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Green Synthesis, Characterization and Antifungal Evaluation of *Combretum roxburghii*-Mediated Silver Nanoparticle-Loaded Topical Gel

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Abstract: The present study aimed to develop a green synthesized silver nanoparticle-loaded topical gel using the hydroalcoholic leaf extract of *Combretum roxburghii* and to evaluate its physicochemical characteristics, in vitro drug release, and antifungal activity. The hydroalcoholic extract was prepared by maceration and subjected to phytochemical screening, total flavonoid, and total phenolic content estimation. Silver nanoparticles were synthesized using different concentrations of silver nitrate and extract-to-metal ion ratios. The prepared nanoparticles were evaluated for percentage yield, flavonoid content, particle size, and zeta potential to identify the optimized formulation. The optimized nanoparticles were incorporated into Carbopol 940 gel formulations and evaluated for physical appearance, homogeneity, pH, spreadability, viscosity, extrudability, flavonoid content, and in vitro drug release. Antifungal activity was assessed against *Candida albicans* and *Aspergillus flavus* using the agar well diffusion method. The hydroalcoholic extract showed a percentage yield of 8.70% and contained flavonoids, phenolic compounds, proteins, saponins, diterpenes, and reducing carbohydrates. Among the six nanoparticle formulations, F3 demonstrated the highest percentage yield (82.68 ± 0.17%) and flavonoid content (0.961 ± 0.016 mg QE/100 mg), with an average particle size of 292.2 nm and a zeta potential of −37.45 mV, indicating good colloidal stability. The optimized nanoparticles were successfully incorporated into a Carbopol gel, and formulation GF2 exhibited suitable physicochemical properties, including a pH of 6.84 ± 0.03, viscosity of 4825 ± 16 cP, spreadability of 12.18 ± 0.64 g·cm/s, and uniform flavonoid content. The gel exhibited sustained drug release, achieving 99.75% cumulative release within 4 h and following zero-order release kinetics (R^2 = 0.9802). The silver nanoparticle gel demonstrated significantly enhanced antifungal activity compared with the crude extract, producing maximum zones of inhibition of 18.6 ± 0.22 mm against *Candida albicans* and 17.5 ± 0.28 mm against *Aspergillus flavus* at 100 mg/ml. The findings suggest that green synthesized silver nanoparticles derived from *Combretum roxburghii* can be successfully formulated into a stable topical gel with sustained release characteristics and improved antifungal efficacy, indicating its potential as a promising topical antifungal drug delivery system.

Keywords: *Combretum roxburghii*; *Green Synthesis; Silver Nanoparticles; Carbopol Gel; Topical Drug Delivery; Antifungal Activity; Flavonoids; in Vitro Drug Release; Zeta Potential; Candida albicans; Aspergillus flavus.*

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I. INTRODUCTION

Fungal infections remain a major global health concern owing to their increasing incidence, recurrent nature, and the emergence of resistance to conventional antifungal agents. Superficial fungal infections caused by *Candida* and *Aspergillus* species are among the most prevalent microbial infections affecting the skin and mucosal tissues [1].

Although several topical antifungal formulations are available, their therapeutic effectiveness is often limited by inadequate skin penetration, short residence time, poor drug stability, and the need for frequent application. These limitations have encouraged the development of novel topical drug delivery systems that can provide sustained release, enhanced antimicrobial activity, and improved patient compliance [2].

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Green nanotechnology has emerged as an environmentally friendly and cost-effective approach for the synthesis of metallic nanoparticles. Among various metallic nanomaterials, silver nanoparticles (AgNPs) have attracted considerable attention because of their broad-spectrum antimicrobial, antifungal, anti-inflammatory, antioxidant, and wound-healing properties [3].

Compared with conventional chemical and physical methods, plant-mediated synthesis of silver nanoparticles offers several advantages, including the elimination of toxic reducing agents, lower energy requirements, improved biocompatibility, and enhanced environmental safety [4].

Plant-derived phytochemicals such as flavonoids, phenolic compounds, terpenoids, proteins, and reducing sugars act as both reducing and stabilizing agents during nanoparticle synthesis, producing stable nanoparticles with enhanced biological activity [5].

Combretum roxburghii is a medicinal plant belonging to the family Combretaceae and is widely distributed in tropical and subtropical regions. The leaves of *Combretum roxburghii* contain several biologically active phytoconstituents, including flavonoids, phenolic compounds, tannins, and terpenoids, which exhibit antioxidant, antimicrobial, anti-inflammatory, and antifungal properties [6].

