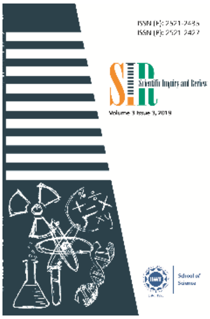

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Green Synthesis of Mixed Metal Oxide (MnO, CuO, ZnO) Nanoparticles (NPs) using Rose Petal Extract: An investigation of their Antimicrobial and Antifungal Activities

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Abstract

In this study, mixed metal oxide (CuO, ZnO, and MnO) nanoparticles (NPs) were synthesized via the green process, which is considered to be simple, cost-effective, eco-friendly, and non-toxic. During the green synthesis method, rose petal extract was used as a reducing and capping agent, while salt solutions (CuCl2, MnCl2, and ZnSO4) were used for the bio-reduction of metal precursors, leading to the formation of mixed metal oxide (CuO, ZnO, MnO) NPs. UV-Visible and FTIR spectroscopy were used for the analysis of mixed metal oxide (CuO, ZnO, MnO) NPs. The results showed maximum absorbance of the mixed metal oxide in the range of 280 to 370 nm. The presence of particular peaks in FTIR verified the synthesis of mixed metal oxide nanoparticles. Subsequently, the antimicrobial and antifungal activities of mixed metal oxide (CuO, ZnO, MnO) NPs were tested by using the disc diffusion method and well diffusion method. Mixed metal oxide (CuO, ZnO, MnO) NPs displayed antimicrobial activity against Escherichia coli and antifungal activity against Candida albicans, Curvularia lunata, Aspergillus niger, and Trichophyton simii. Hence, these mixed metal oxide (CuO, ZnO, MnO) NPs can be effectively used in the pharmaceutical sector.

Keywords: antifungal, antimicrobial, green synthesis, mixed metal oxide nanoparticles (MONPs), rose petal extract

Introduction

Nanoscience is an 'interdisciplinary science', which means that it deals with more than one discipline, such as biotechnology, chemistry, surface science, physics, biology, and biotechnology. [1]. Nanotechnologies are the application of nanoscience that are based on the manipulation, control, and combination of atoms and molecules to form materials, components, structures, devices, and systems at a nanoscale. A nanomaterial is a material that has at least one dimension on the nanometer scale from 1 to 100 nm. Nanoparticles have gained significance due to having a wide range of applications in the field of bio-medical, sensors, antimicrobials, catalysts, electronics, optical fibres, agricultural, bio-labelling, and cosmetics [2]. Techniques used for the synthesis of nanoparticles are mostly categorized into two processes: the "Bottom-up" process and the "Top-down" process. In the Bottom-up process, smaller molecules combine to form new nuclei, which grows into nanoscale particles with the help of various techniques such as aerosol process, spray pyrolysis, laser pyrolysis, sol-gel process, and chemical vapour deposition [3]. In the Top-down process, the material used is fragmented into particles at the nanoscale with several methods, such as chemical etching, laser ablation, mechanical milling, sputtering, and electro-explosion [2].

Traditional production of nanoparticles comprises expensive chemical and physical processes that frequently use poisonous ingredients having potential threats, such as carcinogenicity, cytotoxicity, and environmental toxicity [4]. To prevent undesirable accumulation of the colloids, substances, such as reducing agents, organic solvents, and stabilizers, are used. These substances are harmful and create toxicity problems in the environment. In addition to their size, shape, surface chemistry, and composition, some nanoparticles have also been found to be toxic. The presence of contaminated agents on the manufactured nanoparticles and their toxicity prohibits their medical and biomedical application. The factors can be hypothetically predefined and organized by the manufacturers. For this reason, interest in clean, benign, trustworthy, biologically companionable, and environment-friendly green processes to manufacture nanoparticles is increasing day by day [5]. Agreeing to the EPA definition, green chemistry is a well-defined field of chemistry that deals with biochemical products and processes that are inoffensive to the surroundings. Green chemistry, also known as sustainable chemistry, eliminates or reduces the production of hazardous substances. The products created through green chemistry are sustainable and do not affect the environment.

