RESEARCH ARTICLE

Antibacterial and antioxidant activity of green synthesized Zinc oxide nanoparticles using polyphenol extract from *Mentha piperita* seeds

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Abstract: Currently green nanotechnology presents a smart solution to produce novel nanostructured materials that are highly safe and environmental friendly. In this work, zinc oxide nanoparticles (ZnO NPs) were prepared by employing aqueous mint (*M. piperita*) seeds extract at 60°C, as a green synthesis method. Mint seeds extract was chosen among the 6 plant seeds that were the subject of this study due to it represents the highest content of polyphenols and flavonoids, as well as antioxidant activity. High Performance Liquid Chromatography (HPLC) showed that syringic acid (16%), rutin (22%), and apigenin-7-*O*-glucoside (29%) were the main component of ethanol mint seeds extract. The produced ZnO NPs were examined using an ultraviolet-visible spectrophotometer (UV-VIS), X-ray diffraction (XRD) and transmission electron microscope (TEM). The UV spectrum revealed maximum absorption value at 376 nm, related to green synthesized ZnO NPs. The XRD study demonstrated the creation of ZnO NPs. ZnO NPs were spherical in shape with average particles size of 80±36 nm. The antibacterial activity of aqueous mint seeds extract and green synthesized ZnO NPs were investigated on *E. coli* and *S. aureus* and antioxidant activity as well. Our findings demonstrate a facile approach to improve the antibacterial potential of the ZnO NPs, and therefore could be a promising multifunctional bioactive material for wound healing and other related applications.
Keywords: Flavonoids, HPLC, Mint, aqueous extract, syringic acid, rutin

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1 Introduction

Nanoparticles (NPs) play a crucial role in various medical and nanotechnological applications is specially the green synthesized NPs (Jiménez-Rosado et al., 2022). Various physical and chemical approaches have been reported for the synthesis of NPs (Talam et al., 2012). In chemical approach, NPs are created using a bottom up chemical process using metal salt and reducing agent such sodium borohydride, hydrazine, etc.. Here, the metal cation is reduced by the reaction to a neutral state, creating a nucleation site where the metal atoms can gather and eventually form NPs (Petcharoen and Sirivat, 2012; Saleem et al., 2021). However, such reducing agents frequently result in the production of toxic substances (Sheldon, 2005) and raise the cost of the final product, hindering scientific advancement and increasing carbon emissions (Prabhu and Poulose, 2012). Additionally, they should not be used in food, medical and pharmaceutical applications because of their harmful effects on humans (Varma, 2012).

Polyphenols are naturally substances extracted from plants that are sorted by chemical structure, biological function, and source. Concerning to aromatic rings and their ability for combination to various compounds, they can be classified into two main categories: flavonoid and non-flavonoid (Lipiński et al., 2017; Guneidy et al., 2020). According to Gironi and Piemonte (2011), polyphenols are distinguished by number of phenol groups in each molecule (generally, each molecule contains between 2 and 14-OH groups, which are linked to their antioxidant activity). They can prevent the formation of toxic secondary products and act as reducing agents in the synthesis of NPs due to their abundant availability of -OH groups (Agarwal et al., 2017). By solubilizing these polyphenols in polar solvents like alcohol or water, it is simple to extract them from plants (Prado et al., 2021). Polyphenols extraction parameters such as temperature, concentration and time should be first optimized to

maximize their antioxidant activity, to reduce NP starting materials efficiently (Özbek et al., 2020). Today, metals and metal oxide NPs like silver, gold, selenium, iron, copper, and their oxides have been extensively synthesized using various plant extracts such as *Glycyrrhiza glabra* (Vivekananth et al., 2021), *Calendula officinalis* (Nematollahi et al., 2021), *Phoenix dactylifera* (Abdullah et al., 2020; Rajeswari et al., 2021), and *Capsicum annuum* (Jiménez-Rosado et al., 2022). Among them, nanostructures ZnO NPs were found to be biocompatible and nontoxic for biomedical application including wound healing and drug delivery applications due to their antimicrobial, anti-inflammatory, and antioxidant activities.

In the culture media, the quantitative antimicrobial activities of metal oxide (ZnO) NPs were assessed against Gram-positive and Gram-negative bacteria as well as pathogenic microorganisms that can cause diseases in plants and animals (Luo et al., 2013; Jiang et al., 2020). Reactive oxygen species found in these metal oxide particles may be the main way for destroying bacteria (Hu et al., 2009; Siddiqi et al., 2018). The direct interaction of ZnO NPs with cell surfaces, which influences the permeability of cell membranes, constitutes the ZnO NPs' antibacterial mechanism. After that, NPs enter bacterial cells and cause oxidative stress, which ultimately results in cell death and growth inhibition (Xie et al., 2011; Jain et al., 2020). The demonstration of ZnO NP's antibacterial activity encourages its use in crop seed preservation throughout the period of storage prior to cultivation (Abdelmigid et al., 2022).

The previous studies of Pramila et al. (2012), Sharma et al. (2018), Abdelkhalek and Al-Askar (2020), Koli et al. (2022) and Doğaroğlu et al. (2023) have used mint leaves extract for the green synthesis of ZnO NPs. To the best of our knowledge, the current research is considered the first study to prepare ZnO NPs from mint seeds extract. Consequently, the primary objective of this work was to select the plant seeds with the highest concentration of polyphenol and flavonoids and greatest antioxidant potential to test its application in the green synthesis of ZnO NPs. To accomplish this objective, polyphenol extraction should be firstly optimized to maximize their antioxidant activity, the optimal polyphenol mint extract at 60 ^{o}C was used to obtain a variety of ZnO NPs, and their antibacterial and antioxidant properties were evaluated as well. Synthesized ZnO NPs were confirmed using UV, XRD and TEM spectral analyses.

2 Materials and Methods

2.1 Chemicals

Ethanol, dimethyl sulfoxide (DMSO) and Folin-Ciocateus reagent were obtained from Merck Company (USA). While aluminum chloride, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), L-ascorbic acid, sodium acetate, zinc acetate dihydrate, rutin, quercetin, gallic acid, and sodium hydroxide pellets were obtained by Sigma Aldrich company (USA). All chemicals used in this study were of analytical grade and of high purity.

2.2 Plant materials

The seeds of six plants (Table 1) belong to different families; namely Liliaceae: *Allium cepa, Lamiaceae: Mentha piperita, Rosaceae: Fragaria ananassa, Vitaceae: Vitis vinifera, Solanaceae: Solanum lycopersicum* and *Brassicaceae: Brassica oleracea* were bought from various markets in Egypt. The collected plant seeds were examined by Herbachium Botany Department, National Research Centre (Dokki Cairo, Egypt).

2.3 Preparation of plant extracts

Dry seeds (1 g) of each plant were ground and mixed with 10 ml of solvent (water -70% ethanol) for 2 h at room temperature. The extracted material was centrifuged at 5,000 g for 10 min, filtered through Whatman filter paper No.1, and stored at -20°C for later analyses.

