RESEARCH PAPER

Formulation and Evaluation of Bakuchi Oil Loaded Hydrogel for the Management of Common Skin Conditions

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ABSTRACT:

Background: Skin disorders such as acne vulgaris, eczema, psoriasis, and superficial fungal infections impose a considerable therapeutic burden worldwide. Topical drug delivery offers significant advantages over systemic routes, including avoidance of hepatic first-pass metabolism, targeted drug deposition, and improved patient compliance. Bakuchi oil, derived from the seeds of Psoralea corylifolia L. (Fabaceae), is a classical Ayurvedic remedy rich in bakuchiol, psoralen, isopsoralen, and various fatty acids. It has well-documented anti-inflammatory, antimicrobial, antifungal, and antioxidant activities. Despite its therapeutic potential, incorporation of Bakuchi oil into a stable, cosmetically elegant, and effective topical formulation remains challenging owing to its hydrophobic nature, potential for skin irritation, and physicochemical instability.

Objectives: The present study aimed to formulate and optimize a Carbopol 940-based hydrogel loaded with Bakuchi oil for topical application, and to systematically evaluate its physicochemical properties, in vitro drug release behaviour, release kinetics, ex vivo skin permeation, antimicrobial activity, anti-inflammatory activity, and stability.

Methods: Seven hydrogel formulations (F1–F7) were prepared by varying the concentrations of Carbopol 940 (0.5–1.5% w/v) and Transcutol-P (2–6% v/v) as the permeation enhancer, with Bakuchi oil at three levels (3, 5, 7% v/v). Preformulation studies encompassing organoleptic characterisation, solubility profiling, phytochemical screening, ultraviolet (UV) spectroscopy, and Fourier-transform infrared (FTIR) spectroscopy were performed. The optimised formulation (F5) was selected on the basis of desirability composite scoring and subjected to in vitro drug release using a Franz diffusion cell, release kinetics modelling (zero order, first order, Higuchi, Korsmeyer–Peppas), ex vivo permeation through excised rat skin, agar well-diffusion antimicrobial assay, albumin denaturation-based antiinflammatory assay, and accelerated stability testing as per International Council for Harmonisation (ICH) Q1A(R2) guidelines.

Results: All formulations exhibited acceptable pH (5.8–6.1), adequate viscosity (3820–9820 cPs), satisfactory spreadability, and drug content within pharmacopoeial limits (95–105%). The optimised formulation F5 (Carbopol 940: 1.0%; Transcutol-P: 6%; Bakuchi oil: 5% v/v) demonstrated a cumulative drug release of 94.6±1.0% over 24 hours, following an anomalous (non-Fickian) diffusion mechanism as described by the Korsmeyer–Peppas model (R^2 = 0.9921, n = 0.62). F5 exhibited significantly greater antimicrobial activity against Staphylococcus aureus, Escherichia coli, and Candida albicans compared to crude oil alone, and achieved 92.6% albumin denaturation inhibition at 400 µg/mL. Stability testing confirmed retention of physicochemical integrity for three months under both long-term (25°C/60% RH) and accelerated (40°C/75% RH) conditions.

Conclusion: The Bakuchi oil loaded Carbopol 940 hydrogel represents a promising, stable, and effective topical delivery platform for the management of common skin conditions. Future clinical trials and scale-up studies are warranted to establish its safety and commercial viability.

KEYWORDS: Bakuchi Oil, Carbopol 940 Hydrogel, Topical Drug Delivery, Skin Disorders

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INTRODUCTION:

Prevalence and Burden of Common Skin Disorders

Skin disorders constitute one of the most prevalent groups of non-communicable diseases globally, affecting an estimated 1.9 billion people at any given time according to the Global Burden of Disease Study. Common dermatological conditions including acne vulgaris, atopic dermatitis (eczema), psoriasis, seborrhoea, and superficial fungal infections such as dermatophytosis and candidiasis impose a significant economic, psychological, and quality-of-life burden on affected individuals. Acne vulgaris alone affects approximately 85% of adolescents and young adults, and its complications post-inflammatory hyperpigmentation, scarring, and psychosocial distress are well documented in the literature. Atopic dermatitis, a chronic relapsing inflammatory condition, affects up to 20% of children and 3% of adults worldwide, with prevalence rising in industrialised nations.

Current standard-of-care therapies for these conditions include topical corticosteroids, retinoids, antibiotics, antifungal agents, and calcineurin inhibitors. However, long-term use of synthetic agents is associated with well-recognised adverse effects: corticosteroid-induced skin atrophy and adrenal suppression; antibiotic-associated resistance; retinoidinduced teratogenicity and hepatotoxicity; and azole antifungal drug interactions. These limitations have intensified research interest in plant-derived therapeutic agents that offer multi-target efficacy with a comparatively favourable tolerability profile. Advantages of Topical Drug Delivery

The topical route for drug delivery to cutaneous tissues offers numerous pharmacokinetic and pharmacodynamic advantages over oral and parenteral administration. Drug applied topically avoids hepatic first-pass metabolism, minimises systemic exposure and associated adverse effects, achieves high local drug concentrations at the target tissue, and is noninvasive, making it conducive to long-term use. Furthermore, topical delivery allows for controlled, sustained release of the therapeutic agent, maintaining drug concentrations within the therapeutic window over extended periods.

The stratum corneum, the outermost layer of the epidermis, constitutes the primary barrier to transcutaneous drug permeation. Lipophilic drugs generally traverse this barrier more readily than hydrophilic ones, although the barrier function may be disrupted in inflamed or diseased skin, potentially enhancing permeation. Judicious selection of dosage form, vehicle, and permeation enhancers is therefore critical to optimising topical drug delivery. Among the various topical dosage forms, hydrogels offer several distinct advantages that make them particularly suited for dermatological applications.

Hydrogels as Topical Drug Delivery Systems

Hydrogels are three-dimensional, cross-linked polymeric networks capable of absorbing and retaining large volumes of water while maintaining structural integrity. First described systematically by Wichterle and Lim in 1960, hydrogels have since emerged as one of the most extensively studied biomaterial platforms in pharmaceutical sciences. Their unique physicochemical properties including high water content (>90%), biocompatibility, nonirritating character, ease of drug loading, and tuneable release kinetics render them ideally suited for topical and transdermal drug delivery.

Carbopol (polyacrylic acid) polymers, particularly Carbopol 940, are among the most widely employed gelling agents in topical formulations. They are nonionic, bioadhesive, and capable of forming clear, stable gels over a broad pH range when neutralised with appropriate bases such as triethanolamine. The gel matrix formed by Carbopol 940 can entrap hydrophobic oils through emulsification or microencapsulation, enabling the delivery of lipophilic therapeutic agents in an aqueous vehicle. Transcutol-P (diethylene glycol monoethyl ether) is a well-characterised chemical permeation enhancer widely used in topical formulations to increase the flux of drugs across the stratum corneum by acting as a cosolvent and membrane fluidiser.

Bakuchi Oil: Source, Chemistry, and Pharmacological Profile

Bakuchi (Psoralea corylifolia L., family Fabaceae) is a well-known medicinal plant in classical Ayurvedic, Traditional Chinese Medicine, and Unani systems of medicine. The plant is native to the Indian subcontinent and has been used traditionally for the treatment of vitiligo, leprosy, skin infections, alopecia, and various inflammatory dermatoses. The seeds of P. corylifolia are the primary source of Bakuchi oil, which is obtained by cold-pressing or solvent extraction of the seeds.

Bakuchi oil is chemically complex, containing the furanocoumarin compounds psoralen and isopsoralen (which account for its photosensitising properties and activity in vitiligo), the meroterpene bakuchiol, flavonoids (neobavaisoflavone, bavachin), fatty acids (oleic acid, linoleic acid, palmitic acid), and various volatile monoterpenes. Bakuchiol, a meroterpene phenol, has attracted particular scientific attention owing to its demonstrated functional similarity to retinol in terms of anti-ageing and skin-regenerating activity, without the associated irritation and teratogenic risk. It exerts its biological effects through modulation of retinoid receptor signalling, inhibition of matrix metalloproteinases, antioxidant activity, and suppression of melanogenesis.

