**Green synthesis of cobalt oxide nanoparticles using roots extract of Ziziphus Oxyphylla Edgew its characterization and antibacterial activity**

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**Abstract**

In this modern era, antibiotic resistance is a significant issue that poses a threat to public health. Nanotechnology is an emerging field of science because nanoparticles could be the best alternative to antibiotics. Most nanoparticles are prepared by the green synthesis method because of their less toxicity, low cost, and non-hazardous nature. In this study, cobalt oxide nanoparticles (Co3O4-NPs) were synthesized from roots extract of Ziziphus Oxyphylla Edgew by using cobalt chloride hexahydrate. After the successful synthesis of nanoparticles, various methods were used to analyze these nanoparticles, including Fourier transform infrared spectroscopy, scanning electron microscopy, x-ray diffraction analysis, and energy dispersive analysis of x-ray. Scanning electron microscopy images reveal the spherical and irregular structure of Co3O4-NPs shaving a particle size between 40 to 60 nm. X-ray diffraction analysis revealed the crystalline nature of cobalt oxide nanoparticles with face-centered cubic structure and a size of 15–20 nm. The antibacterial activity of Co3O4-NPs was analyzed for different dilutions against two different bacteria: gram-negative (E. coli) and gram-positive (S. aureus) bacteria. The maximum zone of inhibition against gram-negative E. coli was calculated as 23.1 mm and 14.8 mm against S. aureus at a dilution of 16 mg ml⁻¹ of cobalt oxide nanoparticle. This revealed the wide spectrum of antibacterial activity of the synthesized nanoparticle. It is suggested that root extract of cobalt oxide nanoparticles could be of great importance in pharmaceutical and medical science for their antimicrobial activity.

**1. Introduction**

Nanotechnology is a relatively emerging field of science that involves the synthesis of different nanomaterials. Nanoparticles are those particles whose sizes range from 1–100 nanometers. Different metals like titanium, magnesium, copper, zinc, gold, alginate, and silver can be used to synthesize nanoparticles [1]. Nanotechnology utilizes different physical and chemical techniques to give nanoparticles unique properties. These nanoparticles can be used in different fields such as in the field of agriculture, environment, heavy industry, and the development of nanomedicine, nano-electronics, and biomaterials [2]. With the development of modern nanotechnology processes, it has brought attention to the use of inorganic nanomaterials as antibacterial precursors which are durable for a long time, show resistance to high temperature, and high activity, and have a high selectivity as compared to other organic antibacterial agents [3].

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Based on origin, nanoparticles can be classified into three categories. The first one is incidental nanomaterials, the second is engineered nanomaterials and the third is naturally occurring nanoparticles. Nanoparticles synthesized through the biological method are of great importance because they are multifunctional and can be used for a specific purpose with the least toxicity [4]. Nanoparticles can also be made through laser-ablated techniques by utilizing different metals like gold, silver, and copper. These nanoparticles can be used as nano weapons against bacteria and fungi but the activity of these nanoparticles also depends on the dose and size of the nanoparticle [5].

For the synthesis of nanoparticles, different methods are present which are widely reported in the literature. Some of the methods for the preparation of nanoparticles with uniform size include techniques like the microemulsion technique, high-temperature calcination of the oxide precursors, and solvothermal reaction but these techniques involve the use of noxious organic reagents such as toxic reducing and capping agents which leads to a remarkable reduction in antibacterial activity of the synthesized nanoparticles. Thus, we prefer to use a green synthesis approach for the preparation of nanoparticles [3]. Synthesis of metal oxide nanoparticles through plant-mediated green synthesis has fascinated scientists and researchers from noxious methods because of its environmentally friendly, non-hazardous, and non-toxic fabrication systems [6]. Plant-based nanoparticles are synthesized from the different origins of plants and are widely used in multiple applications from target drug delivery to the treatment of tumor cells. Synthesis of nanoparticles by a green synthesis method is preferred and broadly used nowadays because it is eco-friendly, low cost, and harmless [7]. Plants consist of different phenolic compounds which are highly reactive. These phenolic compounds play a major role in redox reactions during nanoparticle synthesis. During the synthesis of nanoparticles, they reduce the metal compounds which results in the successful synthesis of nanoparticles [8]. Nanoparticles agglomerate and reduce high energy which is associated with a higher surface area to volume ratio. Different phytochemicals that are present in plants act as capping, stabilizing, and reducing ends which reduce the agglomeration of Co$_3$O$_4$-NPs and give these nanoparticles a characteristic feature of controlling the structural morphology and stabilizing the synthesis process of nanoparticles [9]. Plant-based nanoparticles offer several advantages, including being readily accessible, safe to handle, and containing a wide range of biomolecular compounds such as alkaloids, terpenoids, phenols, flavonoids, tannins, and quinines are the compounds that facilitate nanoparticle synthesis [10] [11]. Different plants have been used to extract several types of nanoparticles but none of the research studies has been reported on the green synthesis of cobalt oxide nanoparticles from roots extract of *Ziziphus oxyphylla* Edgew. Co$_3$O$_4$-NPs synthesized by the green synthesis method exhibit good antibacterial and antifungal activities. These activities depend upon the pH, temperature range and duration, reaction time, and clustering of the plant extract that co-occurring the synthesis of Co$_3$O$_4$-NPs [12].