Table 1. Total phenolic content, total flavonoid content and antioxidant activity of different plant seed extracts

Plant name	Water extract			70% ethanol extract		
	Phenolics (mg/g dry seed)	Flavonoids (mg/g dry seed)	Ic_{50} (mg/ml)	Phenolics (mg/g dry seed)	Flavonoids (mg/g dry seed)	Ic_{50} (mg/ml)
A. cepa	1.2±0.18^{c}	0.18±0.02^{b}	4.7±0.20^{a}	1.4±0.26^{b}	0.7±0.13^{b}	3.5±0.15^{a}
M. piperita	2.6±0.50^{a}	1.20±0.15^{a}	0.5±0.03^{b}	6.3±0.67^{a}	3.7±0.34^{a}	0.4±0.02^{b}
F. ananassa	0.5±0.04^{c}	0.13±0.02^{b}	2.4±0.05^{b}	1.0±0.06^{b}	0.5±0.12^{b}	0.4±0.02^{b}
V. vinifera	1.2±0.13^{c}	0.50±0.01^{c}	2.4±0.07^{b}	3.5±0.33^{c}	0.7±0.11^{b}	2.0±0.09^{b}
S. lycopersicum	1.5±0.13^{c}	0.55±0.12^{c}	3.6±0.11^{b}	1.7±0.25^{b}	1.6±0.13^{b}	1.9±0.09^{b}
B. oleracea	1.9±0.20^{d}	0.96±0.16^{d}	1.8±0.04^{b}	2.4±0.26^{b}	2.1±0.20^{b}	0.4±0.02^{b}

Note: Values are presented as means ± SD (n = 4). Means with different superscript letters within the same column are significantly different at $P<0.05$.

2.4 Total phenol content

Total phenol was estimated using the Folin-Ciocateu's reagent (Djeridane et al., 2006). Total phenol content was reported as mg of gallic acid equivalent (GAE) per g of dry seed. Absolute ethanol was used as a blank.

2.5 Total flavonoid content

Lin and Tang (2007), method was used to calculate the total flavonoids present in the extracted samples. Total flavonoids were determined using a calibration curve that was created using quercetin as the reference. The amount of flavonoids was measured in milligrams per gram (mg/g) of dry seed as rutin equivalents (RE).

2.6 DPPH free radical scavenging assay

The DPPH free radical method was used to determine samples' free radical scavenging activities (Blois, 1958; Abdel-Hady et al., 2023). To investigate the antioxidant activity of the plant seed extracts, freshly generated DPPH solution (0.1 mM) was added to a range of sample concentrations, agitated, left for 30 minutes in the dark at room temperature, and the absorbance was measured at 517 nm against a blank. DPPH (IC_{50}) was calculated from the graph of I% (inhibition percentage) versus sample concentration.

2.7 High performance liquid chromatography (HPLC) of phenolic compounds

Mint seeds extract (70% ethanol) was analyzed using HPLC at the National Research Center in Cairo, Egypt. The signal-to-noise ratio was adjusted at three or greater and considered the detection border (Kim et al., 2006).

2.8 Preparation of aqueous mint seeds extract

Aqueous mint seeds extract was made by boiling 10g of powdered dried mint seeds in 100 ml of distilled water for two hours at 60°C. After cooling, it was filtered using Whatman filter paper No.1. For subsequent experiments, the filtrate was kept at 4°C in the refrigerator.

2.9 Synthesis of zinc oxide nanoparticles

An aqueous solution (0.2 M) of zinc acetate dihydrate was prepared. To create zinc oxide nanoparticles (ZnO NPs), the daily prepared zinc acetate solution was combined with 70ml of aqueous mint seeds extract. After the reacted solution was blended, agitated, and the pH was increased to 12 using NaOH (3 M), a pale-white ZnO NPs suspension was created. The two-hour stirring pale-white ZnO NPs were precipitated, 3 times rinsed with sterile distilled water, then washed with ethanol to remove any impurities. After overnight drying at 60°C, a powder of ZnO NPs in the form of pale white crystals was produced (Abdelkhalek and Al-Askar, 2020).

2.10 Characterization of ZnO nanoparticles

2.10.1 UVVis spectra analysis

UV/VIS spectrophotometry was used to characterize the as-prepared ZnO NPs samples. In a 1cm path quartz cell, ultraviolet spectra in the range between 200-800 nm were collected at room temperature and the absorbance was recorded at 376 nm.

2.10.2 Transmission Electron Microscopy (TEM)

A highly integrated compact transmission electron microscope (TEM; JEOL, Peabody, MA, USA) at an accelerating voltage of 80 kV was used to check the morphology of the as-prepared ZnO NPs. A drop (20μl) of the ZnO NP suspensions was applied to create the TEM grids, which were then allowed to dry in air.

2.10.3 X-ray diffraction (XRD)

The XRD analysis was carried out using a Bruker D8 Advance X-ray diffractometer (Germany) at a 2θ (Bragg angle) of 5-80°. The diffraction patterns were collected at a voltage of 40 kV with a current of 40 mA, using copper (Kα) radiation (1.5406 Å).

2.11 Antioxidant activity

Numerous concentrations of green synthesized ZnO NPs were incubated with 0.1 mM DPPH solution to determine Ic_{50} as described above.

2.12 Antibacterial activity study

Human-pathogenic bacterial strains were received from the Marine Toxins Laboratory, Food Toxins and Contaminants Department, National Research Centre, Cairo, Egypt. These strains of pathogenic bacteria included Gram-negative (*Escherichia coli* O157-H7 ATCC) and Gram-positive (*Staphylococcus aureus* ATCC 13,565) bacteria. Dimethyl sulfoxide (DMSO) served as the study's negative control, while Gentamicin which has potent antibacterial action against a variety of bacterial strains, served as the study's positive control (The American Society of Health-System Pharmacists, 2015). Extracts from chemically manufactured ZnO NPs and green generated ZnO NPs were tested for their antibacterial activity using the well-diffusion method on Mueller-Hinton agar (Bauer, 1996). Each tested bacterial suspension (108 CFU/mL) was applied to the surface of the plates in an amount of 0.1 ml. The studied extracts were combined with wells punched in the agar medium, dissolved in DMSO, and then incubated for 18 hours at 37°C. Antibacterial activity was calculated as the

diameter (mm) of distinct growth-inhibition zones against each tested bacterial strain.

2.13 Determination of the minimum inhibition concentration (MIC)

The MIC values for the tested extracts were calculated using the agar dilution diffusion method in accordance with M7-A7 procedures, as advised by the National Committee for Clinical Laboratory Standards (2009) (NCCLS). The wells on Muller Hinton agar media were infected with 10^8 CFU/ml of each pathogenic bacterium, and various doses (5-50 mg/ml) of chemically manufactured ZnO NPs and green synthesized ZnO NPs extracts were added. The wells were then kept at 37°C for 18 hours. A MIC value was known as the minimum extract concentration which inhibits bacterial growth.

