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Antimicrobial Activity of Zinc Oxide-Based *Calotropis Procera* (Aiton) W.T.Aiton Leaf Extract Nanocomposite in Wastewater Remediation

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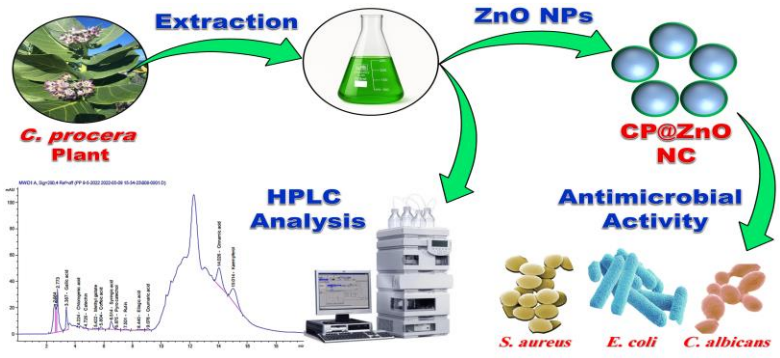
ABSTRACT

In recent years, wastewater treatment technologies have garnered more attention as the world pursues fulfilling the Sustainable Development Goals (SDGs). This study has pointed to the role of wastewater control that plays toward achieving one of the SDGs: clean water and sanitation. In this work, the phytochemical screening of *Calotropis procera* leaf extract detected the appearance of various classes of secondary metabolites involving saponins, phenols, flavonoids, anthocyanins, terpenoids, and tannins. Moreover, HPLC examination revealed that the investigated extract comprises phenolic acids and flavonoids. The average size of semi-spherical zinc oxide nanoparticles is 30.27nm; they were fabricated and conjugated with *C. procera* leaf extract to improve the antimicrobial activity. The results in this study recorded significant antimicrobial performance of ZnO NPs and *C. procera* leaf extract; however, the synthesized CP@ZnO nanocomposite showed maximum antimicrobial activities against the most strains found in wastewater in a relatively low concentration (MIC= 50, 75, and 35µg/ l for *S. aureus*, *E. coli*, and *C. albicans*, respectively). The novel CP@ZnO nanocomposite is considered to be an efficient and safe antimicrobial agent and effective for wastewater treatment in local communities to provide unconventional water sources from water reuse.

Graphical Abstract



INTRODUCTION

Water is the most vital factor in life, and Earth cannot survive without it. Only 3% of about 1351 million km^3 of water is available as freshwater resources that is appropriate for drinking and irrigation **(EC, 2018; Winpenny *et al.*, 2020)**. Unpreserved water sources can be polluted with pathogens through many factors. However, effluents of sewage are the major cause of the pollution of rivers, dams, and wells. Therefore, it became unsuitable for people's consumption because of the existence of pathogenic microorganisms **(Wear *et al.*, 2021)**. Generally, the different sources of sewage effluents, including industries, households, agriculture, and hospitals, contaminate the surface water with poisonous chemicals and microbes **(Phung *et al.*, 2015)**. As a result, there is an increasing demand to study the lack of water resources and the impacts of contaminated water on human health. The wastewater remediation methods have gained great attention, especially in developing countries, where researchers look for effective and safe techniques to combat poor water quality and quantity **(Hanjra *et al.*, 2012; Utzinger *et al.*, 2015; Azzam *et al.*, 2022)**. The conventional wastewater treatment methods like UV, ozonation, and chlorination have many disadvantages, like toxic byproducts and high cost **(Li *et al.*, 2008)**, due to increasing the pathogenic tolerance **(Jin & He, 2011)**. Therefore, new techniques must be improved for this challenge.

Nano-inorganic particles have displayed wonderful antipathogenic efficacy at extremely low levels due to their large surface area and unique chemical and physical characteristics **(Rai *et al.*, 2009)**. Additionally, these nanoparticles exhibit remarkable stability at elevated temperatures and pressures **(Sawai, 2003)**. Metallic and metal oxide nanoparticles, including those of gold, silver, copper, zinc oxide, and titanium dioxide, are the most antibacterial inorganic materials **(Chaudhry *et al.*, 2008; Husen, 2017; Pachaiappan *et al.*, 2020; Padilla-Cruz *et al.*, 2021)**. Zinc oxide, MgO, and TiO_2 nanoparticles are not only considered nontoxic but also include essential minerals for the human body **(FDA, 2011)**. They are considered safe when utilized as medicine deliverers or food additives **(FDA, 2011)**. Zinc oxide nanoparticles are the most promising inorganic particles that have an antimicrobial effect. Furthermore, they can be found in the combination of food packaging, sanitizer, cosmetics, and pharmaceutical drugs procedures **(Kołodziejczak-Radzimska & Jesionowski, 2014)**. Zinc oxide NPs are transitory semiconductor metal oxides that have excellent binding energy, which gives high oxidative features **(Agarwal *et al.*, 2018)**. In this action, the reactive oxygen species appeared in the bactericidal process. Furthermore, the bactericidal reaction can take place by releasing Zn^{2+} ions, which damage the cell membrane as well as the metabolic path **(Burman *et al.*, 2013)**.

