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FORMULATION AND EVALUATION OF SILVER NANOPARTICLES INCORPORATED GEL OF ACONITUM FEROX FOR EFFECTIVE FUNGAL TREATMENT

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ABSTRACT

Fungal infections affecting the skin, hair, and nails are among the most prevalent infectious diseases worldwide and continue to increase due to immunosuppression, antimicrobial resistance, and environmental factors. Conventional antifungal therapies often suffer from toxicity, resistance, and limited efficacy. Therefore, the development of novel antimicrobial systems is essential. Silver nanoparticles (AgNPs) have emerged as potent antimicrobial agents because of their broad-spectrum activity, multi-target mechanisms, and low incidence of microbial resistance. The present study aimed to synthesize silver nanoparticles using *Aconitum ferox* root extract through a green synthesis approach and formulate them into a topical gel for antifungal application. Root extracts were prepared using hydroalcoholic extraction after defatting with petroleum ether. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, phenols, saponins, proteins, carbohydrates, glycosides, and diterpenes. Total flavonoid and phenolic contents were estimated using aluminium chloride and Folin-Ciocalteu methods, respectively. AgNPs were biosynthesized using varying concentrations of silver nitrate and characterized by UV-Visible spectroscopy, FTIR, particle size analysis, zeta potential, and entrapment efficiency studies. The nanoparticle-loaded gel was prepared using Carbopol 940 and evaluated for physicochemical properties including pH, spreadability, viscosity, extrudability, drug content, and in vitro drug release. Antifungal activity was assessed by the well diffusion method using potato dextrose agar medium. The formulated AgNP gel demonstrated promising antifungal activity,

suggesting that *Aconitum ferox*-mediated silver nanoparticles may serve as an effective topical antifungal delivery system.

KEYWORDS: Silver nanoparticles, *Aconitum ferox*, antifungal activity, green synthesis, topical gel, phytochemical screening.

1. INTRODUCTION

Fungal infections of the skin, hair, and nails are major global health concerns affecting approximately 20-25% of the world population. These infections are often chronic, recurrent, and underdiagnosed. The susceptibility to fungal infections is strongly associated with immune status and genetic predisposition. Immunocompromised individuals, including patients undergoing chemotherapy or suffering from prolonged neutropenia, are highly susceptible to opportunistic fungal pathogens such as *Candida*, *Aspergillus*, *Fusarium*, and *Scedosporium* species.

Dermatophytosis is among the most common fungal infections and is caused by keratinophilic fungi including *Trichophyton*, *Microsporum*, and *Epidermophyton*. Common manifestations include tinea pedis, tinea corporis, tinea capitis, and onychomycosis. In addition, candidiasis caused by *Candida albicans* commonly affects mucosal and intertriginous areas, particularly in individuals with weakened immunity or prolonged antibiotic use. *Malassezia* species are also associated with superficial fungal disorders such as pityriasis versicolor and seborrheic dermatitis.

Nanotechnology has emerged as a promising strategy for combating microbial infections. Among various nanomaterials, silver nanoparticles (AgNPs) have gained significant attention due to their potent antimicrobial activity against bacteria, fungi, and viruses. AgNPs exhibit enhanced biological activity because of their nanoscale size (1-100 nm) and high surface area-to-volume ratio. Their antimicrobial action involves multiple simultaneous mechanisms including membrane disruption, DNA damage, protein denaturation, oxidative stress induction, and inhibition of biofilm formation.

Green synthesis of nanoparticles using plant extracts has become increasingly important because it provides an eco-friendly, cost-effective, and sustainable alternative to conventional chemical and physical methods. Plant metabolites act as both reducing and stabilizing agents during nanoparticle synthesis.

Aconitum ferox is a medicinal plant known for its bioactive phytoconstituents and therapeutic properties. The present study focuses on the green synthesis of silver nanoparticles using

Aconitum ferox root extract and their incorporation into a topical gel formulation for antifungal applications.

