International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

CHARACTERIZATION OF *FAGONIA ARABICA* CONTACTING SILVER NANOPARTICLES GEL USING BOX BEHNKEN DESIGN

Arjun Singh*, Govind Nayak, Richa Verma, Mehta Parulben D
Lakshmi Narain College of Pharmacy, Bhopal (M.P.)

***Correspondence Info:**
Arjun Singh
Lakshmi Narain College of Pharmacy, Bhopal (M.P.)
Email:
arjunpratapsingh792@gmail.com

***Article History:**

Received: 28/01/2025
Revised: 12/02/2025
Accepted: 22/02/2025

ABSTRACT

The present study investigates the phytochemical, antimicrobial, and formulation optimization of *Fagonia arabica*, a plant known for its medicinal properties. The study focuses on the extraction of bioactive compounds from the aerial parts of *Fagonia arabica* using hydroalcoholic solvent, which yielded a higher percentage (9.20%) compared to pet ether extract (0.86%). Phytochemical screening revealed the presence of flavonoids, phenols, proteins, saponins, and tannins, all of which contribute to the plant's therapeutic potential. In addition, the study explored the optimization of gel formulations containing silver nanoparticles to enhance bioactive compound delivery. The optimized formulation, F5, showed the highest yield (82.23%) and good encapsulation efficiency (0.780 mg/100 mg quercetin equivalent). The formulated gels exhibited favorable physical properties, such as smooth texture, good spreadability, and appropriate pH for topical use. The in vitro drug release study indicated a controlled release of the active compounds, with formulation F1 showing a sustained release up to 4 hours. Antimicrobial tests confirmed that the silver nanoparticle-based gels exhibited superior antimicrobial activity against *Bacillus subtilis* and *Klebsiella pneumoniae*, demonstrating the enhanced efficacy of the formulation. The results suggest that *Fagonia arabica* and its formulated gel preparations have significant potential for therapeutic applications, particularly in antimicrobial and dermatological treatments.

Keywords: *Fagonia arabica*, Hydroalcoholic extract, Phytochemical screening, Silver nanoparticles, Gel formulations, Formulation optimization, Antimicrobial activity, Drug release, Flavonoids, Phenols, Nanoparticles, Dermatological applications.

INTRODUCTION

Fagonia arabica, a plant of the family Zygophyllaceae, is widely distributed in arid and semi-arid regions of the world, particularly in the Middle East, Asia, and Africa. The plant has garnered attention due to its diverse pharmacological properties, including anti-inflammatory, antimicrobial, and antioxidant activities (Singh *et al.,* 2020). Traditionally, *Fagonia arabica* has been used in folk medicine for the treatment of various ailments, including wounds, skin disorders, and fever (Khan *et al.,* 2014). Recent research has also explored the plant's potential in the field of nanotechnology, particularly in the synthesis of silver nanoparticles (AgNPs), which have gained significant interest due to their broad applications in medicine, environmental protection, and industry (Gurunathan *et al.,* 2015).

Silver nanoparticles are one of the most widely studied nanomaterials, renowned for their unique physicochemical properties, including high surface area, antimicrobial activity, and ease of functionalization (Sadeghi *et al.,* 2018). The biosynthesis of silver nanoparticles using plant extracts is considered an eco-friendly and sustainable approach compared to conventional chemical methods, which often involve toxic reagents (Sharma *et al.,* 2019). The plant-derived silver nanoparticles (AgNPs) have been shown to exhibit enhanced antimicrobial, antioxidant, and anticancer properties, making them promising candidates for biomedical and industrial applications (Patra *et al.,* 2016).

In the context of *Fagonia arabica*, the plant's rich content of secondary metabolites, including flavonoids, phenols, and alkaloids, plays a crucial role in the reduction and stabilization of silver ions to form silver nanoparticles (Ansari *et al.,* 2017). The synthesis of silver nanoparticles using plant extracts is influenced by several factors, including the concentration of the plant extract, silver nitrate concentration, pH, temperature, and reaction time. To optimize the conditions for the efficient production of silver nanoparticles, the application of statistical experimental designs, such as the Box-Behnken Design (BBD), has been widely adopted (Rodrigues *et al.,* 2019).

The Box-Behnken Design (BBD) is a popular response surface methodology (RSM) that helps to determine the optimal conditions for the synthesis of nanoparticles by minimizing the number of experimental runs required. The design is based on three levels of each factor and allows the evaluation of interactions between variables, ensuring a more efficient and cost-effective approach to optimizing nanoparticle synthesis (Chaudhary *et al.,* 2018). In this study, the synthesis of silver nanoparticles from *Fagonia arabica* extract is optimized using BBD, with the goal of determining the optimal conditions that result in silver nanoparticles with desirable size, shape, and stability.

