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Anticancer cytotoxicity and antifungal abilities of green-synthesized cobalt hydroxide ($Co(OH)_2$) nanoparticles using *Lantana camara* L.

Lokesh Ravi*, B. K. Anirudh Sreenivas, G. R. Shree Kumari and O. Archana

Abstract

Background: Green synthesis of metal nanoparticles with pharmaceutical applications is the current focus in the field of nanomedicine. This study aims at use of *Lantana camara* L as a source of green reducing agent toward synthesis of cobalt nanoparticles.

Results: Fe^{3+}-reducing assay demonstrated that Lantana camara methanol extract (LCM) has significant electron transfer potential. Gas chromatography mass spectroscopy (GC–MS) analysis of the crude extracts revealed the presence of 7 known and 17 unknown phytochemicals in LCM. Synthesis of cobalt nanoparticles was confirmed based on color change of reaction mixture from light brown to dark brown. UV–visible spectrometry analysis showed that the synthesized particles had a λ_{max} at 267.5 nm. Based on the two theta (2θ) and Miller indices (*hkl*) values obtained in XRD analysis, the particles were confirmed to be cobalt hydroxide ($Co(OH)_2$) nanoparticles. Further dynamic light scattering (DLS) analysis showed that the average size of the $Co(OH)_2$ nanoparticles is 180 nm. SEM image analysis of the particles revealed that they are spherical mass of feather-like structure, contributing toward increased surface area of the particles. Further, the pharmaceutical potential of the $Co(OH)_2$ nanoparticles was evaluated against eukaryotic cancer and fungal cells. MTT cytotoxicity analysis showed that $Co(OH)_2$ nanoparticles have selective toxicity toward HCT-116 cancer cells with an IC_{50} value of 25 µg/ml and reduced cytotoxicity to non-cancerous VERO cells with an IC_{50} value of 200 µg/ml suggesting that the particles possess selective anti-cancerous cytotoxicity. Additionally, the particles demonstrated significant antifungal activity against 5 human fungal pathogens.

Conclusions: Results of this study conclude that green-synthesized $Co(OH)_2$ nanoparticles using *Lantana camara* L possess excellent eukaryotic cytotoxicity against cancer cells and fungal pathogens.

Keywords: *Lantana camara* L, $Co(OH)_2$ nanoparticles, Green synthesis, Anticancer cytotoxicity, Antifungal

1 Background

Nanotechnology is a dynamic field of synthetic biology which is gaining importance and pace significantly around the world. Nanotechnology broadly refers to production, manipulation and application of nanoscale structures whose size and shape can be controlled through creative and controlled synthesis procedures [1–3]. There are mainly two approaches to synthesize nanoparticles—"top-down" and "bottom-up" approach. Top-down approach basically consists of breaking down of bulk material into finer particles by reduction in size using techniques of grinding, sputtering, milling and thermal/laser ablation; alternatively chemical and biological methods of reduction and electrochemical methods

*Correspondence: lokesh.ravi@sju.edu.in

Department of Botany, School of Life Sciences, St Joseph's University, Bengaluru, Karnataka 560027, India

are used for synthesis of particles in the "bottom-up" approach [4].

It is a known fact that solvents, chemicals and reducing agents used for the synthesis procedures have a major impact on the size, physiochemical nature, utilization, shape and morphological aspects of the synthesized nanoparticles [4, 5]. Their compact size and increased surface area have opened doors for their application in almost all fields of science and technology. Biomedical, chemical industries, cosmetics, catalysis, drug delivery, food and feed, health care, mechanics, optics, space industries, nonlinear optical devices, electronics, environment, energy science, environmental remediation, single-electron transistors and photo-electrochemical sectors are some of the fields which constitute the major applications for nanoparticles [6–8].

1.1 Nanoparticle synthesis

Various methods and methodologies are inculcated for the synthesis of nanoparticles. Different approaches are available to synthesize nanoparticles such as (1) chemical approach, (2) physical approach and (3) greener approach. Synthesis of nanoparticles may be of synthetic origin or natural origin, and these NPs at nanoscale level may exhibit peculiar properties [9].

Classically two basic approaches were used to synthesize nanoparticles, viz. the "top–bottom" approach and the "bottom-up" approach. The top–bottom approach involves many chemical, physical and thermal techniques that provides the required energy for the formation of nanoparticles. This approach calls for decreasing down of solid materials into very small pieces by the application of external forces. On the other hand, bottom-up approach is based on accumulating and combining liquid or gas atoms or molecules. Both the above-mentioned approaches have their own advantages and disadvantages [9]. Some of the commonly used methodologies in synthesizing nanoparticles are the chemical and physical approaches.

1. Chemical approach: This method aims in cooperation of chemicals such as reducing agents, stabilizing agents and metallic precursors. Both inorganic and organics reducing agents are utilized in the synthesis such as sodium citrate, elemental hydrogen and N, N-dimethylformamide (DMF) [10]. Coprecipitation method, chemical reduction of metal salts, electrochemical method, pyrolysis, photochemical (irradiation) method, sol–gel process, etc., are all some of the methods of synthesizing nanoparticles chemically [11].
2. Physical approach: This method mainly uses the top-down approach which calls for reducing the size of materials by various physical approaches such as arc discharge method, electron beam lithography, ultrasonication, mechanical grinding and vapor-phase synthesis are used [11]. This approach utilizes a tube heater at barometrical weight for the integration of nanoparticles by evaporation condensation. Evaporation condensation and laser removal are both considered the most essential physical methodologies. Many nanoparticles have already been synthesized and already reported using dissipation built-up procedure like NPs of Ag, Au and Cd [10].

1.2 Green synthesis

Though traditional methods are used for synthesizing NPs from many years in the past, researchers have proved that there are greener methods available for generation of NPs more effectively with the advantage of less chance of failure, low cost and ease of characterization [12]. Most of the conventional methods used for the production of NPs are expensive, non-environment friendly and toxic. To avoid such problems, researchers have found precise greener routes in synthesizing NPs using naturally occurring sources and their products. Green synthesis is also known as a biological synthesis, as microorganisms, plants, plant extracts and other biological organisms are involved in synthesizing nanoparticles [1]. Green synthesis is a need to develop clean, nontoxic and environment-friendly procedures for nanoparticle synthesis. Green synthesis can be categorized as: (a) utilization of microorganisms like algae, fungi, yeast (eukaryotes), actinomycetes (prokaryotes) and bacteria; (b) using plants and plant extracts; and (c) use of templates like membranes, diatoms and virus DNA.

