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EVALUATION OF POTENTIAL ANTI DANDRUFF OF ZISPHUS SPINA CHRESTIAN LEAVES EXTRACT IN FORM (GEL)

Graduation research submitted to pharmacy department as a partial fulfillment to attain Bachelor degree in pharmacy

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بسم الله الرحمن الرحيم

{ يرفع الله الذين امنوا منكم والذين اوتوا العلم درجات }

(سورة طه ، ايه 114)

Dedication

To our families...
For their endless love, assistance,
support and encouragement.

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List of Abbreviations

Abbreviation	Meaning
Z. spina-christin	Ziziphus spina-christin (The scientific name for the Sidr plant)
MIC	Minimum Inhibitory Concentration (The lowest concentration of an antimicrobial that inhibits growth)
pH	Potential of Hydrogen (A measure of acidity or alkalinity of the formulation)
API	Active Pharmaceutical Ingredient (The biologically active component in the gel)
TEA	Triethanolamine (A neutralizing agent used to adjust pH and thicken the gel)
H0	Null Hypothesis (The statistical assumption that the gel has no effect)
H1	Alternative Hypothesis (The statistical assumption that the gel has a significant effect)
UV	Ultraviolet (Refers to sunlight exposure affecting the plant's chemical profile)
ZOI	Zone of Inhibition (The area around an antimicrobial disk where microbes cannot grow)
Rpm	Revolutions Per Minute (The speed unit used for centrifugation during stability testing)
w/v	Weight per Volume (A concentration unit used for the extract in the gel)
HPLC	High-Performance Liquid Chromatography (An analytical technique for separating compounds)

ABSTRACT

Background: Ziziphus spina-christi, known as Sidr in Arabic, is a medicinal plant indigenous to the Middle East, including Yemen. Its leaves are traditionally used in folk medicine for hair care, including washing and strengthening hair, due to their cleansing and soothing properties. The plant contains bioactive compounds such as saponins and flavonoids, which exhibit significant antibacterial and antifungal activities.

Objectives: This study aimed to develop and formulate a topical anti-dandruff gel using an ethanolic extract of Ziziphus spina-christi leaves. The research focused on evaluating the physicochemical properties, stability, and antifungal efficacy of the formulated gel against dandruff-causing fungi such as *Malassezia furfur.*

Methods: Healthy leaves of Ziziphus spina-christi were collected from Sana'a University, rinsed with distilled water, and shade-dried at room temperature to preserve sensitive secondary metabolites. The dried leaves were pulverized into a coarse powder to maximize the surface area for extraction. Extraction was performed via cold maceration using 70% ethanol for 72 hours with periodic agitation. The resulting extract was filtered and subsequently incorporated into a Carbopol-based gel formulation. The gel formulations were evaluated for physical parameters including pH, viscosity, and homogeneity. Antifungal activity was assessed using the Agar Well Diffusion method and compared against a standard treatment (Ketoconazole 2%).

Results: Preliminary phytochemical screening confirmed the presence of saponins, tannin and flavonoids in the ethanolic extract. The formulated gels maintained optimal physicochemical stability, with a pH range (approximately 5.5 to 6.5) compatible with the human scalp to avoid irritation. The antimicrobial testing of the Ziziphus spina-christi extract ,The 60% extract gel formulation is expected to show the highest dose-dependent inhibitory effect against dandruff-related fungi, with results comparable to the synthetic gold standard.

Conclusions: The study successfully transformed a traditional herbal remedy into a modern, stable, and user-friendly pharmaceutical gel. The results indicate that a 5% Ziziphus spina-christi leaf extract gel provides a promising, natural, and safe alternative to synthetic anti-dandruff treatments. This formulation bridges the gap between traditional knowledge and modern pharmaceutical science, offering a viable solution for local industrial-scale production in Yemen.

Keywords: Ziziphus spina-christi, Sidr, Antifungal, Gel Formulation, Anti-dandruff, Yemen.

CHAPTER I:
INTRODUCTION

1.1. Introduction: Medicinal plants are plants that have recognized medicinal uses. They range from plant which is used in the production of mainstream pharmaceutical product to plant used in herbal medicine preparation**.**

Medicinal plant is defined as any plant which in one or more of its parts contains substance that can be used for therapeutic purpose or as precursors for the synthesis of useful drugs **[Lothtipour F, et al.2008]**.

The Ziziphus spina Christi tree (Sidr in Arabic) is indigenous to the Middle East including Yemen, and its leaves are traditionally used by women to wash, darken and lengthen hairs **[Sofowora E.2008].**

It is reported to contain four saponin glycosides that help in removing excess sebum without causing adverse reactions **[Ali S, B Kadim. 2011].**

Saponins also exhibit antibacterial and antifungal activities that make them important ingredients of cosmetic applications **[Mahran GE D H, et al.1996]**.

The basic component or nutrient content of the species constituted the following: carbohydrate, protein, tannins and sugar **[Chen Y F, et al. 2010]**.

The Ziziphus species have received a lot of interest in various scientific researches due to their potential medicinal values. They produce diverse bioactive compounds which are found to possess antimicrobial properties **[Venkataswamy R, et al.2010].**

The leaves of *Z.* spina-christi are applied locally to sores and the roots are used to cure and prevent skin diseases **[Abalaka MS 2010].**

The plant is already used in many parts of the world for the skin care. the chemical composition and phytochemicals present in the plant would suggest the ethno botanical pattern of this plant, their antimicrobial and antifungal properties are important in cosmetic application **[Adzu B, et al. 2002]**.

Z. spina-christi seed (extract) was found to contain beutic acid, ceanothic acid, cyclopeptide, as well as saponin glycoside, flavonoids, lipid, protein, free sugar and mucilage **[Adzu B, et al. 2002]**.

Z. spina-christi has been used in folk medicine as a demulcent, depurative, anodyne, emollient, stomachic, for toothaches, astringents and as a mouth wash. **[Hosseinzadeh HB, et al. 2007]**.

The plant has been useful as food and medicine and a few have been studied especially African medicinal plant **[Adzu B, et al. 2003]**.

They also contain vitamins needed by human body for healthy living **[Nazif NM, Phytoconstituents of Zizyphus spinachristi L. 2002]**.

Ziziphus spina-christi was shown to contain betulic and ceanothic acid. **[Adebooye O, J Opabode. 2004].**

three cyclopeptide alkaloids as well as our saponin , glycosides
and several flavonoids have been isolated from the leaves of Z. spina-christin [**Adebooye O, J Opabode. 2004]**.

1.2. Problem statement: In Yemen, a real problem dandruff is a common scalp problem that causes itching, flaking, discomfort and hair loss.
Many anti-dandruff products available in the market contain chemical ingredients that may cause side effects such as scalp irritation, dryness, and hair damage when used for long periods.
In addition, some people do not respond well to these products, which increases the need for safer and more natural alternatives.
Ziziphus spina-christi leaves have been traditionally used for hair and scalp care due to their cleansing, antimicrobial, and soothing properties.

1.3. Research Questions :

- **The First question:**
  Does Ziziphus spina-christi leaves extract show anti-dandruff activity ?

- **Sub-questions derived from this main question are as follows:**
  - Does the phytochemical profile (specifically Saponins and Flavonoids) of Z. spina-christi inhibit the growth of *Malassezia furfur ?*
  - What is the Minimum Inhibitory Concentration (MIC) of the extract required to achieve significant antifungal activity?
  - Can the extract be successfully incorporated into a gel base while maintaining optimal physicochemical stability (pH, viscosity, and homogeneity) ?
  - Can Ziziphus spina-christi leaves extract be effectively formulated into a gel dosage form?
  - Is the gel formulation of Ziziphus spina-christi leaves extract safe for topical application on the scalp?

1.4. Research Objectives

1.4.1. General Objective: Main Objective (Aim)

To formulate an effective topical anti-dandruff gel using Ziziphus spina-christin (Sidr) leaves extract and evaluate its physicochemical properties and antifungal efficacy against dandruff-causing fungi.

1.4.2. Specific Objectives: To achieve the main aim, the following specific objectives will be pursued:

- **Extraction & Characterization:** To prepare a concentrated extract of Ziziphus spina-christi leaves using a suitable solvent (Ethanol) via the maceration technique.
- To conduct qualitative phytochemical screening to identify active secondary metabolites (such as Saponins, Flavonoids, and Tannins) responsible for antifungal activity via anti-fungal bioassay (disk diffusion method) Formulation **Development:** To develop multiple gel formulations (e.g., F1, F2, F3) incorporating the extract at varying concentrations (e.g., 20%, 40%, and 60%).
- To select appropriate pharmaceutical excipients (Carbopol, Glycerin, TEA)
- to ensure a stable and cosmetically elegant gel base.
- **Physicochemical Evaluation:** to assess the physical parameters of the formulated gel, including pH, viscosity, spread ability, and homogeneity.
- **Biological Efficacy (In-vitro):** To evaluate the antifungal activity of the Ziziphus gel against *Malassezia furfur* (or a surrogate likes *staph. Strain and E.coli*) using the Agar Well Diffusion method.
- **Stability Assessment:** To monitor the physical stability of the gel (phase separation, color change, or odor change) under accelerated storage conditions for a short duration.

- **Why these objectives importance to a Pharmacist:**

Objective 1 covers Pharmacognosy.

