Cancer Nanotechnology

RESEARCH Open Access

Green adeptness in synthesis of non-toxic copper and cobalt oxide nanocomposites with multifaceted bioactivities

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Abstract

Background: In the present era, we are facing different health problems mainly concerning with drug resistance in microorganisms as well as in cancer cells. In addition, we are also facing the problems of controlling oxidative stress and insect originated diseases like dengue, malaria, chikungunya, etc. originated from mosquitoes. In this investigation, we unfurled the potential of *Achatina fulica* mucus in green synthesis of mucus mediated copper oxide bio-nanocomposites (SM-CuONC) and cobalt oxide bio-nanocomposites (SM-Co_3O_4NC). Herein we carried out the physico-chemical characterization like UV–Vis spectra, X-ray diffraction (XRD), Field Emission Scanning Electron Microscopy (FESEM), Transmission electron microscopy (TEM), Energy Dispersive X-ray Analysis (EDAX) and X-ray photoelectron spectroscopy **(**XPS) of as synthesized bio-nanocomposites. Both the bio-nanocomposites were tested for their potential as antimicrobial activity using well diffusion assay, anticancer activity by MTT assay, antioxidant activity by phosphomolybdenum assay and mosquito larvicidal activity.

Results: The results of this study revealed that, SM-CuONC and SM-Co_3O_4NC were synthesized successfully using *A. fulica* mucus. The FESEM and TEM data reveal the formation of nanoparticles with quasi-spherical morphology and average particle size of ~ 18 nm for both nanocomposites. The EDAX peak confirms the presence of elemental copper and cobalt in the analyzed samples. The X-ray diffraction analysis confirmed the crystalline nature of the CuO and Co_3O_4. The result of anti microbial study exhibited that, SM-CuONC showed maximum antimicrobial activity against *Escherichia coli NCIM 2065* and *Aspergillus fumigatus NCIM 902* which were noted as 2.36 ± 0.31 and 2.36 ± 0.59 cm resp. at 60 µg/well concentration. The result of anticancer activity for SM-CuONC was exhibited as, 68.66 ± 3.72, 62.66 ± 3.61 and 71.00 ± 2.36 percent kill, while SM-Co_3O_4NC exhibited 61.00 ± 3.57, 72.66 ± 4.50 and 71.66 ± 4.22 percent kill against Human colon cancer (HCT-15), Cervical cancer (HeLa), and Breast cancer (MDA-MB-231) cell lines, respectively, at 20 µg/well concentration. Both the nanocomposites also exhibited better antioxidant activity. Total antioxidant activity for SM-CuONC at 50 µg/ml concentration was found to be highest as 55.33 ± 3.72 while that of SM-Co_3O_4Ns was 52.00 ± 3.22 mM of ascorbic acid/µg respectively. Both bio-nanocomposites also exhibited 100% mosquito larvicidal activity at concentration ranging from 40 to 50 mg/l. During cytotoxicity study it is noted that at 5 µg/well

concentration, SM-CuO and SM-Co_3O_4NCs suspension showed more than 97% viability of normal (L929) cell lines. We also studied phytotoxicity of both bio-nanocomposites on *Triticum aestivum*. In this study, 100% seed germination was observed when seeds are treated with SM-CuONC and SM-Co_3O_4NC at 500 mg/l and 250 mg/l concentration respectively.

Conclusions: This study concludes that in future as synthesized SM-CuONC and SM-Co_3O_4NC can be used in pharmaceutical, health care system for betterment and welfare of human life as both bio-nanocomposites exhibits better antimicrobial, anticancer, antioxidant and mosquito larvicidal potential.

Keywords: *Achatina fulica* mucus, Bio-nanocomposites, Characterization, Biological activities, Toxicity

Introduction

Nowadays, nanotechnology is rapidly growing research topic in which nanoparticles specifically metal oxide nanoparticles are widely used in different areas like biological, pharmaceutical, agricultural, environmental, electronics, etc. (Faisal et al. 2021). Due to the diverse applications of nanoparticles, several methods are used for their synthesis including chemical, physical, sol–gel, electrodeposition, etc. (Cuong et al. 2022). Unfortunately, these methods of nanoparticle synthesis are costly, require toxic chemicals and high temperature. In addition, these methods of nanoparticle synthesis lead to production of hazardous by-products. Hence to overcome these problems most recently, green nanotechnology is emerged as a new approach of nanoparticle synthesis and miraculously applied in the fields of biotechnology, health care, cosmetics, food technology, environmental remediation, etc. (Faisal et al. 2022; Dabhane et al. 2023).

There are plethora of reasons why scientists are taking plenty of endeavours on green nanotechnology, rather than physical and chemical methods of nanoparticle synthesis. Environmental concern and energy issues are the main reasons that motivated development of green nanotechnology for synthesis of nanoparticles (Pansambal et al. 2022). An ever-increasing body of literature shows that innovative material and nanotechnology-based products became predominant part of our life. More than 1814 nanoparticle-based products are manufactured till today. Advancement in the field of nanotechnology attracted several researchers, scientists and last few years have witnessed a huge growth in this field. Recently most of the researchers working in the field of nanoscience are influenced by "Green Nanotechnology" (GN) (Griffin et al. 2018; Biswas et al. 2012; Das et al. 2017). In green nanotechnology, there are several studies consistent with using different types of biological material mainly including plants, various plant products like oils and phytochemicals where alkaloids, flavonoids, steroids, phenols, tannins play an important role in reduction and stabilization of nanoparticles. In addition, microorganisms, algae, etc. are also used for the green synthesis of nanoparticles (Gu et al. 2018; Pagar et al. 2019).

Hitherto animal waste products or animal-based products are rarely used in green nanotechnology. Unfortunately, till today not much work was done on synthesis of nanoparticles using animal by-products and their applications in health care or medicinal purposes. In synthesis of nanoparticles using animal-based materials or their metabolites have great opportunity as it contains plenty of biomolecules including proteins and

amino acids which acts as capping and reducing agents. The most important advantage of using animal by-products over plants and their products, microorganisms, algae for nanoparticle synthesis is that, it does not disturbs environment mainly forest ecosystem which happens in case of plants. Secondly, it is economically feasible because in case of microorganisms and algae their mass culturing is required which increases cost of products. Precaution must be taken to avoid any contamination during synthesis of nanoparticles using animal-based by-products as animals may contains dusts, micro-organisms, etc. Till today silver nanoparticles are mostly synthesized using animal products including cobweb, paper wasp net, cockroach wings, honey, cow milk, hen's egg white, etc. These animal product-based nanoparticles are used for different biological applications like antibacterial, antifungal, insecticidal, anticoagulant, thrombolytic and catalytic activities. (Lateef et al. 2016a; Lateef et al. 2016b; Khatami et al. 2019; Balasooriya et al. 2017; Lee et al. 2013; Athreya et al. 2019).

As far as metal oxide nanoparticles are considered, it was observed that copper oxide and cobalt oxide nanoparticles attracted increasing attention (Chattopadhyay et al. 2012a, b). Copper and cobalt are trace metals closely related to human health. Copper is important part of several enzymes like super oxide dismutase and also plays a role to activate the enzymes while cobalt is of paramount concern in metabolic processes of all the animals (Abdullah et al. 2022; Siddiqi et al. 2020; Huang et al. 2021). Due to excellent biological, chemical and pharmaceutical properties of copper and cobalt it was felt that copper oxide and cobalt oxide nanoparticles can be applied to encounter multiple health problems including antimicrobial potential, larvicidal, antioxidant, etc. (Nagore et al. 2021; Kainat et al. 2021).

Recently, the entire world is facing the problem of multidrug resistant bacterial diseases which created sinister situation and led thousands of deaths annually especially patients in Intensive Care Units (Palmer and Kishony 2013).With antibiotics resistance, we are now facing another health issue of increase in cancer cases which is the worldwide leading cause of death. For the treatment of cancer, radiotherapy, surgery, chemotherapy is generally used but these methods are expensive as well as lead to several side effects. Recently, it was felt that nanoparticles can interact with biomolecules hence they can be used in cancer treatment (Liu et al. 2010).

Moreover, to maintain good health for prevention of diseases due to the production of reactive oxygen species one can require antioxidants which can be fulfilled by dietary supplements. But in the fast moving world due to busy schedule, sedentary lifestyle, high intake of carbohydrate, fat and proteins changed the human life resulting in the production of reactive oxygen species. With the dietary supplements, synthetic antioxidants are also available in the market but unfortunately they are carcinogenic and toxic in nature. To overcome all the shortfalls, material science and nanoscience emerged as a ray of hope for the production of nano-antioxidants (Eftekhari et al. 2017, 2018). To achieve the better solution and to overcome the challenges of antioxidants, some researchers are focusing on production of antioxidant functionalized nanocomposites using biological materials. In this concern, cobalt oxide and copper oxide nanoparticles are gaining more importance (Waris et al. 2021).

In some countries, another health-related problem is controlling the disease spreading insects which become resistant to most of the insecticides. In this concern control of

mosquitoes which are the vectors for diseases like malaria, chikungunya, filariasis, dengue, etc. became the subject of prime importance (Mane et al. 2017). It was estimated that 350 million people are at risk of mosquito-borne diseases. Earlier, mosquito-spread Zika virus which causes dengue like symptoms was mostly detected in Asia and Africa. More importantly the death rates due to the mosquito-borne diseases are continuously increasing day by day and is of serious concern to humans (WHO 2010; WHO 2013). More importantly, with the diverse array of nanotechnology in various disciplines like healthcare, chemical and pharmaceutical, one should not forget its adverse effects on aquatic, terrestrial and agricultural ecosystems (Phull et al. 2021).

