Synthesis and characterization of ZnO nanoparticles using aqueous extract of *Becium grandiflorum* for antimicrobial activity and adsorption of methylene blue

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Abstract

Nanotechnology is a recent field of modern research dealing with synthesis, strategy and manipulation of particle’s structure in size range of 1–100 nm. This study introduces one of the methods of synthesis of nanoparticles, i.e., green synthesis of ZnO NPs using aqueous leaf extract of *Becium grandiflorum* (AM: ‘Yedegamentisie’). The biomolecules of the plant extract (such as phenols, flavonoids, saponins, glycosides, steroids, tannins and alkaloids) were used as capping and reducing agent during synthesis of ZnO NPs. Response surface methodology coupled with Box-Behnken design (RSM-BBD) was used to optimize the synthesis of ZnO NPs and adsorption studies of the as-synthesized ZnO NPs. Then, ZnO NPs was characterized using different spectroscopic and microscopic instruments such as UV–Vis spectroscopy, FTIR, XRD and SEM–EDS to consider its purity, shape and crystallinity. UV–Vis analysis showed peaks in the range 305–312 nm due to synthesis of ZnO NPs. FTIR analysis showed the availability of different phytochemicals in the plant extract and synthesis of ZnO NPs at 490 cm⁻¹. Powder XRD patterns confirmed formation of phase pure wurtzite structures of ZnO NPs. The synthesized ZnO NPs were used to remove MB dye from aqueous solution by acting as a photocatalyst and adsorbent as well as, it also showed antimicrobial activity against two gram positive (*Staphylococcus epidermidis*, *Staphylococcus aureus*) and three gram negative (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*) bacteria.

Keywords ZnO NPs · Green synthesis · RSM-BBD · Antimicrobial · Adsorbent · Photocatalyst

Introduction

Cosmetic, food, leather, paper, plastics and textile industries use extensively different types of dyes and pigments to color their products. Textile industries in particular generate 100–170 L dye effluent per Kg of cloth (Mohan et al. 2007). Effluents contaminated with dyes that are discharged into oceans, seas, rivers and ponds from textile factories and the like can bring disturbance to the water eco-system due to two main reasons, i.e., toxic properties of the dyes and blockage of sun light to reach the plankton in the water system.

Dyes are aromatic compounds that have delocalized electron systems that can bind with a material to give color in textile factories (Velmurugan et al. 2011). Methylene blue (MB) was first synthesized in 1876 for use in the textile industry (Berneth 2005). MB is a cationic dye and is commonly used for coloring paper, temporary hair colorant, dyeing cotton wools and so on (Pirkanniemi and Sillanpää 2002). MB is a dye that was synthesized and tested as antimalarial drug before the twentieth century (Schirmer et al. 2003). MB is also used in combination with amodiaquine for falciparum malaria in African adults and children (Mandi et al. 2005; Meissner et al. 2006; Zoungrana et al. 2008). Effluents from textile, paper and pharmaceutical industries containing MB pollute water bodies that adversely affect the eco-system and aquatic life. Inhaling or presence of MB in drinking water is a health hazard as it causes eye and skin irritation, hemolytic anemia, diarrhea and nausea, difficulties in breathing, vomiting and abdominal pain are among the symptoms that can occur in humans (Pirkanniemi and Sillanpää 2002). Now a days about 700 million people of our globe have faced water scarcity for both drinking and domestic purposes due to water pollution mostly from anthropogenic activities, and it is still estimated that people faced these problem will...
increase to 1.8 billion people by 2025 (Water for life: International Decade of Action Begins 2005). According to a World Health Organization (WHO) report, water borne diseases lead to death of nearly 12 million people mostly in developing countries each year (World Health Organization. Regional Office for Europe & International Federation of Red Cross and Red Crescent Societies 2001). Dyes such as MB or cati-onic pharmaceuticals have been treated from industrial efflu-ents/waste water using adsorbents such as poly (vinyl alco-hol) (PVA) (Roufegari-Nejhad et al. 2019), activated carbon (Gimba et al. 2001) and activated charcoal (Zhao et al. 2018) had been reported. Similarly, TiO2/UV-C photocatalytic pro-cess using batch reactor in removing MB from textile synthetic wastewater had been studied by (Rahimi et al. 2013). Omar et al. (2014) reported the use of zinc oxide/reduced graphene oxide (ZnO/rGO) nanocomposites (NCs) as both adsorbent and photocatalyst for removal of MB from aqueous solution, while Kahsay et al. (2020) used magnetic rGO/Fe3O4 NCs as adsorbent to remove crystal violet dye from aqueous solution.

