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# Evaluation of Antimicrobial Activity and Physicochemical Properties of Herbal Acne Face Creams Based on *Euphorbia hirta* L

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**ABSTRACT:**

This study presents a preliminary phytochemical screening and evaluation of aqueous and ethanolic extracts of *Euphorbia hirta Linn*, followed by formulation and assessment of two types of herbal acne face creams. Both extracts were subjected to qualitative phytochemical tests, UV and FTIR analysis, and antimicrobial activity evaluation. The face creams—one based on aqueous extract and another on ethanolic extract—were tested for physicochemical parameters and antimicrobial efficacy. Results indicated the presence of bioactive compounds such as alkaloids, flavonoids, tannins, and terpenoids, contributing to the antimicrobial activity of both formulations. The creams demonstrated promising stability, spreadability, and non-irritant properties, suggesting their potential as safe and effective anti-acne products.

**Keywords:** Acne, Herbal face cream, Acne cream, *Euphorbia hirta Linn,* Anti – Microbial.

## 1. INTRODUCTION:

The concept of beauty and cosmetics is as ancient as mankind and civilization. Indian herbs and its significance are popular worldwide. An herbal cosmetic have growing demand in the world market and is an invaluable gift of nature. Herbal formulations always have attracted considerable attention because of their good activity and comparatively lesser or nil side effects with synthetic drugs. Herbal cosmetics are defined as the beauty products which posses desirable physiological activity such as healing, smoothing appearance, enhancing and conditioning properties because of herbal ingredient. Now-a-days the usefulness of herbs in the cosmaceutical production has been extensively increased in personal care system and there is a great demand for the herbal cosmetics.

Acne cream is a topical skincare product specifically formulated to treat and prevent acne breakouts, such as pimples, blackheads, whiteheads, and inflammatory lesions. These creams work by targeting the root causes of acne—such as excess oil production, clogged pores, bacteria, and inflammation—through active ingredients that are either available over-the-counter (OTC) or prescribed by dermatologists. Acne creams come in various forms, including gels, lotions, ointments, and serums, and are suitable for people with different skin types and acne severity. They are an essential part of many acne treatment routines and can be used alone or in combination with other therapies like oral medications or cleansers.

**Fig: 1 Acne**

**1.1 Types Of Acne:**

Acne is a common skin condition that occurs when hair follicles become clogged with oil and dead skin cells. It can present in various forms, depending on its severity and underlying causes. Here are the main types of acne:

**1. Non-Inflammatory Acne:**

These types are usually milder and not painful.

- **Blackheads (Open Comedones)**
  - Pores clogged with oil and dead skin that remain open.
  - The surface appears dark due to oxidation.
- **Whiteheads (Closed Comedones)**
  - Pores clogged with oil and dead skin that stay closed.
  - Appear as small, white bumps on the skin.

**2. Inflammatory Acne:**

This type is red, swollen, and sometimes painful, indicating infection and inflammation.

- **Papules**
  - Small, red, raised bumps caused by inflamed or infected hair follicles.
  - Do not contain pus.
- **Pustules**
  - Similar to papules, but filled with pus.
  - Red at the base with a white or yellow tip.

**3. Severe Acne Types:**

These require medical attention due to the risk of scarring.

- **Nodules**
  - Large, painful lumps under the skin caused by deep inflammation.
  - Hard and do not contain pus.
- **Cysts (Cystic Acne)**
  - Deep, painful, pus-filled lesions.
  - Most severe form of acne and often leads to scarring.