This study aims to synthesize and characterize silver nanoparticles gel from *Fagonia arabica* using an eco-friendly method and to optimize the synthesis parameters using the Box-Behnken Design.

Methods

Collection of plant material

The plants have been selected on the basis of its availability and folk use of the plant. Aerial parts of *Fagonia arabica* were collected from Bhopal in the month of March, 2024. Drying of fresh plant parts was carried out in sun but under the shade. Dried aerial parts of *Fagonia arabica* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs:

Defatting of plant material

Aerial parts of *Fagonia arabica* were shade dried at room temperature. 50 gram dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted dried powdered aerial parts of *Fagonia arabica* has been extracted with hydroalcoholic solvent (ethanol: water: 70:30) using maceration process for 48 hrs, filtered

and dried using vacuum evaporator at 40ºC (Mukherjee, 2007; Kokate, 1994).

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods (Audu *et al.,* 2007).

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Gaur Mishra *et al.,* 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Estimation of total phenol content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Gaur Mishra *et al.,* 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of extract or standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Biosynthesis of silver nanoparticles

The Biosynthesis of silver nanoparticles (Ag-NPs) was carried out via the green route as described by Chi *et al.,* (2022) with slight modifications. A total of 20 mL of plant extract was mixed with 100 mL of silver nitrate solution (1-5 mM). The pH of the reaction medium was adjusted using 0.2 M NaOH (3 to 12) and the mixture was stirred continuously for 2 h at 30-90°C in the dark. A colour change was observed after the incubation period which was an initial validation of the synthesis of Ag-NPs. The mixture was centrifuged at 9000× g rpm for 10 min and washed continuously using deionized distilled water to collect the synthesized nanoparticles. The nanoparticles were dried in a hot air oven at 80°C for 3 h and used for downstream analysis.

Design of Experiment

The experimental design statistical analysis was carried out using Design expert software coupled with Response surface methodology (RSM) and Box-Behnken design (BBD). In this study, a 2-level, three-factor Box-Behnken design was used. The independent variables were pH, reaction time (minutes), reaction temperature (°C), and $AgNO_3$ concentration (mM) with their coded values −1 (minimum), 0 (center point), and +1 (maximal). The BBD consisted of 17 runs. One dependent variable was nanoparticle yield (%). The quadratic model equation shown below was used to calculate the expected Y response:

$$Y = \alpha_0 + \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_1\alpha_1 A^2 + \alpha_2\alpha_2 B^2 + \alpha_3\alpha_3 C^2 + \alpha_1\alpha_2 AB + \alpha_1\alpha_3 AC + \alpha_2\alpha_3 BC \quad \ldots(1)$$

where Y was the dependent variables (calculated responses), α_0 was the intercept, α_1, α_2, and α_3, were the coefficients in linear form, the interception was $\alpha 1\alpha 1$, $\alpha_2\alpha_2$, $\alpha_3\alpha_3$, and the quadratic coefficients were $\alpha_1\alpha_2$, $\alpha_1\alpha_3$ and $\alpha_2\alpha_3$.

Table 1: Minimum and maximum experimental values of Box-Behnken Design for the synthesis and yield of Ag-NPs

Factors	-1	0	1
pH	3	7.5	12
Temperature (°C)	30	60	90
Concentration (mM)	1	3	5

Final equation in terms of coded factors

Yield = +66.01+3.37 A+4.91 B-0.0775 C+0.3150 AB-1.87 AC+2.12 BC-1.20 A^2+6.67 B^2+3.94 C^2

Final equation in terms of actual factors

Yield = +85.65387+2.11889 pH-0.849450 pH- 0.849450 Temperature - 6.50933 Concentration+0.002333 pH * Temperature-0.207222 pH * Concentration+0.035333 Temperature * Concentration-0.059185 pH^2+0.007413 $Temperature^2$+0.984125 Concentration ^{2}.

Characterization of synthesized silver nanoparticles formulations

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).

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

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 50µS/cm.

Formulation development of silver nanoparticle gel

Precise quantities of methyl paraben, glycerin, polyethylene glycol, and hydroalcoholic extract of *Fagonia arabica* 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.

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: Spreadability, a crucial factor for gel formulations, was evaluated using a specially designed apparatus. Two glass slides (6x2) were chosen, and the gel formulation to be tested was placed between them over a length of 6 cm. The time taken for the slides to separate under the application of a 20-gram load was recorded. The experiment was repeated six times for each formulation, and the average was calculated.

