

# 1 Eco-Friendly Synthesis of Zinc Oxide Nanoparticles Using *Curcuma caesia* 2 Tuber Extract and Their Antioxidant and Anticancer Activities.

3

4 **Abstract:** - The use of environmentally friendly nanomaterials for biomedical applications  
5 has received extensive attention due to their high biocompatibility and therapeutic potential.  
6 In the present study, zinc oxide nanoparticles (ZnO NPs) were synthesized by the green  
7 synthesis approach using *Curcuma caesia* Roxb. tuber extract. The synthesized nanoparticles  
8 were characterized by UV-Visible spectroscopy, Fourier Transform Infrared (FT-IR)  
9 spectroscopy, and Energy Dispersive X-ray (EDX) analysis. Formation of nanoparticles was  
10 confirmed by UV-Visible spectroscopy from the characteristic absorption peak of ZnO NPs.  
11 FT-IR analysis indicated the involvement of different functional groups in the synthesis and  
12 stabilization of nanoparticles. The FESEM analysis confirmed the particle average size  
13 29.25nm. The elemental composition and purity of the synthesized nanoparticles were  
14 confirmed by EDX analysis. The antioxidant potential of the biosynthesized ZnO  
15 nanoparticles was evaluated using free radical scavenging assays, showing concentration-  
16 dependent antioxidant activity. Furthermore, the anticancer activity of the ZnO nanoparticles  
17 was studied against human breast adenocarcinoma (MCF-7) cells by MTT assay. The ZnO  
18 NPs exhibited good cytotoxic activity ( $IC_{50}$  104  $\mu$ g/mL) and significantly reduced cell  
19 viability in a dose-dependent manner. The observed anticancer effect might be due to the  
20 increased generation of reactive oxygen species, leading to oxidative stress and subsequent  
21 induction of apoptotic pathways in the cancer cells. The results of this study showed that  
22 *Curcuma caesia* mediated ZnO nanoparticles exhibit significant antioxidant and anticancer  
23 activities and can be used as a potential candidate for future therapeutic applications based  
24 on nanomedicine.

25 **Keywords:** Zinc-oxide nanoparticles (ZnO NPs), *Curcuma caesia*, Antioxidant activity,  
26 Anticancer activity, Breast cancer- MCF-7 cells.

## 27 **Introduction:**

28 Cancer remains one of the top causes of death worldwide, and breast cancer is the most  
29 common cancer diagnosed in women. Despite great advances in diagnosis and treatment,  
30 conventional therapeutic strategies such as chemotherapy, radiotherapy, and surgery are

31 often associated with adverse side effects, multidrug resistance, and damage to healthy  
32 tissues(Sung *et al.*, 2021). Therefore, the development of new therapeutic agents that are  
33 effective, biocompatible, and environmentally sustainable is gaining increasing interest.  
34 Nanotechnology is a promising interdisciplinary area with broad applications in medicine,  
35 particularly in cancer diagnosis and therapy(Hassan *et al.*, 2022). Among different metal and  
36 metal oxide nanoparticles, zinc oxide nanoparticles (ZnO NPs) have received significant  
37 attention due to their unique physicochemical properties, such as high surface-to-volume  
38 ratio, biocompatibility, semiconducting behavior, and selective toxicity to cancer  
39 cells(Anjum *et al.*, 2021). ZnO NPs have shown various biological activities such as  
40 antimicrobial, antioxidant, anti-inflammatory, and anticancer activities. The anticancer  
41 potential of these compounds is mainly due to the generation of reactive oxygen species  
42 (ROS), induction of oxidative stress, mitochondrial dysfunction, DNA damage, and  
43 activation of apoptosis pathways in malignant cells(Ruddaraju *et al.*, 2019). Green synthesis  
44 of nanoparticles using plant extracts has drawn much attention in recent years as an  
45 environmentally benign alternative to the traditional chemical and physical  
46 methods(Labaran *et al.*, 2024).The plant-mediated synthesis does not require any toxic  
47 reducing and stabilizing agents, and thus it is cost-effective, sustainable, and applicable for  
48 biomedical applications. The plant extract has phytochemicals like phenolics, flavonoids,  
49 terpenoids, alkaloids, and proteins, which act as natural reducing and capping agents during  
50 the nanoparticle formation(Ojoet *et al.*, 2021).

51 *Curcuma caesia*Roxb., is a medicinal plant of the family Zingiberaceae and is popularly  
52 known for its therapeutic properties. *C. caesia* rhizomes are rich in diverse bioactive  
53 compounds, including curcuminoids, flavonoids, essential oils, and phenolic constituents  
54 with antioxidant, antimicrobial, anti-inflammatory, and anticancer activities(Gangal *et al.*,  
55 2025). These phytochemicals may be useful in the synthesis of stable nanoparticles and may  
56 improve their biological efficiency. Oxidative stress is a major factor in the initiation and  
57 progression of many diseases, including cancer(Jayaprakasha, Rao and Sakariah, 2002).  
58 Hence, antioxidant agents that are able to scavenge free radicals are of great therapeutic  
59 interest. The plant-mediated ZnO NPs showed excellent antioxidant activity because of the  
60 synergistic effect of zinc oxide and the phytochemical residues adsorbed on the surface of  
61 the nanoparticles(Yilmaz *et al.*, 2023). Further, a number of studies have demonstrated the  
62 selective inhibition of breast cancer cell proliferation by ZnO NPs through ROS-induced  
63 apoptosis and cell cycle arrest. The human breast adenocarcinoma cell line, MCF-7, is  
64 commonly used as an in vitro model to investigate the anticancer potential of novel

65 therapeutic agents(Harbeck *et al.*, 2019). The cytotoxic and apoptotic effects of  
66 biosynthesized ZnO NPs on MCF-7 cells were evaluated to understand their potential as  
67 anticancer nanotherapeutics(Miri *et al.*, 2019). The physiological behavior of ZnO  
68 nanoparticles is significantly influenced by their size, shape, surface charge, and production  
69 process. Nanoparticles synthesized by eco-friendly methods frequently exhibit enhanced  
70 biological capabilities due to the presence of phytochemical residues on their surfaces.  
71 These bioactive compounds function as natural capping agents to enhance the stability of  
72 nanoparticles and facilitate interaction with biological systems(Fais *et al.*, 2024).  
73 Furthermore, green-synthesized ZnO nanoparticles are normally considered safer for  
74 biomedical applications due to the lack of toxic chemicals commonly used in traditional  
75 synthesis processes. The antioxidant efficacy of plant-mediated ZnO nanoparticles is  
76 typically attributed to the synergistic interaction between zinc oxide and phytochemicals  
77 adsorbed on the nanoparticle surface(Rajeshkumar *et al.*, 2018). This synergistic interaction  
78 may augment free radical scavenging ability and result in improved biological efficacy.  
79 Breast cancer remains a relevant issue for global health, representing a large proportion of  
80 cancer-related morbidity and mortality among women. The emergence of resistance against  
81 conventional chemotherapeutic agents and the development of severe adverse effects  
82 indicate the urgent need for exploration of alternative treatment modalities(GLOBOCAN,  
83 2020). The innovative approaches based on the use of nanotechnology have appeared as an  
84 efficient solution for cancer treatment owing to their ability to selectively target tumor cells  
85 and to minimize damage to healthy tissues. In this context, ZnO nanoparticles have shown  
86 great promise as anti-cancer agents, due to their ability to preferentially accumulate in  
87 cancer cells and induce programmed cell death(Li *et al.*, 2020). Several mechanisms have  
88 been proposed to explain the anticancer properties of ZnO nanoparticles. One of the most  
89 accepted mechanisms is the production of intracellular reactive oxygen species, leading to  
90 oxidative stress, disruption of the mitochondrial membrane, DNA fragmentation, and  
91 activation of apoptotic signaling pathways(Zhang *et al.*, 2015).

92 Recent advances in green nanotechnology have highlighted the significance of combining  
93 medicinal plants and nanoparticle synthesis to create multifunctional nanomaterials(Jain et  
94 al., 2021). The plant-derived nanoparticles show better stability and may also possess the  
95 therapeutic property of phytochemicals involved in their synthesis. The use of *Curcuma*  
96 *caesia*-mediated ZnO nanoparticles is an innovative way to couple the medicinal value of  
97 black turmeric and the unique physicochemical properties of zinc oxide nanoparticles and is

98 expected to result in improved antioxidant and anticancer efficacy. Nanotechnology is a  
99 developing interdisciplinary field that is applied to medicine for the better diagnosis,  
100 prevention, and treatment of disease. The application of nanoscale materials, typically 1-100  
101 nm in size, has transformed biomedical research, resulting in the creation of advanced  
102 therapeutic and diagnostic systems(Shrestha et al., 2024).One of the most studied  
103 applications for cancer therapy has been nanomedicine. Conventional anticancer treatments  
104 are usually characterized by non-specific distribution, systemic toxicity, poor therapeutic  
105 efficacy and the development of drug resistance(Acharya et al., 2024). Therapeutic  
106 approaches based on nanotechnology have important advantages in the selective  
107 accumulation of therapeutic agents at the tumor sites, thus improving the efficacy of  
108 treatment and reducing damage to healthy tissues.

## 102. Methodology

### 110 2.1 Plant material

111 Rhizomes of *Curcuma caesia* are native to Nagaland; they were collected from a farmer in  
112 West Imphal and cultivated in Sangli, Maharashtra, and authenticated by BSI.