2.14 Statistical analysis

The information is shown as the average values from one to three separate experiments together with the standard deviation (SD). In order to analyze the data, Origin 8.0 was used.

3 Results

3.1 Total phenolic and flavonoid content as well as antioxidant capacity

The total phenolic content, flavonoid content and antioxidant capacity for plant seed extracts in both water and 70% ethanol are screened in Table 1. The extracts of 70% ethanol of *M. piperita* had the greatest phenolic and flavonoid values (6.3±0.67 mg GAE/g dry seed and 3.7±0.34 mg RE/g dry seed, respectively) as well as antioxidant capacity (0.4±0.02 mg/ml). The remaining plant seeds under this study had phenolic contents ranging from 0.5 to 2.4 mg GAE/g dry seed and flavonoid contents ranging from 0.13 to 2.1 mg RE/g dry seed.

3.2 Phenolic compounds of M. piperita ethanolic extract (70%) using high performance liquid chromatograph

HPLC analysis was performed to identify and quantify phenolic compounds extracted from *M. piperita* ethanolic extract. Fourteen phenolic compounds were identified as shown in Table 2. HPLC analyses showed the presence of apigenin-7-*O*-glucoside at the highest concentration (5 mg/g dry seed) which accounts for 29% of the total detected phenols followed by rutin (3.8 mg/g dry seed & 22%) and syringic acid (2.8 mg/g dry seed & 16%). Both of vanillic and chlorogenic acids have the same phenolic value (1.2 mg/g dry seed & 7%). Small amounts of rosmarinic acid (0.7 mg/g dry seed) and quercetin (0.58 mg/g dry seed) were found with ratios of 4 and, 3.3%, respectively. Phenolic levels for apigenin, kaempferol *p*-coumaric and ferulic acid are almost equal. The remaining identified phenolic compounds under this study represent less than 4% of total ratio.

Table 2. HPLC identification and quantification of phenolic compounds extracted from *M. piperita* 70% ethanol

Phenolic compound type	Identified compound	(mg/g dry seed)	% (w/w)
Phenolic acids	Gallic acid	ND	ND
	Protocatechuic acid	ND	ND
	p-hydroxybenzoic acid	ND	ND
	Gentisic acid	ND	ND
	Cinnamic acid	ND	ND
	Syringic acid	2.8	16
	Chlorogenic acid	1.2	7
	Vanillic acid	1.2	7
	Rosmarinic acid	0.7	4
	p-coumaric acid	0.34	2
	Ferulic acid	0.33	1.9
	Sinapic acid	0.22	1.2
	Caffeic acid	0.21	1.2
Flavonoids	Catechin	ND	ND
	Apigenin-7-*O*-glucoside	5	29
	Rutin	3.8	22
	Qurecetin	0.58	3.3
	Apigenin	0.38	2.2
	Kaempferol	0.33	1.9
	Chrysin	0.25	1.4
Total phenolics		17.34	100

Note: % (w/w): % calculated relative to the total concentration of identified phenolic compounds. ND: not detected, HPLC: High Performance Liquid Chromatography.

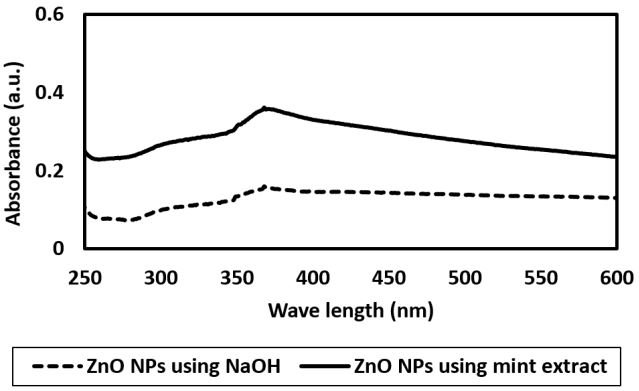

Figure 1. UVVIS spectra of ZnO NPs in the range 250 600 nm.

3.3 Characterization of ZnO nanoparticles

3.3.1 UV/VIS spectroscopy

Adequate quantity of zinc oxide (0.01 g) was dissolved in 10 ml ethanol (96%) and sonicated for 10 min to detect the UVvisible spectra. The UV/VIS spectrum was done in order to specify and ascertain the optical properties of ZnO NPs. Figure 1 depicts the ZnO spectrum with a designated maximum absorption value at 376 nm confirming the successful formation of ZnO NPs using aqueous mint extract. The nanoparticle distribution is monodispersed, as evidenced by this sharp peak, and the majority of the particles are at

nanoscale. The bands of zinc colloids were detected at 376 nm, indicating that the extraction of mint seeds effectively minimizes the zinc ion.

3.3.2 UV/VIS spectroscopy

The phase identity of the as-prepared ZnO NPs was determined by XRD. Figure 2 shows a typical XRD pattern of ZnO NPs in the range of 10° to 80° at a scanning step of 0.01. A number of diffraction peaks was observed for both samples at 2θ values of 31.3°, 34.03°, 35.7°, 47.1°, 56.4°, 62.2°, 66.3°, 67.4° and 68.4°, which are related to (100), (002), (101), (102), (110) (103) (200), (112), and (201) diffraction planes. However, the XRD data clearly illustrate the high purity and development of Zincite phase in the presence and absence of mint extract, according to JCPDS card no. 790208, (a = 2.207 nm and c = 3.379 nm).

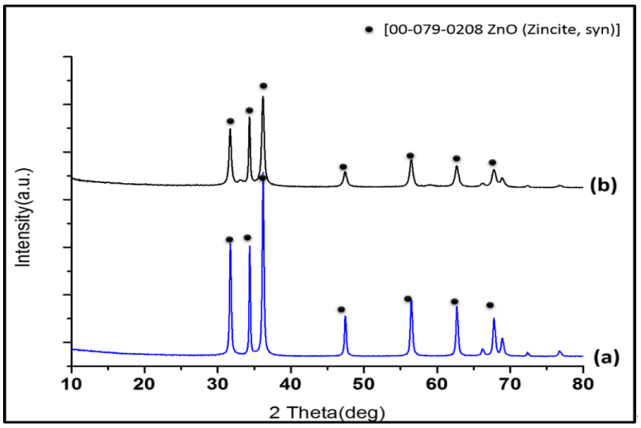

Figure 2. Powder X-ray Diffraction Pattern of the as prepared ZnO NPs (a) using NaOH and (b) mint extract.

