FORMULATION AND EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF HERBAL SHAMPOO

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ABSTRACT
The aim of this study was to study the antimicrobial activity of herbal shampoo against Escherichia coli (gram negative) and Staphylococcus aureus (gram positive) bacteria by using Cup plate method. The formulation was evaluated for product performance which includes organoleptic characters, pH, Physicochemical characterization and for solid content. The abstergent was evaluated by following parameters like pH determination, wetting time, Dirt dispersion, Specific gravity, Determination of solid content, Net Content, Surface tension measurement, foaming ability & stability and Stability study. The antioxidant activity of Herbal Shampoo was determined by using FRAP assay. The antibacterial activity of the herbal shampoo was observed against gram positive and gram negative bacteria at different concentration of shampoo. The % Zone of Inhibition was found to be 0%, 78.12% and 84.37% Antimicrobial (antibacterial) activity against the Gram negative bacteria using cup-plate method. It has been observed that the dose of the Herbal shampoo should not less than 20 g/ml.

KEYWORDS: Hair, Shampoo, Water, Herbal, Weight, Sample, Bacteria, Surface, Density, Antioxidant.

INTRODUCTION:
Hairs are an essential part of human aesthetics. They are protein filaments that originate from follicles within the skin's dermal layer and are scientifically referred to as pili or pilus. As part of the integumentary system, hair extends into the dermal layer where it is housed in the hair follicle. Hair distinguishes mammals from other classes of organisms. In humans, it is a prominent indicator of health, youth, and social status. Additionally, hair serves sensory functions, provides protection from cold and UV radiation, and has significant psychological impacts when its growth or structure is disrupted.
At a microscopic level, variations in hair length, color, diameter, and cross-sectional shape contribute to the diverse profiles seen across different ethnic groups and individuals.

ANATOMY OF HAIR :
Hair grows from follicles located within the scalp's fatty layer. Contrary to the common belief that hair grows as single strands, follicles actually produce groups of 1-4 hairs, known as “follicular units.” At the base of each follicle is the hair bulb, where hair production occurs. Hair follicles are nourished by blood vessels in the dermis. As the cells divide and grow, they form the hair shaft. While developing beneath the epidermis, hair remains soft. Once it emerges from the epidermis, its outer layer hardens into keratin.

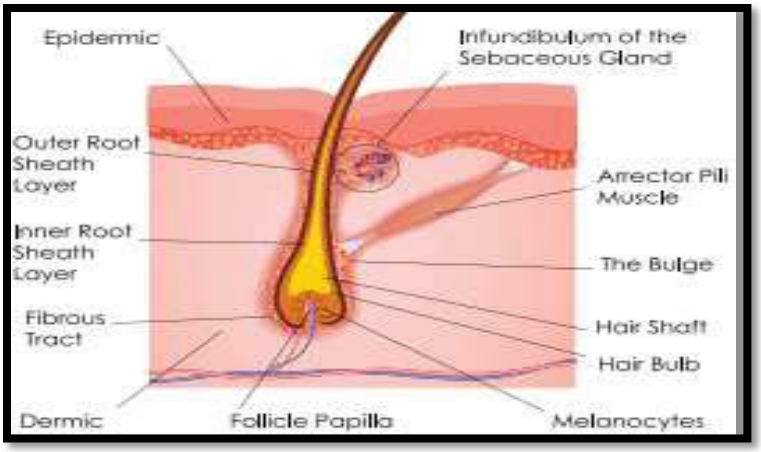

Fig.1. Hair Anatomy

Parts of the Hair:

- **Dermal papillae:** This structure is crucial for regulating the hair cycle and growth. It contains androgen receptors sensitive to the presence of dihydrotestosterone (DHT).
- **Matrix:** Surrounding the dermal papillae, the matrix contains all the active cells necessary for hair growth and the development of different hair parts, including the outer root sheath, inner root sheath, and hair shaft. Together, the matrix and dermal papillae form the hair bulb.
- **Outer root sheath:** Also known as the trichelemma, this is the outermost part of the hair. It is keratinized and covers the entire hair follicle within the dermis, transitioning through to the epidermis and providing an opening for the hair to emerge.
- **Inner root sheath:** Comprising three parts—the Henle layer, Huxley layer, and cuticle—the inner root sheath stabilizes the hair. The Henle and Huxley layers anchor onto each other, while the cuticle, made of dead hardened cells, provides added protection to the hair shaft. Together, these layers secure the hair and support its growth.
- **Hair shaft:** This is the only part of the hair follicle that exits the skin's surface. It consists of three layers: the medulla, cortex, and cuticle. The medulla is an unstructured area in the innermost region of the hair shaft and is not always present. The cortex, highly structured and made of keratin, provides strength, durability, and water uptake. It also contains melanin, determining hair color based on the type and distribution of melanin granules. The cuticle is the hair's outer protective layer, connected to the internal root sheath, with a single molecular layer of lipids that help repel water.