2. MATERIALS AND METHODS

The present study was carried out to synthesize and evaluate silver nanoparticles using *Aconitum ferox* root extract for antifungal applications. The roots of *Aconitum ferox* were collected from Bhopal in February 2024, shade dried, powdered, and stored in airtight containers for further use. The powdered plant material was initially defatted with petroleum ether by maceration to remove non-polar impurities. The defatted marc was subsequently extracted using a hydroalcoholic solvent system consisting of ethanol and water in the ratio of 70:30 through maceration for 48 hours. The obtained extract was filtered and concentrated under reduced pressure using a vacuum evaporator at 40°C. The percentage yield of the extract was calculated based on the weight of dried extract obtained relative to the initial powdered material.

Preliminary phytochemical screening of the hydroalcoholic extract was performed using standard qualitative methods to identify the presence of bioactive constituents such as alkaloids, carbohydrates, glycosides, flavonoids, tannins, phenols, saponins, proteins, and diterpenes. Alkaloids were detected using Mayer's, Wagner's, Dragendorff's, and Hager's tests, while carbohydrates were identified by Molisch's, Benedict's, and Fehling's tests. Glycosides were analyzed using Modified Borntrager's and Legal's tests. Flavonoids were confirmed by alkaline reagent and lead acetate tests, phenols by ferric chloride test, tannins by gelatin test, saponins by froth and foam tests, proteins by xanthoproteic test, and diterpenes by copper acetate test. Total flavonoid content was estimated using the aluminium chloride colorimetric method with quercetin as the standard, and absorbance was measured at 420 nm. Total phenolic content was determined using the Folin-Ciocalteu method with gallic acid as the standard and absorbance recorded at 765 nm using a UV spectrophotometer.

Silver nanoparticles (AgNPs) were synthesized by the green synthesis method using the hydroalcoholic extract of *Aconitum ferox*. Different concentrations of silver nitrate (1-6 mM) were prepared and mixed with the plant extract in varying ratios ranging from 1:1 to 1:2 (v/v) in a total reaction volume of 50 ml. The reaction mixtures were covered with aluminium foil to prevent light exposure and incubated at 60°C for 5 hours in a water bath. Formation of silver nanoparticles was indicated by a visible colour change from pale yellow to dark brown. Various nanoparticle formulations (F1-F6) were prepared using 500 mg of extract with different concentrations of silver nitrate while maintaining a constant ratio.

The synthesized silver nanoparticles were characterized for percentage yield, entrapment efficiency, particle size, particle size distribution, and zeta potential. Entrapment efficiency was determined using the dialysis method in pH 1.2 buffers, where free flavonoids diffused through the membrane and were quantified spectrophotometrically. Particle size and zeta potential analyses were carried out using Dynamic Light Scattering (DLS) and zeta sizer instruments after suitable dilution of samples. UV-Visible spectroscopy was used to confirm nanoparticle formation through surface plasmon resonance, while FTIR analysis was employed to identify the functional groups responsible for reduction and stabilization of nanoparticles.

For topical delivery, silver nanoparticle gel formulations were prepared using Carbopol 940 as the gelling agent along with polyethylene glycol 600, glycerin, methyl paraben, triethanolamine, and distilled water. Carbopol 940 was gradually added to the prepared mixture under continuous stirring followed by neutralization with triethanolamine to obtain a homogeneous gel. Three formulations (F1, F2, and F3) containing varying concentrations of Carbopol 940 (250, 500, and 750 mg respectively) were prepared using 500 mg of *Aconitum ferox* extract.