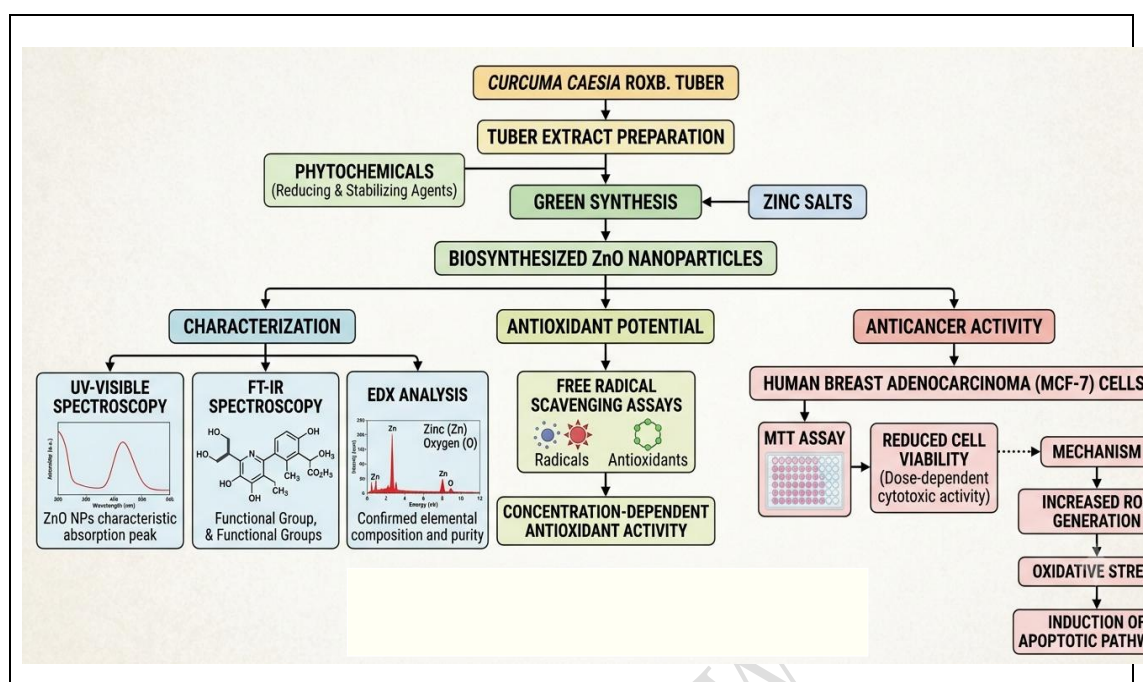
### 113 2.2 Preparation of plant extract:

114 To obtain a uniform range of *Curcuma caesia* rhizome powder, the rhizomes were broken  
115 up and ground into a powder in a mixer before being sieved. A 200 mL flask was filled with  
116 5 grams of powdered plant extract, followed by 100 mL of methanol. The mixture was  
117 shaken thoroughly and then left for 24 hours(Klein *et al.*, 2023). Figure 1 depicts the entire  
118 sample extraction procedure.



119  
120 **Figure 1.** Sample extraction process: (a) Dried *Curcuma caesia* Rhizome, (b) fine powdered  
121 dried *Curcuma caesia*, and (c) methanol extract of *Curcuma caesia*.

122



124 **Figure 2:** Schematic representation of the biosynthesis of zinc oxide (ZnO)  
 125 nanoparticles using *Curcuma caesia* tuber extract, followed by spectroscopic  
 126 characterization (UV–Vis, FT-IR, EDX), evaluation of antioxidant potential via free  
 127 radical scavenging assays, and assessment of anticancer activity against MCF-7 breast  
 128 cancer cells through MTT assay, highlighting ROS generation and apoptosis  
 129 induction.

130

### 131 2.3 Green Synthesis of Zinc Oxide Nanoparticles

132 The extract of *Curcuma caesia* tuber was used as a reducing and stabilizing agent for the  
 133 synthesis of zinc oxide nanoparticles (ZnO NPs). A 0.5 M solution of zinc acetate dihydrate  
 134 was prepared by dissolving the appropriate amount of zinc nitrate in 100mL of distilled  
 135 water under continuous magnetic stirring. The extract was added subsequently to the zinc  
 136 acetate dihydrate solution with continuous stirring. Synthesis of ZnO nanoparticles. The  
 137 reaction mixture was magnetically stirred for 2 h at 70 °C. At the end of the reaction, a pale  
 138 white precipitate was produced and was collected by centrifugation. The resulting precipitate  
 139 was washed several times with distilled water. The product was purified and dried to obtain  
 140 ZnO nanoparticles in the form of pale-yellow powder. The dried powder was annealed in a  
 141 muffle furnace at 400 °C for 2 hr to improve its crystallinity and phase purity (Fig. 2)  
 142 (Reddy *et al.*, 2016).

143

### 144 2.4. Analytical characterization

145 The formation of zinc oxide nanoparticles (ZnO NP) was confirmed preliminarily using  
146 UV–Visible spectrophotometry in the wavelength range of 200–800 nm. Energy Dispersive  
147 X-ray (EDX) spectroscopy was used for elemental analysis of the synthesized nanoparticles  
148 and samples were mounted on a copper grid for analysis. The functional groups responsible  
149 for the synthesis and stabilization of the nanoparticles were confirmed by recording the  
150 Fourier Transform Infrared (FT-IR) spectra. Spectra were recorded from 4000 to 400 cm<sup>-1</sup>  
151 with 128 scans per sample. The results obtained by these characterization techniques  
152 provided useful information on the optical properties, elemental composition, and surface  
153 chemistry of the synthesized nanoparticles, thus giving a complete understanding of their  
154 physicochemical characteristics(Dey *et al.*, 2024).

155

### 156 **2.5 Anti-Oxidant Activity**

157 The antioxidant activity was assessed by using a previous method reported by Puri, Patil,  
158 and Sonawane (Sonawane *et al.*, 2021; Puri and Patil, 2022). 5 µL of each test stock solution  
159 was placed in 0.1 mL of 0.1 mM DPPH solution inside a 96-well plate. The assignment was  
160 completed alongside blanks and samples with various concentrations of the 0.2 mL  
161 methanol sample. The treated wells were for control, but blanks were in the untreated wells.  
162 Following incubation in the dark for 30 minutes, the decolorization was measured at 517 nm  
163 with an iMarkBioRad microplate reader. Scavenging activity was represented as %  
164 inhibition over the control, and IC<sub>50</sub> was determined from GraphPad Prism 6 by plotting  
165 sample concentration on the X-axis and % inhibition on the Y-axis.

166

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of control}} \times 100$$

167

168

### 169 **2.6 Cytotoxic Activity**

170 The toxic impact of ZnO NPs against the MCF-7 breast carcinoma cell line, derived from  
171 NCCS Pune, was assessed using the MTT assay. In DMEM media enriched with a 10% fetal  
172 bovine serum or 1% antibiotic liquid at 37°C with 5% CO<sub>2</sub>, the cells (10000 cells/well) were  
173 grown using 96-well plates for 24 hrs. The cells were then exposed to different formulation  
174 quantities at a later incubation. MTT solution (5 mg/mL) was added to the cultures and were  
175 incubated for adhering, to another 24 hours. The cell layer matrix in 100 µl of dimethyl  
176 sulfoxide (DMSO) was eliminated once the experiment was over. The resulting optical  
177 density at a wavelength of 540 nm was estimated with an ELISA plate reader (iMark, Bio-  
178 Rad, USA). GraphPad Prism 6 enabled the precise calculation of the IC<sub>50</sub> value. An inverted

179 microscope (Olympus EK2) and an AmScope digital camera (10MP Aptina CMOS) served  
180 to record images of excellent quality (Khorrami *et al.*, 2018).

181

## 182 **2.7 Apoptosis Activity**

183 At their IC<sub>50</sub> concentrations, Zinc nanoparticles were added to cells. We washed the  
184 treated and untreated control cells once more with cell staining buffer before putting the  
185 cells back in Annexin V binding buffer at a concentration of  $0.25-1.0 \times 10^7$  cells/ml.  
186 We transferred 100  $\mu$ L of cell suspension to a tube, then added 5  $\mu$ L of FITC Annexin V  
187 and 10  $\mu$ L of Propidium Iodide Solution. The cells were gently mixed and incubated in  
188 the dark at room temperature (25°C) for 15 minutes. Finally, 400  $\mu$ L of Annexin V  
189 Binding Buffer was added to each tube, and they were analyzed with flow cytometry  
190 (Ullah *et al.*, 2020).

## 191 **2.8. Statistical analysis**

192 The cytotoxic activity of plant extract and ZnO NPs were performed in triplicate. The  
193 data were subjected to one way analysis of variance by statistical package SPSS 16.0.  
194 The means were compared by DMRT test at the significance level 0.05.

195

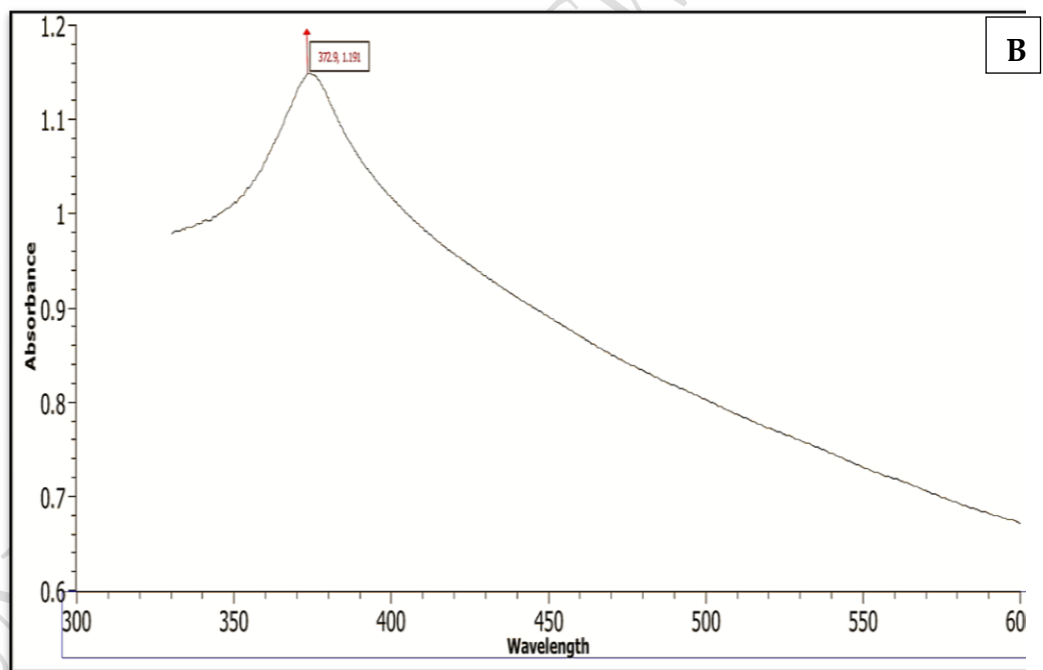
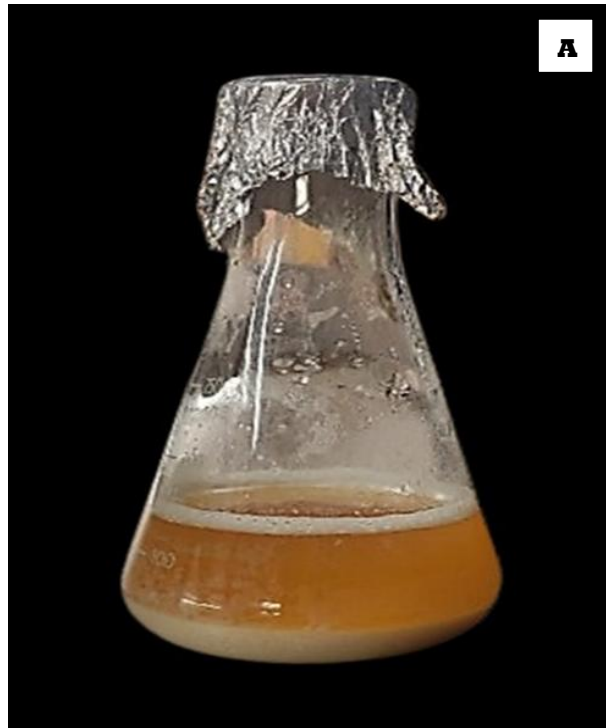
## 196 **3. Results:**

