# Formulation, Physico-chemical evaluation and comparative stability studies of *Dillenia indica* (pulp) based antidandruff and anti hair fall cream with marketed brand


## ABSTRACT

**Background:** *Dillenia Indica* as a medicinal plant is one of the widely used herbs by the various tribes of entire North East (mostly in Assam) and traditionally the jelly like content inside the fruit was used to treat dandruff and falling hairs. **Objectives:** The current work was performed with an objective to assess the antidandruff and anti hair fall property of *Dillenia indica* (pulp), the jelly like content inside the fruit with a marketed brand. **Methods:** With utilization of different concentrations of *Dillenia indica* (pulp) as main drug and with other chemicals different cream formulations are made and optimized. All the formulations along with marketed brand are investigated for their physico-chemical properties. Stability studies of freeze-thaw, elevated temperature exposure and thermal stability study was performed. All the results of the formulations were compared with marketed brand. **Result:** All the result of freeze-thaw, elevated temperature exposure and thermal stability study of all formulations (F1,F2,F3) shows similar characteristic behavior with marketed brand except in elevated temperature exposure where all the formulations fails to withstand the temperature excluding the marketed brand (**BRYLCREEM**). Physico-chemical parameters like $p^H$, FFA, TFM has been studied for formulations F1, F2, F3 and with brand product. All the formulations along with brand product are showing similar kind of result along with similar Spreadability and homogeneity.



**Key words:** Free fatty acid (FFA), Total fatty matter (TFM), Freeze-Thaw stability, Elevated temperature exposure, Spreadability.


## INTRODUCTION

Medicinal plants have long been used in traditional medicine and ethnomedicine all across the world. These medicinal plants' constituents have exhibited a variety of therapeutic qualities that can be employed in drug research and synthesis. Medicinal plants had a significant part in the evolution of human societies all across the world. *Dillenia Indica*, also known as elephant apple [1] or chulta, is a Dillenia species native to southeastern Asia, ranging from

India, Bangladesh, and Sri Lanka through southwestern China (Yunnan) and Vietnam, and south via Thailand to Malaysia and Indonesia. It is a evergreen shrub or small to medium-sized tree that can reach a height of 15 metres. Its branches serve as excellent firewood. The flowers are big, with five white petals and a diameter of 15–20 cm. Its distinctive round fruits are huge, greenish yellow, and contain many seeds. The fruit has a diameter of 5–12 cm and is made up of 15 carpels, each of which contains five seeds embedded in an edible but fibrous pulp. In India, Nepal, Bangladesh, and Sri Lanka, it is widely available [2]. It is mostly found in Assam's north-east region. It is known as Outenga in Assamese, Bharija in Sanskrit, and Indian catman in English [3].

*Image 1:* ***Dillenia Indica***

**TRADITIONAL & MEDICINAL USES OF *Dillenia indica:***

*D.indica* (Outenga) is a commonly utilised herb by numerous tribes throughout the North East (mostly in Assam)

ii. It is an evergreen plant that can be found in the natural forest. It was traditionally used to treat dandruff and falling hairs because of the jelly-like material inside the fruit [4].

iii. It has been discovered that the juice of leaves, bark, and leaves was blended and given orally for the treatment of cancer in many parts of North East India in the past.

iv. A mixture of bark and leaves from *D.indica* is used to cure diarrhoea.

v. The leaves and bark are used as astringents and laxatives.

vi. *D.indica* has antimicrobial action.

vii. It's a word that's employed

Stability tests are carried out under a variety of temperature and humidity settings. Leaving humidity aside, there are two reasons for incubating a product at different temperatures [5]. The first step is to establish product stability at room temperature for various locations of the world; the stability test involves all types of environmental stress. Accelerated-time data, when combined with accelerated conditions, provides a complete view of the cream product's stability issues, which can also be seen in real-time investigations [6]. Physical and chemical stability tests are crucial in describing product properties over time and under various environmental circumstances. Accelerated-time studies

are the only ones that can provide reliable information [7]. The tests are frequently carried out at 37°C, 400°C, and 450°C during 1, 2, 3.....8 months but the temperature used and the duration will depend on the product type. Temperature cycling and/or a "freeze-thaw" test can uncover some types of flaws faster than storage at a constant temperature. For specific types of items, freeze-thaw testing should be considered [8].