PHYSIOLOGY OF HAIR :

- Anagen (growth phase): The majority of hair is in this active growth phase, which lasts for several years.
- Catagen (transitional phase): The majority of hair is in this active growth phase, which lasts for several years.
- Telogen (resting phase): In this phase, which can last for several months, hair growth stops, and the old hair eventually detaches from the follicle. A new hair starts to grow, pushing the old hair out.

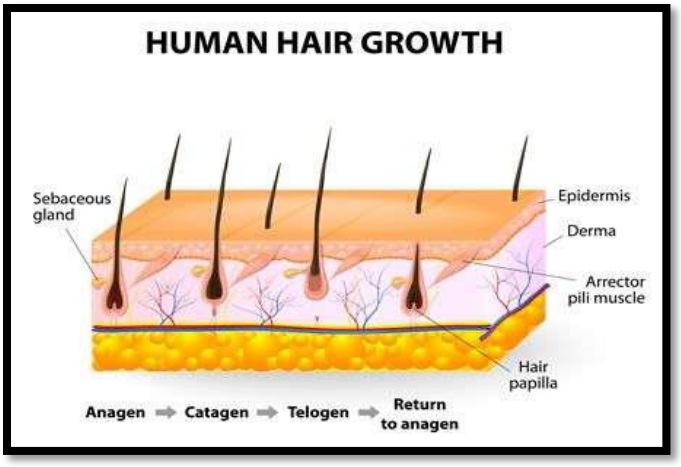

Fig.2. Hair Growth Cycle

PROBLEMS RELATED TO HAIR:

Common hair issues include dandruff, dry hair, split ends, oily hair, frizzy hair, limp hair, hair loss, heat damage, color damage, and greying hair.

HAIRFALL:

Hair fall, also known as alopecia or hair loss, involves excessive shedding of hair from the scalp or other body parts. It can range from mild to severe and can be caused by various factors, including genetics, hormonal changes, stress, nutritional deficiencies, medical conditions, certain medications, and improper hair care practices. Hair fall is a common concern affecting both men and women of all ages.

TYPE OF HAIR FALL

1. Male pattern hair loss
2. Female pattern hair loss
3. Alopecia areata
4. Telogen effluvium

CAUSES OF HAIRFALL:

- Nutritional deficiencies.
- Hormonal Changes
- Severe stress.
- Fungal infection on the scalp.

TREATMENT:

- Maintain a healthy diet.
- Manage stress effectively.
- Consider Hair transplantation
- Explore Scalp treatment
- Use specific shampoos, such as those containing fenugreek, almond hair oil, or egg white shampoo.

SHAMPOO:

Shampoo is a formulation containing surfactants, available in various forms such as liquid, solid, or powder. When applied under specified conditions, it effectively removes surface grease, dirt, and skin debris from the hair shaft and scalp without causing harm to the user.

ADVANTAGES OF SHAMPOO:

- Cleanses hair and scalp.
- Improves hair hygiene.
- Treats scalp conditions
- Helps with dry scalp
- Aids in hair loss treatment.
- Manages greasy or oily hair.
- Relieves itching and irritation
- Repairs damaged hair.
- Keeps hair silky and smooth.
- Keeps your hair beautiful and blossomed.

IDEAL PROPERTIES OF SHAMPOO:

1. Makes hair smooth and shiny.
2. Produces a good amount of foam .
3. Does not cause irritation to scalp, skin and eye.
4. Effectively remove dirt.
5. Imparts a pleasant fragrance.
6. Biodegradable.
7. Low toxicity.
8. Slightly acidic (pH less than 7) to prevent weakening of hair by maintaining the disulphide bonds in keratin.

ACTION OF SHAMPOO:

Fig.3. Mechanism of Shampoo

HERBAL SHAMPOO:

Herbal shampoos are cosmetic products formulated with traditional Ayurvedic herbs for cleansing the hair and scalp, similar to regular shampoos. They effectively remove oils, dandruff, dirt, and environmental pollutants.

ADVANTAGES OF HERBAL SHAMPOO:

- Made from pure and organic ingredients without synthetic additives or surfactants.
- Biodegradable and environmentally friendly.
- Does not cause eye irritation.
- Cost-effective.
- Regular use can improve hair health.
- Balances oil production in the hair and scalp.
- Contains natural antiseptic properties to protect against UV rays and prevent skin infections.

