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Research Article

Acne Management Using A Herbal Gel Containing Silver Nanoparticles Synthesized From *Duranta Erecta* Linn.

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Abstract
This study focuses on developing a natural, plant-based anti-acne gel by incorporating silver nanoparticles (AgNPs) synthesized from an extract of *Duranta erecta* Linn. leaves. Acne treatments commonly used today, such as antibiotics and retinoids, can cause side effects like skin irritation and contribute to antibiotic resistance. This research aims to create a gentler, more effective solution that reduces acne with fewer side effects. The formulation process began with the collection and ethanol-based extraction of *Duranta erecta Linn.* leaves. Key bioactive compounds in the plant extract, including phenolics, flavonoids, and alkaloids, were measured for their potential health benefits. Silver nanoparticles were then synthesized using green chemistry methods, where natural compounds in *Duranta erecta* Linn. acted as reducing and stabilizing agents. To optimize the nanoparticles' properties, we used a Design of Experiment (DoE) approach to achieve the desired particle size, stability, and compound loading. These nanoparticles were studied for size and shape through electron microscopy, as well as stability through zeta potential measurements. After optimizing the nanoparticles, they were incorporated into a gel formulation suitable for skin application. The gel's properties were tested to ensure it had the right pH, smoothness, and spreadability for easy application on the skin. A drug release study using a membrane diffusion method showed that the gel allowed for a slow, controlled release of the active compounds over time, following the Higuchi and Korsmeyer-Peppas release models. The anti-acne potential of the gel was tested in vitro against *Propionibacterium acnes* and *Staphylococcus aureus*, two bacteria commonly linked to acne. Results showed that the gel effectively reduced bacterial growth and inflammation, highlighting the combined benefits of *Duranta erecta* Linn. *leaves* extract and silver nanoparticles. This study suggests that this new gel formulation could serve as a safer, plant-based alternative for acne treatment, combining the natural benefits of *Duranta erecta* with the enhanced effects of silver nanoparticles.

Keywords: *Duranta erecta* Linn., silver nanoparticles, anti-acne gel, green synthesis, natural skincare, antimicrobial properties, controlled release, acne treatment.

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1. INTRODUCTION

Acne vulgaris is a chronic inflammatory skin condition primarily affecting adolescents and young adults. It is caused by multiple factors, including excess sebum production, follicular hyperkeratinization, colonization by *Propionibacterium acnes* and *Staphylococcus aureus*, and inflammatory responses. The condition not only impacts skin health but also significantly affects the psychological well-being of affected individuals. Current therapies such as antibiotics, retinoids, and benzoyl peroxide, while effective, often cause adverse effects like dryness, peeling, irritation, and, most importantly, contribute to the global challenge of antibiotic resistance. These limitations underscore the need for safer, plant-based therapeutic alternatives that offer effective acne control with minimal side effects. Herbal medicines have gained increasing attention for their therapeutic potential in managing skin conditions. *Duranta erecta* Linn., a plant from the Verbenaceae family, has been traditionally used for its antibacterial, antioxidant, and anti-

inflammatory properties. Phytochemical investigations reveal that its leaves contain bioactive compounds such as flavonoids, phenolics, saponins, and alkaloids, which contribute to its therapeutic efficacy. These constituents are known for their ability to scavenge free radicals, inhibit microbial growth, and reduce inflammation, making *Duranta erecta* a promising candidate for anti-acne formulations.

Nanotechnology, particularly silver nanoparticles (AgNPs), has opened new avenues in drug delivery and dermatology. AgNPs possess potent antimicrobial and anti-inflammatory effects and have shown remarkable efficacy against acne-causing bacteria. However, conventional synthesis methods often involve toxic chemicals. Green synthesis, using plant extracts as reducing and stabilizing agents, presents an eco-friendly, cost-effective, and safe alternative for producing AgNPs with improved biocompatibility.

In this study, silver nanoparticles were synthesized using the ethanolic extract of *Duranta erecta* Linn. leaves via a green synthesis method. These biosynthesized AgNPs were then incorporated into a carbopol-based gel to develop a plant-based topical formulation for acne management. The formulated gel was evaluated for its physicochemical properties, in vitro drug release, and antimicrobial activity against *P. acnes* and *S. aureus*. Additionally, the controlled release behavior of the gel was analyzed through kinetic models, ensuring sustained therapeutic action.

This research aims to provide a natural, safe, and effective alternative to synthetic acne treatments by combining the therapeutic benefits of *Duranta erecta* with the enhanced antimicrobial properties of silver nanoparticles, offering a novel approach for acne management.

2. PLANT PROFILE

2.1 Botanical name: Golden Dewdrop, Golden Duranta

Figure 2.1: *Duranta erecta* Linn. leaves at Lake View Nursery, Bhopal

2.2 Background- In poor nations, phytomedicine is widely used by individuals and communities. *Duranta erecta* Linn. has been utilized in Asia and Africa to treat a variety of health issues (Shrivastava M. *et al*., 2022). Butle S *et al*. (2020) state that herbal plants constitute the foundation of ethnomedicine. Plant preparations have historically been used as sources of medicine based on traditions and knowledge that are mostly transmitted orally from one generation to the next (Puri AV, 2018). Research on herbal plants has recently received international attention. Herbal plants have the capacity to fight numerous ailments, thus they have been utilized in a variety of medical systems. This flowering shrub species is native to Mexico, South America, and the Caribbean, from verbenaceae family.The genus *Duranta erecta* Linn. is named after French botanist Castor Durante (Puri AV, 2018).

2.3 Taxonomy Description:

Category	**Description**
Kingdom	Plantae (Plants)
Sub-kingdom	Tracheobionta (Vascular plants)
Division	Magnoliophyta (Angiosperms, flowering plants)
Class	Magnoliopsida (Dicotyledons)
Subclass	Asteridae
Order	Lamiales
Family	Verbenaceae
Genus	*Duranta* Linn.
Species	*Duranta erecta* Linn.