Suspension problems (a tendency to crystallise or cloud), emulsion and cream instability, package design issues (such as wrinkling or loss of label, cracking or distortion), and corrosion of internal lacquers in during storage and transport are examples of problems that can be detected by freeze-thaw testing. Stability testing at these extremes should be considered, for example:

a) Freeze-thaw testing and low-temperature testing

c) Thermal stress testing at high temperatures.

**VARIABILITY IN PARAMETERS DURING THE COURSE OF A PRODUCT'S SHELF LIFE**

It's crucial to keep in mind when building a stability test protocol that as the qualities of a product may alter as it ages. As a result of this, stability testing may require testing of properties other than those that would be reviewed during first release testing Because the test will vary depending on the product category and package type, the following criteria are provided as an example only and are neither exhaustive nor a minimum requirement

1. Appearance, colour, and odour
2. Viscosity
3. Container modifications
4. Weight fluctuations
5. PH
6. Microbial test demonstrating the product's ability to prevent microbial growth during normal use, as well as other tests as needed.
7. Analytical data for a given product type in relation to other criteria [10].

**MATERIAL AND METHODS**

Fruit of *Dillenia indica* was collected in January from Lakhimpur, Assam. The plant material was authenticated by Dr. P.P. Baruah, Head Department of Botany, Gauhati University, Authantication No. Acc.No.18398 dt.15.02.2018

**CHEMICAL REQUIREMENT**

**Table 1: Chemicals required**

| CHEMICAL NAME | COMPANY NAME |
|---|---|
| Olive oil | Himedia |

| | |
|---|---|
| Potassium hydroxide (KOH) | Mark |
| Glycerin | Himedia |
| Stearic acid | Himedia |
| Methyl paraben | Merck |
| Polypropylene glycol | Merck |
| Hydrochloric acid (HCL) | Ranbaxy |
| Methyl orange | Merck |
| Petroleum benzene | Merck |
| Methanol | Merck |
| Phenolphthalein | Merck |

**Drug**

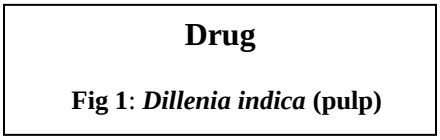


**Fig 1**: ***Dillenia indica*** **(pulp)**

## CHEMICAL USED FOR FORMULATION:

**Table 2:**

| |
|---|
| Olive oil |
| Potassium hydroxide (KOH) |
| Glycerin |
| Steric acid |
| Methyl paraben |
| Polypropylene glycol |
| Water |
| Perfume |
| Drug (Dillenia indica pulp) |

## METHODS:

**1. Parameters for analysis**

- $p^H$
- Total fatty matter
- Free fatty acid
- Spredebility
- Homogeneity

**2. Stability study**

a. Stress stability study

i.Freeze Thaw stability

ii.Elevated temperature exposure

iii.Thermal stability

- **Determination of $P^{H:}$ Procedure:**

**a)** For $p^H$ determination of cream (10% w/v) solution is made.

**b)** For cream 10 gram sample is taken and volume is made up to 100 ml to make 10% w/v solution. After that the electrode of the $p^H$ meter is dipped in the solution to measure the solution $p^H$

**c)** For lotion $p^H$ is determined as such.

- **Determination of TFM (Total Fatty Matter)**

**Procedure:**

**a)** Take 4-5 gm. of sample in 250 ml glass beaker.
**b)** Add 50 ml 1:1 HCl solution and boil. Add 1ml of methyl orange indicator.
**c)** Put the mixture in separating funnel and add 30 ml of petroleum ether. Shake and allow standing for 5 minutes. Repeat the above process twice.
**d)** Fatty matter will dissolve in the upper layer (petroleum ether layer).
**e)** Discard the lower layer & filter the ether layer & take in a previously measured beaker.

Allow to evaporate the petroleum ethers in a water bath. The fatty matter will remain in the bottom of the beaker. Take the wt. of the fatty matter (Residue) [9].

**Calculation:**

**TFM= Residue weight in gm. × 100 / sample wt. in gm.**

- **Determination of % Free Fatty Acid (FFA) of Sample**

**Procedure:**

a) Take 10 gm. of sample in conical flask (W).
b) Add 50 ml of ethanol (95%) previously neutralized with 0.1 (M) KOH to phenolphthalein solution. Boil the mixture for 5 minutes.
c) Add 1 ml of phenolphthalein solution as indicator and titrate with 0.1 M KOH solution until the solution remains faintly pink after shaking for 30 minutes.

