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ORIGINAL ARTICLE

Formulation and Evaluation of Antifungal herbal gel of Indian traditional Herbs

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ABSTRACT
Antimicrobial capability of SNPs allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices. The most important application of silver and SNPs is as tropical ointments to prevent infection against burns and open wounds. In small concentrations, silver is safe for human cells, but lethal for microorganisms. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, Virus without any side effect.The herbal extract contains Monoterpenoids & Sesquiterpenoids which exhibit antimicrobial activity. Hence, the present study is to synthesize silver nanoparticles by using neem seed extract, tulsi extract and turmeric extract, characterization of these silver nanoparticles & Converts its gel formulation as well as to observe antifungal activity.
Keyword: Nanoparticles, Monoterpenoids & Sesquiterpenoids

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INTRODUCTION

Recently considerable attention has been paid to utilize eco-friendly and bio-friendly plant based products for the prevention and cure of different human diseases. It is documented that most of the world's population has taken in traditional medicine, particularly plant drug for the primary healthcare. Antimicrobial properties of certain Indian medicinal plants were reported based on folklore information and only few reports are available on inhibitory activity against certain pathogenic bacteria and fungi.

Herbal drugs are playing a vital role in health care system. This is because they are being cheap and locally available [1]**.** 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 value [2]**.** 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.

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 nanomaterial are emerging rapidly [3]**.**

Silver metal has been used widely across the civilizations for different purposes. Many societies use silver as jewellery, ornamentation and fine cutlery. Silver as jewellery, wares and cutlery was considered to impart health benefits to the users. Silver has a long history of anti-microbial use to discourage contamination of microbes dating back to the Phoenicians who used silver as a natural biocide to coat milk bottles. 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.

MATERIAL AND METHODS

Collection and authentication of plant Material

Plant materials of Azadirachta indica, Curcuma longa &Ocimum santum were collected from Shirpur region of Dhule district (Maharashtra) in July 2018. The plants were authenticated at S S V P S, College, Dhule Dr. D A Patil.

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 [4].

Synthesis of silver nanoparticles

For synthesis of silver nanoparticles, the conical flask containing 100 ml of AgNO3 (1mM) was reacted with 12 ml of themethanolic extract of *A. indica, C. longa* & O. *santum*. This setup was incubated in dark (to minimize the photoactivation of silver nitrate), at 37 ◦C under static condition [5].

Preparation the 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 paraben and propyl paraben 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 [6].

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.

Procedure:

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

1. About 20 ml of 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 fungi were incubated at 30 °C for 48 hours.
5. The positive antifungal activity was read by measuring zone of inhibition (in mm) which was produced after incubation.

Preparation of SNPs

SNPs of all tree extract were prepared using standard method. Reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of color changes due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave.

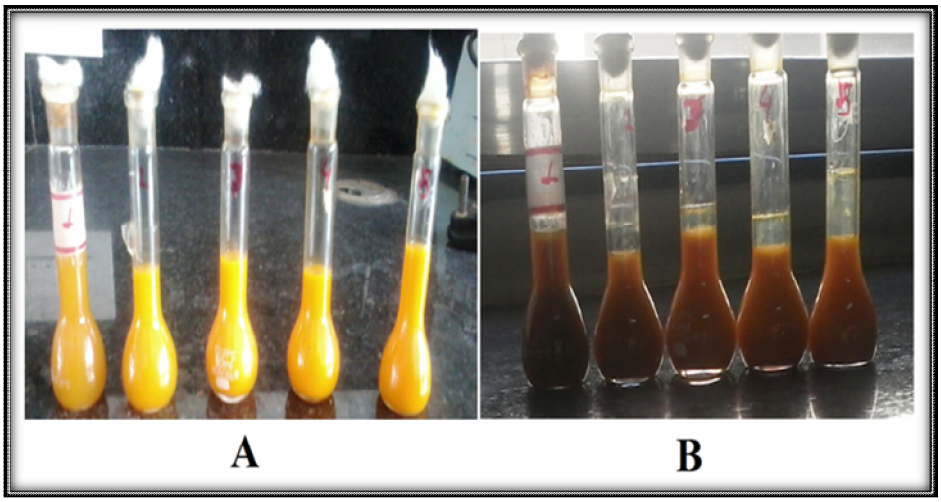

Figure no.1: Color change indicating synthesis of Nanoparticles for Turmeric Extract with respect to time A: Before reaction, B: After 1 hour reaction