197

### 198 **3.1. Characterization:**

#### 199 **3.1.1. UV -Visible spectroscopy:**

200 The first sign of a successful synthesis of ZnO NPs was the formation of a yellow precipitate  
201 after the addition of the plant extract to the zinc acetate dihydrate (Figure 3). UV-visible  
202 spectrophotometric measurement after 2 h of incubation showed the absorption peaks of  
203 extract-to-zinc acetate at 379.6 nm, which confirmed the presence of ZnO NPs.



204 **Figure 3:** The green synthesis of (A) ZnO NPs (B)The UV- spectra graph of ZnO NPs.

205

206

207

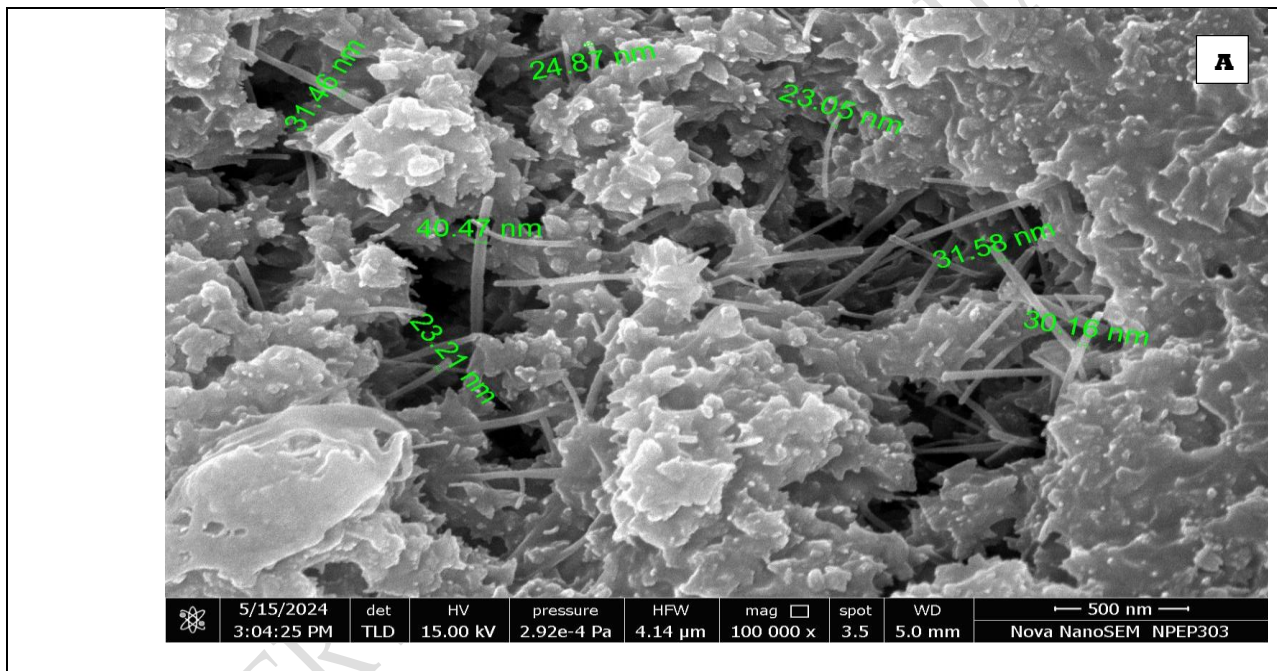
### 3.2.2. FESEM-EDS Analysis:

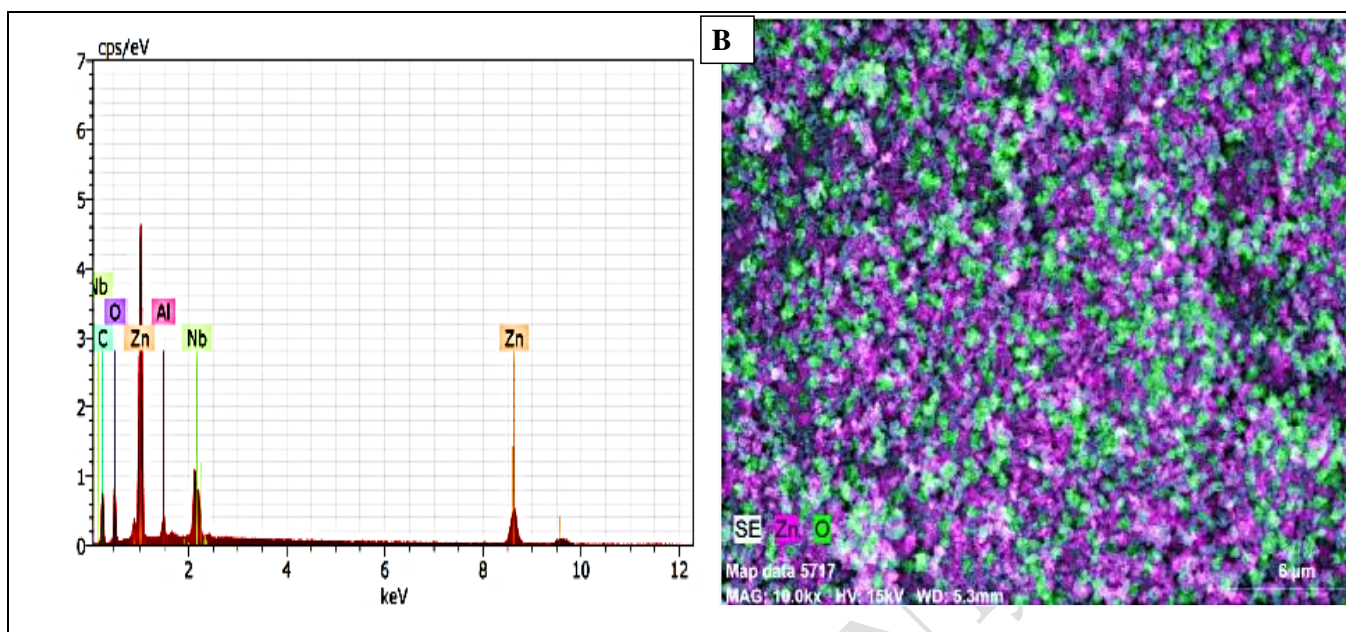
209 The phytochemical-mediated biosynthesized ZnO nanoparticles are spherical shape with

210 average size was 29.25nm (Figure 4A). Although significant aggregation was seen, most

211 likely as a result of the particle surfaces not being fully stabilized. Furthermore, the

212 existence of nanorod-like structures raises the possibility that anisotropic development,  
213 which synthesized heterogeneous particle morphologies, was influenced by an excess of  
214 capping agents in the reaction media. The high purity of ZnO nanoparticles with Zn and O  
215 content of 92.54% was confirmed by SEM-EDS analysis and was further stabilized with  
216 phytochemical residues of the *Curcuma caesia* extract (Figure 4B). The complementary  
217 XRD analysis confirmed the crystalline nature of the nanoparticles with strong diffraction  
218 peaks consistent with the hexagonal wurtzite structure of ZnO. The combined results show  
219 that the green synthesis method produces crystalline ZnO nanoparticles with different  
220 morphologies, which are governed by the interplay of reaction conditions and phytochemical  
221 capping agents with high purity.