Arka is the common name for *Calotropis procera* (Aiton) W.T. Aiton (Asclepiadaceae). Many arid and semi-arid regions are habitat to this xerophytic

perennial shrub or shallow tree. It is native to tropical and subtropical Africa and Asia and is widely distributed in the Middle East **(Parsons & Cuthbertson, 2001; Lottermoser, 2011)**. It is extensively utilized in traditional medicine for the management of various ailments and health conditions **(Rajesh *et al.*, 2014)**. Various plant parts demonstrated a variety of pharmacological characteristics, including antimicrobial **(Sehgal *et al.*, 2005)**, antifungal **(Hassan *et al.*, 2006)**, and hepatoprotective **(Setty *et al.*, 2007)**. Multiple categories of chemical ingredients have been characterized phytochemically in every section of the plant, *viz*., alkaloids **(Israili *et al.*, 1979)**, triterpenes **(Gupta *et al.*, 2000; Gupta *et al.*, 2003)**, cardenolides **(Oluwaniyi & Ibiyemi, 2000)**, flavonoids **(Nenaah, 2013)**, and sterols **(Chundattu *et al.*, 2016)**.

In this study, the most prevalent bacterial strains found in aquatic environments, Gram-positive (*Staphylococcus aureus*), Gram-negative (*Escherichia coli*), and fungi (*Candida albicans*), were investigated for antimicrobial activity using the *C. procera* extract and ZnO NPs, and CP@ZnO nanocomposite. Hence, these types of microbes represent suitable examples of various types of microbial contamination in water. The success of this compound will also be suitable for local application in treating wastewater from local communities, enabling the reuse of water for irrigation and agriculture, thus contributing to a partial solution to Egypt's water problem.

MATERIALS AND METHODS

Plant material

The fresh *Calotropis procera* (Aiton) W.T.Aiton leaves were gathered from Cairo Governorate, Egypt. The specimen was graciously recognized and verified in El-Orman Botanical Garden, Giza, Egypt. A specimen receipt was submitted to the Medicinal Chemistry Department at TBRI and assigned accession number (C.P.l.2021).

Extraction and fractionation

Dry powdered leaves (500 g) were extracted with methanol (99.9%) (3 × 1 L) at room temperature. The collected extracts were filtered and subsequently vaporized under vacuum at 40°C utilizing an evaporator. The raw extract (63g) was drained of fat using petroleum ether (60- 80°C), after which the defatted methanolic extract (55g) was preserved for subsequent investigations **(Mohammed *et al.*, 2019)**.

Primary phytochemical screening

Conventional standard protocols **(Ghareeb *et al.*, 2014)** were employed to identify several chemical classes, such as flavonoids, tannins, phenols, terpenoids, saponins, and anthocyanins in the examined plant extract **(Trease & Evans, 1983; Trease & Evans, 1989; Harborne, 1993; Sofowora, 1993; Edeoga *et al.*, 2005)**.

High-performance liquid chromatography-fingerprint analysis

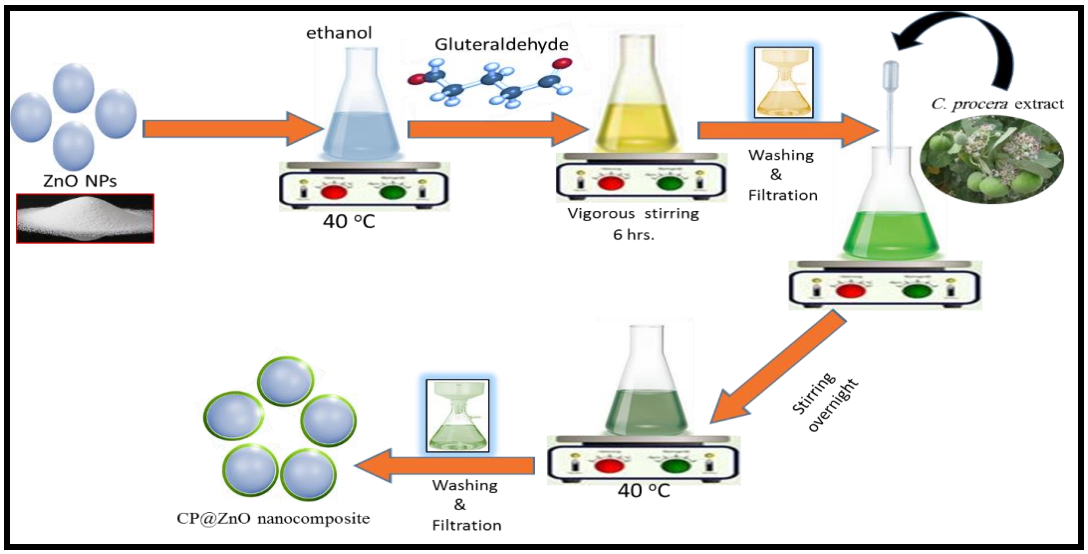
The HPLC-fingerprint technique was utilized for the quantification of phenolic ingredients in the tested extract according to the reported procedures **(Abdel-Wareth & Ghareeb, 2018; Nasr *et al.*, 2018)**.

