Electron Probe Methods for Characterization of Plant-Mediated Nanoparticles

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***Abstract*—Plant-mediated nanoparticles synthesized from leaves, fruits, seeds, flowers, and other organic wastes have gained significant attention because they are eco-friendly, cost-effective, scalable, and non-toxic compared to conventional physical and chemical processes. Because of these advantages, they have multifunctional applications in environmental remediation, agricultural revolutionization, biomedical, textile, rubber, cosmetic, electronics, and several other sectors. Consequently, their immense potential has attracted remarkable attention from researchers worldwide. Nanoparticles are typically defined as particles of size ranging from 1 to 100 nm. Because of their small size, they exhibit unique physical, chemical, optical, and other properties that differ from bulk materials. Such properties vary strongly with their size and shape. So, characterization is needed to determine their versatile properties for effective applications. Various characterization methods are employed to investigate their properties. These are Optical probe methods, electron probe methods, scanning probe methods, spectroscopic probe methods, ion-particle probe methods, and thermodynamic probe methods. Among these, electron probe methods, including SEM, TEM, and EDX, are considered more valuable. These tools provide detailed information about size, shape, surface morphology, internal structure, and elemental composition. They use high-energy electron beams to image nanoparticles for characterization.**

I. INTRODUCTION

Rapid advances in nanotechnology have driven growing interest in diverse nanoparticle applications [1]. They are tiny particles of size between 1 and 100 nm[2], exhibiting versatile optical, electrical, and magnetic properties and less agglomeration. Among various synthesis approaches, including physical, chemical, and microbial methods, plant-based synthesis has proven more sustainable and environmentally friendly [3- 4]. This is because it does not require harmful or toxic chemicals or specialized laboratory equipment, making it more economical and eco-friendlier than other methods [5–7]. Thus, exploring their significant sustainable role in a variety of fields like agriculture [8–11], food processing industries [12], cosmetics [13], environmental pollution remediation [14–19], paint and textiles [15, 20, 21], electronics [22], and so on. The review suggested that nanoparticle properties vary with size and shape [23, 24], which can be characterized by various methods, including electron probe methods [25], optical probe methods, and scanning probe methods. Among these, electron probe methods are considered most significant because they provide detailed information about structure, shape, size, and morphology. These include SEM [26–28], TEM [29–31], and EDX [32, 33], This review covers the synthesis of plant-mediated nanoparticles and their properties. It also explores the working principles, advantages, and applications of some electron probe characterizing tools.

