International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2020.236.243

Research Article
Antimicrobial Activity of Silver and Zinc Nanoparticles Mediated by Eggplant Green Calyx

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Abstract
Background and Objective: Antibiotic resistance is rising to dangerous levels in all parts of the world. Metallic nanoparticles among them; silver and zinc oxide nanoparticles (Ag and ZnONPs) are powerful weapons for fighting microbial resistance. Present study was aimed at green synthesis of silver and zinc oxide nanoparticles using eggplant aqueous methanolic extract, evaluation of their antimicrobial activity against different pathogenic Gram-positive and Gram-negative bacteria and fungi. **Materials and Methods:** Preparation of silver and zinc oxide nanoparticles were performed by reduction of both silver nitrate and zinc acetate by using eggplant green calyx aqueous (aq.) methanolic extract. Characterization and measurement of the prepared particles size and morphology was performed by Transmission Electron Microscopy. Characterization of the functional groups shared in the biosynthesis was done by Infra-red spectroscopy. Agar well diffusion technique was used to determine the *in vitro* antibacterial activity of the eggplant calyx aqueous (aq.) methanol extract, silver and zinc oxide nanoparticles. **Results:** Silver nanoparticles have spherical shape with diameter ranging from 8-20 while zinc oxide nanoparticles have hexagonal shape with diameter range from 8-12 nm. Silver nanoparticles possess potent antimicrobial activity in comparison to prepared zinc oxide nanoparticles and the eggplant extract. **Conclusion:** Eggplant calyx which acts as food waste is potential source for synthesis of potent antimicrobial metallic nanoparticles (Ag and ZnONPs).

Key words: Eggplant calyx, pathogenic microbes, green synthesis, metallic nanoparticles, microbial resistance

Citation: Gouda H. Attia, Hamad S. Alyami, Mohamed A.A. Orabi, Ahmed H. Gaara and Mohamed A. El Raey, 2020. Antimicrobial activity of silver and zinc nanoparticles mediated by eggplant green calyx. Int. J. Pharmacol., 16: 236-243.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

According to WHO, antibiotic resistance is climbing to dangerously high levels in all parts of the world[1]. New resistance mechanisms are about to come out and spread universally, menacing the ability to treat ordinary infectious diseases. An increasing list of infections such as pneumonia, tuberculosis, blood poisoning, gonorrhoea and foodborne diseases are becoming stronger and sometimes untreatable as antibiotics become less effective. Without crucial action, humans will heading for a post antibiotic era in which ordinary infections and minor injuries can once again kill. Therefore, there is crucial need for new strategies for fighting microbial resistance.

Ancient peoples use metals to encounter infectious diseases[2]. Nowadays, metallic nanoparticles have acquiring increased attention as antimicrobial agents due to their wide spectrum inhibitory against bacteria, fungi and viruses[3,4].

The antimicrobial effect of these nanoparticles has been assigned to their small size and high surface area to volume ratio, which allows them to interact with microbe's membranes and is not due to the release of metal ions in solution[3,5].

Silver nanoparticles which are synthesized by chemical method showed various biological risks due to their toxicity. Green syntheses using plant extracts containing reducing agents like phenolic compounds will merit high attention as they exhibiting low toxicity[6].

Solanum melongena (Eggplant) is an economically important edible crop. Egypt produce more than 1.2 million t. *Solanum melongena* fruit has high phenolic compounds content and potent antioxidant activity (one of the top ten highest antioxidant activity containing vegetables). In addition, eggplant is distinct for its content of flavonoids in peel with high amounts of the anthocyanin called nasunin and also high content of phenolic acids in the flesh among them the chlorogenic acid[7,8]. Fortunately, it was reported that eggplant calyx has phenolic content more than the peel and pulp while anthocyanins content less than in the peel and more than in the pulp[9] .

Studies of the eggplant anthocyanins and phenolic acids have a good antioxidant activity, a potential beneficial action on hyperlipidemia in animals and a modest effect on hypercholesterolemia in humans[7]. Eggplant calyx is a food waste, it can be used only in villages as an animal's food. Aim of this study was to use the eggplant calyx to prepare the silver and zinc oxide nanoparticles. In addition, to evaluate antimicrobial activities of the eggplant calyx extracts, silver and zinc oxide nanoparticles.

