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Research on Formulation and Development of Biosynthesized Silver Nanoparticle Incorporated Gel

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ABSTRACT

Nanotechnology is a field where little things collaborate within a scientific framework to develop more useful technologies. For ages, scientists have been fascinated by nanoparticles and have examined them extensively. The advancements in microscope technology have made it possible to see previously hidden details, allowing for the exploration of previously untapped potential and the range of nanoparticles. Drug resistance in pathogenic microorganisms is an emerging and increasing health problem and is a big challenge for the pharmaceutical and biomedical sectors. Multi drug resistant (mdr) bacterial infections lead to significant increase in not only prolonged treatment cost but also morbidity and mortality. Searching and developing novel approach against multi drug resistance bacteria is a priority area for research. Silver is a broad-spectrum antimicrobial agent effective against pathogens. Biological silver nanoparticles have been created, tested for antibacterial activity, and proved to be one of the most effective approaches to address the issue of antibiotic resistance to several drugs. The aim of research work is formulation and development of biosynthesized silver nanoparticle from curry and lemon leaf extract and evaluation of antimicrobial activity against the Streptococcus and E.coli.

Keywords: Lemon, curry leaf extract, silver nanoparticle, UV-spectroscopy, FTIR, Antimicrobial activity,

INTRODUCTION

Nanotechnology is a field where little things collaborate within a scientific framework to develop more useful technologies. For ages, scientists have been fascinated by nanoparticles and have examined them extensively. The advancements in microscope technology have made it possible to see previously hidden details, allowing for the exploration of previously untapped potential and the range of nanoparticles (1). In the past several years, "nanotechnology" has tremendously advanced, is disseminating quickly, has drawn a lot of interest from around the world, and has become a turning point in the scientific revolution. As the subsequent scientific revolution, nanotechnology has gained attention for its potential to speed up computers, treat cancer, and provide solutions for a wide range of medicinal and biomedical problems. Nanoscale organisms can also be found in nature, and this has stimulated a vast field of study in bio nanotechnology. The size spectrum spans macro, micro, and nano sizes (2).

Due to its size and shape, the nanoparticle exhibits a special quality (at dimensions between approximately 1 and 100 nanometers). When compared to bulk material, these nanoscale particles display a number of noteworthy characteristics, such as noticeably lower electrical resistance, lower melting temperatures, or faster chemical reactions (Akbarzadeh et al., 2012; Gericke and Pinches, 2006). Nanoparticles have a number of benefits, including the ability to be stored for longer periods of time, the viability of various administration methods, increased stability and carrier capacity, the usefulness of incorporating both hydrophilic and hydrophobic substances, controlled delivery, and a reduction in the frequency of therapeutic doses (Gelperina et al., 2005). Nanomaterials, nanodots, nanowires, nanoelectronics, and other areas of nano-related research have all been studied by scientists under the more general rubric of nanotechnology. Researchers from all around the world have combined nanotechnology with other scientific disciplines to build and produce a wide range of devices with a variety of applications in daily life after realising the

intelligence and potential of the field. Additionally, NPs have a wide range of applications in biology and medicine, including direct patient applications. The atomic and molecular concepts in chemistry and physics served as the foundation for current understanding in the nanoscale scale sciences, which later included molecular life sciences, medicine, and engineering (3).

The downsizing of devices in electronic engineering has advanced well into the nanometer range, with gate oxides in devices often being 25 nm thick (4). The availability of the first real space images of atomic and molecular processes at surfaces through the development of scanning probe microscopes is directly tied to the recent rise in public awareness of nanoscience (Binnig and Rohrer 1985). (5). The prospect of bottom-up manufacturing of nanoscale materials may lead to some sort of self-assembly of structures comparable to the self-assembly of phospholipid bilayers that resemble cellular membranes as nanotechnology advances (6). However, present knowledge deems it exceedingly unlikely that artificial biological systems may spontaneously form by self-assembly and related processes, as claimed by certain well-known commentators (7). A matter particle with a dimension of one to one hundred nanometers (nm) is typically referred to as a nanoparticle or ultrafine particle (8),(9). When referring to fibres and tubes that are smaller than 100 nm in only two orientations or larger particles up to 500 nm, the phrase is occasionally used (4). Smaller metal particles are typically referred to as atom clusters at the lowest limit, which is smaller than 1 nm. Because of their smaller size, nanoparticles are typically distinguished from microparticles (1-1000 m), "fine particles" (sized between 100 and 2500 nm), and "coarse particles" (ranging from 2500 to 10,000 nm). For example, colloidal properties and ultrafast optical effects or electric properties are driven by nanoparticles (10).

EXPERIMENTAL WORK

I Prefer biosynthesis of silver nanoparticle method for formulation and Development of silver nanoparticle.

Preparation of Extract:
Fresh leaves of curry leaves and lemon leaves powder were collected from the SGRS College, Saswad, Pune, India. The leaves powder extract was prepared by mixing 20 gm of leaf powder with 300 mL of distilled water each (i.e., 66.66 mg/mL) in a 500 mL conical flask. The mixture was stirred and heated between 900C and 1000C for 30 min using heating mantle. After cooling the solution filtered through filter paper twice and finally by using WhatmanNo.1 filter paper. The plant extract was stored at 4^0c for further use. Here onwards curry and lemon leaf extract will be referred to as leaves extract.

