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In-vitro evaluation of biosynthesized zinc oxide (ZnO) nanoparticles from Cassia glauca against *Staphylococcus aureus* and *Escherichia coli*

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Abstract:

Zinc oxide nanoparticles were prepared from the Cassia glauca plant and used as antibacterial agents against multiple drug resistance. *Staphylococcus aureus* and *Escherichia coli* were isolated from urinary tract infections. Leaf extracts of Cassia glauca demonstrated outstanding promise as lowering agents in the NP creation process. Using UV, FTIR, XRD, DLS, and SEM examination, structural and optical investigations verified the production of effective ZnO NPs. ZnO NPs made from leaf extracts have shown a notable capacity to inhibit numerous drug-resistant *S. aureus* and *E. coli*, according to an antibacterial study. The aqueous plant extract was added to the bacteria isolates to inhibit bacterial growth in five various concentrations (0.25, 0.5, 1, 2 and 4) µg/mL, and the results were ineffective on *Staphylococcus aureus* growth with all concentrations that used in current study, except that *E. coli* bacterial growth was effect with The aqueous plant extract (ZnO NPs), in concentrations (0.25, 0.5) µg/mL were more effect than other concentrations.

The treatment of several infectious diseases in humans can benefit from the manufacture of ZnO NPs utilizing extracts of medicinal plants. Further research will be required to verify these NPs' efficacy in medical applications and their capacity to lessen the risks connected to traditional drugs.

Keywords: Zinc oxide (ZnO) nanoparticles, biogreen synthesis, antibacterial activity UV, FTIR, XRD, SEM.

Introduction:

Antibiotic-resistant bacteria have emerged globally as a result of the overuse of antibiotics in a variety of medical and agricultural fields. This trend affects a broad spectrum of microorganisms with a high prevalence that pose a hazard to human health (1,2). The resistance toward currently antimicrobial agents and the need to find alternatives due to the exorbitant expense of producing synthetic substances, as well as their numerous adverse consequences. The plant is a promising target for drug research because of the antibiotics' synergistic effect against resistant bacteria, which creates new possibilities for the treatment of infectious disorders (3,4). For centuries, plants have been considered an important source of medicinal compounds, and a large number of unique medication components have been extracted from natural plant sources (5,6).

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Traditionally, many of these plants and their extracts were utilized in medicine. Secondary metabolites with antimicrobial activities, such as tannins, terpenoids, alkaloids, and flavonoids, are abundant in plants (7,8). Plant-mediated nanoparticle synthesis has garnered a lot of interest lately due to its inherent advantages, such as speed, simplicity, environmental friendliness, and lower costs (9-11). Nanotechnology is a branch of nanoscience that focuses on the nanoscale creation and manipulation of matter (12,13). Nanoparticles have a variety of shapes, including spheres, triangles, hexagons, rods, wires, and tubes, and can be made from a variety of materials. Nanotechnology has recently gained popularity as a discipline with applications in optics, electronics, catalysis, health sciences, mechanics, magnetic energy research, agriculture, and the environment. Nanoparticles are the fundamental building blocks of nanotechnology (14-16).

The biological efficiency of nanoparticles can rise when the specific surface region of the particles increases because of the increase in surface energy. For controlling nanoparticle size and preventing aggregation, three components are used in the manufacture of metallic nanoparticles utilizing plant extract: (1) metal salt, (2) reducing agent, and (3) stabilizing or capping agent (17-19). Other advantages of employing plants to synthesize nanoparticles include the use of safe solvents, the use of fewer harmful chemicals, flexibility, adaptability, and softer reaction conditions in pharmaceutical, medicinal, and surgical applications (20,21). The biosynthesis of NPs utilizing plant extracts from different plant species has made extensive use of some metals, including copper (Cu), gold (Au), silver (Ag), and many more (22,23). They are more harmful to both humans and animals, however, and seriously limit their use in the medical field. Rarely does the inorganic compound zinc oxide appear in nature. It usually exists in crystalline form. Manganese impurities give naturally occurring zinc oxide its characteristic red or orange hue (24,25).

