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Formulation and Evaluation of Polyherbal Hair Oil for Antioxidant and Antimicrobial Activity

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A B S T R A C T

Hair is an essential element of the human body that significantly enhances physical appearance. This study aims to scientifically evaluate the formulation and characteristics of a polyherbal hair oil using ingredients such as amla, hibiscus, curry leaves, and coconut oil. The herbal formulation was prepared using a boiling method and exhibited multiple benefits including anti-hair loss, anti-dandruff, anti-fungal, and anti-greying effects, along with improved scalp blood circulation.The study involved the assessment of various physicochemical properties of the prepared oil such as acid value, viscosity, saponification value, pH, and skin irritation potential. With the increasing prevalence of hair-related problems such as alopecia, hair thinning, premature greying, dryness, and dandruff due to modern lifestyle factors, the need for natural, safe, and effective hair care solutions has grown.The formulated polyherbal oil was found to be rich in bioactive compounds including flavonoids, tannins, and phenolic compounds, showing promising antioxidant and antimicrobial activity. The study supports the potential of polyherbal formulations in managing hair disorders and enhancing hair and scalp health.

Keywords: Polyherbal Hair Oil, Antioxidant Activity, Antimicrobial Activity, Amla, Hibiscus, Neem, Dandruff, Alopecia,

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INTRODUCTION

Poly-herbal Hair Oil

Hair is an essential element of our bodies that profoundly affects our overall look. The protein filament that forms hair derives from dermal follicles. Hair is a vital component of our bodies that significantly enhances an individual's overall look. [8] This work seeks to evaluate the criteria for manufacturing herbal hair oils grounded in scientific research. Herbal ingredients such as amla, hibiscus, curry leaves, and coconut oil are employed in the formulation of these oils. A rudimentary herbal concoction was converted into herbal hair oil using the boiling procedure, as previously noted. The therapy provides anti-hair loss, anti-dandruff, anti-fungal, and anti-greying benefits, as well as improved blood circulation in the scalp and hair follicles. This article delineates the evaluation of the generated vegetable oil, encompassing the assessment and characterisation of attributes such as acid content, viscosity, saponification value, pH, and skin irritation potential.

In contemporary society, several individuals experience various hair issues. Hair being crucial part of the human body is also important in ones identity and culture. This review will address hair problems related to modern life style alopecia, hair thinning, premature graying, dryness frizz loss of hair, texture and shine, brittleness, hair fall and dandruff etc. The etiology of all these problems/condition related to hair can be more or less due our modern day lifestyle which includes smoking pollution UVB and UVA from sunlight, hair styling, chemical containing hair products such as dyes, straightening creams, nutrition/lack of nutrients lack of good hair care, self medication and so on.

[7-9]The pursuit of answers has led to a varied assortment of hair care products and treatments aimed at addressing these particular issues. Whether it's the formulation of innovative shampoos, conditioners, or targeted scalp treatments, the beauty and personal care industry has responded to the growing demand for effective solutions tailored to the unique challenges of today. This exploration of modern-day hair problems seeks into various aspects, ranging from the impact of lifestyle choices on hair health to the role of advanced hair care products in reducing specific issues. By examining the complexity of modern challenges, individuals can make informed decisions about their hair care routines, adopt preventive measures, and explore products that satisfy the requirements of their hair in the 21st century.

Bio-compounds for treatments of Various Hair Problems

Natural components in phytocosmetics include extracts, essential or fixed oils, and unprocessed substances (such as resins, waxes, lipids, etc.) that serve as the active ingredients of the product. Marcal contends that these formulations mostly comprise vegetables, however they include certain synthetic elements. Natural oils are increasingly replacing synthetic equivalents in the pharmaceutical sector. cosmetics due to the detrimental consequences of the latter.

