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Nanomaterials via Green Synthesis: Insights into Biocompatibility and Biotechnological Applications

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Abstract

This review critically examines recent advances in the green synthesis of nanomaterials (NMs) and their diverse biological applications, focusing on antimicrobial, antioxidant, anticancer, and anti-inflammatory properties. The reviewed studies evaluated key characteristics of green-synthesized NMs, including their size, shape, and concentrations, as assessed in both in vitro and in vivo biological models. Compared to traditional chemical methods, green synthesis demonstrated superior efficacy, enhanced biocompatibility, and reduced toxicity. For instance, selenium nanoparticles (SeNPs) and silver nanoparticles (AgNPs) synthesized using plant extracts, such as Zingiber officinale and Syzygium aromaticum exhibited robust antimicrobial activity against Gram-positive and Gram-negative bacteria. Similarly, zinc oxide (ZnO) and iron oxide (FeO) nanoparticles synthesized with extracts, like Ipomoea aquatica and Abutilon indicum, displayed potent anticancer and anti-inflammatory effects. ZnO nanoparticles promoted apoptosis in cancer cells, while FeO nanoparticles exhibited anti-inflammatory activity comparable to standard pharmaceuticals. AgNPs derived from plant extracts, such as Ajuga iva and Pandanus tectorius demonstrated exceptional structural stability and cytotoxic effects against cancer cell lines, including HeLa cells. Furthermore, metallic nanocomposites, like Ag-ZnO, showed enhanced cytotoxicity against breast cancer cells (MCF-7). In vivo studies further highlighted the potential of eco-friendly NMs. AgNPs and SeNPs synthesized via green methods were evaluated in model organisms, including Danio rerio and Biomphalaria glabrata.

Keywords: Nanoparticles, eco-friendly, toxicity, nanotoxicity, plant extracts

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INTRODUCTION

Nanomaterials (NMs) are characterized by their nanoscale size and exhibit unique properties, such as a high surface area-to-volume ratio, enhanced reactivity, quantum confinement effects, and tunable physicochemical characteristics, enabling applications in diverse fields [1–5]. These characteristics position NPs as transformative tools across multiple domains. In medicine, they have revolutionized drug delivery systems, enabled targeted cancer therapies while mitigating the adverse effects associated with conventional treatments [6–8]. In agriculture, NPs demonstrate remarkable potential in detecting and combating phytopathogens [9, 10]. Additionally, their photocatalytic properties make them highly effective agents for wastewater treatment, promoting environmental sustainability [11, 12].

Conventional NMs synthesis techniques, which predominantly involve chemical and physical methods, rely on reducing agents, stabilizers, and solvents. These approaches are often associated with high energy demands, escalating production costs [13–15]. Moreover, these processes generate toxic byproducts, posing substantial risks to both environmental and biological systems [16]. While the nanoscale properties of NMs provide diverse functional benefits, their potential for unintended interactions with biological systems can lead to adverse effects. Improper disposal of NMs and synthesis-derived waste can contribute to environmental contamination and toxicity [17–20]. As such, there is an urgent need to develop environmentally responsible synthesis methodologies that minimize these risks while maintaining the functional integrity of NMs.

Green synthesis, also known as biosynthesis, has emerged as a sustainable and economically viable alternative [21–23]. This approach employs readily available biological resources, including plants, fungi, and microorganisms, as reducing and stabilizing agents. By circumventing the use of hazardous chemical additives [24, 25], green synthesis produces waste that is less toxic to ecosystems and living organisms [26]. Furthermore, it requires lower energy inputs, making it a cost-effective solution for NMs production. Characterization techniques, such as scanning electron microscopy (SEM), zeta potential analysis, and dynamic light scattering (DLS) are instrumental in verifying the properties of green-synthesized NMs [27]. These methodologies ensure that NMs produced through biosynthesis retain the functional attributes of their conventionally synthesized counterparts while aligning with sustainable practices [28, 29].

Despite the exceptional properties of NMs, including their high reactivity, conductivity, and versatility, traditional synthesis methods often fail to align with the principles of environmental sustainability. These methods generate hazardous waste, involve energy-intensive processes, and contribute to the bioaccumulation of NMs in living organisms, resulting in cellular toxicity, oxidative stress, and genetic alterations. Such effects are increasingly concerning as NMs are integrated into a wide range of products, including cosmetics, pharmaceuticals, and electronics. The adverse ecological and health implications of conventional synthesis underscore the necessity of adopting green synthesis approaches. By leveraging renewable biological resources, green synthesis not only mitigates these concerns but also preserves the advantageous properties of NMs. This review synthesizes recent advancements in green nanoparticle synthesis over the past two years, critically examining their advantages and correlating their properties with observed biological effects. Our aim is to provide a comprehensive perspective on the potential of green synthesis to drive safer and more sustainable nanotechnological innovations.

MATERIAL AND METHODS

Initially, a systematic search was conducted in online databases using the keywords “nanomaterials” and “green synthesis.” The pre-selected studies from this search were required to involve biological testing of NMs obtained through the green synthesis method, providing critical information, such as the size and shape of the NMs, as well as the concentrations used *in vitro* and *in vivo* tests. A filtering process was performed, where duplicate studies or those outside the scope of the present review were excluded from the lists compiled for each database used.

