

Synergetic Comparative Study: Synthesis, Characterization, and Biological Investigations of Biosynthesized Zinc Oxide Nanoparticles of Aloe Barbadensis Miller, Citrus Lemon, and Ficus Carica Leaf Extracts

Sajjad Hussain Sumrra, Amina Irfan, Ayesha Siddiqa, Wardha Zafar

Department of Chemistry, University of Gujrat, Gujrat, Pakistan

Abstract: Microbial resistance is an emerging issue, and nano-sized materials have been introduced as a promising solution for this type of resistance. Zinc oxide nano-scale particles (ZnONPs) are good agent for antimicrobial and antioxidant activity. Among all types of synthetic methods, plant-based ZnO nanoparticles have been reported as low-cost, safe to perform, and environmentally friendly. In this research work, extracts of different plants are used to synthesize ZnO nanoparticles from zinc sulphate heptahydrate solution. In the study, extracts of plants, including aloe vera, lemon, and fig, were auspiciously used to synthesize ZnO nanoparticles. Different techniques were used for the analysis of the synthesized ZnO nanoparticles. ZnO nanoparticles produced by using *Aloe barbadensis* Miller, *Citrus lemon*, and *Ficus carica* leaf extract were investigated through ultraviolet visible (UV/Vis), Fourier-transform infrared (FT-IR) spectroscopy, and fluorescence spectrophotometry. Similarly, for antimicrobial activity disc diffusion method was adopted. The presence of the phytochemicals that behave as capping agents surrounding ZnO nanoparticles was proven by Fourier-transform infrared spectroscopy. The ZnO nanoparticles, at a concentration of 50 µg/mL, showed potent antimicrobial activity against *Micrococcus luteus* and *Bacillus halodurans*, and ZnO nanoparticles' antifungal activity was determined against two fungal strains, *Aspergillus niger* and *Aspergillus flavus*. The results revealed that the antimicrobial agent, ZnO nanoparticles, can be synthesized using different plant extracts.

Keywords: ZnO Nanoparticles, Green Synthesis, Aloe Vera, Lemon, Fig, Biological Activities

Email: sajjadchemist@uog.edu.pk

1. Introduction

The idea of nanotechnology was first given by Richard Feynman. Nanotechnology is one of the looming topics of today, and the base of nanotechnology is nanoparticles. Nanoparticles are named nano just because of their small size. The size of the particles ranges from 1-100 nm, so these particles are called nanoparticles [1]. In modern material sciences, the field of nanotechnology is one of the most emerging fields. Nanotechnology is very important due to its wide range of applications in science and technology. Due to its applications, these materials are called the marvel of modern medicine [2]. Nano-scale medicines kill 650 cells while an antibiotic kills a half-dozen disease-causing pathogens. Nanotechnology is one of the environmentally friendly, enduring, and less costly technologies [3]. ZnO nanoparticles have been prepared *via* various procedures, like physicochemical methods. These conventional methodologies for the synthesis of metal oxides are costly and toxic due to the

use of hazardous chemicals [4]. There are different methods for the production of nanoparticles, but we prefer green synthesis for designing metallic nanoparticles because this approach for the synthesis of nanoparticles is environmentally friendly. Other methods, including chemical methods, in which chemicals are used that are hazardous to human beings and other living things [5]. Many physical methods are Pulse laser deposition, Thermal evaporation, and Laser ablation. In these methods, very expensive materials and apparatus are utilized for the synthesis of nanoparticles [6]. While chemical processes are Pyrolysis, Electro-deposition, the Sol-Gel method, and Precipitation. In these methods, hazardous chemicals are used, which are unsafe for human beings as well as other living organisms [7]. But the need for less noxious and less hazardous methods for the production of nanoparticles is required, so we use green synthesis in which plant extracts are used which are nontoxic to living things [8]. Many chemists have used green synthesis for the synthesis of metal/metal oxide nanoparticles through plant leaf extracts to further search for their several implementations [9].

Metal oxide nanoparticles have many biological and biomedical applications, like antibacterial, antifungal, antioxidant, anticancer, antidiabetic, and enzyme inhibition [10]. In the biological synthesis, plant extract, extracts of fruit, vegetables, fungi, and microbes are used for the biosynthesis of metal-based and metal oxide-based nanoparticles [11]. Biological synthesis has many advantages over other methods of synthesizing nanoparticles [12]. Biological synthesis is eco-friendly due to the usage of plants, less toxic due to the usage of plants rather than chemicals, and less costly due to the usage of simple instruments [13]. Many reported ZnO nanoparticles by using different plants are *Monsonia burkeana*, *Aloe barbadensis* Miller, *Citrus aurantifolia*, *Azadirachta indica*, and *Calotropis Gigantea* [14]. In this research work, ZnO nanoparticles were synthesized by using the aqueous extract of three different plants: *Aloe barbadensis* Miller (Aloe vera), *Citrus lemon* (Lemon), and *Ficus carica* (Fig). The main aim of the research work was to check the ability of these nanoparticles against pathogenic microorganisms [15].