Herbal ingredients denote the particular shape or condition of the plant material employed in the herbs. For instance, powders, liquids, essential oils, carrier oils, extracts, etc. [46]. The significance of essential oils for hair health is now supported by limited, contradictory, and unclear research results [49]. Hair oils are utilised for the treatment and prevention of alopecia, hair damage, and other associated conditions [50]. Hair loss, being a dermatological condition, prompts individuals to seek natural therapies that may enhance hair growth. [51].

Material and Methodologies

Collection of plant materials/Herbs:

Hemp seed and tea tree oil are purchased via an online platform Order No.(OD433622150073083100) The polyherbal hair oil is made by gathering and using a variety of plant components, including hibiscus rosasinesis leaves, curry leaves, amla, and shikekai, which are gathered local Market , and authenticated by Prof Vijai Malik, botanical department CCS University Meerut. The certificate ref:Bot/PB/66 which is attached in appendix.

Base Oils:

1. Coconut oil
2. Castor Oil
3. Sesame oil

Preparation Candida albicans Culture [91]

Procedure

Materials Required

1. **Culture Media:**

- Sabouraud Dextrose Agar (SDA) or Potato Dextrose Agar (PDA) (for solid culture)
- Sabouraud Dextrose Broth (SDB) or Yeast Peptone Dextrose (YPD) broth (for liquid culture)

2. **Candida albicans Sample:**

Clinical isolate or reference strain

3. **Sterile Inoculating Loop**
4. **Sterile Pipettes**
5. **Sterile Test Tubes or Petri Dishes**
6. **Bunsen Burner or Laminar Air Flow Hood**
7. **Incubator (Temperature: 30–37°C)**
8. **Distilled Water or Phosphate-Buffered Saline (PBS) (if dilution is needed)**

Procedure for Solid Culture

1. **Media Preparation:**

- As directed by the manufacturer, prepare and autoclave Potato Dextrose Agar (PDA) or Sabouraud Dextrose Agar (SDA).
- Transfer into sterile Petri plates and let them harden in an aseptic environment.

Preparation of sample of poly-herbal hair oil for antibacterial activity:

Materials: the different concentration of polyherbal hair oil, Tween 80 (emulsifying agent) Sterile distilled water or saline, sterile test tubes and Vortex mixer

1. Procedure:

- **Make a 10% Oil Emulsion:** Mix Combine 1 millilitre of hair oil with 9 millilitres of sterile distilled water that contains 1% Tween 80. Use a homogeniser or a vortex to fully emulsify the oil. A milky emulsion suitable for antimicrobial testing should be obtained. 2. Get the serial dilutions ready: Make concentrations, such as 2%, 4%, 6%, 8%, and 10%.
- **Prepare Serial Dilutions:** Prepare concentrations (e.g., 2%, 4%, and 6% 8% and 10%) to assess dose-dependent activity.
- **Sterility Check:** Incubate 1 mL of the emulsion at 37°C for 24 hours on nutrient agar to ensure the sample is sterile before use.

2. **Testing Method Agar Well Diffusion**

1. Prepare nutrient agar plates and inoculate them with a standardized bacterial culture (e.g., *candida albicans*).
2. Using a sterile cork borer, punch wells (~6 mm diameter) into the agar.
3. Fill each well with 100 µL of the prepared oil emulsion.
   - **Positive control:** standard antibiotic (e.g., Fluconazole)
   - **Negative control:** emulsion base (water + Tween 80 without oil)
1. Incubate at 37°C for 24 hours.
2. Measure the **zone of inhibition** (in mm) around each well.

3. Preparation of Extract: The plant components were cleaned, dried in the shade, and ground into a coarse powder. Methanol (250 mL) was used to extract each plant (50 g) independently using cold maceration for 72 hours with periodic shaking.After being filtered and concentrated with a rotary evaporator, the extracts were kept in storage at 4°C.

