RESEARCH PAPER

Green Synthesis and Comprehensive Characterization of Salvadora persica L.-Derived Copper Oxide Nanoparticles

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Abstract

Background: Herbal nanotechnology is an emerging pharmaceutical approach that combines plant-derived bioactive molecules with nanoscale carriers or nanostructures to improve stability, bioavailability, and therapeutic performance.

Salvadora persica L., commonly known as Miswak or toothbrush tree, is a medicinal plant containing salvadorine, benzyl isothiocyanate, alkaloids, flavonoids, tannins, saponins, phenolics, and sulfur-containing compounds that are associated with antimicrobial, antioxidant, anti-inflammatory, antidiabetic, and wound-healing activities.

Objective: The objective of this paper was to prepare and evaluate salvadorine copper nanoparticles by green synthesis using methanolic extract obtained from the leaves and stems of Salvadora persica.

Methods: Fresh leaves and stems were authenticated, shade dried, powdered, extracted by Soxhlet extraction using methanol, and used for the green synthesis of copper oxide nanoparticles through addition to copper sulfate solution, heating at 60 deg C for 1-2 h, pH adjustment to 8-11 using sodium hydroxide, centrifugation, washing, drying, and storage.

Results: Phytochemical screening confirmed alkaloids, flavonoids, tannins, steroids, and glycosides, while physicochemical analysis reported ash value 5%, moisture content 6%, and angle of repose 30 degrees.

FTIR analysis indicated hydroxyl, aliphatic C-H, carbonyl, aromatic C=C, and C-O groups, UV-visible spectroscopy showed a prominent absorption around 391.5 nm, FESEM showed irregular granular and flaky nanoparticles with agglomeration, and zeta potential analysis showed a value of -24.1 mV indicating moderate colloidal stability.

Conclusion: The attached experimental findings support an eco-friendly method for preparing Salvadora persica-mediated copper nanoparticles with functional-group-based phytochemical capping and potential relevance in antimicrobial nanomedicine and herbal drug delivery.

Keywords

Salvadora persica; salvadorine; copper oxide nanoparticles; green synthesis; FTIR; FESEM; zeta potential; herbal nanomedicine.

How to cite this article: Alhat AG, Pathan D, Abdulkhalique SMT, Sawarkar RS, Patel GB, Paravej SS, Chaudhari SR. Green Synthesis and Comprehensive Characterization of Salvadora persica L.-Derived Copper Oxide Nanoparticles. Int J Drug Deliv Technol. 2026;16(76s): 734-743. DOI: 10.25258/ijddt.16.76s.68

Introduction

Herbal medicine has historically been used for the prevention and treatment of several diseases, and recent pharmaceutical research has increasingly explored its integration with nanotechnology [1,2,4].

Nanotechnology can enhance herbal formulations by improving solubility, stability, bioavailability, penetration, controlled release, and therapeutic efficiency [5,7,23].

Plant-mediated nanoparticle synthesis is considered a green synthesis approach because phytochemicals can act simultaneously as reducing, stabilizing, and biologically active agents [7,18-21].

Salvadora persica belongs to the family Salvadoraceae and is widely recognized as Miswak, Peelu, toothbrush tree, or Arak tree [2,4,10].

Different parts of the plant, including leaves, stems, roots, bark, seeds, and twigs, contain phytoconstituents such as salvadorine, benzyl isothiocyanate, flavonoids, tannins, saponins, phenolics, sulfur compounds, and other alkaloid components [4,10,15,17,22].

The traditional and pharmacological importance of Salvadora persica is associated with antibacterial, antifungal, antioxidant, anti-inflammatory, antidiabetic, anti-plaque, and wound-healing actions [1-4,7,10,14].

Conventional herbal formulations may be limited by poor absorption, instability, variable bioavailability, and reduced therapeutic efficiency, which justifies the development of plant-based nanoparticle systems [5,7,23].

Copper oxide nanoparticles have been reported to possess antimicrobial potential through oxidative stress generation, membrane damage, and enhanced surface interactions with microbial cells [19,20].

The present paper therefore reorganizes the attached experimental project into a research-manuscript format focused on preparation, phytochemical evaluation, FTIR characterization, UV-visible analysis, FESEM morphology, and zeta potential assessment of Salvadora persica-mediated copper nanoparticles [23].