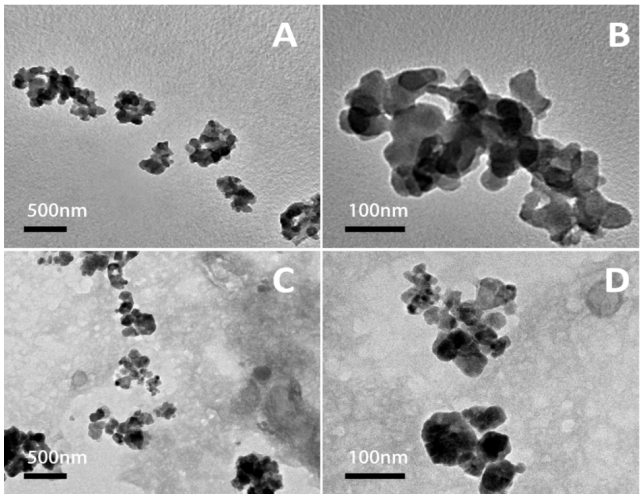

Figure 3. Transmission Electron Microscopy Image of ZnO NPs papered using (A, B) NaOH and (C, D) mint Extract.

Figure 3 shows the TEM micrograph of the as-synthesized ZnO NPs. In the absence of mint extract, the ZNO NPs were mainly spherical with an average particle size 213±89 nm. Although, the green synthesized ZnO NPs were also spherical. The average particles size decreased to 80±36 nm.

3.3.3 Antioxidant activity

The antioxidant activity of test samples is frequently measured using a parameter known as the IC_{50} value, IC_{50} value is the phenolic concentration required to scavenge 50% of either DPPH free radicals. IC_{50} value obtained for green synthesized ZnO NPs was found to be 98 mg/ml. Figure 4 shows that the percent inhibition increases with increasing the concentration of green synthesized ZnO NPs.

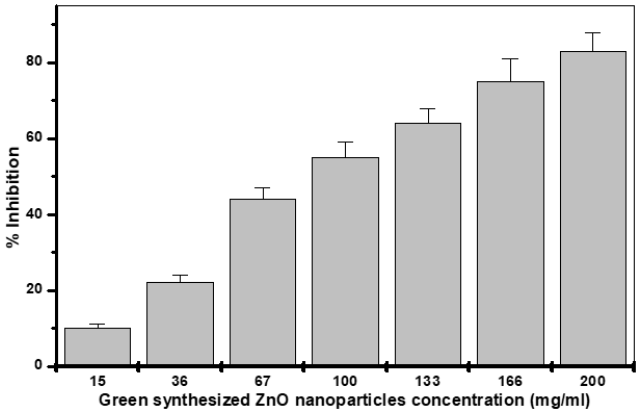

Figure 4. DPPH Scavenging activity of green synthesized ZnO NPs using *M. piperita* ethanolic extract.

3.3.4 Antibacterial activity

The antibacterial activity of the chemical and green synthesized ZnO NPs via *M. piperita* seeds extract were investigated against *E. coli* and *S. aureus* as pathogenic bacteria (Figure 5). The results indicated that green synthesized ZnO NPs was significantly stronger antibacterial activity toward *S. aureus* and *E. coli* with inhibition zones of 20±0.15 and 18±0.11 mm than chemically synthesized ZnO NPs with inhibition zones of 15±0.10 and 16±0.12 mm, respectively (Table 3). Moreover, the green synthesized ZnO NPs exhibited a greater antibacterial capacity (18±0.11 mm-inhibition zone diameter) against the *E. coli* strain than Gentamicin (15±0.10 mm). The minimum inhibitory concentration (MIC) of green synthesized ZnO NPs was 5 and 8 mg/ml while MIC of chemical synthesized ZnO NPs was 12 and 20 mg/ml against *E. coli* and *S. aureus*, respectively.

4 Discussion

Recently, the utilization of plant extracts in the synthesis of nanoparticles (NPs) has been received a lot of attention to reduce the need for pricey and harsh chemicals. Plant biological compounds can be used either extracellularly or

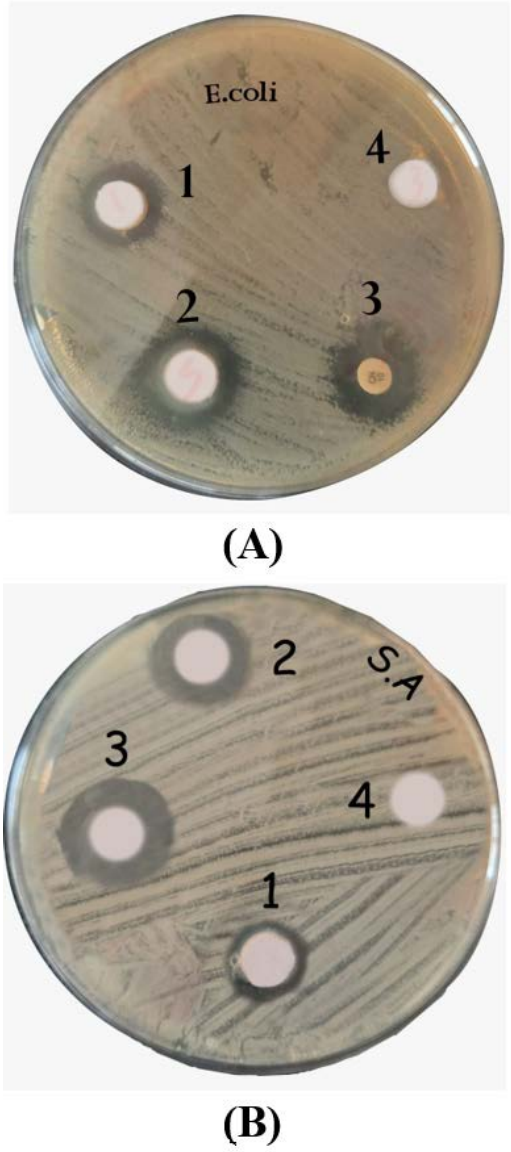

Figure 5. Antibacterial effects of chemical synthesized NPs (1), green synthesized NPs (2), gentamicin (3) and negative control (4) (A against *E. coli*, B against *S. aureus*).

intracellularly to complete the NP synthesis process as reducing or capping agents. According to Debnath and Gupta (2018), utilization of polyphenols as reducing agents could help overcome these disadvantages and reduce production costs while also providing environmentally friendly methods. In this work, *M. piperita* was chosen from plants under this study as it represents the highest one in phenolic and flavonoid contents as well as its antioxidant capacity. All of the plants in this study had the highest overall concentration of phenolic and flavonoid contents when ethanol extraction at 70% was used. Plant phenolics extraction is greatly affected by sample and solvent type, time and storage conditions, and extraction techniques (Eldurini et al., 2021). The ability of phenolic compounds to function as reducing agents is primarily responsible for their antioxidant properties. Phenolics' hydroxyl (OH) groups are efficient reactive oxygen species-scavenging H-donating antioxidants. As a result, they prevent the radical production process (Quideau et al., 2011).