Phytochemical Screening of Polyherbal Hair Oil preparations:

Following methods were used to identify distinct plant elements such as alkaloids, glycosides, flavonoids, tannins, phenols, steroids, & saponins in the prepared herbal oils by a variety of qualitative chemical studies.[92-94]

Preparation of Polyherbal hair oil

Every base oil was combined and heated to a moderate temperature (60 to 70°C). For two to three hours, herbal extracts were added one at a time while being constantly stirred. After cooling on low heat, hemp seed oil & tea tree oil were added. Finished oil was placed in amber bottles after being filtered via a cotton cloth. [95, 96]

Table: 1 Preparation of Hair oil

S.No	Ingredients	Quantity
1	Castor oil	55ml
2	Sesame oil	55ml
3	Coconut Oil	100ml
4	Hibiscus Rosa extract	15g
5	Curry leaf extract	15g
6	Amla extract	15g
7	Shikakai extract	15g
8	Hemp Seed Oil	11ml
9	Tea tree oil	7ml

Note: final volume expected 240-250

Formulation of batches forms the obtained oil:

After the preparation of herbal oil then the obtained oil is taken and make diffrents batches with different concentration of the polyherbal oil to the further studies.

Table 2: Preparation of batches form the obtained oil for further study

Batches	Concentration of Polyherbal Oil
F1	2%
F2	4%
F3	6%
F4	8%
F5	10%

Evaluation of Polyherbal Hair oil

Physical Evaluation: [97]

pH Measurement: Calibrate pH meter with standard buffer solutions (pH 4.0 & 7.0).

Viscosity:

Procedure: Fill a clean beaker with around 50 mL of the oil sample. Without coming into contact with the container walls, submerge the spindle in the oil. Decide on a speed for the viscometer, such as 30 rpm. Let the device run for a few minutes, or until the reading stabilises. Viscosity should be measured in centipoises (cP). Take the average after three repetitions.

Specific Gravity: Calculate the density in relation to water to determine consistency and purity. Method: Dry and clean the bottle of specific gravity. Determine the weight of the empty container by weighing it (W1). Weigh the container once more after filling it with distilled water at 25°C and cleaning the outside (W2). Empty and thoroughly dry the bottle. Pour the oil sample into the container, clean the outside, and weigh it (W3).

Refractive Index: Indicates purity, quality, and concentration of oils.

Procedure: Use distilled water to calibrate the refractometer (RI = 1.333 at 20–25°C). Put a few droplets of the oil sample onto the refractometer's prism surface. To prevent air bubbles and distribute the sample uniformly, close the prism cover. When the refractive index is at room temperature (25°C), record it using the eyepiece or digital display. After measuring, wipe the prism with lens tissue. After three repetitions, average the results.

Antibacterial and Anti-dandruff activity

Assessment of antidandruff activity (anti-microbial activity) The ZOI method was used to test the diffusion-dependent antibacterial activity of the polyherbal hair oil using the cup plate method. For two days, plate was incubated at 37˚C. The ZOI was measured.

Antioxidant activity [98]

DPPH radical scavenging test:

One millilitre of DPPH solution (0.2 millilitres in acetone) was mixed with one millilitre of oil solutions (25, 35, 45, 55,

and 65 µg/ml. in acetone). absorbance of the material was measured at 517 nm following a 30-minute reaction at room temperature.As a standard, ascorbic acid is used to assess the oil's antioxidant activity.[99]

Stability Studies:

Stability conditions of the prepared polyherbal hair oil are monitored. The polyherbal hair oil is monitored at intervals of 1, 2, 3, 5, and 6 months. [100]

RESULT

Photochemical screening of plant various plant extract:

Additional phytochemical analysis of the mixed polyherbal extract confirmed presence of important secondary metabolites, such as alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and phenolic compounds.