According to the literature, there are 12 basic principles of green chemistry (1) prevention, (2) atomic economy, (3) less hazardous chemical syntheses, (4) designing safer chemicals, (5) safer solvents and auxiliaries, (6) design for energy efficiency, (7) use of renewable feedstocks, (8) reduced derivatives, (9) catalysis, (10) design for degradation, (11) real-time analysis for pollution prevention and (12) inherently safer chemistry for accident prevention [13]. Nanoparticles that are biosynthesized also exhibit more therapeutic benefits than the chemically synthesized nanoparticles [14]. Some of the prominent advantages that green synthesis protocols provide over the usual conventionally used physical and chemical methods are.

(a) Nontoxic, clean and eco-friendly method [15].
(b) Reduction in the overall cost of the synthesis process, as the active biological components like

enzyme present act as reducing and capping agents [15].

(c) Considered as a significant energy saver, because external experimental conditions like high pressure and high energy are mostly not required [16].

(d) A vast range of naturally available biological resources like microorganisms such as yeast, fungi, bacteria, algae and viruses, plants and plant extracts can be used for the synthesis of nanoparticles [17].

1.3 Applications of green nanotechnology

Green nanoparticles have differing impact on the utilization of metallic NPs. They assume an imperative part to expanding the utility of NPs in pharmaceutical field particularly. Green NPs are widely used in agricultural engineering such as nanofertilizers, nanopesticides, nanocoating and intertwining nanoherbicides. They are also used in X-ray imaging and drug delivery. Green-synthesized AuNPs showcase a broad spectrum of physicochemical properties, optical properties, viable flexibility and biocompatibility that makes them an excellent nanocarrier in drug delivery [10].

In the new era, green synthesis is gaining importance as it highlights and focuses on the concept of using environmentally friendly approaches to synthesize and obtain nanoparticles. This method accommodates various biological materials including plants, fungi, vitamins, bacteria algae, etc., and the synthesis protocols can also be subjected to various manipulations to suit the specific need. Temperature, pH, pressure and solvent are some of the basic factors that determine the nature of the nanoparticles being produced [18]. The most important biological component currently being used for synthesis is plants attributing to their rich sources of phytochemicals which function as good reducing agents in the process of reduction of metals [19–22]. Based on the same concept, this study focuses on the synthesis of nanoparticles using the extract of *Lantana camara* L.

1.4 *Lantana camara* L.

Lantana camara L is an obnoxious weed as well as an ornamental herb which grows quite well in the tropical and subtropical regions of the world. These plants grow up to a height of 2–3 m and have curved prickles on its branches. They generally have oblong leaves which are quite rough to the touch and have an unpleasant odor. They are also known to cause hepatotoxicity when ingested by many animals [1, 13]. *Lantana* has many reported uses in traditional and folk medicine due to its vast phytochemical diversity. There is also a current emphasis on the use of lantana in modern medicine as well, owing to its antimicrobial, cytotoxic, antifungal, antioxidant, anti-inflammatory and wound healing properties [14]. This study provides a new direction for the use of *Lantana camara* plant in the synthesis of cobalt nanoparticles.

1.5 Cobalt nanoparticles

Cobalt-based nanoparticles are one among the few metallic nanoparticles with wide range of applications in the fields of biology as well as other technologies. They are promising materials in synthetic biology and have procured the interest of researchers since a long time. Typically, cobalt nanoparticles have major applications in media, magnetic sensors, magnetic memories, magnetic fluids, magnetic composites and catalysis due to their excellent magnetic, electrical and catalytic properties. They are also a common choice for use in electromagnetic wave absorption applications including development of wireless communication and high-frequency circuits [15–17]. Cobalt nanoparticles have gained significant importance in the biomedical field with applications in resisting bacterial as well as fungal growth. They are also considered biosafe due to their minimal hemolytic activity [18–20].

On similar lines, this study emphasizes the use of *Lantana camara* L for green synthesis of cobalt nanoparticles and tests its potential in pharmaceutical applications such as anticancer and antifungal agent. Anticancer cytotoxicity is estimated using MTT assay on HCT-116 cell lines, while the antifungal potential of these nanoparticles was estimated using agar-well diffusion method against *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Candida albicans* and *Fusarium oxysporum.* The key objective of this study is to convert the economically insignificant plants such as *L. camara* into a green source for synthesizing CoNPs with pharmaceutical applications, thus giving its economical value.

2 Methods

2.1 Collection of plant sample and extraction of phytochemicals

Lantana camara L plants were collected from the vicinity of Shantinagar, Bangalore, Karnataka, India. The aerial parts of the plant were carefully cut and washed under running water to remove debris and other unwanted materials. The washed plant samples were dried in a hot air oven for 48 h at 60 °C. The dehydrated plant parts were ground to obtain a fine plant powder which was used for the extraction procedure. Chloroform, methanol and petroleum ether were the three organic solvents used for the extraction through

maceration. A weight of 10 g of the plant powder was soaked in 100 ml of each of the solvents for a span of 24 h in a magnetic stirrer for maceration process. The macerated samples were filtered, and a crude extract was obtained upon concentrating through a rotary vacuum evaporator.

2.2 Fe3 +-reducing assay

Five different concentrations of the crude extracts, i.e., 25, 50, 75, 100 and 125 mg/ml, along with ascorbic acid (standard) at five different concentrations, i.e., 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml, were subjected to antioxidant assay. Crude extracts were dissolved in 50% methanol for this assay. Initial reaction was performed in test tubes consisting of 2 ml of phosphate buffer with 2 ml of potassium ferricyanide and 2 ml of crude extract sample. The mixture was incubated at 50 °C for 20 min. To this, 2 ml of trichloroacetic acid was added followed by 0.5 ml of 1% $FeCl_3$. The contents are mixed well, and the intensity of the developed color was read at 680 nm. The observed O.D directly represents the antioxidant potential, with higher absorbance indicating higher antioxidant potential [23–25].

2.3 Gas chromatography mass spectrometry (GC–MS) analysis

The crude extracts of *Lantana camara* L were subjected to GC–MS analysis to identify the secondary metabolites present. Clarus 680 PerkinElmer Gas Chromatography (30.0 m × 0.25 mm × 250 µm) and mass detector turbo mass of EI mode were used. Carrier gas used was helium at a flow rate of 1 ml/min. The temperature of the injector was 200 °C with column temperature set at 60 °C for 2 min and increased at 10 °C/min until 300 °C. The obtained molecular spectrum was matched with NIST library database to identify the secondary metabolites [26–28].

