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Research Article

Green Synthesis of Trimetallic Oxides (CuO-ZnO-MnO) Nanoparticles using *Ocimum basilicum* Aqueous Leaves Extract: Characterization and Antibacterial Activity

Fouzia Majeed, Syeda Shaista Gillani*, and Iram Bashir

Department of Chemistry, Lahore Garrison University, DHA Phase-VI, Lahore 54792, Pakistan

Abstract: Metal oxides nanoparticles (NPs) are becoming more and more significant day by day in a variety of fields, due to their unique physical, biological and chemical properties that have made them popular. In the current study, we developed a green chemistry method that is easy to use, cost-effective, safe for the environment, and produces trimetallic oxide (CuO-ZnO- MnO) nanoparticles using an aqueous leaves extract of *Ocimum basilicum*. The sequential reduction of zinc chloride, manganese chloride, and copper chloride solutions resulted in the production of trimetallic oxide (ZnO-MnO-CuO) nanoparticles within 5 minutes at 35 °C, indicating a faster reaction rate than the chemical techniques used before for such synthesis.Trimetallic oxide nanoparticles (NPs) were charecterized by FTIR, SEM-EDX, XRD, and UV-visible spectropic techniques. A characteristic Surface Plasmon Resonance (SPR) band to confrm trimetallic oxide nanoparticles (NPs) synthesis was observed at ~450 nm of λ light by UV–Visible spectrophotometer. There were noticeable peaks in the FTIR spectra which confirmed the presence of variuos functional groups in the trimetallic oxide NPs. SEM results showed the shape and polydispersive nature of the NPs, the existence of Mn, Cu, Zn, and O atoms was confirmed by EDX. The findings of the XRD results also confirmed the synthesis of nanoparticles. Synthesized trimetallic oxide (CuO-ZnO-MnO) nanoparticles were screened for antibacterial activity against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). The agar well diffusion assay revealed that trimetallic oxide (CuO-ZnO-MnO) nanoparticles have the highest efficacy against *Staphylococcus aureus*. As a result, trimetallic oxide nanoparticles may be effective antibacterial agents in the pharmaceutical sector.

Keywords: Trimetallic Oxide Nanoparticles, Green Synthesis, Structural Characterization, Antibacterial Activity.

1. INTRODUCTION

Nanoscience is a research endeavor aimed at gaining full control over atomic and molecular surfaces. This branch of science describes molecular engineering to create functional structures (at nanoscale). Significant progress has been made in the last 20 years, even though this scientific field is still in its early stages and is expected to grow quickly in the future. The importance of nanoparticles has increased due to the advancement in chemical sensors, microelectronics, and magnetic data storage devices [1]. Planning, synthesis, implementation, and understanding of the basic phenomena and physical properties of nanomaterials are all part of nanotechnology [2, 3]. Nanoparticles are particles that range from 1 to 100 nanometers for laboratory uses and that increase from 1000 nanometers in size are used for industrial purposes [4, 5]. Individual atoms or molecules, as well as bulk materials, are sufficiently distinct from nanoparticles.

Nanomaterials have exceptional chemical, magnetic, and optical properties because of their greater surface area to volume proportion, making them extremely useful [6]. Because of their possible properties in plasmonic, metal oxide nanoparticles have attracted the interest of researchers [7]. Numerous methods were reported for the synthesis of nanomaterials with desired properties [8-13]. Since the properties of nanomaterials are dependent upon their size, hence a suitable method for controlling the nanoparticle's size is needed. Nanoparticles are synthesized by the chemical,

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* Corresponding Author: Syeda Shaista Gillani <shaistaaligillani@gmail.com>

physical, and green approaches. Green chemistry is the key to design chemical products that eliminates the use of hazardous chemicals [14]. Besides various chemical and physical approaches, green synthesis for the production of nanomaterial is gaining popularity. As a reducing and precipitation agent, plant leaves, stem or root extract, or any other biomaterials are used in the green process. Plant used in current study is *Ocimum basilicum* (Figure 1). The common name is Basil, Sweet Basil in English, as Babuli Tulsi in Bengali and Hindi. *Ocimum basilicum* has a lot of medicinal values as pain-relieving activity, anti-swelling action, anti-microbial activity, anti-oxidant action, anti-ulcer activity, cardiac stimulating action, chemo modulatory activity, hepatoprotective activity, and hypoglycemic action.

