

## ORIGINAL ARTICLE

# EVALUATION OF ANTIMICROBIAL EFFECTS OF CITRUS PEEL EXTRACTS AND ITS SILVER NANOPARTICLES AGAINST MULTIPLE PATHOGENS

Maimonah Q. Yahya <sup>1</sup>✉, Khadeeja Y. Abid <sup>2</sup>

<sup>1</sup> Department of Clinical Laboratory Science, College of Pharmacy, Mosul University, Mosul, Iraq

<sup>2</sup> Department of Pharmacognosy, College of Pharmacy, Mosul University, Mosul, Iraq

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### Summary

The majority of nanomaterials have unique properties that make them helpful in a variety of biotechnology applications. The study assesses the phytochemical, antioxidant (using a DPPH radical scavenging assay) and antimicrobial activities and identifies minimum inhibitor concentrations of *Citrus sinensis* (orange), *Citrus Limonum* (lemon), and *Citrus reticulata* (tangerine) extracts and their silver nanoparticles. Fourier Transform Infrared (FTIR) spectroscopy and scanning electron microscopy (SEM) was used to analyze the produced AgNPs. The synthesized AgNPs have a size of less than 100 nm according to SEM examination. Their DPPH radical scavenging activity and reducing power increased in a dose-dependent way that was more than that of their aqueous and alcoholic extracts. In comparison to *Staphylococcus aureus* and *Candida albicans*, silver nanoparticles were found to be more efficient towards *Escherichia coli*. Their activities were increased with increasing dosage. Whereas, no inhibition zones were conducted with the examined plain citrus peel extracts. This finding revealed that the biomolecules that cover nanoparticles can increase metal nanoparticles' biological activity and the organic AgNPs green alcoholic and aqueous extracts from orange, lemon, and tangerine peels could be used as a potential source of new antioxidant and antimicrobial agents.

*Key words: antibacterial; bacteria; citrus; nanoparticle; peel*

### Introduction

Antibiotic resistance is actively expanding over the world, jeopardizing its effectiveness and endangering the health of millions of people (1). Nanoparticle production has connoted a lot of attention in recent years because of its unique features and possible applications. Nanoparticles, whether simple or composite, have unique properties and are becoming an extremely significant material in the development of novel Nano devices that can be used in a wide range of physical, biological, biomedicine, and therapeutic applications (2). As a result, may discovering new antibiotics to replace the ones now in use has urgently become a need (3).

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✉ Mosul University, College of Pharmacy, Department of Clinical Laboratory Science, Mosul, Iraq  
pharm.maymona@uomosul.edu.iq  
☎ Mobile no. +9647518608832

Certain plants have historically been recognized to be a good source of medicinal chemicals, which have been utilized extensively in herbal medicine to address a variety of emergent and existing ailments. This is based on the assumption that natural substances, particularly citrus, are innately less harmful and may be purchased at a lesser cost, especially when eaten fresh or in juice form (4). Phytochemicals constitute one of the most numerous and widely distributed groups of substances in the plant kingdom. Plant-derived compounds have emerged as a promising method for synthesizing metallic nanoparticles (NPs) in an environmentally friendly manner. As a result, preserving particular shape, size, and dispersion parameters while greenly synthesizing NPs remains a difficult challenge (5). Some studies have shown that there is an inhibitory effect on bacteria and fungi of extracts resulting from *Citrus sinensis*– orange, *Citrus Limonum* – lemon, and *Citrus reticulata* – tangerine silver nanoparticles (6, 7, 8). The findings of this research could be useful in biomedical applications based on nanotechnology.

The majority of nanomaterials have unique properties that make them helpful in a variety of biotechnology applications, leading to their usage in the development of extremely effective diagnostic and treatment instruments (9). The demand for environmentally friendly, non-toxic nanoparticle manufacturing techniques has sparked interest in biological approaches that avoid the use of harmful chemicals as by-products (10). The biological technique has a higher chance because it is both environmentally and economically favourable. The biological method involves producing nanoparticles with microorganisms or medicinal plants (11). The antibacterial activities of the synthesized silver nanoparticles were studied and demonstrated to be more effective against gram-negative bacteria than gram-positive bacteria (12). Silver has been utilized as an antimicrobial agent since ancient times, and silver-based compounds are far less expensive than gold-based compounds (13). Due to its unique capacity to fight infectious diseases, prevent the growth of bacteria, mold, and germs, silver nanoparticles is a popular component in many health goods as indicated above (2). The chemical, optical, electrical, magnetic, and mechanical properties of silver nanoparticles (AgNPs) are all distinct. Researchers are interested in AgNPs because of their unique features, which could be useful in nano-medicine applications and has a biological properties like antimicrobial, drug delivery, anticancer and immunomodulatory activities (14, 15). The development of silver nanoparticle synthesis has had a significant impact on a variety of scientific fields.