2. Literature Review and Scientific Rationale

Arshad et al. reported Salvadora persica-mediated synthesis of silver nanoparticles and showed that plant phytochemicals can support nanoparticle formation while enhancing antimicrobial activity [18].

Reyes-Torres et al. described green synthesis of CuO and ZnO nanoparticles and reported antimicrobial activity, cytotoxic effects, and synergistic interaction with ampicillin [19].

Dadi et al. compared CuO and ZnO nanoparticles against Gram-positive and Gram-negative bacteria and linked antibacterial efficacy with oxidative stress and bacterial membrane disruption [20].

Rizg et al. described a Salvadora persica-mediated bimetallic Fe-ZnO nanoparticle system with activity against multidrug-resistant skin pathogens, supporting the suitability of this plant for nanomaterial synthesis [21].

The available literature indicates that phytoconstituents in Salvadora persica can act as capping and reducing agents and that metal-oxide nanoparticles can exhibit antimicrobial potential through surface-mediated mechanisms [18-21].

The need for the present work arises from the increasing concern of microbial resistance, the adverse effects of some synthetic agents, and the limitations of conventional herbal preparations [7,18-21,23].

By converting the plant extract into a nanoparticle-based system, the formulation may offer improved stability, surface area, penetration, and pharmacological activity compared with crude herbal preparations [7,23].

3. Aim and Objectives

The aim of the study was to prepare and evaluate salvadorine copper nanoparticles by green synthesis using salvadorine-rich extract obtained from leaves and stems of Salvadora persica [23].

The objectives included collection and authentication of plant material, preparation of herbal extract by Soxhlet extraction, formulation of nanoparticles by green synthesis, pharmacognostic evaluation, preliminary phytochemical screening, FTIR analysis, FESEM analysis, UV-visible spectroscopy, and interpretation of pharmaceutical significance [23].

Materials and Methods

Plant material and chemicals

Fresh leaves and stems of Salvadora persica were used as plant material and were authenticated before formulation work [23].

Copper sulfate pentahydrate served as the metal precursor, sodium hydroxide was used as the pH-adjusting agent, methanol was used as the extraction solvent, potassium bromide was used for FTIR pellet preparation, and distilled water and ethanol were used during washing steps [23].

The principal instruments included Soxhlet apparatus, rotary evaporator, mechanical grinder, sieves, magnetic stirrer with hot plate, centrifuge, hot air oven, muffle furnace, FTIR spectrophotometer, UV-visible spectrophotometer, analytical balance, pH meter, silica crucibles, Whatman filter paper, and routine laboratory glassware [23].

Table 1. Drug profile of Salvadora persica compiled from the attached report [23].

Parameter	Details
Botanical name	Salvadora persica
Common names	Miswak, Peelu, toothbrush tree, Arak tree
Active constituents	Salvadorine, benzyl isothiocyanate, alkaloids, flavonoids, tannins, saponins, and sulfur compounds
Mechanistic basis	Membrane interaction, increased permeability, cell-wall and membrane disruption, enzyme inhibition, oxidative stress generation, and biofilm inhibition

Parameter	Details
Therapeutic actions	Antimicrobial, anti-inflammatory, analgesic, antioxidant, astringent, anti-plaque, antidiabetic, and anti-ulcer activities
Solubility/extraction profile	Aqueous extract: water-soluble constituents; ethanolic extract: flavonoids and alkaloids; methanolic extract: polyphenols and antioxidant compounds

Table 2. Excipient and chemical profile used for nanoparticle preparation [23].

