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Extraction, Evaluation and Formulation of Herbal V -Wash from Hibiscus Rosa Sinensis

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ABSTRACT

The main aim of the present study is to formulate an effective herbal V Wash of white hibiscus flowers, which can produce a better antibacterial activity against various bacterial infections. Hibiscus rosa sinensis, a plant from the Malvaceae family, is widely distributed in tropical and subtropical climates, with around 40 species found in India. Known for its diverse medicinal properties, hibiscus is used to treat various ailments such as amoebic colitis, abdominal pain, cold, cough, diarrhea, headache, and ulcers. It contains bioactive compounds exhibiting anti-inflammatory, antibacterial, antifertility, antifungal, and antihypertensive activities. The plant's leaves, flowers, roots, and bark are integral in traditional medicine across several cultures. Hibiscus has applications in teas, tinctures, cosmetics, essential oils, and the food and pharmaceutical industries. The therapeutic value of hibiscus extends globally, from anti-diarrheal treatments in Japan to diuretic uses in Egypt, establishing it as a key component in herbal remedies. It was found that F1 batch is pale yellowish in colour and smooth texture with pleasant odour with pH of 3.98, viscosity is 1042 m.Pa.s, foaming height is 10 mm and no irritation found on human skin. The antibacterial activity determines by Disk Diffusion method. F1 batch shows more activity against *E. coli, Bacillus* and it was inactive against *Pseudomonas aeurogenosa and Staphylococcus* aureus.

Keywords: *Herbal V Wash, Hibiscus rosa sinensis, Antibacterial Activity, E. Coli, Bacillus, Tannins.*

1. INTRODUCTION

Hibiscus rosa sinensis has long been utilized in traditional medicine and continues to be widely used today. Teas and tinctures made from its flowers are believed to have therapeutic properties. The plant is also used in the production of cosmetics and essential oils. In West African cuisine, the leaves and powdered seeds are commonly used, while in China, its oil and medicinal benefits are well-recognized. The food and pharmaceutical industries also make use of this versatile plant.In tropical regions, this shrub is often grown as an ornamental plant due to its attractive shapes and vibrant flower colors. A key ingredient in many herbal drinks, hibiscus is valued not only for its flavor but also for its health benefits. Hibiscus sabdariffa is particularly popular around the world; its fresh calyx is consumed raw in salads, used to flavor pastries, or brewed into both hot and cold beverages [2]. It belongs to the subkingdom of flowering plants called Tracheobionta vascular plants that include familiar species like apple trees, roses, and tulips. Within this subkingdom, plants are divided into two classes: Magnoliopsida (dicotyledons) and Liliopsida (monocotyledons)[13].