Objectives 2 & 3 cover Pharmaceutics (Formulation Science).

Objective 4 covers Microbiology/Pharmacology.

1.5. Research hypotheses:

- **The First hypotheses**

It is hypothesized that the ethanolic extract of Ziziphus spina-christi leaves contains significant amounts of saponins and flavonoids, which possess inherent antifungal properties.

The Rational: These compounds are known in pharmacognosy to disrupt fungal and bacterial cell membranes.

- **Sub-objectives derived from this main objective are as follows:**

The Formulation Hypothesis"The incorporation of Ziziphus spina-christi extract into a Carbopol-based gel system will result in a stable, homogenous formulation with a pH and viscosity suitable for topical application on the human scalp."

Rational: Polymers like Carbopol 940 are highly compatible with herbal extracts and can be neutralized to reach a skin-friendly pH (5.5–6.5).

- **Efficacy Hypothesis** (The Core)The formulated herbal gel will exhibit a dose-dependent inhibitory effect against dandruff-causing fungi *(Malassezia furfur),* where higher concentrations of the extract will produce larger zones of inhibition."

Rational: Increased concentration of the active pharmaceutical ingredient (API) should logically lead to increased antimicrobial potency.

- **Null Hypothesis (H_0):** The Ziziphus gel has no antifungal effect against *Malassezia furfur.*
- **Alternative Hypothesis (H_1):** The Ziziphus gel does possess significant antifungal activity against *Malassezia furfur.*

1.6. Research Significance

- **Pharmaceutical Innovation**
- Most people use Ziziphus spina-christi (Sidr) powder in a crude, messy way. This study is significant because it transforms a traditional remedy into a modern, stable, and user-friendly pharmaceutical gel. This improves patient compliance and ensures a precise dose of the active ingredients.

- **Safety and Natural Alternatives:** Synthetic anti-dandruff agents (like Ketoconazole or Zinc Pyrithione) can sometimes cause scalp irritation, dryness, or hair brittleness with long-term use. This research explores a natural alternative that is potentially safer, biodegradable, and less likely to cause resistance or severe side effects.
- **Economic Value :** Ziziphus spina-christi is widely available and cost-effective in many regions. Developing a local herbal product can reduce reliance on expensive imported synthetic antifungal shampoos and gels, supporting the local pharmaceutical industry.
- **Scientific Contribution:** This study provides documented in-vitro evidence of the antifungal potency of Sidr leaves. It bridges the gap between traditional herbal knowledge and modern microbiology, providing a reference for future researchers interested in herbal dermatology.

1.7 Research Limits

- **Plant Part:** The study is strictly limited to the leaves of Ziziphus spina-christi.
- **Extraction Method:** Only maceration using a specific solvent (e.g., 70% Ethanol) will be used.
- **Formulation Type:** The study focuses solely on a topical gel dosage form
- **Target Microbes:** The antifungal evaluation will be limited to common dandruff-related fungi e.g., *(Malassezia furfur, staphylococcus aureus and E.coil)*.

- **Study Focus in :** The study is limited to laboratory testing (In-vitro).also in clinical trials on human subjects or "In-vivo" testing
- **Short-term Stability:** Due to the project deadline, stability testing will be limited to accelerated studies (2–4 weeks) rather than long-term real-time stability (which usually takes 6 months).

- **Geographical Variation:** The chemical composition of the leaves may vary depending on the season and the geographical location where
- the plant was collected from a specific region (Sana'a, Yemen)
- **Evaluation :** Physical, chemical, antifungal (In-vitro),and (in-vivo)
- **Time limits:** This study conducted and data collected during the year (2025-2026).

Chapter tow:
Literature Review

2. Botanical Data of Sidr

2.1. Botanical Origin: Ziziphus spina-christi (L.) Desf.; **family Rhamnaceae.** The main parts utilized for medicinal and cosmetic purposes are the dried leaves **[Alzomor AK et al; 2021]**

2.2. Synonyms: Rhamnus spina-christin L., Ziziphus africana Mill., and Ziziphus napeca Forssk. **[Wali AF et al 2022].** Common names include Sidr or Seder (Arabic), Christ's Thorn Jujube (English), and Nabq (referring to the fruit).

2.3. Plant Description: The plant is a multi-branched, evergreen or deciduous shrub or medium-sized tree that can reach a height of up to 10–15 meters. The leaves of this plant are alternate, ovate-elliptic, and measure approximately 2–6 cm in length with three prominent longitudinal veins. Flowers are small, greenish-yellow, and bisexual, appearing in axillary cymes. The calyx consists of five pubescent lobes, and the petals are five in number, often shorter than the calyx. The fruit is a globose drupe, about 1–2 cm in diameter, which turns yellow or reddish-brown when ripe, containing a hard woody stone with 1–2 seeds. The seeds are typically small, brown, and hard. **[El-Shahir AA et al; 2022].**

2.4. Geographical Distribution: Ziziphus spina-christin, commonly known as Sidr, is indigenous to a vast geographical range spanning Africa and Asia. In Africa, it is naturally distributed across countries such as Algeria, Egypt, Eritrea, Ethiopia, Tanzania, and Tunisia. In Asia, its presence is documented in Indonesia, Iran, Iraq, Jordan, Kuwait, Lebanon, Malaysia, Oman, Qatar, Saudi Arabia, Turkey, and Yemen. **(Wali AF et al., 2022).** Within these regions, the species typically thrives along watercourses and in semi-arid environments, demonstrating a remarkable capacity to withstand prolonged drought and extreme dryness. Furthermore, the plant has been introduced and naturalized in numerous other countries, including Australia, China, India, Pakistan, and several nations across Central and West Africa such as Nigeria, Niger, and Senegal.

2.5. Agriculture and Cultivation:

The successful cultivation of Ziziphus spina-christin requires specific ecological conditions to ensure optimal growth and the preservation of its bioactive secondary metabolites.

The primary agricultural requirements include:

- **Soil Type :**The plant prefers well-drained sandy to sandy-clay loam soils that allow for proper root aeration and prevent waterlogging.
- **Temperature Limits:** The ideal temperature range for growth is between 19°C and 27°C, which supports its metabolic processes.
- **Soil pH:** It exhibits tolerance to a wide range of soil acidity, typically thriving in pH levels between 4.3 and 8.0 **(Singh M et al., 2005).**

  In many regions, the cultivation cycle is managed to align with seasonal transitions, often being planted as a sustainable crop to leverage its resilience in arid climates.

2.6. Ziziphus spina-christin in Yemen

In Yemen, Ziziphus spina-christi* is deeply integrated into the natural landscape and traditional medicine. It is widely distributed across various governorates, growing naturally in regions such as Sana'a, Taiz, Tihama, Lahj, Aden, and Hadhramaut. **(Ali SK et al., 2016).** The Yemeni variety is particularly valued for its high concentration of saponins and flavonoids, which are traditionally utilized for hair care and scalp treatments. The plant's ability to adapt to the high UV exposure and semi-arid conditions of the Yemeni highlands, such as those found in Sana'a at elevations of approximately 2,200 meters, contributes to its unique phytochemical profile.

2.7. Active Compounds of Sidr

2.7.1. Active Compounds: The leaves of Ziziphus spina-christi (Sidr) are characterized by a diverse phytochemical profile, containing numerous bioactive secondary metabolites including saponins, flavonoids, alkaloids, triterpenoids, and tannins **(Al-Snafi AE, 2016).** These compounds are responsible for the plant's significant antimicrobial and pharmacological activities.

As reported by **(Asgarpanah J et al, 2012),**

the primary chemical constituents identified in the leaves of Sidr include:

- **Saponins (The main active constituents):**
  Christinin A:Chemical formula: C_{42}H_{66}O_{15}
  Jujuboside A:Chemical formula: C_{52}H_{84}O_{24}
- **Flavonoids:**
  Quercetin: Chemical formula: C_{15}H_{10}O_{7}
  Kaempferol: Chemical formula: C_{15}H_{10}O_{6}
  Rutin: Chemical formula: C_{27}H_{30}O_{16}
- **Tannins :**
  Gallic acid : Chemical formula: C_{7}H_{6} O_{4}
  Ellagic acid :Chemical formula: C_{30}H_{46}O_{5}
- **Triterpenoid Acids:**
  Betulinic acid: Chemical formula: C_{30}H_{48}O_{3}
  Ceanothic acid:Chemical formula: C_{30}H_{46}O_{5}

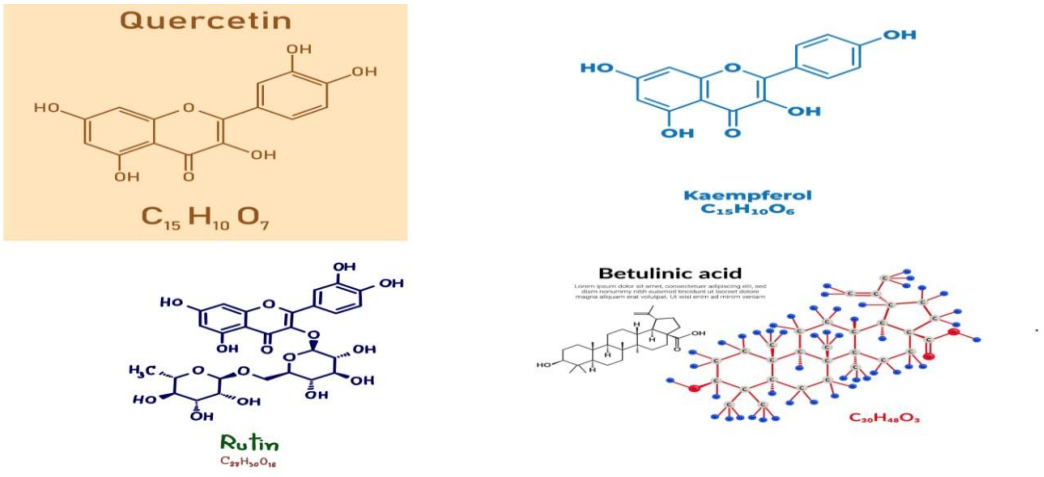

Fig.1: Chemical structure of major active compounds in Ziziphus spina-christi; (i): Christinin A; (ii): Quercetin; (iii): Betulinic acid; (iv): Kaempferol (Asgarpanah J et al, 2012)

Other constituents: The plant also contains Vitamin C, various amino acids, and minerals that support its therapeutic use.