Table 3. Inhibition zone diameter of chemical synthesized ZnO NPs and green synthesized ZnO NPs extracts against some human pathogenic bacterial strains

Sample	Inhibition zone diameter (mm)	
	S. aureus	*E. coli*
Aqueous*M. piperita* seeds extract	11±0.11[a]	10 ± 1.0[a]
Chemically synthesized ZnO NPs	15±0.10 [a]	16 ±0.12[a]
Green synthesized ZnO NPs	20±0.15 [b]	18 ±0.11[b]
Gentamicin	20±0.12 [b]	15 ±0.11[c]

Note: alues are presented as means SD (n = 4). Values with different superscript letters within the same column are significantly different at $p>0.01$.

The correlation between the antioxidant capacity of plant extracts and their phenolic content was evident from our findings, where the lower IC_{50} value, the greater antioxidant activity. According to the findings of this study, *M. piperita* seeds extract (70% ethanol) with the highest concentration of phenolic compounds (6.3±0.67 mg/g dry seed) also possess the highest antioxidant capacity and lower Ic_{50} (0.4±0.02 mg/ml). Antioxidant potential reflects the phenolic and flavonoid contents (Wong et al., 2006; Martins et al., 2015). Strong scavenging abilities of the *M. piperita* and *B. oleracea* extracts (Table 1) could be attributed to the conformation structure of phenolic compound. Despite the fact that phenolic compounds possess large number of OH groups which are able to reduce DPPH free radicals very quickly, they can provide the required component as a radical scavenger (Vadivel et al., 2011). Phenolic compounds identified in *M. piperita* (70% ethanol) extract by HPLC (Table 2) agree with the compounds detected in literatures (Uchenna et al., 2018; Guneidy et al., 2022). Figure 1 depicts the ZnO spectrum with a designated maximum absorption value at 376 nm, which is the typical absorption peak for wurzite hexagonal pure ZnO (Zak et al., 2011). The fact that there were no other peaks in the spectrum indicates that the synthesized compound is only ZnO (Estrada-Urbina et al., 2018).

From XRD analysis, it could be observed that the intensity of diffraction peaks was decreased with mint addition indicating the changes in crystallinity and particle size. Hence, DebyeScherrer and HermansWeidinger methods were used to determine the crystalline size and crystallinity among the samples (Anand et al., 2019; Anand et al., 2020; Avinash et al., 2024). On the bases of θ and full width at half-maximum (FWHM) of the most intense diffraction peak corresponding to (101) diffraction planes found at 35.7°, the crystalline size of the as-prepared synthesized ZnO NPs were found to be 61 nm in the presence of mint and 167 nm in absence of mint. In addition, the mint synthesized ZnO NPs showed an obvious decrease of crystallinity (39.1%) compared to the ones prepared in absence of mint (65.7%). These results indicate that

the active ingredient of mint extract interact with ZnO NPs and prevent their over-growth and aggregation in aqueous environment, thus decreasing the crystalline size of ZnO NP (Arokiyaraj et al., 2016; Khan et al., 2019).

IC_{50} value obtained for green synthesized ZnO NPs are in agreement with that reported by Ananthalakshmi et al. (2019). In general, the antioxidant capacity increases as the concentration of NPs increases. To put it another way, more NPs produced more catalytic surface, which can interact with free radicals and improve antioxidant property (Jiménez-Rosado et al., 2022). Based on our research, the high total flavonoid concentration of rutin (3.8 mg/g dry seed) and syringic acid (5.0 mg/g dry seed) in *M. piperita* represent the most impact on its antioxidant ability. Syringic acid's strong antioxidant properties are due to the two methoxy moieties that are connected to the phenolic nucleus at positions 3 and 5 (Karamac et al., 2005; Cheemanapalli et al., 2018). While the reducing effects of rutin on a variety of oxidizing species, including superoxide, peroxyl, and hydroxyl radicals reflect its antioxidant power (Imani et al., 2021).

Moreover, the green synthesized ZnO NPs exhibited a greater antibacterial capacity against the *E. coli* strain than Gentamicin which may be attributed to the capacity of green synthesized ZnO NPs to destroy bacterial cell wall by producing superoxide and hydroxyl radicals as reactive oxygen species as previously reported (Divya et al., 2013). Interestingly, the small size of ZnO NPs enables them to penetrate the bacterial cell wall easily, affect DNA formation, cell growth and thus providing death of bacteria which in turn will increase antibacterial activity (Kumaresan et al., 2018; Anand et al., 2024). Indeed, the minimum inhibitory concentration (MIC) of green and chemically synthesized ZnO NPs against *E. coli* and *S. aureus* were comparable with that reported by Reddy et al. (2014) and similar to those estimated by Lakshmi et al. (2012) whereas they found that chemically synthesized ZnO NPs had the same effect against some pathogenic bacteria. In like manner, the antibacterial activity of the green synthesized ZnO NPs *Sambucus ebulus* leaf extract, toward *E. coli*, *B. cereus* and *S. aureus* (Alamdari et al., 2020). Ambika and Sundrarajan (2015) obtained the same result from the green synthesized ZnO NPs of *Vitex negundo* extract toward Gram negative and Gram positive.

Due to flavonoid tendency to inhibit the growth of a variety of harmful microorganisms, including multidrug resistant bacteria, flavonoids have also been linked to antibacterial action (Shamsudin et al., 2022). Rutin, for instance, has been demonstrated to have a potent antibacterial activity against a variety of microorganisms (Deepika et al., 2019). According to Akroum et al. (2010), Gram-negative bacteria were more greatly affected by quercetin, while apigenin-7-*O*-glucoside was more potent toward Gram-positive bacteria. In fact, the higher content of apigenin-7-*O*-glucoside (29%) and lower concentration of quercetin (3.3%) which were identified by HPLC analysis may explain the slightly stronger antibacterial action of green synthesized ZnO NPs towards Gram-positive bacteria. These findings are in agreement with Karaoğlan et al. (2023). Indeed, syringic acid play an effective action toward Gram-negative and Gram-positive bacteria due to its phenolic skeleton which possesses antimicrobial activity against several micro-organisms (Cheemanapalli et al., 2018).

5 Conclusion

In this study, ZnO NPs were successfully synthesized utilizing an aqueous extract from *M. piperita* seeds, which displayed intriguing features. The XRD and TEM analyses confirm the formation of ZnO NPs of irregular spherical structure and an average diameter of 80±36 nm. The green synthesized ZnO NPs displayed significant antimicrobial potential against both gram positive and gram negative bacteria that arises from the release of Zn^{2+} ions and generation of reactive oxygen species. The superior antimicrobial performance of green synthesized ZnO NPs arises from its smaller particles size compared the chemically prepared one in addition to the synergetic effect of the phenolic compounds in the plant extract. The findings of our study showed that the green synthesized ZnO NPs, as an alternative to chemical synthesized materials, have promising antibacterial and antioxidant activities. However, additional in vitro cell culture test against different cell types and in vivo animal model are advised to offer more accurate and reliable data for a definitive analysis about their use in clinical wound healing application.