Furthermore, these products are synthesized using sustainable products, rather than using "exotic" reagent, which are not only costly, but also require a lot of energy when used [6]. Green chemistry is an interdisciplinary science that is based on ecological, chemical, and social responsibility, enabling creativity and the improvement of innovative research [6]. As a dynamic region of research, it tries to maintain a balance between the use of economic growth, natural resources, and environmental conservation.

The rose plant is used for various purposes around the world. Anthocyanin occurs in rose petals in high concentrations and has anti-bacterial, anti-oxidant, and anti-inflammatory ability. Furthermore, it is also responsible for providing energy to the plant's vascular system and decreasing blood platelet stickiness [7, 8, 9]. Rose belongs to the Rosacea family. There are around 10,000 species of rose, containing the most aromatic R. Damascena, R. gallica, R. indica, R. centifolia, R. muscatta, R. rugosa, and R. rubiginosa. R. damascena Mill is one of the most popular rose species because it contains beta-damascenone in high concentrations, which improves rose oil quality. Hence, it is generally used for the production of rose essential oil [10, 11].

Prior literature has extensively discussed and investigated the antibacterial, anti-fungal, and anti-oxidant ability of roses [12]. Rosacea has an abundance of phenolic and flavonoids compounds, also known as bioactive agents. Phenolic compounds are used in an extensive variety of biochemical activities, such as antibacterial, free radical foragers, antioxidants [13, 14], antimutagenic, anti-inflammatory [15], and anticancer [16]. Similar to phenolic compounds, flavonoids also display antioxidant activity. They safeguard DNA against oxidative damages. They can also be used for protection against UV radiation having wavelength of 254 nm as well as in sunscreens [17].

The existence of multidrug-resistant (MDR) pathogens has significantly increased the number of infectious diseases, which have also become the main cause of death around the globe [18, 19]. Extensive abuse and misuse of antibiotics are the primary cause of antibiotic resistance in bacteria [20]. A multidrug-resistant bacteria infection may lead to several complications and death. They cause individuals to be sick for longer periods of time, causing huge economic loss for the patient [21]. The bactericidal activities of nanoparticles have been reported to have high surfaces-to-volume ratio and size, which allows them strongly fuse with the microbial membrane. Several mechanisms, such as changing the permeability of membrane or production of reactive oxygen species, result in damage to bacterial cells. In recent years, antimicrobial composites in food packaging systems have gained popularity since it extends the shelf life of products [22]. Zinc oxide (ZnO), due to its high stability, is used in the packaging and processing of vegetables and meat [23, 24]. It is used in food packaging due to its antimicrobial properties [25]. Additionally, the antibacterial activity of ZnO nanoparticles coated polyvinyl chloride has been effectively used against Staphylococcus aureus and Escherichia coli (E. coli). In the same manner, the antimicrobial activity of copper oxide (CuO) nanoparticles was investigated against several strains of E. coli, Klebsiella pneumonia, Pseudomonas aeruginous, Enterococcus faecalis, Shigella flexneri, Proteus vulgaris, Salmonella typhimurium, and Staphylococcus aureus [26]. CuO nanoparticles (100–150 nm) manufactured by Streptomyces sp. were used to develop antimicrobial materials that can be used in hospitals to inhibit or reduce infections from pathogenic bacteria [27]. One study also used Molybdenum (MO) nanoparticles against Gram-negative bacteria (*E. coli, Salmonella typhi*) Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis*), and some funguses (*Aspergillus niger*, *Candida albicans, Curvularia lunata*, *and Trichophyton simii*). MO nanoparticles displayed an extremely high antibacterial and antifungal activity against bacteria and fungus [26].

2. Materials and Methods

2.1. Preparation of Rose Petal Extract

About 20g of rose petals were washed twice with tap water and rinsed twice with distilled water to remove dust and insects. It was put in 100 mL distilled water and boiled at a very low flame until the colour of the solution changes from colourless to dirty pink. After cooling, it was filtered and stored in a refrigerator.