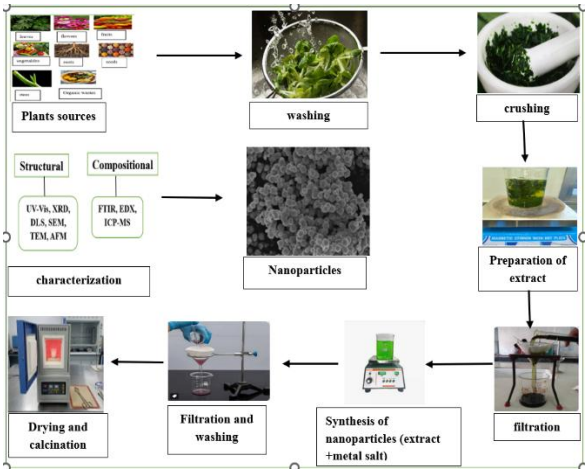


Figure 1: Schematic representation of plant-mediated nanoparticles

II. SYNTHESIS AND ADVANTAGES OF PLANT-MEDIATED NANOPARTICLES

The paper discussed two major approaches to nanoparticle synthesis: bottom-up and top-down [34]. The top-down approach includes physical processes that convert bulk materials into nanoscale materials through ball milling [35–37], laser ablation [38, 39], physical vapor deposition [40, 41], etc. Another approach, the bottom-up methods, includes chemical and biological synthesis processes in which atoms or molecules accumulate into nano-sized particles. The chemical processes are sol-gel [42, 43], co-precipitation method [44, 45], chemical reduction [46, 47], and so on. The biological processes involve microbes such as algae [48], bacteria [49], fungi [50], yeast [51], and plant parts such as leaves [52, 53], fruits [54, 55], roots [56], flowers [57], bark [58], seeds [59], and stems [60] for the synthesis of nanoparticles. The literature review noted that physical and chemical methods are expensive, require toxic chemicals, and are labor-intensive, time-consuming, and hazardous to the environment [34]. In contrast, using microorganisms to synthesize nanoparticles may require sophisticated laboratory facilities and often risks contamination [61]. Plant extracts do not require toxic chemicals; they are readily available, cost-effective, suitable for large-scale production, eco-friendly, and time-efficient [5]. Also, unlike microbial synthesis, it does not require specialized laboratory equipment and is often free from contamination risk [62]. Therefore, plant-based nanoparticle synthesis is considered a vital sustainable approach and has attracted significant attention from researchers. Plants contain a variety of phytochemicals that naturally act as reducing, capping, and stabilizing agents during nanoparticle synthesis [63]. Some of common phytochemicals are flavonoids [64], alkaloids [65], terpenoids [66], saponins [67], tannins [68], steroids [69] and so on.

Thus, the study emphasizes that plant-mediated synthesis requires fewer steps to synthesize nanoparticles [63]. These are:

2.1 Preparation of plant extracts:

Firstly, fresh plant materials were collected and washed properly to remove dust and other impurities. Then, they were either cut into small pieces or crushed and boiled in distilled water for 1-2 hours on a magnetic stirrer. After cooling, the mixture was filtered to obtain a plant extract for nanoparticle synthesis.

2.2 Synthesis of nanoparticles:

The prepared extract was mixed with metal salts of Zn, Ag, Cu, Fe, Mg, etc., which act as precursors, followed by the addition of NaOH solution. The reaction mixture was stirred continuously on a magnetic stirrer with a hot plate using magnetic beads at the desired temperature until a nanoparticle precipitate formed.

2.3 Filtration/centrifugation:

The obtained precipitate was filtered using Whatman ashless filter paper or centrifuged using a centrifuge and washed multiple times to remove NaOH content.

2.4 Drying and calcination:

The filtrate was dried in an oven and then calcined in a muffle furnace at a temperature of 400-500 degrees centigrade to get a powder of nanoparticles.

Before use in specific applications, nanoparticles undergo various characterization methods to determine physicochemical properties such as size, shape, morphology, optical properties, and agglomeration [70, 71]. Such methods include optical probe methods (UV [72], FTIR [73], XRD), electron probe methods (SEM [74], TEM [31], EDX [33]), scanning probe methods (AFM), and so on. This paper focuses on electron probe methods, which are among the most effective characterization tools for determining nanoparticle size, shape, and morphology. This includes scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy-dispersive X-ray.

III. ELECTRON PROBE METHODS

The studies have reported that electron probe methods are the most prominent characterizing tools for defining physical and chemical properties of plant-mediated nanoparticles [25]. Such methods utilize a focused electron beam with high magnification and spatial resolution, enabling accurate characterization of size, shape, morphology, agglomeration, 3-dimensional structure, and elemental composition. Some important electron probe methods are SEM (scanning electron microscopy), TEM (transmission

electron microscopy), EDX (energy-dispersive X-ray), FE-SEM (field emission scanning electron microscopy), HR-TEM (high-resolution transmission electron microscopy), STEM (scanning transmission electron microscopy), Cryo-SEM (cryogenic electron microscopy), and EPMA (electron probe microanalysis). Table 2 in the paper details the various electron probe methods, their uses, nanoparticles, applications, and references.

S. NO.	TECHNIQUE	FULL NAME
1.	SEM	Scanning electron microscopy
2.	TEM	Transmission electron microscopy
3.	FE-SEM	Field emission scanning electron microscopy
4.	HR-TEM	High-resolution transmission electron microscopy
5.	STEM	Scanning transmission electron microscopy
6.	Cryo-SEM	Cryogenic scanning electron microscopy
7.	EDX	Energy-dispersive X-ray analysis
8.	EPMA	Electron probe microanalysis

3.1 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) is the most widely used technique for determining size, shape, 3-dimensional images, agglomeration, structure, and elemental composition in nanoparticles synthesized from different plant sources. It comprises an electron gun, anode, electromagnetic lenses, detector, computer, and sample stage.