Author Contributions

M.Y. and E.T. manuscript revision and carried out TEM and XRD analyses, R.B. and M.W. performed antioxidant and antibacterial assays. E.R. carried out biochemical tests and A.G. experiment design and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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References

Abdel-Hady, B.M., Ekram, B., Müller, W.E.G., Gad, A.A.M., Wang, X., Schrder, H.C., Tolba, E., 2023. Ascorbyl palmitate-PCL fiber mats loaded with strontium polyphosphate nanoparticles for guided bone regeneration. Polymer Bulletin, 1-20. https://doi.org/10.1007/s00289-023-04868-5

Abdelkhalek, A. and Al-Askar, A.A., 2020. Green Synthesized ZnO Nanoparticles Mediated by Mentha Spicata Extract Induce Plant Systemic Resistance against Tobacco Mosaic Virus. Applied Sciences, 10(15): 5054-5068.
https://doi.org/10.3390/app10155054

Abdelmigid, H.M., Hussien, N.A., Alyamani, A.A., Morsi, M.M., AlSufyani, N.M., kadi, H.A., 2022. Green synthesis of zinc oxide nanoparticles using pomegranate fruit peel and solid coffee grounds vs. Chemical method of synthesis, with their biocompatibility and antibacterial properties investigation. Molecules, 27(4): 1236.
https://doi.org/10.3390/molecules27041236

Abdullah, J.A.A., Salah-Eddine, L., Abderrhmane, B., Alonso-Gonzlez, M., Guerrero, A., Romero, A., 2020. Green synthesis and characterization of iron oxide nanoparticles by *pheonix dactylifera* leaf extract and evaluation of their antioxidant activity. Sustainable Chemistry and Pharmacy, 17: 218-228.
https://doi.org/10.1016/j.scp.2020.100280

Agarwal, H., Kumar, S.V. and Rajeshkumar, S., 2017. A review on green synthesis of zinc oxide nanoparticles- an eco-friendly approach. Resource-Efficient Technologies, 3(4): 406413.
https://doi.org/10.1016/j.reffit.2017.03.002

Akroum, S., Bendjeddou, D., Satta, D. and Lalaoui, K., 2010. Antibacterial, antioxidant and acute toxicity tests on flavonoids extracted from some medicinal plants. International Journal of Green Pharmacy, 4(3): 165-170.
https://doi.org/10.4103/0973-8258.69174

Alamdari, S., Ghamsari, M.S., Lee, C., Han, W., Park, H.H., Tafreshi, M.J., Ara, M.H.M., 2020. Preparation and Characterization of Zinc Oxide Nanoparticles Using Leaf Extract of *Sambucus ebulus*. Applied Sciences, 10(10): 3620-3638.
https://doi.org/10.3390/app10103620

Anand, K.V., Kumar, J.A., Keerthana, K., Deb, P., Tamilselvan, S., Theerthagiri, J., Rajeswari, V., Sekaran, S.M.S., Govindaraju, K., 2019. Photocatalytic degradation of rhodamine B dye using biogenic hybrid ZnOMgO nanocomposites under visible light. Chemistry Select, 4(17): 5178-5184.
https://doi.org/10.1002/slct.201900213

Anand, K.V., Anugraga, A.R., Kannan, M., Singaravelu, G., Govindaraju, K., 2020. Bio-engineered magnesium oxide nanoparticles as nanopriming agent for enhancing seed germination and seedling vigour of green gram (*Vigna radiata* L.). Materials Letters, 271: 127792.
https://doi.org/10.1016/j.matlet.2020.127792

Anand, K.V., Mahalakshmi, D., Selvan, S.M., Ravi, M., Kannan, M., Govindaraju, K., Shalan, A.E., 2024. Biomass extract of green macroalga Halimeda opuntia assisted ZnO nanoparticles: Preparation, physicochemical characterization, and antibacterial activity against *Vibrio harveyi*. Biomass Conversion and Biorefinery, 14(2): 2225-2233.
https://doi.org/10.1007/s13399-022-02397-1

Ananthalakshmi, R., Rajarathinam, S.R.X. and Sadiq, A.M., 2019. Antioxidant activity of ZnO Nanoparticles synthesized using *Luffa acutangula* peel extract. Research Journal of Pharmacy and Technology, 12(4): 1569-1572.
https://doi.org/10.5958/0974-360X.2019.00260.9

Ambika, S. and Sundrarajan, M., 2015. Antibacterial behaviour of *Vitex negundo* extract assisted ZnO nanoparticles against pathogenic bacteria. Journal of Photochemistry and photobiology B: Biology, 146: 52-57.
https://doi.org/10.1016/j.jphotobiol.2015.02.020

Arokiyaraj, S., Saravanan, M., Bharanidharan, R., Islam, V.I.H., Bououdina, M., Vincent, S., 2016. Green synthesis of metallic nanoparticles using plant compounds and their applications: Metallic nanoparticles synthesis using plants. In Emerging Research on Bioinspired Materials Engineering, 1-34.
https://doi: 10.4018/978-1-4666-9811-6.ch001

Avinash, B., Ravikumar, C.R., Basavaraju, N., Abebe, B., Naveen Kumar, T., Manjula, S.N., Ananda Murthy, H.C., 2024. Facile green synthesis of zinc oxide nanoparticles: Its photocatalytic and electrochemical sensor for the determination of paracetamol and D-glucose. Environmental Functional Materials.
https://doi.org/10.1016/j.efmat.2024.01.002

Bauer, A.W., 1996. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 45: 149-158.
https://doi.org/10.1093/ajcp/45.4_ts.493

Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature, 181(4617): 1199-1200.
https://doi.org/10.1038/1811199a0

Cheemanapalli.S., Mopuri R., Golla, R., Anuradha, C.M., Chitta, S.K., 2018. Syringic acid (SA)-a review of its occurrence, biosynthesis, pharmacological and industrial importance. Biomedicine and Pharmacotherapy, 108: 547-557.
https://doi.org/10.1016/j.biopha.2018.09.069

Debnath, D. and Gupta, A.K., 2018. Optimizing the fabrication of nanoplasmonic silver nitrogen co-doped zinc oxide (Ag x Zn (1-x) N y O (1-y)) mediated by ammonia template: insight into its enhanced physiochemical and photocatalytic behavior. Journal of Molecular Liquids, 249: 334-345.
https://doi.org/10.1016/J.MOLLIQ.2017.11.050

Deepika, M.S., Thangam, R., Vijayakumar, T.S., Sasirekha, R., Vimala, R.T.V., Sivasubramanian, S., Arun, S., Babu, M.D., Thirumurugan, R., 2019. Antibacterial synergy between rutin and florfenicol enhances therapeutic spectrum against drug resistant Aeromonas hydrophila. Microbial pathogenesis, 135: 103612.
https://doi.org/10.1016/j.micpath.2019.103612

