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DEVELOPMENT, CHARACTERIZATION AND EVALUATION OF HERBAL HANDWASH TABLET

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ABSTRACT

The herbal handwash tablet is formulated using tea tree oil and is sandalwood powder. It is travel friendly, easy to carry, and has a Lavender and sandalwood fragrance. It has antimicrobial properties as well as antifungal properties and also prevents skin related problems such as ringworm, eczema, contact dermatitis, actinic keratosis, etc. It has good antimicrobial activity. A formulation of herbal handwash tablet is based on the crude drug and synthetic chemicals, with the core ingredient tea tree oil and sandalwood powder. It is suitable for human skin and can be a therapeutic alternative to skin problems. Herbal handwash tablet was tested by both pre-evaluation and post compression tests which gave good results for all the batches. The herbal handwash tablet was tested for antimicrobial activity by agar well diffusion assay in various concentration. The sample shows strong antimicrobial activity at different concentration. The herbal handwash tablet was prepared using wet granulation method. Herbal handwash tablet is mild on the skin, avoids harsh chemicals that might cause hand irritation or dryness, and possibly adding plant based moisturizing component. Tea tree oil work on Escherichia coli and Staphylococcus aureus by killing or inhibiting their growth. Tea tree oil is effective for hand disinfection, killing bacteria without antibiotics. Beyond physical health, sandalwood supports emotional and spiritual well-being, marketing it a holistic choice comprehensive wellness.

KEYWORDS: Hand hygiene, Tea tree oil, herbal formulation, antimicrobial activity, sandalwood powder.

INTRODUCTION

Herbal handwash tablet is formulated using tea tree oil and sandalwood powder, which effectively kills germs. It smells of sandalwood and is portable and easy to transport. Additionally, it contains antibacterial qualities and guards against skin conditions like ringworm, eczema, fungal infections, contact dermatitis, and more. Its antibacterial activity is good. The antibacterial and antifungal qualities of herbal extracts are being investigated for potential human use. Tea tree oil is used as the primary ingredient in a herbal handwash tablet formulation that is based on plant extract. It is safe for human skin and may be used as a treatment for skin conditions.

Tea tree oil has antimicrobial, antifungal and skin smoothening properties. It is potent in very small quantity. Sandalwood has a pleasant fragrance and also possess an excellent moisturizing and anti-inflammatory properties. Sandalwood is known for its ability combat bacteria and fungi providing a natural defense against infection that may other herbs lack.

The heartwood of the sandal tree is used to make sandalwood, which has long been valued in traditional and Ayurvedic medicine. It is a popular ingredient in natural skincare products because of its medicinal qualities and pleasant, woodsy scent. Sandalwood is a perfect addition to herbal handwashes because of its well-known antibacterial, anti-inflammatory,. Your hands will be both cleansed and nourished if you use a handwash that contains sandalwood essence. It offers a mild washing experience that honours the skin's natural equilibrium, leaving it feeling moisturized, soft, and subtly fragrant.

Why hand hygiene is important?

The Greek goddess of healing the word "hygiene" comes from the word "hygeia." Nowadays, the word "hygiene" usually refers to cleaning, especially any action that lessens or gets rid of dangerous infectious pathogens. The different spellings of words like "handwashing," "hand washing," and "hand-washing" in the medical literature show how diverse the terminology employed in hand hygiene is. Such variances could lead to irregular article archiving. The definitions of the words are important since standardization of definitions is necessary for reliable comparisons between studies. The most crucial step in preventing infections is washing your hands, and there is proof that clear definitions of terminology help people

follow hand hygiene recommendations more successfully.

Wash your hand thoroughly with soap and water. This will wash away the pollutants. The quantity of pathogenic organisms is significantly decreased by hand washing. Additionally, it should be applied when patients with diarrhea interact with other patients; hand rubs containing alcohol won't be effective in this situation. Hand cleaning with soap and water should be done according to standard operating procedure.

