



Synthesis, Characterization, Anticancer and Antimicrobial Potentials of *Chrysothemis Pulchella* Leaf Extract Mediated Gold Nanoparticles

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Abstract

In this study, *Chrysothemis pulchella* leaf extracts were used to prepare gold nanoparticles (AuNPs) for anticancer and antimicrobial applications. The nanoparticles were characterized using UV-Visible spectroscopy, powder X-ray diffraction method, Scanning and transmission electron microscopic (SEM/TEM) techniques. The UV-vis spectroscopy revealed characteristic absorption band at 527 nm whereas X-ray diffraction analysis showed characteristic crystalline peaks typical of AuNPs and carbon peaks emanating from the plant extract. The energy dispersive X-ray spectroscopic (EDXS) studies showed the following elements: C, O, Na, Mg, Cl, K, Ca and Au. The microscopic analysis using Scanning and Transmission Electron Microscopes showed spherical nanoparticles with average size of 14.7 nm. Cytotoxic tests showed that the AuNPs were toxic to human embryonic kidney (HEK-293) and HELA cells at IC₅₀ dosage of 34.5 and 54.05 µg respectively. The nanoparticles also proved to have broad spectrum antimicrobial properties as they inhibited the growth of some pathogenic micro-organisms.

Keywords Biosynthesis · Anticancer · Antimicrobial

1 Introduction

The synthesis of nanomaterials is ever growing due to the fact that the properties of nanomaterials differ from that of the bulk materials which offers them the prospects for a variety of applications. Noble metal nanoparticles are the most common prepared nanoparticles using physical, chemical and biological reduction methods due to simple synthesis, resistance to oxidation, and surface effects which

enables absorption and scattering of visible light [1, 2]. The chemical method is the most common and well-established method but have shortcomings of being toxic. The use of biological approach is rather getting increased attention more than other conventional methods due to their lower or absence of toxicity, ecofriendly approach and sustainability [3]. Apart from silver nanoparticles, gold nanoparticles are widely explored and they have varieties of biological applications. Gold nanoparticles have demonstrated potential in the detection and treatment of cancer [1, 2]. One of such applications are in photodynamic therapy because of their ability to absorb visible light and precisely provide wavelength-specific energy in just a few picoseconds. They also have the capacity to bond with biological molecules or ligands making them useful for bioimaging under visible light absorption.

Most researchers have reported other biomedical applications of gold nanoparticles which include as antimicrobials, anti-platelet, anti-inflammatory, antioxidants, anti-viral, antidiabetics, anti-angiogenesis, and so on [4–11]. They could perform all these functions resulting from their large surface-to-volume ratio, specificity in action towards target organs and effective enhancement of biological activities [7–11].

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Prihapsara et al. [12] have reported the antimicrobial effect of AuNPs using fruit infusion of *Medinilla speciosa*. Their investigation gave spherical and uniform nanoparticles which according to them is the most desired morphology because of the large surface contact and biocompatibility nature required for efficient drug delivery. In another report by Piktel et al [13], high antimicrobial efficiency of cetrionium bromide mediated AuNPs was recorded using the colony counting assay method. They obtained both peanuts and star-shaped nanoparticles, including porous spherical-like ones. From their observation, there was no significant differences in the antimicrobial activities as regards to the morphologies of the nanoparticles except for *E. coli* and *S. aureus* bacteria which were greatly susceptible to non-spherical AuNPs. Franzolin et al. [14] have reported high antimicrobial activity of silver and gold nanoparticles assisted using leaves and fruit extracts of *Plinia cauliflora* and *Punica granatum*. Their reports presented high inhibition percentages of the AgNPs compared to the AuNPs. Plant materials have been largely employed to create the nanoparticles for these biological functions especially due to the presence of the phytochemical components in them which would not only act as bioreductant, stabilizing or capping agent but contribute to the pharmacological and biological efficiency of the nanoparticles. They offer interesting and straightforward protocol however; one disadvantage of this process is that they cannot be controlled and the nanoparticles are often polydispersed [14].