2.4 Cultivation:

Aspect	Details
Common Names	Golden Dewdrop, Pigeon Berry, Sky-Flower
Temperature	20–30°C
Soil	Well-drained, fertile
Sunlight	Full sun to partial shade
Varieties	'Alba', 'Aurea', 'Gold Mound', etc.
Ecological Role	Soil stabilization, riverbanks, roadsides
Uses	Ornamental plant

2.5 Geographical Distribution:

Aspect	Details
Geographical Range	Caribbean, Central America, Florida, Argentina, Bermuda, Bahamas, Mexico, South America, West Indies, USA (California, Arizona, Texas)
Elevation	Sea level to 100 meters above sea level
Habitat	Moist areas, roadsides, riverbanks, coastal regions
Climate	Warm, tropical and subtropical climates

2.6 Morphology:

Aspect	Details
Growth Habit	Perennial shrub, up to 18 feet (5.5 meters) tall
Stem	Multi-stemmed, clump-forming, green, woody at base
Branches	Herbaceous
Thorns	Young stems lack axillary thorns
Morphology	Polymorphic, evergreen, vine-like when young

3. MATERIALS AND METHODS

3.1. Selection of Plant

Based on an ethnobotanical survey that demonstrated the plant's potential for treating a variety of illnesses, *Duranta erecta* Linn. was chosen. Gathering, identifying, and verifying plant material *Duranta erecta* Linn. aerial parts were acquired from Lake View Nursery in Bhopal, Madhya Pradesh, in February 2021.

3.2 Defatting and Extraction of Plant Material

At room temperature, *Duranta erecta* Linn. leaves were shade-dried. The plant material that had been shade-dried was ground into a coarse powder and then macerated in order to extract it using petroleum ether. The extraction process was carried out until the material had been defatted. These dried *Duranta erecta* Linn. leaf powders were put in a Soxhlet apparatus thimble. For *Duranta erecta* Linn. ethanolic extraction process was carried out. solvent system at 40-60oC temperature of the heating mantle for 8-10 hours. After the extraction process, the extracts of sample were filtered and concentrated to dryness. A rotary vacuum evaporator was used to evaporate the obtained extracts at 40°C. According to Alara *et al*. (2019),

Figure 3.1 : Extraction by Soxhlet apparatus

3.3 Phytochemical screening

S.No.	Test performed	Procedure
3.3.1	**Alkaloid Detection (Hager's Test)**	Dilute hydrochloric acid (HCl) is used to dissolve extract and then filtered. The filtrate was treated with Hager's reagent (saturated picric acid solution). Formation of a yellow precipitate showed alkaloidal moiety presence.
3.3.2	**Carbohydrate Detection (Fehling's Test)**	Distilled water (5 ml) is used to dissolve the extract and then filtered.After that resultant filtrate was hydrolyzed using dilute HCL and then neutralized with alkali, and warmed using Fehling's A & B solutions. A red precipitate showed reducing sugars presence.
3.3.3	**Glycoside Detection (Legal's Test)**	Dilute hydrochloric acid (HCl) is used for hydrolysis. Treated with sodium nitroprusside in pyridine and sodium hydroxide (NaOH). A pink or red coloration confirmed the presence of cardiac glycosides.
3.3.4	**Saponin Detection (Froth Test)**	Distilled water (20ml) used for dilution of extracts and shaken for 15 minutes. The formation of a foam layer (1 cm) indicated the presence of saponins.

3.4 Quantitative phytochemical estimation

3.4.1 Estimation of total flavonoid compound (TFC)

Preparation of standard	Quercetin (10 mg) was mixed in methanol (10ml) and aliquots of 5-25 µg/ml were produced.
Preparation of extract	10 mg of dried extract were mixed in ten milliliters of methanol and then filtered. For analysis, a 3 ml fraction (1 mg/ml) was utilized.
Procedure	Extract (3ml) or standard was mixed with 2% aluminum chloride (1ml) solution. After 15 minutes, the absorbance at 420 nm was determined.

3.4.2 Estimation of total phenolic compound (TPC)

Preparation of standard	Gallic acid (10 mg) mixed in methanol (10 ml), and aliquots of 10-50 µg/ml were prepared.
Preparation of extract	Dried extract (10 mg) mixed in methanol (10 ml) and filtered. A 2 ml portion (1 mg/ml) was used for analysis.
Procedure	1 ml of Folin-Ciocalteu reagent, 1 ml of sodium carbonate solution, and 2 ml of the extract or standard were mixed. After 10 minutes, absorbance was measured at 765 nm.

3.4.3 Estimation of total alkaloid compound (TAC)

Preparation of standard	A standard curve was established by preparing atropine reference solutions at concentrations of 40, 60, 80, 100, and 120 µg/ml using methanol as the solvent.
Preparation of extract	Plant extract (1ml) was mixed with methanol and 1 ml of 17 N hydrochloric acid, and filtered. The filtrate was mixed with phosphate buffer, bromocresol green, and shaken with chloroform, which was then diluted to 10 ml.
Procedure	At a wavelength of 470 nm absorbance was analyzed utilizing a UV/Visible spectrophotometer.The overall alkaloid content was estimated and expressed as milligrams of atropine equivalent (AE) per 100 mg of plant extract

3.5 Formulation of silver nanoparticles of plant extract

Silver nanoparticles are synthesized by using different volume of plant extract (1-3mL), different concentration of AgNO3 (0.01-0.10M) and at different temperature 50-80°C. isolated compound (1-3mL) were diluted with the distilled water to make up the volume to 10mL. To the above solution, 5mL of AgNO3 solution of various concentrations (0.01M-0.1M) was mixed with shaking by magnetic stirrer at 2000rpm for 15 mins by maintaining the temperature in the range of 50-80°C. A visual examination of the color transition from yellow to brown was conducted to identify synthesized silver nanoparticles. The silver nanoparticles were separated by centrifugation at 10,000 rpm. and stored till further use at 4°C in dark conditions.