Synthesis of nanoparticles using biological means has become a new trend in nanoparticle production due to the following advantages: non toxicity, large scale production without the presence of impurities, easy scaling-up, well-defined morphology and has been used as drug carriers, cosmetics and fillings in medical materials (Rosi and Mirkin 2005; Singh et al. 2016; Yuvakkumar et al. 2014). The photochemicals such as proteins, amino acids, organic acid, vitamins, as well as secondary metabolites, such as flavonoids, alkaloids, polyphenols, terpenoids, heterocyclic compounds and polysaccharides, have significant roles in metal salt reduction, and furthermore, act as capping and stabilizing agent for synthesis of nanoparticles (Duan et al. 2015). There are few already identified chemical constituents of plant extracts such as catechin, gallic acid and coumaric acid that have been played a great role in reducing zinc acetate to ZnO NPs (Salam et al. 2014). Some authors have mentioned the role of capping ligand is crucial in pre-venting agglomeration when ZnO NPs are synthesized by precipitation and sol–gel methods. The-OH functional groups of starch and polyethylene glycol (PEG) molecules bind with the surface of ZnO NPs and cap it (Balcha et al. 2016).

Hence, the researcher has used leaf extract of Becium grandiflorum to synthesize ZnO NPs and the as-synthesized ZnO NPs was used for multi applications as adsorbent and photocatalyst to remove MB dye from aqueous solution and as bactericidal against both gram positive and gram negative bacteria.

**Experimental**

**Chemicals**

All chemicals used are analytical grade. Zn(CH₃COO)₂·2H₂O was bought from Chemicals UDYOG-12001 (India), NaOH was acquired from (JHD, China), MB (C₁₆H₁₈ClN₃·xH₂O) was bought from (Abron chemicals, India), HCl (37%) was acquired from (Pentokey, India), CHCl₃ was bought from (Blulux, India), leaves of Becium grandiflorum were collected from Yemrehane Kristos church forest (North Wollo, Ethiopia), and deionized water was used to prepare solutions through out of the experiments.

**Preparation of aqueous leaf extract of Becium grandiflorum**

The leaves of Becium grandiflorum were collected and rinsed with distilled water to remove dust and impurities from the surface of the plant. Then, the leaves were allowed to shed dry at room temperature for 14 days. After drying, the leaves were crushed in to powder with grinder and allowed to pass through 1.4 mm mesh. Finally to prepare 1% plant extract, 1 g leaf powder was immersed in to 100 mL deionized water and heated at 40 °C for 20 min. After cooling, the solution was filtered with Whatman no. 1 filter paper, and extract was collected in volumetric flask for further use.

**Qualitative phytochemical analysis of leaf extract of Becium grandiflorum**

The phytochemical screening of Becium grandiflorum was studied based on the procedure in our previous study (Gebremedhn et al. 2019).

**Synthesis of ZnO nanoparticles**

ZnO NPs were synthesized using biological approach using Zn(CH₃COO)₂·2H₂O precursor and aqueous leaf extract of Becium grandiflorum as reducing and capping agent. The synthesis procedure was optimized (RSM-BBD) using different variables, i.e., Zn(CH₃COO)₂·2H₂O, percent of leaf extract, temperature and reaction time. Best product was collected at the optimized condition of Zn(CH₃COO)₂·2H₂O, pH, leaf extract and reaction time corresponding to 1 mM, 9, 1%, 60 °C and 30 min, respectively. The synthesized product had pale yellow color indicating the formation of ZnO NPs.
The optimization procedure for the synthesis of ZnO NPs was designed by Design expert 11 software and the experimental levels of factors used for RSM-BBD are given in Table 1 below.

**Characterization of ZnO nanoparticles**

To study the optical properties of the synthesized ZnO NPs, ultraviolet–visible spectroscopy (UV–vis) (UV-2450, Shimadzu) was used in the wavelength range 200–800 nm. To identify the functional groups present in the plant extract and ZnO NPs, Fourier transform infrared (FTIR) spectrophotometer (Perkin-Elmer 1725x) in the range 4000–400 cm⁻¹ was recorded by KBr pellet method. The crystallite size and XRD patterns of the as-synthesized ZnO NPs were determined using X-ray diffractometer (Perkin-Elmer spectrum one instrument) Cu–Kα radiations (λ = 0.15406 nm) in 2θ range from 20° to 100°, and morphology of the as-synthesized ZnO NPs was characterized using Scanning electron microscopy (SEM, Zeiss ultra-60) equipped with x-ray energy dispersive spectroscopy (EDS).