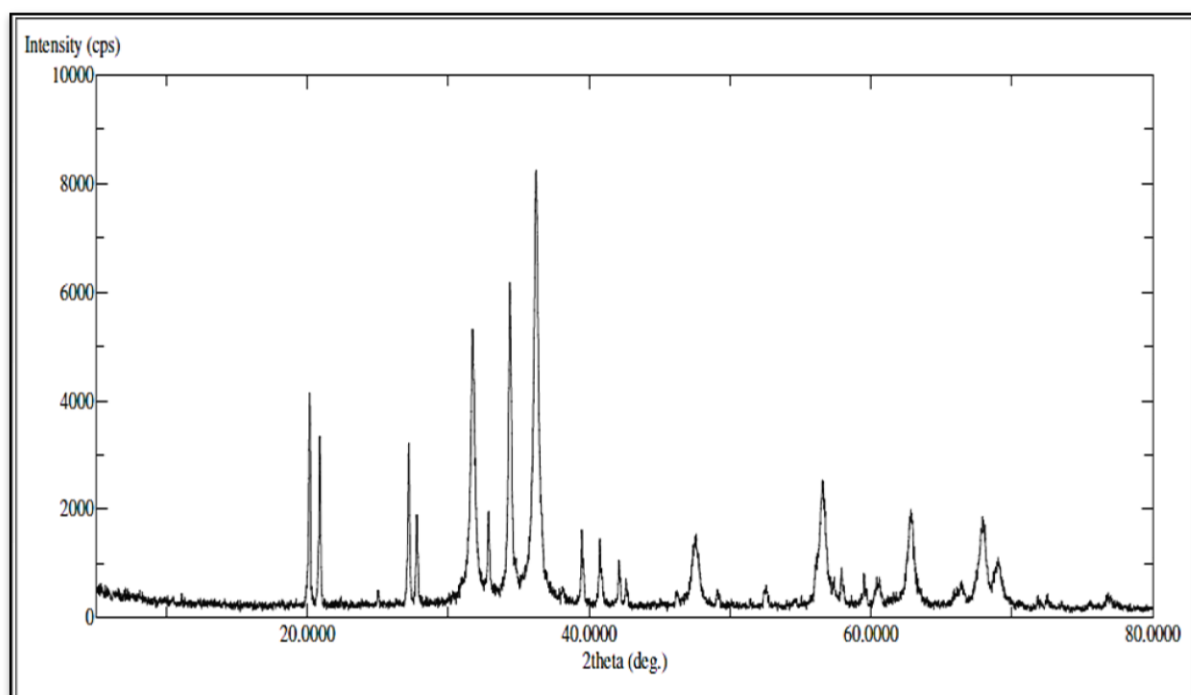


222 **Figure 4:**(A) The FESEM analysis of ZnO NPs (B) EDS analysis of ZnO NPs.

223

3224 **XRD Analysis: -**

225 XRD analysis of ZnO nanoparticles synthesized using zinc acetate and Curcuma caesia  
 226 extract revealed a prominent peak at  $36.2^\circ$  (101 plane), accompanied by minor shifts at  
 227  $36.240^\circ$ ,  $36.200^\circ$ , and  $36.140^\circ$ . These observations indicate potential variations in crystallite  
 228 size or internal strain. The observed pattern matched the JCPDS card number 0361451 (Bala  
 229 et al., 2015), thereby confirming the synthesis of pure ZnO nanoparticles with a rod-like  
 230 structure (Figure 5).



231 **Figure 5:** The XRD photograph of Zinc NP

232

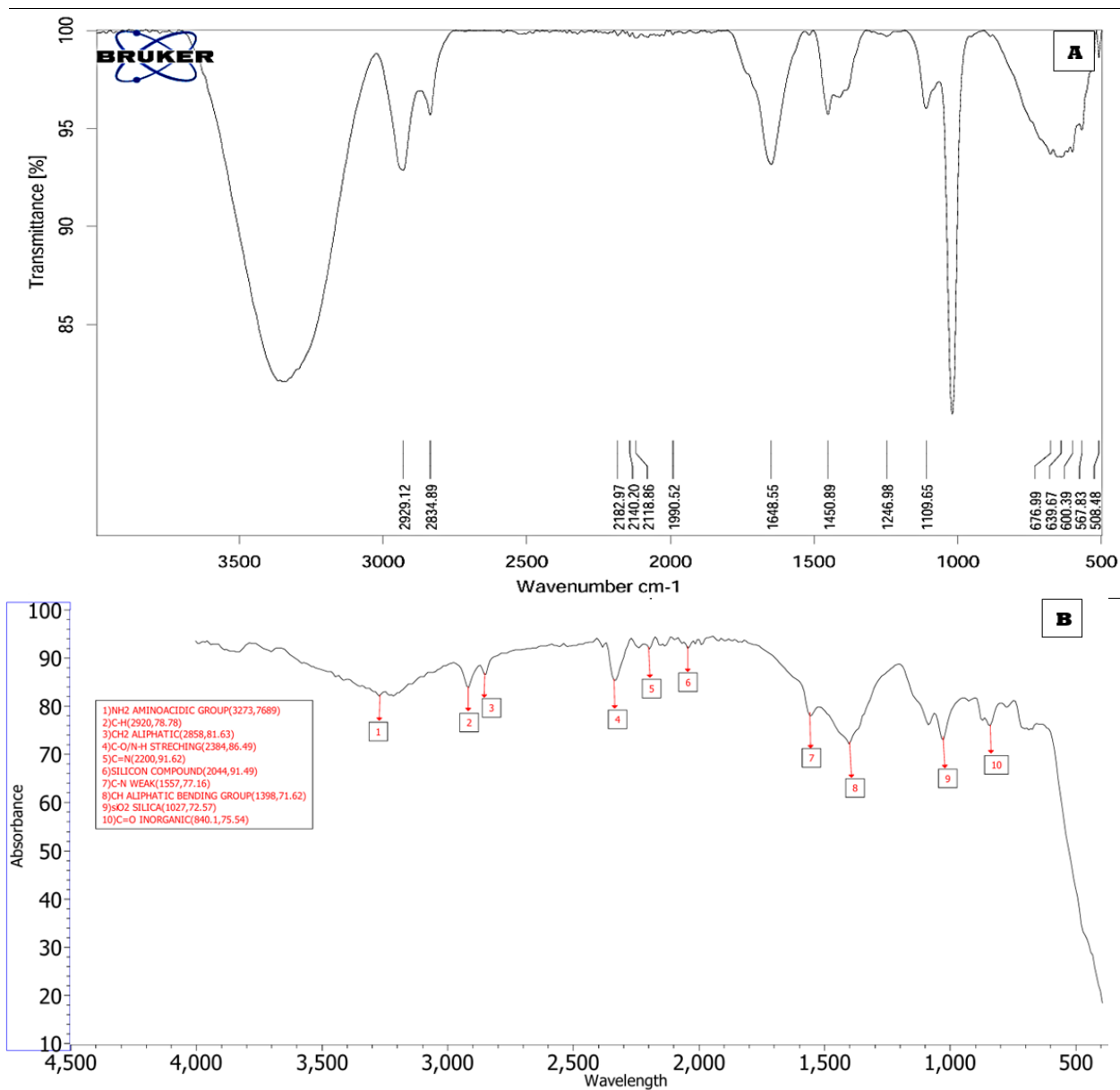
233 **3.1.4. FTIR Analysis:**

234 The FTIR analysis of the plant extract revealed distinct absorption bands at 3290, 2929,  
235 2835, 1646, 1450, 1246, and 1109  $\text{cm}^{-1}$ , which are associated with hydroxyl, aliphatic C–H,  
236 aromatic C=C, and C–O functional groups (Figure 6). These findings reveal the presence of  
237 phenolic compounds, flavonoids, terpenoids, and polysaccharides, which could serve as  
238 reducing and capping agents during nanoparticle biosynthesis. FTIR analysis of ZnO  
239 nanoparticles capped with phytochemicals from *Curcuma caesia* demonstrated the presence  
240 of various functional groups derived from the plant extract, highlighting their significant role  
241 in the reduction and stabilization of the nanoparticles. The findings offer a solid foundation  
242 for comprehending the role of compounds derived from plants.

243

244

245



246 **Figure 6:**The graph of FTIR analysis of (A)Plant Extract and (B) zinc NPs

247

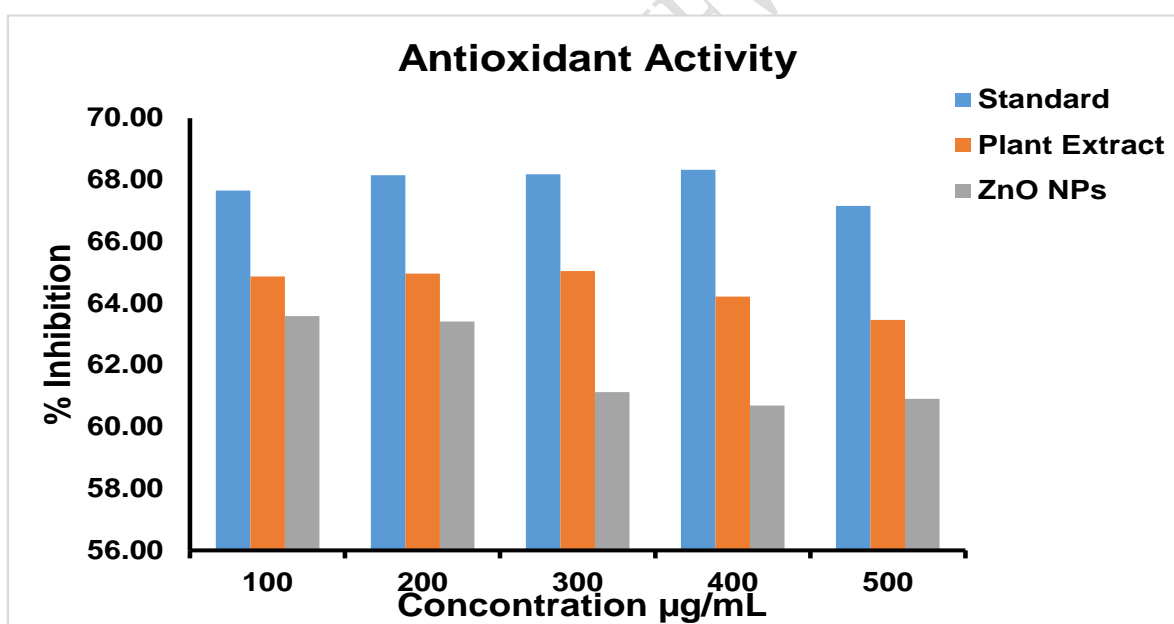
248

### 249.2. Antioxidant Activity:

250 The antioxidant efficacy of the *Curcuma caesia* tuber extract and the green-synthesized ZnO  
 251 nanoparticles (ZnO NPs) was assessed and contrasted with a standard antioxidant across a  
 252 concentration of 100–500  $\mu\text{g/mL}$ . The figure 7 shows that the standard exhibited the greatest  
 253 free radical scavenging activity, with a percentage inhibition ranging from approximately  
 254 67.5% to 68.2%. The plant extract exhibited continuously elevated antioxidant capacity,  
 255 with inhibitory values of approximately 64.8–65.0% at lower concentrations (100–300  
 256  $\mu\text{g/mL}$ ), followed by a minor reduction to 64.1% and 63.4% at 400 and 500  $\mu\text{g/mL}$ ,  
 257 respectively. The ZnO nanoparticles demonstrated modest antioxidant activity, with

258 inhibition values between 60.6% and 63.5%. The peak activity of ZnO nanoparticles  
259 occurred at 100  $\mu\text{g}/\text{mL}$  ( $\approx 63.5\%$ ), followed by a slow decline, resulting in around 60.7–  
260 60.9% at elevated concentrations. The stronger antioxidant activity of the plant extract is due  
261 to the high concentration of bioactive phytochemicals, including phenolics, flavonoids,  
262 terpenoids, and other reducing compounds found in *C. caesia*, recognized for their capacity  
263 to donate electrons or hydrogen atoms and neutralize free radicals. The observed results  
264 indicate that the biosynthesized ZnO nanoparticles exhibit the antioxidant effects conferred  
265 by plant metabolites while also developing distinct physicochemical characteristics linked to  
266 their nanoscale shape. The results demonstrate that both the plant extract and green-  
267 synthesized ZnO nanoparticles exhibit considerable antioxidant activity, with the plant  
268 extract displaying slightly greater efficacy, underscoring the potential of *C. caesia*-mediated  
269 ZnO nanoparticles as a viable natural antioxidant and candidate for nanomedicine in  
270 biomedical applications.

