INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA):IJPS00]
Journal Homepage: https://www.ijpsjournal.com

Research Article

Creation And Evaluation Of Multi Herbal Fairness Cream By Using Carica Papaya And *Phyllanthus Emblica*

Manisha U. Mishra * , Aishwarya A. Jain , Prachi S. Patle , Pratibha U. Thakur , Priyanshi A. Ramteke , Riya A. Jaitwar , Pratiksha I. Damahe

Manoharbhai Patel Intsitute of Bachelor of Pharmacy, Kudwa Gondia

ARTICLE INFO

Received: 14 June 2024
Accepted: 18 June 2024
Published: 09 July 2024
Keywords:
Fairness, Herbal, Cream, Face, Extracts.
DOI:
10.5281/zenodo.12700090

ABSTRACT

The present study was carried out to prepare and evaluate the herbal fairness cream comprising extracts of Aloe barbadensis ,curcuma longa, emblica officinalis,Ocimum sanctum and papaya oil. The various types of formulations oil in water (O/W) base were formulated by incorporating different concentrations Stearic acid and Cetyl alcohol. And different concentration of methyl paraben into the water and glycol in different amount. The pH, viscosity, spreadibilty, and stability of prepared base were investigated. The base was found appropriate for the preparation of cream. The extracts of varying ratio of turmeric , amla , aloe vera and Tulsi were incorporated in base for the preparation of multiherbal fairness cream the herbal cream demonstrated good spreadibilty, good consistency, homogeneity, appearance, pH, irritancy, Wahsability, Homogeneity, Anti-inflammatory,Anti-microbial ease of removal and no evidence of phase separation. All the prepared herbal cream was found to be safe for skin. Mostly people use whitening creams to improve their complexion. Melanin plays main role in the skin colour and pigmentation. The tyrosinase catalyzes melanin synthesis, tyrosinase inhibitors are important in cosmetic skin-whitening. The aim of present study was to formulation and development of whitening poly herbal face cream comprising extracts.

INTRODUCTION

COSMETICS:

Cosmetics are the articles that are meant to be applied topically for cleansing, beauty and attractiveness purposes.

The used rawmaterial should be:

- Highest quality, standardized, distilled, or purified water.
- Tested under all conditions and shelf life.

PROPERTIES OF COSMETICS

- Cleans, beautifies, and alters the appearance.
- Adds fragrance.
- Stops the development of bad odor.

***Corresponding Author:** Manisha U. Mishra
Address: *Manoharbhai Patel Intsitute of Bachelor of Pharmacy, Kudwa Gondia*
Email : payals.patle2002@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

- It does not have any medicinal effect on the body.[1]

HERBAL COSMETICS:

Herbal cosmetics otherwise known as nature cosmetics. Herbal skin care product is most safe and protect the skin from external environment. It provides appearance to the skin.[2]Cosmetics are an substance which used to apply on the human body parts like face, hands to soothing the skin, promoting beauty, enhancing the without any changes in the body functions and body structures.[3]Nowadays use of herbal cosmetics by the people increasing day by day and great need of herbal cosmetics skin care products day to day life. Dermatologist proved herbal cosmetics are safe to use due to lack of side effects and more therapeutic

STRUCTURE AND LAYER OF SKIN :-

The Skin[8,9,10,11,12]

The skin often has been referred to as the largest of the body organs: an average adult's skin has a surface area of about 2m2.The ease with which some drugs can pass through the skin barrier into the circulating blood means that the transdermal route of medication is a possible substitute to the oral route. However, the number of drugs available as marketed transdermal drug products is limited to those that display the correct physicochemical and pharmacokinetic properties which facilitate their effective delivery across the skin.

