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Formulation and Evaluation of Nano Gel containing Biosynthesized Silver Nanoparticles for Antimicrobial Activity

Arun Kumar[1]*, Navneet Verma[2]

*[1]Ph.D. Scholar, IFTM University, Moradabad, U.P. India.
[2]Faculty of Pharmacy, IFTM University, Moradabad, U.P. India.

Corresponding author details: Arun Kumar
Ph.D. Scholar, IFTM University, Delhi Road, Moradabad- 244102 (UP)
Email Id: arunrastogi999@gmail.com

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ABSTRACT
The current work uses liquorice (*Glycyrrhiza glabra* Lin.) root extract to biologically synthesize silver nanoparticles loaded Nano Gel in a unique, quick, environmentally friendly, and simple manner. Nano gel containing biosynthesized silver nanoparticles of liquorice root extract was formulated and evaluated for various parameters. The nano gel prepared with silver nanoparticles of Liquorice root extract were found to have good spreadability, viscous with pH ranged in between 5.25 to 5.47. The optimum pH of the skin is in between 4.75-5.5, therefore, the formulations pH is nearer to this and showed no evidence of dermal irritation. The results of evaluation of nono gel containing biosynthesized silver nanoparticles of liquorice root extract showed the promising results were represented by formulation F4 among other three formulations, thus it was selected as best batch and further evaluated for release kinetic study, stability study, anti-oxidant and anti-microbial activity. The stability study showed that there were no significant changes were seen under stability conditions. Liquorice extract silver nanoparticles loaded nano gel have antioxidant activity that is orders of magnitude nearer to that of ascorbic acid at high doses. At 800 µg/ml, the Liquorice AgNPs nano gel demonstrated 84.25±1.12% inhibition, while ascorbic acid has shown 88.12±1.34% inhibition. The microbial inhibition potential of the freshly prepared AgNPs-Liquorice nano gel against *T. cruris* was more relatively pronounced to that induced by the tested antibiotic, i.e., Terbinafine (18 mm). The magnitude of response for AgNPs-Liquorice nano gel recorded as 88.086%, 66.623%, 56.377%, 53.623%, 52.437% and 40.751% bacterial growth inhibition for *Tinea cruris, S. aureus, S. pyogenes, E. coli, T. corporis* and *T. pedis*, respectively. The experimental results showed that these properties endowed the Liquorice-reduced AgNPs with high antibacterial activity against *Tinea cruris* & *Staphylococcus aureus.*

KEYWORDS: Silver nanoparticles, Nano Gel, Antimicrobial, Nanotechnology, Liquorice, Glycyrrhizinic acid.

INTRODUCTION

The term "antimicrobial resistance" (AMR) refers to the capacity of microbes to continue to exist and function normally despite being subjected to the effects of antimicrobial agents. Antibiotics, disinfectants, and food preservatives are all examples of antimicrobial agents that can be employed against microorganisms to restrict their ability to grow, prevent their multiplication, or even kill them [1, 2]. Antimicrobial agents are available in a variety of forms. There are natural, semi-synthetic, and synthetic agents that possess distinct mechanisms. These agents can cause significant changes on the metabolic and physiological levels. These agents include modifications on cell wall synthesis, such as β-lactams and glycopeptides, protein synthesis inhibition, such as macrolides and tetracyclines, metabolic pathway inhibition, such as sulfonamides, and interference with DNA replication and translation, such as fluoroquinolones [3-5]. It is widely acknowledged that bacterial resistance is a significant concern for healthcare organizations. Because of the increased usage of antibiotics worldwide, there is a greater possibility that bacteria will develop more complex resistance to the antibiotics being used. Since this is the case, it would appear that certain newly changed strains have decreased the likelihood of the treatments being suitably successful in patients, resulting in significant implications that can lead to morbidity and mortality, as well as clinical issues [6-8].

Nanotechnology, particularly through the use of silver nanoparticles (AgNPs), plays a crucial role in combating antimicrobial resistance (AMR). The increasing prevalence of AMR bacteria necessitates innovative approaches, and AgNPs have emerged as promising candidates due to their superior antimicrobial properties against a wide range of pathogens, including both Gram-positive and Gram-negative bacteria [9, 10]. Their effectiveness is attributed to their ultra-small size, which enhances their surface area-to-volume ratio, allowing for direct interactions with bacterial cells and their molecular machinery. AgNPs exert their antimicrobial effects through various mechanisms, such as generating reactive oxygen species (ROS), disrupting bacterial membranes, and facilitating the release of silver ions, which can damage bacterial DNA and proteins [11, 12].

Nanogels are nanoparticles that are typically between 100 and 200 nanometers in size and are constructed of a hydrogel matrix that is made from cross-linked hydrophilic polymers of a certain type. In the case of these nanogels, they can be classified as either biopolymers or synthetic polymers, and they can be cross-linked through either chemical or physical processes.

In addition to their flexible size, huge surface area, and high-water content, their structure consists of pores that can encapsulate either micro-molecules or macromolecules [13].

In this work, we synthesized Nano gel containing AgNPs of Liquorice root extract. Many analytical methods were used to determine the characteristics of the environmentally friendly and optimized Nano gel containing AgNPs. Further optimized Nano gel containing AgNPs, the antimicrobial effectiveness against some human pathogens was evaluated.

MATERIAL AND METHODS

Materials

Carbopol 940, Guar gum, Polysobate-80, Glycerol, Triethanolamine, Methyl Paraben and Propyl Paraben were purchased from various companies of repute. All ingredients used in this study, including the double-distilled water, were of analytical quality. The Liquorice extract silver nanoparticles previously prepared were used for the preparation of nano gel.

Methodology

Formulation Development of Nano Gel Containing AgNPs of Liquorice Root Extract

The measured quantity of AgNPs of Liquorice root extract was dissolved in 50 ml. of D.M. water followed by addition of Polysobate-80 with continuous stirring by using magnetic stirrer. The measured amount of Glycerol, Methyl Paraben and Propyl Paraben were added to the solution. Carbopol 940/Guar gum according to the formulations (F1-F4) and Polyacrylamide was added gradually with continuous stirring for 30 minutes. Then, the preparation was neutralized by adding Triethanolamine solution with slow and constant stirring until the Nano gel preparation formed [14]. The composition of nano gel was given in table 1.

Table 1: Composition of Liquorice root extract AgNPs nano gel

S. No.	Ingredients (mg)	Unit Formula (mg) (For 50 gm Nano-gel)			
		F-1	F-2	F-3	F-4
1	AgNPs of Liquorice root extract	100	100	100	100
2	Guar gum	100	-	200	-
3	Carbopol 940	-	100	-	200
4	Polyacrylamide	750	750	750	750
5	Glycerol	0.5	0.5	0.5	0.5
6	PEG 600	0.2	0.2	0.2	0.2

8	Methyl Paraben	80	80	80	80
9	Propyl Paraben	10	10	10	10
10	Triethanolamine	300	300	300	300
11	Polysorbate 80	250	250	250	250
12	D.M. water (ml) Q.S.	50	50	50	50

Methods of Evaluation of Liquorice Root Extract AgNPs Nano Gel Formulation

Appearance and Consistency

The physical appearance was virtually checked for the texture of silver nano gel and observation reported.

Washability

Prepared formulation was added to the skin and then manually tested for ease and degree of washing with water and findings were recorded.

Extrudability Determination of Formulation

The mixtures were put into flexible aluminum tubes. The tubes were compressed in order to extrude a gel ribbon measuring 0.5 cm within a 10-second timeframe, and the extrudability of the formulations was subsequently assessed.

Determination of pH

The pH of silver nano gel was checked by using numerical pH meter. 1gm of gel was dissolved in 25 ml of purified water and the electrode was then dipped into gel solution until steady reading was achieved. Measurements of pH were noted for all prepared formulations.

Viscosity

The viscosity of the formulation batches was determined using a (Visco QC100) viscometer with spindle L4 at 50 rpm. The spindle was allowed to move freshly into the silver nanogel, and the reading was noted.

Determination of Spreadability

For determined the spreadability of the gel, 0.5 g gel was placed within a circle of 1 cm diameter pre marked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to spreading of the gels was noted.

$$Spreadability\ (S) = m \times lt$$

Where,

S = Spreadability (gcm/sec)
m = weight tied to the higher slide i.e. 20 gm
l = length of glass slides i.e. 6 cm
t = time taken in seconds

Drug Content

The composition of the silver nanogel was measured by taking 1 gm of gel mixed with methanol in 10 ml volumetric flask. The mixture was vortexed for 15 seconds and after that the absorbance in UV at pre-determined λmax was taken.

In-vitro Drug Release

Franz diffusion cell was used to determine the release profile of silver nanogel. A thin membrane (dialysis membrane) was placed over the donor compartment. Capacity of receptor chamber was 18 ml. A magnetic bead was placed in the receptor chamber. The diffusion medium consisted of phosphate buffer pH 6.8. Whole assembly was put on magnetic stirrer at 50rpm stirring speed and temperature was maintained 37.0 ± 0.5 ^{0}C. The 1g samples were kept over the membrane in donor compartment and stirred. 2ml samples were withdrawn from the receptor compartment at predetermined time intervals (0.5h, 1h, 2h, 3h, 4h, 5h, 6h, 8h) and the volume was replenished with same volume of buffer medium. Addition of buffer medium to the receptor compartment was performed with great care to avoid trapping air beneath the diffusion membrane. The samples were analyzed by UV at pre-determined λmax. The % drug release was calculated, and graph of % drug release v/s time was plotted [14].

