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"FORMULATION AND EVALUATION OF HERBAL-BASED SILVER NANOPARTICLES FOR ENHANCED ANTIMICROBIAL ACTIVITY"

Venkatesh*[1], Tanuja[2], Pavan B. M.[3], Manasa M. L.[4], Girish K. M.[5], Sanjan N. H.[6], Hanumanthachar Joshi[7], Mahadevaprasad R.[8]

[1]Head of Department, Department of Pharmaceutics, Sarada Vilas College of Pharmacy, Mysuru, Karnataka, India.

[2]Associate Professor, Department of Pharmaceutics, Sarada Vilas College of Pharmacy, Mysuru, Karnataka, India.

[3,4,5,6,8]8th Semester B Pharmacy Students, Sarada Vilas College of Pharmacy, Mysuru, Karnataka, India.

[7] Principal, Sarada Vilas College of Pharmacy, Mysuru, Karnataka, India.

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Corresponding Author: Venkatesh

Address: Head of Department, Department of Pharmaceutics, Sarada Vilas College of Pharmacy, Mysuru, Karnataka, India. **Email Id:** venkateshkulakarni4u@yahoo.co.in,

ABSTRACT

This study addresses the green synthesis and evaluation of silver nanoparticles (AgNPs) using extracts of neem (*Azadirachta indica*), turmeric (*Curcuma longa*), haritaki (*Terminalia chebula*), and bibhitaki (*Terminalia bellirica*) to increase antimicrobial activity. Particle size analysis and SEM studies revealed that the herbal extracts work as both reducing and stabilizing agents in AgNPs production, resulting in nanoparticle sizes ranging from 120 to 180 nm. Antimicrobial investigations using the agar well diffusion method reveal that herbal-based AgNPs had significantly greater inhibition zones against a variety of bacteria and fungi than individual herbal extracts. These findings suggest that integrating traditional herbal medicine with nanotechnology could yield environmentally safe and effective antimicrobial agents.

KEYWORDS: Silver nanoparticles, green synthesis, herbal extracts. Neem (*Azadirachta indica*), turmeric (*Curcuma longa),* haritaki (*Terminalia chebula),* and bibhitaki (*Terminalia bellirica*), Antimicrobial activities, Nanotechnology, Eco-friendly synthesis. Agar well

diffusion, traditional medicine etc.

INTRODUCTION

The formulation and evaluation of herbal-based silver nanoparticles (AgNPs) has sparked substantial attention because to their improved antimicrobial capabilities and environmentally acceptable manufacturing techniques.[1] Plant-derived bioactive chemicals work as reducing and stabilizing agents, enabling the green synthesis of silver nanoparticles with strong antibacterial, antimicrobial, antifungal, and antioxidant activities.[2] Neem (*Azadirachta indica*), turmeric (*Curcuma longa*), haritaki (*Terminalia chebula*), and bibhitaki (*Terminalia bellirica*) are well-known medicinal herbs that contain phytochemicals such as terpenoids, flavonoids, and phenolic compounds, which aid in the biosynthesis of AgNPs and provide additional therapeutic benefits.[3]

Neem: *Azadirachta Indica* (*A. Indica*), also referred to as neem, is a member of the *Meliaceae* family. It is a source of numerous medicinal compounds utilized in traditional medicine. According to Biswas K et al. (2002), A. indica (leaf, bark, and seed) has antiviral, antifungal, and antibacterial properties against a variety of pathogenic microorganisms, including chikungunya, measles, vaccinia, and coxsackie Viruses.[4] Numerous pharmacological effects, including as antioxidant, antimicrobial, antimalarial, antimutagenic, anticarcinogenic, anti-inflammatory, antihyperglycemic, antiulcer, and antidiabetic qualities.[5]

Application of Neem:

1. Skin care.
2. Oral and Dental Health.
3. Blood Purification and Detoxification.
4. Antimicrobial properties.
5. Anti-inflammatory properties
6. Antidiabetic use.
7. Antimalarial and antipyretic.
8. Promotes liver and digestive health by supporting function and preventing damage.
9. Immune Modulation.[6]

Turmeric: The dietary spice turmeric *(Curcuma longa)* is a member of the *zingiberaceae* family. It adds color and flavor to food and has been shown in in vitro and animal studies to

have antioxidant qualities [7]. Turmeric aqueous extracts exhibited antimicrobial and antioxidant properties because of the polyphenolic component curcumin (5%). It is well known that curcumin's antioxidant qualities are due to its phenolic nature. Vitamin C is present in good amounts in fresh root [8]

Application of Turmeric:

1. Anti-inflammatory properties.
2. Antioxidant properties.
3. Wound Healing and Skin Care.
4. Digestive Health.
5. Antimicrobial and anti-parasitic.
6. Anticancer Potential
7. Antidiabetic Effects
8. Respiratory Disorders.
9. Immune System Support.[9]

Haritaki: Haritaki contains a high concentration of tannins, flavonoids, anthraquinones, and glycosides, all of which contribute to its pharmacological activity. Traditionally, it has been used to treat digestive problems, respiratory issues, wound healing, skin conditions, and as an immunomodulator. Modern pharmacological investigations confirm its antimicrobial, antibacterial, antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, and neuroprotective properties.[10]

Application of Haritaki:

1. Digestive Health.
2. Detoxification and Rejuvenation.
3. Skin and Wound Healing.
4. Respiratory Health.
5. Antimicrobial & Antiviral.
6. Eye Health
7. Oral and dental care
8. Metabolic and Systemic Benefits
9. Anti-inflammatory and pain relief.
10. Cognitive & Nervous System Benefits.[11]

Bibhitaki: Bibhitaki (*Terminalia bellirica*), also called Baheda in Hindi and Vibhitaka in Sanskrit, is a medicinal plant from the *Combretaceae* family. The title Bibhitaki means "fearless of disease," referring to its ancient use in improving health and preventing illness. It has also been noted for its revitalizing and cleansing qualities [12]. Modern pharmacological research supports its diverse biological actions, which include antioxidant, antibacterial, antimicrobial, anti-inflammatory, hypoglycemic, hepatoprotective, and immunomodulatory properties. Because of its high therapeutic potential, Bibhitaki is a popular ingredient in herbal formulations and natural medicine research.[13]

Application of Bibhitaki

1. Promotes respiratory health.
2. Digestive Health.
3. Antimicrobial and antiviral.
4. Liver & Metabolic Support.
5. Eye Health.
6. Skin & Hair Care.
7. Anti-inflammatory and analgesic.
8. Immune System Booster.[14]

MATERIALS AND METHODOLOGY

Materials

- Herbal drug - Bibhitaki fruit
  Haritaki fruit
  Turmeric rhizome
  Neem leaves
- Apparatus - Soxhlet apparatus
- Solvent - Ethanol
- Formulation of - Silver Nitrite ($AgNo_3$)
  Nano-particle
- Filter paper - Whatman No. 1 filter paper

METHODOLOGY

- **Preparation of plant powder:** We Gathered and thoroughly washed the Bibhitaki, Haritaki, Turmeric, and Neem leaves with water. For seven to ten days, let them dry in

the shade rather than the sun. Using a grinder or mortar and pestle, crush the dried ingredients into a fine powder. The powders should be kept in airtight containers.

- **Extraction:** 20–30 g of each plant powder should be weighed, either separately or in the appropriate ratio. Fill a thimble with the powder, then put it inside the Soxhlet extractor. The round-bottom flask should contain 250–500 mL of solvent (such as 70% ethanol or methanol).

  Complete the Soxhlet setup by connecting the solvent flask, extractor, and condenser. Allow the extraction process to continue for 6–8 hours, or until the solvent in the syphon tube is almost colourless, after setting up the setup slowly (e.g., 60–80°C depending on the solvent boiling point). Let the extract chill.

- **Filtration and concentration:** Filter the extract with Whatman No. 1 filter paper. To concentrate the filtrate, slowly evaporate it over a water bath or use a rotary evaporator. The crude extract should be stored at 4°C to preserve it for future use.

- **Formulation of Nano Particle:** Preparation of Silver Nitrate Solution: Prepared 1 mM aqueous $AgNO_3$ solution freshly before use—dissolve the corresponding amount (0.017 g in 100 ml distilled water).

- **Synthesis of silver nanoparticles**

  Mixing & Incubation: In a flask, mix 10 ml of herbal extract dropwise with 50-100 ml of 1 mM $AgNO_3$ solution, stirring continuously. The color change (from pale yellow to brown/dark brown) reflects the creation of AgNPs due to surface plasmon resonance, which typically occurs within 30 minutes to 1 hour. For combination synthesis (e.g., neem + turmeric), combine equal amounts of each herbal extract and proceed as described above.

  Optimization: Incubate at room temperature or at controlled temperature (60–80°C) for 30–60 minutes, protected from direct sunlight to avoid auto-oxidation.

- **Purification of Nanoparticles**

  To pellet AgNPs, centrifuge the reaction mixture at 10,000 rpm for 15-20 minutes. To eliminate unreacted plant metabolites and silver ions, wash the nanoparticle pellet 2-3 times with distilled water (26). Discard supernatant. Dry pellets at 60-100°C for many hours or lyophilize for powdered AgNPs.