In this work, *Chrysanthemum pulchellum* leaves extract were used for syntheses of Au NPs. *Chrysanthemum pulchellum* also known as sunset bells is a tropical perennial plant and the most widespread among the family of Gesneriaceae. The phytochemical components of the plant include polyphenolic compounds (e.g. quercetin, chlorogenic acid), saponins, flavonoid glycosides among others responsible for reduction of the metal ions to their nanoparticles and also intensify the biological function of the as-synthesized nanoparticles [9, 10].

This plant has served as bioreductant, stabilizer, capping effect and used as safe substitutes for an easy, one-step, non-toxic and low-cost way of preparing AuNPs for anticancer and antimicrobial applications. The synthesis is carried out in open vessels showing the possibility of repeating the reaction in large scales.

2 Materials and Methods

2.1 Materials

Chrysanthemum pulchellum leaves from Effurun, Chloroauric acid (HAuCl_4) was purchased from MERCK South Africa and used as supplied.

2.2 Organisms

Bacteria: *Methicillin Resist Staphylococcus* (MRSA), *Staphylococcus aureus*, *Vanomycin Resistant enterococci* (VRE), *Helicobacter pylori*, *Escherichia coli*. Fungi: *Campylobacter jejuni*, *Proteus mirabilis*, *Candida albicans*, *Candida krusei*, and *Candida tropicalis*.

2.3 Preparation of the Aqueous Plant Extract

Chrysanthemum pulchellum leaves were collected from Effurun, Delta state, Nigeria, washed and dried. The dried and powdered leaves (50 g) were added to 500 mL of deionized (DI) water and heated at about 70 °C for 5 min to obtain the aqueous extract. The mixture was further filtered and a reddish-brown colored extract was obtained.

2.4 *Chrysanthemum pulchellum* Mediated Synthesis of the Gold Nanoparticles (AuNPs)

The aqueous leaf extract was used as a substrate to reduce the precursor compound chloroauric acid HAuCl_4 from Au^{3+} to Au^0 . The components of the extract sufficed as stabilizer and capping molecules during the preparation of the nanoparticles. During the synthesis, 100 mL of 1 mM HAuCl_4 was added into 50 mL of aqueous leaf extract of *Chrysanthemum pulchellum*. The reaction of the extract and precursor compound took place at 75–80 °C for 1 h while stirring to give ruby red colloidal solution. The resulting solution was centrifuged at 15,000 rpm for 15 min, washed and dried in an oven at 80 °C to obtain the AuNPs.

2.5 Characterization

The absorbance spectrum of the synthesized AuNPs was determined using UV–Vis spectrophotometer (Germany) whereas the crystallinity was recorded on an automated Röntgen PW3040/60 X'Pert Pro X-ray powder diffractometer (Germany) operating with Ni-filtered $\text{Cu K}\alpha$ radiation ($\lambda = 1.5405 \text{ \AA}$) in a 2θ range of 20°–80° and scanning rate of 2° min^{−1}. The morphology and particle diameter of the as-synthesized nanoparticles were also determined using Quanta FEG 250 Environmental scanning electron microscope operating at an acceleration voltage of 30 kV.

and TECNAI G2 (ACI) transmission electron microscope equipment (Hillsboro, OR, USA) fitted with a LaB 6 electron gun. Gatan Ultrascan digital camera was used to capture the images of the nanoparticles and Image J software was used to obtain the average size of the nanoparticles.

2.6 Cytotoxicity Assay of the AuNPs Against Carcinoma HEK 293 and HeLa Cells

HEK293 cells and Human cervical carcinoma (HeLa) cells were got from the ATCC, Manassas, USA, and cultured using Eagle's minimum essential medium (EMEM) obtained from Lonza BioWhittaker, Verviers, Belgium. The EMEM contains Earle's Balanced Salt Solution, 1 mM sodium pyruvate, non-essential amino acids, 2 mM L-glutamine and 1500 mg/L sodium bicarbonate grown in 5% CO₂. The two cells were cultured at 37°C. Different concentrations (10, 25, 50 and 100 µg) of the AuNPs were prepared, sonicated and added to the growth media following the method of Elemike et al. [15] with slight modifications. Cell viability assays containing 185,000 cells/well were conducted using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay and the IC₅₀ dosage determined. Triplicate experiments were done for consistency and the average recorded.