Phytochemical Evaluation:
Phytochemical analysis (qualitative and quantitative tests) of the crude extracts for the presence of tannins, saponins, cardiac glycosides, alkaloids, anthroquinones, phenols, steroids and flavonoids are done using standard procedures.

Sr.no	Test	Procedure	Observation	Inference
1.	Alkaloids	Reindorf'sreagent	Reddishppt	+
		Mayer'sreagent	Creamcolorppt	+
		Wagner'sreagent	Brownppt	+
		Hager'sreagent	Yellowppt	+
2.	Glycosides	Modified Bontrager'stest	Nopink colorin layer	-
3.	Saponins	Frothformationtest	Formationofforth persist for15min	+
4.	Tannins	Ferricchloridetest	Greencolorobserved indicatecondensedtannins arepresent	+
5.	Flavonoids	Alkalinereagent test	Yellow color formandgetdisappear	+

FTIR Spectroscopy:
FT-IR spectrum of curry and lemon was recorded as potassium bromide (KBr) powder at resolution of 4 cm^{1} over the region of 4000-400 cm^{1} for its authentication and to study principal peaks using FT-IR spectrophotometer (FT-IR 8400S, Shimadzu). The identified peaks were compared with the principle peaks of reported IR spectrum which are given in the IP.

Formulation of Silver nanoparticle:
Silver nanoparticles were synthesis by using biological method. AgNPs were prepared by using optimized condition i.e., 1 mL of leaves extract and 1 mM of AgNO3 for 30 min at 90 0C. as the color change from colorless to brown color indicates silver nanoparticles are formed by reduction of pure silver ions and it was monitored by measuring absorption of the reaction medium in the wavelength range of 400-800 nm using UV spectrophotometry . The AgNP's solutions were centrifuge at 4000-6000 rpm for 15 min. The supernatant was transferred to a clean dry beaker for further settlement of particles and pellets were removed in Petri plate and dried at room temperature to get powder form.

Characterization of AgNps
UV- Visible spectroscopy
Silver nanoparticles were formed by reduction of silver ion; it was monitored by measuring the absorption spectra in the wavelength range of 400-800 nm using Jasco, Japan (Model: V-630) Spectrophotometer. The highest absorption wavelength was calculated once the spectra were recorded**.**

FTIR analysis of the extracellular biosynthesized SNPs
FTIR analysis of the Ag NPs was done using a potassium bromide (KBr) pellet (FTIR grade) method. The spectrum was recorded using Shimadzu (Model: FTIR-8400 S).

Particle Size and Polydispersity Index
The silver ion's ability to decrease and create nanoparticles is determined by particle size. NANOPHOX used particle size analysis to make its findings (NX0088). The size-based heterogeneity of a sample is measured by the polydispersity index (PI). When a sample is isolated or subjected to analysis, it may aggregate or agglomerate, leading to polydispersity.

Standard calibration curve of silver nanoparticles :
a) Preparation of dissolution media (Phosphate buffer 6.8)
16.338 potassium dihydrogen orthophosphate is dissolved in 600 ml in deionized water then took 250 ml of prepared solution and add 113 ml of 0.2 M NaOH and did volume makeup up to 1000 ml.
0.2 M NaOH: 1.6gm in 200 ml water

b) Preparation of standard calibration curve for silver nanoparticle
A known quantity of silver nanoparticle (10 mg) is accurately weighed and dissolved in a small quantity of water and made up to 100 ml with 6.8 phosphate buffer solution. From this primary stock solution, 0.5, 1, 2, 3, 4, 5 ml is taken out and made volume up to 10 ml the concentration become 10, 20, 30, 40, 50 ug/ml respectively. The absorbance of resulting solution is measured at 400-800 nm by UV- visible spectrophotometer using 6.8 buffer solution as blank. The standard curve is plotted by taking concentration in X-axis and absorbance in Y-axis. The calibration curve is used for estimation of the concentration of drug released during the in vitro diffusion studies.

Antimicrobial evaluation of the Ag NPs :
The agar-well diffusion method was used to examine the antibacterial effects of the biosynthesized Ag NPs produced using the aqueous extract of curry and lemon leaf against various pathogenic gram-positive and gram-negative pathogens, including Staphylococcus aureus and E. coli.

Preparation of Mueller-Hinton agar plates :
Using distilled water, the agar solution was made in accordance with the manufacturer's instructions. For 20 minutes, the agar was autoclaved at 20 psi. The agar was autoclaved, allowed to cool to a temperature of roughly 400°C, and then poured into petri dishes. The petri dishes were then given time to set up.

