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Review

Biogenic Synthesis of ZnO Nanoparticles and Their Application as Bioactive Agents: A Critical Overview

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Abstract: Zinc oxide is a safe material for humans, with high biocompatibility and negligible cytotoxicity. Interestingly, it shows exceptional antimicrobial activity against bacteria, viruses, fungi, etc., especially when reduced to the nanometer size. As it is easily understandable, thanks to its properties, it is at the forefront of safe antimicrobials in this pandemic era. Besides, in the view of the 2022 European Green Deal announced by the European Commission, even science and nanotechnology are moving towards "greener" approaches to the synthesis of nanoparticles. Among them, biogenic ZnO nanoparticles have been extensively studied for their biological applications and environmental remediation. Plants, algae, fungi, yeast, etc., (which are composed of naturally occurring biomolecules) play, in biogenic processes, an active role in the formation of nanoparticles with distinct shapes and sizes. The present review targets the biogenic synthesis of ZnO nanoparticles, with a specific focus on their bioactive properties and antimicrobial application.

Keywords: zinc oxide; nanoparticles; antimicrobial; antiviral; bioactive; biogenic synthesis; green synthesis; biosynthesis

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1. Introduction

In recent years, various metal oxide nanoparticles (MONPs) revived interest for a plethora of different applications, specifically when a green experimental approach is proposed [1]. To meet the increasing demand of such NPs, many different methods have been established. Amongst MONPs, zinc oxide NPs (ZnONPs) got unique attention due to their distinctive properties, which make them useful for many different real-life applications. In particular, the biosynthesis of ZnONPs has been proposed, mostly in the last 10 years, as a cheap and environment-friendly option to chemical and physical methods, due to concerns about climate change, water pollution, limited natural resources, toxicology, and so on [2]. A detailed bibliographic search on the Scopus® database confirms the constantly growing interest on the topic (Figure 1). Despite the low cost and need for simple equipment in such processes, long reaction time and the use of nonaqueous media are considerable disadvantages of common chemical routes [3]. In place of these classical methods, "green" and "soft" synthesis approaches are constantly needed to develop tunable ZnO nanomaterials. Ideally, these novel methods should also be cost and time effective in comparison to other available protocols: from a brief analysis of the retrieved literature, research is moving towards these principles [4,5].

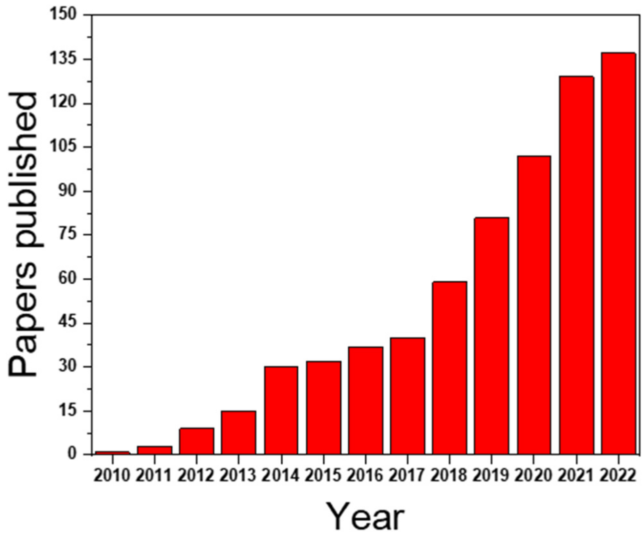

Figure 1. Number of papers published about the biosynthesis of ZnONPs in the last years. Data analysis performed on Scopus® using "Biosynthesis", "Zinc oxide" and "Nanoparticles" as keywords.

The USA Food and Drug Administration (FDA) has registered ZnO as a "GRAS" (generally recognized as safe) material [6]. This is why ZnONPs already find applications in several fields of medicine, industry, food, agriculture, and electronics. Many of them have been recently reviewed by Prasad et al. [5]; thanks to their intrinsic biocompatibility, biogenic ZnONPs have been extensively studied for their biological applications and environmental remediation. The synthesis of NPs, with control over particle size, shape, and crystallinity, has been one of the main objectives in materials chemistry. Nature provides ways and insight into the synthesis of advanced and eco-friendly nanomaterials. About three decades ago, the first reports about biological systems, which could act as "bio-laboratories" for the production of metal and metal oxide particles at the nanometer scale, was envisaged [7,8]. Looking throughout the literature on biogenic synthesis, despite the source of biomolecules that is used, reaction yields are basically never explicated. We believe this is mainly due to the intrinsic variability of each biogenic reaction mixture, which can deeply influence reaction yields and NP properties [9].

Various microorganisms, such as bacteria [10] and fungi [11], along with plant extracts [12] have acted as green chemicals towards NPs. Surprisingly, waste from agrifood industries of food supply chains have been used as chemicals too; these molecules allow for the preparation of NPs without the use of dangerous chemicals and ensure the reuse and reduction in wastes in the view of a circular economy.

