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Biotechnical Synthesis and Characterization of Silvernanoparticles (AgNPs) from *Moringer Oliefera* Leaves in Biu, Borno State Nigeria

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Abstract: *The green synthesis of silver nanoparticles (AgNPs) using Moringa oleifera leaf extract has proven to be an effective, sustainable approach for producing stable and functional nanoparticles. The characterization of these AgNPs using (FTIR, SEM, EDS and XRD) confirmed their desirable size, shape, and stability, while also demonstrating their potent catalytic activity.*

Keywords: Biotechnical Synthesis, Silvernanoparticles (AgNPs), Moringer Oliefera Leaves Biu, Borno State, Nigeria

INTRODUCTION

Industrial activities among other things are a major source of environmental pollution, Water ecosystem for example is majorly affected by various industrial activities such as mining, cement production, soap and detergent productions and textile manufacturing (Venkatesharaju *et al.*, 2010). Developing and especially populated countries like Nigeria has most of its water bodies contaminated by industrial effluents as river water is considered means of industrial effluent disposal (Kayode, 2010).

Waste water treatment is not given the necessary priority it deserves; therefore, industrial waste and domestic sewage are discharged into receiving water bodies without treatment. The consequence of this is increased river pollution, loss of aquatic life and uptake of polluted water by plants and animals which eventually gets into human body resulting in health related problems. The situation is compounded by the fact that the common man in most of these countries does not have access to portable water and in many instances; raw river water is used as source of drinking (Suriptono and Newman, 2000).

Water pollution due to the release of chemicals from industrial sectors has been a major concern in recent times. The major constituents of industrial effluents are heavy metals, pesticides, dyes, aliphatic and aromatic compounds, detergents, chlorophenols, etc. Many conventional methods (such as sedimentation, chlorination, precipitation, etc.) are used to treat these effluents and each method has its own merits and shortcomings (Nagaveni *et al.*, 2005).

Heterogeneous photo catalysis by semiconductor materials such as TiO_2, ZnO, Fe_2O_3, CdS, GaP, and ZnS has indeed been widely used for the degradation of various organic and inorganic pollutants (Faber *et al.*, 2005).

Silver nanoparticles has been applied as a visible light responsive photocatalysts for the remediation of hazardous wastes, contaminated groundwater and control of toxic air contaminants (Jadhav *et al.*, 2010).To enhance the photocatalytic activity of the silver nanoparticles, several methods have been proposed for its synthesis with unique size dependent, physical and chemical properties. Various composites of silver oxide, Ag/TiO_2, Ag/ZnO e.t.c have also been synthesized and their photocatalytic activity is often higher than that of the bare silver (Cao *et al.*, 2007) Nanoparticles (NPs) have garnered significant attention over the past decades due to their unique physicochemical properties, which differ considerably from their bulk counterparts. Nanoparticles are defined as particles with dimensions in the range of 1-100 nanometers (nm), offering a high surface area to volume ratio, quantum effects, and distinct chemical and mechanical properties. This has made them highly applicable across various fields such as medicine, electronics, energy, and environmental remediation.

Methods of Synthesis of Nanoparticles

Physical Methods

Physical methods of nanoparticle synthesis, such as ball milling, laser ablation, and vapor deposition, involve the reduction of bulk materials into nanosized particles. These methods generally offer high precision and produce particles with well-defined sizes and shapes. However, they often require expensive equipment and high energy input (Hassan *et al.*, 2016).

Chemical Methods

Chemical methods include sol-gel synthesis, chemical vapor deposition, and chemical reduction. These methods are widely used due to their simplicity and versatility in producing a wide range of nanoparticles. Chemical reduction is a common method used for metal nanoparticles, where a metal salt is reduced using a reducing agent, forming nanoparticles (Mubarak *et al.*, 2017).

Biological Methods

Biological methods, or "green synthesis," involve the use of biological entities like plants, bacteria, or fungi to synthesize nanoparticles. This method is environmentally friendly and can lead to the production of biocompatible nanoparticles. For example, plant extracts have been successfully used to synthesize gold and silver nanoparticles (Iravani, 2011). Green synthesis methods are gaining popularity due to their low toxicity and eco-friendliness.