ZnO nanoparticles synthesis

Zinc oxide (ZnO) nanoparticles were synthesized according to **Sharma and Ghose (2015)** by a simple homogenous precipitation method as follows: Zinc acetate (7.1 g) was dissolved in 360ml of deionized water. Then, 40 ml of sodium hydroxide 25% was added dropwise. After that, the content was heated at 70- 80°C under constant stirring for 2 hours. A milky white precipitate was formed. The content was filtered and repeatedly cleaned with water to get rid of any contaminants, and then dried in an oven set at 50ºC overnight. For two hours, the produced powder was calcinated in air at 400°C at a heating rate of 2ºC /min in a muffle furnace.

Fabrication of CP@ZnO nanocomposite

Following the technique of **Saad *et al.* (2019)**, ZnO nanoparticles (0.2g) and ethanol (35ml) were mixed under heating at 40°C ± 1 while being stirred for 15 minutes. Then, 25ml of glutaraldehyde solution was added as a cross-linking agent and stirred vigorously for 6 hours at room temperature (Scheme 1). The resulting precipitate was washed three times with ethanol. In a flask, 100ml of ethanol was poured over the formed precipitate, then the *C. procera* extract was added dropwise, while stirring overnight at 40°C. Ethanol was used three times to filter and eliminate any impurities. The mixture was dried in an oven at 70°C for 6 hours.



Scheme 1. *Calotropis procera*@ZnO nanocomposite conjugation

Characterization of ZnO NPs, CP@ZnO nanocomposite and *C. procera* extract

Field emission scanning electron microscopy was used to identify the surface characteristics of ZnO nanoparticles (FESEM, JEOL Model 6500) at 20 kV. SEM measurement was prepared by adding a small amount of nanoparticles sample on a stub using a sticky carbon disc, then the sample was allowed to dry before being measured. TEM has been utilized to detect the shape, size distribution, and scale of nanoparticles. TEM measurement was prepared by adding a drop of nanoparticle solution to a copper grid coated with amorphous carbon. Before measuring, the film was dried for two minutes, and then a filter paper was used to eliminate any excess solution. The size and morphology of the fabricated nanoparticles were assessed by utilizing a transmission electron microscope (Model JEOL Ltd, USA) connected to a high-resolution imaging system. FTIR assessments for ZnO NPs, CP@ZnO nanocomposite, and *C. procera* extract were used to give data about the chemical compositions to understand the transformation of the functional group due to conjugation with antibiotics. In JASCO FT-IR-3600 (Bruker, Germany), nanoparticles and nanoconjugates were dried at 60°C and then analyzed by FTIR spectra (4000-400 cm^{-1}) using the KBr pellet technique.

Antimicrobial activity

By using the standard agar well diffusion procedure, the microbial-resistance performance of *C. procera* extract, ZnO NPs, and CP@ZnO nanocomposite was examined against the most prevalent bacterial strains found in the water environment, such as *Candida albicans* (fungi), *Escherichia coli* (Gram-negative) bacterial organisms, and *Staphylococcus aureus* (Gram-positive) bacterial organisms. Müller-Hinton and Sabouraud Dextrose broth were used for growing fresh and pure cultures of these microorganisms. After pouring, Sabouraud Dextrose Agar (SDA) and bacterial nutritional agar plates were left to stand for 15min. Plates with 6.0mm holes were then used to investigate the antibacterial properties of the carbonaceous materials. Using a micropipette, 100µL of water suspension (10.0mg/ mL) of the tested compounds was added to each plate well. For two days, bacterial organisms were incubated overnight at 37°C to assess the different levels of inhibition zone; for fungus, this was carried out at 28°C. Duplicate plates were used for each organism and concentration **(Hanjra *et al.*, 2012)**.

The lowest concentration of antimicrobial agent that can inhibit visible bacterial growth on agar plates is called the minimum inhibitory concentration (MIC). MIC is essential in diagnostic laboratories to monitor the activity of new antimicrobial agents. ZnO@CP nanocomposite was tested *in vitro* by the broth dilution method to determine its antibacterial activity. A stock of 1000µg/ ml has been set. A serial dilution was prepared for screening. Nutrient broth was used as a nutrient medium to grow and dilute the drug suspension for the tested bacteria **(Gurunathan *et al.*, 2014)**.

RESULTS AND DISCUSSION

Phytochemical screening of *Calotropis procera* leaf extract

Various categories of chemical ingredients, including flavonoids, saponins, phenols, anthocyanins, tannins, and terpenoids, were identified by preliminary phytochemical screening (Table 1). Accordingly, the findings verify the application of *Calotropis procera* leaf extract nanocomposite in water pollution remediation. **Bashyal *et al.* (2017)** stated that the methanolic extract of *C. procera* leaf contains phytoconstituents, *viz*., alkaloids, carbohydrates, saponins, flavonoids, tannins, and proteins. Moreover, it was reported that different solvent extracts of *C. procera* contain phytochemicals like flavonoids, tannins, and saponins **(Kazeem *et al.*, 2016)**.