Excipient /Chemical	Formula / Molecular weight	Role	Key properties	Grade
Copper sulfate pentahydrate, CAS 7758-99-8	CuSO4.5H2O, 249.68 g/mol	Metal precursor for CuO nanoparticle synthesis	Blue crystalline solid; freely soluble in water; pH about 4	AR grade
Sodium hydroxide, CAS 1310-73-2	NaOH, 39.99 g/mol	pH adjusting agent and CuO precipitation promoter	White hygroscopic pellets; highly water soluble; strongly alkaline	AR grade
Methanol	CH3OH, 32.04 g/mol	Extraction solvent for Soxhlet extraction	Colourless liquid; BP 64.7 deg C; polar protic solvent; miscible with water	AR grade
Potassium bromide	KBr, 119.00 g/mol	FTIR pellet diluent /matrix	White crystalline powder; IR-transparent; hygroscopic; melting point about 734 deg C	Spectroscopic grade
Distilled water	H2O, 18.015 g/mol	Solvent and washing medium	Colourless and odourless; neutral pH; free from ions and organic contaminants	Laboratory grade

Powder preparation and Soxhlet extraction

The collected leaves and stems were segregated, washed with distilled water, shade dried at room temperature for 7-14 days, ground using a mechanical grinder, sieved to obtain uniform powder, and stored in airtight containers [23].

For extraction, about 20-50 g of the powdered plant material was packed into a thimble and extracted with methanol in a Soxhlet apparatus for 6-8 h until the siphon solvent became colourless [23].

The extract was filtered through Whatman filter paper No. 1, concentrated by rotary evaporation or water-bath evaporation, dried, and stored at 4 deg C in airtight containers for further evaluation [23].

Figure 1. Soxhlet apparatus used for extraction of Salvadora persica phytoconstituents [23].

4.3 Green synthesis of copper nanoparticles

The herbal extract was added slowly to an aqueous copper salt solution under continuous stirring to initiate phytochemical-mediated reduction and nanoparticle formation [18-21,23].

The reaction mixture was heated at approximately 60 deg C for 1-2 h while stirring continuously until a colour change from light blue to dark brown or black indicated formation of copper oxide nanoparticles [19,20,23].

The pH of the reaction mixture was adjusted between 8 and 11 using sodium hydroxide to enhance nanoparticle formation and precipitation [23].

The synthesized nanoparticles were collected by centrifugation or filtration, washed repeatedly with distilled water and ethanol, dried in a hot air oven at 60-80 deg C, and stored as fine powder in an airtight container [23].

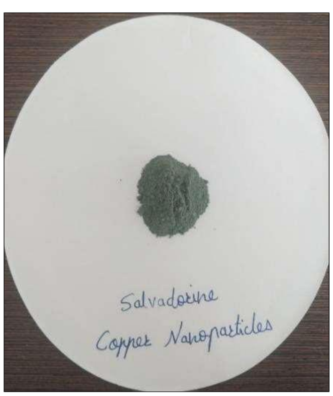

Figure 2. Dried salvadorine copper nanoparticle powder collected after purification .

4.4 Evaluation parameters

Preliminary phytochemical tests were performed using Mayer test, Dragendorff test, ferric chloride test, alkaline reagent test, Salkowski test, Molisch test, and Keller-Killiani test to detect major secondary metabolites [2,4,15,23].

Physicochemical evaluation included ash value, moisture content, and angle of repose to assess quality, purity, and flow behaviour of the plant powder [23].

FTIR spectra of the extract and nanoparticle sample were recorded in the range of 4000-400 cm-1 by the KBr pellet method to identify functional groups [22,23].

UV-visible spectroscopy was performed in the range of 200-800 nm to evaluate the absorption profile of the phytoconstituent-rich extract [23].

FESEM was used to assess morphology and surface features, while zeta potential analysis was used to determine surface charge and colloidal stability of the nanoparticle dispersion [23].

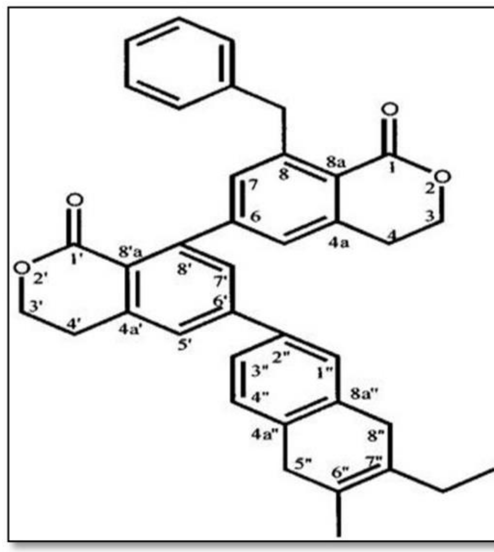

Figure 3. Chemical structure reported as salvadorine in the attached project file [22,23].