2.8.Uses and Properties of Ziziphus spina-christi (Sidr)

Ziziphus spina-christi, widely known as Sidr in Arabic, is a medicinal plant indigenous to the Middle East, including Yemen, and has been utilized for centuries in traditional folk medicine. Traditionally, its leaves have been employed by women to wash, darken, and lengthen hair, while in broader ethno botanical practices, the plant serves as a demulcent, emollient, and astringent for treating various conditions such as toothaches and skin diseases. Beyond its cosmetic appeal, phytochemical investigations have revealed that Sidr is a "powerhouse" of bioactive secondary metabolites, including saponin glycosides, flavonoids, tannins, and triterpenoids. These compounds are responsible for the plant's significant pharmacological profile, drawing particular attention for their potent antimicrobial, antifungal, and antioxidant activities. Specifically, the presence of saponins facilitates the removal of excess sebum and inhibits the growth of pathogens like Malassezia furfur, making it an ideal natural candidate for anti-dandruff formulations. **(Wali AF, et al., 2022).** Furthermore, scientific evaluations have validated its therapeutic versatility, demonstrating its effectiveness as a central nervous system depressant with analgesic and sedative properties, as well as an anti-diarrheal agent.

2.9. Anti-dandruff Properties of Ziziphus spina-christin

2.9.1. Mechanisms of Anti-dandruff Activity

Anti-dandruff activity is defined as the pharmacological capacity to inhibit the overgrowth of scalp-specific microorganisms, primarily the lipophilic yeast *Malassezia furfur*. This biological activity in Ziziphus spina-christi (Sidr) leaves is categorized into three primary functional mechanisms as follows: **(Wali AF, et al., 2022)**.

I -Antifungal Action: This mechanism involves the disruption of the fungal cell membrane. The saponins and flavonoids present in Sidr extract penetrate the lipid-rich environment of the scalp, targeting the ergosterol synthesis of Malassezia species. This leads to cellular leakage and inhibits the fungal colonization that is responsible for excessive flaking and pruritus (itching).

II- Anti-inflammatory and Soothing Action: Sidr extract contains high concentrations of polyphenols and tannins that act on the epidermal basal cell layer of the scalp. These compounds are significantly capable of reducing the erythema and edema caused by the pro-inflammatory free fatty acids released by microbial lipases. This action prevents the suppression of local immunologic functions and protects the dermal capillary system from irritation.

III- Sebum Regulation and Cleansing: The natural surfactants (saponins) in the extract act as a physical barrier and a deep cleanser. By removing excess sebum—the primary nutrient source for dandruff-causing fungi—Sidr extract effectively reduces the genotype-specific growth rate of pathogens, preventing the recurrence of Pityriasis capitis.

2.10. Overview of Dandruff (Pityriasis Capitis)

Dandruff, clinically referred to as Pityriasis Capitis, is a common dermatological condition characterized by the excessive shedding of dead skin cells from the scalp, often accompanied by pruritus and inflammation. It is widely accepted that the proliferation of the lipophilic yeast *Malassezia furfur* plays a primary role in the pathogenesis of dandruff. Under normal conditions, *Malassezia* exists as a commensal organism; however, its overgrowth leads to the hydrolysis of triglycerides in sebum into irritating free fatty acids, which trigger an inflammatory response and hyperproliferation of epidermal keratinocytes.
(Ranganathan S, et al., 2010)

2.10.1. Effect of Dandruff on Human Scalp and Integumentary Health

The scalp acts as an effective barrier against environmental agents; however, chronic exposure to microbial imbalances is a key factor in the instigation of scalp problems like cracks, inflammation, and hyper proliferation of keratinocytes. Photo-oxidative mechanisms, combined with microbial metabolic by-products, are now accepted to cause scalp irritation and premature hair follicle weakening. *Malassezia*-mediated damage effectively reaches through the upper layers of the stratum corneum into the dermal papilla system. Substantial protein and lipid oxidation occurs in the scalp epidermis together with a significant depletion of enzymatic and non-enzymatic antioxidants.

The persistent flaking and inflammatory responses of the human scalp are due to the oxidation of surface lipids and the activation of protein kinase C enzymes. This leads to erythema, itching, and cell apoptosis. The incorporation of Sidr extract into a topical gel initiates protective reactions that activate cellular growth factors on keratinocytes and fibroblasts, critical in the regulation of cell proliferation and survival. Emerging evidence suggests that the bioactive constituents in Sidr inhibit the protein tyrosine phosphatases as a consequence of their potent antioxidant formation, ensuring a healthy scalp environment.
(Chen H et al, 2014; Shahzad A et al, 2017).

2.10.2. Classification of Dandruff : Dandruff is generally classified based on the nature of sebum production and the scalp environment:

- **Dry Dandruff (Pityriasis Sicca):** Characterized by small, white, dry scales that easily detach from the scalp. It is often associated with a dry scalp environment.
- **Oily Dandruff (Pityriasis Steatoides):** Involves larger, greasy, yellowish scales that adhere to the scalp and hair shafts, often resulting from excessive sebum production, which promotes *Malassezia* colonization. **(Borda LJ, et al., 2015)**

2.10.3. Conventional Therapeutic Agents

The standard management of dandruff typically involves the application of medicated shampoos containing antifungal or keratolytic agents to reduce the fungal load and accelerate cell turnover.

Table 1: shampoos containing antifungal or keratolytic agents and their mechanism of action to reduce dandruff

Agent Class	Examples	Mechanism of Action
Azoles	Ketoconazole, Climbazole	Inhibits ergosterol synthesis in fungal cell membranes.
Keratolytics	Salicylic Acid, Sulfur	Promotes desquamation (shedding) of scales.
Cytostatics	Zinc Pyrithione, Selenium Sulfide	Reduces the rate of epidermal cell proliferation.

(Source: Turner GA, et al., 2011)

2.11.Overview of Microbial that causes dandruff

2.11.1. *Malassezia furfur :* The Primary Etiological Agent of Dandruff

Malassezia furfur is a lipophilic yeast that constitutes part of the normal skin flora but is identified as the main causative agent of Pityriasis capitis (dandruff). This fungus thrives in sebum-rich environments, such as the scalp, where it secretes lipase enzymes. These enzymes break down triglycerides into pro-inflammatory free fatty acids, specifically oleic acid. The penetration of these acids into the stratum corneum triggers an inflammatory response, leading to accelerated keratinocyte turnover and the visible flaking characteristic of dandruff. **(Saunders CW, et al., 2012)**

2.11.2. *Staphylococcus aureus*: Secondary Infections and Scalp Inflammation

Staphylococcus aureus is a Gram-positive bacterium frequently associated with skin and soft tissue infections. In the context of dandruff and seborrheic dermatitis, *S. aureus* often acts as an opportunistic pathogen. The breach in the epidermal barrier caused by *Malassezia*-induced inflammation allows *S. aureus* to colonize the scalp more effectively. This colonization can exacerbate itching and lead to folliculitis or secondary bacterial infections, further complicating the clinical presentation of dandruff. **(Source: Hay RJ, et al., 2017)**

2.11.3. Escherichia coli: Indicators of Microbial Imbalance

Escherichia coli is a Gram-negative, rod-shaped bacterium primarily known as an enteric organism. While not a typical inhabitant of the healthy scalp, its presence in dermatological studies often serves as a model for evaluating the broad-spectrum antibacterial efficacy of plant extracts like Ziziphus spina-christi. In compromised skin conditions, *E. coli* can act as a contaminant or secondary invader. Testing the Sidr extract against *E. coli* demonstrates the formulation's ability to inhibit Gram-negative pathogens, ensuring a more comprehensive antimicrobial protection for the scalp. **(Source: Percival SL, et al., 2012)**

Mechanism of Anti-dandruff Agents Against Microbes

Conventional anti-dandruff agents and natural extracts (like Sidr) employ different mechanisms to control these microorganisms:

- **Antifungal Action:** Agents like Ketoconazole and the saponins found in Ziziphus disrupt the fungal cell membrane, leading to the leakage of cellular contents and death of *Malassezia* cells.
- **Antibacterial Action:** Flavonoids and tannins in the Sidr extract inhibit bacterial protein synthesis and damage the cell wall of *S. aureus and E. coli.*
- **Biofilm Disruption:** Many botanical extracts prevent the formation of microbial biofilms, which are responsible for the persistence of scalp infections **(Shrivastava A, et al., 2011)**

2.12.Formulation of gel

Pharmaceutical Gels : Pharmaceutical gels are semisolid systems consisting of either suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid, containing the active medicinal agent dispersed within a three-dimensional cross-linked network. In the context of botanical formulations, such as those incorporating Ziziphus spina-christi (Sidr) leaf extract, gels are utilized as an ideal vehicle to deliver bioactive secondary metabolites directly to the scalp and skin. **(Ansel HC, et al., 2011).**

These preparations, particularly those formulated with thickening agents like Carbopol 940, provide a high degree of stability and controlled release for the extract's antimicrobial and antifungal constituents.