White crystalline powder that is almost insoluble in water is the appearance of purified zinc oxide. Because of their size-dependent characteristics and low toxicity, ZnO NPs have found extensive usage in different fields, including micro-electronics, cosmetics, textiles, and diagnostics. Generally regarded as safe (GRAS) and possessing antibacterial qualities, ZnO nanoparticles (NPs) have a better potential to cure infectious disorders in both people and animals (26,27). The biosynthesis of NPs for therapeutic applications has shown ZnO to be more effective and possibly beneficial than other metals. Numerous investigations have shown how to synthesize ZnO NPs utilizing various plant extracts. For instance, less of the medicinal plant Cassia auriculata extract (28,29). Therefore, the current study is aimed at the In-vitro evaluation of biosynthesized zinc oxide (ZnO) nanoparticles from Cassia glauca against *Staphylococcus aureus* and *Escherichia coli*.

Materials and Methods

Bacteria identification

The 90 urine specimens were collected using the mid-stream method (Alforat hospital in Alnajaf province, in Iraq). The isolates were primary identified by routine bacteriological methods. The confirmed identification was done by the VITEK2 compact system (30).

Extraction of Cassia leaves method

Leaves of cassia trees were collected from the district of Najaf City and repeatedly cleansed with distilled water to get rid of any dust, then let dry in the sun for eighteen days to get rid of any remaining moisture. A 500 ml glass beaker was filled with 200 ml of sterile, distilled water and 10 g of dried, finely chopped cassia leaves to create the extract. After 30 minutes of boiling at 70 °C, the mixture turned from a watery to a yellow aqueous solution. To get rid of the biomaterials, the mixture was centrifuged for two minutes at 1200 rpm after being allowed to settle, let it cool down, and then filtered it through Whatman

No. 1 filter paper. For use in upcoming research, the extract is kept at room temperature (31,32).

Biosynthesis of Zinc oxide nanoparticles:

The steps of the biosynthesis of ZnO nanoparticles include the following: (33,34)

1. To create a light yellow solution, 500 ml of an aqueous zinc sulfate heptahydrate solution containing 0.25, 0.5, 1, 2, and 4 µg/mL is mixed with 10, 20, or 30 ml of the aqueous yellow leaf extract of Cassia glauca. The combination is then given five minutes to sit at room temperature.
2. The mixture's yellow hue began to change to a yellowish-white suspension at pH 12 when 1 molar of sodium hydroxide solution was added to it drop by drop while being constantly stirred at room temperature.
3. The suspended particles underwent three centrifugations and dispersion in sterile distilled water to purify them. To dispose of the contaminants in the finished product, ethanol is next used to wash the white particles. Then, after six hours of drying at 60°C in a vacuum oven, a white powder was obtained.

Characterization of ZnO nanoparticles:

•Infrared spectroscopy using the Fourier transform (FTIR)

It can reveal important details about the molecular structure of organic and inorganic components (35).

• X-ray diffraction

XRD is a non-destructive method for material characterization that can determine the crystal structure and crystallite size (36).

•Scanning electron microscope (SEM)

It was utilized to investigate ZnoNPs morphology and size of particles (37).

Antibiotic susceptibility test:

An antibiotic susceptibility test was conducted by disk diffusion method towered 10 types of antibiotics (38). The results measured the inhibition zone around the antibiotic discs (39).

Antibacterial activity test:

The wells agar diffusion method was utilized to evaluate the antibacterial activity of plant extract (40). The method includes making three wells (6 mm) on the solid nutrient agar medium by Cork Borrer. Extract concentrations were added by 0.1 ml per well. After spreading 0.1 ml of the bacterial suspension on the medium and subsequently incubated for 24 hours at 37 °C, the inhibitory zone's width was determined.