271



272

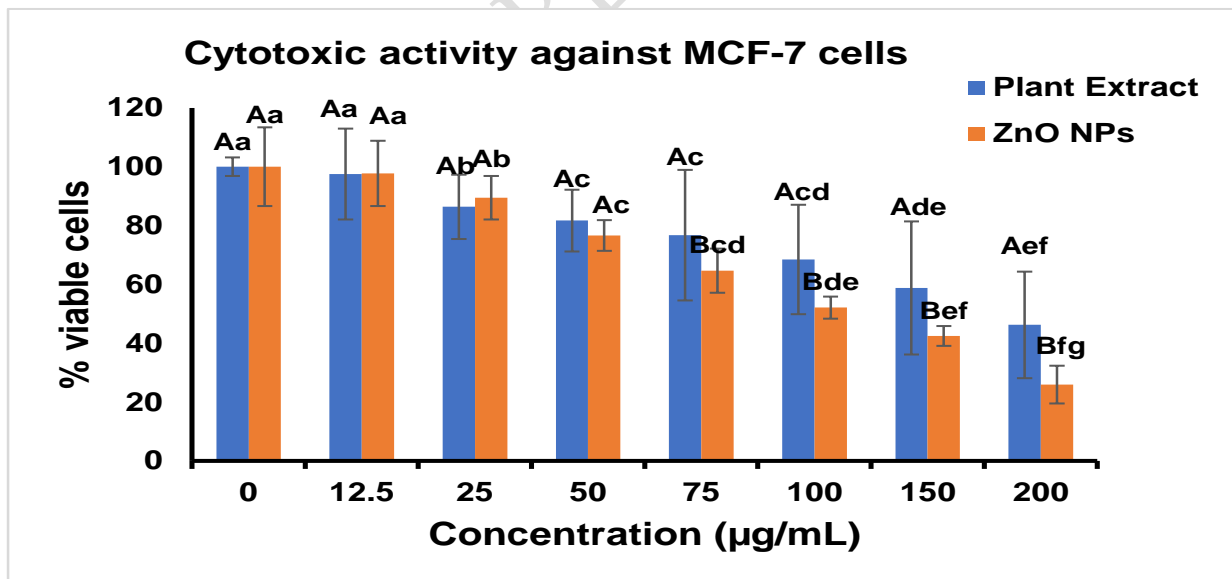
273 **Figure 7.** Antioxidant activity of standard, plant Extract and ZnONPs.

274

### 275 3.3. Cytotoxic activity:

276 The cytotoxic efficacy of the plant extract and biosynthesized zinc nanoparticles (ZnONPs)  
277 against MCF-7 breast cancer cells was assessed via the MTT test at doses between 12.5 and  
278 200  $\mu\text{g}/\text{mL}$ . Both treatments demonstrated a concentration-dependent decrease in cell  
279 viability; however, ZnONPs showed significantly stronger cytotoxic effects than the crude  
280 plant extract (Figure 8 and 9). At the maximum evaluated concentration (200  $\mu\text{g}/\text{mL}$ ), the

281 plant extract inhibited cell viability to 46.24%, whereas ZnNPs significantly lowered  
282 viability to 25.93%. At a concentration of 150  $\mu\text{g/mL}$ , cell viability was 58.78% for the plant  
283 extract and 42.45% for ZnONPs. The increased cytotoxicity of ZnONPs was apparent at all  
284 concentrations beyond 25  $\mu\text{g/mL}$ , demonstrating that nanoparticle manufacturing  
285 significantly augmented the anticancer efficacy of the bioactive phytochemicals. The  
286 superior effectiveness of ZnONPs can be ascribed to their nanoscale dimensions, augmented  
287 surface area, improved cellular absorption, and capacity to produce reactive oxygen species  
288 (ROS), resulting in oxidative stress, mitochondrial impairment, and death in cancer cells.  
289 The findings indicate that green-synthesized ZnONPs have enhanced antiproliferative  
290 activity against MCF-7 cells relative to the associated plant extract, underscoring their  
291 potential as viable nanomedicine candidates for breast cancer treatment. The determined  $\text{IC}_{50}$   
292 value for the plant extract was roughly 182  $\mu\text{g/mL}$ , whereas the ZnONPs demonstrated a  
293 lower  $\text{IC}_{50}$  value of about 104  $\mu\text{g/mL}$ , thereby substantiating the superior cytotoxic efficacy  
294 of the biosynthesized zinc nanoparticles against MCF-7 cells. The reduced  $\text{IC}_{50}$  value  
295 signifies that a lesser concentration of ZnONPs was necessary to impede 50% of cell  
296 proliferation, illustrating their superior therapeutic efficacy compared to the crude extract.  
297

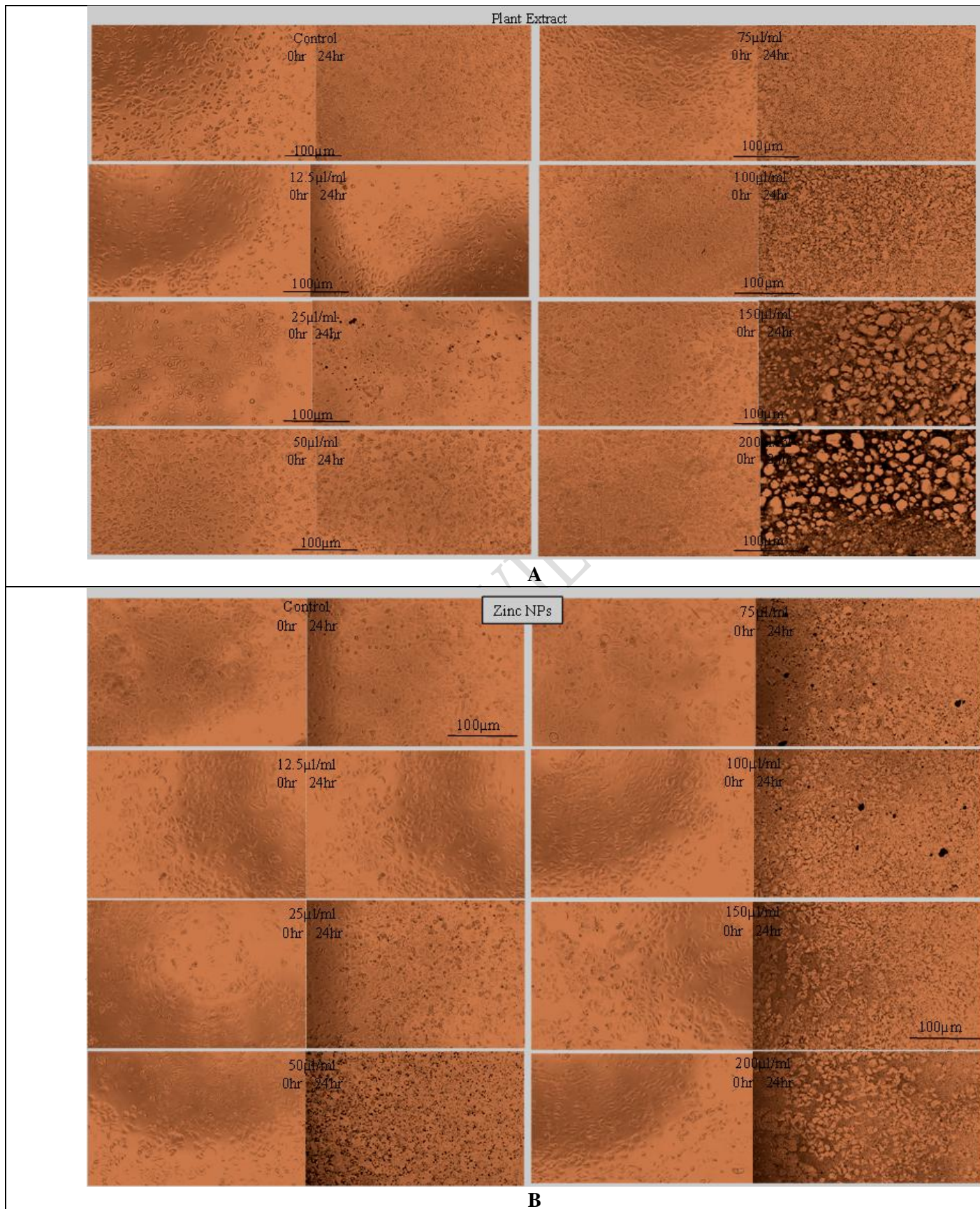


298

299 **Figure 8.** Cytotoxic activity of Plant Extract and ZnONPs.