In the current study, Co$_3$O$_4$-NPs are synthesized using *Z. oxyphylla* Edgew which is a small glabrous tree. *Ziziphus* (Rhamnaceae) is a plant genus that consists of about 100 different species all over the world among which six species are found in Pakistan. In local areas of Pakistan, *Ziziphus* is used for inflammation, allergies, and rheumatism and grows at a higher altitude in cold wet regions [13]. *Z. oxyphylla* Edgew also has anti-diabetic properties [14]. Plant-mediated biological production of nanoparticles is gaining importance day by day because of its cost-effectiveness, simplicity, and eco-friendliness [15].

Co$_3$O$_4$-NPs could be efficient as they acquire good catalytic activity [16]. Cobalt-based nanoparticles are synthesized characterized and used by many researchers for different purposes due to their chemical and physical properties. It also has biomedical and cytotoxic activities, as well as high-performance permanent magnetic characteristics [17]. For the synthesis of cobalt oxide nanoparticles, a complete plant-mediated method is used by using roots extracted from a medicinal plant *Ziziphus* (Rhamnaceae) a plant genus that consists of about 100 different species all over the world among which six species are found in Pakistan. Which acts as an effective stabilizing and chelating agent Co$_3$O$_4$-NPs are eco-friendly and have many applications such as in pigments and dyes, for environmental remediation purposes, cosmetics, and pharmaceutical products [18].

In Pakistan, there is a lot of biodiversity of medicinal plants and the majority of people rely on plant-based remedies for the treatment of different health problems [19]. As for our concern, our main objective was to explore the flora of Pakistan and to find out the medicinal importance of *Z. oxyphylla* Edgew. For this purpose, *Z. oxyphylla* Edgew roots extract has been subjected to different characterization techniques and analyzed for its antibacterial activity.

2. Materials and methods

2.1. Collection of plant materials

Fresh roots of *Z. oxyphylla* Edgew were collected from district Buner KPK Pakistan. 200 g of the collected roots were gently washed with distilled water and then shade dried for 15 days to remove residual moisture.
2.2. Preparation of aqueous plant extract
15 grams of dried root powder were taken in a 500 ml glass flask. 100 ml sterile distilled water was added to the flask and kept at 60 °C for two hours in a water bath with characteristic color of light brown. The solution was then filtered with a filter paper (11 μm) and the filtrate was used in the synthesis of the required Co3O4-NPs. Cobalt chloride salt was added and heated on a magnetic stirrer which change the solution from light to dark brown, this showed the synthesis of cobalt oxide nanoparticles.

2.3. Synthesis of cobalt nanoparticles
Biogenic Co3O4-NPs were synthesized by the plant-mediated ‘green synthesis’ method in which different functional groups present in plants act as reducing ends and result in the formation of nanoparticles. 20 ml of the aqueous leaf extract was taken in a flask and 30 ml of 1 Molar aqueous solution of (CoCl2)2.6H2O was added to it. Then placed it on a magnetic stirrer at 80 °C for 30 min and the color change was observed. The resultant extract solution was centrifuged at 9,000 rpm for 20 min, the supernatant was discarded and the pellet containing precipitates of Co3O4-NPs was collected and washed with ethanol to remove impurities and then transferred into a china dish. The precipitates were kept in an oven at 60 °C for drying. The dried fine powder precursor then was calcinated at 100 °C temperature overnight and further characterized at the Centralized Resource Laboratory university of Peshawar.