A tungsten filament-based electron gun generates a high-energy electron beam, accelerated by the anode. The beam passes through a series of electromagnetic lenses, first a condenser lens and then an objective lens, before focusing on the nanoparticle sample on the sample stage. The interaction between the electron beam and the sample generates various signals, such as secondary electrons, backscattered electrons, and X-rays. The instrument's detectors capture these signals, and the computer converts them into images used to identify the morphological and structural information of the desired nanoparticle sample.

1: Comparative summary of morphological characterization of different plant-mediated nanoparticles using SEM

Table 1 in the paper provides details on various scanning electron microscopy tools, including their uses, nanoparticles, applications, and references.

S. No.	SEM techniques	Nanoparticles	Plants used	Source	Family	Information obtained	Applications	References
1.	FE- SEM	Ag NPs	Morinda citrifolia	root	Rubiaceous	spherical and oval shape; mostly agglomerated	anticancer	[75]
2.	SEM	ZnO NPs	Parthenium hysterophorus	leaves	Asteraceae	spherical and particle size about 10 nm	antimicrobial and vegetative growth	[76]
3.	FE-SEM	ZnO NPs	Cayratia Pedate	leaves	Vitaceae	agglomerated surface horizontal shape	Immobilization of enzyme	[77]
4.	ESEM	Ag NPs	Cannabis sativa	hurd	-	uniform size (200-300 nm); aggregated; spherical or quasi-spherical	anti-bacterial	[78]
5.	SEM	CuO NPs	Moringa oleifera	leaves	Moringacea	nanorod grains with cubic shapes and tube-like holes	supercapacitor	[79]
6.	ESEM	Ag NPs	Ageratum conyzoides	leaves	-	spherical with variable size	antioxidant; H2O2 sensing; DNA binding; photocatalytic degradation	[80]
7.	SEM	MgO NPs	Melia azedarach	seeds	Meliaceae	spherical	larvicidal and antioxidant	[81]
8.	SEM	ZnO NPs	Melia azedarach	seeds	Meliaceae	spherical; agglomerated	cytotoxicity and antimicrobial	[82]
9.	SEM	TiO2 NPs	Melon	peel	Cucurbitaceae	size about 39 nm; agglomerated	Photocatalytic degradation	[83]
10.	FESEM	Ag NPs	Phyllanthus emblica	fruit	-	spherical; not agglomerated; size about 60-80 nm	-	[84]

3.2. Transmission electron microscope (TEM)

TEM is another significant electron probe characterization technique, widely used in defining properties of plant-mediated nanoparticles synthesized from different sources. It provides detailed information about particle size, shape, crystallinity, lattice structure, surface defects, etc., by providing high-resolution images of their internal structure. Examples of TEM types include HR-TEM, cryo-TEM, and STEM. The instrument used in these tools is the transmission electron microscope, which consists of a vacuum, an electron gun, electromagnetic lenses, electrostatic plates, a fluorescent screen, and a CCD camera. The microscope present in the TEM works under high vacuum conditions, where air is pumped Out to allow the free movement of electrons. The electron gun then generates a high-energy, high-intensity electron beam that passes through electromagnetic lenses and is directed onto an ultra-thin specimen placed inside the vacuum chamber. Because of variations in the material's thickness and density, some beams are transmitted and scattered. Electromagnetic lenses then focus and magnify the transmitted electrons before they strike the fluorescent screen, where they are converted into a visible image. The CCD camera captures the images and uses them to evaluate the various properties of the nanoparticle sample. Table 2 summarizes information on different transmission electron microscopy studies reported for characterizing various plant-mediated nanoparticles.

Table 2: Comparative summary of morphological characterization of different plant-mediated nanoparticles using TEM

