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Research article

Physico-chemical characteristics and cytotoxicity evaluation of CuO and TiO_2 nanoparticles biosynthesized using extracts of *Mucuna pruriens utilis* seeds

Nozipho P. Gamedze [1,2], Doctor Mziwenkosi Nhlanhla Mthiyane [1,2], Olubukola Oluranti Babalola [2], Moganavelli Singh [3], Damian C. Onwudiwe [4,5,*]

[1] *Department of Animal Science, School of Agricultural Sciences, Faculty of Natural and Agricultural Science, North-West University (Mafikeng Campus), Private Bag X2046, Mmabatho, South Africa*

[2] *Food Security and Safety Focus area, Faculty of Natural and Agricultural Science, North-West University, Mmabatho 2735, South Africa*

[3] *Nano-Gene and Drug Delivery Laboratory, Department of Biochemistry, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa*

[4] *Material Science Innovation and Modelling (MaSIM) Research Focus Area, Faculty of Faculty of Natural and Agricultural Science, North-West University (Mafikeng Campus), Private Bag X2046, Mmabatho, South Africa*

[5] *Department of Chemistry, School of Physical and Chemical Sciences, Faculty of Natural and Agricultural Science, North-West University (Mafikeng Campus), Private Bag X2046, Mmabatho, South Africa*

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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 (TiO_2) 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 TiO_2 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 TiO_2 NPs. The EDS study indicated that the composition of the samples conformed 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 phytogenic synthesis of various metallic nanoparticles including copper oxide (CuO) and titanium dioxide (TiO_2) (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),

* Corresponding author.
E-mail address: Damian.Onwudiwe@nwu.ac.za (D.C. Onwudiwe).

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Sterculia urens (Karaya gum) (Padil and Černík, 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.

Nanoparticles occur in varied sizes, shapes, compositions, and crystalline phases (Patidar and Jain, 2017), with size being the primary determinant of their difference from their bulk materials (Vithiya and SEN, 2011; Hasan, 2015). 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, nanomaterials 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; Elemike et al., 2019b).

Different types of extracts of plants have been widely utilized for the synthesis of CuO nanoparticles. Ghidan 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. *Azadirachta 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 antidiabetic and anticancer 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 has been synthesized by using aqueous extract of *Jatropha 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 *Cinnamon* (Nabi et al., 2020), *Azadirachta 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 (Trytsman 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-dimethyltryptomine, 5-oxyindole-3-alkylamines, oligosaccharides, beta-carboline, indole-3-alkylamine, and 3,4-dihydroxy-L-phenylalanine (L-DOPA), mucuna seeds has many pharmacological properties such as anti-inflammatory (Natarajan et al., 2012), neuroprotective, antioxidant, antidiabetic, antiprotozoal and antimicrobial activities (Mastan et al., 2009). Also, due to their richness in proteins (23–35%), minerals (Mugendi et al., 2010), and unsaturated fatty acids (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 (Hornykiewicz, 2002; 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 (Pulikkalpura 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 Olajide, 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 (Pulikkalpura 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 (Akbari-vafaii 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 (Osuntokun 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_2O 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 (Arunkumar et al., 2019). During the heating process, the blue solution changed to brown and finally to greyish colour. It was then cooled, and the product was collected by centrifuging at 5000 rpm for 15 min, rinsed three times with a solution of ethanol and distilled H_2O to remove possible remnant ions in the final product. The product was put in a crucible and dried in an oven at 80 °C for 8 h to ensure complete drying (Okpara et al., 2021). After drying, the resultant paste was subjected to calcination in a furnace for 2 h at 450 °C.

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

H_2O 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 H_2O. 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 TiO_2 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 cm^2 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 2.5×10^3 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 TiO_2-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 incubation was allowed further at 37 °C for 4 h. Upon completion of the incubation process, formazan crystals were dissolved in 100 μL of dimethyl sulphoxide, and the absorbance 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).

% Cell viability = (treated cells/untreated cells) × 100.