Results

Organoleptic and physical characteristics

The dried leaves powder and stem powder showed pale greenish-brown to grayish-green colour, fine dry and slightly coarse texture, uniform granular appearance, mild herbal or earthy odour, slightly bitter pungent and astringent taste, and free-flowing hygroscopic behaviour [23].

The powder surface was non-shiny and matte, while particle size was fine to moderately fine depending on the grinding process [23].

Figure 4. Dried powder samples of Salvadora persica leaves and stem as provided in the attached report [23].

Phytochemical screening

Qualitative biochemical tests showed positive reactions for alkaloids, flavonoids, tannins, steroids, and glycosides in the Salvadora persica powder or extract [2,4,15,23].

Mayer test produced a pale yellow precipitate, Dragendorff test produced an orange to reddish-brown precipitate, and the alkaline reagent test produced an intense yellow precipitate [23].

Ferric chloride produced a blue-black colour, Salkowski test produced reddish-brown coloration, and Keller-Killiani test produced a brown ring with a bluish-green upper layer [23].

Table 3. Qualitative phytochemical screening results [23].

Test	Observation
Alkaloids: Mayer test	Pale yellow precipitate formed
Alkaloids: Dragendorff test	Orange/reddish brown precipitate formed
Flavonoids: alkaline reagent test	Intense yellow precipitate appeared
Tannins: ferric chloride test	Blue-black colour observed
Steroids: Salkowski test	Reddish-brown coloration indicated steroids
Glycosides: Keller-Killiani test	Brown ring at the junction with bluish-green upper layer

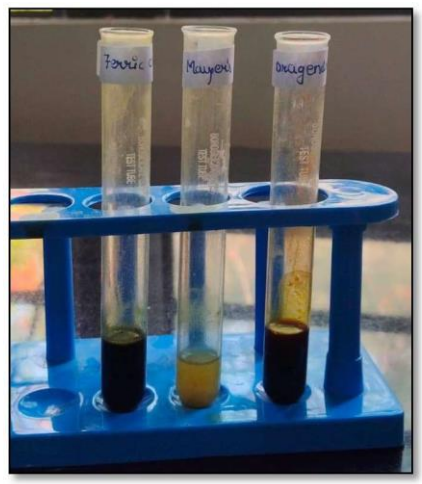

Figure 5. Phytochemical tests showing ferric chloride, Mayer, and Dragendorff reactions [23].

Physicochemical analysis

The ash value of the powder was 5%, the moisture content was 6%, and the angle of repose was 30 degrees [23].

These values were interpreted in the attached report as being within acceptable pharmacognostic limits and supportive of purity, low contamination, acceptable moisture level, and good flow properties [23].

Table 4. Physicochemical analysis of powdered plant material [23].

Paramet er	Ash value	Moisture content	Angle of repose
Definitio n	Indicates inorganic mineral	Expressed as percentage	Depen ds on particl

Parameter	Ash value	Moisture content	Angle of repose
	content and purity of powdered drug.	of total sample weight.	e size, moisture content, and grinding method.
Result	5%	6%	30 degrees
Interpretation	Within acceptable pharmacognostic limits, indicating purity and low contamination.	Within acceptable limits for pharmaceutical processing.	Indicates good flow properties of the powdered material.

FTIR analysis of the extract

The FTIR spectrum of the Salvadora persica extract showed characteristic absorption peaks corresponding to bioactive phytoconstituents and functional groups [22,23].

A broad band around 3550-3200 cm-1 indicated hydroxyl groups associated with phenolic compounds and flavonoids.

Peaks between 2977 and 2853 cm-1 were attributed to aliphatic C-H stretching vibrations, indicating alkane-containing compounds.

A peak near 1691 cm-1 suggested carbonyl C=O groups, while the region around 1618 cm-1 indicated aromatic C=C stretching vibrations.

Peaks in the 1408-1022 cm-1 region were assigned to C-N and C-O stretching vibrations associated with alkaloids, glycosides, alcohols, and ethers.

Lower region peaks between 977 and 536 cm-1 suggested bending vibrations of alkenes and sulfur-containing compounds.

