agronomy

MDPI

Article

The Biogenic Synthesis of Cobalt Monometallic and Cobalt–Zinc Bimetallic Nanoparticles Using *Cymbopogan citratus* L. Leaf Extract and Assessment of Their Activities as Efficient Dye Removal and Antioxidant Agents

Tauheeda Riaz [1], Soha Nayyar [1], Tayyaba Shahzadi [1], Maria Zaib [2], Sammia Shahid [3], Sana Mansoor [3], Mohsin Javed [3,*], Shahid Iqbal [4,*], Murefah Mana Al-Anazy [5], Eslam B. Elkaeed [6], Rami Adel Pashameah [7], Eman Alzahrani [8] and Abd-ElAziem Farouk [9]

1 Department of Chemistry, Government College Women University Sialkot, Sialkot 51310, Pakistan
2 Department of Chemistry, University of Jhang, Jhang 35200, Pakistan
3 Department of Chemistry, School of Science, University of Management and Technology, Lahore 54770, Pakistan
4 Department of Chemistry, School of Natural Sciences (SNS), National University of Sciences and Technology (NUST), H-12, Islamabad 46000, Pakistan
5 Department of Chemistry, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia
6 Department of Pharmaceutical Sciences, College of Pharmacy, AlMaarefa University, Riyadh 13713, Saudi Arabia
7 Department of Chemistry, Faculty of Applied Science, Umm Al-Qura University, Makkah 24230, Saudi Arabia
8 Department of Chemistry, College of Science, Taif University, Taif 21944, Saudi Arabia
9 Department of Biotechnology, College of Science, Taif University, Taif 21944, Saudi Arabia
* Correspondence: mohsin.javed@umt.edu.pk (M.J.); shahidgcs10@yahoo.com (S.I.)

Abstract: In this work, green synthesized cobalt monometallic and cobalt–zinc bimetallic NPs were prepared by bioreduction of metallic salts with *Cymbopogan citratus* plant extract. Biosynthesized cobalt nanoparticles (NPs) and cobalt–zinc bimetallic NPs were characterized using diverse techniques such as FTIR, UV-Visible spectroscopy, SEM, XRD, and EDX analyses. UV-visible spectra for green-synthesized cobalt monometallic and cobalt–zinc bimetallic NPs were in the range between 300 to 350 nm, which confirmed the formation of stable monometallic and bimetallic NPs. The average particle size of CoNPs calculated by XRD analysis was found to be 22.77 nm and that of Co-Zn BMNPs was 14.8 nm. Different functional groups in the *Cymbopogan citratus* plant extract, which served as a reducing and stabilizing agent for NPs, were identified by FTIR spectra. Cobalt NPs and cobalt–zinc bimetallic NPs were used in the evaluation of antioxidant, anti-inflammatory, and dye degradation activity. Green-synthesized cobalt monometallic NPs and cobalt–zinc bimetallic NPs exhibited excellent antioxidant activity with the scavenging of DPPH free radicals. Green synthesized cobalt NPs and cobalt–zinc bimetallic NPs were utilized for the removal of methylene blue (MB) dye. Different parameters such as the effect of temperature, pH, and dye concentration on adsorbent doses were analyzed and optimized. The best dye removal percentage was obtained with Co-Zn BMNPs compared with CoNPs. Cobalt NPs and cobalt–zinc bimetallic NPs did not display good anti-inflammatory activity because of the presence of secondary metabolites which inhibited them to react with proteins.

Keywords: biogenic; leaf extract; dye removal; bimetallic NPs; green synthesis

Citation: Riaz, T.; Nayyar, S.; Shahzadi, T.; Zaib, M.; Shahid, S.; Mansoor, S.; Javed, M.; Iqbal, S.; Al-Anazy, M.M.; B. Elkaeed, E.; et al. The Biogenic Synthesis of Cobalt Monometallic and Cobalt–Zinc Bimetallic Nanoparticles Using *Cymbopogan citratus* L. Leaf Extract and Assessment of Their Activities as Efficient Dye Removal and Antioxidant Agents. *Agronomy* **2022**, *12*, 2505. https://doi.org/10.3390/agronomy12102505

Academic Editors: Laura Siracusa and Rosa Palmeri

Received: 6 September 2022
Accepted: 8 October 2022
Published: 14 October 2022

1. Introduction

Nanotechnology has many applications in the field of science and technology that are used for the synthesis of nanomaterials. Green nanotechnology is the branch of green chemistry that works on the twelve principles of green chemistry and is an effective substitution

for manmade nano-chemistry. Green nanotechnology required nontoxic chemicals, low temperature, benign solvents, and innocuous waste production with safe and clean methods for the synthesis of eco-friendly nanoproducts [1–7]. Now, synthetic nanotechnology is avoided due to different side effects such as harmful chemicals being used, resulting in the production of adverse byproducts that are not eco-friendly. Green nano-chemistry replaces synthetic nano-chemistry due to the usage and production of these deadly chemicals that are toxic to human health [8–12].

There are three different methods used to synthesize NPs. In the chemical method, chemical reagents are required for proper functioning with special temperature and pressure maintenance. This method also produces toxic by-products. Therefore, it is not an eco-friendly method. In the physical method, solvent pollution does not occur, but it also requires very high temperature and pressure for the maintenance and working of the furnace. Therefore, this method is also not good as it pollutes the surrounding environment [13–16]. The biological method is best for the preparation of NPs. In this method, metal ions are converted into metal atoms by the reduction of the metal ion with plant extract. Biomolecules present in plant extract act as the capping agent as well as reducing agents simultaneously. This method is quite simple and carried at normal temperature and pressure. Fungi are also used to produce metallic NPs and their stabilization is done by the proteins. Different types of algae are also used for the reduction of metal ions concentration into metal atoms having rod-like shapes. The maintenance of a culture medium for both fungi and algae is difficult. Therefore, the preferred method for the production of metal NPs is done by the use of metal ions with plant extract [17–19].

The particles having submicron scale entities made up of pure metals such as platinum, gold, zinc, silver, iron, manganese, aluminum, cobalt, nickel, and other such metals, etc. are generally termed metallic NPs. Metals having dimensions (length, breadth, and thickness) between 1 and 100 nm are referred to as metallic NPs. In a reaction between a metal salt solution and a plant extract, the phytochemicals in the plant extract primarily serve as a capping agent, reducing the metal to a nanoparticle size range [20–23]

Cobalt NPs are formed by following the method in which metal salt solution is reacted with plant extract. Polyphenols present in plants can intricate oxidation and reduction reactions. These polyphenols mainly act as the capping agent that maintains the size and morphology of NPs and as oxygen quenchers. The more the phenolic compounds, the more easily the reduction and capping phenomenon of metal ions occurs. These cobalt NPs have many applications, especially in treating wastewater, helping to degrade dyes present in polluted water, and as antibacterial, anti-inflammatory, and anti-microbial agents. It also possesses magnetic properties, which leads to applications in imaging, sensors, and many other areas [24].

Bimetallic NPs have shown more pronounced properties than monometallic NPs in different fields of optics, magnetic and electric. The green method is also used to prepare bimetallic NPs that are eco-friendly and benign. Plant extract is used to react with the solutions of metal salt solutions of equimolar ratios at ambient temperature. Phytochemicals present in plant extract act as a capping agent to control the size of bimetallic NPs [25].

Cymbopogan citratus belongs to the Poaceae family and consists of 500 species. It is commonly called lemon grass because having the aroma of lemon. *Cymbopogan* is a genus comprised of 30 species of grasses. The word *Cymbopogan* is Greek and means "flower spike arrangement", and *citratus* is an ancient word that means "lemony fragrance foliage". Lemon grass is a perennial plant having thin, long and scented leaves [26–28]. It is an aromatic herb, and its aerial parts can be used as medicine in the treatment of flu, fever, malaria, illness, and inflammation. Flavonoids and phenolic compounds are present in lemon grass that show antioxidant and anti-inflammatory properties. Its essential component is its oil which can be extracted through a steam distillation process [29,30].

The essential oil contains many complex vital ingredients mainly terpenes i.e., limonene, elemol, citral, citronellol, geraniol, and oxygenated derivatives. It is mostly used as dried leaves in making herbal tea, and as a masala herb in curries and soups that adds an in-

triguing taste and aroma to it. It is native to Pakistan, Sri Lanka, and India and is widely cultivated throughout the world. It is used as a medicinal herb. It gives a soothing effect to the brain. Lemon grass can act as an antifungal, antibacterial, antidiarrheal, and anti-inflammatory agent [31–34]. It is a good substitute for conventional medicines. It is also used to treat toothache and stomachache etc. [35].