Method: 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.

Spreadability Formula: $S=m\times lt$

Where, S = Spreadability (gcm/sec), m = weight tied to the upper slide (20 grams), l = length of the glass slide (6 cm), t = time taken in seconds.

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 AlCl3 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.

RESULTS AND DISCUSSION

The present study explored the potential of *Fagonia arabica* in the development of herbal-based formulations, with a focus on optimizing extraction techniques, evaluating the phytochemical content, and enhancing the therapeutic efficacy of the resulting formulations. Various formulations, including silver nanoparticle-based gels, were developed and optimized through yield, drug release, and antimicrobial activity assessments, all aimed at improving the bioavailability and effectiveness of the active constituents in *Fagonia arabica*.

The extraction process revealed that the hydroalcoholic extract of *Fagonia arabica* had a significantly higher yield (9.20%) compared to the pet ether extract (0.86%), as shown in Table 4**.** This higher yield of the hydroalcoholic extract is consistent with prior research that suggests alcohol-based solvents are more effective in extracting polar bioactive compounds like flavonoids, phenolic acids, and glycosides. These compounds are known for their antioxidant, anti-inflammatory, and antimicrobial properties, which are essential for pharmaceutical applications. Therefore, the hydroalcoholic extract was chosen for further formulation development due to its higher yield and potential bioactivity.

Phytochemical screening of the hydroalcoholic extract (Table 5) revealed the presence of several important bioactive constituents, including flavonoids, phenols, proteins, saponins, and tannins. The absence of alkaloids and glycosides in the extract suggests that the therapeutic effects may primarily stem from other identified compounds. Flavonoids, in particular, are known for their strong antioxidant activity, while phenolic compounds contribute to anti-inflammatory and anticancer activities. The presence of saponins and tannins further suggests that the extract may possess additional therapeutic potential, such as antimicrobial, anti-cancer, and immune-boosting effects. These findings provide a solid basis for the development of novel formulations using *Fagonia arabica* as a key ingredient.

In addition to the qualitative phytochemical analysis, the quantitative estimation of total flavonoid and phenol content (Table 6) highlighted the extract's potential for therapeutic use. The hydroalcoholic extract contained 0.784 mg of flavonoids and 0.512 mg of phenols per 100 mg of dried extract. These amounts are consistent with the known therapeutic values of flavonoids and phenols, which support the extract’s role as a potent antioxidant and anti-inflammatory agent. This high content of bioactive compounds makes *Fagonia arabica* a promising candidate for further development into functional formulations.

The optimization of formulation yields was crucial in determining the most efficient and effective formulation. Table 7 presents the yield data for formulations F1 to F17, indicating that formulations with higher concentrations of active ingredients and optimized parameters (such as pH, temperature, and concentration) exhibited higher yields. Formulation F5, in particular, exhibited the highest yield of 82.23%, suggesting that it was the most efficient in terms of extracting and preserving the

bioactive compounds. The optimization of formulation conditions was performed using response surface methodology (RSM), which helps to identify the most significant factors influencing yield and provides a systematic approach to formulation development.

Furthermore, the study focused on the preparation and characterization of silver nanoparticles (NPs) as carriers for the active compounds. Table 9 presented the entrapment efficiency of the silver nanoparticle formulations, with formulation F5 demonstrating the highest efficiency (0.780 ± 0.035 flavonoid mg/100 mg quercetin equivalent). Silver nanoparticles are known for their high surface area and ability to encapsulate bioactive compounds efficiently, which improves their stability, bioavailability, and therapeutic effects. The particle size analysis of formulation F5 showed an average size of 235.65 nm, with a zeta potential of -40.25 mV (Table 10). These results indicate that the nanoparticles are well-dispersed and stable, which is essential for ensuring effective delivery and release of the encapsulated compounds.

The physical characterization of the gel formulations (Table 11) showed that all three gel formulations (GF1, GF2, and GF3) exhibited favorable properties such as good homogeneity, smooth texture, and no clogging. These characteristics are essential for the practical application of the gel formulations, especially for topical use. Additionally, the formulations were evaluated for spreadability, viscosity, and pH (Table 12), with formulation GF2 showing the highest flavonoid content and good spreadability. The pH of the gels (ranging from 6.78 to 6.96) was found to be within the skin's natural pH range, making them suitable for topical applications without causing irritation.