During biogenic syntheses, biomolecules generally act both as reducing agents for metal precursors, and capping agent for as-prepared NPs. This "layer" of biological molecules surrounding NPs, is often proposed as a way to confer the high biocompatibility and negligible toxicity in comparison with NPs prepared by classic chemical methods [5]. The biocompatibility of biogenic ZnONPs may offer very interesting applications in biomedicine and prevention of microbial contamination. It is worth pointing out that biogenic procedures may also have some drawbacks. Being, in most cases, the exact biochemical composition of the reaction mixture unknown, it is not possible to foresee which byproducts and wastes could be developed during the process [13]. Additionally, some of these synthetic protocols could need a large amount of solvent (water, in most cases) for obtaining phyto-extracts with a non-negligible environmental impact [14].

Vegetable extracts (from plants, fruits, spices and herbs, flowers), algae and seaweeds, and metal-tolerant bacteria are the most used source for reactants exploited in recent papers on the topic (Figure 2) [15].

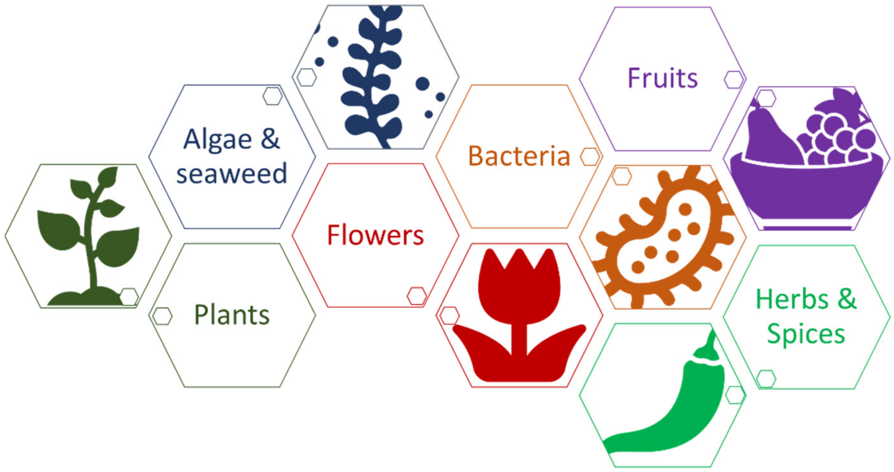

Figure 2. Most used source for reactants exploited in the biogenic synthesis of ZnONPs.

The aim of this review is not a comprehensive listing of all the existing literature about the biosynthesis of ZnONPs, but a critical discussion of the most recent papers, with specific attention towards the use of these NPs as antimicrobial and/or antiviral agents. This review article will not cover heteroatom-doped ZnO nanomaterials. Each reactant source will be discussed in a dedicated paragraph; main results on the bioactive properties of ZnO will be examined as well, lining out the importance of this material during the coronavirus pandemic. A list of recent review papers (2021–2022) on this topic is reported in Table 1.

2. Phyto-Mediated Synthesis: Plants and Flowers

Among green biosynthesis routes, plant-mediated ones got expanding consideration; many plant extracts contain a large amount of phytochemicals, which can act both as reducing agents and capping/stabilizing molecules [16]. Since the 1990s, the use of plants and plant-derived substances, in the ZnONPs synthesis, was proposed to lessen remarkably the requirement of expensive chemicals with limited availability, and hazardous experimental protocols [17]. ZnONP production from plant extracts is easily scalable and then highly appealing for industrial and technological use. Furthermore, this approach is extremely straightforward. Briefly, a zinc salt (mainly zinc nitrate, chloride, or acetate) is added to a plant extract; after a proper reaction time (catalyzed, when appropriate, by sunlight or other energy sources), the produced suspension is washed and subjected to thermal treatments to have stoichiometric ZnO nano- or micro-powders [18]. Preparation of the plant extract is pivotal for a synthesis with sufficient yield. The leaves (or any further plant part) first go through pretreatment processes. The latter are the most important means for the extraction of phytochemicals to deliver the phyto-mediated synthesis. During this step, vegetable cells are disrupted to allow for the release of active molecules. A number of steps are typically necessary [15]:

i. Plant parts are rinsed with water.
ii. Plant parts are then sliced into smaller parts and then grinded in a mortar or ball-milled (the choice depends on the nature of the plant part). The obtained material can either be used itself or subjected to solid–liquid extraction (boiling, soxhlet, etc.).
iii. Mixture is filtered to remove the solid component.
iv.-vi. Plant extract can then be used for NP synthesis (sometimes a pre-concentration step is necessary).

Figure 3 reports a detailed scheme of the process.

Figure 3. Schematic explanation of the phyto-mediated synthesis of ZnONPs. (1) washing, (2) grinding-milling, (3) filtration, (4) pre-concentration (facultative), (5,6) addition of zinc precursor and ZnONPs production. Wiley material reproduced from [15] with permission from John Wiley & Sons Inc, the Wiley Companies®.

A fascinating review was published in 2021 on phytogenic ZnONPs, synthesized using various molecules as reductants of organic and inorganic Zn salts, as well as their production, characterization and biocompatibility, which explains their present request for dermo-pharmaceutical and cosmetic products [19].

