

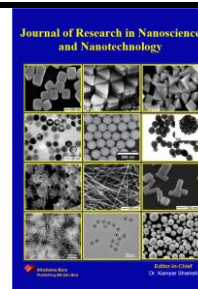


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Green Synthesis of Zinc Oxide Nanoparticles and Its Biomedical Applications: A Review

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ABSTRACT

The rise of nanotechnology has brought to the world a new potential and broader perspective of what humanity can achieve through material manipulation at the nanoscale. In the past two decades, ZnO NPs have become one of the most popular metal oxide nanoparticles in biological applications due to their excellent biocompatibility, economic, and low toxicity. Interestingly, the green method of synthesis using plant sources have been found appropriate for the production of ZnO NPs due to its numerous health, environmental, economic, and medicinal benefits. Due to the large rate of toxic chemicals and extreme environment employed in the physical and chemical production of these nanoparticles, green methods employing the use of plants, fungus, bacteria, and algae have been adopted. Zinc oxide nanoparticles have been successfully obtained by green synthesis using different biological substrates. ZnO NPs have emerged a promising potential in biomedicine, especially in the fields of anticancer and antibacterial fields, which are involved with their potent ability to trigger excess reactive oxygen species (ROS) production, release zinc ions, and induce cell apoptosis. This review summarizes the green synthesis and recent advances of ZnO NPs in the biomedical fields, which will be helpful for facilitating their future research progress and focusing on biomedical fields.

Keywords:

Zinc oxide nanoparticles, green synthesis, cytotoxicity, drug delivery, antibacterial

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1. Introduction

Nanotechnology is an emerging field of modern science and technology which deals with the materials production and manipulation in nanometres [1] [2]. It also has various medical and biomedical applications such as the production of nanoparticles and carbon nanotubes for bio-imaging, cancer therapy, drug delivery and antibacterial[3]. These nanomaterials display unique physicochemical characteristics due to possessing high surface area-to-volume ratio, in comparison

with bulk substances of same compositions [2] [4]. Nanoparticles can easily interact with cell membrane, receptors, proteins, nucleic acids because of their size similarity feature (nanoscale range), which is the key reason of widespread applications of nanoparticles in medicine. Among these metal nanomaterials, ZnO nanoparticles is one of the most extensively employed materials for versatile biomedical applications [2]. Zinc oxide nanoparticles (ZnO-NPs), an FDA approved pharmaceutical excipient, are widely used in drug formulations and cosmetics, due to their stability, biocompatibility and safety [5] [6]. Moreover, chemically, the surface of ZnO is rich in -OH groups, which permit ZnO to slowly dissolve in both acidic (e.g., the tumor cells and tumor microenvironment) and strong basic conditions which approves its potential biomedical applications [7]. ZnO can be synthesized using various methods, such as sol-gel processing, homogeneous precipitation, mechanical milling, organometallic synthesis, the microwave method, spray pyrolysis, thermal evaporation, and mechanochemical synthesis [8]. Nevertheless, these kinds of approaches usually use organic solvents and toxic reducing agents, most of which are profoundly reactive and toxic to the environment. Thus, to limit the effect on the environment, green synthesis method has been used in the production of ZnO nanoparticles. Green synthesis is a method to produce nanoparticles using microorganisms and plants with biomedical applications. This method has numerous advantages, for example, environmentally benign, cost effectiveness, biocompatibility, and safety. This review particularly focuses on the recent advancements in the green synthesis of ZnO-NPs for diverse medicinal applications, including drug delivery.

2. Green Synthesis of Nanoparticles

Green synthesis of nanoparticles is a methodology for the formation of nanoparticles using plants and biopolymers which enhances its biomedical applications. The development of this new approach and the significant interest in it is mainly related to the absence of toxic chemicals or high amount of energy applied to the biological synthesis, which makes the process more cost-effective and eco-friendly [9]. Moreover, the main advantage of this method is that the raw materials used are naturally rich in amino, carboxyl and hydroxyl groups that are often used a stabilizing or capping agents in aqueous medium, triggering the formations of nanoparticles [10]. Many available literatures indicate that the green synthesis of Zn-NPs is more environmentally friendly than the conventional physical or chemical methods used nowadays.