Lemon grass contains many nutritional components such as fats, carbohydrates, proteins, minerals, and many bioactive components. Bioactive components include alkaloids, terpenoids, flavonoids, saponins, tannins, and phenol. β-citronellol is present in lemongrass which shows weight loss activity. Citronella oil is also crucial as it exhibits anti-cancerous and anti-fungal properties [36,37].

In the present study, cobalt monometallic and cobalt zinc bimetallic nanoparticles are synthesized by using plant extract of *Cymbopogan citratus* (lemon grass), which is potentially active for dye removal, having antioxidant and other biochemical activities. This work reflects green synthesis as it is a plant-mediated synthesis with benign and sustainable metal nanoparticles with effective properties.

2. Material and Methods

2.1. Chemicals and Reagents

Cymbopogon citratus leaves were collected from south Punjab, Pakistan. The plant was identified by the botanist of the Botany Department, GCWUS, and voucher specimen number GCWUS-Bot-346 was given to the plant. The chemicals used, cobalt chloride, zinc chloride, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), BHT, sodium carbonate, Folin-Ciocalteu's phenol reagent (FCR), ammonium molybdate, sulphuric acid, NaOH, butylated hydroxytoluene and MB dye were taken from Sigma-Aldrich (Taufkirchen, Germany).

2.2. Preparation of Plant Extract

Cymbopogon citratus leaves were collected, washed properly with distilled H_2O, and then dried under the shade. The dried leaves were ground into a fine powder form by an electronic blender. In the first step, 10 g of plant powder was dissolved in 100 mL of distilled water, and the resulting liquid was constantly swirled for an hour at 70 °C using a magnetic stirring device on a hot plate. The mixture was then allowed to cool at room temperature. The filter paper was used for the filtration of the solution and the solvent was separated. Then, the solution of *Cymbopogon citratus* was kept in the freezer so that it could use for the synthesis of metallic NPs. The same procedure was repeated to prepare the plant extract used during the work [28].

2.3. Synthesis of Cobalt Monometallic NPs

For the synthesis of cobalt NPs, a 0.5 M solution of cobalt chloride was prepared. Then, 50 mL of *Cymbopogon citratus* filtrate was taken with the help of a pipette. This filtrate was added dropwise to a 50 mL solution of 0.5 M cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$). The mixture was heated and stirred continuously by a magnetic stirrer at 60 °C for 3 h. After that, the color change was observed. Then spectral analysis was done by using UV-visible spectroscopy for the confirmation of cobalt NPs. After that, the solution was centrifuged and filtered [18].

2.4. Synthesis of Cobalt–Zinc Bimetallic NPs

For the bimetallic synthesis of NPs, cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) and zinc chloride ($ZnCl_2$) salts were used. For this purpose, an equimolar solution of both salts was prepared. 30 mL of plant extract was heated at about 60 °C. Then 35 mL solution of 0.5 M cobalt chloride hexahydrate was added dropwise by continuous stirring. After 5 min, 35 mL of 0.5 M zinc chloride solution was added. For 2 hours, the mixture was heated to 60 °C and continually swirled with a magnetic stirrer on a hot plate. The hue changed while the reduction took place. When Co-Zn bimetallic NPs were confirmed, spectral analysis was conducted using UV-visible spectroscopy. After the confirmation of biosynthesized

NPs, the solution was centrifuged and filtered. Then it was refined with distilled water three times and again centrifuged. The solid material was dried and obtained finally in powder form [15]

2.5. Characterization of Synthesized NPs

NPs were studied using a variety of methods, including energy-dispersive X-ray spectroscopy, scanning electron microscopy, Fourier transfer infrared spectroscopy, UV-visible spectroscopy, and X-ray diffraction. Through UV-Visible spectroscopic analysis emission and scattering, phenomena can be measured [35]. FTIR was mainly used to analyze the functional groups that are involved in the bio-reduction of metal ions into a metal atom [27]. The structure and morphology of monometallic and bimetallic NPs were assessed with a scanning electron microscope. Crystalline phases and particle size was determined by the X-ray diffraction technique [37]. The elemental composition of synthesized green NPs was ascertained by energy-dispersive X-ray spectroscopic analysis [4].

2.6. Antioxidant Potential

Three methods were followed for the evaluation of the antioxidant potential of monometallic cobalt and bimetallic cobalt–zinc NPs i.e., 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), total phenolic contents, and total antioxidant activity.

2.7. DPPH Free Radical Scavenging Activity

The antioxidant potential of both monometallic and bimetallic NPs was evaluated by using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). This method was reported by Lee and Shibamoto [16]. In this method, different concentrations (1000 µg, 500 µg, and 250 µg) of monometallic and bimetallic were taken and reacted with DPPH methanolic solution. (0.1 mM) DPPH methanolic solution was added to all test tubes. After that, these test tubes were placed in the dark for 30 min at room temperature so that reaction can proceed. After that, the solutions were centrifuged for 5 min at 5000 rpm. Then the supernatant was used to note absorbance on a UV-Visible spectrophotometer at 517 nm wavelength. Methanol was used as a blank [14]. The formula used for the calculation of % scavenging of DPPH free radical is given below:

$$\% \text{ Scavenging} = A_{control} - A_{sample}/A_{control} \times 100$$

where $A_{control}$ was the absorbance of the control reaction and A_{sample} was the absorbance of the sample.

2.8. Total Phenolic Contents

Using Folin–Ciocalteu phenol reagent (Sigma-Aldrich, Taufkirchen, Germany), the total phenolic contents of green-produced monometallic and bimetallic NPs were assessed. 0.1 mL of 2 N Folin–Ciocalteu's phenol reagent was allowed to react with 2.8 mL of 10% Na_2CO_3. Then 0.5 mg of monometallic and bimetallic NPs were taken in different test tubes and reacted with the mixture. After that, the mixture was shaken and placed in the dark for 40 min. UV-visible spectrophotometer was used to note the values of absorbance at 725 nm. By constructing a graph of various gallic acid concentrations, the total phenolic contents were calculated as milligrams of gallic acid equivalent per gram of material [17]

2.9. Total Antioxidant Activity

The phosphomolybdenum complex reagent (Sigma-Aldrich, Taufkirchen, Germany), technique was used to calculate the overall antioxidant activity of green-produced monometallic and bimetallic NPs. 4 mM ammonium hexamolybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid were combined to create the reagent combination. Following this, 500 g of monometallic and bimetallic nanoparticles were collected and placed in various test tubes. The reagent mixture was then poured into these test tubes at a volume of 4 mL. To allow for reaction and prevent impurities from entering the solution, test tubes were

covered with aluminum foil. It was kept at a temperature of 90 °C in a water bath. It was then filled with test tubes. After that, the reaction mixture cooled to a temperature of around 25 °C. UV-visible spectrophotometer was used to note the values of absorbance at 695 nm by using a 4 mL reagent mixture as blank. BHT was taken as standard [25]

2.10. Adsorption of MB Dye

Green synthesized monometallic cobalt and bimetallic cobalt–zinc NPs were used for the removal of MB dye from water. MB dye is basic and cationic. 1000 ppm stock solution of MB dye was prepared. For this purpose, 0.1 g of MB dye was dissolved in a 1000 mL solution of distilled water [6]. Then, using the following dilution formula, solutions of various concentrations (5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm) were created from the stock solution:

$$\text{Dilution Formula} = \text{C(ppm)} \times 100\ \text{mL}/\text{Cs(ppm)}$$

$$\text{C(ppm)} = \text{Solution of required conc. in parts per million}$$

$$\text{Cs(ppm)} = \text{Concentration of stock solution in parts per million}$$

Different factors were studied for the percentage removal of dye from water: temperature, pH, different concentrations of dye, contact time, and NPs dosage. The adsorption process was examined using various graphical and statistical analyses.

2.11. Evaluation of Anti-Inflammatory Activity

The anti-inflammatory activity of green synthesized NPs was assessed to prevent protein denaturation. 50 µL of varying concentrations of NPs were taken. Then, 5 mL of bovine serum albumin (0.2 percent *w/v*) was added. For five minutes, the reaction mixture was heated to 72 °C. The mixture was then allowed to cool for 10 min. Water was added to 5 mL of a 0.2 percent *w/v* bovine serum albumin solution as a control. 100 g/mL of diclofenac sodium solution received 5 mL of bovine serum albumin at a 0.2 percent weight-to-volume ratio. Absorbance was measured on a UV-visible spectrophotometer at 276 nm wavelength [27].

3. Results and Discussion

3.1. Synthesis of NPs

The plant extract was prepared by dissolving plant powder in distilled water. The extract was added to a metal salt solution. Upon continuously heating and stirring the solution color was changed. Dried CoNPs were brownish while Co-Zn bimetallic NPs were blackish.