The prepared gels were evaluated for physical appearance, homogeneity, consistency, washability, extrudability, spreadability, viscosity, pH, drug content, and in vitro drug release. Spreadability was determined using a two-slide method by measuring the time required for separation of slides under a fixed load. Viscosity was measured using a Brookfield viscometer after stabilization of samples at controlled temperature. Drug content was estimated spectrophotometrically following methanolic extraction and reaction with aluminium chloride. The pH of the gel was measured using a digital pH meter. In vitro diffusion studies were carried out using Franz diffusion cells with rat skin as the diffusion membrane and phosphate buffer pH 7.4 as the receptor medium. Samples were withdrawn at predetermined intervals and analyzed spectrophotometrically. The release data obtained were fitted into different kinetic models including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to determine the mechanism of drug release.

The antifungal activity of the prepared silver nanoparticle gel was evaluated using the well diffusion method. Potato dextrose agar (PDA) medium was prepared, sterilized, and poured into sterile Petri plates. Fungal cultures were revived in broth medium and subcultured onto agar plates to obtain pure cultures. Different concentrations of the extract (25, 50, and 100 mg/ml) were introduced into wells made in inoculated agar plates. The plates were incubated

at 25°C for 48 hours, and the antifungal activity was determined by measuring the zones of inhibition around the wells according to the method described by Bauer et al. (1966).

3. RESULTS AND DISCUSSION

3.1 Percentage Yield of Extract

The percentage yield of extraction is an important parameter for evaluating the efficiency of different solvents used in phytochemical extraction. In the present study, the hydroalcoholic extract of roots of Aconitum ferox showed a higher yield (6.9% w/w) compared to the petroleum ether extract (2.5% w/w). The higher yield obtained with the hydroalcoholic solvent indicates its greater ability to dissolve and extract polar phytoconstituents from the plant material.

Table 1: Percentage Yield of Extracts.

S. No.	Extract	% Yield (w/w)
1.	Pet ether	2.5%
2.	Hydroalcoholic	6.9%

3.2 Phytochemical Screening of Extract

Preliminary phytochemical screening of the hydroalcoholic root extract of Aconitum ferox revealed the presence of several important bioactive constituents. The extract tested positive for glycosides, flavonoids, phenols, proteins, carbohydrates, saponins, and tannins, while alkaloids and diterpenes were absent in most of the tests performed. The presence of these phytochemicals indicates the therapeutic potential of the extract, particularly due to flavonoids and phenolic compounds which are known for antioxidant and antimicrobial activities.

Table 2: Phytochemical screening of extract of roots of Aconitum ferox.

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendorff's Test	-ve
	Hager's Test	+ve
2.	Glycosides	+ve
	Legal's Test	
3.	Flavonoids	
	Lead acetate	+ve
	Alkaline test	-ve
4.	Phenol	
	Ferric chloride test	+ve

5.	Proteins Xanthoproteic test	 +ve
6.	Carbohydrates Molisch's Test Benedict's Test Fehling's Test	 -ve +ve +ve
7.	Saponins Froth Test Foam Test	 +ve -ve
8.	Diterpenes Copper acetate test	 -ve
9.	Tannins Gelatin Test	 +ve

[+ve = positive; -ve = negative]

3.3 Estimation of Total Flavonoid and Phenolic Content

3.3.1 Total Flavonoid Content (TFC)

The total flavonoid content of the hydroalcoholic extract was determined using the quercetin calibration curve with the regression equation:

$y = 0.039x + 0.010$, $R^2 = 0.999$

Table 3. Preparation of Calibration Curve of Quercetin.

S. No.	Concentration (µg/mL)	Mean Absorbance
1	5	0.204
2	10	0.412
3	15	0.604
4	20	0.798
5	25	0.971

The calibration curve showed excellent linearity with an R^2 value of 0.999. The total flavonoid content of the hydroalcoholic extract was found to be 0.761 mg/100 mg quercetin equivalent, indicating a considerable presence of flavonoids in the extract.