**Calculation:**

W = Weight in gm. of the sample. N = No. of ml of 0.1 M KOH required.

- **Determination of Spredebility**
- **Procedure:** Through visual observation.
- **Determination of Homogeneity**
- **Procedure:** Through **% FFA= 2.82 × N / W** visual observation.

**STABILITY STUDY:**

**I. Stress Stability Test (Short duration)**

The product is also subjected in the following tests:

- Freeze-Thaw Stability (Colipa Guidelines (ECTPA)
- Elevated temperature exposure (55°c ± 2°c)
- Thermal Stability (IS7679:1978)

**II. Freeze-Thaw Stability**

**a)** Product is subjected to thermal stress.
**b)** It's the sudden temperature shock to the product there by forcing product for destabilization.
**c)** Product is suddenly subjected to two extreme temperature conditions viz. 75°c & 10°c.
**d)** Product is subjected 75°c to 10°c to 75°c. This is considered as one cycle.
**e)** It comprise of minimum 3 cycles.
**f)** Product is visually checked for any separation or DE stability.

**Methodology:**

**a)** Samples were subjected to 75°c for 30 minutes and then suddenly cooled to 10°c for 30 minutes. Allowed to set for 30 minutes. This was considered as one cycle.
**b)** Such 3 cycles were carried out.
**c)** Samples were visually observed after each cycle.

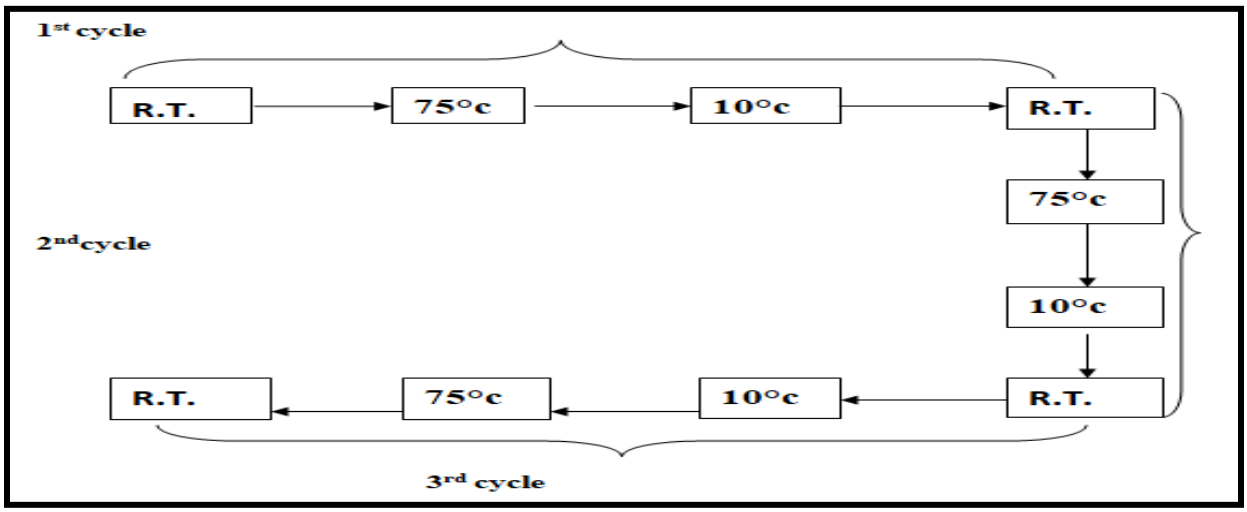



Image 2 : Study method and its application

**III. Elevated Temperature Exposure (55°c)**

**a)** Product is subjected to thermal stress.
**b)** This test accelerates signs of possible instability in the product.
**c)** The product is subjected to 55°c [10].

**Methodology:**

**a)** Samples were placed in transparent glass bottles with a proper lid to avoid any water loss during the test period.
**b)** Test was conducted at constant temperature 55°c ± 2°c.
**c)** Generally one week is sufficient to comment on stability of product during its shelf life of product.
**d)** Samples were visually observed for any phase separation on second, fifth and eighth day.