The literature survey regarding synthesis of nanoparticles using animal by-products lacks sufficient data and very little data are available about pharmaceutical, mosquito larvicidal activities and toxicity of copper and cobalt nanoparticles. Hence, keeping in mind all the above mentioned research gaps related to nanoparticle synthesis and their applications in human health, this investigation puts an emphasis on the aspects of green nanotechnology with different biological activities. Moreover, both the bio-nanocomposites of this study are synthesized with simple, facile, eco-friendly method. These bio-nanocomposites are non-toxic in nature and exhibit multifaceted biological activities including antimicrobial, anticancer, antioxidant and mosquito larvicidal. Most importantly as per our intense literature survey, this is the first report of synthesis of SM-CuONC and SM-Co_3O_4NC using *A. fulica* snail mucus having multiple biological activities which adds novelty to the work.

In this paper, we report the green synthesis of SM-CuONC and SM-Co_3O_4NC using an ingenious material, mucus of *A. fulica*. Additionally, we are also reporting the indisputable potential of as synthesized SM-CuONC and SM-Co_3O_4NC for antimicrobial and anticancer drug development. We also studied the antioxidant and phytotoxicity of *A. fulica* mucus mediated SM-CuONC and SM-Co_3O_4NC. With all above-mentioned applications, we also unfurled the various disease causing mosquito larvicidal activity of both the bio-nanocomposites.

Materials and methods

Collection of snails and mucus extraction

Healthy snails with average weight of 25–30 gm were collected from the college campus, agricultural field and gardens near Junnar tehsil of Maharashtra State, India by hand picking method during monsoon season and were used for this study. The snails were kept in wooden boxes (50 cm width × 30 cm height), containing 30 snails each. The boxes were sprinkled daily with water to maintain humidity and fed with leaves of *Lactuca sativa*. Mucus was collected from 50 healthy individuals, the collected mucus was stored. Before collection of the mucus, snails were starved for 3 days and mucus around 3 ml/snail was collected by stimulating the pedal glands with the fingers in laminar air flow to avoid any contamination. Moreover during collection of mucus latex gloves were used as protection and much caution was taken to avoid the mixing of fecal matter with mucus. The shells of snails were also cleaned with double distilled water to avoid entry of dust or soil particles if present on shell. Afterwards the mucus samples were stored at −20 °C until being used for further experiments (Sallam et al. 2009). After collecting the mucus, the snails were freed back to their original natural habitat.

Synthesis of copper and cobalt oxide bio-nanocomposites

The bio-nanocomposites of copper and cobalt oxides were synthesized within mucus matrix of *A. fulica*. In a typical procedure, 0.2 M copper sulphate and 0.2 M cobalt nitrate was mixed separately with diluted (1:9 v/v) 100 ml of *A. fulica* mucus and the reaction mixture is incubated at room temperature with constant stirring for 1 h. After completion of incubation period, the pH of both reaction mixtures was adjusted to 9 using 0.1 M NaOH and continued stirring for another 1 h. The reaction mixtures containing SM-CuO and SM-Co_3O_4 NCs were separated by centrifugation at 10,000 rpm for 10 min. The obtained NCs were washed thrice with double distilled water and dried at 90 °C (Rangel et al. 2020).

Physico-chemical characterization of the resultant SM-CuO and SM-Co_3O_4NCs

For UV–Visible spectroscopy study, mucus of *A. fulica* and as synthesized SM-CuO and SM-Co_3O_4 NCs were separately suspended in sterile distilled water. The spectra were recorded using UV-visible–NIR spectrophotometer (JASCO V-770) in the wavelength range of 200–400 nm against distilled water as base line solution using optiglass cuvette with path length of 10 mm and bandwidth of 1.0 nm. A PANalytical X'PERT PRO equipment was used to capture the powder X-ray diffraction (XRD) pattern of the synthesized material in the 2θ range of 20°—80° with iron-filtered Cu K_α radiation ($\lambda = 1.54$ Å) and step size of 0.013°. The powdered samples were spread on the glass slide used to record the spectra. The particle size was examined by Field Emission Scanning Electron Microscopy (FESEM: Hitachi S-4200). The material's elemental composition was also determined using Energy Dispersive X-ray Analysis (EDAX). The synthesized powdered samples were dispersed in ethanol and then drop-casted on a silicon wafer to capture the images using FESEM. An accelerating voltage of 200 keV with a Schottky field emitter source with maximum beam current (> 100 nA) and small energy spread (0.8 eV or less) was utilized to assess the morphology and particle size using a Transmission Electron Microscope (TEM) (FEI Tecnai T20). The powder samples obtained were dispersed in ethanol and then drop-casted on carbon-coated copper TEM grids with 200 mesh and loaded into a single tilt sample holder. Thermo Fisher Scientific's X-ray Photoelectron Spectrometer (XPS) K-Alpha + was used to record the X-ray photoelectron spectra for Cu, and O. The monochromatic Al K_α ($h\nu = 1486.6$ eV) was used as the X-ray source was operated in an ultra-high vacuum system with a base pressure of 5 X 10^{-9} Torr, a beam current of 6 mA, and a potential of 12 kV coupled with a Physical Electronics 04–548 dual Mg/Al anode. The XPS measurement made use of a 400 μm spot size. The pressed pallets of the synthesized samples were used to record the XPS spectra and XPS PEAK 4.123 was used to deconvolute the captured XPS data. Information about the functional group bonding between SM-CuO and SM-Co_3O_4NCs with the snail mucus matrix was obtained using Fourier Transform Infra-Red (FTIR) spectroscopy (JASCO FT/IR-6100 FTIR spectrophotometer) recorded in the attenuated total reflection (ATR) mode with an ATR Pro One unit for the powder sample. For XRD and XPS investigations, dried as-prepared powder samples were directly used without any further processing.

Microorganisms and growth conditions

Bacterial strains viz*., Escherichia coli NCIM 2065, Staphylococcus aureus NCIM 5021, Klebsiella pneumoniae NCIM 2957* and *Pseudomonas aeruginosa ATCC 9027* and one fungi *Aspergillus fumigatus* NCIM 902 were used. The pure cultures of all the strains used for study were obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory (NCL), Pune. All four species of bacterial strains were maintained in nutrient agar at 37 ^{0}C and fungi was maintained on potato dextrose agar at 28 ^{0}C.

Antimicrobial activity and MIC/MBC of SM-CuO and SM-Co_3O_4NCs

The antimicrobial activity of SM-CuO and SM-Co_3O_4NCs was ascertained by the agar well diffusion method using nutrient agar (NA) medium. The bacterial inoculum was prepared separately from the 24 h old culture on nutrient agar medium. The final concentration of bacterial inoculum was adjusted to approximately 10^6 CFU/ml. Dispersion of SM-CuO and SM-Co_3O_4 NCs was added in the well of the test media which were previously inoculated with each test micro-organisms. The zones of inhibition were measured after 24 h of incubation at 37 ^{0}C. Minimum Inhibitory Concentration (MIC) was determined by serially diluting SM-CuO and SM-Co_3O_4 NCs with concentration range of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml. All assays were carried out in triplicate and sterilized distilled water was used as a control test. The minimum bactericidal concentration (MBC) / minimum fungicidal concentration (MFC) of SM-CuO and SM-Co_3O_4NCs was determined by sub-culturing the samples from tubes of the MIC assay on freshly prepared nutrient agar plates or potato dextrose agar plates, and incubating at 37 °C or 28 °C for 48 h. (Chin et al. 2008).

Growth curve study

To study the growth curves, bacterial cell concentration of 10^6 CFU/ml was adjusted in Muller-Hinton Broth, then exposed to SM-CuO and SM-Co_3O_4NCs at different concentrations (1/2 MIC, and MIC). Each culture was incubated in a shaking incubator at 37 ^{0}C for 24 h. Growth curves of bacterial cell cultures were attained through repeated measurements of the optical density (OD) at 600 nm after every 2 h and growth curve of OD against time was plotted using Origin 8. (Chin et al. 2008).

Cell membrane leakage

This phenomenon was experimentally investigated using fixed concentration, i.e., MIC of SM-CuO and SM-Co_3O_4NCs. For harvesting cells, fifty milliliters of exponentially growing cells were centrifuged and suspended in the sodium phosphate buffer (50 mM, pH 7.0). These cells were exposed to the test containing functionalized bio-nanocomposites separately for 90 min. while control set lacks SM-CuO and SM-Co_3O_4NCs. The method of Heipieper was followed to determine leakage of cellular material which absorbs at UV_{260} and UV_{280}. An aliquot of 2 ml was withdrawn from the test and control samples with interval of 15 min and optical density were measured using UV-VIS–NIR (Jasco V – 770) spectrophotometer at 260 and 280 nm. The experiments were repeated thrice (Heipieper et al. 1992).

Cell line and culture

Human colon cancer (HCT-15), Cervical cancer (HeLa), Breast cancer (MDA-MB-231), and normal (L929) cell lines were purchased from National Centre for Cell Sciences (NCCS). All the cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 50 U/ml penicillin/streptomycin and 2 mM L-glutamine. The cells were plated in 96-well plates at a density 1×10^4 cells/well and allowed to adhere at 37 °C with 5% CO_2 for 24 h. Cultures were examined using an inverted microscope to evaluate the quality of confluence and confirming absence of bacterial and fungal contaminants.