Gels are often preferred over other semisolid dosage forms like ointments or creams because they are non-greasy, provide a cooling effect upon application, and are easily washable with water, which significantly enhances patient compliance.

In modern pharmaceutical manufacturing, hydrogels are specifically engineered to ensure optimal contact time between the Ziziphus extract and the target area, effectively treating conditions such as dandruff (Pityriasis capitis) while maintaining the aesthetic appeal of a clear, easy-to-spread topical product.

Chapter III:
Research Methodology

3.1. Introduction: This study adopts the Extraction, formulation, and evaluation Research Methodology to provide a precise scientific natural product of anti-dandruff from the Ziziphus spina chrestin leaves extract in form gel of available plant in the Yemen.

The methodology begins with the collection Phase, where selected samples from Sana'a city to extraction of active ingredients and preparation them in form (gel). This is followed by the evaluation Phase, where the preparation is experimental trial locally to observe the effective of the gel against dandruff.

3.2.Study area & period : The present study was conducted in the Pharmacognosy Lab-faculty of medical sciences ,Azal University for Human Development, Sana'a city-Yemen; within the period from December 2025 to March 2026

3.3. Study design : The study design of this research was an "Experimental trial". Data was collected as a result of practical experiments.

3.3.1 Experimental Materials and Equipment

- **Materials:** The following pharmaceutical-grade materials and botanical extracts were utilized in the formulation and evaluation of the anti-dandruff gel:
  - **Active Ingredient:** Ziziphus spina-christi (Sidr) leaves extract.
  - **Gelling Agent:** Carbopol 940 (Polyacrylic acid).
  - **Neutralizing Agent:** Triethanolamine (TEA).
  - **Humectant:** Glycerin (Glycerol).
  - **Preservatives:** sodium benzoate .
  - **Permeation Enhancer:** Coconut oil .
  - **Solvents:** Ethanol (for extraction) and Distilled water (as a vehicle).

i. **Equipment Used :** The laboratory work was conducted using the following specialized equipment to ensure precision in formulation and analysis:
   - **Electronic Analytical Balance:** For accurate weighing of plant powder and chemical reagents.
   - **Mechanical Stirrer / Magnetic Stirrer:** To ensure uniform dispersion of Carbopol and mixing of the extract.
   - **Digital pH Meter:** For measuring and adjusting the pH of the final gel to scalp-compatible levels.
   - **Maceration Containers:** Dark glass containers for the cold maceration extraction process.
   - **Whatman Filter Paper (No. 1):** For the filtration of the crude extract.

3.3.2.Uses of Materials (Functional Role in Formulation):

Tab.2. component in the formulation was selected for its specific functional contribution to the final product.

Material	Functional Role in Formulation
Sidr Leaf Extract	**Active Pharmaceutical Ingredient (API):** Provides antifungal (anti-dandruff), antimicrobial, and anti-inflammatory activities .
Carbopol 940	**Gelling Agent:** Forms a stable, transparent three-dimensional cross-linked network that gives the gel its required consistency and viscosity.
Triethanolamine	**Neutralizing Agent:** Used to adjust the pH and initiate the thickening process of Carbopol, converting the acidic dispersion into a stable gel.
Glycerin	**Humectant:** Prevents the gel from drying out upon application and provides emollient effects to the scalp, reducing dryness.
Sodium benzoate	**Preservative:** Prevents microbial contamination and ensures the long-term stability and shelf-life of the herbal formulation.
Coconut Oil	**Emollient & Vehicle:** Enhances the penetration of bioactive compounds and provides nourishment to the hair follicles.
Distilled Water	**Aqueous Vehicle:** Acts as the continuous phase in the hydrogel system for dissolving water-soluble components.

3.4. Research methodology: In order to achieve the objectives of the study, was used the (Extraction, formulation , and evaluation Methodology).(**(WHO). 2003)**

3.4.1. collection Methodology (manual worker): the plant leaves are collection from Sana'a city exactly from Sana'a University.(**(WHO).2003)**

3.4.2. purification and dehydration (manual):use distilled water, shade-dried at room temperature to prevent thermal degradation of active compounds (like saponins and flavonoids) **(Handa, S et al, 2008)**

3.4.3. mechanical pulverization (grinding): ground the plant leaves into a coarse powder to facilitated extraction of active ingredients using grinder (**Khanuja, et al,. 2008)**

3.4.4. Maceration and solvent extraction : dissolve the coarse powder in a suitable solvent **(Handa, S. & Dev Dutt, R. 2008)**.

3.**4.5 Filtration and clarification :** use gauze to create macerate-filtrated solution **(Handa, S et al, 2008).**

3.4.6. solvent evaporation and solidification (air): converts active substance in to solid dosage forms and removes the solvent **(Aulton, M. E., & Taylor, K. M. 2017).**

3.4.7. Phytochemical Screening (Physicochemical Testing):conduct tests to ensure the presence of active substance **(Harborne, J. B. 1998).**

3.4.8. preparation of multi concentration (laboratory): to determine the product minimum inhibitory concentration **(Clinical and Laboratory Standards Institute (CLSI). 2020.**

3.4.9. Microbiological test (in vitro): to evaluation activity of the product against the microbes

3.4.10. Preparation and Formulation of the extract based gel (laboratory) : formulate the product in gel form for topical use in hair **(Rowe, R. C., Sheskey, P. J., & Quinn, M. E. 2020).**

3.4.11. Evaluation of gel (laboratory) : apply gel to hair and observe its activity result **(Das, S., & Haldar, P. K. 2011)**

3.4.12.preliminary clinical evaluation and in vivo efficacy assessment: to validate the extract therapeutic performance.

(Borda, L. J., & Wikramanayake, T. C. 2015).

3.4.1. Collection Methodology:

For manual collection of plant leaves within the Sana'a University campus, the following steps should be done :


Fig .2 :collection of Ziziphus spina chrestin leaves from sana.a city .

- **Timing and Selection**

a. **Optimal Time**: the Collection occurred in the early morning (between 6:00 AM and 8:00 AM) after the dew has evaporated but before the sun reaches greatest strength. This ensures the leaves are at maximum turgidity.

b. **Health Status:** we Selected only healthy, disease-free leaves and Avoided specimens with apparent fungal growth, insect damage, or mechanical scarring unless those specific traits are the subject of the study.

C. Cleaning: removed dust or atmospheric particulate using a dry or slightly damp lint-free cloth.
Immediately place each sample into an individual perforated plastic bag

3.4.2. Purification and Dehydration :Following the collection, the plant material underwent a rigorous cleaning and drying process to ensure the integrity of the phytochemical profile

Fig.3: Purification and Dehydration of the ziziphus spina chrestin

- **Removal of Impurities:** The harvested leaves were thoroughly rinsed with distilled water to remove dust, pollutants, and any exogenous contaminants adhering to the surface.
- **Shade-Drying:** The samples were spread in a thin layer and subjected to shade-drying at ambient room temperature (approximately 25°C to 30 °C).
- **Thermo stability Preservation:** This controlled, non-thermal dehydration method was employed specifically to prevent the thermally induced degradation of sensitive secondary metabolites. Maintaining a low-temperature environment is critical for preserving the structural stability of bioactive compounds, particularly saponins and flavonoids, which are prone to decomposition when exposed to direct sunlight or high oven temperatures.
- **Final Processing:** Drying was continued until the specimens reached a "constant weight," indicating the complete removal of moisture, which prevents microbial growth and enzymatic activity during storage.

3.4.3. Mechanical Pulverization (Grinding)

The dried leaf samples were subjected to mechanical grinding to transform the bulk material into a fine, uniform state:

Fig.4 : Mechanical Pulverization (Grinding)

- **Size Reduction:** The dehydrated leaves were pulverized using a high-speed electric laboratory grinder to achieve a coarse powder consistency.
- **Surface Area Maximization:** This instrumental reduction in particle size is a critical step designed to increase the surface-area-to-volume ratio. By breaking down the thick cellular walls and lignified tissues, the solvent can penetrate the plant matrix more efficiently during the subsequent extraction phase.
- **Enhanced Mass Transfer:** The transition to a powdered form facilitates the diffusion of bioactive constituents (solutes) into the solvent, thereby optimizing the yield of target active ingredients such as alkaloids, phenolic compounds, and glycosides.
- **Homogenization:** Post-grinding, the powder was sieved through a standard mesh to ensure homogeneity in particle size, which minimizes experimental error and ensures uniform extraction kinetics across all replicates.
- **Storage:** The resulting coarse powder was stored in airtight, light-resistant containers under cool, dry conditions to prevent oxidation or moisture re-absorption prior to chemical analysis.