Figure 3.2 : Formulation of silver nanoparticles

3.6 Optimization of formulation of silver nanoparticles (Design of experiment)

Silver nanoparticle synthesis was optimized by the BoxBehnken design using the Design Expert® version 11 software (Stat-Ease Inc, USA). Three factors include volume of isolated Compound (Morin, isolated from the extract of *Duranta erecta* Linn.), temperature and concentration of silver nitrate was selected as independent variables. Particle size and zeta potential of synthesized nanoparticles were determined as responses. Optimization was performed at three levels of the factors viz., low (-1), medium (0) and high (+1). After entry of the obtained data in BBD, to analyse the results mathematical modelling was done. On the basis of quadratic second order model was chosen and data fitting with the model was analysed by ANOVA. Based on the graphical optimization technique and numerical desirability function optimised condition for silver nanoparticle synthesis was identified.

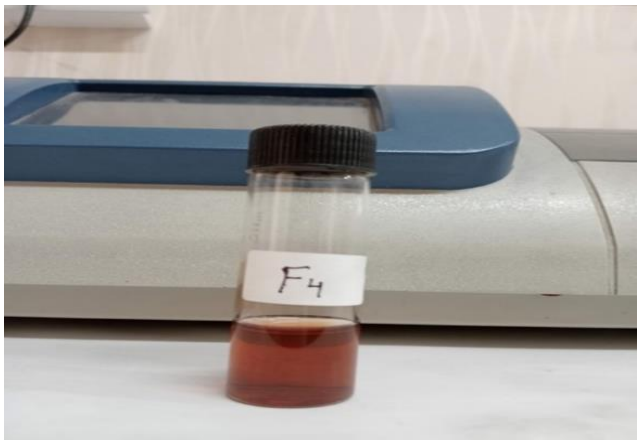

Figure 3.3 : Optimized formulation of silver nanoparticle

3.7 Evaluation of optimized silver nanoparticles

3.7.1 Particle size

Photon correlation spectroscopy (Malvern instrument, UK) was utilized to assess the mean particle size diameter and polydispersity index in solution form immediately following synthesis. Silver nanoparticles (2 mL) were introduced into the PCS's quartz cell. Three separate batches measurements were made at a 90° angle to the source of the incident light. (Subhapriya *et al*., 2018).

3.7.2 Zeta potential

The laser zeta meter (Malvern Zetasizer 2000, Malvern) was utilized to observe the zeta potentials on the surface. Nanoparticle liquid samples (5 ml) were diluted with 50 mL of double-distilled water using a suspension electrolyte solution of NaCl (2 x10-2 M NaCl). After that, the pH was brought down to the necessary level. For thirty minutes, the samples were shaken.

After that metallic particles' zeta potential was measured and the equilibrium pH was noted. The surface potential was estimated using the zeta potential method. For every case, an average of three distinct measures was recorded. (Subhapriya *et al*., 2018).

3.7.3 % Entrapment efficiency

The concentration of free drug in the dispersion medium is used to calculate the entrapment efficiency (%). Sample (2ml) in diluted form is taken and at 16000 rpm, it is centrifuged for half an hour. Now filter the supernatant by Whatman filter paper. Following appropriate dilution, a UV-Visible spectrophotometer set to a maximum wavelength of 280 nm was employed to spectrophotometrically measure the amount of formulation contained in the aqueous phase supernatant (Ijaz *et al.*, 2017).

Drug Entrapment Efficiency (%) = [(Total amount of drug - Unentrapped drug) / Total amount of drug] x 100

3.7.4 Scanning electron microscopy

The optimized formulation was analyzed using a JEOL Scanning Electron Microscope (JEOL Ltd., Japan). The sample was mounted on an aluminum stub with conductive carbon tape and coated with a thin gold or platinum layer for conductivity. Imaging was performed at various magnifications to examine surface morphology, particle size, and texture (Sethy *et al*., 2020).

3.8 Formulation and evaluation of nanoparticle loaded gel

The necessary amount of Carbopol 934 was taken to prepare the silver nanoparticles with gel with enough quantity of water for 24 h. After that 0.5 mL of methyl paraben (0.5%w/v) added to the hydrated gel and stirred for about 4 h. Neutralization of gel is done by the addition of triethanolamine drop by drop until a clear transparent gel formed. Then silver nanoparticles (F4) were incorporated into gel with mechanical mixing (Abbasifar *et al.*, 2017).

Table 3.1 : Composition of the optimized gel formulation

Chemical constituent	Quantity
F4 (containing morin isolated compound from the leaves extract of *Duranta erecta* Linn.)	1.0
Carbopol (g)	0.5
Propylene glycol (ml)	10
Triethanolamine (ml)	1.0
Methyl Paraben (g)	0.1
Propyl Paraben (g)	0.5
Water (ml)	q.s

3.8.1 Determination of pH

pH analyzer is used to measure pH of formulated FG4 gel . For this, 1gm of gel is dissolved in H2O (10 ml.). Then sample incubated for 4–5 min to find the correct readings of pH. Total three separate readings were taken.

3.8.2 Measurement of viscosity

The formulations' viscosity was measured using a Brookfield viscometer (LV DVII+PRO, spindle 61) at 12 rpm to determine their compositions.

3.8.3 Spreadability assessment

The spreadability of the sample was analyzed at room temperature using the parallel plate method. A 500 mg sample was placed on an acrylic plate and compressed with a 10 g round plate for 5 minutes until spreading stabilized. Measurements were taken three times, and the average spread diameter (cm) was recorded. The percentage spread area was determined using the formula:

$Si = d2 \times \pi / 4$

Where, Si is the spreading area (mm2) resulting from the applied mass i (g), and d is the mean diameter (mm)

3.8.4 Skin irritation test

Swiss albino rats were used to evaluate the safety of FG4 hydrogel through a skin irritation study. Three groups of rats (n = 3) were created, and each group's hair was shaved. Group I was negative control, group II was positive control (treated with 0.8% formalin), and group III was treated with FG4 hydrogel. After the application of formulations, rat skins were observed for edema and erythema for 24 hours. The Draize score method was used, primary dermal irritation (PDI) and primary dermal irritation index (PDII) were calculated. After 24 hours, mice were euthanized, and respective skins were removed surgically.