**Thermal Stability Test-** This test determines instability of an emulsion under given condition.

**Methodology:**

a) A 20 mm broad and 5 mm thick stripe of the product was spreaded on the internal wall of the beaker of 100 ml capacity in its total height.
b) The beaker kept for 8 hrs in the humidity chamber at 60-70% relative humidity and temperature of 40°c ± 1°c.
c) On removal from thermostat, oil separation was observed, if any [11].

## RESULT AND DISCUSSION

Physico-chemical parameters like $p^H$, FFA, TFM has been studied for formulations F1, F2, F3 (Table 3, 4 and 5) and with brand product. All the formulations along with brand product are showing similar kind of result along with similar Spreadability and homogeneity (Table 6). The $p^H$ of F1, F2 and F3 are showing similar result compared with brand. However highest $p^H$ is noticed in F1 among all the studied formulation (Fig2). The free fatty acid (FFA) of F1, F2 and F3 are showing similar result compared with brand. However highest FFA is noticed in F1 among all the studied formulation (Fig3). The Total fatty matter (TFM) of F1, F2 and F3 are showing similar result compared with brand. However highest TFM is noticed in F2 among all the studied formulation (Fig4). Fig 5, 6,7 and Table 7 showing the result of complete 1st, 2nd and 3rd cycle of Freeze-Thaw stability study. After completion of all cycle no physical change was observed for all formulations (F1, F2, F3) along with brand product. Fig 8 and Table 8 showing the result of elevated temperature exposure study. After completion of all measured time sign of separation was observed for all formulations (F1, F2, F3) excluding brand product. Brand product shows no sign of separation after elevated temperature exposure study. Fig 9 and Table 9 showing the result of thermal stabilty study. After completion of thermal stability study no change was observed for all formulations (F1, F2, F3) including brand product. After completion of all the stability studies all formulations (F1,F2,F3) including brand product passes the stability test in all stability conditions except in elevated temperature exposure where all the formulations fails to withstand the temperature excluding the marketed brand (Table 10).

## FORMULA OPTIMISATION:

**Table 3. F1:250 ml**

| Chemicals | Quantity |
|---|---|
| Olive oil | 10.5 ml |
| Potassium hydroxide(KOH) | 10.5 ml |
| Glycerine | 7 ml |
| Steric acid | 17.5 gm. |
| Methyl perabin | 3.5 gm. |
| Polypropylene glycol | 35 gm. |
| Water | 140 ml |
| Perfume | 2-3 drops |
| Drug(Dillenia indica) pulp | 25 gm. (10 percent) |

**Table 4. F2:250 ml**

| Chemicals | Quantity |
|---|---|
| Olive oil | 12 ml |
| Potassium hydroxide(KOH) | 12 ml |
| Glycerine | 12 ml |
| Steric acid | 17.5 gm. |
| Methyl perabin | 4 gm. |
| Polypropylene glycol | 40gm. |
| Water | 140 ml |
| Perfume | 2-3 drops |
| Drug(Dillenia indica) pulp | 12.5 gm. (5 percent) |

**Table 5. F3: 250 ml**

| Chemicals | Quantity |
|---|---|
| Olive oil | 12 ml |
| Potassium hydroxide(KOH) | 11 ml |
| Glycerine | 8ml |
| Steric acid | 18 gm. |
| Methyl perabin | 4 gm. |
| Polypropylene glycol | 37 gm. |

Table 6. Physico-chemical parameters:

| PARAMETERS | FORMULATION | | | |
|---|---|---|---|---|
| | F1(10%) | F2(5%) | F3(8%) | BRAND |
| $P^H$ | 7.9 | 7.2 | 6.9 | 7.17 |
| FFA | 0.98 | 0.9 | 0.92 | 0.8 |
| TFM | 21gm | 25 gm. | 20 gm. | 18 |
| SPREADIBILITY | ** | ** | * | *** |
| HOMOGENITY | ** | ** | ** | *** |

*BELOW SATISFATORY ***GOOD ** SATISFACTORY

**TABLE 6: PHYSICO-CHEMICAL PARAMETER**

GRAPHICAL REPRESENTATION:

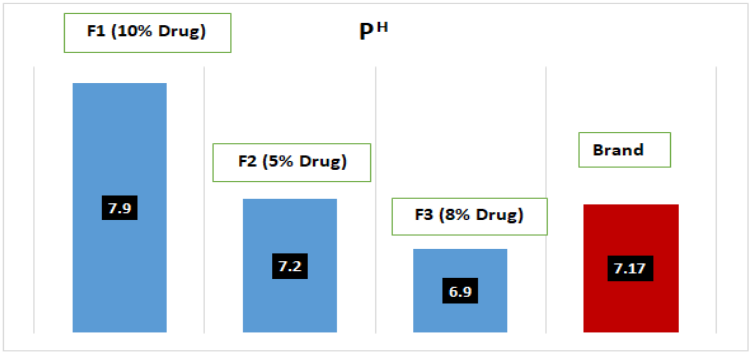



Fig2. The $P^H$ of F1, F2, and F3 is almost closer to the market Brand. But, highest $p^H$ is noticed in F1 among all the studied formulations.

**FIGURE 2: GRAPHICAL REPRESENTATION OF $P^H$ AMONG THE FORMULATIONS (F1, F2, F3 & MARKETED BRAND)**

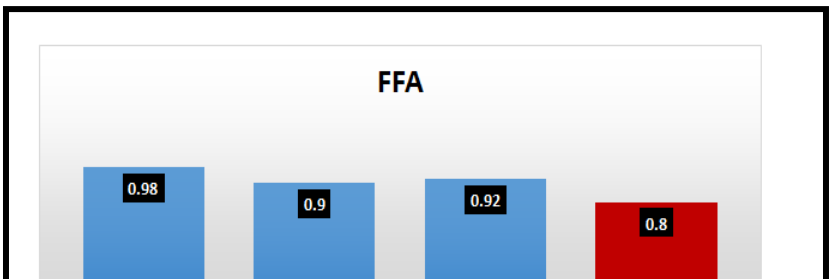


**FIGURE 3: GRAPHICAL REPRESENTATION OF FFA AMONG THE FORMULATIONS (F1, F2, F3 & MARKETED BRAND)**

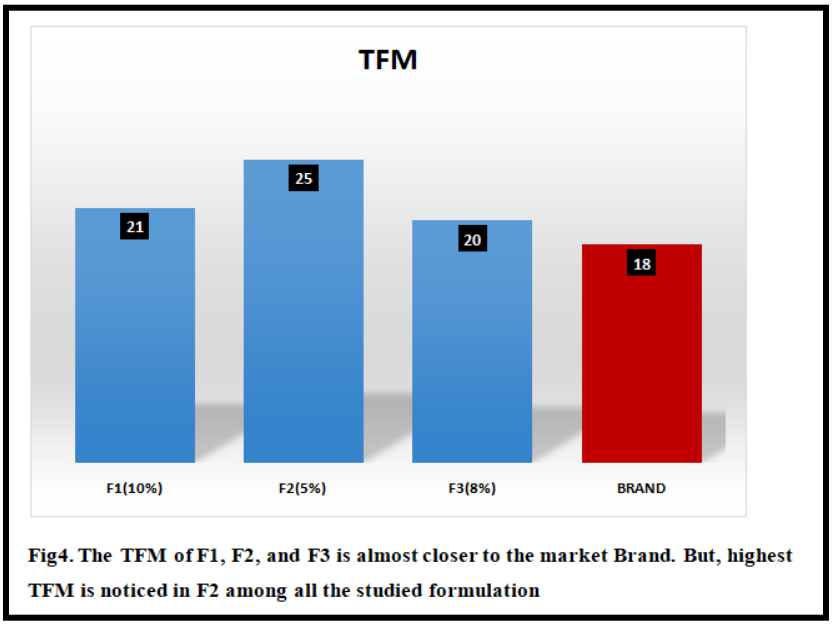



**Fig4. The TFM of F1, F2, and F3 is almost closer to the market Brand. But, highest TFM is noticed in F2 among all the studied formulation**

**FIGURE 4: GRAPHICAL REPRESENTATION OF TFA AMONG THE FORMULATIONS (F1, F2, F3 & MARKETED BRAND)**

## STABILITY STUDY OF FORMULATION (UNDER STRESS CONDITION)

Fig 5. Freeze-Thaw Stability-After First Cycles:

| SI NO | PRODUCT NAME | Observation after completion of 1st cycle<br>Cycle change status observation | |
|---|---|---|---|
| | | 75 °c | 10°c |
| 1 | F1 |  |  |
| 2 | F2 |  |  |
| 3 | F3 |  |  |
| 4 | Brand product |  |  |