2.2. Green Synthesis of Mixed Metal Oxide (CuO, ZnO, and MnO) NPs

Mixed metal oxide (CuO, ZnO, MnO) NPs were synthesized via the green process. 0.05M equimolar salt solution of $CuCl_2$, $MnCl_2$, and $ZnSO_4$ was prepared by dissolving 0.8524g, 0.98955g, and 1.43765g of salt in 50 mL of distilled water and stirred at 35 ^{0} C for 30 minutes to get a clear solution. This mixture of the solution was poured into a separating funnel and added dropwise into the solution of rose petal extract (50mL) with constant stirring. The drop-wise mixture was constantly and gradually added to the rose petal extract. After adding the solution, the rose petal extract's colour changed to ruby/wine red with deep scarlet deposition (blackish appearance) at the bottom. Furthermore, it was subjected to a rotatory evaporator to evaporate the solvent. Subsequently, the black coloured precipitates were obtained and were allowed to dry at room temperature overnight, and then they were placed in the oven at 150 ˚C for 20 minutes. After that, they were kept in a muffle furnace at a temperature of 600˚C for one day. The actual yield of mixed metal oxide (CuO, ZnO, MnO) NPs was 98g. Then the mixed-metal oxides of (CuO, ZnO, MnO) NPs were ground to a fine powder. Figure 1 and Figure 2 illustrate the steps involved in the synthesis of these mixed metal oxide (CuO, ZnO, MnO) NPs.

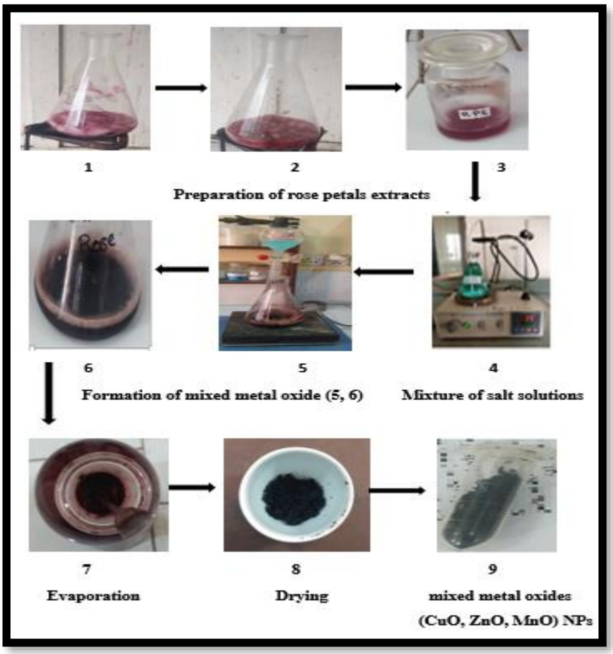

Figure 1. Steps involved for the synthesis of mixed metal oxide (CuO, ZnO, MnO) NPs