These phytochemicals make the plant an excellent candidate for the green synthesis of silver nanoparticles, where they facilitate the reduction of silver ions and stabilize the resulting nanoparticles [7].

Furthermore, phytochemical-coated silver nanoparticles may exhibit synergistic antimicrobial activity due to the combined effects of silver and plant bioactive compounds.

Topical gel formulations are widely preferred for dermal drug delivery because they are non-greasy, easily spreadable, washable, cosmetically acceptable, and capable of maintaining prolonged contact with the skin [8].

Incorporation of silver nanoparticles into a Carbopol-based gel can improve nanoparticle retention at the site of application, provide sustained release of active constituents, and enhance local therapeutic efficacy while minimizing systemic exposure. Such formulations are particularly promising for the management of superficial fungal infections, where localized drug delivery is advantageous [9].

In the present investigation, hydroalcoholic leaf extract of *Combretum roxburghii* was employed for the green synthesis of silver nanoparticles using silver nitrate as the precursor. The synthesized nanoparticles were characterized for percentage yield, flavonoid content, particle size, and zeta potential, and the optimized formulation was incorporated into a Carbopol 940 gel. The developed gel was evaluated for its physicochemical properties, in vitro release behavior, and antifungal activity against *Candida albicans* and *Aspergillus flavus*. The study aimed to develop a stable, eco-friendly, and effective silver nanoparticle-loaded topical gel with enhanced antifungal activity and sustained release characteristics for the treatment of superficial fungal infections.

II. MATERIAL AND METHODS

A. Material

Fresh leaves of *Combretum roxburghii* were collected and authenticated prior to use. Silver nitrate ($AgNO_3$) was procured from Merck Life Science Pvt. Ltd., Mumbai, India. Carbopol 940, polyethylene glycol 600 (PEG 600), propylene glycol, triethanolamine, methyl paraben, ethanol, methanol, and other analytical-grade chemicals and reagents were obtained from Loba Chemie Pvt. Ltd., Mumbai, India. Folin–Ciocalteu reagent, aluminium chloride, quercetin, and gallic acid used for phytochemical estimation were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Double-distilled water was used throughout the study, and all chemicals and reagents employed were of analytical reagent (AR) grade.

B. Methods

The collected plant material was thoroughly washed with distilled water to remove adhering dust and foreign particles and then shade dried at room temperature for about 5-6 days. The dried material was coarsely powdered using a mechanical grinder and stored in an airtight container until further use. About 50 g of the powdered drug was accurately weighed and transferred into a clean, dry conical flask.

To this, 500 ml of suitable solvent such as Hydroalcoholic solvent (ethanol: water; 75:25) was added and the flask was tightly closed. The mixture was kept aside for maceration at room temperature for a period of 72 hours with occasional shaking to facilitate proper mixing and penetration of the solvent into the plant tissues [10]. During this period, the solvent dissolved the active phytochemical constituents present in the crude drug. After completion of maceration, the contents were filtered first through muslin cloth to remove coarse particles and then through Whatman No.1 filter paper to obtain a clear filtrate. The residue was again macerated with fresh solvent to ensure complete extraction, and the filtrates were combined. The combined filtrate was concentrated by evaporating the solvent on a water bath maintained below 40–50°C until a semisolid mass of extract was obtained.

➢ *Extraction By Maceration Process*

- *Determination of Percentage Yield*

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

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

- *Phytochemical Screening*

The Hydroalcoholic extract of *Combretum roxburghii* leaves was subjected to preliminary phytochemical screening

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using standard qualitative methods to detect the presence of various phytoconstituents [11].

- *Estimation of Total Flavonoid Content*

The total flavonoid content of the ethanolic extract of Combretum roxburghii leaves was determined using the aluminium chloride colorimetric method. Quercetin was used as the reference standard for the preparation of the calibration curve. Briefly, 10 mg of quercetin was dissolved in 10 ml of methanol to obtain a stock solution (1 mg/ml), and standard solutions of 5–25 µg/ml were prepared by appropriate dilution with methanol. Similarly, 10 mg of the dried ethanolic extract was dissolved in 10 ml of methanol and filtered. Three millilitres of the extract solution (1 mg/ml) was mixed with 1 ml of 2% aluminium chloride ($AlCl_3$) solution. The reaction mixture was allowed to stand for 15 minutes at room temperature, and the absorbance was measured at 420 nm using a UV–Visible spectrophotometer against a reagent blank. The total flavonoid content was calculated from the quercetin calibration curve and expressed as mg quercetin equivalent (QE) per 100 mg of dried extract [12].

