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FORMULATION AND EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL CREAM OF PAPAYA SEED

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Abstract

Natural plant-based products have gotten a lot of attention in pharmaceutical research because of their potential therapeutic benefits. This research focuses on the development and testing of an antibacterial and antifungal cream made from papaya (Carica papaya) seeds. Bioactive chemicals found in papaya seeds include alkaloids, flavonoids, and phenolic compounds, all of which have antibacterial characteristics. The study entails extracting active chemicals from papaya seeds using proper solvents, then formulating a topical cream. The cream is then examined for its physicochemical qualities, stability, and antibacterial activity against bacterial strains such as Staphylococcus aureus and Escherichia coli, as well as fungal strains such as Candida albicans and Aspergillus niger. Standard antimicrobial testing methods, such as agar well diffusion, are utilized to detect the cream's effectiveness.

Keywords –

Carica papaya seed extracts, benzyl isothiocyanate, antibacterial, antifungal, Zone of inhibition.

INTRODUCTION

Creams are semisolid emulsions that are either oil in water (o/w) or water in oil (w/o), and they are meant for external application. The current research sought to develop a stable o/w herbal cream. The most prevalent cosmetic delivery system is the oil in water (o/w) kind, which provides moisture to the skin and improves its condition by producing an occlusive barrier. It is applied to the outer or superficial layer of skin, and its primary function is to remain at the application site for an extended length of time.

The skin is the biggest organ in the body. It is composed of water, proteins, lipids, and minerals, has a total area of around 20 square feet, and is flexible and self-repairing. The skin protects the body from infections and the elements. Skin aids in the perception of heat and cold sensations as well as the regulation of bodily temperature. Skin requires moisture to stay smooth and conduct its physiological functions adequately. The epidermis is the skin's outermost layer and provides water resistance while also determining our skin tone. The dermis, the second

layer of skin, contains thick connective tissue, hair follicles, and sweat glands. The hypodermis, also known as subcutaneous tissue, is the skin's deeper layer. It consists of fat and connective tissue. The significance of this study is evident because there are several lotions on the market today that contain synthetic chemical compounds that can have negative consequences. Herbal extract that has favorable benefits on the skin, and it has been observed that antibacterial, anti-inflammatory, anti-oxidant, and anti-acne action.

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Fig.1 Papaya Cream

Ideal characteristics of Herbal Cream

1. It should have Good Appearance
2. It should spread easily on the skin
3. Nonirritant to the skin.
4. They should remove oil, sebum, dirt, dead cells from skin
5. They should form emollient film which should remain on skin after application
6. They should also provide softening, lubricating and protecting the skin apart from cleaning
7. They should non-greasy and non-staining