2.1. Plant Mediated Synthesis of ZnO-NPs

Plants are the most common biological substrate used for the green synthesis of nanoparticles with metallic ions [9]. Plant parts like leaf, stem, root, fruit, and seed have been used for ZnO NPs synthesis because of the exclusive phytochemicals that they produce [11]. This may be related to the fact that vegetal substrates are accepted to be more cost-effective, simple and less harmful than microorganisms. Plants are most preferred source of NPs synthesis because they lead to large-scale production and production of stable, varied in shape and size NPs [11].

Generally, green synthesis of ZnO-NPs using plants sources like leaves or flowers is washed thoroughly in running tap water and sterilized using double distilled water (some use Tween 20 to sterilize it). Then, the plant part is dried at room temperature followed by grinding into powder using a mortar and pestle. Next, the weighed powder and distilled water mixed to together under continuous magnetic stirring to prepare plant extract. The solution is filtered using whatman paper to obtain clear solution to be used as plant extract for further process. The specific volume of plant extract is mixed with zinc precursors like zinc nitrate, zinc acetate, zinc sulphate or zinc chloride solution. Later, the mixture is calcined at a higher temperature resulting in the formation of ZnO-

NPs. The obtained ZnO-NPs are visually confirmed by color change and the UV-vis spectroscopy was used to further confirmation. Table 1, summarizes the different plant sources that are utilized for the production of ZnO NPs.

Table 1. Plant mediated synthesis of ZnO nanoparticles.

Plant Source	Description	Precursor	Size & Shape	Ref.
Ferulago angulata (schlecht) boiss	plant	Zinc Acetate Dihydrate	32 and 36 nm with spheroid shape	[12]
Sageretia thea (Osbeck.)	leaf	Zinc Acetate Dihydrate	14 nm	[13]
Plectranthus amboinicus	leaf	Zinc Nitrate	20–50 nm with spherical and hexagonal	[14]
Anisochilus carnosus	leaf	Zinc Nitrate	30 and 40 nm with hexagonal wurtzite structure	[15]
Limonia acidissima L.	leaf	Zinc Nitrate	12 nm and 53 nm with spherical shape	[16]
Syzygium cumini	leaf	Zinc Acetate	71 nm with spherical-like shape	[17]
Punica granatum (pomegranate)	plant	Zinc Nitrate hexahydrate	32.98 nm and 81.84 nm with spherical and hexagonal shapes	[18]
Punica granatum (pomegranate)	plant	zinc nitrate hexahydrate	40-70 nm with spherical shape	[19]
salvia officials	leaf	zinc nitrate	12 nm with hexagonal wurtzite structure	[20]
Carica papaya	leaf	Zinc acetate dihydrate	14 nm with semi-spherical	[21]
Arthrospira platensis	plant	Zinc acetate dihydrate	30.0 to 55.0 nm with spherical shape	[22]
Bergenia ciliata Rhizome	plant	zinc acetate dihydrate	30 nm with flower shaped structure	[23]

2.2. Biopolymer Mediated Synthesis of ZnO-NPs

The use of natural polymers in the synthesis of nanomaterials have a low cost and eco-friendly approach [24]. Many natural polymers have been used in the synthesis of nanoparticles as a green stabilizer [25]. Chitosan (CS) is one of the promising natural biopolymer and has adapted with a suitable properties of biocompatibility, biodegradability, non-hazardous, odorless, metal ion adsorption. Chitosan's primary amine and hydroxyl groups have a very powerful affinity (like a chelating agent) to metal ion to decrease particle size and stop agglomeration [26]. Pullulan is also another biopolymer that is produced from starch by growing yeast like fungus *Aureobasidium pullulans*. The advantage of pullulan is it is water soluble. Pullulan is non-toxic, non-mutagenic, odourless, biocompatible and biodegradable [27]. In addition, tragacanth gum (TG) is a natural, nontoxic and biocompatible polymer widely used as an emulsifier and thickener in the food and drug industries due to stability in a wide range of temperature and pH. There is a study applied the gum

as an eco-friendly and cost effective polymer to synthesize zinc oxide nanoparticles. They synthesized hexagonal zinc oxide with the average diameter of 55–80 nm and length of 240 nm [28]. Moreover, alginate is a naturally occurring poly-anionic polysaccharide derived and commercially extracted from brown marine algae (Phaeophyceae) [29]. As a low-cost, abundantly available, biocompatible and environmentally friendly biopolymer, it has been used as a green stabilizer as stated in numerous studies. Furthermore, carrageenan is an ecofriendly polymer derived from a class of red seaweed. Carrageenan is known to have valuable biological functions, due to the superior gelling and high viscosity properties of the native carrageenan[30]. There are several biological properties of biopolymers such as antiviral activity, anticoagulant activity, antitumor activity, antioxidant activity, anti-inflammation and immunomodulatory activity that might bring more benefits in medical application[31].