BACTERIA

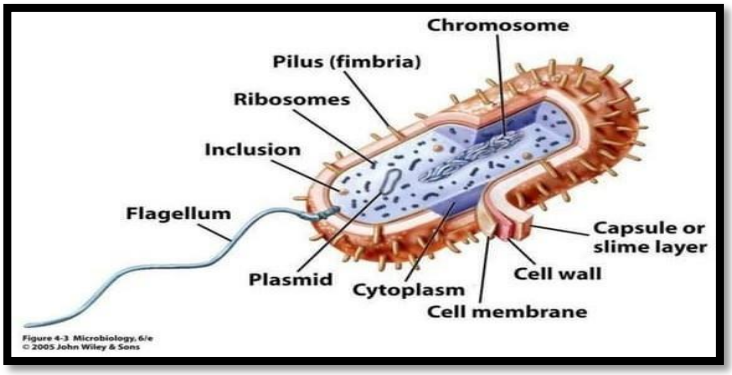

Fig.4. Structure of Bacterial cell

Structure of bacteria :-

Bacteria are single-celled microorganisms characterized by their simple structure, lacking a nucleus and other organelles, thus classifying them as prokaryotic organisms. They are highly adaptable and can survive in extreme environments, often referred to as extremophiles, which include:

1) Thennophiles
2) Alkaliphiles
3) Osmophiles
4) Basophiles
5) Cryophilic

Bacteria have a protective cell wall made of peptidoglycan, a unique protein not found elsewhere in nature. Some bacteria lack this cell wall, while others have an additional protective layer called a capsule. Externally, they may have one or more flagella or pili, which aid in locomotion and attachment to host cells. Bacteria contain ribosomes for protein synthesis and a single loop of DNA, with some having extra circular DNA called plasmids, which can provide antibiotic resistance.

Bacterial Classification:-

Bacteria are single-celled microbes with a simpler structure compared to other organisms, as they lack a nucleus or membrane-bound organelles. Their genetic material is contained in a single DNA loop, with some possessing plasmids that confer advantages such as antibiotic resistance. Bacteria can be classified based on:

1) Shape
2) Composition of the cell wall

3) Mode of respiration
4) Mode of nutrition

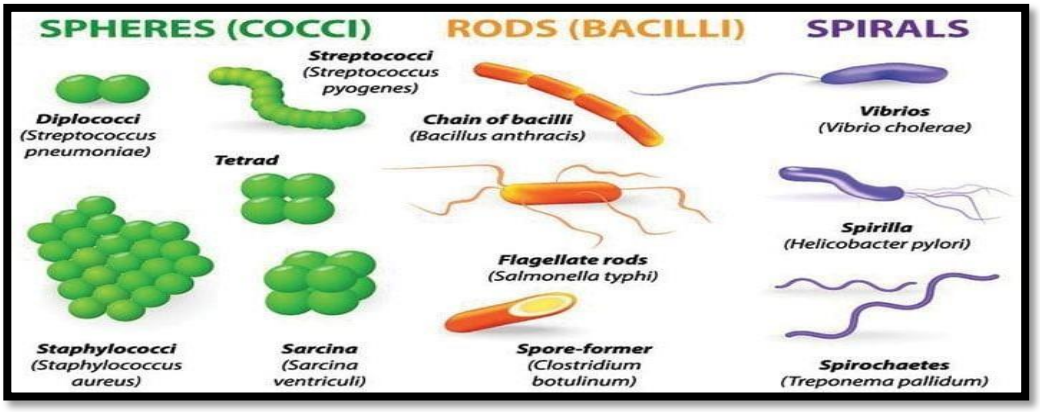

Fig.5. Variants of Bacteria

Antibacterial:-

Antibacterials are used to treat bacterial infections and are classified into groups such as beta-lactams, macrolides, quinolones, tetracyclines, and aminoglycosides. These categories depend on their antimicrobial spectra, pharmacodynamics, and chemical composition. Prolonged use of certain antibacterials can reduce enteric bacteria, potentially impacting health. Probiotics and a balanced diet may help restore gut flora. The discovery and development of antibacterials in the 20th century significantly reduced mortality from bacterial infections.

Bacteria used

1) *Staphylococcus aureus*:

A Gram-positive bacterium found in the anterior nares and skin of mammals. It is an opportunistic pathogen responsible for various diseases, from minor skin infections to severe bacteremia and necrotizing pneumonia. Before antibiotics, the mortality rate from S. aureus infections was over 80%.

Fig.6. Staphylococcus aureus

2) *Escherichia coli*:

A Gram-negative, non-sporulating bacterium typically rod-shaped, about 2.0 µm long and 0.25–1.0 µm in diameter, with a cell volume of 0.6-0.7 µm³. E. coli stains Gram-negative due to its cell wall structure, which includes a peptidoglycan layer and an outer membrane.