Creating the inoculum
The isolates that had been previously kept in slants were taken out and subculture on nutrient agar plates, where they were incubated for 48 hours at 370C. The newly subculture isolate's colony was then placed into a test tube filled with saline solution, and the tube was agitated to create an organism suspension. Then, sterile Mueller Hinton Agar (MHA)

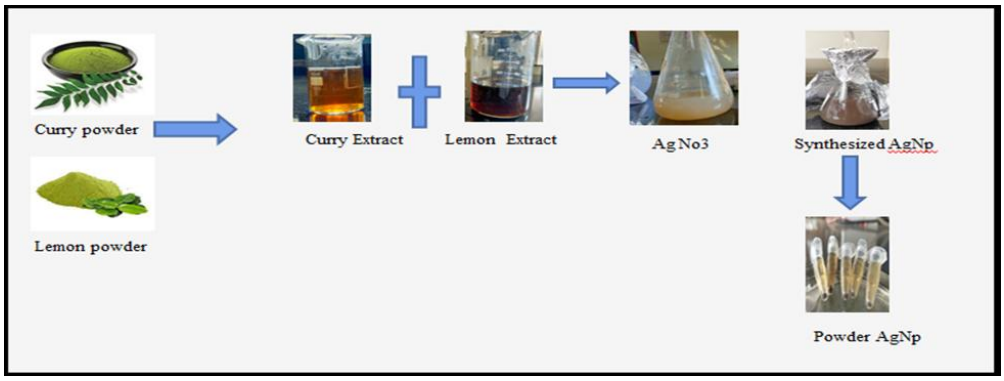
plates are covered with the suspension to test it for bacteria. The Mueller Hinton agar plate is then kept at 370C for 48 hours. After that, inoculation plates were removed, and an 8 mm sterile cork borer was built to bore wells. The wells were filled with plant extract, AgNO3, various concentrations of silver nanoparticles, and the conventional antibiotic ciprofloxacin. Clear zones of inhibition around the wells were measured and reported in millimeters after the plates were incubated.

Minimum inhibitory concentration (MIC) determination:

The MIC of the biosynthesized Ag NPs was determined using two-fold dilutions method according to , that is half of the concentrations of the nanoparticles. A sterile 8 mm cork borer was used to bore seven wells in the already solidified media inoculated with microorganisms which were sensitive to the silver nanoparticles. Different concentrations of silver nanoparticles (25 ug/ml, 50 ug/ml, 100ug/ml), plant extract, AgNO3, and ciprofloxacin as a control were dispensed in each well. The preparation was left to diffuse for 1 h before incubating at 370C for 24 H. The lowest concentration of antimicrobial agent that completely inhibited the growth of the micro-organism was taken as the MIC of the Ag NPs. The zones of inhibition were then measured in millimeter (mm).

RESULT AND DISCUSSION

The plant extract is prepared 60.48 mg/ml and phytochemical screening of aqueous extract of Curry and Lemon leaf is done by using various reagents .



Flow chart of synthesis of Ag Np

Synthesis of AgNp

Ag NP's was synthesis under the optimized condition i.e. using 1 mL of leaves extract and 1 mM of AgNO3 for 30 min at 85^0C. The change in color of liquid from colorless to dark brown in color is determination of nanoparticle synthesis. Figure no UV-vis characterizations.

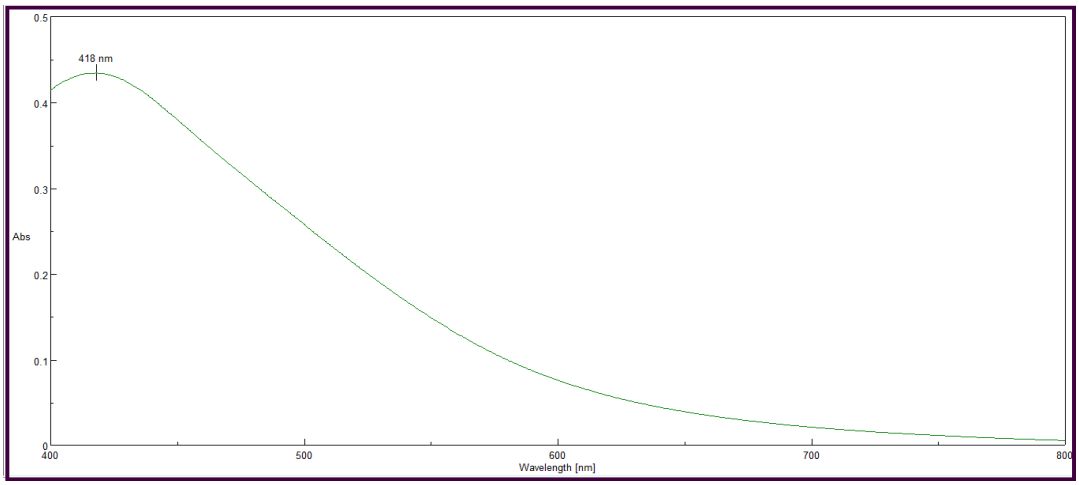


Fig. 1 UV- Vis Spectroscopy of Synthesized Ag Np's.

Quick confirmation of the production and stability of Ag NPs was achieved using UV analysis.. The UV spectra of synthesis Ag NP's was taken in 400-800 nm. The UV–Visible absorption spectrum of biosynthesized Ag NP's using Lemon and curry leaves extract. The absorption spectra of Ag NP's exhibited a strong peak at 418 nm upon incubation for 850C for 30-minutes with 1 mM $AgNO_3$ and 1 mL leaves extract.

