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Eco Friendly synthesis of metal oxide nanoparticles from plant leaf extracts and their applications: A review

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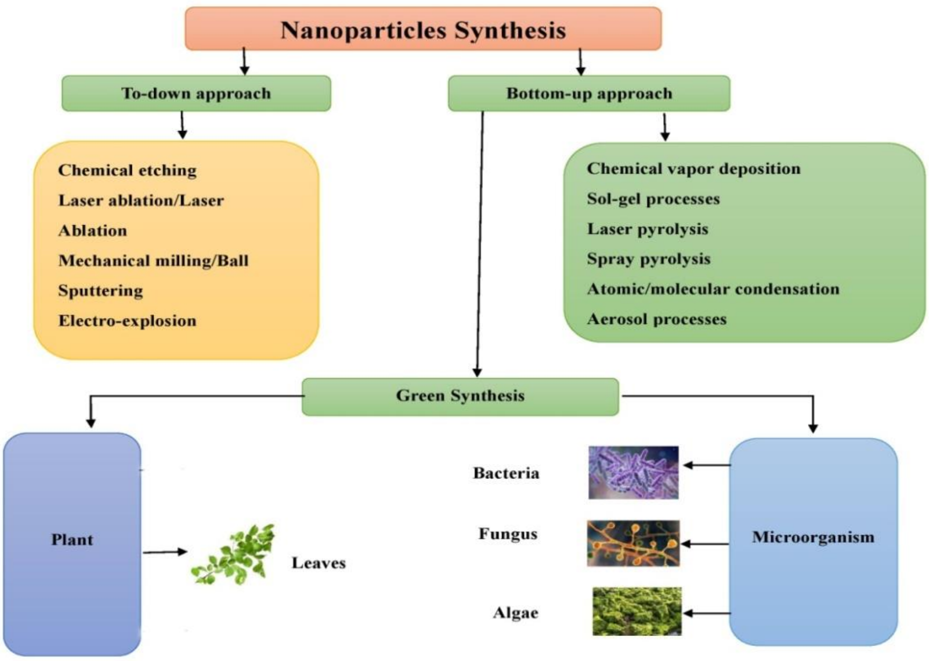
Abstract

In recent years, there has been a growing emphasis on the synthesis of nanoparticles (NPs) using plant extracts, driven by their eco-friendly nature and low toxicity to humans. Metal nanoparticles (MNPs) produced through green synthesis methods have attracted global interest due to their distinctive physicochemical properties and wide-ranging applications in biotechnology. As the demand for sustainable green chemistry practices rises, the production of metal and metal oxide nanoparticles has gained significant traction. Biosynthetic approaches to nanoparticle fabrication are particularly favored for their environmental benefits, simplicity, cost-effectiveness, and cleanliness, as they eliminate the need for hazardous chemicals and generate no harmful byproducts. Among various biological sources, plant extracts have emerged as a prominent choice for their dual role in reducing and stabilizing metal nanoparticles in a single-step process, thanks to their natural properties. Plants, as biological materials, are considered safe and are rich in reducing and capping agents. This review explores the principles of green chemistry and examines recent advancements in plant-mediated nanoparticle synthesis, along with their applications. The nanoparticles covered include gold, silver, copper, palladium, platinum, zinc oxide, and titanium dioxide.

1. Introduction

Nanotechnology is gaining considerable attention as an emerging field of research dedicated to the development of nanomaterials and nanoparticles (NPs) for applications across diverse industries such as catalysis, electrochemistry, biomedicine, pharmaceuticals, sensors, food technology, cosmetics, and beyond.**1–3**Nanoparticles (NPs) are nanometer-sized solid particles (less than 100 nm) made up of atoms or molecules. They possess unique physical properties that distinguish them from bulk materials, with these properties varying based on their size and structural characteristics..**4,5**Among the various types of nanoparticles, metal and metal oxide nanoparticles have been the focus of extensive research in science and technology due to their remarkable properties, including a high surface-to-volume ratio and superior dispersion capabilities in solutions.**6,7**These unique characteristics enable metal and metal oxide nanoparticles to demonstrate enhanced antimicrobial properties, making them highly effective in various applications.**8,9**There are two primary methods for synthesizing nanoparticles: the top-down approach and the bottom-up approach, as shown in Figure 1. In the top-down approach, nanoparticles are produced by reducing the size of bulk materials, breaking them down into smaller particles. This process can be accomplished using physical and chemical methods, such as lithographic techniques. **11**, In the top-down approach, nanoparticles are synthesized using techniques such as mechanical methods (e.g., milling, grinding) **12** sputtering**13** chemical etching **14** thermal evaporation **15** pulsed laser ablation**16** and photoreduction **17** However, a significant limitation of the top-down approach is the imperfection in surface structure **18** In contrast, the bottom-up approach involves wet chemical methods such as the chemical reduction or oxidation of metal ions **19,20** as well as other techniques like sol-gel chemistry **21** chemical vapor deposition (CVD) **22** co-precipitation **23** microemulsion **24** pyrolysis **25** hydrothermal **26** solvothermal **27** radiation-induced **28** and electrodeposition methods **29** In the bottom-up approach, also referred to as the self-assembly method, nanoparticles are built from smaller units, such as atoms, molecules, or smaller particles, which assemble to form the desired structure.**28-30**However, these methods encounter several challenges, such as the reliance on potentially hazardous and toxic chemicals, high costs, environmental toxicity, significant energy consumption, lengthy reaction times, and the production of non-eco-friendly by-products. These limitations highlight the need for more sustainable and efficient approaches to nanoparticle synthesis.**31-34** There are various physical and chemical methods available for synthesizing nanoparticles, including the hydrothermal method, The hydrothermal method is widely utilized for its ability to produce high-quality nanoparticles under controlled conditions **35,** alongside other techniques such as the solvothermal method **36** microemulsion method **37** microwave method **38** and thermal methods **39** While these physical and chemical methods are effective in generating nanoparticles with diverse sizes and morphologies, they are often complex, require expensive equipment, and involve the use of toxic chemicals non-biodegradable stabilizing agents hazardous organic solvents and time-consuming process control As a result, there is a pressing need to develop simpler, safer, and more environmentally friendly approaches to nanoparticle synthesis.**40–42**Thus, biological synthesis through nanobiotechnology processes offers significant potential for improving nanoparticle production. This approach eliminates the need for harsh, toxic, and expensive chemicals commonly used in traditional physical and chemical methods, providing a more sustainable and eco-friendly alternative.**43,44**Due to the abundance of secondary metabolites in plants and their ability to easily reduce metal salts, plants are better suited than other feedstocks for the production of metal oxide nanoparticles Their natural properties make them an ideal choice for efficient and sustainable nanoparticle synthesis..**45-47**.Additionally, nanoparticles synthesized from plant extracts exhibit greater stability and biocompatibility compared to those produced through physical or chemical methods. The green synthesis of metal nanoparticles, utilizing biomolecules present in plant extracts—such as enzymes, proteins, amino acids, vitamins, polysaccharides, saponins, and aromatic compounds—has been widely researched and recognized as a sustainable and efficient approach.**48.**The secondary metabolites found in plant extracts are thought to play a crucial role, acting as both reducing agents and stabilizing agents in the synthesis of nanoparticles. These natural compounds contribute to the efficient and eco-friendly production of nanoparticles while ensuring their stability and functionality..**49,50**.Furthermore, plant-based biosynthesis is a relatively straightforward process that can be easily scaled up for large-scale nanoparticle production. This review highlights the use of plant extracts for synthesizing nanoparticles, as it is one of the most widely adopted methods in green chemistry and is eco-friendly. This approach has garnered considerable attention from scientists and researchers due to the widespread availability and accessibility of plants their diverse range of metabolites and their safety for use in nanoparticle synthesis.

