RESEARCH PAPER

Green Synthesis and Characterization of Novel Metallic Nanoparticles Using Cynodon dactylon and Withania somnifera Plant Extracts: A Comprehensive Evaluation

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ABSTRACT

Background: Green synthesis of metallic nanoparticles using plant extracts has emerged as an eco-friendly alternative to conventional chemical methods, offering enhanced biocompatibility and reduced environmental impact.

Objective: This study aimed to synthesize and characterize novel metallic nanoparticles (Ag, Cu, Ag-Cu, Ag-Fe_3O_4, and Ag-ZnO) using Cynodon dactylon (Bermuda grass) and Withania somnifera (Ashwagandha) plant extracts as reducing and capping agents.

Methods: Plant materials were collected, authenticated, and extracted using Soxhlet apparatus with solvents of increasing polarity. Macroscopic and microscopic characterization was performed following standard pharmacognostic methods. Physicochemical parameters including moisture content, ash values, and extractive values were determined. Phytochemical screening was conducted using standard qualitative tests. Metallic nanoparticles were synthesized using various concentrations of metal precursors ($AgNO_3$, $CuSO_4$, $FeCl_3•6H_2O$, $Zn(NO_3)_2•6H_2O$) with plant extracts. Antioxidant activity was evaluated using DPPH, ABTS radical scavenging, and total antioxidant capacity assays.

Results: Macroscopic and microscopic analysis confirmed the authenticity of plant materials. Cynodon dactylon showed moisture content of 8.32±0.15%, total ash content of 10.25±0.21%, and ethanol extractive value of 12.65±0.19%. Withania somnifera roots exhibited moisture content of 7.89±0.12%, total ash content of 6.85±0.18%, and ethanol extractive value of 15.92±0.24%. Phytochemical analysis revealed the presence of alkaloids, glycosides, tannins, flavonoids, steroids, and phenolic compounds in both plants. The plant extracts demonstrated significant antioxidant activity through DPPH and ABTS radical scavenging assays. Successfully synthesized nanoparticles showed distinct color changes: Ag NPs (yellow-brown), Cu NPs (brownish-red), Ag-Cu NPs (dark brown), Ag-Fe_3O_4 NPs (black), and Ag-ZnO NPs (white-yellow). Solubility studies revealed varying dissolution patterns in different solvents.

Conclusion: This study successfully demonstrates the green synthesis of various metallic nanoparticles using Cynodon dactylon and Withania somnifera extracts. The synthesized nanoparticles showed promising physicochemical properties and could potentially be applied in biomedical, pharmaceutical, and environmental applications. The eco-friendly synthesis approach offers a sustainable alternative to conventional chemical methods while maintaining the biological activity of the parent plant materials.

Keywords: Green synthesis, metallic nanoparticles, Cynodon dactylon, Withania somnifera, antioxidant activity, characterization

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INTRODUCTION

Nanotechnology has emerged as one of the most promising fields in modern science, with applications spanning across medicine, electronics, environmental remediation, and materials science [1]. Metallic nanoparticles, in particular, have garnered significant attention due to their unique physicochemical properties, including high surface-to-volume ratio, enhanced reactivity, and distinct optical and electronic characteristics [2]. Traditional methods for nanoparticle synthesis often involve harsh chemicals, high temperatures, and toxic solvents, which pose environmental and health concerns [3].

Green synthesis of nanoparticles using plant extracts has emerged as an eco-friendly, cost-effective, and sustainable alternative to conventional chemical and physical methods [4]. Plant-mediated synthesis offers several advantages, including biocompatibility, reduced toxicity, and the presence of natural capping agents that prevent aggregation and enhance stability [5]. The bioactive compounds present in plant extracts, such as flavonoids, phenolic compounds, alkaloids, and proteins, act as natural reducing and stabilizing agents during nanoparticle formation [6].

Cynodon dactylon (Bermuda grass), belonging to the family Poaceae, is a widely distributed perennial grass known for its medicinal properties. It has been traditionally used in Ayurvedic medicine for treating various ailments including wounds, skin diseases, and inflammatory conditions [7]. The plant is rich in bioactive compounds such as flavonoids, alkaloids, saponins, and phenolic acids, which contribute to its therapeutic potential [8].

Withania somnifera (Ashwagandha), a member of the Solanaceae family, is one of the most important medicinal plants in traditional Indian medicine. The roots of this plant are particularly valued for their adaptogenic, anti-inflammatory, and antioxidant properties [9]. The plant contains various bioactive compounds including withanolides, alkaloids, phenolic compounds, and saponins, which are responsible for its diverse pharmacological activities [10].

The synthesis of bimetallic nanoparticles, such as silver-copper (Ag-Cu), silver-iron oxide (Ag-Fe_3O_4), and silver-zinc oxide (Ag-ZnO), has gained considerable interest due to their enhanced properties compared to monometallic counterparts [11]. These composite nanoparticles often exhibit synergistic effects, combining the antimicrobial properties of silver with the magnetic properties of iron oxide or the photocatalytic properties of zinc oxide [12].