3.2. Characterization of Synthesized NPs

The characterization of synthesized cobalt monometallic and cobalt–zinc bimetallic NPs was carried out by different techniques. UV-Visible spectra of plant extract, CoNPs, and Co-Zn bimetallic NPs were obtained to assess the formation of NPs. The UV-visible spectrum of plant extract is shown in Figure 1, while the spectra of monometallic and bimetallic NPs are displayed in Figure 2. The formation of biosynthesized NPs was confirmed by the absorption spectrum peak of CoNP and Co-Zn BMNPs between 300 and 350 nm wavelength. After the reduction of monometallic and bimetallic NPs, the reaction mixture color also changed, which indicated the confirmation of the synthesis of NPs [21].

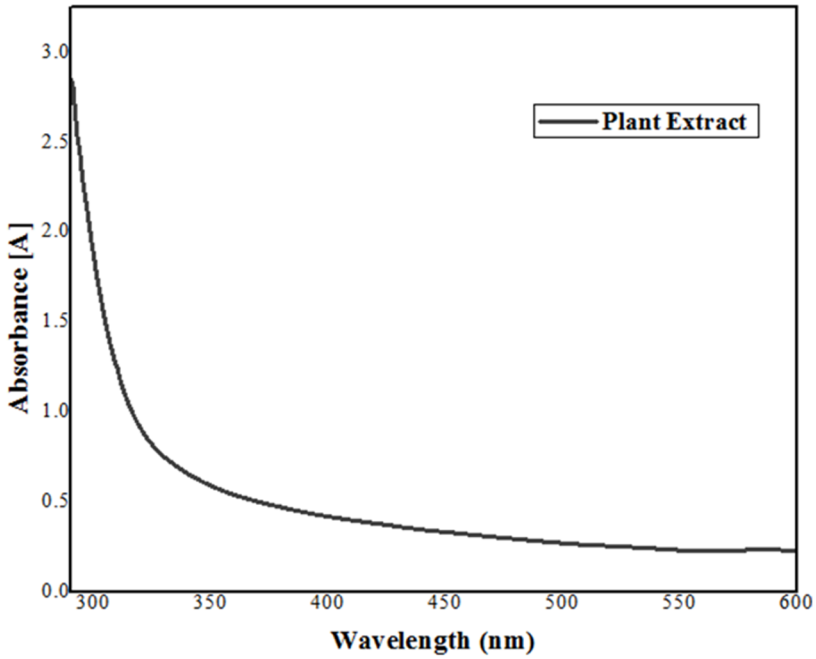


Figure 1. The UV-Visible spectrum of leaf extract.

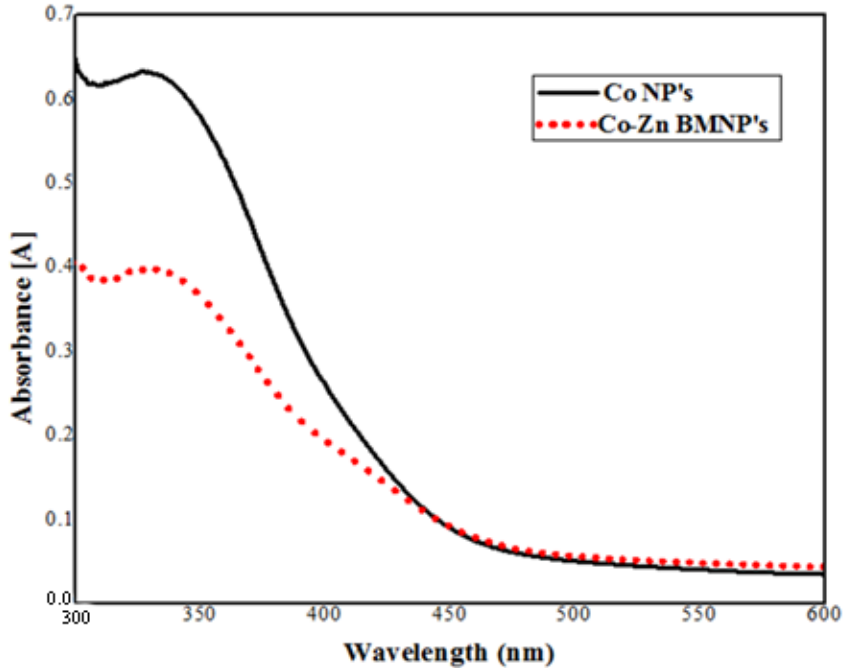


Figure 2. The UV-Visible spectrum of CoNPs and Co-Zn BMNPs.

Fourier transform infrared (FTIR) spectroscopic analysis was performed for leaf extracts of *Cymbopogon citratus* to identify the functional groups participating in the reduction of cobalt and zinc ions. The spectrum showed two peaks at 3279.80 cm^{-1} and 1031.33 cm^{-1} for OH stretching and bending vibrations respectively which indicated the presence of alcoholic or phenolic biomolecules in leave's extract of *Cymbopogon citratus* [8]. Peaks at 2917.56 cm^{-1} and 2849.31 cm^{-1} for sp^3 C-H stretching indicated the presence of sp^3 carbon-containing biomolecules. The peak at 1732.77 cm^{-1} indicated the presence of biomolecules containing carbonyl (C=O) functional groups such as carboxylic acids. The peak at 1605.55 cm^{-1} indicated the presence of an aromatic ring (C=C) containing biomolecules [1]. The peak at 1373.56 cm^{-1} indicated the bending vibration of the methyl

group (CH_3) group as shown in Figure 3. In the FTIR spectrum of cobalt monometallic NPs and Co-Zn bimetallic NPs, the peak at 2849.31 cm^{-1} disappeared which showed a reduction of metal by plant extract. The peak at 1373.56 cm^{-1} for bending vibration of methyl group (CH_3) group also disappeared which indicated the reduction of metal by plant extract. FTIR spectrum of cobalt monometallic NPs showed two peaks at 3289.49 cm^{-1} and 1015.15 cm^{-1} and the spectrum of cobalt–zinc bimetallic NPs also showed two peaks at 3501.64 cm^{-1} and 1020.87 cm^{-1} for OH stretching and bending vibrations respectively which indicated the presence of alcoholic or phenolic biomolecules. The peak in the range of 1500 cm^{-1} indicated the presence of the carbonyl (C=O) group for spectra of Co monometallic and Co-Zn bimetallic NPs. Spectra for Co monometallic and Co-Zn BMNPs showed a peak at 1412.09 cm^{-1} indicating the presence of an aromatic ring (C=C) containing biomolecules. According to changes in peak positions, intensities, and shapes between the biosynthesized NPs and the plant extract's FTIR spectrum, as shown in Figure 3, the plant extract's functional groups played a role in stabilizing and decreasing the NPs' production.

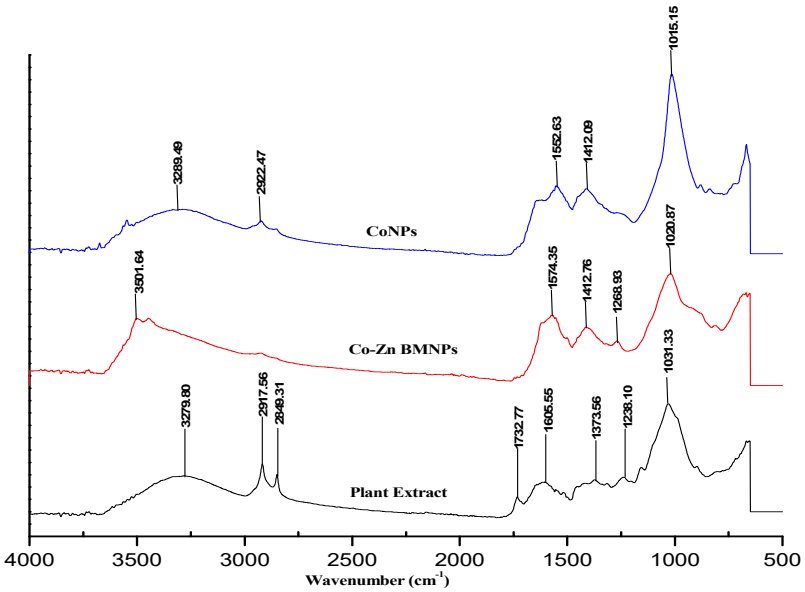


Figure 3. FTIR spectrum of CoNPs, Co-Zn BMNPs, and Plant Extract.

The spectra of green synthesized monometallic and bimetallic NPs revealed that the shape was spherical and almost irregular in cluster form. This cluster or agglomerated form is because of the polymeric nature of nanoparticles [2]. SEM images of monometallic and bimetallic NPs are shown in Figure 4a,b.

