Physico-chemical characteristics and cytotoxicity evaluation of CuO and TiO2 nanoparticles biosynthesized using extracts of Mucuna pruriens utilis seeds

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ABSTRACT
The green synthesis approach to nanoparticles has been widely received as an alternative to the conventional methods, specifically for applications in areas such as biology, agriculture and medicine, where toxicity is of great concern. In this study, copper oxide (CuO) and titanium oxide (TiO2) nanoparticles (NPs) were synthesized using an aqueous extract of Mucuna pruriens utilis seed. The morphology and structural characterization of the NPs were achieved by using scanning and transmission electron microscopy (SEM and TEM), and X-ray diffraction (XRD) measurement, while the elemental composition was studied using electron diffraction X-ray spectroscopy (EDS). A monoclinic phase of CuO and anatase phases of TiO2 with high crystallinity were confirmed from the diffraction patterns of the XRD. Both TEM and SEM micrographs of the CuO confirmed short rod-shaped nanostructure, while spherical morphologies were obtained for the TiO2 NPs. The EDS study indicated that the composition of the samples conform with the identified products in the XRD and attest to the purity of the NPs. The nanoparticles exhibited a dose-dependent profile in MTT cytotoxicity assay with some cell specificity. However, the anticancer potential of these NPs was still lower than that of the standard anticancer drug, 5-fluorouracil.

1. Introduction
Nanoscience is an emerging field of study that involves the synthesis, characterization and development of different nanomaterials with unlimited prospects and applications (Hasan, 2015; Keat et al., 2015; Anbumani et al., 2022). Synthesis of nanoparticles, the reduced size particles with core-shell structures in the nanometer range (1–100 nm), is achieved by physical, chemical and biological (green chemistry) methods (Hasan, 2015; Sardjono et al., 2018; Badawy et al., 2021) Unfortunately, physical and chemical methods are limited by their high costs, energy intensiveness, as well as toxicity and environmental unfriendliness of the reagents used (Rahmani-Nezhad et al., 2017; Singh et al., 2021). For these reasons, biogenic (green) nano-synthesis methods, particularly those involving the use of plant extracts (phyto-nano-biosynthesis), have provided an alternative synthesis strategy due to their low cost, safety, biocompatibility, energy efficiency, sustainability and environmental friendliness (Sardjono et al., 2018). Extracts of multitudinous plants and plant parts (leaf, root, stem, bark, flower and fruits) have previously been employed in phytophagy synthesis of various metallic nanoparticles including copper oxide (CuO) and titanium dioxide (TiO2) (Ahmad et al., 2012; Sunny et al., 2022). The bioactive compounds (phytochemicals) in the extracts serve as natural reducing and capping agents to functionalize and stabilize the synthesized metal nanoparticles in order to prevent their agglomeration (Aslam et al., 2021). Plants including Magnolia kobus (Lee et al., 2013), Syzygium aromaticum (Clove) (Subhankari and Nayak, 2013), Euphorbia nivulia (Common milk hedge) (Valodkar et al., 2011),
Sterculia urens (Karaya gum) (Padil and Cernik, 2013), and many others have been used in this regard. However, there is currently no evidence of use of mucuna seed extract in phyto-nano-biosynthesis of CuO and TiO$_2$ nanoparticles.

Novel properties of mucuna seeds have been identified. They are rich in proteins (Mugendi et al., 2010), carbohydrates, and other leguminous seeds (Alabi and Alausa, 2006; Belewu and Olaoye, 2007). Mucuna seeds are of great interest in agriculture as animal feed. In addition, they have found utility in the treatment of infertility (Ezeagu et al., 2003), mucuna seeds are of great interest in agriculture as animal feed. In addition, they have found utility in the treatment of infertility arising from their ability to recover spermatogenic loss (Horvath et al., 2005). Their nano-size increases their surface reactivity through the enhancement of their surface area to volume ratio (Rahmani-Nezhad et al., 2017), leading to their significantly improved chemical, optical, mechanical and magnetic properties compared to their bulk forms (Vithiya and SEN, 2011) (Gnanajobitha et al., 2012). As a result of these enhanced properties, nanoparticles have found a myriad of applications in numerous fields including, but not limited to, medicine, space industry, food industry, gene delivery, health care, tissue engineering, optics, mechanics, environmental remediation, and agriculture (Ahmed and Ikram, 2016; Elenike et al., 2019).