MATERIALS AND METHODS

Plant material: *Solanum melongena* (Eggplant) were purchased from local market on July, 2019. The plant was cultivated in Al-Sharqia governorate, Egypt. The study was performed at National Research Center, Dokki, Egypt and Faculty of Pharmacy, Najran University, Najran, Saudi Arabia from July-December, 2019.

Plant extract: The dried powder of eggplant calyx (100 g) were macerated 3 times in 500 mL of 70% methanol/ water (v:v). The extract was filtered using Whatman filter paper No. 1 and dried under vacuum to yield 20.6 g of the crude extract which stored at 4°C for further experiments.

Total phenol: Total phenolics in the cultivars extracts were determined according to the method of Souleman and Ibrahim[10]. Briefly, 100 µL of extract was dissolved in 10 mL of methanol and 2 mL of this solution was made up with 0.3% HCl to 5 mL. A 100 µL aliquot of the solution was added to 2 mL of 2% Na_2CO_3 and after 2 min, 100 µL of Folin-ciocalteu reagent (Merck, Darmstadt, Germany) (diluted with methanol 1:1) was added and mixed well. After 30 min incubation, the absorbance of mixtures was recorded spectrophotometrically at 725 nm using UV-Vis Shimadzu (UV-1601, PC) spectrophotometer. The results were expressed as Chlorogenic Acid Equivalents (CAE).

Synthesis of silver nanoparticles (AgNPs): Reduction of $AgNO_3$ (1 mM) solution (10 mL) using green calyx of eggplant aq. methanolic extract (0.4 mL) was performed at room temperature. The mixture was shaken and allowed to stand at room temperature in the dark place. The effect of extract quantity on AgNPs synthesis was tested by mixing varied concentrations of the extract from 100-500 µL per 10 mL of silver nitrate solution (1 mM). The obtained colloidal solution was centrifuged repeatedly at 12,000 rpm for 20 min followed by redispersion of the obtained nanoparticles in the deionized water. This process was repeated twice to isolate the pure AgNPs and exclude the presence of any unbound plant extract residue[6,11].

Synthesis of zinc oxide nanoparticles (ZnONPs): Synthesis of ZnONPs was undergone by modified method of Bala *et al.*[12] in which 100 mg of the plant extract was mixed with 1 g of zinc acetate which was dissolved in 50 mL of distilled water and heated at 80°C for 20 min. Few drops of ammonia solution were added to the reaction mixture up to white colored precipitate was formed. The reaction mixture was left for 30 min for complete reduction to zinc oxide nanoparticles. Then the precipitate was centrifuged at 12000 rpm and washed two times by distilled water followed by ethanol to get off white powder by freeze drying[12].

Characterization of metal nanoparticles

UV-visible spectral analysis: Synthesis of silver and zinc oxide nanoparticles was monitored using a UV spectrophotometer model Shimadzu UV-1601 (Shimadzu Corporation, Japan). The UV-Vis spectra were recorded between 200-500 nm.

FTIR analysis: FTIR 6100 spectrometer (Jasco, Japan) was used for determination the functional groups of the synthesized nanometals and the eggplant extract in the range of 400-4000 cm^{-1}.

Transmission Electron Microscopy (TEM): The morphology and size of the reductive silver and zinc oxide nanoparticles was investigated by TEM (JEOL-JEM-1011, Japan). The samples were prepared by placing drops of the nanoparticles suspension on carbon coated copper grid, followed by allowing the solvent to evaporate slowly before recording the TEM image.

