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FORMULATION AND EVALUATION OF ANTIMICROBIAL HAIR GEL BY USING HIBISCUS ROSA-SINENSIS LINN EXTRACT AND GUAR GUM.

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Abstract: Dandruff is the most typical reason for hair loss, and a microbial infection of the scalp is the primary factor in dandruff. Numerous studies have been focused on discovering phytoconstituentswith antibacterial potential. Due to their excellent therapeutic efficacy, minimal toxicity, and accessibility, medicinal plants are utilized extensively. The goal of this study was to identify the phytoconstituents in Hibiscus rosa-Sinensis Linn that exhibit antimicrobial action. The formulation of a hair gel containing this extract was also evaluated. A phytochemical analysis revealed the presence of proteins, amino acids, alkaloids, cardiac glycosides, and carbohydrates. and the minimuminhibitory concentration (MIC) was examined along with the antibacterial activity of extracts using the agar well diffusion method. development and testing of hibiscus-infused antibacterial hair gel.

Keywords: Hibiscus rosa sinensis, hair gel, extraction, maceration, phytochemicals, formulation, evaluation, antifungal, antibacterial.

Introduction

Worldwide, the number of men and women experiencing hair loss or thinning is rising recently [1].Both men and women can develop androgenetic alopecia, which is characterized by the pattern- defined progressive loss of scalp hair. Alopecia, which affects roughly 70% of men and 30% of women, is the most prevalent issue in contemporary countries and has significant economic and psychological effects [2]. One of the biggest reasons for hair loss is dandruff. But dandruff is a fairlycommon, non-contagious hair issue that can afflict anyone, regardless of age. It is known medicallyas pityriasis simplex capitis, which causes the scalp to slough dead skin. It could be oily or dry [3].Numerous herbal preparations are recognized by the Indian traditional medical system as effectivein promoting hair growth. Herbal cosmetics have recently invaded the global market, and herbs arepriceless gifts from nature [4]. Three ingredients that are good for your hair—hibiscus, guar gum, and vitamin E—increase hair growth and lessen hair loss. Gels are semisolid systems in which a

liquid phase is contained inside a three-dimensional polymeric matrix (containing of natural or synthetic gum) that has been subjected to a significant amount of physical or chemical cross-linking.Gels are a relatively newer sort of dosage form made by trapping larger amounts of aqueous, hydroxy-alcoholic liquids in a network of colloidal solid particles that may be made of organic polymers with natural or synthetic origins or inorganic substances like aluminum salts [5]. Naturalmedicinal substances have mostly replaced many manufactured medications that could have adverseeffects. Alkaloids, flavonoids, tannins, and phenolic compounds are the most significant bioactive substances found in plants [6].

Material and Methods

Plant material preparation and extraction: The genuine vendor Sanchomee Herboveda Private Limited, located in Pune, provided the HRS powder (Hibiscus rosa-sinensis Linn). (Billing number P-CA-2818). Samples that had been ground up were extracted in ethanol using the maceration process for 72 hours. A rotary evaporator was used to concentrate the extract, and a vacuum desiccator was used to dry it. Thedried extract was ground into a powder in a lab mill, and then passed through a sieve with a mesh size of 250.

Phytochemical screening: Standard qualitative tests were used to conduct phytochemical analyses on the extracts.Flavonoids, alkaloids, amino acids, glycosides, carbohydrates, tannins, and other compounds are abundant in hibiscus.

Test for carbohydrates:

A. Molisch's test: A few drops of Molisch's reagent were added to each portion that had been dissolved in distilled water, and 1 mL of concentrated H2 SO4 was added by the test tube's sideafter that. After standing for 2 minutes, the liquid was diluted with 5 mL of pure water. A positive test result was the appearance of a dull violet or red color at the interface of the two layers [7].
B. Benedict's test: Benedict's reagent is mixed with 0.5 ml of filtrate and then added. For two minutes, the mixture is boiled in a bath of boiling water. Sugar is indicated by a distinctively colored precipitate [8].