Different types of extracts of plants have been widely utilized for the synthesis of CuO nanoparticles. Ghdan et al. (2016) reported CuO nanoparticles using the peels extract of Punica granatum. Mono-disperse CuO nanoparticles obtained using extracts of Aloe barbadensis Miller has been reported (Gunalan et al., 2012). Prakash et al. (2018) reported copper oxide nanoparticles that were effective for the photodegradation of BTB as well as high antibacterial potency, from the extracts of Cordia sebestena (C. sebestena) flower. Asadurachta indica plant extract has been found effective for the synthesis of green CuO nanoparticles that were useful for their antibacterial activity for medicinal applications (Sharma et al., 2018). Copper oxide nanoparticles synthesised using Areca catechu leaf extract has been reported for their potential anti-diabetic and anti-cancer activity (Shwetha et al., 2021). Similarly, a wide array of plants has been reported for titanium (IV) oxide nanoparticles whose application ranges from biological, medicinal and lithium ion battery (Kashale et al., 2016). For example, TiO$_2$ nanoparticles have been synthesized by using aqueous extract of Jacaranda curcas L. latex (Hudlikar et al., 2012). Rajakumar et al. (2012) reported the biosynthesis of titanium nanoparticles using Eclipta prostrata leaf aqueous extract. Titanium dioxide nanoparticles with high antimicrobial efficacy have been synthesized using Luffa acutangula leaf extract (Anbumani et al., 2022). Other relevant plants that have also been utilized includes Cinnamomum (Nabi et al., 2020), Asadurachta indica (Sankar et al., 2015), Curcuma longa (Jalill et al., 2016), Morinda citrifolia (Suman et al., 2015), and Psidium guajava (Santhoshkumar et al., 2014).

Mucuna pruriens (L.) DC var. utilis (velvet bean; Fabaceae family) is a dynamic annual climbing legume that is indigenous to Southern Africa (Tryptman et al., 2011) and also occurs in China and eastern India (Capo-chichi et al., 2003). Due to its endowment with numerous bioactive compounds, including polyphenols (tannins), trypsin inhibitors, phytate, cyanogenic glycosides, saponins, lectins, choline, alkaloids, bufotenine, N,N-dimethyltryptamine, 5-oxindole-3-alkylamines, oligosaccharides, beta-carboline, indole-3-alkylamine, and 3,4-dihydroxy-L-phenylalanine (L-DOPA), mucuna seeds have many pharmacological properties such as anti-inflammatory (Natarajan et al., 2012), neuroprotective, antioxidant, anti-diabetic, antiprotozoal and antimicrobial activities (Mastan et al., 2009). Also, due to their richness in proteins (23–35%), minerals (Mogendi et al., 2010), and unsaturated fatty acids (Ezeagu et al., 2005), mucuna seeds are great of interest in agriculture as animal feed. In addition, they have found utility in the treatment of infertility arising from their ability to recover spermatogenic loss (Horvath et al., 2005; Singh et al., 2013).

Notwithstanding, their utility as animal feed is primarily limited by their high concentration (7%) of L-DOPA, a well-known gold standard for the treatment of Parkinson’s disease (Pullikkalpura et al., 2015), and high content of fibre (97–193 g/kg DM relative to 75 g/kg DM in soya bean and other leguminous seeds) (Alabi and Alausa, 2006; Belewu and Olaoye, 2010, Mthiyane et al., 2018). In this regard, its consumption has been reported to cause vomiting, nausea, abdominal distention, and dyskinesia, especially in human beings and non-ruminant animals (Pullikkalpura et al., 2015). Therefore, utilization of this underutilized legume as animal feed requires a strategy to resolve the problem of high L-DOPA and fibre contents (Ajilogba et al., 2021). One of the innovative strategies that have been previously employed to modify the phytochemical composition of plants is nano-priming (do Espirito Santo Pereira et al., 2021). In this regard, a previous study demonstrated that seed priming with a solution of methyl jasmonate decreased the phenolic content of wheat seeds (Akkari-vaiai et al., 2013). Against this background, it is envisaged that mucuna seed priming with CuO and/or TiO$_2$ nanoparticles would decrease the seed content of L-DOPA and fibre, resulting in nutritionally safer and highly digestible seeds for use in animal diets. However, to avoid introducing foreign phytochemicals into mucuna seeds meant for nano-priming and propagation to produce progeny seeds for animal nutrition, it was deemed appropriate to use mucuna seed extract in the phyto-biosynthesis of CuO and TiO$_2$ nanoparticles. Mucuna seeds have previously been used to biosynthesise MgO nanoparticles (Rahmani-Nezhad et al., 2017). However, no studies have employed the seeds in the phyto-biosynthesis of CuO and TiO$_2$ nanoparticles. Since the seeds were meant for use in a biological system, it was necessary to investigate their cytotoxicity. Therefore, this study investigated the physico-chemical characteristics and cytotoxicity of CuO and TiO$_2$ nanoparticles phyto-biosynthesized using extracts of M. pruriens utilis seeds. It is a preliminary study aimed at producing nutritionally safe and more digestible mucuna seeds for beef cattle diets.

