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Formulation, Characterization, and Evaluation of a Novel Herbal Antifungal Oil-in-Water Emulsion Using Karanj Oil (*Pongamia pinnata*)

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ABSTRACT

Fungal infections affecting the skin, nails, and mucosal areas pose a significant public health challenge, constituting nearly 25% of all infections globally. The effectiveness of current antifungal medications is often compromised due to various factors, including systemic toxicity, low patient adherence, and rising drug resistance among clinical fungal strains. Extracted herbal oils containing pongamol, karanjin, and several other flavonoids have demonstrated antifungal properties in multiple studies. In this research, an oil-in-water (O/W) emulsion was developed, incorporating 4% w/w karanj oil. The formulation also included 5% w/w medium-chain triglycerides (MCT) oil, 0.5% w/w Xanthan gum as a thickening agent, 1.5% w/w Polysorbate 60 as the primary non-iconic emulsifier, and 1.2% w/w aqueous Aloe vera extract. A comprehensive physiochemical analysis was performed on both the pure components and the final product, assessing parameters such as pH, viscosity, spreadability, particle size distribution, zeta potential, and in vitro release rate, along with antifungal efficacy against Candida albicans, Candida tropicalis, Aspergillus niger, Aspergillus fumigates, Trichophyton rubrum, and Fusarium solani. The optimized formulation, designated as F1, exhibited a pH of 5.8, a viscosity of 4800 centipoise, an average globule size of 185.2 nm, and a zeta potential of -38.5 mV. Antifungal activity was evaluated using broth microdilution and disc diffusion techniques, revealing significant effectiveness against the six fungal strains mentioned, with minimum inhibitory concentrations (MIC) ranging from 1.56 to 6.25 µg/mL. Stability testing under ICH Q1A(R2) accelerated conditions confirmed that the formulated product maintained its physicochemical stability for 90 days.

Keywords: Pongamia pinnata; Karanj oil; Antifungal emulsion; Xanthan gum; MCT oil; Polysorbate 60; Topical formulation; Candidiasis; Dermatophytosis.

1. INTRODUCTION

Superficial fungal infections are some of the most widespread skin disorders globally, affecting around 1.7 billion individuals annually[1]. These infections can vary from minor skin problems to severe systemic issues, particularly in those with compromised immune systems. Fungi like Trichophyton rubrum and Trichophyton mentagrophytes, as well as non-dermatophyte molds such as Aspergillus spp. And Fusarium spp., are responsible for numerous nail and skin infections. Candida spp. Primarily cause oral and vaginal candidiasis[2].

Conventional antifungal treatments, which include azoles, polyenes, allylamines, and echinocandins, form the basis for managing these conditions[3]. Nonetheless, the increasing resistance to antifungal medications is an alarming issue, particularly concerning candida auris and azole-resistant Aspergillus fumigatus. There are also concerns about potential liver and kidney toxicity and drug interactions associated with systemic treatments[4]. This hasa led researchers to explore alternative treatment strategies. In this context, plant-derived oils and their active compounds are becoming increasingly recognized as potentially safer antifungal solutions.

Karanj oil, derived from the seeds of pongamia pinnata (L.) pierre, is a well- regarded medicinal oil within traditional Ayurvedic practices[5]. The oil is rich in flavonoids (such as karanjin, pongamol, and tannin), fatty acids (including oleic, linoleic, and lignoceric acids), and bitter furanoflavanoids, providing antibacterial, antifungal, anti-inflammatory, and wound-healing properties[6]. However, despite its benefits, the use of raw karanj oil for topical applications poses difficulties due to its dark hue, strong odor, limited cosmetic appeal, and poor skin spreadability.

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Developing an oil-in-water (O/W) emulsion is a practical solution to overcome these issues. An O/W emulsion is a practical solution to overcome these issues. An O/W emulsion encapsulates the oil in tiny structures that are dispersed throughout the water. This configuration enables the controlled release of oil-soluble active substances, enhances skin feel, and promotes deeper absorption of the active ingredients[7]. Incorporating MCT (medium-chain triglyceride) oil as a complementary emollient and penetration enhancer increases the accessibility of oil-soluble actives[2]. Polysorbate 60, which has a high hydrophilic-lipophilic balance, was selected as the primary non-ionic emulsifier due to its excellent compatibility and effectiveness in stabilizing oil-in-water emulsions[7]. Xanthan gum, a high-molecular-weight anionic polysaccharide, was added to enhance viscosity and stabilize the emulsion because of its unique flow characteristics and compatibility with a range of cosmetic and pharmaceutical components[3].

Aloe vera (Aloe barbadensis Miller) gel extract was incorporated as a functional ingredient to utilize its widely recognized anti-inflammatory, moisturizing, wound-healing, and immune-supporting benefits[8]. Acemannan, the main bioactive polysaccharide in aloe vera gel, has been shown to enhance immune responses and support skin repair. This study aimed to develop, optimize, and evaluate a stable, cosmetically appealing, and effective O/W herbal emulsion using Karanj oil, Xanthan gum, MCT oil, Polysorbate 60, and Aloe vera extract. This study aimed to create, improve, and assess a stable, visually appealing, and effective oil-in-water herbal emulsion using Karanj oil, Xanthan gum, MCT oil, Polysorbate 60, and Aloe vera extract.