3. Biomedical Applications

A recent development in the emergence of nanotechnology and ZnO-NPs has led to significant enhancements in biomedical applications of nanomaterials. Use of natural raw materials and living organisms as capping and reducing agents for the synthesis of ZnO-NPs provided better biocompatibility and responses for their interactions with biological tissue which enhances their performance substantially. As a result, green synthesized ZnO-NPs are famous as effective and viable nanoparticles that can be used to target bacterial infections, devastate the membrane of cancerous cells or deliver various compounds to diseased tissue, and to measure concentrations of distinctive biomarkers inside the body. Since numerous literatures is available for different applications of ZnO-NPs in biology and medicine, **Fig. 1** represents the diverse biomedical applications of ZnO-NPs that are elaborated below.



Figure 1. Potential biomedical applications of green synthesized ZnO-NPs.

3.1. Antibacterial Activity

Bacterial diseases pose serious health threats to all humankind. For decades, individual cells within pathogenic bacterial populations have decreased the antibiotic susceptibility, which is linked to reduced metabolic rates. Moreover, antimicrobial resistance continues to increase, bacterial infections that once were easily treated are becoming untreatable. Emergence of new strains intended the researchers to improve this complex problem. ZnO-NPs are extensively explored as an antibacterial agent due to its unique properties like high surface area and its ability to induce oxidative stress [32] [33]. ZnO-NPs release Zn^{2+} ions which interact with the thiol functional group of respiratory enzymes. ZnO-NPs affect the cell membrane and lead to the production of reactive oxygen species (ROS) such as hydrogen peroxides, superoxide anion and hydroxyl radicals. It could cause bacterial cell membrane disintegration, damaging protein membrane, DNA, and mitochondria which ultimately results in the death of bacterial cells [1]. The antibacterial mechanism of ZnO-NPs is displayed in **Fig.2** below.

Many researchers have investigated the antibacterial potency of biosynthesized ZnO NPs against numerous bacterial from which remarkable antimicrobial efficiency have been recorded. For instance, antibacterial activities of *P. granatum*/ZnO-NPs against *Escherichia coli* (*E. coli*) and *Enterococcus faecalis* (*E. faecalis*) were evaluated and compared. Obtained results showed that smaller-sized *P. granatum*/ZnO-NPs are more effective in inhibiting growth of both bacteria lower MIC50 values [18]. Another study also reported on the antibacterial activity of the ZnO-NPs evaluated using *E. coli* and *B. subtilis* as test organisms by the well diffusion method. The results confirmed a larger zone of inhibition as high as 12 mm at a concentration of 100 lg/ml against *E. coli* than *B. subtilis* [34]. Moreover, the antibacterial potential of the biosynthesized ZnO NPs using *B. tomentosa* leaf extract was tested against *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *E. coli*. The bactericidal effect of ZnO NPs was found higher for Gram-negative bacteria than Gram-positive bacteria and was based on the difference in the structural composition of Gram-positive and Gram-negative bacteria [35]. *B. tomentosa* leaf extract-derived ZnO NPs showed a significant zone of inhibition for *P. aeruginosa* (20.3 mm) and *E. coli* (19.8 mm), whereas the zone of inhibition was observed less for *B. subtilis* (8.1 mm) and *S. aureus* (10.7 mm) [36].

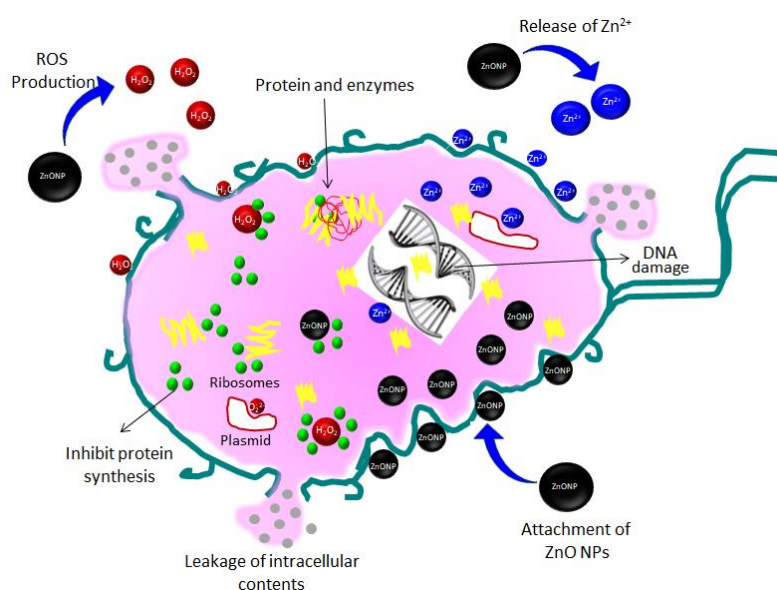


Figure 2. Schematic illustration of antibacterial mechanism of zinc oxide nanoparticles.