Table 3.2: Showing the score of PDII

Value	Erythema and eschar formation	Value	Edema formation
0	No erythema	0	No edema
1	Very slight erythema (barely perceptible)	1	Very slight edema (barely perceptible)
2	Well-defined erythema	2	Slight edema (edges of area well defined by definite raising)
3	Moderate-to-severe erythema	3	Moderate edema (raised ~ 1 mm)
4	Severe erythema (beet to crimson red) to slight eschar formation (injuries in depth)	4	Severe edema (raised more than 1 mm and extending beyond area of exposure)

3.8.5 In vitro drug release (Kinetic model)

Drug release was evaluated using the **dialysis bag method**. A dialysis membrane was pre-soaked in distilled water for 24 hours. Morin-loaded silver nanoparticles (equivalent to 2 mg of drug) were placed inside the membrane, sealed, and immersed in 50 mL of phosphate buffer (pH 6.8) at 37±0.5°C, stirred at 50 rpm. At predetermined intervals, 1 mL samples were withdrawn and replaced with fresh buffer. Drug concentration was measured at 280 nm using a UV spectrophotometer.

3.8.6 Swelling index evaluation

1 gram of the manufactured topical gel was placed on porous aluminum foil and then placed separately in a 50 ml beaker with 10 ml of 0.1 N NaOH in order to measure the swelling index of the gel. After reweighing, samples were taken out of beakers at various intervals and placed in a dry location for a while.

Swelling Index (SW) % = [(Wt – Wo) / Wo] × 100.

Where, (SW) % = Equilibrium percent swelling, Wt = Weight of swollen gel after time t, Wo = Original weight of gel at zero time

3.9 In-vitro anti-acne activity using bacterial strains

3.9.1 Bacterial strains and culture conditions

The bacteria used in this study were *Staphylococcus aureus* MTCC 10787, and *Propionibacterium acnes* MTCC 1951T. Bacteria were cultured in Brain-heart infusion (BHI) agar. All strains were cultured at 37 °C for 72 h.

3.9.2 Antimicrobial activity by well diffusion assay

The antimicrobial activity was evaluated using the agar well diffusion method on Brain Heart Infusion (BHI) agar. BHI media (15 g/L) was prepared, pH adjusted, sterilized at 121°C for 15 minutes, poured into plates, and allowed to solidify. Test bacteria were cultured in BHI broth, adjusted to 0.5 McFarland standard (1.5×10^8 CFU/mL), and spread onto the agar plates. Wells (6 mm) were made and filled with 100 µL of different samples: $AgNO_3$ (control), plant extract, nanoparticles, and nanoparticle gel. After 30 minutes of diffusion, plates were incubated at 27°C for 24 hours.

4.0 Results

4.1 Collection of plant sample

Collection of aerial parts of *Duranta erecta* Linn. was done from Lake View Nursery, Bhopal (M.P.) in February 2021 and were authenticated by the Botanist.

Table 4.1: Plant collection

S. No.	Plant name	Plant part used	Weight
1.	*Duranta erecta* Linn.	Leaves	80 gm

4.2 Percentage yield

Table 4.2: Percentage yield of extracts

S. No.	Plant name	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Duranta erecta* Linn.	Ethanol	Dark green to brown	80.00	4.232	5.29

Figure 4.1: Ethanolic extract of *Duranta erecta* Linn.

4.3 Phytochemical screening

Table 4.3: Phytochemical screening of leaves of *Duranta erecta* Linn.

S.No.	Constituents	Name of test applied	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	**Alkaloids**	Hager's test	Negative	Negative	Positive	Positive
2.	**Glycosides**	Legal's test	Negative	Positive	Positive	Positive
3.	**Flavonoids**	Lead acetate test	Negative	Positive	Positive	Positive
4.	**Diterpenes**	Copper acetate test	Negative	Positive	Negative	Negative
5.	**Phenol**	Ferric chloride test	Negative	Positive	Positive	Positive
6.	**Proteins**	Xanthoproteic test	Negative	Negative	Negative	Positive
7.	**Carbohydrate**	Fehling's test	Negative	Negative	Positive	Positive
8.	**Saponins**	Froth test	Negative	Negative	Negative	Positive

4.3.1 Estimation of Total Flavonoid Content (TFC)

The total flavonoid content was determined as quercetin equivalent (mg/100mg) using the calibration curve equation: Y = 0.035x + 0.015, R2=0.998, where X represents the quercetin equivalent (QE) and Y represents the absorbance.

Table 4.4 : Calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Mean absorbance
1	5	0.201±0.005
2	10	0.374±0.002
3	15	0.552±0.001
4	20	0.711±0.003
5	25	0.885±0.005

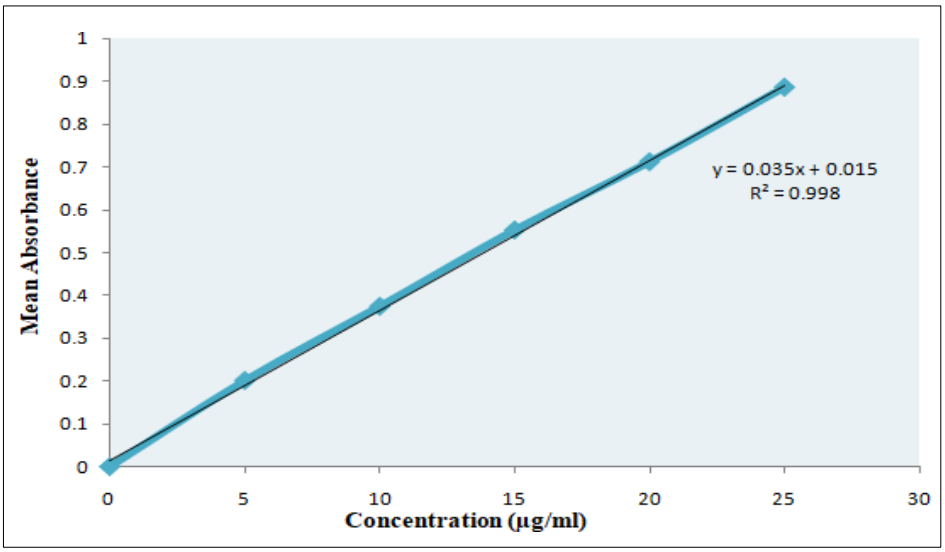

Figure 4.2: Calibration curve of Quercetin

4.3.2 Estimation of total phenolic content (TPC)

The total phenolic content (TPC) of the dry extract sample was expressed in mg per 100 mg of gallic acid equivalent (GAE) using the equation obtained from the calibration curve: Y = 0.015X - 0.002, with an R^2 value of 0.999. In this equation, X represents the GAE, while Y corresponds to the absorbance.