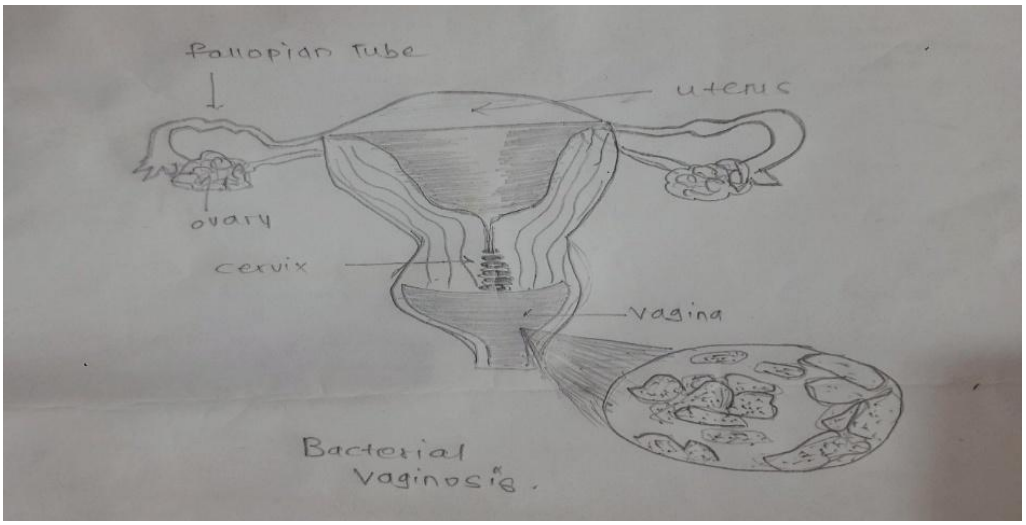
1.1 Leukorrhea

Leukorrhea refers to vaginal discharge, a normal physiological secretion from a woman's vagina. While a clear or whitish discharge is typical, abnormalities in its amount, consistency, color, or odor may suggest an infection or imbalance. Infections caused by bacteria, yeast, or other microorganisms can result in abnormal discharge, but it may also be influenced by hormonal changes, sexual arousal, or disruptions in vaginal bacterial flora.[47]

1.2 Bacterial Vaginosis (BV)

Bacterial vaginosis is caused by an imbalance in the natural bacterial population of the vagina, leading to an overgrowth of certain microorganisms. It often manifests as increased, thin vaginal discharge with a distinct fishy odor, usually gray or white in color. Women with BV are at greater risk for sexually transmitted infections and complications in pregnancy, such

as preterm delivery. Gardnerella vaginalis is the primary bacterium linked to BV, though it can occur in conjunction with other organisms like E. coli or Bacillus species. Clinically, BV is diagnosed by identifying gray, homogeneous discharge, elevated vaginal pH (above 4.5), the presence of "clue cells," and positive bacterial culture.[48]



1.3 Herbal V Wash

Herbal V Wash is an intimate hygiene product formulated to maintain the pH balance and cleanliness of the vaginal area using natural ingredients. Unlike chemical-based products, these herbal formulations are designed to be gentle on sensitive skin. Feminine hygiene is essential to a woman's overall health, particularly in maintaining the balance of vaginal microbiota and pH. Traditional soaps and water may disrupt this balance, prompting the development of specialized hygiene products. Herbal V Washes, in particular, use botanical ingredients such as hibiscus, aloe vera, and neem, which are known for their mild yet effective cleansing properties.

These products have gained popularity as natural alternatives to synthetic washes, reflecting a broader consumer preference for holistic and chemical-free personal care. [24]

1.4 The Importance of Intimate Hygiene

Despite its significance, intimate feminine hygiene remains an under-discussed area in medical literature. Cultural and religious factors often influence personal hygiene practices. While proper vulvar hygiene is undoubtedly beneficial, few formal studies have explored its direct health effects. It is crucial to use intimate care products that are clinically tested and designed specifically for the sensitive vulvar region. Such products should be hypoallergenic, free from soap and harsh chemicals, pH-balanced, and formulated to avoid irritation and dryness. Women should be educated on the importance of intimate hygiene and guided in choosing safe, effective products for their personal care routine. [24]

2. INGREDIENT PROFILE

2.1 Hibiscus (white flower):

Kingdom Plantae: plants

Subkingdom: Tracheobionta-vascular plan

Super division: Spermatophyta-se

Division: Magnoliophyta-Flowering plant

Class: Magnoliopsida-Dicotyledons

Subclass: Dilleniidae

Order: Malvales

Family: *Malvaceae*-Mallow family

Genus: Hibiscus L.-Rose mallow

Species: *Hibiscus rosa sinensis* L.-Shoeblack plant

Synonyms: H. storckii Seem, H. arnottii Griff

Biological source: The flowers obtained from *Hibiscus rosa sinensis linn.*

Geographical source: East Asia , China, Malayasia , Indonesia , India, USA

Chemical constituent: Thiamine, Tannins, Phenols, Alkaloids, Sugars.

Benefits: Antimicrobial Activity [13,24]

2.2 Aloe vera:

Kingdom: Plantae

Subkingdom: Tracheobionta (Vascular plants)

Superdivision: Spermatophyta (Seed plants)

Division: Magnoliophyta (Flowering plants)

Class: Liliopsida (Monocotyledons)

Order: Asparagales

Family: *Asphodelaceae (formerly Liliaceae)*

Genus: Aloe

Species : Aloe vera (L.) Burm.f. Aleo

Synonym: Ghritkumari , Aloe barbadensis Mill, Aloe indica Royle,

Aloe perfoliata var. vera L, Aloe vulgaris Lam.