FTIRanalysisoftheextracellularbiosynthesizedAgNP's

FTIR spectroscopy was used to confirm the presence of residual Phaytomolecules of leaves extract on the surface of Ag NP's which act as stabilizing ligands. Fig no 9.2 (A), (B) shows the FTIR spectra of the aqueous leaves extract of curry and lemon leaves and fig no 9.2 (C) shows the FTIR of prepared silver nanoparticles. For the FTIR investigation, the Ag NP's sample was thoroughly prepared. The IR spectra of the two samples looked to be nearly identical, with the exception that the IR peaks for AgNP's appeared less strong and showed a slight shift.in their peak position. These striking similarities between the IR spectra of both the samples clearly confirm that leaves extract act as bio reducing and stabilizing agent. Besides some other functional groups, Curry and Lemon extract spectra have prominently indicated towards the presence of hydroxyl (O-H stretching, 3572 cm-1), (O-H bending, 1442 cm-1), aromatic (C-H stretching, 3055 cm-1) and (C-O stretching, 1049 cm-1). Hence, this indicates that the phytochemical constituent of Curry and Lemon leaves extract is rich in terpenoids, flavonoids and polyphenols, which are known to possess excellent reducing abilities and stabilizing abilities.

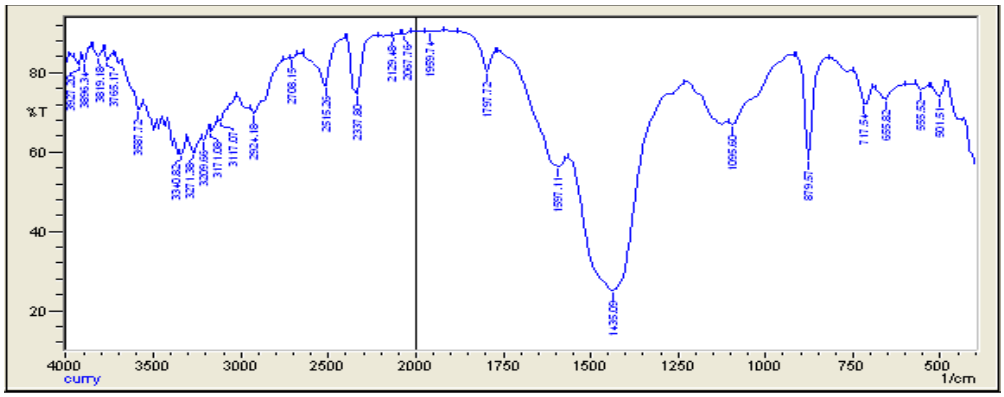


Fig. No.2(A)FTIR spectrum of Curry leaf extract

Table no. 2 (A) FTIR spectra Ranges of Curry Extract

Sr. No.	Standard Range Cm^{-1}	Description	Observed IR peak
1	1725-1800	C=O	1797
2	1000-1300	C-O	1095
3	2950-3600	C-H	3441
4	3000-3670	O-H	3340

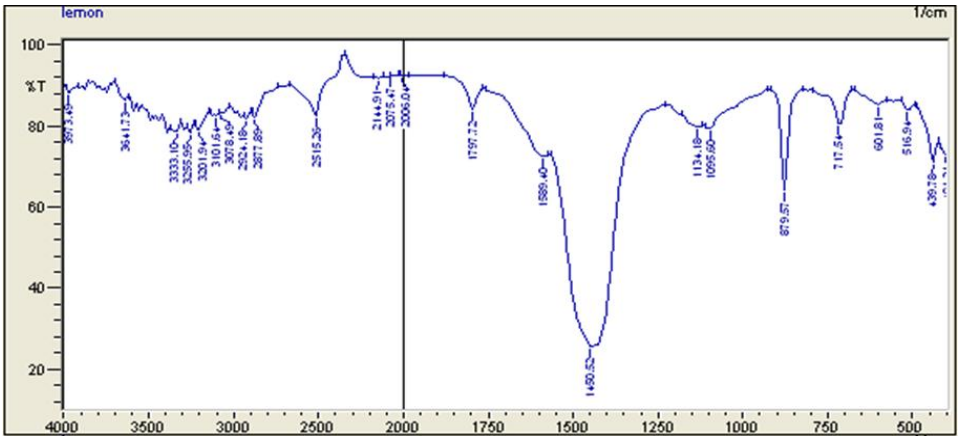


Fig. No. 2 (B) FTIR spectrum of Lemon leaf extract

Table no. 2 (B) FTIR spectra Ranges of Lemon Extract

Sr. No.	Standard Range cm^{-1}	Description	Observed IR peak
1	1725-1800	C=O	1797
2	1000-1300	C-O	1134
3	2950-3600	C-H	3078
4	3000-3670	O-H	3333