2.4 Green synthesis of cobalt nanoparticles

Cobalt chloride (0.1 M) solution was used as precursor for the synthesis. Methanolic extract (1% w/v) of *L. camara* was used as reducing agent. Polyethylene glycol (PEG) was used as stabilizing agent at a concentration of 1% v/v. The mixture was subjected to constant stirring for 2 h at 50 °C. The obtained solution was centrifuged at 7500 rpm for 5 min. Three consecutive acetone washes were done to remove remnants of crude extracts. Obtained particles were placed on a watch-glass and dried in a hot air oven at 120 °C overnight to obtain dry cobalt nanoparticles. The obtained particles were suspended in 10 ml of distilled water and filtered through 0.45 µm pore sized syringe filter. The filtrate was washed thrice with acetone and centrifuged at 7500 rpm for 5 min. The final pellet was dried in hot air oven at 120 °C overnight to obtain the final purified particles.

2.5 Characterization of the nanoparticles

Green-synthesized nanoparticles were subjected to characterization through X-ray diffraction (XRD), UV-spectroscopy, scanning electron microspore (SEM) and dynamic light scattering (DLS). XRD was performed at St. Joseph's College (Autonomous), Bangalore. SEM, DLS and UV–visible spectroscopy were performed at ACIC—St. Joseph's College (Autonomous), Trichy.

X-ray diffraction (XRD) patterns were obtained using a PANalytical X'Pert Pro diffractometer (Cu Kα radiation, secondary graphite monochromator, scanning rate of 1°2θ/min). UV-spectroscopy was performed on the PerkinElmer Lambda 35 model with a range of 190 nm to 1100 nm. DLS was performed on the Micromeritics Nanoplus Particle size analyzer which has a particle size measurement range of 0.1 nm to 12.3 µm for particles suspended in a liquid medium.

2.6 Determination of anticancer cytotoxicity of the $Co(OH)_2$ nanoparticles

The green-synthesized nanoparticles were subjected to in vitro MTT assay to determine its cytotoxicity. VERO cell line (monkey kidney epithelial cells/non-cancerous cells) and HCT-116 cell line (colorectal carcinoma/cancer cells) were used for the assay. The non-cancerous VERO cell lines were used as reference for the study, and the cancerous HCT-116 was used for determining the anticancer activity of the nanoparticles. Standard animal cell culture techniques were used for culture and growth of the cell lines. The cells were maintained in a CO_2 incubator at 37 °C until 80% confluence was obtained. The cells are then treated with various concentrations of cobalt nanoparticles and incubated for 24 h. Following the incubation, the cells were treated with MTT stain, i.e., 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide stain. The color developed was read in UV–Vis spectrometry. The cell viability was calculated based on the absorbance value of the control cells and treated cells [23, 29–31].

2.7 Antifungal activity of the $Co(OH)_2$ nanoparticles

In vitro antifungal activity of the green-synthesized nanoparticles was tested against 5 fungal pathogens, i.e., *Aspergillus flavus* (MTCC 277), *Aspergillus niger* (MTCC 872), *Aspergillus terreus* (MTCC 6324), *Candida albicans* (MTCC 4748) and *Fusarium oxysporum* (MTCC 284). Clotrimazole was used standard for determination of the antifungal activity. The synthesized nanoparticles were

used in concentrations of 25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml. The standard clotrimazole was used at concentration of 30 µg/ml. Antifungal activity was performed using agar-well diffusion method, and the activity was measured through the measurement of the zone of inhibition in mm [32, 33].

3 Results

3.1 Phytochemical extraction

Freshly dried and ground leaves of *Lantana camara* L were extracted in 3 different solvents (i.e., methanol, chloroform and petroleum ether) by maceration technique in a magnetic stirrer at room temperature for 24 h. This was done to extract wide range of phytochemicals present in *L. camara* leaves based on their polarity, with methanol being highly polar solvent, chloroform being mid-polar solvent and petroleum ether being non-polar solvent. The maceration mixture was filtered and concentrated in rotary vacuum evaporator to obtain the crude extract of phytochemicals in different solvent polarity. The extracts are abbreviated as LCM (*L. camara* Methanol), LCC (*L. camara* chloroform) and LCP (*L. camara* petroleum ether).

3.2 Reducing potential of crude extracts

To understand the reducing power of the crude extracts, Fe^{3+} reduction assay was performed. Although previously the literature does confirm the antioxidant property of *L. camara*, this assay was carried out to quantify their reducing potential, thereby to identify the most suitable extract to be used as green reducing agent for nanoparticle (NP) synthesis. The results of the Fe3 + assay are graphically represented in Fig. 1A. Among the 3 different extracts, methanol extract (LCM) demonstrated highest potential for Fe^{3+} reduction. At a lowest concentration of 25 mg/ml, LCM demonstrated reducing potential equivalent to that of the ascorbic acid (1 mg/ml) that was used as positive control and reference standard. Hence, among the 3 extracts, the methanol extract (LCM) was identified as most potent antioxidant source, in regard to the electron transfer potential. Hence, methanol extract was further used as source of reducing agent for nanoparticles synthesis.

3.3 Phytochemical analysis of crude extracts

All 3 extracts were subjected to gas chromatography mass spectroscopy (GC–MS) analysis to identify the phytochemical compounds present. The gas chromatogram of LCM is shown in Fig. 1B. Upon analyzing the individual peaks observed in all 3 extracts, several known phytochemicals were identified, based on their molecular weight and fragmentation pattern. Molecular weights obtained from the GC–MS analysis were compared with list of phytochemicals present in *L. camara*. List of all known phytochemicals present in *L. camara* and the presence of those chemicals in these crude extracts based on GC–MS analysis are summarized in Table 1. A total of 24 different peaks were observed in LCM, among which 7 were observed to be previously reported phytochemicals, i.e., camaric acid, pomolic acid, icterogenin, luteolin, beta-curcumene, lactic acid and theviridoside. Methanol extract of *L. camara* consists of several compounds belonging to phenol and tannin family, which are known to be strong reducing agents, and hence, these molecules contribute toward the strong antioxidant activity observed in LCM extract.

3.4 Synthesis of cobalt nanoparticle

The reaction mixture consisted of 1% LCM (1 g), 1% PEG (1 ml of polyethylene glycol) and 98 ml of 0.1 M Cobalt Chloride ($CoCl_2$) solution. The reaction was carried out at 50 °C for 2 h in a magnetic stirrer at 600 rpm. The reaction was considered complete, when there was no further development or alteration in color of the reaction mixture. The color of the initial reaction was observed to be bright light brown (caramel color), and the color of the end reaction mixture was observed to be dark brown (chocolate color) as shown in Fig. 2A, B, respectively. The end reaction mixture was centrifuged at 7500 rpm for 5 min, and the pellet was washed with acetone thrice. The resultant pellet was dried in hot air oven. The dried particles were resuspended in water and filtered through 0.45 µm syringe filter to remove agglomerates. The filtrate was dried in hot air oven to obtain the final cobalt particles. The final obtained cobalt particle appeared to be gray in color with powdery texture. The appearance of the final extracted cobalt particles is shown in Fig. 2C. This cobalt particle was suspended in distilled water and subjected to UV–visible spectrophotometry analysis. The UV–visible spectrum absorbance of the cobalt particle is shown in Fig. 2D. The lambda max of the cobalt particles was observed to be 267.5 nm. It was already reported that the precursor salt, i.e., cobalt chloride has a lambda max of 510 nm. Thus, the UV–visible spectrum confirms that the cobalt chloride salt has been reduced and the surface Plasmon resonance of the resultant product is different than the precursor salt, suggesting a successful green synthesis-mediated reduction of the cobalt salt.