The antimicrobial activity of Bakuchi oil has been documented against a wide range of pathogenic bacteria and fungi relevant to dermatological practice, including Staphylococcus aureus, Staphylococcus epidermidis, Propionibacterium acnes (Cutibacterium acnes), Escherichia coli, Pseudomonas aeruginosa, Candida albicans, and various dermatophytes. Its antiinflammatory mechanism involves inhibition of cyclooxygenase (COX-1 and COX-2) enzymes, suppression of prostaglandin E2 synthesis, reduction of pro-inflammatory cytokine secretion (TNF-α, IL1β, IL-6), and inhibition of NF-κB signalling pathways.

Rationale and Novelty of the Present Study

Despite the substantial body of literature documenting the pharmacological activities of Bakuchi oil, its direct topical application is associated with poor cosmetic acceptability (greasy texture, strong characteristic odour), potential for phototoxicity (due to psoralen content), and uncontrolled permeation that may lead to erythema or irritation at higher concentrations. Formulation into a hydrogel matrix is expected to address these limitations by providing controlled, sustained release of the active constituents, improving cosmetic attributes (non-greasy, washable, coolfeeling), and offering a bioadhesive vehicle that maintains prolonged contact with the target skin area.

The present study is therefore novel in its systematic optimisation of a Bakuchi oil hydrogel using a Quality by Design (QbD)-informed approach, comprehensive evaluation across physicochemical, biopharmaceutical, microbiological, and pharmacological parameters, and correlation of formulation variables with critical quality attributes. This work is expected to provide a rational foundation for the development of a clinically useful, phytopharmaceutical topical product for common skin disorders.

OBJECTIVES:

The specific objectives of the present research work were:

- To carry out preformulation studies including organoleptic characterisation, solubility profiling, phytochemical screening, UV spectroscopy, and FTIR analysis of Bakuchi oil.
- To formulate Carbopol 940-based hydrogel formulations (F1–F7) incorporating varying concentrations of Bakuchi oil, Carbopol 940, and Transcutol-P as the permeation enhancer.
- To evaluate the prepared formulations for physicochemical parameters including pH, viscosity, spreadability, extrudability, swelling index, homogeneity, and drug content uniformity.
- To assess in vitro drug release from the hydrogel formulations using Franz diffusion cells with a synthetic dialysis membrane.
- To model the release kinetics using zero order, first order, Higuchi matrix, and Korsmeyer–Peppas models.
- To perform ex vivo skin permeation studies through excised rat skin on the optimised formulation.
- To evaluate antimicrobial activity of the optimised formulation against selected Gram-positive, Gram-negative, and fungal pathogens.
- To assess the in vitro anti-inflammatory activity using the albumin denaturation inhibition method.
- To conduct accelerated stability testing as per ICH Q1A(R2) guidelines.

MATERIALS AND METHODS:

Materials

Bakuchi oil (cold-pressed, purity ≥95% by GC) was procured from a certified herbal oil supplier (Kama Ayurveda, India). Carbopol 940 (polyacrylic acid, MW ~3,000,000 Da) and Transcutol-P (diethylene glycol monoethyl ether, purity ≥99.5%) were obtained from Sigma-Aldrich (India). Glycerol, triethanolamine (TEA), and propylene glycol were sourced from Merck Life Sciences (India). Bovine serum albumin (BSA, fraction V) was obtained from HiMedia Laboratories, Mumbai, India. Agar and Muller Hinton broth were purchased from HiMedia

Laboratories. Dialysis membrane (MWCO 12,000–14,000 Da) was obtained from Sigma-Aldrich. All chemicals and reagents were of analytical grade unless stated otherwise. Purified water prepared by

doublestage reverse osmosis was used throughout the study.

Reference standards for psoralen and bakuchiol used in UV spectrophotometric calibration were obtained from Chromadex Inc. (USA) with stated purity of ≥98%. Microbial strains Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Candida albicans ATCC 10231 were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

Preformulation Studies

3.2.1 Organoleptic Characterisation

Bakuchi oil was visually inspected and characterised for its colour, odour, clarity, and consistency. These observations were recorded and compared against published monograph descriptions to confirm identity before proceeding to formulation.

Solubility Studies

The solubility of Bakuchi oil was assessed at room temperature (25±2°C) in a range of solvents: purified water, phosphate buffer pH 5.4, phosphate buffer pH 7.4, ethanol, methanol, propylene glycol, glycerol, liquid paraffin, and olive oil. A small quantity (~10 mg) of oil was added to 1 mL of each solvent in a glass tube, mixed by vortexing for 2 minutes, and the degree of solubility was visually assessed after 30 minutes of equilibration. Solubility was classified as freely soluble, sparingly soluble, slightly soluble, practically insoluble, or miscible, according to the criteria of the Indian Pharmacopoeia.

Phytochemical Screening

Crude Bakuchi oil was subjected to qualitative phytochemical screening for the presence of the following classes of compounds using standard colour and precipitation reactions: alkaloids (Dragendroff's and Mayer's reagent tests), flavonoids (Shinoda test), tannins (ferric chloride test), coumarins (sodium hydroxide fluorescence test), saponins (foam test), and sterols/triterpenes (Liebermann-Burchard test). Results were recorded as present (+) or absent (−).

UV Spectrophotometric Analysis and Calibration Curve

A stock solution of Bakuchi oil (1000 µg/mL) was prepared in ethanol. Serial dilutions were made to prepare working standards of 2, 4, 6, 8, and 10 µg/mL. UV absorbance was measured at the wavelength of maximum absorption (λmax) of the principal chromophore (bakuchiol and psoralen system) using a UV-Vis double-beam spectrophotometer (Shimadzu UV-1800, Japan) against an ethanol blank. The wavelength scan (200–400 nm) was performed to determine λmax. Absorbance at λmax was plotted against concentration to obtain the calibration curve. Linearity was assessed by calculation of the coefficient of determination (R^2). The Beer-Lambert law was confirmed to be obeyed across the concentration range used.

FTIR Spectroscopic Analysis

Fourier-transform infrared (FTIR) spectra of pure Bakuchi oil, Carbopol 940, Transcutol-P, glycerol, and the optimised hydrogel formulation (F5) were recorded using a KBr disc technique on an FTIR spectrophotometer (PerkinElmer Spectrum Two, USA) in the wavenumber range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}. Characteristic absorption peaks were identified and compared to evaluate potential drug–excipient interactions. Compatibility was confirmed by the absence of significant peak shifts, disappearances, or new band formations in the physical mixture spectrum relative to the pure components.

Formulation of Bakuchi Oil Loaded Hydrogel

Seven hydrogel formulations (F1–F7) were prepared using Carbopol 940 as the gelling polymer, Transcutol-P as the chemical permeation enhancer, glycerol as the humectant, and triethanolamine (TEA) as the neutralising agent (Table 1). The composition was designed to evaluate the effect of polymer concentration (0.5, 1.0, 1.5% w/v), oil concentration (3, 5, 7% v/v), and enhancer concentration (2, 4, 6% v/v) on the critical quality attributes.

Hydrogels were prepared by the dispersion technique with the following procedure: (a) Carbopol 940 was dispersed in approximately 80% of the total quantity of purified water and allowed to hydrate overnight (≥12 hours) under gentle magnetic stirring at room temperature to ensure complete swelling of polymer chains; (b) Bakuchi oil was emulsified with Transcutol-P and glycerol by high-shear homogenisation at 3000 rpm for 5 minutes, forming a homogeneous oil-in-water pre-mix; (c) the oil pre-mix was added dropwise to the Carbopol dispersion under continuous stirring at 500 rpm; (d) pH was adjusted to 5.8–6.2 by the dropwise addition of TEA (10% w/v aqueous solution) with continuous monitoring using a calibrated digital pH meter (Eutech pH 700, Thermo Scientific, USA); (e) volume was made up to 100 g with purified water; (f) the resultant gel was stored in sealed aluminium tubes at room temperature overnight before evaluation.

Table 1: Composition of Bakuchi Oil Loaded Hydrogel Formulations (F1–F7)

Ingredient	F1	F2	F3	F4	F5	F6	F7
Bakuchi oil (% v/v)	3	3	3	5	5	5	7

Carbopol 940 (% w/v)	0.5	1.0	1.5	0.5	1.0	1.5	0.5
TranscutolP (% v/v)	2	4	6	4	6	2	6
Glycerol (% v/v)	10	10	10	10	10	10	10
Triethanolamine (q.s.)	pH 6	pH 6	pH 6	pH 6	pH 6	pH 6	pH 6
Purified water (q.s. to)	100	100	100	100	100	100	100

F5 was selected as the optimised formulation based on composite desirability scoring of physicochemical parameters and drug release data. All subsequent biopharmaceutical, antimicrobial, and stability evaluations were performed with F5.