Fig : 1



Abbreviations:

EDAX, Energy Dispersion Analysis of X-ray; FTIR, Fourier Transform Infrared Spectroscopy; MIC, Minimum Inhibitory Concentration; mg, Milligram; nm, Nanometer; PL, Photoluminescence Analysis; SEM, Scanning Electron Microscopy; TEM, Transmission Electron Microscopy; XRD, X-ray Diffractometer; XPM, X-ray Photoelectron Microscopy; ZOI, Zone of Inhibition; DLS, Dynamic Light Scattering; AFM, Atomic Force Microscopy;

2. Principles of Green Chemistry: Green chemistry is guided by twelve principles designed to promote sustainable chemical practices. These principles include waste prevention atom economy safer chemical synthesis and energy efficiency The synthesis of nanoparticles using leaf extractsaligns with these principles by reducing the reliance on toxic chemicals and minimizing environmental impact The use of natural leaf extracts for nanoparticle synthesis supports the principle of utilizing renewable feed stocks and designing safer chemicals Additionally, green synthesis methods are generally more energy-efficient and generate fewer by-products, further enhancing their overall sustainability.

3. Synthesis of nanoparticles: There are numerous physical and chemical methods for successfully synthesizing nanoparticles. These methods can be broadly classified into two main approaches, which are fundamental to research in the field of nanoscale science the top-down approach and the bottom-up approach. Each of these approaches involves distinct characterization techniques and has specific applications tailored to their unique processes and outcomes.

Top-bottom

In top-down approaches, the desired bulk material is broken down into particles within the nanosized range. Examples of commonly used methods in this approach include mechanical milling/alloying and sputtering, which effectively reduce the size of bulk materials to the nanoscale.**53,54**These methods, such as mechanical milling/alloying and sputtering, are examples of techniques used to reduce particle size. However, they can result in surface imperfections in the synthesized nanoparticles, which present significant limitations. This is because the surface structure of a material is critical to its surface chemistry and physical properties, ultimately influencing its overall performance **51.**

Bottom-top

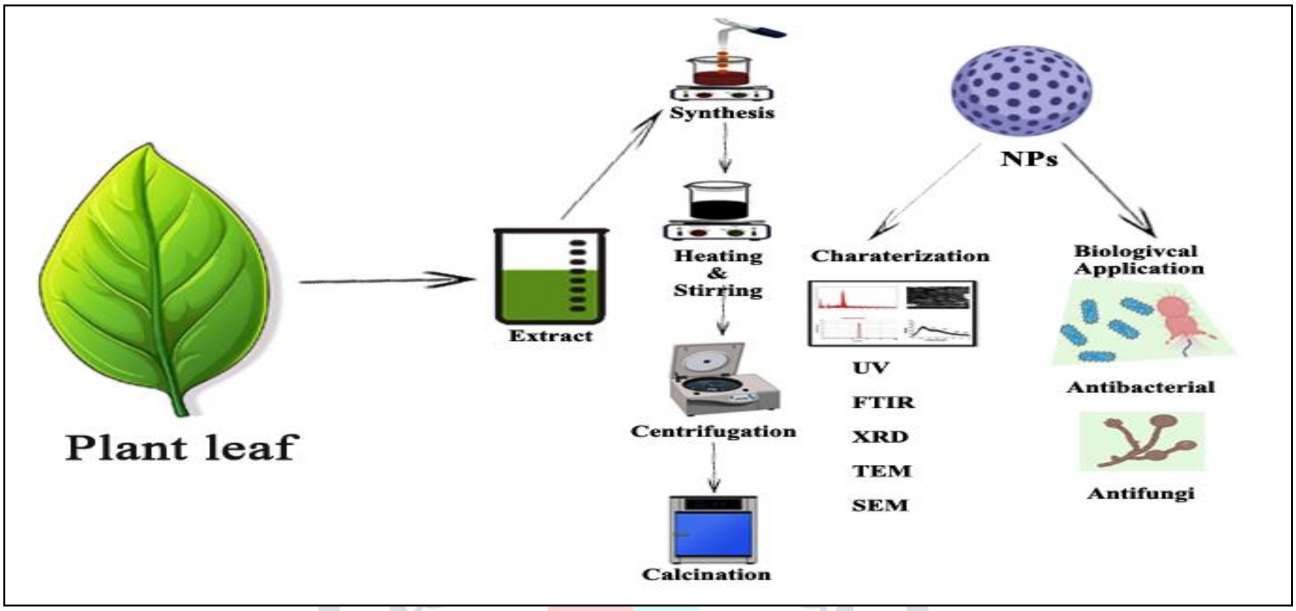
In bottom-up approaches, the synthesis process starts with the self-assembly of atoms or molecules into nuclei, which then grow to form nanoparticles in the nanoscale range. Examples of this approach include co-precipitation, sol-gel, and atomic condensation methods. These techniques build nanoparticles from smaller units, allowing for precise control over their size, shape, and properties.**55–57** These methods are examples of the bottom-up approach, which primarily relies on chemical and biological techniques for nanoparticle production. However, both top-down and bottom-up approaches generally involve the use of chemical and physical methods, which can be costly and potentially harmful to the environment. These methods often require toxic and hazardous chemicals, presenting significant biological and environmental risks. As a result, there is a growing need for greener, safer, and more sustainable alternatives in nanoparticle synthesis.**51,52** Since the plant-mediated nanoparticle synthesis approach does not rely on physical or chemical methods, it is environmentally friendly, biocompatible, and produces highly stable nanoparticles. As a result, this method has garnered significant attention from researchers worldwide, offering a sustainable and efficient alternative to traditional synthesis techniques.

4. Synthesis of Nanoparticles Using Leaf Extracts:

The synthesis of nanoparticles using leaf extracts is a promising green chemistry approach. This process typically involves preparing a leaf extract by boiling or soaking leaves in a solvent to extract phytochemicals, which are then used to reduce metal salts and form nanoparticles. For instance, silver nanoparticles can be synthesized using extracts from neem, mint, and tea leaves. The phytochemicals in the leaf extracts, such as flavonoids and polyphenols, act as both reducing agents and stabilizing agents. This method is highly advantageous as it eliminates the need for harmful chemicals and high-energy processes, making it an eco-friendly alternative to conventional synthesis methods.

Experimental

Fig:2



The leaves were collected and horoughly washed with water to remove any dirt or debris from the surface. The rind of the leaves was carefully peeled off using a sharp knife and discarded. The leaf was then slit longitudinally into halves, and a sharp-edged spoon was used to scrape off the gel, which was placed into a sterile beaker. The gel was stirred well to obtain the extract.Next,0.5 M of metal oxide was taken in a beaker, and 50 mL of distilled water was added. The solution was stirred using a magnetic stirrer until a homogeneous mixture was formed. To this aqueous solution, 50 mL of leaf extract was added, and the reaction mixture was stirred continuously for 30 minutes. The solution was then heated on a hot plate at 80 °C until the supernatant evaporated The resulting product was ground into a fine powder and calcined at 600 °C for 2 hours.The crystal structure of the obtained product was characterized by X-ray diffraction using a PANalytical X'pert Pro with CuKα (λ=1.5406 Å). The morphology and elemental composition of the samples were analyzed using a field emission scanning electron microscope (FEI QUANTA-250). The optical properties of the prepared nanostructures were analyzed using absorption spectra (JASCO V-650 Spectrophotometer) Fourier transform infrared (FTIR) spectra (Bruker IFS 48)were recorded in the range of 4,000–400cm^{-1} to identify the functional groups present in the metal oxide nanoparticles.

5. Synthesis of Metal Oxides Nanoparticles by Using Plants

In this review article, we summarized the basic principles or mechanisms of the green synthesis method, especially for metal oxide (i.e., ZnO, Ag, TiO2, CuO,) nanoparticles.

Zinc Oxide Nanoparticles

Recently, Zinc oxide nanoparticles have emerged as one of the most significant metal oxide nanoparticles due to carrying specific differences in morphology (size, shape, and crystalline nature), applications, lowtoxicity, economic benefits, and bio-compatibility **58–60.** Zinc oxide nanoparticles can be prepared from plant leaf.

5.1 Synthesis of ZnO Nanoparticles Using Leaf:

To date, numerous leaves' extracts have been used for the synthesis of ZnO nanoparticles, as shown in Table 1.