## Material and methods

**Plant material collection:** Fresh citrus fruits (*Citrus sinensis*– orange, *Citrus Limonum* – lemon, and *Citrus reticulata* – tangerine) were purchased from local farmers and fruit dealers in Mosul, Ninevah, Iraq, and validated by a plant taxonomist at the Department of Botany, College of Agriculture and Forestry University of Mosul, Iraq. Analytics of all other chemicals and reagents were utilized and did not require further purification. A slight modification has been followed by the method described by (16). Orange, lemon, and tangerine fruits were transported to the laboratory in College of Pharmacy/ Mosul University, carefully removed the peels from the fruit with a sharp knife, and washed thoroughly with tap water. Washed peels have been cut into little pieces (1-5 cm) and air-dried over two weeks in the shade at room temperature. On a dry basis, the moisture content reached 40 % by weight. To obtain the shape of the powder, a mortar and a pestle were used in the dried fruit peels, then a grinder was employed. The powdered peels passed through sieve No. 40 to get a uniform powder and were stored at room temperature until treatment. Two different extracts (aqueous and alcoholic extract) were prepared from orange, lemon, and tangerine peels powder using two different methods (maceration and soxhlet method) as well as, oil extract was prepared by Hydro-distillation using the Clevenger apparatus.

### *Extracts preparation: Alcoholic and aqueous extract*

**Maceration:** Orange, lemon, and mandarin extracts were prepared in different Erlenmeyer conical flasks according to the procedure described by (11, 17), with some modifications, by macerating 10 g of dried powder of each plant peel in 100 ml of distilled water or absolute ethanol. The stock solution was kept in a refrigerator at 4° C in a sealed storage tube for subsequent phytochemical analysis and silver nanoparticle synthesis.

**Soxhlet methods:** A 10 gm of the powdered sample of each citrus peel was percolated in 100 ml absolute ethanol. The extract was done using the soxhlet method (18). The extracts were placed in air-tight dark bottles and stored in the refrigerator at 4° C in a sealed storage tube for subsequent phytochemical analysis and silver nanoparticle synthesis.

**Phytochemical screening tests:** Each extract of the previously prepared extracts, whether alcoholic or aqueous, will be subjected to the following qualitative phytochemical screening tests according to the standard methods with some modifications:

Alkaloids and Flavonoids tests were assessed by (19), Carbohydrate, Protein and Tannin tests were evaluated by (20), Coumarins, Phenols, Quinones and Saponins tests were estimated by (11) and finally, Steroids and Triterpenoids tests were assessed by (21).

**Silver nanoparticles biosynthesis from plant extracts:** In the green synthesis of AgNPs, aqueous and alcoholic extracts of each plant peel were used as reducing agents as a method prescribed by (22) with some modification. To obtain the best values, the effect of the extract, AgNO<sub>3</sub> concentration, reaction time, and reaction temperature were calibrated. Drop by drop, two millilitres of 10 mmol/L AgNO<sub>3</sub> solution were added to the working plant extract solution while heating (55° C) for 25 minutes and vigorously stirring. After the addition of the AgNO<sub>3</sub> solution, colour changes were indicating the formation of AgNPs. After AgNPs were synthesized, the sample containing the nanoparticles was collected by centrifugation at 8000 rpm for ten minutes to extract the nanoparticles. Analyses were performed on AgNPs sediment. Furthermore, each dried AgNPs mixture was refrigerated in a microtube for future research.

### **Characterization of silver nanoparticles**

**Fourier Transform Infrared (FTIR) Spectroscopy Analysis:** Analyzed with an FTIR spectrophotometer (Bruker-Alpha ATR-FTIR, Germany) was made with each plant extracts and their AgNPs (23).

**Scan electron microscopy (SEM):** It was performed utilizing a Hitachi scanning electron microscope (Model, S-2600N-Tokyo, Japan) (24).

**Antimicrobial activity of extracts and their silver nanoparticles:** It was analyzed using the agar disk diffusion method (25). *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*) and *Candida albicans* (*C. albicans*), as a model for Gram-positive bacteria, Gram-negative bacteria and yeast respectively were used. Different concentrations of each extract were prepared (100, 75, 50, 25, 12.5 mg/ml), a negative (distilled water) and positive (ciprofloxacin or voriconazole (Maxicare Medical Laboratory, Lagos, riNigea) control were used. After incubation at 37 °C for 24-48 hours, clear zone diameter (in mm) was measured in three replications.

**Determination of Minimum Inhibitory Concentration (MIC):** Serial dilutions were carried out for the determination of MIC in citrus peels extracts and their AgNPs following the National Committee for Clinical Laboratory Standards (26). Serial dilutions (100, 50, 25, 12.5, 6.25, 3.125 mg/ml) were done with brain heart infusion (BHI) broth (Lab M Limited Topley House, UK) microdilution assay. From the maintained stock bacterial and *Candida* culture, 5 microliters were added into 2 ml of BHI broth, taking into consideration the number in comparison with standard turbidity of 0.5 MacFarland solution. 200 microliters of such suspension was added to each serially diluted tube and observed the turbidity was after incubation at 37° C for 24 hours.