Test for Saponin:

A test tube containing an aqueous crude plant extract and 5.0 ml of distilled water was forcefullystirred. The presence of saponins was evident in the foam look after the foaming was aggressivelycombined with a few drops of olive oil [9].

Test for flavonoids:

A. NaOH test: An intense yellow color was formed when 2 ml of a 2.0% NaOH combination was combined with aqueous plant crude extract; this color turned colorless when we added 2 drops of diluted acid to the mixture. Flavonoids were present, as evidenced by this finding [9].
B. Lead acetate test: A few drops of lead acetate solution were added to the extracts. The presenceof flavonoids is indicated by the precipitate's yellow color [10].

Test for alkaloids:

A. The Wagner's test involves adding a few drops of Wagner's reagent to a few milliliters of plant extract along the test tube's sidewalls. The test is confirmed to be positive by a reddish- brown precipitate [8].

B. Mayer's test: By the side of the tube, a few drops of Mayer's reagent were added to a few milliliters of filtrate. Alkaloids are confirmed by a creamy white precipitate.[8]

Test for proteins and amino acids:

A. Millon's test: 3 ml of an extract sample's aqueous solution is mixed with 5 ml of Millon's reagent.The presence of proteins is indicated by a white precipitate that gradually turns pink or scarlet when gently warmed [11].

B. Ninhydrin test: To 2 ml of aqueous filtrate, add two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone). The presence of amino acids is shown by the color purple [8].

C. Sulfur test: Add 3 drops of 10% lead acetate solution and 2ml of 40% sodium hydroxide to a sample of 5ml extract. If cysteine is present, the solution turns black or brown when gently heated[11].

D. xanthoproteic test: boil the solution and add sodium hydroxide 40% dropwise after adding 1ml of strong nitric acid to the extract. When a substance is yellow to orange in hue, tyrosine or tryptophane is present [11].

E. Biuret test: One milliliter of a 40% NaOH solution is added to a few mg of extract residue in water. If protein is present, a violet or pink color is produced after adding a drop of a 1% solutionof copper sulfate [11].

Test for glycosides:

A. Liebermann's Test: We combined full aqueous plant crude extract with 2.0 ml of acetic acidand 2 ml of chloroform. After cooling the mixture, concentrated H2SO4 was added. Aglycone, the steroidal component of glycosides, was represented as an entity in green [10].

B. Keller-Kiliani Test: A mixture of 4.0 ml of glacial acetic acid, 1 drop of a 2.0% FeCl3 mixture, 10 ml of aqueous plant extract, and 1 ml of concentrated H2SO4 were combined. Between the layers, a brown ring developed, revealing the presence of cardiac steroidal glycosides [10].

Test for terpenoids:

5 ml of the aqueous plant extract were combined with 2.0 ml of chloroform, which was then added, evaporated on the water path, and heated with 3 ml of concentrated H2SO4. As terpenoids took shape, a gray tint emerged [10].

IR evaluation: The four primary flavonoids found in hibiscus are rutin, quercetin, kaempferol, and myricetin. Out of these 4, quercetin and kaempferol have been shown to have hair-growth-promoting properties. IR spectroscopy was used to find flavonoids. The mull procedure, which entails grinding the powdered analyte with dry potassium bromide and producing a disk, is employed for the examination of solid materials. Typically, the ratio of analyte to potassium bromide is 1:100. Using a tiny ball mill or a mortar and pestle, the components are combined for grinding.After being compressed at 10,000–15,000 pressure in a die to form a tiny, transparent disk, themixture is examined. **[14]**

Antibacterial Activity:[15]

Bacterial culture/inoculation preparation: Using a wire loop, inoculate Staphylococcus aureus into each suspension tube with 1 ml of the prepared nutritional broth (NB) from the prepared 10 ml. After bacterial inoculation, seal the tube's mouth with cotton and place it in anincubator for two to three hours.