Biological source: Dried leaves obtained from Aloe barbadensis

Geographical source: India, china, Africa, US, Spain

Chemical constituent: Acemannan (acetylated glucomannan), cellulose, pectins, hemicellulose

Vitamin A (beta-carotene), C, E, B1, B2, B6, B12, folic acid Contains 20 of the 22 essential and non-essential amino acids.

Enzymes Amylase, lipase, catalase, cellulase, Campesterol, β- sitosterol, lupeol

Benefits: Wound Healing, Moisturizing, Anti-aging, Anti-acne, Soothing Agent [13,24]

2.3 Rose Water

Kingdom: Plantae

Subkingdom: Tracheobionta (Vascular plants)

Division: Magnoliophyta (Angiosperms)

Class: Magnoliopsida (Dicotyledons)

Order: Rosales

Family: *Rosaceae*

Genus: Rosa

Species: Rosa damascena Mill.

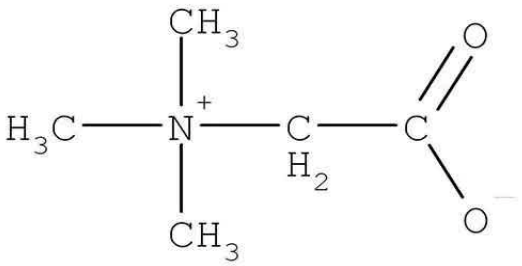
Synonym: Damask rose, Oil-bearing rose, Rose of Castile

Biological source: Fresh petals oil obtained from Rosa damascene Mill.

Geographical source: India, Bulgaria, Turkey, Iran, Morocco.

Chemical constituents: Citronellol, Geraniol, Nerol, Linalool, Geranyl acetate, Farnesol, Phenylethyl alcohol.

Benefits: Natural solvent, Flavouring agent, Source of aromatic compound, Act as natural astringent, Act as anti-oxidant, Anti-inflammatory, Moisturizing agent, Anti-aging [13,24]



2.4 Cocoamidopropyl Betaine:

Appearance: Clear to slightly yellow viscous liquid

Odor: Mild, characteristic

Source: Derived from coconut oil (cocamide) and dimethylaminopropylamine (DMAPA),

then reacted with monochloroacetic acid to form the betaine structure.

Type: Amphoteric surfactant

3. Function in Formulations:

Primary roles: Mild surfactant, Foaming agent, Viscosity builder, Co-surfactant to reduce irritation potential of anionic surfactants like SLS, commonly used in: Shampoos, Body washes, Facial cleansers, Baby products, Hand soaps [13,24].

3. MATERIALS AND METHOD

3.1 Authentication of plant:

The plant specimen was authenticated by the faculty of the Botany Department at K.T.H.M. College, Nashik. The scientific name was cross-verified with standard floras and online botanical databases.

3.2 Extraction of White Hibiscus Flower Powder Using Soxhlet Apparatus

3.2.1 Soxhlet Extraction Method

3.2.2 Introduction to Soxhlet Apparatus

A Soxhlet extractor is a specialized piece of laboratory equipment designed for continuous extraction of soluble components from solid material using a volatile solvent. It was invented in 1879 by Franz von Soxhlet. It is especially useful when the compound to be extracted is only partially soluble in the solvent, and a simple filtration is insufficient [22,31]

3.2.3 Apparatus Description

- The Soxhlet apparatus consists of:
- A round-bottom flask (to hold the solvent),
- A thimble (containing the sample),
- An extraction chamber (with a siphon),
- A condenser (mounted on top).[22,31]

3.3 Advantages of Soxhlet Extraction

- Ensures efficient extraction using a minimal quantity of solvent.
- Continuous recycling of hot solvent improves yield.
- Ideal for heat-stable phytoconstituents.