Metal nanoparticles are classified as monometallic, bimetallic, and trimetallic. Bimetallic and trimetallic nanoparticles have stronger catalytic activity and better performance in various fields than monometallic nanoparticles, as trimetallic nanoparticles can be used as heterogeneous nano-catalysts in a variety of organic processes [15, 16]. Trimetallic nanoparticles have been recently explored, because of their novel physicochemical features caused by the synergetic properties of their monometallic complements [17]. Trimetallic nanoparticles have gained familiarity due to their exceptional characteristics, strong features, as well as creative applications [18-21] in cancer therapy and diagnosis, catalytic applications, antimicrobial activities and active food packing. Nano-scientists and researchers worked for nano-size as well as fine geometry of multi-metallic nanoparticles as a heterodimer, core, and amalgams to improve their catalytic performance [22-24].

The present study aimed the synthesis of trimetallic oxide (CuO-ZnO-MnO) nanoparticles by using green method. Significant antimicrobial activity was also explored against various bacterial stains.

2. MATERIALS AND METHODS

The chemicals utilized were all of analytical grade and purchased from reputable vendors. The plant (*Ocimum basilicum)* was obtained from Jinnah Garden, Lahore, Pakistan.

Fig. 1. *Ocimum basilicum* Plant.

2.1. Preparation of Extract

To prepare plant extract, plant powder (12 g) was mixed to distilled water (100 mL) with continuous stirring at 40 °C for 45 minutes. Prepared plant extract was chilled and filtered.

2.2. Synthesis of Trimetallic Oxide Nanoparticles

Leaf extract was mixed with 0.25 M equimolar salt solution of Zinc chloride, Copper chloride and Manganese chloride in 1:1 ratio. In the reaction mixture 100 mL of leaf extract was added in 100 mL of salt solution at 35 °C. Distilled water was used to wash the synthesized NPs. Reaction medium was left for 30 minutes at 25 °C to settle down nanoparticles. The pellets of nanoparticles were attained after centrifugation at 2000 rpm. Nanoparticles were dried at 45 °C in oven for 23 hours. Dried NPs were calcined in muffle furnace at 550 °C for 1 hour. The flow sheet diagram for the synthesis of trimetallic oxide nanoparticles is shown in Figure 2.

2.3. Characterization Techniques

Different instruments were used for the synthesis and chareterization of trimetallic oxide (CuO-ZnO-MnO) nanoparticles: Electronic balance (FA2004). pH-meter (Cyberscan 500[PH),]Desktop constant temperature drying oven (WHL25A), Centrifuge machine (GY773), Digital autoclave (HICVAVETTI HVE-50), Muffle furnace (SX-2.5-10), UV-Visible (Ultra-3000) spectrophotometer, FTIR (Cray 360 FTIR) spectrophotometer, X-Ray diffractometer (AXS SMART APEX-I), and Scanning Electron Microscope (HITACHI S-3400N).

