Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Formulation Development and Evaluation of Silver Nanoparticles Containing Plant Extract

Prerana Kishor Mahajan[1], Sujit Appasaheb Jadhav[2], Arshi Hussain[3], Prerna chaturvedi[4], Sumeet Dwivedi[5]

[1]Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda, Maharashtra, India
[2]R. G. Sapkal College of Pharmacy, Anjaneri, Nashik, Maharashtra, India
[3]Department of Chemistry, Gurugram University, Gurugram, Haryana, India
[4]Chameli Devi Institute of Pharmacy, Indore, Madhya Pradesh, India
[5]Acropolis Institute of Pharmaceutical Education & Research, Indore, Madhya Pradesh, India
Email: herbal0914@rediffmail.com

The aim of this research was to create a green synthesis of silver nanoparticles using leaves extract of Leonotis nepetaefolia (L.) R.Br. along with excipents were used to prepare Silver nanoparticles and was evaluated for particle size, zeta potential and % EE. The synthesised AgNPs colloidal solution shown superior antibacterial activity against both Gram-positive and Gram-negative bacterial strains i.e., Klebsiella pneumonia, Bacillus subtilis, E. coli and Streptococcus sp. in testing. The diameters of the inhibition zones of AgNPs at 50 g/ml concentrations against Klebsiella pneumonia, Bacillus subtilis, E. coli and Streptococcus sp. were reported and it was found that the Silver Nanoparticles have significant antibacterial activity.

Keywords: Leonotis nepetaefolia (L.) R.Br., Silver Nanoparticles, Anti-bacterial Activity.

1. Introduction

Silver nanoparticles have a long history of antibacterial activities. Silver nanoparticles are actively involved in antibacterial action against a wide range of harmful bacteria and fungi that cause disease in food and drink. Various biological and green materials have been used to synthesise silver nanoparticles, including gramme positive and gramme negative bacteria such as Klebsiella pneumonia and Bacillus subtilis, Cladosporium cladosporioides, marine algae Padina tetrastromatica and Turbinaria conoides, green waste peels of banana fruits, carbohydrate molecules such as polysaccharide and disaccharide starch, sucrose, and maltose. [1-2]

Leonotis nepetifolia (L.) R.Br. is a species of plant in the genus Leonotis and the family Lamiaceae (mint), It is native to tropical Africa and southern India. Leonotis nepetifolia and wild dagga contains several labdane diterpenes including

Hydroxynepetaefolin, Nepetaefuran, Nepetaefolinol, Nepetaefolin, Leonotinin, Leonotin and Dubiin as well as bispirolabdane diterpenes like Leonepetaefolin A-E. Nepetaefuran and leonotinin isolated from Leonotis nepetaefolia plant material demonstrated anti-inflammatory by suppressing NF-κB activation related to proinflammatory Cytokines. Leonotis nepetifolia is known in Trinidad as shandilay and the leaves are brewed as a tea for fever, coughs, womb prolapse, malaria, and suggested to be beneficial to bone and lung health. The roots of L. nepetifolia are considered to be the botanical sources of granthiparna, an ayurvedic herb. [3-5]. The scanty availability of information on this plant facilitates the study on it.

2. Material and Methods

Plant Material Collection

The leaves of the plant was collected from the Malwa region of Madhya Pradesh in the month of October-2024. The plant sample was authenticated as Leonotis nepetaefolia (L.) R.Br. by Botanist and Voucher specimen No. J/Bot/LNL-011 was assigned and it belongs to family Lamiaceae.

Extraction of Plant Material

The dried leaves of Leonotis nepetaefolia was accomplished in a hot continuous mode utilizing ethanol and water as the solvent. The rotary vacuum evaporator was used for absolute remotion of left over dissolvent after collecting the methanol dissoluble components in the receiver. The finished product was moved to a light-resistant container and hermetically sealed. [6]

Biosynthesis of Silver nanoparticles

$AgNO_3$ powder was dissolved in distilled water to prepare 10 mM $AgNO_3$ stock solution from which a series of 1 mM, 2 mM and 3 Mm $AgNO_3$ solutions were prepared. The AgNO3 solutions were mixed with the ethanolic extract of ACL, AAL, C1, and C2 at a ratio of 1:1 and 1:2 v/v to a volume of 50 mL in a flask. The flask was wrapped with an aluminum foil and was then heated in a water bath at 60°C for 5 hours. Furthermore, the mixture was stored in the refrigerator for the further use. [5-6]

Table 1:Composition of Silver Nanoparticles using Plant Extract

Formulation Code	Extract (mg)[LNL-HAE)	AgNO3 (mM)	Ratio
F1	250	1	1:1
F2	250	2	1:1
F3	250	3	1:1
F4	250	1	1:2
F5	250	2	1:2
F6	250	3	1:2

Evaluation of silver nanoparticles [7-8]

Microscopic observation of prepared silver nanoparticles

An optical microscope (Cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared silver nanoparticle formulation.