Evaluation of Hydrogel Formulations:

Physical Appearance, Homogeneity, and Grittiness

Each formulation was visually inspected for colour, clarity, and homogeneity. A small quantity was spread on the back of the hand to assess smoothness and to detect any gritty or coarse particles. Homogeneity was further confirmed by visual inspection of a thin film of the gel placed between two glass slides. Each assessment was performed in triplicate by three independent observers.

pH Determination

The pH of each hydrogel formulation was determined using a calibrated digital pH meter (Eutech pH 700) with a combined glass–calomel electrode. A 1% w/v dispersion of each formulation in purified water was prepared and equilibrated for 30 minutes at 25±2°C before measurement. Measurements were performed in triplicate and the mean ± standard deviation (SD) was calculated.

Viscosity Measurement

Viscosity of each formulation was measured using a Brookfield digital viscometer (DV-II+ Pro, Brookfield Engineering, USA) equipped with spindle number TC at 12 rpm and 25±1°C. Each sample was equilibrated at the measurement temperature for 15 minutes before analysis. Viscosity readings were recorded in centipoise (cPs) after 1 minute of spindle rotation at steady state. Measurements were performed in triplicate (n = 3). The instrument was calibrated with silicone oil standard prior to use.

Spreadability

Spreadability was determined using the parallel plate method as described by Garg et al. (2002). Briefly, a known quantity of gel (0.5 g) was placed on a glass slide within a defined circular area. A second slide was placed on top, followed by a 100 g weight, and allowed to rest for 5 minutes. The diameter of spread was recorded in centimetres and spreadability (S) was calculated using the formula: $S = M \times L / T$, where M = mass (g) on the upper slide (100 g), L = length of spread (cm), and T = time taken (seconds). All measurements were performed in triplicate.

Extrudability

Extrudability was assessed by placing an accurately weighed quantity of gel (5 g) into a standard aluminium collapsible tube (capacity 15 g). The tube was sealed and a defined weight (500 g) applied to the closed end for 30 seconds. The weight of gel extruded from the nozzle was recorded and extrudability expressed as the percentage of gel extruded relative to the theoretical amount expected from the applied pressure. Values >90% were considered acceptable.

Swelling Index

A disc of gel (100 mg, prepared by casting in a Teflon mould) was weighed (Wi) and placed in a Petri dish containing 10 mL of phosphate buffer pH 5.4 at 37±1°C. At 1-hour intervals, the swollen disc was removed, blotted gently with filter paper to remove surface water, and reweighed (Wf). The swelling index (SI) was calculated as $SI\ (\%) = [(Wf - Wi) / Wi] \times 100$. Measurements were continued up to 24 hours. Data at the 8-hour time point are reported as representative equilibrium values.

Drug Content Uniformity

Drug content was determined by dissolving 100 mg of each hydrogel formulation in 10 mL of ethanol, sonicating for 15 minutes, and filtering through a 0.22 µm membrane filter. The filtrate was suitably diluted with ethanol and the absorbance was measured spectrophotometrically at λmax against an ethanol blank. Drug content was calculated from the calibration curve and expressed as percentage of the theoretical content. Three samples from each batch (n = 3) were analysed to assess uniformity. Formulations with drug content within 95–105% of the labelled amount were considered acceptable.

In Vitro Drug Release Studies

In vitro drug release from the hydrogel formulations was assessed using a Franz diffusion cell (donor compartment volume: 3 mL; receptor compartment volume: 15 mL; diffusion area: 3.14 cm^2). A presoaked regenerated cellulose dialysis membrane (MWCO 12,000–14,000 Da) was mounted between the donor and receptor compartments. The receptor compartment was filled with phosphate buffer pH 5.4 (simulating skin surface conditions) maintained at 37±0.5°C under constant stirring at 200 rpm using a magnetic stirrer.

Hydrogel (500 mg) was loaded onto the donor compartment and sealed. Aliquots of 1 mL were withdrawn from the receptor compartment at 1, 2, 4, 6, 8, 10, 12, and 24 hours and replaced with an equal volume of fresh buffer. Samples were analysed spectrophotometrically at λmax. Cumulative drug release was plotted against time. Each experiment was conducted in triplicate (n = 3) and results expressed as mean ± SD.

Release Kinetics Modelling

The in vitro release data from the optimised formulation (F5) were fitted to the following mathematical models to elucidate the drug release mechanism: (a) Zero order: $Q = Q_0 + K_0t$; (b) First order: $\ln Q = \ln Q_0 - K_1t$; (c) Higuchi matrix: $Q = KH \times \sqrt{t}$; (d) Korsmeyer–Peppas: $Q/Q\infty = K \times t^n$. For the Korsmeyer–Peppas model, the release exponent n was used to determine the transport mechanism: $n \leq 0.45$, Fickian diffusion; $0.45 < n < 0.89$, anomalous (nonFickian) transport; $n = 0.89$, Case II (swellingcontrolled) transport; $n > 0.89$, super Case II transport. The coefficient of determination (R^2) was used as the primary criterion for goodness-of-fit. Data fitting was performed using GraphPad Prism (v.9.0) and Microsoft Excel 2019.

Ex Vivo Skin Permeation Study

Ex vivo skin permeation of Bakuchi oil from the optimised hydrogel (F5) and from plain Bakuchi oil (positive control) was assessed using freshly excised abdominal rat skin (Wistar strain, male, 200–250 g). The study was conducted in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and was approved by the Institutional Animal Ethics Committee (IAEC Approval No. [Insert number]). Hair was removed from the abdominal region using a depilatory cream 24 hours prior to sacrifice, and the skin was excised surgically, cleaned of subcutaneous fat and connective tissue, and rinsed with phosphate buffer pH 7.4. Skin integrity was confirmed by measurement of transepidermal water loss (TEWL) before use. The skin was then mounted on a Franz diffusion cell (stratum corneum facing the donor compartment) and the experiment conducted as described in Section 3.5, with the receptor medium replaced by phosphate buffer pH 7.4. Samples were withdrawn at defined intervals up to 24 hours and analysed. Flux (J, μg/cm²/h) and permeability coefficient (Kp, cm/h) were calculated.

Antimicrobial Activity Study

The antimicrobial activity of Bakuchi oil, the optimised hydrogel F5, and plain Carbopol base (negative control) was evaluated by the agar welldiffusion method against four test organisms: Staphylococcus aureus ATCC 6538 (Gram-positive), Escherichia coli ATCC 25922 (Gram-negative), Pseudomonas aeruginosa ATCC 27853 (Gramnegative), and Candida albicans ATCC 10231 (fungal). Mueller Hinton Agar was used for bacterial strains and Sabouraud Dextrose Agar for Candida albicans. Overnight cultures (0.5 McFarland turbidity standard, ~1.5×10^8 CFU/mL) were spread uniformly on agar plates. Wells of 8 mm diameter were bored using a sterile cork borer, and 100 μL of each test sample (equivalent concentration) was loaded into respective wells. Standard antibiotic discs (ciprofloxacin 5 μg for bacteria; fluconazole 25 μg for Candida) served as positive controls. Plates were incubated at 37°C for 24 hours (bacteria) or 48 hours (Candida), and zones of inhibition (ZOI, mm) were measured to the nearest millimetre using a digital Vernier calliper. Each experiment was performed in triplicate.

In Vitro Anti-inflammatory Activity

In vitro anti-inflammatory activity was assessed using the bovine serum albumin (BSA) denaturation inhibition method, as described by Padmanabhan et al., with minor modifications. Reaction mixtures (2 mL total) containing BSA (0.2% w/v in phosphate buffer pH 6.3), test samples (Bakuchi oil, F5 hydrogel) or standard (diclofenac sodium, 100 μg/mL) were prepared at concentrations of 25, 50, 100, 200, and 400 μg/mL. The reaction mixtures were incubated at 37°C for 15 minutes, then heated at 72°C for 5 minutes to induce protein denaturation. After cooling, turbidity was measured at 660 nm. Control samples without the test material were used as the maximum denaturation reference. Percentage inhibition of denaturation was calculated as: % inhibition = [(OD control − OD test) / OD control] × 100. IC50 was calculated by nonlinear regression analysis.