Based on the results obtained from above mentioned evaluation parameters, the formulation F4 was considered as best batch as it showed promising results among all other nano gel formulations. This best batch was selected for performing release kinetic study, stability study, anti-oxidant and antimicrobial activity.

Release Kinetic Study

To characterize the overall release of the medication from dosage forms, a variety of kinetic models exist. Because changes in a formulation's quality and quantity might alter drug release and in vivo performance, it's always a good idea to design approaches that reduce the requirement for bio-studies and make product development easier. Several kinetic equations, including first order, zero order, the Korsmeyer-Peppas model, and the Higuchi model, were used to match the data from the AgNPs release investigations from gel formulation [15].

Stability Testing of the Optimized AgNPs of Liquorice root extract loaded Nano Gel

Stability testing shows how a drug product's quality changes over time when exposed to various environmental conditions such as temperature, humidity, light, and air. It makes it possible to determine the preparation's shelf life and the ideal packing and storage circumstances. The stability study was conducted as per ICH guidelines. The gels were tested on stability to see if they could keep their physicochemical characteristics. The gels were kept in three distinct environments: ambient temperature (25°±2°C with 60% relative humidity), refrigeration (4°±2°C), and accelerated temperature (40°±2°C with 75% relative humidity). The gels' physicochemical characteristics were recorded and tested at predetermined intervals (0, 3, and 6 months). The examined samples' parameters included color, appearance, pH, and percent drug release ICH Harmonized Tripartite guidance.

Antioxidant Activity the Optimized AgNPs of Liquorice root extract loaded Nano Gel

DPPH scavenging assay was used to calculate the total antioxidant content of the Liquorice extract and AgNPs. In a typical procedure, 100 µL of DPPH solution (0.25 mM in methanol) was transferred to each well of a 96-well plate. Then, different concentrations of Liquorice extract and AgNPs (50–400 µg/mL) were added to each well. The plate was incubated in dark for 1–2 hour at 37°C. UV absorbance (A) of the sample was then assessed at 517 nm using a UV–Vis spectrophotometer. The percentage of inhibition was calculated according to following equation [16, 17].

$$\% \text{ inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Antibacterial Activity of AgNPs of Liquorice Root Extract Nano Gel

Agar Disk-Diffusion Method

Agar well diffusion assays were performed to check for antibacterial activity of green synthesised AgNPs-Liquorice nanogel. Green synthesized silver nanoparticles' high surface-to-volume ratio, precision tailoring nanoparticle size with plant bioactive compounds, and close interaction with microbial membranes make them antibacterial. *S. aureus*, *E. coli*, *Streptococcus pyogenes*, *T. corporis*, *T. cruris* and *T. pedis* bacterial cultures were inoculated in sterile petri plates using the spread plate technique and allowed to grow for 24 hours before being used in the experiment. The circular wells used in agar well diffusion was 6 mm in diameter. Then, the well was loaded with 10 µg of Azithromycin or Terbinafine (Standard

drug), while the other wells contained different concentrations of silver nanoparticles loaded Liquorice nanogel and distilled water (negative control). Triplicate plates of each culture were incubated at 37℃ for 24 hours [18, 19]. The antimicrobial activity of prepared gel was then determined.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC of prepared silver nanoparticles of Liquorice extract loaded nano gel was determined by nutrient broth dilution method using test tubes containing sterile Mueller–Hinton broth. The antimicrobial activity of prepared nano gel was tested against *E. coli, S. pyogenes, S. aureus, T. corporis, T. cruris* and *T. pedis*. Azithromycin and Terbinafine were selected as standard drugs. Various concentrations of Liquorice AgNPs loaded nanogel (100, 50, 25, 12.5, 6.25%) used as test samples. The Sub-culturing of strains was done in Broth and incubation was done at 37°C for 5days. A 5X 10^5cfu/ml concentration was used as the test strain. Respective fresh media was used as the Sterility Control. Equal Volume of the Mueller Hinton Broth and Test Strain were used as the Growth Control for the Test Study. Total 5 dilutions at 1:1 with sterile broth were used in duplicate. For Growth control, only 1ml of the broth and 1ml of the test strain and for sterile control, 2 ml was used. All the tubes were incubated at 37°C, for 48 hours in the BOD incubator. At the end of incubation period, end point OD600 was taken and analyzed regarding the sterility control and the Growth control. The lowest dilution which completely inhibits the visible bacterial growth was considered as MIC. The Minimum bactericidal concentration (MBC) was determined as the minimal concentration from which no growth of the microbe could be seen or in the bactericidal concentration, the growth of the test microorganism was completely prevented [20, 21].

RESULT AND DISCUSSION

Formulation Development of Nano Gel Containing AgNPs of Liquorice Extract

The nano gel of optimized batch of silver nanoparticles of Liquorice extract was prepared by utilizing Guar gum and Carbopol 940 and evaluated for physico-chemical parameters. Results of nano gel of silver nanoparticles of Liquorice extract were depicted in following sections (Table 2). During checking of physical characters of prepared silver nanoparticles laden nano gel, it was found that prepared gel was light brown in colour and homogeneous with smooth

texture. No clogging was seen in the nano gel andit was easily washable. Figure 1 is showing the appearance of prepared gel.

Table 2: Physical characteristics of Nano gel formulation

Formulation code	Colour	Clogging	Homogeneity	Texture	Washability	Extrudability
F1	Brown	Absent	Good	Smooth	Good	Good
F2	Brown	Absent	Good	Smooth	Good	Good
F3	Brown	Absent	Good	Smooth	Good	Good
F4	Brown	Absent	Good	Smooth	Good	Good

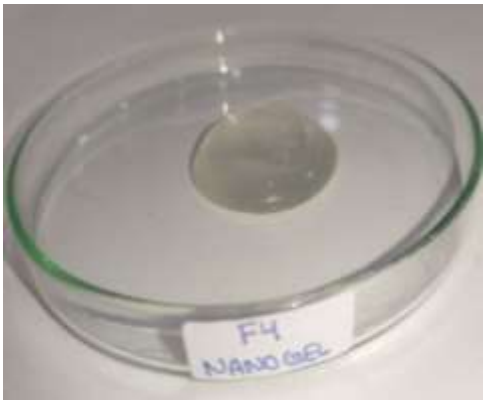

Figure 1: Visual appearance of Liquorice-AgNPs nano gel

The nano gels prepared with silver nanoparticles of Liquorice extract were found to have good spreadability, viscous with pH ranged in between 5.25 to 5.47 (Table 3). The optimum pH of the skin is in between 4.75-5.5, therefore, the formulations pH is nearer to this and showed no evidence of dermal irritation.

Table 3: Results of Spreadability, Viscosity, drug Content and pH of nano gel

Formulation Code	Spreadability* (gcm/sec)	Viscosity* (cp)	Drug (Glycyrrhizin) Content (%)	pH
F1	27.193±0.12	2916.35±0.31	96.84±0.27	5.25±0.24
F2	27.329±0.53	3259.26±0.29	97.12±0.34	5.35±0.19
F3	20.783±0.15	8128.11±0.25	97.86±0.41	5.09±0.25
F4	18.124±0.75	6320.05±0.11	98.98±0.49	5.47±0.32

***In-vitro*Drug Release**

Finally, the *in-vitro* drug release study was conducted on the prepared gel. The *in-vitro* drug release profile of Liquorice-AgNPs best batch was depicted in table 4 and figure 2.

Table 4: *In-vitro* drug release profile of Liquorice-AgNPs nano gel

Time (min.)	% Drug release			
	F1	**F2**	**F3**	**F4**
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
30	6.32±1.23	6.98±1.18	8.72±1.22	10.38±1.15
60	13.53±1.11	14.76±1.11	16.78±1.27	18.93±1.38
120	28.21±1.26	30.17±1.46	32.63±1.12	34.58±1.25
180	46.97±1.52	48.26±1.79	50.41±1.19	52.11±1.47
240	60.18±1.46	61.48±1.28	63.75±1.36	65.86±1.28
300	68.36±1.78	70.23±1.37	72.37±1.28	74.28±1.11
360	76.82±1.35	78.91±1.29	80.61±1.47	83.52±1.39
420	81.45±1.14	82.63±1.62	84.79±1.65	87.19±1.13
480	84.51±1.63	85.93±1.93	88.31±1.17	90.25±1.51

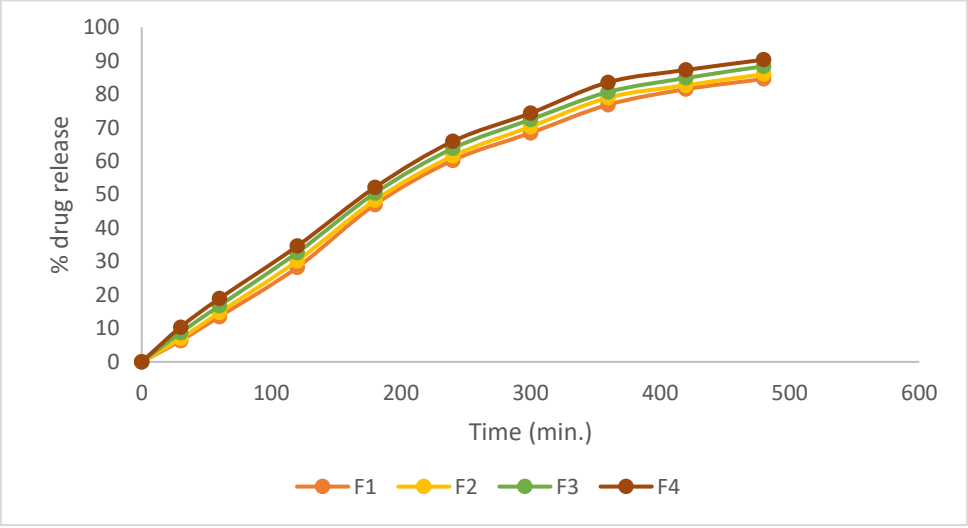

Figure 2: *In-vitro* release profile of optimized nano gel formulation containing AgNPs of Liquorice extract

The drug release from the prepared gel was found to be ranged between 84.51-90.25% at the end of 8 hrs. Maximum drug release was found to be 90.25% from formulation F4. The drug was released in sustained manner from the gel.

