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Formulation and Evaluation of Pharmacological Investigation of Herbal Gel using Antimicrobial Activity

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Abstract

Herbal medicine is still the main stay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents. The field of nanotechnology is one of the most active areas of research in modern material science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of nanoparticles and nano-materials are emerging rapidly. Silver is a well-known antimicrobial agent against a wide range of over 650 microorganisms from different classes such as gram-negative and gram-positive bacteria, fungi or viruses. More recently the metal is finding use in the form of silver nanoparticles. Silver nanoparticles are one of the metal nanoparticles have received significant consideration because they are effective antimicrobial agent/s that exhibits low toxicity. The aim of present study was to synthesize silver nanoparticles by using various extract, characterization of these silver nanoparticles and Converts its gel formulation as well as to observe the antimicrobial activity.

Keywords: nanoparticles, antimicrobial, silver nanoparticles.

Introduction

Herbal drugs are playing a vital role in health care system. This is because they are being cheap and locally available**.** The activity of herbal medicines depends on overall function of a variety of active components, as all the constituents provide synergistic action and thus enhance the therapeutic valu**.**[1]Herbal medicines are now in great demand in the

developing world for primary health care not because of inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects.[2]

The use of plants as the production assembly of silver nanoparticles has drawn attention, because of its rapid, eco-friendly, non-pathogenic, economical protocol and providing a single step technique for the biosynthetic processes. The reduction and stabilization of silver ions by combination of bio-molecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpinoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures.[3] The protocol for the nanoparticle syntheses involves: the collection of the part of plant of interest from the available sites was done and then it was washed thoroughly twice/thrice with tap water to remove both epiphytes and necrotic plants; followed with sterile distilled water to remove associated debris if any. These; clean and fresh sources are shade-dried for 10–15 days and then powdered using domestic blender. For the plant broth preparation, around 10 g of the dried powder is boiled with 100 mL of deionized distilled water (hot percolation method). The resulting infusion is then filtered thoroughly until no insoluble material appeared in the broth. To $AgNO_3$ solution, on addition of few mL of plant extract follow the reduction of pure Ag(I) ions to Ag(0) which can be monitored by measuring the UV–visible spectra of the solution at regular intervals.[4] The medical properties of silver have been known for over 2000 years. Silver is generally used in the nitrate form to induce antimicrobial effect but when silver nanoparticles are used, there is a huge increase in the surface area available for the microbes to be exposed to. Silver nanoparticles synthesized using plant extracts (from different sources) have been used for analyzing their antimicrobial activities against different microbes.[5]

Objective: the objectives of present study is to synthesize silver nanoparticles by using herbals extract, characterization of these silver nanoparticles &converts its gel formulation as well as to observe the antimicrobial activity.

Materials and Methods

Collection of plant Material

Plant materials of *Azadirachtaindica, Curcuma longa* & *Ocimumsantum* were collected from Shirpur region of Dhule district (Maharashtra).

Extraction methodology

The extractions of powdered material were done by using Soxhlet apparatus. In solvent extraction, dried material is extracted with

methanol. For extraction, 250 gm of powdered material were packed in thimble containing filter paper and extracted with methanol in Soxhlet apparatus for the period till all the substances and others were extracted. The extract thus obtained was concentrated with the help of rotary vacuum evaporator.

Synthesis of silver nanoparticles

For synthesis of silver nanoparticles, the conical flask containing 100 ml of AgNO3 (1mM) was reacted with 12 ml of the Methanolic extract of *A. indica, C. longa*&*O. santum*. This setup was incubated in dark (to minimize the photo activation of silver nitrate), at 37^0C under static condition**.**[6]

Preparation of gel formulation

1 g of Carbopol 934 was dispersed in 50 ml of distilled water with continuous stirring. 5 ml of distilled water was taken and required quantity of methyl parabens and propyl parabens were dissolved by heating on water bath. Cool the solution, then to that added Propylene glycol 400. Further required quantity of SNPs was mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. Finally full mixed ingredients were mixed properly to the Carbopol 934 gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency. The same method was followed for preparation of all SNPs & extract **(**Saeed et al., 2003; Das et al., 2011).