The selected studies were initially assessed through a review of their titles and abstracts to ensure they met the established selection criteria. Subsequently, a more detailed reading of the full texts was carried out, focusing on the methods employed and results obtained to confirm their eligibility. The studies were then categorized based on the type of NMs, synthesis method, and relevant information about the biological tests. Finally, the review was constructed by discussing this information and highlighting the main points of each article (Figure 1).

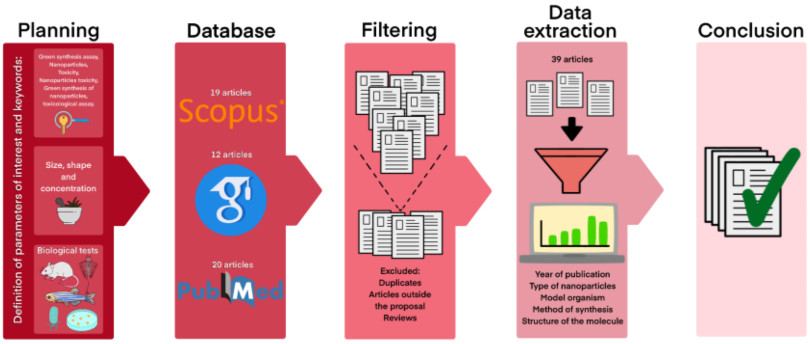

Figure 1. *Flowchart of the methodological process for selecting and analyzing scientific articles.* The diagram outlines the steps from planning, database selection (Scopus, Google Scholar, and PubMed), article filtering, and extraction of relevant data (such as publication year, type of nanoparticles, and biological models), to the conclusion.

GLOBAL TRENDS AND EXPERIMENTAL MODELS IN THE GREEN SYNTHESIS OF NANOMATERIALS

The analysis of the data reveals key trends in the production and use of nanomaterials (NMs) from green synthesis. Figure 2 shows the geographical distribution of scientific articles on NMs published in the last two years (2023–2024), with India leading at 13 publications, followed by China and Iran. This concentration may indicate significant investments and a growing interest in sustainable technologies in India.

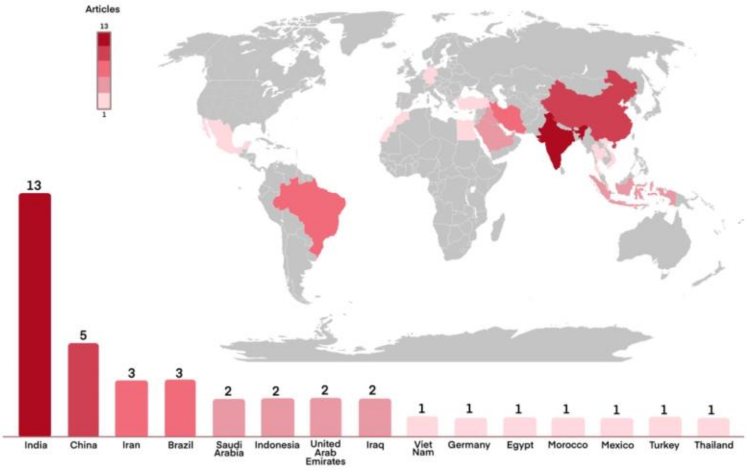

Figure 2. *Geographical distribution of the analyzed scientific articles.* The world map and bar graph shows the number of publications by country, with India leading (13 articles), followed by China (5 articles). The intensity of the colors on the map represents the number of publications, highlighting the regions with the highest scientific contributions.

Figure 3 presents the percentage distribution of experimental models used to study the biological effects of NMs synthesized through green methods. Traditional organisms, such as rats (28.6%) and fish (34.3%), are prominently used, despite ongoing efforts to find alternative toxicity testing methods. Notably, 37.1% of the studies focus on testing the bactericidal activity of new NMs, while in vitro studies evaluate the effects on cancer cells. Despite only two years of research, the scientific community is actively exploring new synthesis methodologies for more responsible applications of NMs, employing diverse approaches to assess their impacts.

These findings underscore the importance of research in green synthesis of NMs and its implications for health and sustainability. As NMs are increasingly integrated into everyday products, it is crucial to support science to evolve towards safer and more sustainable methods, ensuring that benefits do not pose undue risks to health and the environment. This work aims to contribute to this transformation by compiling essential information about NMs and their biological characteristics.

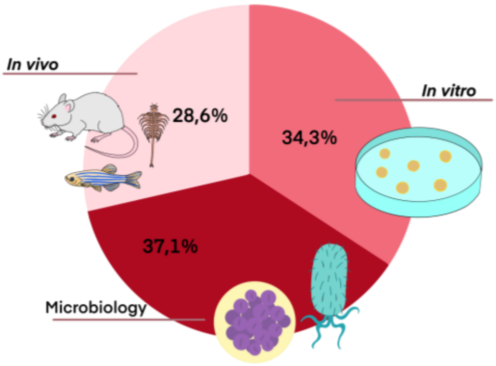

Figure 3. *The proportion of model organisms used in the studies analyzed.* The pie chart illustrates the main biological models employed, including microorganisms (37.1%), cell cultures (34.3%), and animal models, such as mice, zebrafish, and invertebrates (28.6%).