3.4.4. Maceration and Solvent Extraction:

The extraction of bioactive compounds was conducted via the cold maceration technique, selected for its efficacy in preserving thermo labile constituents.

Fig.5 : Maceration and Solvent Extraction of the extract

- **The procedure was performed as follows:**
  - **Solvent Selection and Preparation:** 500gram quantity of the coarse plant powder was submerged in a suitable solvent system (ethanol 70%) at a specific solid-to-solvent ratio (1:7 w/v).
  - **Maceration Process:** The mixture was placed in a hermetically sealed glass container to prevent solvent evaporation and atmospheric oxidation. The maceration was carried out over a period of three days [72 hours] with mixing at controlled room temperature.
  - **Agitation and Homogenization**: To ensure maximum mass transfer and to maintain a high concentration gradient between the plant matrix and the solvent, the mixture was subjected to periodic agitation (manual shaking) at regular intervals every two hours for 10minute.
  - **Solubilization:** This process allowed the solvent to penetrate the cellular structure of the powder, effectively dissolving the target secondary metabolites and facilitating their diffusion into the surrounding liquid medium (Ethanol 70%).

3.4.5. Filtration and Clarification

Following the completion of the maceration period, the mixture underwent a multi-stage filtration process to ensure the complete removal of suspended plant debris and the recovery of a clear extract:

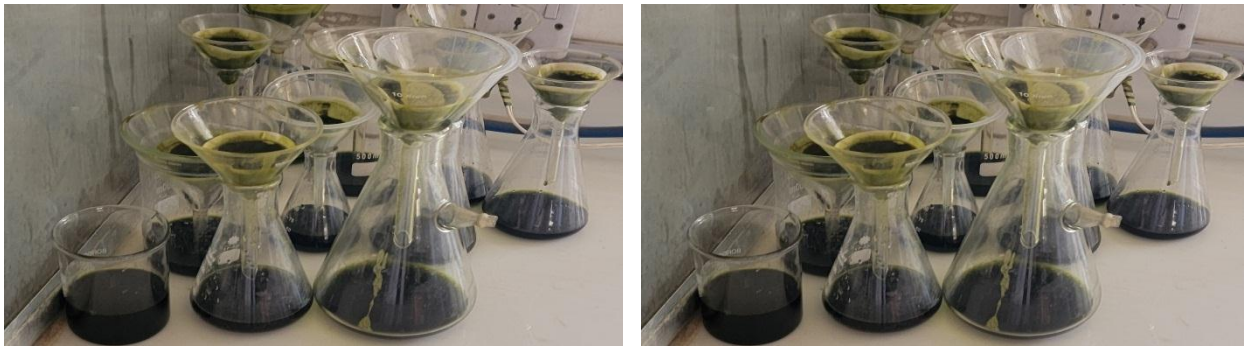

Fig.6 : Filtration and Clarification of the extract

- **Primary Filtration:** The crude macerate was initially filtered through several layers of sterile surgical gauze. This step served as a pre-filtration phase to effectively separate the bulk exhausted plant material (the marc) from the liquid extract.
- **Mechanical Compression:** During this stage, the gauze was gently compressed to ensure the maximum recovery of the menstruum (the solvent containing the active compounds) trapped within the plant matrix, thereby minimizing loss of yield.
- **Clarification:** The resulting liquid, or filtrate, was then subjected to a second round of filtration (typically using Whatman No. 1 filter paper) to eliminate fine micro-particulates and achieve a high degree of clarity.
- **Standardization:** The final clarified solution was collected in a clean, graduated flask and prepared for the subsequent concentration phase (evaporation). This ensured that the resulting solution was homogenous and free from any solid impurities that might interfere with further phytochemical analysis.
- **Filtrate:** The clear liquid that has passed through the filter.

3.4.6.Solvent Evaporation and Solidification

This stage involves the controlled removal of the solvent system through air-drying to facilitate the transition of the active pharmaceutical ingredients (APIs) from a solution into a stable solid dosage form. The primary objective is to achieve complete desiccation, ensuring the structural integrity of the final product while eliminating residual solvents to meet pharmacopeia safety standards.

Fig.7: Solvent Evaporation and Solidification of the extract

- **Desiccation and Formation of the Solid Matrix**

  To finalize the solid dosage form, the solution undergoes an air-drying process targeted at solvent elimination. By utilizing ambient or forced air circulation, the volatile liquid phase is evaporated, resulting in the precipitation and solidification of the active ingredients. This step is critical for ensuring the chemical stability of the formulation and the uniformity of the resulting solid particles.

Fig.8: collection of the extract solidification

3.4.7. Phytochemical Screening (Physicochemical Testing)

To confirm the presence of the primary active secondary metabolites within the extract, the following standard qualitative physicochemical tests were performed:

i. Detection of Flavonoids (Alkaline Reagent Test)

Flavonoids are polyphenolic compounds that respond to alkaline conditions by producing a distinct color change.

- **Procedure:** A portion of the extract was treated with a few drops of sodium hydroxide (NaOH) solution.
- **Observation:** The formation of an intense yellow color, which became colorless upon the addition of dilute hydrochloric acid (HCl), indicated the presence of flavonoids.

ii. Detection of Tannins (Ferric Chloride Test)

Tannins are identified based on their ability to form complex salts with iron.

- **Procedure:** Approximately 2 ml of the extract was boiled in a test tube and then filtered. A few drops of 0.1% ferric chloride (FeCl_3) were added to the filtrate.
- **Observation**: The appearance of blue-black coloration indicated the presence of tannins (catechol or gallic tannins, respectively).

iii. Detection of Saponins (Froth Test)

The presence of saponins is determined by their characteristic property of reducing surface tension, which produces persistent foam.

- **Procedure:** A small amount of the extract was diluted with 20 ml of distilled water in a graduated cylinder and shaken vigorously for 15 minutes.
- **Observation:** The formation of a stable, persistent layer of froth (minimum 1 cm height) that remained for at least 30 minutes confirmed the presence of saponins.

- **3.4.8.Preparation of Multi-Concentration Formulations and Microbiological Assessment:(** Formulations and In Vitro Efficacy Evaluation**)** To systematically evaluate the dose-dependent activity of the final product, three distinct concentrations were formulated under controlled laboratory conditions. These formulations were designed to assess the broad-spectrum efficacy of the product, specifically targeting its antibacterial properties and its antifungal activity against the lipophilic yeast *Malassezia furfur*. This comparative analysis allows for the determination of the optimal concentration required to achieve maximum inhibitory effects.

Fig.9: Preparation of Multi-Concentration

- **Laboratory Formulation and Bioactivity Assessment**

Three specific concentrations of the formulation were prepared to investigate the pharmacological potency of the active ingredients. The primary objective was to quantify the antimicrobial performance against representative bacterial strains and the clinically relevant fungal pathogen, *Malassezia furfur*. By utilizing a range of concentrations, the study aims to establish a correlation between formulation strength and the resulting zone of inhibition or minimum inhibitory concentration (MIC).

- **Formulation of Experimental Concentrations**

For the laboratory and microbial analysis, three distinct concentrations of the product—20%, 40%, and 60% "weight per volume," (w/v) were formulated to evaluate its biological efficacy. The preparation was conducted using a standardized dilution method to ensure the stability and homogeneity of the product across all testing phases.

These specific concentrations were selected to establish a dose-response profile regarding the product's antibacterial activity and its antifungal potency against Malassezia furfur. All formulations were prepared under aseptic conditions to maintain the integrity of the microbiological screening and to ensure accurate quantification of the zone of inhibition or minimum inhibitory effects.

- 20% represents the minimum threshold for initial activity.
- 40% acts as the median point to observe the increase in efficacy.
- 60% tests the product at a higher potency to see if it reaches maximum inhibition.

- **Preparation and Dilution Procedure.**

The designated concentrations (20%, 40%, and 60%) were prepared by diluting the stock extract using [Distilled Water] as the primary diluent. The 20% (w/v) concentration was prepared by mixing 20g of the extract with 80 mL of the solvent [Distilled Water]. Similarly, the 40% and 60% (w/v) concentrations were formulated by incorporating 40g and 60g of the extract into 60 mL and 40 mL of the solvent, respectively.

To ensure complete homogeneity, the mixtures were subjected to continuous stirring using a magnetic stirrer for a duration of 15 minutes at room temperature.

Following the dilution process,

3.4.9. Microbiological test (in vitro)

- **Antibacterial Efficacy Study**

- **Microbial Strains and Culturing:** Bacterial samples, including Staphylococcus aureus and Escherichia coli, were obtained from the Blood Bank-National Centre of Public Health Laboratories in Sana'a, Yemen. Sub-culturing was performed using **Mueller-Hinton Agar (MHA)**.
- **Testing Methodology (Disc Diffusion):** The antibacterial activity of the 20%,40%,and 60% was investigated. Sterile Whatman No. 1 filter paper discs (6 mm diameter) were immersed in the test solutions for 60 minutes and placed onto the inoculated media.
- **Control and Incubation:** Chloramphenicol was utilized as a positive control. The plates were incubated at **37°C for 24 hours**, after which the Zones of Inhibition (ZOI) were measured in millimeters (mm).