Doğaroğlu, Z.G., Uysal, Y., Çaylal, Z., Karakulak, D.S., 2023. Green nanotechnology advances: green manufacturing of zinc nanoparticles, characterization, and foliar application on wheat and antibacterial characteristics using Mentha spicata (mint) and *Ocimum basilicum* (basil) leaf extracts. Environmental Science and Pollution Research, 30:60820-60837.
https://doi.org/10.1007/s11356-023-26827-3

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal, N., 2006. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. Food Chemistry, 97(4): 654-660.
https://doi.org/10.1016/j.foodchem.2005.04.028

Divya, M.J., Sowmia, C., Joona, K., Dhanya, K.P., 2013. Synthesis of zinc oxide nanoparticle from *Hibiscus rosa-sinensis* leaf extract and investigation of its antimicrobial activity. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 4(2): 1137-1142.
http://rjpbcs.com/pdf/2013_4(2)/[123]

Eldurini, S., Shafaa, M.W., Abd-El-Hady, B.M., Gad, A.A.M., Tolba, E., 2021. A multicompartment vascular implant of electrospun wintergreen oil/ polycaprolactone fibers coated with (polyethylene oxide). Biomedical Journal, 44(5): 589597.
https://doi.org/10.1016/j.bj.2020.04.008

Estrada-Urbina, J., Cruz-Alonso, A., Santander-Gonzalez, M., Mndez-Albores, A., Vzquez-Durn, A., 2018. Nanoscale zinc oxide particles for improving physiological and salinity quality of a Mexican landrace of red maize. 8(4): 247-255.
https://doi.org/10.3390/nano8040247

Gironi, F. and Piemonte, V., 2011. Temperature and solvent effects on polyphenol extraction process from chestnut tree wood. Chemical Engineering Research and Design, 89(7): 857-862.
https://doi.org/10.1016/j.cherd.2010.11.003

Guneidy, R.A., Gad, A.M., Zaki, E.R., Ibrahim, F.M., Shokeer, A., 2020. Antioxidant or pro-oxidant and glutathione transferase P1-1 inhibiting activities for *Tamarindus indica* seeds and their cytotoxic effect on MCF-7 Cancer cell line. Journal of Genetic Engineering & Biotechnology, 18(1): 74-89.
https://doi.org/10.1186/s43141-020-00077-z

Guneidy, R.A., Zaki, E.R., Gad, A.A.M., Saleh, N.S.E.D. and Shokeer, A., 2022. Evaluation of Phenolic Content Diversity along with Antioxidant/Pro-Oxidant, Glutathione Transferase Inhibition, and Cytotoxic Potential of Selected Commonly Used Plants. Preventive Nutrition and Food Science, 27(3): 282-298.
https://doi.org/10.3746/pnf.2022.27.3.282

Hu, X., Cook, S., Wang, P., Hwang, H., 2009. In vitro evaluation of cytotoxicity of engineered metal oxide nanoparticles. Science of The Total Environment, 407(8): 3070-3072.
https://doi.org/10.1016/j.scitotenv.2009.01.033

Imani, A., Maleki, N., Bohlouli, S., Kouhsoltani, M., Sharifi, S., Dizaj, S.M., 2021. Molecular mechanisms of anticancer effect of rutin. Phytotherapy Research, 35(5): 2500-2513.
https://doi.org/10.1002/ptr.6977

Jain, D., Bhojiya, A.A., Singh, H., Daima, H.K., Singh, M., Mohanty, S.R., Stephen, B.J., Singh, A., 2020. Microbial Fabrication of Zinc Oxide Nanoparticles and Evaluation of Their Antimicrobial and Photocatalytic Properties. Frontiers in Chemistry, 8: 778. https://doi.org/10.3389/fchem.2020.00778

Jiang, S., Lin, K. and Cai, M., 2020. ZnO Nanomaterials: Current Advancements in Antibacterial Mechanisms and Applications. Frontiers in Chemistry, 8: 580-592. https://doi.org/10.3389/fchem.2020.00580

Jiménez-Rosado, M., Gomez-Zavaglia, A., Guerrero, A., Romero, A., 2022. Green synthesis of ZnO nanoparticles using polyphenol extracts from pepper waste (*Capsicum annuum*). Journal of Cleaner Production, 350: 131541. https://doi.org/10.1016/j.jclepro.2022.131541

Karamac, M., Kosinska, A. and Pegg, R.B., 2005. Comparison of radicalscavenging activities of selected phenolic acids. Polish Journal of Food and Nutrition Sciences, 55(2): 165170.

Karaoğlan, E.S., Hanc, H., Koca, M., Kazaz, C., 2023. Some Bioactivities of Isolated Apigenin-7-O-glucoside and Luteolin-7-O-glucoside. Applied Sciences, 13(3): 1503-1513. https://doi.org/10.3390/app13031503

Khan, R., Inam, M.A., Khan, S., Park, D.R. and Yeom, I.T., 2019. Interaction between persistent organic pollutants and ZnO NPs in synthetic and natural waters. Nanomaterials, 9(3): 472-481. https://doi.org/10.3390/nano9030472

Kim, K.H., Tsao, R., Yang, R., Cui, S.W., 2006. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chemistry, 95(3): 466-473. https://doi.org/10.1016/j.foodchem.2005.01.032

Koli, K., Rohtela, K. and Meena, D., 2022. Comparative study and analysis of structural and optical properties of zinc oxide nanoparticles using neem and mint extract prepared by green synthesis method. In *IOP* International Conference Series: Materials Science and Engineering, 1248(1): 012065. https://doi.org/10.1088/1757-899X/1248/1/012065

Kumaresan, M., Anand, K.V., Govindaraju, K., Tamilselvan, S. and Kumar, V.G., 2018. Seaweed *Sargassum wightii* mediated preparation of zirconia (ZrO_2) nanoparticles and their antibacterial activity against gram positive and gram negative bacteria. Microbial pathogenesis, 124: 311-315. https://doi.org/10.1016/j.micpath.2018.08.060

Venkataraju, J.L., Sharath, R., Chandraprabha, M.N., Neelufar, E., Hazra, A., Patra, M., 2012. Synthesis, characterization and evaluation of antimicrobial activity of zinc oxide nanoparticles. Journal of Biochemical Technology, 3(5): S151-S154.

Lakshmi, J.V., Sharath, R., Chandraprabha, M.N., 2012. Synthesis, characterization and evaluation of antimicrobial activity of zinc oxide nanoparticles. Journal of Biochemical Technology, 3(5): S151-S154.