ADVANCES IN GREEN SYNTHESIS OF NANOMATERIAL ANTIMICROBIAL APPLICATIONS

Green synthesis represents a significant advancement in the field of nanotechnology, showcasing advantages concerning both efficacy and biological safety, particularly when juxtaposed with conventional chemical methods prevalent in industrial and laboratory practices. For instance, selenium nanoparticles (SeNPs) synthesized using *Smilax glabra* extract combined with electrochemical plasma have exhibited remarkable antimicrobial efficacy against a range of pathogens, including *Staphylococcus aureus* and *Escherichia coli*, which are known for their clinical relevance and resistance patterns [30]. In further research, SeNPs that were biosynthesized using aqueous extract from the *Syzygium aromaticum* plant were subjected to rigorous testing against both Gram-negative bacteria, such as *Salmonella* spp. and *Pseudomonas aeruginosa*, along with Gram-positive bacteria including *S. aureus* and *Bacillus subtilis* (Table 1). The research highlighted that the presence of bioactive compounds within the extracts played a crucial role as a reducing and stabilizing agent, consequently enhancing the biological properties and effectiveness of SeNPs, which were observed to be more effective against Gram-negative strains in comparison to their Gram-positive counterparts. Interestingly, the isolated extract of *S. aromaticum*, when tested in the absence of selenium (Se), revealed no significant antibacterial activity, indicating that the synergistic interaction between the extract and Se was vital in amplifying the antimicrobial effects [31].

Table 1. Biotechnological applications of antimicrobials from MNs through green synthesis.

NPs	**Shape Size (nm)**	**Synthesis**	**Tested Concentration**	**Biological Test**	**Reference**
Se	Spherical 100	Combination of *Smilax glabra* root extract (SG) as a reducing and stabilizing agent, together with electrochemical plasma (PLS)	2.5, 25 and 125 µg/mL	*E. coli, Staphylococcus aureus* and *Candida albicans*	[1]
	Spherical–	Leaf extract of *Syzygium aromaticum*	0.437 µg/mL	*Salmonella*	[2]
	Hexagonal 30–150	Stem of *Tridax procumbens*	25 µg/mL	*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*	[3]
TiO_2	Spherical 15–23	Seeds of *Nigella sativa*	500, 750 and 1000 µg/m	*Salmonella typhi*	[9–24]

Ai-FeO	Spherical 10–95	Aqueous extract of *Abutilon indicum*	25, 50, 75 and 100 µg/m	*S. aureus, B. subtilis, E. coli, P. aeruginosa*	[12]
Co-Zn-Ni	Spherical 25.72	Leafs of *Cicer arietinum*	100 µg/mL	*S. aureus*	[4]
Ag–TiO2	Spherical	Maple leaf extract *(Acer palmatum)*	4 mg/ mL in ultrapure water	*S. aureus* and *E. coli*	[10]
AuNPs@AV /AgNPs@AV	Spherical and triangular—	Crude extract of *Aconitum violaceum*	*AuNPs@AV:* 95 and 70 µg/mL *AgNPs@AV:* 90 and 65 µg/mL	*Lactobacillus acidophilus, Escherichia coli*	[8]
Cu(I)-Rl	Spherical 2	Rhamnolipids	10^4 mg/ml	*Staphylococcus aureus ATCC 6538, Bacillus subtilis ATCC 6633, E. coli ATCC 6539 e Salmonella typhi ATCC 8939*	[11]
Ag	Spherical 80–100	Ethanolic extract of *Zingiber officinale*	6 µg/mL	*Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa e Staphylococcus aureus*	[5]
	Spherical 20	Aqueous extract of *Euterpe oleracea Mart* (Açaí)	70–200 µg/mL	*Staphylococcus aureus, Pseudomonas aeruginosa e Acinetobacter baumannii*	[6]
	Spherical 11, 18	Polysaccharides from *Scutellaria baicalensis*	15.6 e 7.8 µg/mL	*Staphylococcus aureus, Escherichia coli*	[7]
α-Mn_2O_3	Spherical 30	Green sol-gel method using Tragacanth (TG) gel	1–5 µg/mL	*Enterococcus faecalis, Staphylococcus aureus, Salmonella typhimurium, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis e Pseudomonas aeruginosa*	[13]

In another study, the stem of *Tridax procumbens* was investigated in conjunction with SeNPs, demonstrating that the bioactive compounds present in the plant – such as flavonoids, alkaloids, and terpenes – exhibited notable efficacy against a variety of bacterial strains, which included those capable of forming biofilms. The formation of biofilms is an important factor contributing to bacterial resistance, thereby complicating the treatment strategies for various infections. In this context, the antibacterial activity displayed by *T. procumbens* was particularly significant when addressing wound infections, especially those attributed to resistant bacterial strains, emphasizing the plant's potential as a resource in combating persistent infections [32]. In Mohammed et al. (2024) [33], cobalt, zinc, and nickel trimetallic oxide (Co-Zn-Ni) nanoparticles were synthesized using *Cicer arietinum* leaf extract, and antibiofilm activity was recorded against the bacterium *Staphylococcus aureus*. The results showed that the nanoparticles inhibited 51.6% biofilm formation, a performance superior to that of the antibiotic ciprofloxacin, which reached 42.5%. Thus, these NPs emerge as excellent alternatives against bacterial resistance associated with the increasing use of antibiotics.