300

301



302

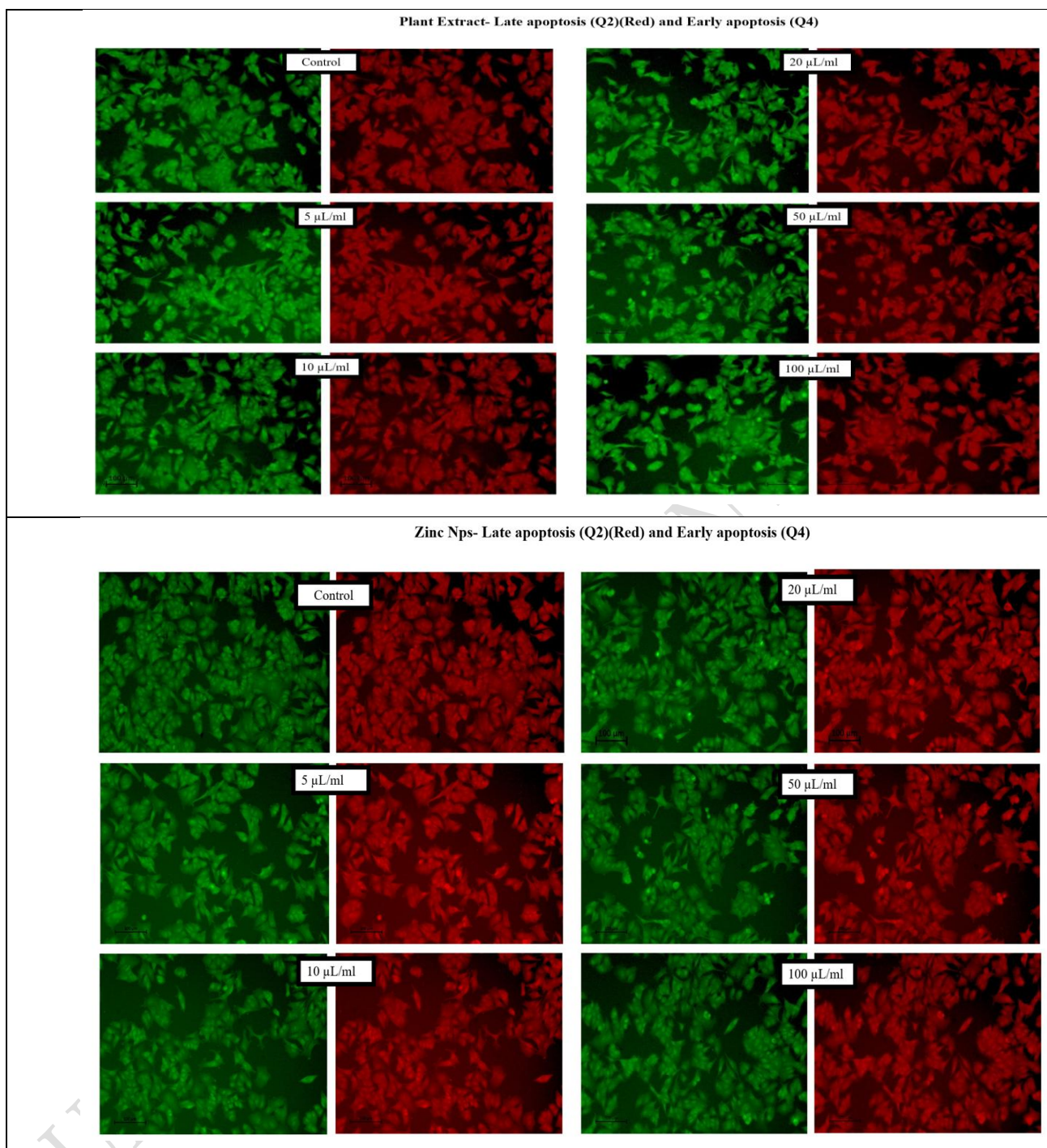
**Figure 9.** Cytotoxic analysis of (A) Plant Extract and (B) zinc NPs

303

#### 303.4. Apoptosis Activity:

305 The apoptotic activity of the plant extract and zinc nanoparticles (ZnO NPs) was determined  
306 through fluorescence staining and apoptotic population analysis. The untreated control cells  
307 exhibited predominantly viable morphology with few apoptotic features, indicating normal  
308 cellular integrity. On the other hand, the treatment with plant extract was found to induce  
309 concentration-dependent stimulation of apoptosis as revealed by increased chromatin  
310 condensation and nuclear fragmentation as characteristic markers of early and late apoptosis,  
311 respectively (Figure 10). Fluorescence micrographs revealed a gradual increase in apoptotic  
312 cells with increasing concentrations of the extract (5–100  $\mu\text{L}/\text{mL}$ ), which was further  
313 confirmed by a significant shift in cell populations towards the apoptotic quadrants in the  
314 scatter plot analysis. Similarly, treated cells with ZnO NPs showed a significant increase in  
315 the apoptotic activity as compared to the control group. The apoptotic response increased  
316 with the concentration of nanoparticles, showing a higher percentage of cells undergoing  
317 early and late stages of apoptosis. Interestingly, the apoptotic effect of ZnO NPs was higher  
318 in comparison to the plant extract at the same concentrations, which revealed a more  
319 efficient cytotoxic effect. The increased activity is attributed to the role of nanoparticles in  
320 causing oxidative stress, impairing mitochondrial function and activating pathways related  
321 to apoptosis. The observed enhancement in chromatin condensation, alteration in membrane  
322 architecture and nuclear fragmentation strongly suggest that the ZnO NPs successfully  
323 induce programmed cell death. Apoptosis was induced by both the plant extract and ZnO  
324 NPs in a dose-dependent manner, but the apoptotic potential of ZnO NPs was higher. These  
325 results suggest that the formulation of nanoparticles can possibly increase bioactivity of  
326 plant-derived components to improve their effectiveness against cancer by inducing  
327 programmed cell death.

328



329 **Figure 10.** The fluorescence microscopic images of apoptosis analysis of plant extract and  
 330 ZnO NPs.

331

### 332 **Discussion**

333 In the current study, zinc oxide nanoparticles (ZnO NPs) were prepared efficiently in an eco-  
 334 friendly manner by a green method using *Curcuma caesia* tuber extract. The extracted  
 335 phytochemicals may be involved in the reduction and stabilization of nanoparticles such as  
 336 phenolics, flavonoids, terpenoids, and alkaloids. The change of colour during synthesis was  
 337 indicative of the formation of ZnO nanoparticles, which was later confirmed by

338 physicochemical characterisation. UV–Visible spectroscopy confirmed the production of  
339 ZnO nanoparticles by a distinct absorption peak. This study synthesized ZnO nanoparticles  
340 using extracts from *Acacia catechu*, *Artemisia vulgaris*, and *Cynodondactylon*. The  
341 produced ZnO nanoparticles exhibited an ultraviolet-visible spectrum at 370 nm, as  
342 reported(Acharya *et al.*, 2024)

343 FTIR analysis revealed the presence of hydroxyl, carbonyl, amine, and aromatic  
344 functional groups, indicating involvement of plant metabolites in the reduction and  
345 stabilization of nanoparticles. The work confirms that ZnO nanoparticles are formed by  
346 using *Lippiaadoensis* extract as a reducing and capping agent(Demissie *et al.*, 2020).The  
347 research indicates that green synthesis significantly affects the bioavailability of seed  
348 germination and growth characteristics in mung beans. *Curcuma longa* tubers were utilized  
349 to produce zinc nanoparticles, which were then analyzed using FT-IR to identify the  
350 numerous distinctive functional groups associated with the synthesized nanoparticles(Reddy  
351 *et al.*, 2016).

352

353 X-ray diffraction study shows the high purity and crystalline nature of the rod-shaped ZnO  
354 structure. SEM photos showed a very homogeneous shape with minor agglomeration, while  
355 EDX spectra confirmed the predominance of zinc and oxygen as the main constituents.The  
356 study highlights the environmentally friendly phytosynthesis of zinc oxide nanoparticles  
357 (ZnONPs) utilizing flower extract from *Candelabra cacti*. Using a variety of instruments, the  
358 structural and functional characteristics of the produced ZnONPs were investigated.  
359 Analysis using transmission electron microscopy showed sphere-shaped ZnONPs with sizes  
360 ranging from 10 to 30 nm(Govarthananet *al.*, 2020).

361 Both *Curcuma caesia* tuber extract and green-synthesized ZnO nanoparticles have  
362 significant free radical scavenging activity, but the plant extract is slightly more effective  
363 due to its high phenolic, flavonoid, and other bioactive phytochemical content. ZnO  
364 nanoparticles have moderate antioxidant activity due to phytochemical capping agents  
365 adsorbed on their surfaces during biosynthesis, which preserve the parent extract's  
366 antioxidant properties. ZnO NPs may have lower activity than crude extract because  
367 nanoparticle formation partially utilizes these compounds. These findings are consistent with  
368 previous reports on plant-mediated ZnO nanoparticles and suggest that *C. caesia*-  
369 *derived*ZnO NPs may be promising biomedical and nanomedicine antioxidants. The study  
370 indicated that the observed results suggest that the free radical scavenging activity may be  
371 ascribed to the high levels of phenolics and flavonoids with enhanced reducing

372 capacity(Tanvir *et al.*, 2017).This review examines the biomedical applications of metal  
373 oxide and ZnO nanomaterials in experimental, preclinical, and clinical stages of  
374 development. The benefits, methods, and restrictions related to the use of metal oxide  
375 nanoparticles for drug delivery and cancer applications are discussed. This article focuses on  
376 ZnO and other metal oxide nanomaterial systems, their suggested mechanisms of cytotoxic  
377 action, and current strategies to enhance their cytotoxicity and targeting against cancer  
378 cells(Tayyeb *et al.*, 2024). The antitumor activity of ZnO nanoparticles was examined on  
379 MCF-7 breast cancer cells by the MTT test. The nanoparticles demonstrated a significant  
380 decrease in cell viability in a dose-dependent manner and were more cytotoxic in  
381 comparison to the plant extract alone. The enhanced anticancer effect might be due to higher  
382 uptake by cells and interaction with intracellular targets, as well as oxidative stress through  
383 ROS generation, causing cellular damage. Annexin V staining was used to confirm the cell  
384 death mechanism for apoptosis studies. Treated MCF-7 cells exhibited typical features of  
385 apoptosis, such as an increased number of Annexin V-positive cells, cell contraction, and  
386 membrane blebbing. The results suggest that apoptosis plays a critical role in the  
387 cytotoxicity of ZnO NPs, and the nanoparticle formulation enhances the pro-apoptotic  
388 effects of phytochemicals of *C. caesia*. The results show the possible antioxidant and  
389 anticancer activities of the ZnO nanoparticles produced in green. The higher biological  
390 activity of crude extract indicates the potential of *Curcuma caesia* mediated ZnO  
391 nanoparticles as a nano therapeutic agent for breast cancer treatment.This is an extensive  
392 overview of recent developments about the application of green ZnO nanoparticles as  
393 nanocarriers for anticancer drugs. The paper will address the manufacturing methods of ZnO  
394 nanoparticles, their characterisation, direct anticancer efficacy, applications in drug delivery,  
395 and documented health hazards(Katarzyna Łukowiak, 2026).However, further in vivo  
396 assessments and molecular pathways are required for validation of their therapeutic  
397 application.