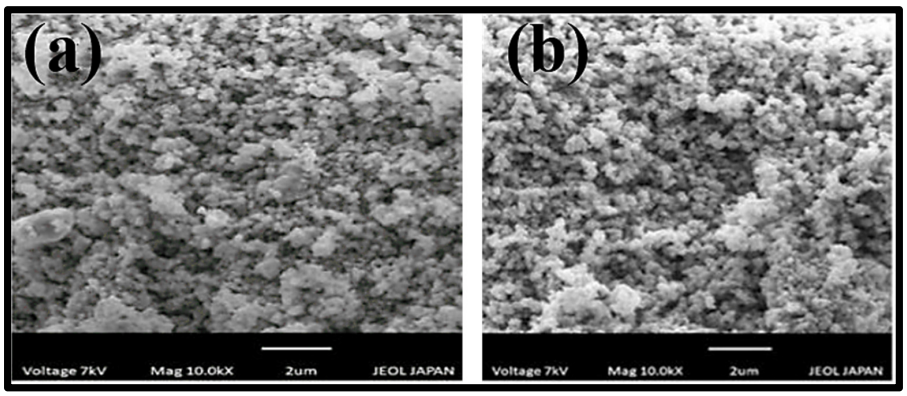


Figure 4. (**a**) SEM images of CoNPs 2 µm, (**b**) SEM images of Co-Zn BMNPs 2 µm.

Energy-dispersive X-ray spectroscopic analysis (EDX) was mainly used to determine the elemental composition of NPs. It analyzed all the fundamental elements present in the synthesized material. The formation of monometallic and bimetallic NPs was confirmed by EDX spectra. EDX spectrum analysis was carried out from 0 keV to 20 keV. Peaks of cobalt and zinc were displayed in the spectra. In Figure 5a, prominent peaks of cobalt were obtained. Elemental analysis of the prepared sample indicated that no other elements were observed except cobalt. Two prominent peaks of cobalt NPs were displayed in spectra at 0 keV and 6–7 keV. EDX spectra confirmed that 100% Co was reduced and changed to NPs. In Figure 5b, prominent peaks of cobalt were obtained at 0 keV and 6.5 to 7 keV. While zinc peaks were obtained at 0.8 keV and 8.5 to 9.5 keV. EDX results showed that cobalt and zinc concentrations were about 69.94% and 30.06%, respectively. EDX analysis of bimetallic NPs confirmed that the sample was enriched with cobalt as compared to zinc [3].

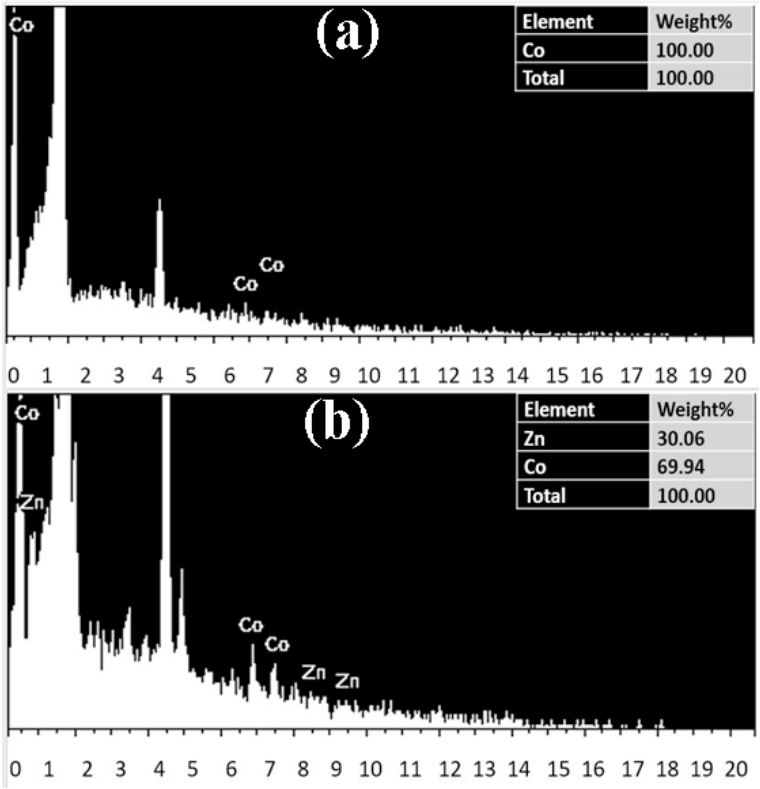


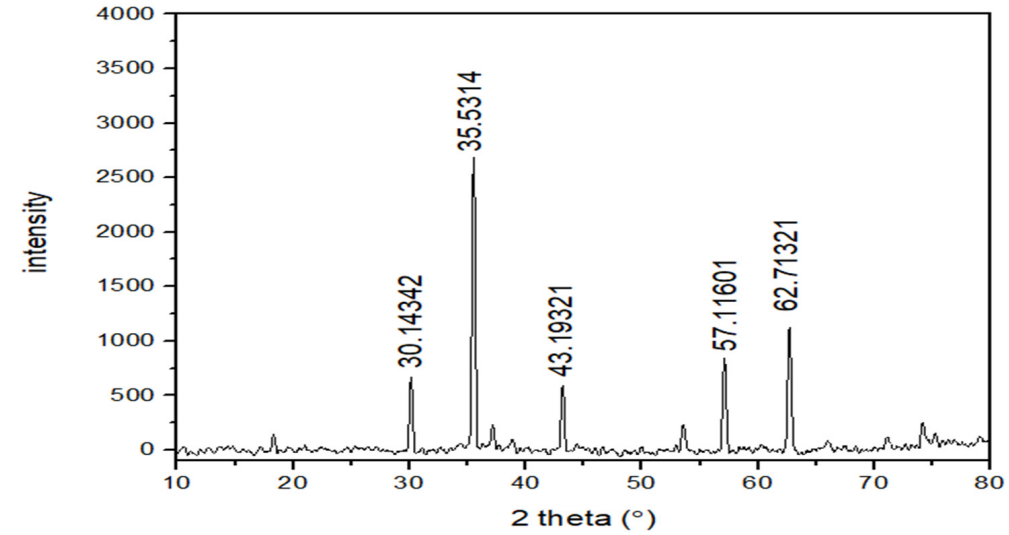
Figure 5. (**a**) EDX spectrum of green synthesized CoNPs. (**b**) EDX spectrum of green synthesized Co-Zn BMNPs.

The average diameter of green synthesized Co and Co-Zn bimetallic NPs was calculated by XRD. The resultant spectrum of cobalt NPs prepared by green synthesis is shown in Figure 6a.

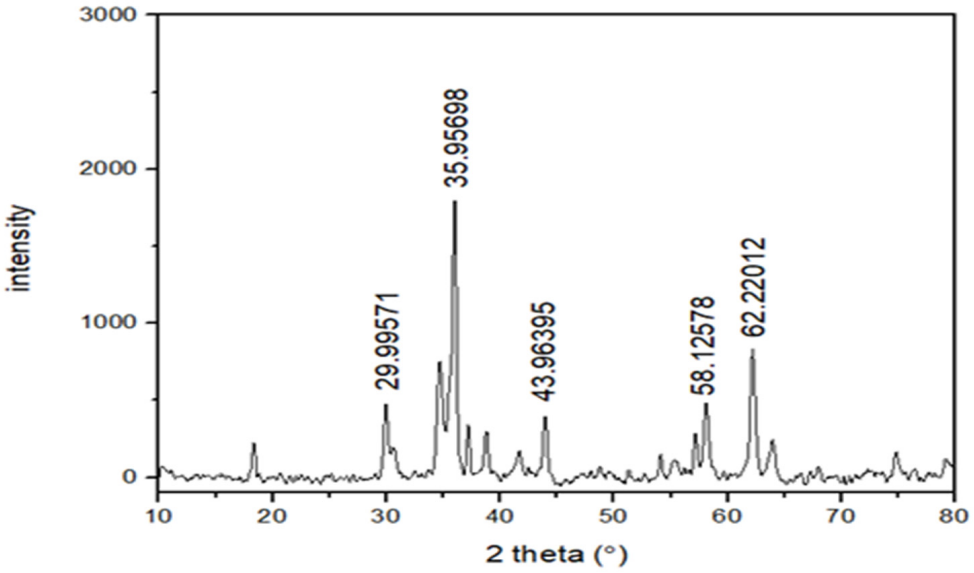
The XRD spectrum displayed noticeable peaks at 2θ values 30.143°, 35.53°, 43°, 57.116°, and 62.71°. Debye–Scherrer's formula was used to calculate the average size (Tables 1 and 2) of the particle:

$$D = \frac{K\lambda}{\beta cos\theta}$$

D = Average diameter of the particle in nm
λ = Wavelength of the X-ray (0.15406 nm)
β = FWHM of the peaks shown at 2θ
K = Scherrer constant (0.9–1)
θ = ($\theta = 2\theta/2$) called Bragg angle



(**a**)



(**b**)

Figure 6. (**a**) X-ray spectrum of green synthesized CoNPs. (**b**) XRD Spectrum of green synthesized Co-Zn BMNPs.