The results of evaluation of Liquorice extract AgNPs showed that promising results were represented by formulation F4 among other three formulations, thus, it was selected as best batch and further evaluated for release kinetic study, anti-microbial activity and anti-microbial activity.

Release Kinetic Study of optimized nano gel formulation containing AgNPs of Liquorice extract

To confirm the drug release mechanism, release kinetic study was conducted. The dissolution profile of the optimized batch of Liquorice-AgNPs nano gel was fitted into different kinetic models viz., Zero order, First order, Higuchi and Korsemeyer-peppas model. Results of kinetic study revealed that the drug release mechanism was found to be Higuchi model followed by Korsemeyer-peppas model. Kinetic release study results were depicted in table 5 & figure 3-6.

Table 5: Release kinetic study of optimized batch of Liquorice-AgNPs nano gel

Formulation batch	**Zero order**		**First order**		**Higuchi model**		**Korsemeyer-peppas model**	
Liquorice-AgNPs optimized batch	**m**	**R^2**	**m**	**R^2**	**m**	**R^2**	**m**	**R^2**
	0.1956	0.935	0.0029	0.645	0.9391	0.971	0.7656	0.995

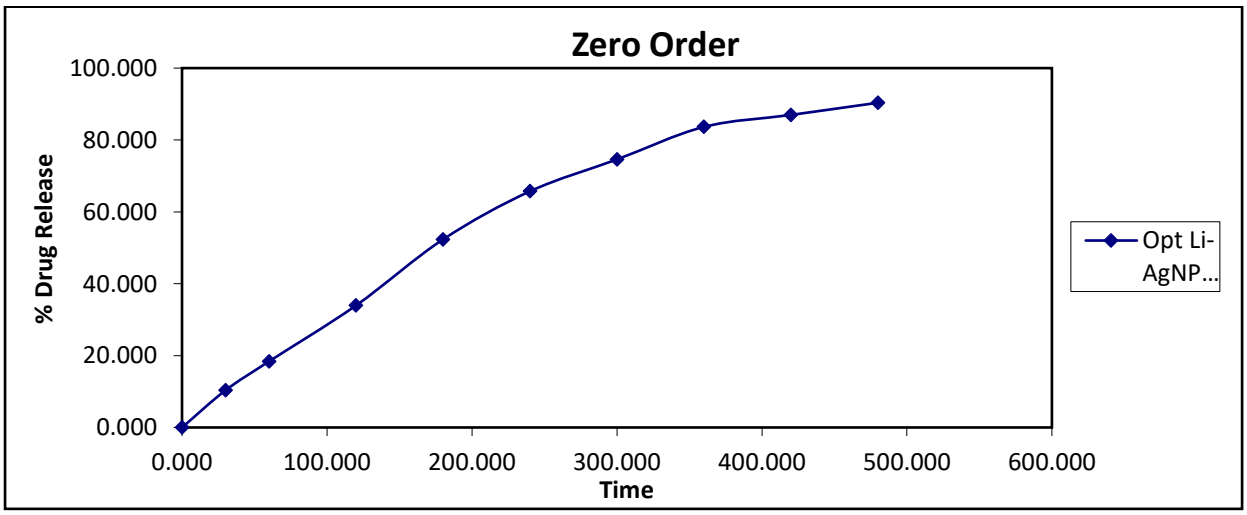

Figure 3: Zero order kinetic model of Optimized batch of Liquorice-AgNPs nano gel

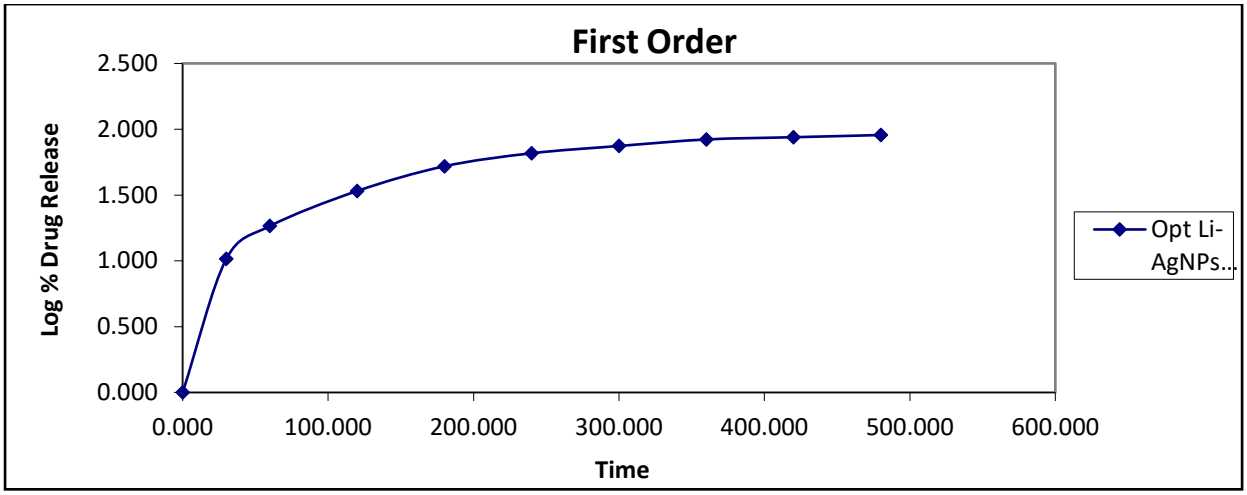

Figure 4: First order kinetic model of Optimized batch of Liquorice-AgNPs nano gel

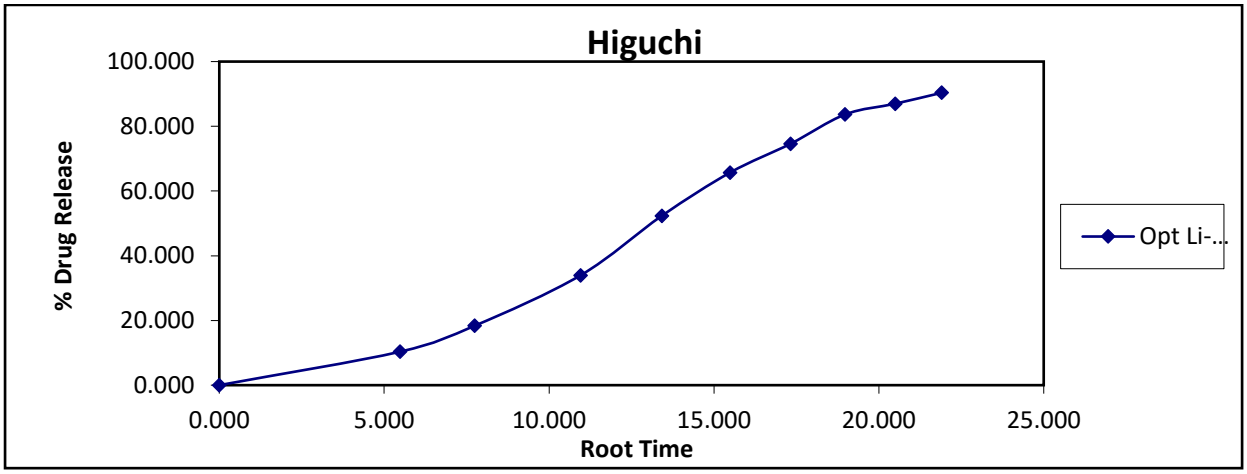

Figure 5: Higuchi kinetic model of Optimized batch of Liquorice-AgNPs nano gel

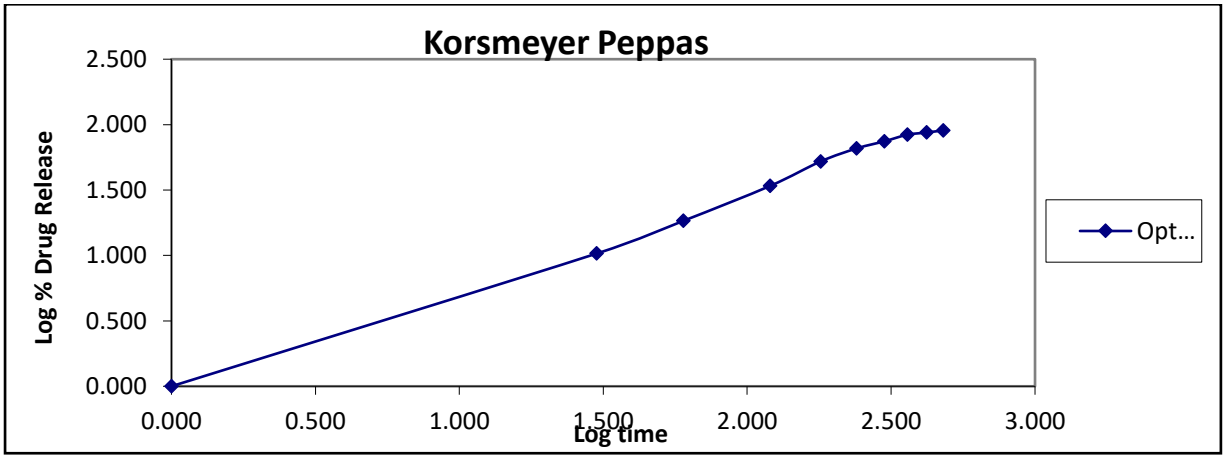

Figure 6: Korsemeyer model of optimized nano gel formulation containing AgNPs of Liquorice extract

Stability Testing of optimized nano gel formulation containing AgNPs of Liquorice extract

The optimized batch of Liquorice-AgNPs nano gel was kept under accelerated stability testing conditions as per ICH guidelines. The samples were withdrawn at periodic time period and analyzed for colour, appearance, pH, and percent drug release. The results were depicted in table 6-7 and figure 7. In-vitro drug release study was conducted at accelerated stability conditions.