2. Materials and methods

2.1. Materials

Copper sulphate (CuSO$_4$), titanium butoxide (Ti(OBu)$_4$) and sodium hydroxide were procured from Merck (Pty) Ltd, South Africa. Mucuna seeds were obtained from The University of Eswatini (UNESWA), Faculty of Agriculture, Luyengo, Eswatini.

2.2. Preparation of the mucuna extract

The mucuna seeds were rinsed with distilled water prior to air-drying in the laboratory (Osontukon et al., 2019). The dried seeds were then ground into powder and sieved through a mesh (40 mm). A total of 8 g of mucuna seed powder was then introduced into 400 mL of distilled water and heated for 1 h at 80 °C. Next, the mixture was cooled to room temperature, and filtered (Whatman No. 1). The obtained filtrate was kept at 4 °C for further use.

2.3. Synthesis of CuO NPs using mucuna extract

About 30 mL of the aqueous mucuna seed extract was measured in a 100 mL flask and subsequently its pH was adjusted to 7.0 by adding 8 mL of sodium hydroxide solution. In another flask, 2.5 g of CuSO$_4$ was dissolved in 10 mL of distilled H$_2$O and the solution was agitated with a magnetic stirrer at 80 °C to get a homogenous solution. Then, the 30 mL of mucuna plant extract was introduced dropwise to the CuSO$_4$ solution and heated for 2 h (Arumugam et al., 2019). During the heating process, the blue solution changed to brown and finally to greyish colour. The solution was then cooled to room temperature, and filtered (Whatman No. 1). The obtained filtrate was kept at 4 °C for further use.

2.4. Synthesis of TiO$_2$ NPs using mucuna extract

The synthesis of TiO$_2$ followed a similar procedure as for CuO. In this case, 2 mL of titanium butoxide was added to 100 mL of distilled water.
H₂O to obtain a clear solution. The pH of the plant extract was adjusted to 7.0 by adding 8 mL of sodium hydroxide solution in dropwise. Then 30 mL of mucuna plant extract was added slowly into the solution of titanium butoxide. A change in colouration from white to orange occurred. The solution was then stirred for 4 h at room temperature and aged for 24 h. Thereafter, the precipitate was then centrifuged at 4350 rpm for 30 min, washed three times with ethanol and distilled H₂O. This was done to remove the by-products, then it was subjected to drying for 6 h at 150 °C, and calcined in a furnace at 500 °C for 2 h (Nabi et al., 2020).

2.5. Characterization of CuO and TiO₂ nanoparticles

The X-ray diffraction (XRD) measurement was obtained using Bruker D8 Advance X-Ray diffractometer over 2θ range of 20–80°. The FTIR spectra of the mucuna plant extract were measured using a Bruker alpha-P FTIR spectrometer. A TECNAI G2 (ACI) electronic microscope was used for the internal morphology of the nanoparticles. Image J software was employed for the measurement of the particle size distribution as well as average size of nanoparticles from the TEM micrographs. Atomic-level compositions were examined using energy-dispersive X-ray spectroscopy (EDS) attached to the SEM.

2.6. Cytotoxicity analysis

The cytotoxic analysis was conducted as reported previously with slight modifications (Adeyemi et al., 2020). The assay utilized two cell lines, namely, HEK293 and HeLa cell lines were procured from the ATCC, Manassas, USA. The culturing of the cells was carried out in 25 cm² tissue culture flasks containing Dulbecco's Modified Eagle's Medium (DMEM), 10% fetal bovine serum, 100 μg/mL streptomycin, and 100 U/mL of penicillin. The 3-(4,5-dimethylthiazol-2-yl)-2,6-diphenyltetrazolium bromide (MTT) assay was conducted using a 96-well plate which contained 2.5 × 10⁵ cells/well in 100 μL DMEM. Before treatment with the samples, incubation of the cells was carried out overnight at 37 °C. Afterward different concentrations that ranged from 10, 25, 50, and 100 μg/mL of CuO and TiO₂-NPs were added to the cells, and further incubation was maintained at 37 °C for 48 h, and subsequently the MTT assay was conducted. The standard anticancer drug, 5-fluorouracil (5-FU) was used for comparison. For the MTT assay, the medium in the wells was replaced with a fresh medium that contained 10% MTT reagent, and absorption of the solution was measured at 570 nm in a Mindray MR-96A microplate reader and DMSO was used as a blank. The assay was conducted three times and the average absorbance was determined. Cell viability was calculated using the equation reported by Ajibade et al. (2020).

