*ejpmr, 2023,10(1), 240-244*

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# SYNTHESIZING AND EVALUATING THE SILVER NANOPARTICLES NEEM EXTRACT INCORPORATED SOAP

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**Article Received on 31/10/2022** **Article Revised on 21/11/2022** **Article Accepted on 11/12/2022**

## ABSTRACT

Improper hand hygiene is the major factor for the various infections and diseases. Washing hands with soap prevents from infections by bacteria, virus and fungus. Azadirachtin in neem possesses anti-microbial activity. The main objective is to bio-synthesize silver nanoparticles and incorporate into a tablet soap which having a consistent property against various Gram-positive and Gram-negative organism. Formation of AgNPs was confirmed by UV, SEM and FTIR analysis. The soap which is eco-friendly, cheap and more effective than common soap, was evaluated for adequate parameters.

**KEYWORDS:** Green synthesis, Silver Nanoparticles, Anti-microbial.

## INTRODUCTION

Improper hand hygiene practice among healthcare providers and public plays a major factor for the origin of various infections like cholera, dysentery, norovirus, airborne illness and nosocomial infections. Hand hygiene practices are important not only in covid situation but also to prevent the spread of other infections. Hand washing with soap has been suggested by WHO, states that approximately 19% of the world population only washes hands with soap after contact with excreta. Also states that handwashing reduces the risk of diseases by 40% by acting against the bacteria and virus. Recently AgNP exhibits a great potential for food storage, cosmetics, biosensor, dental and drug deliver activities. Nanotechnology is the science and technology studied at the nanoscale level at about 1 to 100nm.[1] Nanoscience have been emerging rapidly from past few years ago. Over the past few decades, metal nanoparticles have made an impact in diagnosis and health care practice. Silver nanoparticles have great potential as antimicrobial agents, biomedical devices, drug delivery carriers, anti-inflammatory agents, anti-angiogenic agent and even as anti-cancer.[2]

Current nanotechnology focused on developing silver nanoparticles by various methods like physical, chemical, biological and green synthesis method. But physical, chemical procedures were costly and dangerous.[1] Hence, these methods were substituted by green synthesis method. Green synthesis is one of the promising methods. Nobel metals (Ag, Pt, Au and Pd) are used to make metal nanoparticles.[2] Biosynthesis of nanoparticles using plant extract is the most popular green synthesis of silver nanoparticles.[3] Azadirachtin, quercetin and other secondary metabolites present in neem extract also possess anti-microbial activity.[4] Green synthesized nanoparticles are biocompatible and reduce the environmental toxicity.[5] Bio-synthesized nanoparticles were characterized by UV-Visible, X-ray, SEM and FTIR.[6]

## MATERIALS AND METHODS

Fresh neem leaves, distilled water, $AgNo_3$, deionized water, agar-agar, nutrient broth, ofloxacin, NaOH, coconut oil.

### Preparation of Neem Leaves Extract

20g weighed fresh leaves was taken and washed thrice with distilled water. The leaves were cut into small pieces and extract with distilled water at 70°C. After 20mins the extract was separated and cooled. The extracted sample was subjected to phytochemical screening.[7,8]

### Phytochemical Screening[9]

- **Test for flavonoids**

Alkaline reagent test- To the extract few drops of NaOH and dilute acid added.

- **Test for terpenoids**

Salkowski test- to the extract $CHCl_3$ and conc. $H_2SO_4$ were added.

**Figure 1: Neem leaves extract before the formation of silver nanoparticles.**

**Preparation of Silver Nitrate Solution**

1mM silver nitrate solution was prepared by dissolving 0.0169g of AgNO3 in 100ml of deionized water and stored in an amber coloured bottle. Likewise, 2 to 6mM AgNO3 solution were prepared.[10]

**Green Synthesis of Nanoparticles**

5ml of neem extract was added with 50ml of 1mM AgNO3 solution and incubated for 24 hrs. the solution was further subjected to centrifuge for 30mins at 5000rpm. The sedimented pellets of silver nanoparticles at the bottom were collected and washed thrice with deionized water.[11]

**Preparation of Nanoparticle Soap**

0.9ml of coconut oil was measured and boiled at 60°C in a China dish. Lye solution was prepared by dissolving 4.32g NaOH in 8.4ml water mixed in 60°C. 0.50mg of dried silver nanoparticle was added followed by the addition of colorants and fragrance. Stirred continuously till the saponification reaction was complete. Then the soap solution was poured into tablet moulds.[12]