Evaluation of nano-particle

1. **Organoleptic evaluation**

   Color: The color of the formulation is checked by visual inspection.

   Oduor: The odor of the formulation is determined by smell.

   Texture: The texture of the formulation was checked by touch sensation.

2. **Particle size analysis:** Particle size analysis is critical for analyzing silver nanoparticles made from neem, turmeric, haritaki, and bibhitaki since it has a direct impact on their physical, chemical, and biological properties, including antimicrobial activity, stability, and toxicity.

3. **SEM (scanning electron microscopy) Analysis**

   SEM (Scanning Electron Microscopy) is a technique that uses a focus stream of electrons to scan a sample's surface, creating highly detailed images that show surface topography and composition. The interaction of the electron beam with atoms at the surface produces signals, such as secondary electrons and backscattered electrons, which are detected and used to create the image. SEM (Scanning Electron Microscopy) examination is critical for analyzing silver nanoparticles generated from neem, turmeric, haritaki, and bibhitaki because it offers precise information about the nanoparticles' morphology, size, and surface properties.

4. **Screening of antimicrobial activity**

   An antimicrobial assay is a scientific method for determining a substance's ability to inhibit or kill microorganisms (such as bacteria, fungus, or yeast). The primary goal of an antimicrobial test is to determine the antimicrobial activity of new and existing medicines by measuring how well they inhibit the growth of specific infections. This is commonly assessed by measuring growth inhibition zones or calculating the lowest concentration required to inhibit (MIC) or kill (MBC) microorganisms. Antimicrobial assays are critical for evaluating silver nanoparticles made from neem, turmeric, haritaki, and bibhitaki because they determine their potential to inhibit or kill diverse microorganisms, verifying their efficacy for medical and infection control purposes.

RESULT AND DISCUSSION

1. Physical Evaluation

- **Color:** The color of four formulations was checked by visual inspection. Black color was obtained.
- **Oduor:** The odor of all four formulations was determined by smell.
- **Texture:** The texture of all four formulations was checked by touch sensation respectively.

Table No. 1: Organoleptic Property.

Parameter	Evaluation
Colour: Neem Turmeric Haritaki Bibhitaki	Dark green to brownish-green Deep yellow to orange-yellow Brown to dark brown Light brown to brownish-green
Odour: Neem Turmeric Haritaki Bibhitaki	Characteristic strong, bitter odour Characteristic aromatic odour Slightly unpleasant Mild, characteristic odour
Texture: Neem Turmeric Haritaki Bibhitaki	Smooth, slightly leathery Hard and rough when dried Hard, wrinkled, and rough when dried Hard, smooth outer surface

2. Particle Size Analysis

The particle size distribution of silver nanoparticles (AgNPs) synthesized using various herbal extracts - Neem (*Azadirachta indica*), Turmeric (*Curcuma longa*), Haritaki (*Terminalia chebula*), and Bibhitaki (*Terminalia bellirica*) - was analyzed using the Dynamic Light Scattering (DLS) method.

The average particle sizes obtained for the respective formulations are presented below:

Table No. 2: Average size of Particle size analysis.

Sample	Average Particle Size (nm)
Neem AgNPs	125.8 nm
Turmeric AgNPs	182.0 nm
Haritaki AgNPs	123.0 nm
Bibhitaki AgNPs	129.0 nm

The results showed that all the synthesized silver nanoparticles were within the nanoscale range (<200 nm), confirming the reduction of silver ions by the bioactive phytochemicals present in the plant extracts. Haritaki-based AgNPs had the smallest average particle size

(123 nm) of the samples, followed by Neem (125.8 nm) and Bibhitaki nanoparticles (129 nm). Turmeric-based AgNPs had a significantly larger particle size (182 nm), which could be attributed to the higher presence of curcuminoids, which influenced particle aggregation and growth during synthesis.

Figure No. 1: Sample for particle Size Analysis.

3. SEM Analysis (Scanning electron microscopy)

SEM analysis of Neem nanoparticles: The SEM micrograph revealed that neem nanoparticles have an irregular and porous surface morphology and tend to form agglomerated clusters. The image shows that the individual particle size is in the nanometer to submicron range, or approximately 100-500 nm, confirming the nanoscale nature of the prepared sample. The rough and porous texture observed on the particle surfaces indicates a large surface area, which may improve the material's adsorption and dissolution properties.