Table 1. Main reviews on biogenic synthesis of ZnONPs published in 2021–2022.

Source of Biological Reactants	Year	Applications	Ref.
Microbes/bacteria	2022	Biomedical, agricultural, environmental	[20]
Plant extracts	2022	Antimicrobial	[21]
Microorganisms, plant extracts, algae	2022	Gas Sensing	[22]
Microorganisms, plant extracts	2022	Fertilizers	[23]
Plant extracts	2022	Biomedical	[24]
Microorganisms, plant extracts	2022	Biomedical, (bio)sensing, imaging	[25]
Natural extracts	2022	Pharmacotherapeutics	[26]
Fruit peel	2022	Nutraceutical, biomedical, active coatings, sorbents	[27]
Plant extracts	2022	Anticancer agents	[28]
Microorganisms, plant extracts	2022	Photocatalysis	[29]
Microorganisms, plant extracts, algae	2021	Pollutant removal	[30]
Plant parts	2021	Antimicrobial, anticancer	[31]
Marine organisms	2021	Drug delivery, antimicrobial, (bio)sensing, fertilizers	[32]
Microorganisms, plant extracts, algae	2021	Antibacterial, antioxidant, antidiabetic and tissue regeneration	[33]
Biopolymers, plant parts	2021	Nanocomposite production	[34]
Plant extracts	2021	Environmental	[35]
Plant extracts	2021	Biomedical	[36]
Biopolymers, plant parts	2021	Drug delivery	[37]

One of the most remarkable cases of ZnONPs made for this aim concerns the use of *Salvia Officinalis* [38,39]. It is crucial to state that this is a medicinal plant that grows in most of the continents, and its pharmacologic benefits have been extensively recognized [40]. The major phytochemical composition of *S. officinalis* comprehends glycosidic derivatives (such as flavonoid glycosides, cardiac glycosides, coumarins, tannins, and saponins), steroids, terpenes/terpenoids (including sesquiterpenoids, monoterpenoids, diterpenoids, and triterpenoids), mostly found in leaves and flowers [41]. The synergic antimicrobial effect of ZnONPs prepared from salvia extracts has been recently demonstrated [39].

Still talking about dermo-pharmaceutical and cosmetic interest towards ZnONPs, *Aloe vera* leaf extracts have been used as reducing and capping agents too. Recently, Batool et al. [42] reported about that Aloe leaves contain a gelatinous material (commonly used as balm and humectant) which holds vitamins A and C, folic acid, β-carotene antioxidants, and some trace elements, such as Ca, Cu, Mg, K [43]. All these substances, along with chemicals, such as salicylic acid and anthraquinones, make this extract very attractive for cosmetic and pharmaceutical formulations, guaranteeing a potent reducing power as well [44].

Regarding the specific case of flowers, acetonic extracts are generally used, i.e., extracts made using acetone as solvent. Most of the important chemicals coming from flower petals are, indeed, much more soluble in this solvent rather than in aqueous solutions [45,46]. Flowers have a very high amount of flavonoids (>4%$_{w/w}$), which are crucial in NPs synthesis and have good stabilizing/capping properties [47]. Many flowers have been exploited in 2021 and 2022 for the preparation of ZnONPs: *Geranium robertianum* [47], *Camelia sinensis* and *Datura Stramonium* [48], *Lantana camara* [49], *Rhaponticum repens* [50], *Tagetes erecta* [51], *Gardenia thailandica* [52], *Malva Parviflora* [53], *Parthenium histerophorus* [54], just to cite a few.

Recently, tree bark was also used for the preparation of ZnONPs; in a paper from Parveen et al. [55], ZnO was exploited as nano-fertilizer for the cultivation of different rice varieties.

An enormously wide plethora of flowers, plants, and extracts have been suggested to accomplish the green synthesis of ZnONPs [56]. A comprehensive list of these procedures goes beyond the aim of this review. However, it is worth stating that the ultimate preference of a specific phyto-extract is mostly determined by the local vegetation, i.e., from accessibility of some vegetable types in certain areas. Based on the singular chemical composition of every plant extract, several shapes on ZnO-based nanostructures were achieved, varying from spheroidal particles (which are most common), to rod-like and flower-like ones [57]. Physicochemical properties of the obtained particles are a function of the phyto-extract as well [58].

The exact mechanism of plant-mediated synthesis tightly depends of the (peculiar) chemical nature of each extract and is still unanswered in most cases.

