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Green Synthesis of Zinc Oxide Nanoparticles Using *Moringa oleifera* Leaf Extract and Their Antibacterial Activity

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ABSTRACT

Zinc oxide nanoparticles made with plant extracts are widely reported to kill bacteria, and the leaf of *Moringa oleifera*, a fast-growing tree valued as food and medicine, is a popular source of the reducing and capping agents. This narrative review asks how much weight the published evidence can bear for the claim that zinc oxide nanoparticles synthesised with *Moringa* leaf extract are antibacterial. It draws on work on the chemistry of the plant, on the green synthesis of zinc oxide and other metal nanoparticles, on proposed antibacterial mechanisms, and on the design of susceptibility assays, and it reports no new data. The plausible picture is that particle size, shape, surface chemistry and dissolved zinc together determine activity, and that the extract adds a variable of its own because its composition shifts with cultivar, season and preparation. The common weaknesses are assays that rely on a single zone-of-inhibition reading, a small panel of bacterial strains, missing controls for the extract and the zinc salt, and thin characterisation, all of which make results hard to compare between laboratories. Questions of toxicity to non-target cells and of fate after release receive far less attention than activity. The review concludes that antibacterial activity is plausible and often reported, that its size and cause are not firmly established for the *Moringa* route, and it sets out what a convincing study and a fair comparison would contain.

Keywords: Zinc oxide nanoparticles; *Moringa oleifera*; Green synthesis; Antibacterial activity; Plant extract; Nanotoxicology; Narrative review

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Article Class: Narrative Review

1. Introduction

Antibiotics still do most of the work in treating bacterial infection, but resistance has eroded their reliability, and the problem is now described as a global one

(1). Bacteria resist drugs through a handful of recurring mechanisms, including enzymatic inactivation, altered targets and efflux of the drug (2), and communities growing as biofilms are harder to clear



than free-floating cells (3). It is no surprise that materials with a different mode of attack, such as metal oxide nanoparticles, have attracted steady interest (4, 5).

Zinc oxide is one of the more studied of these materials. It is inexpensive, it is already used in many consumer products and in biomedical research (6, 7), and bulk and nanoscale preparations have been reported to inhibit a range of bacteria (8, 9). Conventional syntheses, however, often use hazardous reagents or energy-hungry steps. Plant-mediated, or green, synthesis offers an appealing alternative in which a leaf extract supplies compounds that help reduce, stabilise and cap the growing particle (10, 11).

Moringa oleifera, the drumstick tree, is a frequent choice. Its leaves are eaten widely and carry a large reputation in traditional medicine (12, 13), and a paper reporting zinc oxide made with its extract appeared in 2017 (14). The claim that such particles are antibacterial is easy to find and easy to repeat. What is less clear is how much of that activity belongs to the zinc oxide, how much to the extract, and how reliably it can be reproduced. A reader weighing a paper in this field needs the answer to one plain question: could another laboratory reproduce it?

This article is a narrative review. It draws on published work on the plant, on green synthesis, on the antibacterial action of zinc oxide and on susceptibility testing, and it reports no new data and no measurements from the authors' own laboratories. Where the literature on the *Moringa* route is thin, we say so, and we describe what a convincing study would need to show instead of quoting figures we cannot check against their sources. The aim is a fair account of what has been shown and what has merely been repeated.

2. Literature Review

2.1 Why Metal Oxide Nanoparticles Interest Microbiologists

Metal and metal oxide nanoparticles act on bacteria in ways that differ from those of conventional antibiotics. Reviews describe contact with the cell envelope, the generation of reactive oxygen species and the release of metal ions as the leading routes, often working together (5, 15). Because no single molecular target is involved, resistance would in principle be harder to acquire, although that is an expectation and not a settled fact. Comparisons across several metal oxides have placed zinc oxide among the materials with measurable activity against both Gram-positive and Gram-negative organisms (4, 8, 16).

2.2 Proposed Antibacterial Mechanisms of Zinc Oxide

Several mechanisms have been proposed (Table 1). Studies on nanocrystalline preparations report that activity rises with the generation of reactive oxygen species and the consequent injury to the cell (17), and imaging of *Escherichia coli* exposed to ultrafine zinc oxide has been read as showing damage to the membrane and entry of particles into the cell (18). Work on *Campylobacter jejuni* likewise reported membrane disruption and signs of oxidative stress (19). Other authors stress the release of zinc ions into the medium and argue that dissolved zinc accounts for much of the effect (15, 20). Such differences look small on a plate, but they decide whether a material is useful at all.

Size matters as well. Experiments with particles of differing dimensions found that smaller particles were generally more active against bacteria, which the authors linked to greater surface area and increased reactive oxygen species production (21, 22). There is also evidence that zinc oxide



nanoparticles can discriminate, to some degree, between bacterial and human cells (23), although the margin may be modest and should not be assumed (24). The honest summary is that all of these mechanisms are plausible and none can be assigned a fixed share of the observed effect.