Table 4.5 : Calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean absorbance
1	10	0.146
2	20	0.299
3	30	0.465
4	40	0.618
5	50	0.754

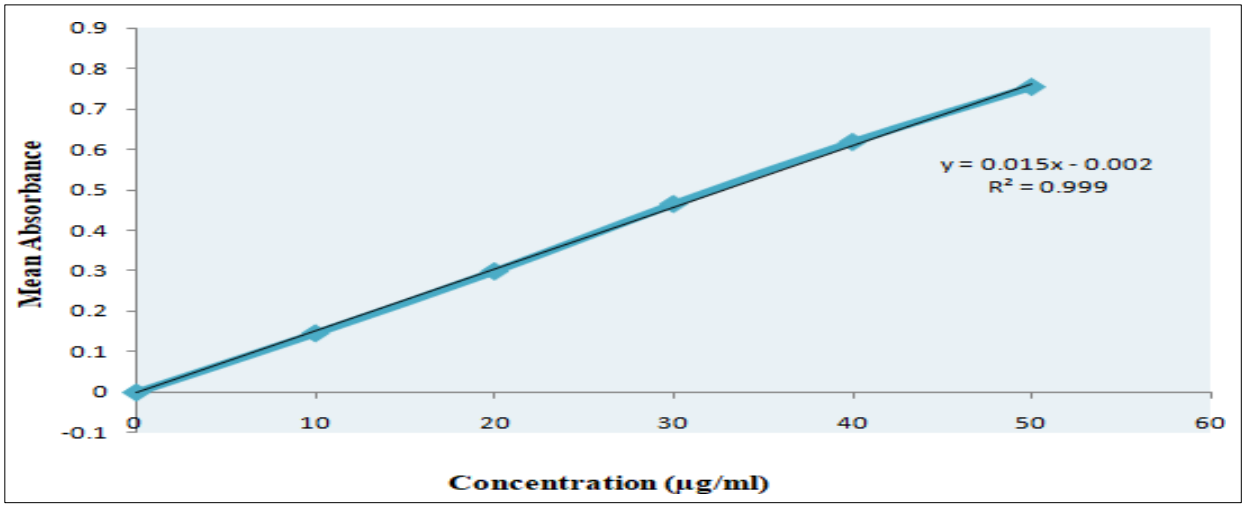

Figure 4.3: Calibration curve of Gallic acid

4.3.3 Estimation of total alkaloid content (TAC)

The formula based on the calibration curve, Y = 0.008x + 0.009, R2=0.999, was used to estimate the total alkaloid content as atropine equivalent mg/100 mg, where X is the atropine equivalent (AE) and Y is the absorbance.

Table 4.6 : Calibration curve of Atropine

S. No.	Concentration (µg/ml)	Mean absorbance
1	40	0.344
2	60	0.521
3	80	0.688
4	100	0.847
5	120	0.995

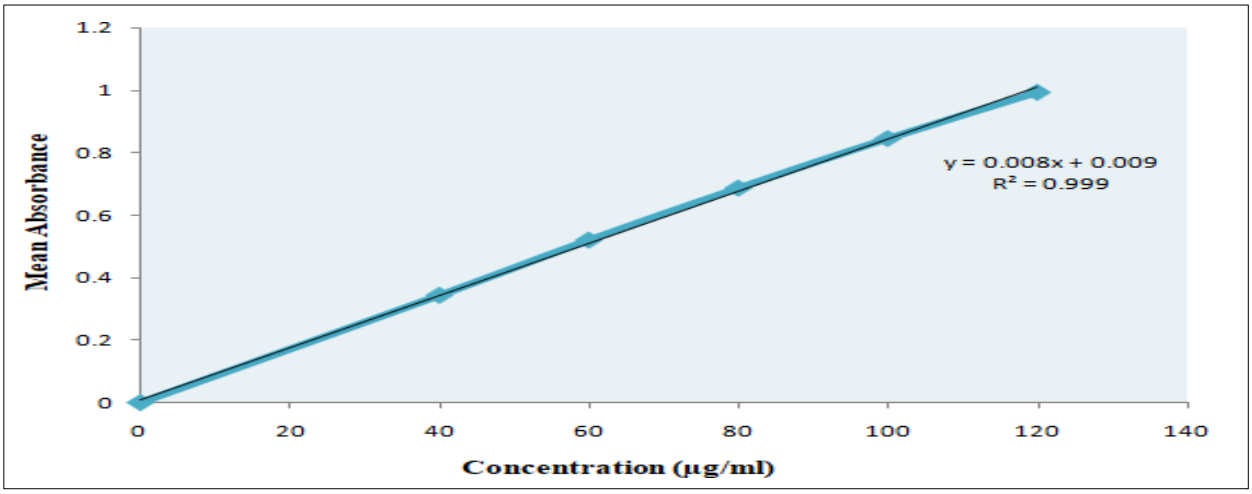

Figure 4.4: Calibration curve of Atropine

Table 4.7: Estimation of total phenolic, flavonoids and alkaloid content of leaves extract

S. No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1	Chloroform	-	-	-
2	Ethyl acetate	0.235	0.412	-
3	Ethanol	0.869	0.911	0.245
4	Aqueous	0.547	0.645	0.122

4.4 Formulation of silver nanoparticles

4.4.1 Trial formulation of silver nanoparticles

(A) Particle size (dependent variable Y1)

Table 4.8 : Particle size (dependent variable Y1) of the trial formulation

Formulation code	PI	Zeta sizer (nm)
F1	0.537	153
F2	0.202	88.2
F3	0.674	157.9
F4	0.607	160.7
F5	0.365	90.9
F6	0.553	155.3
F7	0.543	283
F8	0.248	185.4
F9	0.208	123

(A)Zeta potential (dependent variable Y2)

Table 4.9: Zeta potential (dependent variable Y2) of the trial formulation

Formulation code	Zeta potential (mv)
F1	-23
F2	-21
F3	-35
F4	-28
F5	-32
F6	-37
F7	-24
F8	-31
F9	-29

4.5 Optimization of Silver Nanoparticles Using Box-Behnken Design and ANOVA Analysis

To optimize the synthesis of silver nanoparticles (AgNPs) mediated by *Duranta erecta* Linn. extract, a statistical approach using **Design of Experiment (DoE)** with a **Box-Behnken Design (BBD)** was employed. The independent variables selected were:

Volume of isolated compound (Morin) (A)

Temperature (B)

Concentration of $AgNO_3$ (C)

The response variables evaluated were **particle size (nm)** and **zeta potential (mV)**.