**Evaluation of the antioxidant activity of the extracts and their AgNPs using DPPH radical scavenging assay:** DPPH (2,2-Diphenyl-1-picrylhydrazyl) is a stable organic free radical that has been employed to study free radical activities and thus antioxidant activity in a variety of natural products (24). It was determined using the method of (27). Different volumes (50, 100, and 150) µl of orange, lemon, or tangerine extracts were added to each tube (separately), full the volume to 1 mL by D.W., added 1 mL of DPPH (HiMedia Laboratories Pvt. Ltd, India) solution (0.2 mM in ethanol) to each tube, well mixed, and incubated at room temperature for 30 minutes. To prepare the control without orange, lemon, or tangerine extract, follow the same procedure as before. Positive control was an ascorbic acid solution (0.03 % w/v). The absorbance (A) of the solution was measured at 517 nm with a UV-VIS spectrophotometer (Spectro-UV-VIS double beam, UVD2950, labomed Inc. USA), and the inhibition of DPPH free radical in percent I % was calculated using the following equation:

$$I\% = [(Ac-As)/Ac] \times 100$$

Where I%: percentage of inhibition of DPPH free radical, Ac: absorbance of control measures at 517 nm and As: absorbance of the solution measured at 517 nm.

**Statistical analysis:** Data analysis was done with SPSS statistic package version 19. The results were calculated by percentage and the average and standard variations in the three readings. The data is described as tables and in bar diagrams.

## Results

### Percentage of extracts from citrus peels

Maceration and soxhlet methods were used with aqueous and ethanolic solvent to prepare the citrus peel extracts. For maceration, aqueous, or ethanolic, the greatest yield of phytochemical extract was obtained from *Citrus sinensis*- orange (13.1 %, 14.3 %) followed by *Citrus Limonum* – lemon (12.1 %, 12.3 %) and *Citrus reticulata* – tangerine (8.1 %, 8.9 %) respectively. while the percentage of extracted by soxhlet was higher than the soaking method. The results showed that the highest yield was obtained from *Citrus sinensis*- orange (32.9 %) followed by *Citrus Limonum* – lemon (24.8 %) and *Citrus reticulata* – tangerine (28.5 %) respectively using absolute ethanol

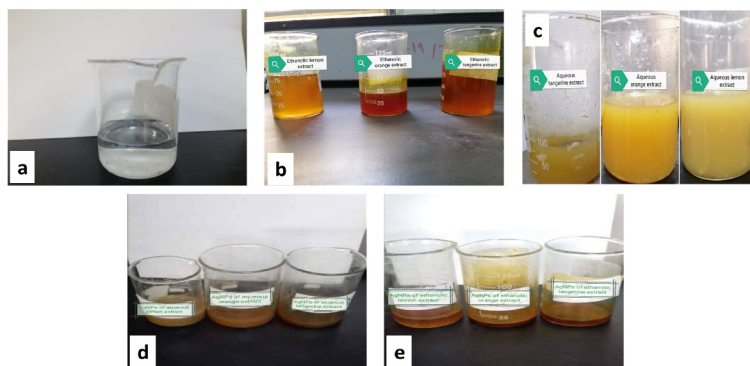
These peels extract either maceration or soxhlet preparation yield a sticky mass after evaporation of a solvent (distilled water or ethanol) and precipitate as a gum which has been used for further biochemical and antimicrobial tests and biosynthesis of AgNPs as green reductants and capping agents. The process produces a yellow solution, which turns into a dark gum after the solvent was being evaporated, with different quantities being higher in soxhlet extraction.

### Phytochemical screening tests

Secondary metabolites have important medicinal qualities for human health. All characterization of phytochemical screening tests, excluding saponins, were positive to all extracts except to aqueous tangerine extracts that showed the absence of carbohydrate, protein, quinines, and steroids.