Fig.7. Escherichia coli

FORMULATION OF HERBAL SHAMPOO :

INGREDIENT	QUANTITY FOR 100ml	PURPOSE
Herbal Extract	20ml	Active Ingredient
Glycerine	2ml	Moisturizing agent
NaCl 0.1M	20ml	Buffering agent
Reetha extract	4.56gm	Foaming agent
Acacia (Gum)	10ml	Viscosity enhancer
Pectin	q.s.	Thickening agents
lemon oil	2ml	Preservative
Water	25ml	Vehicle

Table 1 : COMPOSITION OF HERBAL SHAMPOO INGREDIENTS QUANTITY

Preparation of Herbal Shampoo

Formulation of the herbal shampoo was done as per the formula given in Table 1. Twenty ml of the herbal extract was added to 4.5 gm of reetha extract with 20 ml 0.1M NaCl solution and mixed by shaking gently. The final volume was made to 100 ml by adding 10 ml acacia gum extract, 2 ml of glycerine, and 25 ml of water. To improve Viscosity in the formulation, sufficient quantity (q.s.) of Pectin was added and heated till it dissolves and it is cooled. The shampoo also contains 2 ml of lemon oil as preservative.

Methods:

Collection of plants: The leaves of plants like Rosemary, Neem and Tulsi were collected from the local market. Reetha also collected from local market. These were washed under running water to remove contaminants. They are dried in sunlight, converted into coarse powders and sieved using 60 meshes. The extracts were prepared by decoction method and the prepared extracts were stored in well-closed containers.

Preparation of herbal extract: 2gm of Rosemary Leaves powder, 2gm of Tulsi Powder and 4gm of Neem Powder (Table 2) were mixed with 100 ml water in a stainless steel vessel. The mixture was kept for boiling until the water reduced to one quarter. It was then filtered. The clear extract obtained was used as herbal extract. Reetha extract was prepared by using 3gm of its powder mixed

with 30ml of water and boiled till it reduced till one quarter and after filtering reetha extract was obtained.

PLANT	PART	QUANTITY FOR 100ml	PURPOSE
Rosemary	Leaves	1 to 5% (1 to 5gm)	Smoothens Hair
Tulsi	Leaves	1 to 5% (1 to 5gm)	Prevent Hair thinning
Neem	Leaves	2 to 10% (2 to 10gm)	Prevent graying of hair

Table 2 : INGREDIENTS OF HERBAL EXTRACT

EVALUATION PARAMETERS:

The prepared formulation was evaluated for product performance which includes organoleptic characters, pH, Physicochemical characterization and for solid content. To guarantee the nature of the items, particular tests were performed for surface tension, foam volume, foam stability and wetting time using standard protocol.

1. Physical Appearance

The formulation prepared was evaluated for the clarity, color, odor and foam producing ability and fluidity.

2. Net Content

Mark the bottle upto the Liquid surface. Empty the Bottle, note down the volume required to filled to the mark.

3. Determination of pH

A 10%v/v shampoo solution was constituted in distilled water and the pH of the solution was measured by using a calibrated pH meter.

4. Determination of solid content percentage

A clean dry evaporating dish was weighed and 4gm of shampoo was added to the evaporating dish. The evaporating dish with shampoo was placed on the hot plate until the liquid portion was evaporated. The weight of the solid contents present in the shampoo was calculated after drying.

$$\% \text{ solid content} = \frac{w_3 - w_1}{w_2 - w_1} * 100$$

Where, w_1 = weight of dish

w_2 = weight of dish + sample

w_3 = weight of sample after liquid evaporates

5. Wetting time

Wetting time was calculated by noting the time required by the absorbing paper i.e. filter paper or canvas paper to sink completely. A filter paper was cut into a disc of diameter measuring 1 inch. Over the shampoo (1%v/v) surface, the filter paper disc was kept and the time taken for the paper to sink was measured using stop watch.

6. Dirt dispersion

Two drops of herbal shampoo were added in a wide mouthed falcon tube containing 10ml of distilled water. 1 drop of dirt was added, the falcon tube was covered and shaken for ten times. The amount of dirt in the foam was estimated as None, Light, Moderate, or Heavy.

7. Specific gravity

It is the relative density of the substance to the water.

Determination of specific gravity = Density of shampoo solution/ Density of water

Density of shampoo = $\frac{w_1 - w}{v}$

Density of water = $\frac{w_2 - w}{v}$

where, w = weight of empty bottle

w_1 = weight of empty bottle + weight of sample

w_2 = weight of empty bottle + weight of water

v = volume of liquid

8. Surface Tension Measurement

In order to determine the surface tension of the shampoo the density of shampoo should be known for that purpose first calculate the density of sample by the formula:

w_1= weight of empty bottle

w_2 = weight of empty bottle+ weight of water

w_3 = weight of empty bottle+ weight of sample

$$d_2 = \frac{w_3 - w_1}{w_2 - w_1}$$

To determine surface tension of sample, use the following formula

$$\Gamma_2 = \frac{d_2 \times n_1}{d_1 \times n_2} \times \Gamma_1$$

Where, d_1 is the density of water

d_2 is the density of sample

Γ_1 is the surface tension of water

Γ_2 is the surface tension of sample

n_1 is the number of drops of water

n_2 is the number of drops of sample

9. Foaming ability and stability

Cylinder shake method was used for determining foaming ability. 50ml of the 1% herbal shampoo solution was put into a 250ml graduated cylinder & the cylinder was covered with hands and shaken for 10 minutes. The total volume of the foam content after 1-minute shaking was recorded. Immediately after shaking the volume of foam at 1 minute intervals for 10 minutes were recorded. The foam volume remains same throughout the period of about 5 min showing that the generated foam by the shampoo has good stability and the prepared shampoo exhibits higher foam property which may be due to the presence of Soapnut.