Table 6: Stability study data of optimized nano gel formulation containing AgNPs of Liquorice extract

Stability condition	**0 Day**	**90 Days**	**180 days**
Parameters	**Colour**	**Appearance**	**pH**
25°±2°C, 60% RH	Brown	Homogeneous	5.47
4°±2°C	Brown	Homogeneous	5.49
40°±2°C, 75% RH	Brown	Homogeneous	5.46

Table 7: Effect of Stability conditions on In-vitro drug release

	Percent (%) drug release at Accelerated stability testing conditions		
Time (min.)	**0 day**	**90 days**	**180 days**
0	0±0.00	0±0.00	0±0.00
30	10.38±1.23	10.15±1.35	9.92±1.48
60	18.93±1.11	18.76±1.12	18.38±1.23
120	34.58±1.45	34.17±1.83	33.73±1.41
180	52.11±1.28	51.26±1.62	51.11±1.58
240	65.86±1.25	65.48±1.29	64.75±1.21
300	74.28±1.57	74.13±1.24	73.37±1.32
360	83.52±1.68	82.91±1.22	82.61±1.46
420	87.19±1.34	86.63±1.12	85.79±1.72
480	90.25±1.51	90.15±1.57	89.31±1.85

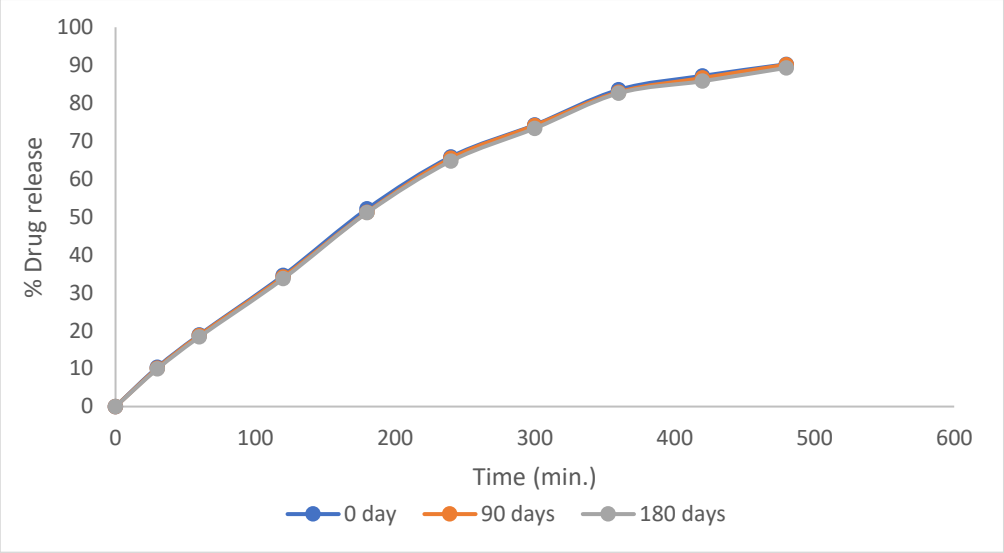

Figure 7: Graph showing effect of stability conditions of *in-vitro* drug release

The stability study showed that there were no significant changes were seen under stability conditions.

Antioxidant Activity of optimized Nano Gel formulation containing AgNPs of Liquorice extract

Free radicals play substantial roles in many different diseases and disorders. To nullify the free radicals and prohibit them from spreading illnesses antioxidants were used. This is done by ROS scavenger mechanism. Natural goods' electron-donating capacity can be measured with the help of the purple DPPH solution bleaching method. Scavenging DPPH via addition of a radical species or antioxidant renders the DPPH solution colourless. The strength of the colour change is controlled by the antioxidant content. A drastic drop is indicative of the molecule's potent free radical scavenging capability. Results were depicted in table 8 & figure 8.

Table 8: DPPH assay for Antioxidant activity of synthesized Opt Liquorice-AgNPs

S.No.	Ascorbic acid		Opt Liq-AgNPs Nano Gel	
	Conc. (mcg/ml)	% scavenging	Conc. (mcg/ml)	% scavenging
1	10	73.27±1.42	10	36.27±1.13
2	50	75.38±1.26	50	51.62±1.56
3	100	77.51±1.74	100	62.53±1.19
4	200	80.46±1.23	200	71.54±1.32
5	400	84.15±1.86	400	77.83±1.46
6	800	88.12±1.34	800	84.25±1.12

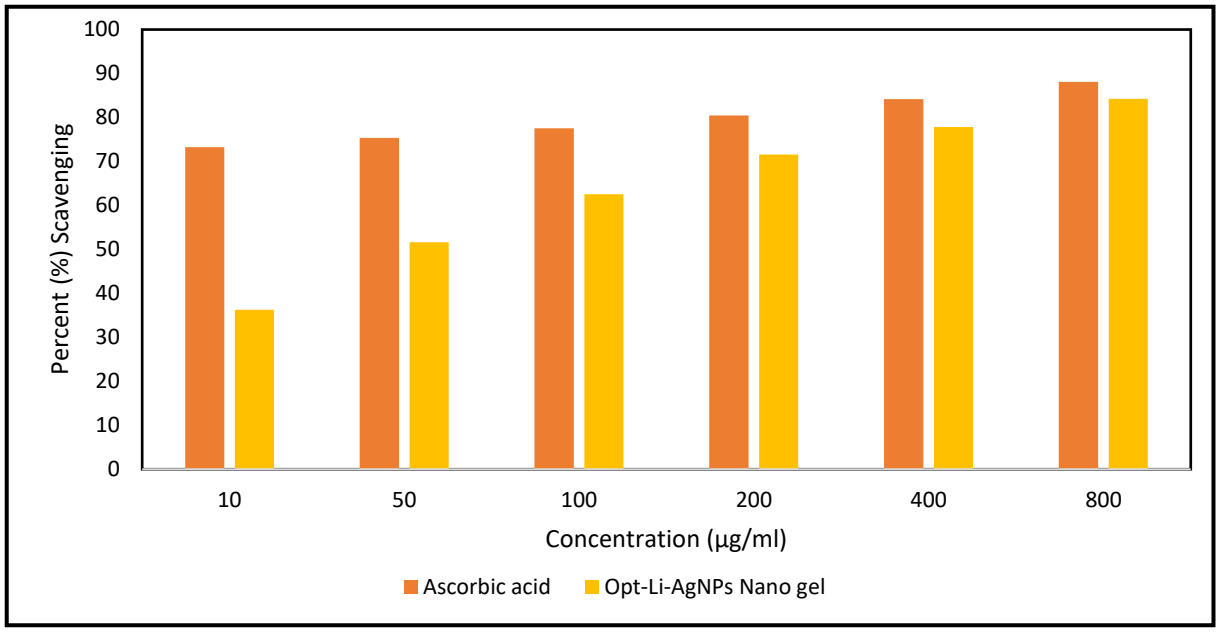

Figure 8: DPPH Antioxidant activity of Opt Liquorice-AgNPs nano gel

The current study demonstrated that Liquorice extract silver nanoparticles loaded nano gel have antioxidant activity that is orders of magnitude nearer to that of ascorbic acid at high doses. At 800 µg/ml, the Li-AgNPs nano gel demonstrated 84.25 ± 1.12% inhibition, while, ascorbic acid has shown 88.12± 1.34% inhibition.