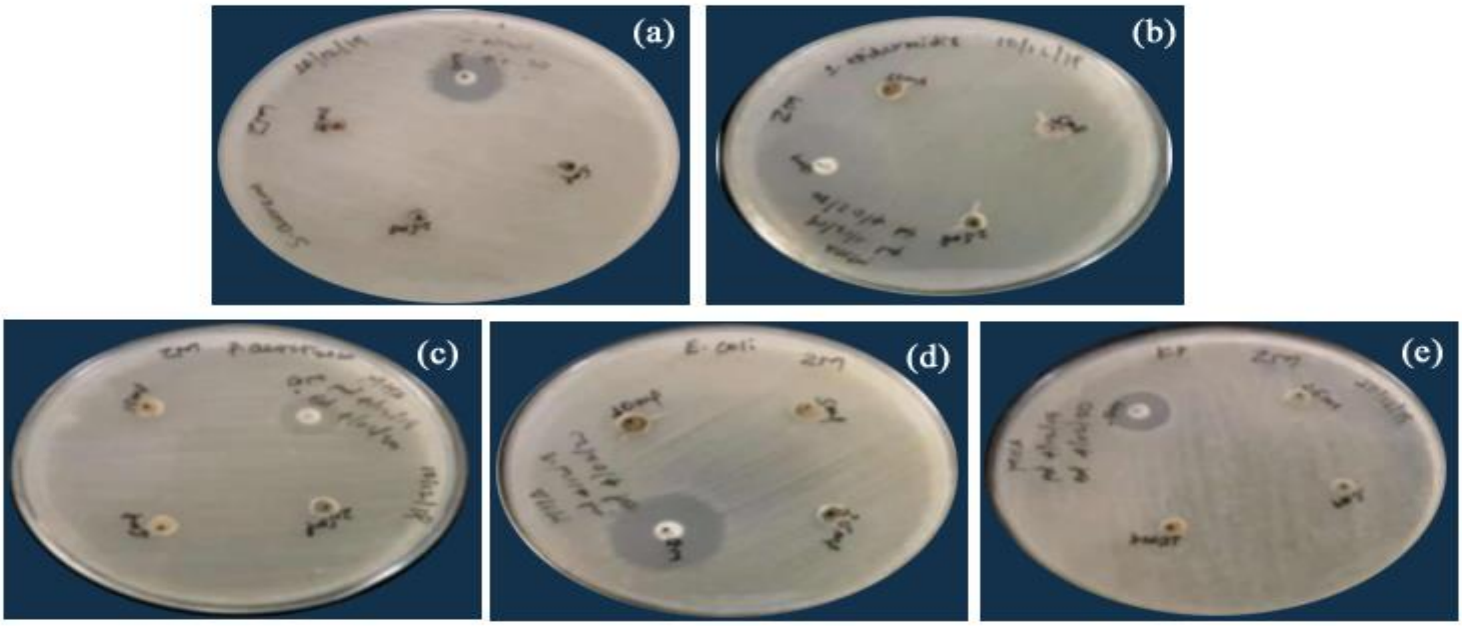
Table 1. Synthesis of ZnO nanoparticles from leaf extract.

Sr. No	Reducing Agent	Part of Plant	Size	Shape	Biological Activities	Year of Publication	Ref.
1.	Walnut leaf	Leaf	15–40 nm	Triangular	E. coli (ZOI = 7–9 mm) and S. aureus	2021	**[61]**
2	Lippia adoensis	Leaf	22.6–26.8 nm	Predominantly spherical	S. aureus (ZOI = 6–14 mm), E. faecalis (ZOI = 6–10 mm), E. coli (ZOI = 6–12 mm) and K. pneumonia (ZOI = 6–12 mm)	2021	**[62]**
3	Cayratia pedata	Leaf	52.24 nm	-	Utilized in the immobilization of the enzyme (Glucose oxidase)	2021	**[63]**
4	Piper betle	Leaf	112 nm	Hexagonal shape and spherical	S. aureus (ZOI = 2–3 mm) and E. coli (ZOI = 1–4 mm)	2021	**[64]**
5	Becium grandiflorum	Leaf	20 nm	-	S. aureus (ZOI = 7 mm) E. coli, (ZOI = 6 mm), K. pneumonia (ZOI = 8 mm), and P. aeruginosa (ZOI = 11 mm) Degradation of methylene blue (69% degraded after 200 min)	2021	**[65]**
6	Achyranthes aspera	Leaf	28.63–61.42 nm	Hexagonal	S. gallinarum (MIC _ 0.195 mg _ 0.00) and S. enteritidis (MIC _ 0.390mg _ 0.00)	2021	**[66]**
7	Arthrospira platensis	Leaf	30–55 nm	Spherical	subtilis (ZOI = 24.1 ± 0.3 mm), S. aureus (ZOI = 21.1 _ 0.06	2021	**[67]**

					mm), P. aeruginosa (ZOI = 19.1 ± 0.3 mm), E. coli (ZOI = 19.9 _ 0.1 mm), and C. albicans (ZOI = 21.6 _ 0.6 mm) Showed significantly high cytotoxic efficacy against cancerous cell		
8	Sambucus ebulus	Leaf	17 nm	Hexagonal	cereus S. aureus, and E. coli Photo-catalytic degradation of Methylene blue ((80% degraded after 200 min)	2020	**[68]**
9	Anacardium occidentale	Leaf	107.03 _ 1.54 nm and 206.58 _ 1.86 nm	Spherical	S. aureus (ZOI = 1.06 ± 0.14mm), E. aquaticum (ZOI = 1.99 ±0.11 mm), K. pneumoniae (ZOI = 2.08 _ 0.03mm), E. coli (ZOI = 1.49 ± 0.09 mm), and A. baumanii (ZOI = 2.99 ± 0.01mm)	2021	**[69]**
10	Broccoli extract	Leaf	4–17 nm	Hexagonal	Catalytic activity against methylene blue (74%) and phenol red (71%).	2019	**[70]**
11	Pandanus odorifer	Leaf	90 nm	Spherical	B. subtilis (ZOI = 26 mm) and Gram-negative E. coli (ZOI = 24 mm).	2019	**[71]**
12	Solanum torvum	Leaf	34–40 nm	Spherical	Decreased serum uric acid level. Could affect hepatic and renal performance in rats.	2019	**[72]**

Walnut aqueous leaf extract was utilized for the bio-synthesis of ZnO nanoparticles with a size range of 15 to 40 nm and evaluated against E. coli (ZOI = 7 to 9 mm) and S. aureus (Gram-positive bacterial stain) **61**. Demissie, Meron Girma, et al. reported Lippia adoensis aqueous leaf extract inspired preparation of ZnO nanoparticles and investigated against Staphylococcus aureus (ZOI = 6–14 mm), Enterococcus faecalis (ZOI = 6–10 mm), Escherichia coli (ZOI = 6–12 mm), and Klebsiella pneumoniae (ZOI = 6–12 mm) **62.** Piper betle aqueous leaf extract was applied for the synthesis of ZnO nanoparticles with an average size of 112 nm. S. aureus (ZOI = 2–3 mm) and E. coli (ZOI = 1–4 mm) are main causes of surgical site infection (SSI). Globally, SSI accounts for 2.5% to 41.9%, and an even higher rate in developing countries. Surgical site infection affects not only the health of patients but also the development of the country. The anti-bacterial agents are a significantly effective solution to lower this rate and Piper betle-mediated ZnO nanoparticles were proven to show excellent antibacterial activity against S. aureus and E. coli **63.** Becium grandiflorum was reported for the biosynthesis of ZnO nanoparticles and evaluated antimicrobial activity against S. aureus (ZOI = 7 mm), E. coli (ZOI = 6 mm),K. pneumonia (ZOI = 8 mm), and P. aeruginosa (ZOI = 11mm) bacteria, shown in Figure 3. Methyl blue dye from an aqueous solution was effectively removed by synthesized ZnO nanoparticles **64.**

Fig:3



Antimicrobial activities of ZnO NPs against (a) Staphylococcus aureus, (b) Staphylococcus epidermidis, (c) Pseudomonas aeruginosa, (d) Escherichia coli, and (e) Klebsiella pneumonia (reused from Ref. **65,** an Open Access Article, Creative CommonsAttribution 4.0 (CC BY 4.0)).