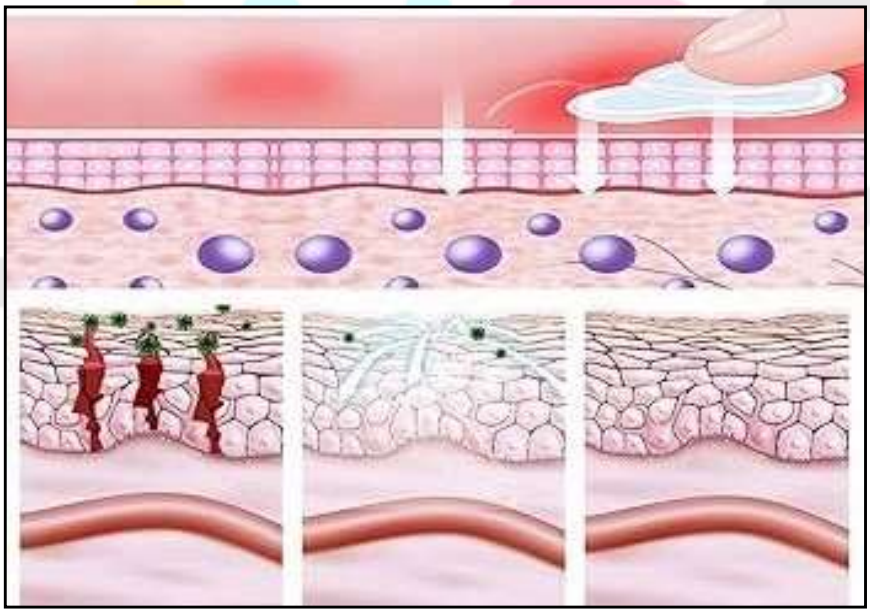

Fig no. 2 Application of cream

The plants grow well in the tropics and can reach up to 10 m high. In 2017, production of papaya reached 13 million metric tonnes with India and Brazil contributing more in the global share, trailed by Mexico, Indonesia, Dominican Republic, and Nigeria. Papaya fruit is consumed either raw or in processed forms as jelly, candy, jam, and pickles. More than 50 papaya varieties have been recorded, but pure breeding varieties are decreasing due to the uncontrolled papaya pollination. Many parts of papaya plant such as roots, leaves, peels, latex, flowers, fruits, and seeds possess nutritional and medicinal significance. The plant has been used as ethno medicine for decades in tropics and subtropics where it is mostly grown The therapeutic motives for use vary, with probably the most common being anthelmintic and as a potential abortifacient agent. Papaya seeds and leaves have been associated with amelioration of diabetes mellitus, hepatic and renal complications, fertility, hyperglycemia, amoebic dysentery, and recently antitumor activities.

Because of the active ingredient isothiocynate, papaya seeds are used to treat bacterial and fungal infections. Isothiocynate possesses antimicrobial properties. The seeds of Carica papaya (Papaya) were extracted using a soxhlet apparatus with ethanol, methanol, and chloroform solvent. Extracts at different concentrations (50, 100, 150µg/ml) were tested for antibacterial and antifungal activity against three gram-positive, three gram-negative, and two fungal organisms using the agar disc diffusion method. Standard drugs used for antibacterial and antifungal activity were gentamicin and fluconazole, respectively.

Fig no.3 Papaya seed

Antibacterial substances are substances that can effectively cure infections caused by various types of bacteria. The frequency of life-threatening diseases caused by microorganisms has increased throughout the world and is becoming a main reason of mortality and morbidity in developing countries. The antibacterial properties of several medicinal substances have been analyzed by a number of studies worldwide, and many of these substances have been used as therapy.

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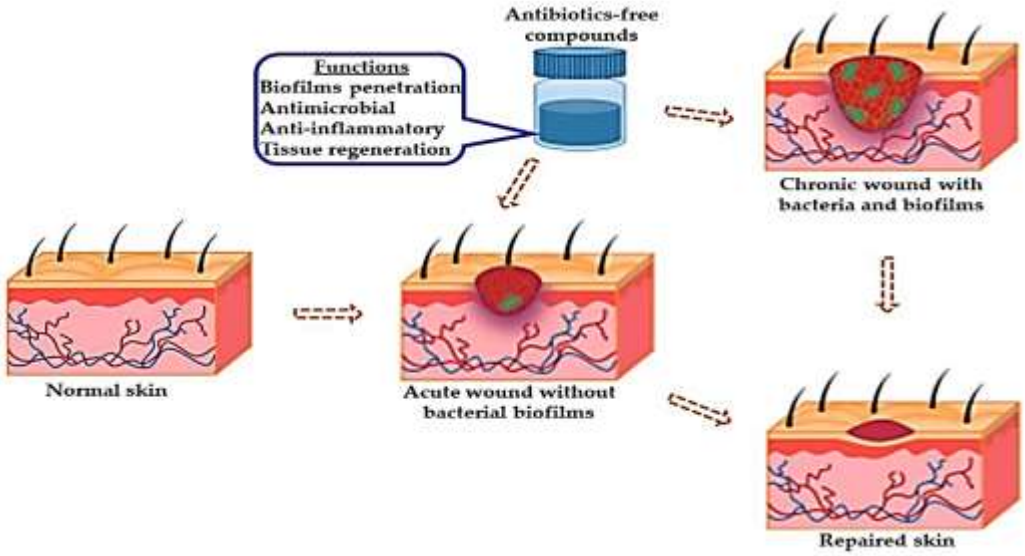