Layers of skin:

A. Epidermis
B. Dermis
C. Hypodermis

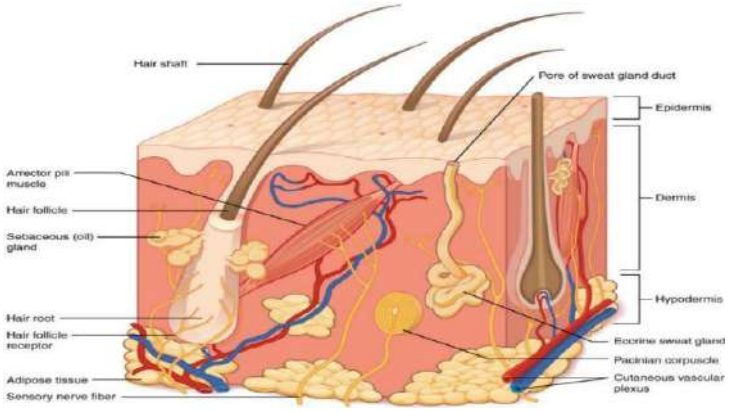


Fig No 1. Anatomy Of Skin

A. Epidermis:

The epidermis is the outermost skin layer. Its thickness depends on where it is on the body.

The following layers comprise the epidermis:

- Stratum Corneum.
- Stratum Lucidium.
- Stratum Granulosum.
- Stratum Spinosum.
- Stratum Basale.

Cells of the Epidermis

- Melanocytes
- Langerhans' cells
- Merkel's cell
- Keratinocytes

B .Dermis

The dermis is connected to the epidermis at the level of the basement membrane and consists of two layers of connective tissue, the papillary and reticular layers which merge together without clear demarcation. The papillary layer is the upper layer, thinner, composed of loose connective tissue and contacts epidermis.

C .Hypodermis

The hypodermis is deep to the dermis and is also called subcutaneous fascia. It is the deepest layer of skin and contains adipose lobules along with some skin appendages like the hair follicles, sensory neurons, and blood vessels.

HERBAL FAIRENESS CREAM:-

India's fairness cream market is evolving at rapid speed, filled by television advertisement by the celebrities and the rapidly changing lifestyles. India's proactive FMCG (Fast Moving Consumer Goods) market has seen the significant growth in the cosmetic market in last two decades and fairness cream accounts for a major part of the cosmetic market with an average growth rate of 20% per annum. The concept of preferring the people with "fair skin" has long been recognized socially and it has been the psychological and social impact on women to be fair. but Emami catered to men with its product Fair and Handsome. Till then fairness cream market dominates the cosmetic market covering male and female segments.. The growth in consumerism and the changing lifestyle of Indian youth have led to strong demand for fairness creams. India's swelling middle class is redefining lifestyle pattern with adoption of western values and growing brand consciousness, creating opportunity for the global players in fairness cream market. [13,14,15] The herbal products claim to have no side effects, commonly seen with products containing synthetic agents. Attractiveness of herbal preparations has socially as well as technologically resulted in flooding of market place in India[16]. So here we planned to select such type of plants which are found to exhibit anti-tyrosinase activity for preparation of polyherbal cream. The plants used in cosmetic preparation have varieties of property like anti-tyrosinase, antioxidant, anti-inflammatory, antiseptic and antibacterial etc. As per this concern we select following plants Aloe barbadensis ,curcuma longa, emblica officinalis,Ocimum sanctum and papaya oil for the formulation of herbal cosmetic. Curcuma longa rhizomes are valued as a topical antioxidant and anti-inflammatory agent, with superior free radical scavenging and lipid peroxidation inhibition efficacy as compared to vitamin E. The curcumin and Tetrahydrocurcuminoids present in C. longa efficiently inhibits the tyrosinase. The parent compound Curcumin is a potent inhibitor of protein kinase C, EGF-receptor tyrosine kinase and IkappaB kinas. Moreover C. longa is reported an effective skin lightening agent with multifunctional topical benefits, without irritant and sensitization side effects. [17]

Fig. No. 2 Herbal Faireness Cream

MATERIAL AND METHOD

INGREDIENTS:

Table no.1: - List of ingredients and their sources

Sr. no.	Ingredients	Sources
1	Amla extract	Lab prepared
2	Aloe-vera extract	Lab prepared
3	Turmeric extract	Lab prepared
4	Tulsi extract	Lab prepared
5	Papaya oil	Salvia papaya oil
6	Glycerol	Burgoyne Burbidge's &Co
7	Cetyl alcohol	Burgoyne Burbidge's &Co
8	Almond oil	Hamdard Laboratories (India)
9	Methyl paraben	Research Lab-fine Chem Industries Mumbai

10	Triethanolamine	Burgoyne Burbidge's &Co
11	Stearic acid	Research Lab-fine Chem Industries Mumbai
12	Distilled water	Lab prepared

Instruments:

Table No.2: List of Instruments

Sr. no	Apparatus	Company
1	Soxhlet apparatus	Shanti scientific industries
2	Digital ph. meter	Globe Instruments
3	Brookfield viscometer	FichaTecnica
4	UV-spectrophotometer	Shimadzu, UV-1700 Pharma Spec
5	Autoclave	ASI 456

Method of preparation: -

Step I: Aqueous phase.

