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Formulation Development of Silver Nanoparticles Gel of Hydroalcoholic Extract of Aconitum Heterophyllum for Antimicrobial Effect

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The present study aimed to develop and evaluate a silver nanoparticle gel formulation containing the hydroalcoholic extract of Aconitum heterophyllum for its antimicrobial effects. Silver nanoparticles were synthesized using the green synthesis method by reducing silver nitrate ($AgNO_3$) with the plant extract. The formulation of the gel involved the incorporation of silver nanoparticles into a gel matrix consisting of Carbopol 940, glycerin, polyethylene glycol, and triethanolamine. The developed formulations (F1, F2, F3) were characterized for physical properties, including color, texture, homogeneity, spreadability, and viscosity. The entrapment efficiency, particle size, and zeta potential were also determined. In vitro drug release studies revealed that formulation GF1 demonstrated rapid release, with nearly 99% drug release in 2.5 hours. Antimicrobial activity was evaluated against Enterococcus faecalis and Klebsiella pneumoniae, and the silver nanoparticle gel showed superior antimicrobial efficacy compared to the plant extract alone. Formulation GF2 exhibited the best antimicrobial activity, with the highest zone of inhibition at 100 mg/mL against both bacterial strains. These findings suggest that the silver nanoparticle gel of Aconitum heterophyllum can be a promising topical antimicrobial formulation.

Keywords: Aconitum heterophyllum, Silver Nanoparticles, Gel Formulation, Antimicrobial Activity, Green Synthesis, Drug Release, Entrapment Efficiency, Enterococcus faecalis, Klebsiella pneumoniae, Topical Application.

1. Introduction

Aconitum heterophyllum, commonly known as Aconite, is an important medicinal plant in traditional herbal medicine, particularly in Ayurveda and Unani systems. Known for its

analgesic, anti-inflammatory, antipyretic, and antimicrobial properties, Aconitum heterophyllum has been used to treat a wide range of ailments, including fever, pain, and infections. The plant contains potent alkaloids such as aconitine, which contribute significantly to its therapeutic effects but also pose a risk of toxicity if not properly prepared or dosed (Sharma et al., 2013). Despite these benefits, the use of Aconitum heterophyllum has been limited due to its toxic effects when consumed inappropriately, which necessitates the development of safe for its medicinal use (Choudhary et al., 2017).

Nanotechnology, particularly the use of silver nanoparticles (AgNPs), has gained significant attention in recent years for its ability to enhance the therapeutic properties of medicinal compounds. AgNPs possess notable antimicrobial, anti-inflammatory, and wound-healing properties due to their small size, high surface area, and ability to interact with microbial cell membranes, leading to increased cell permeability and disruption of cellular processes (Rai et al., 2009). In addition to their intrinsic antimicrobial effects, silver nanoparticles can also serve as carriers for bioactive compounds, improving their stability, solubility, and bioavailability (Hewitt et al., 2012).

Hydroalcoholic extracts, which are prepared using both water and alcohol as solvents, are effective in extracting a broad spectrum of bioactive compounds from plant material. These extracts combine the advantages of both alcohol (for extracting lipophilic compounds) and water (for hydrophilic compounds), making them ideal for the preparation of herbal formulations with enhanced therapeutic potential (Chandrasekaran et al., 2015). By incorporating a hydroalcoholic extract of Aconitum heterophyllum into a silver nanoparticle gel, it is possible to harness the synergistic antimicrobial effects of both the plant extract and silver nanoparticles. Such formulations could provide an effective topical treatment for skin infections, wounds, and other microbial conditions while minimizing the toxicity associated with the direct use of Aconitum heterophyllum.

The combination of silver nanoparticles and hydroalcoholic plant extracts in gel formulations offers numerous benefits, including sustained release, improved stability, and enhanced antimicrobial activity, making it a promising approach for localized drug delivery. This study aims to develop and characterize a silver nanoparticle gel containing the hydroalcoholic extract of Aconitum heterophyllum to explore its potential antimicrobial activity and therapeutic applications in wound healing and infection management.