Anticancer activity

The culture medium containing 10% FBS are aspirated from plate and cells were treated with four different concentrations i.e. 5, 10, 15 and 20 µg/well of SM-CuO and SM-Co_3O_4NCs separately in triplicate. The highest concentration used is 100% of test sample along with positive control, vehicle control and control (blank) which is culture medium in triplicate. The treated and untreated cells were incubated for 24 ± 2 h. at 37 °C with 5% CO_2. After 24 h. treated culture vessel is examined under inverted microscope. After examination culture medium was removed carefully from culture vessel. About 10 µl of MTT solution and 90 µl DMEM culture medium solution are added in culture vessel and incubated for 2–4 h. at 37 °C with 5% CO_2. The solution is decanted and MTT solution is added in culture vessel. The culture vessel is then swayed and absorbance is recorded at 492 nm (Riss et al. 2016).

Total antioxidant activity

For total antioxidant activity assay, the reaction mixture contains 0.1 ml test samples like snail mucus or $CuSO_4$ or $CoNO_3$ or SM- CuO NCs or SM-Co_3O_4NCs with different concentrations (10–50 µg/ml) and 1 ml of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate in a eppendorf tube. The capped tubes were incubated in hot air oven at 95 °C for 90 min. After cooling to room temperature, the absorbance of test solution was measured at 695 nm against blank. Ascorbic acid was used as standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid. The experiment was carried out in triplicate (Prieto et al. 1999).

Mosquito larvicidal activity

For this experiment, *Aedes aegypti* was breed in the laboratory to obtain larvae of uniform and particular age. To carry out the experiment, ten larvae (1st to 4th instar and pupae) were placed in dechlorinated water and different concentrations (10–50 mg/l) of SM-CuONC and SM-Co_3O_4NC were mixed separately with 0.5 mg larval food for each test (WHO 1996; Santhoshkumar et al. 2010). The larvicidal activity of both bio-nanocomposites against each instar and the pupae were replicated thrice in a glass beaker. In each case, the control experiment consists of 10 larvae/pupae in distilled water. The number of dead larvae/pupae are considered as larvicidal activity of bio-nanocomposites and control mortality was corrected using Abbott's formula and average percentage

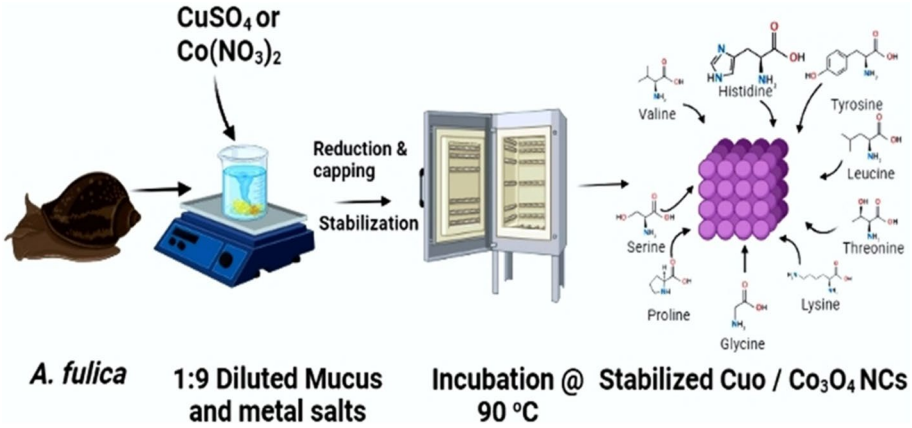

Fig. 1 Schematic representation of bio-nanocomposite synthesis

mortality was calculated (Abbott 1925). The standard error bars are also given in the graphs.

Phytotoxicity study

For phytotoxicity study, seeds of *Triticum aestivum* were treated with 1% sodium hydrochloride and allowed to germinate separately in the presence of 250, 500, 750 and 1000 mg/l of SM-CuONC and SM-Co_3O_4NC, tap water as control and also with solution of copper sulphate and cobalt nitrate (500 mg/l). The treated seeds were then transferred on a filter paper in the petri dishes, (with 10 seeds per dish) and further subjected to incubation for seven days and subsequently placed in a growth chamber maintained at 25 °C with 12 h light and 12 h dark period. Each experiment was replicated thrice. After incubation period number of seeds showing sprouts are considered as germinated seeds and were counted.

Results and discussion

Synthesis of nanoparticles from animal products

In the present research, we synthesized SM-CuO and SM-Co_3O_4NCs using *A. fulica* mucus. There are several reports on use of plant metabolites, microorganisms, etc. to synthesize the nanoparticles. But use of these materials required efforts such as culturing, harvesting, extraction etc. and also leads to extinction of plant species (Griffin et al. 2018). Aforementioned approaches require energy which automatically increases cost of the process. To overcome these shortfalls, alternative strategies need to be developed. In this concern, use of animal derived material is the promising solution. Unfortunately there are very few reports of using animal derived material for synthesis of nanoparticles as compared to plant and microbial materials.

In literature, it was noted that bee honey was used for the synthesis of gold, silver, copper oxide nanoparticles (Philip 2009). Surprisingly, as per our literature survey there is not a single report on synthesis of copper oxide and cobalt oxide using *A. fulica* mucus. The schematic representation of mechanism of bio-nanocomposite synthesis is represented in Fig. 1. In this investigation, for the synthesis of SM-CuO and SM-Co_3O_4NCs diluted *A. fulica* mucus is only used and no any extraction process for bio-molecules is

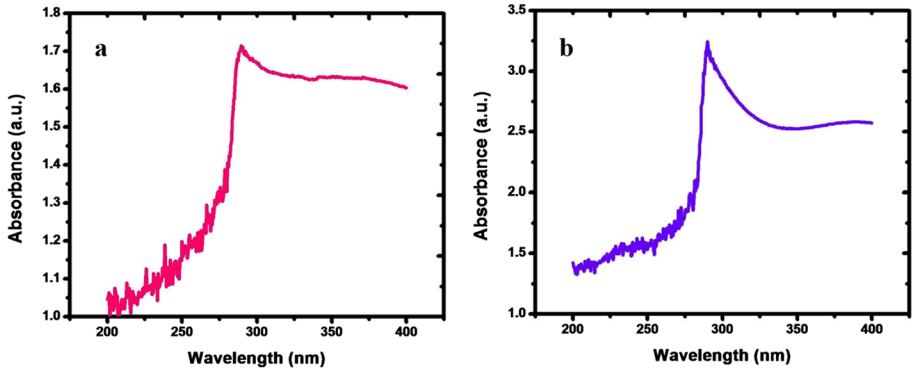

Fig. 2 UV–Vis Spectrum of as synthesized **a** SM-CuO and **b** SM-Co_3O_4NCs

required. Surprisingly, single nanocomposite systems exhibited many biological activities like antimicrobial, antioxidant, mosquito larvicidal, etc. Furthermore, both the bio-nanocomposites exhibited anti-cancer activities against three human cancer lines without showing toxicity against normal cell lines.

Characterization of SM-CuO and SM-Co_3O_4NCs

The mucus of *A. fulica* contains number of proteins, amino acids, carbohydrates, etc. These bio-chemical compounds act as an encapsulating agent and reduce copper and cobalt hydroxide which are generated after reaction between metal precursor with hydroxyl anion generated by water to form copper oxide and cobalt oxide nanocomposites (Nagajyothi et al. 2017). In our previous study, the mucus of *A. fulica* was used for synthesis of silver nanocomposites (Mane et al. 2021).

For confirmation of green synthesized nanoparticles, UV-visible spectroscopy is the perfect tool. In synthesis of green nanoparticles, the preliminary indication of nanoparticles synthesis is the colour change due to the reaction (Siddiquee et al. 2021). In the present investigation, formation of copper oxide bio-nanocomposites was confirmed by change in colour of the reaction mixture from ocean green to blackish brown and for cobalt oxide bio-nanocomposites the colour change was from light brown to dark brown after 2 h of reaction due to surface plasmon resonance phenomenon.

In Fig. 2a, b it was observed that, the absorption spectra of the reaction mixture for copper oxide exhibit the distinct peak at 280 nm and for cobalt oxide it was at 307 nm which is nearer to the other studies for green synthesis of copper and cobalt oxide nanoparticles (Koshy et al. 2011; Farhadi et al. 2016).