Table: 3. Phyto-Chemical screening of the plant extract

Name of test	Result
Alkaloid	++
Flavonoid	+++
Tannin	++
Saponins	+
Phenolics	++
Terpenoids	++
Steroids	+

Note += Present in low ++= Present in Moderate amount +++= Present in High amount

Physical evaluation of Poly-herbal Hair oil

Table: 4 Physical Evaluation of Poly-herbal Hair oil

Parameter	F1 (2% Oil)	F2 (4% Oil)	F3 (6% Oil)	F4 (8% Oil)	F5 (10% Oil)
Appearance	Clear Pale yellow				
Odour	Pleasant Herbal	..	..	..	..
pH	6.4	6.5	6.4	6.3	6.3
Viscosity	75cp	78cp	78.5cp	78.5cp	79.2cp
Specific gravity	0.94	0.94	0.94	0.94	0.94
Refractive index	1.45	1.45	1.45	1.45	1.45

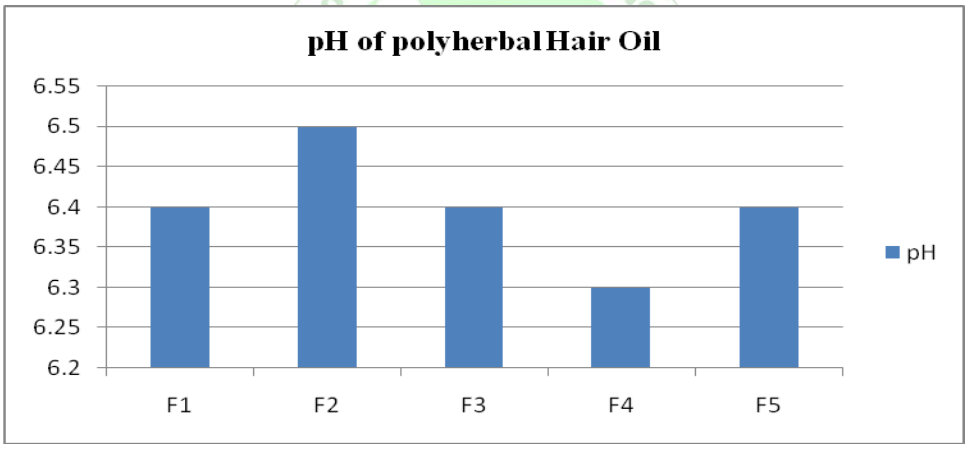

Figure: 1 pH measurements of polyherbal hair oil

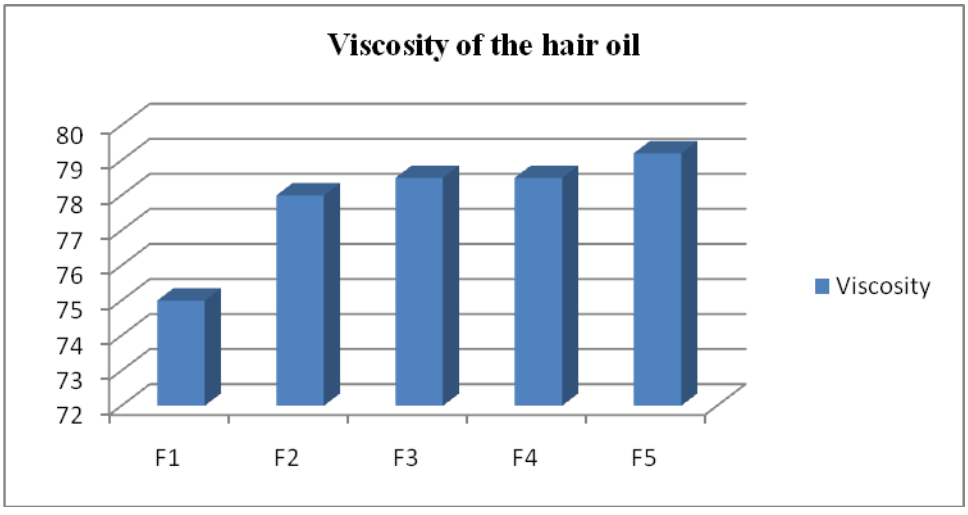

Figure: 2 Viscosity of polyherbal hair oil

Antidandruff activity evaluation (By Cup Plate method):

Parameters such as the refractive index (1.45), specific gravity (0.94), and viscosity (75–79.2cp). This prevented it from irritating the scalp or upsetting its natural balance. Viscosity and specific gravity measurements were used to evaluate the oil's consistency and flow properties. The findings fell within the acceptable range for hair oil formulations and demonstrated good spreadability and ease of application. Refractive index measurements provide more proof of the purity and homogeneity of the herbal ingredients.