**FIGURE 5: FREEZE THAW STABILITY-AFTER 1st CYCLE**

Fig 6. Freeze-Thaw Stability-After Second Cycles:

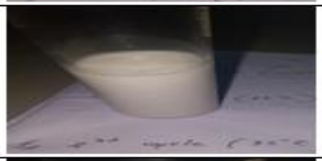
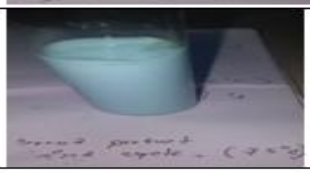
| Si. No. | Product name | Observation after completion of 2nd cycle<br>Cycle change status observation | |
|---|---|---|---|
| | | 75 ° | 10° |
| 1 | F1 |  |  |
| 2 | F2 |  |  |
| 3 | F3 |  |  |
| 4 | Brand product |  |  |

**FIGURE 6: FREEZE THAW STABILITY-AFTER 2nd CYCLE**

Fig 7. Freeze-Thaw Stability-After Third Cycles:

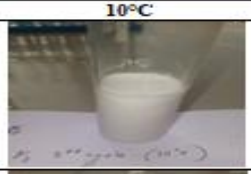
| Si. No. | Product name | Observation after completion of 3rd cycle<br>Cycle change status observation<br>75 °C | <br><br>10°C |
|---|---|---|---|
| 1 | F1 |  |  |
| 2 | F2 |  |  |
| 3 | F3 |  |  |
| 4 | Brand product |  |  |

**FIGURE 7: FREEZE THAW STABILITY-AFTER 3rd CYCLE**

**Table 7**

| After completion of 1st,2nd,3rd cycle , Physical status of the formulation | | | | |
|---|---|---|---|---|
| Si. No. | Product | Observation | Texture | Color |
| 1 | F1 | NO CHANGE | NO CHANGE | NO CHANGE |
| 2 | F2 | NO CHANGE | NO CHANGE | NO CHANGE |
| 3 | F3 | NO CHANGE | NO CHANGE | NO CHANGE |
| 4 | Brand product | NO CHANGE | NO CHANGE | NO CHANGE |

**TABLE 7: PHYSICAL STATUS OF THE FORMULATION AFTER COMPLETION OF 1ST, 2ND AND 3RD CYCLE**

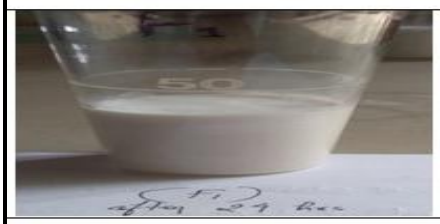
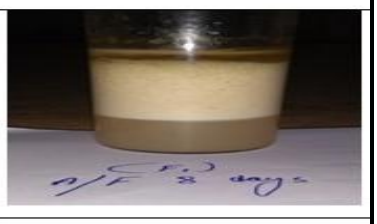
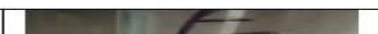
| | | |
|---|---|---|
|  |  |  |
| F1 after 24 hours | F1 after 4th day | F1 after 8 day |
|  |  |  |


**FIGURE 8: STABILITY STUDY OF FORMULATION (UNDER ELEVATED TEMPERATURE EXPOSURE ($55^0$C)**

Table 8.

| | OBSERVATION(After Elevated Temperature Exposure) | | |
|---|---|---|---|
| PRODUCT DETAILS | 24 HOURS | 4TH DAY | 8 TH DAY |
| F1 | NO CHANGE | NO CHANGE | Sign of separation |
| F2 | NO CHANGE | NO CHANGE | Sign of separation |
| F3 | NO CHANGE | NO CHANGE | Sign of separation |
| brand product | NO CHANGE | NO CHANGE | No Sign of separation |