X-ray diffraction (XRD)

As seen in Fig. 3a, b, the X-ray diffraction analysis was done to establish the crystalline nature of the CuO and Co_3O_4. CuO peak observations closely matched the JCPDS card number (JCPDS No. 80-0076) in the XRD pattern. The (hkl) values of Bragg's reflections of the CuO structure were determined to be (110), (-111), (111), (-202), (020), (202), (-113), (-311), and (113) at positions of 32.5°, 35.6°, 38.6°, 48.9°, 53.4°, 58°, 61.4°, 66.3°, 68.1°, 72.2°, and 75.3°. CuO's recorded XRD pattern showed no further impurities, indicating a pure phase. At the same time, the XRD pattern of Co_3O_4 shows the peaks at 2θ

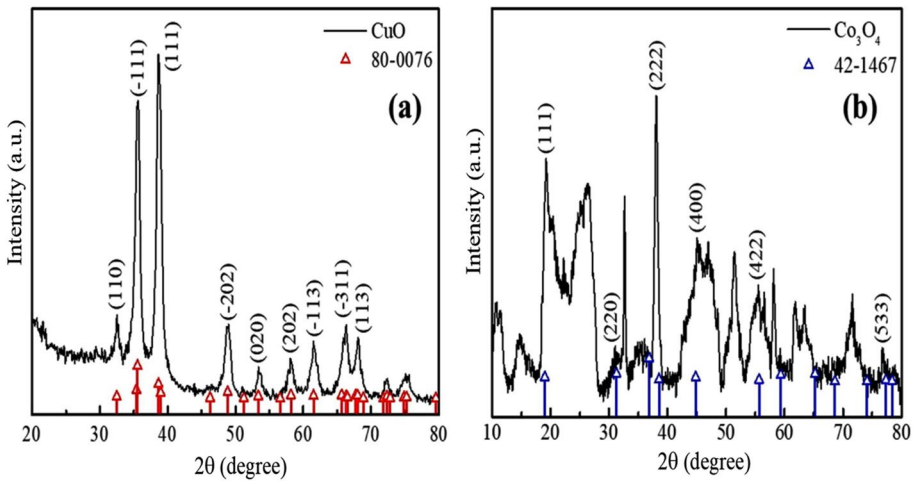

Fig. 3 XRD pattern of **a** CuO matched with the reported JCPDS data [JCPDS No. 80-0076], and **b** Co_3O_4 matched with the reported JCPDS data [JCPDS No. 42-1467]

positions of 19.3°, 30.9°, 38°, 45°, 55.4°, and 76.8° related to the (hkl) values of Bragg's reflections of Co_3O_4 of (111), (220), (222), (400), (422), and (533). The remaining peaks not matched with the JCPDS file in the Co_3O_4 are matched with the bioentity. The sharp peaks in both compounds confirm the crystalline nature of the samples.

Field Emission Scanning Electron Microscopy (FESEM)

FESEM images show the quasi-spherical morphology of the synthesized CuO and Co_3O_4 nanocomposites. The FESEM images were recorded at 1 μm and 500 nm scale to get the particle size, as shown in Fig. 4a, b, d and e. The Co_3O_4 nanocomposites show the flake's morphology made up of a combination of small quasi-spherical particles. At the same time, Fig. 4c, f shows the histogram for particle size calculation. The particle histogram was calculated by taking in accordance of 30 particles. The synthesized CuO and Co_3O_4's average particle size was ~ 18 nm for both nanocomposites. The particle size of the synthesized samples confirms the particle in nm scale i.e., nanoparticles. Figure 5a, b and c shows the EDAX mapping for the Cu and O elements present in CuO nanocomposites. Figure 5d, e and f reveals the presence of Co and O in Co_3O_4 nanocomposites. The EDAX mapping confirms the presence of Cu, Co, and O on the surface of synthesized CuO and Co_3O_4 samples.

Transmission electron microscopy (TEM)

As shown in Fig. 6a, b, c and d, TEM was used to study the morphology of CuO and Co_3O_4 nanocomposites. In some places, nanorods with a quasi-spherical-like shape were seen for the CuO. However, for the Co_3O_4, flakes along with the small particles appeared in the morphology. The FESEM and TEM show the same morphology for both composites. The morphology gives the nanoscale particles confirming the nanoparticle synthesis of samples. TEM images were recorded at 100 nm and 20 nm scales to depict the shape and size of the synthesized samples. Thus the claim of nanoparticle synthesis can be proved from both microscopy images.

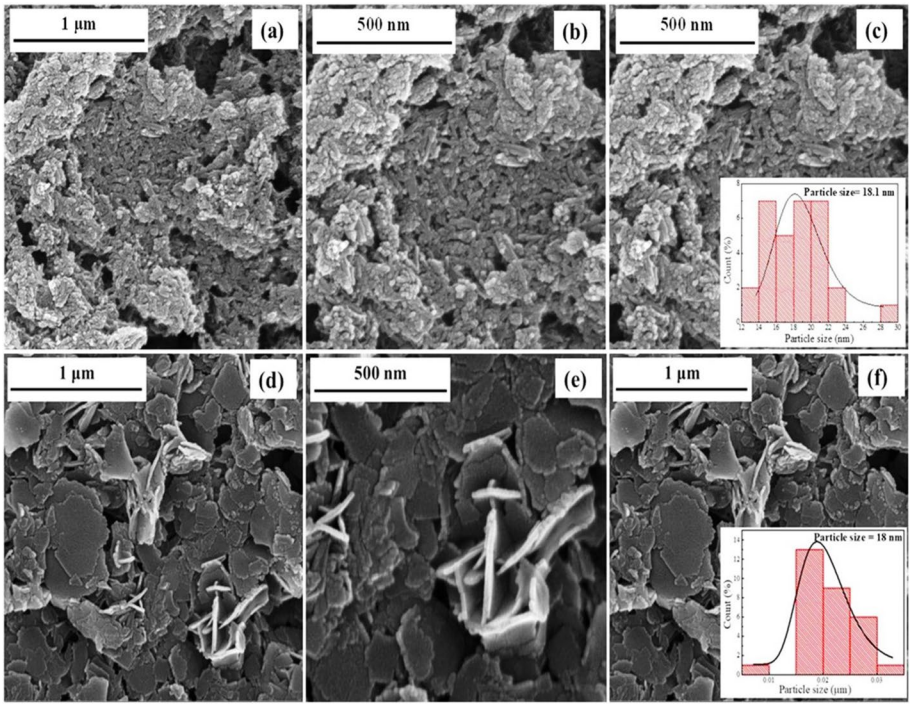

Fig. 4 Field Emission Scanning Electron Microscopy images of **a**, **b** CuO nanocomposites and **d**, **e** Co_3O_4 nanocomposites. The histogram shows the average particle size of **c** the CuO to ~ 18.1 nm and **f** for Co_3O_4 also ~ 18 nm

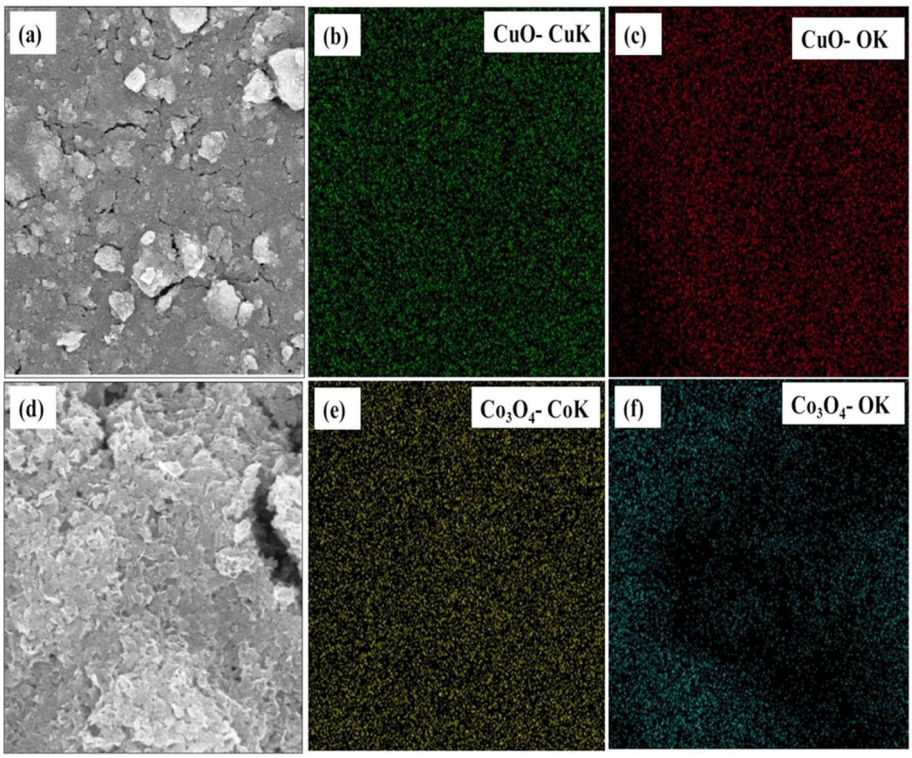

Fig. 5 EDAX mapping for the elements present in **a**–**c** CuO and **d**–**f** Co_3O_4 nanocomposites