2.7 Anti-Microbial Analysis of the As-Synthesized Nanoparticles

The antimicrobial efficacy of gold nanoparticles was determined using some pathogenic microbes obtained from the Department of Medical Microbiology, Ahmadu Bello University (ABU) Teaching Hospital Zaria, Nigeria. The earlier method by Elemike et al., [16] was adopted and modified. For the analysis, 0.001 mg of the AuNPs was dissolved in 10 mL of DMSO to obtain a concentration of 100 µg/mL. Diffusion method of analysis was adopted while Mueller Hinton agar served as the growth medium for the microbes. The manufacturer instructions were carried out during the preparation of the medium while sterilizing at 121°C for 15 min. The medium was thereafter transferred to sterile petri dishes, allowed to cool to solidification and seeded with 0.1 mL of the test organisms. Sterile swab was used to spread the inoculum on the surface of the medium while a 6 mm diameter cork-borer was used to cut the center of each inoculated medium. The AuNPs (0.1 mL, 100 µg/mL) was introduced into the well containing the inoculated medium and incubated at 37°C for 24 h. The plates were thereafter observed for possible microbial growth inhibition which were measured with a metre rule and recorded in millimeter.

2.8 Minimum Inhibitory Concentration (MIC) of the Synthesized AuNPs Against the Test Microorganisms

Broth dilution method was used for the determination of the minimum inhibition concentration of the AuNPs. In a typical procedure, 10 mL of the prepared Mueller Hilton broth was introduced into the test tubes, sterilized at 121°C for 15 min, and allowed to cool. A standard scale number of 0.5 was set by preparing Mc-Farland's turbidity solution. Normal saline was prepared, 10 mL was dispensed into sterile test tube containing the micro-organisms which was inoculated and incubated at 30°C for 6 h. The microbes were continually diluted to turbidity until there is visual semblance to the Mc-Farland's at the set scale. The concentration of the microbes at this point is about 1.5×10^8 cfu/mL. Concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL of the AuNPs were obtained by serial dilution using the sterile broth. The test microbes (0.1 mL) in the normal saline were then inoculated into the different nanoparticles' concentrations, and incubated at 37°C for 24 h. They were further visualized for turbidity (growth) and the concentration of the AuNPs in the sterile broth which did not show turbidity became the minimum inhibition concentration.

3 Results and Discussion

3.1 UV-vis Spectral Analysis

UV-Vis analysis of nanoparticles is one of the major tools for determining the formation of nanoparticles as different nanoparticles exhibit characteristic absorption peaks or surface plasmon bands within a particular wavelength region. During the synthesis, ruby coloured colloidal solution was obtained which gave indication of the formation of AuNPs. The UV-Vis absorption spectrum (Fig. 1) revealed surface plasmon peak at 527 nm which is in tandem with earlier studies [14–16]. Yazdani et al. [17] have recorded absorption peak at 519 nm which increased with reaction time and decrease in pH of the reaction. They also observed that as the absorption peak increased, the solution got darker, and more aggregation of the AuNPs and larger particles were formed.

3.2 Powder X-ray Diffraction (XRD) Studies

The crystallinity and phase structure of the as-synthesized nanoparticles was analyzed using XRD methods. The characteristic spectrum was obtained between 2θ peaks of 20–80° as shown in Fig. 2. The 2θ peaks at 38.25, 46.16°, are strong peaks characteristic of face centered cubic (fcc)

Fig. 1 UV-Vis absorption spectrum for AuNPs synthesized using *Chrysothemis pulchella* extract