Table 1. Proposed antibacterial mechanisms of zinc oxide nanoparticles and the caveats attached to each.

Mechanism	What is proposed	Main caveat
Reactive oxygen species	Oxidative injury to membranes, proteins and DNA (9, 17)	Often inferred from indirect probes
Membrane contact	Particles bind to and disrupt the cell envelope (18, 19)	Hard to separate from ion effects
Dissolved zinc	Released ions interfere with cell function (15, 20)	Depends on medium and pH
Size and surface	Smaller particles tend to act more strongly (21, 22)	Size rarely controlled alone
Selectivity	Some bacteria are affected more than human cells (23)	Window may be narrow (24)

Note: Qualitative summary of the cited literature. The mechanisms are not mutually exclusive and their relative weight is debated.

2.3 Green Synthesis with Plant Extracts

The idea behind plant-mediated synthesis is simple. Metabolites in an extract, including polyphenols, flavonoids, organic acids and proteins, are thought to donate electrons or bind metal ions, steering nucleation and limiting growth (10, 25). An early and much-cited example used neem leaf broth to make gold and silver nanoparticles in minutes at room conditions (26). Later reviews catalogued dozens of plants used for dozens of metals (11, 27), and reviews that focus on silver note its antimicrobial promise as the main motivation (28).

For zinc oxide the pattern is similar. Reviews list extracts from leaves, flowers, fruits and bark that give particles of different sizes and shapes (29, 30). Neem leaf extract has been used to make zinc oxide with reported antibacterial and photocatalytic behaviour (31, 32), a leaf extract was used against bacterial and fungal pathogens (33), and flower extracts have yielded particles tested against bacteria or fungi (34, 35). Each of these reports rests on the premise that a green route is both kinder and at least as effective as a chemical one, a premise that is seldom tested head to head.

2.4 *Moringa oleifera* as a Source Material

Moringa oleifera is a fast-growing tree cultivated across the tropics for food, forage and traditional remedies. Overviews describe leaves rich in protein, minerals and a long list of phytochemicals, among them phenolic acids, flavonoids and glucosinolate derivatives (36, 37). Reviews of its bioactivity discuss antioxidant and anti-inflammatory properties, and some report antimicrobial effects of extracts (12, 38). Chemical profiling of the tissues has shown that glucosinolates and phenolics differ between plant parts and between related species (39).

Two consequences follow for nanoparticle work. First, an extract may contribute antibacterial compounds of its own, which a particle that has been inadequately washed can carry along. Second, an extract made from leaves of a different cultivar, harvest season, age or drying method is not the same reagent, and the product may differ accordingly. Laboratories seldom report these details, which makes the ordinary phrase *Moringa* leaf extract less informative than it sounds. This is why the same plant can seem excellent in one report and unremarkable in the next.



2.5 Moringa Extracts in Nanoparticle Synthesis

The most directly relevant physical study reported the synthesis of zinc oxide using *Moringa oleifera* extract and examined the properties of the product and a proposed mechanism of formation (14). For silver, *Moringa* leaf extracts have been used to make nanoparticles whose antimicrobial potential was examined (40, 41). These papers show that the extract can serve as a reagent. They do not establish that zinc oxide made this way is more, or less, antibacterial than zinc oxide made otherwise, because few reports compare routes under identical conditions.

3. Materials and Methods

3.1 Scope and Question

The review asks a single question in two parts. Is the claim that zinc oxide nanoparticles made with *Moringa oleifera* leaf extract are antibacterial plausible on current evidence, and how securely can the size of any such effect be attributed to the particles themselves?

3.2 Literature Assembly

The review draws on peer-reviewed work in four areas: the chemistry and uses of *Moringa*; green synthesis of metal and metal oxide nanoparticles; the antibacterial action and toxicity of zinc oxide; and the methods used to measure antimicrobial activity. Sources were chosen by the authors for relevance and standing, and all were published before the issue date of this article.

We did not run a formal systematic search and we do not claim one. This is a narrative synthesis, and the selection reflects the authors' judgement. The practical consequence is that the review is a better guide to what a good study should contain than to the exact size of any reported effect, and it deliberately avoids

quoting numerical results from individual papers that could not be checked against the primary sources for this article.

3.3 Appraisal Framework

To judge how far a report can be trusted we used a short framework of five questions (Table 2). Is the product characterised well enough to know what was tested, for instance by X-ray diffraction with crystallite size estimated from line broadening (6, 42)? Are the necessary controls present (36, 39)? Is the assay a recognised one, described fully enough to repeat (43, 44)? Is more than a token panel of strains used (4, 5)? And is any attention paid to harm to non-target cells and to the environment (24, 45)?