***In vitro* antimicrobial activity**

Microorganisms: *In vitro* antimicrobial activity was examined for aq. methanol extract of eggplant calyx. The bacteria used in this study were Gram-positive bacterial strains (*Bacillus cereus* ATCC-6629 and *Staphylococcus aureus* ATCC-29213), Gram-negative bacterial strains (*Escherichia coli* ATCC-51659, *Klebsiella pneumonia* ATCC-10031 and *Pseudomonas aeurinosa* ATCC-27853), fungi (*Aspergillus niger* NRRL-599) and yeast (*Candida albicans* ATCC-10231). The source of these microorganisms is the American Type Culture Collection (ATCC, Rockville MD, USA) and Northern Utilization Research and Development Division, United State Department of Agriculture, Peoria, Illinois, USA (NRRL). The bacterial strains were revived for bioassay by sub-culturing in fresh nutrient broth medium for 24 h before the test. While fungi were cultured on Potato Dextrose Agar (PDA) for 7 days at 28°C before the current experiment was carried out.

Culture media and inoculum preparation

Sabouraud dextrose agar composition (g L^{-1}): Dextrose×20.0, Peptone: 10.0, Agar and final pH (at 25°C) is 5.6±0.2.

Mueller hinton agar composition (g L^{-1}): Beef extract powder×2.0, Acid digest of casein×17.5, Starch×1.5, Agar×17.0 and final pH (at 25°C) is 7.3±0.1.

Inoculum preparation: Stock cultures were k at 4°C on slopes of nutrient agar and potato dextrose agar. Active cultures for experiments were prepared by transferring a lapful of cells from the stock cultures to test tubes of Mueller-Hinton Broth (MHB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungi that were incubated without agitation for 24 h at 37 and 25°C, respectively. To 5 mL of MHB and SDB, 0.2 mL of culture was inoculated and incubated (or diluted) till the turbidity becomes equal to that of the standard 0.5 McFarland solution at 625 nm (A = 0.08-0.1) which is equivalent to 1.5×10^8 CFU mL^{-1}.

Susceptibility testing requires the use of standardized inocula. The 0.5 McFarland standard is recommended for use in the preparation of inocula for performing the antimicrobial disk diffusion susceptibility test.

Principles of the procedure: The 0.5 McFarland turbidity standard is prepared by adding barium chloride to sulfuric acid. The mixture of the two chemical forms a precipitate when in suspension is equivalent to approximately 1.5×10^8 CFU mL^{-1}.

Antibacterial activity: Determination of antibacterial activities of plant extracts. Agar well diffusion technique as described[13] was used to determine the *in vitro* antibacterial activity of the eggplant aq. methanol extract, silver and zinc oxide nanoparticles. A 0.1 mL aliquot of 18 h broth culture of the bacteria that had been adjusted to the turbidity equivalent of 0.5 McFarland standards[14,15] was dispensed into sterile Petri dishes previously labeled with the test bacteria. Molten sterile Muller-Hinton was aseptically poured into the plates and gently rotated for the bacteria to be homogeneously distributed in the medium. The agar plates were left to be solid, after which a sterile cork borer of 9 mm in diameter was used to cut uniform wells in the agar plates. The wells were later filled with 0.1 mL of the each extract which prepared in one concentration 100 mg mL^{-1} for each of three samples which in turn dissolved or suspended in dimethyl sulfoxide (DMSO). DMSO was used as a negative control while rifampicin (15 µg mL^{-1}) used as a positive control for antibacterial activity and cycloheximide (20 µg mL^{-1}) as a positive antifungal agent.

The experiment was conducted in triplicates. All plates were incubated at 37°C for 24 h for bacterial strains and at 28-30°C for 48 h for fungal strain. Clearance zones around the wells were noted and measured in millimeters. Microorganisms with zone size ≥28 mm were classified as strongly sensitive, with a zone diameter of <28-16 mm as moderately sensitive, with azone diameter of <16-12 mm as weakly sensitive and isolates with zone diameter of <12 mm as resistant[16].

RESULTS AND DISCUSSION

Total phenol: Total phenol was expressed as chlorogenic acid equivalent as eggplant is rich in chlorogenic acid[7] and it showed high phenolic content estimated to be 47.3 mg g^{-1} of the extract which facilitate reduction of metal salts to their metal nanoform[6,11] .