1.1 Drug Profiles

a. **Profile :** Karanj oil

Fig.1 Karanj seeds and oil

b. **Description:** Karanj seed oil derived from the seeds of the pongam tree, a plant renowned in ayurvedic medicine for its therapeutic properties. This oil is recognized for its antibacterial, antifungal, and anti-inflammatory characteristics.

c. Taxonomy (Classification):

Table 1. Taxonomy of karanj oil

Taxonomic Rank	Scientific Name
Kingdom	Plantae
Division	Angiosperms
Class	Eudicots
Order	Fabales
Family	Fabaceae (Leguminosae)
Genus	Pongamia
Species	Pongamia pinnata L.
Common Name	Karanj, Pongam, Indian Beech

d. **Pharmacological activities:** Antifungal, antibacterial, anti-inflammatory, larvicidal, and wound-healing

e. **Role in Antifungal cream:** Karanj oil is effective in treating dermatophytes (including Trichophyton rubrum and T. mentagrophytes) as well as Candida species. The fungicidal properties of karanj oil stem from its ability to inhibit growth by causing damage to the cell wall and membrane through the action of karanjin and pongamol, which ultimately leads to a reduction in ergosterol production.

2. Materials

2.1 Drug and Excipients

Karanj oil (cold-pressed, pharmaceutical grade) was obtained from a certified local botanical supplier. MCT oil (fractionated coconut oil, USP grade), cetyl alcohol, stearic acid, and isopropyl myristate (IPM) came from S.D. fine chemicals in Mumbai, India. Purified water (IP grade) was made in-house through double distillation. Glycerol and propylene glycol (AR grade) were provided by merck life sciences pvt. ltd. In India. Xanthan gum, sodium citrate, triethanolamine (TEA), and polysorbate 60 (Tween 60) were purchased from HiMedia Laboratories pvt. Ltd. In India. Lemongrass essential oil was sourced from a certified aromatherapy-grade supplier. Phenoxyethanol (Preservative grade) and aloe vera raw gel were obtained from local suppliers. All reagents used in analytical procedures were of analytical reagent (AR) grade.

2.2 Preparation of Aloe vera aqueous extract

Fresh mature leaves of aloe vera (Linn.) Burm. f. (2-3 years old) were collected, authenticated by a qualified pharmacognosist , and washed thoroughly with distilled water[8]. The yellow latex (aloin-containing exudate) drained for 15-20 minutes by positioning the cut leaves vertically. After that, the green rind was carefully removed using a sterile stainless-steel blade. The transparent inner parenchyma gel was blened in ahigh- speed blender and filtered through muslin cloth and Whatman No. 1 filter paper to get a clear liquid. Aqueous extraction involved suspending 100g of fresh gel in 200 mL of distilled water at 40-50 °C for 1-2 hours in a thermostatically controlled water bath. The extract was filtered, concentrated under reduced pressure at 50 °C, and stored at 4 °C until needed.

3. Formulation Design and Composition

3.1 Rationale for Component Selection

The formulation was designed as a classic O/W emulsion system. The active lipophilic phase, made up of karanj oil and MCT oil, gets emulsified into a hydrophilic aqueous continuous phase. The emulsification mechanism relies on dual action: first, the non-ionic surfactant polysorbate 60 (Tween 60, HLB 14.9) adsorbs at the oil-water interface and stabilizes the globules. Second, triethanolamine stearate forms in situ. This anionic emulsifier comes form the saponification reaction between stearic acid and TEA at 70-75°C And offers electrostatic stabilization[9]. Xanthan gum adds bulk viscosity to the aqueous phase, which helps reduce creaming and Ostwald ripening. IPM improves skin permeation by fluidizing the lipid domains of the stratum corneum.

3.2 Composition Table

Table 2 presents the complete formulation composition for a 50g batch.

Table 2. Complete formulation composition of herbal antifungal O/W Emulsion (50g Batch)

Ingredient	Phase	% w/w	Qty (g/50g batch)	Function
Karanj oil	Oil phase	4.0	2.0	Antifungal active
MCT oil	Oil phase	5.0	2.5	Penetration enhancer
Cetyl alcohol	Oil phase	2.0	1.0	Emulsion stabilizer
Stearic acid	Oil phase	3.0	1.5	Emulsifier (TEA soap)
Isopropyl myristate	Oil phase	2.0	1.0	Emollient
Purified water	Aqueous phase	70.0	35.0	Solvent/Vehicle
Glycerol	Aqueous phase	5.0	2.5	Humactant
Propylene glycol	Aqueous phase	3.0	1.5	Co-solvent
Xanthan gum	Aqueous phase	0.5	0.25	Viscosity modifier
Triethanolamine	Aqueous phase	0.8	0.40	Alkalizing/emulsifier
Sodium citrate	Aqueous phase	0.5	0.25	Buffer
Aloe Vera extract	Aqueous phase	1.2	0.60	Anti-inflammatory
Polysorbate 60	Additive	1.5	0.75	HLB emulsifier
Essential Oil	Additive	0.5	0.25	Fragrance/antifungal
Phenoxyethanol	Additive	1.0	0.50	Preservative

4. Manufacturing Procedure

4.1 Step 1 : Preparation of Oil Phase (Phase A)

Karanj oil (4.0 g), MCT oil (5.0 g), Isopropyl myristate (2.0 g), cetyl alcohol (2.0 g), and stearic acid (3.0 g) were individually measured in a stainless-steel beaker[2]. The oil phase was then heated to a temperature range of 70-75 °C while being stirred gently, which facilitated the dissolution of all solid components (cetyl alcohol and stearic acid) until achieving a homogeneous and clear mixture. The temperature of the oil phase was maintained at 70-75 °C prior to the emulsification process.