Fig no. 4 Mechanism of action of cream

MATERIALS & METHODS

Material:

A. Extraction of Isothiocyanate:

Solvent: Ethanol

Apparatus: Condenser, thimble, round bottom flask, source of heat

Crude Drug: Dried Papaya seed

B. Preparation of cream:

Bee wax, Liquid paraffin, Borax, distilled water, papaya seed extract, Rose oil

Methodology:

Fig no. 5 Procedure of cream

1. Collection and Authentication of Plants:

Extraction of Isothiocyanate

a. Drying of papaya seed for extraction: -

Choose a perfectly ripe, healthy papaya fruit. Use a clean knife to slice the papaya in half. Take a spoon and scoop out the black seeds from the middle. Put the seeds in a dish of water. Rub them lightly to get rid of the gelatinous coating. Rinse well. Spread the cleaned seeds across a paper towel or cloth. Allow them to dry for three to four days in a shaded, airy area. Avoid being under direct sunlight. Once completely dried, put them in an airtight container or paper envelope in a cool, dry location.

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Fig no.6 Papaya seed

Fig no. 7 Papaya seed powder

b. Extraction of isothiocyanate by Soxhlet Extraction technique:

Freshly obtained Carica papaya L seeds were carefully washed with tap water before being rinsed with sterile water. The Carica papaya seeds were rinsed and dried in the shade before being ground into a fine powder. 15gm of dry powdered Carica papya L seeds were extracted with 125ml of ethanol using Soxhlet's equipment for 6 hours (or until the plant material became colorless. To obtain a concentrated extract, the trace amount of solvent was evaporated with a rotating vacuum evaporator. Carica papaya L seeds were extracted using the same manner as methanol and chloroform solvents. Carica papaya seed solvent extracts at several concentrations (50, 100, 150 µg/ml) were produced using Dimethyl Sulfoxide.

Fig no.8 Soxhlet Extraction

Fig no.9 Extract

2. Preparation of cream:

For preparation of W/O Cream, weigh all the ingredients like bee wax, liquid paraffin, borax, distilled water, extract in required quantity and methyl paraben. Melt the 3.2-gram bee wax in porcelain disk and dissolve 0.16 gram of borax in 6ml of distilled water by het on water-bath. Then extract of papaya seed was add into borax solution with gentle shaking (in difficult ratio for checking activity). As the bee wax is melted addition of liquid paraffin with gentle shaking and formation of clear solution. Borax solution was added into beeswax mixture (aqueous phase was added in oil phase to form W/O). The mixture of aqueous phase and oil phase should be done that temp near about 65-70 C. The aqueous phase is slowly added into oil phase in small quantity with gentle shaking. Shake until homogenous mixture is formed. If phase separation is seen, then the cream should homogenizer in mechanical stirrer. At Last soft and proper cream is formed.

Formulation Table: -

Table no.1 Formulation Table

Ingredients	F1	F2	F3	F4
Seed Extract	3ml	4ml	5ml	6ml
Bee wax	4gm	4gm	4gm	4gm
Borax	0.16gm	0.16gm	0.16gm	0.16gm
Methyl paraben	0.02gm	0.02gm	0.02gm	0.02gm
Liquid paraffin	10ml	8ml	8ml	8ml
Rose Essence	QS	QS	QS	QS
Distilled water	QS	QS	QS	QS

Evaluation: -

Evaluation of extract: -

1) Quantity of extract: -

This graph shows how different solvents affect the yield of isothiocyanate extracted from papaya seeds, measured in grams per 100g of dry seeds (g/100g dry seeds)

1. Methanol gave the highest yield of isothiocyanate (14.32 g), suggesting it is the most effective solvent among those tested.
2. Ethanol was the second-best, with a good yield of 12.36 g.
3. Dichloromethane gave a moderate yield (6.27 g).
4. n-Hexane yielded a very small amount (0.71 g), indicating poor solubility or extraction capability.