\[
\% \text{Cell viability} = \left( \frac{\text{treated cells}}{\text{untreated cells}} \right) \times 100.
\]

3. Results and discussion

3.1. X-ray diffraction studies

The X-ray diffraction patterns of both CuO and TiO₂ nanoparticles prepared from the extract of mucuna seeds are presented in Figure 1 (a-b). The CuO nanoparticles give a single phase, which could be indexed to the monoclinic phase of CuO with lattice parameters a = 4.84 Å, b = 3.47 Å, c = 5.33 Å (Rajamma and Nair, 2020). The intensities and positions of the sharp peaks are in good agreement with the reported values (JCPDS file No. 00-048-1548). The absence of other peaks except those indexed to the CuO nanoparticles indicates the high purity of the samples (Velankar et al., 2020). A total of seven diffraction peaks were observed for the TiO₂ nanoparticles indicating well dispersed TiO₂ nanoparticles and impurity-free products that were successfully prepared (Bekele et al., 2020). The entire peaks in the XRD patterns could be indexed as anatase phases of TiO₂ (Etape et al., 2018). The diffraction data were in good agreement with the reported values (JCPDS file No. 00-021-1272). The crystalline size of the CuO and TiO₂ nanoparticles was evaluated using the Scherrer's equation (Siddiqui et al., 2021) (equation 1) and were obtained as 28.4 and 14.15 nm respectively.

\[
D = \frac{0.9\lambda}{β \cosθ}
\]  

3.2. Morphology and EDS studies

The microscopic and electron diffraction measurements were conducted for morphological and compositional analysis of the nanoparticles. Figure 2(a and b) present the SEM and TEM images of CuO nanoparticles, respectively. The SEM images show short rods of relatively uniform morphology which were compactly distributed to form a dense structure with slight agglomeration (Huang et al., 2021). The TEM study was conducted in order to understand the internal morphology of the nanoparticles, which exhibited a rod-shaped nanostructure, and confirmed the morphology obtained from the SEM analysis (Chowdhury et al., 2020). The estimated dimension of the rods is given in the particle size distribution histogram of Fig 2c and d, which showed 70.52 nm length and 31.80 nm width.

Figures 3a and b present the SEM and TEM images of the TiO₂ nanoparticles. The SEM image showed that the prepared nanoparticles were spherically shaped and completely agglomerated (Nabi et al., 2022). Some pores are distinctly visible and are distributed across the entire nanoparticles surface, confirming the high porous nature of the
Figure 2. (a) SEM, (b) TEM images of CuO nanoparticles prepared from the extract of mucuna seed. The corresponding particle size distribution histogram showing (c) length and (d) width.

Figure 3. (a) SEM, (b) TEM images of TiO$_2$ nanoparticles prepared from the extract of mucuna seed, and (c) the corresponding particle size distribution histogram.
nanoparticles (Maurya et al., 2019). TEM micrograph similarly presented spherical morphologies with distinct agglomeration and particle size estimated to be about 11 nm (Figure 3c).

Figure 4 presents the EDS spectra of CuO and TiO2 NPs. The EDS spectra of the nanoparticles showed that they are mainly composed of Cu and O (for the CuO) and Ti and O (for the TiO2), with the carbon arising from the carbon tape of the sample holder stump. Nagajyothi et al. (2017) also confirmed CuO of similar composition in biosynthesized CuO NPs using an aqueous extract of black bean. Some K atoms were also observed in the EDS spectra of TiO2, which might be from the mucuna plant extract.