**RSM-BBD as a method of optimization of synthesis of ZnO NPs and adsorption process**

Optimization parameters were designed by Design Expert 11 software for both synthesis and later adsorption of MB dye using ZnO NPs in order to minimize the cost, time and energy. During synthesis, there are three factors/variables (Zinc acetate, plant extract concentration and pH), while the response is absorption maximum of ZnO NPs. First plant extract (1–5%) is immersed to zinc acetate solution (1–5 mM) in a conical flask and the pH of the mixed solution is adjusted to pH (3, 7, 11) using 0.1 M HCl/0.1 M NaOH solution. Then, the reaction was allowed to react for 30 min at 60 °C, while agitating the reaction mixture at 1000 rpm. However, the adsorption parameters are three variables, i.e., ZnO NPs dose (mg), contact time (min) and initial MB concentration (mg/L), whereas the response is dye removal (%). Selected ZnO NPs dose variables were (25, 62.5, 100 mg); contact time (0–180 min) and initial MB concentration were (25, 65.5, 100 mg/L).
Phytochemical screening of *Becium grandiflorum*

Prior to synthesis of ZnO NPs the qualitative photochemical screening of the plant (Fig. 1a) was studied using different chemicals and procedures. Based on the results the phytochemicals found in aqueous leaf extract of *Becium grandiflorum* were phenols, flavonoids, saponins, glycosides, steroids, tannins and alkaloids (Fig. 1b). These bioactive molecules are believed to reduce Zn(CH$_3$COO)$_2$·2H$_2$O and cap the as-synthesized ZnO NPs afterward to control growth and avoid agglomeration of the nanoparticles.

Characterization of ZnO NPs

Aqueous leaf extract of *Becium grandiflorum* showed characteristic peak at 315 nm due to $n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ transitions of carbonyls, C=C or molecules with heteratoms functional groups from the plant extract. The prepared plant extract was used to synthesize ZnO NPs which was acted as both reducing and capping agent. The UV–vis spectrum results showed the production and distribution of ZnO NPs using different concentrations of Zn(CH$_3$COO)$_2$·2H$_2$O precursor (1, 2, 3, 4, 5 mM), pH and time of reaction that showed maximum absorption at 312, 311, 303, 305 and 308 nm, respectively due to synthesis of ZnO NPs at pH = 9 (Fig. 2) and (Fig. 3). Therefore, ZnO NPs using leaf extract of *Becium grandiflorum* showed minimum absorption peak at 303 nm that has resulted calculated particle size of 1.35 nm using effective mass model and oversimplified mathematical equation, Eq. (2) derived by (Brus 1986). Similarly, (Talam et al. 2012) reported synthesis of ZnO NPs via precipitation method that has a size of 2.07 nm according to the equation below. Hence, size of the synthesized nanoparticles decreased with decrease in the wavelength absorption maximum of nanoparticles (particle size and plasmon resonance absorbance are directly proportional to each other).

The surface plots of the response using the three factors, i.e., concentration of zinc acetate, % of plant extract and pH to obtain optimized absorption maximum of the synthesized ZnO NPs using 2D-3D plots is represented in Fig. 3.

\[
r(nm) = \frac{-0.3049 + \sqrt{-26.23012 + 10240.72/\lambda_p(nm)}}{-6.3829 + 2483.2/\lambda_p(nm)}
\]

where ‘$r$’ is the particle radius and ‘$\lambda_p$’ is peak absorbance wavelength in nm.

Increasing the concentration of the precursor from 1 to 5 mM increased the absorption intensity and wavelength from 298 to 303 nm in acidic medium (pH = 3). Whereas in basic medium (pH = 9) increasing precursor concentration decreased the intensity and wavelength from 312 to 308 nm as in Fig. 3. Synthesis of ZnO NPs was more favored in basic condition (pH = 9) by using minimum concentration of the precursor. More numbers of ZnO NPs were produced at lower concentration of the precursor in basic medium due to precipitation reaction.
Figure 4 shows correlation between predicted (model) $R^2 = -3.4612$ and adjusted (actual) $R^2 = 0.3627$. A negative predicted $R^2$ implies that the overall mean may be a better predictor of your response than the current model. The desirability ramp to yield best absorption maximum for ZnO NPs is shown in Fig. 5.