3.5 X-ray diffraction analysis

Obtained cobalt particles were subjected to X-ray diffraction (XRD) analysis, in comparison with the used precursor salt, i.e., cobalt chloride ($CoCl_2$). This was done to observe the difference in the XRD pattern of the precursor and synthesized particles. The graphical

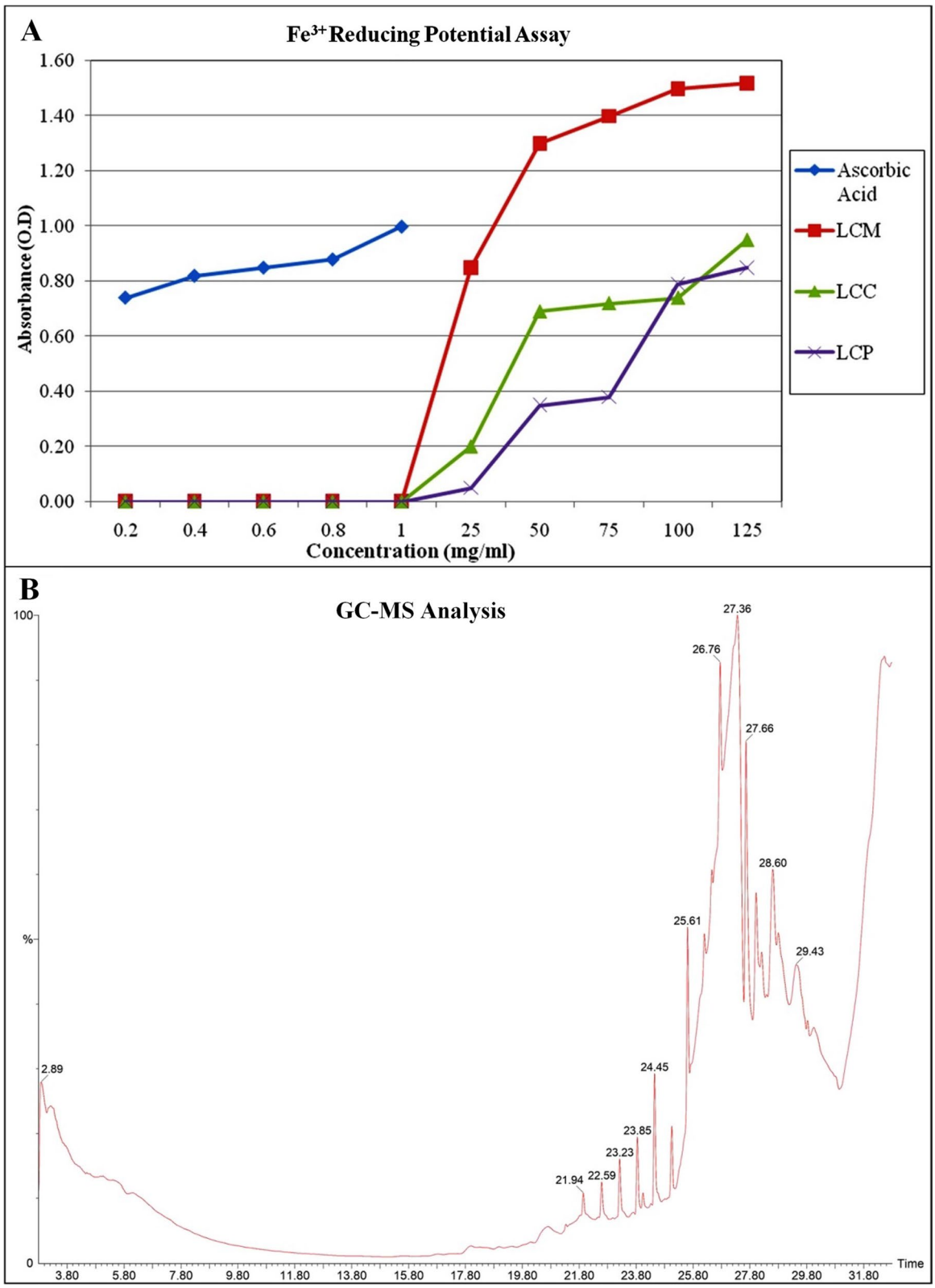

Fig. 1 *Lantana camara* crude extract analysis; **A** Reducing potential assay; **B** gas chromatography mass spectrometry (GC–MS) chromatogram of chloroform extract

Table 1 List of phytochemicals present in *Lantana camara* extracts (✓ = presence)

Phytochemical	Mol. Wgt	LCM	LCC	LCP
Camaric acid	568.8	✓	–	–
Ursolic acid	456.7	–	–	–
Pomolic acid	472.7	✓	–	–
Betulonic acid	454.7	–	✓	–
Betulinic acid	456.7	–	–	–
Lantadene A	552.8	–	✓	✓
Lantadene B	552.8	–	✓	✓
Icterogenin	568.8	✓	–	–
Oleanolic acid	456.7	–	–	–
Katonic acid	456.7	–	–	–
Luteolin	286.24	✓	–	✓
Salicylic acid	138.12	–	✓	–
P-hydroxybenzoic acid	138.12	–	✓	–
P-coumaric acid	164.16	–	–	–
Oleanolic acid	456.7	–	–	–
Beta-curcumene	204.35	✓	✓	✓
(+)-Nuciferol	218.33	–	–	✓
Lantadene C	554.8	–	–	–
Lantadene D	540.8	–	–	–
Lactic acid	470.7	✓	–	–
Theveside	390.34	–	–	–
Theviridoside	404.4	✓	–	–
8-epiloganin	390.4	–	–	–
(*E*)-nuciferal	216.32	–	–	✓
3,7-dimethoxy quercetin	330.29	–	–	–
Verbascoside	624.6	–	–	–
Martynoside	652.6	–	–	–
3-*O*-Methyl quercetin	316.26	–	–	–
Total No. of peaks		24	30	38
No. of known compounds		7	6	6

representation of the XRD phase shift of the precursor and synthesized particles is shown in Fig. 3. The synthesized cobalt particles pattern of phase shift matched with previously reported cobalt hydroxide ($Co(OH)_2$) nanoparticles, as reported by Salman et al. (2014). Three significant 2 theta values along with Miller indices (hkl) that matched with previously reported cobalt hydroxide nanoparticles are 22.85 (*hkl* 100), 34.43 (*hkl* 111) and 48.22 (*hkl* 200), respectively. Based on the observed XRD pattern, it was confirmed that the synthesized cobalt particles are cobalt hydroxide ($Co(OH)_2$) particles.