Antibacterial Activity of optimized Nano Gel formulation containing AgNPs of Liquorice extract

Agar Disk-Diffusion Method

Owing to the observed resistance of many pathogenic bacteria towards the already used antibiotics, exploiting AgNPs may be another urgent choice for controlling proliferation of bacterial human pathogens [22]. For evaluating the antibacterial activity of AgNPs-Liquorice nano gel against *E. coli* (gram-negative bacteria), *S. pyogenes* (gram-positive bacteria), *S. aureus* (gram-positive bacteria), *T. corporis* (pathogenic fungi), *T. Cruris* (pathogenic fungi) and *T. Pedis* (pathogenic fungi), disc-diffusion method was used, in which zone of inhibition around the holes denoted as a function in bacterial growth inhibition (Figure 9-12). It is found that the concerned bactericidal effect may be attributed to Ag^+ [23, 24] who referred this antibacterial action to the binding potentiality of Ag^+ ions with various bacterial cell compartments as DNA molecules and cytoplasm which outflow from the injured cell wall.In the present study, the appearance of clear inhibitory zones confirmed the complete growth inhibition of either *E. coli*, *S. pyogenes*, *S. aureus*, *T. corporis*, *T. Cruris* or *T. pedis*. The results revealed that AgNPs-Liquorice nano gel had effective antimicrobial activity against *E. coli*, *S.*

pyogenes, *S. aureus*, *T. corporis*, *T. cruris* and *T. Pedis* when compared with standard drug. Zone of inhibition was seen against *T. cruris* (15 mm), *S. pyogenes* (14 mm), *S. aureus* (13 mm). *T. corporis* (12 nm) and *T. pedis* (10 mm) and *E.coli* (10mm). The inhibition potential of the freshly prepared AgNPs-Liquorice nano gel against *T. cruris* was more relatively pronounced to that induced by the tested antibiotic, i.e., Terbinafine (18 mm) [25] (Figure 9-12).

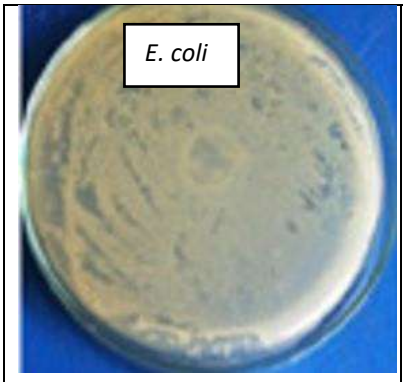

Figure 9A: 25.0% Nano Gel

Figure 9B: 50.0% Nano gel

Figure 9C: 100.0% Nano gel

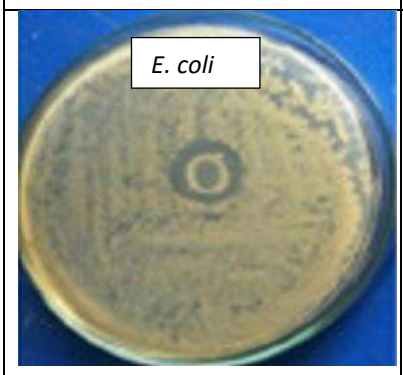

Figure 9D: Azithromycin

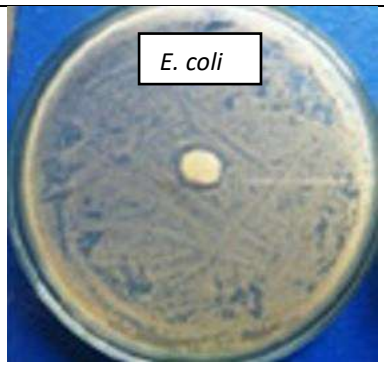

Figure 9E: Negative control

Figure 9: Anti-bacterial activity (zone of inhibition) of AgNPs-Liqorice Nano gel against human pathogenic bacteria E. coli (9A-9E), where 9A is 25% nano gel; 9B is 50% nano gel; 9C is 100% nano gel; 9D is Azithromycin and 9E is Negative control

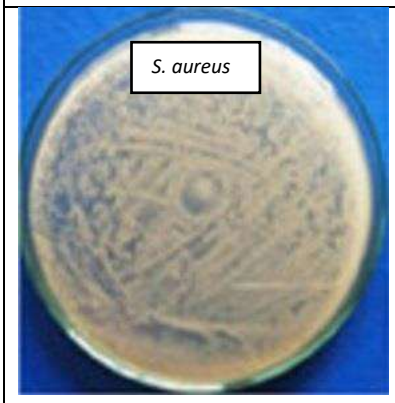

Figure 10A:25.0 % Nano Gel

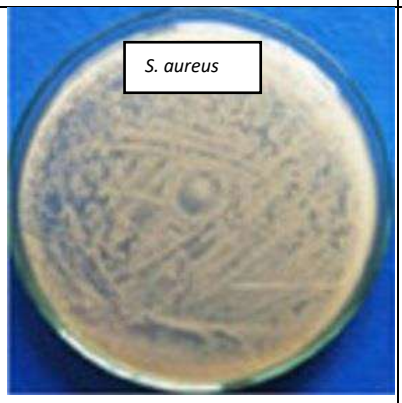

Figure 10B:50.0 % Nano Gel

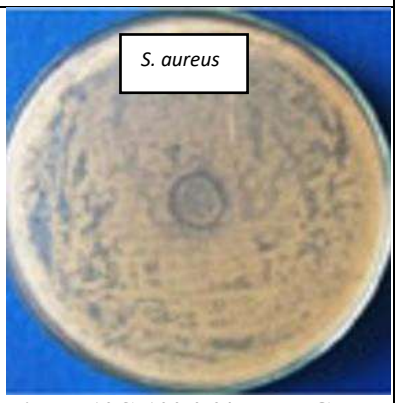

Figure 10C:100.0 % Nano Gel

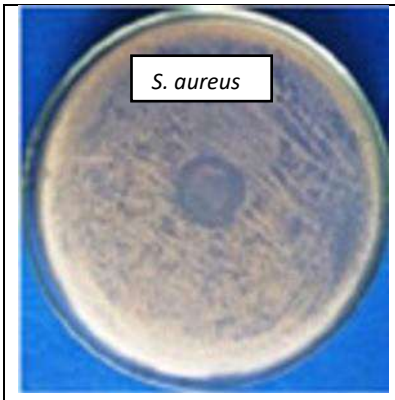

Figure 10D:Azithromycin

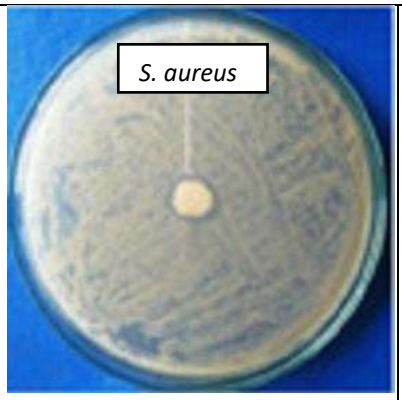

Figure 10E:Negative control

Figure 10: Anti-bacterial activity (zone of inhibition) of AgNPs-Liqorice Nano gel against human pathogenic bacteria S. aureus (10A-10E), where 10A is 25% nano gel; 10B is 50% nano gel; 10C is 100% nano gel; 10D is Azithromycin and 10E is Negative control

Figure 11A: 25.0% Nano gel

Figure 11B: 50.0% Nano gel

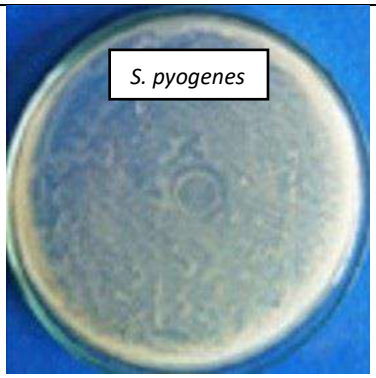

Figure 11C: 100.0% Nano gel

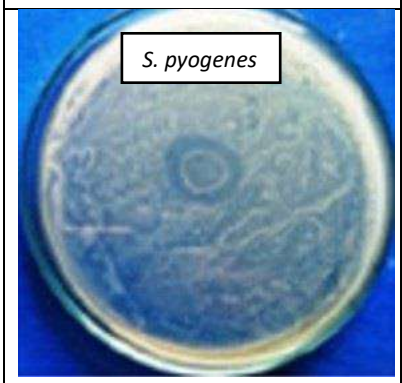

Figure 11D: Azithromycin

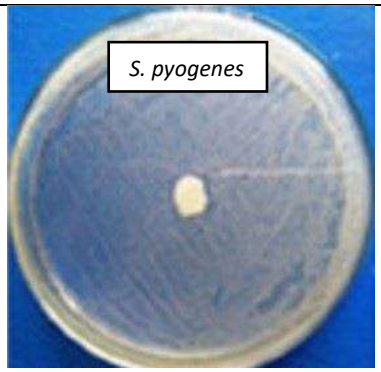

Figure 11E: Negative control

Figure 11: Anti-bacterial activity (zone of inhibition) of AgNPs-Liqorice Nano gel against human pathogenic bacteria S. pyogenes (11A-11E), where 11A is 25% nano gel; 11B is 50% nano gel; 11C is 100% nano gel; 11D is Azithromycin and 11E is Negative control