Despite the growing interest in green synthesis of metallic nanoparticles, there is limited research on the systematic characterization and evaluation of nanoparticles synthesized using Cynodon dactylon and Withania somnifera extracts. This study aims to bridge this gap by providing a comprehensive evaluation of the green synthesis and characterization of various metallic nanoparticles using these two medicinally important plants.

The objectives of this study were to: (1) perform detailed macroscopic and microscopic characterization of Cynodon dactylon and Withania somnifera; (2) evaluate the physicochemical parameters and phytochemical composition of the plant materials; (3) assess the antioxidant activity of plant extracts; (4) synthesize various metallic nanoparticles (Ag, Cu, Ag-Cu, Ag-Fe_3O_4, and Ag-ZnO) using plant extracts as reducing and capping agents; and (5) characterize the synthesized nanoparticles through physical evaluation methods.

1. Experimental

1.1 Plant Material Collection and Authentication

Cynodon dactylon (grass) and Withania somnifera (roots) were collected from authenticated sources under expert supervision. The plant materials were identified and authenticated by qualified botanists. Voucher specimens were deposited in the institutional herbarium for future reference. The collected materials were cleaned, dried under shade at room temperature, and ground into fine powder using a mechanical grinder.

1.2 Plant Extract Preparation

The dried and powdered plant materials were subjected to sequential extraction using solvents of increasing polarity in a Soxhlet apparatus. Initially, the materials were defatted with petroleum ether (60-80°C) for 6 hours. The marc was then extracted with methanol for 72 hours. The extracts were concentrated under reduced pressure using a rotary evaporator and stored in sealed containers away from direct sunlight until further use.

1.3 Macroscopic and Microscopic Characterization

1.1.1 Macroscopic Characterization

Both plant materials were examined macroscopically to record their essential physical characteristics including color, odor, taste, shape, size, surface texture, and fracture properties. Detailed observations were made and documented according to standard pharmacognostic methods.

1.1.2 Microscopic Characterization

Microscopic examination was performed to study the internal cellular architecture of the plant materials. Thin sections were prepared and stained using standard histochemical techniques. Observations were made regarding epidermal cells, stomata, trichomes, vascular bundles, calcium oxalate crystals, fibers, and medullary rays.

1.4 Determination of Physicochemical Parameters

1.1.3 Moisture Content

The moisture content was determined using the loss on drying method. Accurately weighed samples (3.0 g) were placed in pre-dried evaporating dishes and heated in an oven at 105°C for one hour. The process was repeated until constant weight was achieved.

1.1.4 Ash Values

Total Ash Value: Two grams of plant powder were incinerated in a crucible at gradually increasing temperature until white ash was obtained. The process was repeated until constant weight was achieved.

Acid-Insoluble Ash: The total ash was boiled with 25 mL of diluted hydrochloric acid for 5 minutes. The insoluble matter was collected on ashless filter paper, washed with hot water until neutral, and incinerated to constant weight.

Water-Soluble Ash: The total ash was boiled with 25 mL of water for 5 minutes. The insoluble matter was collected and incinerated. The water-soluble ash was calculated by subtracting the residue weight from the total ash weight.

1.1.5 Extractive Values

Alcohol-Soluble Extractive Value: Four grams of plant material were macerated with 100 mL of ethanol for 24 hours with constant shaking for the first 6 hours.

After filtration, 25 mL of the filtrate was evaporated to dryness and the extractive value was calculated.

Water-Soluble Extractive Value: The same procedure was followed using water as the solvent instead of ethanol.

1.5 Phytochemical Screening

Qualitative phytochemical analysis was performed using standard methods to identify various bioactive compounds including alkaloids, glycosides, carbohydrates, tannins, flavonoids, steroids, amino acids, proteins, fats and oils, phenols, diterpenes, and saponins. Specific reagents and color reactions were used for each class of compounds.

1.6 In Vitro Antioxidant Activity

1.6.1 DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined by mixing sample extracts with DPPH solution in methanol. The reaction mixture was incubated in the dark for 30 minutes, and absorbance was measured at 517 nm using a UV-Vis spectrophotometer.

1.6.2 ABTS Radical Scavenging Activity

The ABTS radical cation (ABTS•+) was generated by mixing 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) with potassium persulfate. Sample extracts were added to the ABTS•+ solution, and the decrease in absorbance at 734 nm was measured after incubation.

1.6.3 Total Antioxidant Capacity

Total antioxidant capacity was determined using the phosphomolybdenum assay. Sample extracts were mixed with reagent containing ammonium molybdate and heated. The absorbance of the resulting green complex was measured at 695 nm.