3.6 Characterization of $Co(OH)_2$ particles

The synthesized cobalt hydroxide particles were subjected to particle size analysis, i.e., dynamic light scattering (DLS), to identify the size of individual particles. Result of the DLS analysis is shown in Fig. 4A, which shows that the average particle size is 180 nm.

Based on the DLS analysis, the particles were identified to be in nanometer scale with an average of 180 nm size, and thus, it was confirmed to be nanoparticle in nature. Hence, the synthesized particles were confirmed to be cobalt hydroxide nanoparticles ($Co(OH)_2$NP) by combined interpretation of XRD and DLS results.

Further, the nanoparticles were analyzed using scanning electron microscope (SEM) to observe the morphology and texture of the nanoparticles. The SEM image of the $Co(OH)_2$NP is shown in Fig. 4B. The particles were observed to be spherical in nature with a feathery texture ranging in the size of ~ 180 nm. Agglomerations were observed where particles were observed to be larger. The morphology of the particles was observed with rough surface (irregular surface) and appeared to be ball of fine threads/fibers/feathers. This feathery, rough surface of the nanoparticles contributes toward increased surface area and thereby would lead to higher chemical reactivity and bioactivity.

3.7 Anticancer cytotoxicity of Co(OH)2NP

Green-synthesized $Co(OH)_2$NP was subjected to in vitro cytotoxicity analysis to investigate its anticancer applications. Cytotoxicity MTT assay was performed against VERO cell line (monkey kidney epithelial cells/non-cancerous cells) and HCT-116 cell line (colorectal carcinoma/cancer cells). VERO cells were used as reference to study against non-cancerous healthy cells, while HCT-116 cells were used to study anticancer cytotoxicity against colon cancer. This comparison would provide an understanding on cytotoxicity of the synthesized $Co(OH)_2$NP on both cancerous and non-cancerous cells, thereby to understand its specificity and toxicity.

$Co(OH)_2$NP was subjected to MTT assay at various dilutions, i.e., double dilutions starting from 400 µg/ml up to 1.625 µg/ml. Results of the MTT assay against the two cell lines and the morphology of the treated cells and control cells are shown in Fig. 5. In VERO cell line, $Co(OH)_2$NP demonstrated an IC_{50} value of 200 µg/ml; however, even at highest concentration of 400 µg/ml $Co(OH)_2$NP demonstrated 44% cell viability suggesting that it does not have strong cytotoxicity against the non-cancerous cells. In HCT-116 cell line, $Co(OH)_2$NP demonstrated an IC_{50} value of 25 µg/ml. However, at the highest concentration of 400 µg/ml $Co(OH)_2$NP demonstrated only 6.10% cell viability, suggesting strong cytotoxic activity against the cancer cells. The IC_{50} value against HCT-116 was 8 times lower than the IC_{50} of the VERO cells. This strongly suggests that the synthesized $Co(OH)_2$NP has strong cytotoxicity selectively against