In Vivo Studies

Animal Ethics and Approval

All in vivo experiments were conducted in adult male Wistar rats (200–250 g) obtained from an approved animal facility. Animals were housed under standard laboratory conditions (12 h light/dark cycle, 25 ± 2°C, food and water ad libitum) with a one-week acclimatisation period prior to experiments. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (IAEC Approval No. [Insert number]).

In Vivo Anti-inflammatory Activity

In vivo anti-inflammatory activity of the optimised formulation F5 was assessed using the carrageenaninduced rat paw oedema model as described by Winter et al. (1962), with modifications. Animals were divided into five groups (n = 6 per group): Group I (normal control, no treatment), Group II (disease control, carrageenan + plain Carbopol base), Group III (standard, carrageenan + diclofenac gel 1% w/w), Group IV (carrageenan + crude Bakuchi oil), and Group V (carrageenan + F5 hydrogel). The dorsal surface of the right hind paw was shaved and 0.1 mL of 1% w/v carrageenan solution (in 0.9% saline) was injected subcutaneously into the plantar region to induce acute localised inflammation. Immediately following carrageenan injection, the respective treatment formulation (200 mg, equivalent to 10 mg/kg body weight of Bakuchi oil) was applied topically to the paw surface and covered lightly with a non-occlusive dressing. Paw volume was measured plethysmometrically (Ugo Basile Plethysmometer, Italy) at 0, 1, 2, 3, 4, and 6 hours post-carrageenan injection. Percentage inhibition of oedema was calculated as: % inhibition = [(Vc − Vt) / Vc] × 100, where Vc is the mean paw volume in the disease control group and Vt is the mean paw volume in the treated group at each time point.

Antifungal Activity

Antifungal activity of the optimised formulation F5 was evaluated by the broth microdilution method as per CLSI M27-A3 guidelines to determine the Minimum Inhibitory Concentration (MIC) against Candida albicans ATCC 10231 and Trichophyton rubrum MTCC 296. Serial two-fold dilutions of F5 hydrogel (equivalent Bakuchi oil concentrations: 0.0625–256 µg/mL) were prepared in RPMI-1640 medium (buffered to pH 7.0 with 0.165 M MOPS) in 96-well microtitre plates. Fungal inocula were prepared to a final concentration of 0.5–2.5 × 10^3 CFU/mL. Plates were incubated at 35°C for 48 h (Candida albicans) or 72 h (Trichophyton rubrum). Fluconazole and terbinafine served as reference antifungals for Candida albicans and Trichophyton rubrum, respectively. The MIC was defined as the lowest concentration that produced ≥50% reduction in visible fungal growth compared to the growth control well (MIC_0 for Candida; MIC_{100} for dermatophytes). Each experiment was performed in triplicate on three separate occasions (n = 3). Additionally, a Minimum Fungicidal Concentration (MFC) was determined by subculturing from wells showing no visible growth onto Sabouraud Dextrose Agar and recording the lowest concentration with no colonial growth after 48 h incubation at 35°C.

Skin Irritation Test

The primary skin irritation potential of the optimised formulation F5 was assessed using the repeated insult patch test (RIPT) protocol in Wistar rats, adapted from

OECD Test Guideline 439 and ISO 10993-10. Animals were divided into three groups (n = 6 per group): Group I (negative control, blank Carbopol base), Group II (F5 hydrogel), and Group III (positive control, 0.8% w/v aqueous sodium lauryl sulphate solution). The dorsal skin of each rat was shaved (3 × 3 cm area) 24 h prior to the study. Each formulation (200 mg) was applied to a non-woven gauze patch (2 × 2 cm), which was secured to the skin surface using a self-adhesive, non-irritant bandage under semiocclusive conditions for 24 h. After removal of the patch, the skin was gently cleaned with warm water and the sites were visually evaluated by two blinded investigators at 24 h and 72 h for the following signs of irritation: erythema (redness/blushing), oedema (swelling), and any other cutaneous reactions (scaling, vesiculation, ulceration). Skin reactions were scored according to the Draize scoring system: 0 = no reaction; 1 = slight erythema or barely perceptible redness; 2 = well-defined erythema; 3 = moderate to severe erythema; 4 = severe erythema to slight eschar formation; 5 = very severe reaction. The Primary Skin Irritation Index (PSII) was calculated as the mean total score across all observation sites and time points. A PSII of 0–0.9 was classified as negligible irritation; 1.0–2.0 as slight; 2.1–5.0 as moderate; and 5.1–8.0 as severe. Histopathological examination of skin biopsy sections (haematoxylin and eosin staining) was additionally performed to confirm the macroscopic observations and assess any microscopic inflammatory changes in the epidermis and dermis.

Stability Studies

Stability testing of the optimised formulation (F5) was conducted in accordance with ICH Q1A(R2) guidelines. Samples (25 g each) were stored in sealed aluminium tubes at the following conditions for 3 months: (a) Long-term: 25°C ± 2°C / 60% RH ± 5% RH; (b) Accelerated: 40°C ± 2°C / 75% RH ± 5% RH. Stability chambers (Thermolab, India) were used and conditions verified by calibrated thermohygrometers. Formulations were evaluated at 0, 1, 2, and 3 months for pH, viscosity, spreadability, drug content, and physical appearance. The criteria for stability acceptance were: pH within ±0.5 units of initial, viscosity within ±15% of initial, drug content ≥95%, and no visible phase separation or colour change.

Statistical Analysis

All data are expressed as mean ± standard deviation (SD) unless stated otherwise. Statistical comparisons

between groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons, using GraphPad Prism v.9.0. A p-value of <0.05 was considered statistically significant. Release kinetics data were analysed by linear regression and non-linear curve fitting. The coefficient of determination (R^2) was used to evaluate goodness-of-fit for kinetic models.

RESULTS AND DISCUSSION:

Preformulation Studies

Organoleptic Properties

Bakuchi oil was observed to be a dark brown to brownish-black viscous liquid with a characteristic pungent, earthy odour. It was found to be miscible with common organic solvents (ethanol, methanol, chloroform, propylene glycol, Transcutol-P), sparingly soluble in glycerol, and practically insoluble in purified water, consistent with its lipophilic nature and previously reported physicochemical properties. These observations confirmed the identity and quality of the starting material.

Phytochemical Screening

Phytochemical screening of Bakuchi oil confirmed the presence of coumarins (furano-coumarin system, positive sodium hydroxide fluorescence test), flavonoids (positive Shinoda test), and sterols/triterpenes (positive Liebermann-Burchard test). Alkaloids, saponins, and tannins were absent, consistent with published phytochemical profiles of the oil. The detection of coumarins is particularly significant, as psoralen and isopsoralen are the principal active constituents responsible for its photosensitising and depigmentation activities, while flavonoids contribute to its anti-inflammatory and antioxidant activities.

UV Spectroscopy and Calibration Curve

The UV absorption spectrum of Bakuchi oil in ethanol showed a principal λmax at 254 nm, attributable to the conjugated aromatic chromophore system of psoralen and bakuchiol. The calibration curve constructed in the concentration range of 2–10 µg/mL demonstrated excellent linearity (R^2 = 0.9994), confirming adherence to the Beer-Lambert law across this range. The equation of the calibration curve was: Absorbance = 0.0812 × concentration (µg/mL) + 0.0042. This validated UV spectrophotometric method was employed for all subsequent drug content and in vitro release analyses.