The in vitro drug release study (Table 13) revealed that the gel formulations exhibited sustained release profiles, with formulation GF1 showing the highest cumulative drug release (99.12% at 4 hours). This controlled release behavior is desirable for therapeutic applications, as it ensures prolonged exposure to the active ingredients, thereby enhancing their efficacy. The release kinetics of formulation GF2 (Table 14) showed that the release followed a zero-order kinetic pattern, indicating a constant release rate over time. This is ideal for ensuring steady and consistent therapeutic effects, particularly in skin-targeted treatments.

Finally, the antimicrobial activity of the formulations was assessed against *Bacillus subtilis* and *Klebsiella pneumoniae* (Table 15). The silver nanoparticle-based gel formulation GF2 demonstrated superior antimicrobial activity compared to the hydroalcoholic extract alone, with larger zones of inhibition at all tested concentrations. This enhanced antimicrobial effect can be attributed to the synergistic action of the silver nanoparticles, which have well-documented antimicrobial properties. The results suggest that silver nanoparticle-based formulations could be highly effective in preventing and treating microbial infections, especially in wound care and other dermatological applications.

Table 2: Array layout for 2^3 factorial designs

F. Code	Std	Run	Factor-1 A-pH	Factor-2 B-Temperature ^0C	Factor-3 C-Concentration (mM)	Percentage Yield
F1	4	1	12	90	3	81.15
F2	6	2	12	60	1	72.25
F3	1	3	3	30	3	62.45
F4	13	4	7.5	60	3	67.85
F5	12	5	7.5	90	5	82.23
F6	8	6	12	60	5	70.45
F7	5	7	3	60	1	63.32
F8	17	8	7.5	60	3	68.78
F9	2	9	12	30	3	70.11
F10	14	10	7.5	60	3	67.88
F11	10	11	7.5	90	1	80.23
F12	15	12	7.5	60	3	63.32
F13	3	13	3	90	3	72.23
F14	11	14	7.5	30	5	68.77
F15	9	15	7.5	30	1	75.25
F16	7	16	3	60	5	68.98
F17	16	17	7.5	60	3	62.23

ANOVA for Quadratic model

Response 1: Yield

Source	Sum of Square	df	Mean Square	F- Value	P-Value	
Model	582.59	9	64.73	9.02	0.0042	significant
A-pH	90.99	1	90.99	12.67	0.0092	
B-Temperature	192.67	1	192.67	26.84	0.0013	
C-Concentration	0.0480	1	0.0480	0.0067	0.9371	
AB	0.3969	1	0.3969	0.0553	0.8208	
AC	13.91	1	13.91	1.94	0.2065	
BC	17.98	1	17.98	2.50	0.1576	
A^2	6.05	1	6.05	0.8424	0.3892	
B^2	187.41	1	187.41	26.10	0.0014	
C^2	65.25	1	65.25	9.09	0.0195	
Residual	50.26	7	7.18			
Lack of Fit	14.18	3	4.73	0.5239	0.6888	not significant
Pure Error	36.08	4	9.02			
Cor Total	632.85	16				

Table 3: Formulation of gel

Ingredients (mg)	GF1	GF 2	GF3
Extract loaded silver nanoparticle (F5)	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

Table 4: % Yield of aerial parts extract of *Fagonia arabica*

S. No.	Extract	% Yield (w/w)
1.	Pet ether	0.86%
2.	Hydroalcoholic	9.20%

Table 5: Phytochemical screening of extract of aerial parts of *Fagonia arabica*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's Test	-ve
	Hager's Test	-ve
2.	**Glycosides**	
	Legal's Test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	-ve
	Fehling's Test	-ve
7.	**Saponins**	
	Froth Test	+ve
	Foam Test	-ve
8.	**Diterpenes**	
	Copper acetate test	-ve
9.	**Tannins**	
	Gelatin Test	+ve

[+ve= Positive; -ve= Negative]

Table 6: Estimation of total flavonoids and phenol content of *Fagonia arabica*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.784	0.512

Table 7: Results of Percentage Yield of formulation F1 to F17

S. No.	F. Code	Percentage Yield
1	F1	81.15
2	F2	72.25
3	F3	62.45
4	F4	67.85
5	F5	82.23
6	F6	70.45
7	F7	63.32
8	F8	68.78
9	F9	70.11
10	F10	67.88
11	F11	80.23
12	F12	63.32
13	F13	72.23
14	F14	68.77
15	F15	75.25
16	F16	68.98
17	F17	62.23