S. No.	TEM techniques	Nanoparticles	Plants	Source	Family	Information obtained	Applications	References
1.	TEM	ZnO	Azadirachta indica	leaves	-	spherical shape; size 9.6 nm to 25.5 nm	antibacterial and photocatalytic degradation	[85]
2.	TEM	CuO NPs	madhuca longifolia	flowers	sapotaceae	spherical shape; mostly 120 nm	photocatalytic degradation of dye and antibacterial	[86]
3.	TEM	CuO NPs	madhuca longifolia	seeds	sapotaceae	irregular shape; size about 30 nm; quite aggregated	catalytic degradation of dye and antibacterial	[86]
4.	TEM	Ag NPs	carica papaya	leaves	caricaceae	spherical; average size 5 nm to 40 nm	antibacterial	[87]
5.	HR-TEM	ZnO NPs	Punica granatum	peels	-	spherical; size 40 nm to 70 nm	photocatalytic degradation	[88]
6.	HR-TEM	Ag NPs	petroselinum crispum	leaves	-	Spherical; average size 30-32 nm	antibacterial	[89]
7.	HR-TEM	Au NPs	cacumen platyclade	leaves	-	spherical; average size 15.3 nm	antimicrobial	[90]
8.	TEM	MgO NPs	Mucuna pruriens l.	seeds	-	spherical; average size 50 nm	photocatalytic degradation; antimicrobial; antioxidant	[91]
9.	TEM	Ag NPs	Memecylon umbellatum	leaves	melastomataceae	spherical; range 15-20 nm	antimicrobial	[92]
10.	TEM	Au NPs	memecylon umbellatum	leaves	melastomataceae	spherical, hexagonal and triangular shape; size range 15-25 nm	antimicrobial	[92]
11.	TEM	Ag NPs	Impatiens balsamina	leaves	-	less aggregation; average size 20 nm	antibacterial	[93]
12.	TEM	Ag NPs	lantana camara	leaves	-	less aggregation; average size 12 nm	antibacterial	[93]

3.3. Energy dispersive X-ray (EDX)

Energy-dispersive X-ray (EDX/EDS/EDAX) is also a valuable characterization technique, commonly coupled with SEM or TEM, used to identify and quantify the elements present in the synthesized nanoparticles. It detects elemental composition and distribution, relative atomic and weight percentages, and impurities or contaminants. EDX machine consists of an electron beam source, X-ray detector, pulse processor, and analyzer.

The working principle of such tools is similar to SEM, where a high-energy electron beam source

is produced by an electron gun and passes through a series of electromagnetic lenses before falling on the surface of the sample. This interaction results in the emission of X-rays, which the X-ray detector analyzes. Each element emits characteristic signals that enable identification of the sample's elemental composition. Table 3 below shows the EDX data, including the characteristic peaks for each element, with references.

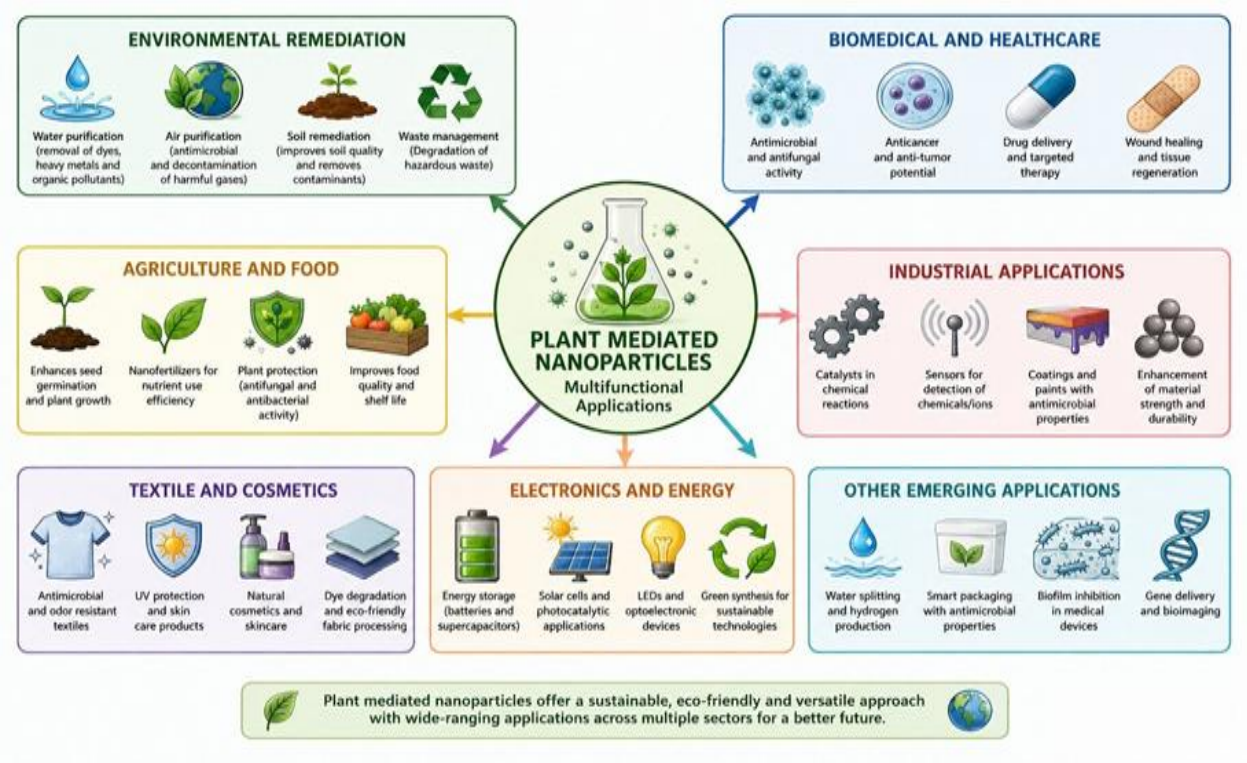
Table 3: EDX elemental analysis and characteristic peak values of phyto-synthesized nanoparticles.