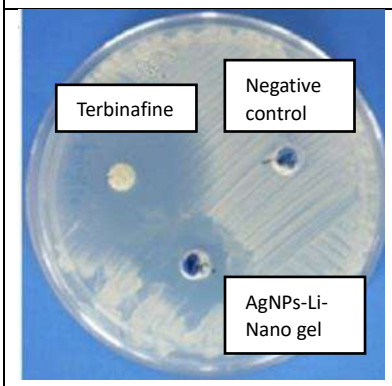

Figure 12 A: Anti-fungal activity (zone of inhibition) of AgNPs-

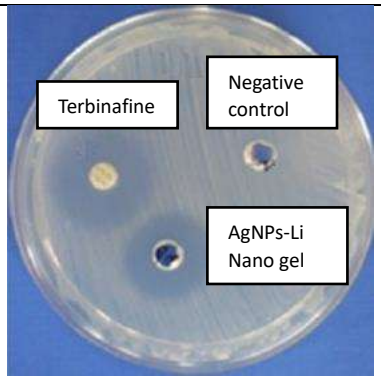

Figure 12 B: Anti-fungal activity (zone of inhibition) of AgNPs-

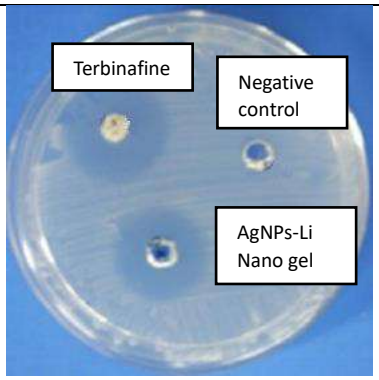

Figure 12 C: Anti-fungal activity (zone of inhibition) of AgNPs-

Liquorice Nano gel against human pathogenicfungi *Tinea cruris*	**Liquorice Nano gel against human pathogenicfungi *Tinea corporis***	**Liquorice Nano gel against human pathogenicfungi *Tinea pedis***

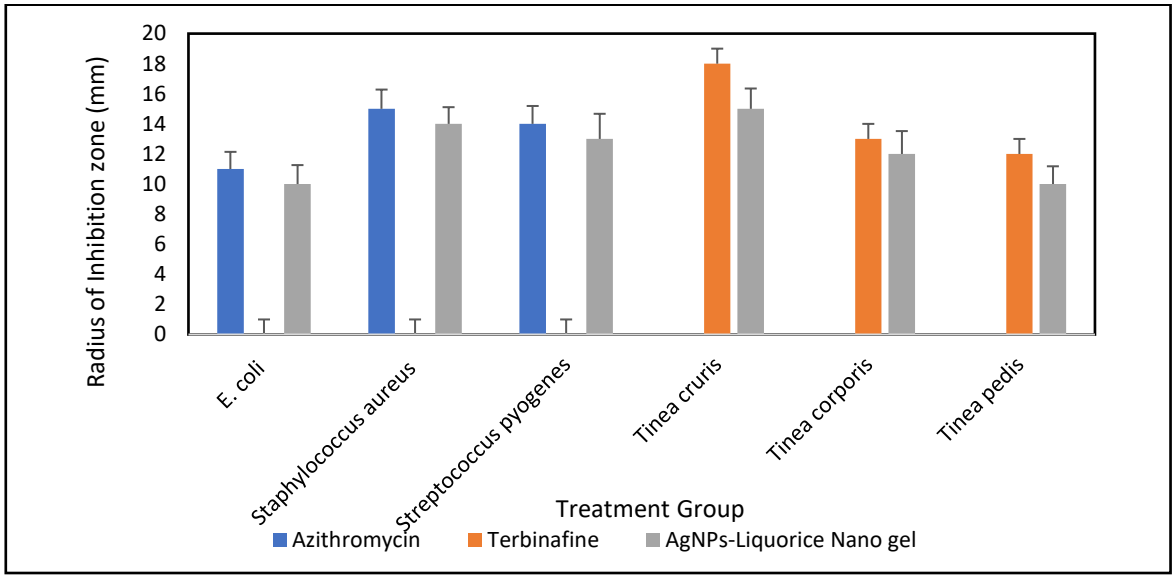

Figure 13: Anti-bacterial activity (zone of inhibition) of AgNPs-Liquorice Nano gel against human pathogenic microbes

Figure 9-13 presented the culture plates showing the anti-microbial activity (zone of inhibition) of AgNPs-Liquorice nano gel and standard drug against pathogenic bacteria and fungi. Figure 13 depicted the graphical representation of zone of inhibition measurements of the standard drugs and AgNPs-Liquorice Nanogel sample. Because of the versatile anti-microbial activity of Liquorice against some pathogenic organisms, it can be beneficial in treating such diseases.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The antibacterial activity of AgNPs and standard drugs, Azithromycin and Terbinafine was determined through serial dilution method. The minimum bactericidal concentration (MBC) is defined as the lowest concentration of an antibiotic killing the majority (99.99%) of bacterial inoculums. The images of serial dilutions were presented in figure 14-19. The results of MIC and MBC were presented in table 9 &10 and figures 14-19.

Table 9: Intergroup comparison of Mean MIC of AgNPs and Standard drugs (Azithromycin and Terbinafine)

Organism	AgNPs-Liquorice nano gel (mean±S.D)	Azithromycin (mean±S.D)	Terbinafine (mean±S.D)
E. coli	25±0.0036	0.25±0.0059	-
Staphylococcus aureus	6.25±0.0016	0.001±0.0042	-
Streptococcus pyogenes	50±0.0036	0.625±0.0039	-
Tinea cruris	12.5±0.0049	-	4±0.0036
Tinea corporis	25±0.0052	-	0.8125±0.0026
Tinea pedis	50±0.0065	-	8±0.0032

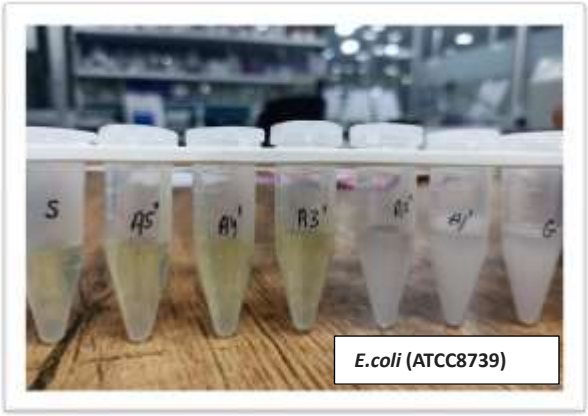

Figure 14 A: Liquorice-AgNPs Nano gel

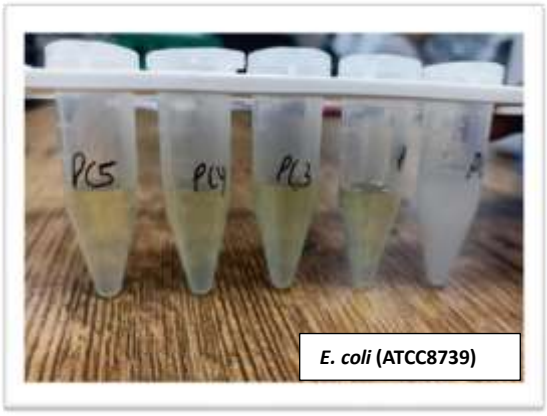

Figure 14 A: Positive Control (Azithromycin)

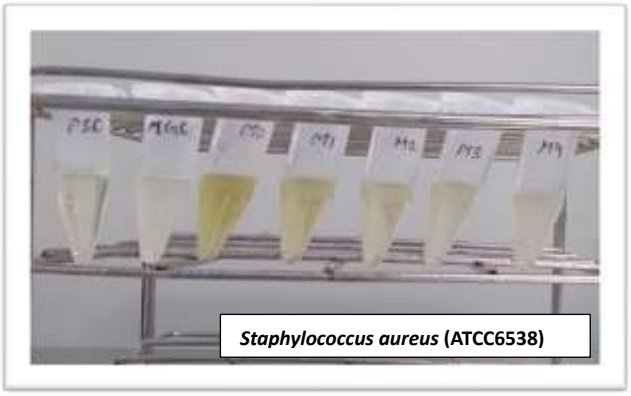

Figure 15 A: Liquorice-AgNPs Nano gel

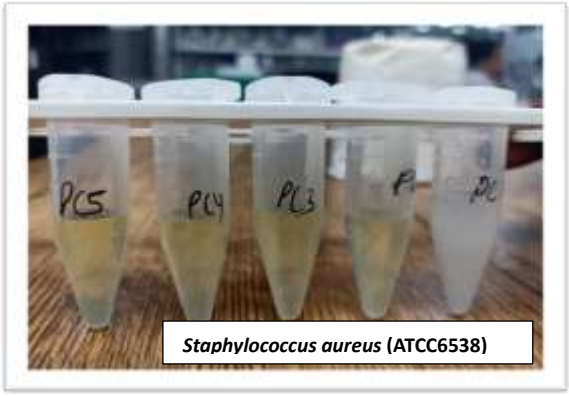

Figure 15 B: Positive Control (Azithromycin)

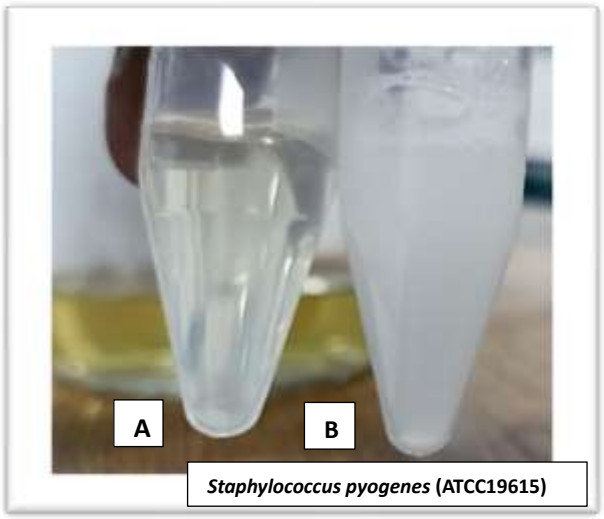

Figure 16A: A. *Streptococcus pyogenes* (ATCC19615); B. Positive Control (Azithromycin)