Figure 6 shows FTIR spectra of both leaf powder of Becium grandiflorum and ZnO NPs. Leaf powder of Becium grandiflorum shows characteristic peaks at 3334, 2915, 1635, 1410, 1261, 1043 and 609 cm$^{-1}$ due to OH stretching, sp$^3$ C–H stretching, C=C stretching, C–H bending, C–N or phenols, C–O stretching and C-H bending (C=C–H), respectively, whereas ZnO NPs using leaf extract of Becium grandiflorum shows characteristic peaks at 3378, 2915, 1560, 1500, 1380, 1043, 939, 826, 691 and 490 cm$^{-1}$ due to OH stretching, C–H stretching of alkane, C=C aromatic ring,
O–N = O nitro group, C–O stretching, C–N stretching, meta disubstitute ring and Zn–O stretching due to formation of ZnO NPs, respectively.

The phase purity and crystallinity of the as-synthesized ZnO NPs were studied using powder x-ray diffractometer, Fig. 7. The XRD patterns of ZnO NPs show 2θ values at 31.74°, 34.58°, 36.43°, 47.71°, 56.79°, 63.20°, 66.58°, 68.18°, 69.40°, 72.96°, 77.13° and 81.92° corresponding to (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202) and (104) planes of hexagonal wurtzite structure (JCPDS card no.: 36-1451 (Tao et al. 2008)). The average crystallite size (D) of ZnO NPs using the Debye–Sherrer formula, Eq. (3) using the intense peak at (101) was calculated to be 20 nm.

\[ D = \frac{0.9 \lambda}{\beta \cos \theta} \]  

where ‘D’ is the crystallite size (nm), ‘λ’ is the wavelength of Cu kα radiation (λ = 0.15406 Å), and ‘β’ is the full width at half maximum of the diffraction peak (in radians).

The morphology of the as-synthesized ZnO NPs was studied using SEM. Figure 8a, b shows SEM image of the as-synthesized ZnO NPs and EDS image of ZnO NPs. EDS image confirmed the elemental composition of ZnO NPs. The percent composition of Zn and O in ZnO NPs was 40.38 and 59.62% by atomic mass, respectively, confirmed synthesis of ZnO NPs.

### Adsorption property of ZnO NPs

RSM-BBD was applied to obtain maximum dye removal efficiency using the as-synthesized ZnO NPs as an adsorbent (Fig. 9). The three independent variables involved according to BBD matrix are ZnO NPs dose (A), contact time (B) and initial MB concentration (C), whereas the response is adsorption (Y), Eq. (4). The quadratic model equation for adsorption capability of ZnO NPs in this study is represented as
In the above equation, positive coefficients increase ZnO NPs adsorption, while negative coefficients affect inversely ZnO NPs adsorption. The Table 2 below shows coefficients in terms of coded factors. Percent of dye removal was calculated to be 71.53% when the adsorbent mass and dye concentration are 25 mg and 25 mg/L, respectively (Fig. 10). Beccium grandiflorum mediated ZnO NPs showed adsorption maximum of 143.6 mg MB/g ZnO NPs adsorbent in this study. Other reports demonstrated ZnO NPs have a removal capacity of 10.70 ± 0.80 mmol/g of MB from aqueous solution (Zhang et al. 2013). Similarly, sawdust-derived cellulose nanocrystals (CNC) incorporated with ZnO nanocomposite showed maximum adsorption capacity of 64.93 mg/g toward MB (Oyewo et al. 2020).

**Photocatalytic degradation of ZnO NPs**

The as-synthesized ZnONPs was used as a catalyst to degrade a model organic dye pollutant, i.e., MB under UV photo irradiation.

The maximum absorption of the dye was observed at 664 nm. 100 ml (100 ppm) MB dye was photodegraded using 0.5 g L\(^{-1}\) of ZnO NPs photocatalyst. MB dye was 69% degraded after 200 min (Fig. 11a) and reaction rate constant (k) for degradation of MB were calculated to be 1.9 × 10\(^{-3}\) min\(^{-1}\) and \(R^2 = 0.983\) (Fig. 11b). However, the reaction rate constant for the photocatalytic degradation of MB using ZnO nanostructures was reported to be 9.14 × 10\(^{-3}\) min\(^{-1}\) (Kahsay et al. 2019). Similarly, in another work MB dye is photocatalytically degraded to 99% at room temperature using 50 mg dose of RGO-Ag/ZnO nanocomposite in the presence of H\(_2\)O\(_2\) (Belachew et al. 2020). The kinetic study of the catalytic degradation of MB dye using the ZnO NPs was performed using the Langmuir–Hinselwood–Hougen–Watson (LH–HW) kinetic model, Eq. (5).

\[
-r_A = \frac{kC_A}{(1 + kC_A)}
\]  

(5)

where ‘\(r_A\)’ is the rate of consumption of MB dye (mol dm\(^{-3}\) s\(^{-1}\)), ‘\(C_A\)’ is an MB dye concentration (mol dm\(^{-3}\)), and ‘\(k\)’ is the rate constant.