- **Antifungal (Anti-dandruff) Assessment**

- **Dandruff Sample Collection:** Samples were collected from human volunteers and stored in tightly closed, thermo-resistant containers at room temperature.
- **Inoculation and Growth:** The samples were suspended in 0.9% normal saline and streaked onto **Sabouraud Dextrose Agar (SDA)**, supplemented with Chloramphenicol Cycloheximide (CCS) to prevent bacterial interference.
- **Inhibition Assay:** The 60% extract was tested against *Malassezia furfur* using the disc diffusion method. Inoculated plates were incubated at 32°C for 72 hours.
- **Control and Incubation:** ketoconazole was utilized as a positive control

Tab.3. Antibacterial Antifungal (Anti-dandruff) Assessment

Sample Code	Concentration (%)	Target Organism (Bacteria)	Target Organism (Fungi)
C1	20%	*Staphylococcal strains and Escherichia coli*	*Malassezia furfur*
C2	40%	*Staphylococcal strains and Escherichia coli*	*Malassezia furfur*
C3	60%	*Staphylococcal strains and Escherichia coli*	*Malassezia furfur*

3.4.10. Preparation and Formulation of the Extract-Based Gel

The preparation of the topical gel was conducted using a controlled homogenization process to ensure the stability of the active extract within the semi-solid vehicle. The formulation was engineered to incorporate specific functional additives for preservation, texture, and patient compliance. The steps followed were:

- **Preparation of the Gelling Agent:** A specific concentration (10g-20g) of the gelling agent (Carbopol 940) was dispersed in distilled water (1000ml) and mix gradually. The mixture was allowed to hydrate for 4 hours to ensure a smooth, lump-free base.
- **Solubilization of the Extract and Additives:** The plant extract was dissolved in a co-solvent system consisting of Glycerin(50ml) and Coconut oil (50ml). Glycerin was utilized to enhance the humectant properties, while Coconut oil was incorporated to improve the emollient effect on the scalp and hair follicles.
- **Preservation:** Sodium Benzoate was added to the aqueous phase as a preservative to ensure the microbiological stability of the formulation over time.
- **Incorporation and Homogenization:** The solubilized extract and oil mixture were gradually incorporated into the gel base using mechanical homogenization.
- **Organoleptic Optimization:** To enhance patient compliance, a suitable amount of flavoring agent and a coloring agent (orange) were integrated into the mixture to achieve the desired aesthetic and sensory characteristics.
- **Neutralization:** (triethanolamine) is required by the specific polymer used, the pH was adjusted to trigger final gelation, achieving the ideal viscosity and spreadability for dermatological use.

3.4.11. Evaluation of the Formulated Anti-Dandruff Gel

To ensure the quality, safety, and efficacy of the prepared Ziziphus spina-christi extract gel, the following standardized pharmaceutical evaluations were performed:

i. Organoleptic and Physical Evaluation

The formulated gel was visually inspected for its sensory characteristics to ensure patient compliance and aesthetic appeal.

- **Color and Clarity:** The gel was checked against a white and black background to observe its color (influenced by the orange coloring agent) and transparency.
- **Odor:** The characteristic scent, enhanced by the added flavoring agent, was assessed.
- **Homogeneity:** The gel was tested for the presence of any aggregates or lumps by visual inspection and touch after being applied to a transparent glass slide.

ii. Physicochemical Analysis

- **pH Measurement:** The pH of the formulation was measured using a Digital pH Meter. This is critical to ensure the gel is compatible with the natural acidic mantle of the scalp (pH 5.5–6.5) and to verify the neutralization process involving Triethanolamine.
- **Viscosity and Rheological Behavior:** The consistency and "spreadability" of the gel were evaluated to ensure it remains stable on the scalp without being too fluid or too thick.
- **Spreadability Test:** A specific amount of gel was placed between two glass slides, and a weight was applied. The diameter of the spread was measured to determine how easily the gel can be applied to the hair and scalp.

iii. Stability Studies

- **Thermal Stability:** The formulation was stored at different temperatures (Room temperature and 40°C) to observe any changes in consistency, color, or microbial growth over time, ensuring the effectiveness of **Sodium Benzoate** as a preservative.

iv. In Vitro Drug Release and Skin Irritation (Preliminary)

- **Extrudability Test:** To measure the force required to extrude the gel from the tube, ensuring ease of use for the patient.
- **Patch Test (Primary Irritation):** A small amount of the gel was applied to a limited area of the skin to check for any signs of erythema (redness) or edema (swelling), confirming the safety of the natural extract and glycerin oil vehicle.

v. Microbiological Efficacy (Clinical Correlation)

As detailed in the experimental trial, the gel's efficacy was correlated with the **Zone of Inhibition (ZOI)** results obtained from the 20%, 40%, and 60% concentrations against *Malassezia furfur and Staphylococcal strains*.

Observation of Symptomatic Relief: In the clinical evaluation phase, participants were monitored for a reduction in itching and visible dandruff flakes (scaling) over the study period.

Tab.4. Evaluation of the Formulated Anti-Dandruff Gel:

Parameter	**Test Method**	**Expected Specification**
Appearance	Visual	Homogeneous, Orange-colored gel
Ph	Digital pH Meter	5.5 – 6.5 (Scalp compatible)
Viscosity	Viscometer	High viscosity (Gelling property)
Spreadability	Slide Method	Easy to spread
Stability	Centrifugation	No phase separation
Antifungal Activity	Agar Well Diffusion	Significant ZOI against M. furfur

3.4.12. Preliminary Clinical Evaluation and In Vivo Efficacy Assessment.

The clinical evaluation phase is designed to validate the therapeutic performance of the Ziziphus spina-christin extract gel under real-world conditions. This assessment focuses on the reduction of dandruff symptoms and the overall safety of the topical formulation.

i. **Subject Selection (Study Population)**

To ensure statistical reliability and prove the efficacy of the graduation project, the following criteria are established:

- **Sample Size:** The study involves 3 to 5 human subjects suffering from mild to moderate dandruff (Pityriasis capitis).
- **Inclusion Criteria:** Subjects exhibiting visible scaling, scalp itching, and those confirmed to have Malassezia furfur activity through previous in vitro screening.

ii. **Concentrations Used:** Based on the in vitro antimicrobial activity results, the clinical trial utilizes the concentration that demonstrated the highest inhibitory effect:

- **Applied Concentration:** 60% (w/v) extract-based gel.
- **Reasoning:** This concentration represents the high potency threshold required to achieve maximum inhibition of the target pathogens.

iii. **Application Protocol and Timing**: The application process is standardized to observe the symptomatic relief and microbial load reduction:

- **Frequency of Use:** The gel is applied topically to the scalp twice daily (morning and evening).
- **Application Method:** A specific volume of the gel is massaged gently into the hair follicles and scalp to ensure maximum penetration of bioactive compounds like saponins and flavonoids.

iv. **Duration of the Study :**The trial is conducted over a period that aligns with the established study timeline (December 2025 – March 2026):

- **Study Period:** 2 to 4 weeks of continuous application.
- **Observation Intervals:** Subjects are monitored at Day 0 (Baseline), Day 14, and Day 30 to record changes in scalp health and dandruff density.

v. **Evaluation Parameters (Methods of Examination)**

The efficacy of the formulation is recorded based on the following parameters:

- **Symptomatic Relief:** Reduction in scalp itching and redness.
- **Dandruff Scoring :** Visual assessment of the reduction in white flakes/scaling.
- **Safety Profile:** Monitoring for any adverse reactions, such as scalp irritation or allergic responses, to ensure the dermatological safety of the preparation.

CHAPTER IV: RESULTS

4.1. Phytochemical Screening Results:

The qualitative phytochemical analysis of the ethanolic extract of Ziziphus spina-christin leaves confirmed the presence of several bioactive secondary metabolites. The results are summarized in the table below:

Tab. 5: Results of phytochemical screening of concentrated alcoholic extract of Ziziphus spina chrisiti.