10. Stability Study

The stability of the formulation was studied for a period of four weeks by keeping at temperature of 25-30°C.

ANTIMICROBIAL ACTIVITY OF SHAMPOO :

To study the antimicrobial activity of prepared herbal shampoo using Cup Plate Method.
Material: Petri plate, Nutrient agar media, Bacterial cultures (+ ive and – ive). Antibiotic standard (Streptomycin)

Procedure:

Preparations of nutrient agar

Chemicals: Beef extract, peptone, sodium chloride, agar, glucose.

Equipment: Autoclave, oven, pH meter, balance.

Principle: Nutrient agar is nutrient broth solidified by the addition of 1 to 2 % agar. In Addition to liquid media, solid & semisolid media are prepared with agar at concentration of 0.5% or less & are useful for the cultivation of microaerophilic bacteria & for detection of bacterial motility.
Composition of Nutrient Agar are as follows:
Beef extract ---- 10gm
Peptone --- 10gm
Sodium chloride --- 5.0gm
Distilled water to make ----- 1000ml
Agar --- 20gm
(PH 7.0)
Agar is a complex polysaccharide Consisting of 3,6 - unhydro-L- galactose and D-Galactopyranose produced from various red algae belonging to Delirium, Gracilaria, Gigartina and pterocladia. It melts at 95° C to 100° C and solidifies at 40° C to 45° C. It does not metabolize by any pathogenic bacteria. Agar can be replaced with gelatin (10% w/v) which is prepared by hydrolysis of collagen with boiling water. It is in liquid form at 37°C. It forms transparent gel below 25°C or with proteolytic microbes.

Observation and results:

It is clean transparent yellow colored nutrient agar.

1. **Preparation of plates**

Prepare the plates by adding 15ml of sterile nutrient agar to each Petri plates aseptically. Allow to set and incubate the plates for 24hrs as a test of sterility.

2. **Cup Plate Method**

In a prepared agar plates, add 5ml of nutrient agar medium to which 1ml of culture is already added. Allow to set and then cut out cylinder cups of diameter approximately 1cm using an alcohol sterilized borer. Remove the cups using a sterile forceps. Place test solution into the cups. Fill the cups to the Brim taking care that the solution doesn't overflow or spill in the area nearby. Incubate the Plates at a 37°C. Measure the zone of inhibition after 18hrs.

ANTIOXIDANT ACTIVITY OF SHAMPOO :

The antioxidant activity of Herbal shampoo was determined by using FRAP assay

Chemical:

Herbal shampoo, phosphate buffer (monobasic sodium phosphate and dibasic sodium phosphate 6.6 pH) 0.2 M1% potassium ferrocyanide,10 % trichloro acetic acid,0.1 % ferric chloride.

• PROCEDURE OF MAKING CHEMICALS

1. Phosphate buffer pH 6.8, 0.2M mixed Dissolve 13.872g of potassium dihydrogen phosphate and 35.084g of disodium hydrogen phosphate in suffer water to produce 1000ml store in cold place.
2. Potassium Ferrocynate- Potassium hexacyanoferrate (III) K3Fe(CN)6 = 329.25 Analytical reagents grade of commerce. Ruby red crystal.
3. Trichloroacetic acid- Cl3COOH=163.40 colourless, very deliquescent crystals or crystalline masses; odour, slight or pungent & characteristic; MP about 56 degrees. Store protected from light.

Trichloroacetic acid solution-dissolve 10g of Trichloroacetic acid in sufficient water to produce.

4. 0.1% FeCL3

Solution I: Dissolve wing of # Ng hexahydrate in 100ml of art of hydrochloricacid.

Solution II: Dissolve 3.5g of potassium ferricyanide in 100ml of water

PROCEDURE:

1. Take plant extract of various concentration 5,10,15 and 20. Add 2.0 ml of phosphate buffer then add 1% of Ferro cyanide (2.0 ml).
2. Mix well cover it with aluminium foil and incubate then boil in water bath for 20 min (maintain 50º Celsius) and cool it.
3. Shake and add 2.0 ml of trichloroacetic acid. Centrifuge and then take upper layer extract about 2.0 ml from test tube and place it in another test tube and add 2.0 ml distilled water and add ferric chloride blue colour will absorb.