4.2 Step 2 : Preparation of Aqueous phase (Phase B)

Distilled water (70 g per 100 g of batch) was accurately measured in a stainless steel beaker designated for temperature assessment. Xanthan gum (0.5 g) was gradually dispersed using a high shear rate stirrer set at a speed of 200 rpm to prevent the formation of lumps and to ensure complete hydration of the polymer chain[3]. Subsequently, glycerol (5 g) and propylene glycol (3 g) were added in succession to the xanthan gum dispersion, which was stirred until achieving a homogeneous mixture. Sodium citrate (0.5 g) was incorporated as a buffering agent to maintain the pH of the resulting emulsion within the range of 5.5 to 6.5. At this stage, aloe vera extract (1.2 g) was introduced into the aqueous phase. The mixture was then heated to a temperature of 70-75°C in a thermostatic water bath while being continuously stirred. Finally triethanolamine (0.8 g) was introduced into aqueous phase at the maintained temperature of 70-75°C.

4.3 Step 3: Emulsification

Both phases were maintained at temperatures ranging from 70 to 75°C simultaneously to prevent solidification or phase separation prior to emulsification. The aqueous phase was gradually introduced into oil phase while under high shear mechanical agitation (500-700 rpm) utilizing an overhead stirrer[9]. The gradual addition of the aqueous phase into the oil phase, rather than the reverse, was employed to establish a primary water-in-oil (W/O) emulsion that would subsequently undergo phase inversion to form an oil-in-water (O/W) emulsion as additional water was incorporated; this phenomenon is known as phase inversion. Agitation persisted for a duration of 15 to 20 minutes at 70 to 75°C until a triethanolamine-stearate complex emulsifier was formed in situ. During the emulsification process, 1.5 g of polysorbate 60 was added[7].

4.4 Step 4 : Cooling and Finishing (Phase C)

The heated emulsion was subsequently cooled to approximately 40°C while maintaining constant mild agitation at a rate of 150-200 rpm to avert the introduction of air into the emulsion. At this temperature, lavender essential oil (0.5 g) and phenoxyethanol (1.0 g) were incorporated, followed by comprehensive mixing to ensure uniform distribution[10]. The pH of the emulsion was measured using a calibrated pH meter, and adjustments were made to maintain it within the range of 5.5 to 6.5 by adding triethanolamine (TEA) when the pH fell below 5.5, or citric acid solution when it exceeded 6.5.

5. Evaluation of Raw Materials

A comprehensive and systematic analysis of all raw materials is a critical prerequisite in the intricate process of developing pharmaceutical formulations. This ensures that each excipient and active pharmaceutical ingredient adheres to established quality standards prior to their intentional blending into a finished medicinal product. In the current study, a thorough evaluation has been conducted on all excipients utilized- specifically karanj oil, medium-chain triglyceride (MCT) oil, polysorbate 60 (commonly known Tween 60), and xanthan gum – in accordance with defined pharmacopoeial specifications and supported by relevant peer – reviewed literature concerning their identity, chemical, purity and various physical properties[2,3,5,7]. This meticulous application of a Quality by design (QbD) approach is intended to maintain and enhance batch-to batch reproducibility, which is essential for ensuring the therapeutic efficacy, safety, and stability of the final dosage form presented as a pharmaceutical emulsion throughout its market shelf life.

5.1 Evaluation of Karanj Oil

The primary active ingredient in the formulation is derived from karanj oil, which is obtained through a cold-pressing method that extracts oil from the seeds of the pongamia pinnata tree. This substance exhibits a color ranging from yellowish to reddish – brown and possesses a distinctive bitter-nutty aroma. Its composition includes furanoflavanoids such as karanjin and pongamol, as well as oleic acid, linoleic acid, and lignoceric acid[5]. Evaluation parameters encompass organoleptic properties, acid value, and saponification value, which collectively establish the identity and freshness of the oil while determining the length of its fatty acid chains. The acid value assesses hydrolytic rancidity by measuring the content of free fatty acids, while the saponification value

indicates average molecular weight of the fatty acids present in the mixture[11]. The Ayurvedic pharmacopoeia of India mandates that these tests be performed on fixed oils.

5.1.1 Organoleptic Test

Approximately 5 mL of karanj oil was examined in a transparent glass test tube under standard laboratory lighting conditions. The characteristics of the sample, including color, odor, clarity, and physical state, were assessed in accordance with API guidelines[5]. Observation : The oil appeared clear, exhibiting a yellow to reddish-brown hue and a bitter, nutty aroma, consistent with the values reported in the literature.

5.1.2 Acid value determination

The acid value of karanj oil was determined in accordance with the standard procedures outlined by IP/BP[11].

The methodology is briefly described as follows : A precise measurement of 0.5 g of karanj oil was placed in a 250 mL conical flask and diluted with 50 mL of a solution consisting of ethanol and diethyl ether in a 1:1 ratio. Subsequently, two to three drops of phenolphthalein indicator solution were added. The mixture was then titrated using a standardized alcoholic KOH solution (0.1 N) until the endpoint was reached, indicated by a pink coloration. The volume of KOH solution utilized, measured in millilitres (V mL), was recorded. The formula for calculating the acid value is as follows: Acid Value = (V x N x 56.1)/W, Where N represents the normality of the KOH solution, and W denotes the weight of Karanj oil. Observation : 3.2 mg KOH/g (range : 2-5 mg KOH/g).

