Pak. j. sci. ind. res. Ser. A: phys. sci. 2025 **68A**(2) 161-168

Synthesis of Metallic Oxide Nanoparticles Using *Cymbopogon citratus* Through Conventional Sol-gel Method

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(received March 9, 2023; revised May 27, 2024; accepted September 2, 2024)

Abstract. Metallic oxide nanoparticles are synthesized through various methods for research and commercial purposes, with significant improvements observed over time. The sol-gel process is among the chemical methods employed for nanoparticle synthesis. Lemongrass (*Cymbopogon citratus*) was selected for synthesizing metallic nanoparticles using acetate salts of copper, cobalt and magnesium through the conventional sol-gel method. This technique is relatively new for direct nanoparticle synthesis. The nanoparticles have undergone characterization using various spectroscopic techniques (FT-IR, UV/Vis, SEM, EDS), along with the addition of their antibacterial activities. The EDS spectra have been confirmed through EDS mapping for all nanoparticles. The antibacterial activities of these nanoparticles have also exhibited a positive response to *E. coli* bacteria, revealing clear zones of inhibition against the tested micro-organisms.

Keywords: metallic oxide nanoparticles, green plant, FT-IR, UV/Vis, SEM, EDS, antibacterial activities

Introduction

The synthesis of metallic oxide nanoparticles is classified into three basic types that is physical, chemical and biological processes. Chemical and physical methods for the synthesis of NPs are hazardous, energy consuming and involve contaminated elements that can be harmful to health (Ahmad *et al.,* 2019; Ahmed *et al*., 2010). In contrast, biological methods are carried out in eco-friendly conditions and consume minimal energy, although they may require more time for the preparation of metallic nanoparticles compared to other methods (Banerjee *et al*., 2014; Begum, *et al*., 2009). Synthesizing metallic NPs from plant extracts is cost effective and easy to handle, making it beneficial for large scale production (Bharti *et al*., 2013; Bhutkar *et al*., 2012).

The sol-gel method is considered an effective technique for modifying the surface of substrates (Ajitha *et al*., 2016). One of its most significant advantages is the ability to obtain a high surface area and stable surfaces. This method forms metallic NPs through a wet chemical process involving two types of reactions that is hydrolysis of monomers to prepare a sol (a colloidal suspension) and poly-condensation reactions. The sol-gel process begins with the formation of an organic precursor solution, such as metal alkoxides and acetyl acetonates, which is hydrated with water, alcohols or an acid/base solution to form a sol. Subsequently, a poly-condensation reaction produces a wet gel with minimal water content which is then dried to yield nanoparticles with specific properties (Sajjadi *et al*., 2017).

The main advantages of the sol-gel method include the production of small sized nanoparticles at lower temperatures, precise control of particle size (ranging from 10 to 100 nm) and the ability to synthesize mixed oxide networks (Farzaneh *et al*., 2021; Markus *et al*., 2007). For example, it is used in the synthesis of glasses, ceramics or ceramic based materials and boasts a high manufacturing percentage, rapid production of fine homogeneous solids, low cost and energy efficiency (Cherian *et al*., 2019; Amit *et al*., 2015).

However, the sol-gel method also has some drawbacks, such as the difficulty of separating the gel due to its porosity, resulting in a crude product (Prakash *et al*., 2018). Additionally, the gel formation is a time-consuming process compared to other methods like the combustion method. Nevertheless, the sol-gel method remains highly beneficial for the synthesis of metallic nanoparticles (Ali *et al*., 2019).

Numerous green plants have been studied for the development of various metallic and metallic oxide nanoparticles (Haseeba *et al.*, 2024; Irfan Ali. *et al.*, 2020). Similarly, some research has used Ag and Cu metals as precursors in nanoparticle manufacture through various methods (Asghar *et al*., 2017; Suarez-Cerda *et*

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al., 2017). Furthermore, different metals can also be used as materials for making nanoparticles (Tsade *et al*., 2019; Kashif *et al*., 2018). Some nanoparticles are more effective and efficient in killing fungi or lichens due to their antibacterial activities (Imtiaz *et al*., 2016).