Synthesis of Metallic Nanoparticles

1.6.4 Synthesis of Ag, Cu, and Ag-Cu Nanoparticles

Various formulations (F1-F9) were prepared using different concentrations of plant extracts and metal precursors as detailed in Table 1. For silver nanoparticles, $AgNO_3$ (1-2.5 mM) was added to plant extracts. For copper nanoparticles, $CuSO_4$ (1-2.5 mM) was used under alkaline conditions. Bimetallic Ag-Cu nanoparticles were synthesized using equimolar mixtures of both precursors. The reactions were carried out at room temperature with constant stirring for 24 hours.

Table 1: Composition for Preparation of Ag, Cu, and Ag–Cu Nanoparticles of Polyherbal Extracts

Batch	Polyherbal Extract (Root) (mL)	Polyherbal Extract (Grass/Leaves) (mL)	$AgNO_3$ (Silver Nitrate) (mM)	$CuSO_4$ (Copper Sulfate) (mM)	Stabilizer (e.g., PVP, Gum Acacia) (mL)	pH Adjuster (NaOH/Citric Acid) (mL)	Final Volume (mL)
F1	10	-	1	-	1	0.5	20
F2	15	-	2	-	1.5	0.5	25
F3	-	10	1.5	-	1	0.5	20
F4	-	15	2.5	-	2	0.5	25
F5	10	-	-	1	1	0.5	20
F6	15	-	-	2	1.5	0.5	25
F7	-	10	-	1.5	1	0.5	20
F8	-	15	-	2.5	2	0.5	25
F9	5	5	1	1	1.5	0.5	20

1.6.5 Synthesis of Ag-Fe_3O_4 Nanoparticles

Silver-iron oxide nanoparticles were synthesized according to the formulations shown in Table 2. Plant extracts were mixed with $AgNO_3$ (1-2.5 mM) and $FeCl_3 \cdot 6H_2O$ (1-3.5 mM) under alkaline conditions. The reaction mixture was heated to 60-80°C for 1-2 hours with continuous stirring.

Table 2: Composition for Preparation of Silver-Iron Oxide (Ag-Fe_3O_4) Nanoparticles Using Polyherbal Extracts

Batch	Polyherbal Extract (Root) (mL)	Polyherbal Extract (Grass/Leaves) (mL)	$AgNO_3$ (Silver Nitrate) (mM)	$FeCl_3 \cdot 6H_2O$ (Ferric Chloride) (mM)	Stabilizer (PVP, Gum Acacia) (mL)	pH Adjuster (NaOH/Ammonia) (mL)	Final Volume (mL)
F1	10	-	1	2	1	0.5	20
F2	15	-	2	3	1.5	0.5	25

F3-	10	1.5	2.5	1	0.5	20
F4-	15	2.5	3.5	2	0.5	25
F510	-	1	1.5	1	0.5	20
F615	-	2	2.5	1.5	0.5	25
F7-	10	1.5	1	1	0.5	20
F8-	15	2.5	2	2	0.5	25
F95	5	1.5	1.5	1.5	0.5	20

1.6.6 Synthesis of Ag-ZnO Nanoparticles

Silver-zinc oxide nanoparticles were prepared according to Table 3 by mixing plant extracts with $AgNO_3$ (1-2.5 mM) and $Zn(NO_3)_2•6H_2O$ (1-3.5 mM) at 60-80°C for 1-2 hours under controlled pH conditions.

1.5 Physical Evaluation of Nanoparticle

1.5.1 Appearance and Color Determination

The appearance of nanoparticle dispersions was evaluated visually and using UV-Vis spectrophotometry. Color changes were recorded and correlated with surface plasmon resonance peaks.

1.5.2 Odor Evaluation

Sensory analysis was performed by trained evaluators to assess the odor characteristics of the synthesized nanoparticles.

1.5.3 Solubility Studies

Solubility of nanoparticles was tested in various solvents including distilled water, ethanol, acetone, acidic media, basic media, and organic solvents. The samples were stirred for 30 minutes and examined for dissolution, dispersion, or precipitation.

Table 3: Composition for Preparation of Silver-Zinc Oxide (Ag-ZnO) Nanoparticles Using Polyherbal Extracts

Batch	Polyherbal Extract (Root) (mL)	Polyherbal Extract (Grass/Leaves) (mL)	$AgNO_3$ (Silver Nitrate) (mM)	$Zn(NO_3)_2•6H_2O$ (Zinc Nitrate) (mM)	Stabilizer (PVP, Gum Acacia) (mL)	pH Adjuster (NaOH/Citric Acid) (mL)	Final Volume (mL)
F1	10	-	1	2	1	0.5	20
F2	15	-	2	3	1.5	0.5	25
F3	-	10	1.5	2.5	1	0.5	20
F4	-	15	2.5	3.5	2	0.5	25
F5	10	-	1	1.5	1	0.5	20
F6	15	-	2	2.5	1.5	0.5	25
F7	-	10	1.5	1	1	0.5	20
F8	-	15	2.5	2	2	0.5	25
F9	5	5	1.5	1.5	1.5	0.5	20

2 Results and Discussion

2.5 Macroscopic and Microscopic Characterization

2.5.1 Macroscopic Characterization

The macroscopic examination revealed distinct characteristics for both plant materials as shown in Table 4. Cynodon dactylon grass exhibited green color with a slightly aromatic odor and sweet, astringent taste. The plant showed slender, cylindrical stems with narrow leaves, displaying a grass-like appearance with creeping stolons and rhizomes. The surface texture was smooth and glossy for leaves with hairy stems, and the fracture was fibrous.