UV spectroscopy: Synthesis of the eggplant Ag and ZnONPs in aqueous solution was monitored by UV spectroscopy at a wavelength range of 200-500 nm (Fig. 1Sa-b). It was observed the changing of silver nitrate color to dark brown after addition of eggplant aq. methanolic extract due to formation of silver nanoparticles and it showed characteristic UV absorption peak at 393 nm due surface plasmon resonance phenomena[6] while Zinc oxide nanoparticles mediated by Eggplant extract showed UV absorption peak at 280 and 365 nm[17-19].

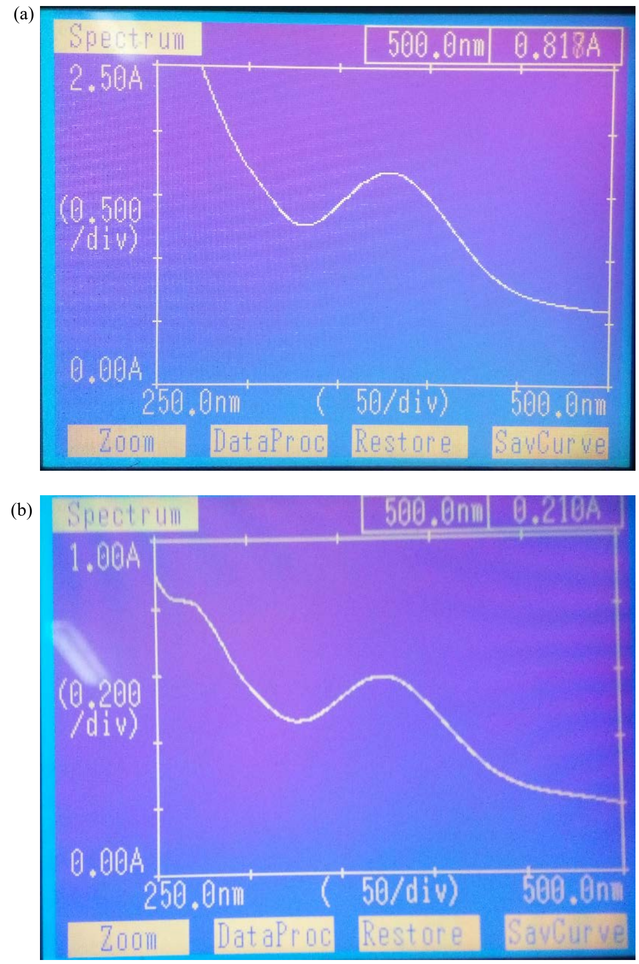

Fig. 1S(a-b): UV spectrum of (a) AgNPs and (b) ZnONPs

Infrared spectroscopy: Fourier transform infrared spectroscopy spectrum (Fig. 1) of the pressed powder eggplant extract, ZnO and AgNPs in the spectral width range from 400-4000 cm^{-1}. It showed peaks at 3421 cm^{-1} attributed to the O-H stretching mode of the total extract with high intensity. There was an observed decrease the intensity of this peak in case of ZnONPs and AgNPs which interpreted the role of phenolic hydroxyl moieties in the reduction of both silver nitrate and zinc acetate to their metallic nanoparticles[6,11,20]. The characteristic bands corresponding to the ZnO nanoparticles stretching mode[18,21] are observed at 445.5 and 878.4 cm^{-1}.

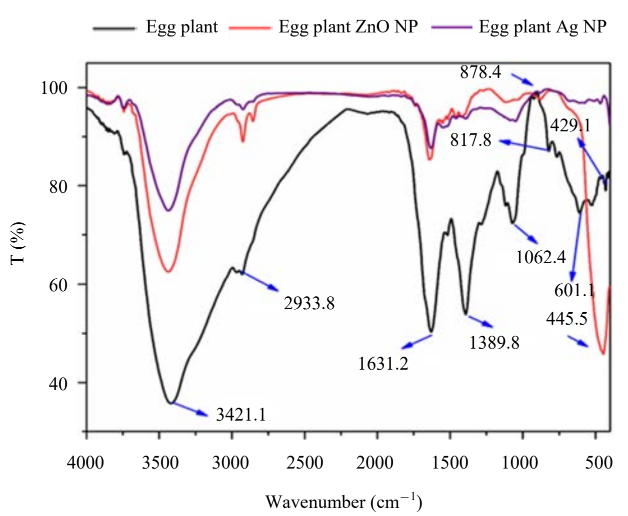

Fig. 1: IR spectrum of eggplant extract mediated zinc oxide and silver nanoparticles

Transmission electron microscopy: It was reported that the antimicrobial activity of both zinc oxide and silver nanoparticles increased as their particle size decreased[22,23].