Moreover, the specific characteristics of these nanoparticles depend on their size, distribution, morphology, and phase (Mohammad *et al*., 2019; Shah *et al*., 2019). Smaller particle size can lead to a greater antimicrobial effect. During synthesis, factors such as the temperature of solutions, metal concentrations, reducing agents and reaction time can also influence the properties of the nanoparticles (Reddy *et al*., 2018; Shah *et al*., 2014).

In this study some metallic oxide nanoparticles were formed from lemongrass (*Cymbopogon citratus*) with different metals (Cu, Co, Mg) using the conventional sol-gel method. These nanoparticles have been characterized using several spectroscopic techniques (FT-IR, UV/Vis, SEM, EDS) and as an application work, had been observed their biological activities against selected bacteria.

Materials and Methods

Chemicals and samples. All chemicals used in this study were of analytical or equivalent grade. These included cupric acetate, cobalt acetate and magnesium acetate (Fluka, Switzerland), as well as sodium acetate, methanol, ethanol and acetone (E. Merck, Germany). Stock solutions (1000 mg/L) of Cu (II), Co (II) and Mg (II) were prepared by directly dissolving appropriate amounts of their respective acetate salts. The freshly prepared metal ion solutions were derived from these stock solutions. Buffer solutions with different pH values ranging from pH 3 to 6 and pH 7 to 10 were prepared using solutions of acetic acid, sodium acetate, ammonia and NH4Cl, respectively. Two different brands of *Cymbopogon citratus* (lemongrass) were procured from the local market in Hyderabad.

Preparation of *Cymbopogon citratus* (lemongrass) extract. The *Cymbopogon citratus* (lemongrass) extract was obtained using a simple refluxing technique. Initially, 25g of dried lemongrass leaves were finely chopped. They were then refluxed in 50mL of aqueous media for approximately 60 min. After refluxing, the hot mixture was initially filtered and the pure extract was separated from the water by adding anhydrous sodium carbonate through filtration. At this point, the lemongrass extract is ready for further practical work.

Synthesis and optimization of metallic nanoparticles (CuONPs, CoONPs and MgONPs) of *Cymbopogon citratus* (lemongrass). The synthesis of CuONPs from lemongrass was evaluated by monitoring the characteristic UV-visible absorbance band in the range of 180-650 nm under various concentrations of the precursor (lemongrass) (20, 40, 60, 80 and 100% v/v) and pH values (4.0, 6.0, 8.0, 10.0 and 12.0). The reaction mixture was added and mixed homogeneously on low flame to form a colloidal suspension. The colloidal suspension (Sol) was further heated and transformed into a thick gel on chilled water. The obtained gel was dried in an oven at 90 °C and converted into a precipitate of corresponding nanoparticles. After the formation of metallic CuONPs, a clear change in colour was observed from green to greyish black. This change was related to the fervour of inter-band transitions, reflecting the metallic nature of NPs from lemongrass. The peak of CuONPs appeared at 519 nm (Fig. 1a). A hypochromic shift was observed with increasing concentrations of lemongrass from 20 to 100%, while the concentration of copper acetate throughout the reaction was fixed at 0.25 mmol. This hypochromic shift indicates a peak with 20% concentration of precursor which is due to the reduction in NPs size (Ali *et al*., 2019).

The case of pH optimization, the concentration of the precursor (lemongrass) was kept at 20% and the concentration of copper acetate was fixed at 0.25 mmol (Fig. 1b). The UV-vis spectra presented a hyperchromic shift at different pH values (6.0, 6.2, 7.4 and 9.2) due to an increase in the size of NPs at pH 4.0, 6.0, 8.0 and 10.0, respectively. Therefore, the optimized pH of 10 has been marked for further studies (Cherian *et al*., 2020; Shah *et al*., 2019). Similarly, other metallic particles from lemongrass (CoONPs and MgONPs) were optimized at 536 nm and 584 nm with a precursor concentration of 20%, synthesized by the same mechanism.