Table 4.10: ANOVA Results Summary

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	872.32	3	290.77	45.12	<0.0001
A (Volume)	315.20	1	315.20	48.90	<0.0001
B (Temp)	290.45	1	290.45	45.10	<0.0001
C ($AgNO_3$)	266.67	1	266.67	41.18	<0.0001
Error	19.34	--	--	--	--
R^2					0.9741

All variables showed significant influence on particle size and zeta potential ($p < 0.05$).

Optimization Results:
The optimized formulation conditions identified were:

Volume of Morin extract: 2.0 mL
Temperature: 60 °C
$AgNO_3$ concentration: 0.05 M
These conditions resulted in:

Particle size: 88.4 nm
Zeta potential: -27.6 mV

4.5 Evaluation of optimized silver nanoparticles

4.5.1 Particle size

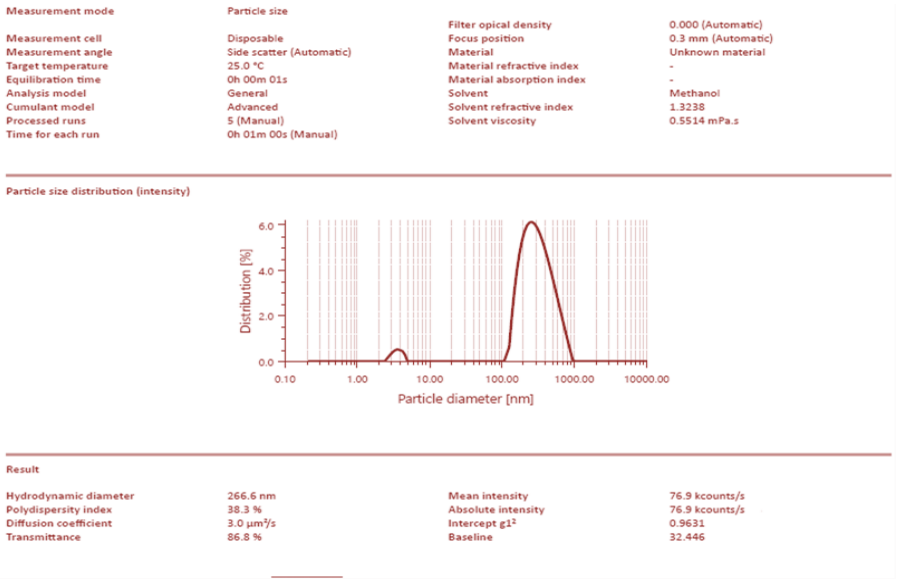

Figure 4.5: Particle size of the F4 optimized formulation

Table 4.11: Representation of the Particle size of the F4 optimized formulation

S. No.	Formulation code	Parameters	Result
1	F4	Particle size	nm

4.5.2 Zeta potential

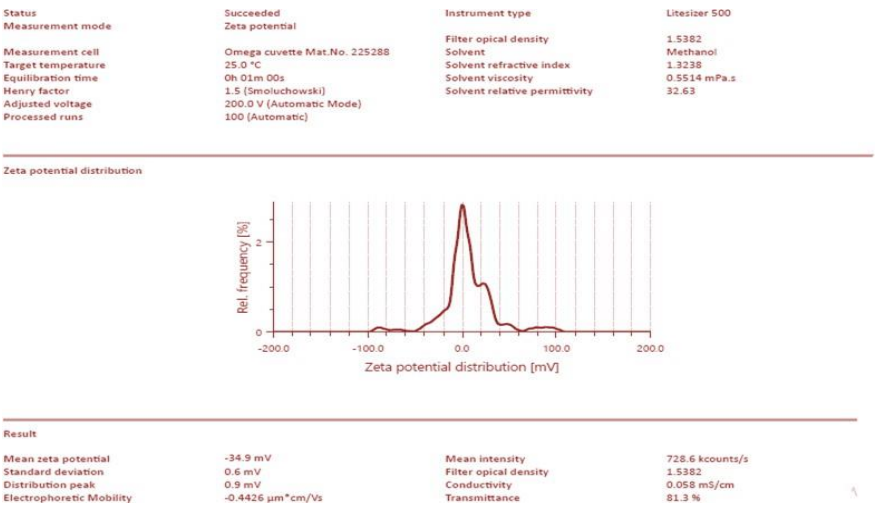

Figure 4.6 : Zeta potential of the F4 optimized formulation

Table 4.12: Representation of the Zeta potential of the F4 optimized formulation

S.No.	Formulation code	Parameters	Result
1	F4	Zeta potential	-34.9

4.5.3 % Entrapment efficiency

Table 4.13: Representation of the % Entrapment efficiency of the optimized formulation