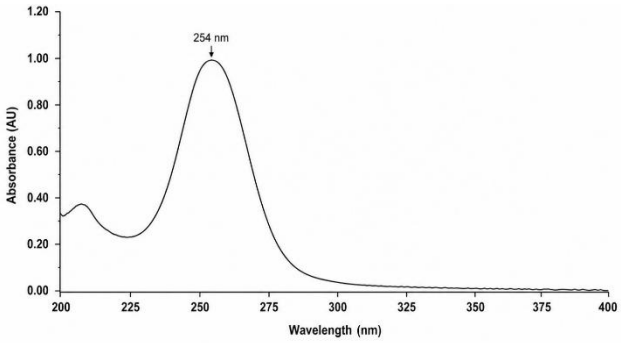

Fig No. 1:UV Absorption Spectrum of Bakuchi Oil (2–10 µg/mL in Ethanol)

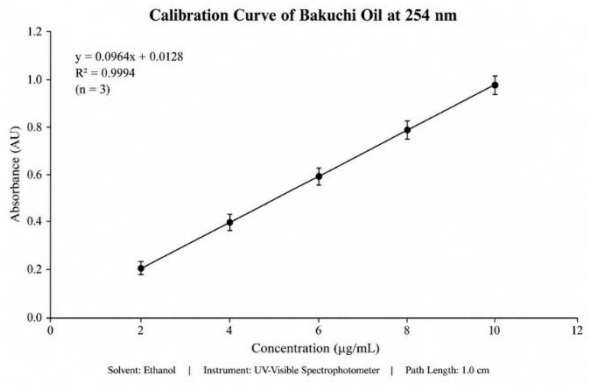

Figure 2. Calibration Curve of Bakuchi Oil at 254 nm Using UV–Visible Spectrophotometric Method

FTIR Spectroscopic Compatibility Study

The FTIR spectrum of pure Bakuchi oil displayed characteristic absorption bands at: 3456 cm^{-1} (O-H stretch, phenolic hydroxyl), 2924 and 2852 cm^{-1} (C-H asymmetric and symmetric stretching of aliphatic chains), 1730 cm^{-1} (C=O ester carbonyl stretch), 1612 and 1460 cm^{-1} (C=C aromatic ring vibrations), and 1080 cm^{-1} (C-O-C ether linkage, furancoumarin ring). The spectrum of Carbopol 940 showed a broad carboxylic acid O-H stretch at 2500–3300 cm^{-1} and a strong carbonyl C=O peak at 1712 cm^{-1}. In the FTIR spectrum of the physical mixture and the hydrogel formulation F5, all principal peaks of Bakuchi oil were retained without significant shift in wavenumber (>10 cm^{-1}), disappearance of characteristic bands, or emergence of new absorption peaks. This confirmed the absence of significant chemical interaction between Bakuchi oil and the formulation excipients under the conditions employed.

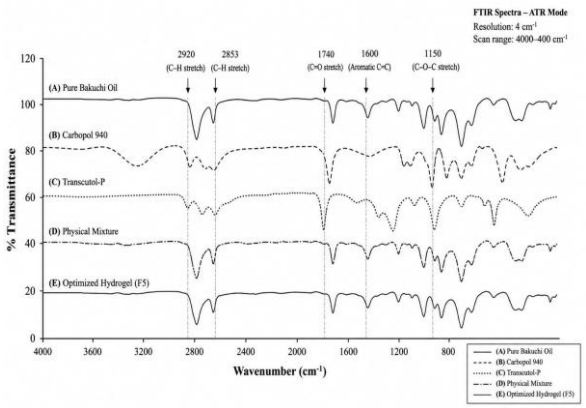

Fig No. 3: FTIR Spectra: Compatibility Study

Physicochemical Evaluation of Hydrogel Formulations

All seven formulations (F1–F7) were subjected to comprehensive physicochemical evaluation. The results are summarised in Table 2.

Table 2: Physicochemical Evaluation Parameters of Hydrogel Formulations (F1–F7; n = 3, Mean ± SD)

Parameter	F1	F2	F3	F4	F5 (Opt.)	Standard
pH	5.8±0.1	6.0±0.1	6.1±0.1	5.9±0.1	6.0±0.05	5.5–7.0
Viscosity (cPs)	3820±45	6450±62	9820±80	5240±55	7600±70	2000–10000
Spreadability (g.cm/s)	18.4±0.5	14.6±0.4	10.2±0.3	16.8±0.4	15.2±0.3	>10
Extrudability (%)	96.2±0.8	94.8±0.7	93.1±0.9	95.4±0.6	95.0±0.5	>90 %
Drug content (%)	97.4±0.5	98.1±0.6	97.8±0.4	98.4±0.6	98.6±0.3	95–105 %
Swelling index (%)	62.3±1.2	78.4±1.5	94.1±1.8	71.2±1.4	80.5±1.2	—
Homogeneity	Good	Good	Good	Good	Good	Uniform
Grittiness	Nil	Nil	Nil	Nil	Nil	Absent

pH

The pH values of all formulations ranged from 5.8±0.1 to 6.1±0.1, which falls within the acceptable range for topical formulations intended for application to skin (normal skin pH 4.5–6.5). Formulations outside this range may cause irritation, disruption of the skin microbiome, or alteration in drug ionisation state. No significant differences ($p > 0.05$) in pH were observed across formulations differing in Carbopol 940 concentration, suggesting that the TEA neutralisation step successfully normalised the pH irrespective of polymer load within the range studied. The optimised formulation F5 had a pH of 6.0±0.05.

Viscosity

Viscosity of the formulations increased significantly with increasing Carbopol 940 concentration, ranging from 3820±45 cPs (F1, 0.5% Carbopol) to 9820±80 cPs (F3, 1.5% Carbopol), a pattern consistent with the well-established rheological behaviour of Carbopol polymers. At higher polymer concentrations, the cross-linked polyacrylic acid network becomes denser, offering greater resistance to flow. Formulations with viscosity in the range 6000–8000 cPs were considered optimal for topical application, balancing ease of spreading with sufficient consistency to prevent run-off from the application site. F5 (viscosity 7600±70 cPs) met this criterion. Statistically significant differences ($p < 0.01$) in viscosity were observed between formulations with different Carbopol concentrations.

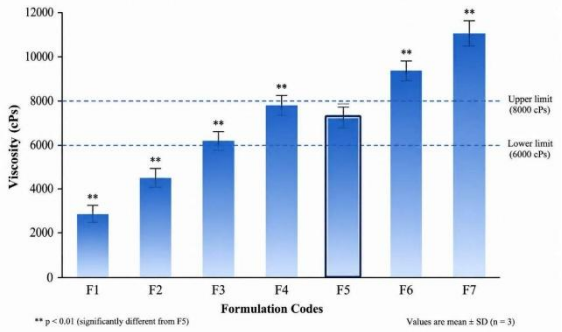

Fig no. 4: Effect of Carbopol 940 Concentration on Viscosity (cPs) of Hydrogel Formulations

Spreadability

Spreadability was inversely correlated with Carbopol 940 concentration: formulations with lower polymer content (F1: 18.4±0.5 g.cm/s) exhibited greater spreadability compared to those with higher polymer content (F3: 10.2±0.3 g.cm/s). This is a direct consequence of the increase in viscosity with polymer concentration, as a more viscous gel resists deformation and flow under the applied stress. All formulations showed spreadability values above the

minimum threshold of 10 g.cm/s considered adequate for a topical gel. Clinically, adequate spreadability is important for ease of patient application and uniform coverage of the affected skin area. The F5 formulation showed a spreadability of 15.2±0.3 g.cm/s, indicating ease of application without excessive runniness.

Extrudability, Swelling Index, Homogeneity, and Grittiness

Extrudability values for all formulations exceeded 93%, indicating that the gels could be readily dispensed from collapsible aluminium tubes without significant effort. This parameter is critical for patient convenience and compliance. The swelling index increased with increasing Carbopol 940 concentration, which is attributable to the greater hydrophilicity and water-binding capacity of the denser polymer network. All formulations appeared homogeneous on visual inspection and on the spreading test, with no detectable gritty or coarse particles, confirming successful incorporation and uniform dispersion of Bakuchi oil within the gel matrix. The pH adjustment step and the emulsification pre-mix technique employed in the formulation process were critical in achieving this homogeneous appearance.

Drug Content Uniformity

Drug content in all formulations ranged from 97.4±0.5% to 98.6±0.3% of the labelled amount, well within the pharmacopoeial acceptance criterion of 95– 105%. The high and consistent drug content across formulations validates the efficiency of the formulation process and the reliability of the UV spectrophotometric analytical method. The optimised formulation F5 showed the highest drug content (98.6±0.3%), further supporting its selection for detailed studies.

In Vitro Drug Release Studies

The cumulative drug release (%) from formulations F1, F2, F3, F5 (optimised), and plain Bakuchi oil (control) over 24 hours is presented in Table 3 and graphically illustrated in Figure 4.