3.2. Anticancer Activity

Cancer may be a conjunction of illnesses described by the irregular development of tissue that might lead to the development of tumors that can spread into other tissues and cause extreme impacts in the patient, with complications and severities possibly causing death [37]. In 2019, disease was indexed as the subsequent driving reason for death in the US with roughly 2 million individuals being analysed each year [10]. Current methodologies, for example, chemotherapy and radiotherapy, albeit useful, are not totally viable and present huge disadvantages as serious results, for example, immunosuppression, anemia, sickness, or even death. Indeed, it has been mentioned in the literature that some cancer cells have become resistant to treatments, prompting the presence of chemotherapy-resistant tumors, making those medicines not a viable alternative for this sort of patient. As a result, important efforts have been made in the development of new approaches. Thus, the use of nanotechnology has become more popular, because it tends to be applied towards cancer treatment and beat considerable downsides (of the conventional treatment methods) without experiencing toxicity to normal tissues. Among several biomedical applications, the use of ZnO-NPs has been explored as biocompatible and biodegradable nanoplatforms for cancer treatment [38]. ZnO-NPs are known to initiate the generation of ROS upon contact with the cells, which leads to mitochondrial damage, activating cell death in cancer tissue.

The anticancer activity of ZnO NPs prepared by green synthesis method has been validated against a variety of cancerous cell lines. For example, the anticancer activity of the synthesized nanoparticles against A549 lung cancer cells was evaluated, revealing an inhibitory concentration (IC₅₀) of 15.6 lg/ml [34]. The MTT assay used for cytotoxicity evaluation also depicted the significant dose-dependent cytotoxic effect of ZnO NPs against the A549 lung cancer cell line [39]. In addition, the potential anticancer activity of the synthesized ZnO-curcumin nanocomposites was studied on the rhabdomyosarcoma RD cell line via MTT assay, while their cytotoxic effects were tested against human embryonic kidney cells using the resazurin assay [40]. The results exhibited the best balance between the two, showing the lowest toxicity against healthy cells and good anticancer activity. Another study reported similarly that ZnO-NPs synthesized using *Deverra tortuosa* plant showed a profound selective cytotoxic effect on the Caco-2 and A549 cancer cell lines with appreciable lower cytotoxic activity on the normal WI38 cells [7]. Noticeably, the ZnO-NPs had the most potent cytotoxic activity and Caco-2 was more sensitive than A549. This study also described that significantly higher IC₅₀ values 902.83 and 434.60 µg/ml obtained from the treatment of the normal lung epithelial cell (WI38) with the respective ZnO-NPs [7]. ZnO-NPs are with its all promising characteristics offering a satisfying, safer and cheaper alternative to conventional therapy protocols.

3.3. Antifungal Activity

The antimicrobial potential of ZnO-NPs is not restricted to microbes or bacteria but also to different kinds of microorganisms like fungi. Various reports are available for its antifungal activity studies for the treatment of yeasts and fungi because of its widely usage as antifungal additives in food industry. Increase of fungal pathogens is one of the main issues in agriculture and causes high financial losses to farmers [41]. Many groups have reported the antifungal efficacy of ZnO-NPs against plant pathogens. For example, nanoparticles were tested against fungal phytopathogens, namely *A. alternata*, *A. niger*, *B. cinerea*, *F. oxysporum* and *P. expansum* [42]. Nanoparticles used in the study exhibited good antifungal activity. Nanoparticles positively influenced the inhibitory effects of fungicides. *P. expansum* was observed to be the most sensitive fungus. Moreover, the synthesized ZnO-NPs have demonstrated an acceptable antifungal effect against *C. albicans* with the

minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were 128 and 256 $\mu\text{g/ml}$, respectively [43]. Antifungal action of ZnO NPs was also studied against *Aspergillus* and *Penicillium* by well dispersion strategy. The antifungal activity showed that ZnO-NPs constitute as an effective fungicidal agent against both *Aspergillus* (4 ± 0.5 mm) and *Penicillium* ($3 \text{ mm} \pm 0.4$ mm) at 30 $\mu\text{g/mL}$ fixation [44]. Another study reported that MICs were measured as the lowest concentration of nanoparticle that resulted in $\geq 90\%$ decrease in growth inhibition of *Candida* isolates compared with the control growth levels (wells without ZnO NPs) [45]. The lowest concentration of ZnO NPs that inhibited $\geq 99.9\%$ of yeast cell growth was considered as minimum fungicidal concentration (MFC) [45].