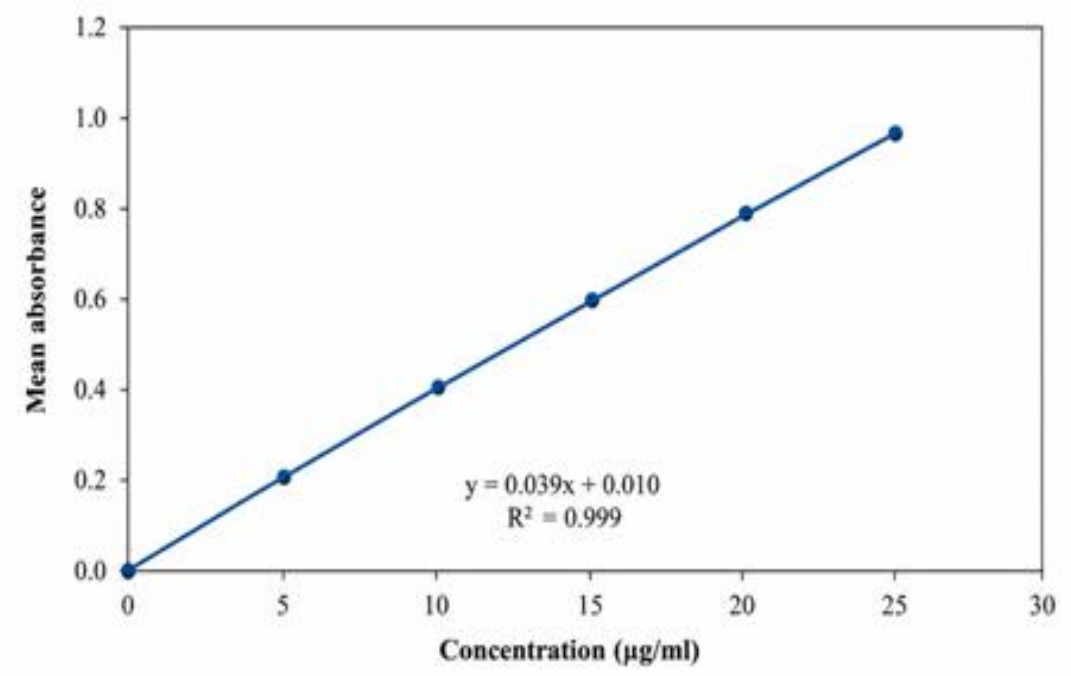

Fig 1. Calibration Curve of Quarcetin.

3.3.2 Total Phenolic Content (TPC)

The total phenolic content was estimated using gallic acid as standard with the regression equation:

Table 4. Preparation of calibration curve of Gallic acid.

S. No.	Concentration (µg/ml)	Mean absorbance
1	10	0.185
2	20	0.347
3	30	0.514
4	40	0.676
5	50	0.857

$y = 0.016x + 0.006$, $R^2 = 0.999$

The calibration curve also exhibited excellent linearity ($R^2 = 0.999$). The total phenolic content of the hydroalcoholic extract was found to be 0.523 mg/100 mg gallic acid equivalent, suggesting the extract possesses significant phenolic constituents responsible for antioxidant activity.