- Water-soluble components like methyl paraben, amla extract, aloe vera extract, turmeric rextract, tulsi extract were added in desired amount of water.
- Stir well until they are completely dissolved in water.

Step II: Oil phase.

- Glycerin and triethanolamine were taken in a beaker.
- Oil phase was prepared by heating stearic acid and cetyl alcohol at suitable temperature.
- Natural oils were added.

Step III: Emulsion

- Oil phase was added to aqueous phase at 80 ℃ with continuous stirring.
- It was homogenized till uniform emulsion is formed.
- The finished product was poured into the wide mouth container.
- The container was well-labelled and stored at room temperature.[18]

Formulation of Herbal Fairenesscream: -

Table no. 3: - Formulation Of Herbal Fairness Cream

Sr.no.	Ingredients	Formula % w/w(quantity for 30g)		
		F1	F2	F3
1	Amla extract	1gm	1.2g	1.5g
2	Aloe- vera extract	1.2g	1g	1.3g
3	Turmeric extract	1g	1g	1g
4	Tulsi extract	1.2g	1.59g	1.3g
5	Papaya oil	4ml	3ml	4ml
6	Glycerol	3ml	3ml	3ml
7	Cetyl alcohol	1.8ml	1.9ml	2ml
8	Almond oil	4ml	4ml	4ml
9	Methyl paraben	0.02ml	0.02ml	0.02ml
10	Triethanolamine	2.8ml	2.4ml	1.9ml
11	Stearic acid	10ml	11ml	10ml
12	Distill Water	q.s	q.s	q.s

EXPERIMENTAL METHOD

EXTRACTION PROCESS:

EXTRACTION OF TURMERIC: -

Turmeric extracts were prepared by Soxhlet apparatus method: -

1. The rhizomes of turmeric were dried in oven at 105 °C for 3 h.

2. Dried rhizomes were triturated using mortar and screened through a sieve with mesh 80 to obtain uniform powder with particle size of 0.18 mm. The turmeric powder was stored in refrigerator to prevent moisture uptake.
3. The Soxhlet extraction, as the reference method, was performed as follows: 50g ground turmeric powder was weighed and embedded in a thimble and put in the Soxhlet apparatus which was gradually filled with ethanol350 ml in RBF as a extraction solvent.
4. The extraction experiment was carried out at 60 °C within 24 hr until clear colourless extraction was obtained.
5. Extract was obtained after 24 hrs.
6. Extract kept in hot air oven for drying till 48 hrs. at 500C.
7. Extract obtained in powder form. [19]

EXTRACTION OF AMLA: -

Amla extracts were prepared by maceration method: -

1. Fresh amla were taken from the central market.
2. Amla was washed and dried in oven at 1050 For 3 h.
3. Dried amla were triturated using mortar and pastel.
4. Afterthat grind it and screened through a sieve with mesh 80 to obtain uniform powder with particle size 0.18 mm.
5. Amla powder was stored in refrigerator to prevent moisture uptake.
6. In this process the amla powder (50 g) is placed with the whole of the alcohol (360 ml) in a closed vessel for 7 days.
7. During this period shaking is done occasionally. After 7days the liquid is strained, and marc is pressed.
8. The extract was obtained and dried in hot air oven at 500 C for 24 hr. [20]

EXTRACTION OF TULSI:-

Tulsi extracts were pared by Soxhlet apparatus method: -

1. Fresh Tulsi leaves were plug from garden.
2. Tulsi leaves were washed and dried at hot air oven at 1050 C for 3 hr.
3. The dried Tulsi (50g) powder was placed in the thimble of Soxhlet apparatus.
4. 450ml ethanol was used for extraction.
5. The extraction was continued till clear solvent or water was seen in the thimble.
6. The extract was concentrated using rotary evaporator.
7. Then the extract was dried at hot air oven at 500C for 24 hours.