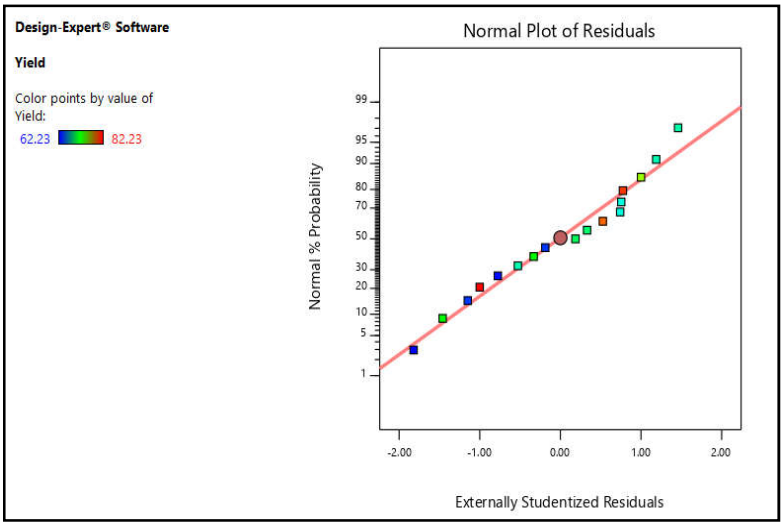

Figure 1: Response Surface Plots for Percentage Yield (Normal Plots of Residuals)

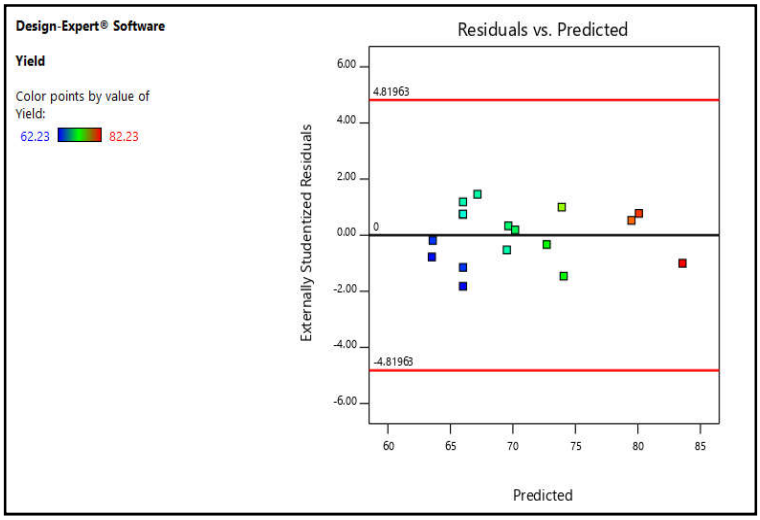

Figure 2: Response Surface Plots for Percentage Yield (Residuals vs Predicted)