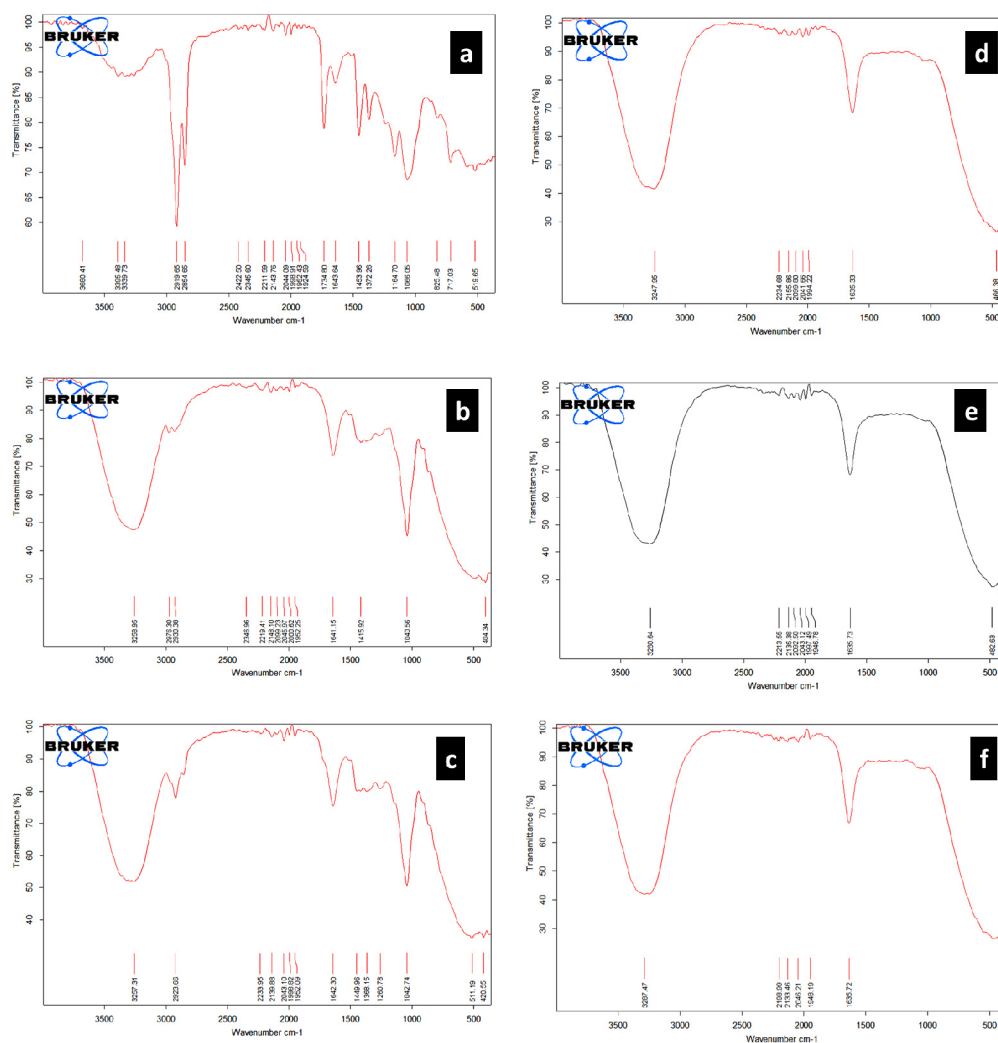
### Biosynthesis of AgNPs by Using the Plant Extracts

After the addition of the  $\text{AgNO}_3$  solution, the yellowish solution of the diluted plant extract transformed into a colloidal brownish of the reaction solution was confirmed. This colour change is a morphological indicator of the colloidal solution formation of AgNPs. Figure-1 shows the colour change in the mixture after mixing citrus extracts with  $\text{AgNO}_3$  when silver ions ( $\text{Ag}^+$ ) are reduced to silver nanoparticles ( $\text{Ag}^0$ ). The green synthesis of AgNPs from each extract was collected after drying the mixture of each extract and  $\text{AgNO}_3$ . It's worth noting that aqueous orange peel extract needs 15 minutes to convert to brown colour and formation of precipitate while tangerine needs more than 30 minutes at  $55^\circ\text{C}$  with vigorously stirring.



**Figure 1.** Colour change in the mixture after mixing citrus extracts with  $\text{AgNO}_3$ , (a): colorless silver nitrate ( $\text{AgNO}_3$ ) solution, (b): aqueous citrus peels extracts, (c): ethanolic citrus peels extracts, (d): silver nanoparticles of aqueous citrus peels extracts, and (e): silver nanoparticles of ethanolic citrus peels extracts.

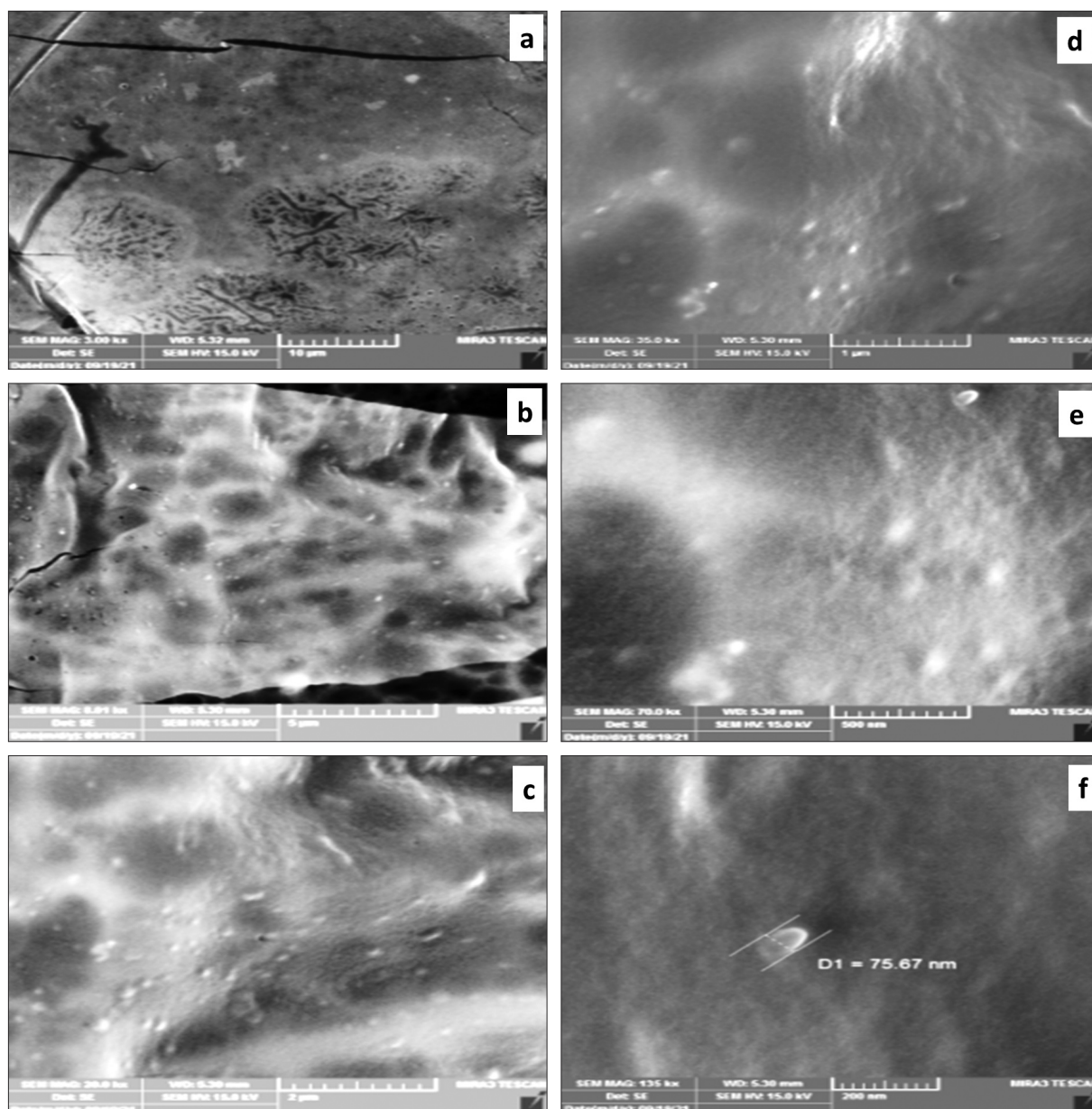
### Fourier Transform Infrared (FTIR) Spectroscopy Analysis