Preparation and incubation test for the antibacterial activities. *Chemicals*. Agar (2 g), Peptone (2 g), Yeast extract (1.5 g), NaCl (0.7 g), glucose (2 g) and distilled water (200 mL) used for antibacterial activity.

Procedure. All equipment used for the incubation test were thoroughly washed, rinsed with 96% alcohol, wrapped in paper and then sterilized using an oven at 180°C for 2 h.

In the Biotechnology Department of the University of Sindh, Jamshoro, agar (2 g), peptone (2 g), yeast extract

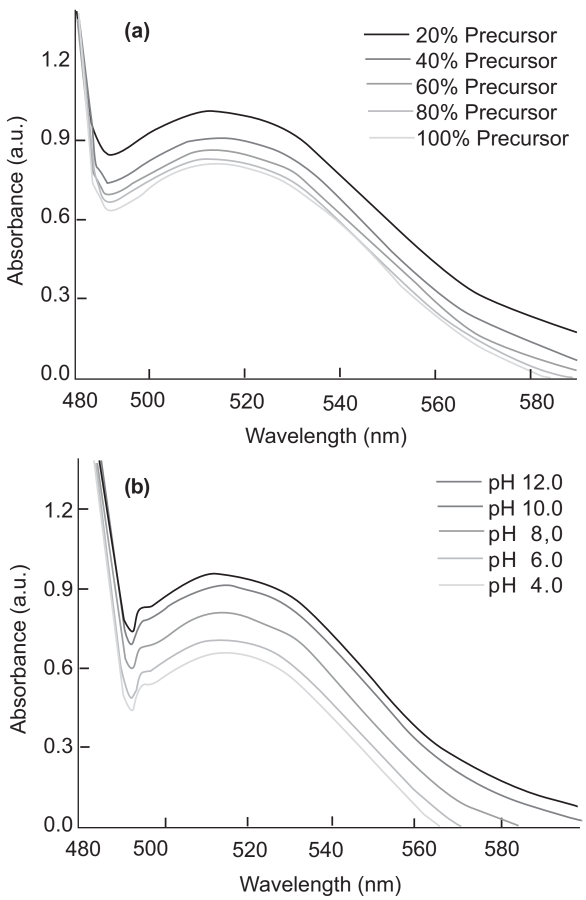

Fig. 1. The optimization of concentration of various precursor % extract **(a)** and, at various pH **(b)**, for the synthesis of CuONPs of lemon grass.

(1.5 g), NaCl (0.7 g) and glucose (2 g) were dissolved for media preparation in an autoclaved conical flask. The media were subjected to autoclaving to eliminate microbial species for 45 min. After autoclaving, they were placed in a laminar airflow cabinet to create a particulate free environment for bacteria in petri dishes, which were used for the counting method.

In the disc diffusion technique, Petri dishes were utilized for each reading. Agar plates were prepared, with one plate containing inoculums and the others containing inoculum and nanoparticle solutions, which were spread on it using a sterilized spreader. This plate was then incubated for 24 h at 37 °C and the colonies on each plate were counted. Subsequently, the inhibition percentage growth was calculated for each reading (Chatterjee *et al*., 2012).

Characterizations of MNPs. The newly synthesized MNPs were characterized using various spectroscopic techniques. Infrared spectra were recorded on a Nicolet Avatar 330 FTIR (Thermo Nicolet Electron Corporation, USA) with an attenuated total reflectance (ATR) accessory (smart partner) within the range of 4000-600/cm. UV-Vis Spectrophotometer, a double-beam Hitachi 220 spectrophotometer (Tokyo, Japan) with dual 1 cm silica cuvettes within the range of 185-700 nm. Scanning electron microscope-energy dispersive X-Ray (SEM-EDX) (Phenom-World), USA.