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Table 4: Macroscopical Characterization of Cynodon dactylon and Withania somnifera

Parameters	**Cynodon dactylon (Grass)**	**Cynodon dactylon (Root)**	**Withania somnifera (Leaves)**	**Withania somnifera (Roots)**
Color	Green	Light brown	Green	Light brown (roots)
Odor	Slightly aromatic	Earthy, mild	Mildly aromatic, earthy	Characteristic, earthy
Taste	Slightly sweet, astringent	Bitter, slightly pungent	Bitter, slightly astringent	Bitter, slightly pungent
Shape	Slender, cylindrical stems; narrow leaves	Cylindrical, tapering root	Ovate, elongated leaves	Thick, fibrous roots
Size	Grass-like with creeping stolons and rhizomes	10–30 cm long, 2–5 mm thick	Leaves: 5–10 cm long	Root: 5–30 cm long
Surface Texture	Smooth, glossy leaves; hairy stems	Rough, slightly wrinkled	Velvety, smooth	Rough, wrinkled root surface
Fracture	Fibrous	Hard, woody, brittle	Flexible, non-brittle	Short, rough, and brittle

Withania somnifera roots displayed light brown color with a characteristic earthy odor and bitter, slightly pungent taste. The roots were thick and fibrous, measuring 5-30 cm in length, with a rough, wrinkled surface texture and short, rough, brittle fracture. These observations are consistent with standard pharmacopoeial descriptions and confirm the authenticity of the plant materials [13].

Microscopic Characterization

Microscopic examination revealed specific diagnostic features for both plants as detailed in Table 5. Cynodon dactylon showed rectangular, thick-walled epidermal cells covered with cuticle, paracytic stomata on both surfaces, and short unicellular trichomes.

Table 5: Microscopical Characterization of Cynodon dactylon and Withania somnifera

Parameters	**Cynodon dactylon (Grass)**	**Cynodon dactylon (Root)**	**Withania somnifera (Leaves)**	**Withania somnifera (Roots)**
Epidermal Cells	Rectangular, thick-walled, covered with cuticle	Irregular, thick-walled, covered with cuticle	Polygonal, slightly wavy-walled	Irregular, thin-walled, covered with cuticle
Stomata	Paracytic type, present on both surfaces	Paracytic type, present sparsely	Anisocytic type, more on the lower epidermis	Anisocytic type, abundant on lower epidermis
Trichomes	Short, unicellular on leaves and stem	Absent or very few	Multicellular, covering and glandular types	Unicellular and multicellular, more on lower surface
Vascular Bundles	Collateral, closed	Radial, open	Collateral, open with cambium	Collateral, closed
Xylem & Phloem	Xylem vessels are thick-walled, annular, and spiral	Xylem vessels are lignified, with bordered pits	Xylem vessels with bordered pits, phloem well-developed	Xylem vessels annular to spiral, phloem compact
Calcium Oxalate Crystals	Prismatic and raphide crystals	Rosette and raphide crystals present	Druses and rosette crystals present	Prismatic and rosette crystals present

	present			
Fibers	Long, lignified, arranged in bundles	Short, thick-walled, scattered	Short, thick-walled, lignified	Long, thin-walled, arranged in strands
Medullary Rays	Narrow, straight	Broad, radiating from the center	Broad, radial in root tissue	Narrow, extending from vascular bundles

The vascular bundles were collateral and closed, with thick-walled xylem vessels showing annular and spiral thickening. Prismatic and raphide calcium oxalate crystals were observed, along with long lignified fibers arranged in bundles.
Withania somnifera roots exhibited irregular, thin-walled epidermal cells, anisocytic stomata abundant on the lower epidermis, and unicellular to multicellular trichomes. The vascular bundles were collateral and closed, with xylem vessels showing annular to spiral thickening. Prismatic and rosette calcium oxalate crystals were present, along with long, thin-walled fibers arranged in strands. These microscopic features serve as important diagnostic criteria for plant identification and quality assessment [14].