**TABLE 8: OBSERVATION AFTER ELEVATED TEMPERATURE EXPOSURE**

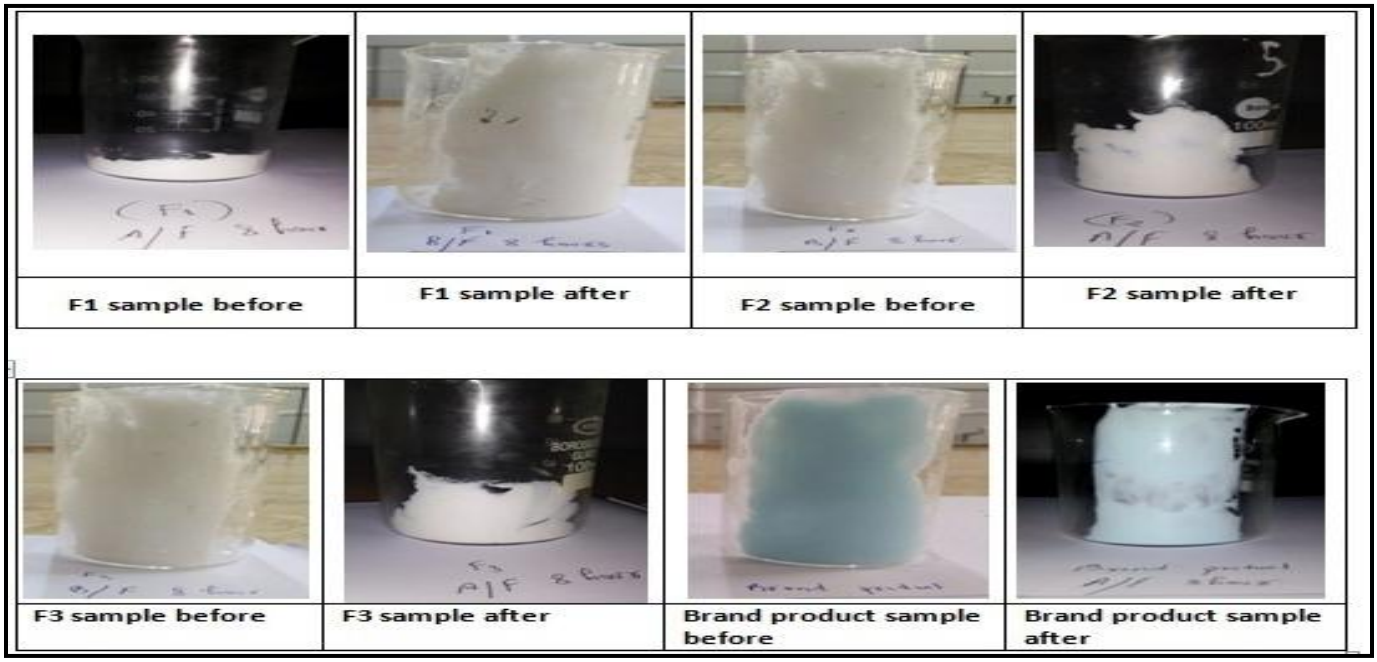



**FIGURE 9: STABILITY STUDY OF FORMULATION (UNDER THERMAL STABILITY STUDY)**

Table: 9

| PRODUCT DETAILS | OBSERVATION(Thermal Stability Study) | |
|---|---|---|
| | BEFORE | AFTER |
| F1 | No Change | No Change |
| F2 | No Change | No Change |
| F3 | No Change | No Change |
| Brand Product | No Change | No Change |

**TABLE 9: THERMAL STABILITY STUDY**

**FINAL OBSERVATION OF STABILITY STUDY:**

Table 10

| Sr. No. | Formulation | Freeze-Thaw Stability | Elevated temperature exposure (55°C ± 2°C) | Thermal Stability |
|---|---|---|---|---|
| 1 | *F1* | ***PASSES*** | ***FAILS*** | ***PASSES*** |
| 2 | *F2* | ***PASSES*** | ***FAILS*** | ***PASSES*** |
| 3 | *F3* | ***PASSES*** | ***FAILS*** | ***PASSES*** |
| 4 | *Brand* | ***PASSES*** | ***PASSES*** | ***PASSES*** |

**TABLE 10: OBSERVATION OF STABILITY STUDY**

# CONCLUSION

Formulation F1 (Formulation F1), F2 (Formulation F2), F3 (Formulation F3) given in (Table 3,4,5) are showing almost similar physico-chemical characteristics with the marketed brand (Brylcreem)and their stability standards are quite same except in elevated temperature exposure where all the formulations fails to withstand the temperature excluding the marketed brand. For further study of all formulations chemical test also can be done in different stability conditions. At last microbiological test also can be done.

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