Applications of Nanoparticles

Medicine

Nanoparticles are revolutionizing the medical field, particularly in drug delivery, imaging, and diagnostics. Drug delivery systems using nanoparticles can target specific tissues, reducing side effects and enhancing therapeutic outcomes. For example, liposomes and polymeric nanoparticles are used to encapsulate chemotherapeutic drugs, reducing their toxicity while improving efficacy (Farokhzad & Langer, 2009). Furthermore, nanoparticles like iron oxide are utilized in magnetic resonance imaging (MRI) for enhanced contrast and diagnosis (Gupta & Gupta, 2005). Nanoparticles are also essential in cancer treatment. Gold nanoparticles, for instance, can be used in photothermal therapy, where they are targeted to cancer cells and heated using light to destroy tumors (Huang *et al.,* 2008).

Energy

In the energy sector, nanoparticles play a critical role in enhancing the efficiency of solar cells, batteries, and fuel cells. Quantum dots and metal oxide nanoparticles are widely researched for their ability to improve the efficiency of photovoltaic cells by allowing better light absorption and energy conversion (Nozik, 2002). In addition, lithium-ion batteries incorporate nanoparticles like silicon to enhance storage capacity and reduce degradation (Zhang *et al.,* 2014).

Environmental Applications

Nanoparticles are highly useful in environmental remediation, especially for removing pollutants from water and soil. Titanium dioxide nanoparticles are employed in photocatalysis to degrade organic pollutants in wastewater (Zhang *et al.,* 2018). Similarly, iron nanoparticles are used in the remediation of contaminated groundwater through the reduction of toxic substances like chlorinated hydrocarbons (Zhang, 2003).

Electronics In the field of electronics, nanoparticles are pivotal in the development of smaller, faster, and more efficient devices. Nanoparticles like quantum dots are used in displays and lighting systems due to their tunable optical properties (Kim *et al.,* 2011). Carbon nanotubes are also being integrated into transistors and sensors, offering better performance than traditional materials.

Potential Risks and Toxicity of Nanoparticles

Despite their numerous applications, nanoparticles pose potential risks to human health and the environment. The small size of nanoparticles allows them to penetrate biological membranes, leading to possible toxic effects in cells and tissues. Studies have shown that inhaled nanoparticles, such as those from silver or titanium dioxide, can cause oxidative stress, inflammation, and even genotoxicity (Oberdörster *et al.*, 2005).

In addition, the environmental impact of nanoparticles is still a concern. When nanoparticles are released into the environment, they may accumulate in ecosystems, posing risks to aquatic organisms and soil health. The long-term effects of nanoparticles on the environment remain largely unknown, necessitating further research (Klaine *et al.,* 2008). Silver nanoparticles (AgNPs) are among the most widely studied and applied nanomaterials due to their remarkable antimicrobial, catalytic, optical, and electrical properties. Traditionally, silver nanoparticles have been synthesized using chemical and physical methods, but concerns about the environmental and health impacts of these techniques have led to the exploration of more sustainable alternatives. Green synthesis, also known as eco-friendly or biogenic synthesis, involves the use of biological entities such as plant extracts, microorganisms, and enzymes to produce nanoparticles, offering a more environmentally benign approach. This literature review explores various green synthesis techniques, the biological mechanisms behind the synthesis, and the characterization methods employed to study the properties of green-synthesized silver nanoparticles.

Moringaoleifera, often referred to as the drumstick tree, miracle tree, or simply moringa, is a versatile and highly valued plant known for its exceptional nutritional and medicinal properties.
Moringaoleifera belongs to the family Moringaceae and is native to the Indian subcontinent and parts of Africa.