3.4 PROCEDURE:

15 grams of dried white Hibiscus flower powder was accurately weighed and placed into a thimble made of filter paper. The thimble was inserted into the central chamber of the Soxhlet extractor. 250 of distilled water was measured and poured into a round-bottom flask. Water served as the extraction solvent (menstruum) to extract hydrophilic bioactive constituents from the flower powder. The round-bottom flask containing distilled water was gently heated using a heating mantle. As water evaporated, it rose into the condenser where it condensed and dripped into the [7] containing the flower powder. The powder was repeatedly soaked with hot condensed water, facilitating the extraction of water-soluble phytochemicals [23]. Once the chamber filled to the siphon level, the aqueous extract was siphoned back into the flask. This cycle was allowed to continue for 24 hours, ensuring thorough extraction. Extraction was considered complete when the solvent in the siphon appeared nearly colorless, indicating exhaustive extraction. The aqueous extract accumulated in the flask. The water was partially removed by gentle heating under reduced pressure, concentrating the extract. Care was taken to avoid overheating, preserving heat-sensitive constituents. The concentrated aqueous extract was collected and stored in a clean, airtight container (preferably amber-colored) at 4°C for further phytochemical analysis. [22,31]

Fig: 1

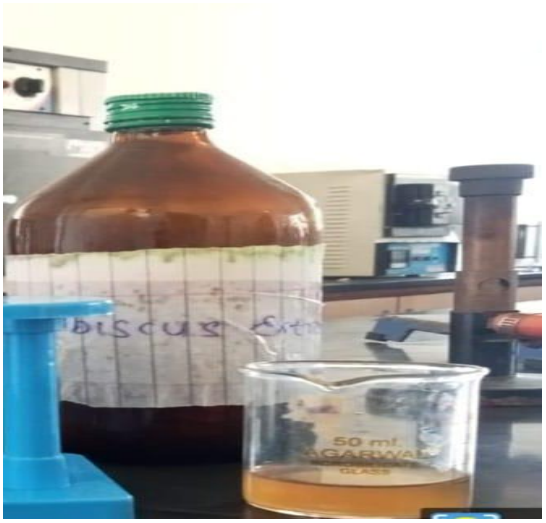


Fig:2

3.5 Herbal V wash Formulation Table:

3.6 Formulation Procedure:

1.(Phase A): Combine D.M. water & Rose water in beaker.

2.Slowly add White Hibiscus flowers extract & mix well until fully dissolved.

3.(Phase B): In a separate beaker, add cocamidopropyl betaine.

4.Add Aloe vera gel to the surfactant & mix gently to avoid foaming.

5.Slowly add Phase B into Phase A while stirring gently.

6.Adjust the pH. Ideal range is 3.8 - 4.5.

7.Finally, add preservative & stir thoroughly.

8.Pour into sterilized bottles [22,31]

3.7 Formulation Table

Content	F1H	F2H	F3H	F4H
Hibiscus Extract	6 mL	6 mL	4 mL	4 mL
Aloe vera gel	4 mL	5 mL	2 mL	3 mL
Cocoamidopropyl betaine	10 mL	8 mL	12 mL	14 mL
Rose water	12 mL	15 mL	11.5 mL	14.5 mL
Citric acid	1 mL	1 mL	0.5 mL	0.5 mL
D.M. water	67 mL	65 mL	70 mL	64 mL

Table 2: -Formulation Batches

F2H: The phase separation was observed. The V Wash unstable due to aloe gel

F3H: The V Wash was more scented due to increase in amount of rose water

F4H: The foaming agent quantity increase due to this V Wash become unstable

F1H: Phase separation, instability, low greasiness was not observed. The V Wash was stable

3.8 Optimized formula:

Content	Quantity (for 100ml)
Hibiscus Extract	6 ml
Aloe vera gel	4ml
Cocoamidopropyl betaine	10ml
Rose water	12ml
Citric acid	1ml
D.M.Water	67ml

4. PHYTOCHEMICAL TEST

Qualitative techniques for the determinations of phytochemicals.

4.1 ALKALOIDS TEST

1) Mayer's test:

A little quantity of plant extract is put to the test tube's sidewalls along with two drops of Mayer's reagent. A white, creamy precipitate is a sign that alkaloids are present [31].

4.2 FLAVANOID TEST

1) Sulphuric acid test:

On adding of H2SO4 (66% or 80%) flavonoid dissolve into it and gives a deep yellow solution [31].