2.4. Characterization
Characterization of Co3O4-NPs was carried out through x-ray diffraction analysis (XRD), Scanning Electron microscopy (SEM), Fourier transform Infrared Spectroscopy (FTIR), and Energy dispersive analysis of x-ray (EDX) at the Centralized Resource Laboratory (CRL), University of Peshawar, Pakistan.

2.5. Anti-bacterial activity analysis
Biochemically identified E. coli and S. aureus were obtained from Nowshera Medical Complex. By using the well diffusion technique, the antibacterial activity of Co3O4-NPs was investigated. Different dilutions of Co3O4-NPs were made by dissolving 8, 12, and 16 mg of Co3O4-NPs in 1 ml of 1% DMSO. Nanoparticles were properly mixed with the help of vortex until the disappearance of any suspended particles. A loop full of 24 h old culture was taken and dissolved in 0.9% autoclaved normal saline and properly mixed at room temperature. The lawn of selected test bacteria was made on MHA with the help of a sterilized spreader at room temperature (37 °C). Then wells were made in media by cork-borer and 100 μl of nanoparticles were poured into each well with the help of a micropipette. One well was filled with water as a negative control. The Petri plates were incubated for 24 h and then checked for the zone of inhibition. The zone of inhibition was measured by a graduated scale after 24 h.

3. Results

3.1. FTIR
FTIR analysis was performed for the characterization of different functional groups that act as capping and stabilizing agents presented in Ziziphus Oxyphylla Edgew roots extract. The FTIR spectrum of Z. oxyphylla Edgew root extract show peaks at the wavelength of 3286 cm\(^{-1}\), 1639 cm\(^{-1}\), 1392 cm\(^{-1}\), 1067 cm\(^{-1}\) and 575 cm\(^{-1}\) which show the presence of hydroxyl group, carbonyl group, amide group, C–O of alcohols or phenols and CH groups respectively. The FTIR spectrum of Co3O4-NPs solution exhibits different peaks at the wavelength of 3250 cm\(^{-1}\), 1636 cm\(^{-1}\), 1395 cm\(^{-1}\), 1061 cm\(^{-1}\) and which show the presence of hydroxyl group, carbonyl group, amide group, C–O of alcohols or phenols respectively and the peak at 562 cm\(^{-1}\) show C-H out of the plane that confirms the synthesis Co3O4-NPs. There is a difference in spectra of the plant extract and Co3O4-NPs containing plant extract shown in figure 1. Different researchers observed variation in results and a possible reason for this might be the difference in the manufacturing process (chemical, physical and biological methods) due to which variation occurred in our results.

3.2. XRD
XRD analysis was performed to confirm the synthesis of Co3O4-NPs. To know the crystalline structure XRD analysis was carried out and major peaks were observed at Bragg’s angle or 2 Theta values of 16.8, 30.1, 36.4, 37.9, 43.8, 54, 60.1, and 65.8 corresponded to lattice plains of (111), (220), (311), (222), (400), (422), (511) and (440) respectively that could be indexed to the face centered-cubic phase of Co3O4-NPs, in reference with the previously reported literature data and Joint Committee on Powder Diffraction Standards database (JCPDS card file no. 76–1802) indicating the biosynthesis of pure Co3O4-NPs. Table 1 shows the different parameters calculated for Co3O4-NPs including d-spacing, FWHM, hkl, dislocation density, and lattice strain. The lattice constant (a\(_0\)) of Co3O4-NPs was determined using the following relationship.
The $a_0$ calculated from the XRD pattern is 8.2606 Å which is close to the literature value (JCPDS card file no. 76–1802). The diffraction pattern obtained from XRD analysis has strong peaks (using origin software for peak analysis) which reveals that the synthesized particles are crystalline with a size of 15 nm. The size was calculated by the Scherrer equation given below and was found in a range of 15–20 nm. XRD diffraction pattern is shown in figure 2.

$$a_0 = \frac{d_{hkl}(h^2 + k^2 + l^2)^{1/2}}{2}$$

The $a_0$ calculated from the XRD pattern is 8.2606 Å which is close to the literature value (JCPDS card file no. 76–1802). The diffraction pattern obtained from XRD analysis has strong peaks (using origin software for peak analysis) which reveals that the synthesized particles are crystalline with a size of 15 nm. The size was calculated by the Scherrer equation given below and was found in a range of 15–20 nm. XRD diffraction pattern is shown in figure 2.