Evaluation of Gel Formulation

Physical Evaluation

Physical parameters such as Color and Appearance& Homogeneity were checked.

Measurement of pH

pH of the gel was measured by using pH meter.

Viscosity

Viscosity of gel was measured by using Brookfield viscometer with spindle.

Spreadability

A sample of 0.5 g of each formula was pressed between two slides (divided into squares of 5 mm sides) and left for about 5 minutes where no more spreading was expected. Diameters of spreaded circles were measured in cm and were taken as comparative values for spreadability. The results obtained are average of three determinations

In vitro diffusion studies

The in vitro release of SNPs was carried out in phosphate buffer saline (pH 5.5) using Cellophane dialysis membrane, Franz diffusion cell**.** The diffusion cells were thermo regulated

with a water jacket. 0.5 gm gel of SNPs was loaded into dialysis membrane of donor compartment at it mounted on receptor compartment**.** Receptor compartment consist of 12.5 ml of capacity fill with phosphate buffer saline (pH 5.5) at maintain temperature 32ºC, which was constantly stirred at 200 rpm with small magnetic bar. At the predetermined time intervals, samples were withdrawn from the receptor compartment, replaced with fresh medium in order to maintain sink conditions. The quantity of SNPs released was determined using UV spectro-photometry at 427 nm, 230 nm & 210 nm for Tulsi SNP, Turmeric SNP & Neem seed SNP respectively (Helal et al., 2012; Saeed et al., 2003; Das et al., 2011).

Drug Release Kinetics

To study the drug release kinetics, the data obtained from in vitro drug release study was fitted to various kinetic models such as zero order (Equation 4) as cumulative amount of drug released versus time; first-order (Equation 5) as log cumulative percentage of drug remaining versustime and Higuchi's model (Equation 6) as cumulative percentage of drug released versus square root of time.

Procedure

Evaluation of Antimicrobial & Anti-fungal activity by agar diffusion method[7]

1. About 20 ml of Muller Hinton agar medium for bacteria and Potato dextrose agar for fungi was allowed to set in empty sterile Petri plate.
2. About 0.1 ml of fungal inoculums was made in petri plates preset for spore count, cell density and bacterial inoculums in respective Medias.
3. The well of 6 mm diameters were bored on the agar media using sterile borer and each plate was filled with 0.5 ml of plant extracts.
4. The plates containing bacteria were incubated at 37ºC for 24 hours and those containing fungi were incubated at 30ºC for 48 hours.
5. The positive antimicrobial activity was read by measuring zone of inhibition (in mm) which was produced after incubation.
6. All the tests were repeated in triplicates.

Experimental Requirements: (Sen and Batra, 2012; Zhao et al., 2010)

Equipment's:

Nichrome wire loop, Petri plates, Autoclave, Test tubes, Micropipette with number of tips. Borer (6 mm), Surgical cotton roll, Aseptic cabinet etc.

Chemicals	Dimethyl sulfoxide (DMSO), 70% ethyl alcohol etc.
Media used	For bacteria **:** Muller Hinton agar medium (Hi Media)
Inoculums size	For bacteria**:** 1 x 10^8 bacteria / ml
Conc. of standard solution	1mg/ ml, prepared in dimethyl sulfoxide
Conc. of extracts	1mg/ml, prepared in DMSO
Method used	Agar diffusion assay (Well size, 6mm)

Results and Discussion

Antimicrobial activity

SNPs & extract of The Neem Seed, Turmeric & Tulsi were screened for antimicrobial activity by agar-well diffusion method.