3. Results and discussion

3.1. X-ray diffraction studies

The X-ray diffraction patterns of both CuO and TiO_2 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 (Velsankar et al., 2020). A total of seven diffraction peaks were observed for the TiO_2 nanoparticles indicating well dispersed TiO_2 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

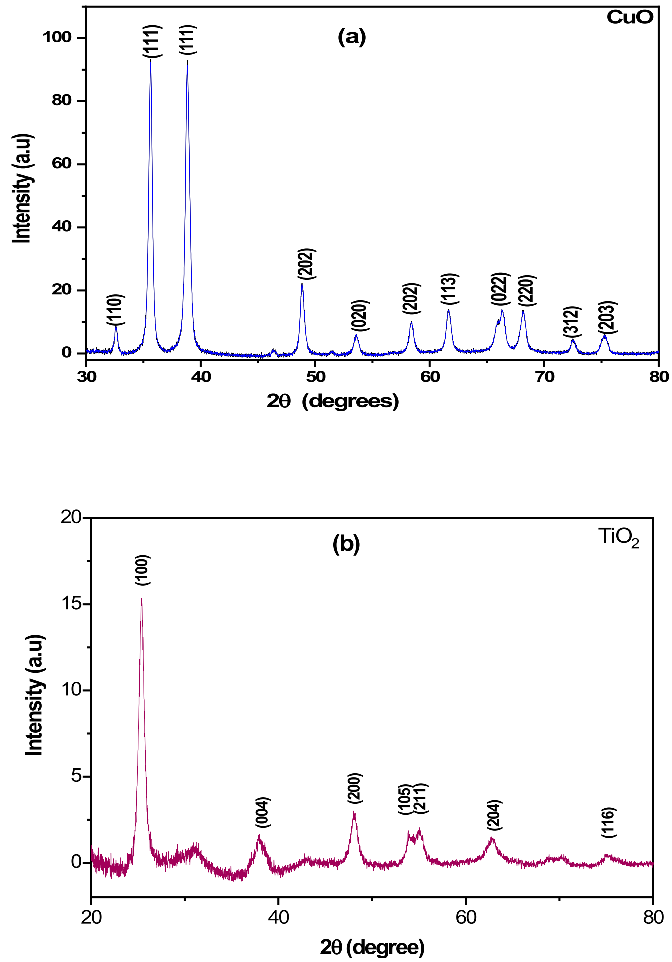

Figure 1. XR diffraction patterns of (a) CuO and (b) TiO_2 NPs prepared from the extract of *mucuna* seed.

phases of TiO_2 (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 TiO_2 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.

$$D = (0.9\lambda)/(\beta \cos\theta) \qquad (1)$$

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 TiO_2 nanoparticles. The SEM image showed that the prepared nanoparticles were spherically shaped and completely agglommerated (Nabi et al., 2022). Some pores are distinctly visible and are distributed across the entire nanoparticles surface, confirming the high porous nature of the

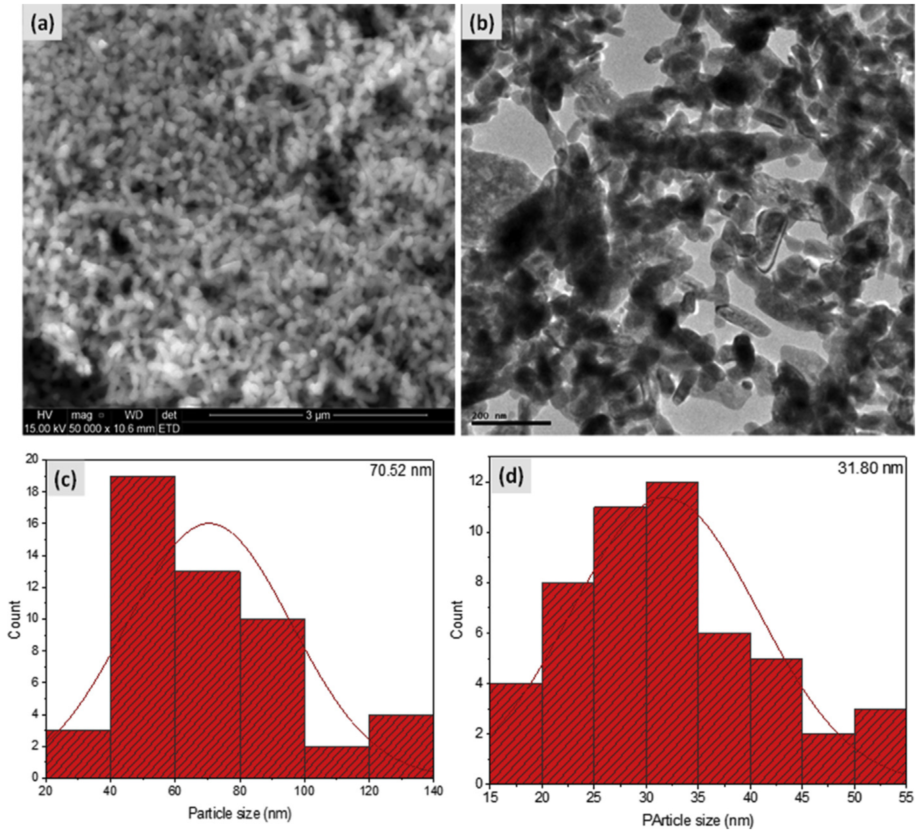

Figure 2. (a) SEM, (b) TEM images of CuO nanoparticles prepared from the extract of mucuna seed. The corresponding particle size distribution histogram showing (c) length and (d) width.