Results and Discussion:

Identification of Isolates

The findings showed that out of 90 (70 positive growth bacteria) urine specimens only 20 specimens tested positive for *E. coli* isolates (28%) and 10 specimens tested positive for *Staphylococcus aureus* isolates (14%) while findings 40 isolates (57%) for different types of bacteria Fig: (1). These results consist with finding obtained by (41) that demonstrated the uropathogenic *E. coli* (UPEC) are the primary cause of UTIs; they are responsible for over half of complex UTI cases and over 75% of simple UTI cases.

Studies have shown that most occurrences of *S. aureus* bacteriuria are just colonization and do not correspond with UTI symptoms. For instance, (42) discovered that UTI symptoms were present in just 33% of individuals whose urine included *S. aureus* (43). The most common pathogenic pathogen for UTIs is the UPEC of concern. To gain a deeper comprehension of the pathophysiology of UPEC UTI, the following characteristics of UPEC are enumerated: adherence, motility, toxin generation, metal acquisition, intracellular bacterial communities (IBCs), and the ability to evade host immune systems. Furthermore, discussed are conventional medications, complementary and alternative therapies, antibiotic resistance, the enormous potential of vaccinations, and medicinal plants in the treatment of urinary tract infections (44,45).

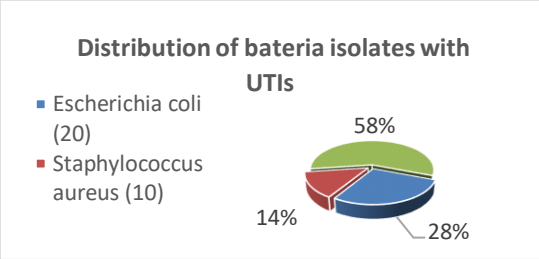


Figure 1: Distribution of bacteria isolates with UTIs

Antibiotic Susceptibility

In vitro, an antibiotic susceptibility test was conducted on both *E. coli* (G-ve) and *Staphylococcus aureus* (G+ve) isolates. However, *E. coli* isolate (fig:2) showed a resistance to antimicrobial agent class including Amoxicillin (70%), Cefixime (65%), Cefotaxime (65%), Levofloxacin(60%), Chloramphenicol(60%), Kanamycin(60%), Nalidixic acid (60%) Ciprofloxacin (55%), Ampicillin(50%) and Amoxicillin clavulanate(45%) while, E. coli isolate showed susceptible to Meropenem (85%), Imipenem(75%), Amikacin (50%).

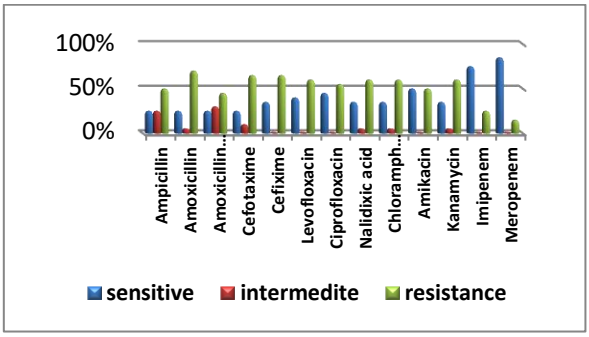


Figure 2: Antibiotic susceptibility results of E. coli isolates.

Staphylococcus aureus isolate (fig 3) showed a resistance to antimicrobial agent class including Amikacin (80%), Amoxicillin (60%), Cefixime (60%), Cefotaxime (60%), Chloramphenicol (60%), Ampicillin (50%), Kanamycin (50%), Levofloxacin (50%) and Imipenem (40%) while, *S. aureus* isolate showed susceptible towered, Meropenem(70%), Amoxicillin Clavulanate (60%), Ciprofloxacin (60%), and Nalidixic acid (50%)

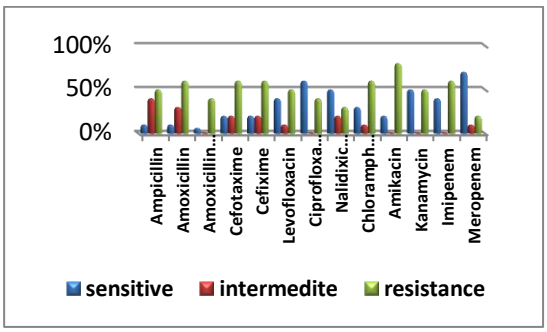


Figure 3: Antibiotic susceptibility test of S. aureus isolates.