**Evaluation**

**Anti-Microbial Assay**

Antimicrobial property of silver nanoparticle was performed against Gram positive and Gram-negative bacteria (*Bacillus subtilis, Escherichia coli, Klebsiella nemoniae, Staphylococcus aureus*) by cup plate method. 3.8gm of agar and 2.6gm of nutrient broth was completely dissolved in 200ml of distilled water and sterilized by autoclaving. The agar medium was poured into the sterilized Petri dish and allow to solidify. Bacterial culture was streaked thoroughly. Well of 2mm diameter was bored. Ofloxacin and distilled water was used as standard and blank respectively. Different concentration of silver nanoparticle solution from 1mM to 6mM concentration were evaluated with the standard and the blank.[13] Kept in refrigerator for diffusion and the plates were incubated. After 24 hours, zone of inhibition was measured and compared.

**RESULTS**

The presence of flavonoids and terpenoids were confirmed by phytochemical screening, which responsible for reducing and capping action leads to formation of AgNP from silver nitrate.

**Table 1: Preliminary Test FOR Neem Leaves.**

| S. No | TEST | Constituent | Inference |
|---|---|---|---|
| 1 | Alkaline reagent Test | Flavonoids | Present |
| 2 | Salkowski test | Terpenoids | Present |

**Visual Examination**

Observed the color change from yellowish green to dark brown which confirms the formation of silver nanoparticles.

**Figure 2: Neem leaves extract after the formation of silver nanoparticle (Dark brown).**

**UV-Visible spectra analysis**

0.4 ml,0.6 ml,0.8 ml and 1 ml of nanoparticle solution was diluted to 10ml and observed under 350-500 nm for the presence of AgNP and stability studies were performed after the storage of 48hrs.

**Table 2: UV Spectrophotometry measurement of silver nanoparticles after 24hours of preparation.**

| Standard Solution | λ max(nm) | Absorbance |
|---|---|---|
| 0.4ml | 443 | 0.10046 |
| 0.6ml | 443 | 0.13751 |
| 0.8ml | 443 | 0.20976 |
| 1.0ml | 443 | 0.22829 |

**Table 3: UV Spectrophotometry measurement of silver nanoparticles after 48 hours of preparation.**

| Standard Solution | λ max(nm) | Absorbance |
|---|---|---|
| 0.4ml | 445 | 0.1044 |
| 0.6ml | 445 | 0.1474 |
| 0.8ml | 445 | 0.1873 |
| 1.0ml | 445 | 0.2242 |

**SEM Analysis**

SEM images of soap impregnated with silver nanoparticles exhibited spherically agglomerated particles due to presence of soap.