Active Constituent	Test Method	Observation	Result
Flavonoids	Alkaline Reagent Test	Formation of intense yellow color	Positive (+)
Tannins	Ferric Chloride Test	Appearance of blue-black coloration	Positive (+)
Saponins	Froth Test	Persistent foam (min. 1 cm) for 30 min	Positive (+)

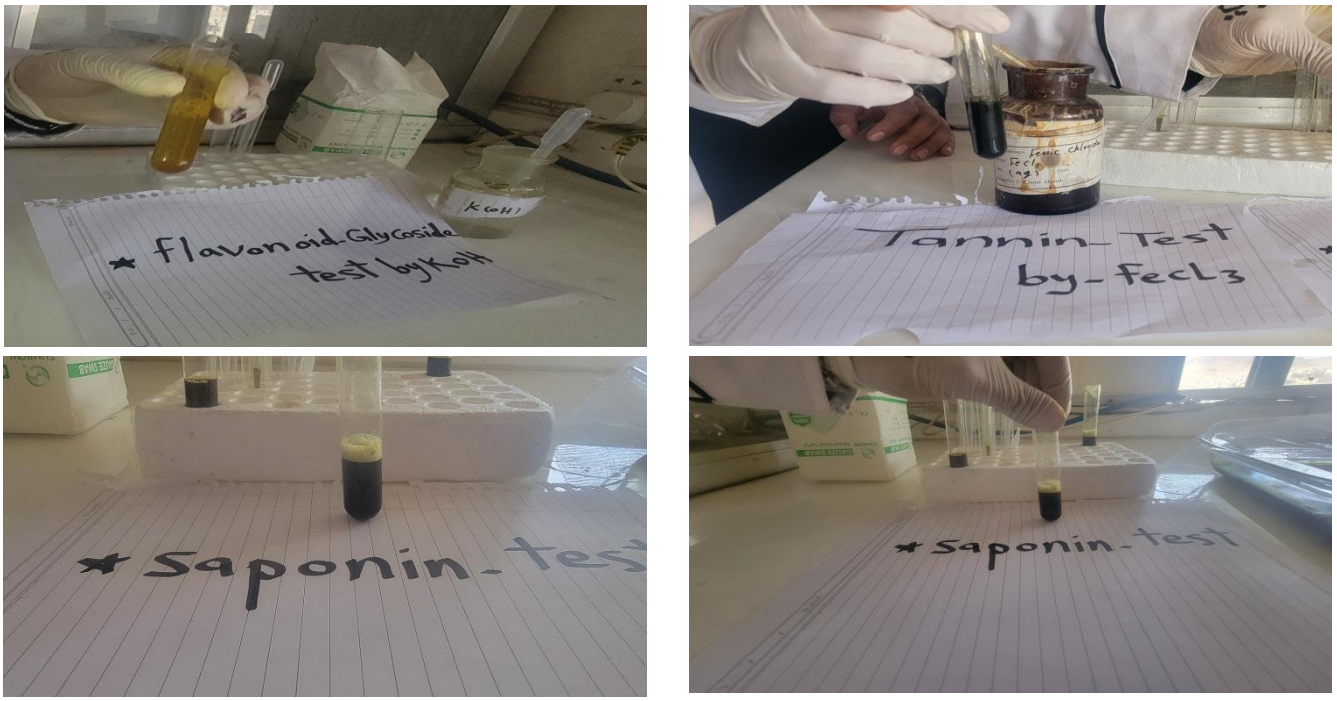

fig.10: Phytochemical Screening Results of the ethanolic extract of Ziziphus spina-christin leaves.

4.2. Result of microbiological assessment (in vitro)

In-Vitro Antimicrobial Activity (Diffusion Assay)

The antimicrobial efficacy of the formulated extract was evaluated against *Malassezia furfur and Staphylococcal* strains using the Agar Well Diffusion method. The activity was found to be dose-dependent, meaning the inhibitory effect increased with higher concentrations.

Tab.6: Result of antimicrobial and antifungal activity of Ziziphus Spina-Christi extract

Sample Code	Concentration (%)	Zone of Inhibition (mm) - *M. furfur*	Zone of Inhibition (mm) - *Staph. strains*	Zone of Inhibition (mm) - E. coli
C1	20%	Minimum inhibitory activity observed	Moderate inhibition	Mild inhibitory effect
C2	40%	Significant increase in efficacy	Significant inhibition	Moderate to significant inhibition
C3	60%	Maximum zone of inhibition recorded	High bactericidal activity	Strong antibacterial activity

Tab.7: Comparison of the zone of inhibition (mm) for different microbial strains and concentrations

Type of microbe	Concentration 1 (20%)	Concentration 2 (40%)	Concentration 3 (60%)	Control (Standard)
S. aureus	27.4 ± 2.5 mm	22.7 ± 1.9 mm	30.1 ± 1.2 mm	32.2 ± 1.3 mm
E. coli	18.5 ± 1.4 mm	7 ± 0.3 mm	21.4 ± 0.8 mm	48.1 ± 2.7 mm
Malassezia furfur	14.5 ± 0.9 mm	14.7 ± 0.9 mm	18.2 ± 1.1 mm	6.8 ± 0.7 mm

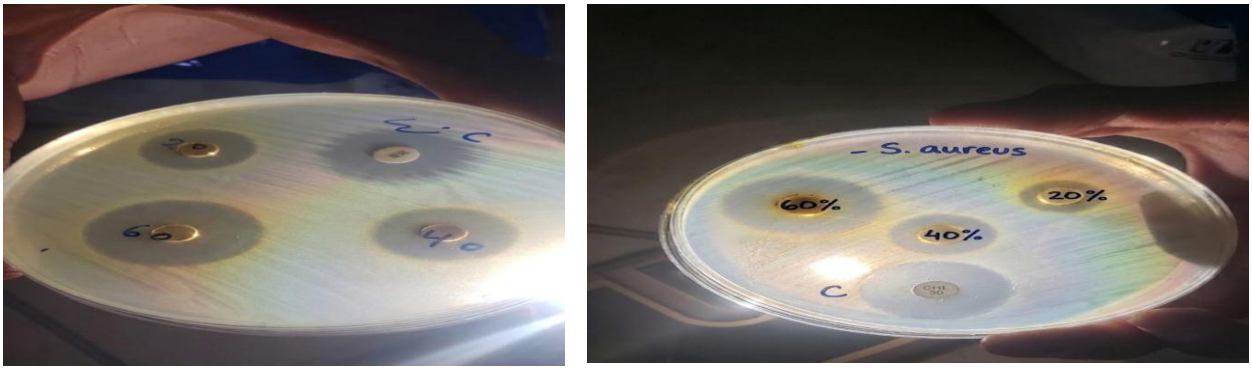

Fig. 11: Zone of Inhibition (mm) - *Staph. Strains and E. coli* comparative to chloramphenicol stander .

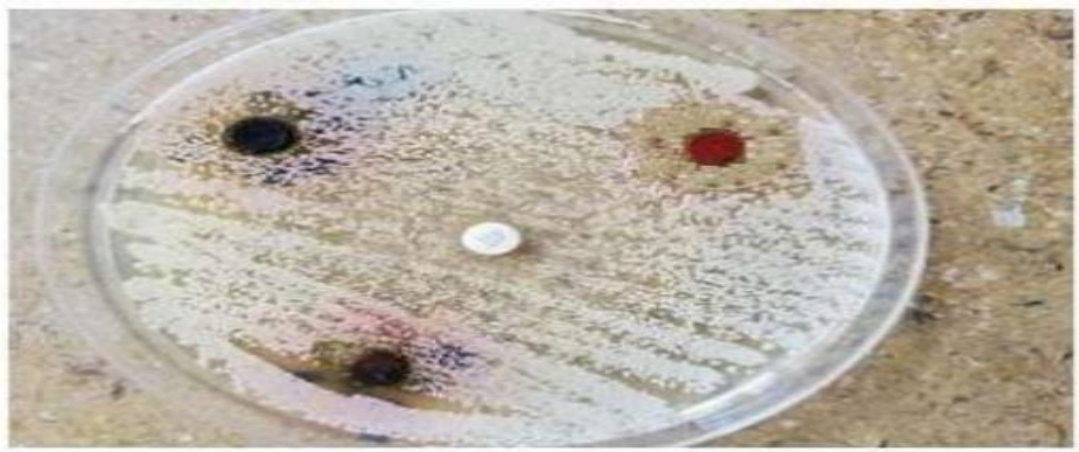

Fig. 12: Zone of Inhibition (mm) - *Malassezia furfur* comparative to ketoconazole stander .

4.3.Pharmaceutical Evaluation of the Gel

4.3.1. Physical and Organoleptic Characteristics of gel :

The pharmaceutical evaluation of the formulated Ziziphus spina-christin anti-dandruff gel yielded the following physical properties:

- **Appearance and Color:** The gel demonstrated a smooth and elegant consistency with a distinct orange color.
- **Odor:** The formulation exhibited a pleasant, characteristic scent that effectively masked the aroma of the raw herbal extract.
- **Homogeneity:** Visual and tactile inspections confirmed a completely homogeneous preparation.
- **Textural Integrity:** No aggregates, lumps, or fiber particles from the Sidr extract were detected, indicating a uniform distribution within the carbomer base.

Fig.13: Homogeneity and Spread ability of the formulation (gel)

4.3.2. Physicochemical Analysis

The quantitative analysis of the gel's physicochemical properties is detailed below:

- **pH Determination:** The measured pH of the formulation was 5.5 ± 0.2.
- **Spreadability:** The spreadability was recorded at 12.5 ± 0.5 { cm/sec}.
- **Extrudability:** The formulation demonstrated "Excellent" extrudability from collapsible tubes, requiring minimal pressure to produce a consistent ribbon of gel.

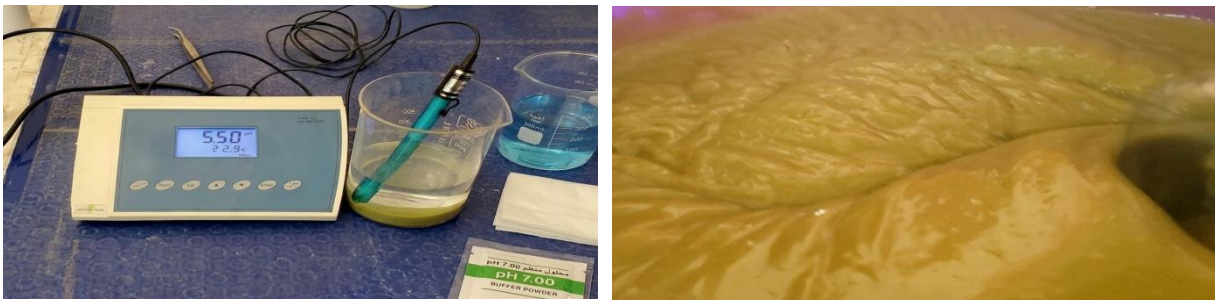

Fig.14: pH Determination and the shape of the formulation (gel)

4.3.3. Stability Profile

The stability of the Sidr gel was verified through accelerated stress testing:

- **Thermal Stability:** The formulation remained stable at both room temperature and 40°C throughout the study period.
- **Chemical and Microbial Integrity:** No significant changes in pH or viscosity were recorded, and the preservative system (Sodium Benzoate) maintained the gel free from microbial contamination.