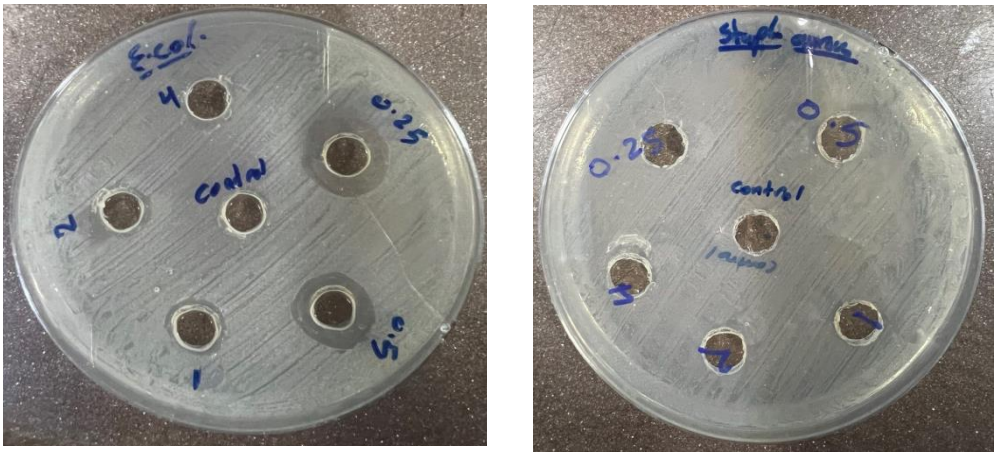
These results are closely consisting of (46), they demonstrated *E. coli*, most often, isolates from urine samples taken from humans were resistant to Kanamycin (80%), amoxicillin and ampicillin (70%), ciprofloxacin (50%), cefixime (25%), amoxicillin/clavulanic acid, gentamicin (10%), and amoxicillin/sulbactam (5%). According to the data, the isolates of Escherichia coli showed resistance to cefixime and amoxicillin/clavulanic acid, yet they appeared to be sensitive to levofloxacin and norfloxacin (47).

Antibacterial activity of Aqueous Extract

As shown in Figure 4, the results of aqueous plant extract in five various concentrations (0.25, 0.5, 1, 2 and 4) µg/mL showed that the concentrations (0.25, 0.5, 1, 2 and 4) µg/mL of plant extracts were not inhibit the growth of Staphylococcus isolates, while the concentration (0.25, 0.5) µg/mL of the plant extract showed a clear effect and inhibited the growth of *E. coli*,, while concentration (1, 2) µg/mL showed a mild effect and its ability to inhibit bacterial growth was weaker than concentration, except *E. coli* bacteria, concentration (4 µg/mL) of the plant extract showed no effect on bacterial growth. This result agrees with the study by Ansari et al. (2020), they demonstrated that produced zinc oxide nanoparticles using plant extract from Cinnamomum verum and found that the minimum inhibitory concentration (MIC) was 125 µg/mL and 62.5 µg/mL for *Escherichia coli* and *Staphylococcus aureus*, respectively. Numerous research predominatingly support the idea that ZnO nanoparticles also exhibit

antibacterial activity that is concentration-dependent in addition to particle size dependence. According to (48).