3. Algae and Seaweeds

Although less common, seaweeds and cellular algae have been employed for the green synthesis of metal oxide NPs [59] as well. Algae are sea microorganisms that have been reported not only to uptake heavy metals from the environment, but also to synthesize metal NPs [60]. Many seaweed species possess similar properties [61]. These sources are environmentally amenable and are the proficient biological sources for the preparation of ZnONPs [62]. Seaweeds are characterized by a higher amount of polysaccharides [63], as compared to plant extracts; the presence of these compounds ensures a very high capping and/or chelating capacity on produced NPs [64]. Polysaccharides are thought to be involved in the conversion of −OH (alcoholic groups) into –CHO (aldehydic groups) through oxidation, which results in the reduction of precursor zinc ions into elemental oxidation state. Spontaneous oxidation of the as-prepared NPs brings the final chemical state to ZnO (Figure 4) [62]. The powdered extract of *Gracilaria edulis* was proficiently applied for the preparation of ZnO nanorods (NRs). Quinine, highly present in the aqueous extract of this seaweed, is regarded as the main reducing agent in the production of ZnONPs. Quinines are biomolecules with a very high redox potential, successfully used as reducing agents for metal ions in various papers [65]. In 2021, Alsaggaf et al. proposed the synthesis of ZnONPs using a green phenol-rich extract from *Ulvaceae*, a widely available macroalga in the Mediterranean Sea; these ZnONPs were successfully used as active layers for the preservation of seafoods [66]. A similar approach was also followed by Anjali et al. [67] and Thirumoorthy et al. [68] for antibacterial, antifungal, and anticancer purposes [69]. Algae-mediated synthesis of ZnONPs was also described by Subramanian et al. [70]; brown seaweed *Sargassum muticum* was collected at a marine biodiversity hotspot area along the Gulf of Mannar coastline, in the eastern coastal region of Tamil Nadu, India. ZnONPs were used here for both the photo-degradation of methylene blue dye under different light conditions, and as wide-spectrum antimicrobial agents against multidrug-resistant bacteria [71]. Another brown alga named *Dictyota dichotoma* was analogously exploited to prepare antimicrobial ZnONPs [72].

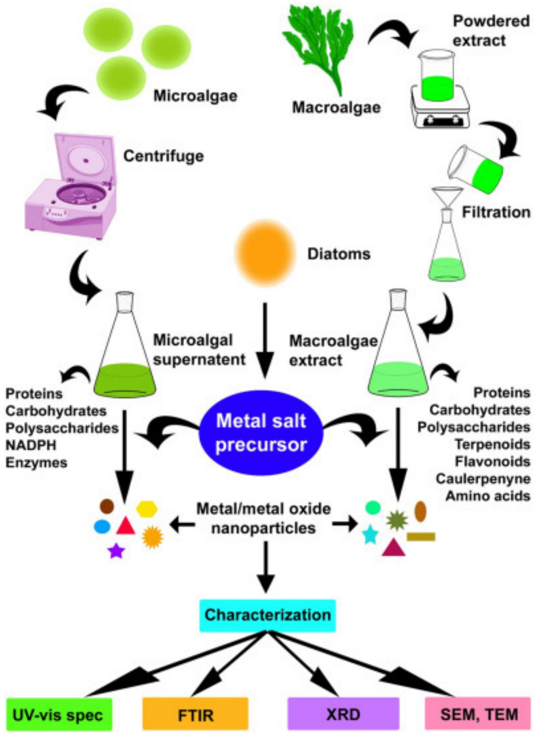

Figure 4. Synthesis route and characterization of algae-based metal and metal oxide NPs. Reprinted from [62] with permission from Elsevier.

To the best of our knowledge, one of the few systematic studies on the effect of synthetic parameters and provenance of seaweed extracts on the final physicochemical properties of ZnONPs is that from Nagarajan et al., published in 2013 [73]. They rationalized the effect of seaweed extract concentration, temperature, pH, and reaction time. The composition of extracts coming from three different species were compared, e.g., green *Caulerpa peltata*, red *Hypnea valencia*, and brown *Sargassum myriocystum*.

The rich biodiversity and easy availability of algae and seaweeds, which do not require any direct "plant care" from humans, has not been exploited exhaustively for nanomaterials synthesis yet, and we believe that this approach will flourish in the near future.

4. Foods and Herbs

Another class of bio-mediated syntheses regards the use of food wastes and herbs. Most wastes contain phenolic compounds, low-molecular-weight secondary metabolites, which act as protective agents from oxidative damage and possess antimicrobial properties. These phenolic compounds include anthocyanins, flavonoids, tannins, alkaloids, gallic acid, ferulic acid, chlorogenic acid, catechin, epicatechin, saponins, and their content in waste depends on the type and profile of the (agri-)waste itself [74]. More specifically, fruits peel and pulp generally contain reducing sugars [75], which can effectively reduce metal precursors. Similarly, edible vegetables and nuts contain terpenoids, polysaccharides, and aromas with both reducing and stabilizing properties [76]. Lignin is another polyphenolic compound (present in high amount in grape stalks, as an example) which has been successfully used for the production of metal oxide NPs [74].

The active components can be extracted by the use of aqueous, organic or mixed solvents. The solvent is added to the food waste, and batch extraction is performed at a low temperature (generally about 50 °C, with some molecules being thermolabile). The liquid-solid suspension is generally made by a 20:1 ratio between solvent and solid waste and is stirred at fixed temperature for about 1–2 h. The solution is then filtered, centrifuged, and the extract is then ready for NPs synthesis. The extract can also be stored for future use at a temperature of about 0–4 °C [77].