Table 2. Appraisal questions applied to green-synthesis antibacterial reports.

Question	Why it matters	Typical gap
Is the product characterised?	Identity and size set the activity (6, 42)	Single technique only
Are controls present?	Extract and salt may act alone (36, 39)	Extract control missing
Is the assay standard?	Comparability between sites (43, 44)	Zone width only
How many strains?	Species respond differently (4, 5)	Two or three strains
Is toxicity examined?	Safety limits use (24, 45)	Rarely addressed

Note: The last column is the authors' reading of the cited methodological and reporting literature; it is not attributed to any single study.

4. Results

4.1 What the Plant-Mediated Route Involves

Across the reports reviewed, the route follows a common outline (Figure 1). Leaves are washed and extracted, usually in water; the extract is mixed with a zinc salt; the pH and temperature are adjusted until a precipitate forms; and the solid is



separated, washed, dried and often heated (14, 29). The product is then characterised, most often by X-ray diffraction, electron microscopy and spectroscopy (6, 42).

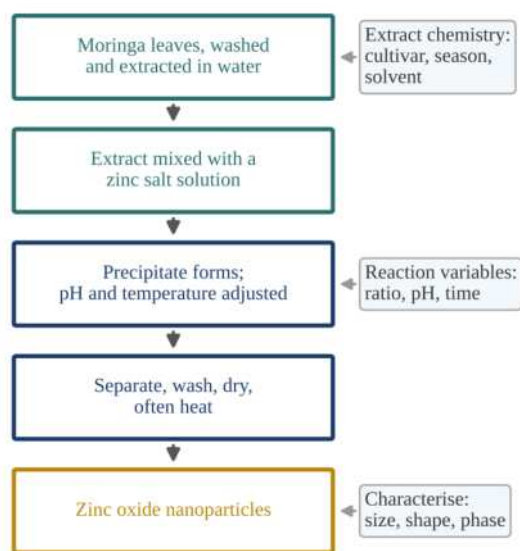


Figure 1. Typical stages of the plant-mediated route and the checks that surround them (schematic, not data).

Each stage hides a choice. The ratio of extract to salt, the pH, the temperature and the heating step can all alter size and shape, and the extract adds a second source of variation in its own chemistry (10, 36). Reports that name the cultivar, the harvest date, the solvent and the ratio are the exception. One consequence is that two laboratories describing the same Moringa route may in fact be producing different materials.

4.2 What Characterisation Can and Cannot Tell Us

Diffraction patterns identify the crystalline phase and, through line broadening, give an estimate of crystallite size (42). That estimate is not the same as the size of a particle or an aggregate in the test medium, which can be quite different. Electron microscopy shows shape but samples only a few particles. Surface chemistry, including how much of the extract remains on the surface, is the least often described,

yet it is the part of the particle that meets the bacterium (15, 25).

This matters because activity is sensitive to size and surface (21, 22). A report that characterises the dry powder and then tests a suspension in broth is making an assumption about aggregation that it has not checked. A suspension that has been given time to settle behaves differently from one that has not.

4.3 Assays, Controls and Comparability

The antibacterial assays used in this literature fall into a small number of formats (Table 3). Diffusion methods are simple and popular but depend on how far particles move through agar, so a small zone does not necessarily mean a weak material (43). Broth dilution gives a minimum inhibitory concentration, a more transferable measure when performed to a standard method (44), though turbid particle suspensions can make visual reading difficult.

Table 3. Common antibacterial assay formats and what each can and cannot show.

Assay	What it reads out	Limitation
Disc or well diffusion	Zone of clearing around the sample (43)	Depends on diffusion of particles in agar
Broth dilution (MIC)	Lowest concentration that stops visible growth (44)	Particles can cloud the reading
Viable counts	Survivors over time	Labour intensive, seldom done
Biofilm assays	Activity against attached communities (3)	Rarely included
Membrane or ROS probes	Hints at mechanism (17, 19)	Indirect, medium dependent

Note: Qualitative comparison; formats are described in the cited methods reviews.

Controls are the weakest link. A test of the nanoparticles alone cannot say whether the effect comes from zinc oxide, from



residual extract or from leftover zinc salt (Figure 2). Moringa extract has been described as bioactive (36, 38), and the raw salt can release zinc ions with their own effects (20). A study that includes all three controls, and a reference antibiotic, can isolate the contribution of the particles; one that omits them cannot. Read this way, a null result from one small assay is not evidence of absence.

	Zinc oxide itself	Extract residue	Zinc salt residue
Untreated medium	-	-	-
Extract alone	-	may act	-
Zinc salt alone	-	-	may act
Reference antibiotic	-	-	-
Test nanoparticles	may act	may act	may act

Shaded cells: what each control isolates (schematic, not data)

Figure 2. Controls that an antibacterial report needs, and the question each one answers (schematic matrix, not data).