$$D = \frac{k\lambda}{\beta \cos \theta}$$

| No. | 2θ  | d-Spacing | FWHM (β) | hkl values | Dislocation density \((\text{lines nm}^{-2})\) | Lattice strain (ε) |
|-----|-----|-----------|-----------|------------|----------------------------------------|------------------|
| 1   | 16.8| 5.273029  | 1.36956   | 1 1 1      | 29.10917749                           | 16.5598          |
| 2   | 30.1| 2.96655   | 8.02303   | 2 2 0      | 0.600167976                           | 20.8168          |
| 3   | 36.4| 2.466263  | 1.44984   | 3 1 1      | 6.897115123                           | 14.8700          |
| 4   | 37.9| 2.37203   | 0.6941    | 2 2 2      | 22.03899787                           | 85.3531          |
| 5   | 43.8| 2.065215  | 7.40902   | 4 0 0      | 76.06628591                           | 12.5244          |
| 6   | 54  | 1.696732  | 5.97054   | 4 2 2      | 44.3463358                           | 17.7561          |
| 7   | 60.1| 1.538275  | 2.3871    | 5 1 1      | 67.1740797                           | 6.25641          |
| 8   | 65.8| 1.418145  | 0.89965   | 4 4 0      | 6.897115123                           | 66.0969          |

Figure 1. Shows absorption spectra for the different functional groups.

Figure 2. XRD graph shows non-crystalline Co$_3$O$_4$-NPs.
Where D indicates the size of the particle (in nm), k is a constant having value of 0.9, λ is the wavelength of x-ray radiation (1.541Å), and b is the full width at half maximum (FWHM) of the peak (in radians) and 2θ is the Bragg angle (in degrees).

3.3. SEM
The morphology of Co$_3$O$_4$-NPs was determined using an SM6510LV scanning electron microscope. The SEM figure represents several irregulars and spherical Co$_3$O$_4$-NPs. The SEM picture of Co$_3$O$_4$-NPs also revealed that spherical and irregular nanoparticles with diameters ranging from 40 to 60 nm were accommodated and their size was calculated by using ImageJ software by taking a 10 μm line as a scale given in figure 3. The average size calculated for Co$_3$O$_4$-NPs was found 46 nm. The smooth surface of Co$_3$O$_4$-NPs helps it to come in contact with bacterial peptidoglycan thus facilitating bacterial killing. SEM images at different magnifications are given in figure 3.

3.4. EDX
EDX analysis was performed to know about the estimation of the elemental composition of Co$_3$O$_4$-NPs. Cobalt oxide nanoparticles show the major peaks of CO, C, and O and minor peaks of calcium, aluminum, silicon, phosphate, and chlorine. Cobalt Oxide was found at 4% and oxygen at 25% by weight. Peaks were observed for Calcium, Potassium, Chlorine, and Oxygen at different Kilo electron Volts (KeV). The Additional EDX peaks were due to the plant roots extract that reduced the Cobalt Chloride to Co$_3$O$_4$-NPs. Different extra elements which include Ca, Al, Si, P, K, Cl, and oxygen-like contaminants originated from the natural root extract of Ziziphus Oxyphylla Edgew. Due to the small ionic radius, these elements can easily diffuse along with Co$_3$O$_4$-NPs. EDX results are shown in figure 4.

3.5. Antibacterial activities
Results from the Co$_3$O$_4$-NPs antibacterial activity are presented in table 1. The antibacterial activity was performed in triplet for all three dilutions and the mean value was calculated for each dilution. Antibacterial activity was performed against the gram-negative bacteria *E. coli* and gram-positive bacteria *S. aureus*. Results revealed that Co$_3$O$_4$-NPs show a greater antibacterial activity against gram-negative as compared to the gram-positive bacteria used in antibacterial activity analysis. The zone of inhibition for *E. coli* was calculated as $23.1 \pm 1.2$ mm for 16 mg ml$^{-1}$, $19.6 \pm 1.5$ mm for 12 mg ml$^{-1}$ 14.1 $\pm$ 1.0 mm for 8 mg ml$^{-1}$ of Co$_3$O$_4$-NPs.
solution, and 0 ± 0 for water used as a negative control. For \textit{S. aureus} as 14.8 ± 1.0 mm for 16 mg ml\(^{-1}\), 11.6 ± 0.5 mm for 12 mg ml\(^{-1}\), 8.6 ± 1.5 mm for 8 mg ml\(^{-1}\) of Co\(_3\)O\(_4\)-NPs solution, and 0 ± 0 for water used as a negative control are shown in figure 5. Graphical representation of antibacterial activity results of Co\(_3\)O\(_4\)-NPs against \textit{E. coli} and \textit{S. aureus} can be seen in figure 6.