In another study, ZnO nanoparticles were prepared from aqueous leaf extract of Achyranthes aspera and evaluated for antibacterial activity against S. gallinarum and S. enteritidis using the agar wall diffusion method. The author also observed that Achyranthesaspera-mediated ZnO nanoparticles showed zone of inhibition (ZOI) of 31 mm against S.enteritidis and S. gallinarum showed 30mm **66**. Spherical-shaped ZnO nanoparticles with a size range of 30–55 nm were fabricated using an aqueous leaf extract of Arthrospira platensis. The results showed that antimicrobial activities of Arthrospira platensis-mediated nanoparticles were dose-dependent. Their application as an anti-microbial agent was studied and formed clear zones of 24.1 ± 0.3, 21.1 ± 0.06, 19.1 ±0.3, 19.9±0.1, and 21.6 ± 0.6 mm, at 200 ppm against B. subtilis, S. aureus, P. aeruginosa, E. coli, and C. albicans, respectively. These antibacterial activities were reduced as synthesized ZnO concentration decreased. ZnO nanoparticles showed significantly higher cytotoxic efficacy against cancerous cells than normal cell lines **67**. Hexagonal-shaped ZnO nanoparticles with a crystallite size of 17 nm were produced from ethanol leaf extract of Sambucus ebulus. The synthesized ZnO nanoparticles showed acceptable photo-catalytic degradation of Methylene blue dye. Sambucus ebulus-mediated ZnO nanoparticles explain efficient antioxidant and antibacterial activity **68.** Droepenu, Eric Kwabena, et al. reported the biosynthesis of ZnO nanoparticles using Anacardium occidentale and tested against S. aureus (ZOI = 1.06 ± 0.14mm),E. aquaticum (ZOI = 1.99±0.11mm), K. pneumoniae (ZOI = 2.08 ± 0.03 mm), E.

coli (ZOI = 1.49 ± 0.09 mm), and A. baumanii (ZOI = 2.99±0.01mm) **69**. From 2019, several publications reported ZnO nanoparticles synthesized using leaf extract of various plants, e.g., Broccoli extract**70**, Pandanus odorifer **71.**, and Solanumtorvum **72.**

6. Synthesis of Silver Nanoparticles Using Leaf Extracts:

Synthesis of silver nanoparticles, as shown in **Table 2.** Rauf, Abdur, et al. reported the formation of AgNPs using Mentha longifolia aqueous leaves' extracts. The round oval morphology of silver nanoparticles with a mean size of 10.23 ± 2 nm was revealed by TEM. Mentha longifoliabased silver nanoparticles showed tremendous antibacterial effect toward S. aureus (ZOI = 12 ± 0.03 mm), B. subtilis (ZOI = 10 ±0.01 mm), and K. pneumonia (ZOI = 0) and antioxidant activities **73.** In another research, the biological fabrication of silver nanoparticles was explained by Ocimum Americanum, with a particle size of 48.25 nm. The biologically prepared silver nanoparticles showed anti-bacterial activity against S. aureus (ZOI = 18.33 ± 0.33 mm), P. aeruginosa (ZOI = 17.66 ± 0.66 mm), V. cholera (ZOI = 15.66 ± 0.88 mm),Aeromonas sp (ZOI = 13. 33 ± 0.33 mm), Bacillus sp (ZOI = 16.33 ± 0.33 mm), and E. coli (ZOI = 7.66 ± 0.33 mm). The anti-oxidant activity was examined by H2O2 and DPPH. Silver nanoparticles showed excellent photocatalytic degradation of Eosin dye **74.** By utilizing Clerodendrum inerme as both a capping and reducing agent, Khan, Shakeel Ahmad, et al., synthesized silver nanoparticles and evaluated them for various biological activities, e.g., anti-mycotic, i.e., A. niger (ZOI = 17 mm) and A. flavus (ZOI = 22 mm), and antibacterial,i.e., B. subtilis (ZOI = 15 mm) and S. aureus (ZOI = 14 mm), activities. The antioxidant and cytotoxic activities of prepared gold nanoparticles were also examined by utilizing DPPH free radical scavenging (78.8 ± 0.19%) and the MTT process **75.** In another study, Salvia officinalis hexane, ethyl acetate, and ethanol leaf extract were utilized in the formation of Ag-NPs. The biologically produced silver nanoparticles exhibited less cytotoxicity toward theHeLa cells' line and exhibited excellent anti-plasmodial efficiency (IC50 = 3.6 lg/mL) **76**.A rapid and eco-friendly approach for preparing spherical silver nanoparticles with sizeof 27–36 nm by utilizing Alstonia venenata was performed. The larvicidal efficiency onearly-third-instar larvae was sufficiently higher for silver nanoparticles as compared toextract. The larvicidal activity was tested toward Culex quinquefasciatus with IC50 equivalent to 14.50 lg/mL, Anopheles stephensi with IC50 equivalent to 12.28 lg/mL, and Aedes aegyptiwith IC50 equivalent to 13.49 lg/mL **77.** Previous work reported the bio-fabrication ofspherical silver nanoparticles with a diameter of 20–40 nm, utilizing Sida retusa and testedtoward S. aureus (ZOI = 17 mm), B. subtilis (ZOI = 14 mm), E. coli (ZOI = 15 mm), and S. typhi (ZOI = 15 mm). **78.** In 2021, Singh, Surya P., et al. reported the formation ofAgNPs from Carica papaya aqueous leaf extract and its anticancer activity toward varioushuman cancer cells. The cytotoxic commotion was performed toward various human celland non-tumorigenic keratinocytes' cells. Cure of DU145 cell with C. papaya-mediatedsilver nanoparticles (0.5–5.0 _g/mL) for 1 or 2 days decreased the total cell number by21–36% **79.** In another research, the author achieved silver nanoparticles with particles'sizes of 35 ± 2 nm and 30 ± 3 nm using Carissa carandas aqueous leaf extract. Biologicallysynthesized silver nanoparticles exhibited excellent anti-oxidant activity throughDPPH assay. Prepared silver nanoparticles also showed remarkable ant-bacterial activitytoward human pathogenic bacteria, e.g., E. faecalis (ZOI = 7.0 ±0.0 mm), S. flexneri (ZOI= 8.0 ± 1.0 mm), S. typhimurium (ZOI = 8.0

± 1.0 mm), and gonococci spp (ZOI = 6.0 ±0.0 mm) **80.** Malva parviflora ethanol and waterleaf extract were utilized to synthesizespherical silver nanoparticles. The biologically synthesized silver nanoparticles inhibitedthe growth of F. oxysporum (81%), A. alternate (82%), H. rostratum (89%), and F. solani (81%) **81.** Ziziphus nummularia aqueous leaf extract was utilized to synthesize silvernanoparticles. These silver nanoparticles exhibited efficient anti-microbial commotionagainst S. aureus, C. rubrum, S. typhimurium, P. aeruginosa, C. neoformans, C. albicans, and C. glabrata. Silver nanoparticles also exhibited good DPPH activity (IC50 = 520 mg/mL)and ABTS activity (IC50 = 55 mg/mL) **82.** A previous study confirmed for first time thecapability of Otostegia persica for the bio-synthesis of silver nanoparticles. These particles exhibited excellent anti-oxidant activity compared to the Otostegia persica leaf extract. These particles also showed potential anti-bacterial activity toward S. pyogenes (ZOI = 14 _ 0.4 mm), S. aureus (ZOI = 16 ± 0.1 mm), B. subtilis (ZOI = 15 ± 0.3 mm), P. aeruginosa (ZOI = 21±0.5 mm), S. typhi (ZOI = 19 ± 0.4 mm), and E. coli (ZOI = 17 ± 0.1 mm) **83.** In another study, Abdallah, Basem M., et al. aimed to produce silver nanoparticles from Lotuslalambensis aqueous leaf extract and their anticandidal activity toward C. albicans (MIC = 125 _g/mL) **84.** Spherical silver nanoparticles were produced utilizing Symplocos racemosa. Anti-microbial activity of biologically prepared silver nanoparticles was studied on P. aeruginosa (ZOI = 22 mm) **85.** And In the period of 2019–2020, several publications reported Silver nanoparticles synthesized using leaf extract of various plants, e.g., Borago officinalis **86**,Melia azedarach **87**,Mentha aquatica **88**,Ganonerion polymorphum **89** and Piper betle **90.**