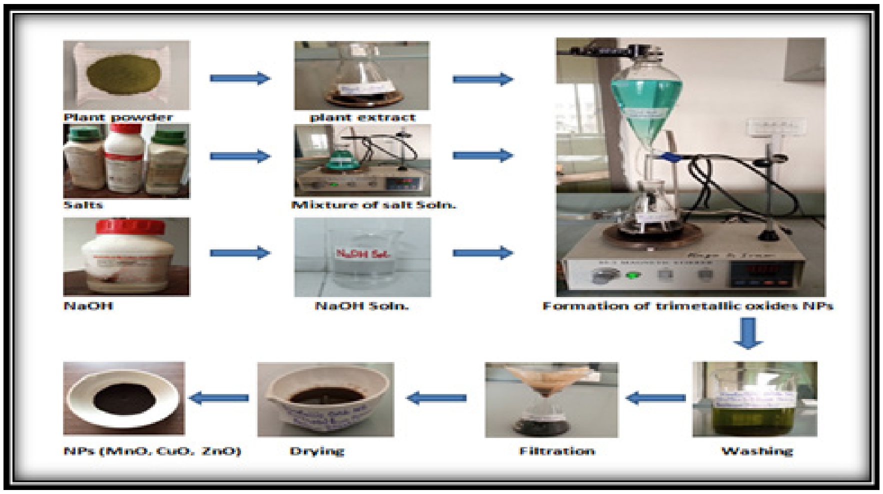

Fig. 2. Flow sheet diagram representing green synthesis of trimetallic oxide nanoparticles.

3. RESULTS AND DISCUSSION

Various spectroscopic techniques, such as UV-Visible, FTIR, XRD, SEM-EDX were employed to characterize the synthesized trimetallic oxides (CuO-ZnO-MnO) nanoparticles.

3.1. UV-Visible

Trimetallic oxide (CuO-ZnO-MnO) NPs were characterized by UV-Vis spectrophotometer. This technique was also used to monitor the production of trimetallic oxide nanoparticles. The colour of reaction medium was changed from dark brown to colloidal light brown, demonstrating the surface plasmon resonance effect (SPR) of trimetallic oxide nanoparticles. After the formation of nanoparticles, the clear aqueous extract solutions transformed into colloidal solutions. The results showed the absorption maximum at 280-370 nm that matches with ranges reported (CuO-ZnO-MnO) [25]. The absorption peak given in the Figure 3, represents the surface plasmon resonance phenomenon that occurs when nonbonding electrons were excited to an upper energy state, from the ground state as indicated by the shift in colour from dark brown to light brown. The reasonably strong absorption peaks of trimetallic oxide NPs specify the NP distribution's monodispersed character [22, 23].

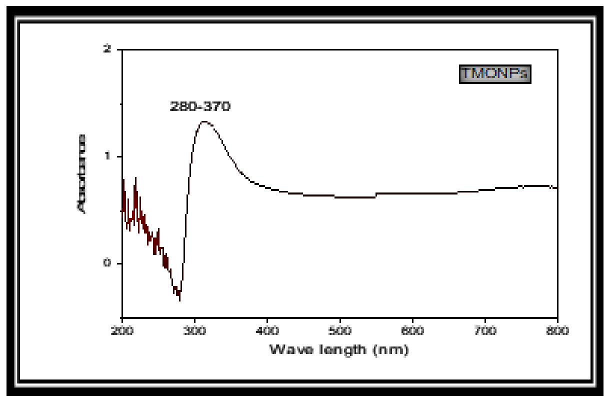

Fig. 3. UV-Visible spectrum of trimetallic oxide nanoparticles.

3.2. FTIR

FTIR has been employed to confirm the different functional groups present in plant extracts that are responsible for bio-reduction of metal ion to trimetallic oxide NPs and consequently their stabilization. Figure 4 and 5 showed FTIR spectra of studied plant extracts (*Ocimum basilicum*) as well as trimetallic oxide nanoparticles derived from the extract, respectively. The O-H stretching vibrations for alcohols and phenols at 3500-3200 cm^{-1} appeared as broad band in extract [25], while the same appeared as narrowed band in nanoparticles. The peak at 1640 cm^{-1} confirmed the stretching vibrations of C=O in carboxyl and bending vibrations of C=N present in the amide functional group of flavonoids, phenolic acids, and other compounds [26]. The bands around 424, 568, and 635cm^{-1} are assigned to trimetallic bonding with oxygen (ZnO, CuO [27], and MnO [28] bands from hydroxyl groups [29, 30]. The FTIR spectrum of the trimetallic oxide nanoparticles attained from leaf extracts showed the occurrence of a number of functional groups (Table 1) connected with active phytochemicals like as flavonoids, aromatic