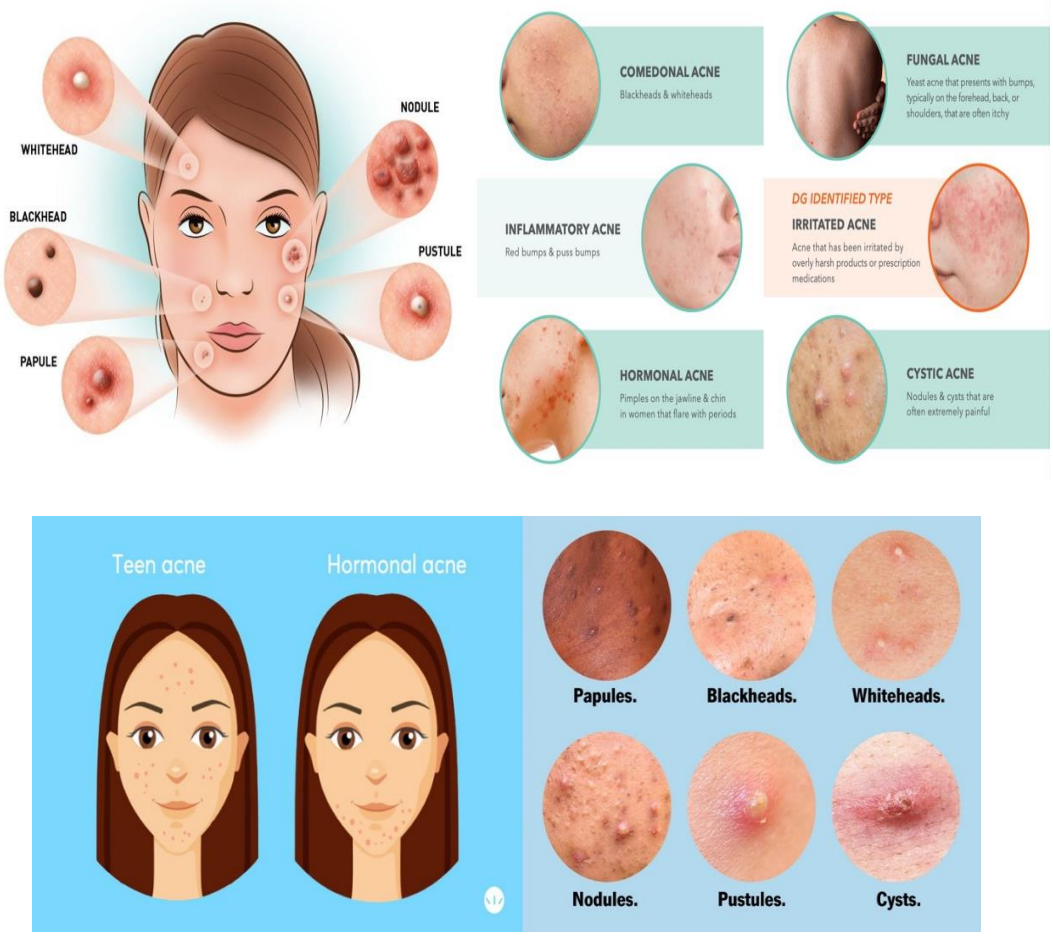


**Fig: 2 Types Of Acne & Pimples**

**1.2 Types Of Acne Creams:**

1. **Over-the-Counter (OTC) Creams**

Best for mild to moderate acne.

- Target blackheads, whiteheads, and small pimples.
-

2. **Prescription Creams**

Stronger formulas for moderate to severe or persistent acne.

- Often contain antibiotics, retinoids, or stronger anti-inflammatory agents.

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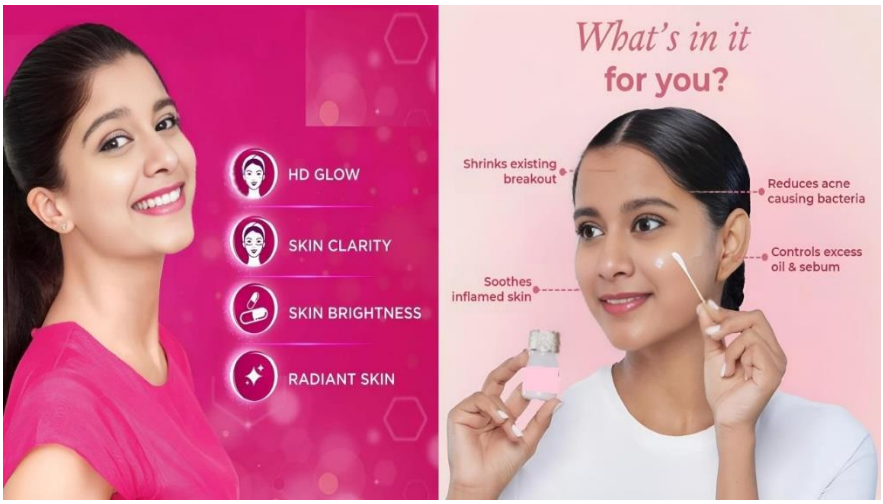


**Fig: 3 Acne Cream**

## 2. PLANT PROFILE:

### 2.1 *Euphorbia Hirta Linn:*

Euphorbia hirta**,** commonly known as asthma plant**,** is a widely distributed tropical herb belonging to the Euphorbiaceae family. It is used in traditional medicine across Asia, Africa, and the Americas for various ailments, especially respiratory, skin, and gastrointestinal issues.

### 2.2 Scientific Classification:

- **Scientific Name**: *Euphorbia hirta* Linn.
- **Common Names**: Asthma plant, snakeweed, pill-bearing spurge, tawa-tawa (Philippines)
- **Family**: Euphorbiaceae
- **Genus**: *Euphorbia*

### 2.3 Botanical Description:

**Table: 1 Botanical Description**

| Feature | Description |
|---|---|
| **Habit** | Small, branched, hairy annual herb |
| **Height** | Up to 60 cm |
| **Stems** | Erect or prostrate, reddish, with many branches |
| **Leaves** | Simple, opposite, oblong to elliptical, toothed margins |
| **Flowers** | Small, yellowish or pinkish, in dense clusters |
| **Latex** | Milky and sticky; typical of many Euphorbia species |

### 2.4 Phytochemical Constituents:

- Flavonoids (quercetin, kaempferol)
- Tannins
- Alkaloids
- Terpenoids
- Saponins
- Phenolic compounds

**2.5 Medicinal Uses In Traditional Medicine:**

**Table: 2 Medicinal Uses**

| Condition | Use |
|---|---|
| **Respiratory conditions** | Used to treat asthma, bronchitis, and cough |
| **Skin disorders** | Applied to wounds, warts, and skin infections |
| **Digestive issues** | Acts as a laxative, treats diarrhea and dysentery |
| **Fever and malaria** | Decoctions used to reduce fever |
| **Urinary infections** | Acts as a diuretic and antiseptic |


**Fig: 4 *Euphorbia Hirta Linn***

**3. MATERLALS AND METHODS:**

**3.1 ETHANOLIC EXTRACTION:**

The seed from *Euphorbia Hirta Linn* were shade dried for a week. The dried plant material was powdered, and 50g of *Euphorbia Hirta Linn* powder mixed well and subjected to different methods of extraction. 99% of ethanol were macerated for 48 hours.

**3.2 AQUEOUS EXTRACTION:**

The seed from *Euphorbia Hirta Linn* were shade dried for a week. The dried plant material was powdered, and 50g of *Euphorbia Hirta Linn* powder mixed well and subjected to different methods of extraction. 99% of ethanol were macerated for 72 hours.