3.3. FTIR studies of mucuna seed

Fourier transform infrared spectroscopy was utilized to ascertain the different functional groups present in the extract and offer an idea of the

| Sample | Concentrations | IC50 μg mL⁻¹ |
|--------|----------------|--------------|
|        | 10 μg mL⁻¹ | 25 μg mL⁻¹ | 50 μg mL⁻¹ | 100 μg mL⁻¹ |
| 5-FU   | 78.40 ± 0.034 | 58.89 ± 0.037 | 50.47 ± 0.015 | 37.99 ± 0.017 | 17.48 |
| CuO    | 85.62 ± 0.090 | 38.05 ± 0.048 | 13.61 ± 0.024 | 7.84 ± 0.012 | 22.48 |
| TiO2   | 89.39 ± 0.046 | 70.99 ± 0.010 | 58.98 ± 0.025 | 14.03 ± 0.023 | 43.85 |

Figure 5. Overlapped FTIR spectra of the plant extract, (a) CuO and (b) TiO2 nanoparticles.

Table 1. Viability (%) of HeLa cell line at different concentrations of CuO and TiO2 nanoparticles.
chemical constituents present in the mucuna seed. Furthermore, it could also provide information on the biomolecules that might have mediated the formation of CuO and TiO2 nanoparticles. Overlaid FTIR spectra of the mucuna plant and CuO nanoparticles are shown in Figure 5a, while Figure 5b shows the overlaid spectra of the extract and TiO2. In the spectra of the plant extract, the broad peak observed around 3273 cm⁻¹ could be attributed to O–H stretching vibration. This O–H group has also been reported on mucuna seed shell, and are associated with proteins and glycerides (Nwabanne et al., 2018). The broadness of this peak is usually associated with the strong hydrogen bonding that exist in them due to the wide difference in the electronegativity of hydrogen and the oxygen atom. The peak observed around 2922 and 2859 cm⁻¹ are characteristic of the symmetrical and asymmetrical stretching vibration of C–H of a sp³ hybridized carbon. The low-intensity peak around 1740 cm⁻¹ is typical of the C=O stretching vibration of an aldehyde (Agatonovic-kustrin et al., 2020).

The strong intensity peak around 1634 cm⁻¹ is due to the carbonyl group (C=O) of amide group (Shooto et al., 2020). The mucuna seed was reported to have one of the best vibration due to peptide linkage, which mainly originates from the stretching vibration of the carbonyl C=O (~80%) group as well as from both the CN stretching and NH bending vibrations (~20%) (Stani et al., 2020). Further, these proteins may be the enzymes that facilitated the formation of CuO and TiO2 nanoparticles (Arulkumar and Sabesan, 2010). The peak due to the stretching of C–H possibly from the aromatic ring of amino acid was observed at 1397 cm⁻¹. Stretching vibration band due to the C–O bending vibration was observed at 1244 cm⁻¹ and 1146 cm⁻¹.

The FTIR spectrum of the CuO NPs showed an intense peak around 500 cm⁻¹ which is ascribed to the Cu–O vibration. The absence of other peaks from the plant extract was due to the calcination process that led to the removal of the biomolecules from the plant extract. A similar pattern was observed in the spectrum of the TiO2 which showed some peaks due to Ti–O.

3.4. MTT cytotoxicity assay

The CuO and TiO2 nanoparticles synthesized using *M. pruriens utilis* seed extract were used to evaluate their *in vitro* cytotoxic potency in the HeLa and HEK293 cells at various concentrations. Their activity was compared with that of fluorouracil (5-FU), a commonly used standard anti-cancer drug (De angelis et al., 2006). The percentage cell viability and the minimum inhibitory concentration (IC₅₀) of the test samples against cancer cells were recorded (Tables 1 and 2). The results showed that the death of both the HeLa and HEK293 cells is concentration-dependent with high concentrations of both nanoparticles resulting in over higher cell death. The 5-FU drug exhibited the highest concentration-dependent with high concentrations of both nanoparticles shown in Table 1 and 2.

| Sample       | Concentrations | IC₅₀ μg mL⁻¹ |
|--------------|----------------|-------------|
|              | 10 μg mL⁻¹      | 25 μg mL⁻¹  | 50 μg mL⁻¹ | 100 μg mL⁻¹ |
| 5-FU         | 77.36 ± 0.048   | 51.92 ± 0.003 | 35.38 ± 0.010 | 11.33 ± 0.017 | 6.05 |
| CuO          | 72.79 ± 0.055   | 61.86 ± 0.059 | 48.15 ± 0.017 | 33.40 ± 0.041 | 42.33 |
| TiO2         | 79.99 ± 0.006   | 58.94 ± 0.044 | 40.23 ± 0.014 | 25.47 ± 0.047 | 35.09 |