Table 1. The average size of synthetic CoNPs is calculated.

Sr.	FWHM	B	θ	$D = K\lambda/\beta\ cos\theta$	Average D (nm)
1.	0.29	0.0051	15.07	25.59	22.77
2.	0.31	0.0054	17.76	23.67	
3.	0.29	0.0051	21.59	24.37	
4.	0.32	0.0057	28.55	20.78	
5.	0.34	0.0059	31.35	19.44	

Table 2. Calculations of the average size of synthesized Co-Zn BMNPs.

Sr.	FWHM	B	2θ	θ	$D = K\lambda/\beta\ cos\theta$	Average D (nm)
1.	0.49	0.0086	29.99	14.99	15.49	14.87
2.	0.56	0.0098	35.95	17.97	13.37	
3.	0.39	0.0069	43.96	21.98	18.54	
4.	0.52	0.0091	58.12	29.06	13.30	
5.	0.49	0.0087	62.22	31.11	13.64	

The average diameter of cobalt monometallic NPs was found to be 22.77 nm.

The spectrum of Co-Zn bimetallic NPs is shown in Figure 6b.

The spectrum showed a pattern of 4 prominent peaks appeared at 2θ values 29.99°, 35.95°, 43.96°, 58.12° and 62.22°. Co-Zn Bimetallic nanoparticles have a crystallite size of 14.98 nm, while the average diameter of cobalt monometallic NPs was found to be 22.77 nm.

3.3. Antioxidant Potential

DPPH Free Radical Scavenging Activity

The antioxidant potential of CoNPs and Co-Zn BMNPs produced from *Cymbopogan citratus* plant extract was assessed using the free radical DPPH scavenging technique (Table 3). Antioxidant activity is mainly based on electron transfer and the solution's color also changed from purple to yellow. A Stable molecule of DPPH was formed by gaining hydrogen or electrons from CoNPs and Co-Zn BMNPs. Three different concentrations (1000 µg/mL, 500 µg/mL and 250 µg/mL) of CoNPs and Co-Zn BMNPs were analyzed, and it was noted that as the concentrations of CoNPs and Co-Zn BMNPs increased, the percentage scavenging of the DPPH free radical also increased. NPs reacted with DPPH and donated electrons so the absorbance decreased and color changes occur from purple to pink. In Figure 7, results suggested that at a concentration of 1000 µg/mL both CoNPs and Co-Zn BMNPs exhibited good free radical inhibition capacity. The highest percentage of scavenging of DPPH free radical for CoNPs and Co-Zn BMNPs was found to be 75.85% and 70.02% respectively at 1000 µg/mL. Bimetallic NPs gave a better percentage of scavenging of DPPH free radicals than monometallic NPs [14].

Table 3. % Scavenging of DPPH free radicals.

Sr. No.	Sample	Concentration in the Assay (µg/mL)	% Scavenging of DPPH Radical ± S.E.M
1.	Co NPs	1000	70.02 ± 0.54
		500	59.19 ± 0.43
		250	38.01 ± 0.78
2.	Co-Zn Bimetallic NPs	1000	75.85 ± 1.18
		500	64.41 ± 1.30
		250	39.5 ± 0.19
3.	BHT	60	94 ± 0.13
		30	73 ± 0.07
		15	49 ± 0.06

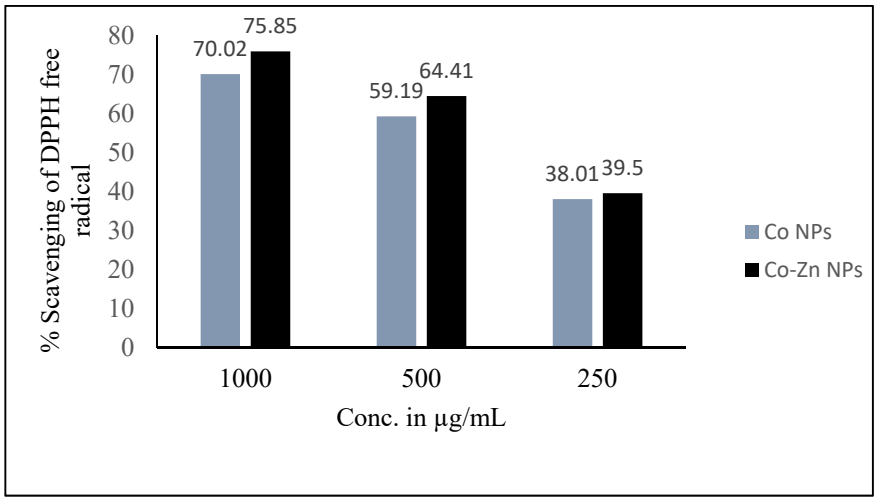


Figure 7. % Scavenging of DPPH radical by CoNPs and Co-Zn BMNPs.

3.4. Total Phenolic Contents

The plant extract of *Cymbopogon citratus* contains a large number of polyphenols and flavonoids. Using the Folin–Ciocalteu reagent, the total phenolic content was calculated. The standard was gallic acid. Phenols and reducing species may cause a reaction with Folin's reagent. Polyphenols have more ability to capture free radicals and prevent the oxidation process. In this method total phenolic contents were measured by the reaction of the Folin–Ciocalteu reagent with monometallic and bimetallic NPs [32]. A prominent change in color confirmed that the reaction occurs between the Folin–Ciocalteu reagent and CoNPs and Co-Zn BMNPs. In Table 4 total phenolic contents for CoNPs and Co-Zn BMNPs were shown as Gallic acid equivalents (GAE). Results showed more phenolic contents were found in bimetallic NPs as compared to monometallic NPs. Higher antioxidant properties are directly related to higher phenolic contents. CoNPs and Co-Zn BMNPs exhibited excellent antioxidant capacity as the phenolic content scavenged the free radical. The mean value of total phenolic contents determined for CoNPs and Co-Zn BMNPs was found to be 70.25 mg/g GAE and 82.4 mg/g GAE respectively which was found good as compared with a blank (4.8 mg/g).

Table 4. IC_{50}, Total phenolic contents and total antioxidant activity.

Sample	DPPH-Radical Scavenging Activity (IC_{50}; µg/mL) ± S.E.M	TPC (GAE mg/g of Sample) ± S.E.M	Total Antioxidant Activity ± S.E.M
Co NPs	64.165 ± 0.79	70.25 ± 2	0.11 ± 0.02
Co-Zn Bimetallic NPs	361.97 ± 0.233	82.4 ± 0.07	0.32 ± 0.007
BHT	12.1 ± 0.91	-	0.70 ± 0.06
Blank	-	4.8 ± 0.09	-

3.5. Total Antioxidant Activity

The total antioxidant activity of monometallic and bimetallic NPs was evaluated by the phosphomolybdenum complex formation process. With this method, antioxidants such as polyphenols, terpenes, and flavonoids were confirmed. The oxidation of antioxidants occurs that give electrons to molybdenum and change its oxidation state from molybdenum (VI) to molybdenum (V) [30]. CoNPs and Co-Zn BMNPs showed good antioxidant activity. The total antioxidant activity of CoNPs and Co-Zn BMNPs was found to be 0.11 and 0.32 respectively. BHT was used as a standard having a value of 0.70. Co-Zn BMNPs exhibited moderate antioxidant capacity when compared to standard (BHT). The results of the antioxidant activity are displayed in Table 4.

3.6. Adsorption of Dyes

3.6.1. Adsorption of MB Dye

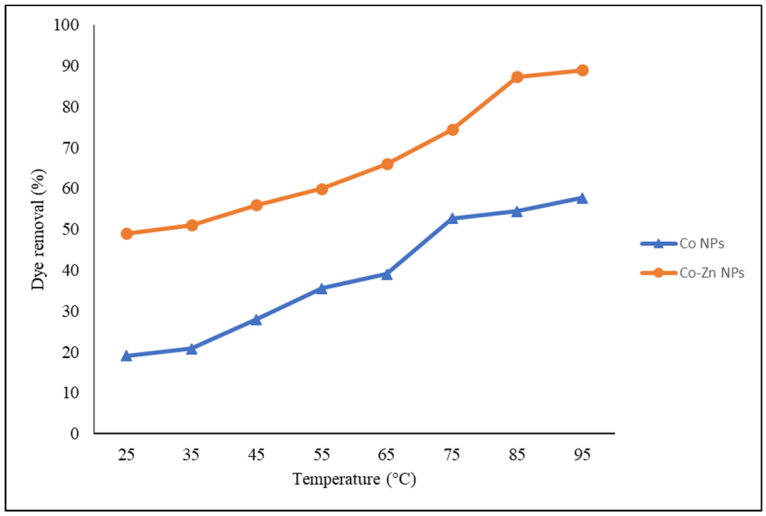
The following explanations describe the effects of temperature, pH, dye concentration, contact duration, and NPs dose on the % clearance of MB dye.

