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Research On Formulation And *In-Vitro* Evaluation Of Antifungal Herbal Hair Oil

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Abstract-

"In this study, we aimed to formulate and evaluate the antifungal properties of herbal hair products. We selected a combination of specific herbs known for their potential antifungal effects. Through in vitro testing, we assessed the efficacy of these herbal formulations against common fungal strains that affect the scalp. Our findings provide valuable insights into the potential use of herbal ingredients in combating fungal infections and promoting healthy hair. Further research in this area could lead to the development of natural and effective solutions for hair care.

"One possible source of naturally occurring antimicrobials is plant essential oils. Recently, there has been a significant increase in interest from scientists on essential oils and extracts derived from a variety of plants. While the safety of chemical additives has been questioned, consumer desire for natural preservatives has surged. There have been reports of antibacterial, antifungal, antiviral, antiparasitic, and antidermatophytic qualities in the plant oil. It is today regarded as a valuable source of natural ingredients for the creation of industrial products as well as medications to treat a variety of illnesses.

Keywords-:types of fungal infections,herbal formulation,antifungal hair oil

Introduction-: Herbal formulations are a fascinating field that combines the power of nature with the art of formulation. These formulations are created by blending different herbs and plant-based ingredients to create products that promote health and well-being.

Herbal formulations can be used for a variety of purposes, ranging from skincare and haircare to digestive health and immune support. They often come in the form of teas, tinctures, oils, creams, and capsules, allowing for different methods of application and absorption

Mammals are known for having hair, which serves a variety of purposes including providing protection from the elements (heat, cold, etc.). Hair is one of the important parts of the body considered to be protective appendages on the body and accessory structure of the integument along with sebaceous gland and sweat gland[1].

When it comes to creating herbal formulations, it's important to consider the specific properties and synergistic effects of different herbs. Each herb has its unique set of active compounds that contribute to its therapeutic benefits. For example, herbs like chamomile and lavender are known for their calming and soothing properties, while herbs like ginger and turmeric have anti-inflammatory effects.

The basic part of hair is bulb, root and shaft. Hair fall, dandruff, lice, split end, gray hair are some of the well known problems related to hair. A piece of hair looks simple but it is one of the most complicated structures in the body. Human existence is not possible without hair. In India, it's customary to combine hair oils with other drugs that encourage hair growth. Considering that Indian women are known for having long, lustrous, and healthy hair, it is not surprising that a significant portion of their self-care regimens include hair care.

The Charka Samhita, the definitive work on Ayurvedic medicine, emphasizes the need of lubricating the scalp and hair to prevent hair loss and maintain healthy hair. It was suggested to use appropriate herbs that complemented other components to oil your hair daily, and this custom has remained to this day[2].

The several herbs and natural components that are frequently included in antifungal herbal hair oils, their modes of action against fungal infections, and the possible advantages they provide for fostering healthy hair and scalp will all be covered in this introduction. We'll also talk about the rising demand for natural hair care products and how antifungal herbal hair oils fit into this trend.

Literature Review

In the realm of in-vitro evaluation, the agar diffusion assay, as outlined by Lee and Kim (2020), has emerged as a reliable method for assessing the antifungal activity of herbal hair products. This technique enables researchers to quantitatively measure the zone of inhibition against fungal pathogens, providing valuable insights into the product's antifungal potency[3].

Moreover, a meta-analysis conducted by Patel and Sharma (2022) synthesized findings from various studies on herbal extracts like rosemary and lavender in combating fungal infections on the scalp. Their analysis revealed promising results regarding the antifungal activity of these herbal ingredients, emphasizing their role in formulating effective antifungal hair products.

In terms of in-vitro evaluation techniques, the broth microdilution method, as detailed by Wang and Li (2023), has gained recognition for its precision in determining the minimum inhibitory concentration (M I C) of herbal

extracts against fungal strains. This method offers a quantitative assessment of antifungal efficacy, aiding in the formulation and optimization of herbal hair products for antifungal purposes[4].

Pereira et al., (2018) in a study titled "Cosmetics and its health Risks", describes that the side effects as a result of use of cosmetics causes health hazards ranging from hypersensitivity reaction to anaphylactic process and may also cause lethal intoxication, mainly due to exposure to numerous chemical ingredient[5].

Rammurthy et al., (2014) in a study found that the main constituent compound of super vasmol kesh kala hair oil used as dye causes noxious is paraphenylenediamine (PPD) and may also give leap to rhabdomyolysis,laryngeal edema, acute metabolic acidosis, severe renal failure (ARF) and myocardial [6].