4.3 CARBOHYDRATE TEST

1) Benedicts test:

Mix equal volume of Benedict's reagent and test solution in test tube, heat in boiling water bath for 10-15minute, solution appears green, yellow colour [31].

4.4 SAPONINS TEST

1) Foam test:

Take a small amount of extract in test tube and same quantity of water, shake the solution their foam generates and confirm presence of saponin[31].

4.5 TANNINS TEST

1) Lead acetate solution:

Take small amount extraction add lead acetate then form of white ppt [31].

4.6 RESULT:

SR.NO	IDENTIFICATION TEST	OBSERVATION	INFERENCE
1	ALKALOID TEST: Mayer's test		It shows the presence of alkaloid
2	FLAVANOID TEST: Sulfuric acid test		It shows the presence of flavonoid

3	CARBOHYDRATE TEST: Benedict's test		It shows the presence of carbohydrate
4	SAPONINS TEST: Foam test		It shows the presence of saponins
5	TANNINS TEST: Lead acetate test		It shows the presence of tannins

5. EVALUATION OF FORMULATION

5.1 Evaluation Parameters

1. Appearance

Colour - Pale yellow

Odour - Pleasant

Texture – Smooth [19,23]

2. Viscosity Determination

Ensure the viscometer is clean, calibrated, and on a level surface. Set up the viscometer on a stable stand. Attach Spindle No. 2 to the viscometer., Pour 50 mL of the sample into a clean, dry 100 mL beaker (or suitable container). Ensure the sample is homogeneous. Stir gently if needed (without introducing air bubble) Measure the temperature of the sample. If a specific temperature 25°C is required, use a water bath or temperature control [19]. Lower the viscometer head so that Spindle No. 2 is immersed in the sample up to the immersion mark. Avoid trapping air bubbles around the spindle. Set the viscometer to 38 RPM. Allow the spindle to rotate for about 30–60 seconds until the reading stabilizes. Once the reading stabilizes, record the viscosity value displayed in 1042 m.Pa·s. Take 2–3 readings and calculate the average for better accuracy. The viscosity of the sample was found to be 1042 using Brookfield Viscometer with Spindle No. 2 at 38 RPM at 25°C. The viscosity was found to be 1042 m.Pa.s [23].

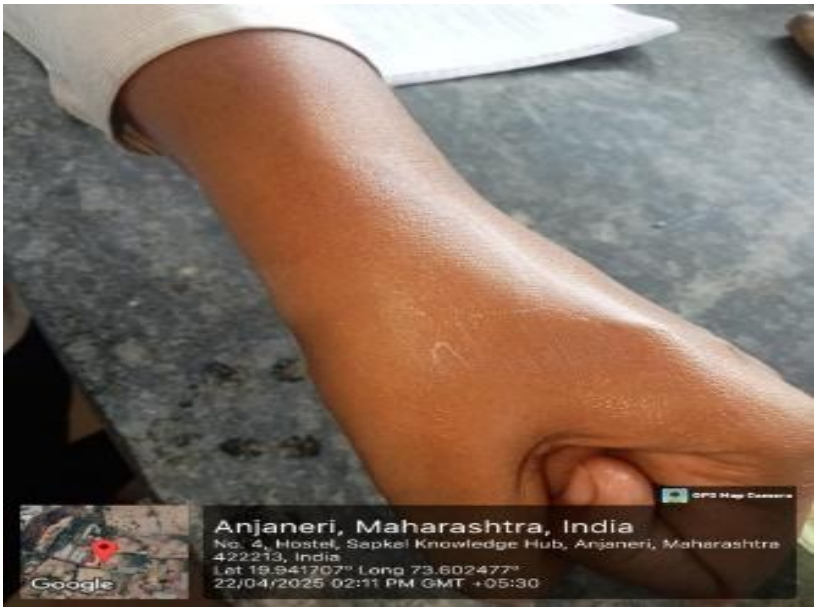
3. pH Determination

The prepared formulation was transferred to a clean beaker. The pH meter was set to pH mode, and the electrode was first immersed in distilled water. The meter was calibrated by adjusting the pH to 7 using a standard buffer solution.