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**3.3 FORMULATION OF *EUPHORBIA HIRTA LINN* HERBAL ACNE FACE CREAM:**

**Table: 3 Formulation Herbal Acne Face Cream**

| S.NO | INGREDIENTS | HAFC 1 | HAFC 2 |
|---|---|---|---|
| 1 | Ethanolic Extraction | 5ml | - |
| 2 | *Aqueous Extraction* | - | 5ml |
| 3 | Beeswax | 3.5g | 3.5g |
| 4 | Liquid Paraffin | 15ml | 15ml |
| 5 | Stearyl Alcohol | 3g | 3g |
| 6 | Borax | 2g | 2g |
| 7 | Methyl Cellulose | 1.5g | 2g |
| 8 | Purified Water | 10ml | 10ml |

**Procedure:**

**Formulation Procedure (Cold Cream Base with Herbal Actives):**

***Phase 1: Oil Phase***

1. In a beaker, add:
   - Beeswax (3.5 g)
   - Liquid paraffin (15 ml)
   - Stearyl alcohol (3 g)
2. Heat the mixture to 70–75°C using a water bath until fully melted.
3. Stir continuously to ensure all ingredients are well combined.

***Phase 2: Aqueous Phase***

1. In another beaker, heat 10 ml of purified water **to** 70°C**.**
2. Dissolve borax (2 g) in the hot water completely.
3. Sprinkle and disperse methyl cellulose (2 g) slowly while stirring to avoid lumps.
4. Once fully hydrated and mixed, maintain the temperature at 70°C.

***Phase 3: Emulsification***

1. Slowly add the hot aqueous phase into the oil phase with continuous stirring**.**
2. Stir vigorously until an emulsion forms. Continue stirring as it cools to room temperature**.**
   - A hand blender or overhead stirrer can help ensure proper emulsification.

***Phase 4: Active Addition***

1. Once the cream base is lukewarm (below 40°C), add the herbal extract (5 ml)**.**
   - Stir gently and thoroughly to incorporate without degrading active compounds.

***Phase 5: Final Adjustments***

- **Check pH**: Should be between 5.5 and 6.5 (ideal for skin).
- Adjust if needed with citric acid (to lower) or sodium hydroxide (to raise).
- **Preservative**: If you want a longer shelf life, consider adding a preservative like phenoxyethanol or ethylhexylglycerin (optional).

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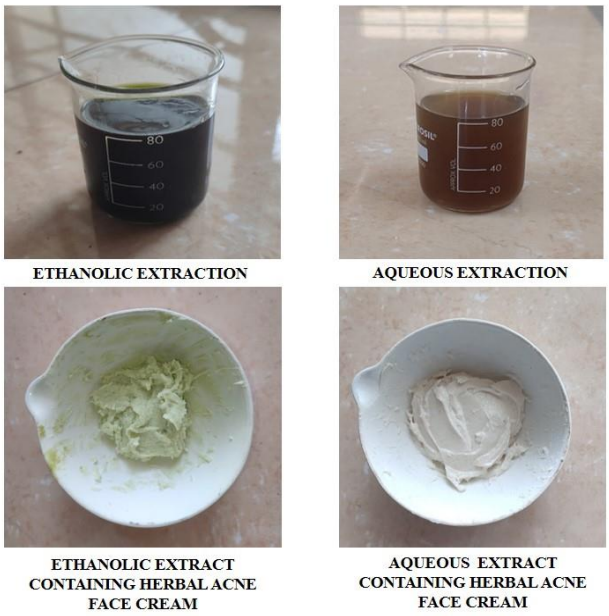


**Fig: 5 Extract & Herbal Acne Face Cream**

### 3.4 CHEMICAL TEST:

- Test For Alkaloids
- Test For Phenol
- Test For Carbohydrate
- Test For Flavanoids
- Test For Tannis
- Test For Terpenoids
- Test For Proteins
- Test For Steroids
- Test For Glycosies

### 3.5 EVALUATION OF HERBAL ACNE FACE CREAM:

**3.5.1 Physical Evaluation Of The Formulation:** The formulations were inspected visually for their appearance, colour and odour.

**3.5.2 Measurement Of PH:** The pH was measured using a pH meter, which was calibrated before each use with standard buffer solutions at pH 4, 7, 9. The electrode was inserted into the sample 10 minutes prior to taking the reading at room temperature.

**3.5.3 Viscosity:** The viscosity of the creams was obtained by multiplying the corresponding dial reading with the factor given in the Brookfield Viscometer catalogue.

**3.5.4 Test For Thermal Stability:** Thermal stability of the formulation was determined by the humidity chamber controlled at 60-70% RH and 37 ± 1°C.

**3.5.5 Spreadability:** Spreadability is measured in terms of time in seconds taken by two slides to slip off from the cream when placed in between the slides under the direction of a certain load. The excess amount of sample was placed between the two glass slides and a definite amount of weight was placed on these glass slides to compress the glass slides of uniform thickness. A weight of 70 g was added and the time required to separate the two slides was noted. Spreabability was calculated using the formula:

$$S = M.L / T$$

where,

M = wt tied to upper slide,

L = length of glass slides,

T = time taken to separate the slides.

**3.5.6 Irritancy:** Mark 1cm2 area on left hand dorsal. Cream was applied on that area and note that time. After interval up to 24 hours it is checked for irritant effect, erythema and edema if any then reported.

**3.5.7 Washability:** Apply small amount of cream on hand and wash it under running tap water.

**3.5.8 Phase Separation:** Prepared cream is kept in tightly closed container at room temperature away from sunlight and observed for 24 hours for 30 days for phase separation.

**3.5.9 Greasiness:** The cream is applied in the form of smear on the surface of skin and observed if smear was oily or grease like. According to result, we can say that all 5 formulations.