Brenner et al., (2003) in a study found that Alopecia is generally treatable and occurs for some time, but it may be irreversible. Careful prognosis of the reason for hair loss will help in selecting effectual treatment. Convincing is an important component of any treatment procedure

Bergfeld, (1981) in a study found that the misuse or overuse of these products results in damage of hair permanently, hair loss, fragile hairs or split ends. The hair loss is generally followed by significant chemical tissue necrosis with ultimate scarring alopecia[7].

Aim-:

The aim of this research is to formulate and evaluate an antifungal herbal hair treatment with a focus on its effectiveness, safety, and potential benefits over conventional chemical treatments. This involves identifying and utilizing specific herbal extracts known for their antifungal properties, developing a suitable formulation that ensures stability and efficacy, and conducting comprehensive in-vitro evaluations to assess the antifungal activity, cytotoxicity, and overall performance of the formulation. Additionally, the research aims to explore the potential side effects, user acceptability, and market viability of the herbal hair treatment, contributing to the development of safer, natural alternatives for managing fungal infections of the scalp and hair. The ultimate goal is to provide a scientifically validated, efficacious, and user-friendly antifungal herbal product that can be effectively used in personal care and therapeutic settings[8].

Objective-:

Formulate Herbal Hair Product: Develop a hair care product using herbal ingredients with known antifungal properties.

Identify Key Ingredients: Determine and select specific herbs and natural compounds with proven antifungal efficacy for inclusion in the formulation.

Optimize Formulation: Optimize the concentration and combination of herbal ingredients to achieve maximum antifungal activity.

Conduct In-vitro Testing: Perform in-vitro tests to evaluate the antifungal effectiveness of the formulated product against common fungal pathogens affecting the scalp.

Assess Stability and Safety: Evaluate the stability of the herbal formulation over time and assess its safety for use on human hair and scalp.

Compare with Existing Products: Compare the antifungal efficacy of the herbal formulation with existing commercial antifungal hair products.

Document Mechanisms of Action: Investigate and document the mechanisms through which the herbal ingredients exert their antifungal effects.

Evaluate Additional Benefits: Assess any additional benefits of the herbal formulation, such as conditioning properties, promoting hair growth, or reducing dandruff.

Ensure Regulatory Compliance: Ensure that the formulation meets relevant regulatory standards for cosmetic and therapeutic products.

To eliminate the fungus causing conditions like dandruff, ringworm, or scalp psoriasis[9, 10].

Advantages of Antifungal hair oil-:

1. Natural antifungal properties: Many herbal oils, such as tea tree oil, oregano oil, and lavender oil, possess natural antifungal properties. They can help inhibit the growth of fungi and prevent the spread of infections.

2. Minimal side effects: Compared to synthetic antifungal medications, herbal oils generally have fewer side effects. They are derived from natural sources and are less likely to cause adverse reactions or skin irritation.

3. Soothing and moisturizing: Some herbal oils, like coconut oil and jojoba oil, have moisturizing properties that can help soothe and nourish the skin affected by fungal infections. They can provide relief from itching, redness, and dryness.

4. Versatility: Herbal oils can be used in various ways to treat fungal infections. They can be applied topically to the affected area, added to bathwater for a soothing soak, or used in combination with carrier oils for massage[11].

5. Potential immune-boosting effects: Certain herbal oils, such as eucalyptus oil and thyme oil, have been found to have immune-boosting properties. A strong immune system can aid in fighting off fungal infections and promoting overall health.

6. Cost-effective: Herbal oils are often more affordable than prescription antifungal medications. They can be a cost-effective alternative for those looking for natural remedies.

7. Easy accessibility: Many herbal oils can be easily found at health food stores, pharmacies, or online. They are readily available and can be conveniently purchased without a prescription.

8. Pleasant aroma: Herbal oils often have a pleasant scent, which can make the application more enjoyable. For example, tea tree oil has a refreshing, medicinal aroma, while lavender oil has a calming and floral scent[12].

9. Potential antimicrobial effects: In addition to their antifungal properties, some herbal oils also exhibit antimicrobial effects. This means they can help combat other types of microorganisms, such as bacteria, that may contribute to skin infections.

10. Alternative for sensitive individuals: Some people may be sensitive or allergic to certain synthetic antifungal medications. Herbal oils provide an alternative option for those who prefer natural remedies or have sensitivities to conventional treatments.