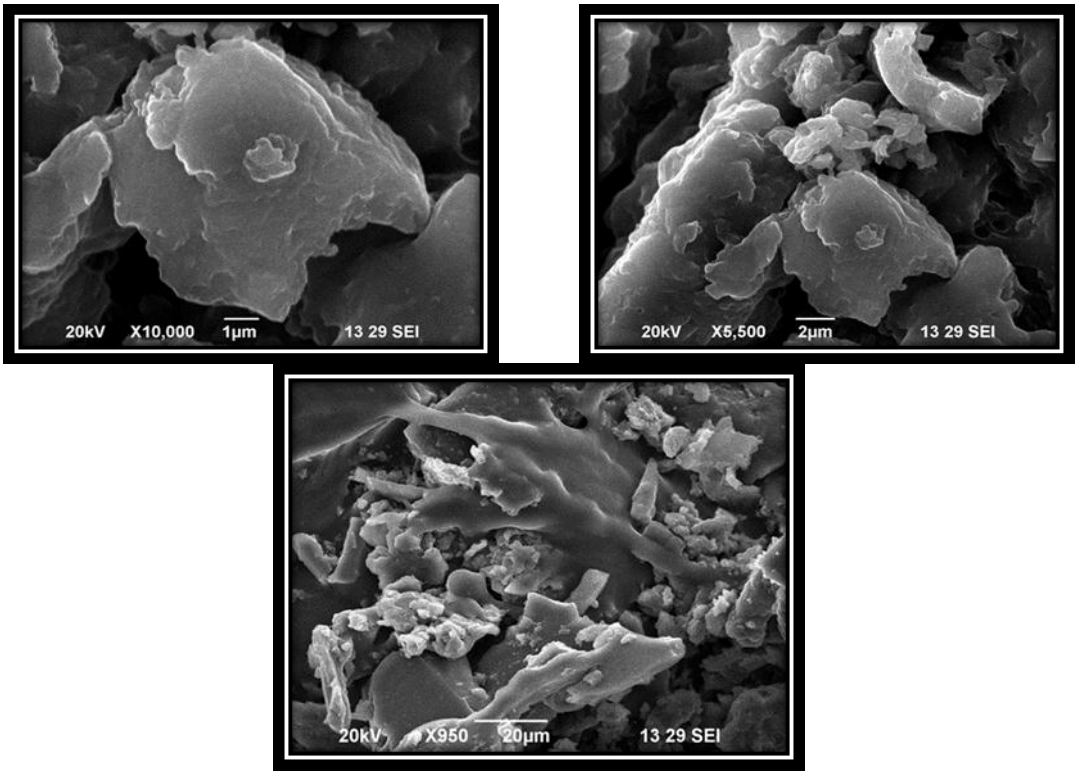


**Figure 3: SEM analysis of silver nanoparticle formulation.**

**FTIR Analysis**

Sample was observed under FT-IR for surface absorption of functional groups in silver nanoparticle.

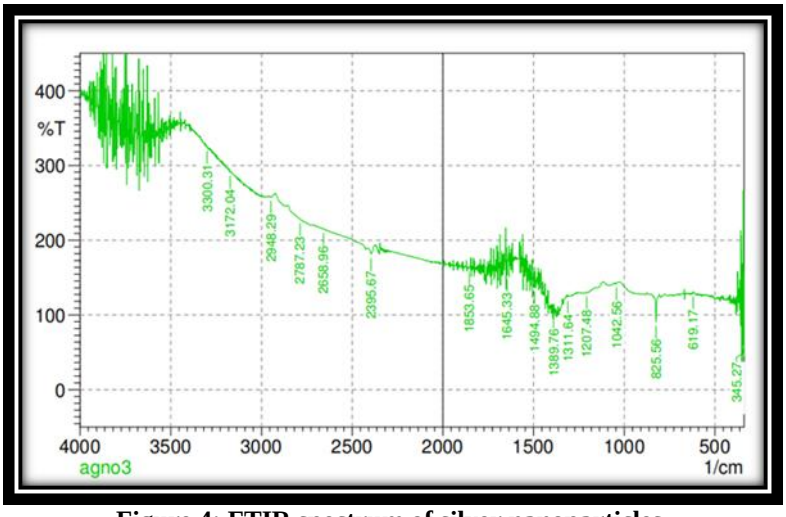


**Figure 4: FTIR spectrum of silver nanoparticles.**

| Materials | Functional group | Type of vibration | Characteristic vibration ($cm^{-1}$) | Test absorption ($cm^{-1}$) |
|---|---|---|---|---|
| Silver nano particles | C-H | stretching | 2850 - 3000 | 2998.29 |
| | $-NH_2$ | stretching | 3200-3500 | 3300.31 |
| | Ag ions | Shoulder peak | - | 1645.33, 1389.76 |

**Antimicrobial Assay**

From prepared 1mM to 6mM nanoparticles solution antimicrobial activity was observed after 24hrs of incubation.

**Table 4: Anti-microbial activity shown the zone of inhibition for the organisms (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*).**

| S. no. | *Bacillus subtilis* | *Staphylococcus aureus* | *Escherichia coli* | *Klebsiella pneumoniae* |
|---|---|---|---|---|
| Ofloxacin | 35mm | 40mm | 42mm | 33mm |
| Blank | No growth | No growth | No growth | No growth |
| 1mM | 17mm | 19mm | 20mm | 11mm |
| 2mM | 19mm | 18mm | 20mm | 14mm |
| 3mM | 23mm | 29mm | 34mm | 27mm |
| 4mM | 20mm | 21mm | 25mm | 22mm |
| 5Mm | 18mm | 18mm | 21mm | 10mm |
| 6Mm | 18mm | 19mm | 24mm | 10mm |