2. Material and Methods

Preparation of Aconitum heterophyllum extracts solution

For preparation of solution 10 g extract of Aconitum heterophyllum was added into 100 mL deionized water. Then it was heated at 100°C for 20 to 30 minutes. The obtain solution was filtered using filter paper (Whatman no. 1) and stored at 4°C for further use.

Precursor Preparation

Silver nitrate ($AgNO_3$) was used as precursor for the synthesis of silver nanoparticles from the Aconitum heterophyllum extract. 1 mM solution of silver nitrate was prepared using double distilled water and stored at 4°C in refrigerator.

Biosynthesis of Silver Nanoparticles

The green synthesis method was followed for the synthesis of silver nanoparticles. For the reduction of Ag^+ ions, 1 mL hydroalcoholic extract of Aconitum heterophyllum was added drop wise into 100 mL of 1 mM aqueous solution of $AgNO_3$ and heated at 60–80°C for 1 hour. The change in color was observed from dark brown to reddish brown which indicated the formation of silver nanoparticles. Similarly, 0.5, 1.0, 1.5 and 2.0 mL of Aconitum heterophyllum extract were suspended for the preparation of F1, F2, F3, and F4 respectively (Asif et al., 2022).

Table 1: Different formulation of silver nanoparticles

F. Code	1mM $AgNO_3$ (ml)	Extract (ml)	Ratio $AgNO_3$/ Extract (ml)	Reaction Time (Min.)	Temperature (^{0}C)
F1	50	0.1	1/100	60	60-80
F2	50	1.0	1/50	60	60-80
F3	50	1.5	1/25	60	60-80
F4	50	2.0	1/20	60	60-80

Characterization of synthesized silver nanoparticles formulations

UV-VIS Absorption Spectra Analysis

UV-VIS spectrophotometer was used for the determination of optical properties of M. oleifera leaves mediated AgNPs. The formation of the AgNPs was followed by measuring the spectra over the range of 200–800 nm (Labindia 3000+). Prior studies displayed the silver nanoparticles (AgNPs) contributed to the absorption at around the 390–470 nm in the UV-VIS spectra.

Percentage yield

The silver nanoparticles, prepared with a size range of 200-300 nm, were gathered and quantified from various formulations. The calculated weight was then divided by the total quantity of all non-volatile components utilized in the microsphere preparation (Vanaja et al., 2013; Umashankari et al., 2012).

$$\% \text{ Yield } = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} \text{x } 100$$

Entrapment efficiency

The entrapment efficiency of the drug was defined as the ratio of the mass of the drug associated with the formulations to the total mass of the drug. The entrapment efficiency was assessed using the dialysis method, where the silver nanoparticle-entrapped extract was separated from the free drug. For this purpose, the aforementioned formulations were loaded into dialysis bags, and the free drug was dialyzed for 24 hours in 50 ml of buffer at pH 1.2. The absorbance of the dialysate was measured against a blank buffer at pH 1.2, and the absorbance of the corresponding blank was measured under the same conditions. The concentration of free flavonoids was determined based on the absorbance difference using a standard curve (Banerjee et al., 2014).

Surface charge and vesicle size

The particle size, size distribution, and surface charge were determined using the Dynamic Light Scattering method (DLS) with a Malvern Zetamaster, ZEM 5002 instrument from Malvern, UK, at SAIF RGPV Bhopal. Zeta potential measurements for the silver nanoparticles were conducted based on the Helmholtz–Smoluchowsky equation derived from electrophoretic mobility. For zeta potential measurement, a zetasizer was employed with a field strength of 20 V/cm in a large bore measurement cell. Samples were appropriately diluted with 0.9% NaCl and adjusted to a conductivity of 50 µS/cm.

Formulation development of silver nanoparticle gel

Precise quantities of methyl paraben, glycerin, polyethylene glycol, and hydroalcoholic extract of Aconitum heterophyllum were dissolved in approximately 100 ml of water in a beaker. The mixture was vigorously stirred using a mechanical stirrer or sonicator, following the method described by Raut et al. in 2009. Subsequently, Carbopol 940 was gradually introduced into the beaker containing the aforementioned liquid while maintaining continuous stirring. The solution was neutralized by slowly adding a triethanolamine solution, stirring constantly, until the gel formation occurred.