2. Materials and Methods

All the chemicals, solvents, reagents, and standards used in the activity were obtained from Sigma-Aldrich. These reagents were utilized without any refining. All glassware was carefully washed, dried, and rinsed with distilled water. The water used throughout the research was double-distilled.

2.1 Gathering the Plant Material

Leaves of the species of plants, including *A. barbadensis*, *C. lemon*, and *F. carica*, were

assembled in January 2021 from the countryside of Wazirabad. The leaves of these plants (Figure 1) were thoroughly washed to remove all dust particles with distilled water [16].

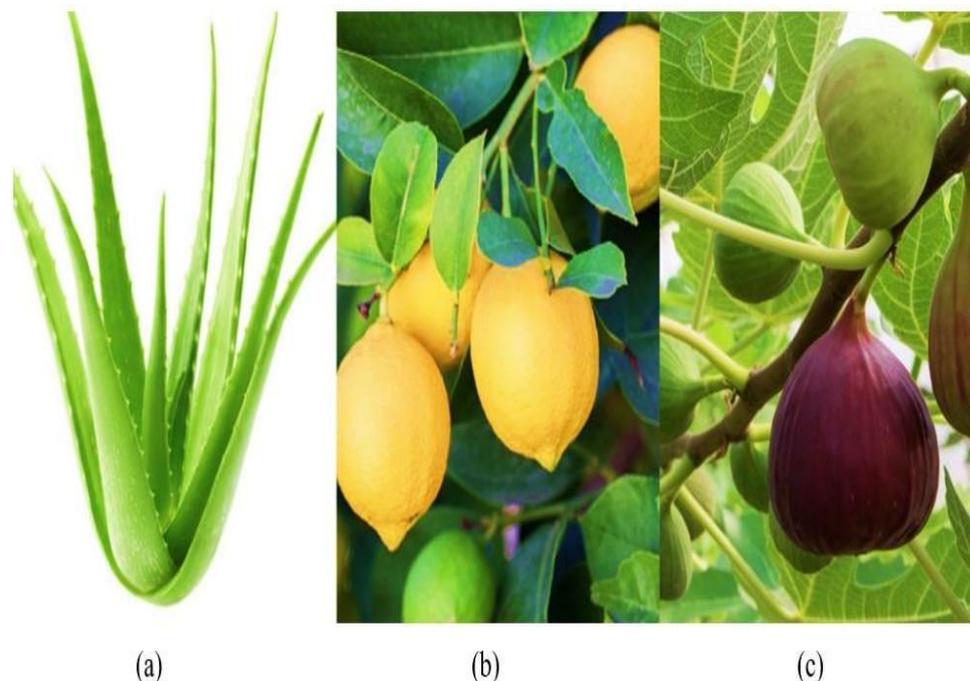


Figure 1. (a) Aloe vera, (b) lemon, (c) fig plants used for the biosynthesis of zinc oxide nanoparticles

2.2 Preparation of Leaf Extracts

The leaves of lemon and fig were washed and dried in the shade, but the leaves of aloe vera were used after removing the gel. The extract of all the mentioned plants was synthesized by taking 10 grams of leaves of these plants in three different 250 mL beakers containing 100 mL of distilled water. For 15 minutes, the mixture was then boiled at 70°C until the color of the solution was changed from transparent to pale yellow [17]. These extracts of the plants were allowed to cool at ambient temperature and sieved using the Whatman filter paper. These extracts were stored at 4°C in a refrigerator.

2.3 Plant's Extract Phytochemicals Analysis

The purpose of phytochemical analysis was to identify various classes of naturally occurring compounds by *A. barbadensis*, *C. lemon*, and *F. carica*. Different tests for plant phytochemicals were performed to confirm their presence [18]. To identify alkaloids, a sample solution of 3 mL in water with a few droplets of Wagner's reagent was added, and there was formation of rusty colour precipitates exhibited the existence of alkaloids. Furthermore, for tannin analysis, to 3 mL water solution of the sample, a few droplets of 1%

lead acetate solution were added, and the emergence of a yellow-coloured precipitate indicated its presence. Similarly, for flavones confirmation, to the 3 mL sample solution of the plant extract, concentrated H₂SO₄ was added dropwise; a dark yellow colour appeared that showed the presence of flavones and flavonols [19]. The Shinoda test showed the presence of flavonoid, Liebermann's test showed the presence of glycosides, and the Salkowski test indicated the presence of terpenoids. The Molisch test indicated that there were no carbohydrates in the plant extracts, and the foam test was for saponin confirmation, indicating their existence in *A. barbadensis*, *C. lemon*, and *F. carica* [20].