Table 1. Preliminary phytochemical screening of *Calotropis procera* leaf extract

Chemical classes	*C. procera* leaf extract Presence/ Absence of phytoconstituents
Flavonoids	+++
Tannins	++
Terpenoids	++
Saponins	++
Anthocyanins	+
Phenols	+++

+: Low; ++: Moderate; +++: High

On the other side, a proper HPLC-fingerprint approach has been established to determine the chemical components in the *Calotropis procera* leaf extract. The obtained HPLC chromatogram of the investigated extract was contrasted to nineteen standard phenolic compounds (Table 2 & Figs. 1, 2). HPLC examination revealed that the investigated extract comprises seven phenolic acids, viz. gallic acid (Conc.: 522.09 µg/g), chlorogenic acid (Conc.: 27.82µg/ g), caffeic acid (Conc.: 73.98µg/ g), coumaric acid (Conc.: 19.43µg/ g), syringic acid (Conc.: 303.93µg/ g), ellagic acid (Conc.: 146.51 µg/g), and cinnamic acid (Conc.: 323.72µg/g). While flavonoidal compounds were determined as rutin (Conc.: 84.40µg/ g), kaempferol (Conc.: 1585.33µg/ g), and catechin (Conc.: 87.27µg/ g). Moreover, phenolic acid derivatives and others were determined as methyl gallate (Conc.: 31.00µg/ g), and pyrocatechol (Conc.: 57.69µg/ g). Previous phytochemical studies revealed that the aqueous extract of the whole *C. procera* plant contains quercetin, *p*-coumaric acid, gallic acid, sinapic acid, and chlorogenic acid **(Raza *et al.*, 2019)**. The aqueous leaf extract of *C. procera* contains polyphenolic ingredients like caffeic acid, gentisic acid, catechol, syringic acid, ellagic acid, resorcinol, *p*-hydroxybenzoic acid, gallic acid, and *p*-coumaric acid **(Gulzar *et al.*, 2016)**. UHPLC-QTOF-MS/MS profiling of aqueous ethanol leaf extract of *C. procera* led to the identification of hydroxybenzoic acid, quinic acid, myrciacitrin, quinic acid derivative, and Kaempferol glucoside derivative **(Nadeem *et al.*, 2019)**.

Table 2. Areas under peaks and concentrations of the identified phenolic compounds in the *C. procera* leaf extract compared to nineteen standard phenolic compounds

				Sample		
Standards	**Conc. µg/ml**	**Area %**	**R_t (min)**	**Area %**	**Conc. µg/ml**	**Conc. µg/g**
Gallic acid	15	2.28	3.38	7.83	9.87	522.09
Chlorogenic acid	50	4.81	4.22	0.26	0.53	27.82
Catechin	75	3.88	4.72	0.44	1.65	87.27
Methyl gallate	15	3.46	5.42	0.70	0.59	31.00
Caffeic acid	18	3.43	5.80	1.38	1.40	73.98
Syringic acid	17.2	2.93	6.51	5.09	5.74	303.93
Pyrocatechol	40	3.80	6.87	0.54	1.09	57.69
Rutin	26	2.45	7.50	0.78	1.60	84.40
Ellagic acid	120	8.29	8.44	0.99	2.77	146.51
Coumaric acid	20	9.09	9.07	0.86	0.37	19.43
Vanillin	12.9	4.62	9.62	ND	0.00	0.00
Ferulic acid	20	4.64	10.12	ND	0.00	0.00
Naringenin	30	4.66	10.41	ND	0.00	0.00
Daidzein	35	7.50	12.18	ND	0.00	0.00
Quercetin	40	4.59	12.65	ND	0.00	0.00
Cinnamic acid	10	6.70	14.02	21.35	6.12	323.72
Apigenin	50	9.56	14.38	ND	0.00	0.00
Kaempferol	60	7.87	15.01	20.47	29.96	1585.33
Hesperetin	20	5.34	15.42	ND	0.00	0.00