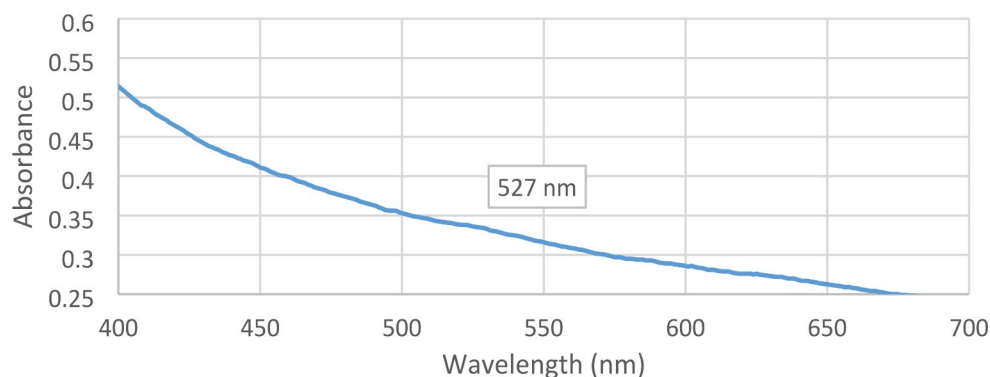
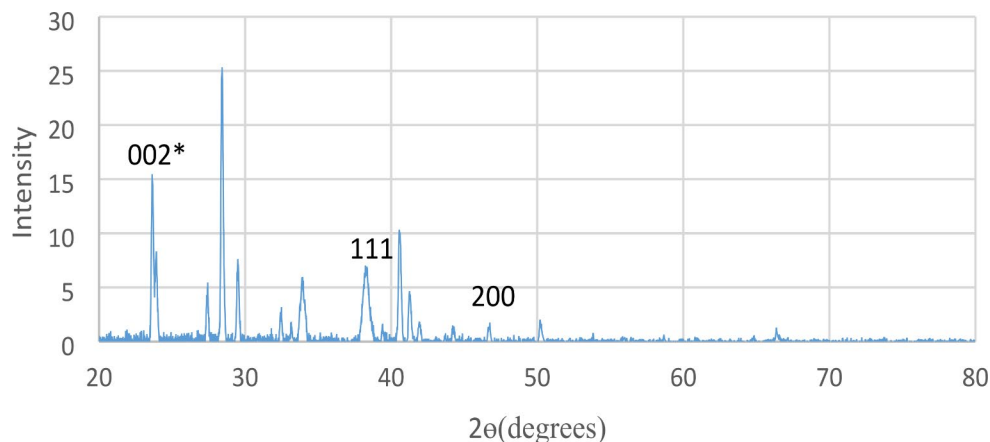


Fig. 2 pXRD spectrum of the as-synthesized AuNPs



gold nanoparticles corresponding to miller indices (111) and (200). Other peaks manifesting in the spectrum could be attributed to carbon materials from the plant extract that have contributed to stabilization and capping effect of the nanoparticles. The Braggs reflection near $2\theta = 23.6^\circ$ is indexed as (002) which reveals the presence of amorphous carbon nanomaterial's [18].

3.3 Energy Dispersive X-ray Spectroscopy (EDXS) and Microscopic Analysis

The EDXS analysis showed different elemental composition of the nanoparticles as follows: C (32.06%), O (35.99%), Au (13.39%), Cl (3.41%), K (10.48%), Mg (1.48%), Ca (1.56%), Si (0.75%), Na (0.88%) (Fig. 3a). There was confirmation of presence of AuNPs of about 13.39% which reflected as peak in the spectrum characteristic of registered energy due to gold nanoparticles. The presence of the other elements could be attributed to the plant extracts used in the synthesis which were deployed in crude form [19]. Our results corroborate that of Correa et al., [20] who reported 12.88% Au NPs in their synthesis using *Tamarindus indica* leaves with highest intensity of carbon and small percentage of other elements such as O, Ca, Al, Si, Na.

The scanning electron microscopy revealed spherical morphology of the nanoparticles as shown in Fig. 3b.

The image showed nanoparticles with less aggregation and some kind of oriented attachment. SEM is of lower resolution than TEM and therefore could just give the morphology of the nanoparticles and hence further image analysis was carried out using TEM to determine the size of the nanoparticles. The nanoparticles were sonicated in aqueous medium, studded in a copper grid, dried and visualized with transmission electron microscope to obtain the particle size. Figure 4a shows the spherical distribution of the nanoparticles as depicted by the TEM images and the average particle sizes was resolved using image J software as also shown by the histogram distribution (Fig. 4b). Careful analysis of the TEM image showed average particle size of 14.7 nm. The nanoparticles were majorly spherical with proper distribution and less aggregation. The distribution of the synthesized NPs in aqueous media would give an idea about their inherent performance in biological systems, solubility, motion dynamics, and stability. However, in natural media which typically contain mixed salts, there could be an increase in nano size and sedimentation of aggregates for the biological performance of nanomaterials. Dissolutions may be impacted by pH of nanomaterials leading to alteration of surface charges. Nanomaterial stabilities are also impacted by the cation/anion valence concentrations in reaction media [21–23].