The electrode was then gently wiped with a tissue to remove excess water. Next, the electrode was immersed in the sample, and the pH was measured. The pH reading was recorded. The pH was found to be 3.96 [19,23].

4. Skin Irritation Testing

The formulation was applied to the forearm of the hand to assess any signs of skin irritation. The area was observed for redness, itching, or other reactions are seen. The skin irritation test yielded a positive result. No irritation was observed in the skin irritancy test. There were no other visible reactions on the skin [19,23,25].



5. Foaming Ability

10 mL of distilled water was added to the sample. The cylinder was shaken vigorously. Foam formation 1 mL of the sample was taken in a graduated cylinder was observed, and the height of the foam was measured in millimeters. The foam height was recorded. Foam height was found to be 1 cm i.e. (a) [19,23,28].

Foaming Index = 1000/ a

Where a = volume in ml of the stock sol. Used in the tube

Where foaming to a height of 1 cm is observed.

Foaming Index = 1000/ 1

= 1000

5.2 OBSERVATION

SR.NO	PARAMETER	RESULTS
1.	pH	3.98
2.	Viscosity	1042 m Pa.s
3.	Foaming ability	10 mm height foam formation
4.	Skin Irritancy Test	No irritation

6. ANTIMICROBIAL ACTIVITY OF V WASH FORMULATION

6.1 Procedure:

Materials Required: Mueller-Hinton Agar (MHA), Bacterial strains (e.g., E. coli, S. aureus, F Pseudomonas, Bacillus.), Sterile filter paper disks (6 mm), Test compound or extract, Sterile Petri dishes, pipettes, forceps, and borer Inoculating loop, Sterile cotton swabs. Incubator (set to 37°C), Laminar air flow cabinet or biosafety cabinet.

Sterilization: Glassware & Instruments: Autoclave all glassware (Petri dishes, test tubes, pipette tips, forceps) at 121°C for 15 minutes at 15 psi, Dry items in a hot air oven if needed.

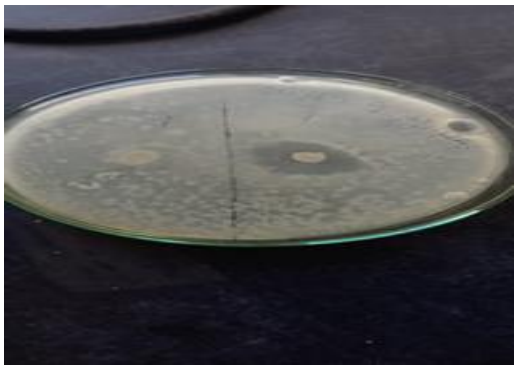
Media Preparation: Prepare Mueller-Hinton Agar (MHA) as per manufacturer instructions (usually 38 g/L). Take 3.8 g Mueller-Hinton Agar. Heat to dissolve completely, autoclave at 121°C for 15 min. Cool to ~45–50°C before pouring into sterile Petri dishes (20 mL per plate).

Disks & Solutions: Sterilize blank paper disks or purchase pre-sterilized one [17,19,21,27].

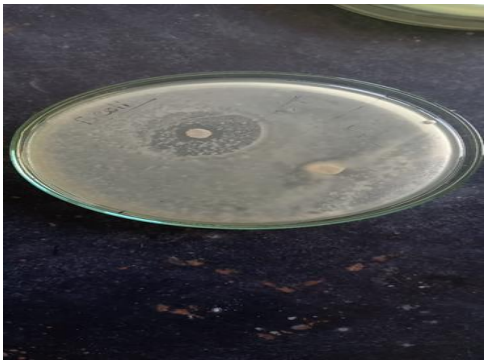
Preparation of Bacterial Inoculum: a single colony of the bacterial strain from a fresh culture plate. Inoculate into 5 mL of nutrient broth. Incubate at 37°C for 3–5 hours until turbidity matches 0.5 McFarland Standard (~1.5×10^8 CFU/mL).