5.1.3 Saponification Value

The saponification value was determined utilizing the reflux saponification method in accordance with the guidelines of the Indian Pharmacopoeia[11]. Two grams of karanj oil were subjected to heating under reflux with 25 mL of 0.5 N alcoholic KOH solution for a duration of 30 minutes. Subsequently, the remaining solution was neutralized with 0.5 N HCl until a color change was observed in phenolphthalein. The result obtained was 187.4 mg KOH/g, which falls within the required range of 180-195 mg KOH/g. It can be inferred that karanj oil consists of C18 fatty acids.

5.2 Evaluation of MCT oil

Medium-chain triglyceride (MCT) oil, which is derived through the fractionation of coconut oil, primarily consists of triglycerides of caprylic acid (C8:0) and capric acid (C10:0). This formulation serves as both a co-emollient and a penetration enhancer[2]. The evaluation process is based on three critical parameters, namely specific gravity, dynamic viscosity, and refractive index, measured at a temperature of 25°C. The primary identity tests involve specific gravity measurements ranging from 0.94 to 0.96 and refractive index readings between 1.440 and 1.450, while viscosity measurements between 25 and 35 cP indicate its characteristics as a low – viscosity fluid, facilitating skin penetration and the formation of emulsion microstructure[2]. The researchers utilized USP-grade MCT oil to ensure both product quality and compliance with biocompatibility standards.

5.2.1 Specific Gravity

The specific gravity test was conducted at 25°C using a clean and dried Gay-Lussac's pycnometer. We measured the weights of the pycnometer first with distilled water (W_w) and then with MCT oil (W_o)[2]. The formula for specific gravity is (W_o -W_{empty})/(W_w -W_{empty}). The result we got was 0.952, which is close to the specified value of 0.94.

5.2.2 Viscosity

The dynamic viscosity of MCT oil was determined at a temperature of 25°C using a Brookfield DV-II+ pro viscometer equipped with an SC4-18 spindle operating at a speed of 50 rpm[3]. The measured viscosity was recorded at 28.5 cP, with a range of 25 to 35 cP. This measurement indicates that the viscosity is notably low, making it appropriate for applications requiring penetration enhancement.

5.2.3 Refractive index

The refractive index was determined at a temperature of 25°C utilizing an Abbe refractometer. One to two drops of MCT oil were applied to the prism, and the measurement was taken subsequent to achieving thermodynamic equilibrium[2]. Observation: 1.445 (specification: 1.440-1.450).

5.3 Evaluation of Tween 60 (Polysorbate 60)

Polysorbate 60 is classified as a non-ionic polyoxyethylene sorbitan ester that serves as an oil-in-water (O/W) emulsifying agent, characterized by a hydrophilic-lipophilic balance (HLB) value of approximately 14.9[7]. It was assessed for the pH of its 1% aqueous solution (with specifications of 5.5-7.5), HLB value verification (through emulsion stability comparison using blends of span 20 and tween 80), and water solubility (according to USp criteria). The findings its capacity to form stable emulsions in water while exhibiting no indications of hydrolytic degradation that could compromise emulsion stability or safety for the skin[7]. The high HLB value (greater than 8) confirms its hydrophilic dominance and propensity to form oil-in water emulsions.

5.3.1 pH Determination

Polysorbate 60 (1% W/V) was dissolved in dissolved in distilled water to produce an aqueous solution at a temperature of 25°C. The pH was measured using a calibrated pH meter (pH-Spear, Eutech Instruments) after calibration against buffer solutions[7]. The result obtained was a pH of 6.2, which falls within the specified range of 5.5 to 7.5.

5.3.2 HLB Value

The HLB value of Polysorbate 60 was determined through experimental methods by creating O/W emulsions at various HLB values via the combination of span 20 (HLB = 8.6) and Tween 80 (HLB = 15.0), followed by an assessment of their stability[7]. The result indicated that the HLB value was approximately 14.9, signifying that the substance possesses a high degree of hydrophilicity and functions effectively as an O/W emulsifying agent.

5.3.3 Solubility

One gram of polysorbate 60 was combined with 10 mL of distilled water utilizing a glass stirring rod for a duration of 5 minutes at room temperature. The solubility was assessed according to USP standards[7]. Solubility Test Results : The substance is freely soluble in water, resulting in a clear to slightly opalescent liquid that possesses an oily odor.

5.4 Evaluation of Xanthan Gum

Xanthan gum is an anionic heteropolysaccharide of high molecular weight that is produced through the fermentation of xanthomonas campestris. This substance serves a dual purpose as both a viscosity-enhancing agent and an emulsion-stabilizing compound[3]. The evaluation process employs three parameters, including solubility behavior to assess polymer hydration and colloidal solution formation, as well as the viscosity of a 1% aqueous dispersion, which ranges from 1200 to 1600 cP and indicates chain entanglement alongside pseudoplastic material flow. Additionally, the swelling index quantifies polymer expansion in water at a rate of 15 to 25 mL per gram[3]. The shear-thinning pseudoplastic flow characteristics of xanthan gum solutions are critical for quality control in topical products, as then enable ease of application while ensuring that the product retains its appropriate viscosity post-application.