Gram-negative bacteria were more sensitive to plant-based ZnO-NPs than Gram-positive bacteria. The reason for this is that the Gram-positive bacteria's cell walls include a thick coating of peptidoglycan that can slow down ZnO-NP penetration (49). Additionally, the Gram-negative bacteria have large quantities of negatively charged lipopolysaccharide, which attracts positively charged NPs electrostatically (50,51). Furthermore, they note that *P. aeruginosa* and *E. coli* bacterial strains were more susceptible to phyto-synthesized ZnO-NPs



A B

Figure 4: Antibacterial activity of Aqueous Extract on A: *E. coli* growth, B: *Staphylococcus aureus* growth, Control: Free nuclease water.

FTIR of ZnoNPs

ZnO samples prepared at different concentrations using Cassia glauca leaf extract were examined using FTIR analysis in a range of wavelengths (400-4000 cm-1). Figure (6) showed a wide absorption peak at 3446.79,1838.46 in extract plants while figure (7) showed a wide absorption peak at 3473.8 cm-1 because of the (O-H) bond's stretching vibration because of the absorption of water molecules. The peaks at 1624.89, 1500.52, 1395.38, 1076.21, 927.76, and 650 cm-1 were assigned to the vibration of C-H, C=O (amide), C=N and C-N (aliphatic and aromatic amines), and N-H (primary amine and amide) bonds, respectively. The appearance of absorption peaks confirming the existence of functional groups is a result of the biomolecules present in the Cassia glauca leaf extract that act as a reducing agent such as proteins. The characteristic peak at 457.13 cm-1 is due to the stretching vibration of the (Zn-O) bond (52). The identical peaks of the spectrum for all the prepared concentrations indicate the success of biosynthesis ZnO nanoparticles. The results are similar to previous research prepared by (53,54).

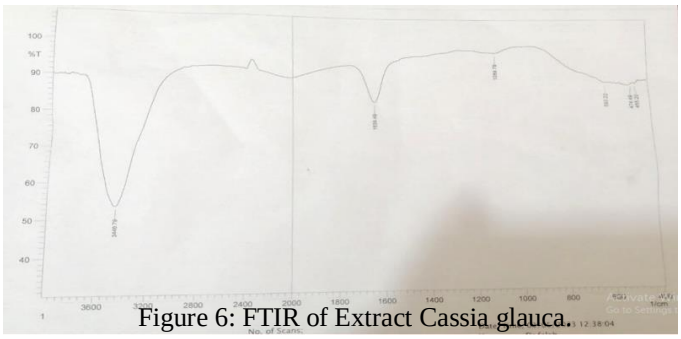


Figure 6: FTIR of Extract Cassia glauca.

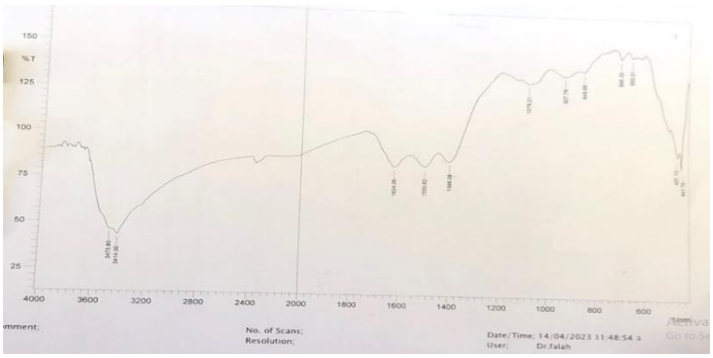


Figure 7: FTIR of different concentrations of ZnoNPs

XRD of ZnoNPs:

ZnO nanoparticles were analyzed using X-ray diffraction as shown in Figures (8). The XRD spectra of ZnO NPs at concentrations 0.25 µg/mL, 0.5 µg/mL, 1 µg/mL, 2 µg/mL, and 4 µg/mL showed low crystallinity with the presence of impurities due to the appearance of several peaks in the spectrum. In contrast, the XRD spectra of the ZnO NPs showed sharp and high diffraction peaks at 2θ = 30.26, 32.42, 35.80, 47.10, 56.20, 62.40 and 67.50 assigned to the hkl planes (100), (002), (101), (102), (110), (103), (112). respectively. It was found that there is a great be identical between the apparent peaks with the standard data (JCPDS card 36-1451), and this confirms that the structure of ZnO NPs is hexagonal wurtzite (55).