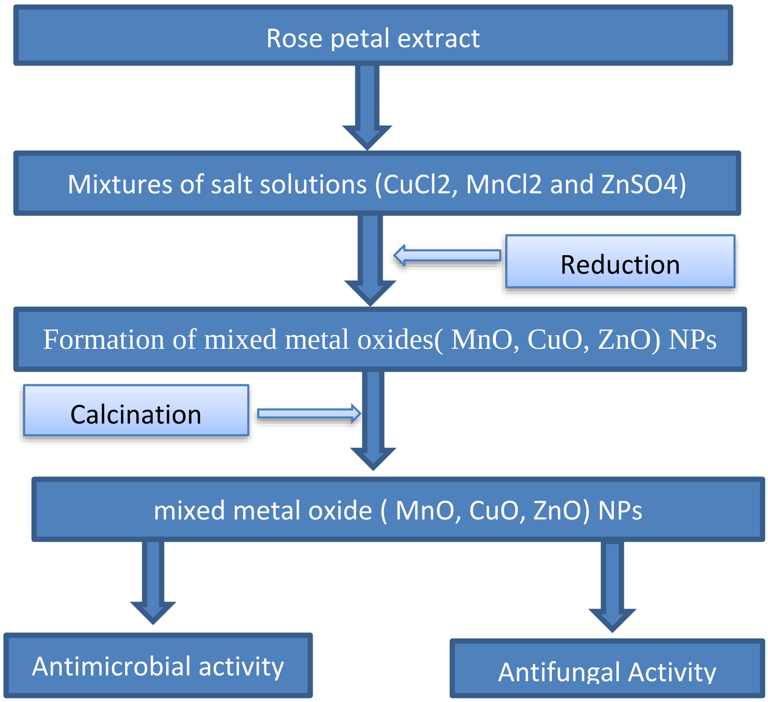

Figure 2. Flowsheet for the synthesis of mixed metal oxide (CuO, ZnO, MnO) NPs

2.3. Antimicrobial Activity

2.3.1. Preparation of Agar Media

Agar media was prepared by dissolving 1g tryptone, 0.5g yeast, 0.5g NaCl, and 1. Agar in 100 mL distilled water. Its pH was maintained at 7. Agar media was autoclaved for sterilization.

2.3.2. Protocol for Testing Antimicrobial Activity

TestingTwo protocols were used to test the antibacterial activity. The first was the agar well diffusion method, while the other was the one-disc diffusion method. These methods were used to detect the presence of antibacterial activity of the green synthesized mixed metal oxide (CuO, ZnO, MnO) NPs extracted from rose petal extract. Nutrient Agar was prepared and then autoclaved. After autoclaving, media was poured into two sterile Petri plates labelled as plate A and plate B. After media solidified on plates, both plates were wiped with *E. coli* suspension. Subsequently, the test strains (gram-negative bacteria) were uniformly spread over the media.

The disc diffusion method was performed on plate A. Four small discs of a diameter of 0.5 mm were prepared. Each disc was dipped in a different solution. One disc was dipped in salts solution, the next one was dipped in plant extract, and the other two discs were dipped in a mixture of mixed metal oxide (CuO, ZnO, MnO) NPs solution. These discs were allowed to dry and then put these discs were placed over the plate with the help of tweezers at four labelled points.

Then four 0.5mm diameter wells were made in plate B for the good diffusion method. Salts solution was added in the first well. In the second well, the plant extract was added. In the rest of the two wells, a 10µl solution containing 500µg mixed metal oxide (CuO, ZnO, MnO) NPs solution was added.

Both plates were incubated at 37^0C for 24 hours. After 24 hours, results were observed.

The antifungal activity testing was done using the agar well diffusion method. For this method, approximately 108 CFU/ml of inoculum suspension was prepared using *Sabouraud's* dextrose agar by suspending the fungal strains, such as *C. Albicans*, *C. lunata*, *A. niger*, *and T. simii* for 6 hrs. A total of 6 mm diameter wells were perforated into the agar. The solvent blanks (hydro alcohol and hexane) and samples were filled in the wells of the agar and the fungal plates were incubated overnight at 37°C.

3. Results and Discussion

The mixed metal oxide (CuO, ZnO, MnO) NPs were synthesized by the green process. The actual yield calculated for mixed metal oxide (CuO, ZnO, MnO) NPs was 82.624%.

3.1. Characterization Techniques

The mixed metal oxide (CuO, ZnO, MnO) NPs were characterized by UV-Visible and Fourier transforms infrared spectroscopy (FTIR).