Table 2: Formulation of gel

Ingredients (mg)	F1	F 2	F3
Extract loaded silver nanoparticle	500	500	500
Carbopol 940	250	500	750
Polyethylene Glycol 600	0.2	0.2	0.2
Methyl Paraben	0.08	0.08	0.08
Triethanolamine	1.0	1.0	1.0
Distilled Water	100 ml	100ml	100ml

Evaluation of gel

Appearance and Consistency

The physical appearance and texture of gel formulations were visually inspected.

Washability

Formulations were applied to the skin and manually assessed for ease and degree of washing with water.

Extrudability Determination

Gel formulations were filled into aluminum collapsible tubes, sealed, and pressed to extrude the material. Extrudability of the formulation was noted.

Determination of Spreadability

Two glass slides were selected, and the gel formulation was placed over one slide. The second slide was placed over the formulation, sandwiching it over a length of 6 cm. A 20-gram weight

was applied, forming a thin layer. The time taken for the slides to separate under the weight was recorded.

$S = M \times L / T$

(S) weight in the pan (M), the length moved by the glass slide (L), and the time it takes to separate the slide completely from each other (T)

Viscosity: The viscosity of the gel was determined using a Brookfield digital viscometer with spindle no. 6 at 10 rpm and at a room temperature of 25-30°C. Measurements were taken after allowing the gel samples to settle for more than 30 minutes.

Drug Content: The drug content was measured by dissolving 1g of gel in methanol in a 10 ml volumetric flask. A mixture of 3 ml of stock solution and 1 ml $AlCl_3$ solution (2%) was vortexed, and the color production was allowed to stand at 40°C for 30 minutes. Absorbance was measured at 420 nm using a spectrophotometer.

Determination of pH: The pH of the gels was measured using a digital pH meter. One gram of gel was dissolved in 25 ml of purified water, and the electrode was dipped into the gel solution until a steady reading was obtained. pH measurements were repeated twice for each formulation.

In vitro Diffusion Profile (In vitro Permeation in Rat Skin): In vitro diffusion experiments were conducted using Franz diffusion cells. Rat abdominal skin was used as the membrane for dialysis, tied to the diffusion cell. Isotonic phosphate buffer solution (pH 7.4) served as the substrate for receptors. A weighed quantity of the formulation equivalent to 1g of gel was applied to the rat skin, and aliquots were withdrawn at different time intervals, measured at 295 nm. The total percent release was calculated for each time period, and the diffusion media were replaced with fresh medium after each withdrawal.

Antimicrobial activity of silver nanoparticle gel

Three concentrations (25, 50, and 100 mg/ml) of silver nanoparticle gel were used. Wells containing silver nanoparticle gel were placed on the agar surface immediately after inoculation with the test organism. Undiluted overnight broth cultures were avoided as inoculums. The plates were then incubated at 37°C for 24 hours and examined for clear zones of inhibition around the wells with specific concentrations of the drug, following the standard procedure by Bauer et al. (1966).

3. Results and Discussion

The development of silver nanoparticles (AgNPs) incorporated into a gel formulation containing the hydroalcoholic extract of Aconitum heterophyllum has been successfully achieved using green synthesis methods. The process of synthesizing AgNPs with the plant extract involved the reduction of silver ions (Ag^+) using the bioactive compounds present in the extract, which resulted in the formation of silver nanoparticles. The color change from colorless to reddish-brown in the reaction mixture confirmed the formation of AgNPs, as is typically observed in green synthesis methods.

The formulation of the silver nanoparticle gel was designed to improve the antimicrobial efficacy of the Aconitum heterophyllum extract by utilizing the properties of silver nanoparticles, which are known for their potent antimicrobial activity. The preparation of the gel involved the dissolution of excipients such as Carbopol 940, glycerin, and polyethylene glycol, with the silver nanoparticles being incorporated to achieve the final gel formulation. The process was optimized by maintaining appropriate stirring and using triethanolamine for gel formation, ensuring consistency and stability in the formulation.