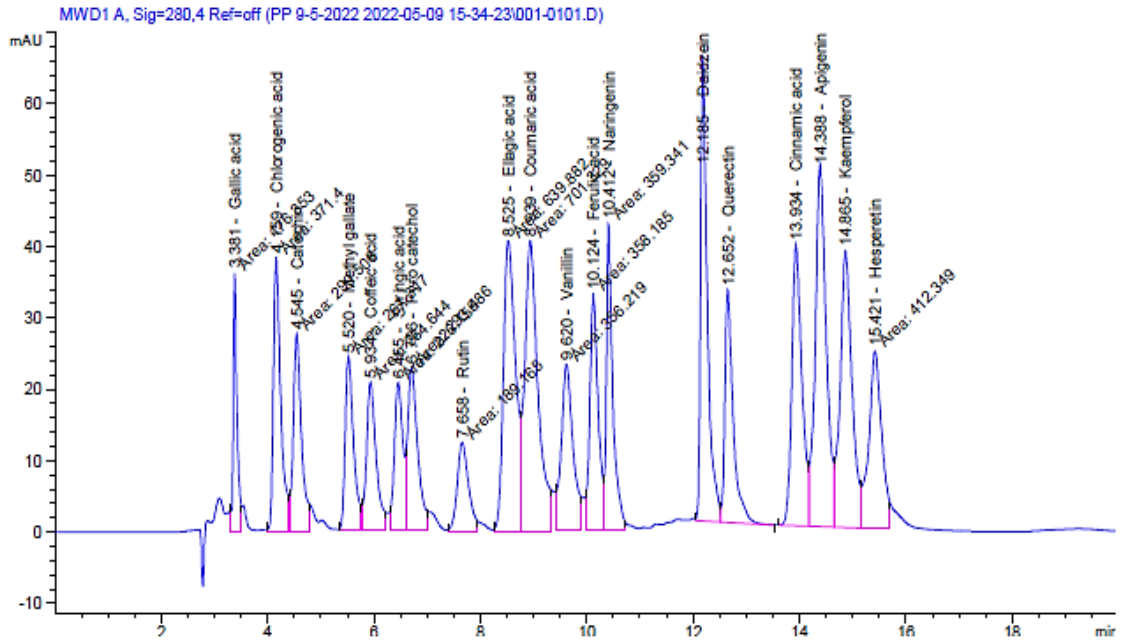


Fig. 1. HPLC chromatogram of nineteen standard phenolic compounds (Gallic acid, Chlorogenic acid, Catechin, Methyl gallate, Caffeic acid, Syringic acid, Pyrocatechol, Rutin, Ellagic acid, Coumaric acid, Vanillin, Ferulic acid, Naringenin, Daidzein, Quercetin, Cinnamic acid, Apigenin, Kaempferol, and Hesperetin)

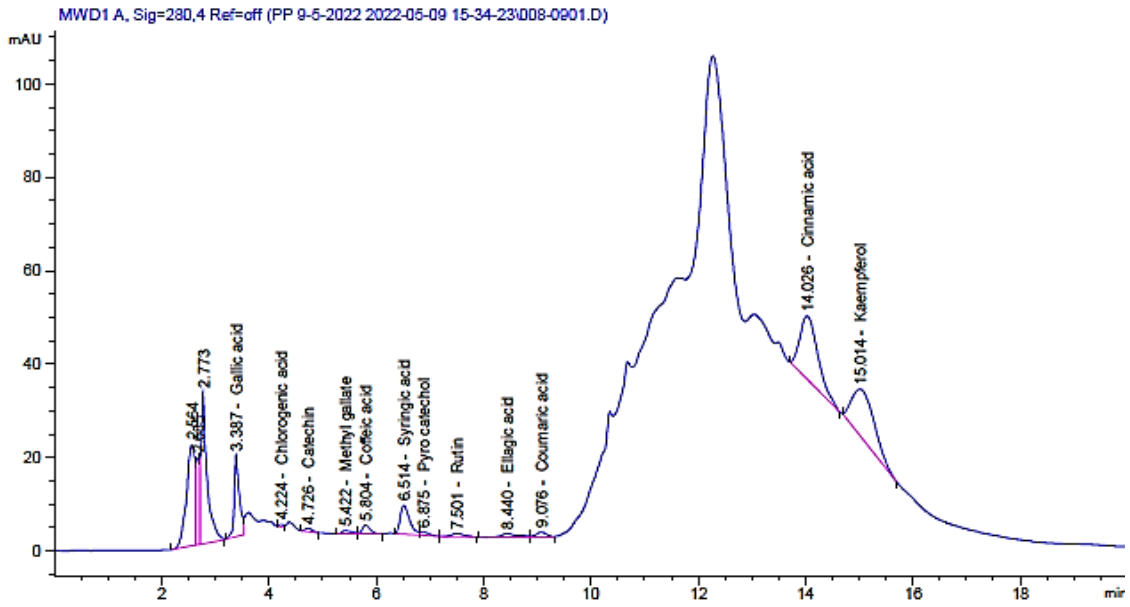


Fig. 2. HPLC chromatogram of *C. procera* leaf extract

Characterization of the synthesized nanomaterials

In the present study, zinc oxide nanoparticles were synthesized and characterized by SEM, TEM, and FTIR spectroscopy. The surface morphology of fabricated ZnO NPs was observed by SEM micrograph in Fig. (3A); accordingly, the ZnO nanoparticles showed semi-globular aggregation with nanocrystallite appearance. Furthermore, the morphology is confirmed by a TEM micrograph in Fig. (3B), in which the synthesized zinc oxide nanoparticles are indicated as approximately spherical particles with an average size of 28.2nm. This finding is in good agreement with **Hedayati (2015)** and **Mendes *et al.* (2021)**. Other findings revealed ZnO NPs in other shapes and sizes **(Mahamuni *et al.*, 2019; De Souza *et al.*, 2023)**.