4. Discussion

Nanoparticles are particles that have a size range of 0–100 nm. They have various applications in different fields such as medical biology and catalysis. Synthesis of Cobalt oxide nanoparticles can be done through ultrasonic spray pyrolysis, thermal decomposition, and electrochemical procedures. Recently green method for nanoparticle preparation is preferred because of its simplicity, less expensive, and eco-friendly. Co\(_3\)O\(_4\)-NPs nanoparticles exhibit catalytic activity and biomedical and cytotoxic activity. They have good catalytic activity and could be efficient nanoparticles as they have high-performance permanent magnetic properties [20]. Besides this cobalt nanoparticles produced through the green method also possess anti-microbial activity that’s why they can be used in pharmaceutical products [21].

In one of the previously reported literature synthesis of Co\(_3\)O\(_4\)-NPs was performed by using leaves extract of a plant known as \textit{Populus Ciliata} (Safaida). In a recent study cobalt nitrate, and hexahydrate was utilized for the preparation of cobalt oxide nanoparticles, as a source of cobalt. Different characterization methods were used for the synthesized nanoparticles. In its FTIR analysis, various peaks were discovered, revealing distinct functional groups. A peak was obtained at a wavelength of 3458 cm\(^{-1}\) which identifies the OH group of phenolic
compounds. Some of the other major peaks which were found include 1622 cm\(^{-1}\), 1381 cm\(^{-1}\), 1082 cm\(^{-1}\), and 533 cm\(^{-1}\) which show the presence of the carbonyl group, amide group, C–O of alcohols or phenols, and Co\(_3\)O\(_4\) respectively [22].

According to an already reported literature study, the root extract of *Z. oxyphylla* Edgew had not been used in the synthesis of Co\(_3\)O\(_4\)-NPs to date. In our study, we synthesized Co\(_3\)O\(_4\)-NPs from the roots extract of *Z. oxyphylla* Edgew and perform different characterization techniques such as FTIR, SEM, XRD, and EDX for finding the nature, size, and morphology of the target nanoparticles. The FTIR spectrum of *Z. oxyphylla* Edgew root extract exhibits different peaks at the wavelength of 3458 cm\(^{-1}\) which identifies the OH group of phenolic compounds. Some of the other major peaks which were found include 3250 cm\(^{-1}\), 1636 cm\(^{-1}\), 1395 cm\(^{-1}\), 1061 cm\(^{-1}\), and 562 cm\(^{-1}\) which show the presence of hydroxyl group, carbonyl group, amide group, C–O of alcohols or phenols and Co\(_3\)O\(_4\)-NPs respectively. Different researchers observed variation in results and the possible reason for this might be differences in the manufacturing process (chemical, physical and biological methods) due to which variation occurred in our results [23].

Co\(_3\)O\(_4\)-NPs are widely used in catalysis activities and have much other biological importance. In a recent research study, cobalt oxide nanoparticles synthesis and their characterization were performed. In characterization results, it was found that in the diffraction pattern of XRD, there is no sharp peak which reveals the non-well crystalline nature of nanoparticles [22]. Using the Scherrer formula, the particle size was determined in the range of 40–50 nm. In this study, we attained a diffraction pattern that has sharp peaks which reveals that the cobalt nanoparticle is crystalline having a crystallite size of 15 nm. The size was calculated by the Scherrer equation and was found between 15–20 nm. The size of Co\(_3\)O\(_4\)-NPs in the current study is less as compared to the results of relevant literature which might be due to different manufacturing conditions like temperature, time of incubation, nature of plant extract, and handling procedures. In recent literature it is reported that increasing the temperature for calcination of Co\(_3\)O\(_4\)-NPs improves the crystallinity and structural parameters of biosynthesized Co\(_3\)O\(_4\)-NPs. High annealing temperature also results in the existence of only CO and o in EDX analysis without the presence of any other bioactive compound [24]. In This study, the XRD pattern of Co\(_3\)O\(_4\)-NPs revealed the face-centered-cubic structure of Co\(_3\)O\(_4\)-NPs, about already reported literature and JCPDS database (JCPDS card file no. 76–1802) which is indicating the synthesis of pure Co\(_3\)O\(_4\) Co\(_3\)O\(_4\)-NPs [25].