Fig. 3 (a) EDX spectrum (b) SEM image of the as-synthesized AuNPs

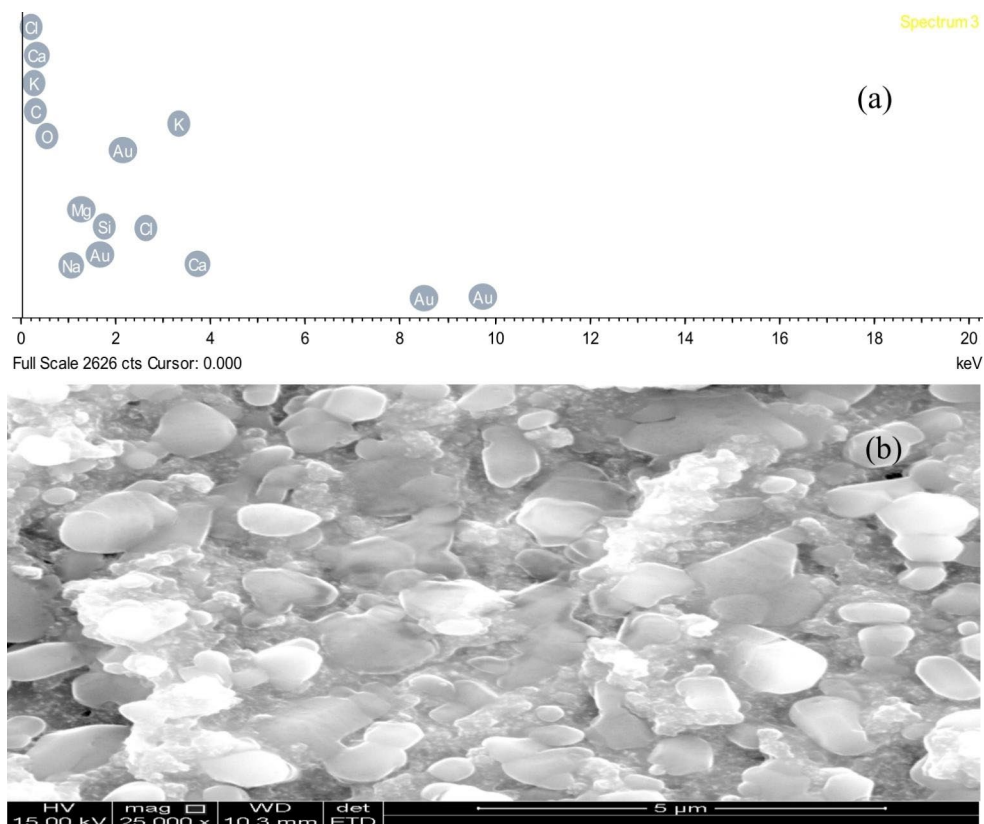
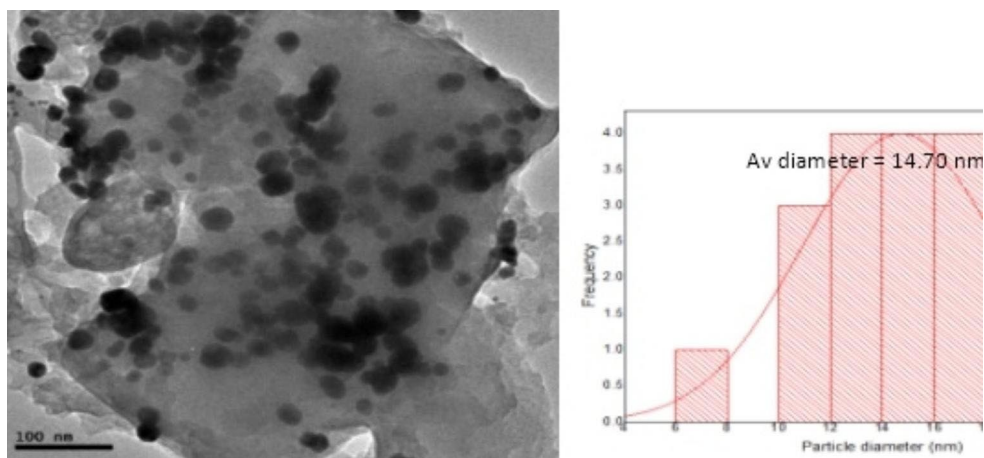