Plate preparation: To prevent contamination, plates are first wrapped in paper and put in an autoclave. To eliminate moisture off plates, a little flame is used to warm the plates. Each platewas filled with prepared nutrient agar to create a thick layer, and the plates were then left for 1to 2 hours to allow the agar to properly settle. In order to prepare a spread plate, 100ul of infected bacterial culture is distributed on a plate using a glass spreader. The plates are then given some time to settle properly. Agar Well technique To accommodate three different volumes of the Carica papaya extract, create four circular wells. Each of these three petri dishes contains four portions or holes/Wells,one of which is standard and the other three contain various amounts of Carica papaya extract.Split lamps were used to keep the area around the Petri dishes sanitary. After that, the plates underwent a 48-hour aerobic incubation at 37°C. The zone of inhibition was measured with a Vernier caliper after 48 hours.

Preparation of formulation:[12] Five different herbal hair gel formulations were created using a Carbopol gel basis and a straightforward gel formulation production procedure. Vitamin E oil, guar gum, citric acid, glycerol,rose oil, and rose water are all ingredients in the gel recipe. Two grams of guar gum were placed in a mortar and pestle. Rose water was then slowly added, and the mixture was agitated in a circular motion until it took on the consistency of a thick gel. The hibiscus flower extract and vitamin E oil were then added in the specified amounts. As a preservative, citric acid was added after everything had been blended [12]. Glycerol was added and rose oil as a fragrance. Up until a homogenous gel was created, mixing was continued. To achieve a homogeneous consistency in the gel formulation, several concentrations of the Gaur gum as stated in table no. 1 were added.

Table 1: Formulation of herbal hair gel.

Formulation	F1	F2	F3	USE
Guar gum	1 g	1.5 g	2 g	Gel base
Hibiscus extract	0.5 g	0.5 g	0.5 g	Antibacterial, promotes hair growth, gives thickness and shine
Vitamin E oil	0.5 ml	0.5 ml	0.5 ml	Increase shine, enhance growth.
Citric acid	0.03g	0.03g	0.03g	Preservative
Glycerol	5 ml	5 ml	6 ml	Thickening agent, viscosity enhancer
Rose oil	Q. S	Q. S	Q. S	Fragrance
Rose water	85 ml	80 ml	75 ml	Vehicle

Physicochemical evaluation of hair gel formulation [13]**:**

1. **Physical appearance:** The gel formulation was evaluated in terms of physical character like phase separation and change in color, odour and rheological parameters.
2. **Homogeneity:** After the developed gel had been placed in the container, it was visually inspected to determine its homogeneity. For appearance, the presence of any aggregates, and flocculates, it was examined.

3. **pH:** A digital pH meter was used to ascertain the pH of the gel composition. The pH of one gram ofgel was measured in triplicate using 100 ml of distilled water, and the average value was computed.
4. **Washability:** After applying the prepared hair gel formulation to the skin, the ease and extent of water washing are assessed as usual.
5. **Spreadability:** The gel (2g) was placed between two glass slides and weighed out. Weight of 500g was applied to the slides. The weight was applied for a predetermined duration of five minutes. The spread circle's diameter was then measured at various locations after the weight was removed. An equation was used to calculate spreadability. S=M.L/T where S is spreadability, M is weight appliedto the slide, L is circle diameter in centimeters, and T is time in seconds..
6. **Skin irritation test:** Apply the herbal hair gel formulation to your skin and check for rashes, irritation, or redness. 8. Functions against fungi. The therapeutic effectiveness of antifungal medications may be demonstrated by the inhibition of fungal growth under controlled settings. The cup-plate method, which relies on drug diffusion from the gel contained in the cup through a solidifiedagar layer in the petridish to an extent such that development of the introduced microbe is completelystopped in a zone surrounding the cup, was used to evaluate the microbiology of gels. A wider zoneof inhibition is a sign that the medication is released from the base more effectively.
7. **Antimicrobial study:** The agar plate method was used to assess antimicrobial activity. Fungi and bacteria were cultivated on agar. Using the cup-plate method, which relies on drug diffusion from the extract and gel contained in the cup through a solidified agar layer in the petridish to a degree such that growth of the added microorganism is completely prevented in a zone around the cup, the microbiological evaluation of extract and gels was conducted. A wider zone of inhibition is a sign that the medication is released from the base more effectively.Malassezia furfur species and Staphylococcus aureus bacterium were the microorganisms used in this study.