Results and Discussion

FT-IR Analysis. The FT-IR analysis of CuONPs produced from lemongrass provided essential evidence for the synthesis of metallic oxide nanoparticles. The wavenumbers indicated at 3395 to 3422/cm corresponded to the absorption of the OH functional group, while 2916-2923/cm, 1451 and 1377/cm and 1013/cm represented the absorption of –CH and CO alcohol group (Shah *et al*., 2023). The strong peak at 609.32/cm indicated the monoclinic structure of the CuONPs, signifying the Cu-O bond's stretching vibration due to metal-oxygen stretching. Peaks at 852.16/cm and 921.35/cm might be attributed to C-H vibrations in CuONPs (Fig. 2a,b,c and d).

EDS Analysis. The EDS spectrum of green synthesized CuO nanoparticles from *Cymbopogon citratus* (Lemongrass) displayed peaks of carbon, copper and oxygen along with their atomic and weight percentages: carbon (C) 22.47%, copper (Cu) 32.21% and oxygen (O) 45.31% in CuONPs nanoparticles from lemongrass Fig. 3a. Furthermore, the EDS spectra were confirmed by EDS mapping, confirming the presence of Cu and O in the synthesized green nanoparticles. This EDS result aligns with previously reported CuO nanoparticles synthesized using lemongrass leaf extract (Wang *et al*., 2016).

The EDS spectra for the weight percentage of cobalt oxide nanoparticles were represented as: carbon (C) 22.90%, cobalt (Cu) 35.31% and oxygen (O) 41.79% in lemongrass at low and high magnifications. The EDS spectra for the weight percentage of magnesium oxide nanoparticles were represented as: carbon (C) 30.30%, magnesium (Mg) 16.04%, oxygen (O) 52.63%, with traces of aluminum found. The EDS spectra of CoONPs and MgONPs from *Cymbopogon citratus* (lemongrass) are also shown in Fig. 3(b) and 3(c).