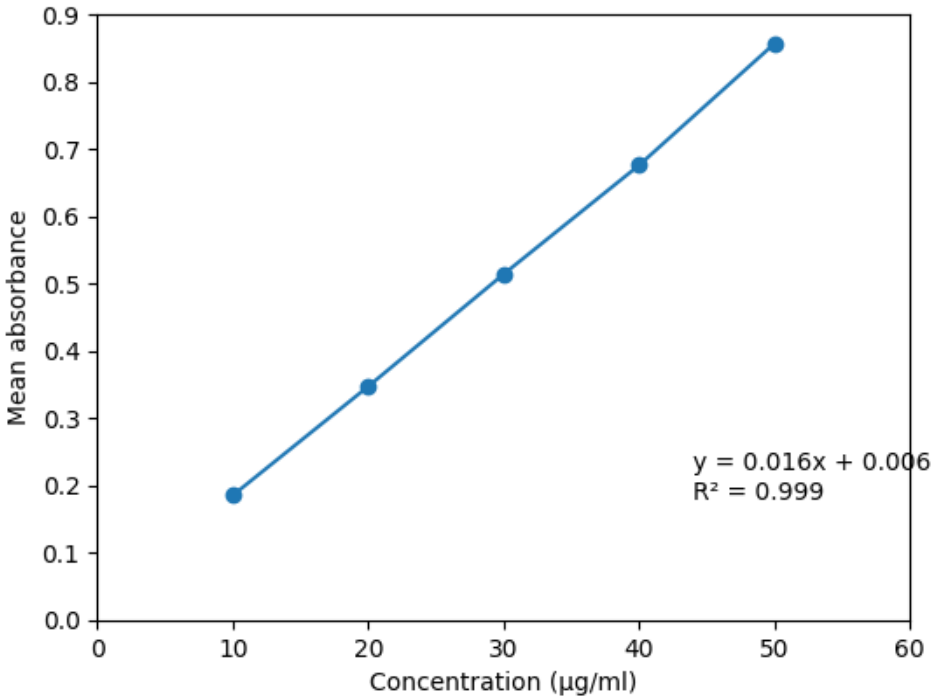

Fig 2. Graph of calibration curve of Gallic acid.

Table 5: Estimation of total flavonoids and phenol content of *Aconitum ferox*.

S. No.	Extract	Total flavonoids content (mg/100 mg of dried extract)	Total phenol content (mg/100 mg of dried extract)
1.	Hydroalcoholic	0.761	0.523

3.4 Characterization of Optimized Silver Nanoparticles

3.4.1 Percentage Yield of Silver Nanoparticles

Among all the prepared silver nanoparticle formulations (F1–F6), formulation F3 exhibited the highest percentage yield (78.85 ± 0.15%), indicating optimum synthesis conditions for nanoparticle production. Formulations F2 and F4 also demonstrated satisfactory yields, whereas F1, F5, and F6 showed comparatively lower yields. The variation in yield may be attributed to differences in reaction conditions such as concentration of reducing agents, pH, temperature, and stabilization efficiency. The results indicate that formulation F3 was the most efficient formulation for silver nanoparticle synthesis.

3.4.2 Entrapment Efficiency

Entrapment efficiency studies revealed that formulation F3 showed the highest flavonoid entrapment efficiency (0.785 ± 0.004 mg/100 mg quercetin equivalent), indicating superior encapsulation capability. Formulations F4 and F5 also showed good entrapment efficiency, whereas F1, F2, and F6 exhibited comparatively lower values. The improved entrapment

efficiency of F3 may be due to optimized formulation variables and better interaction between flavonoids and the nanoparticle matrix.

3.4.3 Particle Size and Zeta Potential

The optimized formulation F3 showed an average particle size of 225.5 nm, which is considered suitable for drug delivery applications. The zeta potential was found to be -35.65 mV, indicating good physical stability due to strong electrostatic repulsion between particles. These findings suggest that formulation F3 possesses desirable nanoparticle characteristics with reduced aggregation tendency and enhanced stability.

3.5 Evaluation of Gel Formulation

3.5.1 Physical Characteristics

The prepared gel formulations (GF1, GF2, and GF3) were evaluated for physical appearance and performance characteristics. All formulations were brown in color, homogeneous, smooth in texture, free from clogging, and showed good washability and extrudability. These findings indicate uniform formulation quality and suitability for topical application.

3.5.2 Spreadability

Spreadability studies demonstrated that formulation GF1 had the highest spreadability (12.32 ± 0.45 g·cm/sec), followed by GF2 and GF3. The higher spreadability of GF1 indicates ease of application and better distribution over the skin surface, whereas the comparatively lower spreadability of GF3 may be due to higher viscosity.

3.5.3 Viscosity

Viscosity evaluation revealed that GF3 exhibited the highest viscosity (3650 ± 15 cp), followed by GF2 and GF1. The increase in viscosity from GF1 to GF3 suggests improved gel consistency and stability; however, highly viscous formulations may reduce ease of spreading. Thus, viscosity directly influenced the spreadability characteristics of the gels.