Only during 2021 and 2022, many different examples can be found in the literature, ranging from fruit peels, such as papaya [78], pomegranate [79], myrica [80], coconut [81], dates [82], mulberry [83], orange [84,85], and banana [86], to olives [87], grounded coffee [79], turnip and raphanus discards [88,89], spinach [90], nut shells and leaves [91,92], and so on.

A wide variety of herbs has been used to the same aim. In this case, odorous molecules can play the role of reductants and stabilizing agents to ZnONPs. These MONPs are widely used as food-preserving packaging to extend the shelf life of perishable foods; some examples have been recently published about guava fruit [93] and ichthyic products [94], exploiting nettle leaf extracts [95]. Lemongrass leaves were successfully used for the production of active ZnONPs against human ticks with slight toxicity for people [96]. Thanks to its semiconducting properties, ZnO can be also applied for sunlight protection in both cosmetics and industrial fields. An interesting piece of research from Asmat-Campos et al. reported on the biosynthesis of ZnONPs with coriander as a reducing agent with high biosafety [97]. Biomedical applications can be found for food-derived ZnO nanostructures with thymus [98] and onion [99]; curcumin-stabilized NPs were used for sensing applications [100] and mint-produced ones for energy storage [101] as well. S. Vijayakumar used paprika extracts for rod-shaped ZnONPs with strong antimicrobial activity [102]. El Golli et al. [103] prepared wurtzite ZnONPs using garlic bulb extracts, with a good morphological control (Figure 5) for photocatalytic applications. Mbenga et al. [104] prepared ZnONPs from garlic extracts as well, which displayed a high cytotoxicity on human liver cells.

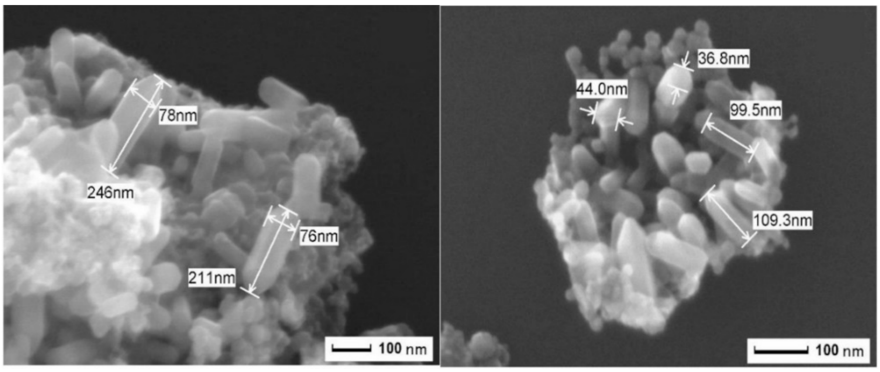

Figure 5. FESEM (field emission scanning electron microscopy) images of ZnO synthesized using garlic bulb extracts. Reprinted from [103] with permission from Elsevier.

Recently, onions were used for the preparation of small ZnONPs [105]; they were revealed to be extremely toxic for the aqueous environment, with massive bioaccumulation in carp gills and alteration of antioxidants gene expression, in addition to a 100% mortality recorded at the maximum tested concentrations of 10 mg/L at 96 h. This example demonstrates how "biogenic" is not necessarily equivalent to "safe".

The valorization of food waste, e.g., through conversion into useful chemicals for NPs synthesis, is a beneficial option in terms of economics, sustainability, and social and environmental impacts. This important contest requires methodical and complex biochemical process design and optimization, to ensure that the extraction methods are energy efficient, economically viable, and with a negligible environmental impact. This requires an interdisciplinary approach, involving experts in food science, chemists, materials scientists, etc., to find a suitable design with minimum cost and maximum benefit [106].

5. Bacteria and Microorganisms

The bacteria-mediated synthesis of ZnONPs is much less common. In this case, a metal precursor should be added to a bacterial culture in planktonic state. Enzymes and proteins exert the reducing action [57]. Recently, studies demonstrated the spotlight role of extracellular enzymes in biogenic synthesis, recognized specifically in nicotinamide adenine dinucleotide (NADH) and its reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). These enzymes are fundamental for electron transfer between cofactor NADH to NADH-dependent enzyme (in the reduction process), thus acting as electron carriers (Figure 6) [107]. Elemental Zn^0, produced by the reduction of salt precursors is then spontaneously oxidized again to form water-insoluble ZnO, by dissolved oxygen.