3.1.1. UV-Visible Analysis

The Ultra 3000 UV-Vis spectrophotometer was run to record the UV-visible spectrum of green synthesized mixed metal oxide (CuO, ZnO, MnO)

NPs. The spectrophotometer was permitted to scan at wavelengths ranging from 200 to 800nm. UV–Vis spectroscopy was used to monitor the production of mixed metal oxide nanoparticles. The reaction medium's colour changed from dark brown to colloidal light brown, demonstrating the surface plasmon resonance effect (SPR) of mixed metal oxide nanoparticles. After the creation of nanoparticles, the clear aqueous extract solutions transformed into colloidal solutions. It is important to highlight that attaining distinct peaks for individual metal oxides (CuO, ZnO, MnO) present in the structure of mixed metal oxide may not be possible, owing to the presence and interaction of several compounds in the reaction mixture. The absorption peak seen in Figure 2 represents the surface plasmon resonance (SPR) phenomenon, which occurs when the ground state nonbonding electrons are excited to an upper energy state. This process was indicated by the shift in colour from dark brown to light brown. The relatively strong absorption peaks of mixed metal oxide NPs indicate the NP distribution's monodispersed character [28].

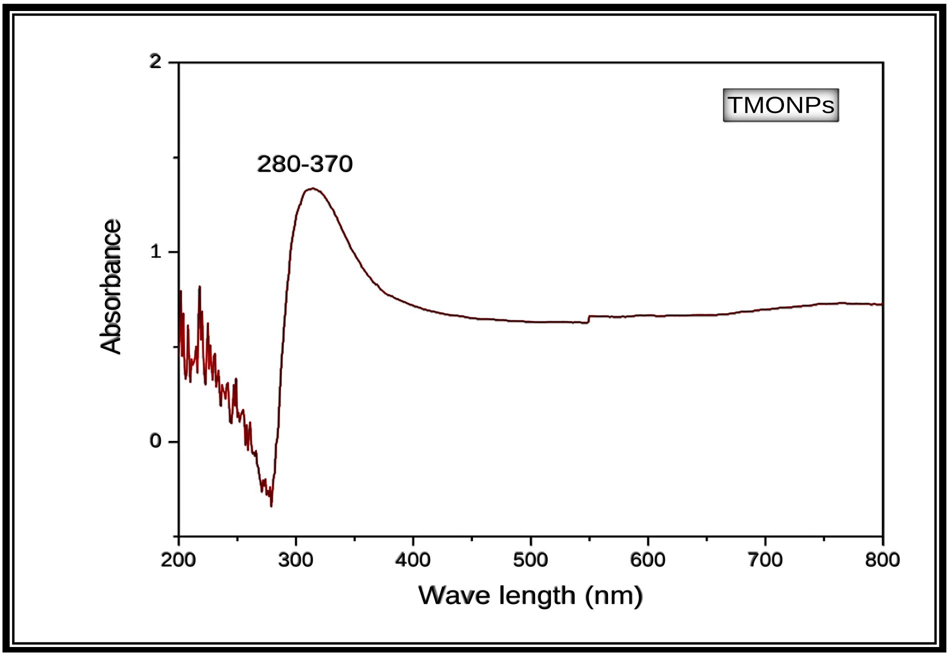

Figure. 2. UV-Visible spectrum of mixed metal oxide (CuO, ZnO, MnO) nanoparticles (MONPs)

3.1.2 FTIR Spectroscopy

FTIR was performed to identify potential functional groups present in the rose petal extract. These groups are accountable for the bio-reduction of metal ions to mixed metal oxide NPs and subsequently their stabilization. Figure 3 and Figure 4 illustrate FTIR spectra of rose petal extract and mixed metal oxide (CuO, ZnO, MnO) NPs, respectively. The presence of wide bands in the range of 3500-3200 cm^{-1} in rose petal extract and nanoparticles refers to the O-H stretching of alcohols and phenols [29]. This band narrowed in the nanoparticles' spectra as alcohols or phenols were consumed for the bio-reduction of metals into corresponding metal oxide nanoparticles. The peak at 1639 cm^{-1} showed stretching vibrations of C=O in carboxyl or bending vibrations of C=N in the amide functional group of flavonoids, phenolic acids, and other compounds [30]. Hence, the bands around 424, 564 and 635 cm^{-1} were allocated to mixed metal oxide bonding with oxygen ZnO, CuO [31], and MnO [29] bands from hydroxyl groups [32, 33].