- *Estimation of Total Phenolic Content*

The total phenolic content of the ethanolic extract of Combretum roxburghii leaves was estimated by the folin–ciocalteu colorimetric method using gallic acid as the reference standard. A stock solution of gallic acid (1 mg/ml) was prepared by dissolving 10 mg of gallic acid in 10 ml of methanol, and standard solutions of 10–50 µg/ml were prepared by serial dilution. For sample preparation, 10 mg of the dried ethanolic extract was dissolved in 10 ml of methanol and filtered. Two millilitres of the extract solution (1 mg/ml) was mixed with 1 ml of folin–ciocalteu reagent (previously diluted with distilled water in the ratio of 1:10 v/v), followed by the addition of 1 ml of sodium carbonate solution (7.5 g/L). The reaction mixture was vortexed for 15 seconds and incubated at room temperature for 15 minutes for colour development. The absorbance was recorded at 765 nm using a UV–Visible spectrophotometer. The total phenolic content was calculated from the gallic acid calibration curve and expressed as mg gallic acid equivalent (GAE) per 100 mg of dried extract [13].

- *Green Synthesis of Silver Nanoparticles*

Silver nanoparticles (AgNPs) were synthesized by the green synthesis method using the ethanolic leaf extract of *Combretum roxburghii* as a natural reducing and capping agent. A 10 mM silver nitrate ($AgNO_3$) stock solution was prepared in double-distilled water and diluted to obtain working concentrations of 1, 2, and 3 mM. The required quantity of *Combretum roxburghii* leaf extract (500 mg) was dissolved in distilled water and filtered through Whatman No. 1 filter paper to obtain a clear extract solution.

Different formulations were prepared by mixing the plant extract with $AgNO_3$ solutions at extract-to-silver nitrate ratios of 1:1 and 1:2 (v/v) as shown in Table 1. The total reaction volume for each formulation was maintained at 50 ml. The reaction mixtures were continuously stirred using a magnetic stirrer at 500 rpm for 30 minutes and then incubated in a water bath at 60 ± 2°C for 4–5 hours. The flasks were covered with aluminium foil throughout the reaction to prevent photoreduction of silver ions.

The formation of silver nanoparticles was initially confirmed by the gradual colour change of the reaction mixture from light yellow to dark brown, indicating the reduction of Ag^+ ions to Ag^0 nanoparticles. After completion of the reaction, the suspensions were cooled to room temperature and centrifuged at 12,000 rpm for 20 minutes. The collected nanoparticles were washed three times with double-distilled water to remove unreacted plant constituents and excess silver ions. The purified nanoparticles were dried in a hot air oven at 45–50°C, stored in airtight amber-coloured containers at 4°C, and used for further characterization and biological evaluation [14].

Table 1 Different Formulation of Silver Nanoparticles

F. Code	*Combretum roxburghii* Extract (mg)	$AgNO_3$ Concentration (mM)	Extract$_3$ Ratio (v/v)	Total Volume (ml)	Stirring Time (min)	Temperature (°C)
F1	500	1	1:1	50	30	60 ± 2
F2	500	2	1:1	50	30	60 ± 2
F3	500	3	1:1	50	30	60 ± 2
F4	500	1	1:2	50	30	60 ± 2
F5	500	2	1:2	50	30	60 ± 2
F6	500	3	1:2	50	30	60 ± 2

➢ *Characterization of Synthesized Silver Nanoparticles Formulations*

- *Percentage Yield*

The synthesized silver nanoparticles were collected after completion of the reaction by centrifugation at 12,000 rpm for 20 minutes. The nanoparticle pellet was washed three times with double-distilled water to remove unreacted silver ions and residual plant constituents. The purified nanoparticles were dried in a hot air oven at 45–50°C until a constant weight was obtained [15]. The dried silver nanoparticles were accurately weighed, and the percentage yield was calculated by comparing the weight of the dried nanoparticles with the total weight of the initial non-volatile components (silver nitrate and *Combretum roxburghii* leaf extract solids) used in the formulation. The percentage yield was calculated using the following equation:

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

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- *Entrapment Efficiency*

The entrapment efficiency of silver nanoparticles was determined by estimating amount of flavonoids associated with the nanoparticles after separation of unentrapped extract. Briefly, 5 ml of prepared silver nanoparticle dispersion was centrifuged at 12,000 rpm for 20 minutes. The supernatant containing unentrapped phytoconstituents was carefully collected, while the nanoparticle pellet was retained. The concentration of free flavonoids present in the supernatant was determined using the $AlCl_3$ colorimetric method by measuring absorbance at 420 nm with a UV–Visible spectrophotometer against a suitable blank. The flavonoid concentration was calculated from the quercetin calibration curve.

The amount of flavonoids entrapped within the silver nanoparticles was calculated by subtracting the amount of free flavonoids from the total flavonoid content initially added during nanoparticle preparation [16]. The percentage entrapment efficiency was calculated using the following equation:

Entrapment Efficiency (%) = [(Total flavonoid content − Free flavonoid content) / Total flavonoid content] × 100

- *Surface Charge and Vesicle Size*

The average particle size, polydispersity index (PDI), and zeta potential of the optimized silver nanoparticle formulation were determined using Dynamic Light Scattering (DLS) with a Malvern Zetasizer (Malvern Instruments Ltd., UK) available at SAIF, RGPV, Bhopal. Prior to analysis, the silver nanoparticle dispersion was diluted appropriately with double-distilled water to avoid multiple light scattering effects. The diluted sample was gently sonicated for 2–3 minutes to obtain a uniform dispersion before measurement.

For particle size analysis, the sample was transferred into a disposable polystyrene cuvette and analyzed at 25 ± 1°C with a scattering angle of 90°. The average hydrodynamic diameter and polydispersity index (PDI) were recorded [17].

For zeta potential measurement, the diluted nanoparticle dispersion was filled into a folded capillary zeta cell, and the electrophoretic mobility of the particles was measured. The zeta potential was calculated automatically by the instrument using the Helmholtz–Smoluchowski equation. All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation (SD).

Topical gels containing *Combretum roxburghii* leaf extract-loaded silver nanoparticles were prepared using the Carbopol 940 gel base. The required quantity of Carbopol 940 was accurately weighed and slowly dispersed in approximately 70 ml of distilled water under continuous stirring at 700–800 rpm using a mechanical stirrer. The dispersion was allowed to hydrate for 1 hour to obtain a uniform polymer solution.

In a separate beaker, the optimized silver nanoparticle formulation (500 mg) was dispersed in 20 ml of distilled water, followed by the addition of polyethylene glycol 600 as a humectant and methyl paraben as a preservative. The mixture was stirred until a homogeneous dispersion was obtained [18].

The nanoparticle dispersion was then gradually incorporated into the hydrated Carbopol solution under continuous stirring. Triethanolamine was added dropwise to neutralize the dispersion and adjust the pH to 6.5–7.0, resulting in the formation of a transparent and homogeneous gel. The final volume was adjusted to 100 ml with distilled water, and stirring was continued for 15–20 minutes to ensure uniform mixing.

Table 2 Formulation of Silver Nanoparticle Gel

Ingredients	GF1	GF2	GF3
Optimized ***Combretum roxburghii*** loaded silver nanoparticles (mg)	500	500	500
Carbopol 940 (mg)	250	500	750
Polyethylene Glycol 600 (ml)	0.2	0.2	0.2
Methyl Paraben (mg)	0.08	0.08	0.08
Triethanolamine (ml)	q.s.	q.s.	q.s.
Propylene Glycol (ml)	5	5	5
Distilled Water	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml

➢ *Evaluation of Silver Nanoparticle Gel*

- *Physical Appearance and Homogeneity*

The prepared *Combretum roxburghii* silver nanoparticle gel formulations were visually examined for colour, clarity, homogeneity, consistency, smoothness, grittiness, and phase separation. The gels were inspected for the presence of lumps, coarse particles, or any signs of syneresis. The formulations were gently rubbed between the fingers to assess texture and uniformity. The observations were recorded for comparative evaluation.

- *Washability*

The washability of the prepared gel formulations was evaluated by applying approximately 1 g of gel onto the dorsal surface of the hand and allowing it to remain for 5 minutes. The applied gel was then washed with running tap water, and the ease of removal, presence of any residue, and skin feel after washing were visually observed and recorded. Formulations exhibiting easy removal without leaving a sticky residue were considered satisfactory [19].