SEM of ZnoNPs:

The surface morphology of ZnO nanoparticles with different concentrations was determined using SEM analysis. Figure (10) shows the morphology of ZnO nanoparticles, as it shows irregular shapes with a high physical contact between the particles, and this leads to the distribution of the particles in the form of lumps. Figure 9.

EDX of ZnoNPs:

The elemental analysis of zinc oxide samples was carried out with concentrations 1 µg/mL and 2 µg/mL as displyed in the Figures (10,11). The EDX analysis of ZnO with a concentration of 1mM shows an increase in the weight ratio of zinc element up to 60.78% and the appearance of several additional peaks due to the presence of calcium, oxygen, sodium, and sulfur elements. The appearance F D D E 65 of pattern peaks for 4 µg/mL, where the zinc and oxygen elements were observed in weight ratios that are 42.47% and 46.12, respectively. This shows the completion of ZnO synthesis. The existence of calcium, sodium, and sulfur elements in weight ratios 1.46%, 1.35%, 9.74%, and 0.21 is due to the materials that constitute the Cassia glauca leaf extract as a reducing agent. The high weight ratios of zinc and oxygen elements indicate the success of the biosynthesis of zinc oxide nanoparticles. The presence of sodium in the pattern is a result of using sodium hydroxide during the synthesis process. In EDX analysis of ZnO with a concentration of 4 µg/mL, an increase in the weight ratio of zinc was observed due to the increase in the concentration of zinc sulfate heptahydrate solution (56,57).

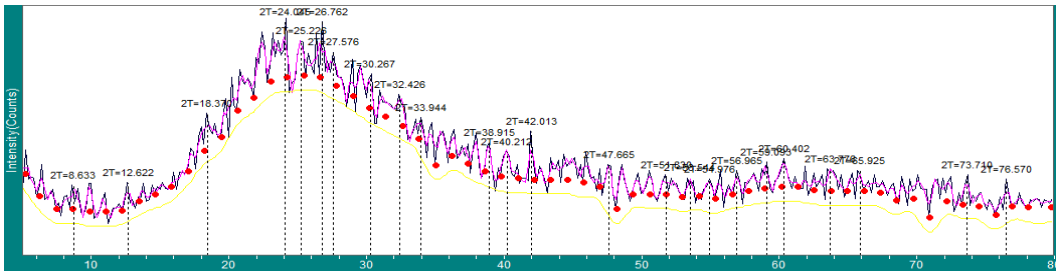


Figure 8: XRD of different concentrations of Zno

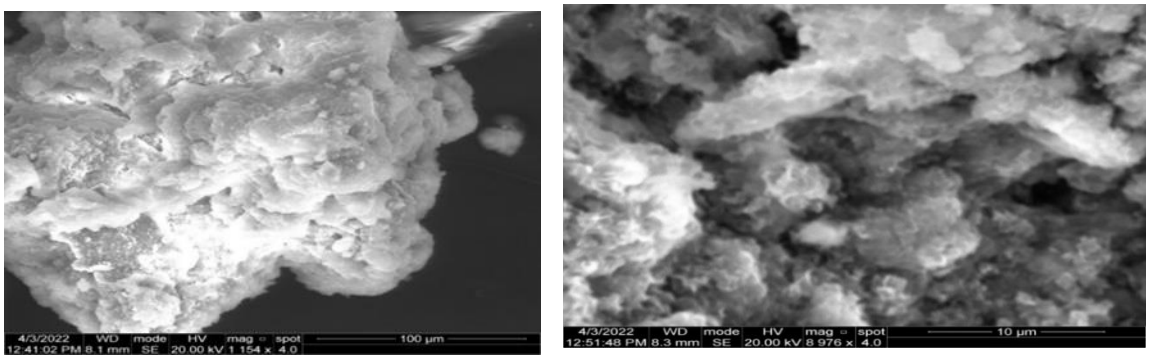


Figure 9: SEM images for ZnO nanoparticles at different concentrations.