Fig. 4 (a) TEM image showing the as-synthesized AuNPs and (b) histogram of size distribution of the nanoparticles



3.4 Cytotoxic Analysis of the AuNPs

Cancer is one of the deadliest diseases facing humanity and treatment methods including chemotherapy and radiotherapy have been utilized, but they have shown undesirable side effects. The use of plant-based nanoparticles has been applied in the synthesis of AuNPs towards cancer treatment and some are showing huge successes. MTT-based cytotoxicity assay was used to verify the cytotoxic nature of the AuNPs against HEK 293 and HeLa cells at different concentrations of the nanoparticles and IC_{50} was also calculated as shown in Table 1. 5-fluorouracil was used as

a standard for this investigation. Triple measurements were done for each of the samples to ensure consistency of the results. Intriguingly, the nanoparticles exhibited a high level of cytotoxicity against the HEK 293 and HeLa cell lines. It was found that with an increase in the concentration of the nanoparticles, the cells viability continued to decrease. The IC_{50} value was found to be 34.5 and 54.05 μg on HEK 293 and HeLa cells respectively. The nanoparticles were not selective as they were also toxic to normal HEK 293 cells as against the earlier report by Clarence et al., [24] where there was no significant toxicity on the HEK 293 cells.

Table 1 Cytotoxic analysis of the synthesized nanoparticles using HEK 293 and HeLa cells

Specimen	10 µg	25 µg	50 µg	100 µg	IC ₅₀
Cell only	100±0.017	100±0.017	100±0.017	100±0.017	
5-Fluorouracil	-	36.57±4.15	27.47±6.14	23.54±2.90	5.46 µg
HEK 293					
AuNPs	77.64±0.103	57.68±0.016	38.18±0.005	29.23±0.004	34.5 µg
HeLa cells					
Au NPs	84.54±0.04	78.05±0.04	55.77±0.06	28.62±0.06	54.05 µg

Table 2 Zone of inhibition of synthesized AuNPs and some orthodox drugs against the test microorganisms

Test Organism	AuNPs	Sparfloxacin	Ciprofloxacin	Fluconazole
MRSA	0	30	0	0
VRE	25	0	34	0
Staphylococcus aureus	27	40	0	0
Escherichia coli	0	0	37	0
Helicobacter pylori	24	0	35	0
Campylobacter jejuni	28	35	0	0
Proteus mirabilis	27	30	0	0
Candida albicans	0	0	0	32
Candida krusei	24	0	0	35
Candida tropicalis	25	0	0	33

Botha et al., [25] also reported the non-toxicity of AuNPs towards normal H4IIE-luc and HuTu-80 cells at concentration of 50 µg/mL but the Ag and Ag-Au bimetallic nanoparticles obtained from the same plant extract were toxic.

In general, the cytotoxic effects of AuNPs whether in vitro or in vivo could be attributed to dosage, size, morphology, sampling points, surface effects of the nanoparticles and cell lines or animal models and the results are inconsistent and somewhat confusing [26].

3.5 Antimicrobial Test Analysis

The antimicrobial activities of AuNPs against some clinical pathogenic microbes such as *Methicillin Resist Staph aureus* (MRSA), *Staphylococcus aureus*, *Vanomycin Resistant enterococci* (VRE), *Helicobacter pylori*, *Escherichia coli*, *Campylobacter jejuni*, *Proteus mirabilis*, *Candida krusei*, *Candida tropicalis* and *Candida albicans* were determined using Mueller Hinton agar medium. The synthesized nanoparticles showed antimicrobial properties against all strains tested except for *Methicillin Resist Staph aureus*, *Escherichia coli* and *Candida albicans* as shown in Table 2; Fig. 5. The MIC of AuNPs against the organisms was 25 µg/mL for *Vanomycin Resistant enterococci*, *Candida krusei* and *Candida tropicalis* while 12.5 µg/mL was the MIC against *Staphylococcus aureus*, *Campylobacter jejuni* and *Proteus mirabilis* as shown in Table 3. The minimum bacterial/fungicidal concentration (MBC/MFC) of AuNPs against all the organisms occurred at 50 µg/mL except for *Campylobacter jejuni* which occurred at 25 µg/mL (Table 4). Folorunso et al., [27] reported that the potency and effectiveness of synthesized nanoparticles against test pathogens increases with increase in the concentration of the nanoparticles which is in tandem with the results of the present investigation. The antibacterial and antifungal investigation of the synthesized AuNPs mediated using *Chrysothemis pulchella* in this research work reveals that the synthesized AuNPs have broad-spectrum antimicrobial activities.

