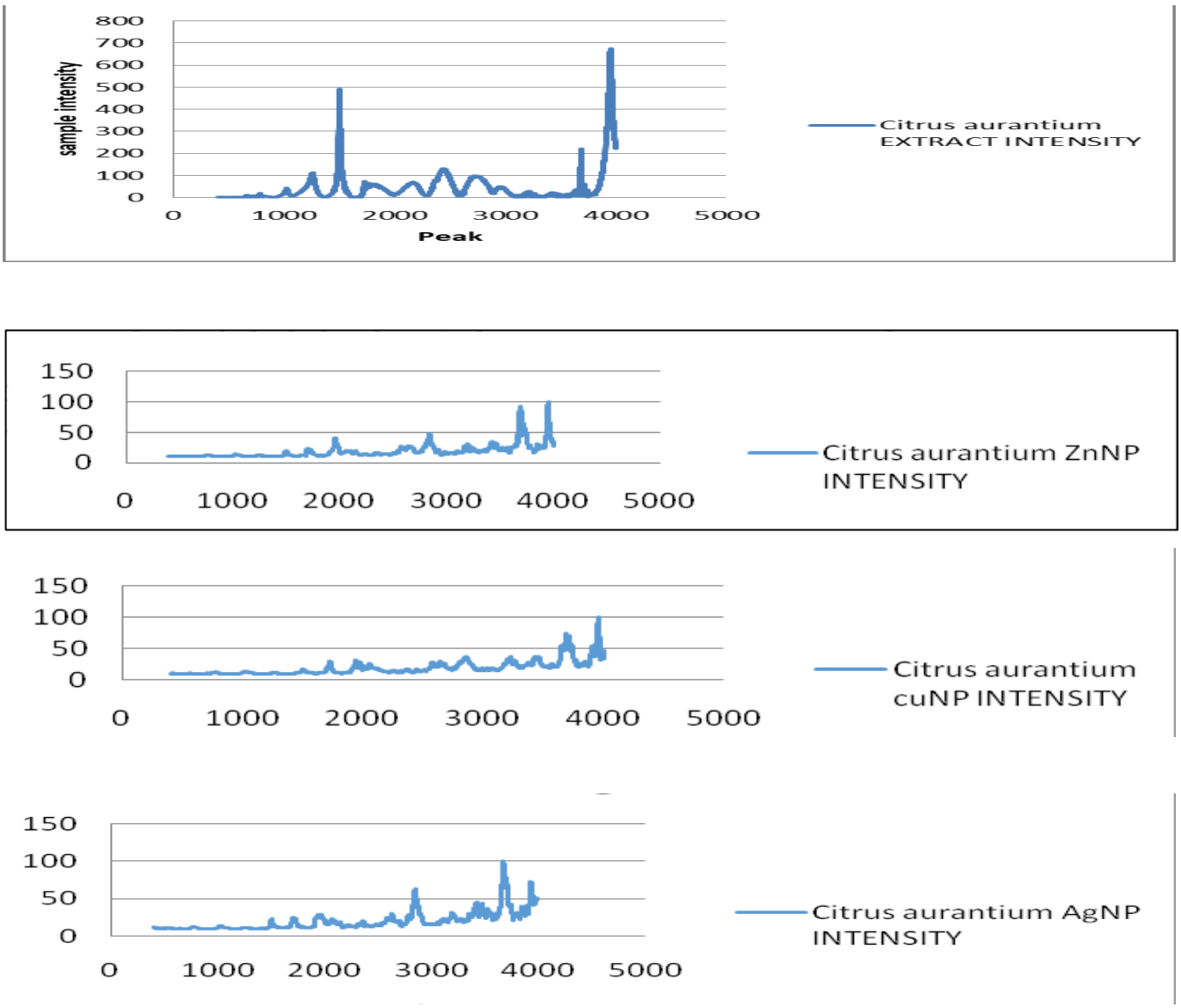


**Figure 4.0:** The FTIR spectra of *C. aurantium extract*, copper, silver and zinc nanoparticles

This is consistent with the findings of Aibinu et al., [38], as ZnNP and AgNP only inhibit *Ralstonia salanaceanum*. ZnNP and AgNP were not as effective against bacteria as the extract and CuNP. This plant's extract and CuNP can be used as substitute sources to prevent the growth of bacteria. Based on the aforementioned outcome, the standard antifungal agent (ketoconazole and trimethoprime) (Table 8) was compared to *C. aurantium* leaf extract in combination with nanoparticles.

**Table 8.0:** Activities of *C. aurantium* extract, silver, zinc and copper nanoparticles. against fungi

| | *Trichophyton verrycosum* (%) | *Epidermophyton floccosum (%)* |
|---|---|---|
| *C. aurantium extract* | 80.77 | 14.28 |
| ZnNP | 75.00 | 29.59 |
| AgNP | 67.31 | 22.45 |
| CuNP | 61.54 | 56.16 |
| Ketokonazole | 80.00 | 70.00 |
| Trymethroprime | 80.77 | 89.80 |

It was generally observed here that the *C. aurantium* extract has the same potency as the standard antifungal drug when used on *Trichophyton verrycosum*. This implies that the plant can be used in the management of fungal infection caused by *T. verrycosum.* The earlier studies have shown that citrus extract has an inhibitory effect against other fungi like *Macrophomina phaseolina*. [39] In another related study, it was reported that *C. aurantium* demonstrated inhibitory activity against *Fusarium graminearum*, a fungus responsible for crown rot of wheat. Makhlouf et al., [40] Additionally, it was found that the extract for *Trichophyton verrycosum* had the largest inhibition zone, 80.77%, followed by ZnNP (75.00%), AgNP (67.31%), and CuNP (61.54%) in ascending order of inhibition. Consistent with this investigation, 41 additionally documented the antifungal impact of citrus peel green extraction on *Aspergillus flavus*. In the case of *Epidermophyton floccosum*, CuNP exhibits the largest zone of inhibition, measuring 56.16%. ZnNP comes in second, with a zone of inhibition of 29.59%, followed by AgNP at 22.45% and the plant extract at 14.28%. Thus, using plants and nanoparticles together can effectively inhibit the growth of fungi.

Comparable to the results of [38], who investigated the antimicrobial qualities of Citrus aurantifolia (lime fruit), the plant extract's activity was examined in the absence of nanoparticles.

## Conclusion

This study's conclusion, however, states that *C. aurantium* is a plant and that, in addition to the AEs, the synthesized AgNP, ZnNP, and CuNP nanoparticles showed strong antimicrobial and therapeutic potential. The plant was found to contain flavonoids, saponin, alkaloids, phenolic substances, and steroids. Its antioxidant potential is effective as a primary and secondary antioxidant. The plant's vitamin C content was found to be 24.30 mg/100 g, making it a good source of the vitamin when compared to other plants and vegetables. The results of *C. aurantium* nanoparticles' antibacterial and antifungal activity showed how effective they were against the human infections that were the subject of this study. The results of this research validate the application of this plant in the ethnomedical management of microbial illnesses caused by some of the organisms used in this study.

## Conflict of Interest

The authors declare no conflict of interest.

## Author's Declaration

The authors hereby declare that the work presented in this article is original. Any liability for claims relating to this article will be borne by us.

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