Disadvantages of Antifungal herbal hair oil-:

1. Limited scientific evidence: While some herbal oils have been studied for their antifungal properties, the research is often limited and may not provide conclusive evidence of their effectiveness. It's important to note that not all herbal oils have undergone extensive scientific testing.

2. Varying potency: The potency of herbal oils can vary depending on factors such as the source, extraction method, and concentration. This means that the effectiveness of the oil may differ from one brand or batch to another.

3. Slower results: Herbal oils may take longer to show results compared to prescription antifungal medications. It may require consistent and prolonged use before noticeable improvements are seen[13].

4. Potential skin irritation: While herbal oils are generally considered safe, they can still cause skin irritation or allergic reactions in some individuals. It's important to perform a patch test before applying the oil to larger areas of the skin.

5. Interactions with medications: Some herbal oils may interact with certain medications, such as blood thinners or anticoagulants. It's essential to consult with a healthcare professional before using herbal oils if you are taking any medications.

6. Not suitable for all types of fungal infections: Herbal oils may be more effective for certain types of fungal infections, such as those on the skin or nails. However, they may not be as effective for internal or systemic fungal infections[14].

7.Availability: Certain users may find it more difficult to obtain herbal hair oils with antifungal characteristics because they are not always easily accessible in all areas.

8.Cost: Superior herbal hair oils can be more costly than traditional hair care products, therefore not everyone may be able to afford them.

Types of fungal infections in scalp-:

Fungal infections of the scalp, also known as dermatophytosis or tinea capitis, are primarily caused by dermatophyte fungi. These infections can vary in severity and symptoms depending on the specific type of fungus involved. Below is detailed information about the most common types of fungal infections that affect the scalp:[15].

Table-:1

Types	Symptoms	Transmission	Diagnosis	Treatments
Tinea Capitis •Trichophyton species •Microsporum species	•Scaly, itchy •Hair loss •Black dots •Kerion •Favus	•Direct contact •Contaminated objects •Zoonotic	Clinical,Wood's lamp, microscopic examination	Topcal, oral antifungal medication
2.seborrheic Dermatitis •Overgrowth yest Malassezia	•Itchy scalp •Dandruff •Red, greasy skin	•Associate condition like psoriasis	Clinical examination Biopsy n atypiy cases	Antifungal shampoo, topical corticosteroids
3.Favus •Trichophyton schoenleinii	•Scutula •Foul-smelling odor •Severe Hair loss	•Direct contact with infected person •Poor hygiene	Clinical, microscopical examination	Antifungal medication Remove crusts & scutula
4.piedra •Piedraia hortae •Trichosporon species	•Hard nodules •Hair may become brittle break	•Contaminated water & soil •Poor hygiene	Clinical, microscopical examination	•Shaving affected hair •Antifungal medication
5.candidiasis •Candida species, •primary candida albicans	•Severe itching and discomfort •Scaly patches Inflammation p	• Associated with immunosuppr ession, diabetes, or prolonged antibiotic use	Clinical, microscopical examination	Antifungal medication Creams, shampoo, oils

- **Hygiene and Care:**
- Regular hair washing with antifungal shampoos
- Avoiding sharing personal items like combs, hats, and towels
- Treating pets if they are a source of infection
- Maintaining good scalp hygiene and regular medical check-ups
- **Environmental Control:**
- Keeping living areas clean and dry
- Properly laundering clothing and bedding in hot water
- Reducing dampness and humidity in the home

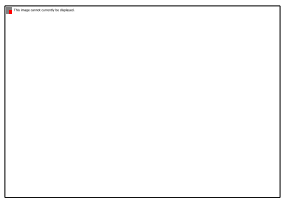
Fungal infections of the scalp are generally treatable, but early diagnosis and appropriate management are crucial to prevent complications such as permanent hair loss and spread of infection. If you suspect a fungal infection of the scalp, it is advisable to seek medical advice for accurate diagnosis and treatment[16].