3.4. Anti-inflammatory Activity

Inflammation is an extreme response reaction by living tissue because of any sort of injury. Redness, pain, heat, and swelling are the primary indicators of inflammation. Inflammation is essential for the complex organic response of body tissues to destructive stimuli, for example, pathogens, harmed cells, or irritants [33]. The arterioles in the surrounding tissue dilate when there is harm to any part of the body. This gives redness because of the raised blood circulation toward the area [46]. Inflammation could be either acute or chronic inflammation. Acute inflammation might be an initial response of the body to harmful stimuli. In chronic inflammation, the inflammatory response may be out of proportion which brings about harming the body over a long time. Since the emergence of nanoparticles and considering these biological applications of zinc ions, the anti-inflammatory effects of ZnO-NPs have gained much attention.

ZnO NPs exert their anti-inflammatory activity through various mechanisms namely, inhibition of pro-inflammatory cytokines release, myeloperoxidase inhibition, inhibition of inducible nitric oxide synthase (iNOS) enzyme expression, and inhibition of the NF- κ B pathway and inhibition of mast cell degranulation [1]. The anti-inflammatory activities of green synthesized ZnO NPs are summarized in **Table.2**.

Table 2. Anti-inflammatory activity of ZnO nanoparticles.

Type of NPs	Model/Activity	Effects	Reference
ZnO-NPs using <i>Amla</i> fruit extract synthesized	Bovine serum albumin (BSA) makes up approximately 60% of all proteins in animal serum	The synthesized nanoparticles show the quality of anti-inflammatory activity in the range of 80–90%, respectively. All these data with results show that ZnO nanoparticle produced from <i>Amla</i> fruit potent anti-inflammatory property	[46]
ZnO-NPs using <i>Kalanchoe pinnata</i> leaf extract	LPS induced murine macrophage RAW 264.7 cells	RAW 264.7 cells were incubated with different concentrations of ZnO NPs (1, 0.5, 0.25, and 0.1) mg/mL for 24 h and cell viability relative percentage with control was calculated. Cells showed no significant toxicity up to the concentration of 1 mg/mL	[47]
ZnO-NPs	ketoprofen in rats	Ketoprofen at all the examined doses (5, 10, and 20 mg/kg) inhibits the carrageenan-induced paw edema by 40%–65% at the second and third hour after the administration of carrageenan. Research	[48]

			also showed that ZnO-NPs efficiency to reduce acute pain in hot plate test is higher than their conventional form	
ZnO-NPs using <i>Andrographis paniculata</i> leaf extract	In vitro: protein denaturation		The result was interpreted in terms of the IC50 values (effective concentration showing 50% inhibition activity).	[49]
ZnO-NPs using <i>H. officinalis</i> extract	In vivo: inflammatory genes (IL-1B and IL-10)		Evaluation of mouse paw edema showed that the NPs are able to decrease inflammation.	[50]
ZnO-NPs using <i>Vernonia amygdalina</i>	Different pain and inflammation-induced mice		All the outcomes revealed the effective anti-inflammatory of zinc oxide nanoparticles from <i>V. amygdalina</i> in mice models.	[51]

3.5. Wound Healing

Skin is an important part of our body which functions to protect the body from external invasions like harmful chemical and physical environments, and invasion of microorganisms. Any damage caused to the skin results in wounds. The wound will heal automatically, often wound healing might delay because of microbial infection. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are few such microorganisms which cause severe wound infections [1]. As nanoparticles are known for their antibacterial activity and metal oxides nanoparticles generate hydrogen peroxide (H₂O₂) which could cause cell damage. Therefore, metal oxide nanoparticles can be used to kill these pathogenic organisms and thus can enhance the process of wound healing. Several literatures reported the use of ZnO-NPs as successful wound healing agent.