Lin, J.Y. and Tang, C.Y., 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chemistry, 101(1): 140-147. https://doi.org/10.1016/j.foodchem.2006.01.014

Lipiński, K., Mazur, M., Antoszkiewicz, Z., Purwin, C., 2017. Polyphenols in monogastric nutrition-a review. Annals of Animal Science, 17(1): 4158. https://doi.org/10.1515/aoas-2016-0042

Luo, Z., Wu, Q., Xue, J., Ding, Y., 2013. Selectively enhanced antibacterial effects and ultraviolet activation of antibiotics with ZnO nanorods against *Escherichia coli*. Journal of Biomedical Nanotechnology, 9(1): 69-76. https://doi.org/10.1166/jbn.2013.1472

Martins, N., Ferreira, I.C., Barros, L., Carvalho, A.M., Henriques, M., Silva, S., 2015. Plants used in folk medicine: The potential of their hydromethanolic extracts against Candida species. Industrial crops and products, 66: 62-67. https://doi.org/10.1016/j.indcrop.2014.12.033

Nematollahi, F., Konjini, F.T. and Hergalani, F.Z., 2021. Determination of the antioxidant activity of *Calendula officinalis* extract and its role in synthesis of ZnO nanoparticles. Journal of Food Technology and Nutrition, 18(4): 127-134.

Özbek, H.N., Halahlih, F., Göğüş, F., Yank, D.K., Azaizeh, H., 2020. Pistachio (*Pistacia vera* L.) Hull as a potential source of phenolic compounds: evaluation of ethanolwater binary solvent extraction on antioxidant activity and phenolic content of Pistachio Hull extracts. Waste and Biomass Valorization, 11(2): 21012110. https://doi.org/10.1007/s12649-018-0512-6

Petcharoen, K. and Sirivat, A.J.M.S., 2012. Synthesis and characterization of magnetite nanoparticles via the chemical co-precipitation method. Materials Science and Engineering B, 177(5): 421-427. https://doi.org/10.1016/j.mseb.2012.01.003

Prabhu, S. and Poulose, E.K., 2012. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International Nano Letters, 2: 1-10. https://doi.org/10.1186/2228-5326-2-32

Prado, J.M., Vardanega, R., Debien, I.C.N., et al, 2021. Conventional extraction. In Food waste recovery, pp. 109127. Academic Press.

Pramila, D.M., Marimuthu, K., Kathiresan, S., Khoo, M.L., Sathya, K., 2012. Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (*Minta piperita*: Lamiaceae). Journal of Medicinal Plants Research. 6(2): 331-333. https://doi.org/10.5897/JMPR11.123

Quideau, S., Deffieux, D., Douat-Casassus, C., Pouysgu, L., 2011. Plant polyphenols: Chemical properties, biological activities, and synthesis. Angewandte Chemie International Edition, 50(3): 586-621. https://doi.org/10.1002/anie.201000044

Rajeswari, V.D., Khalifa, A.S., Elfasakhany, A., Badruddin, I.A., Kamangar, S., Brindhadevi, K., 2021. Green and ecofriendly synthesis of cobalt oxide nanoparticles using *Phoenix dactylifera* L: antimicrobial and photocatalytic activity. Applied Nanoscience, 13(2): 1367-1375. https://doi.org/10.1007/s13204-021-02038-5

Reddy, L.S., Mary, M.N., Mary, J., Shilpa, N.P., 2014. Antimicrobial activity of zinc oxide (ZnO) nanoparticle against *Klebsiella pneumoniae*. Pharmaceutical biology, 52(11): 1388-1397. https://doi.org/10.3109/13880209.2014.893001

Saleem, S., Hasnain-Jameel, M., Akhtar, N., Nazir, N., Ali, A., Zaman, A., Rehman, A., Butt, S., Sultana, F., Mushtaq, M., Zeng, J.H., Amami, M., Althubeiti, K., 2021. Modification in structural, optical, morphological, and electrical properties of zinc oxide (ZnO) nanoparticles (NPs) by metal (Ni, Co) dopants for electronic device applications. Arabian Journal of Chemistry, 15(1):103518. https://doi.org/10.1016/j.arabjc.2021.103518

Shamsudin, N.F., Ahmed, Q.U., Mahmood, S., Shah, S.A.A., Khatib, A., Mukhtar, S., Alsharif, M.A., Parveen, H., Zakaria, Z.A., 2022. Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. Molecules, 27(4): 1149-1191. https://doi.org/10.3390/molecules27041149

Sharma, S., Kumar, K., Chauhan, S., et al, 2018. Synthesis and characterization of ZnO nanoparticles using mint plant leaves. CPUH-Research journal, 3(2): 1-4.

Sheldon, R.A., 2005. Green solvents for sustainable organic synthesis: state of the art. Green Chemistry, 7(5): 267-278. https://doi.org/10.1039/B418069K

Siddiqi, K.S., Rahman, A. and Husen, A., 2018. Properties of Zinc Oxide Nanoparticles and Their Activity Against Microbes. Nanoscale Research Letters, 13(1): 1-13. https://doi.org/10.1186/s11671-018-2532-3

Talam, S., Karummuri, S.R. and Gunnam, N., 2012. Synthesis, characterization and spectroscopic properties of ZnO nanoparticles. International Scholarly Research Network, 2012:1-6. https://doi.org/10.5402/2012/372505

Uchenna, U.E., Shori, A.B. and Baba, A.S., 2018. *Tamarindus indica* seeds improve carbohydrate and lipid metabolism: An in vivo study. Journal of Ayurveda and integrative medicine, 9(4): 258-265. https://doi.org/10.1016/j.jaim.2017.06.004

Vadivel, V., Nandety, A. and Biesalski, H.K., 2011. Antioxidant, free radical scavenging and type II diabetes related enzyme inhibition properties of traditionally processed Jequirity bean (*Abrus precatorius* L.). International Journal of Food Science & Technology, 46(12): 2505-2512. https://doi.org/10.1111/j.1365-2621.2011.02774.x

Varma, R.S., 2012. Greener approach to nanomaterials and their sustainable applications. Current Opinion in Chemical Engineering, 1(2): 123-128. https://doi.org/10.1016/j.coche.2011.12.002

Vivekananth, G.G., Poonkothai, M. and Alaguprathana, M. 2021. Sustainable root mediated synthesis of iron oxide nanoparticles from *Glycyrrhiza glabra* and its environmental applications. SPAST Abstracts, 1:(01).

Wayne, P.A., 2009. National Committee for Clinical Laboratory Standards, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard M7A7, vol.26 (Clinical and Laboratory Standards Institute).

Wong, C., Li, H., Cheng, K., Chen, F., 2006. Systematic survey of antioxidant of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chemistry, 97: 705-711. https://doi.org/10.1016/j.foodchem.2005.05.049

Xie, Y., He, Y., Irwin, P.L., Jin, T., Shi, X., 2011. Antibacterial activity and mechanism of action of zinc oxide nanoparticles against *Campylobacter jejuni*. Applied and environmental microbiology, 77(7): 23252331. https://doi.org/10.1128/AEM.02149-10

Zak, A.K., Abrishami, M.E., Majid, W.H.A., Yousefi, R., Hosseini, S.M., 2011. Effects of annealing temperature on some structural and optical properties of ZnO nanoparticles prepared by a modified solgel combustion method. Ceramics International, 37(1): 393398. https://doi.org/10.1016/j.ceramint.2010.08.017