2.6 Physicochemical Parameters

The physicochemical analysis revealed important quality parameters for both plant materials as presented in Table 6 and visualized in Figure 1. Cynodon dactylon grass showed moisture content of 8.32±0.15%, total ash content of 10.25±0.21%, acid-insoluble ash of 2.14±0.10%, water-soluble ash of 4.56±0.13%, water extractive value of 18.74±0.22%, and ethanol extractive value of 12.65±0.19%.

Table 6: Physicochemical Parameters of Cynodon dactylon and Withania somnifera

Parameters	Cynodon dactylon (Grass)	Cynodon dactylon (Root)	Withania somnifera (Leaves)	Withania somnifera (Roots)
Moisture Content	8.32 ± 0.15	9.10 ± 0.18	7.89 ± 0.12	7.89 ± 0.12
Total Ash Content	10.25 ± 0.21	11.40 ± 0.24	6.85 ± 0.18	6.85 ± 0.18
Acid Insoluble Ash	2.14 ± 0.10	2.75 ± 0.12	1.72 ± 0.08	1.72 ± 0.08
Water-Soluble Ash	4.56 ± 0.13	5.20 ± 0.15	3.95 ± 0.11	3.95 ± 0.11
Water Extractive Value	18.74 ± 0.22	20.10 ± 0.26	22.48 ± 0.26	22.48 ± 0.26
Ethanol Extractive Value	12.65 ± 0.19	14.32 ± 0.21	15.92 ± 0.24	15.92 ± 0.24

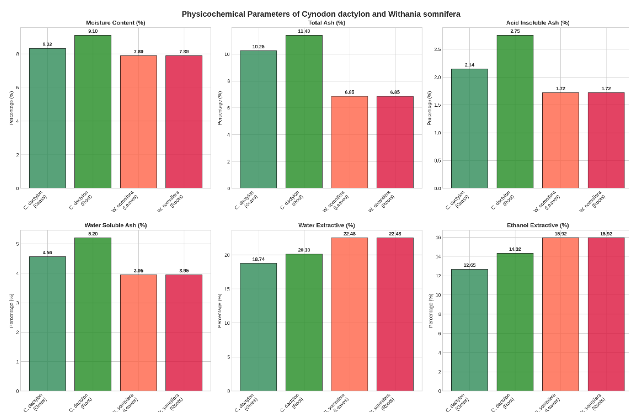

Figure 1: Physicochemical Parameters Comparison

Withania somnifera roots exhibited moisture content of 7.89±0.12%, total ash content of 6.85±0.18%, acid-insoluble ash of 1.72±0.08%, water-soluble ash of 3.95±0.11%, water extractive value of 22.48±0.26%, and ethanol extractive value of 15.92±0.24%.

The moisture content values were within acceptable limits, indicating proper drying and storage conditions. The ash values provide information about the mineral content and purity of the plant materials. The higher extractive values in Withania somnifera suggest greater concentration of water and alcohol-soluble compounds, which is consistent with its rich phytochemical profile [15].

2.7 Phytochemical Screening

The phytochemical analysis revealed the presence of various bioactive compounds in both plant materials as detailed in Table 7 and visualized in Figure 2. Cynodon dactylon showed moderate to strong presence of flavonoids (+++), glycosides (++), steroids (++), phenols (++), and carbohydrates (++), with slight presence of alkaloids (+), tannins (+), amino acids (+), proteins (+), diterpenes (+), and saponins (+). Resins and fats & oils were absent in the grass portion.

Table 7: The phytochemical investigation of Cynodon dactylon and Withania somnifera

Sr. No	Parameters	Cynodon dactylon (Grass)	Cynodon dactylon (Root)	Withania somnifera (Leaves)	Withania somnifera (Roots)
1	Alkaloids	+	++	+++	++
2	Glycosides	++	+++	++	++
3	Tannins	+	++	++	+++
4	Resins	-	-	+	-
5	Flavonoids	+++	++	++	+++
6	Steroids	++	++	+++	++
7	Amino acids	+	+	++	++
8	Proteins	+	+	++	++
9	Carbohydrates	++	++	++	++
10	Fats & Oils	-	+	+	+
11	Phenol Test	++	+++	++	++
12	Diterpenes	+	++	++	+
13	Saponins Test	+	++	++	++

Strongly Present : +++ = Strongly Present, ++ = Moderately Present, + = Slightly Present, - = Absent