Furthermore, the cytotoxicity of CuO and TiO2 nanoparticles could also be ascribed to the reactive oxygen species (ROS)-inducing ability which in turn might bring about a variation. This can be attributed The nanoparticles might have undergone a Fenton-type reaction that gives rise to ROS resulting to DNA damage and ultimately to the cell death. (Adeyemi et al., 2019; Maheswari et al., 2020). The mechanism of action of the TiO₂-NPs on HeLa cells could also be associated with the shape, size of the nanoparticles as well as surface functionalization. In this regard, previous studies have shown that nanoparticles with a small particle size are able to penetrate into cells much easier to effect cytotoxicity (Ajjibade et al., 2020). TEM provided evidence of the spherical nature and small size of these nanoparticles especially the CuO-NPs, which produce higher cytotoxicity in the HeLa cells. Nagaijothi et al. (2017) on HeLa cells treated with CuO-NPs. On the other hand, the HEK293 cell viability results in the current study are in agreement with a previous study of TiO2 cytotoxicity (Meena et al., 2012).

4. Conclusion

In this study, CuO and TiO2 NPs were successfully synthesized using the green method, and were structurally and morphologically characterized using analytical techniques. Well crystalline and pure phase nanoparticles were obtained with fair similarity in their distribution of the particles. The crystalline sizes of the CuO and TiO2 nanoparticles obtained were 28.4 and 14.15 nm respectively. SEM and TEM micrographs showed uniform short rod nanostructures for CuO and spherical shaped nanostructures with some agglomeration in the case of TiO2. Both nanoparticles showed similar cytotoxic profiles in the human cell lines tested with the CuO nanoparticles possessing a better anticancer potential in the cervical carcinoma cells. This however, was still lower than that of the standard anticancer drug, 5-FU but exhibited better efficiency compared to similar samples obtained via the same approach but with different plants. *Mucuna* is a promising plant that has great potential and has various uses due to the phytochemicals that are present in it. The green approach to nanoparticle synthesis using Mucuna seed extract could be extended to other nanoparticles for application in biology, medicine and agriculture.

Declarations

**Author contribution statement**

Nozipo P. Gamedze: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Doctor M.N. Mthiyane: Conceived and designed the experiments; Edited the paper.

Olabukola O. Babalola: Analyzed and interpreted the data; Edited the paper.

Moganavelli Singh: Contributed reagents, materials, analysis tools or data.

Damian C. Onwudiwe: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Edited the paper.

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Data availability statement
Data will be made available on request.

Declaration of interest's statement
The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

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References
Adeyemi, J.O., Elemike, E.E., Onwudike, D.C., Singh, M., 2019. Bio-inspired synthesis and cytotoxic evaluation of silver-gold bimetallic nanoparticles using Kei-Apple (Diospyros caffra) fruits. Inorg. Chem. Commun. 109, 107569.

Adeyemi, J.O., Onwudike, D.C., Nundkumar, N., Singh, M., 2020. Diorganoctin (IV) benzylindolocarbazole complexes: synthesis, characterization, and thermal and monomericity study. Open Chemistry 18 (1), 453-462.

Amanovski-Kustin, S., Ristivojević, P., Gegechko, V., Livinova, T.M., Morton, D.W., 2020. Essential oil quality and purity evaluation via HR spectroscopy and pattern recognition techniques. Appl. Sci. 10, 7294.

Ahmad, F., Siddiqui, M.A., Babalola, O.O., Wu, H.F., 2012. Biofunctionalization of Nanomaterials 11 (2), 267.

Ajilogba, C.F., Babalola, O.O., Nikoro, D.O., 2021. Nanotechnology as Vehicle for characterization, and its application on drug resistance bacteria. J. Nanomater. 2020 (1), 2817037.

Aslam, M., Abdullah, A.Z., Rafatullah, M., 2021. Recent development in the green biochemical responses of wheat seedlings infected by Fusarium culmorum. Arch. Phytopathol. Plant Protect. 47, 1893–1904.

Adeyemi, J.O., Elemike, E.E., Onwudike, D.C., Singh, M., 2019. Bio-inspired synthesis and cytotoxic evaluation of silver-gold bimetallic nanoparticles using Kei-Apple (Diospyros caffra) fruits. Inorg. Chem. Commun. 109, 107569.

Adeyemi, J.O., Onwudike, D.C., Nundkumar, N., Singh, M., 2020. Diorganoctin (IV) benzylindolocarbazole complexes: synthesis, characterization, and thermal and monomericity study. Open Chemistry 18 (1), 453-462.

Agatonovic-Kustin, S., Ristivojević, P., Gegechko, V., Livinova, T.M., Morton, D.W., 2020. Essential oil quality and purity evaluation via HR spectroscopy and pattern recognition techniques. Appl. Sci. 10, 7294.