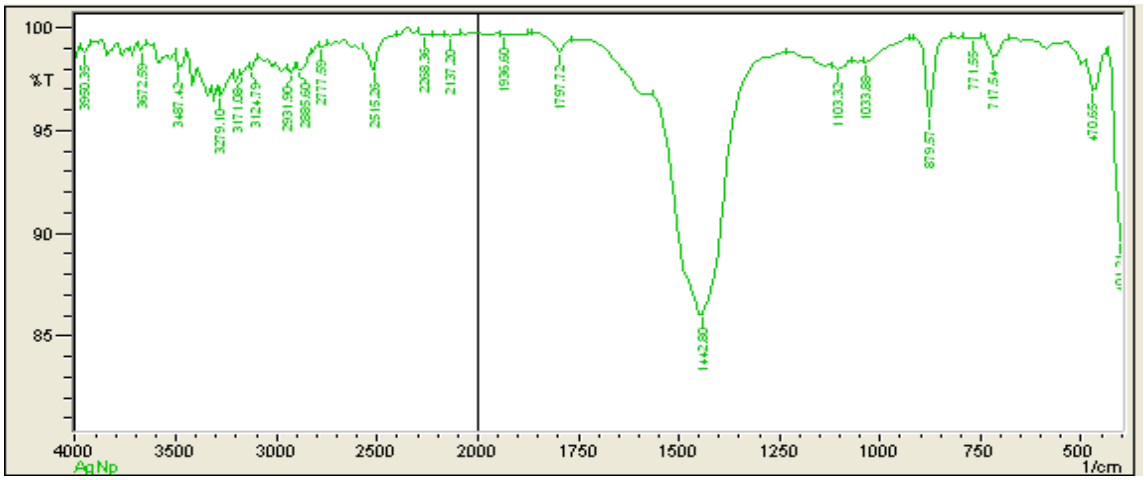


Fig.No.2 (C)FTIR spectrum of biosynthesized silver nanoparticle

Particle size and polydispersity Index

The particle size analysis was done by measuring the average particle size in the aqueous reaction mixture. It was observed that the average particle size was 129.73 nm which confirmed that the silver ions were reduced into nanoparticles. The polydispersity index (PDI) of the silver nanoparticle was found to be 0.38 mid-range polydispersity which indicates the broad distribution of globules and its homogeneity.

Polydispersity index: X90-X10

$$\frac{X50}{} = 8.8$$

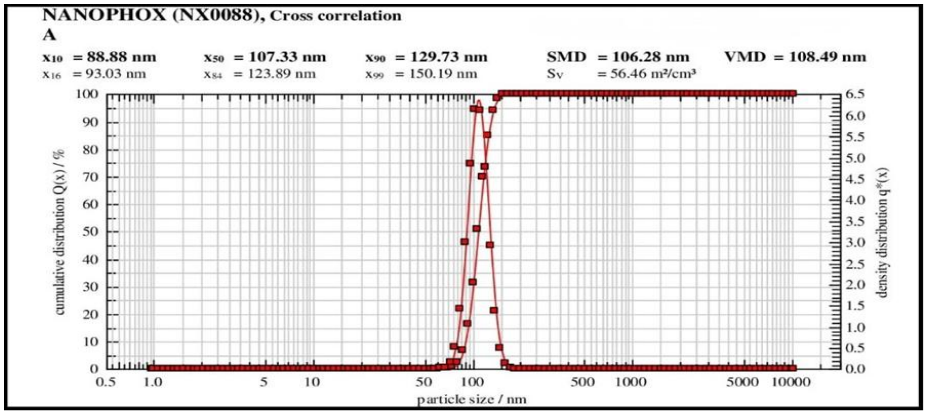


Fig.no.4 Particle size determination.

Calibration curve using phosphate buffer 6.8

Tabale no. 3 calibration curve

Srno	Concentration5-80µg/ml	Absorbance
1	0	0
2	10	0.1757
3	20	0.2764
4	30	0.4991
5	40	0.6585
6	50	0.8751

Calibration curve of biosynthesized silver nanoparticle

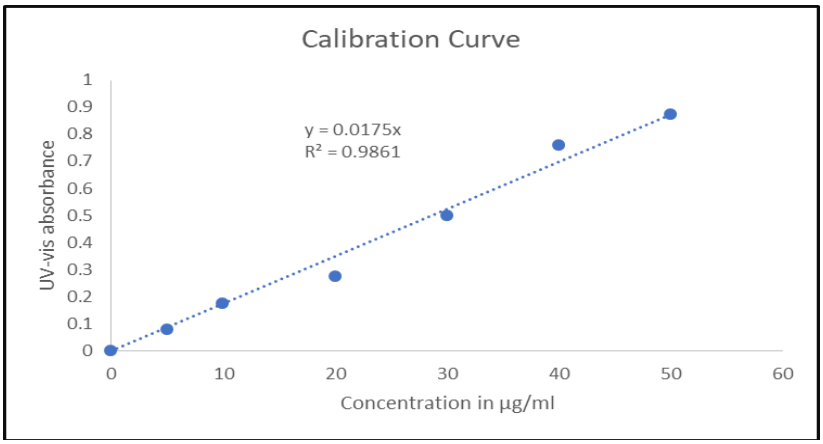
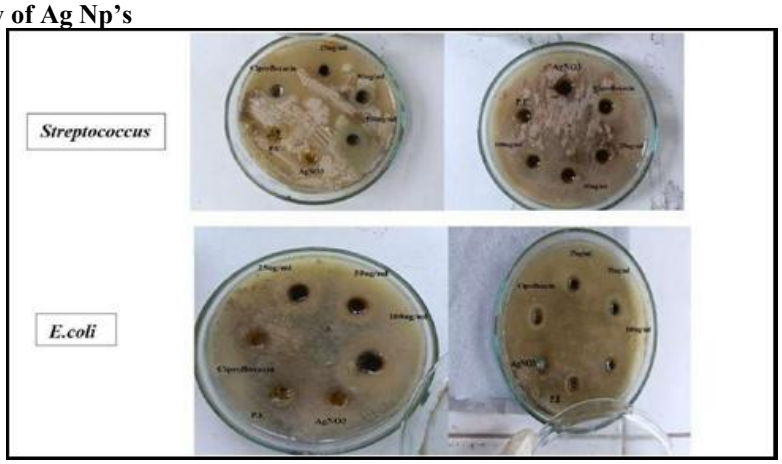