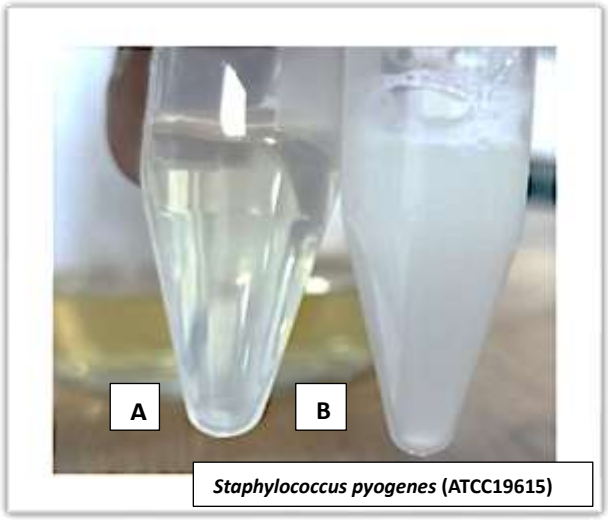

Figure 16 B: A. *Streptococcus pyogenes* (ATCC19615); B. Liquorice-AgNPs Nano gel

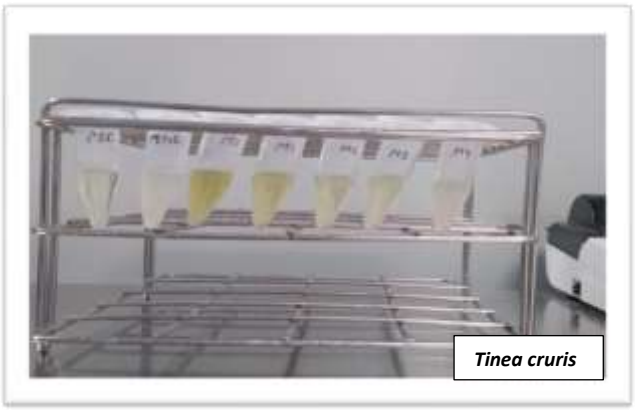

Figure 17 A: Liquorice-AgNPs Nano gel

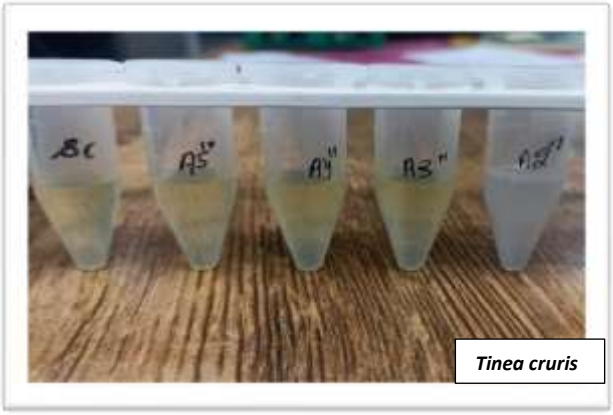

Figure 17 B: Positive Control (Terbinafine)

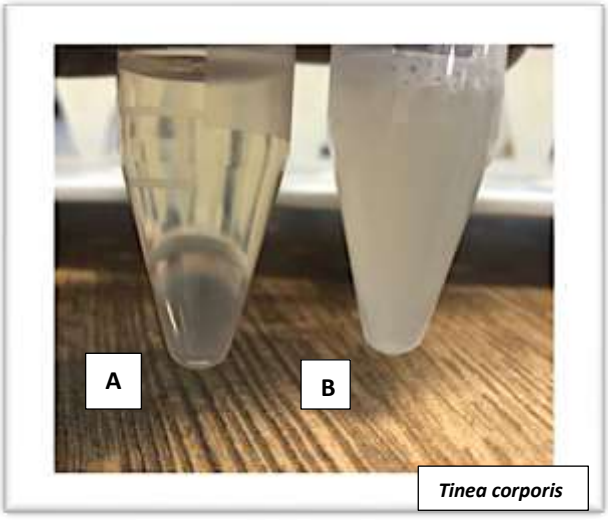

Figure 18 A:A. *Tinea corporis*; B. Positive control (Terbinafine)

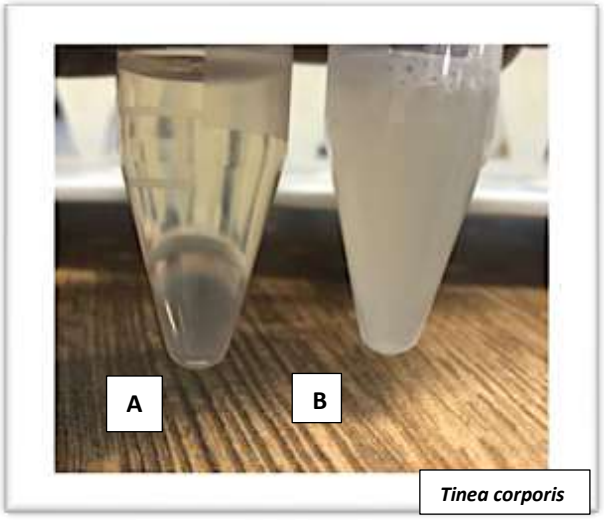

Figure 18 B: A. *Tinea corporis*; B. Liquorice-AgNPs Nano gel

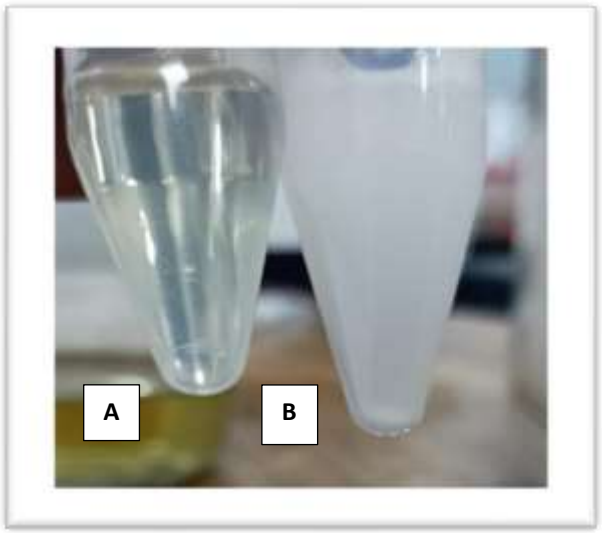

Figure 19 A: A. *Tinea pedis* B. Positive Control (Terbinafine)

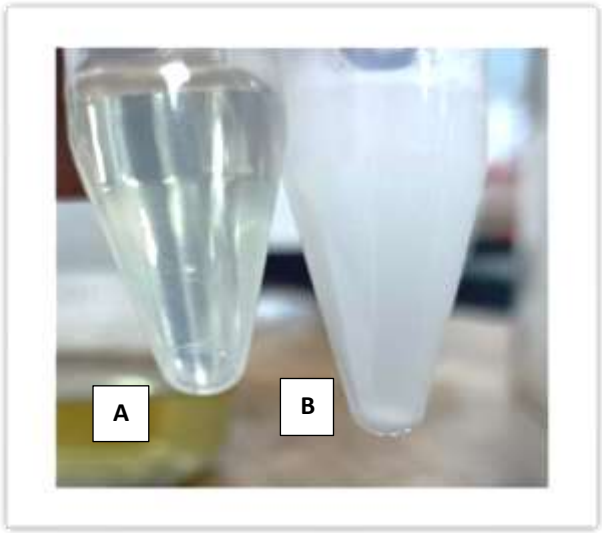

Figure 19 B: A. *Tinea pedis* B. Liquorice-AgNPs Nano gel

Figure 14 A-B to 19 A-B: Determination of MIC of Liquorice-AgNPs by broth dilution method

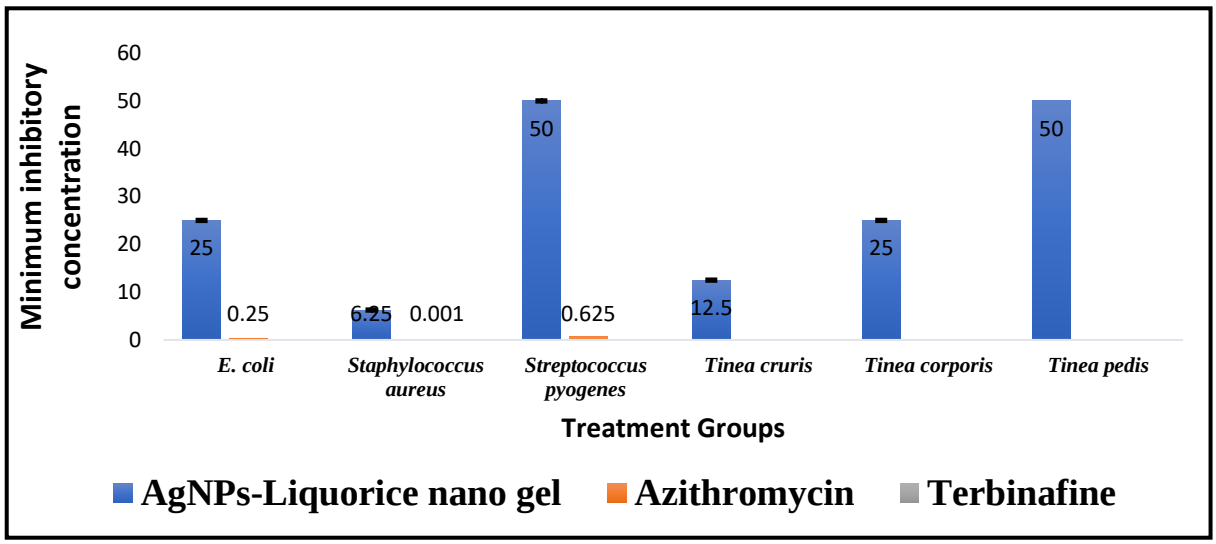

Figure 20: Mean MIC of AgNPs-Liquorice nano gel and Standard drugs against pathogenic organisms