### Scan electron microscopy (SEM)

The antimicrobial property of Ag nanoparticles may be influenced by a variety of factors, including content, morphology, size, and diameter. As a result, SEM was used to determine the shape and size of synthesized nanoparticles. The SEM images of the nanoparticles Synthesized were shown in figure-3. The size of the produced AgNPs was less than 100 nm, according to SEM data, which correspond well with DLS results.



**Figure 3.** Measurements of silver nanoparticles from (a) alcoholic orange, (b) alcoholic lemon, (c) alcoholic tangerine, (d) aqueous orange, (e) aqueous lemon and (f) aqueous tangerine peels extracts using scanning electron microscopy (SEM).

The PDI measurements suggested that all nanoparticles were circular and polydisperse. Nevertheless, some silver nanoparticle clumps and particle diameter fluctuations were observed, which could be attributed to sampling preparation solvent evaporation. Around the AgNPs, a faint, thin coating was observed.

**In Vitro Antimicrobial Activity of Citrus Peel Extracts and Their AgNPs**

Different concentrations of *Citrus sinensis*– orange, *Citrus limonum* – lemon, and *Citrus reticulata* – tangerine and their AgNPs plant extract solution whether aqueous (maceration) or alcoholic (maceration or soxhlet) citrus peel crude extracts (orange, lemon, or tangerine) were determined. The results showed that various measurements were recorded with different concentrations of their AgNPs crude peel extracts. Whereas, no inhibition zones were conducted with the examined plain citrus peel extracts against *S. aureus*, *E. coli* and *C. albicans*. The antibacterial effectiveness of standard antibiotics was checked that ZOI of Ciprofloxacin for *S. aureus* and *E. coli* and Voriconazole for *C. albicans* was 40, 37, and 25 mm respectively.

As with orange, lemon, and tangerine peel extracts, the data reveal that AgNO<sub>3</sub> and DMSO have no particular antimicrobial effect against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. *Escherichia coli* was shown to be more sensitive to AgNPs than *Staphylococcus aureus* and *Candida albicans*. Furthermore, their microbial activity was observed to increase with increasing dosage. The inhibition of silver nanoparticles generated from tangerine peel extract was higher than that of the other nanoparticles. The bactericidal function of silver nanoparticles was more active against Gram-negative bacteria than Gram-positive bacteria, according to the literature. All citrus AgNPs extracts at 100 mg/ml exhibited strong inhibitory action against three pathogens examined than their other concentrations as recorded in table-1.

**Table 1.** The antimicrobial activity of different silver nanoparticles from citrus (orange, lemon, and tangerine) peel either alcoholic or aqueous extracts on the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.