Ahmad, F., Siddiqui, M.A., Babalola, O.O., Wu, H.F., 2012. Biofunctionalization of nanoparticle assisted mass spectrometry as biosensors for rapid detection of plant associated bacteria. Biosens. Bioelectron. 35, 235–242.

Ahmed, S., Ikram, S., 2016. Biosynthesis of gold nanoparticles: a green approach. J. Photochem. Photobiol. B 161, 141–153.

Ajiabade, P.A., Oluwalana, A.E., Skakima, B.M., Singh, M., 2020. Structural, photocatalytic and anticancer studies of hydroxyethylamine capped ZnO nanoparticles. Chem. Phys. Lett. 755, 137813.

Ajilore, C.F., Babalola, O.O., Wu, H.F., 2012. Biofunctionalization of Nanomaterials 11 (2), 267.

Alabi, D., Alusa, A., 2006. Evaluation of the mineral nutrients and organic food contents of the seeds of Lablab purpureus, Leucaena leucocephala and Mucuna utilis for domestic consumption and industrial utilization. World J. Agric. Sci. 2, 115–118.

Ambunani, D., Vizhi Dhansapani, K., Manoharan, J., BabuJanthanam, R., Bashir, A., Muthusamy, K., Alfaran, H., Kanimozhi, K., 2022. Green synthesis and antimicrobial efficacy of titanium dioxide nanoparticles using Luffa acutangula leaf extract. J. King Saud Univ. Sci. 34 (3), 018966.

ArulKumar, S., Sabesan, M., 2010. Rapid preparation process of antiparkinsonian drug Mucuna pruriens silver nanoparticle by bioreduction and their characterization. Pharmacoheg. Res. 2 (4), 233–236.

Bare, A., Chudzinski, S.J., Tomishis, M., 2019. A sol-gel approach to the synthesis of CuO nanoparticles using Lantana camara leaf extract and their photo catalytic activity. Optik 183, 698–705.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

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Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.