SEM analysis is used to find out the structure of the nanoparticles. The SEM image of Co\(_3\)O\(_4\)-NPs show spherical and irregular-shaped nanoparticles with a diameter range of 20–60 nm [26]. In our study SEM analysis shows several irregulars and spherical cobalt chloride nanoparticles. The SEM image of Co\(_3\)O\(_4\)-NPs shows that the nanoparticles are of irregular shape with a diameter range between 40–60 nm. The same images for Co\(_3\)O\(_4\)-NPs are also reported by many researchers [27].

Co\(_3\)O\(_4\)-NPs can cross the plasma membrane of the bacterial cell through the nonendocytic pathway and then enters the cytoplasm. Co\(_3\)O\(_4\)-NPs help in killing bacterial cells by producing free radicals inside the cell like 2, 2-diphenyl-1-picrylhydrazyl (DPPH). They can also stimulate the inhibition of protein kinase and alpha-amylase [28]. There is also another possibility as some bacteria possess negative charges on their surface and can interact with a positive charge of nanoparticles thus inhibiting the growth of bacteria. Due to the smaller size of nanoparticles, they can pass through the permeable membrane of the bacterial cell and show antibacterial activity due to the accumulation of nanoparticles in the cell membrane. It is reported that aging of the metal
oxide nanoparticles reduces their toxicity and increases their antibacterial activity. The antibacterial activity also greatly varies with the size of particles and their aggregates in colloidal solutions, the chemical composition of the media, and the suspension’s storage time[29]. Co₃O₄-NPs due to their properties can be widely used as an effective precursor against both gram-negative, and gram-positive bacteria along with other applications in other fields including the food and agriculture industry, biosensing, cosmetics, and sterilization processes[30].

Biologically synthesized Co₃O₄-NPs from Sageretia the leaves were tested for its biocompatibility analysis and it was found that Co₃O₄-NPs exhibit good biocompatibility towards RBCs as compared to macrophages that show a good free radical scavenging property. As our synthesized Co₃O₄-NPs are closely related to these biogenic synthesized nanoparticles we assume that our synthesized nanoparticles are biocompatible and can be used in several medical application including treatment of leishmaniasis and anti-cancer therapy. However, we suggest that further biocompatibility studies should be carried out for the biogenic Co₃O₄-NPs to explore more knowledge about the biocompatibility and cytotoxicity of Co₃O₄-NPs toward human cells[27].

5. Conclusion

It was concluded that root extract of Z. oxyphylla Edgew showed its ability to successfully synthesize nanoparticles at room temperature. FTIR analysis indicates confirmation about the capping of Co₃O₄-NPs and reveals reduction done by phenolic, carboxyl, and hydroxyl groups. The SEM images show that Co₃O₄-NPs have spherical and irregular morphology with a diameter range of 40–60 nm. EDX analysis showed the extent of carbon, oxygen, sodium, chlorine, calcium, and potassium in the synthesis of cobalt oxide nanoparticles. The XRD peaks reveal the nature as well the non-crystalline shape of Co₃O₄-NPs with crystallite size calculated as 15 nm. Furthermore, the synthesized Co₃O₄-NPs exhibit good antibacterial activity against the bacterial pathogen (E. coli and S. aureus). It is suggested that Co₃O₄-NPs could be of great importance in medical science for their antimicrobial activity antioxidant, antileishmanial, cytotoxic, hemolytic, larvicidal activities, anticholinergic, anti-diabetic, and anti-cancer activities.

Data availability statement

The data that support the findings of this study are openly available at the following URL/DOI: https://doi.org/https://iopscience.iop.org/article/10.1088/2053-1591/ab70dd/meta.

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Competing interests

The authors declare no competing interest.

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