*Moringaoleifera*is a genus of the fast-growing tropical deciduous plant of the Moringaceae family, with thick, tuberous roots, light green leaves and abundant flowering with elongated, pendulous fruits and seeds (Asensi *et al.,* 2017) It is a native crop of northern India, although it is found in southwest Asia, southwest and northwest Africa and Madagascar. It has long been a part of traditional horticulture, used mainly for ornamental purposes in cities along the Pacific coast of Mexico (Olson *et al.*, 2011), as well as plantations in Bolivia, Argentina and elsewhere in the world (José and García, 2016). It has 13 known species, with *Moringaoleifera*, native to India, being one of the most studied and used for its nutritional, phytochemical and pharmacological properties. According to ayurvedic medicine (traditional and alternative medicine of India) (Singh and Panchakarma, 2012), it is attributed properties for the treatment of some diseases, such as asthma, epilepsy, eye and skin diseases, fever and haemorrhoids (Sanjay *et al.*, 2015). In fact, it is a medicinal plant traditionally known in the approach to malnutrition and other diseases (Ma *et al.*, 2020). It can withstand long periods of drought, growing well in arid and semi-arid areas. According to researchers, it tolerates soils with a pH between 4.5 and 8, although neutral or slightly acidic pH is more favourable (Padilla *et al.*, 2017); it is a very adaptable species, lives about 20 years and reaches a height of 5 to 10 m in a short period of time, reaching 4 m in 6 months (García, 2016). It is considered a very versatile plant due to its great capacity to provide edible food, which includes different vegetative structures, such as leaves, pod shells, stem, flowers, fruits and seeds. These structures contain bioactive compounds and nutrients, such as phenolic compounds, fatty acids, carbohydrates, fibre, minerals, vitamins and functional peptides with a wide potential to be used in food. However, not all of the plant is probably safe, as toxic compounds have been found (Martínez *et al.*, 2019). On the other hand, its uses are varied, as the seed is used to purify water and the oil from the seeds can be used as a fertiliser (Folkard and Sutherland, 1996). Considering that previous studies have shown that bioactive compounds fromherbs and plants could be used for functional food product innovation (Nieto *et al.*, 2017). e compounds from herbs and plants could be used for functional food product innovation. Moringaoleifera plants could be used for functional food and other industrial food applications (Oyeyinka and Oyeyinka, 2018). Therefore, Moringaoleifera provides nutrients that benefit health, making it a key food for food security in areas with fewer economic resources (Sagona *et al.*, 2020), this review summarises recent knowledge on bioactive compounds from Moringaoleifera plants and their potential use in the formulation of food products, especially bakery products. The objective of this review is to know the uses and applications of Moringaoleifera in bakery products, in order to know what the quantity w the uses and applications of *Moringaoleifera*in bakery products, in order to know what the quantity or concentration of *Moringaoleifera*is that allows maintaining the sensory characteristics of the product.

Figure 1: Moringa leaf

Equipment

Table 1: Equipment that were used

S/NO	Names	Model	Manufacturer	Location
1	Hot Plate and Magnetic Stirrer	C1202	Thermocool, England	NAUB
2	Digital weighing balance	AB 204 (0 -180g)	Metter Teledo, Switzerland	NAUB
3	Halogen lamp	100 W	China	NAUB
4	Oven	Thermally Protected Varistors – 420(0 – 260^{0}C)	Gallenkamp, England	NAUB
5	Furnace	Lufthansa120/14(30-3000^{0}C)	Nebertherm, GmbH, Germany	NAUB
7	X-Ray Diffractometer	Empyrel	PANalytical, Netherland	Multiuser ABU, Zaria
8	Scanning Electronic Microscopic	Phenom pro-X	Phenomworld, Netherland	Chemical Eng Dept, ABU
9	UV-Vis	Ultaviolet – 2500S	Shimazu	NARICT
10	FTIR	Empyrel	Shimazu	NARICT

Key: NARICT = National Research Institute for Chemical Technology, Zaria, Kaduna State, Nigeria.
ABU = Ahmadu Bello University Zaria , Kaduna state Nigeria.
NAUB = Nigeria Army University Biu, Borno State Nigeria.

METHODOLOGY

Collection and handling of samples

The sample was collected in Tabra Biu local Government Area of Borno state, the leaves (moringa) were washed thoroughly with running water, rinsed with distilled water to remove impurities, then dried at room temperature in an open shade for two weeks kept in a labelled container for further use in the laboratory (Ezike*et al.*, 2012). The sample was transported immediately to the laboratory for further analysis (Patrungo *et al.,* 2007).

Sample preparation (Moringa Extracts)

The grounded moringa leave was weighed 50 grams and transferred in to 500 Ml beaker containing 200 mL distilled water, boiled for a n hour to collected an extract which was left undisturbed for 20 minutes, filtered through whatman filter paper under a vacuum condition. The filtered sample was refrigerated at 4 °C to maintain accuracy and effectiveness in the result for further experiment (Sathyavathhi, *et al.,* 2011).