From the results obtained, the physical properties of the gel formulations were evaluated, including color, homogeneity, texture, washability, and extrudability. All formulations exhibited good homogeneity, smooth texture, and satisfactory washability and extrudability, indicating that the gels were well-prepared and suitable for topical application. The spreadability of the gel formulations was also tested, and formulation F1 showed the highest spreadability (12.25±0.25 gcm/sec), which indicates its ease of application on the skin.

The viscosity of the gel formulations (GF1, GF2, GF3) was found to be in the range of 3125-3465 cP, which suggests that the gels had an adequate thickness for topical application, offering a smooth application without being too runny. Viscosity is an important parameter for gel formulations, as it affects both the spreadability and the retention of the gel on the application site. The pH of the formulations was found to be slightly acidic (6.59–6.80), which is ideal for skin applications as it matches the natural pH of human skin, thus minimizing the risk of skin irritation.

The entrapment efficiency, which reflects the ability of the formulation to incorporate and retain bioactive compounds, was evaluated for each formulation. Formulation F3 exhibited the highest entrapment efficiency (0.715±0.016 mg flavonoid/100mg), indicating that this formulation could effectively deliver a higher amount of active compounds, potentially enhancing the therapeutic effects of Aconitum heterophyllum.

The release study of the silver nanoparticle gel showed that the cumulative drug release increased with time across all formulations, with formulation GF1 demonstrating the fastest release profile. Formulation GF1 released 30.25% of the drug in the first 0.25 hours and reached 98.88% release at the 2.5-hour mark, suggesting that it could provide rapid relief when applied topically. This rapid release of bioactive compounds could be beneficial for infections that require immediate antimicrobial action.

The antimicrobial activity of the silver nanoparticle gel against Enterococcus faecalis and Klebsiella pneumoniae was significantly higher than the activity of the plain plant extract. At 100 mg/mL, the silver nanoparticle gel (GF2) exhibited the largest zone of inhibition against Enterococcus faecalis (16.0±0.25 mm) and Klebsiella pneumoniae (15.0±0.10 mm), demonstrating the enhanced antimicrobial effect of the gel. The incorporation of silver nanoparticles in the formulation significantly improved the antimicrobial efficacy compared to the hydroalcoholic extract alone, as silver nanoparticles possess intrinsic antimicrobial properties. The improved activity can be attributed to the synergistic effect between the Aconitum heterophyllum extract and the silver nanoparticles, both of which act on microbial cells by disrupting their membrane integrity and inhibiting growth.

The results of this study suggest that silver nanoparticle gels containing the hydroalcoholic extract of Aconitum heterophyllum offer promising antimicrobial activity and could be considered for further development in topical formulations for the treatment of skin infections. The formulation not only enhances the antimicrobial effects of the plant extract but also ensures the controlled release of bioactive compounds, improving their therapeutic potential.

Results of UV-VIS Absorption Spectra Analysis

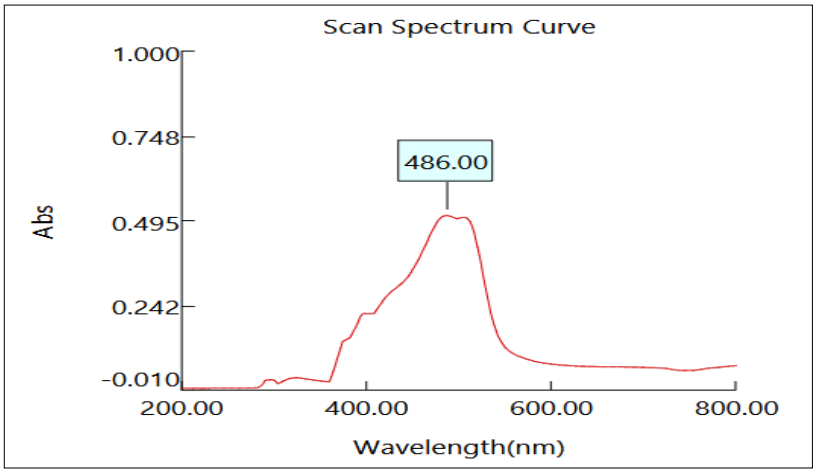

Figure 1: Spectra of UV-VIS Absorption Spectra Analysis of Aconitum heterophyllum silver nanoparticles at 486nm