2.4 Synthesis of ZnO Nanoparticles

Aqueous extracts of aloe vera, lemon, and fig were used to synthesize zinc oxide nanoparticles as per the method given by Chaudhary *et al.*, with a bit modifications. ZnO nanoparticles were synthesized from the aqueous extract of these plants. Then 10 mM zinc sulphate (ZnSO₄.7H₂O) solution and a solution of sodium hydroxide (NaOH) in distilled water were made ready [21]. After that, 15 mL of extracts of these three plants in separate beakers were added to 100 mL of zinc sulphate solution, then sodium hydroxide was added dropwise, and waited for the formation of a white suspension of nanoparticles. This mixture was then centrifuged. And then the product was dried at 60°C in an oven [22].

2.5 Ultraviolet-Visible and Fourier-Transform Infrared (FTIR) Spectroscopy

For the confirmation of the synthesis of nanoparticles of zinc oxide, UV-Vis spectroscopy was performed for these three sample solutions in the quartz cuvettes, and the absorption wavelength was in the range of 200-800 nm. A high-tech Shimadzu UV-1800 spectrophotometer was used for this purpose. An FTIR spectrophotometer was used to obtain FTIR spectra of ZnO nanoparticles. The samples prepared from all the above-mentioned plant extracts were placed in the specimen parts of the spectrometer for analysis, and a spectrum was plotted in the range of 4,000–500 cm⁻¹. FT-IR was used for the recognition of functional groups present in the extract of these plants [23].

2.6 Fluorescence Spectrophotometry

Fluorescence spectrophotometry is one of the technique in which a material absorb light and emits light at a lower energy. In this technique, we examine the emission of light in the visible region. The samples were prepared in DMF solvent. This is performed by using a Shimadzu RF-6000 spectrofluorophotometer.

2.7 Antimicrobial Studies

Antibacterial activities were done by using the disc diffusion method for the antibacterial profile of all the synthesized ZnO nanoparticles against some bacterial strains, including

Micrococcus luteus and *Bacillus halodurans*. The standard drug used in this process was azithromycin. The agar solution was prepared, mixed with bacterial strains, and poured into petri dishes. The filter papers were punched to form small discs used in this activity. These discs were introduced in petri dishes by taking 10 μL of the sample solutions, DMSO (negative control), and standard drug azithromycin (positive control) with the help of a sterile micropipette.

The *in vitro* antifungal activities were done by using the reported method against some fungal strains, including *Aspergillus niger* and *Aspergillus flavus*. The standard drug used in this process was Terbinafine. The agar solution was prepared, mixed with fungal strains, and poured into petri dishes. The filter papers were punched to form small discs used in this activity. These discs were introduced in petri dishes by taking 10 μL of the sample solutions, DMSO (negative control), and standard drug terbinafine (positive control) with the help of a sterile micropipette.

2.8 Antioxidant Activity

Antioxidant activity was carried out by using two methods. i) DPPH ii) Total Phenolic Content.

a) DPPH Method

In method 2,2-Diphenyl-1, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is used, which is a free radical that does not disintegrate and can be used for analyzing the antioxidant activity of nanoparticles. In this method, a 500 μM methanolic solution of DPPH was prepared by dissolving 10 mg in 50 mL of methanol. The 1 mg/mL solutions of all tested samples were also prepared. Then the reaction mixture was prepared by adding 4 mL of methanol to 1 mL of DPPH solution, and then mixed with 500 μL of each test sample in a separate test tube for each test sample. Then, at the end, the absorbance of all the test sample solutions was noted at 517 nm wavelength with the help of a Shimadzu UV-4000 spectrophotometer. The activities were compared with BHT (butylated hydroxytoluene). The percentage inhibition was determined by applying this formula:

$$(\%) \text{ Inhibition} = \frac{\text{blank} - \text{sample}}{\text{blank}} \times 100 \quad 1$$

b) Total Phenolic Content

1 mg/mL of sample solutions were prepared in this procedure. Then, 0.1 milliliter of the sample solutions was added to 2 mL of sodium carbonate solution (7.5%) and 1 mL of Folin–Ciocalteu reagent solution (10%) in three different test tubes. Then the size was made up to 10 milliliters by adding 7 mL of distilled water. Finally, after half an hour, the absorbance was noted at 760 nanometers by employing an ultraviolet-visible spectrophotometer. For reference, gallic acid was used in this procedure to compare the results of the synthesized nanoparticles.

3. Results and Discussion

A. Barbadosensis, *C. lemon*, and *F. carica* are abundant in bioactive compounds and are renowned for their remarkable healing properties, medicinal benefits, and use in skincare products. Consequently, a phytochemical investigation of *A. barbadensis*, *C. lemon*, and *F. carica* was carried out, alongside the synthesis of zinc oxide nanoparticles using extracts derived from these plants.