[21]

EXTRACTION OF ALOE-VERA: -

Aloe-vera extract were prepared by maceration method:-

1. Fresh Aloe vera leaves plug from garden.
2. Leaves of aloe barbadense were cut.
3. To avoid biodegradation of aloe vera leaves kept in the ice box at 4-50 C
4. The leaves were thoroughly wash with fresh water and dry in hot air oven at 1050 C for 48 hours.
5. Removed from hot air oven with the help of tongs.
6. Domestic blender was used to grind the aloe vera.
7. After the grinding screened through a sieve with mesh 80 to obtain uniform powder.Aloe powder was stored in refrigerator to prevent moisture uptake.
8. In this process the aloe powder (25 g) is placed with the whole of the ethanol (250 ml) in a closed vessel for 7 days.
9. During this period shaking is done occasionally. After 7days the liquid is strained, and marc is pressed.
10. The extract was obtained and dried in hot air oven at 500 C for 24 hr.[22]

EVALUATION PROCES

PHYTOCHEMICAL TESTS

Test For Carbo hydrates

Molisch's Test:

2-3 ml extract + few drops of α- napthol solution in alcohol + conc. H_2SO_4. Violet ring is formed at junction of two liquid.

Test For Proteins

1. **Biuret Test:**

3 ml extract + 4% NaOH + few drops 1% $CuSO_4$. Voilet or Pink color.

2. **Millon's Test:**

3 ml extract + 5ml Millon's reagent. White ppt turn brick red ppt dissolves giving red color solution

Test For Amino Acid

1. Ninhydrin Test: Heat 3 ml extract + 3 drops 5% ninhydrin solution in boiling water bath for 10 min. purple or bluish color appears.
2. Tyrosine Test: Heat 3 ml extract + 3 drops Million's reagent. Solution shows dark red color.
3. Cysteine Test: 5 ml extract + few drops of 40% NaOH + 10 % lead acetate solution. Boil solution. Black ppt formed.

Test For Glycoside

Baljet's Test:

Extract + Sodium Picrate Yellow to orange color. 3 Keller- Killiani Test: 2 ml extract + glacial acetic acid + 1 drop 5% $FeCl_3$ & conc. H_2SO_4. Reddish brown color appears at junction of 2 liquid layers & upper layer appears bluish green.

Test For Flavonoids

Sulphuric Acid Test:

Extract + sulphuric acid (66% or 80%) flavones &flavano dissolve into it gives deep yellow solution. Chalcones & Aurones give red or red bluish solution flavanes give orange to red colors.

Test For Tannins

1. **5% $FeCl_3$ Solution:**

2-3 ml extract + few drops of 5% $FeCl_3$ Solution. Deep blueblackcolor.

2. **Lead Acetate Solution:**

2-3 ml extract + few drops of lead acetate solution. White ppt.

Test For Alkaloids

1. **Dragendroff's Test:**

2-3 ml extract + few drops of Dragendroff's reagent.Orange brown ppt is formed.

2. **Mayer's Test:**

2-3 ml extract + few drops of Mayer's reagent ppt is formed.

3. **Hager's Test:**

2-3 ml extract + few drops of Hager's reagent yellow ppt is formed.[23-24]

Thin layer chromatography of Extract:

The phytochemical determine in the extract by the phytochemical test where analyzed by thin layer chromatographic method.TLC plate where prepared by using silica gel as absorbent .silica Gel G(15) gram was mixed with 30mL of distilled water to make slurry ,slurry immediately poured on the plate ,plate where allowed to air dry for one hour and layer was fixed by drying at 100°C in hot air oven for half hour.Using a micropipette about 10 mil extract where loaded gradually over the plate and air dried.The plate were developed by using two different solvent system, Take 10 ml Solvent in beaker as mobile phase for Tulsi take solvent consist chloroform :methanol (12 :2).