High Resolution Transmission Electron Microscopy (HRTEM) image of ZnONPs (Fig. 2a-b) mediated by the Eggplant calyx showed relatively small hexagonal nanoscaled particles with dimensions ranging from 8-12 nm diameter. Moreover, the Selected Area Electron Diffraction (SAED) image of zinc oxide nanoparticles indicates the highly crystallized structure of the prepared ZnONPs (Fig. 3).

On the other hand, HRTEM of AgNPs mediated by the eggplant calyx also showed relatively small spherical shaped nanoparticles with dimensions ranging from 8-20 nm diameter (Fig. 4a-c). Furthermore, the Selected Area Electron Diffraction (SAED) image of eggplant mediated AgNPs indicates the highly crystallized structure of the AgNPs (Fig. 5).

Antimicrobial activity: Interactions of bacteria with AgNPs and ZnONPs were reported to depend on their size, shape and dose[4,22,23].

Antimicrobial activity of aqueous methanolic extract of eggplant calyx, eggplant mediated ZnONPs and AgNPs were determined and compared with each other and with Rifampicin as a standard antibacterial against certain pathogenic strains of gram-positive and Gram-negative bacteria. Also, their antifungal activity against *Aspergillus niger* and *Candida albicans* was determined and compared with Cycloheximide as a standard antifungal, using Agar well diffusion technique.

From the obtained results, it is clear that both Ag and ZnO nanoparticles positively affect the examined bacteria and fungi more than eggplant calyx aqueous methanolic extract with the highest activity of AgNPs.

Silver nanoparticles (AgNPs) showed strong antibacterial activity against multi-resistant Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli* and *Klebsiella pneumonia*, while yeast (*Candida albicans*), fungi (*Aspergillus niger*), Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Bacillus cereus*) bacteria showed moderate sensitivity with eggplant AgNPs.

Following eggplant AgNPs, the selected strains of Gram-negative, Gram-positive bacteria and fungi exhibited moderate sensitivity to eggplant ZnONPs, while their sensitivity to eggplant calyx aqueous methanolic extract range from moderate to weak with nill activity against *Aspergillus niger* (Table 1).

Unfortunately, ZnONPs negatively affected *Staphylococcus aureus* ATCC-29213. This finding was not aligned with the previously published data[24] which showed reactivity of ZnONPs against some *S. aureus* strains, in turn this difference in activity may be interpreted due to the difference in nanoparticle size, shape and the method of preparation[24]. On the other hand, antimicrobial activity of AgNPs matched with the reported data[25]. So, the eggplant AgNPs has the highest antibacterial and antifungal activity against the selected strains of bacteria and fungi.