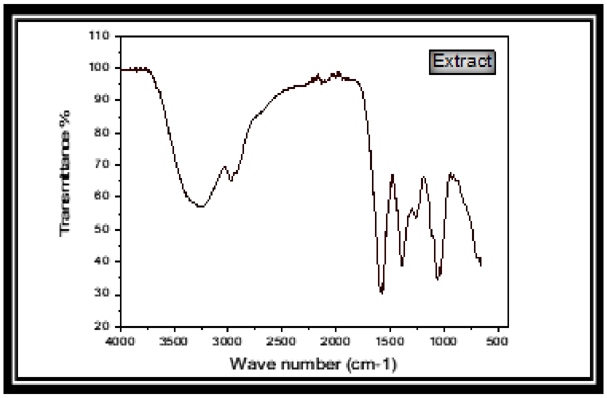

Fig. 4. FTIR spectrum of Plant Extract.

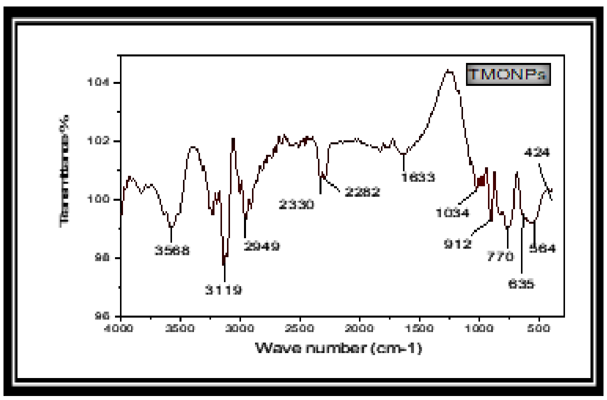

Fig. 5. FTIR spectrum of trimetallic oxide nanoparticles.

Table 1. FTIR peaks of trimetallic oxide nanoparticles.

Wavenumber (cm^{-1})	Functional groups
3568	O-H stretching
3119	Aromatic C-H stretch
2949	C-H_3 stretch
2330	Stretching of CO_2, CH
1633	C=N, C=O stretching vibration in carboxyl
912	O-H Bending
424, 564, 635	Cu-O, Zn-O, Mn-O

compounds, and phenolic acids, among others, which were most probably to be responsible for bio reduction of metal precursor and capping of synthesized trimetallic nanoparticles.

3.3. XRD

This technique was used to measure the crystallinity of nanoparticles. The XRD measurements was carried out by using Bruker AXS D8 Discover diffractometer at 40 kV, with 35 mA at room temperature. The diffracted angle 2θ was selected from 10° to 80° (Figure 6). The interplanar spacing between atoms of nanoparticles was studied by using Bragg's law:

$$n\lambda = 2d \sin\theta \qquad (1)$$

Where, θ is diffraction angle, λ is wavelength of X-ray radiations of Cu K-α which is 1.5406 Å, d is interplanar spacing and n = 1, 2, 3, 4….

The XRD peaks of green synthesized trimetallic oxide (CuO, MnO, ZnO) nanoparticles of *Ocimum basilicum* leaf, at diffracted angles 20.18°, 35.6°, 39.26° and 56.22°were indexed as (100), (021), (111) and (160) planes for MnO, respectively (JCPDS card No. (04-0326)), which indicated Orthorhombic symmetry for MnO nanoparticles. For ZnO nanoparticles, diffracted peaks at 31.77°, 35.6°, 47.16°, 56.22°, and 67.43° were indexed as (100), (101), (102), (110) and (112) indexes planes, respectively. These peaks showed resemblance with those described in JCPDS card No. (36-1451) which indicated Hexagonal ZnO nanoparticles Barzinjy and Azeez [32]. For CuO nanoparticles, diffracted peaks at 31.77°, 35.6°, 39.26°, 47.16°, 49.88° and 67.43° were indexed as (-110), (002), (200), (-112), (-202) and (113) indexes planes, respectively. These peaks showed resemblance with those described in JCPDS card No. (45-0937) which indicated Monoclinic shape for CuO nanoparticles crystal.