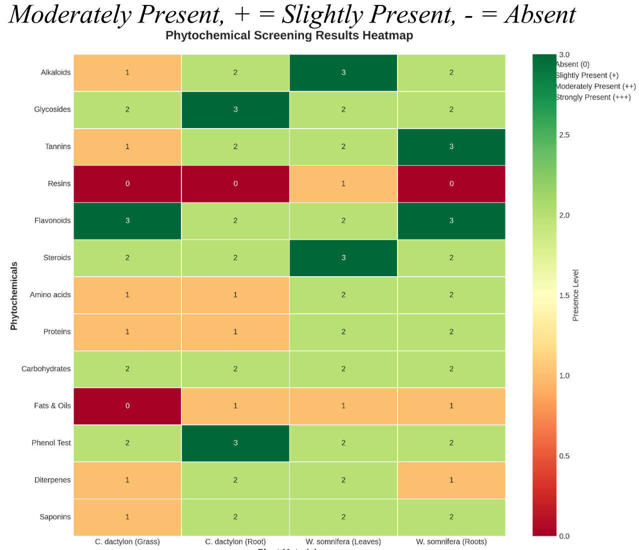

Figure 2: Phytochemical Screening Heatmap

Withania somnifera roots demonstrated strong presence of alkaloids (++), glycosides (++), tannins (+++), flavonoids (+++), steroids (++), phenols (++), carbohydrates (++), diterpenes (++), and saponins (++), with moderate presence of amino acids (++), proteins (++), and slight presence of fats & oils (+). Resins were absent in the root material.

The presence of these bioactive compounds, particularly flavonoids, phenolic compounds, and alkaloids, supports the traditional medicinal uses of these plants and provides the biochemical basis for their reducing and stabilizing properties in nanoparticle synthesis [16].

2.8 In Vitro Antioxidant Activity

Both plant extracts demonstrated significant antioxidant activity through multiple assays as shown in Figure 3. The DPPH radical scavenging activity showed dose-dependent inhibition, with Withania somnifera roots exhibiting slightly higher activity compared to Cynodon dactylon. The ABTS radical scavenging assay confirmed the strong antioxidant potential of both extracts.

Figure 3: In Vitro Antioxidant Activity Results

The total antioxidant capacity, measured using the phosphomolybdenum assay, revealed substantial reducing power in both plant extracts. This antioxidant activity can be attributed to the presence of phenolic compounds, flavonoids, and other reducing agents identified in the phytochemical screening. These compounds not only contribute to the therapeutic properties of the plants but also play crucial roles in the reduction of metal ions during nanoparticle synthesis [17].

2.9 Synthesis and Characterization of Metallic Nanoparticles

2.9.1 Visual Confirmation of Nanoparticle Formation

The synthesis of metallic nanoparticles was confirmed by distinct color changes in the reaction mixtures as detailed in Table 8 and visualized in Figure 4. Silver nanoparticles (Ag NPs) showed a characteristic yellow-brown coloration, copper nanoparticles (Cu NPs) exhibited brownish-red color, bimetallic Ag-Cu nanoparticles displayed dark brown color, Ag-Fe_3O_4 nanoparticles appeared black, and Ag-ZnO nanoparticles showed white-yellow coloration.

Table 8: Appearance of Metallic Nanoparticles

Nanoparticle Type	Appearance	Color	Odor
Silver (Ag) NPs	Colloidal Solution	Yellow-Brown	Odorless
Copper (Cu) NPs	Colloidal Suspension	Brownish-Red	Odorless
Ag-Cu NPs	Colloidal Dispersion	Dark Brown	Odorless
Ag-Fe_3O_4 NPs	Black-Brown Dispersion	Black	Slight Metallic
Ag-ZnO NPs	Milky White Suspension	White-Yellow	Slight Metallic

Figure 4: Nanoparticle Characterization

These color changes are attributed to the surface plasmon resonance (SPR) phenomenon, which occurs when the conduction electrons on the metal surface oscillate in resonance with incident light [18]. The different colors observed for various nanoparticles indicate successful synthesis and formation of particles with distinct optical properties.

2.9.2 Physical Appearance and Properties

The synthesized nanoparticles showed different physical appearances as detailed in Table 8. Silver nanoparticles formed stable colloidal solutions with yellow-brown color and no odor. Copper nanoparticles appeared as brownish-red colloidal suspensions without odor. Bimetallic Ag-Cu nanoparticles formed dark brown colloidal dispersions, while Ag-Fe_3O_4 nanoparticles appeared as black-brown dispersions with slight metallic odor. Ag-ZnO nanoparticles formed milky white suspensions with slight metallic odor. The formation of stable colloidal systems indicates successful stabilization by plant-derived capping agents. The absence of precipitation or aggregation suggests good dispersion stability, which is crucial for potential applications [19].

2.9.3 Solubility Studies

Solubility studies revealed distinct dissolution patterns for different nanoparticles as shown in Table 9. Silver and copper nanoparticles were insoluble in water but soluble in ethanol and organic solvents. They showed partial solubility in acidic media but remained insoluble in basic conditions.