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- *Extrudability*

The prepared gels were filled into aluminium collapsible tubes, sealed properly, and stored at room temperature. Extrudability was determined by applying constant finger pressure on the tube and observing the ease with which the gel was extruded. The amount and uniformity of gel extruded from the tube were visually assessed. Based on the ease of extrusion, the formulations were graded as Poor, Good, Very Good, or Excellent [20].

- *Determination of Spreadability*

Spreadability of the gel formulations was determined by the glass slide method. Approximately 1 g of gel was placed between two clean glass slides (6 × 2 cm). A weight of 20 g was placed over the upper slide for 5 minutes to produce a uniform film of gel between the slides. The upper slide was then allowed to move freely by applying the specified weight, and the time required for complete separation of the slides was recorded. Each formulation was evaluated in triplicate, and the average value was calculated [21].

Spreadability was calculated using the following equation:

$S = (M \times L) / T$

Where:

S = Spreadability (g·cm/sec)

M = Weight applied (20 g)

L = Length of glass slide (6 cm)

T = Time taken for separation of the slides (sec)

Higher spreadability values indicated better ease of application of the gel on the skin.

- *Determination of Viscosity*

The viscosity of the gel formulations was determined using a Brookfield Digital Viscometer fitted with Spindle No. 6 operating at 10 rpm. Prior to measurement, the gels were allowed to equilibrate at 25 ± 2°C for 30 minutes to eliminate entrapped air bubbles. Approximately 50 g of gel was transferred into the sample container, and viscosity was recorded in centipoise (cP). Each measurement was performed in triplicate, and the mean value was reported [22].

- *Determination of Total Flavonoid Content in Gel*

The flavonoid content of the silver nanoparticle gel formulations was estimated by the aluminium chloride colorimetric method. Accurately 1 g of gel was transferred into a 10 ml volumetric flask, dissolved in methanol, sonicated for 10 minutes, and the volume was adjusted with methanol. The solution was filtered through Whatman No. 1 filter paper. From the filtrate, 3 ml was transferred into a test tube, and 1 ml of 2% aluminium chloride solution was added. The reaction mixture was incubated at room temperature for 15 minutes, after which the absorbance was measured at 420 nm using a UV–Visible spectrophotometer. The flavonoid content was determined from the quercetin calibration curve and expressed as mg quercetin equivalent (QE) per 100 mg of gel. All analyses were carried out in triplicate [23].

- *Determination of pH*

The pH of the prepared gel formulations was measured using a calibrated digital pH meter. One gram of gel was dispersed in 25 ml of distilled water and stirred until a uniform dispersion was obtained. The electrode was immersed into the sample, and the pH was recorded after the reading became stable. The measurements were performed in triplicate, and the average pH value was reported [24].

- *In-Vitro Drug Release Study*

The *in vitro* release of phytoconstituents from the silver nanoparticle gel formulations was carried out using a Franz diffusion cell. Freshly prepared egg membrane (or dialysis membrane) was mounted between the donor and receptor compartments. The receptor compartment was filled with phosphate buffer (pH 7.4) maintained at 37 ± 0.5°C and continuously stirred at 100 rpm using a magnetic stirrer to maintain sink conditions. Approximately 1 g of gel was evenly spread over the membrane in the donor compartment. At predetermined intervals (0.5, 1, 2, 4, 6, 8, and 10 hours), 5 ml aliquots were withdrawn from the receptor compartment and immediately replaced with an equal volume of fresh phosphate buffer maintained at the same temperature [25]. The withdrawn samples were analyzed for total flavonoid content by the aluminium chloride method at 420 nm using a UV–Visible spectrophotometer.

- *In Vitro Antifungal Activity of Silver Nanoparticle Gel*

The antifungal activity of the *Combretum roxburghii* silver nanoparticle gel was evaluated by the agar well diffusion method. Sterile PDA plates were inoculated with the fungal suspension using a sterile cotton swab. Wells of 6 mm diameter were made in the agar, and gel formulations at 25, 50, and 100 mg/ml were introduced into the respective wells. A standard antifungal drug and plain gel base served as positive and negative controls, respectively. The plates were allowed to stand for 1 hour for diffusion and then incubated at 37°C for 24–48 hours [26]. After incubation, the zone of inhibition (mm) around each well was measured using a Vernier caliper. The experiment was carried out in triplicate, and the results were expressed as mean ± SD.