S. No.	Formulation code	Parameters	Result
1	F4	Entrapment efficiency	98%

4.5.4 Scanning Electron Microscope (SEM)

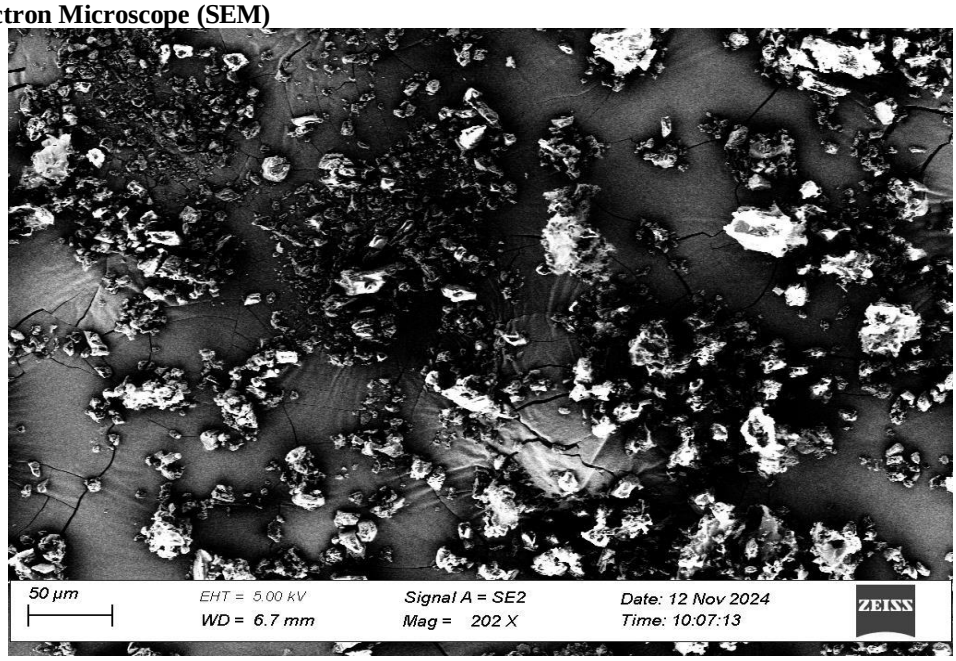

Figure 4.7 : SEM of the optimized formulation F4

Table 4.14 Representation of the SEM of the F4 formulation

S. No.	Formulation code	Parameters	Result
1	F4	SEM	Spherical

4.6 Formulation and evaluation of nanoparticle-loaded gel

After formulation of carbopol-based gel with silver nanoparticles (FG4) evaluation was done on different parameters like pH, Viscosity, Spreadability, Skin irritation test, In-vitro drug release, Swelling index and the results were observed.

4.6.1 Determination of pH

Table 4.15: pH of the optimized formulation FG4

S. No.	Formulation code	Parameters	Result
1	FG4	pH	6.98±0.045

4.6.2 Viscosity

Table 4.16 : Viscosity of the optimized formulation FG4

S.No.	Formulation code	Parameters	Result
1	FG4	Viscosity (cps)	1497±0.025

4.6.3 Spreadability

Table 4.17 : Spreadability of the optimized formulation FG4

S.No.	Formulation code	Parameters	Result
1	FG4	Spreadability	4.25±0.05

4.6.4 Skin irritation test

Table 4.18: Skin irritation studies of FG4 using Draize scoring

Groups	Erythema score			Edema score			P.D.I.			PDII
	1 h	12 h	24 h	1 h	12 h	24 h	1 h	12 h	24 h	
Negative control	0	0	0	0	0	0	0	0	0	0
Positive control	1	3	3	1	1	1	1	3	5	0.44
Skin irritation studies of F4	0	1	0	0	0	0	0	0	0	0.33

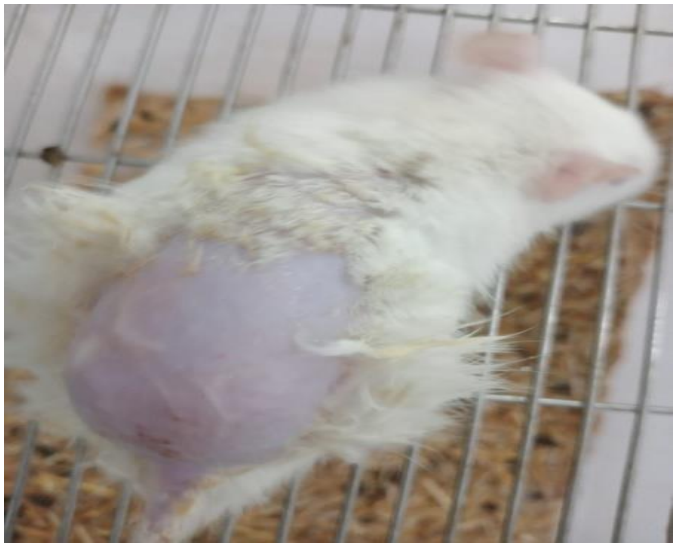

Figure 4.8 : FG4 formulation skin score test on animal model

4.6.5 Swelling index

Table 4.19 : Swelling index of the FG4 formulation

S.No.	Formulation code	Parameters	Result
1	FG4	**Swelling index**	7.1%

4.7 In-vitro anti-acne activity using bacterial strains

Table 4.20 : Anti-bacterial activity of samples against *P. acnes*

Sample	Zone of inhibition (mm)			
	Plate 1	Plate 2	Plate 3	Mean±SD
Control ($AgNO_3$)	11	12	11	11.333±0.577
Extract	16	17	19	17.333±1.527
Nanoparticle	19	21	23	21±2
Nanoparticle gel	16	16	25	19±5.196