5. Acetonitrile gave no yield (N/A), meaning it was not effective for this extraction.

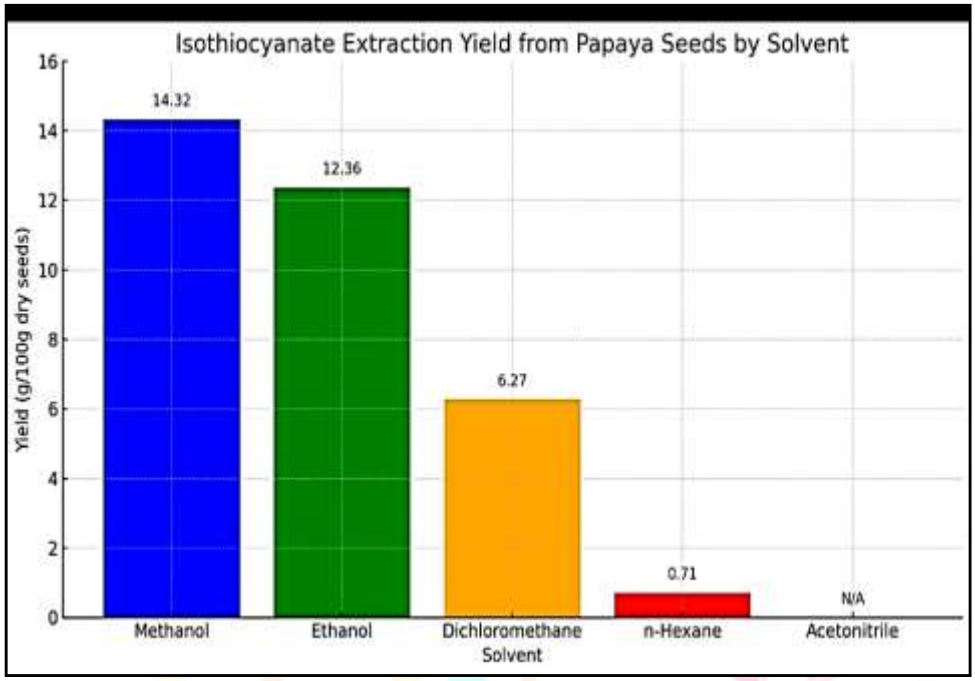

Fig no. 10 Quantity of extract isolated by diff. solvent

Polar solvents like Methanol and Ethanol are more effective in extracting isothiocynates from papaya seeds. Non-polar solvents (like n-Hexane and Acetonitrile) are not suitable for this compound. Methanol would be the preferred solvent for maximum extraction efficiency.

2) IDENTIFICATION OF ISOTHIOCYANATE: -

a. Chemical Test: -

Tab no. 2 chemical test

Sr no.	Chemical test	Observation Inference	Inference
1.	2ml of plant extract + 3ml silver nitrates	precipitate of silver sulfide	presence of the isothiocyanate.
2.	Plant extract + few drop addition of bismuth(III) nitrate or chloride solution	yellow precipitate.	presence of the isothiocyanate.

3) TLC Chromatography: -

This image shows a chromatogram, likely from HPLC (High-Performance Liquid Chromatography) analysis, of papaya seed extract.

a. X-Axis (t/min): Retention time in minutes (how long each compound takes to pass through the column). Peaks at specific times indicate different compounds.

b. Y-Axis: Intensity or abundance (not labeled here, but typically shows detector response). Higher peaks = higher concentration of that compound.