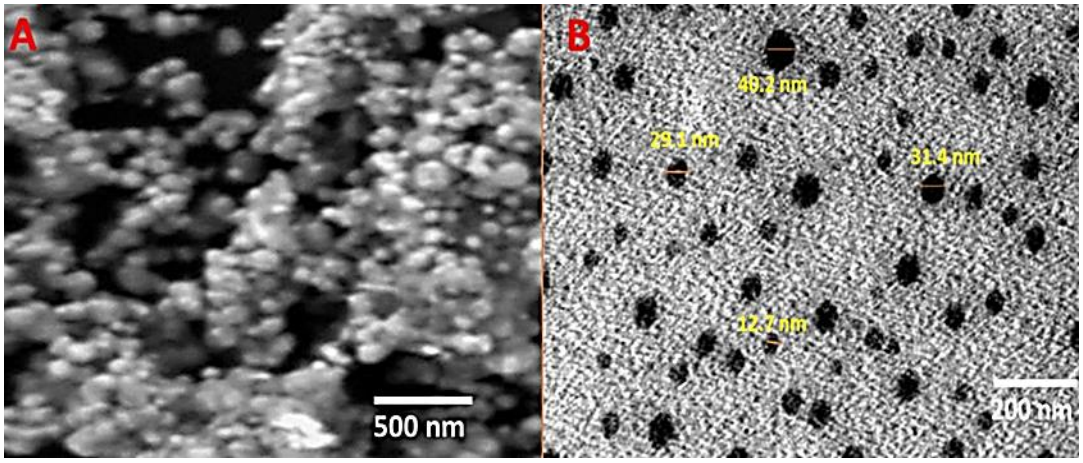


Fig. 3. SEM **(A)** and TEM **(B)** images of ZnO nanoparticles

FTIR spectrum is a fingerprint for molecules that provides precious knowledge about chemical composition and their modifications made by interactions with other materials **(Mendes *et al.*, 2021)**. Fig. (4A) shows the FTIR of *C. procera* extract; the bands observed at 544 and 1047cm^{-1} refer to the N–H and C–N stretching vibrations of amines, respectively **(Elumalai &Velmurugan, 2015)**. The absorption bands in the location 1603cm^{-1} always refer to an aromatic rings **(Sathayavathi *et al.*, 2010)**. On the other hand, the absorption band at 2933 is owing to C≡C stretching vibration. The intense and broad band at 3341cm^{-1} is due to O–H stretching **(Dinesh *et al.*, 2014)**. Broad FTIR bands at 3341, 1603, and 2933cm^{-1} referred to the existence of hydroxyl groups, aldehydes, amines, and aromatic ring and revealed that the plant extract could increase the stabilization of ZnO NPs. Moreover, the hydroxyl group (-OH) affects photocatalytic reactions in zinc oxide by producing superoxide radicals, which act as an antimicrobial **(Sirelkhatim *et al.*, 2015)**.

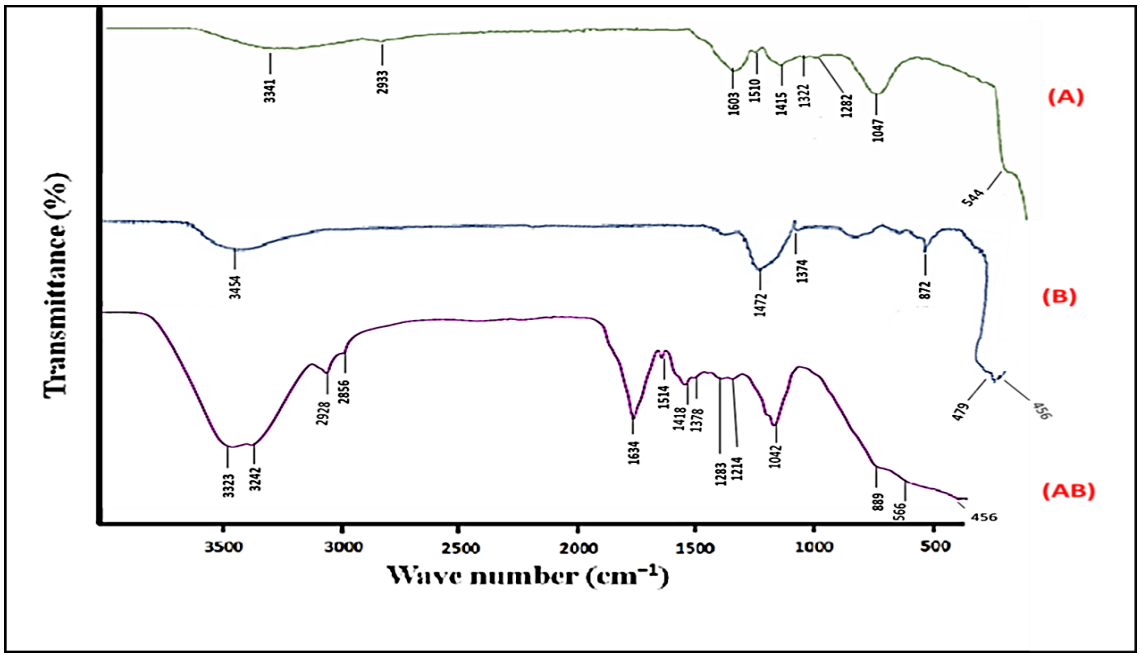


Fig. 4. FTIR spectrum of **A)** *C. procera* extract, **B)** ZnO NPs and **AB)** CP@ZnO NC