Table 3: Determination of % yield of prepared silver nanoparticles formulations

Formulation code	% Yield
F1	69.98±0.25
F2	72.25±0.13
F3	76.65±0.22
F4	71.12±0.15

Table 4: Determination of entrapment efficiency of prepared formulations

Formulation code	Percentage entrapment efficiency (Flavonoid mg/100mg quercetin equivalent)
F1	0.558±0.012
F2	0.612±0.015
F3	0.715±0.016
F4	0.632±0.022

Table 5: Characterization of average particle size and zeta potential of optimized formulation F3

Formulation code	Average Particle size (nm)	Zeta Potential (mV)
F3	18.52	- 42.5 mV

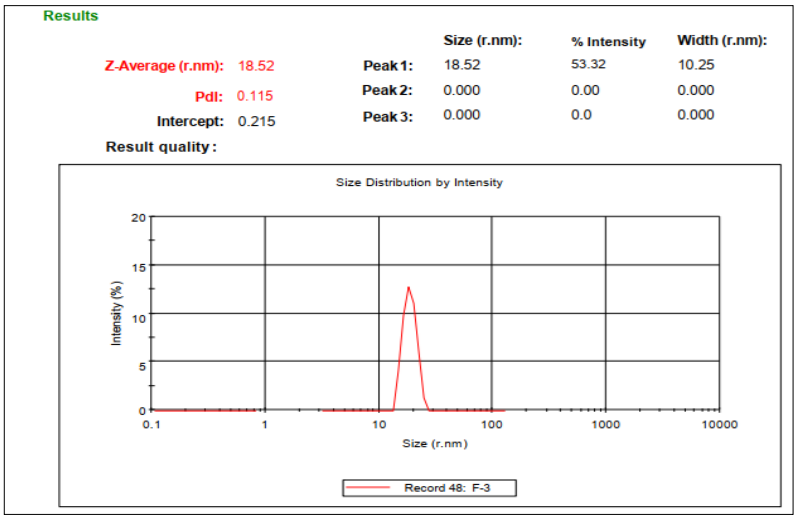

Figure 2: Graph of average particle size of formulation F3

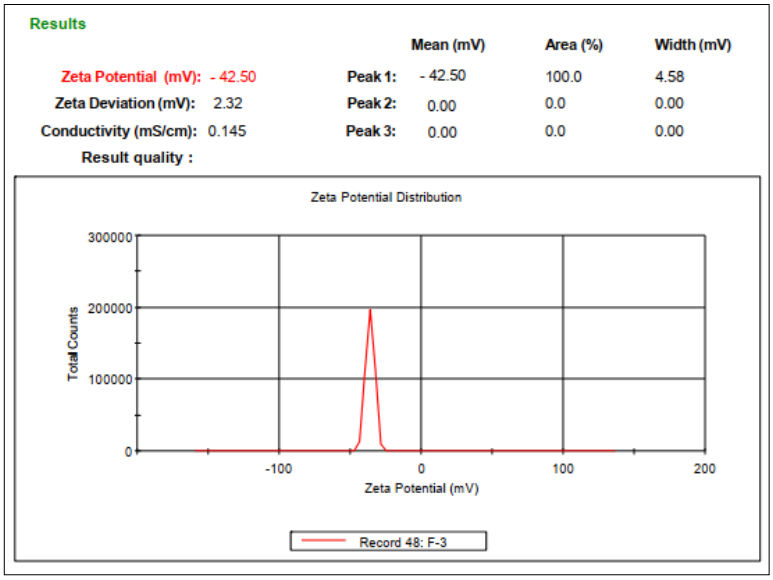

Figure 3: Graph of Average vesicle size formulation F3

Table 6: Results of physical characteristics

Formulation code	Colour	Clogging	Homogeneity	Texture	Washability	Extrudability
GF1	Brown	Absent	Good	Smooth	Good	Good
GF2	Brown	Absent	Good	Smooth	Good	Good
GF3	Brown	Absent	Good	Smooth	Good	Good

Table 7: Results of spreadability of gel

Formulation code	Spreadability* (gcm/sec)
GF1	12.25±0.25
GF2	11.32±0.15
GF3	10.56±0.23