Furthermore, silver nanoparticles (Ag) have emerged as another critical focus within this research domain. Various studies have successfully demonstrated the efficacy of green synthesis methods employing *Zingiber officinale*, which effectively targeted pathogens like *E. coli*, *Bacillus subtilis*, *P. aeruginosa*, and *S. aureus* [34]. Other studies explored the use of *Euterpe oleracea* against *S. aureus, P. aeruginosa, and Acinetobacter baumannii* [35]. Additionally, polysaccharides derived from *Scutellaria baicalensis* proved effective against both *S. aureus* and *E. coli* [36]. AgNPs are widely recognized for their potent antimicrobial activity, and the green synthesis approach further amplifies this efficacy, rendering them even more effective against a broad spectrum of bacterial strains that pose significant healthcare challenges. Notably, gold (Au) and silver (Ag) nanoparticles – specifically, AuNPs@AV and AgNPs@AV – prepared with an extract from *Aconitum violaceum* also exhibited impressive antibacterial activities at concentrations of 95 and 70 µg/mL against *Lactobacillus acidophilus* and 90 and 65 µg/mL against *E. coli* [37].

Moreover, studies investigating titanium dioxide (TiO_2) nanoparticles synthesized from *Nigella sativa* indicated a larger zone of inhibition against *Salmonella typhi* than those created through traditional inorganic synthesis methods [38]. Another innovative study proposed an eco-friendly technique for producing silver-doped TiO_2 nanoparticles (Ag–TiO_2) using an extract from maple leaves (*Acer palmatum*). This method of doping silver into TiO_2 has been shown to significantly enhance the inhibition of growth for both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacterial strains [39–40].

NPs of copper (Cu) also play a vital role in exhibiting broad-spectrum antibacterial properties that are effective against both Gram-positive and Gram-negative bacteria. Habibah, F. et al. (2024) introduced a new type of nanoparticle combining Cu ion NPs with ecological glycolipid biosurfactants, specifically rhamnolipids (Cu(I)-Rl NPs). This combination demonstrated enhanced antibacterial activity attributed to the synergistic effects observed between Cu(I) ions and rhamnolipids, especially noted in their effectiveness against *E. coli* and *Bacillus subtilis.*

Additionally, iron oxide (FeO) NPs have been successfully synthesized through green methods using parts of the *Abutilon indicum* (*Malvaceae*), which is known to contain a diverse array of phytochemicals, including alkaloids, proteins, amino acids, and flavonoids. The resulting Ai-FeO nanoparticles exhibited efficacy against both Gram-positive (*S. aureus, Bacillus subtilis*) and Gram-negative (*E. coli, P. aeruginosa*) bacteria, particularly at the highest tested concentration of 100 µg/mL [41].

Another finding refers to α-Mn_2O_3 (manganese III oxide) nanoparticles, highlighted by their antibacterial activity and photocatalytic capacity under visible light. These nanoparticles were synthesized by the sol-gel method, using gum tragacanth as a stabilizing agent. The antibacterial activity was evaluated against Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) strains, using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods. The results indicated greater efficacy against Gram-positive bacteria, with MIC ranging from 1 to 3.5 µg/mL, attributed to the structural simplicity of their cell membranes [42].

Therefore, these studies underscore the tremendous potential of green synthesis as a progressive and promising approach to produce nanoparticles with enhanced antimicrobial properties. This innovative method offers new perspectives and opportunities for developing alternative therapies aimed at effectively combating bacterial infections that present considerable challenges in contemporary medicine.

NANOPARTICLE STABILITY AND APPLICATIONS *IN VITRO* CELLS

The growing demand for sustainable methods in the synthesis of nanoparticles (NPs) has led researchers to explore the use of plant extracts as reducing agents. This approach not only offers ecological alternatives to conventional chemical methodologies but also enables the creation of NPs with diverse characteristics and potential applications in assays *in vitro*, as observed in Table 2. In the synthesis process, it is essential to consider the phytochemicals present in plant extracts, as they play a crucial role in the formation and stabilization of these NPs. Furthermore, they can enhance their biological activities, such as antioxidant and anticancer properties, which may provide promising perspectives for applications in biomedicine and other fields.

Mohammadi, E. and Amini, S. (2024) [43] point out that the synthesis of metallic NPs from whole plant extracts can result in NPs with improper structures. Therefore, they opted to synthesize silver nanoparticles (AgNPs) using the flavonoid apigenin as a reducing agent (Api@AgNPs). The authors suggest that using phytochemicals from whole plant extracts allows for the synthesis of NPs with more precise shapes. In this context, when comparing the structural characteristics of Api@AgNPs with NPs obtained from a chemical reducing agent (citrate – Ci@Ag), equivalent stabilities were observed. In addition, Api@AgNPs exhibited lower cytotoxicity even at high concentrations (256 µg/ml) and antioxidant activity at low concentrations (>32 µg/ml). On the other hand, by using the full extract of aerial roots from *Pandanus tectorius*, AgNPs with a well-defined crystalline structure, as demonstrated by SEM analysis [44]. The authors indicate that primarily the flavonoids and phenols present in the whole extract contributed to the reduction of metal salts during the synthesis of NPs, demonstrating the effectiveness of the synthesis method. The qualities of the obtained NPs were also reflected in their ability to inhibit the growth of cancer cells (HeLa) in a concentration-dependent manner through the production of reactive oxygen species (ROS).