3.1 Analysis of Bioactive Compounds in Plant Extracts

For the analysis of phytochemicals, some standard tests were performed for carbohydrates, tannins, alkaloids, flavonols, flavonoids, terpenoids, and saponins. The tests confirmed the presence of alkaloids, tannins, saponins, glycosides, terpenoids, carbohydrates, and flavonoids, as detailed in Table 1. Previous studies have shown that these phytochemicals play a key role in the reduction of metal ions, facilitating the formation of nanoparticles. These phytochemicals were also responsible for the capping of nanoparticles. These phytochemicals are called capping agents, which are responsible for the surrounding of metallic ions.

Table 1. Bioactive compounds identified in the plant extracts

Phyto-chemicals	Plant samples	Phyto-chemicals	Plant samples
Alkaloids	+ I've	Terpenoids	+ I've
Tannins	+ I've	Flavonoids	+ I've
Saponin	+ I've	Carbohydrates	+ I've
Glycosides	+ I've	Flavonols	+ I've

3.2 Bio-synthesis of ZnO Nanoparticles

The ZnO nanoparticles formed a creamy pellet, which was thoroughly rinsed with distilled water to yield purified nanoparticles. 1st step was the extraction of plants, where the phytochemicals were extracted, and the phytochemicals are flavonoids, limonoids, and carotenoids. 2nd step was the reaction of zinc sulphate with some amount of extract, and in this

step, nanoparticles were formed (Figure 2). In this step, a ZnO complex was formed with these phytochemicals. In 3rd step, ZnO nanoparticles were dried at 60°C, and water molecules were evaporated.

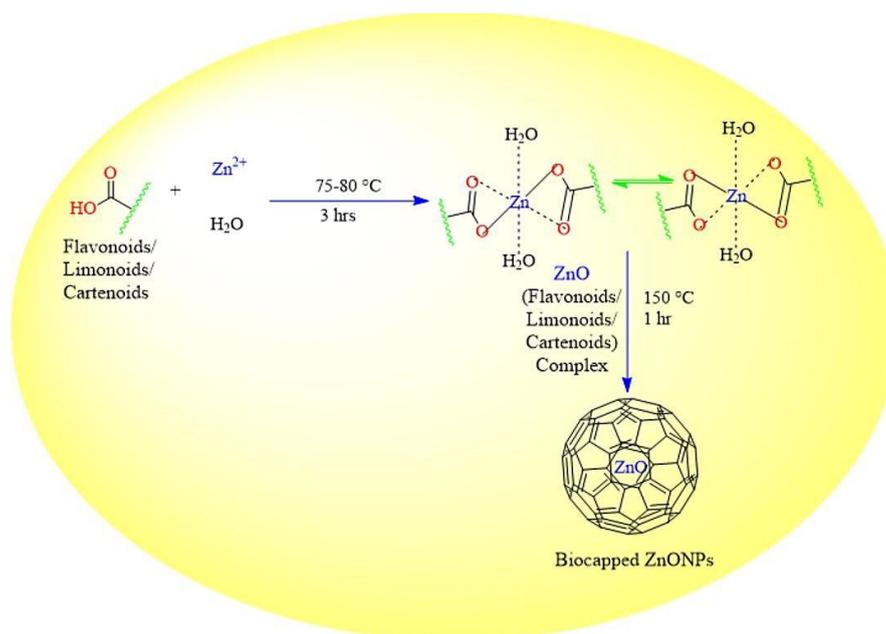


Figure 2. Mechanism of reaction for ZnO nanoparticles

3.3 UV-Visible Analysis of all Synthesized Nanoparticles

ZnO nanoparticles were synthesized using plant extracts from *A. barbadensis*, *C. lemon*, and *F. carica*. Phytochemicals such as terpenoids, flavonoids, alkaloids, and other bioactive compounds present in the leaf extracts facilitated the reduction of zinc ions into ZnO nanoparticles. This transformation was initially indicated by a visible color change, serving as a preliminary sign of successful nanoparticle formation. The observed color change, corresponding to a single absorption peak in the UV-Vis region, resulted from energy transitions within the electron energy levels of the nanoparticles. The maximum absorption peaks for ZnO nanoparticles synthesized from different plant extracts were recorded as follows: 344 nm for the aloe vera extract, 373 nm for the lemon extract, and 356 nm for the fig extract, within the 200–800 nm range, as shown in Figure 3.