Green Synthesis of Silver Nanoparticles

10 mL of the Moringa oleifera leaf extract was mixed with 90 mL of the 1 mM silver nitrate solution. The mixture was stirred using a magnetic stirrer for uniform mixing . (Girish, 2011).
The reaction mixture was heated to 70 °C to facilitate the reduction of silver ions (Ag^+) to silver nanoparticles (Ag^0). The reaction was observed over time (20 min) for a change in color.The reduction of silver ions by the leaf extract was confirmed by a color change from light yellow to dark brown. This change indicated the formation of silver nanoparticles, which occurs due to the surface plasmon resonance (SPR) effect of the nanoparticles (Song & Kim, 2009)

Characterization of the Synthesized Silver Nanoparticles

X-Ray diffraction (XRD) spectroscopy

Empyrel X-Ray diffraction (XRD) machine was used to examine the crystal structures of the synthesized nanoparticles. It is a method of determining the arrangement of atoms within a crystal, in which a beam of X-ray strikes a crystal and diffracts into many specific directions using a diffractometer. It provides detailed information on the crystallographic structure and physical properties of materials and thin films.

Scanning electron microscope (SEM) analysis

A scanning electron microscope (SEM) is a type of electron microscope that produces image of a sample by scanning it with a focused beam of electron. The electrons interact with atoms in the sample, producing various signals that contain information about the sample surface topography and composition. The electron beam is generally scanned and the beam's position is combined with detected signal to produce an image. (Daneshvar *et al.,* 2007).

UV-Visible spectroscopic analysis

A computer based UV-Vis spectrophotometer was used for the determination of concentration of samples. The system was switched on and warm up to 30 minutes, thoroughly clean quartz cuvettes was use. One of the cuvettes was fill with the reference compound and the other one with compound whose absorbance were measure at maximum wavelength. To get the relationship between concentration and absorbance of the compound, a calibration curve was plotted. Calibration solutions were made from standard solutions of known concentration. The absorbance was plotted against concentration of the calibrated samples. These calibration curves was stored in the system itself and the concentration of the unknown sample was calculated directly from the absorbance.

RESULT AND DISCUSSIONS

Synthesis of silver Nanoparticles;

Formation of Ag NPs was easily noticed due to changes in color of the solution. The color changes arise due to excitation of surface plasmon resonance in the metal nanoparticles indicating the formation of AgNPs. When 10 ml of Moringa oleifera extract was added to 90ml of 1mM solution of $AgNO_3$ while stiring and heating (70 ^{0}C) for 20 minutes,the reaction mixture changed its color from pale yellow to brown indicating the formation of AgNPs.

Characterization of silver nanoparticles (AgNPs):

Scanning Electrone microscopy (SEM):
The SEM image reveals that the synthesized silver nanoparticles (AgNPs) are within the nanoscale, with most particles appearing to be less than 100 nm in size. This matches the typical range expected for green-synthesized silver nanoparticles, which often fall between 10-100 nm.The size of the particles can be attributed to the effectiveness of biomolecules from the Moringa oleifera leaf extract, which act as reducing and stabilizing agents. The nanoparticles' small size is advantageous for applications like antimicrobial activity due to the large surface area-to-volume ratio.

The image shows a mixture of shapes, with particles appearing largely irregular and agglomerated. This aggregation of particles may be due to the biomolecules surrounding the nanoparticles, causing them to clump together during synthesis. Such agglomeration is common in green synthesis, where capping agents like proteins and polyphenols from the plant extract create a somewhat non-uniform coating around the particles.

A study by Singh *et al. (2015)* reported that silver nanoparticles synthesized using Azadirachta indica leaf extract exhibited irregular shapes and agglomeration due to the presence of capping biomolecules from the plant extract .

The surface of the nanoparticles appears rough, with a granulated texture. This roughness is often associated with nanoparticles that are capped or coated by organic molecules, such as proteins, polysaccharides, or flavonoids from the plant extract. These molecules stabilize the particles and prevent excessive growth during synthesis.

Similar findings were reported in a study by Anandalakshmi *et al. (2016)*, where SEM images of silver nanoparticles synthesized using Moringa oleifera leaf extract showed rough, agglomerated particles due to the bio-organic molecules from the extract acting as both reducing and capping agents .

Many studies have documented the synthesis of AgNPs using plant extracts, with particle sizes generally falling between 10-100 nm, similar to the results observed here. For instance, an investigation by Patel *et al.* (2015) reported silver nanoparticles synthesized using Moringa oleifera leaf extract to have sizes ranging from 20-80 nm, with irregular shapes and aggregation, aligning closely with the current SEM findings.