The observed FTIR groups support the role of plant phytochemicals in reduction, capping, and stabilization during nanoparticle synthesis.

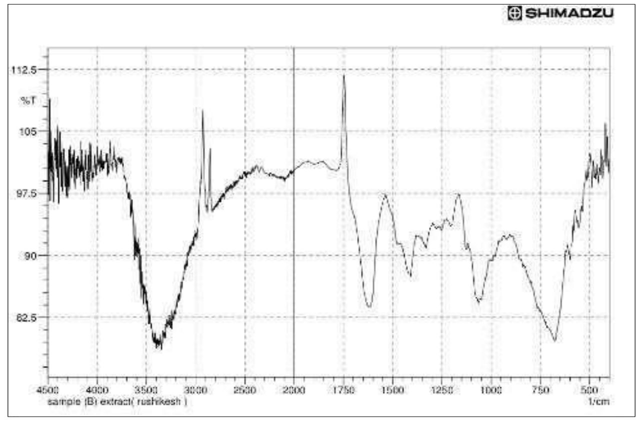

Figure 6. FTIR spectrum of Salvadora persica extract without marked peak table .

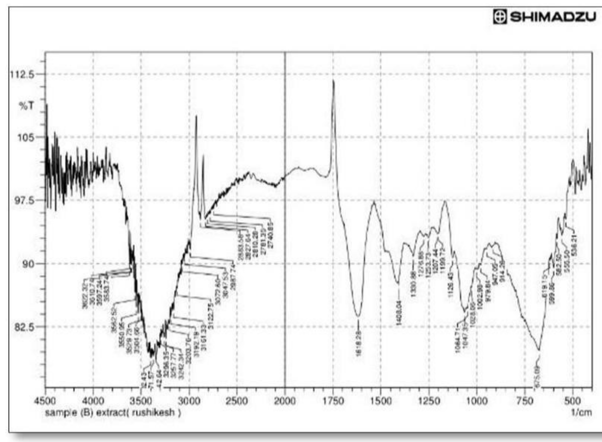

Figure 7. FTIR spectrum of Salvadora persica extract with peak table.

FTIR analysis of the nanoparticle sample

The FTIR spectrum of the sample showed absorption bands corresponding to O-H, C-H, C=O, C=C, and C-O functional groups, confirming the phytochemical and lipidic nature of the sample.

The broad region around 3400-3200 cm-1 indicated O-H stretching vibrations from hydroxyl-containing compounds such as phenolics, alcohols, or flavonoids.

The prominent peaks around 2920 and 2850 cm-1 corresponded to asymmetric and symmetric C-H stretching of aliphatic CH2 and CH3 groups.

The band around 1740-1710 cm-1 was attributed to C=O stretching of ester or carbonyl-containing phytoconstituents.

The 1600-1500 cm-1 region indicated aromatic C=C stretching associated with aromatic phytochemicals including flavonoids and phenolic derivatives.

The 1250-1050 cm-1 region corresponded to C-O stretching of alcohols, ethers, and esters.

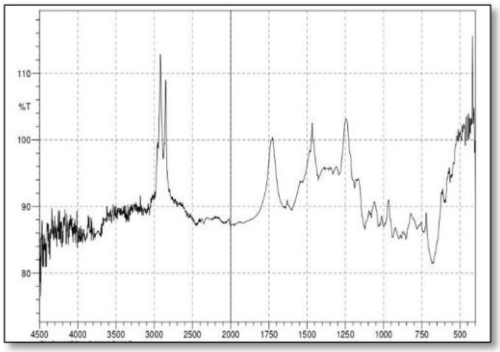

Figure 8. FTIR spectrum of the nanoparticle/sample without peak table .

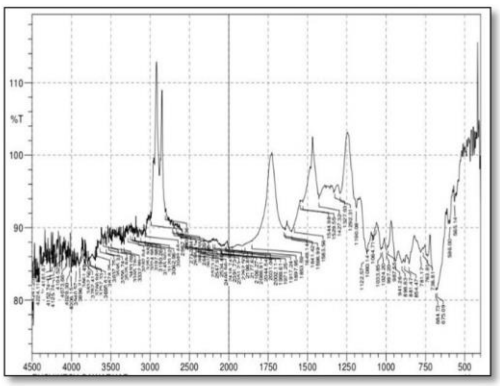

Figure 9. FTIR spectrum of the nanoparticle/sample with peak table [23].