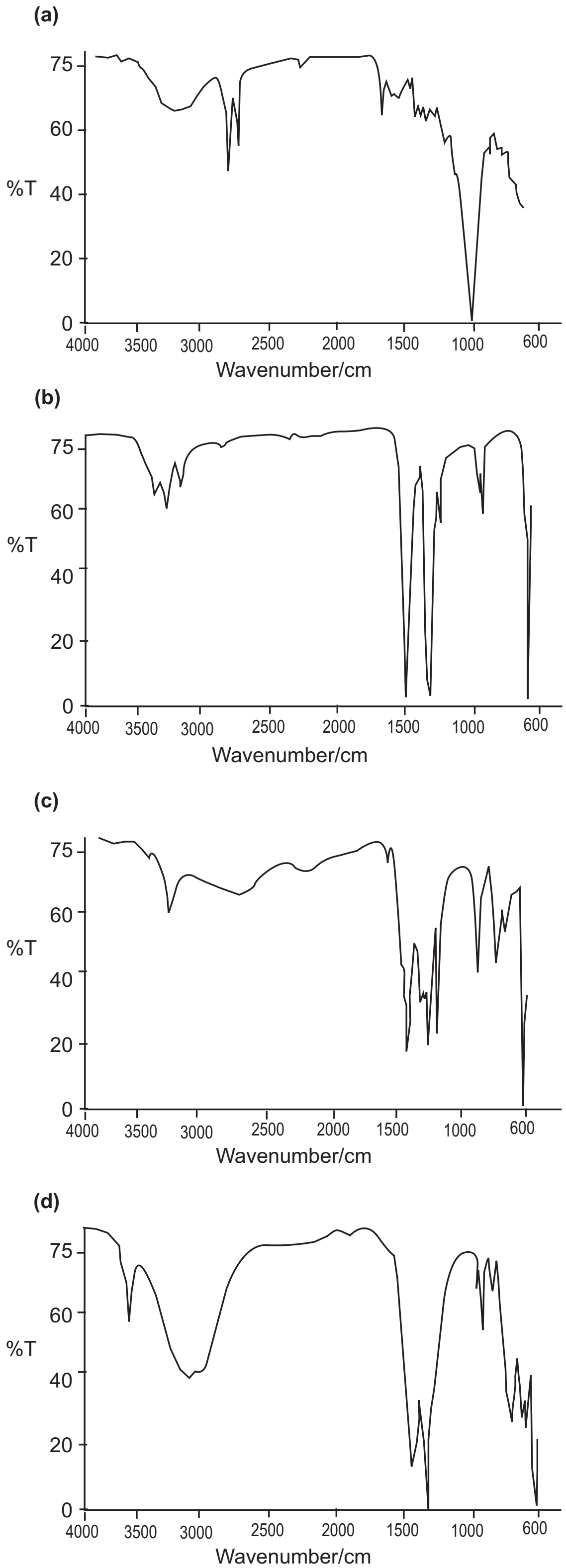

Fig. 2. FTIR Spectra of **(a)** *Cymbopogon citratus* (lemon grass) and its nanoparticles **(b)** CuONPs, **(c)** CoONPs, **(d)** MgONPs.

SEM Analysis. SEM analysis is a technique that generates images of samples by directing an electron beam from the instrument source onto a specific sample. Scanning electron microscopy (SEM) is used to determine particle size, shape and texture with minimal quantities of material.

The SEM images represent the synthesized nanoparticles at various magnifications. In the morphology photographs of the green synthesized CuONPs and CoONPs from *Cymbopogon citratus* (lemongrass), the nanoparticles appear agglomerated or irregularly spherical and are seen as scattered clusters. The particle size is smaller, exhibiting resolution in micrometers.

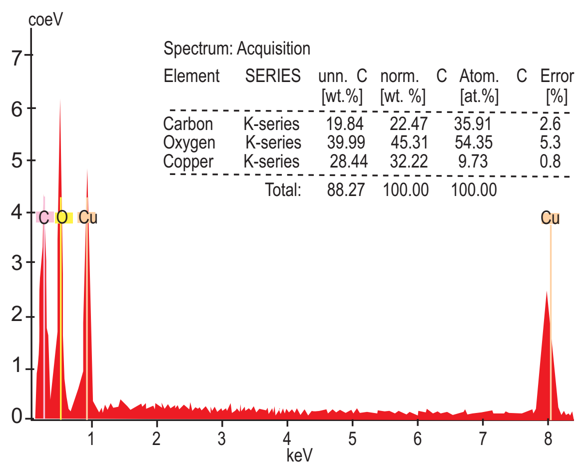

Fig. 3a. EDS Spectrum of CuO NPs of (*Cymbopogon citratus*) lemon grass.

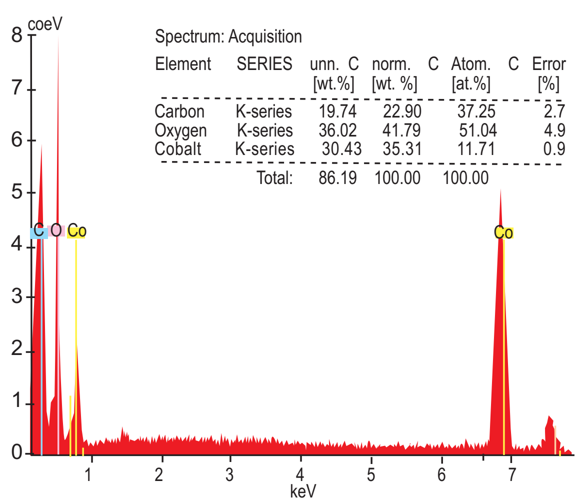

Fig. 3b. EDS Spectrum of CoO NPs of (*Cymbopogon citratus*) lemon grass.