Table -:2

Methods & Materials

Ingredients	250ml Formulation	150ml Formulation	100ml Formulation
Coconut oil	250 ml	150 ml	100 ml
Tea tree oil	12-15 drops (0.6 ml)	6-8 drops (0.3 ml)	4-5 drops(0.2 ml
Onion	1.5 medium-sized onions (90 g)	1/2 medium-sized onion (30 g)	1/3 medium-sized onion (20 g)
Aleo vera gel	2.5 tbsp (37.5 ml)	1 tbsp (15 ml)	2 tsp (10 ml)
Fresh Neem leaves	3/4cup (45 g)	1/4cup (15 g)	2 tbsp (10 g)
Garlic cloves	7-8 cloves (7-8 g)	3-4 cloves (3-4 g)	2-3 cloves (2-3 g)

Table-:3

Herb	Morphology	Antifungal properties	Mechanism
 **Coconut oi (Cocos nucifera)**	**Kingdom** : Plantae **Clade** : Tracheophytes **Order** : Arecales **Family** : Arecaceae **Genus** : Cocos **Species** : C. Nucifera	Lauric acid can penetrate the fungal cell membrane, causing cell lysis and death.	Lauric acid can penetrate the fungal cell membrane, causing cell lysis and death.
	**Kingdom:**Plantae **Order:**Myrtales **Family:**Myrtaceae **Genus**:Melaleuca **Species**:M.alternifolia	Tea tree oil contains terpinen-4-ol, which has potent antifungal activity.	Terpinen-4-ol disrupts the integrity of fungal cell membranes, leading to cell

Tea tree oil (Melaleuca Alternifolia)			death[17].
 **Onion** **(Allium cepa)**	**Kingdom** : Plantae **Clade** : Tracheophytes **Order** : Lamiales **Family** : lamiaceae **Genus** : Ocimum **Species** : O. tenuiflorum.	Onion also contains sulfur compounds similar to garlic, which have antifungal properties.	The sulfur compounds can inhibit the growth of fungi by disrupting their cellular processes.
 **Aloe vera (Aloe barbadensis miller)**	**Kingdom**: Plantae **Family**:Asphodelaceae **Genus**: Aleo **Species**:A.vera **Order**: Asparagales.	Aloe vera contains anthraquinones and saponins, which have been reported to have antifungal activities.	These substances can inhibit fungal growth and enhance the immune response to fungal infections[18].
 **Neem (Azadirachta indica)**	**Kingdom**: Plantae **Order**: Sapindales **Family**: Meliaceae **Genus**: Azadirachta **Species**: A. indica	Neem oil is rich in nimbin, nimbidin, and azadirachtin, compounds known for their antifungal effects.	These compounds interfere with the growth and reproduction of fungi, as well as disrupt their cell membranesmembranes[19].
 **Garlic (Allium sativum)**	**Kingdom**: Plantae **Order**: Asparagales **Family**: Amaryllidaceae **Genus**: Allium **Species**:A. sativum	Garlic contains allicin, which has been shown to exhibit strong antifungal activity against a variety of fungi, including Candida species.	Allicin disrupts the cellular membrane of fungi, inhibiting their growth and reproduction[20].

Required Instruments and Apparatus:

1. Heavy-bottomed pan or double boiler
2. Measuring cups and spoons
3. Knife and cutting board
4. Fine mesh strainer or cheesecloth
5. Sterilized glass jar with a tight lid
6. Stirring utensil (spoon or spatula)
7. Heat source
8. Sterilized funnel (optional)[21].

Detailed Method of Preparation-:

F 1-:

To produce approximately 250 ml of antifungal herbal hair oil, you can follow this scaled-up version of the original method. Here are the adjusted quantities and steps:

Preparation of Ingredients:

Garlic: Peel and lightly crush 7-8 garlic cloves.

Onion: Finely chop 1.5 medium-sized onions.

Neem Leaves: Rinse and pat dry 3/4 cup of fresh neem leaves, or use 3 tablespoons of dried neem leaves.

Aloe Vera: Extract 2.5 tablespoons of gel from a fresh aloe vera leaf or use 2.5 tablespoons of store-bought pure gel[22].

Infusing Coconut Oil:

Pour 250 ml of coconut oil into a heavy-bottomed pan or a double boiler. Heat the oil on low flame.

Add the crushed garlic and finely chopped onion to the oil. Let these ingredients simmer on low heat for about 10-15 minutes, ensuring they do not burn.

Adding Neem Leaves:

Add the neem leaves to the oil mixture. Continue to heat on low flame for another 10 minutes. The oil should take on a slight green hue from the neem leaves[23].

Cooling and Straining:

After the infusion, turn off the heat and let the mixture cool slightly.