Strain choice and replication add further doubt. Reviews of nanoparticle antibacterial work note that species differ in their response and that a handful of laboratory strains is a thin basis for general claims (4, 5). Biofilm assays, which are more relevant to persistent infection (3), are rarely reported. The same is true of exposure over time, which would show whether an early reduction in counts is sustained. The point is not that a paper is poor, only that its result travels less far than its abstract suggests.

4.4 Safety, Selectivity and Fate

A material that kills bacteria is not automatically safe to use. Reviews of mammalian toxicity of zinc oxide nanoparticles describe effects in cell and animal studies, and they stress that findings depend on particle properties and

dose (24). The general concern that engineered nanomaterials can injure cells through oxidative stress is long established (45). In one study zinc oxide nanoparticles were more toxic to bacteria than to human cells (23), but a single comparison cannot define a safe window.

What happens after release matters too. Nanomaterials in the environment dissolve, aggregate, react with ligands and change their behaviour (46), and zinc oxide in particular is known to be harmful to some aquatic organisms, with much of that effect attributed to dissolved zinc (20). Almost none of the green-synthesis antibacterial reports reviewed here address these points, and a plant origin gives no assurance on either count.

5. Discussion

5.1 Interpretation of the Evidence

At face value, the literature says that zinc oxide nanoparticles made with plant extracts, including Moringa, can inhibit bacteria in laboratory assays. That much is believable. It is consistent with what is known about the activity of zinc oxide generally (8, 9) and with the single physical study that confirms that the Moringa route yields zinc oxide (14).

What the literature does not yet say is how large the effect is, how much of it belongs to the particle rather than to the extract or to dissolved zinc, or how it compares with chemically made material of the same size. The evidence can be pictured as a ladder (Figure 3), and most reports occupy its lower and middle rungs, with characterisation and a single assay, while the upper rungs of varied strains, safety and application are seldom reached. That is a modest claim, and it is the one the evidence currently bears. Nothing in this reading argues against the green route; it argues for measuring what the product actually is.



Figure 3. Layers of evidence from material to application; widths are not counts (schematic).

5.2 Limitations

This review is narrative and not systematic, and its selection of sources is ours. The set of Moringa-specific zinc oxide reports available up to the date of this article is small, and several relevant observations come from silver or from other plants, which can inform but cannot substitute for direct evidence. Readers should treat the conclusions as an appraisal of the state of the evidence and not as measured effect sizes. Reviews of this kind are more useful when they say where the evidence stops than when they smooth it over.

5.3 Directions for Future Work

A convincing study would document the plant material, including cultivar, origin, harvest date, drying and solvent, and would report the extract's own activity. It would characterise the particles by more than one method, report size in the test medium as well as in the dry state, and say how much organic material remains (15, 42). It would use a recognised susceptibility method, a broader set of strains and a reference antibiotic, with all three controls shown in Figure 2 (43, 44). It is a habit worth keeping even when the answer seems obvious.

Beyond the assay, useful work would compare green and chemical routes at matched size, test activity in biofilms (3), and examine toxicity to mammalian cells and to aquatic organisms alongside activity (20, 24). Replication of published Moringa syntheses by independent laboratories would do more for the field than another single-laboratory report. These points apply with equal force to any plant-mediated nanomaterial, not only to zinc oxide.

A short reporting checklist would cost little. Authors could state the plant part and its origin, the extraction conditions, the zinc salt and the reaction pH and temperature, and show the extract and salt controls beside the nanoparticles (43, 44).

6. Conclusion

Zinc oxide nanoparticles made with Moringa oleifera leaf extract are plausibly antibacterial, and the broader literature on zinc oxide gives reason to expect some activity. The evidence, as we can responsibly summarise it, does not yet establish how large that activity is, how much of it is due to the particles, or how it compares with other routes. What the literature does show is where the uncertainty lies: in the variable extract, in thin characterisation, in assays without full controls and in the near absence of toxicity and fate studies.

For the practitioner the message is practical rather than discouraging. The green route can be pursued, provided the extract is documented, the product is characterised in the medium in which it is tested, and the controls are written down. For the researcher, the opportunity is a set of carefully controlled and independently replicated studies that turn a plausible claim into a measured one. Until such studies exist, the sensible reading of the



Moringa literature is cautious optimism: the particles may well help, but the size of that help, and the share owed to the particles rather than to the extract or to dissolved zinc, remain open questions that the next round of experiments, and not the next round of reviews, will have to settle. Readers, reviewers and editors can help by asking for the controls and the plant details that make a result checkable.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

O.T. conceived the review and wrote the sections on synthesis and characterisation. A.Ci. wrote the sections on bioprocess aspects and the plant material. A.Ch. wrote the sections on antibacterial assays and safety. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No human participants or animals were used and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

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