Table :2

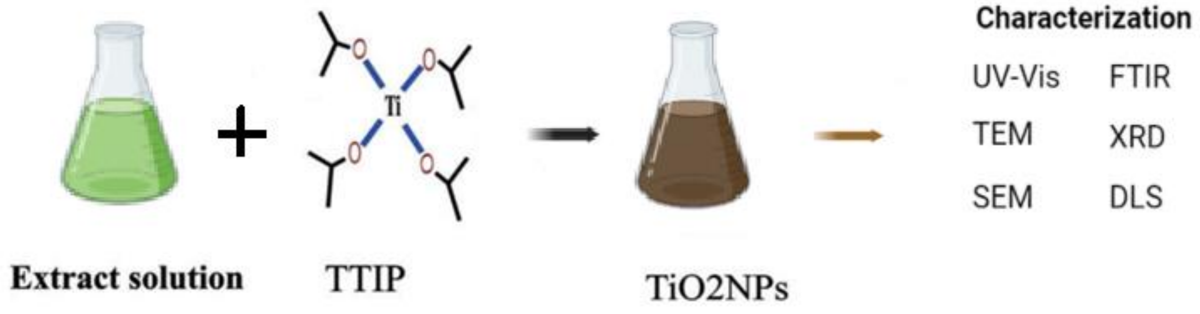
Sr. No	Reducing Agent	Part of Plant	Size	Shape	Biological Activities	Year of Publication	Ref.
1.	Mentha longifolia	Leaf	10.23 _ 2 nm	Round oval	At 2.0 (_g/mL) Concen of AgNPs S.aureus(ZOI=12± 0.03mm),B.subtilis (ZOI =10±0.01mm), and K.pneumonia (ZOI = 0).	2021	**[73]**
2	Ocimum americanum	Leaf	48.25 nm	-	At 100 (_g/mL) Concen of AgNPs S. aureus (ZOI = 18.33 ± 0.33 mm), P.aeruginosa (ZOI = 17.66 ± 0.66 mm), V. cholera (ZOI = 15.66 ±0.88 mm), Aeromonas sp (ZOI = 13. 33 ± 0.33 mm), bacillus sp (ZOI = 16.33 ± 0.33 mm), and E. coli (ZOI = 7.66± 0.33 mm) Anti-oxidant activity was examined by H2O2 (58.71%) and DPPH (75%). Photocatalytic degradation of Eosin dye (91.17%)	2021	**[74]**

3	Clerodendrum inerme	Leaf	-	-	A. niger (ZOI = 17 mm), A. flavus (ZOI = 22 mm), and antibacterial i.e., B. subtilis (ZOI = 15 mm) and S. aureus (ZOI = 14 mm) The anti-oxidant and cytotoxic activities were alsoexamined by utilizing DPPH free radical scavenging and MTT process (78.8 _ 0.19%)	2020	**[75]**
4	Salvia officinalis	Leaf	41 nm	Spherical	Exhibited less cytotoxicity toward HeLa cells' line and exhibited excellent anti-plasmodial efficiency (IC50 = 3.6 lg/mL)	2021	**[76]**
5	Alstonia venenata	Leaf	27–36 nm	Spherical	The larvicidal efficiency on early-third-instar larvae was sufficiently higher for silver nanoparticles as compared to extract. The larvicidal activity was tested toward Culex quinquefasciatus with IC50 equivalent to 14.50 lg/mL, Anopheles stephensi with IC50 equivalent to12.28 lg/mL, and Aedes aegypti with equivalent to LC5013.49 lg/mL	2021	**[77]**
6	Sida retusa Leaf		20–40 nm	. Spherical	S. aureus (ZOI=17 mm), B. subtilis (ZOI=14 mm),E. coli (ZOI = 15 mm), and S. typhi(ZOI = 15 mm)	2021	**[78]**
7	Carica papaya	Leaf	-	-	Anticancer activity toward various human cancer cells. The cytotoxic commotion was performed toward various human cells and non-tumorigenic keratinocytes' cells. Cure of DU145 cell with papaya-mediated silver nanoparticles (0.5–5.0 _g/mL) for 1	2021	**[79]**

					or 2 days decreased the total cell number by 21–36%		
8	Carissa carandas	Leaf	35 _ 2 nm at 25 _C and 30 _ 3 nm at 60	-	E. faecalis (ZOI = 7.0 ±0.0 mm), S. flexneri (ZOI = 8.0±1.0 mm), S. typhimurium (ZOI = 8.0 _ 1.0 mm), and gonococci spp (ZOI = 6.0±0.0 mm) Exhibited excellent antioxidant activity through DPPH assay (IC50 = 68.12±1.27).	2021	**[80]**
9	Malva parviflora	Leaf	50.6 nm	Spherical	Inhibited the growth of F. oxysporum (81%), A. alternate (82%), H. rostratum (89%), and F. solani (81%).	2021	**[81]**
10	Ziziphus nummularia	Leaf	25.6 nm	Oval and Spherical	Exhibited good DPPH activity (IC50 = 520 mg/mL) and ABTS activity (IC50 = 55 mg/mL)	2021	**[82]**
11	Otostegia persica	Leaf	36.5 _ 2.0 nm	Spherical	S. pyogenes (ZOI = 14 _ 0.4 mm), S. aureus (ZOI = 16 _ 0.1 mm), B. subtilis (ZOI = 15 _ 0.3 mm), P. aeruginosa (ZOI = 21 _ 0.5 mm), S. typhi (ZOI = 19 _ 0.4 mm), and E. coli (ZOI = 17 _ 0.1 mm) Exhibited excellent anti-oxidant activity (84%) compared to Otostegia persica leaf extract (64%).	2021	**[83]**
12	Lotus lalambensis	Leaf	-	-	C. albicans (MIC = 125 _g/mL)	2021	**[84]**
13	Symplocos racemosa	Leaf	-	-	P. aeruginosa (ZOI = 22 mm)	2021	**[85]**
14	Borago officinalis	Leaf	40 nm	Irregular	The bio-synthesized silver nanoparticles were hazardous to Spodoptera littoralis	2020	**[86]**
15	Melia azedarach	Leaf	18–30 nm	Spherical	Verticillium dahlia	2020	**[87]**
16	Mentha aquatica	Leaf	41 nm	Spherical	P. aeruginosa (MIC = 2.2_g/mL), E. coli (MIC = 58_g/mL), B. cereus (MIC = 20),	2020	**[88]**

					and S. aureus (MIC = 198_g/mL)		
17	Ganonerion polymorphum	Leaf	20–60 nm	Hexagonal and Spherical	B. Cereus (99.75%) and E. coli (99.94%)	2019	**[89]**
1	Piper betle	Leaf	6–14 nm	Spherical	At 1000 (_g/mL) Concen of AgNPsF. Solani (ZOI = 3.13 _ 0.25 mm) and A. brassicae (ZOI = 67. 21 _ 3.15 mm)	2019	**[90]**

7. Synthesis of Titanium Oxide Nanoparticles from Leaves,



Among biological extracts, plants are considered one of the most preferred sources for synthesizing titanium oxide nanoparticles, as illustrated in **Table 3.** Various phytochemicals, such as phenols, amino acids, carbohydrates, and flavonoids, play a key role in the biosynthesis of titanium oxide nanoparticles by stabilizing and reducing the precursor material. The reaction becomes noticeable when a titanium salt (precursor) is combined with a plant extract, with a color change from light green to dark serving as the initial indication of titanium oxide nanoparticle formation, as shown in. **92.**

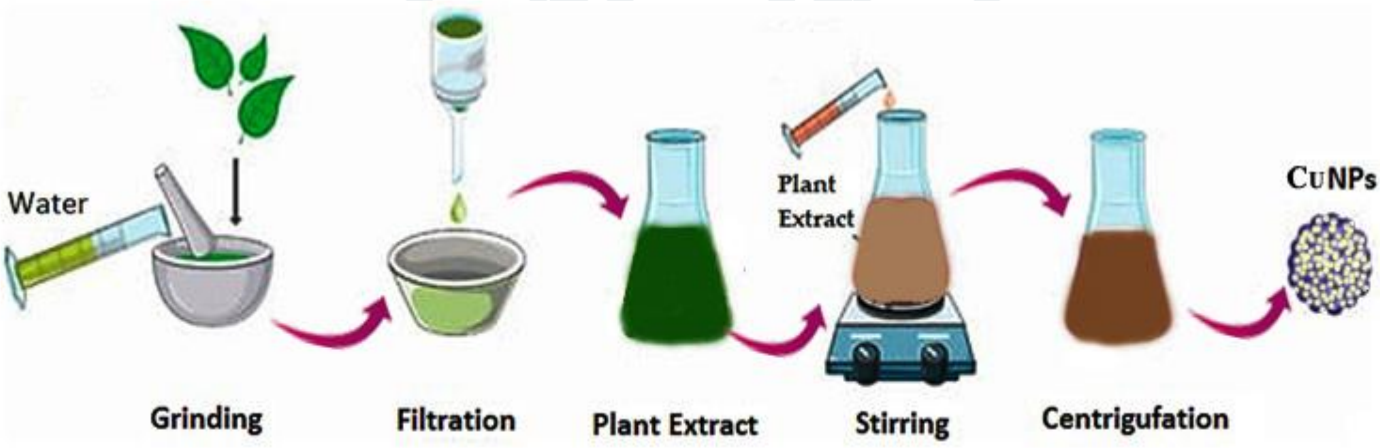
Synthesis of titanium oxide nanoparticles using various parts of plant is shown in

Table 3.