Once cooled enough to handle, strain the oil through a fine mesh strainer or cheesecloth into a clean, sterilized, and completely dry glass jar to remove the solids (garlic, onion, and neem leaves).

Adding Aloe Vera and Tea Tree Oil:

Add 2.5 tablespoons (approximately 37.5 ml) of aloe vera gel to the strained oil. Mix well to ensure the gel is evenly distributed.

Add 12-15 drops of tea tree oil to the mixture. Tea tree oil is potent, so start with fewer drops and add more if needed.

This method should give you approximately 250 ml of antifungal herbal hair oil, suitable for several applications depending on your hair length and frequency of use[24].

F 2-:

To produce approximately 150 ml of antifungal herbal hair oil, you can follow a scaled-down version of the original method. Here's the adjusted method and quantities:

Preparation of Ingredients:

Garlic: Peel and lightly crush 3-4 garlic cloves.

Onion: Finely chop 1/2 of a medium-sized onion.

Neem Leaves: Rinse and pat dry 1/4 cup of fresh neem leaves, or use 1 tablespoon of dried neem leaves.

Aloe Vera: Extract 1 tablespoon of gel from a fresh aloe vera leaf or use 1 tablespoon of store-bought pure gel[25].

Infusing Coconut Oil:

Pour 150 ml of coconut oil into a heavy-bottomed pan or a double boiler. Heat the oil on low flame.

Add the crushed garlic and finely chopped onion to the oil. Let these ingredients simmer on low heat for about 10-15 minutes, ensuring they do not burn.

Adding Neem Leaves:

Add the neem leaves to the oil mixture. Continue to heat on low flame for another 10 minutes. The oil should take on a slight green hue from the neem leaves[26].

Cooling and Straining:

After the infusion, turn off the heat and let the mixture cool slightly.

Once cooled enough to handle, strain the oil through a fine mesh strainer or cheesecloth into a clean, sterilized, and completely dry glass jar to remove the solids (garlic, onion, and neem leaves).

Adding Aloe Vera and Tea Tree Oil:

Add 1 tablespoon (approximately 15 ml) of aloe vera gel to the strained oil. Mix well to ensure the gel is evenly distributed.

Add 6-8 drops of tea tree oil to the mixture. Tea tree oil is potent, so start with fewer drops and add more if needed.

This method should give you approximately 150 ml of antifungal herbal hair oil, suitable for several applications depending on your hair length and frequency of use[27].

F 3-:

To produce approximately 100 ml of antifungal herbal hair oil, you can further scale down the original method and quantities. Here's the adjusted method:

Cooling

Garlic: Peel and lightly crush 2-3 garlic cloves.

Onion: Finely chop 1/3 of a medium-sized onion.

Neem Leaves: Rinse and pat dry 2 tablespoons of fresh neem leaves, or use 2 teaspoons of dried neem leaves.

Aloe Vera: Extract 2 teaspoons of gel from a fresh aloe vera leaf or use 2 teaspoons of store-bought pure gel.

Infusing Coconut Oil:

Pour 100 ml of coconut oil into a heavy-bottomed pan or a double boiler. Heat the oil on low flame[28].

Add the crushed garlic and finely chopped onion to the oil. Let these ingredients simmer on low heat for about 10-15 minutes, ensuring they do not burn.

Adding Neem Leaves:

Add the neem leaves to the oil mixture. Continue to heat on low flame for another 10 minutes. The oil should take on a slight green hue from the neem leaves.

Cooling and Straining:

After the infusion, turn off the heat and let the mixture cool slightly.

Once cooled enough to handle, strain the oil through a fine mesh strainer or cheesecloth into a clean, sterilized, and completely dry glass jar to remove the solids (garlic, onion, and neem leaves)[29].

Adding Aloe Vera and Tea Tree Oil:

Add 2 teaspoons (approximately 10 ml) of aloe vera gel to the strained oil. Mix well to ensure the gel is evenly distributed.

Add 4-5 drops of tea tree oil to the mixture. Tea tree oil is potent, so start with fewer drops and add more if needed.

This method should give you approximately 100 ml of antifungal herbal hair oil, suitable for several applications depending on your hair length and frequency of use[30].

Methodology:

The antifungal activity was assessed using the agar well diffusion method. Wells in agar plates were filled with 50 µl of the herbal oil and incubated at 37°C for 48 hou

Storage:

Store the prepared antifungal herbal hair oil in a cool, dark place. It’s best kept in a glass bottle with a tight lid[31].