**3.5.10 Homogeneity:** It was found that the cream was homogeneous and smooth and consistent in nature.

**3.5.11 Dilution Test:** In this test the emulsion is diluted either with oil or water. If the emulsion is o/w type and it is diluted with water, it will remain stable as water is the dispersion medium" but if it is diluted with oil, the emulsion will break as oil and water are not miscible with each other. Oil in water emulsion can easily be diluted with an aqueous solvent, whereas water in oil emulsion can be diluted with an oily liquid.

**3.5.12 Dye Solubility Test:** In this test an emulsion is mixed with a water soluble dye (amaranth) and observed under the microscope. If the continuous phase appears red, it means that the emulsion is o/w type as the water is in the external phase and the dye will dissolve in it to give color. If the scattered globules appear red and continuous phase colorless, then it is w/o type. Similarly, if an oil soluble dye (Scarlet red C or Sudan III) is added to an emulsion and the continuous phase appears red, then it is w/o emulsion.

**3.5.13 Fourier Transform Infrared Spectroscopy (FTIR):** In order to check the integrity (Compatibility) of drug in the formulation, FT-IR spectra of the formulations along with the drug and other excipients were obtained and compared using FT-IR spectrophotometer. In the present study, Potassium bromide (KBr) pellet method was employed. The samples were thoroughly blended with dry powdered potassium bromide crystals. The mixture was compressed to form a disc.

The disc was placed in the spectrophotometer and the spectrum was recorded. The FT-IR spectra of the formulations were compared with the FT-IR spectra of the pure drug and the polymers. An infrared spectrum of pure drug, mixture of drug with each retardant and physical mixture of optimized formulation was recorded using FTIR Spectrophotometer. The scanning range was 500–4000 $cm^{-1}$ and the IR spectra of samples were obtained using KBr disc method. Any change in spectrum pattern of drug due to presence of polymers was investigated to identify any chemical interaction.

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**3.6 ANTI – MICROBIAL ACTIVITY:**

**3.6.1 ANTI – FUNGAL:**

***Microorganisms: Aspergillus flavus, Pityrosporum folliculitis (*Malassezia *species)***

***Control Compound: Amphotericin B***

***A. Culture Media and Procedure:***

**Table: 4 Anti – Fungal Activity**

| Microorganism | Recommended Media | Preparation Instructions | Notes |
|---|---|---|---|
| **Aspergillus flavus** | Sabouraud Dextrose Agar (SDA) | - Dissolve 65 g of SDA powder in 1 L distilled water.<br>- Sterilize by autoclaving at 121°C for 15 minutes.<br>- Cool to ~50°C and pour into Petri dishes. | Incubate at 25–30°C for 3–5 days. |
| **Pityrosporum folliculitis** (*Malassezia*) | Modified Dixon's Agar OR Leeming & Notman Agar | - Prepare medium containing lipids (e.g., Tween 60, glycerol monostearate).<br>- Sterilize lipid components separately, combine under sterile conditions.<br>- Autoclave the rest of the components at 121°C for 15 mins. | Incubate at 32–37°C for up to 7 days in humidified conditions. |

***B. Antifungal Susceptibility Testing:***

- **Control:** Amphotericin B
- **Method:** Disc diffusion (CLSI M44-A) or broth microdilution (CLSI M38-A2)
- **Concentration of Amphotericin B for Testing:** Typically 0.25–16 µg/mL
- Zone of inhibition measured around Amphotericin B disc to determine sensitivity.

**3.6.2 ANTI – BACTERIAL ACTIVITY:**

***Microorganisms: Propionibacterium spp. (*Cutibacterium acnes*), Escherichia coli***

***Control Compound: Ciprofloxacin***

***A. Culture Media and Procedure:***

**Table: 5 Anti – Bacterial Activity**

| Microorganism | Recommended Media | Preparation Instructions | Notes |
|---|---|---|---|
| **Propioni bacterium spp.** | Anaerobic Blood Agar or Brain Heart Infusion (BHI) with supplements | - Dissolve 40 g BHI in 1 L distilled water.<br>- Add 5% defibrinated sheep blood (if required).<br>- Sterilize by autoclaving.<br>- Incubate anaerobically at 37°C for 48–72 hrs. | Use anaerobic jars or Gas Pak system. |
| **Escherichia coli** | Nutrient Agar or Mac Conkey Agar | - Dissolve 28 g of Mac Conkey or 23 g of nutrient agar in 1 L water.<br>- Sterilize at 121°C for 15 min.<br>- Pour into plates. | Incubate at 37°C for 18–24 hrs. |

***B. Antibacterial Susceptibility Testing:***

- **Control:** Ciprofloxacin
- **Method:** Kirby-Bauer disc diffusion (CLSI M100)
- **Ciprofloxacin Disc Concentration:** 5 µg
- Measure zone of inhibition (≥21 mm = Sensitive for E. coli).

**4. RESULTS AND DISCUSSION:**

**4.1 PRELIMINARY PHYTO CHEMICAL INVESTIGATION OF AQUEOUS & ETHANOLIC EXTRACT OF PLANTS:**

**Table: 6 Chemical Test**

| S.NO | CHEMICAL TEST | ETHANOLIC EXTRACT | AQUEOUS EXTRACT |
|---|---|---|---|
| 1 | Carbohydrates | - | + |
| 2 | Alkaloids | + | - |
| 3 | Steroids and Sterols | + | + |
| 4 | Glycosides | + | + |
| 5 | Flavonoids | + | + |
| 6 | Saponins | - | - |
| 7 | Amino acid | + | - |
| 8 | Protein | + | + |
| 9 | Tri-terpenoids | - | - |
| 10 | Terpenoids | + | + |
| 11 | Gums and Mucilage | - | - |
| 12 | Phenolic compound | + | + |
| 13 | Tannins | + | + |
| 14 | Fixed Oils and Fatty acids | - | - |