- For Aloevera as mobile phase takes solvent consists chloroform: methanol (12:2)
- For Amla as mobile phase takes solvent Consist butanol : acetic acid :water 8 :2:2
- For turmeric as mobile phase takes solvent consist butanol : acetic: water 8:2:2 .

now the plate is inserted in solvent which is mobile phase after and half hour remove the plate from the mobile phase and place were allowed to air dry ,and determined the distance covered by solute and solvent and calculate Rf value. [25-26]

Organoleptic evaluation:-

Parameters like Appearance, Color, Odor, Homogeneity, Consistency and Texture were evaluated by visual interpretation

pH:

The pH of the preparation was determined by using digital pH meter. The pH meter was initially calibrated at different pH using suitable buffer solution. A 10 % (w/v) dispersion of the preparation was prepared in distill water and pH was determined directly without any further dilutions.

Spreadability:

Excess of sample was placed between two glass slides. 10g of weight was placed on the glass slide for 5 min to compress the sample to a uniform thickness. The time required to separate the two slides was taken as a measure of spread ability. The results were recorded. Spread ability is calculated by using formula-

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

where S=spread ability, M=weight tied to the slide, T=time, L=length of glass slide

Washability:

It was determined by rubbing the little amount of cream on hand which was later washed under the running tap water

Viscosity:

Viscosity of the cream was determined by Brookfield viscometer. The correct spindle was selected (spindle no.4) for the given product and the operating conditions were set up. Then, the viscosity was directly measured at 6 rpm speed by keeping the torque constant. The mean was obtained. The viscosity is determined by the formula-

$$\text{Viscosity}=\text{Dial Reading}\times\text{Factor}$$

(for LV-4 at 6 rpm, factor is 1M)

Rancidity:

For evaluation, 10 ml of melted cream wax taken and added to 10 ml of concentrated hydrochloric acid along with 10 ml Phloroglucinol solution. The mixture was shaken well for one minute. The material shall be taken to have passed the test if no pink color is developed

Homogeneity:-

The formulations were tested for the homogeneity by visual appearance and by touch.

Type of smear :-

After application of cream, the type of film or smear formed on the skin were checked.

Removal:-

The ease of removal of the cream applied was examined by washing the applied part with tap water.

Irritancy test :-

Mark an area (1sq.cm) on the left hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24 hrs and reported.[27]

Anti-inflammatory activity:-

Protein denaturation test (pain killer):-

Preparation of reference drug (positive control):

NSAID (ibuprofen) were used as reference drug Ibuprofen was crushed into fine powder. About 0.2 g of Ibuprofen drug powder was measured using a digital analytical balance and was added to 20.0 ml of distilled water. The solution was mixed well.

Serial dilutions: Serial dilution from 1000 ug/ml to 0.01 µg/ml was performed for 3 sample extract and for reference drugs (prednisolone and ibuprofen). All samples contained 5.0 ml of total volume. Reaction mixtures were prepared using 2.8 ml of phosphate-buffered saline (pH 6.4) and 0.2 ml of egg albumin (from fresh hen's egg). Then 2 ml of extract from each different concentration were mixed gently with reaction mixtures. A similar procedure was used for reference drugs (prednisolone and ibuprofen) and they were used.

Inhibition of protein denaturation: Reaction mixtures were incubated in a water bath at 37℃ ± 2℃ for 15-20 min, and later, it was heated at 70℃ at which the reaction mixture was maintained for 5 min. Then, the reaction mixture was allowed to cool down at room temperature for 15 min.

Absorbance of mixture before and after denaturation was measured for each concentration at 680 nm using a colorimeter. Each test was repeated thrice and the mean absorbance was recorded. The percentage of inhibition of protein was determined on a percentage basis with respect to control using the following formula

Percent inhibition (%) = Absorbance control – Absorbance sample/ Absorbance control[28]

Anti-microbial test:-

Preparation of nutrient broth:

For the preparation of nutrient broth take the weighted amount of beef extract, sodium chloride and peptone in 500 ml of distilled water. Heat the mixture and agitate with glass rod to dissolve the ingredients. Add distilled water to make up the final volume. Adjust the pH of the medium to 7.0 by adding acid or alkali. Pour 10 ml medium in each test tube or a conical flask. Apply cotton plugs to all test tube or conical flask.

Sterilize in autoclave at 121 C̊ under 15 lb. pressure for 15 min. Allow the autoclave cool. Remove the broth tubes or flasks and store at room temperature for use.