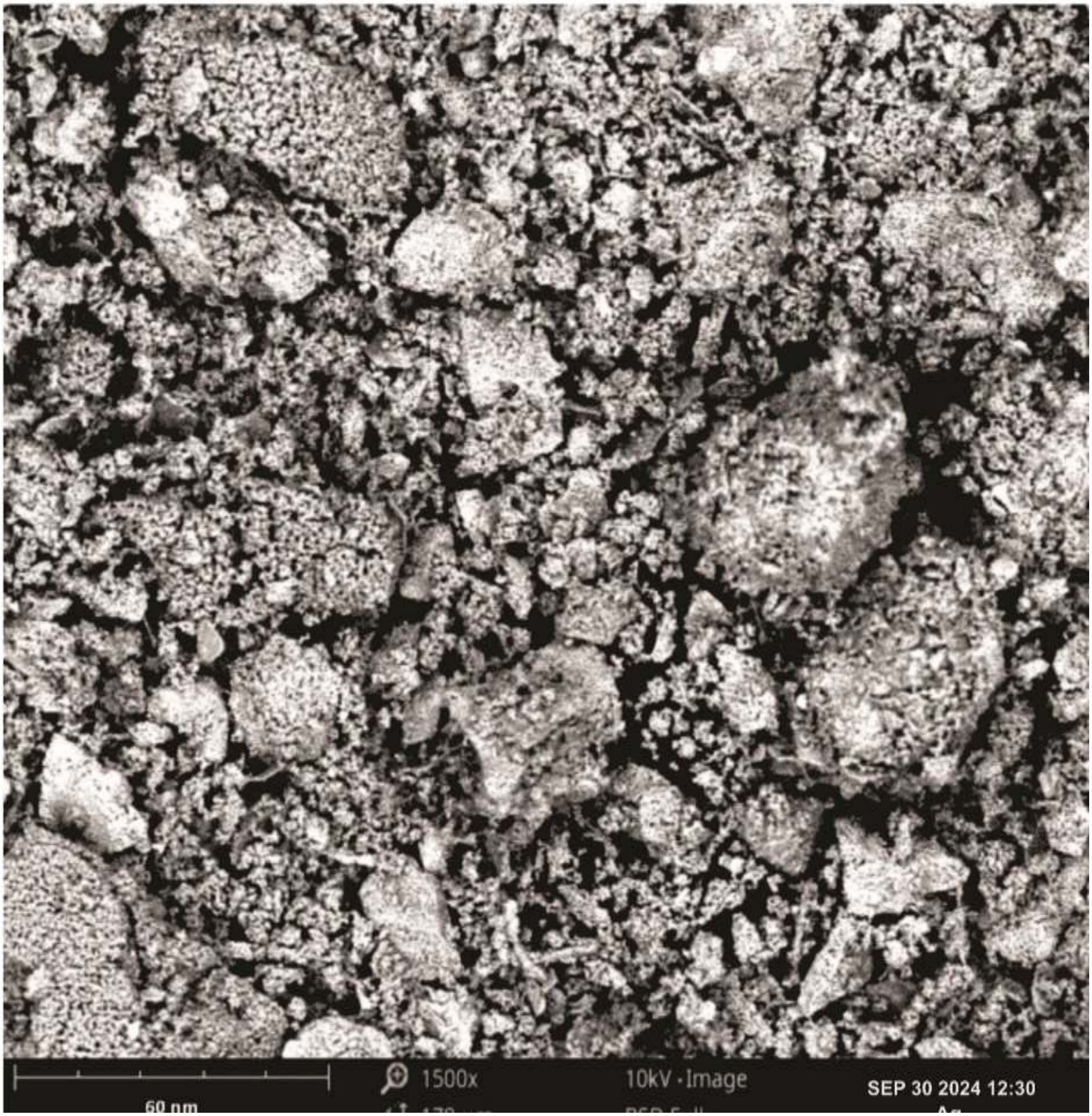


Fig. 2. SEM image

Energy disperse spectroscopy (EDS)

The Energy Dispersive X-ray Spectroscopy (EDS) results for silver nanoparticles synthesized using Moringa oleifera leaf extract in (table 1). and fig (2) shows the elemental composition of the sample. The table provides atomic and weight concentrations of various elements, while the spectrum provides a visual representation of the elements detected.

Key Findings from the EDS Table:

Silver (Ag): The predominant element, with an atomic concentration of 65.96 % and a weight concentration of 29.21 %. This indicates that the synthesized nanoparticles are rich in silver, which is expected since the nanoparticles were synthesized using silver salts.