5.4.1 Solubility Test

0.5 grams of xanthan gum was slowly incorporated into 50 millilitres of distilled water while maintaining constant stirring at a rate of 200 rotations per minute (rpm) and permitted to hydrate for a duration of 30 minutes[3]. Observation : This process resulted in a highly viscous, slightly turbid colloidal solution.

5.4.2 Viscosity

The preparation of a 1 % W/V solution of xanthan gum in deionized water involved dispersing 1 g of xanthan gum in 100 mL of deionized water, followed by stirring for a duration of 2 hours. The viscosity of the xanthan gum was assessed using a Brookfield LVT Viscometer with spindle No. 3, operating at 30 rpm and a temperature of 25°C[3]. The recorded viscosity was 1420 cP, which is within the specified range of 1200-1600 cP.

5.4.3 Swelling Index

A quantity of 1.0 g of xanthan gum was combined with 25 mL of distilled water within a measuring cylinder. The volume of the hydrated polymer was measured after a period of 24 hours of static standing at a temperature of 25°C[3]. Result : 18.5 mL/g (Specification range: 15-25 mL/ g).

Table 3. Summary of Raw materials Evaluation Results

Ingredient	Test Parameter	Standard Range	Observed Result	Status
Karanj oil	Organoleptic	Yellowish-brown, nutty odor	Yellowish-brown characteristic	Pass
Karanj oil	Acid Value	2-5 mg KOH/g	3.2 mg KOH/g	Pass
Karanj oil	Saponification Value	180-195	187.4	Pass
MCT oil	Specific Gravity	0.94-0.96	0.952	Pass
MCT oil	Viscosity	25-35 cP	28.5 cP	Pass
MCT oil	Refractive Index	1.440-1.450	1.445	Pass
Tween 60	pH (1% Solution)	5.5-7.5	6.2	Pass
Tween 60	HLB Value	~14.9	14.9	Pass
Tween 60	Solubility	Freely soluble	Freely soluble	Pass
Xanthan Gum	Solubility	Viscous colloidal solution	Colloidal solution formed	Pass
Xanthan Gum	Viscosity (1%)	1200-1600 cP	1420 cP	Pass
Xanthan Gum	Swelling Index	15-25	18.5	Pass

6. Evaluation of the Finished Emulsion

A comprehensive physicochemical analysis of the final emulsion product is essential for three primary objectives: to ascertain product quality, to predict medical efficacy, and to safeguard patient health. The finished karanj oil O/W emulsion was assessed against several critical quality attributes (CQAs), including pH, viscosity, spreadability, globule/particle size, zeta potential, drug content, and in vitro drug release testing[9,11]. Three independent batches (F1, F2, F3) were prepared and examined to evaluate intra-batch reproducibility. Specifications for each parameter were defined based on literature reports, pharmacopoeial guidelines, and clinical relevance. The targeted pH range of 5.5 to 6.5 was selected as it corresponds with the normal skin pH levels of 4.5 to 6.5. A viscosity range of 3000 to 6000 cP was established to ensure optimal spreadability , preventing excessive runniness[9]. The zeta potential must be greater than -30 mV, while the globule size should remain below 300 nanometers to establish benchmarks for colloidal stability in emulsion systems[4]. Furthermore, drug content uniformity ranges from 98 to 102 percent, confirming dosage accuracy, while in vitro release is expected to reach 80 percent or more within 8 hours, thereby validating the sustained-release profile of the formulation.

6.1 Physical Appearance and Organoleptic Properties

Each of the three formulation batches (F1, F2, F3) resulted in a smooth, consistent, creamy-white emulsion with a subtle lemongrass fragrance. There was no evidence of phase separation, creaming, or visible oiling-off immediately after preparation or after being stored at room temperature for 24 hours[11]. The emulsion applied effortlessly on the skin and felt non-greasy, indicating it is suitable for cosmetic applications.

6.2 pH, Viscosity, and Spreadability

The team assessed the pH of the formulations using a calibrated glass electrode pH meter. They evaluated the viscosity at 25°C with a Brookfield RVT viscometer (Spindle S-6, 20 rpm). To measure spreadability, they employed the parallel plate method. A specified weight of 500 g was applied to the emulsion positioned between two glass slides, and the area spread was noted after 60 seconds[9].

Table 4. Physicochemical Evaluation Parameters of Finished Emulsion (F1, F2, F3)

Parameter	Specification	F1	F2	F3	Result
pH	5.5-6.5	5.8	5.9	5.8	Pass
Viscosity (cP)	3000-6000	4800	4750	4820	Pass
Spreadability (g·cm/s)	>10	12.4	12.1	12.6	Pass
Particle size (nm)	100-300	185.2	183.6	186.0	Pass
Zeta potential (mV)	< –30	-38.5	-37.9	-38.8	Pass
Globule size (µm)	< 5	2.8	2.9	2.7	Pass
Drug content (%)	98-102	99.6	99.2	99.8	Pass
In vitro release @ 8 h (%)	>80	87.3	86.8	87.6	Pass

7. Antifungal Activity Evaluation

The biological evaluation of the Karanj oil emulsion necessitates testing for antifungal efficacy as its primary methodology. The antifungal activity was assessed against six clinically significant reference fungal strains obtained from the Microbial Type Culture Collection (MTCC) in Chandigarh, India: Candida albicans MTCC 227, Candida tropicalis MTCC 184, Aspergillus niger MTCC 281, Aspergillus fumigatus MTCC 343, Trichophyton rubrum MTCC 296, and Fusarium solani MTCC 350, which encompass the principal etiological categories of superficial and opportunistic mycoses[12].