Preparation of nutrient Agar:

For the preparation of nutrient agar take the weighted amount of beef extract, sodium chloride and peptone in distilled water except ager. The pH of the fluid medium is determined with the pH meter and adjust by using 1N HCL or 1N NaOH. Add agar powder and medium is heated to dissolve the agar to form clear liquid. The medium is dispensed into tubes or flasks. Plug the flasks containing medium by using non-absorbent cotton.

Thin layer chromatography of Extract: Table no. 05:- Thin layer chromatography Sterilize in autoclave at 121 C̊ under 15 lb. pressure for 15 min in an autoclave. Allow the flasks to cool up to 50℃ and pour the medium quickly into sterile petri plates under aseptic condition. Allow the medium to cool and to produce solid agar plates.[29

RESULT AND DISCUSSION:

The percentage yield for alcoholic extraction was 20%,15% 25% 17.5% was obtained with 75% ethanol for Aloevera Extract ,Tulsi Extract, Turmericextract,Amla Extract Respectively The difference in the percentage yields could be traced to either the components extracted or the different methods of extraction: Soxhlet extractor was employed for ethanolic extraction and this gave a higher percentage yield because Soxhlet extraction gave complete extraction and cold maceration employed for extraction may not give complete extraction.

Phytochemical Test.

Table no.04:- Phytochemical test of Extract:

Sr no	Plant constituent	Test /Reagent	Aloe barbadensis mill	Ocimum tenuiflorum	Curcuma Longa	Phyllanthus Emblica
1	Carbohydrate	Molish Test	Present	Present	Present	Present
2	Carbohydrate	Fehling's test	Present	Present	Absent	Present
3	Carbohydrate	Benedict test	Present	Present	Absent	Present
4	Proteins	Millions test	Present	Absent	Absent	Absent
5	Amino acid	Ninhydrin test	Present	Present	Present	Absent
6	Amino acid	Tyrosin test	Present	Present	Present	Present
7	Amino acid	Cysteine test	Absent	Present	Absent	Absent
8	Glycoside	Beljet test	Present	Present	Absent	Absent
9	Glycoside	Killer killani test	Present	Present	Absent	Absent
10	Flavonoid	Shinoda test	Present	Absent	Absent	Absent
11	Flavonoid	Sulphuric acid test	Present	Absent	Absent	Present
12	Tannins test	Lead acetate solution	Present	Present	Present	Present

13	Tannins test	Acetic acid test	Present	Present	Absent	Present
14	Alkaloid	Dragendr off test	Present	Present	Present	Present
15	Alkaloid	Hagers test	Present	Present	Present	Present
16	Alkaloid	Mayers test	Present	Absent	Present	Present
17	Phenol	Lead acetate test	Absent	Absent	Present	Present

Thin layer chromatography of Extract:

Table no. 05:- Thin layer chromatography

Sr no	Extract	Stationary Phase	Mobile Phase	Spraying Reagent	Retension Factor (Rf) Value
1	Tulsi Extract	Silica gel G	Chloroform:Ethano l (12:2)	Dragendroff reagent	0.74
2	Aloevera Extract	Silica gel G	Chloroform:Ethano l (12:2)	Dragendroff reagent	0.66
3	Amla Extract	Silica gel G	Butanol:Aceticacid: Water (8:2:2)	Ninhydrin n reagent	0.40
4	Turmeric Extract	Silica gel G	Butanol :Aceticacid:Water (8:2:2)	Ninhydrin n reagent	0.85

Fig no.3:-Turmeric TLC

Fig no.4:- Amla TLC

3. Physicochemical evaluation:

The prepared cream were found to be good in appearance along with easily acceptable colour

and odour. The odour of the different batches with the extract has a characteristic smell of the extract, It was also found that the formulated preparations that the prepared cream were easily washable under the running water or tap water. Over-all wash ability was found toA be good. The spread ability value indicates that the cream formulation is easy to apply. The viscosity of the different batches ranges from 10018, to 20000 mPas. Batch BF3 with viscosity of 10064 mPas was the most viscous. Batch BF2with viscosity of 9808 mPas was the least viscous cream. The prepared formulations were found to be easily applicable on are uniform and homogenous in nature with good consistency and smooth texture .It was observed

topical administration along with good absorbing property.Cream is not rancid according to rancidity test no pink colur form after testing. physical properties of aqueous herbal cream formulated from ethanolic herbal extracts are represented in Table 06. The different batches of creams had different colours based on the quantity of the extract added .The different batches were homogeneous and stable for over 30 days that they were observed