Sr. No	Reducing Agent	Part of Plant	Size	Shape	Biological Activities	Year of Publication	Ref.
1.	Mentha arvensis	Leaf	20–70 nm	Spherical	At 10 (_g/mL) Concen of AgNPs P. Vulgaris (ZOI = 25 mm), E. coli (ZOI = 20 mm), S. aureus (ZOI = 21 mm), A. niger (ZOI = No), A. fumigates (ZOI = 6 mm), and A. cuboid ZOI = No)	2021	**[93]**
2	Pouteria campechiana	Leaf	-	Spherical	Exhibited larvicidal activity toward Aedes aegypti	2021	**[94]**
3	Coleus aromaticus	Leaf	12–33 nm	Hexagonal	S. boydii (ZOI = 30 mm) and E. faecalis	2021	**[95]**

					(ZOI = 33 mm) Larvicidal activity toward fourth stages of instars' larvae of Aedes aegypti. Cytotoxic activity toward HeLa cell line		
4	Ochradenusarabicusis	Leaf	20–40 nm	-	S. aureus (MIC = 31.25 _g/mL) and P. aeruginosa (MIC = 128 _g/mL)	2021	**[96]**
5	Aegle marmelos	Leaf	150 nm	Spherical	Removed ornidazole from wastewater	2020	**[97]**
6	Azadirachta indica	Leaf	25–87 nm	Spherical	B. subtilis (MIC = 25 _g/mL), E. coli (MIC = 10.42 _g/mL), K. pneumonia (MIC = 16.66 _g/mL), and S. typhi (MIC = 10.42 _g/mL).	2019	**[98]**
7	Carica papaya	Leaf	20 nm	Spherical	Photocatalytic activity (91.19%) against degradation of RO-4 dye	2019	**[99]**
8	Aloe barbadensis	Leaf	8 ~20 nm	Spherical	Anti-biofilm activity toward P. aeruginosa (ZOI = 30.69 _ 3.78 mm)	2019	**[100]**

8. Synthesis of Copper Oxide Nanoparticles Using Leaves:



Plants produce a variety of secondary metabolites and phytochemicals, which serve as valuable bioresources for the synthesis of copper oxide nanoparticles **(Table 4).** Flavonoids and phenols are among the most prominent phytochemicals found in different parts of the plant, including leaves, fruits, seeds, and flowers. These phenolic compounds contain ketone and hydroxyl groups that participate in iron chelation, contributing to their strong antioxidant activity. Nanoparticles synthesized through this green synthesis method exhibit improved stability, preventing deformation and agglomeration, and enhancing the adsorption of phytochemicals onto the nanoparticle surface, which increases the reaction rate. A common method for producing copper and copper oxide nanoparticles involves mixing a stoichiometric amount of plant extract with copper salt, heating the solution to an appropriate temperature, and continuously stirring..

Fig: 4 showa the mechanism of formation of copper oxide nanoparticles

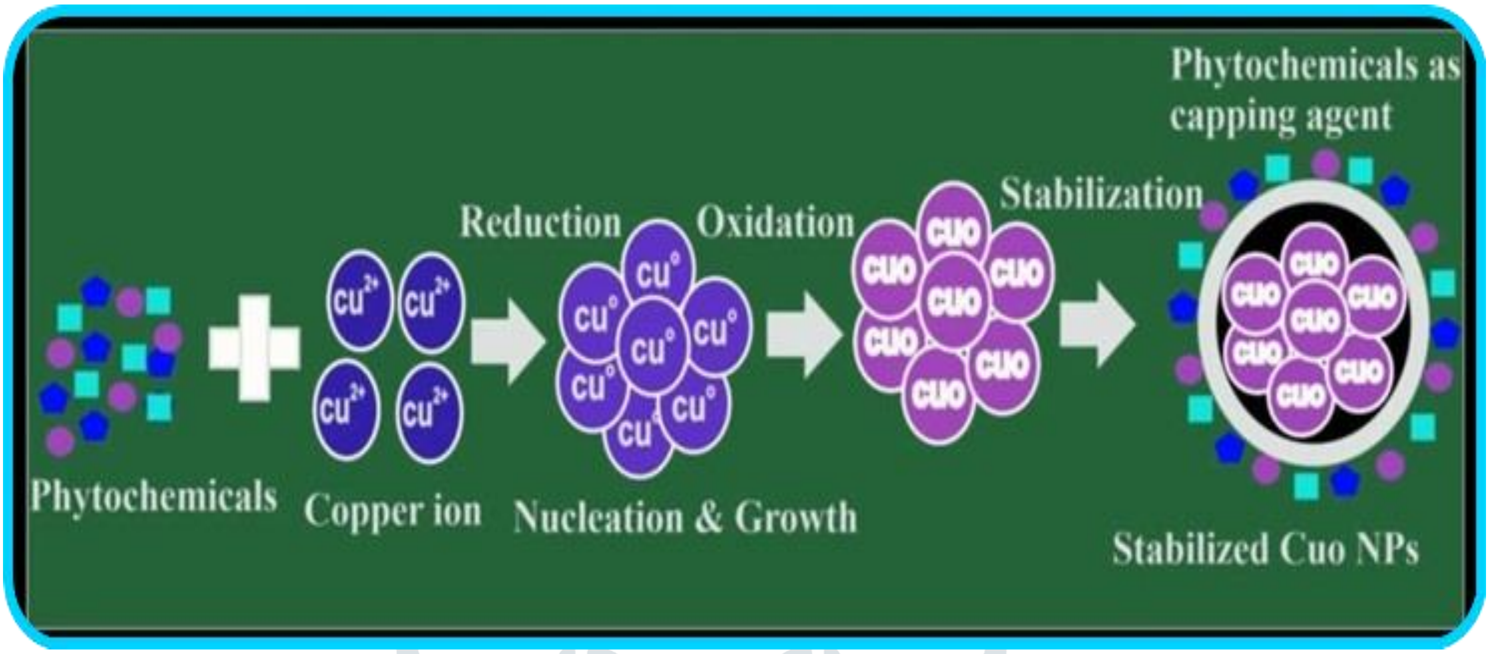


Table 4:

Sr. No	Reducing Agent	Part of Plant	Size	Cu/CuO NPs	Shape	Biological Activities	Year of Publication	Ref.
1.	Terminalia chebula	Leaf	100 nm	CuO	Rod-like shape	Applications on diesel engine.	2021	**[103]**
2	Cedrus deodara	Leaf	100 nm	CuO	Spherical	S. aureus (MIC = 25 lg/mL) and E. coli (MIC = 150 lg/mL)	2021	**[104]**
3	Psidium guajava	Leaf	40–150 nm	CuO	Oval	epidermis (ZOI = 1.8 mm), E. coli (ZOI = 2 mm), S. pneumoniae (ZOI = 1.4 mm), and P. aeruginosa (ZOI = 3 mm)	2021	**[105]**
4	Celastrus paniculatus	Leaf	2–10 nm	CuO	Spherical	Oxysporum (maximum mycelial inhibition =76.29 mm)	2020	**[106]**
5	Catha edulis	Leaf	-	CuO	Spherical	K. Pneumonia (ZOI = 29 _0.03 mm), E. coli (ZOI =32 _ 0.02 mm), S. aureus (ZOI = 22 _ 0.01 mm), and S. pyogenes (ZOI = 24_ 0.02 mm)	2020	**[107]**