Percentage Yield

The prepared silver nanoparticle with a size range of 200-300 nm were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres[3].

$$\% \text{ Yield } = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} x\ 100$$

Entrapment efficiency

The entrapment efficiency of the drug was defined as the ratio of the mass of formulations associated drug to the total mass of drug. Entrapment efficiency was determined by dialysis method. Silver nanoparticle entrapped extract were isolated from the free drug using dialysis method. The above said formulations were filled into dialysis bags and the free drug dialyzed for 24 hr. into 50 ml of buffer pH 1.2. The absorbance of the dialysate was measured against blank buffer pH 1.2 and the absorbance of the corresponding blank was measured under the same condition. The concentration of free flavonoids could be obtained from the absorbance difference based on standard curve.

Surface charge and vesicle size

The particle size and size distribution and surface charge were obtained by Dynamic Light Scattering method (DLS) (SAIF RGPV Bhopal, Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the silver nanoparticles was based on the zeta potential that was estimated according to Helmholtz–Smoluchowsky from electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50 lS/cm. pH, drug content and drug release was determined for optimized formulation using standard procedure.

Antibacterial Activity of Synthesized Silver Nanoparticles [9-10]

The antibacterial activity of synthesized silver nanoparticles was performed by agar well diffusion method against pathogenic bacteria, Klebsiella pneumonia, Bacillus subtilis, E. coli and Streptococcus sp. Fresh overnight culture of each strain was swabbed uniformly onto the individuals' plates containing sterile Luria Bertani agar and 5 wells were made with the diameter of 6 mm. Then 25 μL of purified silver nanoparticles, extract, and silver nitrate solution were poured into each well and commercial antibiotic discs are placed as control and incubate for 24 h at 37^0C. After incubation the different levels of zonation formed around the well and it was measured. This experiment was repeated for three times.

3. Results and Discussion

The synthesized silver nanoparticles containing hydro-alcoholic leaves extracts of Leonotis nepetaefolia (L.) R.Br. was evaluated. The results were presented in table 2. From the results

obtained it was showed that formulation code F3 showed maximum % entrapment efficiency, therefore these formulation were selected to determine the drug content and pH. The pH, drug content and in vitro drug release was given in table 3 & 4. The results of anti-microbial activity were given in table 5.

Table 2: Evaluation of Silver Nanoparticles

Formulation Code	% Yield	% Entrapment efficiency	Average Particle size (nm)	Zeta Potential (mV)
F1	79.58±0.12	80.12	126.25	- 35.5
F2	74.26±0.18	79.20	121.22	-34.39
F3	82.39±0.05	86.20	133.43	-30.11
F4	81.62±0.26	85.18	135.43	-34.78
F5	80.28±0.18	80.28	130.29	-32.02
F6	79.02±0.11	79.39	129.49	-31.19

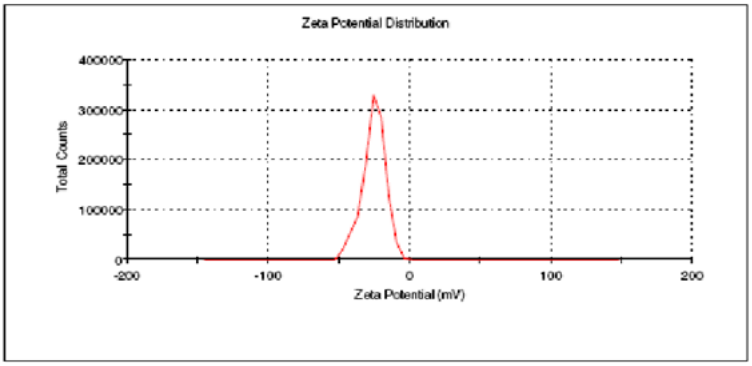

Fig. 1: Zeta Potential of F3

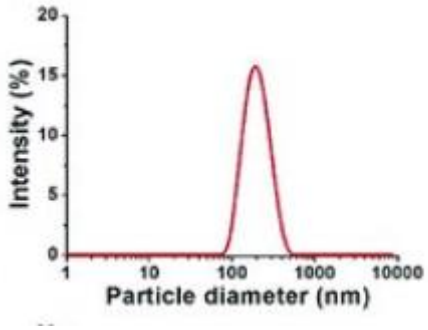

Fig. 2: PDI of F16

Table 3: Evaluation parameters of Silver Nanoparticles (F3)

Formulation Code	pH	Drug content (%)
F3	6.2	89.14

Table 7:In-vitro drug release of Silver Nanoparticles (F3)

Time (mts)	% Drug release
0	0
10	28.10
20	32.29
30	47.21
40	60.10
50	63.28
60	80.26
70	86.13