The photogenerated charge carriers (h\(^+\) in the valence band and e in conduction band) are able to interact with the adsorbed MB dye to form hydroxyl or superoxide radicals to facilitate the degradation of MB dye molecules to CO\(_2\) and water (Herrmann et al. 1997); (Vignesh et al. 2012).

**Antimicrobial activity**

Figure 12 shows the antibacterial activity of ZnO NPs against gram positive and gram negative bacteria using standard well-diffusion. The higher zone of inhibition was measured to be 12 mm by *Staphylococcus epidermidis*, whereas *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed minimum zone of inhibition of 7, 6, 8 and 11 mm, respectively. The release of Zn\(^{2+}\) from ZnO NPs is one of the main propositions in antibacterial mechanisms which are known to inhibit several bacterial cells activities (such as active transport, bacteria metabolism and enzymes activity) which leads to the formation of reactive oxygen species (ROS) which leads to oxidative stress and subsequent cell damage or death (Soren et al. 2018).

**Conclusions**

ZnO NPs was successfully synthesized by using leaf extract of *Beccium grandiflorum*. Based on the FTIR results phytoconstituents such as phenols, flavonoids, saponins, glycosides, steroids, starch, tannins and alkaloids were used as both reducing and capping agent. Similarly, synthesis of ZnO NPs was confirmed by showing characteristic peak at 490 cm\(^{-1}\). The as-synthesized ZnO NPs showed adsorbent property to remove MB dye from aqueous solution. Adsorption behavior best fitted to Langmuir isotherm with \(r^2 = 1\) and \(q_{\text{max}} = 143.6\) mg MB/g ZnO NPs adsorbent (not shown here). Maximum percent of MB dye removal was calculated 71.53% when the adsorbent mass and dye concentration were 25 mg and 25 mg/L, respectively, at 180 min. Similarly, ZnO NPs degraded MB by 69% within 200 min. Moreover, ZnO NPs showed antimicrobial property against five standard bacteria strain and showed maximum zone of inhibition of 12 mm by *Staphylococcus epidermidis*. Hence, ZnO NPs using leaf extract of *Beccium grandiflorum* has a potential to remove organic
Table 2  Coefficients in terms of coded factors

| Factor                  | Coefficient estimate | df | Standard error | 95% CI low | 95% CI High | VIF |
|-------------------------|----------------------|----|----------------|------------|-------------|-----|
| Intercept               | 54.60                | 1  | 3.41           | 46.54      | 62.66       |     |
| A-ZnO NPs dose          | 6.09                 | 1  | 2.69           | −0.2814    | 12.46       | 1.0000 |
| B-contact time          | 39.38                | 1  | 2.69           | 33.01      | 45.74       | 1.0000 |
| C-MB conc.              | 1.04                 | 1  | 2.69           | −5.33      | 7.41        | 1.0000 |
| AB                      | 1.25                 | 1  | 3.81           | −7.76      | 10.26       | 1.0000 |
| AC                      | −6.58                | 1  | 3.81           | −15.58     | 2.43        | 1.0000 |
| BC                      | −3.50                | 1  | 3.81           | −12.51     | 5.51        | 1.0000 |
| A²                      | 3.61                 | 1  | 3.71           | −5.17      | 12.39       | 1.01  |
| B²                      | −19.46               | 1  | 3.71           | −28.24     | −10.68      | 1.01  |
| C²                      | 4.86                 | 1  | 3.71           | −3.92      | 13.64       | 1.01  |

Fig. 10  The desirability ramp for the optimization of three factors (viz. ZnO NPs dose, contact time and MB conc.) to yield maximum dye removal (%) using the as-synthesized ZnO NPs adsorbent

Fig. 11  a Photocatalytic degradation of MB using ZnO NPs catalyst and b pseudo first order kinetics
dye pollutants and pathogenic bacteria from contaminated water.

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Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

Data availability The author declares that the data supporting the findings of this study are available within the article, and other supplementary data are available from the corresponding author upon request.

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Fig. 12 Antimicrobial activities of ZnO NPs against a Staphylococcus aureus, b Staphylococcus epidermidis, c Pseudomonas aeruginosa, d Escherichia coli and e Klebsiella pneumonia
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