Moreover, the XRD spectrum showed few impurity peaks in addition to distinctive CuO, ZnO and MnO nanoparticles peaks, which indicated the presence of impurities in synthesized nanoparticles.

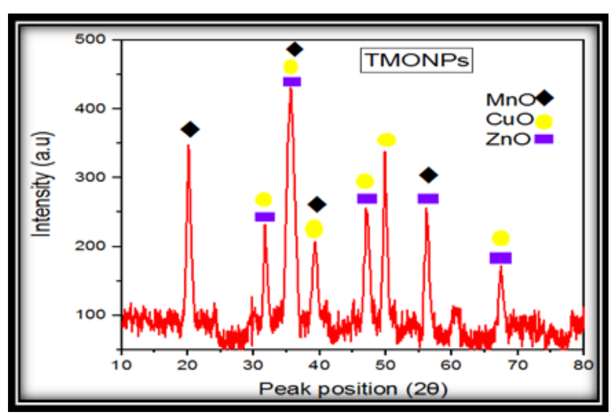

Fig. 6. XRD spectrum of trimetallic oxide nanoparticles.

Moreover, the robust and relatively broad diffraction peak showed that some other moieties were also present [30, 31].

3.4. SEM-EDX

The monoclinic (CuO), orthorhombic (MnO) and hexagonal (ZnO) shapes of trimetallic oxide nanoparticles are shown by SEM (Figure 7). The SEM results were in good agreement with XRD results. From the EDX spectra (Figure 8) Copper, zinc, manganese, and oxygen are found in trimetallic nanoparticles synthesized from *Ocimum basilicum* leaf extracts. However, trace components such as potassium, sulphur, and chlorine have also been found, which were more likely due to the presence of minerals in the soil in the form of metals and transported into various parts of the plant such as the leaf. In contrast to zinc and manganese, the resulting weight percentage for copper is lower than the feed stoichiometric ratio Table 2). Furthermore, the proportion of oxygen is considerable (about 66.36% in atomic and 44.71% in weight percent composition in trimetallic oxide nanoparticles

Fig. 7. SEM of nanoparticles.

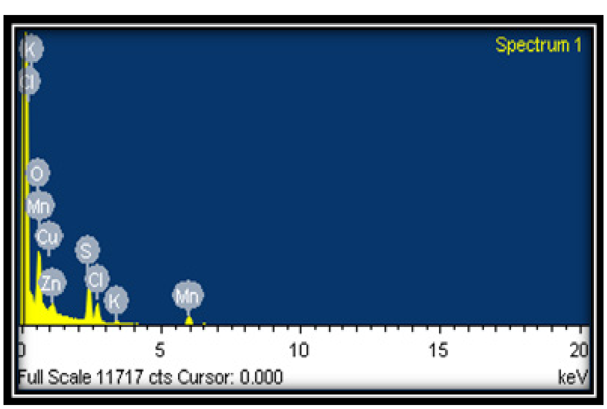

Fig. 8. EDX of nanoparticles.

Table 2. Results of EDX analysis of nanoparticles.

Element	Weight%	Atomic%
Oxygen	41.71	66.36
Manganese	18.48	5.96
Sulfur	14.46	2.48
Chlorine	10.06	2.76
Zinc	8.98	1.20
Copper	4.90	1.14
Potassium	1.42	0.93

produced from leaf extracts), that was most likely owing to its absorption from the reaction media throughout the bio-reduction procedure.

3.5. Antimicrobial Activity

Ocimum basilicum extracts have long been used for their powerful antibacterial and antifungal activities, both traditionally and commercially [9]. The varied chemical profile of *Ocimum basilicum* and high concentration of phenolic (-OH) components present in it, indicated that the therapeutic capabilities of plants were dependent on the precise chemical groups separated. Tannins, terpenoids, quinones, polysaccharides, phenols, and flavonoids are examples of antimicrobial compounds. In the present work, the green production of Trimetallic oxide NPs capped with these essential phytochemicals adds to the phytochemical mixture's improved characteristics. This is especially important for overcoming antimicrobial resistance with higher benefits and lesser harm to people.