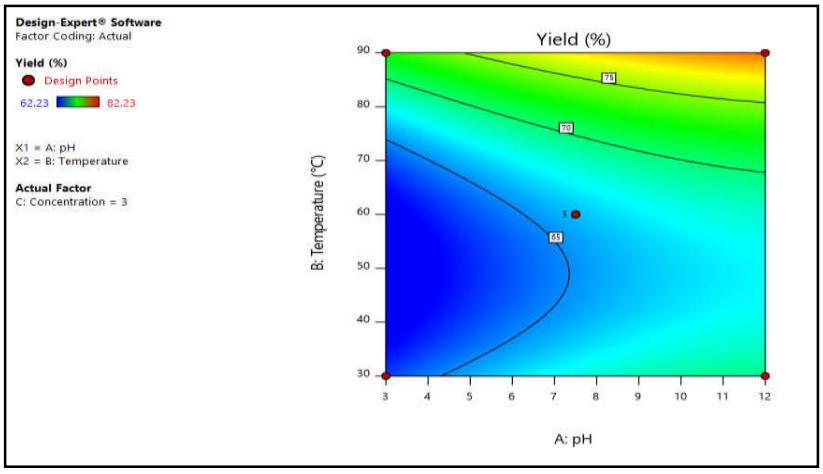

Figure 3: Contour plots for Percentage Yield between pH and Temperature

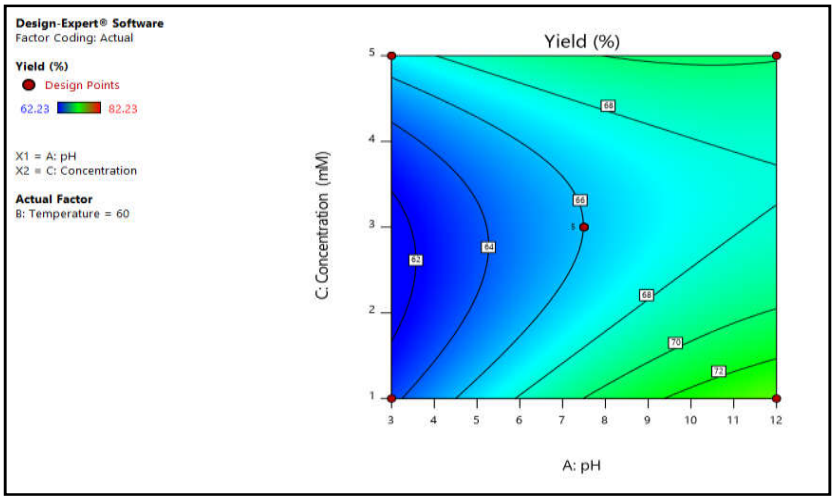

Figure 4: Contour plots for Percentage Yield between pH and Concentration

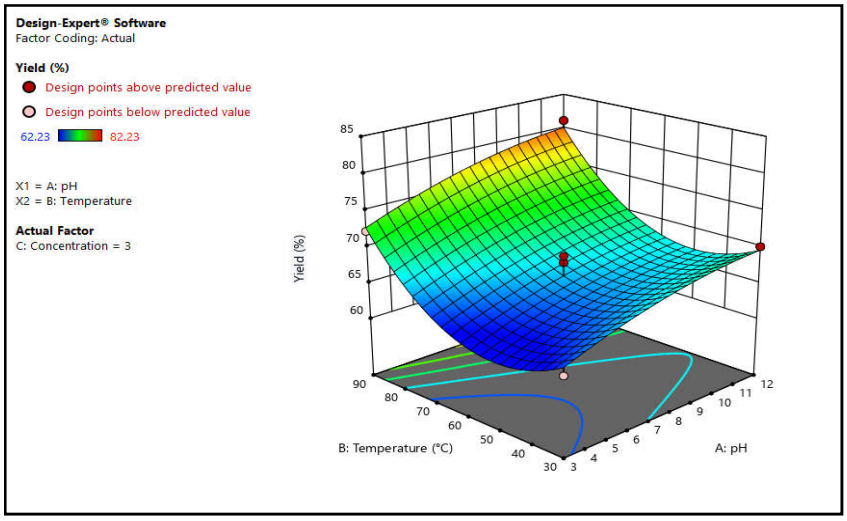

Figure 5: 3D plots for Percentage Yield between pH and Temperature

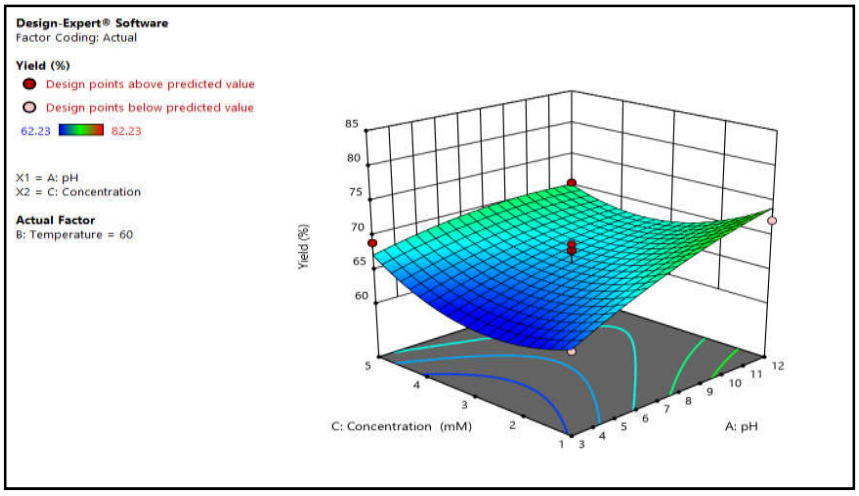

Figure 6: 3D plots for Percentage Yield between pH and Concentration

Report

F. Code	Run Order	Actual value	Predicted value	Residuals	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cooks Distance	Influence on fitted value DFFITS	Standard order
F1	**1**	**81.15**	**80.08**	**1.07**	**0.750**	**0.799**	**0.776**	**0.191**	**1.343**	**4**
F2	2	72.25	74.07	-1.82	0.750	-1.355	-1.460	0.551	-2.529(1)	6
F3	3	62.45	63.52	-1.07	0.750	-0.799	-0.776	0.191	-1.343	1
F4	4	67.85	66.01	1.84	0.200	0.767	0.742	0.015	0.371	13
F5	**5**	**82.23**	**83.57**	**-1.34**	**0.750**	**-1.000**	**-1.000**	**0.300**	**-1.732**	**12**
F6	6	70.45	70.18	0.2700	0.750	0.202	0.187	0.012	0.324	8
F7	7	63.32	63.59	-0.2700	0.750	-0.202	-0.187	0.012	-0.324	5
F8	8	68.78	66.01	2.77	0.200	1.155	1.189	0.033	0.594	17
F9	9	70.11	69.64	0.4750	0.750	0.355	0.331	0.038	0.574	2
F10	10	67.88	66.01	1.87	0.200	0.779	0.755	0.015	0.378	14
F11	**11**	**80.23**	**79.48**	**0.7450**	**0.750**	**0.556**	**0.527**	**0.093**	**0.912**	**10**
F12	12	63.32	66.01	-2.69	0.200	-1.123	-1.149	0.032	-0.574	15
F13	13	72.23	72.70	-0.4750	0.750	-0.355	-0.331	0.038	-0.574	3
F14	14	68.77	69.52	-0.7450	0.750	-0.556	-0.527	0.093	-0.912	11
F15	**15**	**75.25**	**73.91**	**1.34**	**0.750**	**1.000**	**1.000**	**0.300**	**1.732**	**9**
F16	16	68.98	67.16	1.82	0.750	1.355	1.460	0.551	2.529(1)	7
F17	17	62.23	66.01	-3.78	0.200	-1.578	-1.820	0.062	-0.910	16