Table 3: Cumulative In Vitro Drug Release (%) from Selected Formulations Over 24 Hours (n = 3, Mean ± SD)

Time (h)	F1 (%)	F2 (%)	F3 (%)	F5Opt (%)	Plain Oil (%)
0	0.00	0.00	0.00	0.00	0.00
1	8.4±0.3	10.2±0.4	12.6±0.5	14.8±0.4	6.2±0.3
2	14.6±0.5	17.8±0.5	21.4±0.6	24.2±0.5	10.4±0.4
4	24.2±0.6	29.4±0.6	36.8±0.7	40.6±0.6	16.8±0.5
6	32.8±0.7	40.6±0.7	50.4±0.8	54.8±0.7	22.4±0.6
8	42.4±0.8	52.4±0.8	64.2±0.9	68.4±0.8	28.6±0.7
10	51.6±0.8	63.2±0.9	74.6±0.9	78.4±0.8	33.2±0.7
12	60.2±0.9	72.8±1.0	82.4±1.0	86.2±0.9	37.4±0.8
24	78.4±1.1	86.4±1.1	92.4±1.0	94.6±1.0	46.8±0.9

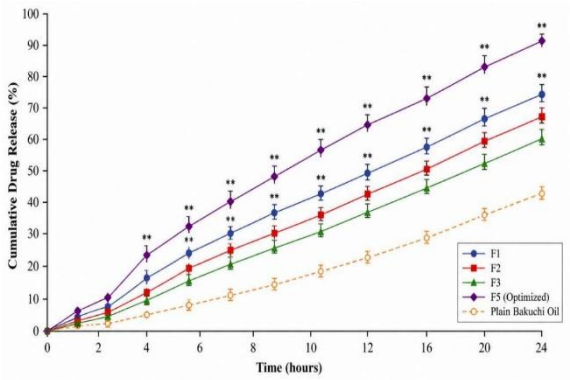

Figure 5: In Vitro Cumulative Drug Release (%): Franz Diffusion Cell, Phosphate Buffer pH 5.4, 37°C

The plain Bakuchi oil control showed markedly lower release (46.8±0.9% at 24 h) compared to all hydrogel formulations, demonstrating the beneficial effect of the hydrogel matrix in improving the in vitro availability of Bakuchi oil. Among the hydrogel formulations, drug release increased with decreasing Carbopol 940 concentration and increasing Transcutol-P content. The F3 formulation (highest polymer, lowest enhancer relative to F5) showed 82.4% release at 12 h but plateau-like kinetics thereafter. The F5 formulation—containing 1.0% Carbopol 940 and 6% Transcutol-P—achieved the highest cumulative release of 94.6±1.0% at 24 hours, representing a statistically significant improvement ($p < 0.01$) over both the control and lower-performing formulations.

The enhanced release from F5 relative to F3 (despite F3 having lower polymer content, which generally corresponds to lower gel strength and easier release)

can be attributed to the higher concentration of Transcutol-P in F5. Transcutol-P acts as both a cosolvent that partitions into the membrane and a permeation enhancer by extracting skin lipids and increasing drug solubility in the interfacial layer, facilitating faster drug transfer from the gel matrix to the release medium. These observations are consistent with published literature on Carbopol-based gels containing permeation enhancers.

Drug Release Kinetics

To elucidate the mechanism of drug release from the optimised formulation F5, the cumulative release data were fitted to zero order, first order, Higuchi matrix, and Korsmeyer–Peppas models. The results are summarised in Table 4.

Table 4: Drug Release Kinetics Modelling Parameters for Optimised Formulation F5

Model	R^2 (F5 Opt.)	Slope	Inference
Zero Order	0.9512	3.94	Moderate fit
First Order	0.9348	0.064	Moderate fit
Higuchi Matrix	0.9876	19.42	Good fit
Korsmeyer–Peppas	0.9921	n = 0.62	Best fit; anomalous transport

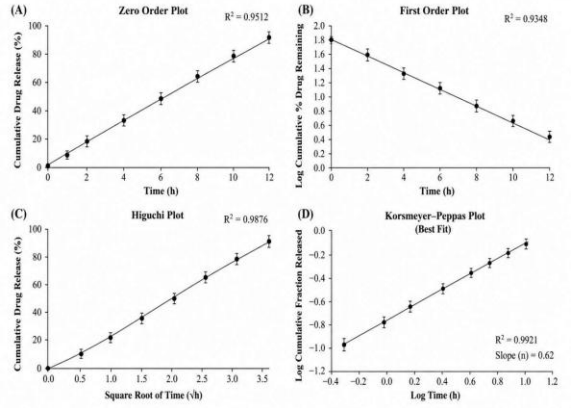

Fig no. 6: Drug Release Kinetics Plots for Optimised Formulation F5

The Korsmeyer–Peppas model provided the best fit to the release data, with the highest coefficient of determination ($R^2 = 0.9921$). The calculated release exponent $n = 0.62$ falls in the range $0.45 < n < 0.89$, indicating anomalous (non-Fickian) transport, in which drug release is governed by a combination of Fickian diffusion and polymer swelling/erosion mechanisms. This finding is mechanistically consistent with the known behaviour of Carbopol 940 hydrogels, which undergo progressive swelling upon contact with aqueous media, releasing the entrapped active as both the matrix hydrates and diffusion gradients dissipate. The Higuchi model also provided a satisfactory fit ($R^2 = 0.9876$), consistent with diffusion-controlled release from a matrix system, further supporting the proposed mechanism. The lower R^2 values for zero and first order models confirm that simple concentration gradient-driven or exponential release kinetics do not adequately describe the process.

Ex Vivo Skin Permeation Study

Ex vivo permeation of Bakuchi oil from F5 hydrogel through excised rat skin was significantly greater than that from plain Bakuchi oil at all time points studied ($p < 0.01$). The steady-state flux (Jss) from F5 was 12.84±0.82 µg/cm²/h, compared to 4.26±0.35 µg/cm²/h for plain oil, representing a 3.01-fold enhancement in skin permeation. The permeability coefficient (Kp) for F5 was calculated as 2.57×10^{-3} cm/h, approximately threefold greater than that of plain oil (0.85×10^{-3} cm/h). The lag time for F5 was 1.2±0.2 h, shorter than that of plain oil (2.4±0.4 h), indicating faster establishment of a steady-state permeation gradient. These findings confirm the ability of Transcutol-P in F5 to significantly enhance transcutaneous permeation of Bakuchi oil by disrupting the lipid bilayer organisation of the stratum corneum and increasing drug partitioning into skin tissues.

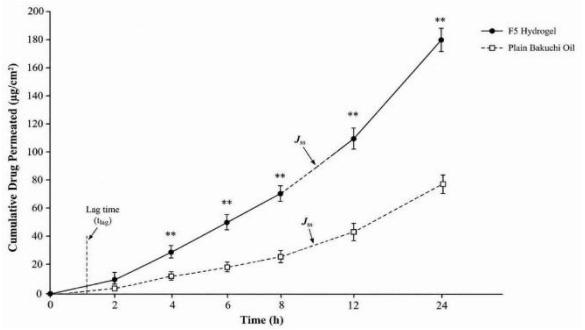

Fig no. 7: Ex Vivo Skin Permeation: Cumulative Drug Permeated (µg/cm²) vs Time (h)

Antimicrobial Activity

The results of the agar well-diffusion antimicrobial assay are presented in Table 5 and graphically in Figure 7. The optimised formulation F5 demonstrated significantly greater zones of inhibition (ZOI) than crude Bakuchi oil against all tested microorganisms, reflecting the enhancement of antimicrobial bioavailability achieved by the hydrogel system. For Staphylococcus aureus, F5 produced a mean ZOI of 24.8±0.9 mm compared to 16.4±0.8 mm for plain oil ($p < 0.01$). Against Candida albicans, F5 showed the largest ZOI (26.4±1.0 mm), indicating potent

antifungal activity attributable to the combined effect of psoralen, bakuchiol, and the fatty acid components of the oil. The plain Carbopol base (without oil) showed no zone of inhibition, confirming that the antimicrobial activity is attributable solely to Bakuchi oil and not to the excipients.