Significance of herbal handwash

Many of the antiseptic hand washes available on the market contain alcohol-based sanitizers, which might be problematic. An attempt has been undertaken to develop a polyherbal hand wash employing extracts from synthetic handwashes in order to prevent these side effects, which include dermatitis, itching, dryness, and irritation. The produced polyherbal hand wash's efficacy against volunteer skin infections was evaluated using the Cup Plate Method. The Cup Plate Method showed that hand wash made from an alcoholic extract and an aqueous extract of ginger rhizomes is efficient because of the combined action of the phytoconstituents included in the extracts. The results of the present study lend support to the fusion and the usage of spices in definitions to enhance their impact.

A test was used to evaluate herbal hand wash.

The herbal medicine is also known as phytomedicine or botanical treatment. The use of any plant's seeds, roots, leaves, bark, flowers, or aerial parts for medicinal purposes is known as herbal medicine. Since the skin is the most exposed part of the body, it needs to be protected from skin pathogens. Herbal medicine has been used to treat and care for many diseases.

Numerous chemical antiseptics, such as alcohol-based sanitizers and chlorhexidine products, are now available on the market. These soaps or solutions help prevent contagious disease transmission in healthcare settings more effectively, but they have some drawbacks or side effects. They can irritate the skin and make pathogens resistant if used frequently.

Practicing good hand hygiene can help stop the spread of harmful bacteria. Research has demonstrated the efficacy of different cleansers. The development of resistant microorganisms and antibacterial soaps have drawn criticism. The FDA limits triclosan and triclocarban-containing antiseptic wash products from being marketed. To lower the hand microbial burden, foam soaps are more frequently used and more affordable in the food

sector, healthcare, and educational environments. In order to prevent nosocomial infections, skin pathogens must be avoided. To lessen the spread of MDRs, hand care workers should wash their hands with an antiseptic. In medical environments, antiseptic chemicals decrease the spread of infectious diseases. Regular usage of these may result in infection resistance and skin discomfort. Microorganisms including Pseudomonas aeruginosa and Staphylococcus aureus are responsible for skin infections.

Some research claim that epidemics have been caused by resistance to chemical antiseptics. Antimicrobials derived from plants are safe and effective in treating infectious illnesses. It has been discovered that flavonoids and polypeptides, which are present in plants used in traditional medicine, are effective against a variety of bacteria. Herbal medicine promotes a healthy lifestyle by using plants for therapeutic purposes. It was frequently utilized to supply common and first-line healthcare providers. In India, herbal remedies have been utilized for centuries to heal and cure illnesses. Herbal remedies can be used to treat a wide range of ailments. One of the most crucial defences against dangerous bacterial infections and diseases is practicing good hand hygiene. Hand washing removes soil, dirt, and microorganisms to prevent transmission.

Surfactants are frequently employed in cleaning solutions, but repeated use of their emulsifying or solubilizing properties might harm the skin's dermal oils. Natural sources are cheaper, more readily available, and safer than chemical products. Research is needed to find novel, safe, and efficient antimicrobial medicines. This work creates herbal hand soap using a variety of plant extracts that may have antibacterial properties. Traditional uses of ocimum sanctum include treating illnesses, their consequences, and microorganisms. One 2017 research that appeared in the International Journal of Current Microbiology and Applied Sciences examined the antibacterial efficacy of neem, tulsi, and aloe vera-based herbal handwash solutions. The study came to the conclusion that these natural substances might be employed as viable substitutes for synthetic antimicrobial agents.

Why tea tree oil?

Complementary and alternative medicines such as tea tree (melaleuca) oil have become increasingly popular in recent decades. This essential oil has been used for almost 100 years in Australia but is now available worldwide both as neat oil and as an active component in an array of products. The primary uses of tea tree oil have historically capitalized on the antiseptic and anti-inflammatory actions of the oil. This review summarizes recent

developments in our understanding of the antimicrobial and anti-inflammatory activities of the oil and its components, as well as clinical efficacy. Specific mechanisms of antimicrobial and anti-inflammatory action are reviewed, and the toxicity of the oil is briefly discussed.

Many complementary and alternative medicines have enjoyed increased popularity in recent decades. Efforts to validate their use have seen their putative therapeutic properties come under increasing scrutiny in vitro and, in some cases, in vivo. One such product is tea tree oil (TTO), the volatile essential oil derived mainly from the Australian native plant *Melaleuca alternifolia*. Employed largely for its antimicrobial properties, TTO is incorporated as the active ingredient in many topical formulations used to treat cutaneous infections. It is widely available over the counter in Australia, Europe, and North America and is marketed as a remedy for various ailments.