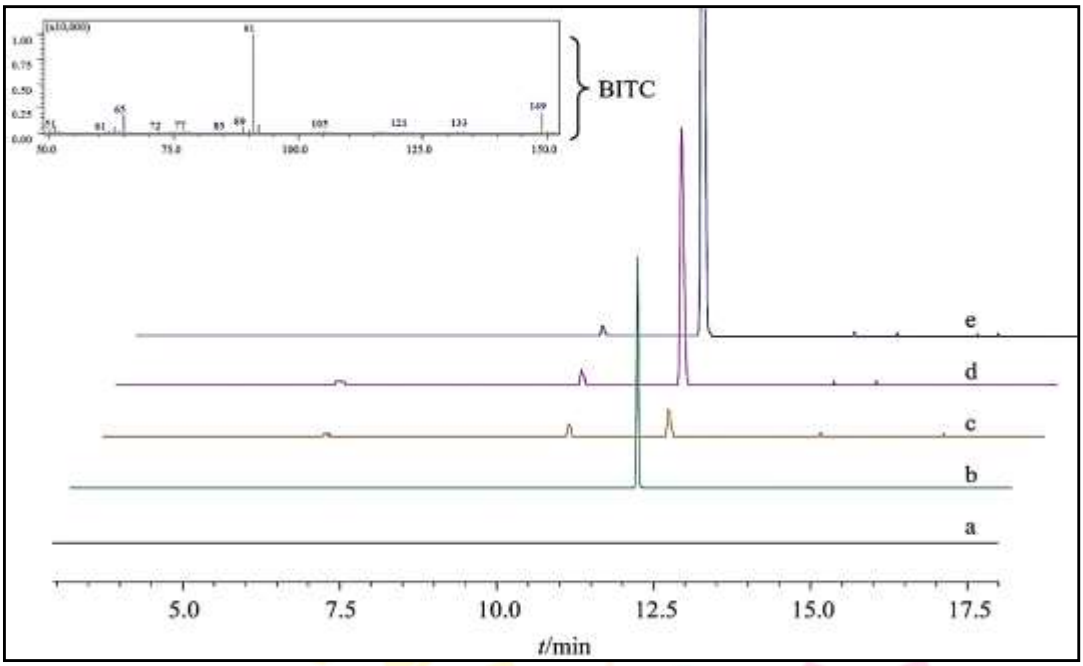

Fig no.12 TLC chromatogram

c. Lines a–e: These are different samples or stages of the extract:
   a: Likely a blank (no peaks).
   b: Raw extract or unprocessed.
   c: After partial processing.
   d: Further refined.
   e: Possibly the final product or a standard for comparison.

d. BITC (Benzyl isothiocyanate): The bracketed peak and the mass spectrum at the top-left show the identification of BITC.BITC is a bioactive compound known for anticancer, antimicrobial, and anti-inflammatory properties. Found in papaya seeds, it is the focus compound in this analysis.

e. Inset (Top-Left): Mass spectrum of BITC (m/z values from 50–150), confirming its identity. The base peak at m/z 91 is characteristic of BITC.

Conclusion:

This figure demonstrates the presence and increasing concentration of benzyl isothiocyanate (BITC) in different samples (from b to e) of papaya seed extract, using chromatographic and mass spectrometric analysis. It highlights BITC as the key active compound.

Evaluation of Cream

1. **Physical Evaluation:** - Formulated herbal creams was further evaluated by using the following physical parameters like color, odor, consistency and state of the formulation.
   a. **Color: -** The color of the cream was observed by visual examination.
   b. **Odor: -** The odor of cream was found to be characteristics.
   c. **State: -** The state of cream was examined visually.
   d. **Consistency: -** The formulation was examined by rubbing cream on hand manually.
2. **pH Measurement: -**For some cream types, a 50% dilution with distilled water may be necessary.
   - Prepare the dilute solution of cram to check pH

- Immerse the pH meter's electrode into the sample (either the undiluted cream or the diluted solution).
- Ensure the electrode is completely covered by the cream.
- Allow the reading to stabilize.
- Record the pH reading.

Alternative Method (pH Paper):

a. **Dilution (if needed):** Dilute the cream sample with water if necessary.

b. **Application:** Apply a drop of the diluted sample onto the pH paper using a clean dropper.

c. **Comparison:** Compare the color of the pH paper with the standard pH chart to determine the pH.