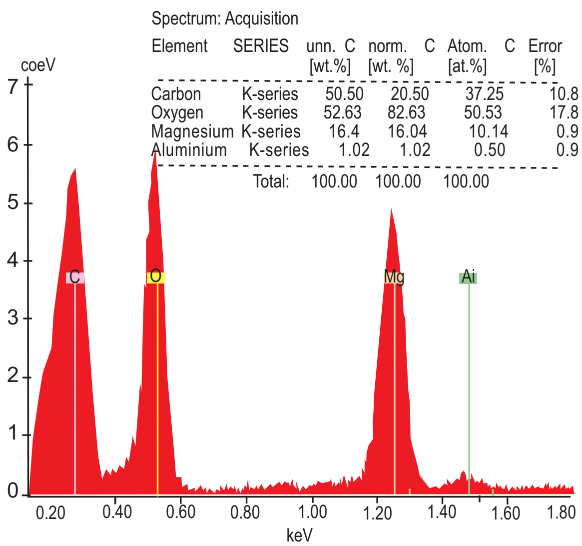

Fig. 3(c). EDS Spectrum of MgONPs of (*Cymbopogon citratus*) lemon grass.

The morphological photographs of CuONPs and CoONPs are presented with low and high resolution in Fig. 4(a and b) and 5(a and b).

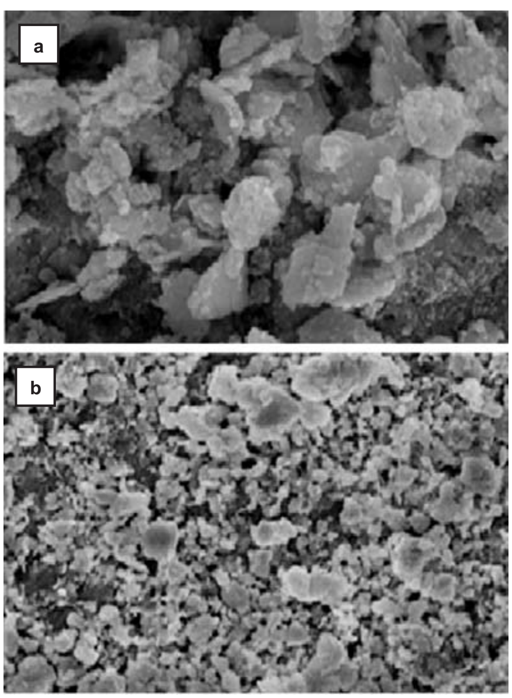

Fig. 4. Typical **(a)** high-magnification and **(b)** low-magnification of SEM images of synthesized CuONPs of (*Cymbopogon citratus*) lemon grass.

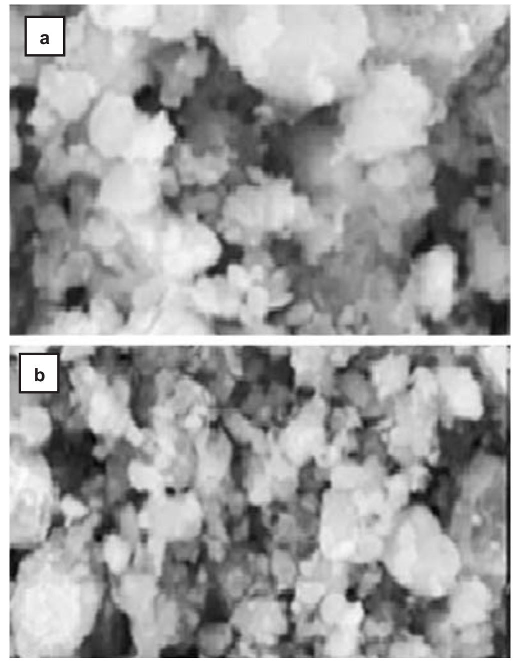

Fig. 5. Typical **(a)** high-magnification and **(b)** low- magnification of SEM images of synthesized CoONPs of (*Cymbopogon citratus*) lemon grass.