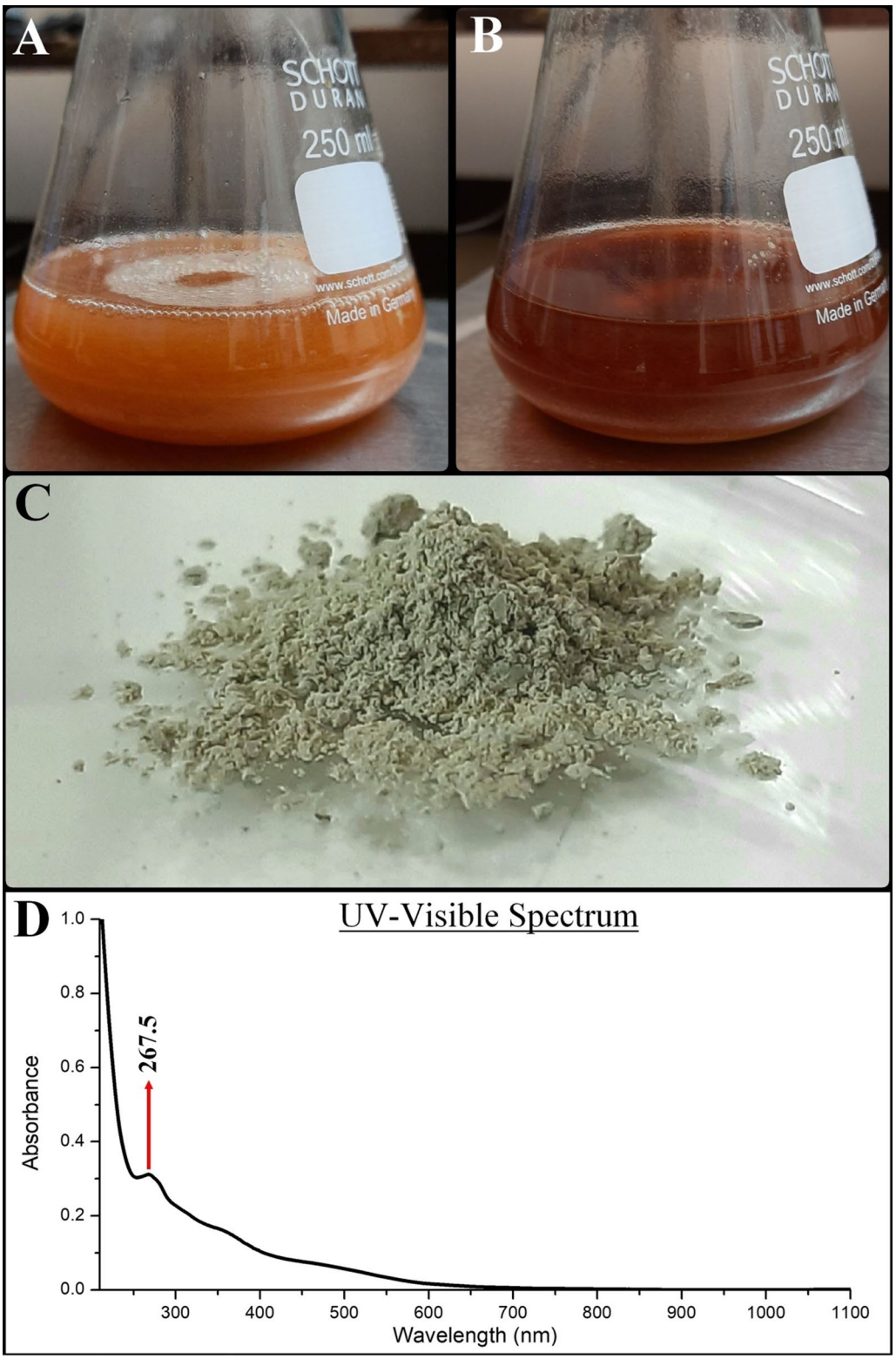

Fig. 2 Green synthesis of cobalt nanoparticles

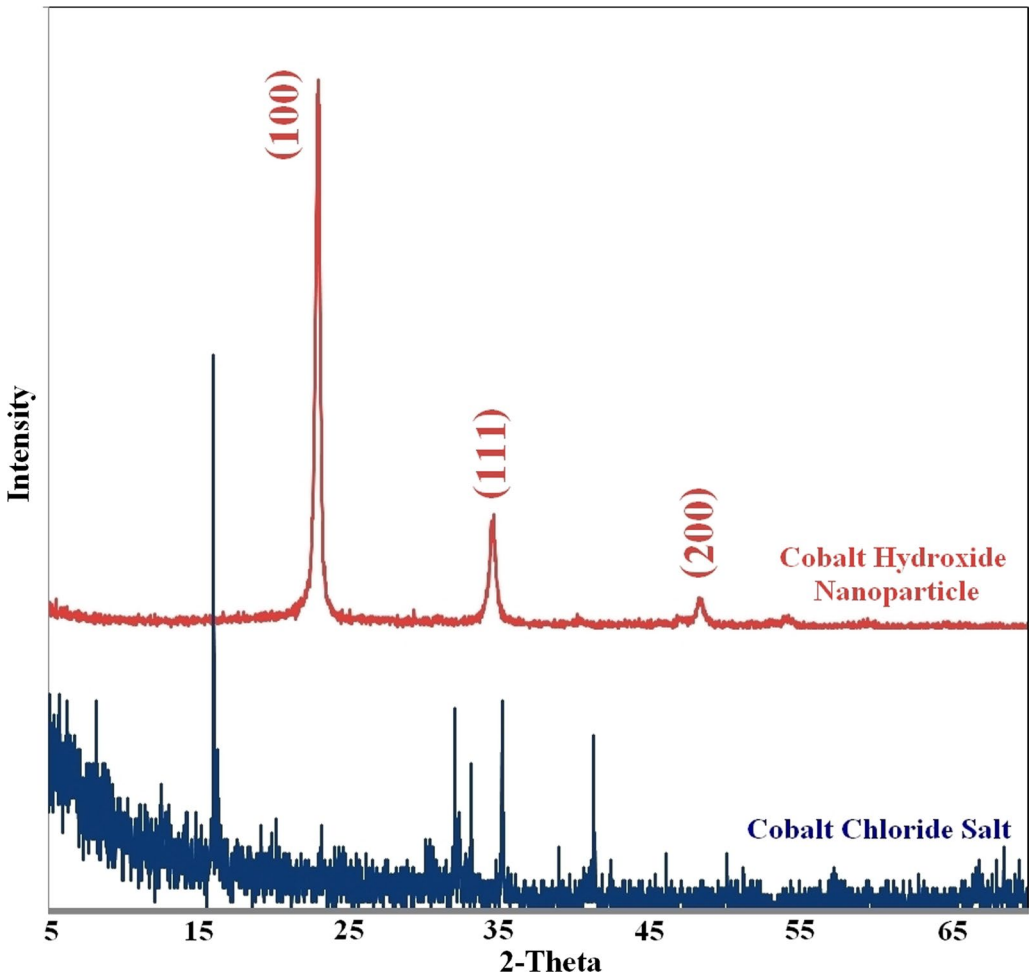

Fig. 3 XRD analysis of green-synthesized $Co(OH)_2$NP and its precursor $CoCl_2$ salt

cancerous cells, demonstrating significant potential as anti-cancerous cytotoxic agent. The eightfold difference in the IC50 value strongly suggests that the synthesized $Co(OH)_2$NP has great potential for pharmaceutical applications, as it has very low impact on the non-cancerous cells.

3.8 Antifungal activity of $Co(OH)_2$NP

Green-synthesized $Co(OH)_2$NP was subjected to in vitro antifungal activity analysis via agar-well diffusion assay to investigate its antifungal applications. The results of the agar-well diffusion assay are summarized in Table 2. Clotrimazole was used as standard reference drug. The $Co(OH)_2$NP demonstrated significant antifungal activity against the tested fungal pathogens (*Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Candida albicans* and *Fusarium oxysporum*). Among the 5 tested fungal pathogens, $Co(OH)_2$NP demonstrated highest antagonism against *A. flavus* and *F. oxysporum* with a ZOI of 10 mm and 18 mm, respectively, at 100 µg/ml concentration (as shown in Fig. 6A, B, respectively). Based on the agar-well diffusion assay, it was confirmed that the synthesized $Co(OH)_2$NP is a potent antifungal agent with significant antagonism against human fungal pathogens.

4 Discussion

The current study reports synthesis of cobalt hydroxide nanoparticles using *Lantana camara* methanol extract (LCM) as a source of reducing agent (antioxidant). *L. camara* is a commonly available weed plant that is usually uprooted and destroyed. *L. camara* is reported to contain significant antioxidant activity that can be exploited in green synthesis reactions such as nanoparticle synthesis. This study aims at providing a commercial value for currently what is considered to be a green