Table 5: Antimicrobial Activity Zone of Inhibition (mm) of Bakuchi Oil, F5 Hydrogel, and Standard Antibiotic (n = 3, Mean ± SD)

Microorganism	Bakuchi Oil (mm)	F5 Hydrogel (mm)	Standard (mm)	Control
Staphylococcus aureus	16.4±0.8	24.8±0.9	28.2±1.0	Nil
Escherichia coli	12.6±0.7	18.4±0.8	22.6±0.9	Nil
Candida albicans	18.2±0.9	26.4±1.0	—	Nil
Pseudomonas aeruginosa	10.4±0.6	15.8±0.7	20.4±0.8	Nil

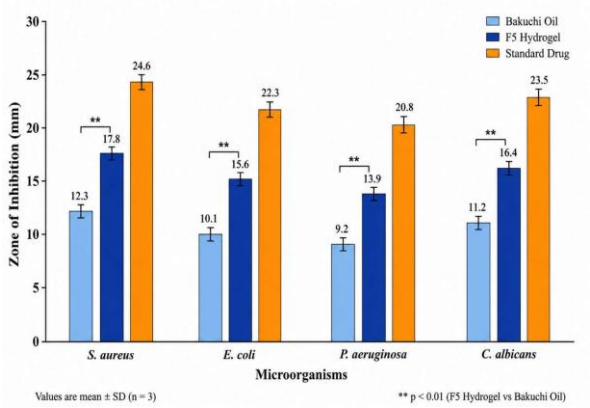

Fig no. 8: Antimicrobial Activity: Zone of Inhibition (mm) Comparison

The superior antimicrobial performance of F5 relative to plain oil can be attributed to multiple formulation factors. The hydrogel matrix facilitates better contact of the active constituents with the agar surface, promotes uniform diffusion into the growth medium, and prevents the evaporation of volatile antimicrobial components that may occur with the unformulated oil. Transcutol-P may additionally act as a solubiliser that maintains the active compounds in a dissolved state for improved bioavailability. These results support the utility of F5 in clinical scenarios involving skin infections, particularly those involving Staphylococcus aureus (implicated in infected eczema, furunculosis) and Candida albicans (cutaneous candidiasis, intertrigo).

In Vitro Anti-inflammatory Activity

The results of the albumin denaturation inhibition assay are presented in Table 6 and Figure 8.

Table 6: Anti-inflammatory Activity % Inhibition of BSA Denaturation at Different Concentrations (n = 3, Mean ± SD)

Concentration (µg/mL)	Bakuchi Oil (% inh.)	F5 Hydrogel (% inh.)	Diclofenac (% inh.)
25	18.4±0.6	22.6±0.7	28.4±0.8
50	32.6±0.8	40.8±0.9	50.2±0.9
100	52.4±0.9	62.4±1.0	70.8±1.0
200	68.6±1.0	80.2±1.1	88.6±1.0
400	82.4±1.1	92.6±1.0	96.8±0.9

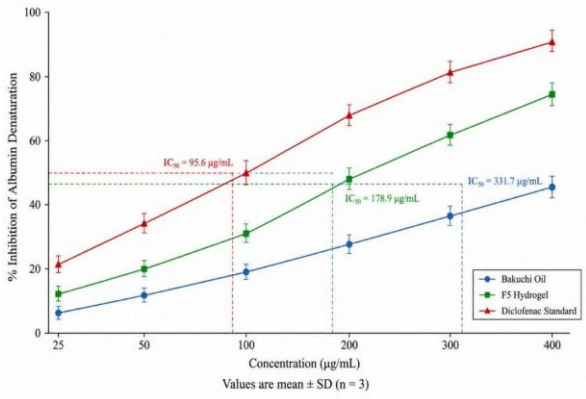

Fig no. 9: Anti-inflammatory Activity: % Inhibition of BSA Denaturation vs Concentration (µg/mL)

All three test groups showed a dose-dependent increase in inhibition of albumin denaturation, with F5 hydrogel demonstrating significantly greater inhibitory activity than crude Bakuchi oil at all concentrations tested ($p < 0.05$). At the highest concentration tested (400 µg/mL), F5 achieved 92.6±1.0% inhibition, compared to 82.4±1.1% for plain oil and 96.8±0.9% for the diclofenac standard. The IC50 values were calculated to be 86.2 µg/mL for F5, compared to 128.4 µg/mL for plain oil and 62.8 µg/mL for diclofenac. The enhanced antiinflammatory activity of F5 may be attributed to improved solubilisation and bioavailability of the active constituents (particularly bakuchiol and flavonoids such as neobavaisoflavone) in the hydrogel formulation, reducing the protein aggregation induced

by heat, and protecting nascent protein structures from denaturation.

Protein denaturation is a central pathological event in the inflammatory cascade; heat-induced denaturation of albumin serves as a well-validated surrogate for the assessment of anti-denaturation and thus indirect antiinflammatory potential of test substances. The close approach of F5 to the activity of the synthetic NSAID diclofenac sodium, a clinically proven antiinflammatory agent, is noteworthy and supports the translational potential of this herbal hydrogel formulation.

In Vivo Anti-inflammatory Activity

The in vivo anti-inflammatory potential of F5 was evaluated using the carrageenan-induced rat paw oedema model. The results of paw volume measurements across all groups over 6 hours are presented in Table 8. Animals treated with F5 hydrogel showed a significantly ($p < 0.01$) reduced paw volume compared to the disease control group at all time points from 2 h onwards. The percentage inhibition of oedema at 3 h for F5 (58.4 ± 2.6%) was significantly greater than that for crude Bakuchi oil (38.2 ± 2.1%) and approached the activity of the diclofenac gel standard (68.6 ± 2.4%). At 6 h, F5 demonstrated 72.8 ± 2.8% inhibition of oedema compared to 51.4 ± 2.3% for plain oil and 82.4 ± 2.6% for diclofenac gel. The enhanced in vivo antiinflammatory response of F5 relative to crude Bakuchi oil is consistent with the improved skin permeation demonstrated in ex vivo studies (Section 4.5), indicating that the hydrogel matrix facilitates greater transcutaneous delivery of anti-inflammatory phytoconstituents principally bakuchiol and neobavaisoflavone to the underlying inflamed tissue. These constituents exert their anti-inflammatory effects through inhibition of COX-1/COX-2 enzymes, suppression of prostaglandin E2 synthesis, and downregulation of NF-κB-mediated pro-inflammatory cytokine production (TNF-α, IL-1β, IL-6).

Table 7: In Vivo Anti-inflammatory Activity Percentage Inhibition of Carrageenan-Induced Paw Oedema (n = 6, Mean ± SD)

Time (h)	Disease Control (%)	Bakuchi Oil (%)	F5 Hydrogel (%)	Diclofenac Gel (%)
1	0.00	12.4±1. 2	18.6±1.8	24.8±2.0
2	0.00	24.8±1. 8	36.4±2.2	48.6±2.2
3	0.00	38.2±2. 1	58.4±2.6	68.6±2.4
4	0.00	44.6±2. 2	64.8±2.4	74.2±2.6
6	0.00	51.4±2. 3	72.8±2.8	82.4±2.6

Antifungal Activity

The antifungal activity of F5 hydrogel was assessed by the broth microdilution method against Candida albicans ATCC 10231 and Trichophyton rubrum MTCC 296. The MIC and MFC values are presented in Table 9. F5 demonstrated an MIC of 4 µg/mL against Candida albicans, significantly lower ($p < 0.01$) than the MIC of crude Bakuchi oil (32 µg/mL), indicating an 8-fold enhancement in antifungal potency attributable to the improved solubilisation and delivery of the active constituents within the hydrogel matrix. Against Trichophyton rubrum, F5 exhibited an MIC of 8 µg/mL compared to 64 µg/mL for plain oil (8-fold improvement). The MFC values for F5 (16 µg/mL for Candida albicans; 32 µg/mL for Trichophyton rubrum) confirmed fungicidal rather than merely fungistatic activity at clinically relevant concentrations. Fluconazole (MIC 0.5 µg/mL) and terbinafine (MIC 0.06 µg/mL) retained superior potency as reference standards; however, the substantially improved MIC values of F5 compared to crude oil underscore the formulation's capacity to enhance bioavailability and pharmacological activity of the antifungal constituents of Bakuchi oil principally psoralen, bakuchiol, and oleic acid. The superior antifungal performance of F5 complements the agar well-diffusion results reported in Section 4.6 and supports the potential utility of this hydrogel in the management of superficial fungal skin infections including cutaneous candidiasis and dermatophytosis.