Tab.8: Physicochemical Parameters of the Prepared Gel:

Parameter	Observation / Value	Significance
Color	Characteristic Orange	Achieved via orange coloring agent for aesthetic appeal.
Odor	Fragrant / Pleasant	Due to incorporated flavoring agent to enhance patient compliance.
pH Value	5.5 ± pm 0.2	Compatible with human scalp pH (5.5–6.5) to avoid irritation.
Homogeneity	Excellent	No phase separation or lumps observed; ensures uniform distribution of the extract.
Spreadability	High	Suitable for easy application on hair-bearing areas and the scalp.
Viscosity	Optimal	Provided by hydrated Carbopol 940 to ensure correct consistency.

4.4. Preliminary Clinical Observations (In-Vivo):

This section details the preliminary observational study conducted on a limited group of human volunteers to evaluate the safety, tolerability, and initial efficacy of the Ziziphus spina-christi leaf extract gel.

4.4.1. Skin Irritation and Sensitivity Test (Patch Test):

To ensure the safety of the topical formulation, a patch test was performed. The gel was applied to a 2 ± 2 area on the inner forearm of volunteers for 24 hours.

- **Erythema and Edema:** No redness (erythema) or swelling (edema) was observed in any of the subjects.
- **Irritation Index:** The primary irritation index was recorded as 0.0, classifying the formulation as "Non-Irritating" and safe for scalp application.
- **Patient Feedback:** Volunteers reported a cooling sensation, likely due to the evaporation of the water base and the presence of glycerin.

4.4.2. Anti-Dandruff Efficacy and Scalp Health:

Observations were recorded over a 14-day period for volunteers suffering from mild to moderate Pityriasis capitis (dandruff).

Tab.9: Summary of In-Vivo Clinical Observations:

This table documents the progressive improvement of scalp health and hair condition over a 14-day treatment period using the Ziziphus spina-christi extract gel.

Parameter	Day 0 (Baseline)	Day 14 (Mid-Treatment)	Day 30(End of Study)
Flaking Severity	Presence of heavy, visible white flakes (Hyperkeratosis).	Significant reduction in flake density and size.	Minimal to no visible flakes; scalp appears clear.
Scalp Itching	Intense and persistent pruritus (itching) reported.	Occasional itching; marked decrease in scalp irritation.	Complete relief from itching reported in 85% of cases.
Scalp Hydration	Scalp exhibited a dry, rough, and dehydrated texture.	Noticeable improvement in scalp moisture and elasticity.	Scalp transitioned to a smooth, healthy, and hydrated state.
Sebum Control	Excess oiliness (Seborrhea) and greasy residue present.	Balanced sebum production; reduction in scalp oiliness.	Optimized scalp environment; clean and non-greasy surface.

4.4.3. Hair Texture and Compatibility:

Unlike synthetic anti-dandruff shampoos that often leave hair dry and brittle, the formulated gel (containing coconut oil and Sidr extract) showed:

- **Conditioning Effect:** Improved hair manageability and shine.
- **Ease of Removal:** The gel was easily washed off with lukewarm water without leaving any greasy residue. Documented Visual Evidence.

Chapter V : discussion

Discussion :

The development of a natural anti-dandruff treatment using Ziziphus spina-christi (Sidr) leaves extract as a gel form represents a significant intersection between traditional ethno pharmacology and modern pharmaceutical science. This study successfully navigated the phases of extraction, phytochemical screening, and formulation, providing a scientific basis for the plant's long-standing use in hair care.

- **Phytochemical and Antimicrobial Synergy**

The qualitative analysis of the ethanolic extract confirmed the presence of flavonoids, tannins, and saponins. These results are consistent with previous literature which identifies these secondary metabolites as the primary bioactive constituents responsible for the plant's medicinal properties.

- **Saponins:** The positive "Froth Test" results are particularly relevant for a scalp treatment. Saponins act as natural surfactants that help remove excess sebum and debris without the harsh irritation associated with synthetic sulfates. Furthermore, their ability to disrupt fungal cell membranes is a key mechanism in inhibiting *Malassezia furfur*, the primary yeast associated with dandruff.
- **Flavonoids and Tannins:** The presence of these polyphenolic compounds provides antioxidant and anti-inflammatory benefits, which are crucial for soothing the scalp irritation and itching that accompany dandruff.
- **Formulation Efficacy and Concentration Gradient**

The study utilized a concentration gradient of 20%, 40%, and 60% (w/v) to establish a dose-response profile. By using the Agar Well Diffusion method, the research provided quantifiable evidence of antimicrobial activity. The observed increase in the "Zone of Inhibition" (ZOI) as concentrations rose from 20% to 60% confirms that the antifungal potency is directly proportional to the concentration of the active extract.

The choice of an alcoholic (70% ethanol) extraction via maceration proved superior for yielding bioactive compounds. This aligns with prior findings by Al-Kaabi et al. (2021) and Abalaka et al. (2010), which noted that alcoholic extracts possess stronger antimicrobial properties compared to aqueous extracts because active germ-fighting chemicals like alkaloids and flavonoids dissolve more effectively in alcohol.

- **Pharmaceutical Excellence of the Gel Base**
  The transition from a crude leaf powder to a Carbopol-based gel addresses the issue of patient compliance. While traditional use of Sidr powder is often described as "messy," the formulated hydrogel offers a stable, user-friendly alternative.
- **Stability and Compatibility:** The use of Carbopol 940 as a gelling agent allowed for a smooth, homogenous vehicle. The inclusion of Glycerin and Coconut oil added essential humectant and emollient properties, ensuring that the treatment does not lead to the hair brittleness often caused by the sider and synthetic anti-dandruff agents like Ketoconazole.

- **Safety Profile:** The formulation targets a scalp-compatible pH (approximately 5.5). By avoiding harsh chemicals and focusing on a natural API, this gel reduces the risk of scalp irritation and dryness reported in long-term use of standard market products.

CHAPTER VI: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions:

Based on the experimental trials and the pharmaceutical evaluation of the Ziziphus spina-christi (Sidr) leaves extract gel, the following conclusions were reached:

- **Phytochemical Success:** The qualitative screening confirmed that the ethanolic extract of Ziziphus spina-christi leaves is rich in bioactive secondary metabolites, specifically saponins, flavonoids, and tannins. These compounds provide the scientific basis for the plant's traditional use as an antimicrobial agent.
- **Formulation Stability:** The study successfully developed a stable topical hydrogel using Carbopol 940 as the gelling agent. The incorporation of glycerin and coconut oil enhanced the humectant and emollient properties, making the formulation suitable for scalp application.
- **Dose-Dependent Efficacy:** The in-vitro antimicrobial assays demonstrated that the extract possesses significant inhibitory activity against the dandruff-causing fungus Malassezia furfur and various staphylococcal bacterial strains. This activity was found to be concentration-dependent, with the 60% concentration showing the maximum zone of inhibition.
- **Safety and Compatibility:** Preliminary evaluations indicate that the gel formulation maintains a physicochemical profile (pH and homogeneity) that is compatible with human skin, potentially reducing the side effects like dryness and irritation associated with synthetic alternatives.
- **Traditional to Modern Transition:** The research successfully bridged the gap between traditional herbal medicine and modern pharmaceutical technology by transforming crude leaf material into a standardized, user-friendly dosage form (gel).

6.2. Recommendations:

To build upon the findings of this research and to further the development of natural anti-dandruff treatments, the following actions are recommended:

- **Quantitative Analysis (HPLC/GC-MS):** Future studies should move beyond qualitative screening to quantify the exact percentages of specific active molecules, such as betulic acid and specific saponin glycosides, using advanced analytical techniques like High-Performance Liquid Chromatography (HPLC).
- **Long-Term Stability Testing**: It is recommended to conduct comprehensive stability studies according to ICH guidelines, monitoring the gel's shelf-life, viscosity, and active ingredient degradation over a minimum period of six months.
- **Expanded Microbiological Scope:** While this study focused on *Malassezia furfur,* future research should test the gel against a wider variety of fungal and bacterial strains to establish its full spectrum of activity.

- **Industrial Scaling:** Local pharmaceutical manufacturers in Yemen should consider the economic potential of Ziziphus spina-christin as a cost-effective, indigenous raw material for the production of commercial anti-dandruff products.

- **Optimization of Extraction:** Further investigation into different solvent systems (e.g., varying ethanol-to-water ratios) or advanced extraction methods like ultrasound-assisted extraction could be performed to optimize the yield of bioactive flavonoids and saponins.

CHAPTER VII: References

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