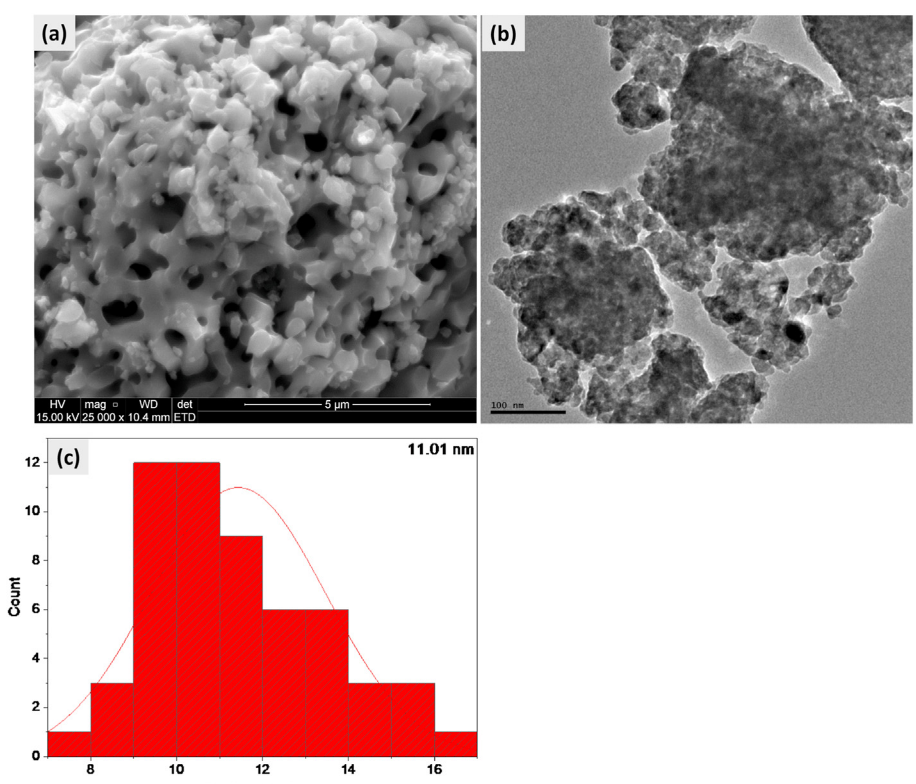

Figure 3. (a) SEM, (b) TEM images of TiO_2 nanoparticles prepared from the extract of mucuna seed, and (c) the corresponding particle size distribution histogram.

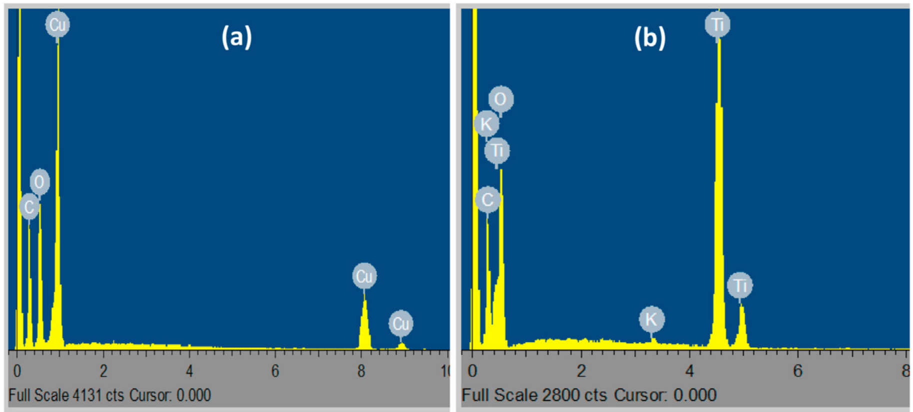

Figure 4. EDS spectra of synthesized (a) CuO and (b) TiO_2 nanoparticles prepared from the extract of mucuna seed.