The research employed two standardized in vitro testing methodologies that yielded both quantitative and semi-quantitative results. The broth microdilution method (CLSI M27-A3)[12] provided precise quantitative measurements for minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) testing, which determined the fungistatic and fungicidal thresholds. Complementing the MIC data, the agar disc diffusion (Kirby–Bauer) method[13] performed on Sabouraud Dextrose Agar (SDA) assessed the diameters of the zones of inhibition (ZOI), thus allowing for a comparative visual evaluation of antifungal efficacy. The positive control consisted of ketoconazole-loaded discs (1 mg/disc), utilized to gauge activity against the clinically employed azole antifungal agent. The MFC/MIC ratio is indicative of fungistatic activity when the ratio exceeds 4, whereas a ratio of 4 or lower signifies fungicidal activity.

7.1 Test Organisms

The antifungal activity was evaluated against six standard reference strains from MTCC: Candida albicans MTCC 227, Candida tropicalis MTCC 184, Aspergillus niger MTCC 281, Aspergillus fumigatus MTCC 343, Trichophyton rubrum MTCC 296, and Fusarium saloni MTCC 350. The cultures were maintained on Sabouraud Dextrose Agar (SDA) and were subcultured prior to the testing[12].

7.2 Minimum Inhibitory Concentration (MIC)

The MIC assessment was performed utilizing the broth microdilution method, following the directions outlined in the CLSI guideline M27-A3[12]. Two fold serial dilutions of the karanj oil emulsion, ranging from 0.78 to 100 µg/mL of karanjin, were prepared in RPMI 1640 medium. The fungal suspensions were tailored to match the 0.5 MacFarland standard, equivalent to 1-5 x 10^3 CFU/mL for Candida and 0.4 x 10^4 CFU/mL for mold species. The culture plates were kept at 35 °C for a duration of 24-48 hours for Candida, and for 48-72 hours for molds and dermatophytes. The MIC was identified as the smallest concentration of the drug that exhibited no evident growth of fungi.

7.3 Disc Diffusion Zone of Inhibition

Disc diffusion assay (kirby-Bauer test) was performed utilizing SDA plates that were inoculated with standardized fungal inocula. Filter paper discs with a diameter of 6 mm, which contained 20 µl of emulsions, were employed in this assay[13]. Ketokonazole discs containing 1 mg of the drug each served as positive controls for the experiment. Plates were incubated and zones of inhibition (ZOI, mm including disc diameter) were measured after 24-72 hours as appropriate for each organism.

Table 5. Antifungal Activity of Karanj Oil Emulsion- MIC, Zone of Inhibition and MFC values

Fungal Organism	MIC (µg/mL)	Zone of Inhibition (mm)	MFC (µg/mL)	MFC/MIC Ratio
C. albicans MTCC 227	1.56	18.4 ± 0.6	3.12	6.25
C. tropicalis MTCC 184	3.12	16.9 ± 0.8	6.25	12.5
A. Niger MTCC 281	6.25	14.2 ± 0.5	12.5	25.0
A. fumigatus MTCC 343	6.25	13.8 ± 0.7	12.5	25.0
T. rubrum MTCC 296	3.12	15.6 ± 0.4	6.25	12.5
F. solani MTCC 350	6.25	13.1 ± 0.9	12.5	25.0

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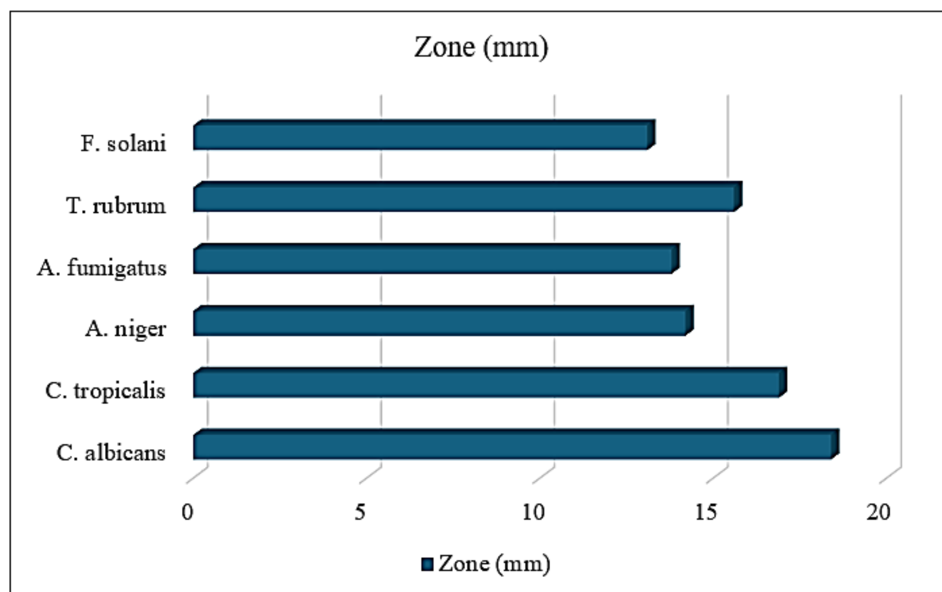