Ferrous reducing antioxidant capacity assay:

Formula to calculate Antioxidant effect:-

$$\text{Antioxidant effect (\%)} = \frac{\text{(control absorbance)-(sample absorbance)}}{\text{(control absorbance)}} \times 100$$

RESULT AND DISSCUSSION:

1. Evaluation Parameters

➢ **Physical Appearance**

Test	Result
Colour	Light brown
Odour	Pleasant smell
Clarity	Clear
Foam ability	Yes

Table 3 : Test for Physical appearance

Fig.8.Physical appearance

- **Net Content**

The Net Content was found to be 90 ml.

- **Determination of pH**

The pH of the shampoo was found to be 5.45.

Fig.9. pH Calibaration of Shampoo

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- **Determination of solid content percentage**

The weight of the solid contents present in the shampoo was calculated after drying.

Weight of evaporating dish = w_1 =71.80

Weight of sample in dish = w_2 = 75.91

Weight of dish after liquid evaporates = w_3 = 72.11

$$\% \text{ solid content} = \frac{w_3-w_1}{w_2-w_1} * 100$$

$$= \frac{72.11-71.80}{75.91-71.80} * 100$$

$$= 0.075*100 = 7.5\%$$

The percentage solid content of shampoo was found to be 7.5%

- **Dirt dispersion**

Two drops of herbal shampoo were added in a wide mouthed falcon tube containing 10ml of distilled water. 1 drop of dirt was added, the falcon tube was covered and shaken for ten times. The amount of dirt in the foam was estimated as Light.

- **Wetting time**

Wetting time of shampoo was found to be 3sec.

- **Specific gravity**

It is the relative density of the substance to the water.

$$\text{Determination of specific gravity} = \frac{\text{Density of shampoo solution}}{\text{Density of water}}$$

Using this formula, the specific gravity of shampoo can be determined.

$$\text{Density of shampoo } (d_1) = \frac{w_1-w}{v}$$

$$\text{Density of water } (d_2) = \frac{w_2-w}{v}$$

Put the values, w =17.87 gm

w_1= 45.25 gm

w_2= 44.98 gm

$$d_1 = \frac{45.25-17.87}{25} = 1.09 \text{ kg/m}^3$$

$$d_2 = \frac{44.98-17.8725}{25} = 1.08 \text{ kg/m}^3$$

$$\text{Specific gravity} = \frac{d_1}{d_2} = \frac{1.09}{1.08} = 1 \text{ kg/m}^3$$

The Specific gravity of Shampoo was found to be 1.00 kg/m^3

- **Surface Tension Measurement**

In order to determine the surface tension of the shampoo the density of shampoo should be known for that purpose first calculate the density of sample by the formula:

w_1= weight of empty bottle (17.87gm)
w_2 = weight of empty bottle+ weight of water (44.98 gm)
w_3 = weight of empty bottle+ weight of sample (45.25 gm)

$$d_2 = \frac{w_3 - w_1}{w_2 - w_1} = \frac{45.25 - 17.87}{44.98 - 17.87} = \frac{27.38}{27.11} = 1.00 \text{ gm/ml}$$

Solution	No. of drop count (1)	No. of drop count (2)	Average No. of drop count	Density (gm/ml)	Surface Tension (dyne/ cm)
Water	31	29	30	1 gm/ml	72 dyne/ cm
Shampoo Solution	68	66	67	1 gm/ml	32.23 dyne/ cm

Table 4 : Observation table for Surface Tension Measurement

$$\Gamma_2 = \frac{d_2 \times n_1}{d_1 \times n_2} \times \Gamma_1$$

Where, d_1 is the density of water (1g/ml)
d_2 is the density of sample (1 g/ml)
Γ_1 is the surface tension of water (72 dyne / cm)
Γ_2 is the surface tension of sample (?)
n_1 is the number of drops of water (30)
n_2 is the number of drops of sample (67)

$$\Gamma_2 = \frac{1 \times 30}{1 \times 67} \times 72$$

$$= \frac{30}{67} \times 72$$

= 32.23 dyne/cm

The surface tension of the sample was found to be 32.23 dyne/ cm

- **Foaming ability and stability**

Cylinder shake method was used for determining foaming ability. 50ml of the 1% herbal shampoo solution was put into a 250ml graduated cylinder & the cylinder was covered with hands and shaken for 10 minutes. The total volume of the foam content after 1 minute shaking was recorded. Immediately after shaking the volume of foam at 1 minute intervals for 10 minutes were recorded. The foam volume remains same throughout the period of about 5 min showing that the generated foam by the shampoo has good stability and the prepared shampoo exhibits higher foam property which may be due to the presence of Reetha.
The foaming ability and stability of shampoo was studied and observed.

- **Stability Study**

The stability of the formulation was studied for a period of four weeks by keeping at temperature of 25-30°C.

After keeping at certain temperature for four weeks it is observed that the Formulated shampoo is Stable.