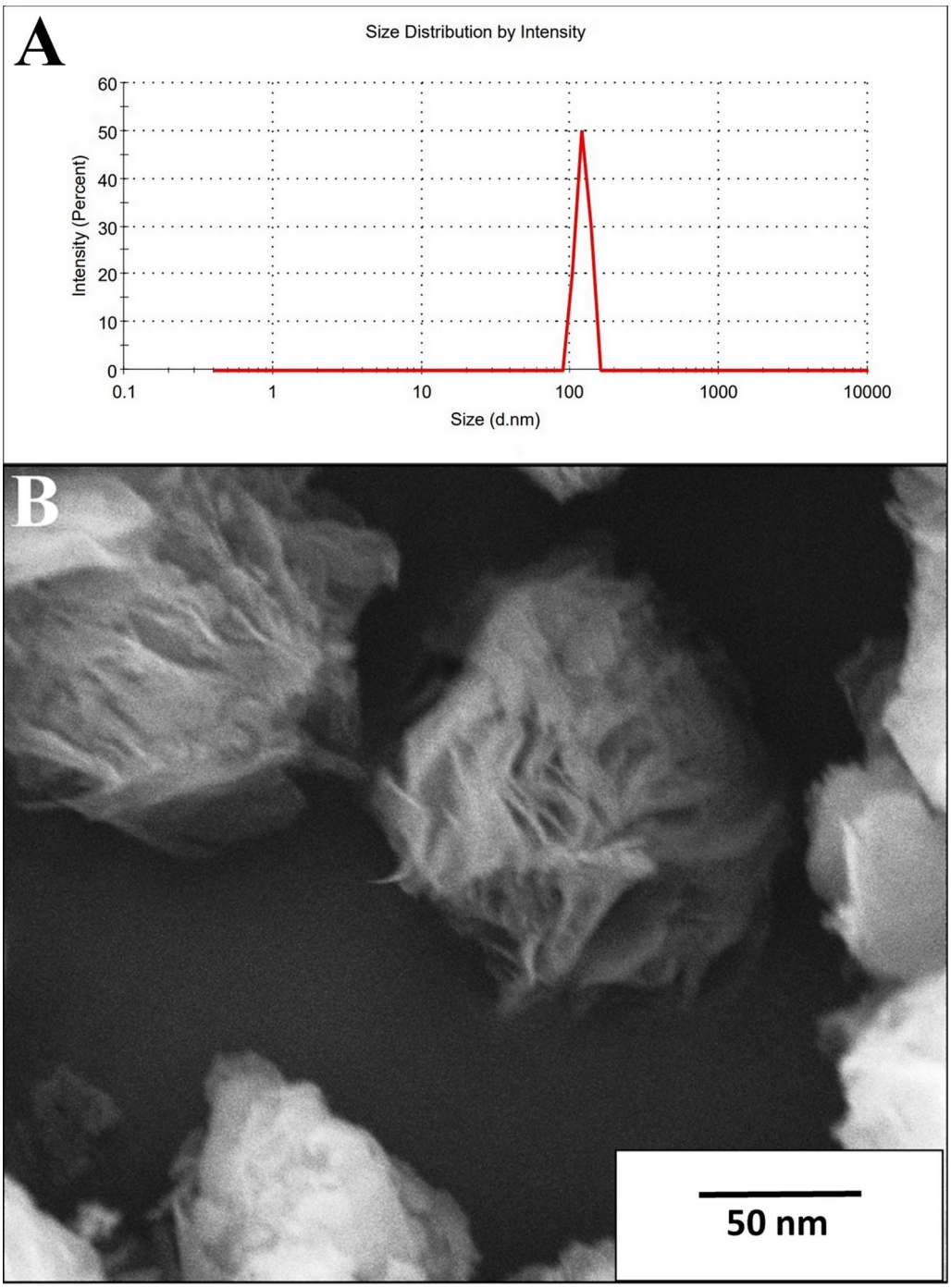

Fig. 4 Cobalt hydroxide nanoparticles $Co(OH)_2$NP characterization; **A** particle size analyzer; **B** scanning electron microscope analysis

waste, i.e., weed plants. *L. camara* is a fast-growing plant and can be cultivated in large quantity in short period of time and space. This study opens up opportunities for use of *L. camara* as source of antioxidant for synthesis of several bioactive nanoparticles such as silver, gold, copper and iron.

The synthesized nanoparticles demonstrated an average of 180 nm size and feathery sphere-like (ball of feather-like) morphology in SEM analysis. The size of the $Co(OH)_2$NPs synthesized in this study is well between the range of already commercially available cobalt nanoparticles that are 80 nm to 200 nm in size as proven by

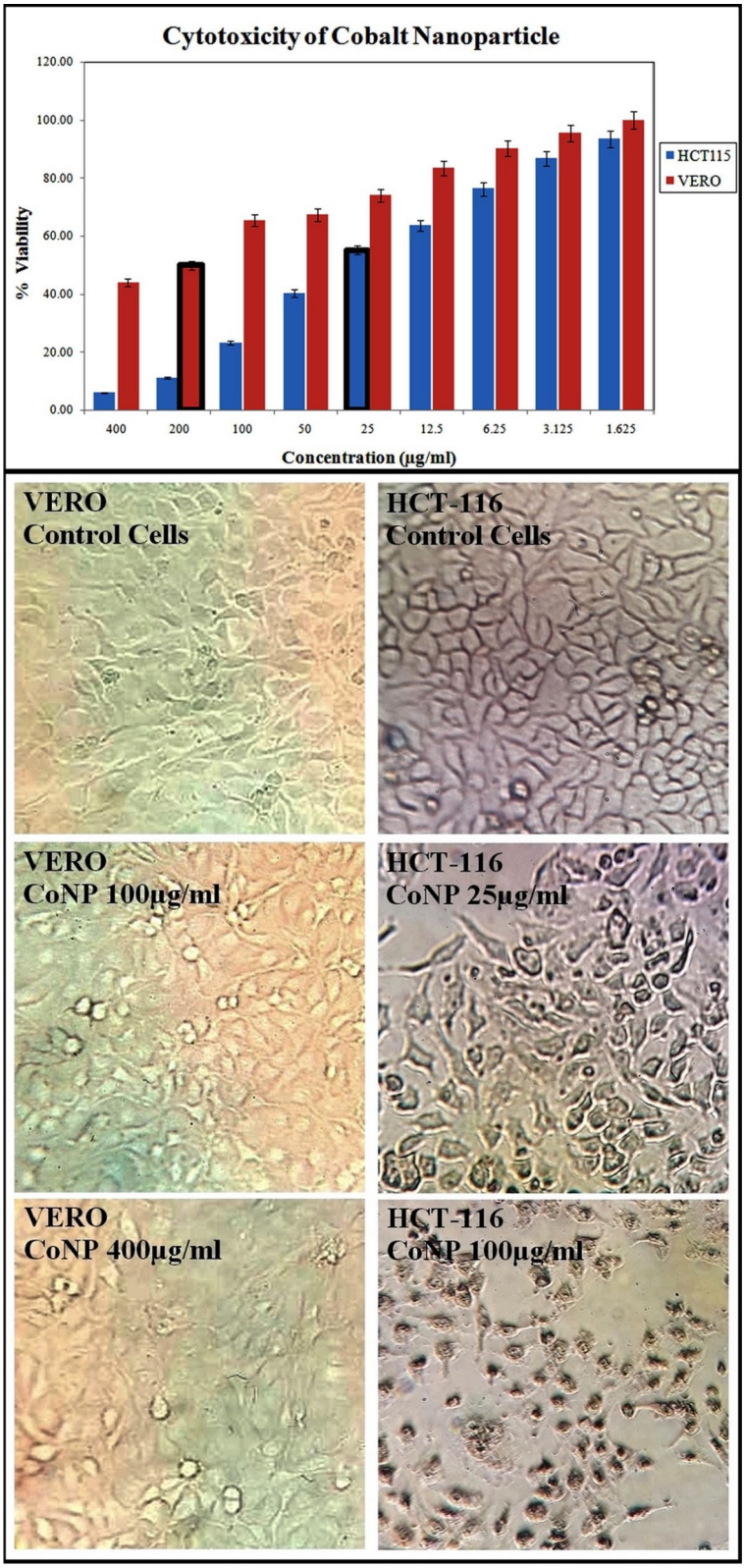

Fig. 5 Cytotoxicity of $Co(OH)_2$NP against VERO cells and HCT-116 cells

Table 2 Antifungal activity of $Co(OH)_2$NP against selected fungal pathogens

Test organisms	CoNP ZOI (mm)				Clotrimazole ZOI (mm)
Concentration	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	30 µg/ml
Aspergillus flavus	–	7	8	10	25
Aspergillus niger	–	–	6	8	27
Aspergillus terreus	–	–	6	7	30
Candida albicans	–	–	8	9	20
Fusarium oxysporum	–	7	12	18	25