Table no. 06: Evaluation Parameter

Sr. No.	Evaluation Parameter	Observation		
		F1	F2	F3
1	Appearance	Cream like	Cream like	Cream like
2	Colour	Pale Yellow	Pale Yellow	Pale Yellow
3	Oduor	Floral	Floral	Floral
4	Texture	Fine	Fine	Fine
5	Smoothness	Smooth	Smooth	Smooth
6	Homogeneity	Homogeneous	Homogeneous	Homogeneous
7	Smear test	aqueous	aqueous	aqueous
8	Removal test	Easily removable	Easily removable	Easily removable
9	Rancidity	No pink Colour no rancid	No pink Colour no rancid	No pink Colour no rancid
10	Spreadability test	Easily spreadable	Easily spreadable	Easily spreadable
11	Washability test	Easily washable	Slightly washable	Slightly washable
12	Viscosity	10018	9808	10064

4. pH:

The pH values of all the formulations were in the close range of Ideal pH values for skin of face. The pH of the different batches of cream ranged from 4.54 to 6.84, which was in the acidic region and similar to the pH of the stratum corneum layer of the skin. Thus, the cream will not irritate the skin and also the pH is sufficient to prevent the growth of fungi or bacteria in the formulation.

Table no. 07:- pH

Sr. No.	Evaluation parameter	Observation		
		F1	F2	F3
1	pH	5.40	5.39	5.93

5. Irritancy test:

No any kind of skin irritation or lesions were noticed in human skin when tested for skin

irritancy. Moreover, the prepared cream were found to be safe & effective. It also provides a cooling effect on topical application to the skin

Table.no. 08 :-Irritancy Test

Sr. No.	Evaluation parameter	Observation		
		F1	F2	F3
1	Irritancy Test	No reaction	No reaction	No reaction
2	Erythema Test	No reaction	No reaction	No reaction
3	Edema Test	No reaction	No reaction	No reaction

6. Anti-inflammatory test:

Table No.09:- Percentage protein denaturation of Sample (F1)

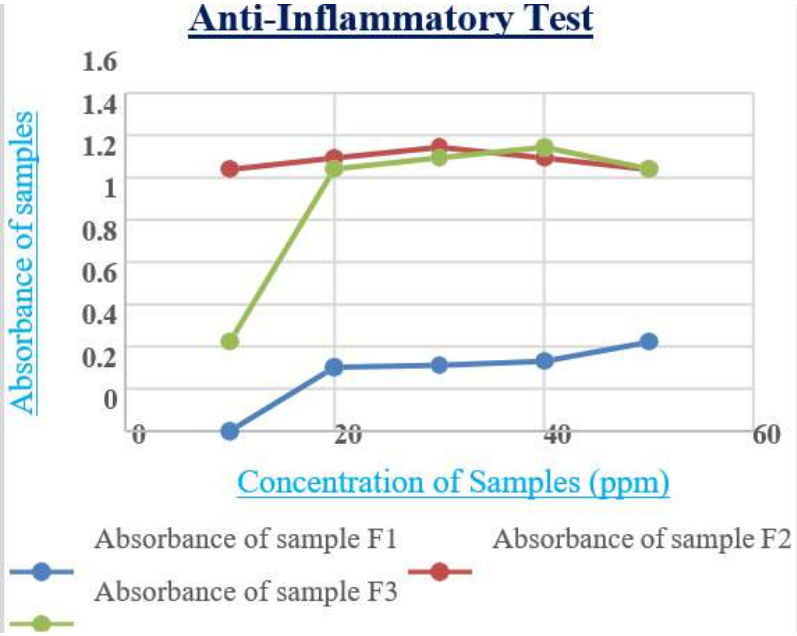
Sr.no.	Concentration of sample F1 (ppm)	Absorbance of blank	Absorbance of sample	Percentage protein denaturation
1	10	0.483	0.234A	67%
2	20		0.304A	58%
3	30		0.314A	56%
4	40		0.333A	54%
5	50		0.424A	41%