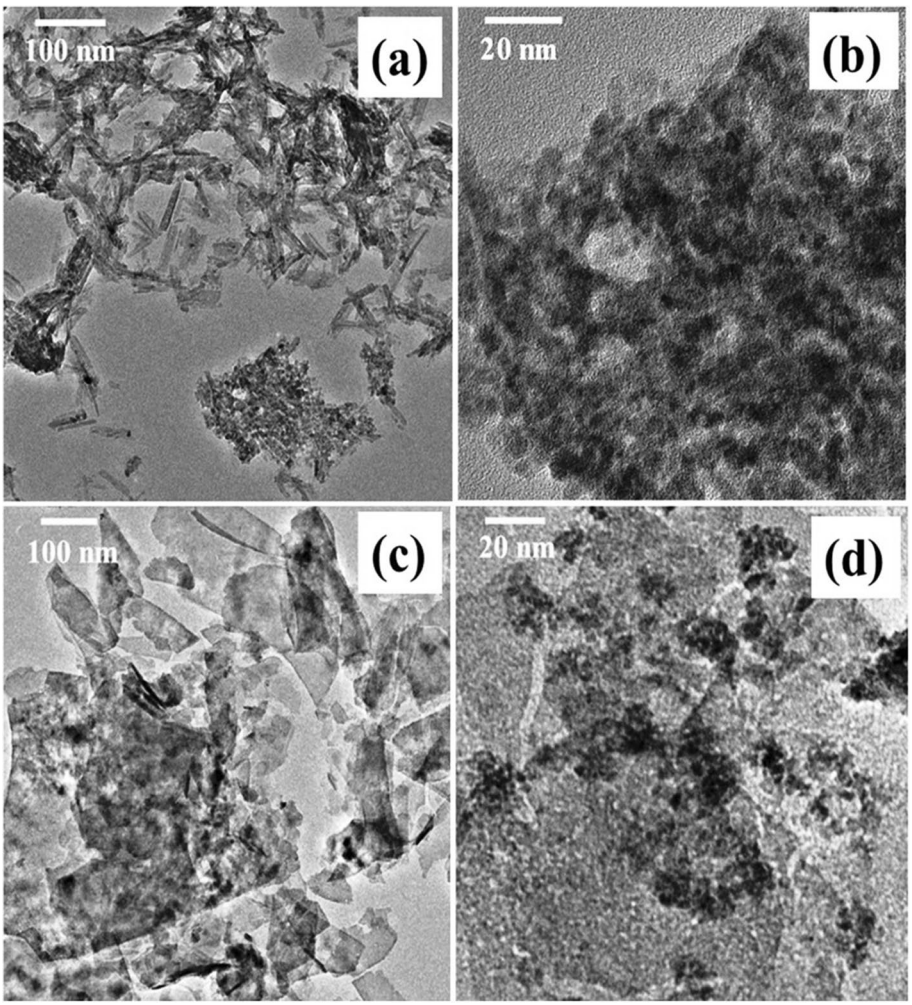

Fig. 6 Transmission electron microscopy images of **a**–**b** CuO nanocomposites and **c**–**d** Co_3O_4 nanocomposites

X-ray photoelectron spectroscopy (XPS)

CuO nanopowder's chemical makeup, purity, and oxidation level have all been verified using the potent surface-sensitive method known as XPS analysis. As a reference, the binding energies were calibrated using the C 1 s (carbon 1 s) peak at ~ 284.8 eV. Cu *2p* and O*1s*' high-resolution (core XPS) spectra are displayed in Fig. 7a, b, c, and d. By having the prominent shake-up peak, as seen in Fig. 7a, the XPS results can distinguish CuO from Cu_2O and metallic copper (Yuji et al. 2012; Muhammad et al. 2020). Cu*2p*'s core level or limited energy range spectra exhibit a significant shake-up peak at the higher binding BE side of the Cu $2p_{3/2}$ and Cu $2p_{1/2}$. This finding suggests that the Cu$3d^9$ shell is unfilled. The discovery of an unfilled Cu$3d^9$ shell further supported the presence of Cu^{2+} in the sample of CuO. Additionally, the peaks at ~ 954 eV and ~ 934 eV in the core level spectra of Cu *2p* might be attributed to Cu$2p_{1/2}$ and Cu$2p_{3/2}$ of CuO, respectively. Cu $2p_{3/2}$ and Cu $2p_{1/2}$, which are the properties of Cu^{2+} ions, can be attributed to the peaks at ~ 934 eV and ~ 954 eV with a spin-energy separation of 20 eV. At the same time, satellite peaks with binding energies of ~ 937 eV, ~ 943 eV, ~ 957 eV, and ~ 962 eV provide

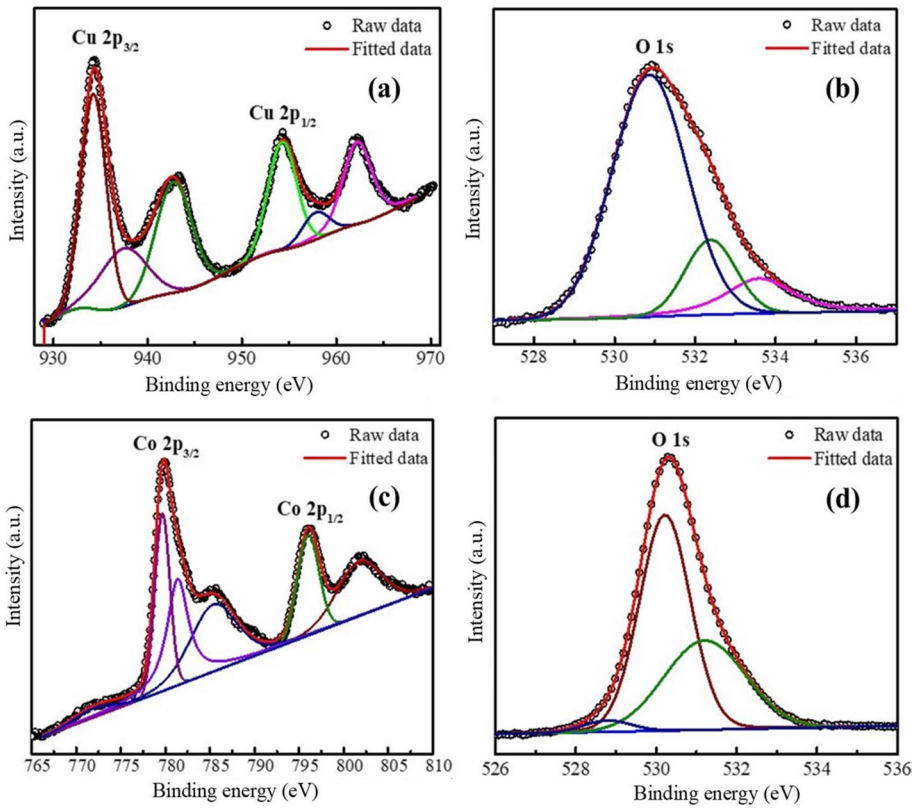

Fig. 7 Core level XPS spectra of **a** Cu 2p and **b** O 1 s in CuO nanocomposite and **c** Co 2p and **d** O 1 s in Co_3O_4 nanocomposite

further evidence of the presence of CuO in the composites (Yuji et al. 2012; Muhammad et al. 2020; Jing et al. 2018; Wenbo et al. 2020). High-resolution XPS spectra for O*1s* in CuO nanocomposites are de-convolved in Fig. 7b. The high-resolution XPS spectra of O *1 s* in CuO have three peaks at ~ 530 eV, ~ 532 eV, and ~ 533 eV (Fig. 7b). The peak at about ~ 530 eV, which is stronger, can be attributed to the binding energy of the O^{2-} ion at the metal oxide sites (Cu^{2+}- O^{2-}) and is in good accord with the binding energy of lattice oxygen (O^{2-}) in the CuO lattice. The ~ 532 eV and ~ 533 eV peaks may indicate that water has been physically or chemically absorbed on or within the surface (Muhammad et al. 2020; Jing et al. 2018; Wenbo et al. 2020). Figure 7c, d shows the core level XPS spectra of Co *2p* and O *1 s* of Co_3O_4 nanocomposites. The Co *2p* spectra were divided into two main peaks located at ~ 780 eV and ~ 796 eV, corresponding to Co $2p_{3/2}$ and Co $2p_{1/2}$, respectively, with a spin energy interval of 16 eV. The deconvolution of core level spectra shows the peaks at ~ 781 eV and ~ 796 eV corresponding to the Co^{2+} state, whereas the peak at ~ 779 eV shows the presence of Co^{3+}. The other peaks in the spectra show the satellite peaks at ~ 785 eV and ~ 801 eV (Xiao-Ming et al. 2021; Jingjing et al. 2018; Wu et al. 2015). The O *1 s* spectra in Co_3O_4 nanocomposites show three peaks over deconvolution at ~ 528 eV, ~ 530 eV, and ~ 531 eV, corresponding to different oxygen-containing chemical bonds. The peak positioned at ~ 528 eV and ~ 530 eV can be attributed to the binding energy of the O^{2-} ion at the metal oxide sites (Co–O),

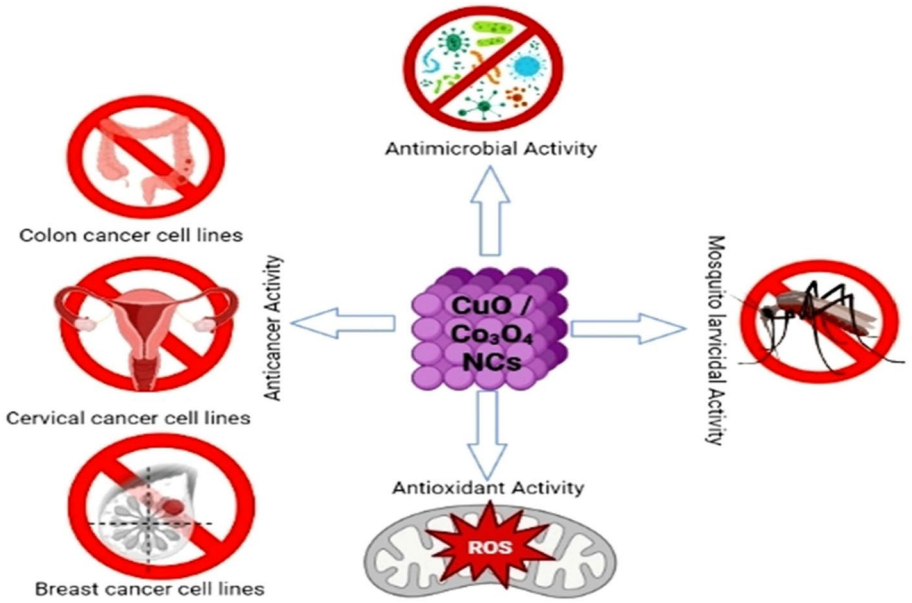

Fig. 8 Schematic representation of biological activities of SM-CuO and SM-Co_3O_4NCs

and ~ 531 gives the presence of hydroxide (Xiao-Ming et al. 2021; Jingjing et al. 2018; Wu et al. 2015).