Regarding AgNPs, Kocakaya, S., Dokan, F. and Karatoprak, G. (2024) [45] chose to synthesize Ag-NiO, Ag-SrO, and Ag-ZnO nanocomposites from whole extracts of the lichen *Diploschistes scruposus*. In their study, the properties of the bioactives present in the extract were highlighted as excellent reducing agents and contributing to the stability of nanomaterials (NMs). Furthermore, the authors point out that the phytochemicals present in NMs obtained from lichen extracts significantly influence the surface properties of these NMs. Consequently, it was found that the Ag-ZnO nanocomposites achieved the highest anticancer levels against breast cancer cells (MCF-7), with an excellent cytotoxicity index (IC50) of 4.25 µg/mL, compared to the other tested NMs. The anticancer activity of AgNPs on MCF-7 cells was also tested by Al Baloushi, K. et al. (2024) [46], where the NPs obtained from the aqueous extract of *Moringa peregrina* achieved an IC of 26.93 µg/mL compared to 1.725 µg/mL for the positive control dexorubicin, a chemotherapeutic agent that has aggressive side effects. Among the phytochemical constituents of *M. peregrina,* the presence of quinone was noted as an important feature in the immediate reduction of nitrate, which, in the synthesis of AgNPs, is directly related to the stability characteristics of the NPs, such as rapid formation and decreased aggregation. AgNPs synthesized from the aqueous extract of *Ajuga iva* revealed significant biological interaction, where no cytotoxicity was identified in immune cells; conversely, exposure to the NPs resulted in the stimulation of thymocyte proliferation [47]. The antioxidant, anticancer, and antidiabetic activities of AgNPs synthesized from the extract of *Phlebopus portentosus* demonstrated the positive biological applications of the substance [48].

The toxicity of zinc oxide (ZnO) NPs is contradictory and considers various factors, such as concentration, size, and crystallinity. Therefore, it becomes essential to find ways to minimize potential negative interactions of ZnO with biological systems. In this sense, the synthesis of ZnO from the aqueous extract of *Ipomoea aquatica* demonstrated concentration-dependent cytotoxicity, indicating the need for careful consideration regarding the use of the substance [49]. On the other hand, ZnO NPs obtained through *Streptomyces plicatus* showed positive results against KB cells, stimulating apoptosis processes and contributing to the death of cancer cells [50]. Such results enhance the discussion of the toxicity of ZnO NPs obtained from different green synthesis methods and highlight the need for studies regarding the impacts of this substance on biological systems.

The biological applications of nickel oxide (NiO) nanoparticles (NPs) are demonstrated through the stimulation of cell death by apoptosis in cancer cells. However, the impacts following the interactions of these NPs with biological systems remain uncertain. Aiming to bypass possible negative effects and reduce the adverse impacts caused by synthesis through chemical methods, the cobalt (Co)-doped NiO NPs from the extract of *Salvadoran persica* [51]. The doping with Co significantly contributed to enhancing the properties of the NPs, where the inhibitory capacity related to cancer cells was greater after doping. Alongside the biological potential of these NPs, green synthesis emerges as an ecological alternative.

Combining biological potential with safe synthesis alternatives, Ramya, R. et al. (2024) [38] suggest synthesis using the extract of *Nigella sativa* as an excellent alternative to reduce costs and impacts from conventional chemical methods. Thus, the authors compared the structural properties of NPs obtained through both methods, revealing structural similarity, which indicates the quality of the performed green synthesis method. Furthermore, TiO_2 NPs obtained from the extract of *N. sativa* and Ag NPs obtained from the *Zingiber officinale* showed higher anticancer activity and potential anti-inflammatory activity. Demonstrating the importance of the method, TiO_2 NPs obtained from the extract of *Terminalia bellirica* exhibited low toxicity to zebrafish embryos, as well as excellent antioxidant activity. Therefore, the green synthesis method has become an ally not only in reducing the residual damages of chemical synthesis but also in enhancing the properties of the NPs [52].

Table 2. *In vitro* evaluation of nanomaterials synthesized from green synthesis.