RESULT AND DISCUSSION

Antifungal activity

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

Table no. 1: Antifungal activity of SNPs & Extract

Micro-organisms	Diameter of Zone of inhibition(mm)						
	Silver Nanoparticles			Extract			Standard
	Neem Seed	Turmeric	Tulsi	Neem Seed	Turmeric	Tulsi	
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. 2: Antimicrobial activity of SNPs&Extract of Tulsi, Neem Seed & Turmeric against: Aspergillus niger

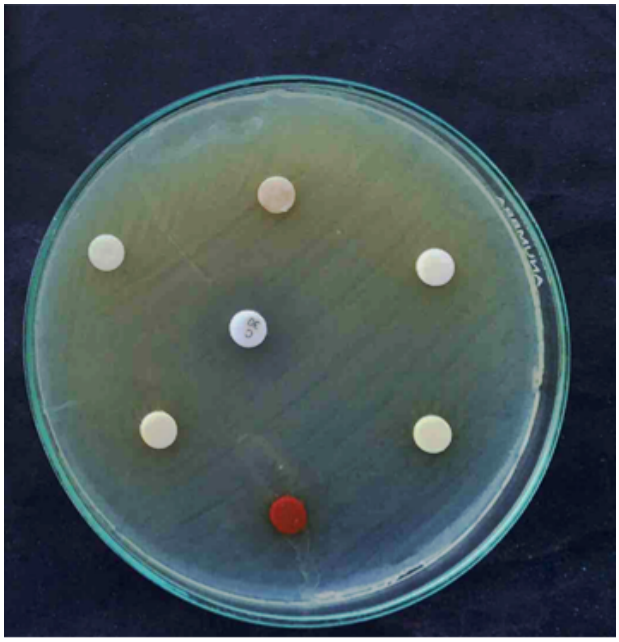

Figure no. 3: Antimicrobial activity of SNPs& Extract of Tulsi, Neem Seed & Turmeric against: Candida albicans

Tulsi SNPs most effective against both the fungi i.e. *Candida albicans* & *Aspergillus niger*. Turmeric extract most effective against Candida albicans &Tulsi extract gives more positive against Aspergillus niger.

The silver nanoparticles showed efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrated inside the bacteria. The bacterial membrane contains sulfur containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity.

Evaluation of Gel Formulation

Spreadability

The spreadability is very much important as show the behavior of gel comes out from the tube. The values of spreadability shown in table 13 indicate that all the polymers used gave gels spread by small amount of shear. Data in table revealed that increasing the concentration of any of the gelling agents was always associated with a decrease in the spreadability as expressed by the lower diameter of the spreaded circle[8].

Measurement of pH

The pH values of all developed gel formulation was in range 4-6.5 which is considered acceptable to avoid the risk of irritation upon application to the skin.

Table no. 2: Physical Properties of SNPs Gels

Gels	Color	Spreadability (cm)	pH	Homogeneity
SNP_2	Brown	0.48	6.1	Completely Homogenize
SNP_1	Brown	0.45	6.1	Completely Homogenize
Ext_1	Yellow	21.6	4.28	Completely Homogenize

Table no. 3: Shows the rheological properties of Gels

Sample	CP	Tork	Spindle No
SNP_2	10160	25.6	7
SNP_1	8200	82	6
Ext_1	7440	74.4	6

CONCLUSION

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 show considerable antifungal activity.

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