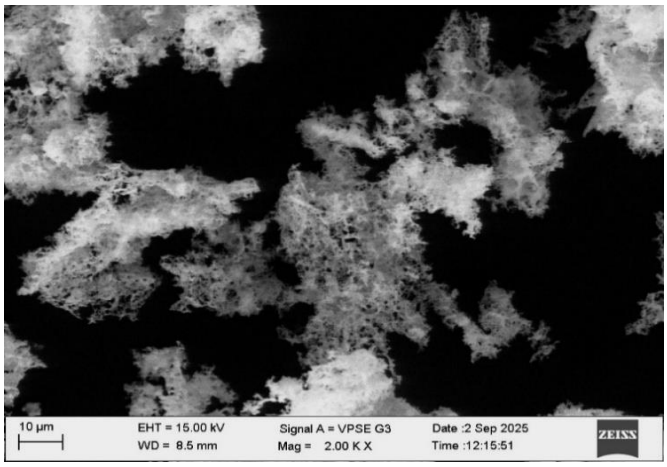

Figure No. 2: Neem.

SEM Analysis of Turmeric Nanoparticles: The obtained SEM micrograph clearly demonstrated the formation of nanosized particles with diameters ranging from 85.5 to 176.4

nm. The particles appeared irregularly shaped and agglomerated, as is typical of phytochemical-based nanoparticles derived from natural sources such as turmeric. The presence of natural curcuminoids and other bioactive compounds in turmeric most likely served as reducing and stabilizing agents, facilitating nanoparticle formation while also causing partial clustering due to van der Waals forces and moisture adsorption.

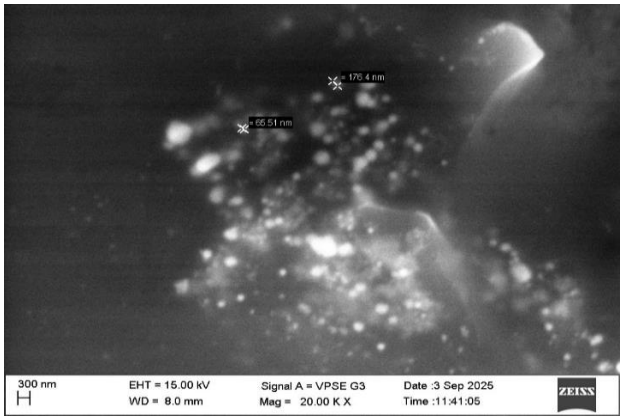

Figure No 3: Turmeric

SEM analysis of Haritaki nanoparticle: The SEM image revealed that the Haritaki-mediated silver nanoparticles were spherical to irregular in shape and exhibited slight aggregation, as expected of biosynthesized nanoparticles due to the presence of natural capping and stabilizing agents derived from the Haritaki extract. The particle size observed in the micrograph ranged between 93.3 nm and 177.0 nm, confirming the successful formation of nanosized particles in the expected nanometer range.

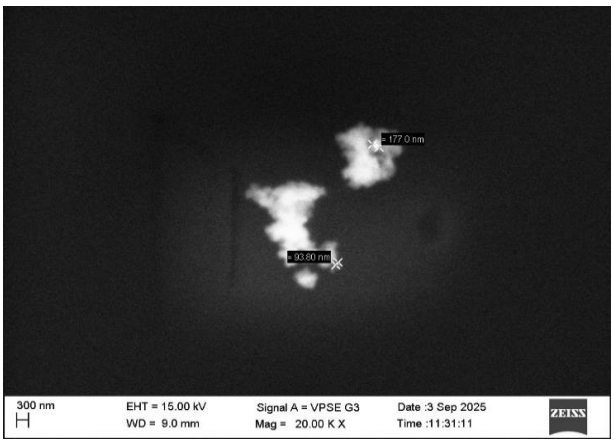

Figure No 4: Haritaki.

SEM analysis of Bibhitaki nanoparticles: The SEM micrograph shows a smooth, layered, and wavy surface morphology with clearly defined folds and continuous sheet-like structures.

The lack of cracks or granular roughness indicates the formation of uniform and compact nanoparticles with high surface integrity. Based on surface appearance and magnification, the nanoparticles are estimated to be in the nanometer range (roughly 100-400 nm). This nanoscale dimension and smooth morphology are advantageous for pharmaceutical applications, as they allow for enhanced surface area, improved solubility, and controlled release of bioactive compounds.