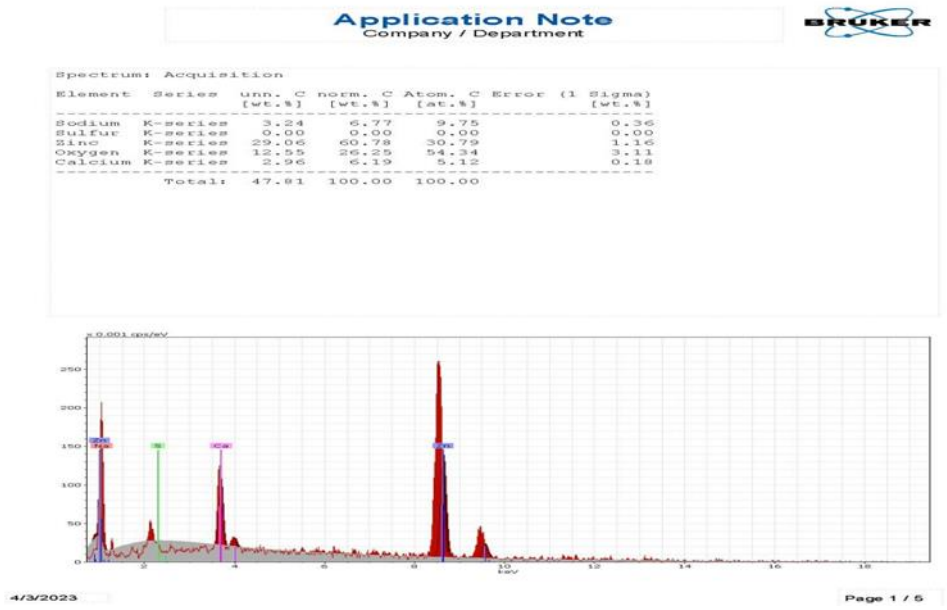


Figure 10: EDX analysis for ZnO at 1µg/mL concentrations

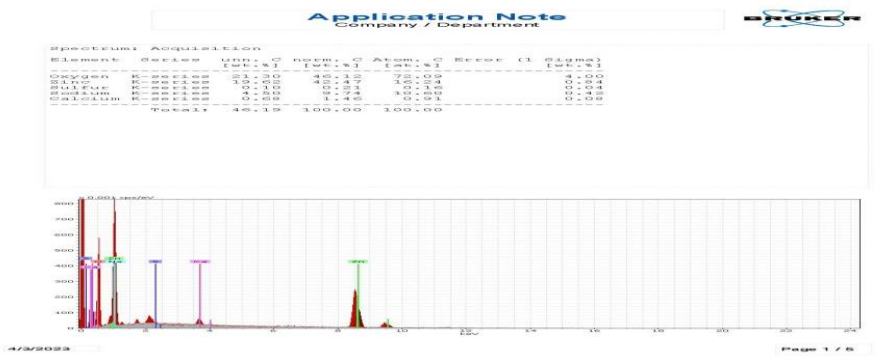


Figure 11: EDX analysis for ZnO at 2 µg/mL concentrations

Conclusions

To prevent the spread of bacterial resistance, local health officials should be alerted to step up monitoring efforts and implement antimicrobial stewardship programs. However, Zno NPs (Green synthesis by plant extract) constitute a potential antimicrobial agent against both *S. aureus* and *E. coli*.

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Conflict of interest: NIL

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