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

Table 8: Results of viscosity of gel

Formulation code	Viscosity* (cp)
GF1	3125±10
GF2	3365±12
GF3	3465±13

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

Table 9: Results of flavonoid content in gel using $AlCl_3$ method

Formulation code	Flavonoid Content (mg/100mg)
GF1	0.552±0.012
GF2	0.615±0.015
GF3	0.485±0.018

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

Table 10: Results of pH of gel

Formulation code	pH
GF1	6.78±0.06
GF2	6.80±0.03
GF3	6.59±0.07

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

Table 11: In vitro drug release study of prepared gel formulation

S. No.	Time (hr)	% Cumulative Drug Release		
		GF1	GF2	GF3
1	0.25	30.25	22.25	15.65
2	0.5	40.25	36.65	23.32
3	1	62.23	43.32	36.65
4	1.5	71.15	55.45	49.98
5	2	88.85	68.85	62.23
6	2.5	98.88	79.98	73.32
7	3		87.74	85.65
8	4		98.58	93.32

Table 12: Antimicrobial activity against selected microbes

S. No.	Microbes	Zone of inhibition (mm)		
		25mg/ml	50mg/ml	100mg/ml
		Hydroalcoholic extract		
1.	Enterococcus faecalis	7.0±0.57	7.4±0.94	10.0±0.84
2.	Klebsiella pneumoniae	6.0±0.5	7.0±0.47	8.0±0.57
		Silver nanoparticles gel (GF2)		
1.	Enterococcus faecalis	10.0±0.2	11.0±0.15	16.0±0.25
2.	Klebsiella pneumoniae	11.0±0.11	13.0±0.13	15.0±0.10

Figure 4: Antimicrobial activity of hydroalcoholic extract against selected microbes

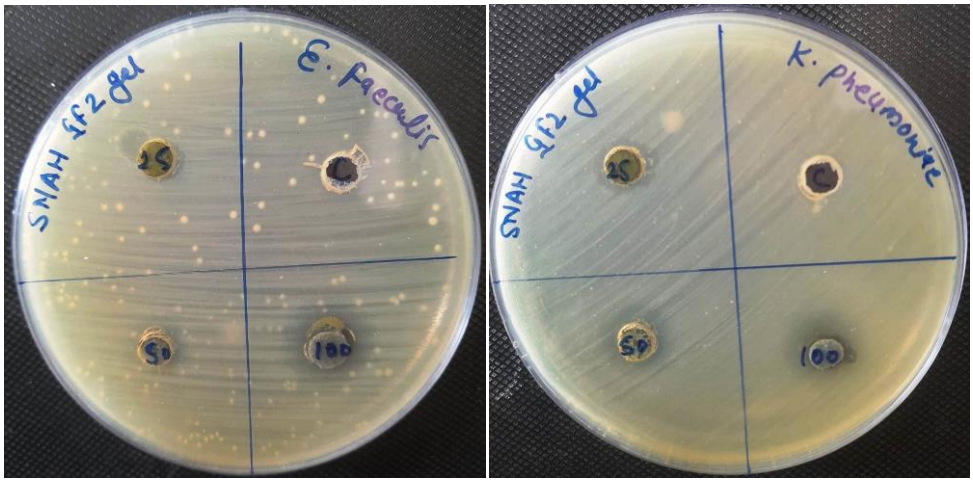

Figure 5: Antimicrobial activity of silver nanoparticles gel (GF2) against selected microbes

4. Conclusion

In conclusion, the formulation of silver nanoparticles loaded with the hydroalcoholic extract of Aconitum heterophyllum in gel form has demonstrated excellent antimicrobial properties, with formulation GF2 showing the most promising results. This formulation exhibited good physical characteristics, high entrapment efficiency, and effective drug release, along with superior antimicrobial activity against common pathogens. The synergistic effect of silver nanoparticles and the plant extract makes this formulation a potential candidate for future clinical applications, particularly for the treatment of topical infections. Further studies, including in vivo testing, are warranted to evaluate the safety and efficacy of the formulation in real-world scenarios.

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