3.6.2. Effect of Temperature

Different temperature ranges (25 °C to 95 °C) were used to study the impact of temperature on dye degradation. The adsorbent dose was 25 mg. It was added in an MB dye solution having a 5 mg/L concentration. The results have been shown in Figure 8a of percentage dye removal at different temperatures for CoNPs and Co-Zn BMNPs. As an increase in 15 °C temperature, the process of adsorption increased which showed that adsorption was an endothermic process. With the increase in active sites on CoNPs and Co-Zn BMNPs, more dye molecules adhere to the surface of NPs and maximum removal occurred at 95 °C temperature [23]. The results demonstrated that both CoNPs and Co-Zn BMNPs exhibited excellent percentage removal of dyes at 95 °C temperature. The highest percentage removal of MB dye was 88.98% found at 95 °C for Co-Zn BMNPs.

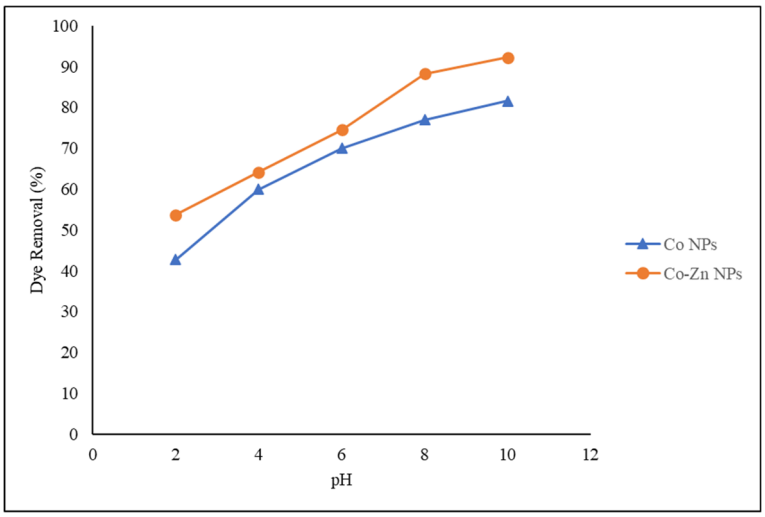
3.7. Effect of pH

The influence of pH on the removal of dye is also considered an important factor. It was analyzed at different pH values from 2 to 10 as shown in Figure 8b. The adsorbent concentration was 25 mg in a dye solution having a fixed concentration of 5 mg/L as MB dye is cationic. By increasing the pH of the solution, deprotonation of the functional group of CoNPs and Co-Zn BMNPs occurred, due to which OH^{-1} ions adsorbed on the active surface of CoNPs, and Co-Zn BMNPs and become negatively charged. So adhesion between NPs and cationic dye occurs more strongly. Thus, better removal efficiency was achieved by increasing pH [7]. The findings of the % MB dye removal at various pHs of CoNPs and Co-Zn BMNPs are shown in Figure 8b. The highest percentage of MB dye removal for both CoNPs and Co-Zn BMNPs occurred at pH 10 according to the findings. Co-Zn BMNPs had the greatest clearance rate of MB dye, at 92.19%.

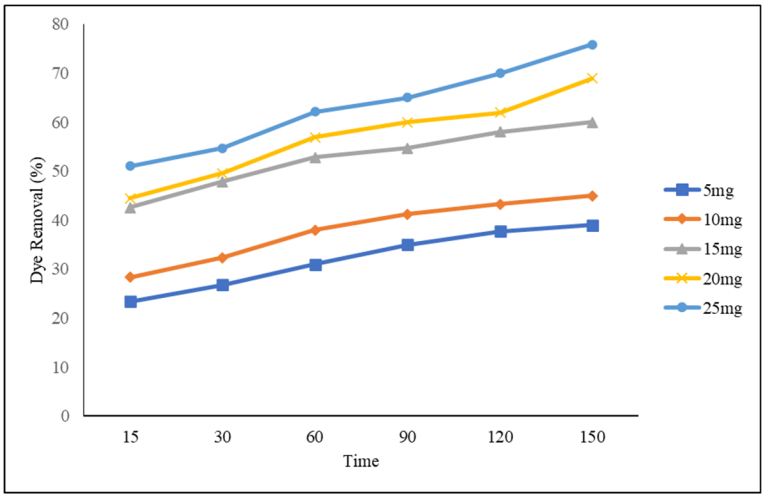


(**a**)

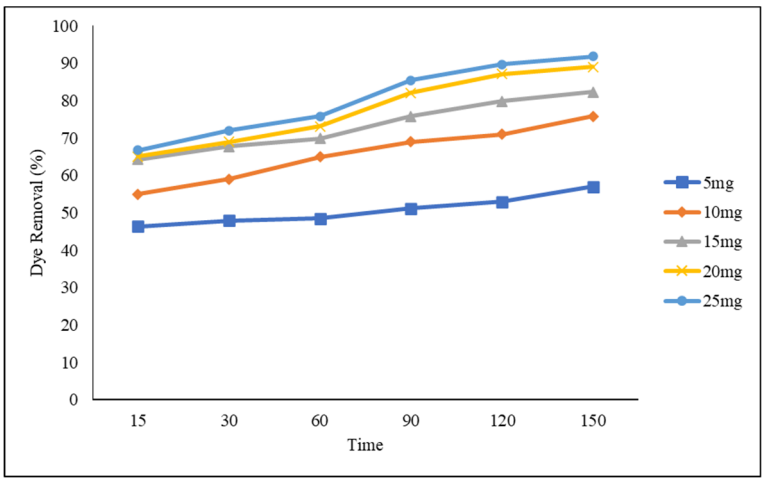
Figure 8. *Cont.*



(**b**)

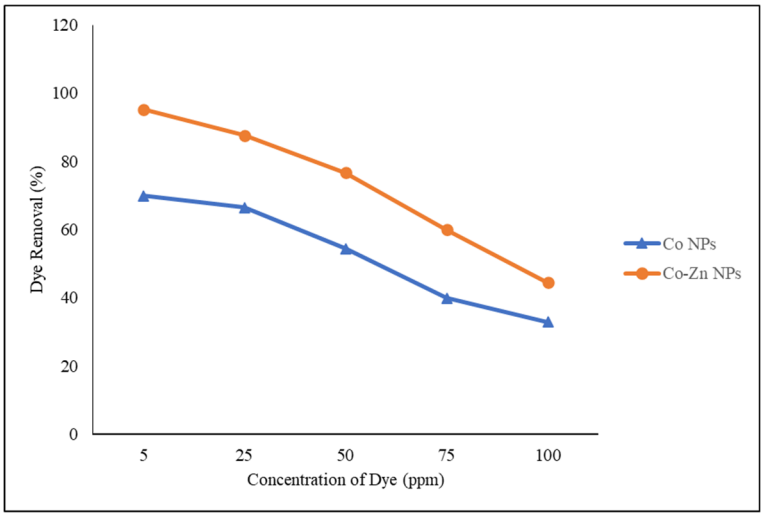


(**c**)



(**d**)

Figure 8. *Cont.*



(**e**)

Figure 8. (**a**) Effect of temperature on percentage removal of MB dye using CoNPs and Co-Zn BMNPs. (**b**) Effect of pH on MB dye removal efficiency using CoNPs and Co-Zn BMNPs. (**c**) Effect of contact time on adsorbent doses to the percentage removal MB dye using CoNPs. (**d**) Effect of contact time on adsorbent doses to the percentage removal MB dye using Co-Zn BMNP€ (**e**) Effect of dye concentration on the amount of MB dye removed by CoNPs and Co-Zn BMNPs.

3.8. Effect of Contact Time on Adsorbent Doses

To analyze the removal of dye, different quantities of monometallic and bimetallic NPs were taken as 5 mg, 10 mg, 15 mg, 20 mg, and 25 mg. These adsorbent doses were mixed with the specific concentration of dye (5 mg/L). Contact time also varied from 15 to 150 min. As the contact time increased more removal of MB dye occurred. The maximum percentage removal of dye was observed by giving maximum contact time and at a high adsorbent dosage. As the concentration of adsorbent increased, more interaction of MB dye occurred with the active sites of CoNPs and Co-Zn BMNPs. Maximum degradation was obtained by giving maximum contact time at a high adsorbent dosage [22]. The results suggested that a concentration of 25 mg of both CoNPs and Co-Zn BMNPs exhibited excellent percentage removal of MB dye. The highest percentage removal of dye was 75.9% and 91.9% for CoNPs and Co-Zn BMNPs respectively as shown in Figure 8c,d.