**4.2 EVALUATION OF HERBAL ACNE FACE CREAM:**

**Table: 7 Evaluation Of Herbal Acne Face Cream**

| S.NO | PARAMETER | HAFC 1 | HAFC 2 |
|---|---|---|---|
| 1 | Color | Light Green | White |
| 2 | Odour | Characteristic | Characteristic |
| 3 | Appearance | Semi-solid | Semi-solid |
| 4 | pH | 6.8 | 6.9 |
| 5 | Thermal Stability | Stable, No Separation | Stable, No Separation |
| 6 | Phase Separation | No phase separation | No phase separation |
| 7 | Greasiness | Non-greasy | Non-greasy |
| 8 | Homogeneity | Homogeneous | Homogeneous |
| 9 | Wash ability | Easily Washable | Easily Washable |
| 10 | Dilution Test | No Separation | No Separation |
| 11 | Dye Test | O / W Type | O / W Type |
| 12 | Spreadability( g cm / sec ) | 10.9 | 11.3 |
| 13 | Viscosity | 8635 | 8213 |
| 14 | Irritancy | Nil | Nil |

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**4.3 UV ANALYSIS:**

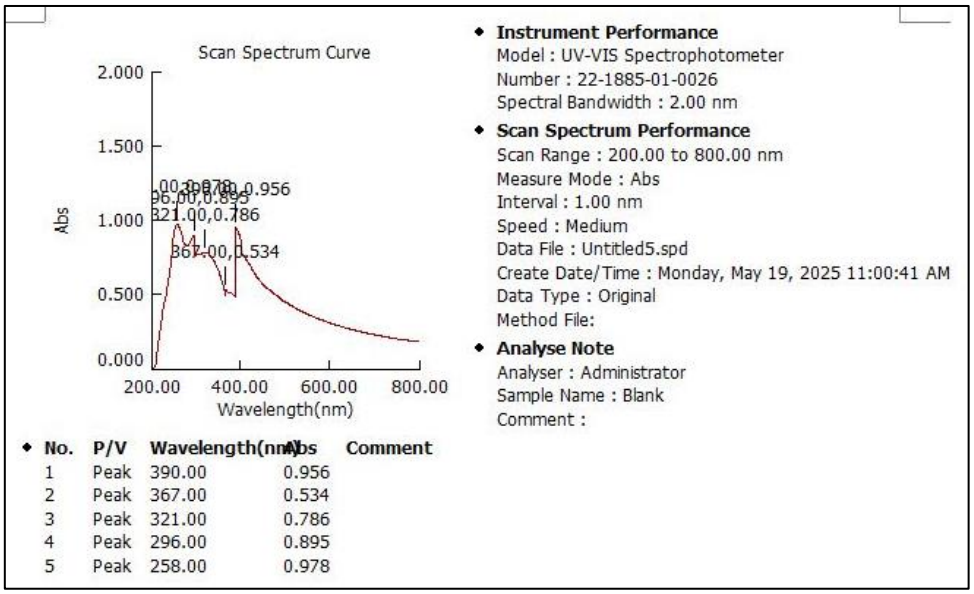


**Fig: 6 Aqueous Extract Of *Euphorbia Hirta Linn***

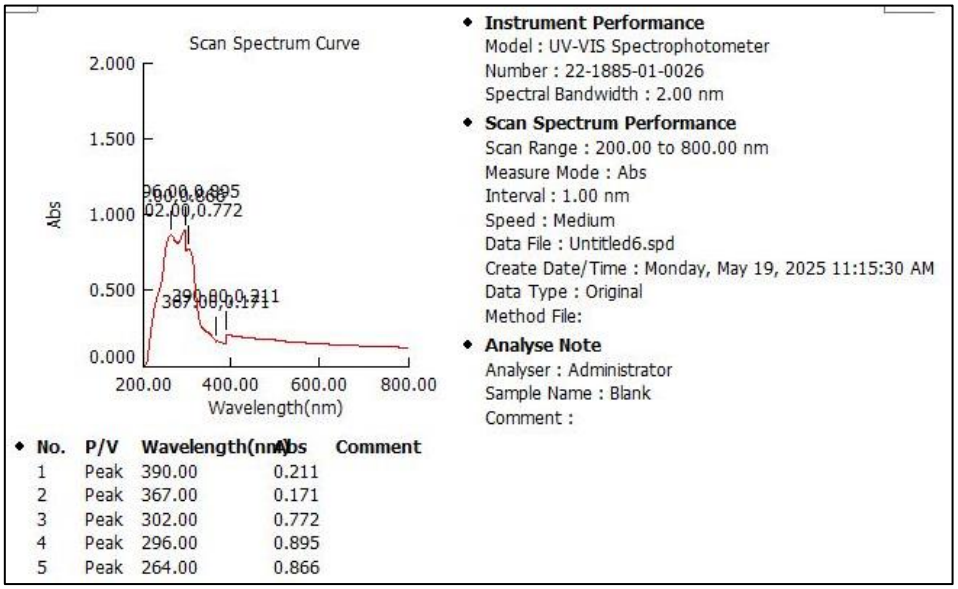


**Fig: 7 Aqueous Extract Containing Herbal Acne Face Cream**

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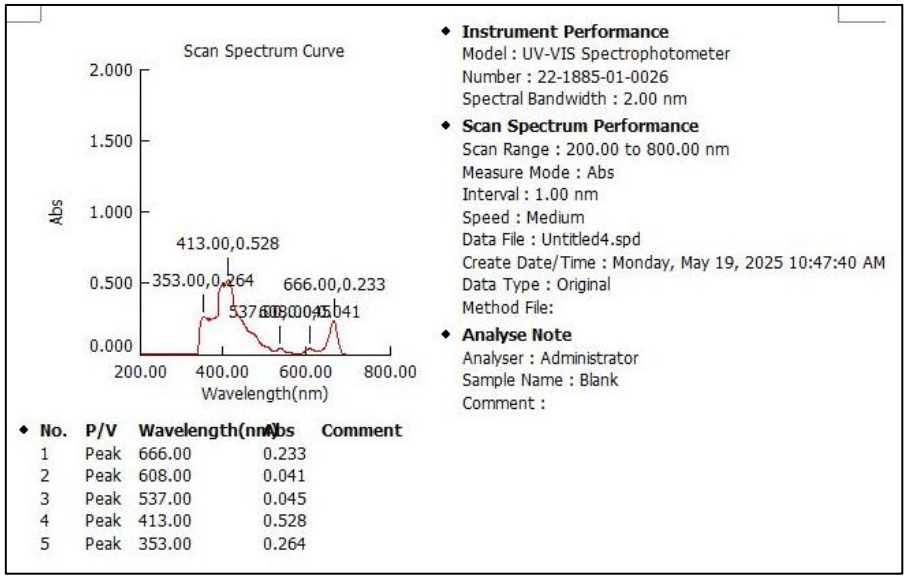