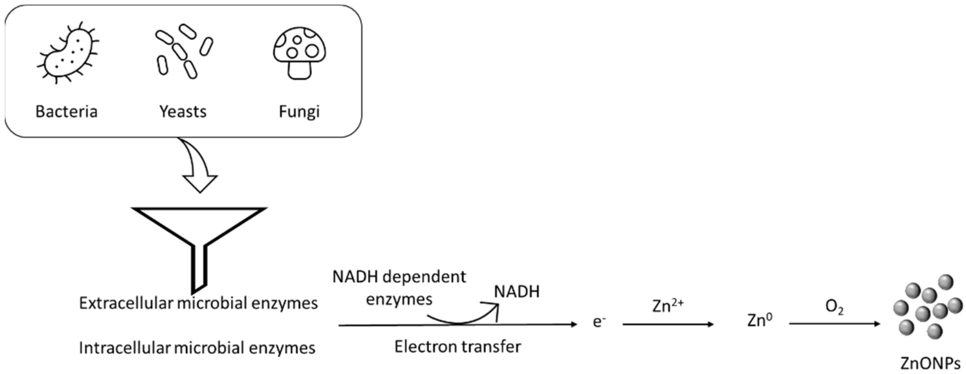

Figure 6. Role of NADH and NADH-dependent microbial enzymes in the synthesis of ZnONPs. Adapted from [107] with permission from Elsevier.

Due to a certain antimicrobial action exerted by metal/metal oxide NPs, it is necessary to use metal-tolerant [108] and thermophilic [109] bacterial strains. In order to respect the non-harmful principle of green chemistry, bacterial strains should be non-pathogenic, too. For example, in the last year, ZnONPs were prepared through *Pseudochrobactrum* sp. suspension for the degradation of organic dyes in the textile industry [110]. A similar approach was also used for the preparation of ZnONPs against parasites of rice cultures with *Bacillus cereus* [111] and cyanobacteria, such as spirulina (i.e., *Arthrospira platensis*) [112].

Recently, Faisal et al., reported on the bio-mediated synthesis of ZnONPs using a novel bacterial strain named *Paraclostridium* sp. [113]. They demonstrated the efficacy of the produced NPs in a wide plethora of in vivo biomedical applications: *Helicobacter* eradication, anti-inflammatory, anti-diabetic, anti-arthritic, and anti-diabetic efficacy.

An up-to-date, comprehensive listing of all the microorganisms used for ZnONP synthesis has been recently published in a specific review paper on microorganism-mediated NPs syntheses [114].

From the aforementioned papers, it is clear that only few microorganisms have the ability to synthesize ZnONPs [115]. Hence, there is a demand to discover more prospective biogenic substrates for the synthesis of these nanomaterials. Biological synthesis using bacteria/yeasts offers an advantage over plants, fruits, etc., since microbes are easily reproduced. Nonetheless, there are many drawbacks pertaining to the isolation and screening of potential microbes. The main disadvantage however resides in the fact that the process is time consuming and involves the use of expensive chemicals for microorganisms growth medium. The presence of various enzymes, proteins, and other biomolecules from microbes plays a crucial role in NP production process. These multiple organic components, secreted in the suspension or growth medium, are responsible for the formation of NPs with multiple sizes and shapes. Moreover, some proteins produced from microbes could behave as capping agents, thus increasing the stability of ZnONPs [116]. The specific mechanism of nanoparticle formation by microbial extracts is the most critical unanswered issue in the biosynthesis approach. It is striking that identifying specific biomolecules present in microbes, responsible for NPs formation, may support improvements to the synthetic method [117]. Large scale production with lower reaction times and reduced solvents amount is researchers' final goal. However, most of the papers available in the literature show that NPs are produced thanks to the synergistic co-action of several biomolecules or metabolites present in the microbial extracts or growth medium [118].

The biological synthesis of ZnONPs needs more time to reach pre-commercialization steps and then coming to the market. Pilot scale demonstrators are pivotal in transforming the results of the (nano)biotechnological research into competitive manufacturing. Hence, as biogenic nanotechnology is at its nascent stage, there are understandably still few investors taking the risk in early stage innovation [119].

6. Application of ZnONPs as Antimicrobial and Antiviral Agents

Thanks to the presence of natural compounds, ZnONPs synthesized by biogenic methods are generally considered highly biocompatible; this evidence lays beyond the large use of these NPs as antimicrobial agents with negligible cytotoxicity [120–129]. For the same reasons, they have been widely investigated in recent years as new-generation anti-cancer and antioxidants drugs [130] with very promising results and much less side effects in respect to other metal-based medications [131–138]. A detailed study confirming the high antimicrobial and antifungal activity of these NPs against food pathogens, with limited cytotoxicity, was recently published, exploiting food derivatives for their biogenic synthesis [139]. It is worth underlining that not all biogenic ZnONPs show antimicrobial activity; a peculiar case was shown by Dey et al., who prepared ZnONPs with leaf extracts and tested them against both gram-positive and negative bacteria with no success [140].

Some other papers reported on the technological application of biosynthesized ZnONPs, such as photocatalytic wastewater treatment [141,142], gas [143], and electrochemical [49] sensors, catalysis [144], etc. This kind of application is less diffuse because it generally requires a good morphological control and a well-known chemical composition; these conditions are quite complex to be achieved with biogenic-mediated synthetic routes.