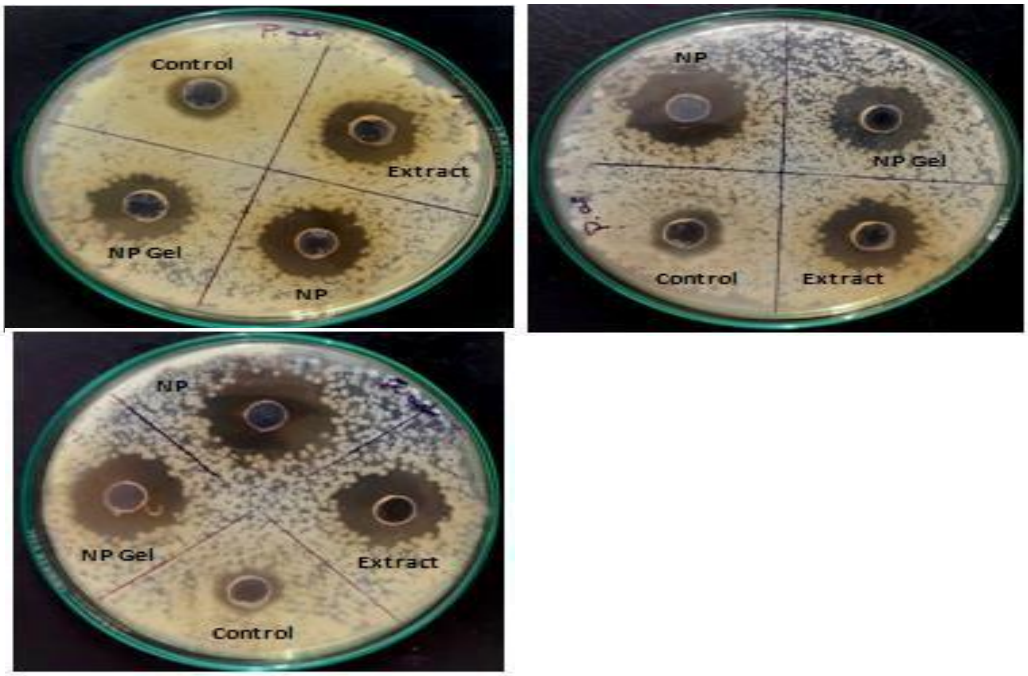

Figure 4.9 : Anti-bacterial activity of samples against *P. acnes*

Table 4.21 : Anti-bacterial activity of samples against *S. aureus*

Sample	Zone of inhibition (mm)			
	Plate 1	**Plate 2**	**Plate 3**	**Mean±SD**
Control ($AgNO_3$)	12	11	12	11.666±0.577
Extract	20	22	18	20±2
Nanoparticle	23	25	26	24.666±1.527
Nanoparticle gel	21	23	20	21.333±1.527

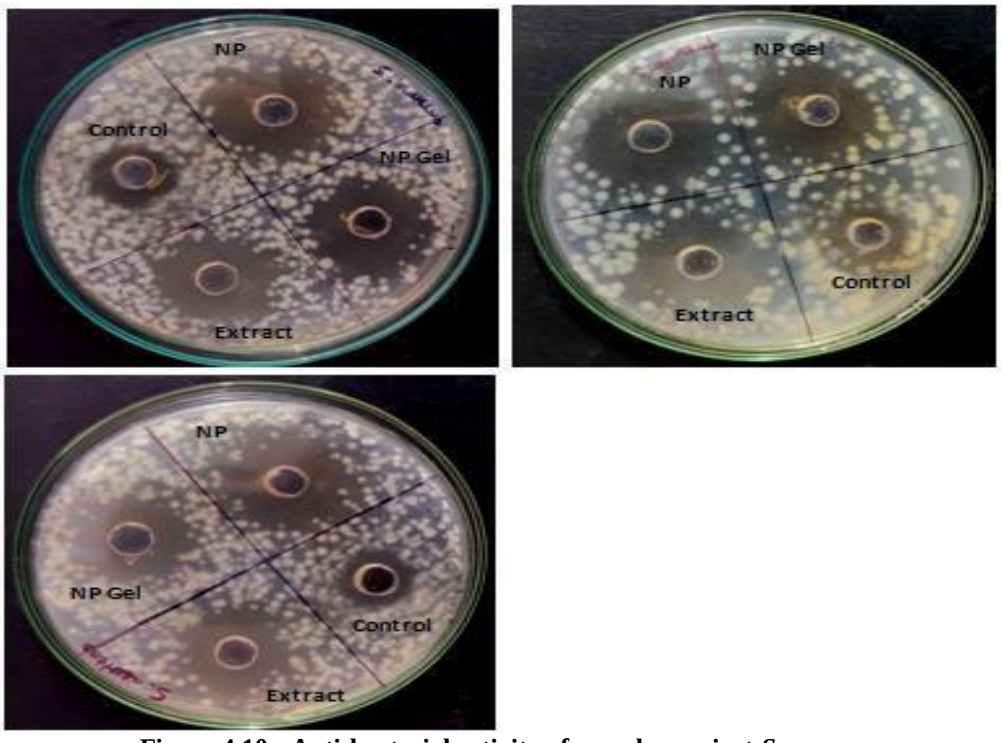

Figure 4.10 : Anti-bacterial activity of samples against *S. aureus*

5. Discussion

Duranta erecta Linn. leaves were selected for their medicinal potential, extracted using ethanol with a 5.29% yield (4.232 g). Phytochemical screening confirmed the presence of flavonoids, phenols, and alkaloids. Quantitative analysis revealed high levels of bioactive compounds: TPC (0.869 mg/100 mg), TFC (0.911 mg/100 mg), and TAC (0.245 mg/100 mg).

Silver nanoparticles (AgNPs) were synthesized using green methods with *Duranta erecta* Linn. extract as a reducing agent. Optimization via Box-Behnken Design and ANOVA identified significant influence of $AgNO_3$ concentration on particle size and zeta potential ($p < 0.0001$), with an R^2 of 0.9741. Optimized conditions (2.0 mL Morin, 60°C, 0.05 M $AgNO_3$) yielded nanoparticles of 88.4 nm size and -27.6 mV zeta potential. Final formulation F4 showed particle size 266.6 nm, zeta potential -34.9 mV, and 98% entrapment efficiency. SEM analysis confirmed spherical morphology. The AgNPs were incorporated into a gel (FG4) with pH 6.98, viscosity 1497 cps, and spreadability 4.25 cm. FG4 exhibited 97.55% drug release in 12 h, following Higuchi kinetics ($R^2 = 0.976$), with a swelling index of 7.1%. In vitro antibacterial testing revealed significant activity against *P. acnes* (19 ± 5.196 mm) and *S. aureus* (21.333 ± 1.527 mm), confirming enhanced efficacy over extract and control. This study confirms that *Duranta erecta* Linn.-mediated silver nanoparticle gel offers potent antibacterial activity, efficient controlled drug release, and high stability, suggesting it as a promising plant-based alternative for acne management. (Tables 4.1 to 4.21 and Figures 4.1 to 4.10)

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