The FTIR image for ZnO NPs is shown in Fig. (4B), in which the spectrum showed a characteristic peak at 3454cm^{-1}, which was ascribed to stretching vibrations of the hydroxyl group (O-H) **(Gavade *et al.*, 2016; Meshram *et al.*, 2017)**. Also, the two peaks at 1374 and 1472cm^{-1} align with COO– (carboxylate group) and C=C bond, respectively **(Chithra *et al.*, 2015)**. As a result of interatomic vibrations, metal oxides indicate absorption bands in the fingerprint area below 1000cm^{-1} **(Janaki *et al.*, 2015)**. In the synthesized nanoparticles spectrum, the peaks at 453 and 479cm^{-1} correspond to ZnO, which showed the stretching vibration of Zn-O **(Khana *et al.*, 2015)**. Additionally, the

peak at 873cm^{-1} is also due to Zn-O bonds. Fig. (4AB) shows the FTIR spectrum of CP@ZnO nanocomposite, in which the peaks appeared at 889, 566, and 456cm^{-1} attributed to the metal–oxygen bond of ZnO. On the other side, the absorption band in the region between 3323 and 3242cm^{-1} is detected, which aligns with the O–H stretching. Furthermore, the powerful connection of ZnO NPs to amide groups of *C. procera* was detected. The new band at 2928 and 2856cm^{-1} showed the presence of C–H species and was referred to asymmetric stretching of CH3 and CH2 of the plant **(Elumalai *et al.*, 2015)**. The peak at 1634cm^{-1} is due to the H–O–H bending **(Vijayakumar *et al.*, 2013)**. According to the FTIR spectrum of the synthesized nanocomposite, a shift and broadening of bands was noticed after loading, which indicated that the contribution of hydroxyl groups, aldehydes, amines, ketones, and carboxylic acid in the loading reaction; moreover, this confirmed the successful bond between plant and nanoparticles **(Li *et al.*, 2010)**.

Antimicrobial activity

The agar well diffusion experiment was used to examine the plant extract's inhibition zone. In this experiment, the antimicrobial activity of Cp, ZnO NPs, and CP@ZnO NC was examined versus some of the most common microbial strains found in the water environment, including *S. aureus* (Gram-positive), *E. coli* (Gram-negative) bacterial organisms, and *C. albicans* (fungi). The antimicrobial activity, which means the microorganism's growth prevention, was visible in the shape of the clear zone. In this work, the three tested compounds showed activity against all strains but at various grades (Fig. 5).

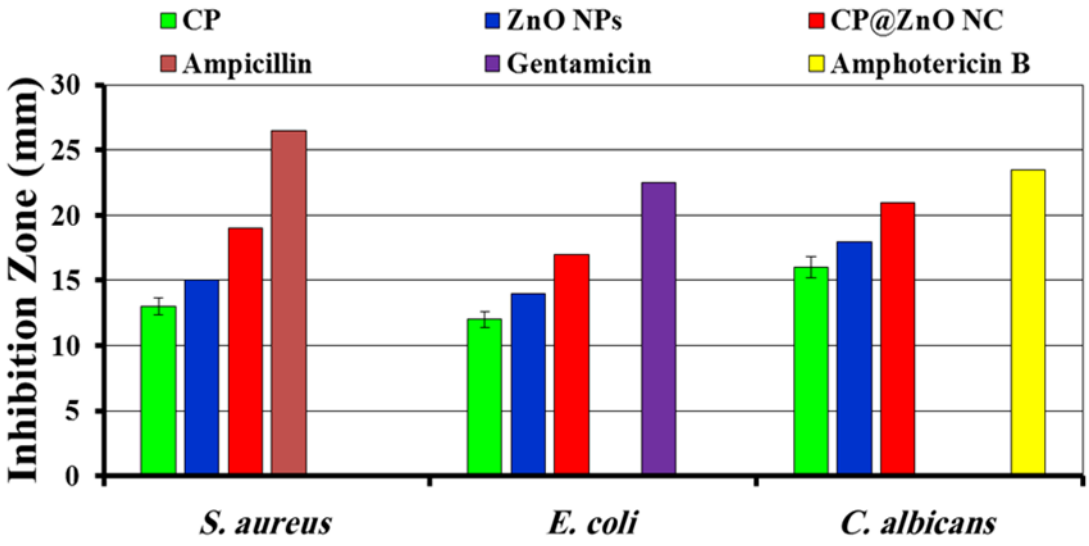


Fig. 5. Inhibition zones of plant extract (CP), ZnO NPs and CP@ZnO nanocomposite against tested bacteria and fungi

The findings indicated that the diameter of the inhibition zone for CP@ZnO NC was greater than that of the ZnO nanoparticles. Specifically, the inhibition zone diameters were 19mm for *S. aureus*, 17mm for *E. coli*, and 21mm for *C. albicans*, compared with the selected commercial antibiotics: Ampicillin for *S. aureus* (26.5mm), Gentamicin for *E. coli* (22.5mm), and the antifungal Amphotericin B for *C. albicans* (23.5mm). Furthermore, the fabricated ZnO nanoparticle also exhibits an excellent inhibition zone. However, the inhibition zone diameter for plant extract indicated the lowest diameter for all tested microorganisms (Fig. 5). The ZnO NPs have recently demonstrated antibacterial efficacy against a variety of microbial species. Many studies showed these antimicrobial effects, such as **Kadiyala *et al.* (2018)**, who examined its antimicrobial action versus methicillin-resistant *S. aureus*; **Alekish *et al.* (2018)**, who investigated its effects on *S. aureus* and *E. coli;* and others who studied its antifungal action **(Djearamane *et al.*, 2022)**.