S. No.	Nanoparticles	Plants	Source	Family	EDX data (elements and characteristic peak value in Kev)	Applications	References
1.	Au NPs	Bauhinia variegata	bark	fabaceae	Au (1.3,1.5, 1.7, 1.2, 2.6) 1.7, 1.2, 2.6)	antibacterial; anticancer	[94]
2.	Ag NPs	citrus aurantifolia	fruits peel	Rutacea	Ag (3.0)	phytochemical constituents	[95]
3.	Ag NPs	decalepis hamiltonii	roots	-	Ag (2.5,3.0,3.5 kev)	antioxidant; antimicrobial	[96]
4.	Ag NPs	artemisia nilagirica	leaves	asteraceae	Ag (2-4 kev)	antibacterial	[97]
5.	CuO NPs	morinda citrifolia	leaves	rubiaceae	Cu (1.0 kev); O (0.5 kev)	antibacterial; antifungal	[98]
6.	CuO NPs	camelia sinensis	leaves	-	Cu (1.0 kev); O (0.5 kev)	anticancer; apoptotic mechanism	[99]
7.	CuO NPs	portulaca olracea	leaves	portulacaceae	Cu (1.0,8.0.,9.0 kev); O (0.5 kev)	antibacterial; wastewater treatment; heavy metal sorption	[100]
8.	ZnO NPs	achillea nobilis	flowers	-	Zn (1.0 kev); O (0.5 kev)	antioxidant and antibacterial	[101]
9.	ZnO NPs	solanum nigrum	leaves	solanaceae	Zn (1.0 kev); O (0.5 kev)	antibacterial	[102]
10.	ZnO NPs	sapindus mukorossi	fruits	sapindaceae	Zn (1.0 kev); O (0.5 kev)	antioxidant	[103]

IV. APPLICATIONS OF NANOPARTICLES

Thus, the study shows that nanoparticles, owing to their versatile physicochemical properties, are valuable materials in various sectors with diverse applications [104, 105]. For example, De et al. explored the various applications of nanoparticles in biology [106]. Another study by Singh and Nalwa discussed the role of nanoparticles in biological imaging, cell labeling, and as antimicrobial and anticancer agents [107]. Khot et al. explained the application of nanomaterials in agricultural production and crop protection [108]. Also, Rehmanullah, in their review, explained the role of nanoparticles as fertilizers and pesticides [109]. Prasad et al. explained the effect of zinc oxide nanoparticles at different concentrations on peanut germination, growth, and yield [110]. The study also reveals that nanoparticles have variable applications in cosmetics, as discussed in [13, 111, 112], and so on. Matsui et al. described various applications of nanoparticles in electronic devices [113]. Magdassi et al. discussed using copper nanoparticles in printed electronics [114]. Nanoparticles also play a significant role in the paint and textile industries, as discussed in [20, 21, 115–118].

The diagram below presents the diverse applications of nanoparticles in different fields.



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