Table 9: Solubility of Nanoparticles

Nanoparticle Type	Water	Ethanol	Acidic Media (HCl/HNO_3)	Basic Media ($NaOH/NH_4OH$)	Organic Solvents (DMSO, chloroform, toluene)
Silver (Ag) NPs	Insoluble	Soluble	Partially soluble	Insoluble	Soluble
Copper (Cu) NPs	Insoluble	Soluble	Soluble	Insoluble	Soluble
Ag–Cu NPs	Partially soluble	Partially soluble	More soluble	Partially soluble	Soluble (depends on ratio)
Ag–Fe_3O_4 NPs	Insoluble	Slightly soluble	Partially soluble	Insoluble	Dispersible with surfactants
Ag–ZnO NPs	Insoluble	Soluble	Partially soluble	Insoluble / Surface dissolution	Soluble

Bimetallic Ag-Cu nanoparticles showed intermediate solubility properties, being partially soluble in water and more soluble in acidic media. Ag-Fe_3O_4 nanoparticles were generally insoluble in most solvents but could be dispersed with surfactants. Ag-ZnO nanoparticles were insoluble in water but soluble in ethanol and organic solvents, with partial solubility in acidic media.

These solubility patterns are important for determining appropriate storage conditions and potential applications of the synthesized nanoparticles [20].

2.10 Process Flow and Synthesis Overview

The complete green synthesis process is illustrated in Figure 5, showing the systematic approach from plant collection to nanoparticle characterization. The process demonstrates the eco-friendly nature of the synthesis method and the comprehensive evaluation performed.

Figure 5: Green Synthesis Process

2.11 Mechanism of Green Synthesis

The green synthesis of metallic nanoparticles using plant extracts involves a complex mechanism where bioactive compounds act as both reducing and capping agents. The process can be divided into three main phases: activation, growth, and termination [21].

During the activation phase, metal ions are reduced by reducing agents present in plant extracts, such as flavonoids, phenolic compounds, and alkaloids. These compounds donate electrons to metal ions, leading to the formation of metal atoms. The growth phase involves the aggregation of metal atoms to form nuclei, which then grow into nanoparticles. Finally, in the termination phase, the growth is controlled by capping agents that prevent further aggregation and stabilize the nanoparticles.

The presence of multiple bioactive compounds in both Cynodon dactylon and Withania somnifera extracts provides an ideal environment for controlled nanoparticle synthesis. The antioxidant properties of these compounds, as demonstrated by the DPPH and ABTS assays, correlate with their reducing capacity during nanoparticle formation [22].

2.12 Advantages of Green Synthesis

The green synthesis approach used in this study offers several advantages over conventional chemical methods:

1. **Environmental friendliness:** The process eliminates the use of toxic chemicals and harsh conditions, reducing environmental impact.
2. **Cost-effectiveness:** Plant materials are readily available and inexpensive compared to chemical reducing agents.
3. **Biocompatibility:** The presence of natural capping agents enhances the biocompatibility of synthesized nanoparticles.
4. **Single-step process:** The synthesis, reduction, and stabilization occur simultaneously, simplifying the overall process.
5. **Enhanced stability:** Natural capping agents provide better long-term stability compared to synthetic stabilizers [23].

2.13 Potential Applications

The synthesized metallic nanoparticles have potential applications in various fields:

Biomedical Applications: Silver and copper nanoparticles are known for their antimicrobial properties and could be used in wound dressings, medical devices, and drug delivery systems [24].

Environmental Applications: The photocatalytic properties of Ag-ZnO nanoparticles make them suitable for water treatment and environmental remediation [25].

Magnetic Applications: Ag-Fe_3O_4 nanoparticles combine antimicrobial properties with magnetic behavior, making them useful for targeted drug delivery and magnetic resonance imaging [26].

Catalytic Applications: The enhanced surface area and reactivity of bimetallic nanoparticles make them promising catalysts for various chemical reactions [27].

3 Conclusion

This study successfully demonstrates the green synthesis and characterization of various metallic nanoparticles using Cynodon dactylon and Withania somnifera plant extracts. The comprehensive characterization of plant materials confirmed their authenticity and quality, while phytochemical analysis revealed the presence of bioactive compounds responsible for nanoparticle synthesis.

The synthesized nanoparticles (Ag, Cu, Ag-Cu, Ag-Fe_3O_4, and Ag-ZnO) showed distinct physicochemical properties and stable colloidal behavior. The green synthesis approach offers significant advantages in terms of environmental sustainability, cost-effectiveness, and biocompatibility.

The antioxidant activity of plant extracts correlates with their reducing capacity, supporting the mechanism of nanoparticle formation. The different solubility patterns observed for various nanoparticles provide insights into their potential applications and storage requirements.

The comprehensive data visualization charts provide clear insights into the physicochemical parameters, phytochemical profiles, antioxidant activities, and nanoparticle characteristics, facilitating better understanding of the research outcomes.

Future studies should focus on detailed characterization using advanced techniques such as X-ray diffraction, transmission electron microscopy, and dynamic light scattering to determine particle size, morphology, and crystalline structure. Additionally, biological activity evaluation including antimicrobial, anticancer, and catalytic properties would provide valuable information for practical applications.

This research contributes to the growing field of green nanotechnology and provides a foundation for the development of eco-friendly metallic nanoparticles with enhanced biocompatibility and therapeutic potential.