Advantages of herbal handwash tablet

1. No adverse effects.
2. We can reduce the number of bacteria on our hand.
3. It also aids in the resolution of antiseptic and fungal issues.
4. It also aids in the efficient removal of oil and debris form the skin.
5. Easy to use in contrast to soap and water.
6. The simplest method for eliminating microbes.
7. Washing our hands keeps pathogens out of our bodies.

Ideal characteristic of herbal handwash tablet

1. Ideal herbal handwash tablets should be effective at killing germs, easy to carry, and have antimicrobial properties.
2. They should also be suitable for human skin and help prevent skin conditions.
3. Should minimize potential side effects.
4. Should thoroughly clean hands.
5. Should aid in the prevention of skin disorders such as contact dermatitis, ringworm, eczema, and fungal infections.
6. It needs to be appropriate for human skin.
7. Should be a medicinal substitute for skin issues
8. Herbal handwash's main goal is to thoroughly clean hands while reducing any possible negative effects.

Tablet

A tablet, sometimes referred to as a pill, is a solid unit dosage form or oral solid dosage (OSD) used in pharmaceuticals. A solid unit dose form of medication with appropriate excipients is called a tablet.

Tablet manufacturing procedures

1. Wet Granulation
2. Dry granulation
3. Hot melt extrusion

1) Wet Granulation

Using a liquid binder to gently agglomerate the powder combination is known as wet granulation. Since too much liquid will make the granules too hard and too little would make them too soft and friable, the amount of liquid must be carefully regulated. Although aqueous solutions are safer to work with than solvent-based systems, they might not be appropriate for medications that hydrolyze.

Procedure

1) Excipients and the active substance are combined and weighed.

2) The liquid binder-adhesive is added to the powder mixture and well mixed to create the wet granulate.

3) Aqueous cornstarch preparations, natural gums like acacia, and cellulose derivatives like methyl cellulose, gelatin, and povidone are a few examples of binders and adhesives.

4) Form pellets or granules by passing the moist mixture through a mesh screen.

5) Granulation drying. The most widely used dryers are the traditional tray-dryer and the fluid-bed dryer.

6) To produce granules of consistent size, the dried granules are run through a screen that is smaller than the one used for the wet bulk.

Low shear wet granulation methods might take a long time to reach a uniformly mixed state and require very basic mixing equipment. Equipment used in high shear wet granulation procedures rapidly combines the liquid and powder, accelerating the manufacturing process. In order to pre-heat, granulate, and dry the powders, fluid bed granulation is a multi-step wet granulation procedure carried out in the same vessel.

Because it enables precise control over the granulation process, it is utilized.

2) Dry granulation

By lightly compacting the powder mixture at low pressures, dry granulation methods produce granules. Granules (agglomerates) are created by gently breaking up the soformed compacts. When the product to be granulated is heat and moisture sensitive, this method is frequently employed. Dry granulation can be done on a roll press known as a roller compactor or on a tablet press using slugging tooling. A variety of pressures are available with dry granulation equipment to achieve the right densification and granule formation. Because dry granulation is easier than wet granulation, it is less expensive. Dry granulation, on the other hand, frequently results in a higher proportion of fine granules, which may lower tablet quality or cause issues with yield. Drugs or excipients having cohesive qualities are necessary for dry granulation, and in order to promote granule formation, a "dry binder" may need to be added to the formulation.

3) Hot melt extrusion

In pharmaceutical solid oral dosage manufacturing, hot melt extrusion is used to deliver medications with low solubility and bioavailability. It has been demonstrated that hot melt extrusion can molecularly distribute poorly soluble medications in a polymer carrier, accelerating their rates of dissolution and bioavailability. In order to combine materials and "extrude" them via a die, heat, pressure, and agitation are applied. High shear extruders with twin screws combine materials and break up particles at the same time. Following extrusion, the particles can be combined and compacted into tablets or capsules.

- **Advantages of tablet**

1. Compared to alternative solid and liquid dosage forms, tablets provide a variety of potential benefits
2. Unit dosage form (accuracy of dosing).
3. It's easy to swallow.
4. Highly soluble, easy to produce.
5. Economical for packing, shipping, and storage.