3.9. Effect of Dye Concentration

By using various concentrations of MB dye (5 ppm, 25 ppm, 50 ppm, 75 ppm, and 100 ppm), the effect of dye concentration on the % elimination of dye was examined. The dose of NPs was 25 mg. Because the dye interacts more with the active sites of CoNPs and Co-Zn BMNPs, the highest % removal of MB dye was found to be attained at lower dye concentrations [36]. Figure 8e shows the results of the % removal of MB dye at various dye concentrations with a given quantity of CoNPs and Co-Zn BMNPs. It was determined that BMNPs demonstrated excellent percentage elimination of MB dye at concentrations of 5 ppm for both CoNPs and Co-Zn. The Co-Zn BMNPs showed the greatest percentage of dye removal, 95.26 percent, at a dye concentration of 5 ppm. The Mechanism of Adsorption of Dye is shown in Figure 9.

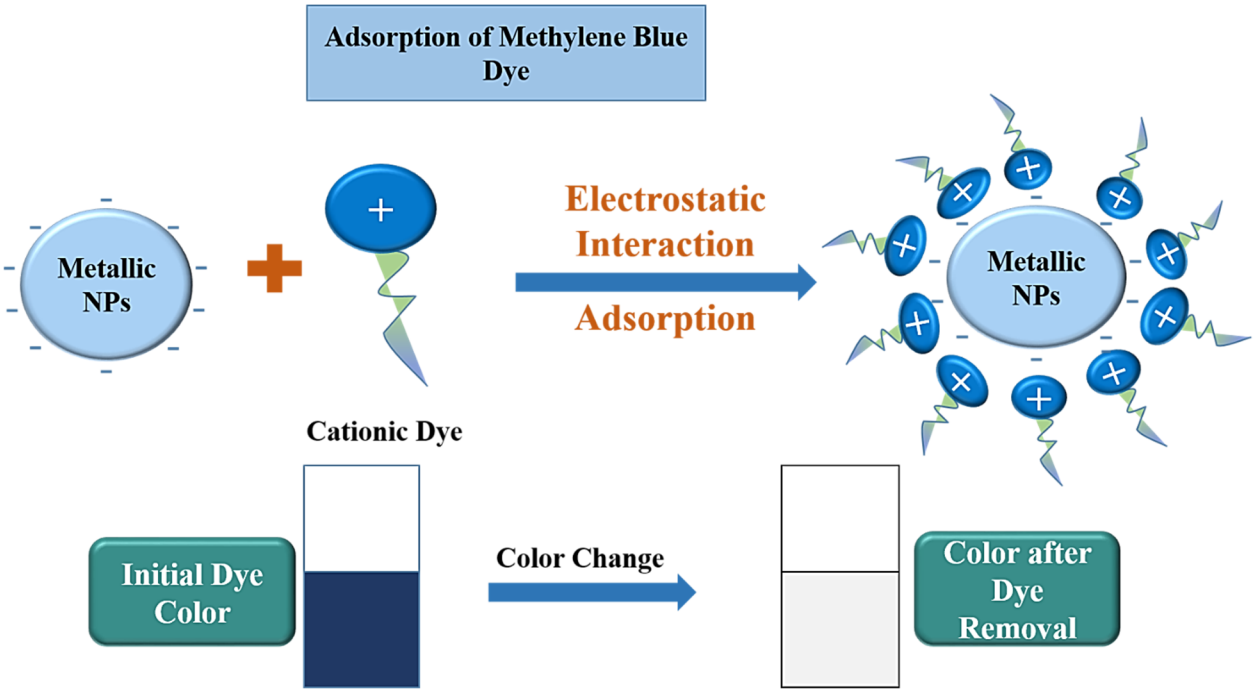


Figure 9. Mechanism of adsorption of MB dye.

3.10. Evaluation of Anti-Inflammatory Activity

NPs were used to measure the anti-inflammatory effect. Inflammation occurs when the proteins are denatured. In response to denatured proteins, self-antigens can produce that cause inflammation. So monometallic and bimetallic NPs were used to inhibit inflammation. In this method, the denaturation of protein, albumin. NPs obstructed inflammation of protein compared with the reference drug. The standard drug was diclofenac sodium salt [27]. The results shown in Table 5 indicate very low anti-inflammatory activity. In CoNPs and Co-Zn BMNPs, there are secondary metabolites that inhibited them from reacting with proteins and caused denaturation, and for this reason, they did not show anti-inflammatory activity.

Table 5. Anti-inflammatory activity of CoNPs and Co-Zn BMNPs.

Treatments	Absorbance	Positive Control (Diclofenac)	% Inhibition of Protein Denaturation	% Anti-Inflammatory Activity
Co NPs	0.24	0.31	20	1.5
Co-Zn Bimetallic NPs	0.23	0.31	25	18.32

4. Conclusions

Cobalt monometallic and cobalt–zinc bimetallic NPs were efficiently synthesized via the green route, which was an eco-friendly, cost-effective and non-toxic method. *Cymbopogan citratus* plant extract was used along with metal salt solutions for the bioreduction of NPs. The *Cymbopogan citratus* plant extract included many phytochemicals that served as reducing and capping agents for the stability and reduction of metallic NPs. NPs were characterized by UV-Vis, FTIR, SEM, XRD, and EDX. CoNPs and Co-Zn BMNPs both exhibited excellent antioxidant potential, but these NPs were found to be less successful anti-inflammatory agents as secondary metabolites were present that inhibit their interaction with proteins. IC_{50} values also calculated for cobalt monometallic nanoparticles that were 64.165 ± 0.79 µg/mL and for cobalt–zinc bimetallic nanoparticles was 361.71 ± 0.233 µg/mL. CoNPs and Co-Zn BMNPs showed good percentage removal of MB dye. Different factors such as temperature, pH, adsorbent dosage, and dye concen-

tration were studied for the removal of dye by using synthesized NPs. Although both the CoNPs and Co-Zn BMNPs were found efficient Co-Zn BMNPs were found to be more effective for dye removal and also showed better antioxidant activity than cobalt monometallic NPs. It was concluded that green synthesized CoNPs and Co-Zn BMNPs were prepared easily by the effective method. They were handled easily and had good capacity to remove the dyes with minimum production of toxic waste.

Author Contributions: Conceptualization, writing—original draft Preparation, T.R., S.N. and T.S.; methodology, writing—original draft Preparation, M.Z., S.S. and S.M.; software, writing review and editing, funding, M.J. and S.I.; validation, project administration, critical revision, funding, M.M.A.-A., E.B.E. and R.A.P.; writing review and editing, resources, funding, E.A. and A.-E.F. All authors have read and agreed to the published version of the manuscript.

Funding: The authors would like to thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work by Grant Code: (22UQU4320141DSR53). This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2022R7), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data will be available on request.