UV-visible spectroscopy

The UV-visible spectrum exhibited strong absorption in the region of approximately 200-400 nm followed by a gradual decrease in absorbance beyond 400 nm [23].

The observed lambda max was reported as 391.5 nm in the attached experimental file [23].

The absorption profile was interpreted as being consistent with alkaloid-containing phytoconstituents such as salvadorine in Salvadora persica extract [22,23].

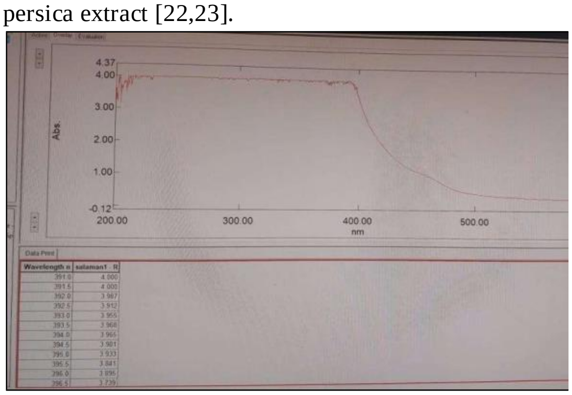

Figure 10. UV-visible spectroscopy graph of the Salvadorine-rich extract showing lambda max 391.5 nm [23].

FESEM morphology

FESEM analysis was performed at 10,000x magnification with a 5 um scale bar to observe the morphology of the nanoparticle powder [23].

The particles appeared irregular rather than perfectly spherical and showed granular or flaky morphology with rough surfaces [23].

Primary particles were estimated to be in the nanometer to submicron range, with smaller particles around 100-500 nm and larger agglomerates extending into the micron range [23].

The agglomeration was attributed to high surface energy and sample preparation or drying effects [23].

The rough and porous appearance suggested increased surface area, which is relevant for surface-mediated biological activity of metal-oxide nanoparticles [19,20,23].

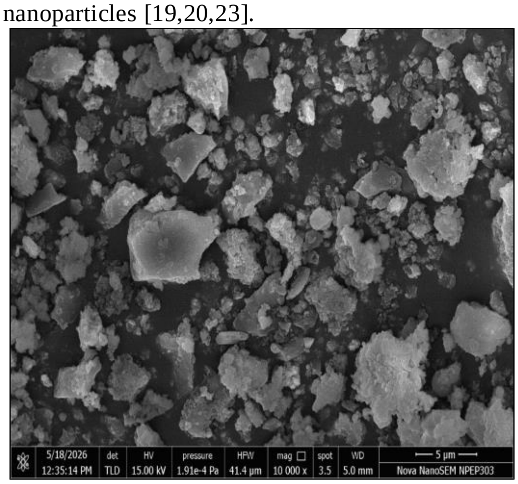

Figure 11. Field emission scanning electron microscopy image of the prepared nanoparticles [23].

Zeta potential and colloidal stability

Zeta potential analysis was used to evaluate the surface charge and colloidal stability of the herbal copper nanoparticle dispersion [23].

The measured zeta potential was -24.1 mV, indicating that the nanoparticle surface carried a negative charge [23].

The negative charge was interpreted as being associated with adsorbed phytochemicals such as phenolics, flavonoids, alkaloids, and tannins from the Salvadora persica extract [2,4,16,23].

Using the stability classification included in the attached report, a value between +/-20 and +/-30 mV indicates moderate colloidal stability [23].

The single sharp peak centered around approximately -24 mV suggested relatively uniform particle distribution with limited aggregation in the measured dispersion [23].

Table 5. General zeta potential stability range used for interpretation [23].