- **Drawbacks**

1. Compressing highly amorphous materials is quite challenging
2. Drugs with poor wetting and slow dissolving cannot be put in tablet form.

3. Bitter-tasting chemicals require specific compression care.

AIM AND OBJECTIVE

Aim

- Developing a handwashing solution that effectively kills germs using natural, plant-based extracts.
- To minimize skin irritation and promoting a safer alternative to conventional chemical-based hand wash tablet.
- Because of the tablet format, these products frequently focus on portability and convenience while attempting to provide thorough hand cleansing with potential added.
- benefits like skin conditioning from herbal ingredients.
- To show how well herbal extracts work against a variety of bacteria and fungus, guaranteeing good hygiene by eliminating dangerous microbes on hands.

OBJECTIVES

- To create a handwash tablet that is mild on the skin, avoiding harsh chemicals that might cause hand irritation or dryness, and possibly adding plant-based moisturizing component
- To promote a more ecologically friendly choice by using easily accessible plant extracts with well-known antibacterial qualities, such as neem, sandalwood powder, tea tree oil, etc.
- To create tablets that are small, portable, and easily dissolved in water, making them appropriate for use on the go or in locations with restricted access to liquid soap.
- To keep the emphasis on herbal qualities while adding natural essential oils for a pleasing aroma.
- To investigate cost-effective alternatives by using herbal compounds that are produced locally, which could open up the product's market.
- Comparing various plant extracts' antibacterial properties to those of conventional chemical hand soaps.
- Investigating various herbal extract and excipient combinations to attain the best possible cleansing and skin compatibility.
- Obtaining opinions on the herbal handwash tablets' overall acceptability, efficacy, and scent.

Need of Investigation

- **Efficacy of active Ingredient**

➢ **Tea tree oil**

Antimicrobial Effectiveness: Tea tree oil is widely recognized for its antibacterial, antifungal, and antiviral properties. Investigate the level of antimicrobial activity that tea tree oil retains in a solid tablet form. Does it effectively kill germs, viruses, or bacteria when used as a handwash?

Compatibility with Sandalwood: Explore how tea tree oil interacts with sandalwood powder. Are there any conflicting properties that may reduce the effectiveness of either ingredient, or do they complement each other in their antimicrobial and soothing benefits?

Concentration: Tea tree oil is potent in very small quantity. Ensure to use tea tree oil in an effective concentration.

- **How does it work?**

Bacteria such as Escherichia coli and Staphylococcus aureus can be killed or their growth inhibited by tea tree oil.

The components of the oil, particularly terpinen-4-ol and -terpineol, cause bacterial membranes to lose their structural and functional integrity, which ultimately results in their death.

Tea tree oil germ fighting properties make it a valued natural remedy for treating bacterial and fungal skin conditions preventing infection and promoting healing.

- **Use of Tea tree oil in handwash tablet**

1. Tea tree oil is effective for hand disinfection, killing bacteria without antibiotics.
2. Tea tree oil prevents infection in cuts and abrasions and when combined with traditional methods may also encourage wound healing.
3. Tea tree oil may help reduce inflammation and trigger the activity of white blood cells that are essential in the healing process.

- **Activities of tea tree oil**

1. Antibacterial activity
2. Antifungal activity
3. Insect repellent

4. Natural deodorant
5. Antiseptic for minor cuts and scrapes
6. May support wound healing
7. Reduce acne

- **Side effects of Tea tree oil**

1. Tea tree oil can cause skin irritation, allergic contact dermatitis.
2. Swallowing tea tree oil can be serious.
3. Redness on the skin.
4. Can dry out the skin.

- **How to overcome side effects of tea tree oil:**

1. Dilute it with a carrier oil like coconut or almond oil before applying to the skin.
2. Perform a patch test before widespread use and discontinue use immediately if any irritation occurs.

➢ **Sandalwood powder**

▪ **Efficacy of Active Ingredient**

Antiseptic and Anti-inflammatory Properties: Investigate how effective sandalwood powder is in cleansing and soothing the skin. It is known for its calming properties.

Skin Nourishment: Explore the moisturizing and skin-repairing effects of sandalwood powder.