3.5.4 Flavonoid Content

The flavonoid content analysis showed that GF2 contained the highest flavonoid concentration (0.745 ± 0.032 mg/100 mg), indicating better incorporation of active constituents within the gel matrix. GF1 and GF3 also showed appreciable flavonoid content but were comparatively lower than GF2.

3.5.5 pH Determination

The pH values of all gel formulations ranged between 6.72 and 6.82, which are close to the physiological pH of skin. This indicates that the formulations are suitable for topical application and are less likely to cause skin irritation.

3.5.6 *In-Vitro* Drug Release Study

The in vitro drug release study demonstrated that GF1 showed the highest cumulative drug release (99.02%) over 4 hours, followed closely by GF2. GF3 exhibited comparatively slower drug release, possibly due to its higher viscosity. Kinetic analysis of GF2 indicated that the drug release followed zero-order kinetics with a regression coefficient of 0.992, suggesting a controlled and concentration-independent release profile. In comparison, the first-order model showed a lower regression value (0.9044), indicating a less suitable fit.

3.5.7 *In-Vitro* Antifungal Activity

The antifungal activity against Candida albicans infection demonstrated that the optimized silver nanoparticle gel formulation GF2 exhibited greater antifungal efficacy compared to the crude extract at all tested concentrations (25, 50, and 100 mg/ml). The zones of inhibition produced by GF2 were consistently larger than those of the extract, indicating enhanced antimicrobial activity due to the presence of silver nanoparticles and improved delivery through the gel system. The antifungal activity increased with increasing concentration in both formulations, with GF2 showing maximum inhibition at 100 mg/ml (13.65 mm). These findings suggest that the silver nanoparticle gel possesses significant potential as an effective topical antifungal formulation.

4. CONCLUSION

The present study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using the hydroalcoholic root extract of *Aconitum ferox* and their incorporation into a topical gel formulation for antifungal therapy. Preliminary phytochemical screening confirmed the presence of important bioactive constituents, including flavonoids, phenolics, glycosides, saponins, tannins, proteins, and carbohydrates, which likely contributed to the reduction and stabilization of the synthesized nanoparticles.

Among the prepared nanoparticle formulations, formulation F3 exhibited the highest percentage yield, optimum entrapment efficiency, desirable particle size (225.5 nm), and a zeta potential of −35.65 mV, indicating good stability. The nanoparticle-loaded gel formulations prepared using Carbopol 940 showed satisfactory physicochemical properties, including acceptable pH, homogeneity, viscosity, spreadability, extrudability, and drug content, making them suitable for topical application.

The optimized gel formulation GF2 demonstrated an excellent balance between physicochemical characteristics and controlled drug release behavior. Furthermore, the in vitro antifungal study against *Candida albicans* revealed that the silver nanoparticle-

incorporated gel produced significantly greater zones of inhibition than the crude extract, confirming the enhanced antifungal efficacy of the nanoparticle delivery system. The improved activity may be attributed to the synergistic effects of the phytoconstituents of *Aconitum ferox* and the broad-spectrum antimicrobial properties of silver nanoparticles.

Overall, the findings suggest that *Aconitum ferox*-mediated silver nanoparticles incorporated into a topical gel represent a promising, eco-friendly, and effective antifungal drug delivery system. However, further studies involving detailed toxicity evaluation, skin permeation studies, stability testing, and in vivo pharmacological and clinical investigations are necessary to establish the safety, efficacy, and therapeutic potential of this formulation for future clinical applications.

Author Contributions

Mr. Ved Prakash Patel performed the research and drafted the manuscript, while other authors supervised the work. All authors reviewed and approved the final version of the manuscript.

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Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors affirm that none of their interests conflict.

Conflict of Interest

The authors declare no conflicts of interest.

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