Table No.1: Antimicrobial activity of SNPs and Extract

Micro-organisms	Diameter of Zone of inhibition(mm)						
	Silver Nanoparticles			Extract			Standard
	Neem Seed	Turmeric	Tulsi	Neem Seed	Turmeric	Tulsi	
Gram Negative bacteria							
Pseudomonas aeruginosa	**8.3**	7.4	6.7	4.3	**5.7**	4.9	12.46
Escherichia coli	6.5	7.9	**8.8**	3.7	4.9	**5.1**	29.12
Gram Positive bacteria							
Staphylococcus aureus	**9.1**	7.7	8.4	5.4	**6.1**	4.6	15.32
Bacillus subtilis	7.5	6.6	**7.9**	**4.9**	3.9	4.4	18.02
Fungi							
Candida albicans	3.8	4.2	**4.6**	2.1	**3.4**	2.6	11.59
Aspergillus niger	4.6	5.2	**5.8**	3.5	2.4	**3.7**	12.10

Diameter in mm calculated by Vernier Caliper; '-' means no zone of inhibition; Well diameter= 6 mm; NCIM-National Collection of Industrial Micro-organisms; Standard- Chloramphenicol

Figure No. 1: Antimicrobial activity of SNPs and Extract of Tulsi, Neem Seed and Turmeric against: *Escherichia coli*

Figure No. 2: Antimicrobial activity of SNPs and Extract of Tulsi, Neem Seed and Turmeric against: *Pseudomonas aeruginosa*

Figure No. 3: Antimicrobial activity of SNPs and Extract of Tulsi, Neem Seed and Turmeric against: *Staphylococcus aureus*

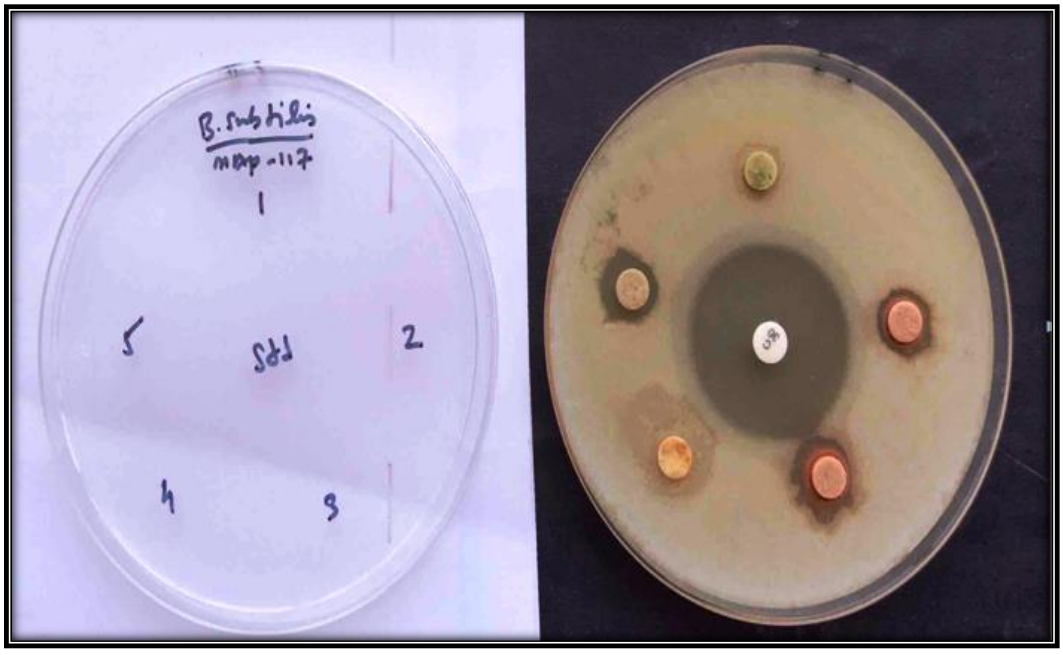

Figure No.4: Antimicrobial activity of SNPs and Extract of Tulsi, Neem Seed & Turmeric against: *Bacillus subtilis*