Long-Term Benefits: Research whether the benefits of sandalwood powder are preserved when it's mixed with other ingredients, and how it performs in a dry tablet form versus a liquid handwash.

- **How does it work?**

Sandalwood is known for its ability to combat bacteria and fungi providing a natural defence against infections that may other herbs lack.

- **Cultural Significance**

Sandalwood has a rich history in traditional medicine and rituals across various cultures lending it a sense of depth and tradition that may other herbs may not possess.

- **Holistic Benefits**

Beyond physical health, sandalwood supports emotional and spiritual well-being, marketing it a holistic choice comprehensive wellness.

- **Activities of sandalwood**

1. Reduces Inflammation
2. Treats Acne and Blemishes
3. Lightens Dark Spots and Pigmentation
4. Reduces Skin Irritation
5. Reduces Sunburn

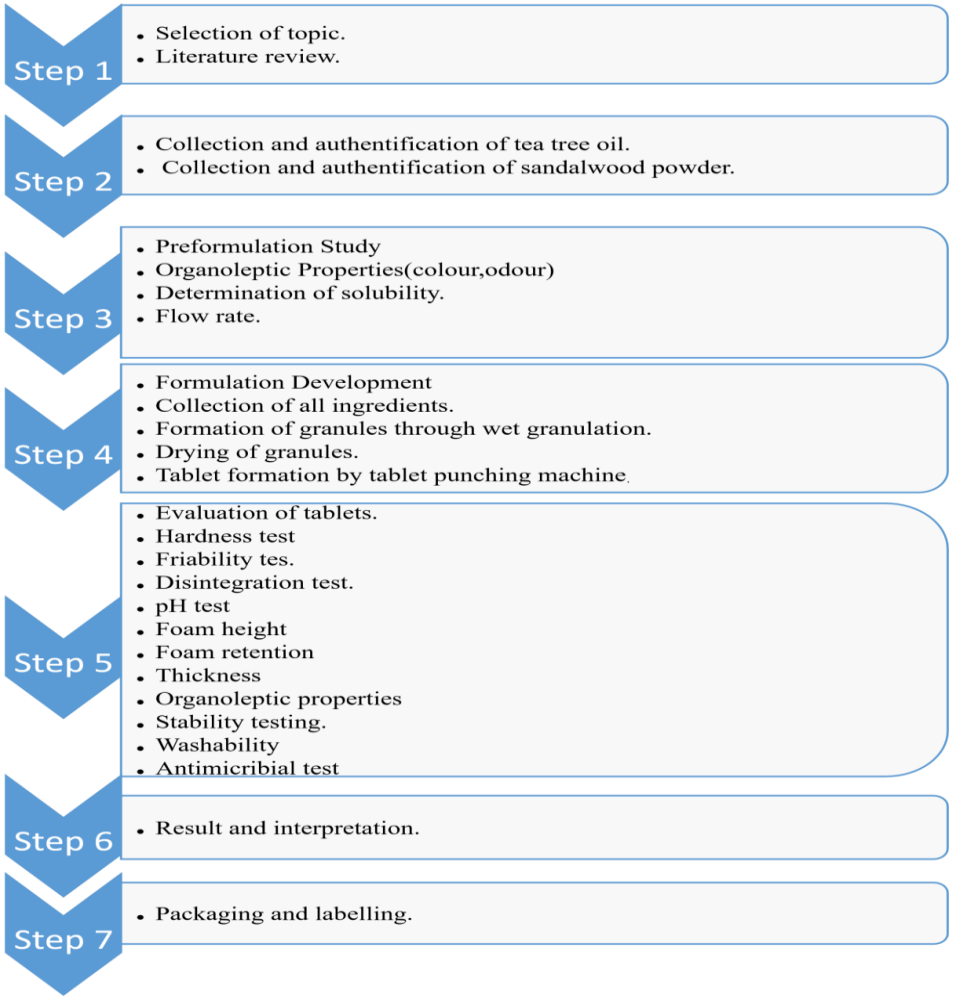
- **Side effects of sandalwood**

1. Skin Irritation
2. Allergic Reaction
3. Contact Dermatitis

- **How to overcome side effects**

1. Conduct patch test before using it on the skin.
2. Be cautious with sensitive areas such as around eyes and mucous membrane.