NPs	**Shape Size (nm)**	**Synthesis**	**Tested Concentration**	**Cell Type**	**Reference**
Ag	Spherical 37.8–5.6	Apigenin	*Hemolysis* <1%: 64 µg/ml <5%: 128 µg/mL. *Toxicity* <256 µg/mL. *Antioxidant potential* – 32 µg/mL	Fibroblastos dérmicos	[14]
	Spherical <30	Aqueous extract of the aerial root of *Pandanus tectorius*	IC50: 47.58 µg/mL	HeLa	[15]
	Spherical 25–60	Aqueous extract of leaves of *Moringa peregrina*	*IC50:* *MCF-7* – 26.93 µg/mL *Caco-2* – 41.59 µg/mL.	MCF-7 Caco-2	[17]
	Polygonal 100–300	Extract of *Ajuga iva*	Thymocyte immunostimulant: 2 mg/mL.	Lymphocytes, splenocytes and thymocytes	[18]
	Spherical 80–100	*Zingiber officinale*	IC50 – 0.6 µg/mL	Vero and HeLa	[24]
	Spherical 68–78	*Phlebopus portentosus*	*Antioxidant activity:* IC50 – 0.1082 mg/mL *Antidiabetic activity:* IC50 – 11.1 µg/mL *Anticancerous activity:* IC50 (HEPG2) 14.36 µg/mL IC50 (MDA-MB-231) 40.05 µg/mL	DPPH/HEPG2 and MDA-MB-231	[19]
Ag–ZnO Ag–SrO Ag–NiO	Spherical *Ag-NiO*: 361.1 *Ag-SrO:* 138.6 *Ag-ZnO:*131.4	*Diploschistes scruposus*	*IC50:* Ag–ZnO 4.25 µg/mL Ag–SrO 37.28 µg/mL Ag–NiO 18.45 µg/mL.	breast cancer cells (MCF-7 and MDA-MB-231)	[16]

Au	Spherical 20–40	Extract of *Coffea arabica* conjugated Doxorrubicin	?	MRC-5 and H69	[28]
Au /Ag	Spherical triangular –	*Aconitum violaceum*	Au-IC50: 114.03 μg/mL Ag-IC50: 161.20 μg/mL	DPPH	[29]
NiO Co-NiO (1, 3 and 5%)	Spherical/ NiO: 35.19 Co-NiO:37.9, 38.95, and 41.18	Aqueous extract of *Salvadoran persica*	IC50: *Co-NiO (5%)* – 640 μg/mL	MCF-7 HUVEC	[22]
FeO	Spherical 10–95	Aqueous extract of leaves of *Abutilon indicum*	IC50: 85 μg/mL. *Anti-inflammatory activity:* 120 μg/mL–92.71 %.	MDA-MB-231	[26]
CuO	Various shapes 20–90	Aqueous extract of *Artemisia annua*	*IC80 (A375):* 2.9 μg/mL. For PC12, there was no significance.	A375 and PC12	[27]
ZnO	Spherical 67.70	Aqueous extract of leaves of *Ipomoea aquatica*	*Concentration-dependent cytotoxic effects:* 5, 25, 50, 75 and 100 μl/mL.	L-929	[20]
	Hexagonal 41.76	Extract of *Streptomyces plicatus*	IC50: 22.06 μg/ml	KB and erythrocytes	[21]
Se	Spherical –	Aqueous extract of *Syzygium aromaticum*	*Antioxidant activity:* IC50 – 0.437 μg/mL *Antiangiogenic effects:* 0.5 mg	DPPH and antiangiogenic activity in embryonated eggs	[2–30]
	Spherical < 100	Leaf extract of *Smilax glabra*	*Antioxidant activity:* >94% – of 125 μg/mL *Cytotoxicity:* 10, 25, 50, 100, and 200 μg/mL – were not toxic at any concentration	DPPH/HaCaT	[1–31]
TiO_2	Spherical 9	*Nigella sativa* seed extract	Antioxidant potential and anti-inflammatory action – 400 μg/mL IC50 – 40 μg/mL	MCF-7	[9–23]
		Aqueous extract of *Terminalia bellirica*	2, 4, 6, 8, and 10 μg/mL	*Danio rerio* (zebrafish) embryos	[25]

In this perspective, the biological effects of iron oxide (FeO) nanoparticles (NPs) obtained from the extract of *Abutilon indicum* were extremely positive. Regarding their anti-inflammatory activity, the NPs exhibited concentration-dependent activity (120 μg/mL–92.71%), with indices like that of the commercial anti-inflammatory cisplatin, used as a positive control (120 μg/mL–97.15%). When compared to cisplatin, the FeO NPs achieved an excellent stimulation of cell death by apoptosis in cancer cells. The cytotoxic effect on these cells was equally positive, with the IC50 of the NPs relative to the commercial anti-inflammatory being 85 and 66 μg/ml, respectively [41]. Promising biological effects were also observed in copper oxide (CuO) NPs obtained from the aqueous extract of *Artemisia annua*. In this study, cytotoxic assays against melanoma cells were also conducted, where concentration- and time-dependent activity was observed; the greater the exposure time and concentration of the NPs, the lower the viability of cancer cells [53].