6	Citrofortunella microcarpa	Leaf	-	CuO	-	Photocatalytic activity against Rhodamin B (98%)	2020	**[108]**
7	Enicostemma axillare	Leaf	330 nm	CuO	-	-	2019	**[109]**
8	Camelia sinensis	Leaf	60 _ 6 nm	CuO	Spherical	Photocatalytic degradation (83.7%) of prepared copper nanoparticles was tested by utilizing bromophenol blue	2019	**[110]**

9. Characterization of Leaf Extract Nanoparticles :

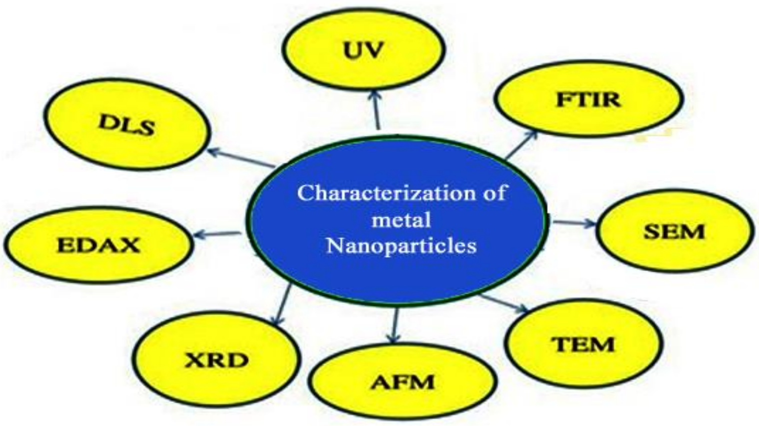


Fig:5

The nanoparticles can be analyzed using a variety of spectroscopic techniques, such as UV-Visible absorption spectroscopy, X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Energy Dispersive X-ray Analysis (EDAX). These methods provide valuable insights into the elemental composition structure, and concentration of the nanoparticles. By combining these techniques, more comprehensive and detailed information can be obtained compared to using each method in isolation. **111**.

UV-visible spectroscopy is a technique used to analyze the scattering and absorption of light as it passes through a sample. The sample is placed between the light source and the photodetector, and the intensity of light is measured both before and after it passes through the sample. A plot of the extinction coefficient versus wavelength is then generated. This method is particularly useful for identifying and characterizing nanomaterials, as well as providing insights into the stability of the synthesized nanoparticles.

112,113,114. The X-ray technique, particularly X-ray Diffraction (XRD), is primarily used to determine particle size,lattice dimensions, and to characterize the crystal structure of a material. This method provides detailed information about the crystalline phases, orientation, and structural properties of nanoparticles, making it a crucial tool in nanomaterial analysis..**115,116**. This technique, particularly X-ray Diffraction (XRD), is primarily focused on determining the three-dimensional structures and crystallinity of nanoparticles. It provides detailed insights into the atomic arrangement,crystal phases, and structural properties of the material, making it an essential tool for characterizing nanomaterials.**117,113**.

Fourier Transform Infrared Spectroscopy (FTIR) is a technique used to identify organic materials, such as polymers and polymer compounds, by analyzing how infrared radiation affects the rotation and vibration of molecules. FTIR analysis can be conducted on both solid and liquid samplesand has also been utilized to characterize bacteria exposed to nanoparticles. This method provides valuable information about the chemical bonds and functional groups present in a sample, making it a versatile tool for material characterization.**118**. FTIR can effectively detect the properties of functional groups and metabolites present on the surface of nanoparticles. These functional groups and metabolites often play a role in the reduction and stabilization of nanoparticles during synthesis. This capability makes FTIR a valuable tool for understanding the surface chemistry and interactions involved in nanoparticle formation and stability..**119, 120**.

The size and morphology of synthesized nanoparticles can be determined using both Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Transmission Electron Microscopy (TEM) provides detailed information on the size, internal structure, and morphology of nanomaterials, offering excellent image quality and high resolution for nanoscale samples. Scanning Electron Microscopy (SEM), on the other hand, offers subnanometer-scale resolution, making it highly effective for characterizing nanoparticles. SEM microscopes achieve resolution in the range of 1-3 nm and capture surface images by scanning the sample with a focused electron beam. SEM also enables the detection of various signals produced by the interaction of the electron beam with the sample. Additionally, Environmental SEM (ESEM) instruments are capable of examining wet samples, allowing for the observation of specimens in their natural, hydrated state without the need for extensive sample preparation. This makes ESEM particularly useful for studying biological or environmentally sensitive materials.**122**.

AFM offers several advantages over SEM and TEM, including requiring less laboratory space, being more cost-effective, and having a simpler operational process. The technique typically uses substrates like mica and clean silicon, and it can be applied to characterize nanoparticles such as TiO2 and CdS, providing valuable chemical insights into the samples. AFM is particularly useful for studying surface properties and interactions at the nanoscale.**121,122**

Energy Dispersive X-ray Spectroscopy (EDX),Dynamic Light Scattering (DLS), and Thermal Gravimetric Analysis (TGA)are also valuable techniques for characterizing nanoparticles. EDX provides information about the elemental composition of nanoparticles and is commonly used to determine the metal composition of metal nanoparticles, as well as to analyze the elemental makeup of nanomaterials. DLS measures particle size distribution and zeta potential, offering insights into the stability and dispersion of nanoparticles in a solution. TGA helps assess the thermal stability and composition of nanoparticles by analyzing weight changes as a function of temperature. These techniques, when used together, provide a comprehensive understanding of the physical, chemical, and thermal properties of nanoparticles.**120,122.121** Dynamic Light Scattering (DLS) is used to analyze nanoparticles with sizes ranging from 0.1 nm to 10 nm . This technique helps determine the aggregation state and the hydrodynamic diameter of nanoparticles in solution, offering valuable insights into their stability, dispersion, and behavior in different environments. DLS is particularly useful for studying nanoparticles in liquid suspensions, making it a key tool for understanding their interactions and performance in various applications.**123,124.** This method, known as Thermal Gravimetric Analysis (TGA), is commonly used to determine the thermal stabilityof nanoparticles by analyzing weight changes as a function of temperature. TGA provides valuable insights into the material's composition and stability under different thermal conditions, making it a crucial technique for understanding the decomposition and thermal behavior of nanoparticles.**121**. Thermal Gravimetric Analysis (TGA) helps determine the presence of volatile components, moisture, and the amount of coating on the nanoparticle surface. It also provides valuable information about the thermal stabilityof nanoparticles by measuring weight changes as the temperature increases. This technique is essential for understanding the decomposition behavior, composition, and thermal properties of nanomaterials under varying temperature conditions..**121,124**.

10. BIOLOGICAL APPLICATIONS OF LEAF EXTRACT NANOPARTICLES

Leaf extract nanoparticles have shown promising applications in various biological fields due to their unique properties and biocompatibility. Their applications include:

10.1 Antibacterial Activities

Ongoing research has produced a substantial body of literature on the antimicrobial properties of metal oxide nanoparticles. Silver nanoparticles are particularly effective at breaking down the polymer components of microbial cell membranes. When synthesized using plant-based methods, these silver nanoparticles further disrupt the cell membrane, ultimately hindering the bacteria's ability to synthesize proteins. **125**. A higher concentration of silver nanoparticles increases membrane permeability more rapidly than a lower concentration, leading to the eventual disruption of the bacterial cell wall, as demonstrated in **126**. Rhizophora apiculata-mediated silver nanoparticles demonstrated the highest conductivity and resulted in fewer bacterial colonies on the experimental plate compared to cells treated with silver nitrate. This enhanced antimicrobial effect may be attributed to the nanoparticles' smaller size and larger surface area, which facilitate greater membrane permeability and subsequent cell destruction. **127.** Silver nanoparticles synthesized through a green method using *Citrus sinensis* peel extract were tested for their antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. **128.**