Results

Hibiscus flower extract quality phytochemical analysis: The findings of the initial hibiscus ethanolic extract phytochemical analysis are presented below.

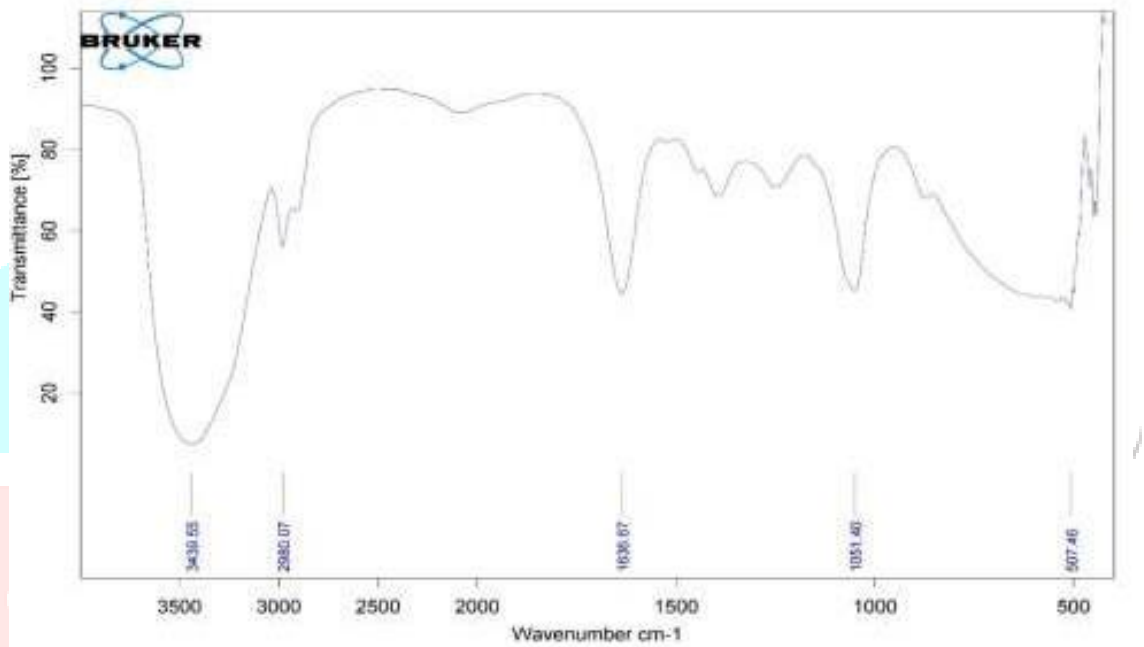
Table 2: Observation table of phytoconstituent evaluation

PHYTOCHEMICALS	TEST	RESULTS FOR HIBISCUS
Carbohydrates	Molisch Test: Benedict Test:	+ +
Saponin:	Foam test:	-
Flavonoids:	NaOH test Lead acetate test:	+ +
Alkaloids:	Wagner Test: Mayer Test:	+ +

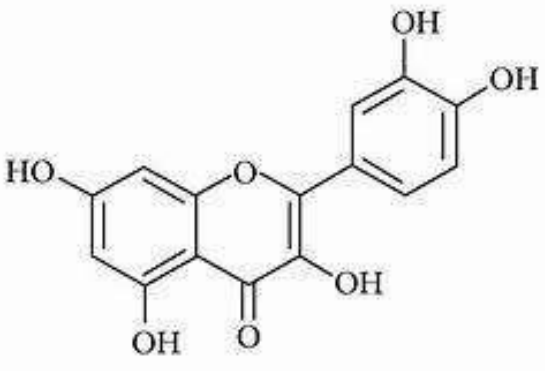
Proteins and amino acids:	Millon Test: Ninhydrin test: Sulphur test: Biuret test: Xanthoproteic Test:	+ - + - +
Glycosides:	Liebermann's Test: Keller-Kiliani test	+ -
Terpenoids:	Chloroform test:	+