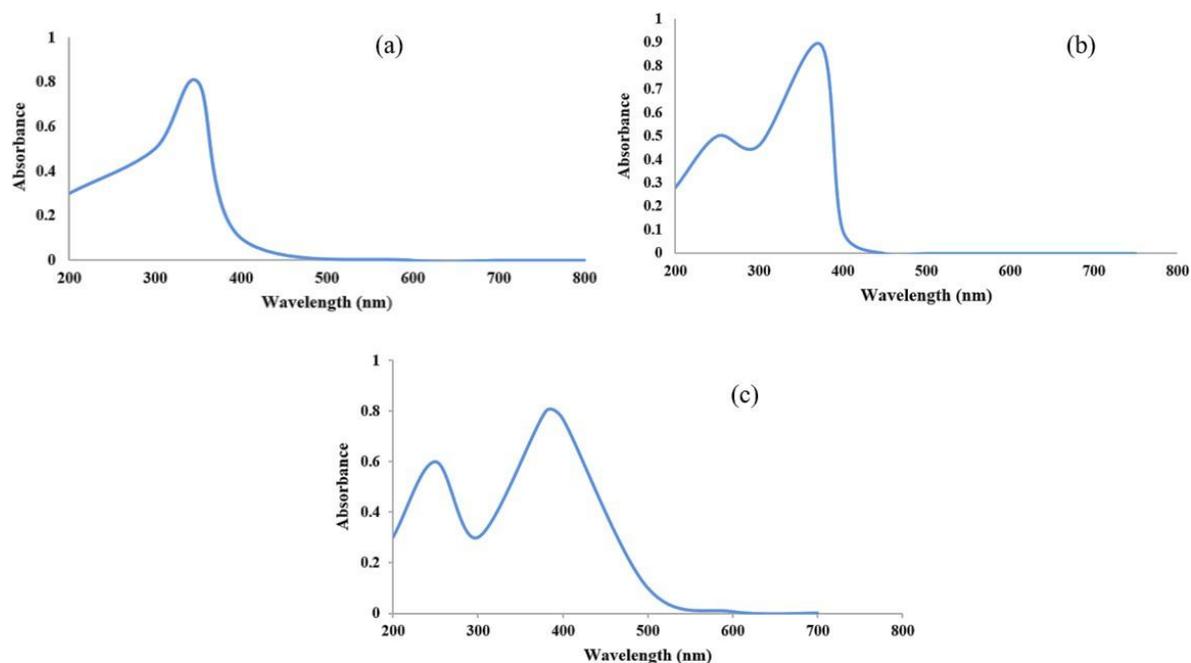


Figure 3. UV-Vis spectra (a) aloe vera-based ZnO nanoparticles, (b) lemon-based ZnO nanoparticles, and (c) fig-based ZnO nanoparticles

3.4 FTIR Analysis of all Synthesized Nanoparticles

The stabilizing and reducing capacities of these agents were assessed by comparing the FTIR spectra of the synthesized nanoparticles with those of the corresponding plant extracts. To eliminate unbound residual organic molecules, the metallic nanoparticles were dispersed in deionized water. The FTIR spectra of the aloe vera, lemon, and fig plant extracts closely resembled those of the aloe vera-ZnO, lemon-ZnO, and fig-ZnO nanoparticles, with only slight differences in a few peaks (Figure 4). This suggests that some metabolites from the plant extracts remained on the surface of the ZnO nanoparticles. The phytochemicals responsible for reducing ZnO and stabilizing the nanoparticles through bonding vibrations were identified in the spectra. Peaks between $500\text{-}650\text{ cm}^{-1}$ were attributed to ZnO stretching and deformation. Key phytochemical peaks are discussed in Table 2.

Table 2. FTIR spectral data of the synthesized nanoparticles

No.	Plants	Nanoparticles	IR (cm^{-1})
(1)	Aloe vera	ZnO	3128 hydroxyl group, 2321 saturated hydrocarbon, 1655 amide group, 980-1141 alcoholic and phenolic, 591 ZnONPs
(2)	Lemon	ZnO	3180 hydroxyl group, 2052, 1151 phenyl group, and 606 ZnONPs
(3)	Fig	ZnO	3238 hydroxyl group, 1577 nitro group, 1212 phenyl group, 596 ZnONPs