Fig.no 13. pH Meter

3. Spread ability test: (Slide Method)

1. Take a fixed amount of cream (e.g., 1 gram) and place it between two glass slides.
2. Place a known weight (e.g., 500 g) on the top slide for 5 minutes to allow even spreading.
3. Remove the weight and measure.
4. Spread diameter (in cm) using a ruler.
5. Time (in seconds) taken by the upper slide to slip off when a small weight (e.g., 20 g) is applied on it.

Formula: -

$$S = M \times L/T$$

Where, M = weight tide 50 g with upper slide,

L = length moved on the glass slide

T = time taken.

Fig no 14. Spread ability

4.Washability: - To determines how easily a cream can be removed from the skin using water.

1. Apply a fixed amount of cream (e.g., 1 gram) on a defined area of skin or glass slide.
2. Let it stay for 5–10 minutes.
3. Rinse the area gently with running tap water or dip in water for a set time (e.g., 1–2 minutes).
4. Use cotton/gauze to gently wipe the area.
5. Observe and record.

Result: - Completely washable

Partially washable

Not easily washable

5. Irritancy test:

a. Preparation: Clean the skin (typically the upper arm or back) with alcohol. Mark 2–3 sites on the skin (test cream, base/control, blank).

b. Application: Apply a small amount of cream (0.5 g or less) to the marked area. Cover with a patch and keep it in place for 24 hours.

c. Patch Removal & Observation: After 24 hours, remove the patch. Observe the skin at 24, 48, and 72 hours post-application.

6.Viscosity Testing:

Take about 50–100 grams of cream in a beaker.

a. Place the appropriate spindle (e.g., spindle no. 4 for semisolid) into the cream.

b. Set the rotational speed (usually 10, 20, or 50 rpm).

c. Allow the spindle to rotate and wait for a stable reading.

d. Record the viscosity in centipoise (cP or mPa·s).

e. Repeat the test at different speeds if required to observe shear-thinning or thickening behavior.

Fig no. 15 Viscometer

6. Determination of Moisture Content: -

Weigh 5g of sample into a porcelain dish containing 6-8cm in diameter and 2-4cm depth. Dry the sample in oven at 150 C

Calculate: -

$$\% \text{ by mass} = 100\ M1/M$$

Where, M1 is loss of mass (in grams) on frying;

M is mass (in grams) of material taken for test

7. Microbial testing: -

a. **Antibacterial Studies:** The antibacterial activity was evaluated using the agar well diffusion method. Mueller-Hinton Agar (MHA) plates were inoculated with a 24-hour culture of Pseudomonas aeruginosa. Mueller-Hinton Broth cultures were prepared in test tubes. Using a sterile cotton swab, the surface of the MHA plates was evenly swabbed to prepare a uniform lawn culture. Once the agar surface had dried (approximately 5 minutes), wells were aseptically created using a sterile cork borer. The wells were then impregnated with different concentrations of the papaya seed extract under study. A standard ciprofloxacin antibiotic disk was included on the same plate as a reference control.The plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured using a scale to the nearest millimeter for each formulation.

Table no. 4 extract and zone of inhibition

Seed Extract	S.aureus (mm)
50(ug/ml)	10
100(ug/ml)	14
150(ug/ml)	15

b. **Antifungal studies:** The antifungal activity was evaluated using the slice prolixity system. Sabouraud's Dextrose Agar (SDA) plates were inoculated with a 72-hour culture of Candida albicans. Sabouraud's Dextrose Broth cultures were prepared in test tubes. Using a sterile cotton swab, the surface of the SDA plates was evenly swabbed to prepare a uniform field culture. Once the agar surface had dried (approximately 5 minutes), wells were aseptically created using a sterile cork borer. The wells were then impregnated with three formulations of the nail cream under study. A standard fluconazole disk was included on the same plate as a reference control. The plates were incubated at 28°C for 24–48 hours. After incubation, the zones of inhibition were measured using a scale to the nearest millimeter for each formulation.