❖ **Plan of Work:**



❖ MATERIAL AND METHODS

Table No. 02.

Ingredient	F1 (g)	F2 (g)	F3 (g)	F4 (g)	F5 (g)
Tea tree oil	0.4	0.5	0.6	0.7	0.8
Sandalwood powder	2	2.4	2.8	3.2	3.6
Corn starch	3.6	3.4	3.2	3	2.8
Glycerine	1	1	1	1	1
Sodium lauryl sulphate	1.6	1.6	1.6	1.6	1.6
Magnesium stearate	1	1	1	1	1
Citric acid	1.4	1.4	1.4	1.4	1.4
Sodium bicarbonate	4	4	4	4	4
Lavender oil	0.4	0.4	0.4	0.4	0.4
Colouring agent (Beetroot)	2.6	2.7	2.4	2.7	2.4

Procedure

Weigh the required quantities of tea tree oil, sandalwood powder and other excipients.

↓

Pass sandalwood powder, fillers and disintegrants through a fine sieve to ensure uniform particle size.

↓

Mix dry ingredient (sandalwood powder, fillers, disintegrants)

↓

Prepare liquid binder and gradually add the liquid binder to the dry mix while stirring to form a damp mass with a uniform consistency.

↓

Add tea tree oil to the damp mass during the wet mixing stage.

↓

Pass the damp mass through the sieve to form granules, allow the granules to air dry or hot air oven.

↓

Once dried mix the granules with lubricant and use a tablet press to the compress granules into tablets.

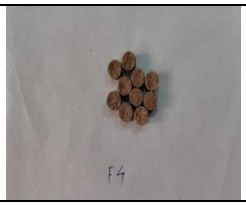
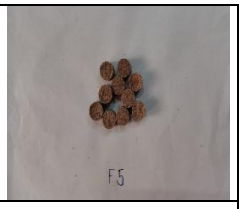
RESULT AND DISCUSSION

Pre-evaluation Tests of Tablet

Table No. 03.

Tests	F1	F2	F3	F4	F5
Bulk Density(g/ml)	0.45	0.47	0.47	0.4	0.4
Tapped Density (g/ml)	0.55	0.58	0.52	0.52	0.45
Loss on Drying (%)	0.03	0.01	0.03	0.03	0.016
Angle of Repose	25.64	26.10	27.02	23.26	25.17
Carr's Index (%)	0.18	0.19	0.09	0.23	0.11
Hausner's Ratio(g/ml)	1.22	1.23	1.10	1.3	2

Table No. 05: Formulation Batches

				
F1	**F2**	**F3**	**F4**	**F5**

Evaluation Tests

1) pH Detection Test

Dissolve one handwash tablet in 100ml of purified water and measure the pH using digital pH meter.

Table No. 06.

Formulation	Attachment	pH of The Tablet
F4		6.27
F5		6.28

2) Foam Height and Foam Retention Test

Foam Height

Dissolve 0.5 gram of sample in 25 ml of distilled water and pour it into 50ml measuring cylinder. Shake the measuring cylinder for 30 seconds and measure the height.

Foam Retention Test

Dissolve 1g of herbal handwash tablet in 50ml of distilled water and shake the water for 30 sec.

Volume of foam was measured at two-minute interval for 6 minutes.

Table No. 07.

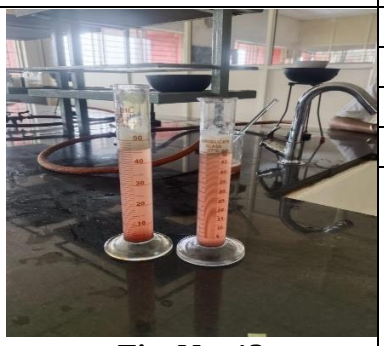
Formulation	Attachment	Foam Height	Foam Retention		
			2 min	4 min	6 min
F1		5cm	4.7cm	4.2cm	4cm
F2		7cm	7cm	6.5cm	5cm
F3		3cm	3cm	2.7cm	2cm
F4		5cm	4.5cm	4cm	3.5cm
F5		3.5cm	3cm	2.5cm	2cm

Fig. No. 13

3) Disintegration Test

Fill each of the basket rack assembly's six tubes with one dosage unit (tablet).