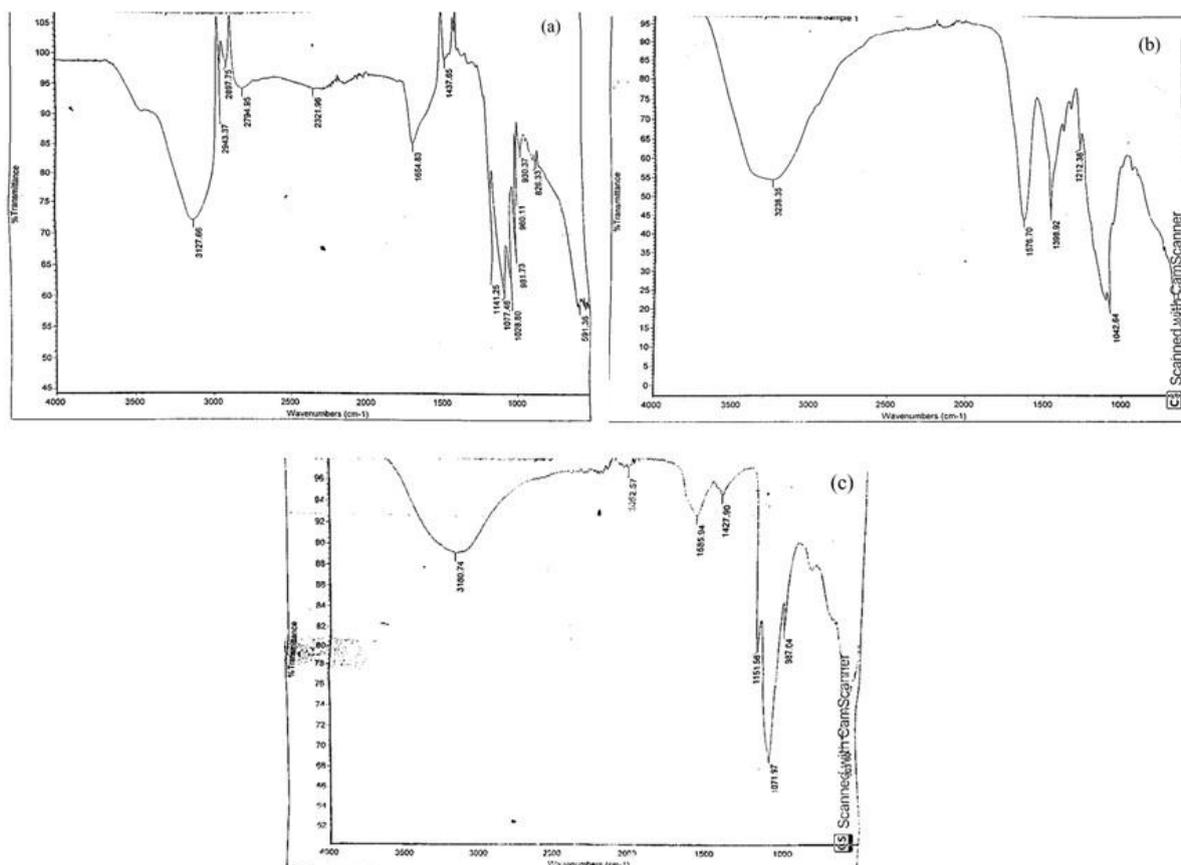


Figure 4. FTIR spectra (a) Aloe vera-based ZnO nanoparticles, (b) lemon-based ZnO nanoparticles, and (c) fig-based ZnO nanoparticles

3.5 Fluorescence Analysis of all Synthesized Nanoparticles

In this analysis, dilute solutions of these newly synthesized nanoparticles were prepared in DMF solvent. The emission wavelength of the synthesized nanoparticles was recorded by a spectrofluorophotometer. In the fluorescence spectral analysis, the emission peaks of ZnO nanoparticles (1, 2, 3) were determined by providing an excitation wavelength, which was the same value that was obtained as absorption maxima from UV-Vis spectra. In this analysis, emission peaks were at 745, 780, and 740 nm (Figure 5).