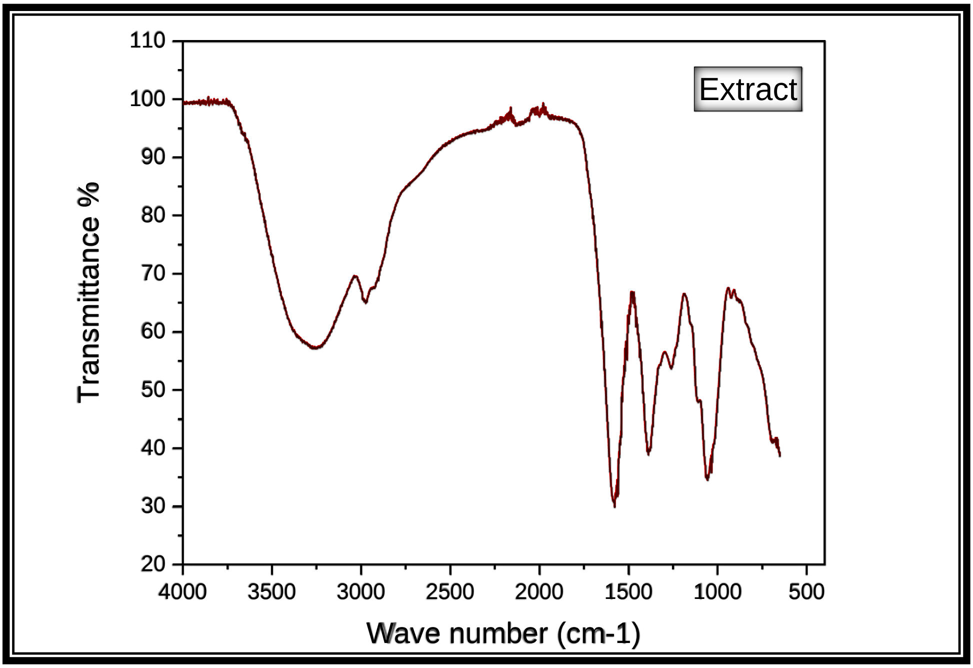

Figure. 3. FTIR spectrum of rose petal extract

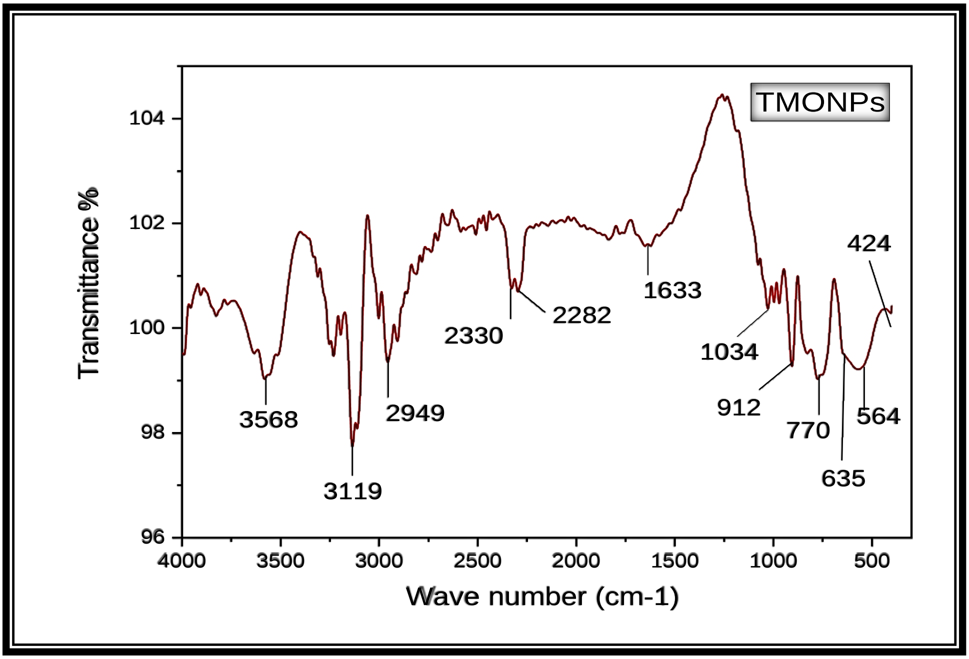

Figure 4. FTIR spectrum of mixed metal oxide (CuO, ZnO, MnO) nanoparticles (MONPs)