Antimicrobial activity of SM-CuO and SM-Co_3O_4NCs

After successful synthesis of SM-CuO and SM-Co_3O_4NCs, the nanocomposites were used to observe their multifaceted bioactivities as shown in Fig. 8. In the present investigation, the well diffusion method was employed to explore the antimicrobial potential of as synthesized SM-CuO and SM-Co_3O_4NCs against four human pathogenic bacteria and fungi and the results are elicited in Table 1. The suspension of SM-CuO and SM-Co_3O_4NCs with varying concentrations ranging from 20 to 60 µg/well was employed against both Gram Positive and Gram Negative bacteria and fungi (Additional file 1: Figure S1a, b). In addition, the antimicrobial activity of 1:9 diluted snail mucus was also carried out to confirm that the present activity is due to as synthesized bio-nanocomposite only. Furthermore, the result of this study was compared with standard antibiotic i.e., Azithromycin against bacteria and Clotrimazole against fungi (Table 1). The results demonstrated that the antimicrobial activity depends on concentration of both bio-nanocomposites and exhibit significant antimicrobial activity against *E. coli* and *A. fumigatus* at 60 µg/well concentration. However, SM-CuONC exhibited maximum antimicrobial activity against *E. coli* and *A. fumigatus*. In literature it is mentioned that, the antimicrobial activity of nanoparticles depends on the shape of nanoparticles. It was further elaborated that the rod or wire-shaped nanoparticles penetrates easily into the cells and hence exhibits more antimicrobial activity than spherical shapes (Yang et al. 2009). In the present study also SM-CuONCs are rod-shaped while SM-Co_3O_4NCs are quasi-spherical, hence SM-CuONCs exhibited more antimicrobial activity as compared to SM-Co_3O_4NCs. The antimicrobial activity of both bio-nanocomposites was further reconnoitered using MIC and MBC. It was also noted that the minimum inhibitory

concentration of SM-CuONC against *E. coli* was 20 µg/ml while in case of SM-Co_3O_4NC it was 60 µg/ml (Table 2). In recent literature related to global death burden associated with bacterial pathogens, it was prominently mentioned that, during 7.7 million deaths are associated with 33 bacterial pathogens. It was also noted that from the list of bacterial pathogens, there are five leading bacteria which caused 54.9% of total deaths. These five leading bacteria include *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Ikuta et al. 2022). Surprisingly in the present study our both bio-nanocomposite systems exhibited excellent antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

In literature it is noted that copper oxide nanocomposites consist of significant antimicrobial action on both Gram Positive and Gram Negative bacteria which is because of attraction of copper ions towards amines and carboxyl groups present on microbial cell surface. The copper ion released from CuO nanocomposites also binds with DNA and disturbs the helical structure and biochemical processes when it enters into the cell (Kim et al. 2000).

While in case of cobalt oxide nanocomposites, cobalt ions react with the thiol group of enzymes which lead to inactivation and cell death. The ions from cobalt oxide nanocomposites react with negatively charged bacterial cell surface and disturb vital life processes such as cell reproduction, breathing, etc. (Haq et al. 2021).

In literature, it is noted that antimicrobial activity in fungi is less as compared to bacteria which is due to its more firm cell wall. The fungal cell wall is made up of chitin hence most of the nanoparticles cannot easily enter into the cell. In case of bacterial cell wall which is made up of peptidoglycan and is not firm like that of fungi hence nanoparticles can easily enter into the cell and exhibit more antimicrobial activity (Qamar et al. 2020). Recently WHO released the first ever list of life threatening fungi in which drug resistant *A. fumigatus* is included in priority category on which more action is needed. Noteworthy, as shown in Additional file 1: Figure S2 the present study confirms that both SM-CuO and SM-Co_3O_4NCs exhibited promising antifungal activity against *A. fumigatus* (Alastruey-Izquierdo 2022).

Another important aspect of this study is that, both the bio-nanocomposites exhibited potent antimicrobial activity against Gram negative bacteria also. In most of the studies, it is mentioned that the cell wall of Gram positive bacteria is thick but porous in nature with maximum permeability while Gram negative bacteria consist of thin cell wall with less permeability. Due to this difference in porosity nature of bacteria, most of the time the antibacterial activity is less in Gram negative bacteria (Bankier et al. 2019).

Growth curve assay

Furthermore, to elaborate the antimicrobial study of both bio-nanocomposites, we carried out the growth curve assay using *E. coli* as a model organism. In this assay, it was found that at 20 µg/ml of SM-CuONC, the growth of *E. coli* was completely inhibited while in case of SM-Co_3O_4NC the growth of *E. coli* was completely inhibited at 60 µg/ml (Fig. 9a, b).

The growth of *E. coli* was inhibited due to the interaction of copper and cobalt ions released from copper oxide and cobalt oxide bio-nanocomposites with intracellular proteins (Bossi et al. 2016). As copper and cobalt are essential trace elements, but elevated intracellular concentration leads to pro-oxidative reactions which cause cell death (Meghana et al. 2015). The generation of reactive oxygen species (ROS) which inhibits the growth, more precisely in case of Gram-negative strains is the mechanism involved in inhibition of bacterial growth (Nair et al. 2009). As *E. coli* is Gram-negative strain, hence the mechanism of ROS generation is presumed to be responsible for the cell death.

Cell integrity study

The bacterial cell membrane acts as a protective component which will be compromised during exposure of cells to antibiotics, nanocomposites, biocides, etc. Hence for the study of cell integrity, the release of intracellular compounds will be considered as indicator of cell integrity. When any biocidal compounds lead to loss of membrane integrity, the small ions like potassium and phosphate, etc. and other biochemical materials including DNA, RNA leach out (Santo et al 2008).

In the present investigation, the cells of *E. coli* exposed to SM-CuO and SM-Co_3O_4NCs at MBC concentration and UV–Visible study on the release of 260 and 280 nm was observed and results are shown in Table 3. Moreover, the results of the empirical studies showed that after exposure of *E. coli* cells to CuO NCs and Co_3O_4 NCs, there is sudden increase in the optical density (OD) of bacterial suspension after 15 min. This quick increase at 260 and 280 nm indicates the fast killing potential of both the studied NCs.

Anticancer activity

In recent years, there is growing interest in development of nano-based anticancer drugs. The present anticancer study contemplates about potential of SM-CuO and SM-Co_3O_4NCs against colon, cervical and breast cancers. From the results of this study, it is evident that both the bio-nanocomposites showed clear cytotoxic effects against colon, cervical and breast cancer cell lines with concentration- response dependent manner and maximum percent kill was noted at 50 µg/well concentrations (Fig. 10a, b and c). The present investigation is the first report on synthesis of SM-CuO and SM-Co_3O_4NCs using *A. fulica* mucus representing anticancer as well as antimicrobial activities. Most important thing about the present anticancer study is that the estimated new cases of cancer are related to colon, cervical and breast cancer cells and death rate of these cancer is also high (Siegel et al. 2017).

In case of SM-CuONC, at 20 µg/well concentration, 68.66 ± 3.72, 62.66 ± 3.61 and 71.00 ± 2.36 percent kill was achieved against HCT, HeLa and MDA-MB-231 cell lines, respectively. While SM-Co_3O_4NC exhibited 61.00 ± 3.57, 72.66 ± 4.50 and 71.66 ± 4.22 percent kill against HCT, HeLa and MDA-MB-231 cell lines, respectively. This investigation also substantiates that SM-CuONC exhibits better results against MDA-MB-231 cell lines while SM-Co_3O_4NC shows better anticancer activity against HeLa cell lines.

For thousands of year, copper compounds are used for the treatment of several diseases including cancer (Hajra and Liu 2004). Copper is toxic to mammalian cells but due

to nanotechnology, copper nanoparticles can be used to treat specific cells with low toxicity towards the normal cells (Nagajyothi et al. 2017; Phull et al. 2021).

Nowadays, for killing cancerous cells, chemotherapy is widely used but chemotherapeutic agents work non-specific which leads to toxicity towards the normal cells also. It was reported that copper nanoparticles attracting many researchers for the management of cancer with biological synthesis method (Harne et al. 2012).

Additional file 1: Figures S3a, b, S4a, b and S5a, b show the significant reduction in the number of HCT, HeLa and breast cancer cells treated with different concentrations of SM-CuO and SM-Co_3O_4NCs as compared to control after 24 h.

Like copper, cobalt is also considered as an essential trace element for human being as well as for other animals. The most important fact about cobalt is that, it shows lower toxicity as compared to other non-essential elements, hence cobalt comes in limelight for its application in anticancer drug discovery (Munteanu and Suntharalingam 2015).

As compared to normal cells, tumor cell membrane contains more amount of phospholipids and the presence of acids in phospholipids leads to release of cobalt ions from cobalt oxide nanocomposites into the cells. This leads to damage of DNA and causes apoptosis (Chattopadhyay et al. 2012a, b).