**Fig: 8 Ethanolic Extract Of *Euphorbia Hirta Linn***

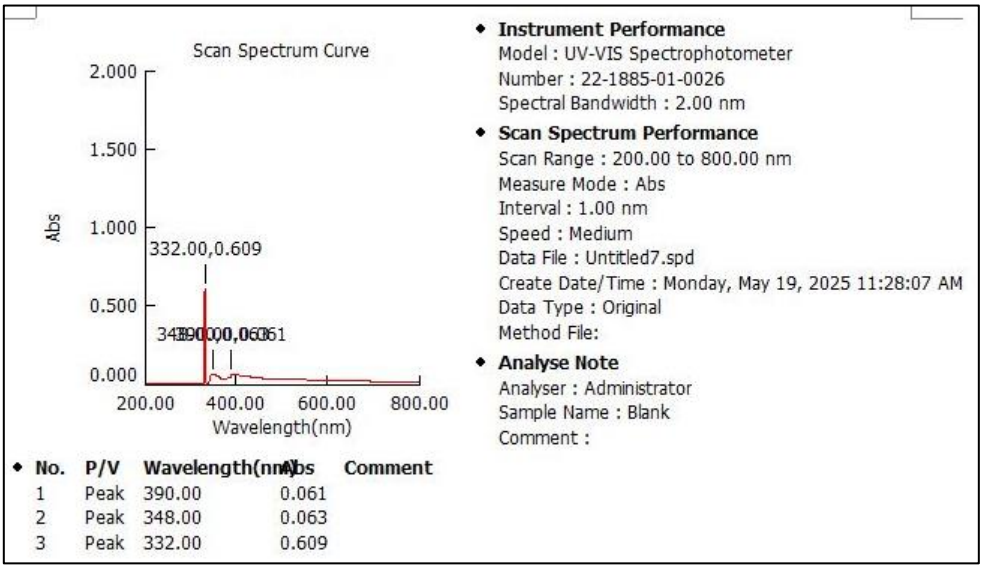


**Fig: 9 Ethanolic Extract Containing Herbal Acne Face Cream**

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**4.4 FTIR ANALYSIS:**

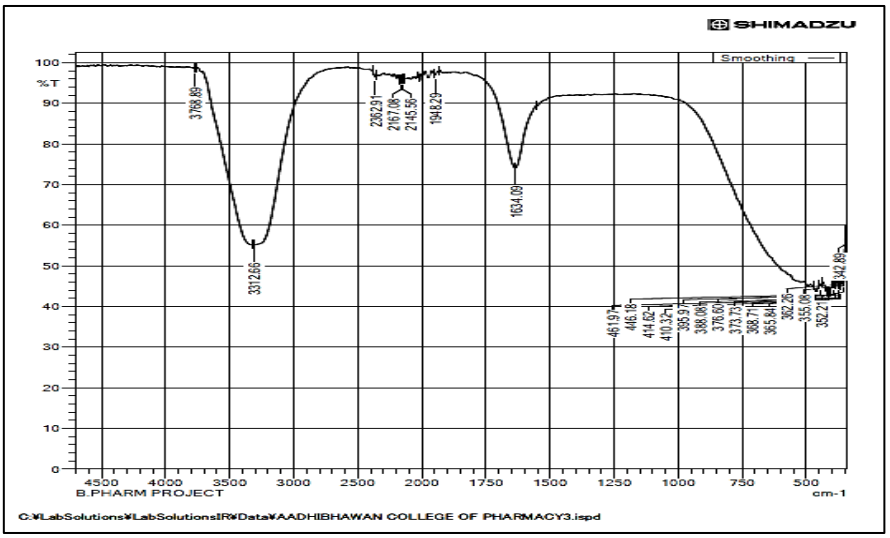


**Fig: 10 Aqueous Extract Of *Euphorbia Hirta Linn***

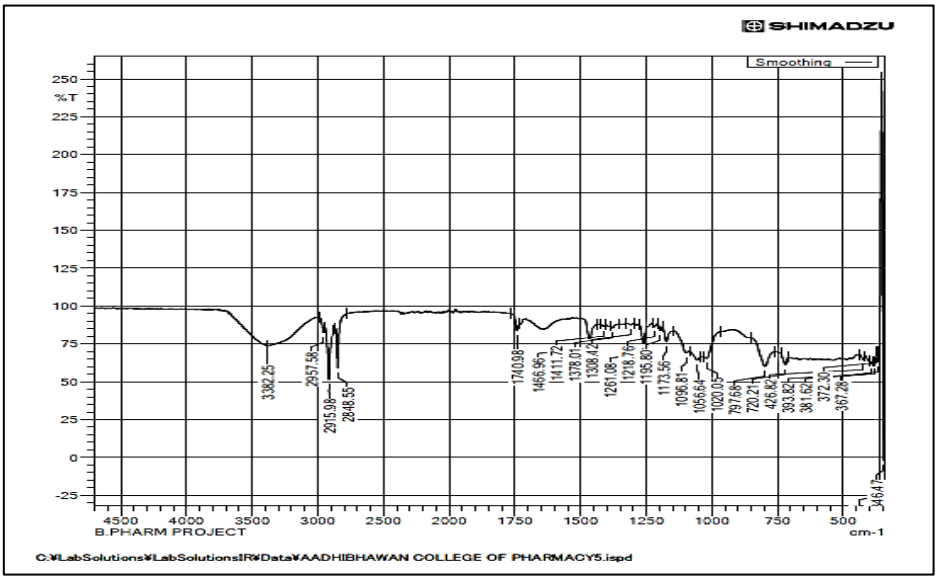