In 2016, the proficiency of ZnO-NPs for wound healing was assessed in vivo by topical application of a matrix with EPCs on wounds in mice [52]. In vivo evaluation in this mice model, GPZ scaffold enriched with EPCs demonstrated faster wound healing in comparison to all other groups. Recently, wound healing properties of chitosan/poly(vinyl alcohol)/zinc oxide (CS/PVA/ZnO) beads were tested in mice skin wound [53]. In vivo evaluation in mice skin confirmed that the CS/PVA/ZnO dressings appeared faster wound healing, and more complete when compared with that of pure chitosan, and CS/PVA. In addition, the influences of ZnO-NPs on fibroblast cell growth (NIH3T3) as a wound healing activity was conducted [54]. The results proved that the growth of fibroblast cell is higher with ZnO nanoparticles of larger particle size. Another study also reported the healing efficiency of mouse wound using ZnO-NP/silica gel (ZnO-NP/SG) dressings due to its extensive evidence on healing property [55]. ZnO-NP/ SG-30 ppm exhibited a maximum 95% wound contraction of skin mice. These findings elaborated that ZnO-NPs enhanced the skin repairing on the wound surface and support its usage in the future.

4. Drug Delivery

Using nanoparticles in targeted drug delivery provides exciting opportunities for much more safety and effective cancer treatment. Targeted drug delivery is an important biomedical application that aims to deliver anticancer drugs to the specific site of the tumor and avoid damage to surrounding healthy cells. Compared with other nanomaterials, ZnO NPs are attractive because of their low toxicity and biodegradable characteristics. ZnO NPs have obtained enormous interest in cancer drug delivery. Different types of drugs such as doxorubicin, paclitaxel, curcumin, and baicalin or DNA fragments could be loaded onto the ZnO NPs to show better solubility, higher toxicity compared with individual agents, and effective delivery into cancer cells [33]

Recently, pectin-based gel and its nanocomposite with zinc oxide have been compared for their capacity to release Donepezil for the possible use as an implantable drug delivery platform for the treatment of Alzheimer's disease. The parent polymer released about 46% whereas the nanocomposite released 88% of the drug during 120 h [56]. The result explored pectin based nanocomposite as a potential platform for the development of implantable drug delivery systems for chronic diseases. Another study functionalised nanohybrid hydrogel using L-Histidine (HIS) conjugated chitosan, phyto-synthesised zinc oxide nanoparticles (ZNPs) and dialdehyde cellulose (DAC) was formulated as a sustained drug delivery carrier for the polyphenol drugs – Naringenin (NRG), Quercetin (QE) and Curcumin (CUR). A maximum loading efficiency of 90.55 %, 92.84 % and 89.89 %, respectively were optimised for NRG, QE and CUR in the hybrid hydrogel [57]. The maximum drug release was favoured for the optimum drug loading and at pH-5 which makes it a promising drug carrier towards targeted anti-cancer applications. Moreover, the phenylboronic acid (PBA) conjugated Zinc oxide nanoparticles (PBA-ZnO) synthesized, loaded with quercetin and conducted drug delivery test for breast cancer cell [58]. Most interestingly, both the *in vitro* and *in vivo* results indicate that ZnO based nanoformulations can significantly enhance the anticancer effect of quercetin by increasing its bioavailability. These results demonstrate that ZnO-NPs could be a drug delivery system to deliver the drug more specifically to the colon.

5. Conclusion

The rise of nanotechnology has brought about a tremendous enhancement in several scientific fields, particularly for biomedical applications of materials. Biosynthesis of nanoparticles utilizing eco-friendly approach has been the area of focused research in the last decade. Green sources act as both stabilizing and reducing agent for the synthesis of nanoparticles controlled shape and size. These green-synthesized nanostructures have been effectively implemented as biomedical agents, offering considerable performance enhancements, compared to their traditionally produced counterparts. These enhancements include a better cytocompatibility and clearance from the organisms, and a mild synthetic process which is both environmentally friendly and cost-effective. ZnO NPs have displayed promising biomedical applications dependent on its biocompatibility and anticancer, antibacterial, anti-inflammatory, drug delivery, as well as wound healing activity. ZnO NPs are one of the significant nanomaterials utilized widely in biomedical field. Because of inherent toxicity of ZnO-NPs, they possess strong inhibition effects against cancerous cell and bacteria, by inducing intracellular ROS generation and activating apoptotic signaling pathway, which makes ZnO-NPs a potential biomedical agent in the field of medicine world.

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