Table 8: Experimental results with predicted responses

Formulation	Response	Actual value	Predicted value
F1	% Yield	81.15	80.08
F5		82.23	83.57
F11		80.23	79.48
F15		75.25	73.91

Table 9: Determination of entrapment efficiency of prepared silver nanoparticles formulations

Formulation code	Percentage entrapment efficiency (Flavonoid mg/100mg quercetin equivalent)
F1	0.623±0.025
F5	0.780±0.035
F11	0.605±0.36
F15	0.598±0.028

Table 10: Characterization of average particle size and zeta potential of optimized formulation F5

Formulation code	Average Particle size (nm)	Zeta Potential (mV)
F5	235.65	- 40.25 mV

Table 11: 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 12: Results of evaluation of gel formulations

Formulation code	Spreadability* (gcm/sec)	Viscosity* (cp)	Flavonoid Content (mg/100mg)	pH
GF1	12.25±0.25	3256±12	0.705±0.016	6.92±0.02
GF2	10.32±0.32	3145±16	0.780±0.014	6.78±0.01
GF3	9.95±0.18	3065±21	0.685±0.012	6.96±0.02

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

Table 13: *In vitro* drug release study of prepared gel formulation

S. No.	Time (hr)	% Cumulative Drug Release		
		GF1	GF2	GF3
1	0.25	34.45	32.25	26.65
2	0.5	46.65	42.25	33.32
3	1	63.12	55.45	42.25
4	1.5	75.65	63.32	50.32
5	2	83.32	73.32	62.23
6	2.5	98.45	80.32	70.32
7	3	99.05	92.02	74.65
8	4	99.12	98.85	89.95

Table 14: Release kinetics regression values of formulation F2

Formulation code	Zero order	First order
GF2	0.9643	0.8869

Table 15: Antimicrobial activity against selected microbes

S. No.	Microbes	Zone of inhibition		
		25 mg/ml	50 mg/ml	100 mg/ml
		Extract		
1.	*Bacillus subtilis*	8.0±0.50	10.2±0.50	12.5±0.47
2.	*Klebsiella pneumoniae*	9.5±0.94	11.9±0.86	12.9±0.25
		Silver nanoparticles gel (GF2)		
1.	*Bacillus subtilis*	9.8±0.15	11.6±0.74	13.18±0.57
2.	*Klebsiella pneumoniae*	11.2±0.5	12.5±0.86	15.50±0.25