During the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, nanomaterials, and ZnONPs specifically, were revealed to be powerful allies in the prevention of viral infections [145]. Beside the novel SARS-CoV-2, there are other viruses responsible for respiratory diseases, such as other coronaviruses, syncytial virus, rhinovirus, and influenza virus, whose contagion capacity have caused former pandemic outbreaks, as well as viral seasonal outbreaks where prophylaxis and prevention play a determining role [146]. In this scenario, the search for wide-spectrum antiviral agents, with restrained side effects for humans, becomes fundamental. Since the size of these viruses falls in the nanoscale, nanotechnology and materials science possess an enormous potential to interact with viruses lifecycle [147]. Additionally, they can deliver new chances in the development of safer personal protective equipment (PPE) and new effective therapeutic solutions [148]. Besides, in antiviral strategies aimed at preventing viral infection (disinfecting or anti-contamination materials, blocking viral docking to host cells, self-cleaning common touch surfaces, etc.), NPs, which are generally able to carry proteins, drug molecules, and a variety of other chemical compounds, are also used for drug delivery and diagnostic or therapeutic tools [149]. This pandemic revealed the need to keep surfaces clean and uncontaminated. Due to the inability of many viruses to spread outside the body (e.g., human immunodeficiency virus, HIV), viral transmission through surfaces have attracted little attention in the past. However, SARS-CoV-2 can remain viable on surfaces for days [150], and this poses a great risk for transmission via surface route, highlighting the critical need for efficient solutions that avoid the survival of viruses on surfaces [151]. Anti-infective surfaces can have different mechanisms of action, which are direct disinfection, indirect disinfection, and receptor inactivation [152]. Zn-based nanomaterials were proven to be efficient antimicrobials that offer various significant photocatalytic, surface, and morphological properties to inhibit and deactivate viruses at all the aforementioned levels. In other words, ZnONPs exhibit tunable antibacterial, antifungal, and antiviral capacities. Although the mechanism of action of ZnONPs as an antibacterial and antifungal agent has been determined [153,154], the antiviral one is still under study. A recent report hypothesized virus inactivation by Zn^{2+} release and ROS formation (Figure 7).

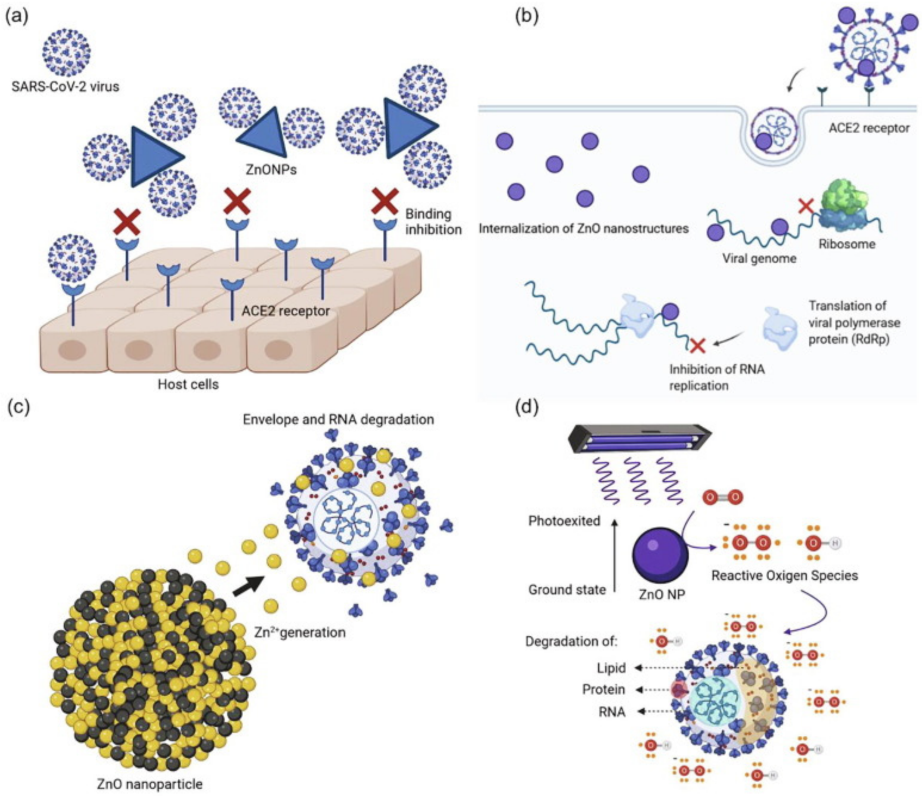

Figure 7. Possible mechanisms of antiviral activity of ZnO against SARS-CoV-2. (**a**) Design of ZnO nanostructures for the possible anchoring of SARS-CoV-2 virions, thus inhibiting interactions with host cell receptors. (**b**) Internalization of ZnO nanostructures for the inhibition of early stages of the viral replication cycle. (**c**) Ion release as a surface attack mechanism to disrupt the plasmid and RNA virus integrity. (**d**) Photocatalytic generation of reactive oxygen species for the possible degradation of the lipid, protein, and nucleic structure of SARS-CoV-2. Reproduced from [155]; article distributed under the terms of the Creative Commons CC-BY license.

The authors pointed out that the same mechanisms for antibacterial activity are also responsible for damaging the lipid membrane and RNA, thereby inactivating the virus [155]. These same pathways were hypothesized for ZnONPs prepared by *Plumbago indica* alcoholic leaf extract, against Herpes Simplex Virus Type 1 (HSV-1). *Plumbago indica* leaf extract is considered a valuable source for various types of active compound, such as alkaloids, phenolics, and saponins [156].