Fig no.5 Calibration curve of biosynthesized silver nanoparticle

Antimicrobial Activity of Ag Np's

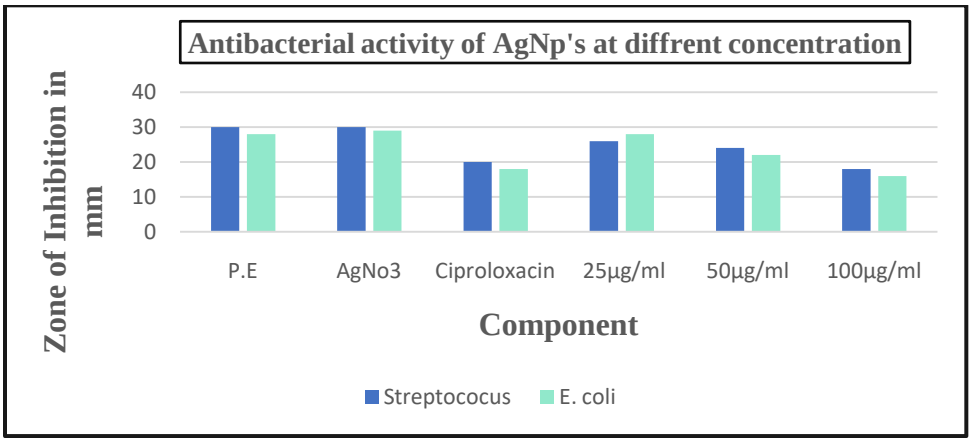


Figno 6: Antibacterialactivity of the AgNPs

The antimicrobial activity is shown in table no 4. The silver nanoparticles are tested against ciprofloxacin as a control. Different concentration of nanoparticles was used (25ug/ml, 50ug/ml, 100ug/ml).

Table no 4: Antibacterial activity of AgNPs at different concentration

Sr. no	Component	Streptococcus	E.coli
1	Plantextract	30 ± 0.6 mm	28± 0.4 mm
2	AgNO3	30 ± 0.6 mm	29± 0.4 mm
3	Ciprofloxacin	20 ± 0.4 mm	18 ± 0.8 mm
4	25ug/ml	26± 0.6 mm	28± 0.4 mm
5	50ug/ml	24± 0.8 mm	22 ± 0.7 mm
6	100ug/ml	18±0.4 mm	16 ± 0.6 mm



Figno7: Antibacterial activity of Ag Np's at different concentration

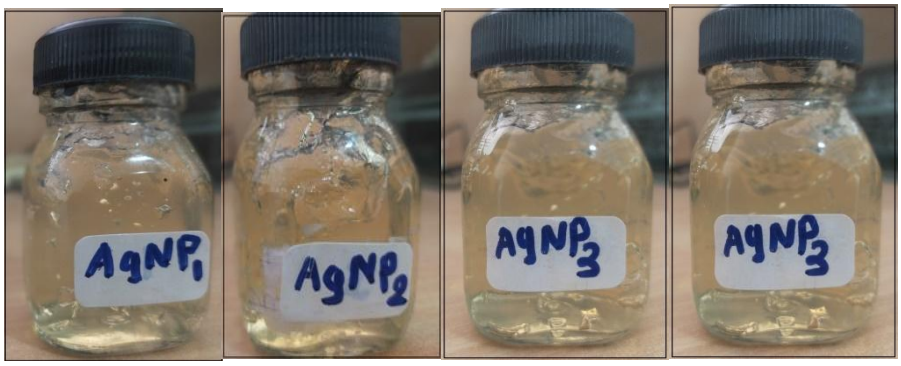
Preparation of Topical Gel Formulation

Table no 5: Topical gel formulation's

Sr.no	Ingredients	Ag Np 1	Ag Np 2	Ag Np 3	Ag Np 4
1.	Carbopol 934	125 mg	250 mg	375 mg	500 mg
2.	Glycerin	1 ml	1 ml	1 ml	1 ml
3.	Silvernanoparticles	0.02gm	0.02gm	0.02gm	0.02gm
4.	Waterup to	50 ml	50 ml	50 ml	50 ml