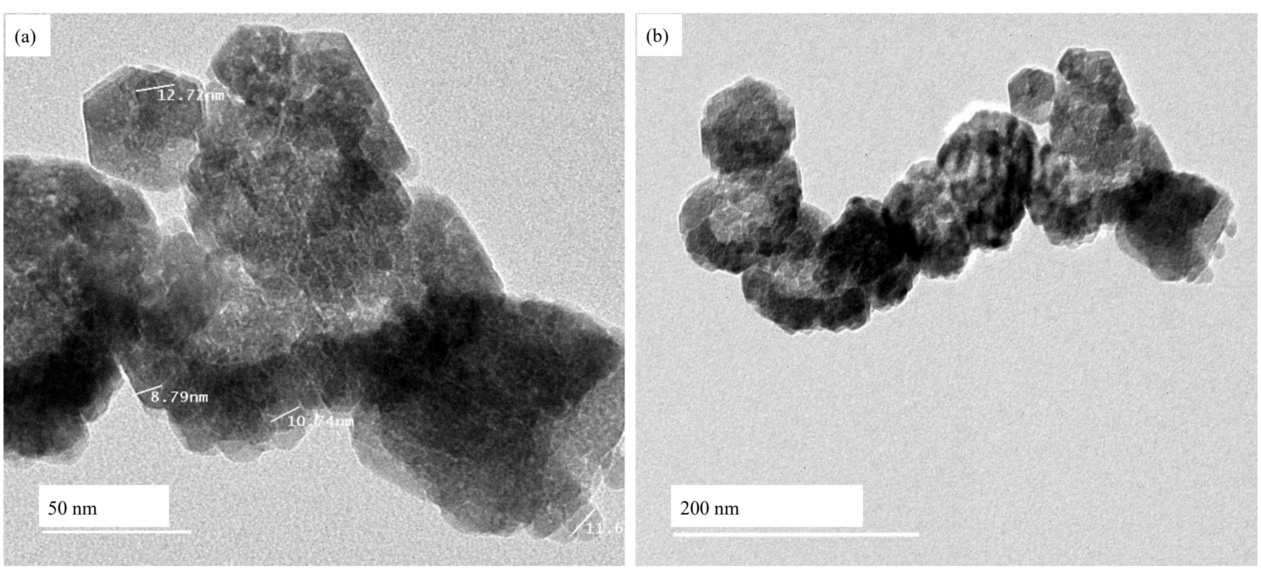

Fig. 2(a-b): TEM spectrum of eggplant mediated ZnONPs at (a) 50 nm and (b) 200 nm