IR evaluation:

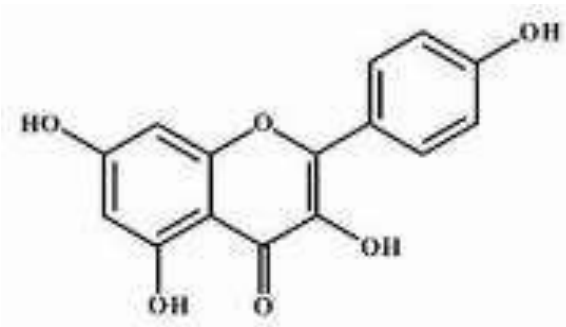
FT-IR was used for detection of various functional groups present in extracts.



Structures of active phytoconstituents of hibiscus:



Quercetin



Kaempferol

Table no: 3 Functional groups detected from IR graph

Functional Group	IR standard Ranges	IR observed Value
C=O	1625-1650	1636.67
C-OH	3300-3700	3439.55
C-H	2880-2900	2900
C=C	1650-1566	1570
Phenolic-OH	1390-1310	1370
=C-O-C=	1225-1200	1220

The various functional groups observed in the extracts probably indicate the presence of quercetin. By using FT-IR spectrum, we can confirm the functional constituent's presence in the extract.

Antimicrobial test of extract:

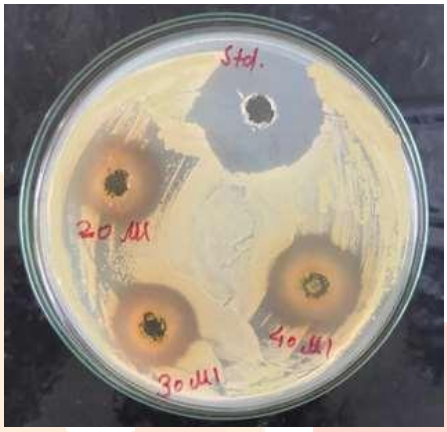


Figure. 1 Antimicrobial study by using Staphylococcus aureus Bacteria

The prepared extract showed a zone of inhibition, Streptomycin used as standard.

Table no:4 anti-bacterial activity of extract

Samples	Zone of inhibition (mm)
standard Streptomycin	31
20mg extract	16
30mg extract	19
40mg extract	21

Evaluation of gel:

Table no:5 Evaluation study of gel

TEST	OBSERVATION		
	F1	**F2**	**F3**
Colour	Red	Red	Red
Odour	Rose odour	Rose odour	Rose odour
Consistency	Semisolid	Semisolid	Semisolid
pH	5.9	5.9	5.9
Washability	Easy to wash	Easy to wash	Easy to wash
Spreadability	13.6gm.cm/sec	12.9gm.cm/sec	12.1gm.cm/sec
Skin irritation	No irritation (Scale 0)	No irritation (Scale 0)	No irritation (Scale 0)

Evaluation of F1, F2 and F3 batches was done. Evaluation study such as appearance, consistancy, F2 batch was selected and it shows good antimicrobial activity as shown in below figure 2.

Antimicrobial and antifungal activity:

A) B)

Figure 2: The prepared Gel showed zone of inhibition A) S.Aureus B) Malassezia

Table no: 6 Anti-microbial activity of prepared gel

Effecct of Prepared Gel on	Zone of inhibition (mm)
S.Aureus	16
Malassezia	18

Discussion

The results of phytochemical screening in the present study have shown that Hibiscus flower has antimicrobial potentials. Hibiscus flower useful for repairing damage caused by microbialinfection of scalp may be because of hair products, cosmetic product, disease condition etc. Hibiscus can strengthen and improve the condition of hair cuticle and boost shine by preventingmicrobial infection of scalp. Hibiscus flowers contain a high amount of mucilage, which acts as a natural conditioner. Hibiscus contains amino acids & vit.C which improves blood circulation under the scalp & boosts hair growth.

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