**Fig. 5** (a) Diagram of fungi plates (b) bacteria plates showing zone of inhibition

Table 3 Minimum inhibitory concentration (MIC) of the AuNPs synthesized from *Chrysothemis pulchella* plant extract against the test microorganism

Test Organism	Au-NPs				
	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL
<i>MRSA</i>					
<i>VRE</i>	-	-	0*	+	++
<i>Staphylococcus aureus</i>	-	-	-	0*	+
<i>Campylobacter jejuni</i>	-	-	-	0*	+
<i>Proteus mirabilis</i>	-	-	-	0*	+
<i>Candida krusei</i>	-	-	0*	+	++
<i>Candida tropicalis</i>	-	-	0*	+	++

Key -=> No turbidity (no growth), 0*=>MIC, +=>turbid (light growth), ++=>Moderate turbidity, +++=>High turbidity

Table 4 Minimum bactericidal/ fungicidal concentration of the AuNPs synthesized from *Chrysothemis pulchella* plant extract against the test microorganism

Test Organism	Au-NPs				
	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL
<i>MRSA</i>					
<i>VRE</i>	-	0*	+	++	+++
<i>Staphylococcus aureus</i>	-	0*	+	++	+++
<i>Campylobacter jejuni</i>	-	-	0*	+	+++
<i>Proteus mirabilis</i>	-	0*	+	++	+++
<i>Candida krusei</i>	-	0*	+	++	+++
<i>Candida tropicalis</i>	-	0*	+	++	+++

Key=- No colony, 0*=>/MFC, +=>Scanty colony growth, ++=>Moderate colony growth, +++=>Heavy colony growth

3.6 Minimum bactericidal/ Fungicidal Concentration (MBC/MFC) of the Synthesized AuNPs Against the test Microorganisms

Growth inhibition or death of the micro-organisms were determined by the MBC/MFC assay. As earlier stated, the sterilized Mueller Hilton agar was introduced into petri dishes and left to cool and solidify. The prepared medium was used to sub-culture the contents of the MIC in the serial dilutions, incubated at 37° C for 24 h, kept to monitor for colony growth. The plates with lowest concentration of the

nanoparticles that did not form colony growth are regarded as the MBC/MFC and their results are shown in Table 4.

4 Conclusion

The green synthesis of AuNPs using *Chrysothemis pulchella* approach was easy, eco-friendly and could easily be scaled up. In this research work, we achieved the synthesis and characterization of gold nanoparticles using *Chrysothemis pulchella* aqueous extract while verifying their cytotoxic behaviour against normal HEK 293 and Hela cell line and antimicrobial activities. The nanoparticles were formed by appearance of ruby colouration which was confirmed with surface plasmon band at 527 nm. XRD spectrum showed crystalline peaks characteristic of AuNPs and carbon emanating from the plant extract while EDXS gave elemental composition of 13.39% Au among others. The SEM and TEM analysis revealed spherical morphology and particle size of about 14.7 nm respectively. Gold nanoparticles was synthesized and characterized using *Chrysothemis pulchella* plant extract for the first time which has anticancer potentials against Hela cells but not selective in their properties as the nanoparticles were toxic against HEK 293 normal cell lines. The use of normal (non-cancerous) cell cultures is a closer test to an in vivo situation which in this case does not support the clinical applicability of the nanoparticles as anti-cancer agents. The antimicrobial test of the nanoparticles gave interesting results showing broad spectrum activities of the nanoparticles. More investigations are recommended to ascertain the best way for the clinical application of the nanoparticles and vary some properties in order not to harm normal cells.

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Declarations

Competing Interests The authors declare no competing interests.

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