Table 10: Intergroup comparison of Mean MBC of AgNPs and Standard drugs (Azithromycin and Terbinafine)

Organism	AgNPs-Liquorice nano gel	Azithromycin	Terbinafine
E. coli	50±0.0036	1±0.0059	
Staphylococcus aureus	25±0.0016	1±0.00424	
Streptococcus pyogenes	100±0.0036	5±0.0039	

Tinea cruris	50±0.0049	-	8±0.0036
Tinea corporis	100±0.0052	-	0.8125±0.0026
Tinea pedis	100±0.0065		16±0.0032

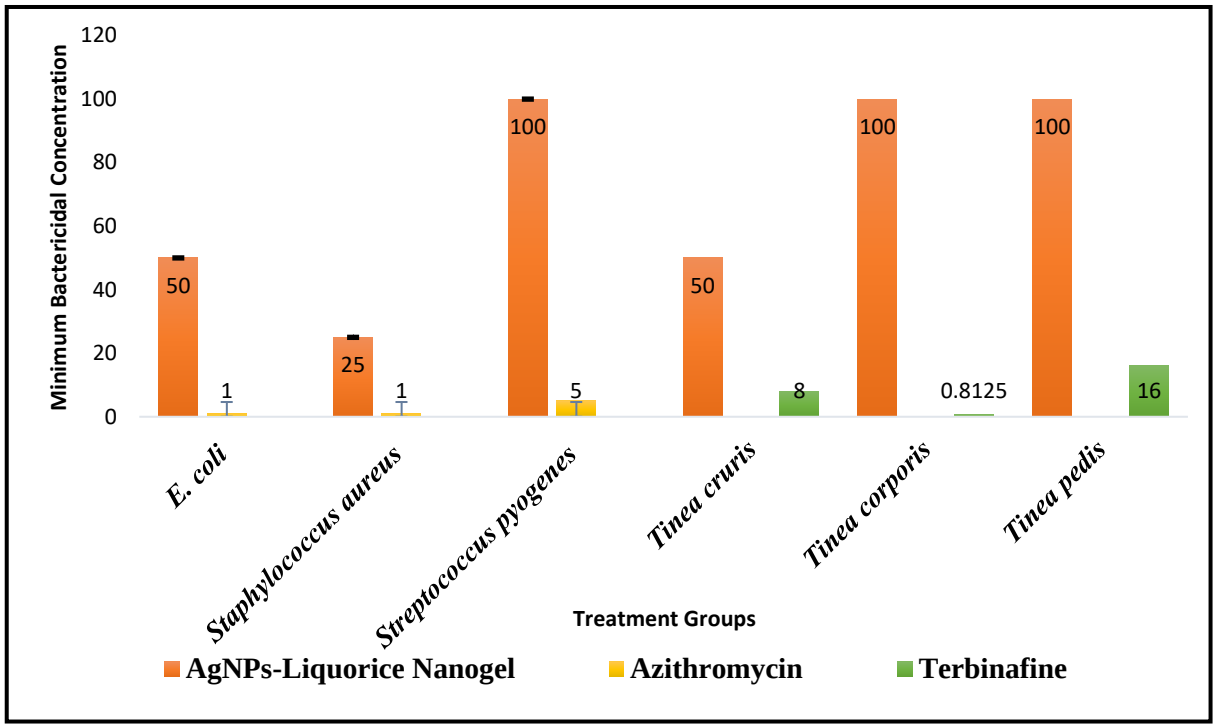

Figure 21: Mean MBC of AgNPs-Liquorice nano gel and Standard drugs against pathogenic organisms

It was observed that AgNPs-Liquorice nano gel induced inhibitory effect on growth of the tested microorganisms as stated above, was comparable to the standard drugs i.e., Azithromycin and Terbinafine. The magnitude of response for AgNPs-Liquorice nano gel recorded as 88.086%, 66.623%, 56.377%, 53.623%, 52.437% and 40.751% bacterial growth inhibition for *Tinea cruris, S. aureus, S. pyogenes, E. coli, T. corporis* and *T. pedis*, respectively. Azithromycin and Terbinafine were used as standard drugs and results showed that Azithromycin has shown 79.948%, 72.307% & 70.281% inhibition against *S. pyogenes, S. aureus* & *E.coli*, respectively, while, Terbinafine has shown more profound percent inhibition against *T. cruris* & *T. corporis* (89.938% & 83.222%, respectively) and about 48.775% inhibition against *T. pedis*. The results were summarized in table 11 & figure 21.

Table 11: Bacterial growth inhibition (culture optical density) of Liquorice-AgNPs nano gel against human pathogen

Organism	% Bacterial Growth inhibition		
	AgNPs-Liquorice Nano gel	Azithromycin	Terbinafine
E. coli	53.623±0.00367	70.281±0.0059	-
Staphylococcus aureus	66.623±0.00353	72.307±0.00424	-
Streptococcus pyogenes	56.377±0.0041	79.948±0.00395	-
Tinea cruris	88.086±0.0016	-	89.938±0.0036
Tinea corporis	52.437±0.0052	-	83.222±0.0026
Tinea pedis	40.751±0.0049	-	48.775±0.0041

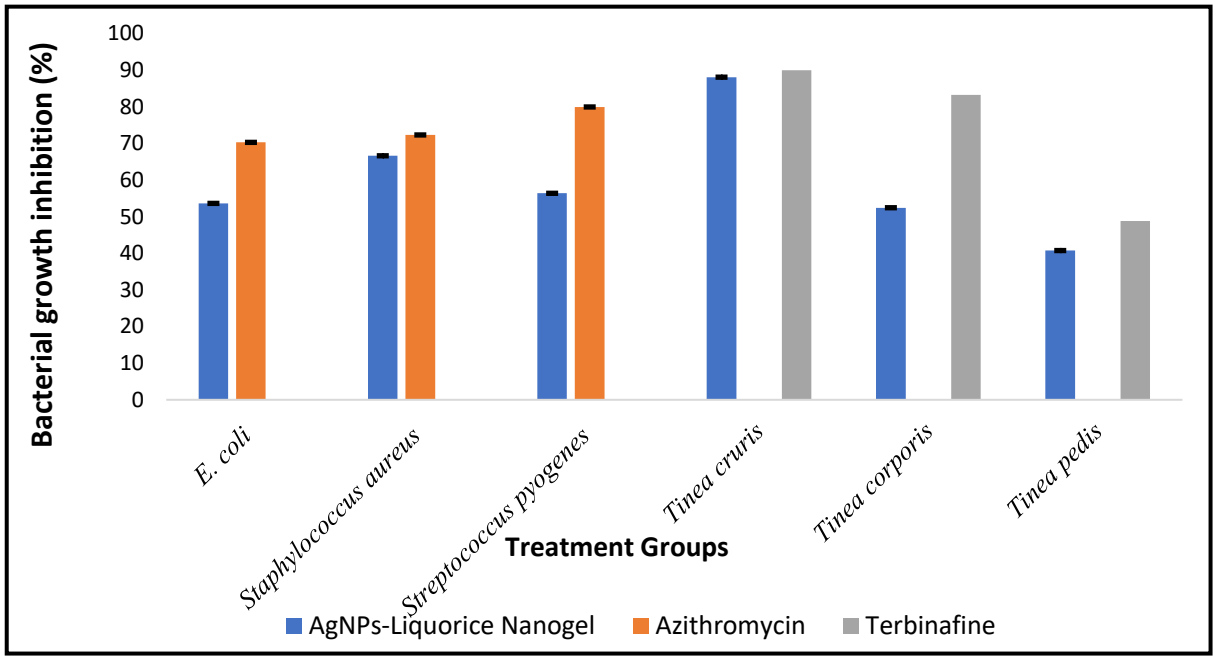

Figure 22: Bacterial growth inhibition (culture optical density) of optimized Nano Gel formulation containing AgNPs of Liquorice extract against human pathogen

CONCLUSION

The study uses Liquorice (*Glycyrrhiza glabra* Lin.) root extract to synthesize silver nanoparticles (AgNPs) and formulate a nano gel. The resulting Liquorice silver nano gel showed good spreadability and viscosity, with a pH between 5.25 and 5.47, close to the skin's optimal pH of 4.75-5.5, indicating no dermal irritation. Among four formulations, F4 was the most promising and underwent further in-vitro drug release, kinetic studies, and anti-microbial activity tests. Stability studies showed no significant changes.The nano gel demonstrated

antioxidant activity comparable to ascorbic acid at high doses, with 84.25 ± 1.12% inhibition at 800 µg/ml. It also exhibited significant antibacterial activity, particularly against Tinea cruris and Staphylococcus aureus, with inhibition rates of 88.086% and 66.623%, respectively. The AgNPs-Liquorice nano gel also inhibited other bacteria, showing potential as an effective antibacterial agent.

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