Inhibition zone (mean (mm) ±SD)							
		Citrus sinensis	Citrus Limonum	Citrus reticulata	Citrus sinensis	Citrus Limonum	Citrus reticulata
<i>Candida albicans</i>	12.5	6±0.5bA	6.5±0.00aBA	5.5±0.00aA	0±0.00a	5.5±0.00b	5±0.4bA
	25	6±0.00aA	7±0.00bB	6±0.00aB	3±0.5aB	6±0.00b	5.5±0.00bA,B
	50	7±0.00aB	8.5±0.00bC	7±0.26aC	5.5±0.00aC	7±0.00c	6±0.2bB,C
	75	8±0.45aC	10±0.2bD	8±0.00aD	6±0.5aC	8±0.00b	6.5±0.0aC,D
	100	8±0.5aC	11.5±0.00bE	12±0.3bE	8±0.00bD	8.5±0.00b	7±0.5aD
<i>Escherichia coli</i>	12.5	6.5±0.26aA	6±0.28aA	6±0.00aA	5±0.00bA	0.0±0.00aA	6±0.36cA
	25	10±0.00bB	7±0.00aB	7.5±0.00aB	5.5±0.25aB	5.5±0.00aB	6±0.00bA
	50	13±0.5bC	8±0.00aC	8±0.00aC	6±0.5aC	8.5±0.00bC	6.5±0.2aB
	75	14±0.00bD	9±0.45aD	10±0.00bD	6.5±0.00aD	9±0.26cD	7.5±0.00bC
	100	14.5±0.00bE	11±0.36aE	15±0.5bE	8±0.00aE	12±0.34cE	8.5±0.00bD
<i>Staphylococcus aureus</i>	12.5	6.5±0.17bA	6.5±0.00bA	6±0.3aA	5±0.00bA	7.5±0.00cA	0.0±0.00aA
	25	7±0.00aB	7±0.00aB	7±0.17aB	5.5±0.00aB	8.5±0.1cB	6±0.2bB
	50	9±0.00aC	7±0.50bB	8±0.50cC	6±0.00aC	9.5±0.00cC	6.5±0.2bC
	75	10±0.10aD	8.5±0.00bC	12±0.00cD	6.5±0.1aD	10±0.00bD	7±0.5aD
	100	10.5±0.00aE	9±0.30bD	12.5±0.10cD	10±0.00aE	10.5±0.17bE	11.5±0.00cE
Extracts		Citrus sinensis	Citrus Limonum	Citrus reticulata	Citrus sinensis	Citrus Limonum	Citrus reticulata
Concentrations (mg/ml)		alcoholic extracts			Aqueous extracts		

- SD: standard deviation.

- significant at p≤0.05.

- Different letters vertically (a), (b), (c), (d), (e) indicate that the mean is different significantly at p≤0.05 between different groups at different concentrations.

- Different letters horizontally (A), (B), (C), (D), (E) indicate that the mean is different significantly at p≤0.05 between each group at different concentrations.

The result showed the inhibition zone on 100 mg /ml concentration of alcoholic AgNPs extracts of tangerine by the soxhlet method against *S. aureus*, *E. coli*, and *C. albicans* was greater than other extracts used, ZOI was 12.5, 15, and 12 respectively. The results demonstrated that the compounds of all AgNPs extracts of citrus peel were effective in inhibiting *S. aureus*, *E. coli*, and *C. albicans*. The strongest antimicrobial activity for inhibiting the growth of *S. aureus*, *E. coli*, and *C. albicans* was found in the alcoholic AgNPs extracts of tangerine by the soxhlet method at 100 mg/ml, the inhibition zone was 12.5, 15, and 12 mm respectively, followed by the extracts of alcoholic orange AgNPs extracted by the soxhlet method on *S. aureus* and *E. coli*, inhibition zone was 10.5 and 14.5 mm respectively, while for *Candida albicans*, the inhibition zone of alcoholic lemon AgNPs extracted by the soxhlet method was 11.5 mm.

### Calculation of minimum inhibitory concentration (MIC)

Determination of MIC for silver nanoparticles of ethanolic and aqueous extracts of *Citrus sinensis*- orange, *Citrus limonum* – lemon, and *Citrus reticulata* – tangerine against *S. aureus*, *E. coli*, and *C. albicans* was represented in table-2.

**Table 2.** The minimum inhibitory concentration (MIC) of silver nanoparticles extracted from different citrus peels.

MIC concentration (mg/ml)	Citrus Peel AgNPs Extracts					
	Alcoholic orange	Alcoholic lemon	Alcoholic tangerine	Aqueous orange	Aqueous lemon	Aqueous tangerine
<i>S. aureus</i>	12.5	12.5	50	25	25	50
<i>E. coli</i>	12.5	25	12.5	50	50	12.5
<i>C. albicans</i>	50	12.5	25	50	12.5	25

The study indicated that the minimum inhibitory concentration for alcoholic extraction of silver nanoparticles from orange, lemon, and tangerine citrus peels were either equal or less than that used for aqueous extraction against the three pathogens used.