398

### 399. **Conclusion:**

400 The research showed how to synthesize zinc oxide nanoparticles (ZnO NPs) in an  
401 environmentally benign manner by employing *Curcuma caesia* tuber extract as a natural  
402 stabilizing and reducing agent. The synthesis of pure, crystalline ZnO NPs with desired  
403 physicochemical characteristics was confirmed by characterization. In comparison to the  
404 pure extract, the nanoparticles demonstrated high, concentration-dependent antioxidant  
405 activity and increased cytotoxicity against MCF-7 breast cancer cells. Apoptosis was

406 identified as the main mechanism of cell death by Annexin V staining. These results  
407 demonstrate how ZnO and phytochemicals produced from plants work in concert to produce  
408 higher biological activity and therapeutic potential. ZnO NPs mediated by *Curcuma caesia*  
409 thus show promise for antioxidant and anticancer applications; however, more mechanistic,  
410 toxicological, and in vivo research is needed to confirm therapeutic value.

411

#### 416. **Acknowledgement:**

413 The authors would like to express their appreciation to the Central Instrument Facility (CIF)  
414 at Savitribai Phule Pune University (SPPU) as well as the research centre in botany at Prof.  
415 Ramkrishna More College in Akurdi, Pune, for providing the research resources and  
416 support. When it comes to the examination of nanoparticles, Pune.

417

#### 418 **References:**

419 Acharya, R. *et al.* (2024) 'Bioinspired synthesis and characterization of zinc oxide  
420 nanoparticles and assessment of their cytotoxicity and antimicrobial efficacy',  
421 *Discover Applied Sciences*, 6(3). doi:10.1007/s42452-024-05719-2.

422 Anjum, S. *et al.* (2021) 'ZnO-cancer drugs.pdf'.

423 Demissie, M.G. *et al.* (2020) 'Synthesis of Zinc Oxide Nanoparticles Using Leaf  
424 Extract of *Lippia adoensis* (Koseret) and Evaluation of Its Antibacterial Activity',  
425 *Journal of Chemistry*, 2020. doi:10.1155/2020/7459042.

426 Dey, S. *et al.* (2024) 'A critical review on zinc oxide nanoparticles: Synthesis,  
427 properties and biomedical applications', *Intelligent Pharmacy*, 3(1), pp. 53–70.  
428 doi:10.1016/j.ipha.2024.08.004.

429 Fais, G. *et al.* (2024) 'Cytotoxic Effects of ZnO and Ag Nanoparticles Synthesized in  
430 Microalgae Extracts on PC12 Cells', *Marine Drugs*, 22(12).  
431 doi:10.3390/md22120549.

432 Gangal, A. *et al.* (2025) 'Green nanotechnology: nanoparticle synthesis using  
433 *Curcuma amada*, *Curcuma caesia*, *Curcuma longa*, and *Curcuma zedoaria*', *Green*  
434 *Chemistry Letters and Reviews*, 18(1), pp. 1–18.  
435 doi:10.1080/17518253.2024.2449122.

436 GLOBOCAN (2020) 'The Global Cancer Observatory - CANCER FACT SHEETS',

437 *International Agency for Research on Cancer - WHO*, 419, pp. 199–200. Available at:  
438 <https://gco.iarc.fr/today/home>.

439 Govarathanan, M. *et al.* (2020) ‘Utilization of funnel-shaped ivory flowers of  
440 Candelabra cactus for zinc oxide nanoparticles synthesis and their in-vitro anti-cancer  
441 and antibacterial activity’, *Materials Letters*, 273, p. 127951.  
442 doi:10.1016/j.matlet.2020.127951.

443 Harbeck, N. *et al.* (2019) *Breast cancer, Nature Reviews Disease Primers*.  
444 doi:10.1038/s41572-019-0111-2.

445 Hassan, T. *et al.* (2022) ‘Nanoparticles in Cancer Treatment: A Narrative Review’,  
446 *Proceedings of the Pakistan Academy of Sciences: Part B*, 58(3), pp. 1–18.  
447 doi:10.53560/PPASB(58-3)664.

448 Jain, N. *et al.* (2021) ‘Green synthesized plant-based silver nanoparticles: therapeutic  
449 prospective for anticancer and antiviral activity’, *Micro and Nano Systems Letters*,  
450 9(1). doi:10.1186/s40486-021-00131-6.

451 Jayaprakasha, G.K., Rao, L.J.M. and Sakariah, K.K. (2002) ‘Improved HPLC method  
452 for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin’,  
453 *Journal of agricultural and food chemistry*, 50(13), pp. 3668–3672.  
454 doi:10.1021/JF025506A.

455 Katarzyna Łukowiak, E.U.S. (2026) ‘Green Synthesis of Zinc Oxide Nanoparticles  
456 and Their Application in Anticancer Drug Delivery – A Review’, (January).

457 Khorrami, S. *et al.* (2018) ‘Selective cytotoxicity of green synthesized silver  
458 nanoparticles against the MCF-7 tumor cell line and their enhanced antioxidant and  
459 antimicrobial properties’, *International Journal of Nanomedicine*, 13, pp. 8013–8024.  
460 doi:10.2147/IJN.S189295.

461 Klein, W. *et al.* (2023) ‘Green-Synthesized Silver Nanoparticles: Antifungal and  
462 Cytotoxic Potential for Further Dental Applications’, *Journal of Functional*  
463 *Biomaterials*, 14(7). doi:10.3390/jfb14070379.

464 Labaran, A.N. *et al.* (2024) ‘Biosynthesis of copper nanoparticles using *Alstonia*  
465 *scholaris* leaves and its antimicrobial studies’, *Scientific Reports*, 14(1), pp. 1–10.  
466 doi:10.1038/s41598-024-56052-y.

467 Li, Z. *et al.* (2020) ‘Zinc oxide nanoparticles induce human multiple myeloma cell  
468 death via reactive oxygen species and Cyt-C/Apaf-1/Caspase-9/Caspase-3 signaling  
469 pathway in vitro’, *Biomedicine and Pharmacotherapy*, 122(July 2019), p. 109712.  
470 doi:10.1016/j.biopha.2019.109712.

471 Miri, A. *et al.* (2019) ‘Zinc oxide nanoparticles: Biosynthesis, characterization,  
472 antifungal and cytotoxic activity’, *Materials Science and Engineering C*, 104(June).  
473 doi:10.1016/j.msec.2019.109981.

474 Ojo, O.A. *et al.* (2021) ‘Nanoparticles and their biomedical applications’, *Biointerface*  
475 *Research in Applied Chemistry*, 11(1), pp. 8431–8445.  
476 doi:10.33263/BRIAC111.84318445.

477 Puri, A. and Patil, S. (2022) ‘Tinospora cordifolia Stem Extract-mediated Green  
478 Synthesis of Selenium Nanoparticles and its Biological Applications’, *Pharmacognosy*  
479 *Research*, 14(3), pp. 289–296. doi:10.5530/pres.14.3.42.

480 Rajeshkumar, S. *et al.* (2018) ‘Biosynthesis of zinc oxide nanoparticles  
481 using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic  
482 properties in lung cancer (A549) cells’, *Enzyme and Microbial Technology*, 117, pp.  
483 91–95. doi:10.1016/j.enzmictec.2018.06.009.

484 Reddy, P.R. *et al.* (2016) ‘Zinc Oxide Nanoparticles Synthesized From Curcuma  
485 Longa Extract for Seed Germination’, *World Scientific Research*, 3(1), pp. 70–74.  
486 doi:10.20448/journal.510/2016.3.1/510.1.70.74.

487 Ruddaraju, L.K. *et al.* (2019) ‘Antibiotic potentiation and anti-cancer competence  
488 through bio-mediated ZnO nanoparticles’, *Materials Science and Engineering C*, 103,  
489 p. 109756. doi:10.1016/j.msec.2019.109756.

490 Shrestha, D.K. *et al.* (2024) ‘ Synthesis of Silver and Zinc Oxide Nanoparticles Using  
491 *Polystichum lentum* Extract for the Potential Antibacterial, Antioxidant, and  
492 Anticancer Activities ’, *Journal of Chemistry*, 2024(1). doi:10.1155/2024/1876560.

493 Sonawane, H. *et al.* (2021) ‘Rhizoctonia bataticola: From plant pathogen to a potential  
494 source of pharmaceutically relevant metabolites’, *Current Research in Green and*  
495 *Sustainable Chemistry*, 4(October), p. 100171. doi:10.1016/j.crgsc.2021.100171.

496 Sung, H. *et al.* (2021) ‘Global Cancer Statistics 2020: GLOBOCAN Estimates of

497 Incidence and Mortality Worldwide for 36 Cancers in 185 Countries', *CA: A Cancer*  
498 *Journal for Clinicians*, 71(3), pp. 209–249. doi:10.3322/caac.21660.

499 Tanvir, E.M. *et al.* (2017) 'Antioxidant properties of popular turmeric (*Curcuma*  
500 *longa*) varieties from Bangladesh', *Journal of Food Quality*, 2017.  
501 doi:10.1155/2017/8471785.