It was also observed that cobalt nanoparticles can lead to cell apoptosis rapidly as compared to other nanoparticles as cobalt nanoparticles easily enters into the cell membrane of cancer cells. It was also noted that the engulfment of copper into cells is carried out through the process of endocytosis. In this process cells uptake the nanoparticles like other macromolecules and proteins as by normal cells. In case of cancer cells uptake of nanoparticles is more incessant (Gal et al. 2015). Hence, applications of nanoparticles in cancer treatment are perfect asset. In the present study all cancer cell lines revealed that growth inhibition and cell death were increased in a dose-dependent manner. Micronuclei were mostly responsible for the rise in cell death, and both the bio-nanocomposites may prevent DNA strand breaks from being repaired. Thus, at all tested concentrations, SM-CuO and SM-Co_3O_4NCs showed strong cytotoxicity against cancer cell lines while negligible against normal cells (Wahab et al. 2011).

Antioxidant activity

It is well known that antioxidants are very important to human being as it protect our body from free radicals. In recent years, some progress was made in the field of nanotechnology for synthesis of nanomaterials endowed with antioxidant activity (Valgimigli et al. 2018). In the present study, we carried out antioxidant potential of SM-CuO and SM-Co_3O_4NCs and compared with metal salts and biomaterial, the results are presented in Additional file 1: Figure S6a, b, c, d and e. In case of SM-CuONC, the total antioxidant activity was found to be highest 55.33 ± 3.72 at concentration of 50 µg/ml while that of SM-Co_3O_4Ns was 52.00 ± 3.22 mM of ascorbic acid/µg of sample at same concentration.

The antioxidant activity of nanoparticles is mainly due to presence of proteins and some other biomolecules present in the solution of nanoparticles (Netala et al. 2016). In present investigation we synthesized SM-CuO and SM-Co_3O_4NCs using *A. fulica* mucus which contains plenty of proteins with several biomolecules and hence exhibits better antioxidant activity.

Mosquito larvicidal activity

As far as mosquito larvicidal activity of Cuo and Co_3O_4 nanoparticles are concerned, very few reports are present till today indicating the novelty of work. In the present study, we used SM-CuONCs and SM-Co_3O_4NCs against the mosquito larvae. We evaluated different concentrations of SM-CuO and SM-Co_3O_4 NCs ranging from 10 to 50 mg/l. The result of this study exhibited that both bio-nanocomposites have dose-dependent larvicidal potential against all the instars (I–IV) and pupae of *A. aegypti* but SM-CuONC shows 100% mortality of all the instars and pupae at the concentration of 40 mg/l while Co_3O_4 NCs shows 100% mortality for first three instars at 50 mg/l (Additional file 1: Figure S7a, b).

In one study, at higher concentrations (200 mg/l) of cobalt oxide nanoparticles only 67.2% mortality of third instar larvae of *A. aegypti* were observed and 18.3% mortality was observed at lower concentration (25 ppm) (Khan et al. 2021). It is of paramount concern to control such disease spreading insect vectors for the comfort of human beings. The recent strategy to control insect vector is application of synthetic insecticides which leads to certain threats to the different ecosystems. Unfortunately, very few studies have been carried out on unfurling the use of nanotechnology for developing insect control weapon (Mane et al. 2017). In the present study, we explored the potential of bio-nanotechnology by synthesizing SM-CuO and SM-Co_3O_4NCs from novel biomaterial to control *A. aegypti*.

There are some studies which used nanoparticles of selenium (Cittrarasu et al. 2021), zinc oxide (Amuthavalli et al. 2021), Au–Pd bimetallic (Minal and Prakash 2020), but for these studies they used higher concentration of nanoparticles to get 100% mortality. As compared to above studies, our present investigation revealed 100% mortality of all the larval instars and pupae at very low concentrations of bio-nanocomposites.

Cytotoxicity study

Additionally with biological activities of SM-CuO and SM-Co_3O_4NCs we carried out cell viability assay during the course of present investigation. The concentration dependent cell viability of normal cell line (L929) was determined and the results are exhibited in Table 4 and Additional file 1: Figure S8a, b. From the results, it is clear that upto 20 µg/well concentration, SM-CuO and SM-Co_3O_4NCs suspension does not significantly affect the cell viability of L929 cells and showed around 14% inhibition by SM-CuONC and 12% inhibition by SM-Co_3O_4NC.

In literature, it was also pointed out that cobalt nanoparticles exhibits low cytotoxicity against normal cells. It was further observed that even at higher concentrations, cobalt nanoparticles doesn't exhibit high toxicity towards normal cells and surprisingly it shows toxicity against cancer cells even at low concentrations. Due to all these factors, cobalt nanoparticles are at top-spot for cancer treatment (Rauwel et al. 2020).

Phyto-toxicity study

When we talk about development in nanotechnology, we must focus on its phyto-toxicity also because nanoparticles can enter into the plants via root epidermis and distributed in all parts and exhibits toxicity (Szollosi et al. 2020).

Table 1 Antimicrobial activity of SM-CuO and SM-Co_3O_4NCs

Name of the microorganisms	Zone of inhibition (cm)			
	Snail mucus (1: 9) diluted	Azithromycin/ Clotrimazole (60 µg/ well)	SM-CuONC (60 µg/well)	SM-Co_3O_4NC (60 µg/well)
Escherichia coli	0.00	2.30 ± 0.26	2.36 ± 0.31	1.60 ± 0.17
Klebsiella pneumoniae	0.00	1.10 ± 0.17	1.93 ± 0.27	1.30 ± 0.17
Pseudomonas aeruginosa	0.00	2.12 ± 0.21	1.50 ± 0.26	1.33 ± 0.31
Staphylococcus aureus	0.00	0.98 ± 0.34	1.06 ± 0.18	1.36 ± 0.22
Aspergillus fumigatus	0.00	0.00 ± 0.00	2.36 ± 0.59	1.56 ± 0.22

All the values are mean of three experiments, ± indicates S. D

Table 2 MIC/MBC of SM-CuO and SM-Co_3O_4 NCs

Name of the microorganisms	MIC/MBC (µg/ml)	
	SM-CuONC	SM-Co_3O_4NC
Escherichia coli	20	60
Klebsiella pneumoniae	40	70
Pseudomonas aeruginosa	60	70
Staphylococcus aureus	70	90
Aspergillus fumigatus	20	60

Hence, we assessed phytotoxicity of SM-CuO and SM-Co_3O_4NCs against *Triticum aestivum*. Here we studied the effects of SM-CuO and SM-Co_3O_4NCs on seed germination, root, shoot, chlorophyll and protein content of *Triticum aestivum* crop plant. It is interesting to note that in case of seed germination, 100% seeds were germinated up to 500 mg/l concentrations of SM-CuONC while in case of SM-Co_3O_4NC, 100% cells were germinated at 250 mg/l concentrations (Additional file 1: Figure S9a, b).

After germination we also observed root and shoot length. The results of this study revealed that root length and shoot length have stimulatory effects upto 500 mg/l when treated with SM-CuONC while in case of SM-Co_3O_4NC, root length and shoot length does not have any stimulatory effects (Additional file 1: Figure S10a, b).

This study further investigated the effects of both bio-nanocomposites on photosynthetic parameters like chlorophyll a, chlorophyll b, total chlorophyll and protein content of *Triticum aestivum*. The results of this investigation revealed that at 200 mg/l of SM-CuONC and 250 mg/l of SM-Co_3O_4NC total chlorophyll content was found to be higher as compared to control (Additional file 1: Figure S13a, b). The results of chlorophyll a and chlorophyll b are represented in Additional file 1: Figures S11a, b and S12a, b. Outcome of this phytotoxicity study reveals that the present SM-CuO and SM-Co_3O_4NCs did not exhibit any acute toxicity on crop plants like *Triticum aestivum* and thus it is environmentally safe.

In another study it is mentioned that greater phytotoxicity was observed on plants with smaller seeds like cabbage, radish, etc. when compared to plants with larger seeds like wheat (Ma et al. 2010).

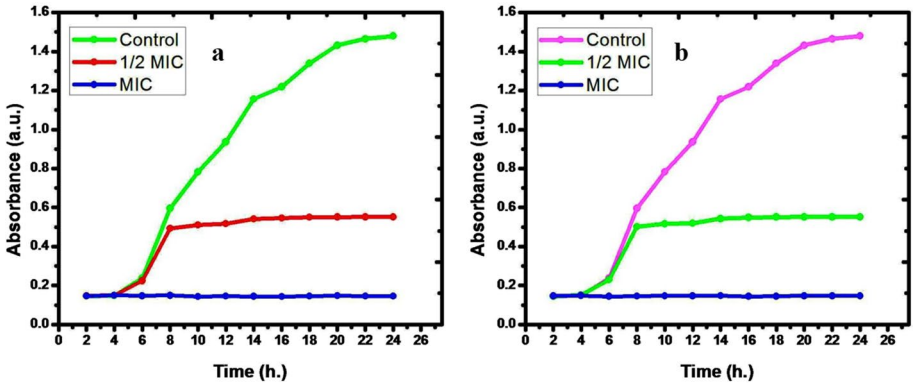

Fig. 9 Growth curve assay of as synthesized **a** SM-CuO and **b** SM-Co_3O_4NCs

In one more study, it was mentioned that copper oxide nanoparticles at concentration of 500 mg/kg reduce the root growth and lead to negligible phytotoxicity to the plants. It also does not affect chlorophyll production in *Coriandrum sativum* at low concentration (Siddiqi and Husen 2017).

Chlorophyll is the only important parameter which can be correlated with photosynthetic rate. Here we found that at lower concentrations of both the bio-nanocomposites, chlorophyll content was enhanced which could be due to increased accumulation of soluble proteins playing important role in plant metabolic activities (Arora et al. 2012).