Table No.10:- Percentage protein denaturation of Sample (F2)

Sr.no.	Concentration of sample F2 (ppm)	Absorbance of blank	Absorbance of sample	Percentage protein denaturation
1	10	0.483	1.242A	70%
2	20		1.295A	77%
3	30		1.345A	84%
4	40		1.295A	77%
5	50		1.242A	70%

Table No.11:- Percentage protein denaturation of Sample (F3)

Sr.no.	Concentration of sample F2 (ppm)	Absorbance of blank	Absorbance of sample	Percentage protein denaturation
1	10	0.483	1.242A	70%
2	20		1.295A	77%
3	30		1.345A	84%
4	40		1.295A	77%
5	50		1.242A	70%



7. Anti-microbial test:-

The results of antimicrobial sensitivity to formulated herbal cream are presented in Table 3, while the minimum inhibitory concentrations (MICs) of thecreams are presented in Table 12. The negative control and the cream containing 1.25%w/w, 2.5%w/wand 5.0%w/w of the ethanolic extract had no activity against Staphylococcus aureus and E.coli. However the batch containing 15%w/w of extract showed activity against Staphylococcus aureus and E.coli with a zone of inhibition of 25mm for Staphylococcus aureus and 25 mm for E.coli .

Table no. 12:- Anti-microbial test of herbal faireness cream

1.	**For E. coli**			
Conc. (µg/ml)	**Formulation**			**Standard (ampicillin)**
	F1	**F2**	**F3**	
	Zone of inhibition(mm)			
100	24	23	22	25

2	**For S. aureus**			
Conc. (µg/ml)	**Formulation**			**Standard (ampicillin)**
	F1	**F2**	**F3**	
	Zone of inhibition(mm)			
100	25	23	23	28

DISCUSSION:

In the present work papaya, turmeric, aloe-vera, amla, tulsi which gives skin whitening anti-oxidant and anti-microbial. The formulation F1 and F2 shows the best result maybe because of constituents present in it such as papaya, turmeric, aloe-vera, Amla, Tulsi. Amla and turmeric is known for its anti- microbial and anti-bacterial properties that may aid in infections in small quantity of Amla have inhibitory effects on bacteria Based on the findings of the present study the relevance of Amla, Tulsi, turmeric, aloe-vera and papaya are safe and effective for skin whitening anti -wrinkle anti-microbial and anti-inflammatory For our research study we have formulated three batches from which F1 and F2 possess good quality of ideal fairness cream which helps them to perform for the study formulation F3 did not pass the common evaluation test such as anti-inflammatory and anti-microbial later on formulation F1 and F2 are kept under observation for time period of a week everyday it was watched weather they were stable or not. From the above cited study it was found that the formulation F1 where superior andF2 and F3 was found to be optimum batch among of them the other formulation.

CONCLUSION:

From the above discussion of it is concluded that on combining the extracts of aloe vera, turmeric, tulsi, amla in different ratio to get multipurpose effect such as whitening, antiwrinkle, antiaging and sunscreren effect of skin. as we known that it not possible to increases the extend of efficiency of medical and cosmetics properties of single plant extract but by combining the plant extract it can be possible to increases the efficacy of extract in this regard .we mix the extract of alo vera turmeric amala tulsi to improve as well as synerizes the cosmetic properties of prepared product compare to individual extract further research will carry out to cheak scientific action of selected formulation. the studies suggest that composition of extract of base cream are more stable and safe it may produced synergistic action All three herbal ingredients showed significant different activities. Based on the results, we can suggest that all the three formulations F1, F2, F3 were stable and can

be safely used on the skin..From the above based result it is concluded the F1 is the ideal and stable batch as compared to F2 and F3

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HOW TO CITE: Manisha U. Mishra , Aishwarya A. Jain , Prachi S. Patle, Pratibha U. Thakur , Priyanshi A. Ramteke , Riya A.Jaitwar , Pratiksha I. Damahe, Creation And Evaluation Of Multi Herbal Fairness Cream By Using Carica Papaya And *Phyllanthus Emblica*, Int. J. of Pharm. Sci., 2024, Vol 2, Issue 7, 579-593. https://doi.org/10.5281/zenodo.12700090