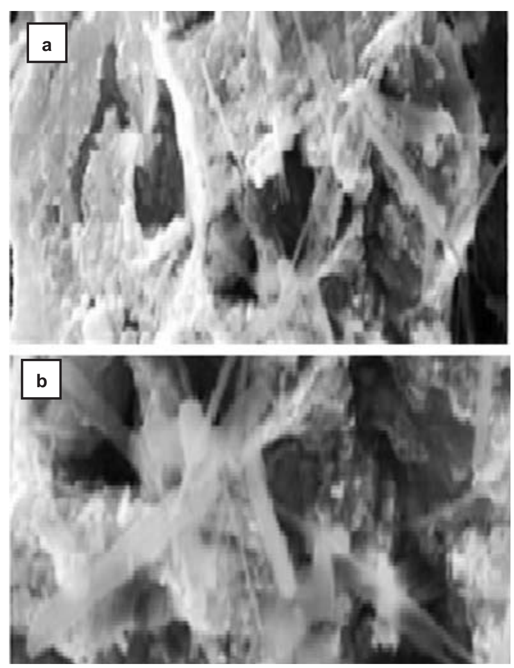

Fig. 6. Typical **(a)** high-magnification and **(b)** low- magnification of SEM images of synthesized MgONPs of (*Cymbopogon citratus*) lemon grass.