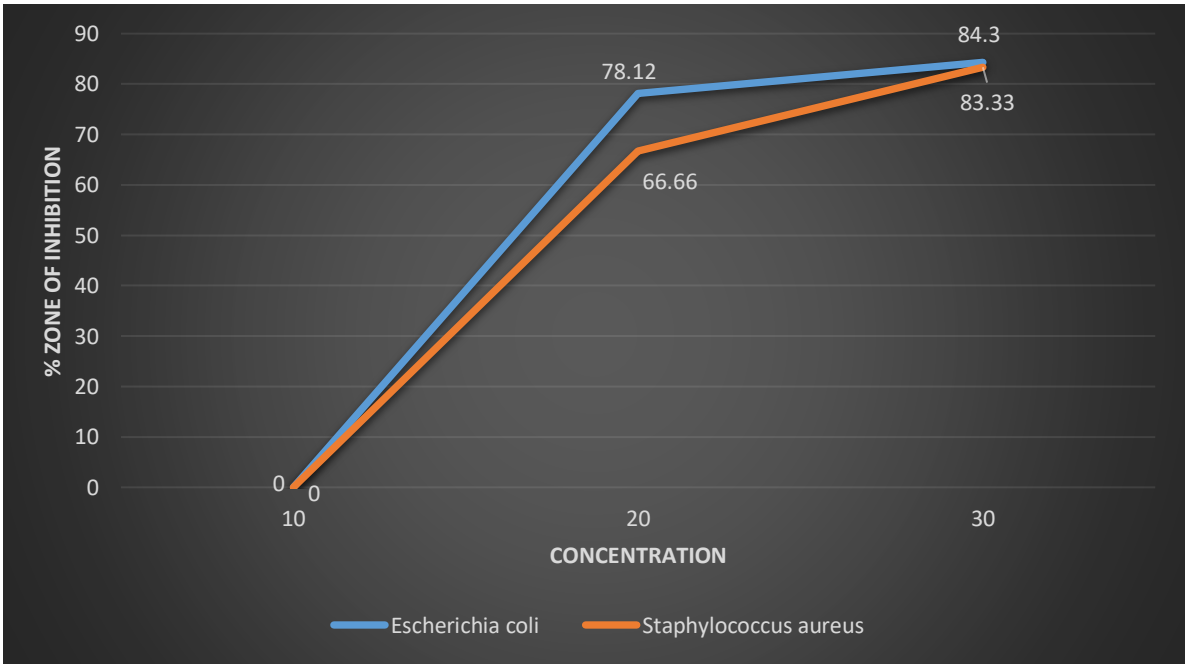
2. Antimicrobial Observation and Result: -

Organisms	Sample Conc. (µg/ml)	Zone of Inhibition (in mm)	Zone of Inhibition (in %)
Escherichia coli	10	NZ	00
	20	25	78.12
	30	27	84.37
	Standard (Streptomycin)	32	100
Staphylococcus aureus	10	NZ	00
	20	20	66.66
	30	25	83.33
	Standard (Streptomycin)	30	100

Table 5 : Zone of Inhibition (in mm) and (in %) for S. Aureus and E. Coli

Considering control zone of inhibition (i.e. streptomycin) as 100% calculated all the zone of inhibition (mm) as zone of inhibition (mm) in %.

Graphical representation of Antimicrobial activity



Graph 1 : % Zone of Inhibition vs Concentration of Shampoo

Fig.10. Antimicrobial activity of Shampoo Against E.coli and S.aureus

3. Antioxidant Observation Table

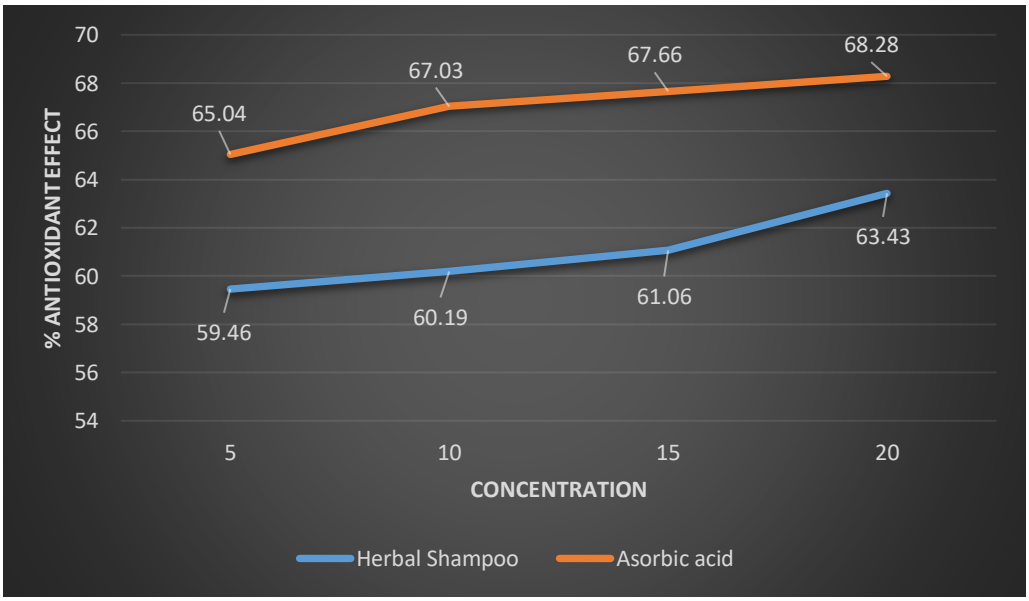
Recorded under UV spectrophotometer at a wavelength of 600nm.