The FTIR spectrum of the mixed metal oxide (CuO, ZnO, MnO) NPs attained from rose petal extract showed the occurrence of many functional groups (Table 1). They were probably responsible for the bio-reduction of metal precursors and the capping of synthesized mixed metal oxide (CuO, ZnO, MnO) NPs.

Table 1. FTIR Peaks Analysis

Wavenumber (cm)	**Functional groups**
3567	O-H stretching
3119	Aromatic C-H stretch
2949	$C-H_3$ stretch
2304	Stretching of CO_2, CH
1639	C=N, C=O stretching vibration in the carboxyl
912	O-H Bending
424, 564, 635	Cu-O, Zn-O, Mn-O

3.1.2. Antimicrobial Test

Plate A indicated negative results since no zones appeared. However, zones of inhibition appeared on plate B around the well having mixed metal oxide (CuO, ZnO, MnO) NPs indicating antimicrobial activity of mixed metal oxide (CuO, ZnO, MnO) NPs synthesized from rose petal extract (Figure. 5).

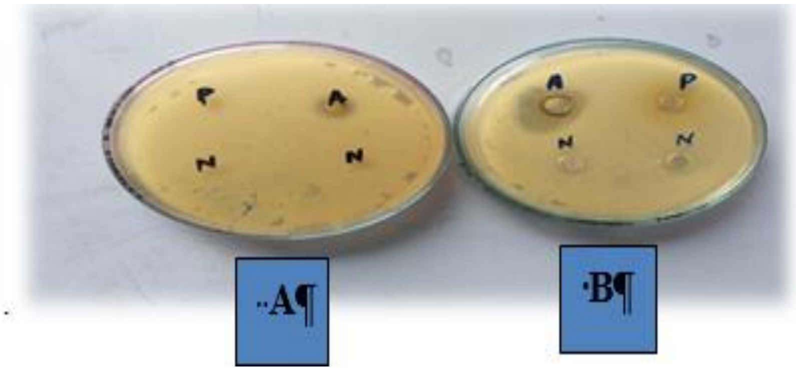

Figure 5. No zone of inhibition on plate A (negative result) Zone of inhibition on plate B (positive result)

3.1.4 Antifungal Activity

The antifungal activity of mixed metal oxide (CuO, ZnO, MnO) NPs was checked against four funguses, that is, *Candida albicans*, *Curvularia lunata*, *Aspergillus niger*, and *Trichophyton simii.* According to the zone of inhibition, illustrated in Table 2, mixed-metal oxide (CuO, ZnO, MnO) NPs show maximum antifungal activity against *C. lunata,* moderate to mild activity against *T. simii* and *A. niger*, and the least activity against *C. Albicans.*

Table 2. Estimation of antifungal efficacy using zone of an inhibition test method

Fungal Species	Zone of inhibition diameter (mm/sample)
C. albicans	25
T. simii	19
C. lunata	15
A. niger	18

4. Conclusion

In this study, green synthesis of mixed metal oxide (CuO, ZnO, MnO) nanoparticles was carried out by using rose petal extract. It is an efficient, sustainable, and low-cost approach. UV-Visible and FTIR spectroscopy was used to examine the specific functional groups attached to the surface of nanoparticles. They were also used to verify the existence of stable nanoparticles. Mixed metal oxide (CuO, ZnO, MnO) NPs displayed antimicrobial activity against gram-negative bacteria (*Escherichia coli*) and antifungal activity against *Candida albicans*, *Curvularia lunata*, *Aspergillus niger*, and *Trichophyton simii*. Hence, these mixed metal oxide (CuO, ZnO, MnO) NPs can be effectively used in the pharmaceutical sector

Conflict of Interest

The authors declare no conflict of interest.

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