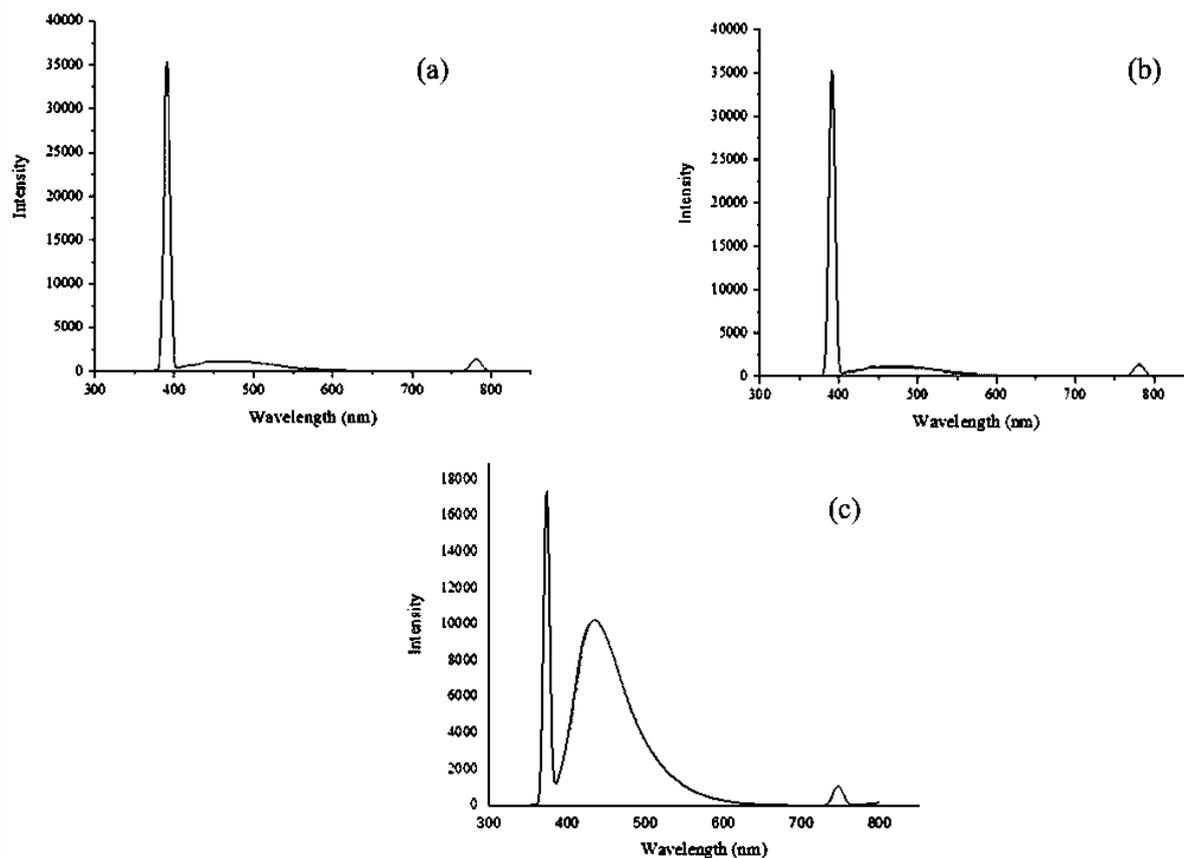


Figure 5. Fluorescence spectra (a) Aloe vera-based ZnO nanoparticles, (b) lemon-based ZnO nanoparticles, and (c) fig-based ZnO nanoparticles

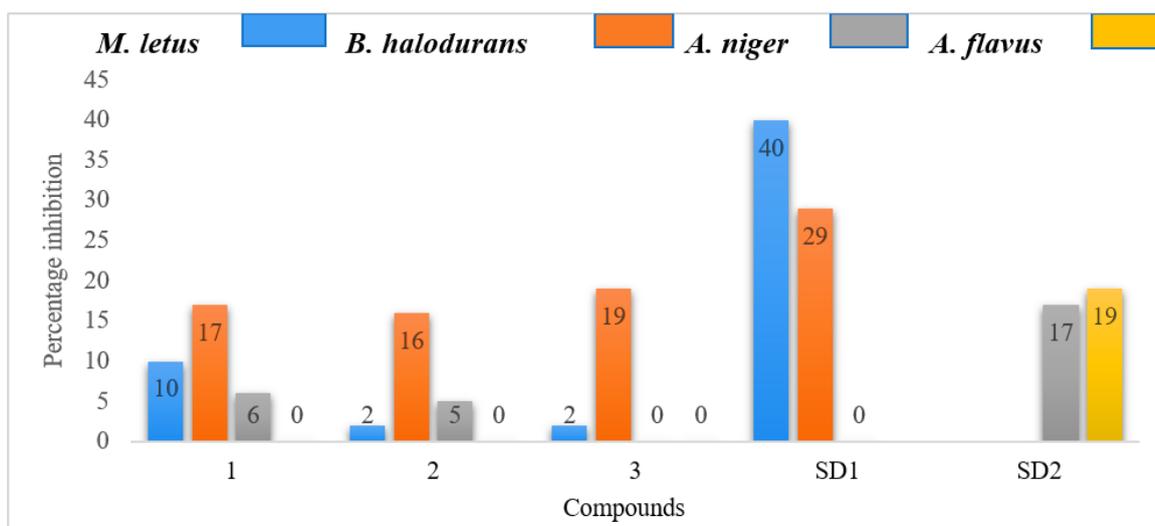
3.6 *In vitro* Antimicrobial Assay

In vitro antibacterial activities of ZnO nanoparticles were assessed against Gram-positive bacteria, *M. luteus* and *halodurans*, using the disc diffusion method. Azithromycin as a standard is discussed in Table 3. ZnO nanoparticles synthesized from the extract of the fig plant showed the highest activity against the bacterial strain, which was 19 mm (Figure 6). The antifungal activity of ZnO nanoparticles was evaluated against two fungal strains, *A. niger* and *A. flavus*. Terbinafine was used as a standard drug. ZnO nanoparticles synthesized from aloe vera extract showed greater activity against *A. niger* with an inhibition zone area of 6 mm. All the samples for the *A. flavus* fungal strain are inactive.