The effectiveness of ZnONPs was firstly predicated by in-silico models in early 2021; specifically, the effect of hesperidin (from food wastes) in combination with ZnONPs was demonstrated theoretically, with a significant synergistic effect (e.g., hesperidin-mediated ZnONPs exhibited higher antiviral activity than hesperidin itself) [157]. Hamdi et al. [158] performed a detailed computational analysis of the possible interaction between ZnONPs and SARS-CoV-2 targets, including the ACE2 receptor, RNA-dependent RNA polymerase, and main proteases. ZnONPs cellular internalization in human lung fibroblasts was also assessed. The highest antiviral activity was predicted for hexagonal and spherical ZnO nanostructures with a crystallite size of around 11 nm and positive z-potential. Interestingly, successful binding between ZnONPs and viral molecular targets, via hydrogen bond formation, was detected. Based on this evidence, ZnONPs have been extensively used for the production of face masks or (respiratory) filters for the inactivation of virions before their entry in human cells.

ZnONPs were immobilized in polyethylene oxide (PEO) matrix, for the modification of common touch surfaces, with exceptional results of virus inactivation; in [159] we demonstrated that ZnO nano-powders were effective in lowering the quantification of nucleocapsid (N) protein in virus samples from nasopharyngeal swabs.

Composites, based on polyacrylonitrile (PAN) nanofibers modified with ZnONPs, were used to remove air pollutants and microbes (bacteria and respiratory viruses) with application in masks, cleanrooms, and indoor air purification [148]. ZnONPs were also grown directly within textile and face mask materials, including polypropylene (PP) and nylon–cotton. This novel filtering material achieved a $\geq$99.9% reduction in SARS-CoV-2 titer within a contact time of 10 min, by disintegrating the viral envelope. Additionally, the new ZnO-modified textile could retain its antiviral properties even after 100 laundry cycles, and was dermatologically tested as non-irritant and hypoallergenic [160]. Analogously, surface modification of both touching surfaces and air filters was performed by Merkl et al. with significant limitation of airborne viral transmission from aerosols [161].

We believe it is worth describing the paper by El-Megharbel and co-workers [162], which despite the synthesis of ZnONPs cannot be considered specifically green. They reported on the production of ZnO-based nano-sprays for surfaces disinfection against SARS-CoV-2, with negligible cytotoxicity.

Berberine-capped ZnONPs were successfully prepared by Ghareeb et al. [163]. Berberine is a quaternary ammonium salt found in the roots of some plants, such as barberry or turmeric, which possesses pharmacological properties, including antioxidant and antimicrobial ones. In the above mentioned work, berberine-capped ZnONPs were found to be effective in the treatment of bacterial nosocomial infections associated with SARS-CoV-2. Still talking about pharmacological applications, a vaccine against SARS-CoV-2 based on the antiviral properties of Zn^{2+} ions was recently proposed by Ishida [164].

Safety worries related to shopping in supermarkets during the SARS-CoV-2 pandemic has headed to a predilection for fresh-food packaged in plastic containers by consumers and sellers, as well as the usage of disposable food packaging and plastic bags to carry groceries. In order to address these concerns, active packaging with antiviral properties was proposed. ZnONP-modified packaging material was described in 2021 [165]; specifically, NPs surfaces were here functionalized with geraniol and carvacrol thus obtaining an antimicrobial material with synergistic action against common food pathogens and SARS-CoV-2, simultaneously.

Nanotechnology-based tools play a key role in improving infections treatment and prevention. These materials can effectively help in the current global public health challenge, by delivering exactly the type of wide-ranging, easily scalable, low-harmful, combined tactics that are indispensable to manage and control the SARS-CoV-2 plague. Nanotechnology can offer appropriate and more efficient approaches to dealing with SARS-CoV-2, or other emerging viral or bacterial pandemics, which could occur in the future.

7. Conclusions

This review outlines how the biogenic and green synthesis of (ZnO) nanomaterials is becoming more and more important in an industrial and scientific context. Our purpose was not to provide a comprehensive enumeration of all available studies on this topic; we meant, instead, to describe selected and extremely recent examples, elucidating which synthetic route could be more suitable for a precise application, or to address a specific problem. We focused purposely quite exclusively on papers published during the last two years, in order to provide a point of view in steps with the times.

We believe that the research field reviewed here will certainly undergo further growth in the next years, and we hope the readers will find the information provided useful. Among the reviewed approaches to the biogenic production of ZnO nanocolloids, the syntheses based on renewable and waste-reuse sources might receive massive attention in the coming years, due to their scalability to industrial processes and the invaluable advantages in reducing energy consumption and environmental impact related to organic solvents and harmful reagents.

Thanks to scientific research, and to a strong collaboration between industries and academia, it will be possible to deepen the knowledge about green synthetic routes for the preparation of helpful nanomaterials, such as ZnO, with reduced risks for humans and environments.

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