3.5.1. Inhibitory effects of trimetallic oxide NPs on the growth of bacterial strains

The clinical pathogenic strains used in the study were E. coli (gram-nagative) and S. aureus (gram-positive). The antibacterial activity of trimetallic oxides (ZnO, CuO, MnO) nanoparticles against the studied pathogenic strains is shown in Figure 9. The antimicrobial activity of synthesized trimetallic nanoparticles (100µg/mL) was studied against pathogenic strains and their zone of inhibition was found as 25 mm for nanoparticles and 28 mm for standard (sulfamethoxazole) against E. coli. Zone of inhibition against S. aureus was 33 mm for both nanoparticles and standard (Table 3). Solvent

Table 3. Zone of Inhibition (ZOI) NPs against *E. coli* and *S. aureus*.

Clinical pathogenic strains	Zone of inhibition (mm)		
	Standard (Sulfamethoxazole) positive control	Trimetallic oxides (CuO, ZnO, MnO) NPs	Solvent (Double distilled water) negative control
Escherichia coli	28	25	0
Staphylococcus aureus	33	33	0

Table 4. Minimum Inhibitory Concentration (MIC) of NPs against *E. coli* and *S. aureus*.

Clinical pathogenic strains	Minimum inhibitory concentration (µg/mL))	
	Standard (Sulfamethoxazole) positive control	Trimetallic oxides (CuO, ZnO, MnO) NPs
Escherichia coli	5	10
Staphylococcus aureus)	8	12

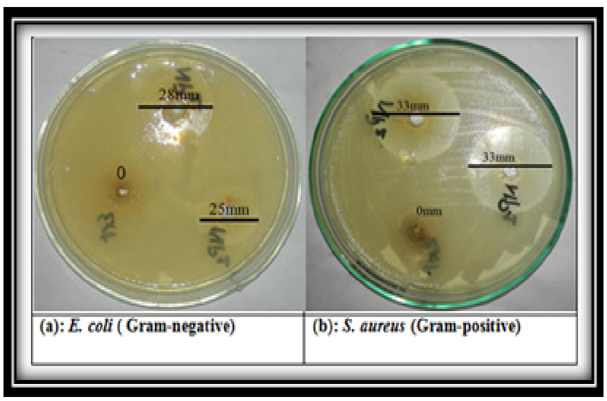

Fig. 9. Zone of inhibition of growth by NPs against E. coli (a) and S. aureus (b).

(double distilled water) showed no antimicrobial activity. Minimum Inhibitory Concentration (MIC) of nanoparticles for *E. coli* was 12 µg/mL and for *S. aureus* was 8 µg/mL. MIC of sulfamethoxazole was 10µg/mL for *E. coli* and 5µg/mL for *S. aureus* (Table 4).

4. CONCLUSIONS

Green synthesis method was used to effectively synthesize trimetallic oxides (CuO, MnO, ZnO) NPs using *Ocimum basilicum* leaf extract, NaOH solution, and equimolar precursor salt solution ($ZnCl_2$, $MnCl_2$, and $CuCl_2$). The sequential reduction of metal ions resulted in the production of nanoparticles within 5 minutes at 35 °C, indicating a faster reaction rate than the chemical techniques used before for such synthesis. UV-Visible spectroscopy, SEM-EDX, XRD, and FTIR confirmed the prominent functional groups and verified the existence of stable, monoclinic (CuO), orthorhombic (MnO) and hexagonal (ZnO) nanoparticles with well-defined dimensions. Moreover, nanoparticles showed significant antibacterial action against *Escherichia coli* and *Staphylococcus aureus*.

5. ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

The authors declare no competing conflict of interest.

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