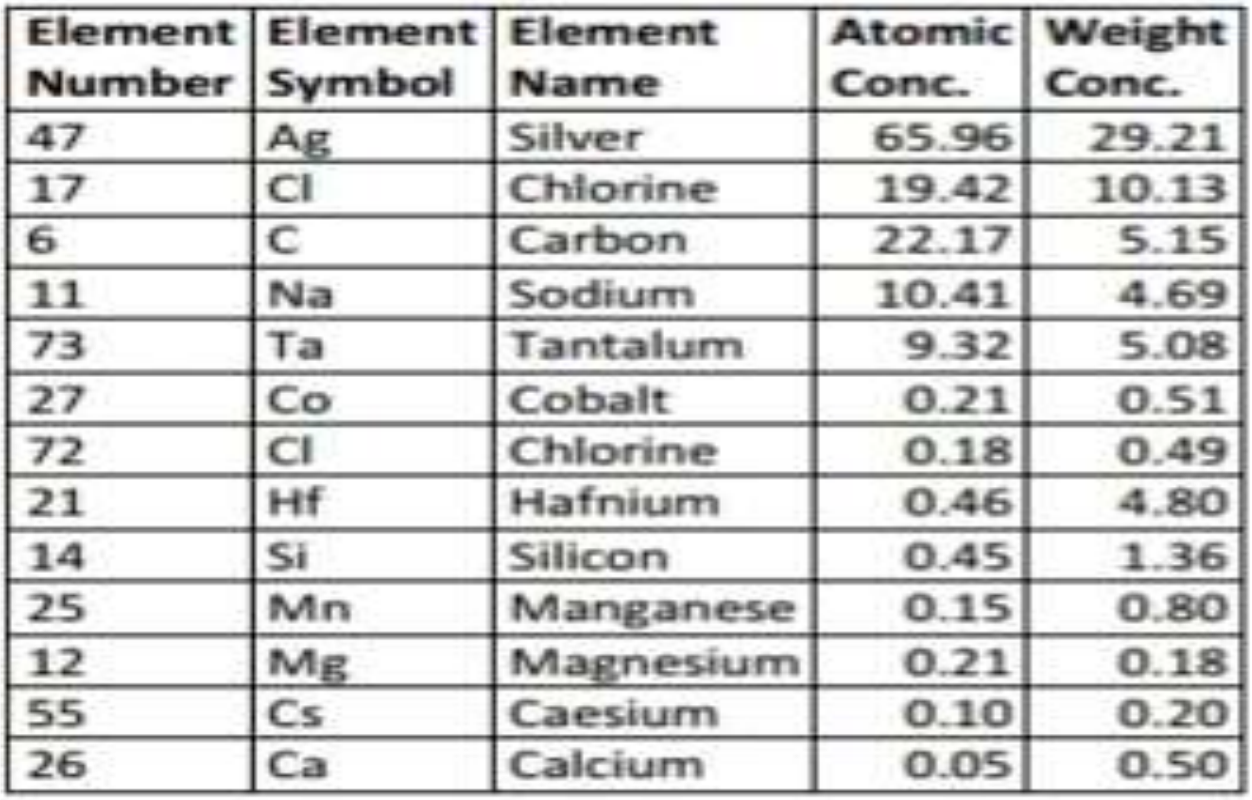
Chlorine (Cl): The second most abundant element, with an atomic concentration of 19.42 % and a weight concentration of 10.13 %. Chlorine could be present due to the silver precursor used (e.g., silver nitrate) or compounds in the Moringa oleifera extract.

Carbon (C): with a Significant presence (22.17 % atomic concentration), which could be attributed to the organic compounds in the Moringa oleifera leaf extract used as the reducing agent.
Other elements such as Sodium (Na), Tantalum (Ta), Cobalt (Co), and Silicon (Si) are present in smaller amounts, likely as impurities or residuals from the synthesis process or extract composition.

The EDS spectrum shows a sharp peak for silver at lower energies, corresponding to its dominant presence in the sample. There are smaller peaks corresponding to other elements, but they are much less intense than the silver peak.

Green synthesis of silver nanoparticles (AgNPs) using plant extracts, including Moringa oleifera, typically shows silver as the dominant element, with trace amounts of elements such as chlorine and carbon due to the natural organic matter present in the extract. Arya *et al*. (2018) reported high silver content and a similar presence of organic elements like carbon and oxygen, supporting the role of plant metabolites in nanoparticle stabilization .