Acknowledgments: The authors would like to thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work by Grant Code: (22UQU4320141DSR53). This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2022R7), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ahmed, S.; Annu; Ikram, S.; Yudha, S. Biosynthesis of gold NPs: A green approach. *J. Photochem. Photobiol. B Biol.* **2016**, *161*, 141–153. [CrossRef] [PubMed]
2. Ahmad, S.; Munir, S.; Zeb, N.; Ullah, A.; Khan, B.; Ali, J.; Bilal, M.; Omer, M.; Alamzeb, M.; Salman, S.M. Green nanotechnology: A review on green synthesis of silver NPs—An ecofriendly approach. *Int. J. Nanomed.* **2019**, *14*, 5087–5107. [CrossRef]
3. Akinsiku, A.A.; Dare, E.O.; Ajanaku, K.O.; Ajani, O.O.; Olugbuyiro, J.A.O.; Siyanbola, T.O.; Ejilude, O.; Emetere, M.E. Modeling and synthesis of Ag and Ag/Ni allied bimetallic NPs by green method: Optical and biological properties. *Int. J. Biomater.* **2018**, *2018*, 9658080. [CrossRef] [PubMed]
4. Bala, N.; Saha, S.; Chakraborty, M.; Maiti, M.; Das, S.; Basu, R.; Nandy, P. Green synthesis of zinc oxide NPs using *Hibiscus subdariffa* leaf extract: Effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity. *RSC Adv.* **2014**, *5*, 4993–5003. [CrossRef]
5. Boukhatem, M.N.; Ferhat, M.A.; Kameli, A.; Saidi, F.; Kebir, H.T. Lemon grass (*Cymbopogon citratus*) essential oil as a potent anti-inflammatory and antifungal drugs. *Lib. J. Med.* **2014**, *9*, 25431. [CrossRef] [PubMed]
6. Cai, Z.; Sun, Y.; Liu, W.; Pan, F.; Sun, P.; Fu, J. An overview of nanomaterials applied for removing dyes from wastewater. *Environ. Sci. Pollut. Res.* **2017**, *24*, 15882–15904. [CrossRef] [PubMed]
7. Dawood, S.; Sen, T.K. Removal of anionic dye Congo red from aqueous solution by raw pine and acid-treated pine cone powder as adsorbent: Equilibrium, thermodynamic, kinetics, mechanism and process design. *Water Res.* **2012**, *46*, 1933–1946. [CrossRef]
8. Fakhari, S.; Jamzad, M.; Fard, H.K. Green synthesis of zinc oxide NPs: A comparison. *Green Chem. Lett. Rev.* **2019**, *12*, 19–24. [CrossRef]
9. Figueirinha, A.; Paranhos, A.; Pérez-Alonso, J.J.; Santos-Buelga, C.; Batista, M.T. Cymbopogon citratus leaves: Characterization of flavonoids by HPLC–PDA–ESI/MS/MS and an approach to their potential as a source of bioactive polyphenols. *Food Chem.* **2008**, *110*, 718–728. [CrossRef]
10. Francisco, V.; Figueirinha, A.; Costa, G.; Liberal, J.; Lopes, M.C.; García-Rodríguez, C.; Geraldes, C.F.; Cruz, M.T.; Batista, M.T. Chemical characterization and anti-inflammatory activity of luteolin glycosides isolated from lemongrass. *J. Funct. Foods* **2014**, *10*, 436–443. [CrossRef]
11. Fujimoto, T.; Mizukoshi, Y.; Nagata, Y.; Maeda, Y.; Oshima, R. Sonolytical preparation of various types of metal NPs in aqueous solution. *Scr. Mater.* **2001**, *44*, 2183–2186. [CrossRef]
12. Guo, K.W. Green nanotechnology of trends in future energy: A review. *Int. J. Energy Res.* **2011**, *36*, 1–17. [CrossRef]
13. Iravani, S.; Korbekandi, H.; Mirmohammadi, S.V.; Mekanik, H. Plants in Nanoparticle Synthesis. *Rev. Adv. Sci. Eng.* **2014**, *3*, 261–274. [CrossRef]

14. Kayani, Z.N.; Saleemi, F.; Batool, I. Effect of calcination temperature on the properties of ZnO NPs. *Appl. Phys. A* **2015**, *119*, 713–720. [CrossRef]
15. Kumar, V.; Singh, S.; Srivastava, B.; Bhadouria, R.; Singh, R. Green synthesis of silver NPs using leaf extract of Holoptelea integrifolia and preliminary investigation of its antioxidant, anti-inflammatory, antidiabetic and antibacterial activities. *J. Environ. Chem. Eng.* **2019**, *7*, 103094. [CrossRef]
16. Lee, K.-G.; Shibamoto, T. Antioxidant property of aroma extract isolated from clove buds [*Syzygium aromaticum* (L.) Merr. et Perry]. *Food Chem.* **2001**, *74*, 443–448. [CrossRef]
17. Makkar, H.P.S.; Blümmel, M.; Borowy, N.K.; Becker, K. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J. Sci. Food Agric.* **1993**, *61*, 161–165. [CrossRef]
18. Malaikozhundan, B.; Vaseeharan, B.; Vijayakumar, S.; Thangaraj, M.P. Bacillus thuringiensis coated zinc oxide nanoparticle and its biopesticidal effects on the pulse beetle, Callosobruchus maculatus. *J. Photochem. Photobiol. B Biol.* **2017**, *174*, 306–314. [CrossRef]
19. Mittal, A.K.; Chisti, Y.; Banerjee, U.C. Synthesis of metallic NPs using plant extracts. *Biotechnol. Adv.* **2013**, *31*, 346–356. [CrossRef]
20. Mohamad, N.A.N.; Arham, N.A.; Jai, J.; Hadi, A. Plant Extract as Reducing Agent in Synthesis of Metallic NPs: A Review. *Adv. Mater. Res.* **2013**, *832*, 350–355. [CrossRef]
21. Nagajyothi, P.; An, T.N.M.; Sreekanth, T.; Lee, J.-I.; Lee, D.J.; Lee, K. Green route biosynthesis: Characterization and catalytic activity of ZnO NPs. *Mater. Lett.* **2013**, *108*, 160–163. [CrossRef]
22. Nandi, B.; Goswami, A.; Purkait, M. Adsorption characteristics of brilliant green dye on kaolin. *J. Hazard. Mater.* **2009**, *161*, 387–395. [CrossRef]
23. Nourmoradi, H.; Nikaeen, M.; Hajian, M.K. Removal of benzene, toluene, ethylbenzene and xylene (BTEX) from aqueous solutions by montmorillonite modified with nonionic surfactant: Equilibrium, kinetic and thermodynamic study. *Chem. Eng. J.* **2012**, *191*, 341–348. [CrossRef]
24. Okwunodulu, F.U.; Chukwuemeka-Okorie, H.O.; Okorie, F.C. Biological synthesis of cobalt NPs from mangifera indica leaf extract and application by detection of manganese (II) ions present in industrial wastewater. *Chem. Sci. Int. J.* **2019**, *27*, 1–8. [CrossRef]
25. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal. Biochem.* **1999**, *269*, 337–341. [CrossRef]
26. Rajakumar, G.; Thiruvengadam, M.; Mydhili, G.; Gomathi, T.; Chung, I.M. Green approach for synthesis of zinc oxide NPs from *Andrographis paniculata* leaf extract and evaluation of their antioxidant, anti-diabetic, and anti-inflammatory activities. *Bioprocess Biosyst. Eng.* **2017**, *41*, 21–30. [CrossRef] [PubMed]
27. Rajput, S.; Kumar, D.; Agrawal, V. Green synthesis of silver NPs using Indian Belladonna extract and their potential antioxidant, anti-inflammatory, anticancer and larvicidal activities. *Plant Cell Rep.* **2020**, *39*, 921–939. [CrossRef] [PubMed]
28. Ramesh, M.; Anbuvannan, M.; Viruthagiri, G. Green synthesis of ZnO NPs using Solanum nigrum leaf extract and their antibacterial activity. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2015**, *136*, 864–870. [CrossRef]
29. Shah, G.; Shri, R.; Panchal, V.; Sharma, N.; Singh, B.; Mann, A. Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf (*Lemon grass*). *J. Adv. Pharm. Technol. Res.* **2011**, *2*, 3. [CrossRef]
30. Santhoshkumar, T.; Rahuman, A.A.; Jayaseelan, C.; Rajakumar, G.; Marimuthu, S.; Kirthi, A.V.; Velayutham, K.; Thomas, J.; Venkatesan, J.; Kim, S.-K. Green synthesis of titanium dioxide NPs using Psidium guajava extract and its antibacterial and antioxidant properties. *Asian Pac. J. Trop. Med.* **2014**, *7*, 968–976. [CrossRef]
31. Sorbiun, M.; Shayegan Mehr, E.; Ramazani, A.; Mashhadi Malekzadeh, A. Biosynthesis of metallic NPs using plant extracts and evaluation of their antibacterial properties. *Nanochem. Res.* **2018**, *3*, 1–16. [CrossRef]
32. Stevanato, R.; Fabris, A.S.; Momo, F. New enzymatic method for the determination of total phenolic content in tea and wine. *J. Agric. Food Chem.* **2004**, *52*, 6287–6293. [CrossRef] [PubMed]
33. Turakhia, B.; Turakhia, P.; Shah, S. Green synthesis of zero valent iron NPs from *Spinacia oleracea* (spinach) and its application in waste water treatment. *J. Adv. Res. Appl. Sci.* **2018**, *5*, 46–51.
34. Uddin, S.; Bin Safdar, L.; Iqbal, J.; Yaseen, T.; Laila, S.; Anwar, S.; Abbasi, B.A.; Saif, M.S.; Quraishi, U.M. Green synthesis of nickel oxide NPs using leaf extract of *Berberis balochistanica*: Characterization, and diverse biological applications. *Microsc. Res. Tech.* **2021**, *84*, 2004–2016. [CrossRef] [PubMed]
35. Xia, B.; He, F.; Li, L. Preparation of bimetallic NPs using a facile green synthesis method and their application. *Langmuir* **2013**, *29*, 4901–4907. [CrossRef] [PubMed]
36. Zhang, L.; Zhang, H.; Tian, Y.; Chen, Z.; Han, L. Adsorption of methylene blue from aqueous solutions onto sintering process red mud. *Desalination Water Treat.* **2012**, *47*, 31–41. [CrossRef]
37. Zhang, X.-F.; Liu, Z.-G.; Shen, W.; Gurunathan, S. Silver NPs: Synthesis, characterization, properties, applications, and therapeutic approaches. *Int. J. Mol. Sci.* **2016**, *17*, 1534. [CrossRef] [PubMed]