- Triethanolamineaddeddropwisetoadjustthe pH

Image of Bottle



Gelformulationofsilver nanoparticles

Table 6 : Physical appearance of formulated gel

Codes	**Color**	**Texture**	**Homogeneity**	**Easeof application**	**Easeofremoval**
SNG1	White	Smooth	Homogenous	Easytoapply	Easyto remove
SNG2	white	Smooth	Homogenous	Easytoapply	Easyto remove
SNG3	Light Yellow	Smooth	Homogenous	Easytoapply	Easyto remove
SNG4	Light Yellow	Smooth	Homogenous	Easytoapply	Easyto remove

Evaluation of Gel

pH was found in range of 6.4 ± 0.17 to 6.6 ± 0.12 for gel prepared by Carbopol as gel base which is near to the pH of the skin and hence is found to be compatible with skin. The outcomes of the results are discussed in the table. The viscosity was performed to assess the effect of the type and concentration of the gelling agent on the physical properties of the final silver nanoparticle loaded gel products and their viscosity (171). Viscosity was shown in table no 5 as the pH increase the viscosity also increase pH and viscosity is directly proportionally to each other. Bioavailability and therapeutic property of the topical formulation depends upon the spredability. The spredability is expressed of time in seconds based on the slip off from the gel by upper slide under certain load. Time taken for the separation of the two slides is less which indicates the topical formulation has better Spredability. The Spredability value was found to be 6.8 ± 0.3 to 5.5 ± 0.2. The observed results were comparable with the earlier literature (172). Shown in table no 7

Tableno7: Evaluationpara meters of formulated gel

Srno.	**Formulation**	**pH**	**Viscosityin cps**	**Spredabilitygm.cm/sec**
1	Ag Np 1	6.4 ± 0.2	2273 ± 0.4	6.8 ± 0.3
2	Ag Np 2	6.5± 0.5	2279 ± 0.8	6.0± 0.2
3	Ag Np 3	6.5± 0.6	2285 ± 0.6	5.9± 0.3
4	Ag Np 4	6.6± 0.4	2294 ± 0.4	5.5± 0.2

*Average of three determinations (n=3±SD)

Antimicrobial Evaluation of Formulated Gel

The antibacterial activity study results of the formulated silver nanoparticle incorporated gel showed antibacterial activity against Streptococcus and E. coli. In antimicrobial study it is conclude that the silver nanoparticle synthesized from Curry and Lemon shows greater activity than plant Extract and AgNO3. For the control Silverex ionic marketed gel is used in which 0.2% w/w silver nitrate is present.

Table no. 8 Evaluation of antimicrobial activity of formulated gel

Sr no	**Microbes**	**Silverex ionic gel**	**Ag Np 1**	**Ag Np 2**	**Ag Np 3**	**Ag Np 4**
1	STREPTOCOCCUS	18± 1.6 mm	12±0.8mm	13±1.2mm	12 ± 0.2 mm	16±1.2mm
2	E.COLI	16 ± 0.4 mm	16 ±0.6mm	15±1.4mm	16 ± 0.4 mm	18±0.4mm

*Average of three determinations (n=3 ±SD)

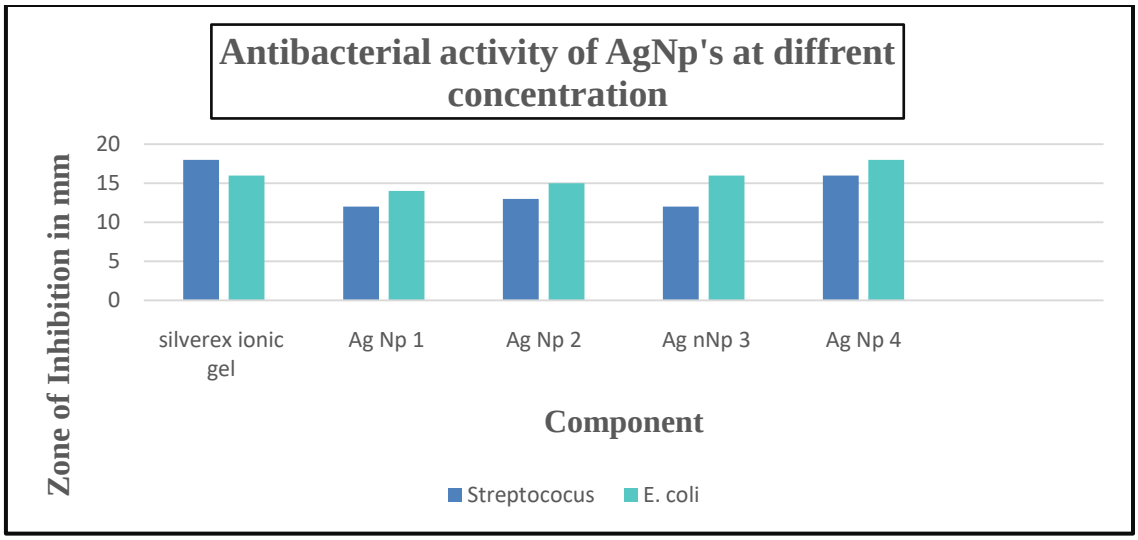


Fig. No. 9 Antimicrobial activity against selected microbes

The antibacterial activity of optimum batch Ag Np 1 formulated silver nanoparticle loaded gel showed antibacterial activityis studied against Streptococcus and E. coli at different quantity if gel . In antimicrobial study it is conclude that the 2 gm quantity of silver nanoparticle showed MIC between 12± 4 to 11 ± 0.4 mm.