Zeta potential value	Stability
0 to +/-10 mV	Highly unstable

Zeta potential value	Stability
+/-10 to +/-20 mV	Relatively unstable
+/-20 to +/-30 mV	Moderately stable
> +/-30 mV	Highly stable

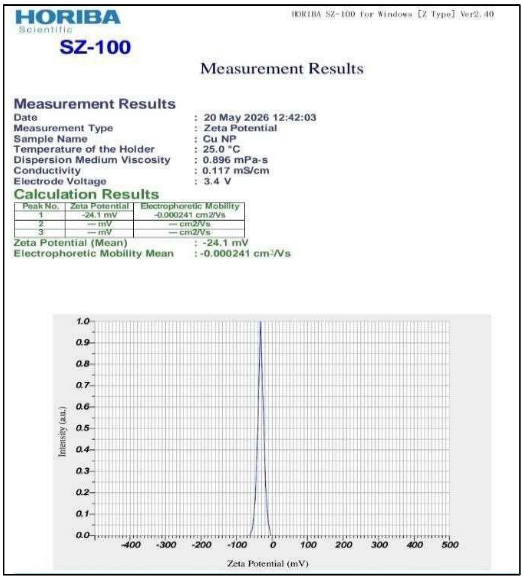

Figure 12. Zeta potential result showing mean value of -24.1 mV [23].

Discussion

The present data indicate that Salvadora persica extract contains multiple phytochemical classes capable of supporting green synthesis of copper nanoparticles.Alkaloids, flavonoids, tannins, glycosides, and phenolic compounds can contribute to reduction and stabilization because they contain electron-rich functional groups capable of interacting with metal ions and nanoparticle surfaces.The colour change during heating and alkaline pH adjustment supported formation of copper oxide nanoparticles through phytochemical-mediated reduction and precipitation. The ash value, moisture content, and angle of repose results suggested that the plant powder was suitable for processing and nanoparticle preparation.

The FTIR results were consistent with the presence of hydroxyl, carbonyl, aromatic, C-O, and C-N groups, which can contribute to both phytochemical activity and nanoparticle stabilization. The UV-visible absorption maximum of 391.5 nm was interpreted in the attached file as supportive of a salvadorine-rich alkaloid profile in the extract.The FESEM image confirmed irregular nanosized and submicron particles with agglomerated clusters, which is common for dried nanoparticles because high surface energy promotes particle-particle association.The zeta potential value of -24.1 mV suggested moderate electrostatic stabilization, meaning the particles possess enough surface charge to reduce but not completely eliminate aggregation. The findings agree with previous reports that plant-mediated metal and metal-oxide nanoparticles can show improved biological relevance because phytochemicals may act as reducing, capping, and bioactive components.

Although the attached report included formulation and characterization data, it did not provide quantitative antimicrobial zone-of-inhibition, MIC, cytotoxicity, or in-vivo activity data for the final copper nanoparticle sample.

Future work should therefore evaluate antimicrobial activity, antioxidant activity, particle-size distribution by DLS, XRD crystallinity, thermal analysis, batch reproducibility, and long-term stability to strengthen pharmaceutical interpretation.

7. Conclusion

Salvadora persica leaves and stems were successfully processed into methanolic extract and used for green synthesis of copper nanoparticles according to the attached experimental protocol. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, glycosides, and steroids, which supports the reducing and capping potential of the extract.

Physicochemical parameters showed ash value 5%, moisture content 6%, and angle of repose 30 degrees, indicating acceptable quality and flow characteristics of the plant powder.

FTIR analysis confirmed the presence of key functional groups including O-H, C-H, C=O, aromatic C=C, C-O, and C-N groups in the extract and sample.

UV-visible spectroscopy showed an absorption maximum at 391.5 nm, which was reported as supportive of the salvadorine-rich phytochemical profile. FESEM demonstrated irregular granular and flaky particles with nanoscale to submicron dimensions and agglomerated clusters. Zeta potential analysis showed -24.1 mV, indicating negative surface charge and moderate colloidal stability. Overall, the findings support a cost-effective and eco-friendly method for preparing Salvadora persica-mediated copper nanoparticles with potential application in antimicrobial herbal nanomedicine and drug-delivery research.

8. Declarations

Data availability: all experimental data, figures, graphs, and tables in this manuscript were taken from the attached project report.

Conflict of interest: no conflict of interest statement was provided in the attached project report.

Funding: no funding information was provided in the attached project report.

Limitation: this manuscript does not add new experimental values beyond the attached file and should be verified by the investigator before journal submission.

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