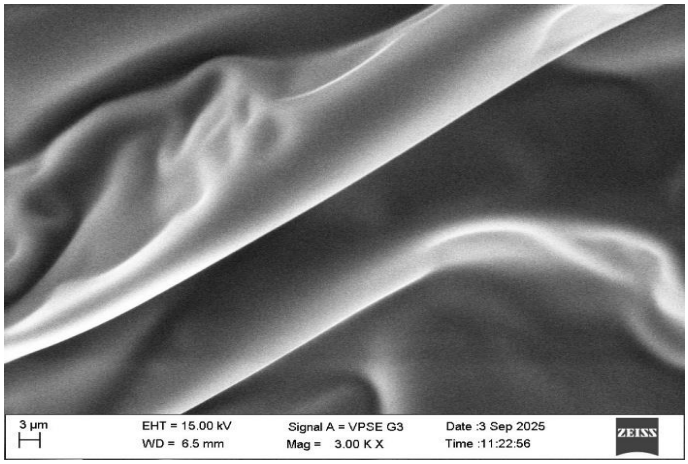

Figure No 5: Bibhitaki

4. Antimicrobial activity using the well diffusion method

The microorganism's inoculum was created using bacterial cultures. In sanitized Petri plates, pour 15ml of nutrient agar (Hi media) medium and let it cool and harden. Using a pipette, uniformly spread 100 µl of bacterial strain broth over the medium with a spreading rod until dry. A sterile cork borer was used to bore wells of 6mm diameter. The compounds (100µl/ml) were produced in DMSO, followed by 100µl of test solutions (1mg/ml) and standard added to the wells. The petri plates were incubated for 24 hours at 370C.Streptomycin (1mg/ml) was used as the positive control and DMSO as the negative control. Antimicrobial activity was assessed by measuring the diameters of the zone of inhibition (ZI) in triplicate.

RESULTS

Table No: 01

Antimicrobial activity of test compound against B. Subtilis

SR.NO	SAMPLES	ZONE IN DIAMETER (mm)
1	Control	00
2	Standard (Streptomycin)	26
3	MH 1	21

Table No 3: Antimicrobial activity of test compound against B. Subtills

Table No: 02

Antimicrobial activity of test compound against E. coli

SR.NO	SAMPLES	ZONE IN DIAMETER (mm)
1	Control	00
2	Standard (Streptomycin)	27
3	MH 1	19

Table No 4: Antimicrobial activity of test compound against E. coli

CONCLUSION

The present study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using aqueous extracts of neem (*Azadirachta indica*), turmeric (*Curcuma longa*), haritaki (*Terminalia chebula*), and bibhitaki. Visual confirmation of nanoparticle formation was provided by a distinct color change from pale yellow to dark brown, indicating that the phytoconstituents present in the extracts reduced silver ions to silver nanoparticles.

The particle size analysis confirmed that all of the synthesized nanoparticles were in the nanoscale range (120-180 nm). Haritaki-based AgNPs had the smallest average particle size (123 nm), followed by Neem (125.8 nm) and Bibhitaki (129 nm), while Turmeric-based AgNPs were relatively larger (182 nm). The smaller particle sizes of the Haritaki and Neem formulations suggested their potential for increased surface reactivity and biological activity, whereas the slightly larger size of Turmeric nanoparticles indicated a tendency to aggregate due to the extract's higher organic content.

Scanning Electron Microscopy (SEM) analysis confirmed the formation of spherical to near-spherical nanoparticles with smooth morphology and minimal aggregation. The observed morphology was consistent with particle size analysis, indicating that the phytochemicals in the extracts worked as natural reducing and stabilizing agents, ensuring nanoparticle uniformity and stability.

The antimicrobial activity of the synthesized AgNPs against Bacillus subtilis (ATCC 19659) and Escherichia coli (ATCC 25922) was determined using the well diffusion method, and all formulations exhibited significant antimicrobial properties. Haritaki-based AgNPs had the largest zone of inhibition, followed by Neem-based AgNPs, both of which were highly effective against both Gram-positive and Gram-negative bacteria. Bibhitaki-based AgNPs also demonstrated moderate antimicrobial activity, indicating a good inhibitory effect, whereas Turmeric-based AgNPs showed noticeable but lower activity, most likely due to

their larger particle size and partial aggregation, which may have reduced the available reactive surface area.

The overall results confirmed that smaller and more uniform nanoparticles had higher antimicrobial efficiency, owing to increased surface area, improved cell membrane interaction, and increased silver ion release.

Finally, the study concluded that herbal-mediated synthesis of silver nanoparticles is a green, cost-effective, and environmentally friendly approach to the development of biologically active nanomaterials. Haritaki- and Neem-mediated AgNPs had the most promising morphological characteristics, nanoscale uniformity, and antimicrobial potency of any formulation, while Bibhitaki and Turmeric formulations also had significant antibacterial effects, demonstrating their potential as natural antimicrobial agents. These findings indicate that herbal silver nanoparticles could be effectively used in pharmaceutical and topical formulations to combat microbial infections.

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