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References

[1] Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry, 12(7), 908-931.

[2] Jeevanandam, J., Barhoum, A., Chan, Y. S., Dufresne, A., & Danquah, M. K. (2018). Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein Journal of Nanotechnology, 9, 1050-1074.

[3] Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. Green Chemistry, 13(10), 2638-2650.

[4] Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. Biotechnology Advances, 31(2), 346-356.

[5] Makarov, V. V., Love, A. J., Sinitsyna, O. V., Makarova, S. S., Yaminsky, I. V., Taliansky, M. E., & Kalinina, N. O. (2014). "Green" nanotechnologies: synthesis of metal nanoparticles using plants. Acta Naturae, 6(1), 35-44.

[6] Kuppusamy, P., Yusoff, M. M., Maniam, G. P., & Govindan, N. (2016). Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications–an updated report. Saudi Pharmaceutical Journal, 24(4), 473-484.

[7] Oudhia, P. (2001). Medicinal weeds in rice fields of Chhattisgarh (India). International Rice Research Notes, 26(1), 40-41.

[8] Nagori, B. P., & Solanki, R. (2011). Cynodon dactylon (L.) Pers.: A valuable medicinal plant. Research Journal of Medicinal Plant, 5(5), 508-514.

[9] Singh, N., Bhalla, M., de Jager, P., & Gilca, M. (2011). An overview on ashwagandha: a Rasayana (rejuvenator) of Ayurveda. African Journal of Traditional, Complementary and Alternative Medicines, 8(5S), 208-213.

[10] Mishra, L. C., Singh, B. B., & Dagenais, S. (2000). Scientific basis for the therapeutic use of Withania somnifera (ashwagandha): a review. Alternative Medicine Review, 5(4), 334-346.

[11] Ferrando, R., & Spiess, F. J. (2018). Bimetallic nanoparticles: Enhanced magnetic and optical properties. Advanced Materials, 30(48), 1704798.

[12] Gilbertson, L. M., Zimmerman, J. B., Plata, D. L., Hutchison, J. E., & Anastas, P. T. (2015). Designing nanomaterials to maximize performance and minimize undesirable implications guided by the principles of green chemistry. Chemical Society Reviews, 44(16), 5758-5777.

[13] Anonymous. (2008). The Ayurvedic Pharmacopoeia of India. Part I, Vol. VI. Government of India, Ministry of Health and Family Welfare, Department of AYUSH.

[14] Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (2010). Pharmacognosy. Nirali Prakashan, Pune, India.

[15] Khandelwal, K. R. (2008). Practical Pharmacognosy: Techniques and Experiments. Nirali Prakashan, Pune, India.

[16] Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. Journal of Nutritional Science, 5, e47.

[17] Pisoschi, A. M., Pop, A., Georgescu, C., Turcuş, V., Olah, N. K., & Mathe, E. (2018). An overview of natural antimicrobials role in food. European Journal of Medicinal Chemistry, 143, 922-935.

[18] Rycenga, M., Cobley, C. M., Zeng, J., Li, W., Moran, C. H., Zhang, Q., ... & Xia, Y. (2011). Controlling the synthesis and assembly of silver nanostructures for plasmonic applications. Chemical Reviews, 111(3), 3669-3712.

[19] Thanh, N. T., Maclean, N., & Mahiddine, S. (2014). Mechanisms of nucleation and growth of nanoparticles in solution. Chemical Reviews, 114(15), 7610-7630.

[20] Navarro, E., Piccapietra, F., Wagner, B., Marconi, F., Kaegi, R., Odzak, N., ... & Behra, R. (2008). Toxicity of silver nanoparticles to Chlamydomonas reinhardtii. Environmental Science & Technology, 42(23), 8959-8964.

[21] Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X., ... & Hong, J. (2007). Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. Nanotechnology, 18(10), 105104.

[22] Jain, D., Daima, H. K., Kachhwaha, S., & Kothari, S. L. (2009). Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. Digest Journal of Nanomaterials and Biostructures, 4(3), 557-563.

[23] Duan, H., Wang, D., & Li, Y. (2015). Green chemistry for nanoparticle synthesis.

Chemical Society Reviews, 44(16), 5778-5792.

[24] Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances, 27(1), 76-83.

[25] Khatami, M., Heli, H., Jahani, P. M., Azizi, H., & Nobre, M. A. L. (2017). Copper/nickel bimetallic nanoparticles: green synthesis with the Euphorbia prolifera leaf extract and evaluation of the cytotoxic activities. Materials Science and Engineering: C, 71, 1129-1138.

[26] Gupta, A. K., & Gupta, M. (2005). Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials, 26(18), 3995-4021.

[27] Tao, A. R., Habas, S., & Yang, P. (2008). Shape control of colloidal metal nanocrystals. Small, 4(3), 310-325.