Table 9 Evaluation of antimicrobial activity of formulated gel at different quantity

Sr.no	Ag Np 1	Streptococcus	E.Coli
2	250 mg	20 ± 0. 8 mm	17± 0.4 mm
3	500mg	19 ± 0.6 mm	18± 0.5 mm
4	750mg	16 ± 0.4 mm	16 ± 0.3 mm
5	1gm	15 ± 0.4 mm	14 ± 0.8 mm
6	2gm	12 ±0.6 mm	11 ±0.4 mm

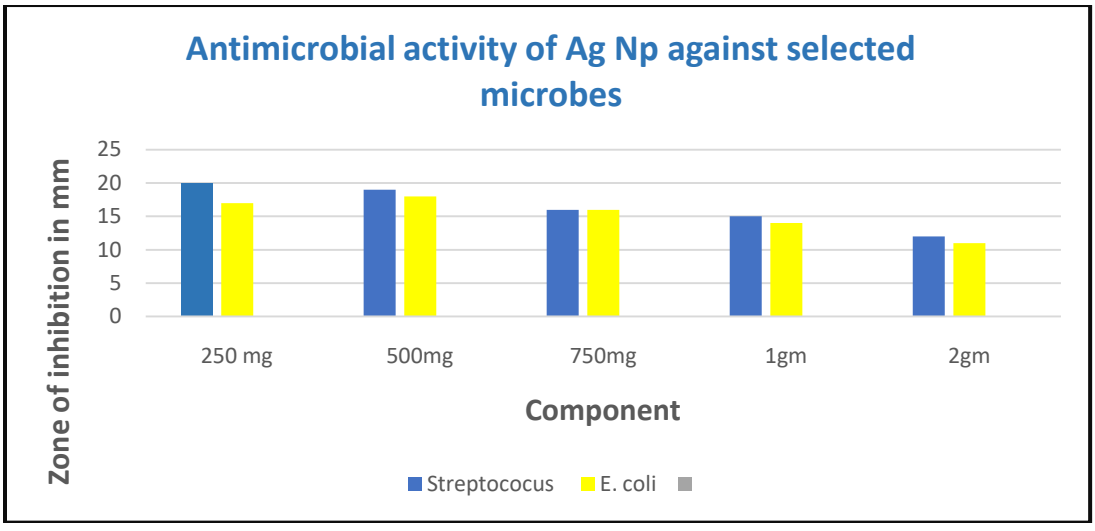


Fig. 10 Antimicrobial activity against selected microbes

From the present study, it can be concluded that the preparation of silver nanoparticle incorporated gel of Curry and lemon proved to be a new and successful approach to obtain stable silver nanoparticle loaded gel. The optimized batch of silver nanoparticle incorporated gel showed optimum particle size of nanoparticles. Thus, silver nanoparticle incorporated gel proved the potential for topical delivery over the conventional formulations.

CONCLUSION

Drug resistance in pathogenic microorganisms is an emerging and increasing health problem and is a big challenge for the pharmaceutical and biomedical sectors. Multi drug resistant (mdr) bacterial infections lead to significant increase in not only prolonged treatment cost but also morbidity and mortality. Searching and developing novel approach against multi drug resistance bacteria is a priority area for research. Silver is a broad-spectrum antimicrobial agent effective against pathogens. Biological silver nanoparticles have been created, tested for antibacterial activity, and proved to be one of the most effective approaches to address the issue of antibiotic resistance to several drugs. Biological synthesis approach is cost effective, hazardous free, environment friendly. Curry and Lemon leaves extract has tremendous medicinal properties s; hence it was used for the synthesis of silver nanoparticles. Silver nanoparticle is synthesized by adopting optimized condition. The IR spectra of the two samples show strong similarities in FTIR, which amply supports the leaf extract's dual function as a bio reducing and stabilizingagent.

The formation of Ag NPs was confirmed by UV–vis analysis, where the UV spectrum exhibited a highest absorption peak at 418 nm. The particle size was found to be 129.73 and polydispersity index was found to be0.38 i.e., mid-range polydispersity. Biosynthesized AgNP's was investigated and the inhibitory concentrations were found to be 50 µg/ml to 100 µg/ml for Streptococcus and Escherichia coli.

Gel with silver nanoparticle incorporatedAg Np1 shows better result like pH-6.4 and Spredabilty 6.8, Viscosity 2273 ± 0.4. The antibacterial activity of gel compared with standard shows the better result against both gram negative and gram positive bacteria. So the formulation and development of biosynthesized silver nanoparticle incorporated gel was found to be effective against both gram negative and gram positive bacteria.

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