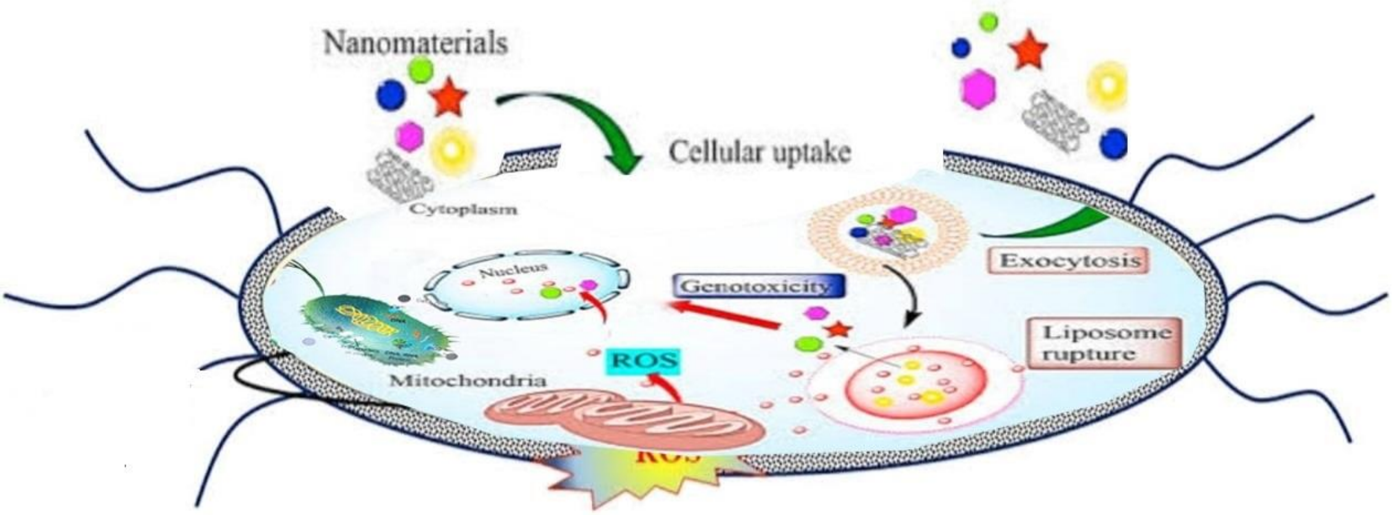


Fig:6

10.2 Antifungal Activity

The fungicidal action of plant-derived metal oxide nanoparticles shows greater potential than that of conventional antibiotics such as amphotericin. Plant-mediated silver nanoparticles have been shown to cause membrane disruption in *Candida* species, along with damage to intracellular fungal components, ultimately leading to the loss of cellular function. **129.** Many commercial antifungal drugs have limited clinical utility due to their associated side effects. These adverse reactions can include liver and kidney damage, as well as symptoms such as nausea, diarrhea, and elevated body temperature following drug administration. The fungal cell wall, primarily composed of proteins and fatty acids, is a critical target. Plant-mediated silver nanoparticles have shown strong antifungal activity, particularly against spore-forming fungi, by effectively inhibiting fungal growth. Interaction with metal oxide nanoparticles has been observed to cause significant alterations in the structure and composition of the fungal cell membrane. **130**

10.3 Antimicrobial Activity

Leaf extract-derived nanoparticles possess strong antimicrobial capabilities. Among them, silver nanoparticles have been thoroughly investigated for their effectiveness in suppressing bacterial and fungal growth. Their antimicrobial activity is mainly due to the release of silver ions, which interact with microbial cell membranes, causing structural damage and cellular disruption. Silver nanoparticles synthesized using leaf extracts have shown effectiveness against various pathogens, including those resistant to antibiotics.In a similar vein, gold and zinc oxide nanoparticles obtained from leaf extracts have shown promise in fighting microbial infections, thereby broadening the potential applications of natural nanoparticles in antimicrobial treatment.

10.4 Cancer Therapy

Leaf extract-derived nanoparticles are increasingly used in cancer therapy for targeted treatment and imaging. Gold nanoparticles, produced from leaf extracts such as pomegranate or mint, have been applied in photo thermal therapy, where they absorb light and produce heat to destroy cancer cells. Additionally, these nanoparticles can be coupled with therapeutic agents or imaging dyes to enhance diagnostic precision and treatment effectiveness. The biocompatibility and potential for fictionalization of leaf extract nanoparticles make them promising candidates for developing advanced cancer therapies, providing a more focused and efficient treatment approach.

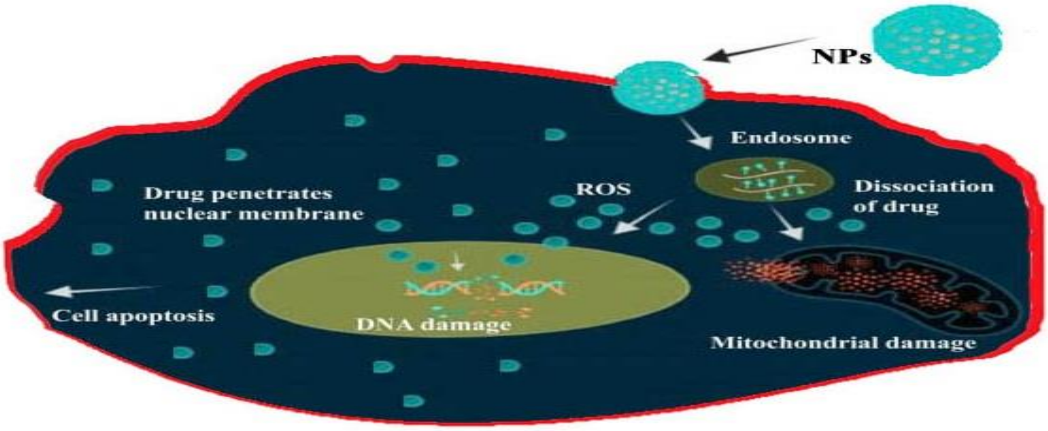


Fig:7

10.5 Antioxidant and Anti-inflammatory Effects:

Nanoparticles derived from leaf extracts exhibit notable antioxidant and anti-inflammatory properties, primarily due to the presence of phytochemicals such as polyphenols and flavonoids. Antioxidant nanoparticles work by neutralizing free radicals, reducing oxidative stress, and preventing cellular damage. Anti-inflammatory nanoparticles have the ability to regulate inflammatory pathways, offering potential therapeutic benefits for conditions like arthritis and cardiovascular diseases. Ongoing research into these applications is expanding, highlighting the versatility and potential of leaf extract nanoparticles in a wide range of biological uses.

11. CHALLENGES AND LIMITATIONS

Although leaf extract nanoparticles offer numerous advantages, several challenges and limitations must be overcome. A key issue is the variability in the composition of leaf extracts, which can result in inconsistencies during nanoparticle synthesis, affecting their quality and performance. Scalability is another major concern, as methods effective at the laboratory scale may not easily be adapted for large-scale industrial production. Furthermore, the potential toxicity of nanoparticles and their long-term environmental effects require comprehensive evaluation. Ensuring the safety, effectiveness, and environmental sustainability of these nanoparticles is essential for their successful use in biological and medical applications.

12. FUTURE DIRECTIONS

Future research on leaf extract nanoparticles should aim to refine synthesis methods to improve reproducibility and scalability. Exploring new plant sources and their distinct phytochemical compositions could lead to the development of nanoparticles with enhanced properties and broader applications. Combining leaf extract nanoparticles with cutting-edge technologies like nanotechnology and bioengineering offers great promise for creating innovative solutions in medicine and environmental management. Moreover, adopting principles of a circular economy—such as recycling and reusing nanoparticles—could support their sustainability and long-term viability, making them more eco-friendly and economically viable for widespread use.

13. CONCLUSION

Nanoparticles derived from leaf extracts mark a major step forward in green synthesis, offering a sustainable and eco-friendly alternative to conventional methods. Their distinct properties, coupled with their wide range of applications in antimicrobial activity, drug delivery, cancer therapy, and antioxidant effects, demonstrate their immense potential in various biological fields. By tackling existing challenges, such as inconsistencies in leaf extract composition, scalability, and potential toxicity, while focusing on future research opportunities, the field of leaf extract nanoparticles can continue to progress and contribute significantly to advances in green chemistry and biotechnology.

Abbreviation

S. aureus	Staphylococcus aureus
E. coli	Escherichia coli
B. Subtilis	Bacillus subtilis
P. aeruginosa	Pseudomonas aeruginosa
C. Albicans	Candida albicans
E. faecalis	Enterococcus faecalis
B. cereus	Bacillus cereus
K. Pneumoniae	Klebsiella pneumoniae
S. typhi	Salmonella typhi
P. mirabilis	Proteus mirabilis

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