III. RESULTS AND DISCUSSION

The hydroalcoholic extract of Combretum roxburghii leaves was successfully utilized for the green synthesis of silver nanoparticles and their incorporation into a topical gel formulation. The extraction process yielded 8.70% w/w extract, indicating satisfactory recovery of phytoconstituents (Table 3). Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, proteins, saponins, diterpenes, and reducing carbohydrates, while alkaloids, glycosides, and tannins were absent (Table 4).

Quantitative estimation showed that the hydroalcoholic extract contained 0.851 ± 0.02 mg QE/100 mg of total flavonoids and 0.724 ± 0.01 mg/100 mg of total phenolics,

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct947

confirming the presence of bioactive constituents responsible for the reduction and stabilization of silver nanoparticles (Table 5).

Six nanoparticle formulations (F1–F6) were prepared by varying the concentration of silver nitrate and the extract-to-silver nitrate ratio while keeping other processing parameters constant (Table 1). Among these, formulation F3 exhibited the highest nanoparticle yield (82.68 ± 0.17%) and the maximum flavonoid content (0.961 ± 0.016 mg QE/100 mg), indicating that 3 mM $AgNO_3$ with a 1:1 extract-to-silver nitrate ratio provided optimum conditions for nanoparticle formation (Table 6). Characterization of the optimized formulation showed an average particle size of 292.2 nm and a zeta potential of −37.45 mV, suggesting good colloidal stability and low aggregation tendency of the nanoparticles (Table 7).

The optimized silver nanoparticles were incorporated into Carbopol 940 gel formulations containing different polymer concentrations (Table 2). The prepared gels showed acceptable physical characteristics, including good homogeneity, smooth texture, absence of clogging, satisfactory washability, and good extrudability (Table 8). Increasing Carbopol concentration resulted in increased viscosity and improved consistency of the gel formulations.

Among the gel formulations, GF2 exhibited an optimum balance between spreadability, viscosity, texture, and extrudability, making it the most suitable formulation for topical application (Tables 8 and 9). The pH values of all formulations ranged from 6.72 to 6.93, which is compatible with the pH of normal skin and may minimize the risk of irritation (Table 9). Drug (flavonoid) content analysis confirmed uniform distribution of nanoparticles throughout the gel matrix, with GF2 containing 0.904 ± 0.018 mg QE/100 mg gel (Table 9).

The in vitro release profile of GF2 demonstrated a sustained release pattern, with cumulative drug release increasing from 15.84% at 0.25 h to 99.75% at 4 h (Table 10). This controlled release behavior is advantageous for topical delivery as it can prolong the residence time of the active constituents at the site of application.

Release kinetic analysis showed that GF2 followed zero-order kinetics (R^2 = 0.9802) more closely than first-order kinetics (R^2 = 0.7759), indicating an approximately constant release rate throughout the study period (Table 11).

The antifungal study demonstrated concentration-dependent activity against *Candida albicans* and *Aspergillus flavus* (Table 12). The silver nanoparticle gel (GF2) produced larger zones of inhibition than the crude extract at all tested concentrations. At 100 mg/ml, GF2 showed inhibition zones of 18.6 ± 0.22 mm against Candida albicans and 17.5 ± 0.28 mm against *Aspergillus flavus*, whereas the extract showed 13.2 ± 0.31 mm and 11.8 ± 0.29 mm, respectively (Table 12).

Table 3 % Yield of Leaves Extract of *Combretum roxburghii*

S. No.	Plant Materials	Solvent Used	Weight of Dried Powder (g)	Weight of Extract Obtained (g)	Percentage Yield (%)
1.	*Combretum roxburghii* leaves	Hydroalcoholic	50	4.35	8.70