### Evaluation of the antioxidant activity of the extract and its AgNPs

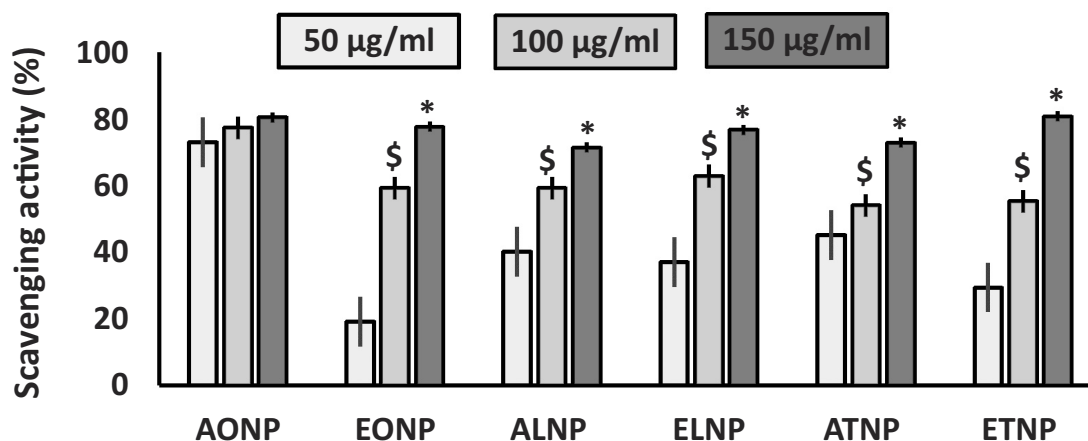
*In vitro* antioxidant activity of citrus peel extracts and their synthesized AgNPs based on DPPH radical scavenging activity of extracts and synthesized AgNPs at 50, 100, and 150 g/ml. Antioxidants react with DPPH to convert it to 1,1-diphenyl-2-picryl hydrazine, which stops free radical oxidation chains from propagating and produces stable end products that do not cause more lipid oxidation (28). Orange peel showed the best antioxidant capacity in DPPH testing for ethanolic extract, whereas tangerine peel had the lowest antioxidant capacity. On the other hand, Aqueous lemon extract had the highest antioxidant activity. Independent of extraction solvent, orange peel extract demonstrated the best antioxidant activity in all experiments as represented in Table-3.

**Table 3.** Antioxidant activity (%) of citrus peels extracts by in vitro assays DPPH radical methods.

Citrus peel extracts	Scavenging activity (%)								
	orange			Lemon			tangerine		
Conc. (µg/ml)	AqE-M	AcE-M	EE-S	AqE-M	AcE-M	AcE-S	AqE-M	AcE-M	AcE-S
50	40.5	78.6	74.9	61.1	68.6	65.7	18.5	38.6	27.9
100	50.9	85.6	80	71.8	77.5	71.3	31.6	58.9	34.6
150	78	86	84.5	83.5	85.9	83	58.5	70	70.7
Aq=aqueous; Ac=alcohol; M=maceration; S=soxhlet; E=extraction									



At concentrations of (50, 100, 150 µg/mL), the DPPH free radical scavenging activity of orange, lemon, and tangerine-silver nanoparticles was assessed. The biosynthesized orange AgNPs peel extract demonstrated the best antioxidant activity in all experiments in this study, regardless of extraction solvent, as shown in Figure-4.



**Figure 4.** antioxidant scavenging potential of the biosynthesized aqueous and ethanolic extract of citrus silver nanoparticles against DPPH radical. Whereas AONP: aqueous orange nanoparticles, EONP: ethanolic orange nanoparticles, ALNP: aqueous lemon nanoparticles, ELNP: ethanolic lemon nanoparticles, ATNP: aqueous tangerine nanoparticles and ETNP: ethanolic tangerine nanoparticles. Data expressed as Mean±SD, \*p<0.05 extract 150 µg/ml as compared to 100 µg/ml or 50 µg/ml; \$p<0.05 extract 100 µg/ml as compared to 50 µg/ml.

The results of the research showed that the antioxidant activity of nanoparticles for the used citrus peels is much more than that of their plain peels extracts, whether they are aqueous or alcoholic extracts.

## Discussion

In these results, the greatest yield of phytochemical extract, whether aqueous or ethanolic maceration, was obtained from *Citrus sinensis*-orange (13.1 %, 14.3 %) followed by *Citrus limonum*-lemon (12.1 %, 12.3 %) and *Citrus reticulata* – tangerine (8.1 %, 8.9 %) respectively. The same results were obtained with soxhlet extraction using absolute ethanol as a solvent that showed the highest yield about (32.9 %) followed by (24.8 %) and (28.5 %) respectively which agrees with Shakya A, *et al* 2019 (29). Secondary metabolites present like phenoles, terpenoid, alkaloid, and flavonoid compounds are used as medicinal products and as dietary supplements, provide essential human health pharmaceutical properties (29).

The green synthesized silver nanoparticles from citrus peels extracts using AgNO<sub>3</sub> were optimized. During this study, silver nitrate ions (Ag<sup>+</sup>) were reduced to Ag<sup>0</sup> quickly within thirty minutes. The colour of the mixture changes due to the collective oscillation of free conduits the colour of the silver colloid is supported by the surface plasma resonance (SPR) as seen with Niluxsshun, *et al.* 2021 and Reda M, *et al.* 2019 results (8, 22). This Zero Valente of silver metal clustered around bioactive compounds present in extracts. The transformation of a yellowish solution of diluted plant extract into a colloidal brownish reaction solution was confirmed as seen in the results of a study conducted by Niluxsshun, *et al.* 2021 (8).