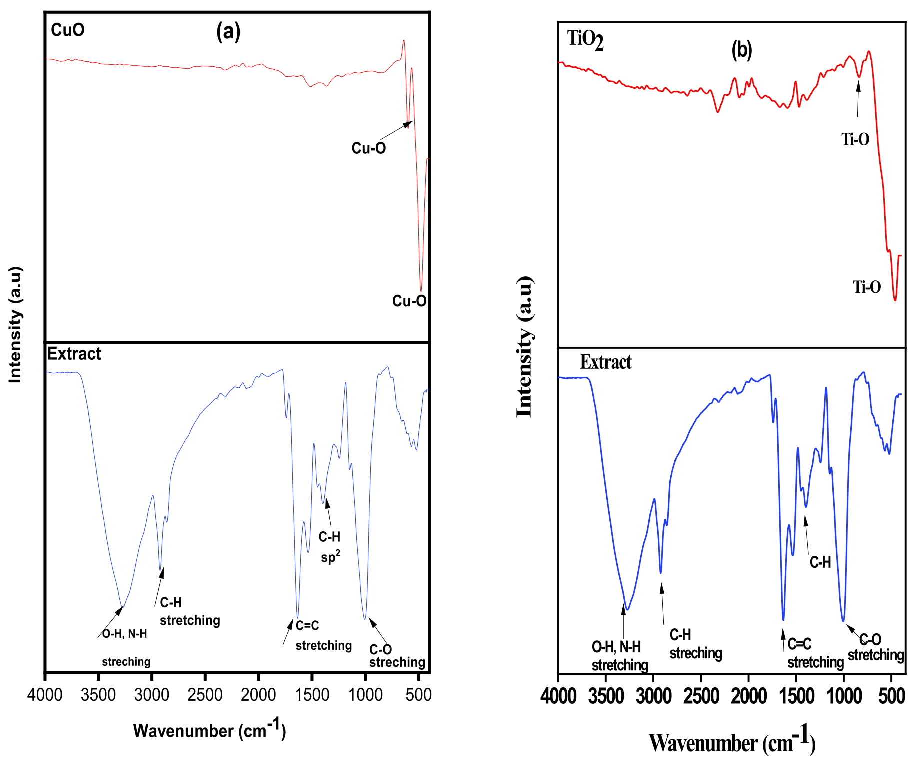

Figure 5. Overlapped FTIR spectra of the plant extract, (a) CuO and (b) TiO_2 nanoparticles.

Table 1. Viability (%) of HeLa cell line at different concentrations of CuO and TiO_2 nanoparticles.

Sample	Concentrations				IC_{50} 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
TiO_2	89.39 ± 0.046	70.99 ± 0.010	58.98 ± 0.025	14.03 ± 0.023	43.85

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 TiO_2 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 TiO_2), 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 TiO_2, 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

Table 2. Viability (%) of HEK293 cell line at different concentrations of CuO and TiO_2 nanoparticles.

Sample	Concentrations				IC_{50} 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
TiO_2	79.99 ± 0.006	58.94 ± 0.044	40.23 ± 0.014	25.47 ± 0.047	35.09

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 TiO_2 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 TiO_2. 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 sp^3 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 TiO_2 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 TiO_2 which showed some peaks due to Ti–O.

3.4. MTT cytotoxicity assay

The CuO and TiO_2 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 (IC_{50}) 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 cytotoxicity in both cell lines compared to the CuO and TiO_2 nanoparticles. However, the CuO nanoparticles were more cytotoxic compared to TiO_2 nanoparticles in the HeLa cells, while the TiO_2 nanoparticles were more cytotoxic in the HEK293 cells. This was evident from the IC_{50} values obtained for CuO (22.48 μg mL^{-1}) and TiO_2 (43.85 μg mL^{-1}) in HeLa cells, and CuO (42.33 μg mL^{-1}) and TiO2 (35.09 μg mL^{-1}) in HEK293 cells. However, these CuO and TiO_2 synthesized using *M. pruriens utilis* showed better IC_{50} values when compared to the CuO NPs (112 μg mL^{-1}) reported by Elemike et al. (2019a) and CuO (45.31 48 μg mL^{-1}) reported by Rehana et al. (2017) at a similar concentration.

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 TiO_2-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 (Ajibade 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. Nagajyothi 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 TiO_2 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 TiO_2 nanoparticles obtained were 28.4 and 14.15 nm respectively. SEM and TEM micrographs showed uniform short rod nanostructures for CuO and spherically shaped nanostructures with some agglomeration in the case of TiO_2. 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

Nozipho 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.

Olubukola 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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