Ashok, M., Ahmad, A.Z., Yen, M.S., 2018. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. J. Ind. Eng. Chem. 98, 1–16.
Patidar, V., Jain, P., 2017. Green synthesis of TiO2 nanoparticle using Moringa oleifera leaf extract. International Res. J. Eng. and Technol. 4 (3), 470–473.
Prakash, S., Elavaranan, N., Venkatesan, A., Subashini, K., Soundharya, M., Sujatha, V., 2018. Green synthesis of copper oxide nanoparticles and its effective applications in Bignell reaction, BTB photodegradation and antibacterial activity. Adv. Powder Technol. 29 (12), 3315–3326, 0921-8831.
Prakulakpura, H., Kurup, R., Mathew, P.J., Baby, S., 2015. Levodopa in Mucuna pruriens and its degradation. Sci. Rep. 5, 1–9, 11078.
Rahmani-Nezhad, S., Dianat, S., Saeedi, M., Hadjiakhoondi, A. Synthesis, 2017.
Rajamma, R., Nair, S.G., 2020. Antibacterial and anticancer activity of biosynthesised CuO nanoparticles. IET Nanobiotechnol. 14 (9), 833, 833.
Sharma, B.K., Shah, D.V., Roy, D.R., 2018. Green synthesis of CuO nanoparticles using Sardjono, R., Khoerunnisa, F., Musthopa, I., Qowiyah, A., Khairunisa, D., Er Sankar, R., Rizwana, K., Shivashangari, K.S., Ravikumar, V., 2015. Ultra-rapid Rehana, D., Mahendiran, D., Kumar, R.S., Rahiman, A.K., 2017. Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts. Biomed. Pharmacother. 89, 1067–1077, 0753-3322.
Sankar, R., Rizwana, K., Shivaashangari, K.S., Ravikumar, V., 2015. Ultra-rapid photocatalytic activity of Azadirachta indica engineered colloidal titanium dioxide nanoparticles. Appl. Nanosci. 5, 731–736.
Santhoshkumar, T., Rahuman, A.A., Jayaseelan, C., Rajakumar, G., Marimuthu, S., Kirthi, A.V., Velayutham, K., Thomas, J., Venkatesan, J., Kim, S.K., 2014. Green synthesis of titanium dioxide nanoparticles using Psidium guajava extract and its antibacterial and antioxidant properties. Asian Pac. J. Trop. Med. 7 (12), 968–976, 1955-7645.
Sardjono, R., Khoerunnisa, F., Musthapa, I., Qowiyah, A., Khairunisa, D., Erfianty, D., Rachmawati, R., 2018. Biosynthesis, characterization and anti-Parkinson activity of magnetite-Indonesian velvet beans (Mucuna pruriens L.) nanoparticles. J. Eng. Sci. Technol. 13 (12), 4258–4270.
Sharma, B.K., Shah, D.V., Roy, D.R., 2018. Green synthesis of CuO nanoparticles using Azadirachta indica and its antibacterial activity for medicinal applications. Mater. Res. Express 5, 095033.
Shooto, N.D., Thabede, P.M., Bhila, B., Moloto, H., Naidoo, E.B., 2020. Lead ions and methylene blue dye removal from aqueous solution by mucuna beans (velvet beans) adsorbents. J. Environ. Chem. Eng. 8 (2), 2213–3437, 105537.
Shwerba, U., Latha, M., Kumar, C.R., Kiran, M., Onkarappa, H., Betageri, V.S., 2021. Potential antidiabetic and anticancer activity of copper oxide nanoparticles synthesised using Areca catechu leaf extract. Adv. Nat. Sci. Nanosci. Nanotechnol. 12, 025008.
 Siddiqui, V.U., Ansard, A., Chauhan, R., Siddiqui, W.A., 2021. Green synthesis of copper oxide (CuO) nanoparticles by Punica granatum peel extract. Mater. Today Proc. 36 (3), 751–755, 2214-7853.
Singh, A.P., Sankar, S., Tripathi, M., Rajender, S., 2013. Mucuna pruriens and its major constituent L-DOPA recover spermatogenic loss by combating ROS, loss of mitochondrial membrane potential and apoptosis. PLoS One 8 (1) e54655.
Singh, P., Singh, K.R., Singh, J., Das, S.N., Singh, R.P., 2021. Tunable Electrochemistry and Efficient Antibacterial Activity of Plant-Mediated Copper Oxide Nanoparticles Synthesized by Amomum Spumans Seed Extract for Agricultural Utility. 11. Royal Society of Chemistry, pp. 18050–18060.
Stani, C., Vaccari, L., Mitri, E., Birarda, G., 2020. FTIR investigation of the secondary structure of type I collagen: new insight into the amide III band. Spectrochim. Acta Mol. Biomol. Spectrosc. 229, 1386–1425, 118006.
Subbankari, I., Nayak, S., 2013. Synthesis of copper nanoparticles using Syzygium aromaticum (Cloves) aqueous extract by using green chemistry. World J. Nucl. Sci. Technol. 2 (1), 14–17.
Suman, T.Y., Ravindranath, R.R.S., Elumalai, D., Kaleena, P.K., Ramkumar, R., Perumal, P., Arangananathan, L., Chitrarasa, P.S., 2015. Larvicidal activity of titanium dioxide nanoparticles synthesized using Morinda citrifolia root extract against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus and its other effect on non-target fish. Asian Pac. J. Trop. Dis. 5 (3), 224–230, 2222-1808.
Sunny, N.E., Mathew, S.S., Chandel, N., Saravanan, P., Rajeshkannan, R., Rajasimman, M., Vaseeghan, Y., Rajamohan, N., Kumar, S.V., 2022. Green synthesis of titanium dioxide nanoparticles using plant biomass and their applications-A review. Chemosphere 300, 45-6535, 134612.
Trytsman, M., Van wyk, A.E., Masemola, E.L., 2011. Systematics, diversity and forage value of indigenous legumes of South Africa, Lesotho and Swaziland. Afr. J. Biotechnol. 10 (63), 13777–13779.
Valdokar, M., Jadeja, R.N., Thounaojam, M.C., Devkar, R.V., Thakore, S., 2011. Biocompatible synthesis of peptide capped copper nanoparticles and their biological effect on tumor cells. Mater. Chem. Phys. 128 (1-2), 83–89, 0254-0584.
Velsankar, K., Aswin Kumar, R.M., Preethi, R., Muthulakshmi, V., Sudharah, S., 2020. Green synthesis of CuO nanoparticles via Allium sativum leaf aqueous extract mediated synthesis of titanium dioxide nanoparticles synthesized using medicinally important plant extracts. Biomed. Pharmacother. 89, 1067–1077, 0753-3322.
Vithiya, K., Sen, S., 2011. Biosynthesis of nanoparticles. Int. J. Pharmaceut. Sci. Res. 2 (11), 2781–2785, 0975-8232.