Chlorine Presence: Chlorine is often detected in EDS analyses of AgNPs synthesized with plant extracts, especially when silver salts such as silver nitrate are used as the precursor. Similar results were observed in the study by (Mittal *et al. (2020)*, where chlorine peaks were attributed to the use of silver chloride during synthesis.



Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
47	Ag	Silver	65.96	29.21
17	Cl	Chlorine	19.42	10.13
6	C	Carbon	22.17	5.15
11	Na	Sodium	10.41	4.69
73	Ta	Tantalum	9.32	5.08
27	Co	Cobalt	0.21	0.51
72	Cl	Chlorine	0.18	0.49
21	Hf	Hafnium	0.46	4.80
14	Si	Silicon	0.45	1.36
25	Mn	Manganese	0.15	0.80
12	Mg	Magnesium	0.21	0.18
55	Cs	Caesium	0.10	0.20
26	Ca	Calcium	0.05	0.50

Table 2. EDS Table

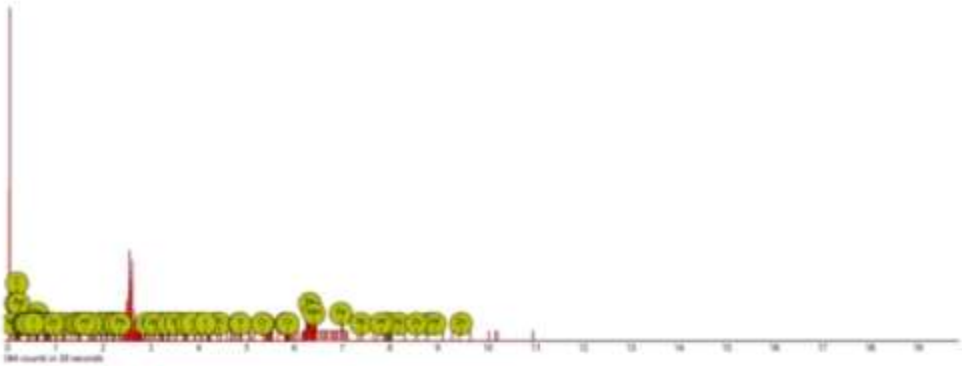


Fig 3. EDS Spectra

X-RAY DIFFRACTION SPECTROSCOPY (XRD)

The significant sharp peak around 5° indicates the presence of crystalline structures. However, this peak is not typical of silver nanoparticles, suggesting that other organic compounds or residual plant biomolecules from the Moringa oleifera extract may be present.

Broadened Peaks: The broader peaks in the range of 15° to 80° indicate smaller crystallite sizes, a common characteristic of nanoparticles. This broadening is often attributed to the nanoscale dimensions of the particles and the strain in their crystal lattice.

The XRD peaks appear at approximately 38°, 44°, 64°, and 77° (2θ values) correspond to the (111), (200), (220), and (311) planes of the face-centered cubic (FCC) structure of silver.

The data shows peaks at positions around 38°, 44.9°, 64°, and 82°, suggesting the formation of crystalline silver nanoparticles.

The table provides detailed fitting data for each peak, including:

2θ values: The angle at which diffraction peaks occur.

FWHM (Full Width at Half Maximum): This value indicates the peak width, which is inversely related to crystallite size.

Height (cps): The intensity of each peak, which relates to the abundance of the corresponding crystallographic planes.

D-spacing: The distance between atomic layers, which can help confirm the crystal structure.

Key findings from the table

The peak around 38° (Peak 21) corresponds to a height of 137 cps and a d-spacing of 2.015 Å, confirming the (111) plane of silver.

The peak around 44.99° (Peak 23) is sharp with a height of 137 cps, further indicating the (200) plane of silver nanoparticles.

Other smaller peaks align with the (220) and (311) planes, suggesting the polycrystalline nature of the silver nanoparticles.

Mittal *et al.* (2013) reported that on the biosynthesis of silver nanoparticles using *Moringa oleifera*, plant extracts typically exhibit XRD peaks matching the FCC structure of silver. Peaks corresponding to the (111), (200), (220), and (311) planes were observed at similar 2θ values. This comparison shows that the nanoparticles in this AgNPs exhibit similar diffraction behavior, further confirming their identity as silver nanoparticles.