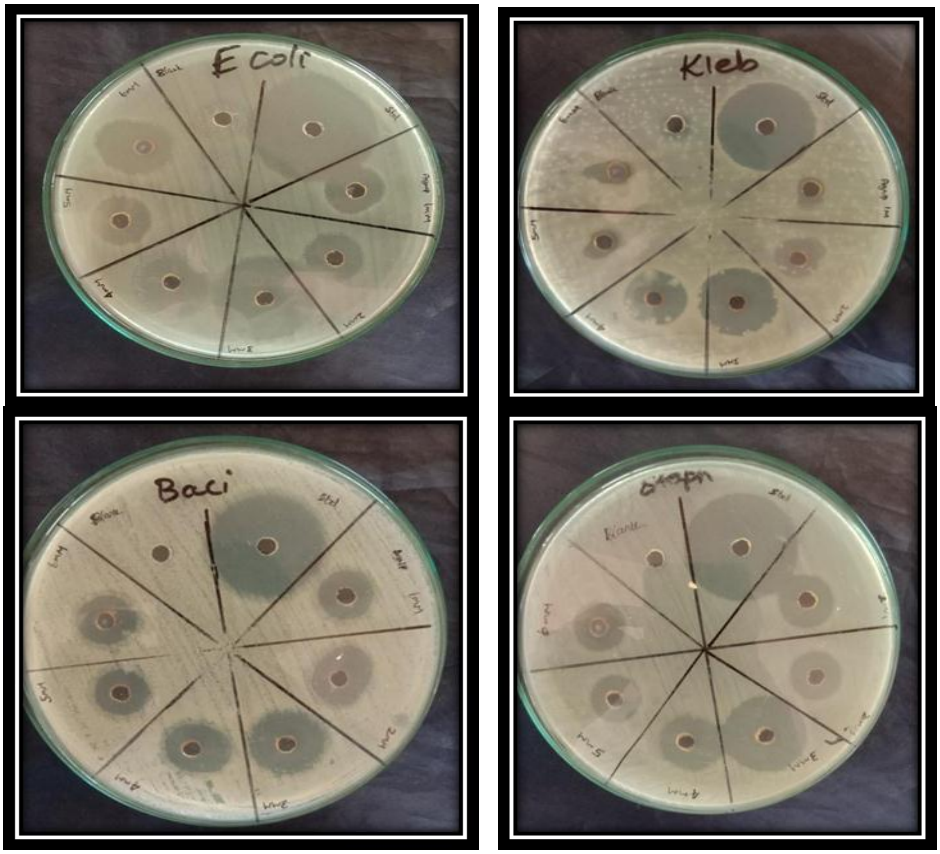


**Figure 5: Zone of inhibition for the organisms (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*).**

**Evaluation of Soap**

The physicochemical parameters of the prepared soap were evaluated for Parameters such as color, odor, appearance and pH was tested.

**Table 5: Evaluation Parameters for Soap.**

| CHEMICAL PARAMETER | SOAP | STANDARDS |
|---|---|---|
| COLOUR | WHITE | - |
| PH | 7.3 | 6.5-8 |
| FOAM HEIGHT | 3cm | 2.5-3 |
| FOAM RETENTION | 2.5cm | 0.5-2.5 |
| TOTALFATTYM ATTER | 77.6 | 70-90 |
| SAPONIFICATION VALUE | 0.184 | - |

## CONCLUSION

It was found that the biosynthesized AgNP with neem leaf extract had potential antimicrobial properties. A biosynthesis of AgNP with neem leaves extract was uncovered in the current study. In UV analysis, AgNP was formed at lambda max 443. Based on SEM analysis, the nanoparticles range in size from 2-10nm. Synthesized green nanoparticles contained 1mM to 6mM. The 3 mM solution was effective against Gram-positive and Gram-negative bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, and *Klebsiella pneumoniae*. 4mM solution performed better than 3mM. In all four strains, the zone of inhibition decreases with increasing concentration. 2mM and 1mM had less activity than 3mM. The activity also decreased at higher concentrations like 5mM and 6mM. E. coli is more susceptible to standard drugs and green synthesized nanoparticles among the four species. Green synthesized nanoparticles and standard drugs are less effective against *Klebsiella pneumoniae. E. coli* Gram negative bacteria have more inhibition zones for standard than prepared nanoparticles. In contrast, *Klebsiella pneumoniae* from clinical isolates has a smaller zone of inhibition. The 3mM nanoparticle solution was effective against *Klebsiella pneumoniae*, followed by *E. Coli, Staphylococcus aureus and Bacillus subtilis*. A nanoparticle solution prepared against Gram positive bacteria was found to be more active than Gram negative bacteria. Among Gram positive bacteria, staphylococcus aureus is more likely to be active. Similarly, Gram-negative *E. coli* showed a larger zone of inhibition than *Klebsiella pneumonia*. Tablet soap containing biosynthesized AgNP was developed for travel friendly and economy.

## CONFLICT OF INTREST

The authors found no conflict of interest.

## ACKNOWLEDGMENT

All the authors sincerely thanks to T.K Ravi sir, principal of SRIPMS college, Dr. Venkatasaamy working at SRIPMS and senior Navineeth.

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