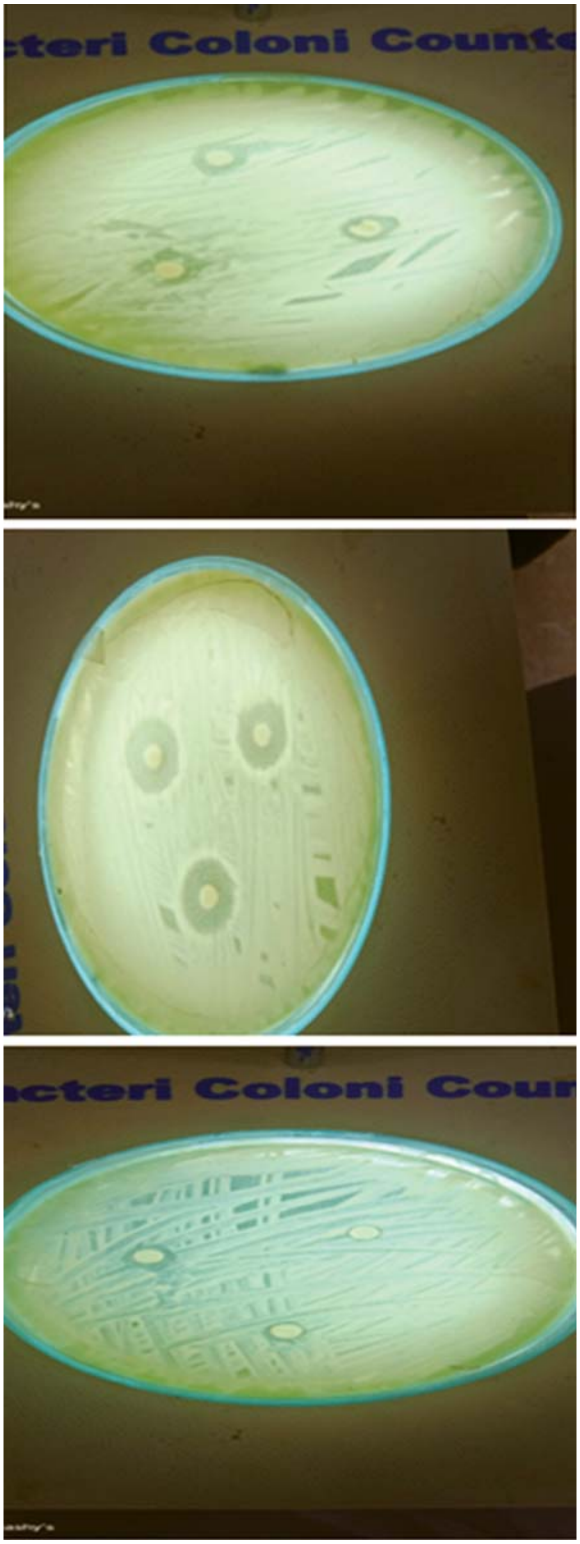

Fig. 7. Zone of inhibition of CuONPs **(a)**, CoONPs **(b)** and MgONPs of **(c)** *Cymbopogon citratus*) lemon grass against Escherichia coli cells.

Table 1. Zone of inhibition of copper, cobalt and magnesium oxide nanoparticles against Escherichia coli cells

Sample of metallic NPs	Reading 1	Reading 2	Reading 3	Mean
CuONPs	11.4 mm	10.7 mm	10.1 mm	10.7 mm
CoONPs	18.3 mm	17.8 mm	17.1 mm	17.73 mm
MgONPs	10 mm	6.9 mm	10.5 mm	9.1 mm

On the other hand, metallic nanoparticles MgONPs exhibit an aggregative cylindrical shape from the pictures, it is evident that the size of the nanoparticles is less than 100 nm, which is in good agreement with the particle sizes and crystalline nature. The morphological photographs of MgONPs are presented with low and high resolution in (Fig. 6a and b).

Biological activity of metallic nanoparticles (CuO, CoO and MgO) of *Cymbopogon citratus* (lemon grass). Some new metallic nanoparticles (CuO, CoO and MgO) were synthesized, characterized, and their biological activities were observed as an application of these metallic nanoparticles.

Antibacterial mechanism of copper, cobalt and magnesium oxide nanoparticles. In this research, CuONPs, CoONPs and MgONPs derived from *Cymbopogon citratus* (lemongrass) were employed to study the antibacterial mechanism. The size of chemically synthesized Cu, Co and MgONPs without any stabilizer rapidly increased. Antibacterial activity was also observed using the Disk diffusion method (Prakash *et al*., 2018).

Determination of zone of inhibition against *E. coli*. The zone of antimicrobial activity of CuONPs, CoONPs and MgONPs was calculated against *E. coli* in millimeters using a digital vernier caliper scale. These metallic oxide nanoparticles exhibited a clear zone of inhibition against the tested micro-organisms. The zone of inhibition ranged from 17~18 mm for Co, 6~10 mm for Mg and 10~11 mm for Cu against tested bacteria (Table. 1). The zone of inhibition of CuONPs, CoONPs and MgONPs from *Cymbopogon citratus* against *Escherichia coli* cells is provided in Fig. 7(a), (b) and (c).

Conclusions

This study establishes a simple conventional sol-gel method for the synthesis of newer metallic nanoparticles from the leaves of *Cymbopogon citratus* (lemongrass) with different metals. Initially, these metallic oxide nanoparticles were synthesized by optimizing their maximum absorption in the visible region. The structure of these newer nanoparticles (CuONPs, CoONPs and MgONPs) was elucidated through various spectroscopic techniques (FT IR, UV/Vis, SEM, EDS). Finally, their biological activities were also observed, confirming the validity of metallic oxide nanoparticles. Antimicrobial screening results showed that these metallic oxide nanoparticles exhibited an effective response against *Escherichia coli*. The antibacterial activity of these NPs increased with higher concentrations, resulting in an enhanced area of inhibited growth as the sample solution concentration increased. Therefore, these metallic oxide NPs demonstrated positive behavior toward bacterial species with acetate groups of Cu, Co and Mg salts.

Conflict of Interest. The authors declare that they have no conflict of interest.

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