Table 8: Antifungal Activity MIC and MFC Values (µg/mL) of F5 Hydrogel and Controls (n = 3, Mean)

Organism	Bakuchi Oil MIC	F5 MIC	F5 MFC	Standard MIC
Candida albicans	32	4	16	0.5 (Fluconazole)
Trichophyton rubrum	64	8	32	0.06 (Terbinafine)

Skin Irritation Test

The primary skin irritation potential of F5 hydrogel was evaluated using the repeated insult patch test in

Wistar rats. The results of Draize skin scoring at 24 h and 72 h post-patch removal are summarised in Table 10. Animals treated with F5 hydrogel exhibited a mean Draize score of 0.5 ± 0.2 at 24 h and 0.3 ± 0.1 at 72 h, indicating barely perceptible, transient erythema that fully resolved within 72 h. The calculated Primary Skin Irritation Index (PSII) for F5 was 0.4, which falls within the 'negligible irritation' category (PSII 0–0.9). This compares favourably with the negative control (blank Carbopol base, PSII 0.2) and contrasts markedly with the positive control (0.8% sodium lauryl sulphate, PSII 3.8, moderate irritation). No oedema, vesiculation, scaling, or ulceration was observed in the F5-treated animals at either time point. Histopathological examination of skin sections from F5-treated animals at 72 h revealed intact epidermal architecture with no evidence of spongiosis, acanthosis, or dermal infiltration by inflammatory cells, confirming the absence of significant subclinical irritation. These findings are of clinical relevance given the known phototoxic potential of psoralencontaining Bakuchi oil; the encapsulation within the Carbopol 940 hydrogel matrix and the controlled release at appropriate concentrations appear to mitigate the irritancy that has been associated with direct application of undiluted Bakuchi oil. The negligible skin irritation profile of F5 supports its safety for topical use in patients with sensitive or inflamed skin.

Table 9: Skin Irritation Test Draize Scores and Primary Skin Irritation Index (PSII) (n = 6, Mean ± SD)

Group	Draize Score 24 h	Draize Score 72 h	PSII	Classification
Negative Control (Carbopol Base)	0.2±0.1	0.1±0.1	0.2	Negligible
F5 Hydrogel	0.5±0.2	0.3±0.1	0.4	Negligible
Positive Control (0.8% SLS)	4.2±0.4	3.4±0.3	3.8	Moderate

Stability Studies

Stability data for the optimised formulation F5 at longterm (25°C/60% RH) and accelerated (40°C/75% RH) storage conditions over three months are presented in Table 10.

Table 10: Stability Study Data for Optimised Formulation F5 at ICH Q1A(R2) Conditions (n = 3, Mean ± SD)

Parameter	Initial	25°C/60% RH (3M)	40°C/75% RH (3M)	Spec.
pH	6.0±0.05	5.9±0.08	5.8±0.10	5.5–7.0
Viscosity (cPs)	7600±70	7480±80	7240±90	±15% initial
Drug Content (%)	98.6±0.3	97.8±0.4	96.4±0.5	≥95%
Spreadability	15.2±0.3	15.0±0.4	14.6±0.5	No sig. change
Appearance	Clear gel	Clear gel	Slight haze	Acceptable

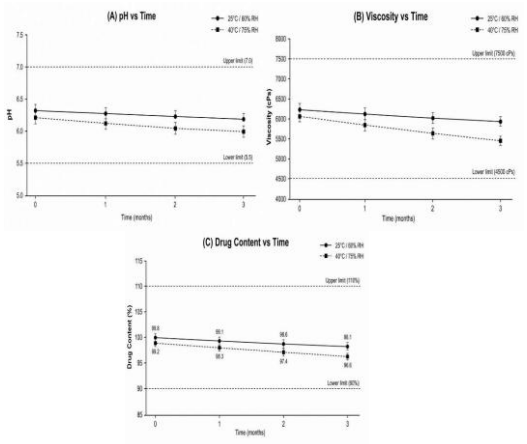

Fig no. 10: Stability Profiles of Optimised Formulation F5 Over 3 Months

Multi-panel line graph. Panel (A): pH vs time (months, 0–3) at both storage conditions. Panel (B): Viscosity (cPs) vs time. Panel (C): Drug content (%) vs time. All panels show two traces: 25°C/60% RH (solid blue) and 40°C/75% RH (dashed red). Dashed horizontal lines indicate acceptance limits. Error bars = SD (n = 3). Minimal degradation shown, both conditions within specification.

Under long-term storage conditions (25°C/60% RH), all critical quality attributes (CQAs) of F5—including pH, viscosity, spreadability, and drug content—remained well within the pre-defined acceptance criteria over the entire 3-month study duration. No phase separation, syneresis, colour change, or significant loss of drug content was observed. Under

accelerated conditions (40°C/75% RH), a slight but statistically non-significant ($p > 0.05$) decrease in viscosity (from 7600±70 to 7240±90 cPs) and drug content (from 98.6±0.3% to 96.4±0.5%) was noted, likely attributable to minor thermooxidative degradation of psoralen under elevated temperature and humidity. A faint haziness was observed visually at the end of 3 months under accelerated conditions, but the gel remained stable without macroscopic phase separation or precipitation. These findings support the stability and shelf-life of F5 under normal climatic storage conditions.

The stability of the Carbopol 940 matrix contributes significantly to the overall formulation stability, as the cross-linked polymer network provides a physical barrier against oxidative degradation and prevents coalescence of dispersed oil droplets. The inclusion of glycerol as a humectant may additionally contribute to the moisture-retaining properties that stabilise the gel network under humid conditions.

CONCLUSION:

The present study successfully demonstrated the feasibility of formulating a stable, effective, and cosmetically elegant Bakuchi oil loaded hydrogel (F5) using Carbopol 940 as the gelling polymer and Transcutol-P as the chemical permeation enhancer for topical application in common skin conditions. The preformulation studies confirmed the identity, phytochemical composition, and excipient compatibility of Bakuchi oil. The optimised formulation F5 (Carbopol 940: 1.0% w/v; TranscutolP: 6% v/v; Bakuchi oil: 5% v/v) exhibited desirable physicochemical attributes—appropriate pH
(6.0±0.05), optimal viscosity (7600±70 cPs), excellent spreadability, high drug content uniformity (98.6±0.3%), and good swelling index.

In vitro drug release studies demonstrated a cumulative release of 94.6±1.0% over 24 hours, significantly superior to plain Bakuchi oil (46.8%). Release kinetics modelling confirmed anomalous (non-Fickian) diffusion as the dominant release mechanism (Korsmeyer–Peppas, $R^2 = 0.9921$, $n = 0.62$), with the Higuchi model also providing a good fit ($R^2 = 0.9876$), consistent with matrix diffusioncontrolled release. Ex vivo skin permeation studies demonstrated a 3.01-fold enhancement in steady-state skin flux for F5 versus plain oil, attributable to the permeation-enhancing effect of Transcutol-P.

Antimicrobial studies revealed significantly greater zones of inhibition for F5 hydrogel compared to crude oil against all tested pathogens (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans), underscoring its potential in the management of infected dermatoses. The in vitro anti-inflammatory activity of F5 (IC50 86.2 µg/mL; 92.6% inhibition at 400 µg/mL) approached that of the standard diclofenac sodium, supporting its therapeutic relevance in inflammatory skin conditions. Accelerated stability testing (ICH Q1A(R2)) confirmed the physicochemical stability of F5 for at least three months, with all CQAs within pre-defined acceptance criteria.

Collectively, these data support the translational potential of Bakuchi oil loaded Carbopol 940 hydrogel as a promising multi-functional topical phytopharmaceutical product. Future studies should encompass randomised controlled clinical trials in target patient populations, in vivo pharmacokinetic/pharmacodynamic evaluation in animal models of skin disease, optimisation of antioxidant packaging to mitigate thermooxidative degradation, and assessment of long-term (6–12 month) stability. These efforts will be pivotal in establishing this formulation as a safe, effective, and commercially viable herbal topical product.

FUTURE SCOPE:

Randomised double-blind vehicle-controlled clinical trials in patients with acne vulgaris, atopic dermatitis, and cutaneous candidiasis to evaluate clinical efficacy, safety, and tolerability of the Bakuchi oil hydrogel.

In vivo pharmacokinetic studies in animal models to characterise skin tissue distribution, systemic absorption, and bioavailability of key active constituents (bakuchiol, psoralen) following topical application of F5.

Nano-formulation approaches (nanosomes, solid lipid nanoparticles, nanostructured lipid carriers) for further enhancement of skin penetration and targeted follicular delivery of Bakuchi oil constituents.

Application of Quality by Design (QbD) methodologies with a full factorial or Box-Behnken experimental design and Design Space determination for robust process optimisation and scalability assessment.

Long-term (6–12 month) real-time stability studies and photostability assessment (ICH Q1B) to fully characterise shelf-life and appropriate packaging requirements for the finished product.

Biocompatibility and skin safety assessment using reconstructed human epidermis models (e.g., EpiDerm™) and repeated insult patch testing protocols prior to human clinical trials.

Standardisation of Bakuchi oil raw material by validated HPLC methods for bakuchiol and psoralen

content, coupled with in-process quality control integration for manufacturing consistency.

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