% Antioxidant activity:

Sample (µg/ml)	% Antioxidant effect (Herbal Shampoo)	% Antioxidant effect (Ascorbic acid)
5	59.46	65.04
10	60.19	67.03
15	61.06	67.66
20	63.43	68.28

Table 6 : % Antioxidant Effect of Herbal Shampoo and Ascorbic acid

Graphical representation of % Antioxidant effect



Graph 2 : % Antioxidant effect of Herbal Shampoo and Ascorbic acid

DISCUSSION

1. Evaluation Parameters

The evaluation parameters for an ideal shampoo is been checked. The Evaluation parameter includes physical appearance, Net content, Determination of pH, Determination of solid content, wetting time, Dirt Dispersion, Specific gravity, Surface tension measurement, foaming ability & stability and Stability study has been carried out. By Considering all the evaluation parameter, the product i.e. Herbal Shampoo has passed the test successfully.

2. Antimicrobial Activity

The antibacterial activity of Prepared Herbal Shampoo against the Escherichia coli (gram negative) and Staphylococcus aureus (gram positive) bacteria by using Cup plate method and the result are as follows:

- The antibacterial activity of herbal shampoo was observed against gram positive and gram negative bacteria at different concentration of shampoo.

i. As per the cup-plate method, the maximum Zone of inhibition was found to be 27mm for 30µg/ml, 25mm for 20 µg/ml and No Zone of Inhibition for 10 µg/ml against Escherichia coli i.e. gram negative bacteria.

ii. As per the cup-plate method, the maximum Zone of inhibition was found to be 25mm for 30µg/ml, 20mm for 20 µg/ml and No Zone of Inhibition for 10 µg/ml against Staphylococcus aureus i.e. gram positive bacteria.

iii. The % Zone of Inhibition for 10 µg/ml, 20 µg/ml and 30 µg/ml by considering Streptomycin Zone of Inhibition as 100% was found to be 0%, 78.12% and 84.37% Antimicrobial (antibacterial) activity against Escherichia coli (Gram negative) using cup-plate method.

iv. The % Zone of Inhibition for 10 µg/ml, 20 µg/ml and 30 µg/ml by considering Streptomycin Zone of Inhibition as 100% was found to be 0%, 66.66% and 83.33% Antimicrobial (antibacterial) activity against Staphylococcus aureus (Gram positive) using cup-plate method.

v. By Considering the above results it is concluded that the dose of the Herbal Shampoo should not less than 20 µg/ml.

vi. It has been observed that the Shampoo has the capability to show antimicrobial effect against bacteria like Escherichia coli (Gram Negative) and Staphylococcus aureus (Gram Positive) at 20 µg/ml or more than 20µg/ml.

3. Antioxidant Activity

The Scavenging activity of formulated Herbal Shampoo was determined by method using ascorbic acid as a Standard. While comparing the no effect ascorbic acid was taken as a standard which shows the 68.28% antioxidant effect at 20µg/ml of Sample Similarly the formulated Sample shows the 63.43% antioxidant effect at 20µg/ml. So from the above information we can definitely say that the herbal shampoo is showing the potent antioxidant effect of ascorbic acid. Similarly, from the graphical representation the starting point of the ascorbic acid is from 65.04% and that of formulated Shampoo is from 59.46% so the origin of ascorbic acid is above the formulated herbal shampoo is indicating that the prepared sample is effective against oxidation. Hence we can say that it exhibits antioxidant property.

CONCLUSION:

The vegetal abstergent was formulated using the herbal or grassy ingredients such as rosemary, neem, tulsi, which strengthens and makes the hair healthy and secondly the abstergent was evaluated by following parameters like pH determination, wetting time, Dirt dispersion, Surface

tension measurement, Specific gravity, Determination of solid content, Net Content, Stability study, Foaming ability and its stability, etc. Antimicrobial properties against Escherichia coli and Staphylococcus aureus have been observed and the Antioxidant effect which can be concluded that this shampoo is effective against scalp and hair related issues. We had used the oldest and effective drug such as neem for itchy scalp it shows Anti hair fall property due to the presence of rosemary. Here the main focus or objective is on the treatment of hair related issues by evaluating all the parameters we can say that the formulation is capable of giving the appropriate results.

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