Conclusion

This is the first study on the production of SM-CuO and SM-Co3O4NCs utilizing the mucus of *A. fulica* and their use in various pharmaceutical processes. The novel use of *A. fulica* mucus for environmentally friendly, low-cost synthesis of SM-CuO and SM-Co3O4NCs was shown in the current study. *A. fulica* mucus includes strong biomolecules that are essential for the bio-reduction of metal salts and the creation of nanocomposites, as shown by the study. In this work, two bio-nanocomposites were synthesized as, SM-CuONCs are rod shaped and SM-Co3O4NCs are quasi-spherical in shape. The average size of both bio-nanocomposites is approximately 18 nm. Both

Table 3 Cell membrane leakage at UV_{260} and UV_{280} nm after SM-CuO and SM-Co_3O_4 NCs treatment

Time (min.)	Absorbance					
	UV_{260}nm			UV_{280}nm		
	Control	SM-CuO NCs	SM-Co_3O_4 NCs	Control	SM-CuONCs	SM-Co_3O_4 NCs
15	0.07	0.39	0.40	0.10	0.41	0.43
30	0.07	0.41	0.40	0.10	0.44	0.44
45	0.07	0.42	0.41	0.11	0.61	0.59
60	0.08	0.43	0.42	0.11	0.63	0.61
75	0.08	0.44	0.43	0.12	0.75	0.72
90	0.09	0.44	0.43	0.12	0.79	0.80

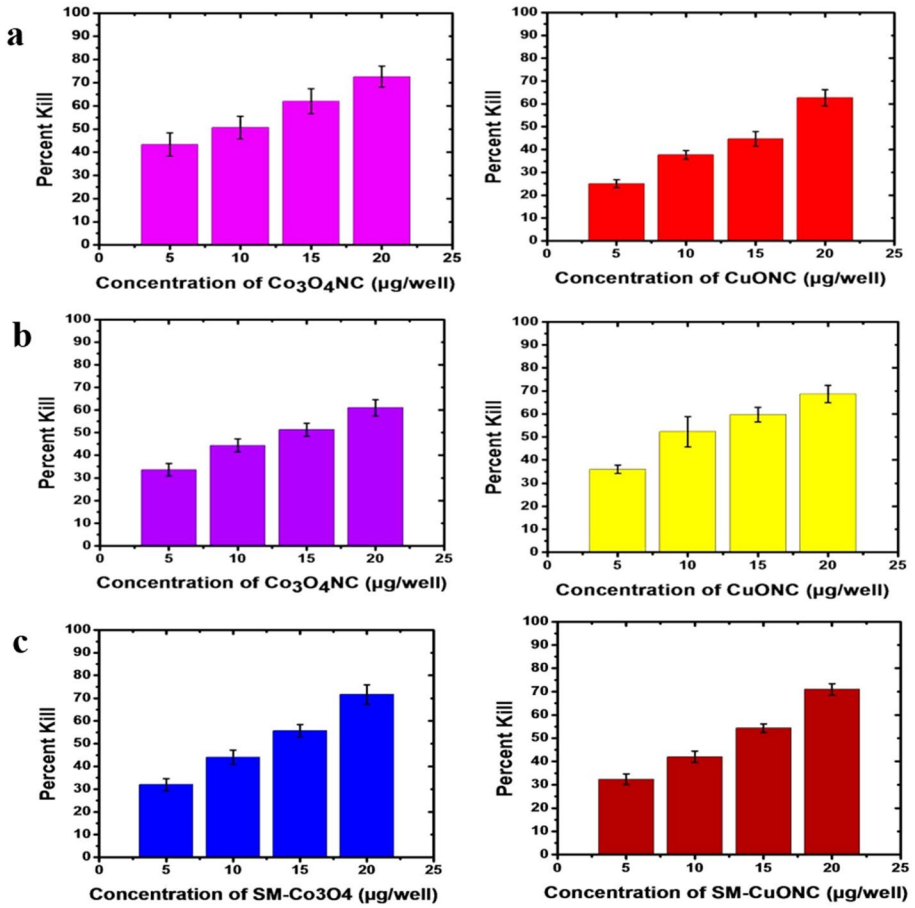

Fig. 10 Anticancer activity of as synthesized SM-CuO and SM-Co_3O_4NCs against Human **a** Cervical cancer (HeLa), **b** Colon cancer (HCT-15), and **c** Breast cancer (MDA-MB-231)

bio-nanocomposites in this investigation shown remarkable antibacterial, anticancer, antioxidant, and mosquito larvacidal properties. Most importantly, both bio-nanocomposites neither exhibit any phytotoxicity nor cytotoxicity. The antimicrobial, anticancer, and antioxidant properties of SM-CuO and SM-Co3O4NCs can also be applied to a variety of biomedical applications. Due to the fact that both bio-nanocomposites were

Table 4 Cyto-toxicity study of SM-CuO and SM-Co_3O_4NCs against normal cell line

Sr. No	Concentrations of bio-nanocomposites (µg/well)	Cell viability (%) after 24 h	
		SM-CuONC	SM-Co_3O_4NC
1.	Blank	100.0 ± 0.00	100.0 ± 0.00
2.	Vehicle control	98.33 ± 1.03	98.33 ± 1.03
3.	Positive control	28.00 ± 4.09	28.00 ± 4.09
4.	5	97.33 ± 1.86	97.33 ± 1.36
5.	10	96.00 ± 3.22	94.00 ± 2.68
6.	15	92.67 ± 5.24	91.67 ± 3.14
7.	20	86.33 ± 6.59	88.00 ± 6.26

All the values are mean of three experiments, ± indicates S. D

found to be mosquito larvicidal against *A. aegypti*, they can also be employed to prevent diseases that are transmitted by insects, especially mosquito-borne diseases. As both bio-nanocomposites are non cytotoxic and non phytotoxic, they can also be used for other purposes including cosmetics, drug delivery and also environmental applications in future. Still more in vitro and in vivo research on copper and cobalt oxides bio-nanocomposites is needed.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12645-023-00226-2.

Additional file 1: Figure S1. Digital photograph of antibacterial activity of a) SM-CuO and b) SM-Co_3O_4NCs against human pathogenic bacteria. KP—*Klebsiella pneumoniae*, EC—*Escherichia coli*, PA—*Pseudomonas aeruginosa* and SA—*Staphylococcus aureus*. **Figure S2.** Digital photograph of antifungal activity of a) SM-CuO and b) SM-Co_3O_4NCs against *Aspergillus fumigatus*. **Figure S3.** Cancer cell apoptosis observed under inverted microscope (a) – SM-CuONC and (b) – SM-Co_3O_4NC HeLa (Cervical cancer). **Figure S4.** Cancer cell apoptosis observed under inverted microscope (a) – SM-CuONC and (b) – SM- Co_3O_4NC HCT-15 (Colon cancer). **Figure S5.** Cancer cell apoptosis observed under inverted microscope (a) – SM-CuONC and (b) – SM- Co_3O_4NC MDA-MB-231 (Breast cancer). **Figure S6.** Antioxidant activity of a) copper sulphate, b) cobalt nitrate, c) snail mucus, d) SM-CuONC and e) SM-Co_3O_4NCs treatments at different concentrations (10 to 50 µg/ml). **Figure S7.** Mosquito larvicidal activity of a) SM-CuO and b) SM-Co_3O_4NCs. **Figure S8.** Cyto-toxicity evaluation of a) SM-CuONC and b) SM-Co_3O_4NC on normal (L929) cell line. **Figure S9.** Effect of bio-nanocomposites on seed germination of *Triticum aestivum* a) SM-CuO treated and b) SM-Co_3O_4NCs treated. **Figure S10.** Effect of bio-nanocomposites on root & shoot length of *Triticum aestivum* a) SM-CuO treated and b) SM-Co_3O_4NCs treated. **Figure S11.** Effect of bio-nanocomposites on Chlorophyll a content of *Triticum aestivum* a) SM-CuO treated and b) SM-Co_3O_4NCs treated. **Figure S12.** Effect of bio-nanocomposites on Chlorophyll b content of a) SM-CuO treated and b) SM-Co_3O_4NCs treated. **Figure S13.** Effect of bio-nanocomposites on Total chlorophyll content of a) SM-CuO treated and b) SM-Co_3O_4NC treated.

Acknowledgements
The authors would like to express their sincere thanks and gratitude towards the Chairman and Trustees, JTSSPM, Junnar and the Principal, Shri Shiv Chhatrapati College, Junnar for providing laboratory facilities. Deepali D. Kadam gratefully acknowledges to Department of Science and Technology, Ministry of Science and Technology, India. Dr. Sachin B. Agawane gratefully acknowledges the Director, CSIR-National Chemical Laboratory, Pune and CSIR, New Delhi for funding this work under Health Care Project MLP 101826.

Author contributions
RDC and PCM devised the broad experimental framework; RDC directed the research work; DDK, ANK and ARC conducted the antimicrobial experiments; PCM conducted synthesis of bio-nanocomposites, anticancer, biocompatibility, antioxidant and mosquito larvicidal activities. SBA and SPU carried out physico-chemical characterization of bio-nanocomposites and its interpretation. PCM, DDK and ANK drawn the figures, wrote the first draft of the manuscript which was duly modified, improved and finalized by RDC and PCM with the help of SBA. All the authors read and approved the final contents of the manuscript.

Funding
This research was funded with the support by Govt. of India, Department of Science and Technology, Ministry of Science and Technology, India under DST/INSPIRE Fellowship/2013/557 and CSIR, New Delhi with National Chemical Laboratory, Pune under Health Care Project MLP 101826.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 4 February 2023 Accepted: 14 September 2023
Published online: 16 October 2023

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