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Formulation and Evaluation of Antibacterial Silver Nanoparticles containing herbal extract of *Leonotis nepetaefolia* (L.) R.Br.

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Abstract

The green synthesis method of nanomaterial us developing in the field of nanotechnology, it changes the way and duration of toxic chemicals. In the present investigation green synthesis of silver nanoparticles using plant extract of *Leonotis nepetaefolia* (L.) R.Br. was reported. The plant is commonly known as barchibuti belongs to family Lamiaceae and contains Alkaloids, Labdane diterpenes, Flavonoids, Iridoid glycosides.Every part of the plant is medicinally used. The plant is claimed to be used in pain, inflammation, microbial infection, as contraceptives and gynecological disorders. The formulated silver nanoparticles were evaluated to reveal the percentage yield, entrapment efficiency and surface charge. Antibacterial activity of synthesized silver nanoparticles was done by agar well diffusion method against different pathogenic bacteria. The green synthesized silver nanoparticles can be used in the field of medicine, due to their high antibacterial activity.

Keywords: *Leonotis nepetaefolia* (L.) R.Br., Silver Nanoparticles, Formulation

Introduction

Nanoparticles possess high surface area to volume ratio. Nanoparticles such as silver, gold, cadmium sulfide, zinc sulfide, and zinc oxide play important role in various fields. Recently fabrication of silver nanoparticles has drawn considerable attention due to their physical and chemical properties and application in biomedicine, antiangiogenic activity against bovine retinal endothelial cells, anticancer activity against lung carcinoma cells, controlling HIV infection, detection of bacterial pathogens, and good catalytic activity. Silver nanoparticles are having good history in the field of antimicrobial properties. [1] The silver nanoparticles are vigorously involved in the antimicrobial activity against a lot of disease causing food borne and water borne pathogenic bacteria and fungus Synthesis of silver nanoparticles has been proved by various biological and green materials such as bacteria both gram positive and gram negative like *Klebsiella pneumonia* and *Bacillus subtilis*, *Cladosporium cladosporioides*, marine algae *Padina tetrastromatica* and *Turbinariaconoides*, the green waste peels of banana fruits, carbohydrate molecules like polysaccharide and disaccharides starch, sucrose, and maltose, and monosaccharides like glucose and fructose. In the green materials mediated nanoparticles synthesis

plant sources have major role in the past ten years. Many plants are used for synthesizing nanoparticles ncluding *Cinnamomum camphora*, *Azadirachta indica*, *Nelumbo nucifera*, *Garciniamangostana*, pomegranate, and grape fruit extracts. Keeping the above fact in mind the present work was undertaken to formulate and evaluate silver nanoparticles of selected plant extract. [2-3]

Material and Methods

Plant Material Collection

The plant part (Flowers) was collected from the Malwa region of Madhya Pradesh in the month of October-2022. The plant sample was authenticated as *Leonotis nepetaefolia* (L.) R.Br. by Botanist and Voucher specimen No. J/Bot/LNF-039 was assigned and it belongs to family Lamiaceae.

Extraction of *Leonotis nepetaefolia* (L.) R.Br.

The crude drug material was prepared by dehydrating and pulverising *Leonotis nepetaefolia* flowers. The extraction process was accomplished in a hot continuous mode utilizing ethanol and water as the solvent. The rotary vacuum evaporator was used for absolute remotion of leftover dissolvent after collecting the methanol dissoluble components in the receiver. The finished product was moved to a light-resistant container and hermetically sealed.

Formulation Development and evaluation of Silver nanoparticle

Biosynthesis of Silver nanoparticles

$AgNO_3$ powder wasdissolved in distilled water to prepare 10 mM $AgNO_3$ stocksolution from which a series of 1 mM, 2 mM and 3 Mm $AgNO_3$ solutions were prepared. The AgNO3solutions were mixed with the hydroalcoholic extract of *Leonotis nepetaefolia* flowers at a ratio of 1:1 and 1:2 v/v to a volume of 50 mLin a flask. The flask was wrapped with an aluminum foil andwas then heated in a water bath at 60°C for 5 hours. Furthermore, the mixture was stored in the refrigerator for the further use. [4-5]

Optimization of formulation of Silver nanoparticles

Table 1: Different formulation of Silver nanoparticles

Formulation Code	Extract (mg)	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

Characterization of synthesized silver nanoparticles formulations [5-6]

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 200-300nm 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.

Antibacterial Activity of Synthesized Silver Nanoparticles[5-6]

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, leaf 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.

Results and Discussion

The synthesized silver nanoparticles containing herbal extract of *Leonotis nepetaefolia* (L.) R.Br., was characterized. The results were presented in table 12. From the results obtained it was showed that formulation code F4 having maximum entrapment efficiency i.e., 89.21 %. The results of antibacterial acitivy were presented in table 3.

Table 2: Characterization of Silver Nanoparticles

Formulation	% Yield	% Entrapment efficiency	Average Particle size (nm)	Zeta Potential (mV)
F1	58.32±0.01	69.28	179.29	-30.28
F2	62.21±0.12	72.84	181.20	-31.27
F3	65.28±0.10	80.39	183.48	-33.48
F4	78.39±0.02	89.21	186.28	- 35.5
F5	70.39±0.04	84.26	184.29	-32.20
F6	68.25±0.02	79.20	180.47	-30.20

Table 3: ZOI of prepared Silver Nanoparticles

Antibacterial agents	Zone inhibition (mm in diameter)			
	B. subtilis	***Streptococcus* sp.**	***E. coli***	***K. pneumonia***
Silver nitrate solution	9.8±0.24	13.88±0.18	11.43±0.21	12.48±0.02
Silver nanoparticles (F4)	11.8±0.11	14.18±0.11	16.32±0.18	13.23±0.11
Commercial antibiotic disc	10.2±0.14	13.24±0.21	10.11±0.08	12.12±0/15

Conclusion

From the results it was concluded that the Silver Nanoparticles prepared from hydroalcoholic extract of *Leonotis nepetaefolia* flowers having extract and AgNO3 in the ration of 1:2 v/v Formulation Code F4 showed better efficacy in term of yield and % EE, therefore the formulation code F4 was consider as best. Furthermore the antibacterial activity of F4 was determined using four bacterial strains and it was noted that the prepared Silver nanoparticles possess significant antibacterial activity. This green synthesized nanoparticle could be used in the medical field against human diseases due to their high efficiency as antibacterial agent.

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