502 Tayyeb, J.Z. *et al.* (2024) 'Multifunctional curcumin mediated zinc oxide nanoparticle  
503 enhancing biofilm inhibition and targeting apoptotic specific pathway in oral  
504 squamous carcinoma cells', *Molecular biology reports*, 51(1). doi:10.1007/S11033-  
505 024-09407-7.

506 Ullah, I. *et al.* (2020) 'Green-Synthesized Silver Nanoparticles Induced Apoptotic Cell  
507 Death in MCF-7 Breast Cancer Cells by Generating Reactive Oxygen Species and  
508 Activating Caspase 3 and 9 Enzyme Activities', *Oxidative Medicine and Cellular*  
509 *Longevity*, 2020. doi:10.1155/2020/1215395,.

510 Yılmaz, G.E. *et al.* (2023) 'Antimicrobial Nanomaterials : A Review', pp. 269–290.

511 Zhang, J.Y. *et al.* (2015) 'Combinational treatment of curcumin and quercetin against  
512 gastric cancer MGC-803 cells in vitro', *Molecules*, 20(6), pp. 11524–11534.  
513 doi:10.3390/molecules200611524.

514 Acharya, R. *et al.* (2024) 'Bioinspired synthesis and characterization of zinc oxide  
515 nanoparticles and assessment of their cytotoxicity and antimicrobial efficacy',  
516 *Discover Applied Sciences*, 6(3). doi:10.1007/s42452-024-05719-2.

517 Anjum, S. *et al.* (2021) 'ZnO-cancer drugs.pdf'.

518 Demissie, M.G. *et al.* (2020) 'Synthesis of Zinc Oxide Nanoparticles Using Leaf  
519 Extract of *Lippia adoensis* (Koseret) and Evaluation of Its Antibacterial Activity',  
520 *Journal of Chemistry*, 2020. doi:10.1155/2020/7459042.

521 Dey, S. *et al.* (2024) 'A critical review on zinc oxide nanoparticles: Synthesis,  
522 properties and biomedical applications', *Intelligent Pharmacy*, 3(1), pp. 53–70.  
523 doi:10.1016/j.ipha.2024.08.004.

524 Fais, G. *et al.* (2024) 'Cytotoxic Effects of ZnO and Ag Nanoparticles Synthesized in  
525 Microalgae Extracts on PC12 Cells', *Marine Drugs*, 22(12).  
526 doi:10.3390/md22120549.

- 527 Gangal, A. *et al.* (2025) 'Green nanotechnology: nanoparticle synthesis using  
528 *Curcuma amada*, *Curcuma caesia*, *Curcuma longa*, and *Curcuma zedoaria*', *Green*  
529 *Chemistry Letters and Reviews*, 18(1), pp. 1–18.  
530 doi:10.1080/17518253.2024.2449122.
- 531 GLOBOCAN (2020) 'The Global Cancer Observatory - CANCER FACT SHEETS',  
532 *International Agency for Research on Cancer - WHO*, 419, pp. 199–200. Available at:  
533 <https://gco.iarc.fr/today/home>.
- 534 Govarathanan, M. *et al.* (2020) 'Utilization of funnel-shaped ivory flowers of  
535 *Candelabra cactus* for zinc oxide nanoparticles synthesis and their in-vitro anti-cancer  
536 and antibacterial activity', *Materials Letters*, 273, p. 127951.  
537 doi:10.1016/j.matlet.2020.127951.
- 538 Harbeck, N. *et al.* (2019) *Breast cancer*, *Nature Reviews Disease Primers*.  
539 doi:10.1038/s41572-019-0111-2.
- 540 Hassan, T. *et al.* (2022) 'Nanoparticles in Cancer Treatment: A Narrative Review',  
541 *Proceedings of the Pakistan Academy of Sciences: Part B*, 58(3), pp. 1–18.  
542 doi:10.53560/PPASB(58-3)664.
- 543 Jain, N. *et al.* (2021) 'Green synthesized plant-based silver nanoparticles: therapeutic  
544 prospective for anticancer and antiviral activity', *Micro and Nano Systems Letters*,  
545 9(1). doi:10.1186/s40486-021-00131-6.
- 546 Jayaprakasha, G.K., Rao, L.J.M. and Sakariah, K.K. (2002) 'Improved HPLC method  
547 for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin',  
548 *Journal of agricultural and food chemistry*, 50(13), pp. 3668–3672.  
549 doi:10.1021/JF025506A.
- 550 Katarzyna Łukowiak, E.U.S. (2026) 'Green Synthesis of Zinc Oxide Nanoparticles  
551 and Their Application in Anticancer Drug Delivery – A Review', (January).
- 552 Khorrami, S. *et al.* (2018) 'Selective cytotoxicity of green synthesized silver  
553 nanoparticles against the MCF-7 tumor cell line and their enhanced antioxidant and  
554 antimicrobial properties', *International Journal of Nanomedicine*, 13, pp. 8013–8024.  
555 doi:10.2147/IJN.S189295.
- 556 Klein, W. *et al.* (2023) 'Green-Synthesized Silver Nanoparticles: Antifungal and

557 Cytotoxic Potential for Further Dental Applications’, *Journal of Functional*  
558 *Biomaterials*, 14(7). doi:10.3390/jfb14070379.

559 Labaran, A.N. *et al.* (2024) ‘Biosynthesis of copper nanoparticles using *Alstonia*  
560 *scholaris* leaves and its antimicrobial studies’, *Scientific Reports*, 14(1), pp. 1–10.  
561 doi:10.1038/s41598-024-56052-y.

562 Li, Z. *et al.* (2020) ‘Zinc oxide nanoparticles induce human multiple myeloma cell  
563 death via reactive oxygen species and Cyt-C/Apaf-1/Caspase-9/Caspase-3 signaling  
564 pathway in vitro’, *Biomedicine and Pharmacotherapy*, 122(July 2019), p. 109712.  
565 doi:10.1016/j.biopha.2019.109712.

566 Miri, A. *et al.* (2019) ‘Zinc oxide nanoparticles: Biosynthesis, characterization,  
567 antifungal and cytotoxic activity’, *Materials Science and Engineering C*, 104(June).  
568 doi:10.1016/j.msec.2019.109981.

569 Ojo, O.A. *et al.* (2021) ‘Nanoparticles and their biomedical applications’, *Biointerface*  
570 *Research in Applied Chemistry*, 11(1), pp. 8431–8445.  
571 doi:10.33263/BRIAC111.84318445.

572 Puri, A. and Patil, S. (2022) ‘*Tinospora cordifolia* Stem Extract-mediated Green  
573 Synthesis of Selenium Nanoparticles and its Biological Applications’, *Pharmacognosy*  
574 *Research*, 14(3), pp. 289–296. doi:10.5530/pres.14.3.42.

575 Rajeshkumar, S. *et al.* (2018) ‘Biosynthesis of zinc oxide nanoparticles  
576 using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic  
577 properties in lung cancer (A549) cells’, *Enzyme and Microbial Technology*, 117, pp.  
578 91–95. doi:10.1016/j.enzmictec.2018.06.009.

579 Reddy, P.R. *et al.* (2016) ‘Zinc Oxide Nanoparticles Synthesized From *Curcuma*  
580 *Longa* Extract for Seed Germination’, *World Scientific Research*, 3(1), pp. 70–74.  
581 doi:10.20448/journal.510/2016.3.1/510.1.70.74.

582 Ruddaraju, L.K. *et al.* (2019) ‘Antibiotic potentiation and anti-cancer competence  
583 through bio-mediated ZnO nanoparticles’, *Materials Science and Engineering C*, 103,  
584 p. 109756. doi:10.1016/j.msec.2019.109756.

585 Shrestha, D.K. *et al.* (2024) ‘ Synthesis of Silver and Zinc Oxide Nanoparticles Using  
586 *Polystichum lentum* Extract for the Potential Antibacterial, Antioxidant, and

587 Anticancer Activities ', *Journal of Chemistry*, 2024(1). doi:10.1155/2024/1876560.

588 Sonawane, H. *et al.* (2021) 'Rhizoctonia bataticola: From plant pathogen to a potential  
589 source of pharmaceutically relevant metabolites', *Current Research in Green and*  
590 *Sustainable Chemistry*, 4(October), p. 100171. doi:10.1016/j.crgsc.2021.100171.

591 Sung, H. *et al.* (2021) 'Global Cancer Statistics 2020: GLOBOCAN Estimates of  
592 Incidence and Mortality Worldwide for 36 Cancers in 185 Countries', *CA: A Cancer*  
593 *Journal for Clinicians*, 71(3), pp. 209–249. doi:10.3322/caac.21660.

594 Tanvir, E.M. *et al.* (2017) 'Antioxidant properties of popular turmeric (*Curcuma*  
595 *longa*) varieties from Bangladesh', *Journal of Food Quality*, 2017.  
596 doi:10.1155/2017/8471785.

597 Tayyeb, J.Z. *et al.* (2024) 'Multifunctional curcumin mediated zinc oxide nanoparticle  
598 enhancing biofilm inhibition and targeting apoptotic specific pathway in oral  
599 squamous carcinoma cells', *Molecular biology reports*, 51(1). doi:10.1007/S11033-  
600 024-09407-7.

601 Ullah, I. *et al.* (2020) 'Green-Synthesized Silver Nanoparticles Induced Apoptotic Cell  
602 Death in MCF-7 Breast Cancer Cells by Generating Reactive Oxygen Species and  
603 Activating Caspase 3 and 9 Enzyme Activities', *Oxidative Medicine and Cellular*  
604 *Longevity*, 2020. doi:10.1155/2020/1215395,.

605 Yılmaz, G.E. *et al.* (2023) 'Antimicrobial Nanomaterials : A Review', pp. 269–290.

606 Zhang, J.Y. *et al.* (2015) 'Combinational treatment of curcumin and quercetin against  
607 gastric cancer MGC-803 cells in vitro', *Molecules*, 20(6), pp. 11524–11534.  
608 doi:10.3390/molecules200611524.

609  
610  
611  
612  
613  
614  
615  
616  
617