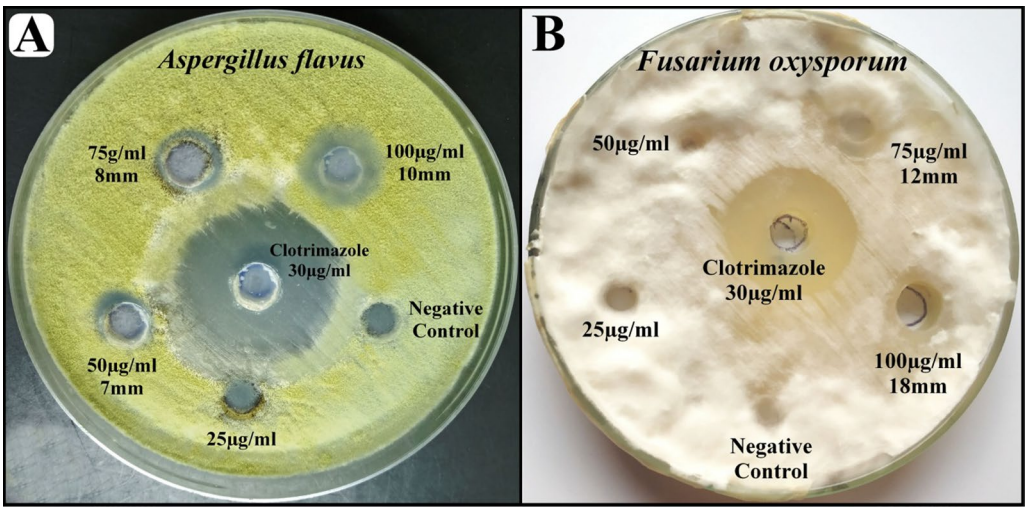

Fig. 6 Antifungal activity of $Co(OH)_2$NP via agar-well diffusion; **A** against *Aspergillus flavus*; **B** against *Fusarium oxysporum*

Kong et al. [23]. Cobalt is one of the few unexploited elements in the nanoparticle synthesis, as this element is difficult to be reduced into a nanoscale structure. This study would be one of the few green synthesis approaches in cobalt nanoparticles adding value to the field.

The synthesized $Co(OH)_2$ nanoparticles demonstrated strong potential against eukaryotic cells, i.e., cancer cells and fungal cells, suggesting the $Co(OH)_2$NP as potent pharmaceutical products.

Similar studies in the recent past have also shown that green-synthesized cobalt nanoparticles possess strong pharmaceutical applications. Cobalt nanoparticles (29.08 nm size) synthesized using the leaves of *Ziziphora clinopodioides* demonstrated strong antibacterial and antifungal activities. Cobalt nanoparticles were antagonistic to *Salmonella typhimurium*, *Streptococcus pneumonia*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and on *Staphylococcus aureus* at a concentration of 2–4 mg/mL and also demonstrated antifungal activity against *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Candida guilliermondii* at a concentration of 1–4 mg/mL. Cobalt nanoparticles can also be used as a cutaneous wound healing agent as it reduced the wound contracture, hydroxyl proline, hexosamine, hexuronic acid, fibrocyte in rat [34].

Cobalt nanoparticles (40 to 200 µm size) from leaf extract of *Cardiospermum halicacabum* demonstrate significant anticancer activity [35]. Cobalt oxide nanoparticles (25 to 35 nm size) synthesized using *Populus ciliata* leaf extract demonstrated strong antibacterial activities against Gram-positive and Gram-negative bacteria [36].

Cobalt nanoparticles (27.42 nm size) green synthesized using *Celosia argentea* whole plant extract demonstrated remarkable DPPH antioxidant potential and antibacterial activities against *Bacillus subtilis* (Gram positive) and *Escherichia coli* (Gram negative) bacteria. NPs were also found to be more biocompatible to red blood cells because of its nontoxic feature [37]. Cobalt oxide nanoparticle (40–80 nm size) synthesized by using *Punica granatum* peel extract demonstrated effective photocatalytic potential [38].

These previous literature evidences strongly suggest that green-synthesized cobalt nanoparticles (CoNPs) possess great potential for application in pharmaceutical industries for various medicinal ailments. The results of the current study provide additional proof for cobalt nanoparticles application in anticancer and antifungal treatment. Further proof for in vivo effectiveness and efficacy is in demand to justify these observations for clinical use.

5 Conclusion

Results of this study conclude that cobalt hydroxide ($Co(OH)_2$) nanoparticles green synthesized using *L. camara* methanol extract have potential applications in pharmaceutical industry as anticancer and antifungal agent. Bioactivity of synthesized $Co(OH)_2$ nanoparticles shows significant antagonism against eukaryotic cells, i.e., human colon cancer cells and human fungal pathogens, without having strong effects on non-cancerous VERO cells. This strongly suggests that the $Co(OH)_2$NPs would not affect normal human cells, when applied in treatment of cancer or fungal infections. Thus, $Co(OH)_2$NPs are a potent nanomedicine in the field of cancer and fungal treatment, with suitable drugability properties. Further in vivo investigations are in demand to justify these results.

Abbreviations

$Co(OH)_2$: Cobalt hydroxide; NPs: Nanoparticles; VERO: Kidney epithelial cells (*Verda reno*); HCT-116: Human colon carcinoma; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; ZOI: Zone of inhibition; LCM: Lantana camara methanol extract; LCC: Lantana camara chloroform extract; LCP: Lantana camara petroleum ether extract; GC-MS: Gas chromatography mass spectroscopy.

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Author contributions

LR provided the basic conception of idea and execution plan for the research study. LR supervised and interpreted the results at all stages of the research work. AS, SK and AO executed the research work under the guidance of LR. AS, SK and AO contributed to preparation of the manuscript. All authors read and approved the final manuscript.

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No known competing interest for this research work.

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