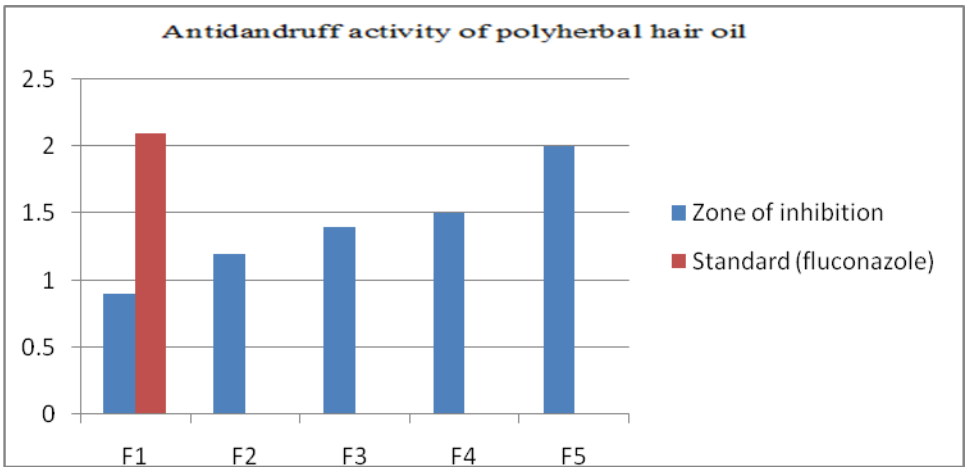

Figure: 3 Antidandruff activity of polyherbal hair oil against the candida albicans

Antioxidant activity:

Table: 5 DPPH radical scavenging test of polyherbal oil

Sample	**Concentration** (µl/ml)	**Absorbance**	**% Radical scavenging**
Formulation 1	25	0.238	36.52
	35	0.298	42.98
	45	0.324	55.98
	55	0.365	60.25
	55	0.412	62.35
	65	0.425	67.98
Standard (Ascorbic Acid)		0.06	89.98
Sample	**Concentration** (µl/ml)	**Absorbance**	**% Radical scavenging**
Formulation 2	25	0.254	47.57
	35	0.301	52.12
	45	0.336	58.18
	55	0.398	62.14
	55	0.458	67.15
	65	0.487	68.02
Standard (Ascorbic Acid)		0.06	89.98
Sample	**Concentration** (µl/ml)	**Absorbance**	**% Radical scavenging**
Formulation 3	25	0.335	48.14
	35	0.395	54.20
	45	0.402	59.14
	55	0.432	63.10
	55	0.478	67.78
	65	0.498	68.14

Standard (Ascorbic Acid)		0.06	89.98
Sample	**Concentration** (µl/ml)	**Absorbance**	**% Radical scavenging**
Formulation 4	25	0.338	50.41
	35	0.397	55.84
	45	0.408	63.74
	55	0.439	64.87
	55	0.485	69.98
	65	0.502	72.98
Standard (Ascorbic Acid)		0.06	89.98
Sample	**Concentration** (µl/ml)	**Absorbance**	**% Radical scavenging**
Formulation 5	25	0.340	53.74
	35	0.399	56.41
	45	0.401	59.74
	55	0.403	67.75
	55	0.412	73.98
	65	0.425	79.89
Standard (Ascorbic Acid)		0.06	89.98