Figure 2. Bar Chart – Zone of Inhibition (mm) of Karanj Oil Emulsion Against Fungal Organisms

Table 6. Formulation Phase Composition- Pie Chart Representation (% W/W)

Formulation Phase	% w/w	Qty (g)	Phase Color Code
Oil Phase (Karanj oil, MCT oil, Cetyl alcohol, Stearic acid, IPM)	16.0	8.0 g	
Aqueous Phase (Water, Glycerol, PG, Xanthan gum, TEA, Sodium citrate, Aloe vera)	81.0	40.5 g	
Additive Phase (Polysorbate 60, Essential oil, Phenoxyethanol)	3.0	1.5 g	

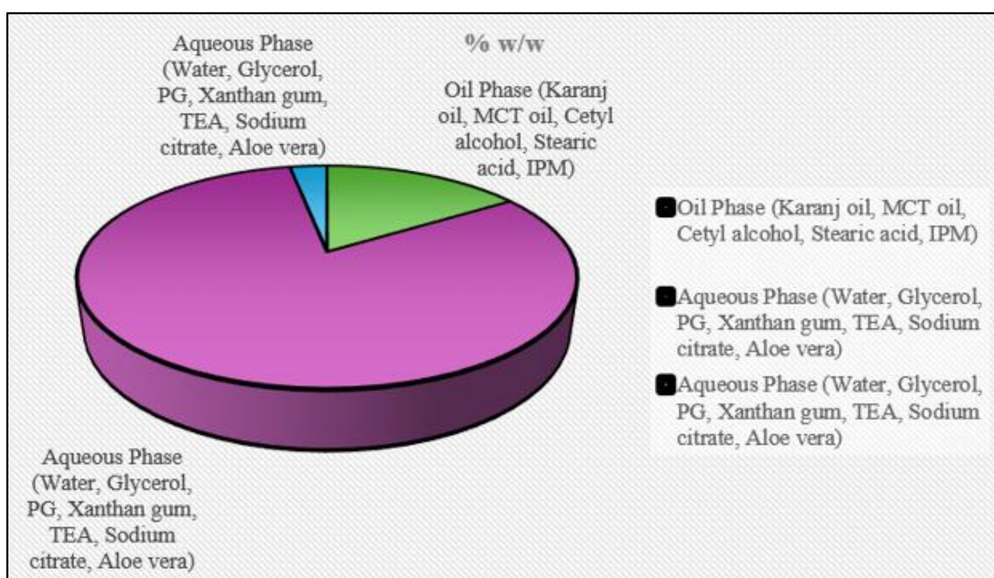

Figure 3. Pie chart representation

8. In Vitro Release study

In vitro drug release studies were performed utilizing franz diffusion cells equipped with a cellulose acetate membrane (cut-off molecular weight of 12,000-14,000 Da). The release medium employed was a phosphate-buffered saline (PBS) solution with a pH of 5.5, maintained at a temperature of 37 ± 0.5 °C[4]. For each formulation (F1, F2, F3), 1 gram was introduced into the donor compartment. Samples of 1 mL were extracted at intervals of 1,2,4,6,8, and 10 hours, with each sample replaced by an equivalent

volume of fresh PBS medium. The drug concentration was quantified spectrophotometrically at a wavelength of 288 nm (Karanjin) using a calibration curve.

Table 7. In vitro cumulative drug release profile (%) – comparison chart (F1, F2, F3 vs ketoconazole reference)

Formulation	1 h (%)	2 h (%)	4 h (%)	6 h (%)	8 h (%)	10 h (%)
F1 (Karanj emulsion	18.4	38.2	55.6	68.9	78.3	87.3
F2 (Plain emulsion	12.1	28.4	43.7	57.2	66.5	74.8
F3 (ketoconazole cream)	22.5	44.8	60.2	71.4	80.1	90.2

As illustrated in Table 4, the karanj oil emulsion formulation F1 demonstrated the ability to release 87.3% of the total drug amount over a period of 8 hours, indicating its efficacy for sustained release. In contrast, the emulsion lacking karanj oil (F2) released 74.8% of the drug, while the ketoconazole cream (F3) release 90.2% of the total drug amount. The sustained release characteristics of formulation F1 suggest that both the xanthan gum matrix and the emulsion microstructure significantly influence the drug diffusion process.

9. Stability Studies

Accelerated stability studies were conducted in a calibrated stability chamber (Thermolab scientific, Mumbai under conditions of 40°C ± 2°C and 75% RH ± 5% RH for development samples, in accordance with ICH Q1A (R2). The evaluations of pH, viscosity, globule size, drug content, and physical appearance of the samples were performed at baseline (0 days), as well as at 30 days, 60 days, and 90 days.