Make that the water used for immersion is at the proper temperature (37°C).

Using a predetermined frequency (e.g., 28–32 times per minute), raise and lower the basket rack assembly in the immersion fluid. Throughout the test, keep an eye on the dosage units.

Table No. 08.

Formulation	Attachment	Disintegration Time
F1		15 min
F2		12 min
F3		16 min
F4		14 min
F5		13 min

Fig. No. 14

4) Friability Test

The likelihood that a tablet will shatter or crumble during handling, packaging, shipping, or distribution is determined by a friability test.

The equipment used to perform a friability test is called a friabilator or friability tester.

Table No. 09.

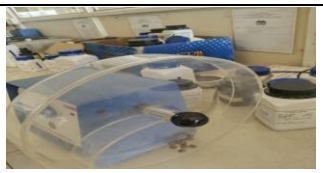
Formulation	Attachment	Percent Friability
F1		0.59
F2		0.60
F3		0.49
F4		0.59
F5		0.74

Fig. No. 15

5) Hardness Test

Hardness testing is a critical method used to evaluate how resistant a material is to indentation, scratching, or permanent deformation under load. By pressing a harder material (often a specific indenter) into the test material's surface and measuring the depth or size of the resulting indentation, hardness tests give valuable insights into several key material properties.

Table No. 10.

Formulation	Attachment	Hardness (kg/cm2)
F1		4.38
F2		4.28
F3		4.12
F4		4.08
F5		4.34

Fig. No. 16

6) Thickness Test

The thickness test of a tablet is an important quality control measure in the pharmaceutical industry.

The tablet is placed between the jaws of the calliper, and the reading is taken in millimetres.

Table No. 11

Formulation	Attachment	Thickness
F1		0.6
F2		0.6
F3		0.6
F4		0.6
F5		0.6

Fig. No. 17

7) Organoleptic properties

Appearance: The herbal handwash tablet was inspected for its colour, odour, shape and size.

Aroma: Aroma of the tablet was assessed by smelling it. Tablet has a plesant smell. Texture: Texture was assessed by rubbing it on hands.

Table No. 12.

Formulation	Attachment	Appearance	Odour
F1		Brown	Pleasant
F2		Brown	Pleasant
F3		Brown	Pleasant
F4		Brown	Pleasant
F5		Brown	Pleasant

Fig No. 18

8) Washability

Dissolve one herbal handwash tablet in 50ml distilled water.

Use this solution to wash hands and rate the washability of the tablet (Good or bad) based on its cleansing effect.

Table No. 13.

Formulation	Attachment	Washability
F1		Excellent
F2		Very Good
F3		Good
F4		Good
F5		Good

Fig. No.19

9) Stability

As part of an expedited stability test, a herbal handwash tablet was kept at 40°C and 75% relative humidity for three months. At different points of time, the tablet's appearance, texture, scent, and dissolvability were evaluated. The results showed that the tablet's organoleptic properties did not change substantially, nor did its stability.

Table No. 14

Formulation	Attachment	Stability
F1		Good
F2		Good
F3		Good
F4		Good
F5		Good

Fig. No.20

10) Anti-microbial test

Antimicrobial activity of topical agent was determined by agar well diffusion assay, in this various concentration i.e. 25 µg, 50 µg,75 µg and 100 µg of topical agent directly taken from final formulation aseptically by sterile spatula and loaded in wells which are prepared by using sterile cork-borer on Luria Agar plates having diameter 0.7cm. After that plates were keep at 27 ˚C for 24 hrs for incubation, after incubation plates were observed for zone of inhibition around the well.