The antimicrobial activity of silver nanoparticles was evaluated against Gram negative *Escherichia coli & Pseudomonas aeruginosa* and Gram positive bacteria namely, *Staphylococcus aureus*& *Bacillus subtilis* by disc method. The zone of inhibition of synthesized SNPs was found to be more than that of plant extract. Neem and Tulsi SNPs exhibited good antimicrobial activity against gram negative bacteria such as *Escherichia coli & Pseudomonas aeruginosa* with zone of inhibition 8.3 mm & 6.7 mm and 6.5 mm & 8.8 mm respectively. Looking toward the activity of extracts Turmeric extract was most effective against *Pseudomonas aeruginosa* &Tulsi extract gives positive effect against *Escherichia coli*. Neem and Tulsi SNPs exhibited good antimicrobial activity against gram positive bacteria such as *Staphylococcus aureus*& *Bacillus subtilis* with zone of inhibition 9.1 mm & 8.4 mm and 7.5 mm & 7.9 mm respectively. Looking toward the activity of extracts Turmeric extract was most effective against *Staphylococcus aureus*& Neem seed extract gives positive effect against *Bacillus subtilis.*

Conclusion

In the present study, dried powdered Tulsi leaves, Neem seeds & Turmeric rhizomes were subjected to extraction with methanol. SNPs of all tree extract were prepared using standard method.

Since the antimicrobial effects of silver and its compounds are known from several years back, the antimicrobial activity of the synthesized silver nanoparticles was studied using the renowned agar well diffusion method. All SNPs were evaluated for its possible anti-microbial & antifungal properties by standard *in vitro* models. The antibacterial efficacy was tested against the Gram negative bacteria namely, *Pseudomonas aeruginosa & Escherichia coli*as well as Gram positive bacteria namely, *Staphylococcus aureus*& *Bacillus subtilis*. The results of antibacterial study suggest that the synthesized SNPs are very effective antibacterial agents. They exhibited very good activity against all tested bacterial strains.

In conclusion, it has been demonstrated that the extract of Tulsi leaves, Neem seeds & Turmeric rhizomes are capable of producing Ag nanoparticles extracellularly and the Ag nanoparticles are quite stable in solution. The formed silver nanoparticles shows considerable antimicrobial & antiseptic activity compared to the respective antibiotics. These biosynthesis silver nanoparticles prove

to be potential candidates for medical applications where antimicrobial activity is essential.

References

1. *Shah CS, Qadry JS. A Text Book of Pharmacognosy, 216-220,1998.*
2. *Pal, S., Tak, Y. K., & Song, J. M. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. Applied and environmental microbiology, 73(6), 1712-1720, 2007.*
3. *Kulkarni, N., & Muddapur, U. Biosynthesis of metal nanoparticles: a review. Journal of Nanotechnology, 2014.*
4. *Sahayaraj, K., & Rajesh, S. Bionanoparticles: synthesis and antimicrobial applications. Science against microbial pathogens: communicating current research and technological advances, 228-244, 2011.*
5. *Ankanna, S., TNVKV, P., Elumalai, E. K., & Savithramma, N. Production of biogenic silver nanoparticles using Boswellia ovalifoliolata stem bark. Dig J Nanomater Biostruct, 5(2), 369-372, 2010.*
6. *El-Nour, K. M. A., Eftaiha, A. A., Al-Warthan, A., & Ammar, R. A. Synthesis and applications of silver nanoparticles. Arabian journal of chemistry, 3(3), 135-140, 2010.*
7. *Dubey, D., Sahu, M. C., Rath, S., Paty, B. P., Debata, N. K., &Padhy, R. N. Antimicrobial activity of medicinal plants used by aborigines of Kalahandi, Orissa, India against multidrug resistant bacteria. Asian pacific journal of Tropical biomedicine, 2(2), 846-854, 2012.*