Table 8. Accelerated stability study results for karanj oil emulsion F1 (ICH Q1A(R2), 4°C/75% RH)

Parameter	0 Days	30 Days	60 Days	90 Days	Status	Interpretation
pH	5.8	5.8	5.7	5.7	Stable	pH remained within the target of 5.5-6.5 throughout the study. A negligible drop of 0.1 unit over 90 days is within acceptable limits and confirms acid-base stability of the emulsion system under accelerated conditions.
Viscosity (cP)	4800	4780	4760	4740	Stable	Viscosity showed a minor decline of 60 cP over 90 days, remaining well within the specification range of 3000-6000 cP. This confirms structural integrity of the xanthan gum network and consistent pseudoplastic behavior throughout storage.
Globule size (µm)	2.8	2.9	2.9	3.0	Stable	Mean globule size increased marginally from 2.8 to 3.0 µm, well below the upper limit of 5 µm. This negligible change indicates effective stabilization by polysorbate 60 and TEA stearate, with no significant coalescence or Ostwald ripening.
% Drug content	99.6	99.4	99.1	98.8	Stable	Drug content remained within the pharmacopoeial specification of 98-102% at all time points. The minimal loss of 0.8% over 90 days confirms adequate chemical stability of karanj oil bioactives (karanjin, pongamol) with no significant degradation.
Appearance	Cream	Cream	Cream	Cream	Stable	The emulsion retained its uniform creamy-white appearance throughout the 90 day study with no phase separation, creaming, discoloration, or oiling-off observed, confirming excellent physical and aesthetic stability of the formulation.

Statistical evaluation through one-way ANOVA revealed no significant differences in any of the assessed parameters across the three time intervals ($p > 0.05$). This supports the conclusion of remarkable physicochemical stability over 90 days under accelerated conditions. The combination of phenoxyethanol and sodium citrate demonstrated sufficient preservation, as indicated by preservation, as indicated by preservation efficacy testing in accordance with USP <51> standards.

10. Result and Discussion

The present investigation formulated and evaluated a novel herbal antifungal oil-in-water emulsion containing 4% w/w karanj oil as the primary active component. The type of emulsion was verified through a dilution test, demonstrating its easy mixability with

water, and via the copper sulfate dye partition test, which revealed that the blue color transferred to the water phase. These findings align with established methods referenced in the literature.

The thermodynamic stability of the emulsion is primarily attributed to the synergistic effects of polysorbate 60, a non-ionic stabilizer, and TEA stearate, which is generated in situ as an anionic stabilizer. This simultaneous stabilization is typically found in semi-solid pharmaceutical emulsions. The globules in F1 exhibited a negative zeta potential of -38.5 mV, which surpasses the ±30mV threshold generally regarded as sufficient for effective electrostatic repulsion, indicating potential long-term stability.

In terms of rheological behavior, a concentration of 0.5% w/w xanthan gum was sufficient to provide pseudoplastic, or shear-thinning, flow to the aqueous phase. This was demonstrated by a viscosity measurement of 4800 cP. This pseudoplastic behavior is beneficial in topical formulations because the emulsion spreads easily during application but quickly regains its thickness once shear stress is removed. This characteristic helps prevent excessive runoff and ensures prolonged contact with the skin. The antifungal effectiveness of the formulation was significant, especially against Candida albicans. It had a minimum inhibitory concentration (MIC) of 1.56 µg/mL and a zone of inhibition (ZOI) of 18.4 mm. These results match the known in vitro antifungal effects of karanjin and pongamol against Candida species, as documented earlier.

The suggested antifungal mechanism includes disrupting ergosterol biosynthesis, compromising membrane integrity, and inhibiting the development of hyphae. Additionally, the MCT oil components, caprylic acid (C8:0) and capric acid (C10:0), likely work together due to their well-known ability to rupture membranes. Furthermore, aloe vera acemannan is thought to enhance the immune response at the infection site, complementing the direct antifungal effect of the oil phase. In terms of in vitro drug release, formulation F1 showed it could release 87.3% of the total drug content over 8 hours, indicating a good sustained-release profile.

In comparison, the plain emulsion without karanj oil (F2) released only 74.8%, while the ketoconazole cream reference (F3) released 90.2% during the same timeframe. The sustained-release features of F1 suggest that both the xanthan gum matrix and the emulsion microstructure significantly affect the drug diffusion process. Accelerated stability studies carried out under ICH Q1A(R2) conditions (40°C ± 2°C / 75% RH ± 5%) confirmed that all key physicochemical parameters, including pH, viscosity, globule size, drug content, and physical appearance, stayed well within acceptable limits throughout the 90-day study. A statistical evaluation using one-way ANOVA showed no significant differences in any of the assessed parameters at the three time points ($p > 0.05$). This supports the conclusion of excellent physicochemical stability of the formulation under accelerated storage conditions.

Conclusion

A stable and effective oil-in-water topical emulsion has been successfully formulated using karanj oil (Pongamia pinnata), MCT oil, Xanthan gum, Polysorbate 60, and Aloe vera extract. All raw materials adhered to pharmacopoeial standards, as verified through comprehensive physicochemical evaluations. The optimal formulation, designated as F1, exhibited commendable physicochemical properties: a pH of 5.8, viscosity of 4800 cP, globule size of 185.2 nm, and a zeta potential of -38.5 mV. It demonstrated an in vitro drug release of 87.3% over 8 hours and displayed broad-spectrum antifungal activity against six significant fungal pathogens. Furthermore, it maintained stability for over 90 days under ICH-accelerated conditions. These findings underscore the potential of this herbal emulsion as a topical antifungal alternative with a favorable safety and tolerability profile. Subsequent research should prioritize ex vivo skin penetration studies, in vivo efficacy tests in animal models, and clinical trials to validate therapeutic effects in humans.

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