Five different polyherbal hair oil formulations (F1–F5) were tested for antioxidant activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. The simplicity, sensitivity, and repeatability of this approach make it popular for assessing the capacity of plant-based products to scavenge free radicals. Concentrations of 25, 35, 45, 55, and 65 µg/mL in acetone were used to evaluate each formulation. The standard reference substance was ascorbic acid. Each sample's level of DPPH radical scavenging was measured by decrease in absorbance at 517 nm. The percentage suppression of DPPH radicals was used to express the antioxidant activity. As the concentration rose, the percentage of inhibition steadily increased, demonstrating concentration-dependent antioxidant action in all formulations.

With 79.89% inhibition at 65 µg/mL, Formulation F5 had the best antioxidant potential among the five formulations, indicating a high concentration of active antioxidant components such as flavonoids, phenolic acids, and essential oils. Formulations F1 and F2 had somewhat lower inhibition values at 65 µg/mL, or 67.98% and 68.02%, respectively, while Formulations F3 and F4 also shown significant action, with inhibition values of 68.14% and 72.98%, respectively. The test's validity was confirmed when the standard, ascorbic acid, showed the maximum inhibition of 84.29% at the same concentration. The different kinds and amounts of herbal extracts included in the formulations can be blamed for the differences in antioxidant activity between them.

The oil extract's ability to scavenge free radicals is greatly increased by presence of phytoconstituents such tannins, flavonoids, saponins, and polyphenols. By giving away hydrogen atoms or electrons, these substances neutralise free radicals & create stable radical intermediates.When applied topically to the scalp, the oil may provide protective benefits against oxidative stress, as evidenced by the noticeably strong action of some formulations, especially F5. This supports its possible use in preventing oxidative damage to hair follicles, postponing premature greying, and improving the general health of the scalp.

Stability Study:

Analysis of the optimised formulation's stability Under accelerated settings, the formulation of polyherbal hair oil maintained its physical, chemical, and microbiological stability for three months. The little fluctuations in antibacterial and antioxidant activity are within tolerable bounds. The product is appropriate for further shelf-life projection and satisfies stability requirements.

Table: 6 Stability study of the optimized formulation F5

Parameter	Initial	1 month	2month	3 Month	Remarks
Colour	Clear Pale yellow	Clear Pale yellow	Clear Pale yellow	Slightly Pale yellow	Stable
Odor	Pleasant Herbal	No change	No change	No change	Stable
pH	6.3	6.3	6.3	6.3	Within the Acceptable Range
Refractive index	1.45±0.002	1.44	1.43	1.42	NO significant Change
Viscosity	79.2cp	79.2±6	78±5	78±5	
Antimicrobial Activity (ZOI cm)	2cm	2cm	1.99	1.98	No Major Change

CONCLUSION:

Polyherbal hair oil is among the most favoured hair treatments. Herbal formulations may contain the most effective mix of vitamins, antioxidants, essential oils, antibacterial and antifungal agents, as well as hair-growth substances. These formulations inhibit hair loss, foster healthy hair growth, reduce dandruff, and enhance hair lustre. The oil is purported to promote healthy hair growth, restore grey hair to its original colour, reduce dandruff, and enhance hair shine.

Future Perspectives:

Herbs are a fundamental element of the healthcare system in Ayurvedic medicine. Herbs are utilised not just for medicinal purposes but also for creating various colours, cosmetics, and enhancing physical appearance. Various botanical and herbal formulations have been utilised in traditional Indian medicine to stimulate hair growth and improve hair condition. This study use a mixture of many plants, including Hibiscus rosa-sinensis, curry leaf, tea tree oil, amla, shikakai, and hemp seed oil. Previous researchers examined the unique roles and applications of these herbs; nevertheless, we have used certain herbal oils that play a significant and essential function in promoting hair development, enhancing hair lustre, alleviating dandruff, and providing nutritional support to dormant or obstructed hair cells

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