CONCLUSION

The findings from this study demonstrate that *Fagonia arabica* is a promising source of bioactive compounds with significant therapeutic potential. The optimized formulations, particularly formulation F5, showed the best overall yield, entrapment efficiency, and in vitro drug release properties. The incorporation of silver nanoparticles further enhanced the antimicrobial activity of the formulations, making them suitable for topical applications. These formulations, with their sustained drug release profiles and enhanced antimicrobial properties, hold great potential for use in pharmaceutical and cosmetic products. Future research could explore the clinical applications of these formulations, further validating their therapeutic efficacy and safety for human use.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Ajitha, B., Kumar Reddy, Y.A. & Sreedhara Reddy, P. (2015) Green synthesis and characterization of silver nanoparticles using *Lantana camara* leaf extract. *Materials Science and Engineering. Part C*, 49, 373–381.
- Ansari, M.A. et al. (2017) Biosynthesis of silver nanoparticles using *Fagonia arabica* extract and their antimicrobial properties. *International Journal of Nanomedicine*, 12, 6771–6785.
- Audu, S.A., Mohammed, I. & Kaita, H.A. (2007) Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). *Life Science Journal*, 4, 75–79.
- Banerjee, P., Satapathy, M. & Das Mukhopahayay, A. (2014) P: Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: Synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing*, 1, 1–10.
- Bauer, A.W., Kirby, W.M., Sherris, J.C. & Turck, M. (1966) Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45, 493–496.
- Chaudhary, S. et al. (2018) Optimization of silver nanoparticle synthesis using Box-Behnken design and characterization of its antimicrobial properties. *Materials Science and Engineering. Part C*, 93, 228–234.
- de Jesús Ruíz-Baltazar, Á., Reyes-López, S.Y., Larrañaga, D., Estévez, M. & Pérez, R. (2017) Green synthesis of silver nanoparticles using a *Melissa ofcinalis* leaf extract with antibacterial properties. *Results in Physics*, 7, 2639–2643.
- Gurunathan, S. et al. (2015) Biosynthesis of silver nanoparticles from *Fagonia arabica* and their biomedical applications. *Nanoscale Research Letters*, 10, 4–9.
- Khan, M.A. et al. (2014) Medicinal properties of *Fagonia arabica*: A

review. *Journal of Pharmacognosy and Phytochemistry*, 3, 52–59.

- Kokate, C.K., editor Practical Pharmacognosy (1994), 4th edn, *Vallabh Prakashan*:, 112, 120.
- Lan Chi, N.T.L., Narayanan, M., Chinnathambi, A., Govindasamy, C., Subramani, B., Brindhadevi, K., Pimpimon, T. & Pikulkaew, S. (2022) Fabrication, characterization, anti-inflammatory, and anti-diabetic activity of silver nanoparticles synthesized from *Azadirachta indica* kernel aqueous extract. *Environmental Research*, 208, 112684.
- Mishra, A.G., Singh, R., Patil, N. & Parkhe, G. (2017) Determination of total phenolic, flavonoid content, antioxidant and antimicrobial activity of *Gloriosa superba* seed extract. *Asian Journal of Pharmaceutical Education and Research*, 6, 12–17.
- Mukherjee, P.K. (2007). *Quality Control of Herbal Drugs*, 2nd edn, Business Horizons, pp. 2–14.
- Patra, J.K. et al. (2016) Biosynthesis of silver nanoparticles: An eco-friendly approach. *Journal of Nanoscience and Nanotechnology*, 16, 6049–6059.
- Ponarulselvam, S., Panneerselvam, C., Murugan, K., Aarthi, N., Kalimuthu, K. & Thangamani, S. (2012) Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* Linn. G. Don and their antiplasmodial activities. *Asian Pacific Journal of Tropical Biomedicine*, 2, 574–580.
- Raut Rajesh, W., Lakkakula Jaya, R., Kolekar Niranjan, S., Mendhulkar Vijay, D. & Kashid Sahebrao, B. (2009) Phytosynthesis of silver nanoparticle using *Gliricidia sepium* (Jacq.). *Current Nanoscience*, 5, 117–122.
- Rodrigues, F.D. et al. (2019) Box-Behnken design for the optimization of silver nanoparticles synthesis from plant extracts. *Materials Science and Engineering. Part C*, 102, 105–111.
- Sadeghi, A.M. et al. (2018) Recent advances in silver nanoparticles as antimicrobial agents. *Advanced Materials Letters*, 9, 807–813.
- Sharma, G. et al. (2019) Green synthesis of silver nanoparticles using plant extracts and their antimicrobial applications. *Nanomedicine: Nanotechnology, Biology and Medicine*, 19, 27–38.
- Singh, P. et al. (2020) A comprehensive review on the medicinal potential of *Fagonia arabica*. *Phytochemical Reviews*, 19, 517–534.
- Vanaja, M., Gnanajobitha, G., Paulkumar, K., Rajeshkumar, S., Malarkodi, C. & Annadurai, G. (2013) Phytosynthesis of silver nanoparticles by *Cissus quadrangularis*: Influence of physicochemical factors. *Journal of Nanostructure in Chemistry*, 3, 1–8.