FT-IR spectroscopy confirmed the possibility of biological reduction and adequate stabilization of green synthesized silver nanoparticles. All three FT-IR spectra of AgNPs produced from alcoholic citrus extracts showed little changes. Flavanoids and terpenoids, which are abundant in plant extract and are used as reductants during the manufacture of silver nanoparticles, are primarily responsible for these IR peaks (23). The FT-IR results indicate that various bioorganic chemicals found in fruit sp. peel isolates, such as flavonoids, alkaloids, coumarins, and phenolics, may be implicated in the reduction of silver ions to Ag<sup>0</sup> leading to prevent the accumulation of silver nanoparticles. The variances in single-particle growth rates during the nucleation process, some AgNPs clusters and particle size

fluctuations were observed, which could be attributed to the evaporation of the solvent during sample preparation. The AgNPs were surrounded by a light, light coating generated by other plant elements, such as proteins and flavonoid compounds, which prevented them from clumping, as demonstrated by FTIR data.

The study showed that the inhibition zones of Ciprofloxacin and Voriconazole against bacteria and fungi were agree with Al-Wahaibi, *et al.*, 2021 and Al-Nima, *et al.*, 2020 results respectively (30, 31). No inhibition zones were conducted with the examined plain citrus peel extracts against *S. aureus*, *E. coli* and *C. albicans*, this may be due to the extraction medium or the low concentration used in the research. Whereas, their silver nanoparticles showed antimicrobial activity against pathogens used. Furthermore, their biological properties were reported to increase significantly with increasing dosage, which is consistent with Niluxsshun, *et al.* 2021 and Reda M, *et al.* 2019 results (8, 22). The results revealed that the mean ZOI were highest with AgNPs extracted from alcoholic extracts in comparisons with their aqueous. Furthermore, when compared to gram-negative bacteria, gram-positive bacteria were significantly ( $p \leq 0.05$ ) more responsive to AgNPs extracts that agree with Khan, S. S., *et al.* 2011 (32). The difference could be due to the cell wall structures of different bacterium groups. There is also no outer layer on the cell wall of gr<sup>+</sup>ve bacteria, they do, however, interact in different ways with the charged AgNPs (22, 27). One factor could be differences in the nature and depth of the peptidoglycans in the bacterial cell. Gram-positive bacteria have an 80-nanometer thick tri peptidoglycan coating (ten times thick versus gr<sup>-</sup>ve bacteria) that makes them less vulnerable to silver nanoparticle attacks. Silver is thought to interact with thiol groups of proteins on cell membranes, causing respiration to be blocked and eventually death. Extracts contain a variety of naturally occurring chemicals including proteins, tannins, terpenoids, and flavonoids, that interact with the microbial membrane causing growth inhibition. Otherwise, this could be due to the release of Ag<sup>+</sup> ions which could adhere to the positively charged cell wall, causing protein deformation and cell death (22). Some research, on the other hand, proposes that the inhibition zone around the disk is caused by AgNPs releasing diffusible inhibitory reactive oxygen species (ROS). Metal-derived free radicals have been shown to damage bacterial membranes, mitochondria, and DNA, resulting in cell bursting and death (33).

According to published studies, fungi can be affected in the same way. Silver nanoparticles extracts may influence cell membranes by disrupting it and also can infiltrate the cytoplasm and react with sulfur- and phosphorus-containing proteins and molecules, disrupting DNA replication (22).

Orange peel showed the highest antioxidant capacity in DPPH tests for ethanolic extract that agree with Zahoor S, *et al.* 2016 (34), while tangerine peel had the lowest antioxidant capacity, which is consistent with Hegazy AE, *et al.* 2012 and Shehata MG, *et al.* 2021 findings (16, 28). Aqueous lemon extract, on the other hand, exhibited the highest antioxidant activity, which contradicts the findings (28). These results are most likely linked to phenolic concentration depending on plant sp. and solvent extraction (34). As previously documented, Variances in antioxidant component solubilization explain differences in antioxidant properties among extracts (35). The presence of phenolic chemicals and flavonoids in citrus peels could explain their antioxidant activity like flavanones, flavanone glycosides, and polymethoxylated flavones (28).

## Conclusion

*Citrus sinensis* – orange, *Citrus limonum* – lemon, and *Citrus reticulata* – tangerine are types of citrus fruit that contains a variety of natural components, including alkaloids, tannins, coumarins, phenols, triterpenoids, flavonoids, and others, and has pharmacological effects. In this study, AgNPs were synthesized using an eco-friendly, simple, and limited green synthesis strategy. We assumed that adding AgNO<sub>3</sub> to aqueous and alcoholic citrus peel extracts forming their AgNPs would boost its potential activity. The antioxidant and antimicrobial activities of different citrus plant peels extracts and their AgNPs were all investigated. Furthermore, the presence of biological components of plant origin surrounding the AgNPs was confirmed by FTIR. Silver nanoparticles of citrus peel extracts have antioxidant activity higher than their aqueous and alcoholic plain peel extracts. Moreover, they have antibacterial and antifungal activities with higher effect against *E. coli* than *S. aureus*. This study's findings that silver nanoparticles of Citrus peel extracts could be useful in nanotechnology-based biomedical applications. Further characterization and quantitative testing may be performed for various pharmacological and therapeutic activities with these extracts.

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## Adherence to Ethical Standards

Not applicable. This article does not contain any studies involving animals performed by any of the authors. This article does not contain any studies involving human participants performed by any of the authors.

## Conflict of Interest

The authors declare that no conflict of interest exists for this research.

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