**Fig: 11 Aqueous Extract Containing Herbal Acne Face Cream**

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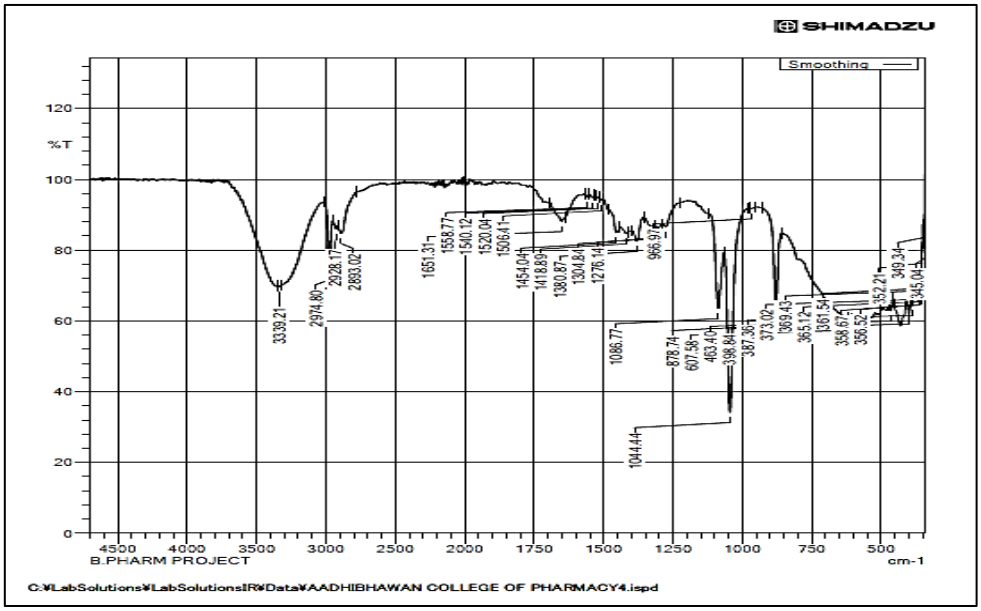


**Fig: 12 Ethanolic Extract Of *Euphorbia Hirta Linn***

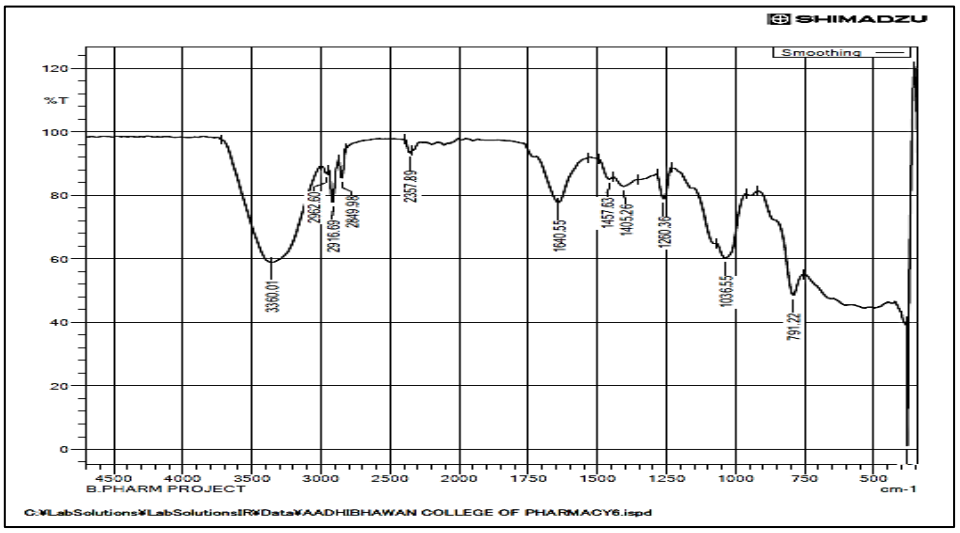


**Fig: 13 Ethanolic Extract Containing Herbal Acne Face Cream**

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**4.5 ANTI MICROBIAL ACTIVITY:**