Table no. 5 extract and zone of inhibition

Seed Extract	*C.Albaican*(mm)
50(ug/ml)	8
100(ug/ml)	9
150(ug/ml)	12

8. **Test of stability:**The stability assessment of the final product was carried out by keeping it under a constant temperature (room temperature) for 1 to 3 months. Further the physical instabilities such as changes in color, odor and constituency of the formulation and any signs of bacterial or fungal growths were checked. The stability of the final product was evaluated by storing it at a constant room temperature for a period of 3 months. During this time, the formulation was regularly monitored for physical instabilities, including changes in color, odor, and consistency. Additionally, the samples were inspected for any signs of bacterial or fungal growth.

RESULT

A. Evaluation of Extract-

1. Quantity of extract: - 10 grams of sample was to be taken and 170 ml of ethanol was taken according to that small quantity of isothiocyanate was extracted.

2. Identification test for isothiocyanate: - From below reaction, Isothiocyanate is present in papaya seed extract

Fig no 16. Chemical test

B. Evaluation of cream –

1. Organoleptic characteristic: - Physicochemical properties of cream have a significant impact on its efficacy and application on skin. Physicochemical properties of herbal cream which was prepared

Table no. 7 Organoleptic characteristic

Organoleptic	F1	F2	F3	F4
color	Yellowish white	Yellowish white	Yellowish white	Yellowish white
Odor	Sweet	Sweet	Sweet	Sweet
Appearance	Semi-solid	Semi-solid	Semi-solid	Semi-solid

9. **pH Measurement: -** The pH of an antibacterial and antifungal cream typically ranges between 4.5 and 6.5, which is close to the natural pH of human skin.

Table no. 8 pH Measurement

F1	**4.5**
F2	**6.06**
F3	**7.1**
F4	**7.5**

Fig no. 17 pH meter

3. **Washability: -**After the Evaluation of cream it was intercepted that cream was washable easily but for complete removal it required soap application

Fig no. 18 Washability testing

4. **Irritancy test: -** (Non-Irritant)

This means the cream did not cause any redness, itching, burning, or swelling on the skin. It is considered safe for use.

Table no. 9

Evaluation	F1	F2	F3	F4
Phase separation	No	No	No	No
Irritancy	No	No	No	No
Washability	Yes	Yes	Yes	Yes
After Feel	Emollient	Emollient	Emollient	Emollient
Homogeneity	Yes	Yes	Yes	Yes

5. **Viscosity Testing:** Viscosity of papaya seed cream is tested using a Brookfield Viscometer, ensuring the cream is smooth, stable, and easy to apply. This helps assess quality and consistency of the formulation.

6. **Microbial Testing: -**

   a. **Antibacterial testing:** clear zones are observed; it confirms the antibacterial potential of the papaya seed cream. This supports the presence of active compounds like benzyl isothiocyanate in the formulation.

   b. **Antifungal testing:** The appearance of clear zones around the papaya seed cream wells indicates antifungal activity, likely due to bioactive compounds like benzyl isothiocyanate present in the extract.

a. Antifungal testing:

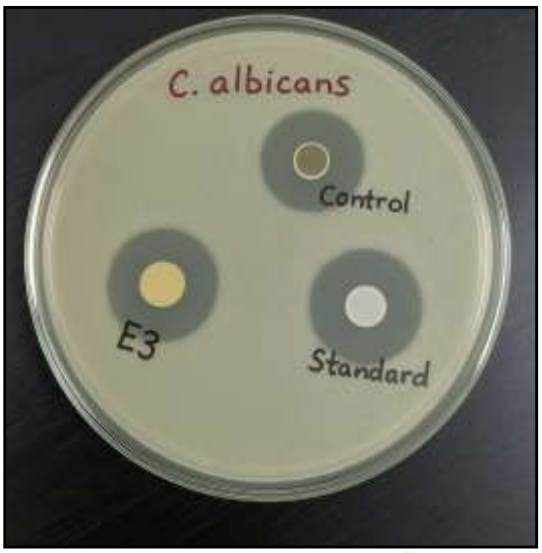

Fig no. 20 Antifungal testing

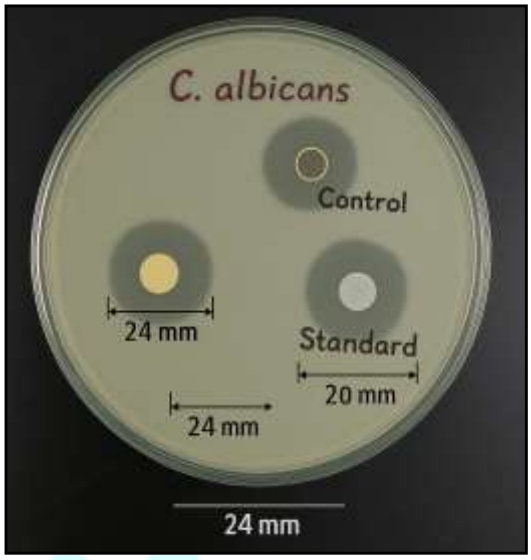

Fig no. 21 counting zone of inhibition

DISCUSSION

The antibacterial and antifungal cream of papaya cream is W/O type emulsion, hence can be easily washed with plane water that gives better customer compliance. Products formulated with phase inversion technique had produced finer internal phase and showed more physical stability in long storage condition. These formulations had almost constant pH, homogeneous, emollient, non-greasy and easily removed after the application. The stable formulations were safe in respect to skin irritation and allergic sensitization. Viscosity and shear time functions can help to analyses the processibility and material transport of the creams. The small extent of initial structure break observed in the creams, which is not followed by significant viscosity decrease, ensure good processibility.

Isothiocyanates are biologically active compounds naturally found in papaya seeds, primarily benzyl isothiocyanate (BITC). These compounds are formed by the enzymatic breakdown of glucosinolates in the seeds. During the extraction process using ethanol, especially under Soxhlet conditions, isothiocyanates are effectively extracted due to their solubility in organic solvents. Incorporating this extract into a cream allows isothiocyanates to be delivered topically, where they exhibit strong antibacterial, antifungal, and anti-inflammatory properties. Studies have shown that BITC has broad-spectrum antimicrobial activity, particularly effective against Gram-positive and Gram-negative bacteria, as well as fungi like *Candida albicans*. Their presence in the cream formulation enhances its therapeutic potential, especially for treating minor skin infections or fungal conditions.

However, because isothiocyanates are volatile and sensitive to heat and pH, their stability in the cream must be carefully monitored. Proper pH adjustment (around 5.5–6.5) and storage conditions are essential to preserve their activity. Overall, the presence of isothiocyanates in the papaya seed cream significantly contributes to its natural antimicrobial and antifungal effectiveness.

CONCLUSION

The concept of above formulation was to incorporate the extract of Papaya Seeds extract form in the cream, as cream as widely accepted and better absorb by skin with is Moisturizing and emollient effect Hence in the present investigation we prepared papaya seed extract cream by using conveniently Excipients. Results of evaluation demonstrate the pH of the cream we're normal range of the skin with good Viscosity, Spread-ability, wash ability, greasiness, stability, irritancy, antimicrobial activity, phase separation which indicated cream were capable to remain in the site of application for prolonged time. Thus we concluded that papaya seed extract cream would provide safe and healthy germ free skin. In conclusion, the formulation of the herbal antimicrobial cream demonstrated promising results in inhibiting microbial growth. Through a comprehensive evaluation process involving various tests such as antimicrobial activity, stability, and sensory analysis, the formulated cream exhibited significant efficacy and stability. The incorporation of herbal extracts not only enhanced the antimicrobial properties but also provided potential benefits such as skin hydration and soothing effects.