In addition to being an ecologically safe alternative, the synthesis of gold nanoparticles (Au) obtained from the extract of *Coffea arabica* was essential to ensure the stability of the NPs due to the presence of phytochemicals, such as flavonoids and terpenes. These NPs, obtained in the work by Trejo-Tenient, I. et al. (2024) [54], were conjugated with the drug doxorubicin (DOX), an excellent anticancer agent, with the aim of increasing the drug's specificity. Upon observing that the pH levels caused unwanted drug release, destabilizing the obtained conjugation, mercaptoundecanoic acid (MUA) was used as a linker, stabilizing the interaction. The combination of Au NPs with DOX and the commercial organic linker MUA resulted in increased specificity and cytotoxicity for cancer cells, leveraging the unique properties of each component. Positive results from Au and Ag NPs obtained through the extract of *Aconitum violaceum* were noted, where the antioxidant and photocatalytic activities were considered excellent [37]. The positive contribution of the method was also documented by Behera, A. et al. (2024) [31] and Nguyen, P. et al. (2024) [30], where the use of the green synthesis method as an alternative was important in enhancing the anti-angiogenic effects and antioxidant activity of selenium (Se) NPs obtained from *Syzygium aromaticum* and *Smilax glabra*, respectively.

The results compiled here suggest that by opting for green synthesis, research and applications involving the use of NPs can reduce possible negative interactions with living beings. It is demonstrated that the constituents of plant extracts play a fundamental role in the stabilization of NPs, significantly contributing to the quality of the synthesis. Moreover, the properties of these constituents can combine with the pre-existing properties of the NPs, enhancing them.

IN VIVO APPLICATIONS AND TOXICOLOGY EVALUATION OF NMS

Recent studies have demonstrated the efficacy of silver nanoparticles (AgNPs) synthesized using plant extracts in controlling medically relevant organisms. For instance, AgNPs produced with *Croton urucurana* extract showed significant activity against embryos and newly hatched snails of *Biomphalaria glabrata*, resulting in delayed hatching and a high frequency of hydropic embryos, without adverse effects associated with the leaf extract used in the synthesis. On the other hand, exposure to the isolated leaf extract caused no adverse effects, highlighting G-AgNPs as potential agents for the control of medically significant snails [55]. Similar results were observed with AgNPs synthesized from *Senna alexandrina*, which demonstrated high larvicidal efficacy against *Culex quinquefasciatus* and *Aedes aegypti*, with controlled toxicity in brine shrimp larvae at doses below 30 μg [56]. In the case of $ZnFe_2O_4$ NPs synthesized using *Nyctanthes arbor-tristis*, larvicidal activity was also highlighted, with an LC50 of 43.529 μg/mL against *C. quinquefasciatus* [57]. These findings demonstrate that green synthesis can produce highly effective materials for controlling pests of epidemiological relevance.

In the experimental model *Caenorhabditis elegans*, AgNPs synthesized with *Euterpe oleracea* indicated low toxicity levels [35]. *C. elegans* is a nematode widely used as a model organism in toxicological and biological studies due to its simplicity, short life cycle, and high degree of genetic conservation with mammals. Its use enables the investigation of the effects of various substances at cellular and molecular levels, contributing to the preliminary safety evaluation of new materials (Table 3).

Despite the positive results and low toxicity observed in some studies, green-synthesized NPs require thorough investigation as they may still present toxicity concerns. For example, high concentrations of selenium (Se) NPs synthesized using *Syzygium aromaticum* exhibited adverse effects on *Artemia franciscana*, emphasizing the importance of detailed dose evaluations to avoid significant ecotoxicological impacts [31]. Such findings are crucial for delineating the toxicological and ecotoxicological profiles of NPs, especially in aquatic environments.

Table 3. *In vivo* toxicity testing of nanomaterials.

NPs	**Shape Size (nm)**	**Synthesis**	**Tested Concentration**	**Organism**	**Organ Life Stage**	**Reference**
	Spherical 32	Aqueous and hydroethanolic extract of *Croton urucurana*	0.012; 0.024; 0.048; 0.097; 0.195 mg/L	*Biomphalaria glabrata*	Embryos and newborn snails	[32]
Ag	Spherical 20–35	*Senna alexandrina leaf*	100–500 μg/mL 5–30 μg	*Cx. quinquefasciatus*, brine shrimp		[33]
	Spherical 23.6	Dihidromiricetina	25.0 μg/mL	*Zebra fish*	Embryos	[38]
	Spherical 62.18	*Thymus vulgaris*	250.500.750 and 1000 (ug/mL)	Rats	Edema in the paws	[58]
	Spherical 20	*Euterpe Oleracea Mart*	10 μg/L	*Caenorhabditis elegans*		[35]
ZnO	Spherical 67.70	*Ipomoea aquatica*	2 μg/ml, 6 μg/ml, 10 μg/ml	*Zebrafish and* mice	not alterations in vital organs	[21–37]
Se	**?**	*Syzygium aromaticum*	100, 200, 300, 400, 500 μg/mL	*Artemia franciscana*	Larvae	[36]
Chitosan gum acacia	227.7	Ionic interaction/ Curcumin	51.1 mg / L	*Zebrafish*	Embryos	[59]
Au	Spherical 5–30	*Euterpe oleracea, Croton lechleri and Aloe vera*	12.5 μg/mL	Wistar rats	Epitélio	[39]
$ZnFe_2O_4$	**?**	*Nyctanthes arbor-tristis*	LC50 43.529 μg/mL LC90 276.867 μg/mL	*Culex quinquefasciatus*	Larvae	[34]

The biocompatibility of NPs synthesized through green methods has also been a focus of investigation. For instance, exposure of *Danio rerio* and Swiss albino mice to ZnO NPs synthesized with *Ipomoea aquatica* leaf extract showed no negative impacts, with no toxicity observed in vital organs, such as the liver and heart [49]. Encapsulation is another promising strategy to minimize toxicity, as demonstrated by AgNPs encapsulated by dihydromyricetin and gum-chitosan nanoparticles loaded with curcumin, which exhibited low toxicity in aquatic models [58, 59].