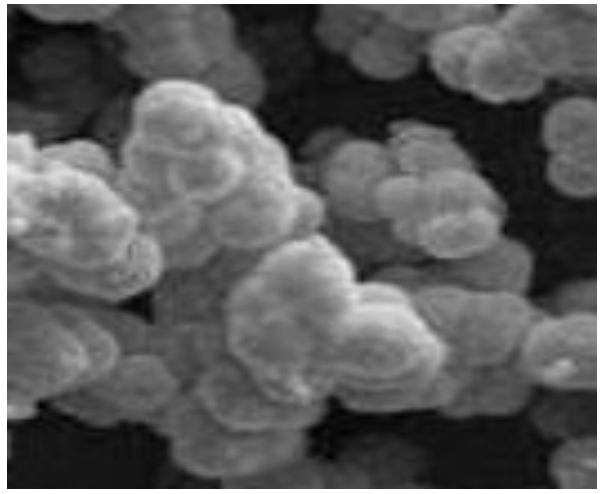

Fig. 3: SEM of F3

Table 8: ZOI of prepared Silver Nanoparticles

Antibacterial agents	Zone inhibition (mm in diameter)			
	B. subtilis	Streptococcus sp.	E. coli	K. pneumonia
Silver nitrate solution	9.9±0.22	12.86±0.17	11.29±0.22	12.18±0.62
Commercial antibiotic disc	10.8±0.16	13.39±0.32	10.18±0.06	12.87±0.03
F3	14.21±0.10	16.11±0.16	14.39±0.18	14.10±0.12

4. Conclusion

According to the findings, silver nanoparticles made from leaves extract and AgNO3 in a 1:3 ratio. Formulation F3 demonstrated superior efficacy in terms of yield and % EE, hence it was chosen as the best formulation. F3 antibacterial activity was further tested using four different bacterial strains, and it was discovered that the generated silver nanoparticles have strong antibacterial activity. Because of its remarkable efficiency as an antibacterial agent, this green synthesised nanoparticle could be exploited in the medical field to treat human ailments.

References

1. El Khoury E, Abiad M, Kassaify Z, Patra D. (2015). Green synthesis of curcumin conjugated nanosilver for the applications in nucleic acid sensing and anti-bacterial activity. Colloids Surf B Biointerfaces 2015; 127: 274-80.
2. Veerasamy, R., Xin, T. Z., Gunasagaran, S., Xiang, T. F. W., Yang, E. F. C., Jeyakumar, N. and Dhanaraj, S. A. (2011). Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. Journal of Saudi Chemical Society. 15(4): 113– 120.
3. Barbosa, Jackson de Menezes; Cavalcante, Noelly Bastos; Delange, David Marrero; Almeida, Jackson Roberto Guedes da Silva (2018). "A review of the chemical composition and biological activity of Leonotis nepetifolia (Linn.) R. Br.(lion's ear)". Revista Cubana de Plantas Medicinales. 23 (4).
4. Ueda, Fumihito; Iizuka, Keito; Tago, Kenji; Narukawa, Yuji; Kiuchi, Fumiyuki; Kasahara, Tadashi; Tamura, Hiroomi; Funakoshi-Tago, Megumi (1 October 2015). "Nepetaefuran and leonotinin isolated from Leonotis nepetaefolia R. Br. potently inhibit the LPS signaling pathway by suppressing the transactivation of NF-κB". International Immunopharmacology. 28 (2): 967–976.
5. Dwivedi, S., Chandekar, A., Tripathi, A., Mishra, R., Chhajed, M., Sharma, K., & Dubey, A. (2024). In-Vivo Anti-diabetic activity of Leonotis nepetaefolia (L.) R. Br. Root in Alloxan induced Diabetic Model. NATURALISTA CAMPANO, 28(1), 2190-2193.
6. Vaidya, A., Gehlot, S., Dwivedi, S., & Shrivastava, S. (2024). Evaluation of Wound Healing Activity of Syzygium cumini root extract in rats. International Journal of Pharmacy & Life Sciences, 15(3).
7. Patel N., Jain U., Yadav Y. and Jain S. (2019). Formulation and Evaluation of Silver Nanoparticles of Methanolic extract of Solanum virginianum L. for Antimicrobial and Antioxidant potential. J. Drug Del. Theu., 9(2-A):20-8.
8. Dwivedi, S., Gandhi, S., Lamale, J. J., Satpute, B. T., Chourasiya, R., & Bijwar, R. S.(2023). Formulation and Evaluation of Antibacterial Silver Nanoparticles containing herbal extract of Leonotis nepetaefolia (L.) R. Br. BioGecko, 12(3): 4640-4643.
9. Vanaja M., Paulkumar K., Gnanajobitha G., Rajeshkumar S., Malarkodi C. and Annadurai G. (2014). Herbal Plant Synthesis of Antibacterial Silver Nanoparticles by Solanum trilobatum and Its Characterization, International Journal of Metals, Article ID 692461, 8.
10. Guzman M, Dille J, Godet S. Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. Nanomedicine: Nanotechnology, biology and medicine. 2012 Jan 1;8(1):37-45.