Moreover, the broadening of peaks, especially around the (111) plane, is consistent with the nanoscale size, as seen in other studies using green synthesis methods. The use of Moringa oleifera extract often results in biocompatible silver nanoparticles with small sizes and stabilized by phytochemicals from the extract.

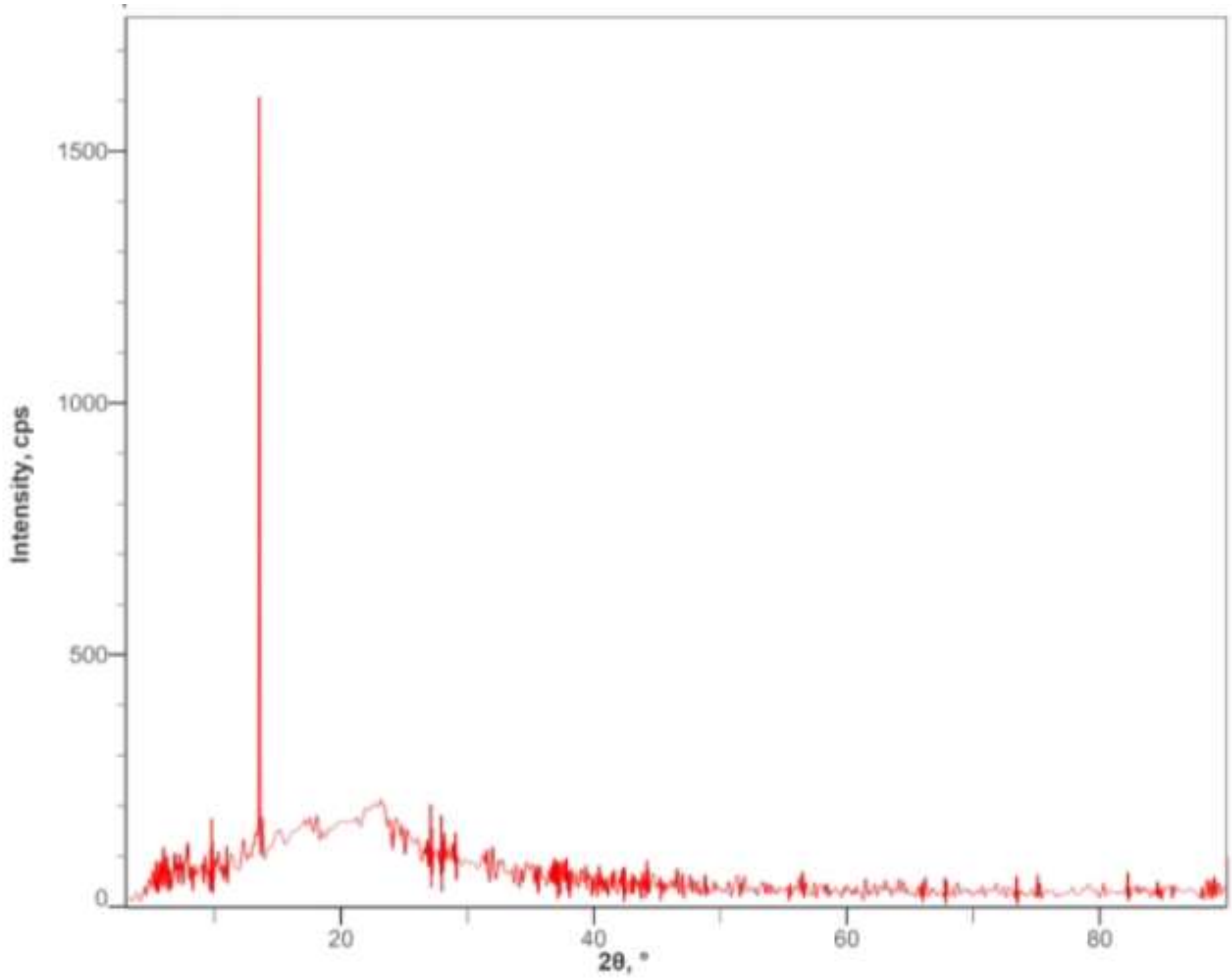


Fig 4. XRD Spectra

CONCLUSION

Aqueous Moringaoleifera leave extract is effective at reducing aqueous methylene blue. silver nanoparticles synthesized from the different type of plant extracts resulted in having great applications in various fields like pharmaceuticals, cosmetics, plastics, textiles, drug transporters in cancer therapy, biosensors for metabolites and pollutants, water purification and food packaging.

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