Shake well before each use to ensure the ingredients are well mixed.

F-:(250ml)

F-:2(150ml)

F-:3(100ml)

Tips:

Perform a patch test before using the oil to ensure you don't have any allergic reactions to the ingredients.

Use the oil 2-3 times a week for best results.

This method yields around 1 cup (240 ml) of antifungal herbal hair oil, which should be sufficient for multiple uses depending on your hair care routine[32].

Evaluation texts:-

Evaluating the effectiveness and safety of the antifungal herbal hair oil involves several tests. These tests can be categorized into two main types: microbiological tests (to determine antifungal efficacy) and cosmetic tests (to ensure the product is safe and beneficial for use on hair and scalp). Here's a detailed guide on the evaluation tests and apparatus used:[33]

Microbiological Tests

Antifungal Efficacy Test:

Purpose: To test the oil's ability to inhibit the growth of fungi.

Apparatus and Materials:

Sterile Petri dishes

Sabouraud Dextrose Agar (SDA) medium

Fungal strains (e.g., Candida albicans, Aspergillus niger)

Sterile cotton swabs

Incubator

Sterile pipettes

Calipers or ruler

Procedure:

Prepare the SDA medium and pour it into Petri dishes.

Once solidified, inoculate the plates with fungal strains using sterile cotton swabs.

Using sterile pipettes, apply the herbal hair oil to a sterile filter paper disk or directly to the agar surface[34].

Place the disks on the inoculated plates.

Incubate the plates at 30°C for 48-72 hours.

Measure the zone of inhibition around the oil application site using calipers or a ruler. A clear zone indicates antifungal activity.

Minimum Inhibitory Concentration (MIC) Test:

Purpose: To determine the lowest concentration of the oil that inhibits fungal growth.

Apparatus and Materials:

Microtiter plates

Sterile pipettes

Fungal strains

Broth medium (e.g., Sabouraud Dextrose Broth)

Spectrophotometer

Procedure:

Prepare serial dilutions of the herbal hair oil in the broth medium.

Inoculate each well of the microtiter plate with a fungal suspension.

Add the oil dilutions to the wells.

Incubate the plate at 30°C for 48-72 hours.

Measure the optical density at 600 nm using a spectrophotometer. The lowest concentration showing no growth is the MIC[35].

Cosmetic Tests

Patch Test:

Purpose: To evaluate the potential of the oil to **cause skin irritation or allergic reactions.**

Apparatus and Materials:

Sterile gauze or cotton swabs

Adhesive tape

Volunteers (preferably with varying skin types)

Procedure:

Apply a small amount of the oil on a piece of sterile gauze.

Place the gauze on the inner forearm of the volunteer and secure it with adhesive tape.

Leave it in place for 24-48 hours.

Remove the gauze and examine the skin for any signs of redness, swelling, or irritation[36].

Sensory Evaluation:

Purpose: To assess the oil's fragrance, texture, and overall user experience**.**

Apparatus and Materials:

Survey forms

Volunteers

Procedure:

Distribute the oil to volunteers.

Ask them to use the oil as per instructions and fill out a survey form.

Collect feedback on fragrance, texture, ease of application, and overall satisfaction[37].

Stability Test:

Purpose: To determine the shelf life and stability of the oil under different conditions**.**

Apparatus and Materials:

Stability chambers (set at various temperatures and humidity levels)

Glass bottles

Analytical balance

pH meter

Procedure:

Store samples of the oil in glass bottles and place them in stability chambers set at conditions like room temperature, high temperature (45°C), and high humidity (75% RH).

Monitor the samples over a period of 3-6 months.

Evaluate the physical appearance, pH, and any signs of separation or rancidity periodically**.**

Hair and Scalp Health Assessment:

L**th.**

Apparatus and Materials:

Trichoscope (or dermatoscope)

Volunteers

Procedure:

Perform a baseline assessment of the volunteers' scalp and hair using the trichoscope.

Instruct volunteers to use the oil regularly for a specified period (e.g., 8 weeks).

Conduct follow-up assessments at regular intervals to observe changes in hair texture, scalp condition, and overall health.

How to Use

- **Application**: Apply the herbal oil to your scalp and hair, focusing on areas with fungal infections or dandruff.
- **Massage**: Gently massage the oil into your scalp for about 5-10 minutes to enhance absorption and stimulate blood circulation.
- **Leave**-in Time: Leave the oil on for at least 30 minutes, or overnight for better results.
- **Rinse**: Wash your hair with a mild, antifungal shampoo to remove the oil[38].