Table 4 Phytochemical Screening of Extract of Leaves of *Combretum roxburghii*

S. No.	Phytoconstituents	Test Performed	Observation	Result
1.	Alkaloids	Mayer's Test	No cream colored precipitate formed	-ve
		Wagner's Test	No reddish brown precipitate formed	-ve
		Dragendorff's Test	No orange precipitate formed	-ve
		Hager's Test	No yellow precipitate formed	-ve
2.	Glycosides	Legal's Test	No pink to red coloration observed	-ve
3.	Flavonoids	Lead Acetate Test	Yellow precipitate formed	+ve
		Alkaline Reagent Test	Intense yellow coloration observed	+ve
4.	Phenolic Compounds	Ferric Chloride Test	Deep bluish green coloration developed	+ve
5.	Proteins	Xanthoproteic Test	Yellow to orange coloration observed	+ve
6.	Carbohydrates	Molisch's Test	No violet ring formed at junction	-ve
		Benedict's Test	No reddish precipitate formed	-ve
		Fehling's Test	Brick red precipitate observed	+ve
7.	Saponins	Froth Test	Persistent froth observed	+ve
		Foam Test	No stable foam formed	-ve
8.	Diterpenes	Copper Acetate Test	Emerald green coloration observed	+ve
9.	Tannins	Gelatin Test	No white precipitate formed	-ve

Table 5 Estimation of Total Flavonoids and Phenol Content of Leaves of *Combretum roxburghii*

S. No.	Extract	Total flavonoids content	Total phenol content
		(mg/ 100 mg of dried extract)	
1.	Hydroalcoholic	0.851± 0.02	0.724± 0.01

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Table 6 Determination of % Yield and Flavonoids Content of Prepared Silver Nanoparticles Formulations

S. No.	Formulation Code	% Yield*	Flavonoids Content (QE mg/100 mg
1	F1	64.85 ± 0.31	0.748 ± 0.018
2	F2	71.24 ± 0.28	0.826 ± 0.021
3	**F3**	**82.68 ± 0.17**	**0.961 ± 0.016**
4	F4	76.15 ± 0.24	0.889 ± 0.019
5	F5	72.86 ± 0.21	0.854 ± 0.017
6	F6	69.94 ± 0.26	0.798 ± 0.020

*Average of Three Determinations (n=3 ±SD)

Table 7 Characterization of Average Particle Size and Zeta Potential of Optimized Formulation F3

Formulation code	Average Particle size (nm)	% Yield	Zeta Potential (mV)
F3	292.2	**82.68 ± 0.17**	- 37.45 mV

Table 8 Results of Physical Characteristics

F. Code	Colour	Clogging	Homogeneity	Texture	Washability	Extrudability
GF1	Light Brown	Absent	Good	Smooth	Good	Good
GF2	Brown	Absent	Very Good	Uniform and Smooth	Very Good	Excellent
GF3	Dark Brown	Absent	Excellent	Smooth and Homogeneous	Excellent	Excellent

Table 9 Results of Evaluation of Gel

Formulation Code	Spreadability* (g·cm/sec)	Viscosity* (cP)	Flavonoid Content (mg QE/100 mg Gel)*	pH*
GF1	14.32 ± 0.82	3850 ± 18	0.812 ± 0.021	6.72 ± 0.05
GF2	12.18 ± 0.64	4825 ± 16	0.904 ± 0.018	6.84 ± 0.03
GF3	10.24 ± 0.38	5728 ± 19	0.958 ± 0.015	6.93 ± 0.02

Values are Expressed as Mean ± SD (n = 3)

Table 10 In-Vitro Drug Release Data for Gel GF2

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.25	0.500	-0.602	15.84	1.200	84.16	1.925
0.5	0.707	-0.301	25.68	1.410	74.32	1.871
1	1.000	0.000	37.95	1.579	62.05	1.793
1.5	1.225	0.176	50.38	1.702	49.62	1.696
2	1.414	0.301	63.75	1.804	36.25	1.559
2.5	1.581	0.398	76.82	1.885	23.18	1.365
3	1.732	0.477	88.64	1.948	11.36	1.055
4	2.000	0.602	99.75	1.999	0.25	-0.602

Table 11 Release Kinetics Regression Values of Formulation GF2

Formulation code	Zero order	First order
GF2	0.9802	0.7759

Table 12 Antifungal Activity of Extract and GF2 Gel Formulation against Selected Microbes

Microorganism	Extract (Zone of Inhibition, mm)			GF2 Gel Formulation (Zone of Inhibition, mm)		
	25 mg/ml	50 mg/ml	100 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
Candida albicans	7.8 ± 0.28	10.4 ± 0.35	13.2 ± 0.31	12.6 ± 0.24	15.8 ± 0.27	18.6 ± 0.22
Aspergillus flavus	6.5 ± 0.30	9.1 ± 0.26	11.8 ± 0.29	11.8 ± 0.25	14.9 ± 0.30	17.5 ± 0.28

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