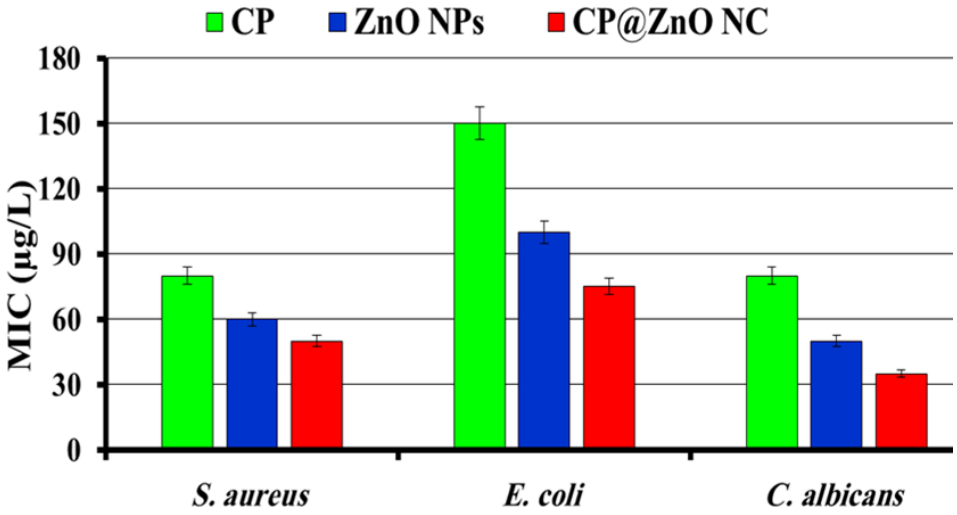


Fig. 6. Minimum inhibitory concentration (MIC) of plant extract (CP), ZnO NPs and CP@ZnO nanocomposite against tested bacteria and fungi

As a qualitative test, the agar diffusion technique was used to monitor and predict the antibacterial and antifungal activity for the tested compounds (Cp plant extract, ZnO NPs, and CP@ZnO NC). Numerous benefits of this approach include its ease of use, affordability, and capacity to test a large variety of microbes and antibiotics. By the consideration, since the antimicrobial agent's diffusion in the agar cannot be determined, it cannot detect the MIC **(Balouiri *et al.*, 2016)**. Because of the greater probability of nanoparticle-microbe interactions in the liquid phase, the broth medium approach can be regarded as more accurate than the agar diffusion experiment **(Negi *et al.*, 2012)**. The particles must enter microbial cells to exhibit their antibacterial properties **(Mirhosseini *et al.*, 2013)**. Consequently, the broth dilution procedure provided an

appropriate estimation of the MIC value. In the current work, the synthesized CP@ZnO NC showed maximum antimicrobial activities to all tested strains in a relatively low concentration (MIC = 50, 75, and 35µg/ l for *S. aureus, E.coli*, and *C. albicans*, respectively) (Fig. 6). Furthermore, the MIC values for ZnO NPs and Cp plant extract were lower than those of CP@ZnO NC but also, zinc oxide nanoparticles still showed good value at low concentrations (MIC= 60, 100 and 50µg/ l for *S. aureus, E.coli*, and *C. albicans*, respectively). In the present study, the CP@ZnO NC MIC values for the three tested microorganisms were smaller than ZnO NPs MIC in this work and in other previous studies **(Tayel *et al.*, 2011; De Souza *et al.*, 2023)**.

The structural features of the ZnO NPs and the HPLC analysis of *C. procera* extracted that have a reactive surface clarify the type of CP@ZnO–microbes interaction **(Selim *et al.*, 2024)**, which occurred through the following:

(i) Electrostatic interaction-induced external adsorption of CP@ZnO-NC on microbial cell membrane.

(ii) Production of ROS through the ZnO NPs surface and toxic active components of *C. procera* extract that were loaded on the ZnO NPs surface.

(iii) The microbial cells are inactivated by the interaction between CP@ZnO NC and cell wall protein, leading to disturbance in portability and decomposition of internal cellular components, such as cytoplasm, DNA, and mitochondria, due to ROS reactions.

CONCLUSION

In this work, CP@ZnO nanocomposite was prepared by conjugation between ZnO nanoparticles and *C. procera* leaf extract to improve the antimicrobial activity against waterborne microbes. The highest inhibition zones (19, 17, 21 mm) and the lowest MIC (50, 75, 35 µg/l) were recorded by Cp@ZnO NC against *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative), and fungi (*Candida albicans*), respectively. The CP@ZnO nanocomposite recorded maximum antibacterial and antifungal efficiency compared with ZnO NPs and *C. procera* plant extract itself. Finally, the CP@ZnO nanocomposite is considered to be an efficient, safe, economic bactericidal and fungicidal agent and can be used in the purification of different wastewater sources, and is effective for use as a disinfectant agent in small communities.

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