Danio rerio, commonly known as zebrafish, is a highly relevant model organism in modern science due to its ease of maintenance in laboratory settings, rapid reproduction, and transparent embryos, which allow real-time observation of development and chemical agent responses. This model is widely used in toxicological studies to evaluate the impacts of substances on aquatic systems and vertebrate organisms. Its well-characterized genetics and similarities with the human genome make zebrafish an indispensable tool for studying the toxicity and sublethal effects of nanoparticles in aquatic and terrestrial organisms [60]. Results, such as those reported by Fahaduddin and Trishna B. (2024) [49] reinforce its utility, especially in demonstrating the absence of negative impacts upon exposure to ZnO NPs.

In mammals, AuNPs synthesized from *Euterpe oleracea*, *Croton lechleri*, and *Aloe vera* were tested on Wistar rat epithelium, showing excellent anti-inflammatory and antioxidant activities [61]. Similarly, AgNPs obtained from *Thymus vulgaris* reduced paw edema in rats, highlighting the excellent biological properties of these materials [62].

Due to the benefits associated with green synthesis as an alternative to traditional chemical methods, it is imperative to comprehensively investigate the toxicity of NPs produced through this alternative method. Interaction with biological systems is critical for outlining the toxicological profile of these substances, ensuring their safe use. In this context, tests evaluating interactions with aquatic organisms provide critical insights into the ecotoxicological impacts of NPs.

Alternative models, such as *Danio rerio* (zebrafish), have gained prominence due to their ease of handling, embryo transparency, and high genetic similarity to humans [60]. Similarly, *Drosophila melanogaster* (fruit fly) has emerged as a valuable tool in nanotoxicity studies and biomedical applications. This model organism offers significant advantages, including a short life cycle, low cost, and well-characterized genome. The ease of genetic manipulation in *Drosophila* and its robust physiological response to various chemical agents make it a useful platform for investigating the mechanisms of toxicity and biocompatibility of nanoparticles [63]. Furthermore, in vitro models and organoid-based systems are being developed as promising alternatives, enabling the analysis of cellular and molecular effects without the use of animals. In the case of green-synthesized NPs, studies utilizing *D. melanogaster* and *D. rerio* have shown consistent results in identifying potential environmental and therapeutic impacts while reducing the reliance on murine models.

The use of alternative models is essential for advancing research on nanomaterials synthesized through sustainable methods. Organisms, such as *D. melanogaster* and *D. rerio* enable robust safety and efficacy assessments of nanoparticles, aligning with modern ethical and scientific guidelines. However, continuous efforts are needed to integrate different experimental approaches that ensure a comprehensive toxicological profile for both biomedical applications and environmental impacts.

The exceptional properties of nanomaterials play a fundamental role in increasing their applications across a wide variety of sectors, ranging from medicine and electronics to the food and environmental industries. These unique and advantageous characteristics make nanomaterials highly desirable in various technological and scientific innovations. However, this accelerated growth in the use of nanomaterials inevitably results in the generation of waste from their synthesis. Studying their properties and interactions with the environment and biological systems is essential for safe technological applications.

To overcome the negative implications associated with the synthesis of nanomaterials, green synthesis emerges as a promising and innovative ecological tool. This approach aims to reduce the potential adverse impacts generated by inorganic-synthesis nanoparticles and their resulting waste. Green synthesis utilizes renewable biological sources, such as plants and microorganisms, which play a crucial role in maintaining the stability and desirable properties of nanomaterials. The phyto-constituents and metabolites present in plant extracts not only act as reducing and stabilizing agents but also significantly contribute to the efficacy and functionality of the produced nanomaterials. Therefore, it is crucial that the benefits of green synthesis are continuously explored and promoted, offering a sustainable alternative and the possibility of producing nanomaterials with superior characteristics.

CONCLUSIONS

The works collected here reveal green synthesis of nanoparticles (NPs) as a more cost-effective and environmentally friendly alternative to conventional chemical methods. The constituents present in the extracts used in this type of synthesis have demonstrated a crucial role in stabilizing the NPs, highlighting the quality of the method. Therefore, it is essential that NPs synthesized through this alternative method are compared to those synthesized using conventional methods to verify whether the process has preserved the inherent structural characteristics of the nanomaterials.

The results obtained from various studies clearly indicate that the size and shape of the nanoparticles differ depending on the organism used in the synthesis process. The biological properties of the NPs are also species-dependent, meaning that the choice of the biological source used in the synthesis must align with the intended outcomes. Due to this diversity, it is vital to analyze the interactions between biological systems and the constituents of the extracts, supporting the safe use of these substances, especially for biological applications. Furthermore, the number of studies utilized in the construction of this review highlights the need for more research to be conducted in this area, fostering discussion about the toxicity of NPs obtained from green synthesis.

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