Inoculation of Plates: Dip a sterile cotton swab into the bacterial suspension. Remove excess liquid by pressing against the tube wall. Swab the entire surface of the MHA plate horizontally, vertically, and diagonally to ensure even coverage. Let the

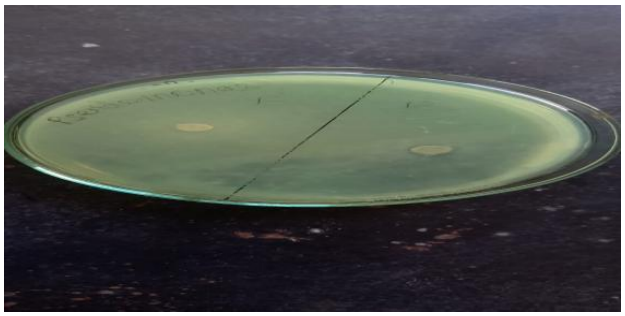
plate sit for 5 minutes to absorb the inoculum. V. Application of Disks. Impregnate disks with the test compound: Add 10–20 µL of test solution onto sterile paper disks using a micropipette. Allow disks to dry under sterile conditions to remove solvent. Place disks on the inoculated agar plate using sterile forceps. Include: Test disk(s) with compound. Slightly press the disks to ensure contact with the agar [17,19,21,27].



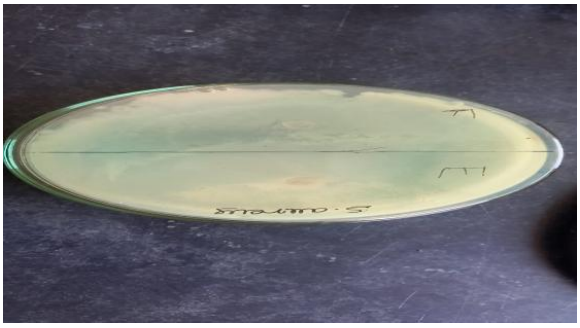
1.E.coli



2.Bacillus



3.Pseudomona aeruginosa



4. Staphylococcus aureus

SAMPLE	RADIUS (mm)	ZONE OF INHIBITION AREA ($mm^{2)}$	INTERPRETATION
E. coli	13	530.66	Active
Bacillus	10	314	Active
Pseudomonas aeruginosa	----	----	In Active
S. aureus	----	----	In Active

Incubation: Invert the plates. Incubate at 37°C for 18–24 hours in an incubator. Finally, results are noted.

6.2 RESULT

The test compound demonstrated antibacterial activity against E. Coli, Bacillus, Pseudomonas, S. aureus as indicated by a clear zone of inhibition measuring. The formulation F1 batch shows positive results against E. coli and Bacillus. This suggests the compound has potential as an antimicrobial agent. However, the activity was lower compared to the standard antibiotic. The negative control showed no inhibition, confirming that the observed antibacterial effect was due to the active compound, not the solvent. The results suggest that while the test compound has promising antibacterial activity, further purification, formulation, or combination therapy may enhance its effectiveness. Additional studies including time-kill assay and mechanism of action are recommended to better understand its therapeutic potential.

The Formulation Batch F1 was found to be the best as follows:

F2H: The phase separation was observed. The V Wash unstable due to aloe gel.

F3H: The V Wash was more scented due to increase in amount of rose water.

F4H: The foaming agent quantity increase due to this V Wash become unstable.

F1H: phase separation, instability, low greasiness was not observed. the V Wash was stable.

7. CONCLUSION

The present study successfully formulated a herbal vaginal V Wash utilizing white *Hibiscus rosa sinensis* flower extract, which is rich in tannins includes gallic acid, methyl gallate, ellagic acid, in combination with Aloe vera gel, cocoamidopropyl betaine, rose water, and demineralized water. The formulation was developed to target *Gardnerella vaginalis, E. coli, Bacillus* a key causative agent of leukorrhea, a condition commonly affecting women of reproductive age. The tannins includes gallic acid, methyl gallate, ellagic acid in *Hibiscus rosa sinensis* known for their antibacterial properties, along with the soothing and healing properties. Aloe Vera and rose water, contribute synergistically to the efficacy of the formulation. The resulting V-Wash is a gentle, natural alternative to chemical-based feminine hygiene products, offering both antibacterial action against *E coli, Bacillus* and also for vaginal flora support

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