Table 3. Antimicrobial activities of the nanoparticles

*Compound ^s	Antibacterial activity (Zone of Inhibition, mm)		Antifungal activity (Zone of Inhibition, mm)	
	<i>M. luteus</i>	<i>B. halodurans</i>	<i>A. niger</i>	<i>A. flavus</i>
(1)	17	10	06	-
(2)	16	-	05	-
(3)	19	-	-	-
(SD ¹)	29	40	-	-
(SD ²)	-	-	17	20

* 1 = Aloe vera based ZnO nanoparticles, 2 = lemon based ZnO nanoparticles, 3 = fig based ZnO nanoparticles, SD¹ = azithromycin, SD² = terbinafine

**Figure 6. Antimicrobial activities of the synthesized nanoparticles and standard drugs**

3.7 Anti-oxidant activity

All the synthesized nanoparticles showed significant antioxidant activity, which was determined by two types of assays: DPPH radical scavenging and concentration of total phenolic content (Figure 7, Table 4). The activity values of all the nanoparticles are listed below:

a) DPPH Radical Scavenging Assay

Butylated hydroxytoluene (BHT) was used as a standard in this assay for comparing the

results of the synthesized nanoparticles. Among all the tested nanoparticles, sample (3) showed maximum activity of 48 %. Whereas, sample (2) has shown a minimum activity of 08 %.

b) Total Phenolic Contents

The concentration of total phenolic contents was recorded for all the nanoparticles using gallic acid as the standard. The highest (72%) value was observed for the sample (1), and the lowest activity (70%) was noted for the sample (3).

Table 4. Antioxidant activity of the nanoparticles

Nanoparticles	DPPH (%)	Total Phenolic Content (%)
(1)	41	72
(2)	08	71
(3)	48	70
(SD ¹)	74	-
(SD ²)	-	88

SD¹ = Butylated hydroxytoluene, SD² = Gallic acid.

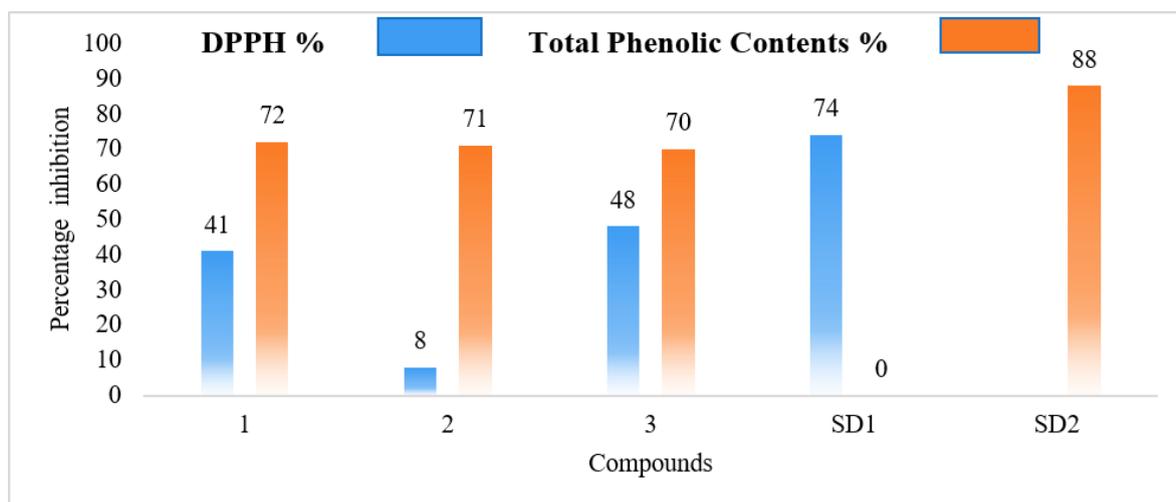


Figure 7. Antioxidant activities of the synthesized nanoparticles and standard drugs

4. Conclusion

ZnO nanoparticles were prepared by using zinc sulphate heptahydrate (ZnSO₄.7H₂O) and aqueous extract of aloe vera, lemon, and fig leaves separately. All the plant extracts had phytochemicals. These synthesized nanoparticles were characterized using the following characterization techniques UV UV-Visible (UV-Vis), Fourier transform infrared (FTIR) spectroscopy, and fluorescence spectrophotometry. Antibacterial activity was checked against

two bacterial strains, *M. luteus* and *B. halodurans*, and antifungal activity was determined against two fungal strains.

A. niger and *A. flavus*. The nanoparticles prepared using fig extract showed the highest activity against the *M. luteus* bacterial strain. Aloe vera extract-based ZnO nanoparticles were most active against *A. niger* fungal species. ZnO nanoparticles prepared from fig-based extract exhibited the highest antioxidant property against the DPPH assay, while the nanoparticles synthesized from aloe vera extract exhibited the highest antioxidant activity in the total phenolic content assay.

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