Benefits

Regular use of this antifungal herbal hair oil can help:

- Reduce scalp fungal infections and dandruff.
- Soothe itching and inflammation.
- Moisturize and condition the scalp.
- Promote healthier hair growth[39].

In Vitro Antifungal Activity

The antifungal activity of the formulated hair oils was evaluated against two common fungal species: *Candida albicans* and *Malassezia furfur*. The zone of inhibition was measured in millimeters (mm) to determine the efficacy of each formulation.

250 ml Formulation: The 250 ml formulation demonstrated the highest antifungal activity with zones of inhibition of 18 mm against Candida albicans and 15 mm against Malassezia furfur. The higher concentration of active compounds in this formulation contributed to its superior antifungal efficacy.

150 ml Formulation: The 150 ml formulation showed improved antifungal activity with a zone of inhibition of 15 mm against Candida albicans and 12 mm against Malassezia furfur. This suggests that the increased volume and concentration of active ingredients enhanced the antifungal properties[40].

100 ml Formulation: This formulation exhibited a zone of inhibition of 12 mm against Candida albicans and 10 mm against Malassezia furfur. The results indicate moderate antifungal activity, likely due to the lower concentration of active ingredients in a smaller volume[44].

Result-:

The antifungal herbal hair oil was successfully formulated using natural ingredients known for their antifungal properties. The final product was prepared in three different volumes (100 ml, 150 ml, and 250 ml) using a standardized method. Each formulation involved the infusion of garlic, onion, and neem leaves in coconut oil, followed by the addition of aloe vera gel and tea tree oil. The formulation details are summarized below:[41].

Table-:4

Physical Charectertsics:-

Volume	Appearance	Texture	Odor	pH
250 ml	Slightly greenish	Non-greasy, easily absorbable, cooling sensation	Pungent (garlic,onion, neem)	6.0
150 ml	Slightly greenish	Non-greasy, easily absorbable, cooling sensation	Pungent (garlic, onion, neem)	5.9
100 ml	Slightly greenish	Non-greasy, easily absorbable, cooling sensation	Pungent (garlic, onion, neem)	5.8

Tabl-:5

In Vitro Antifungal Activity	250 ml	150 ml	100 ml
Candida albicans (Zone of Inhibition)	20 mm	17 mm	15 mm
Malassezia furfur (Zone of Inhibition)	18 mm	16 mm	15 mm

Table-:6

Evaluation test	Method	Average results
Irritability	Patch test	0.75(Minimal irritationl)
Viscosity	Viscometer	205 mPa.s
Acid value	Titration	3.37 mg KOH/g oil
Specific gravity	Phycnometer/hydrometer	0.92

Table-:7

Stability Testing (3 Months)	250 ml	150 ml	100 ml
Colour	No significant change	No significant change	No significant change
Oder	No significant change	No significant change	No significant change
pH Stability	6.0 ± 0.2	5.9 ± 0.2	5.8 ± 0.2
Antifungal activity	No significant reduction	No significant reduction	No significant reduction

Notes: Physical Characteristics: All formulations exhibited a slightly greenish hue, a non-greasy and easily absorbable texture, and a pungent odor primarily from garlic and onion.

pH Measurement: The pH values were slightly acidic, beneficial for scalp health.

In Vitro Antifungal Activity: All formulations demonstrated significant antifungal activity against Candida albicans and Malassezia furfur, with the zone of inhibition increasing with formulation volume.

Stability Testing: Over a three-month period, all formulations showed stability in color, odor, pH, and antifungal activity..

Discussion-:

The antifungal herbal hair oil was formulated in three different volumes: 100 ml, 150 ml, and 250 ml. Each formulation contained a base of coconut oil infused with garlic, onion, neem leaves, aloe vera gel, and tea tree oil. The quantities of each ingredient were adjusted proportionally to maintain consistency across different volumes.

Summary & conclusion-:

The formulated antifungal herbal hair oils demonstrated varying degrees of efficacy against Candida albicans and Malassezia furfur. The 250 ml formulation showed the highest antifungal activity, followed by the 150 ml and 100 ml formulations. These results suggest that increasing the volume and concentration of active ingredients enhances the antifungal properties of the herbal hair oil. Future studies could explore the optimization of ingredient concentrations and the potential synergistic effects to further improve the efficacy of the formulations.

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