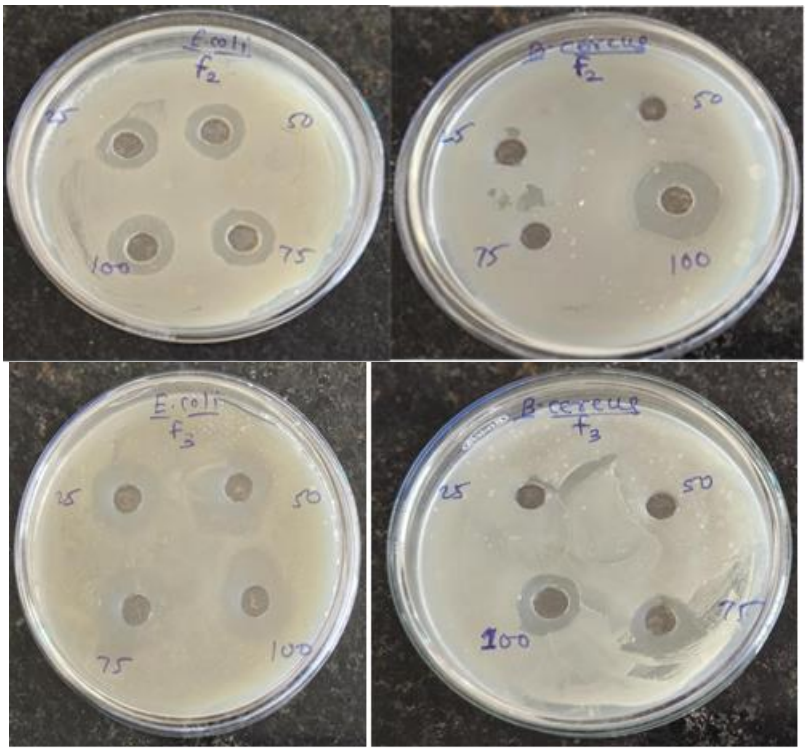
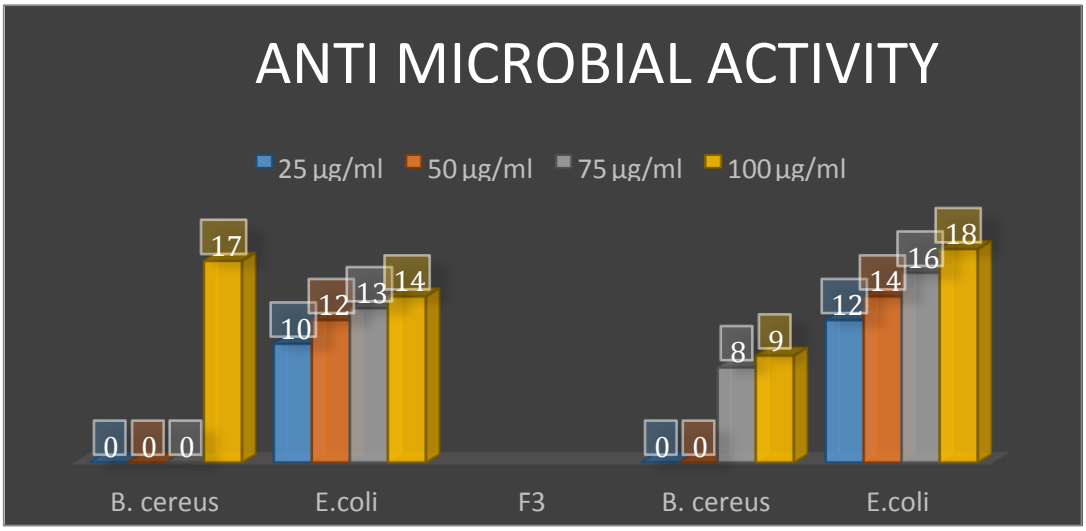


Fig. No 21: Anti-microbial Test.



RESULT AND CONCLUSION

The given sample exhibited strong antimicrobial activity at different concentrations.

❖ CONCLUSION

From above all observation it was found that F2 batch of herbal handwash tablet is more effective and potent. It has a good washability and stability. Batch F2 contain 2.5% tea tree oil and 12% sandalwood powder. The main source of illnesses affecting the skin, respiratory system, gastrointestinal tract, etc., is the hands. The bar soap becomes contaminated as a result of several illnesses and bacteria, which could cause the germs to spread. The main advantage of herbal handwash tablet is they are travel-friendly, easy to store, and can be more effective in killing germs with natural ingredients like tea tree oil and sandalwood powder. Additionally, herbal handwashes are less likely to cause skin irritation and offer an eco-friendlier option due to reduced packaging waste.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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