**4.5.1 Anti – Fungal:**

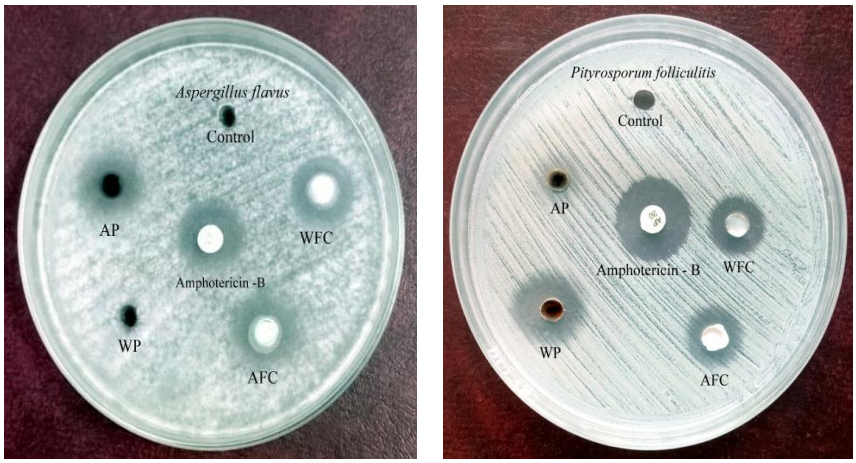


**Fig: 14 Anti – Fungal Activity**

**Table: 8 Anti – Fungal Activity**

| S.No. | Microorganisms | Control | AP | WP | AFC | WFC | Amphotericin – B |
|---|---|---|---|---|---|---|---|
| | | Zone of inhibition in mm | | | | | |
| 1. | *Aspergillus flavus* | - | 09 | - | 10 | 12 | 12 |
| 2. | *Pityrosporum folliculitis* | - | 05 | 15 | 13 | 12 | 15 |

**4.5.2 Anti – Bacterial:**

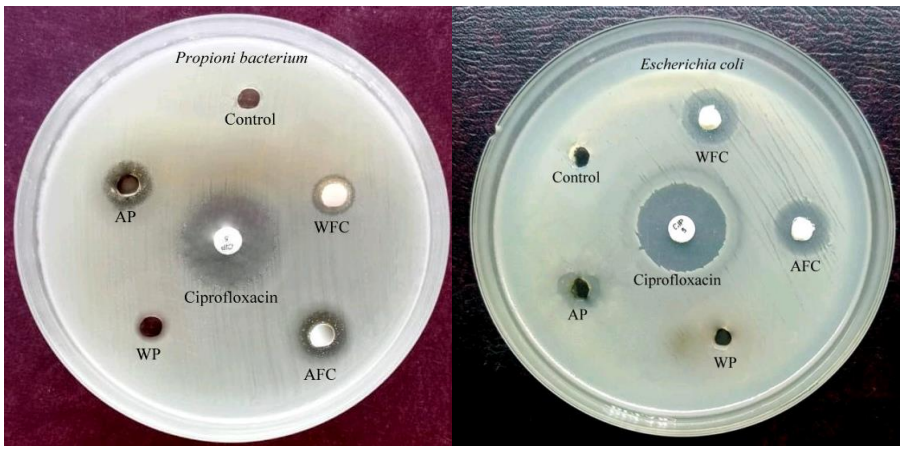


**Fig: 15 Anti – Bacterial Activity**

**Table: 9 Anti – Bacterial Activity**

| S.No. | Microorganisms | Control | AP | WP | AFC | WFC | Ciprofloxacin |
|---|---|---|---|---|---|---|---|
| | | Zone of inhibition in mm | | | | | |
| 1. | *Propionibacterium sps* | - | 10 | 05 | 08 | 07 | 18 |
| 2. | *Escherichia coli* | - | 10 | - | 13 | 15 | 20 |

AP – Alcohol Plant Extract

WP - Water Plant Extract

AFC – Alcohol Face Cream

WFC – Water Face Cream

**7.6 LABEL & INSTRUCTION:**


**Fig: 16 Label**


**Fig: 17 Product Advertisement**

**DISCUSSION:**

The phytochemical analysis revealed distinct variations between aqueous and ethanolic extracts. Alkaloids and amino acids were only detected in the ethanolic extract, while carbohydrates were exclusive to the aqueous extract. Both extracts tested positive for

flavonoids, glycosides, tannins, and terpenoids—phytochemicals known for their antimicrobial and anti-inflammatory properties, which are desirable for acne treatment.

Evaluation of the formulated Herbal Acne Face Creams (HAFC 1 and HAFC 2) showed consistency in physical attributes such as color, odor, pH, stability, and spreadability. HAFC 1 (ethanolic-based) showed slightly higher viscosity, while HAFC 2 (aqueous-based) demonstrated marginally better spreadability. Both formulations passed the irritation test, making them suitable for topical application.

UV and FTIR analyses confirmed the presence of functional groups corresponding to bioactive compounds in both extracts and creams, supporting the presence of phytoconstituents responsible for therapeutic activity.

Antimicrobial testing revealed that both face creams exhibited activity against fungal (e.g., *Aspergillus flavus* and *Pityrosporum folliculitis*) and bacterial strains (e.g., *Propionibacterium sps* and *Escherichia coli*). The ethanolic extract and corresponding face cream demonstrated superior antifungal activity, while the aqueous formulations showed better antibacterial effects against certain strains.

## 5. CONCLUSION:

The study supports the potential of *Euphorbia hirta Linn* as a natural source for developing herbal acne face creams. The formulations derived from both aqueous and ethanolic extracts showed favorable physical properties, were non-irritating, and possessed significant antimicrobial activity. These findings highlight the therapeutic viability of the plant for dermatological applications, particularly in managing acne-related microbial infections. Further in vivo studies and long-term stability tests are recommended to confirm the efficacy and safety of the developed products.

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