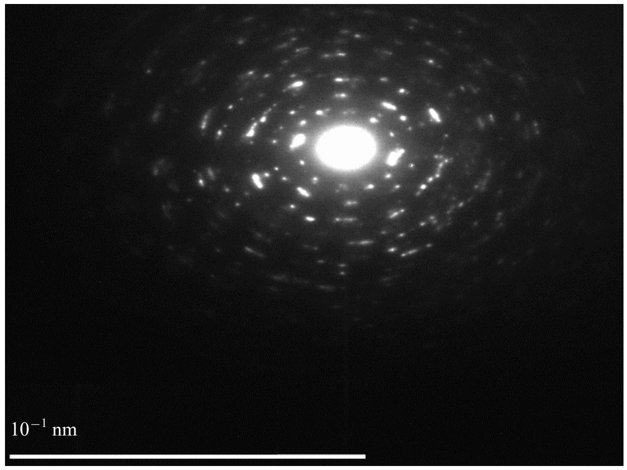

Fig. 3: SAED spectra of eggplant calyx zinc oxide nanoparticles

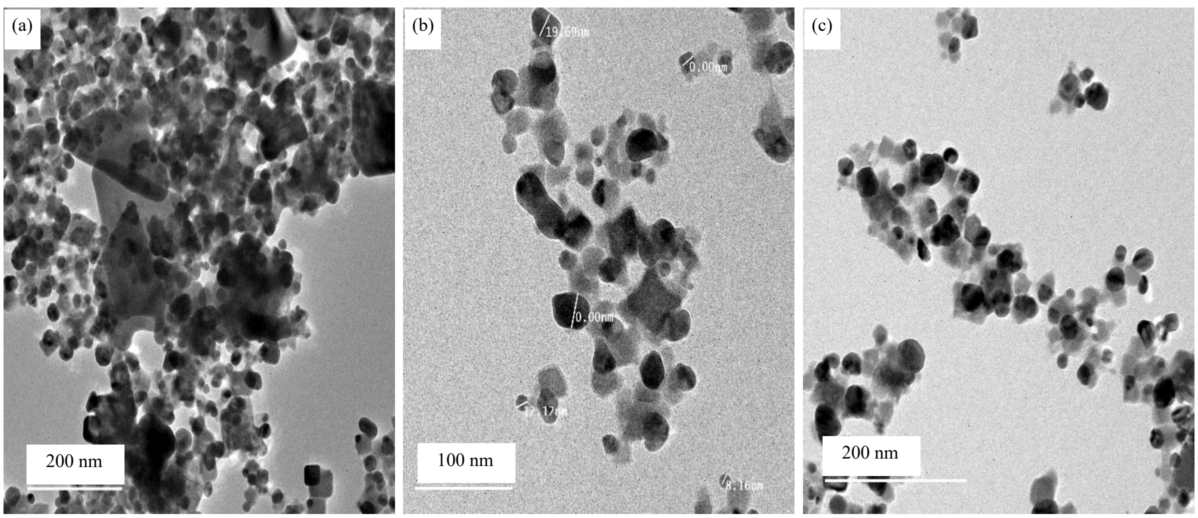

Fig. 4(a-c): TEM spectrum of eggplant calyx mediated AgNPs at nanoscale, (a) 200 nm, (b) 100 nm and (c) 200 nm (aerial view)

Table 1: Inhibition zones of eggplant extract, ZnONPs and AgNPs

	Microorganisms						
	Gram-positive bacteria		Gram-negative bacteria			Yeast	Fungi
Samples codes	*Bacillus cereus* ATCC-6629	*Staphylococcus aureus* ATCC-29213	*Pseudomonas aeruginosa* ATCC-27853	*Escherichia coli* ATCC-25922	*Klebsiella pneumonia* ATCC-10031	*Candida albicans* ATCC-10231	*Aspergillus niger* NRRL-599
	Inhibition zones diameter (mm)						
	Rifampicin (15 μg mL^{-1})					Cycloheximide (20 μg mL^{-1})	
Standards	29	36	21	32	35	32	35
Samples (20 mg mL^{-1})							
Eggplant calyx extract	12	16	14	14	17	11	NIL
Eggplant ZnONP	16	2.1	18	21	22	19	16
Eggplant AgNP	24	32	26	29	31	27	24

*$\leq$28 mm Strong sensitive, *<28 to 16 mm moderately sensitive, *<16-12 mm weakly sensitive

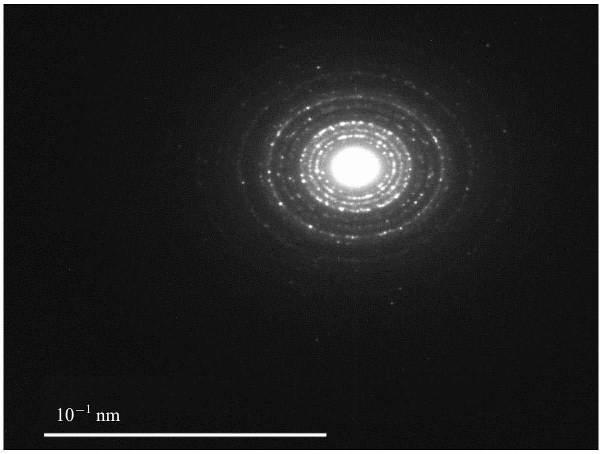

Fig. 5: Selected Area Electron Diffraction (SAED) spectra of eggplant mediated AgNPs

CONCLUSION

Eggplant green calyx which is a food waste and reported for its high concentration of phenolic constituents is capable of synthesizing ZnO and Ag nanoparticles. Synthesized Ag and ZnONPs were characterized by different spectroscopic UV, FTIR and TEM techniques, with a nano-size range 8-12 nm for ZnONPs and 8-20 nm for AgNPs, that refer to the increased surface area and hence the biological activity, which showed higher antimicrobial activity of AgNPs than ZnONPs of eggplant green calyx against the pathogenic microbes of gram-positive, gram-negative and fungi under examination.

SIGNIFICANCE STATEMENT

This study discovers new antimicrobial agents from green synthesis of silver and zinc oxide nanoparticles using eggplant calyx for the first time that can be beneficial for overcoming microbial resistance. This study will help the researcher to manufacture new safe, economic and environment friendly creams for treating burn infections and bed sores, antimicrobial clothing for medical staff and antimicrobial food additives that many researchers were not able to explore. Thus, a new industrial and pharmaceutical products may be arrived at.

ACKNOWLEDGMENT

The authors thank the Deanship of Scientific Research, Najran University, for supporting this work and providing the fund to perform this research (NU/MID/16/38).

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