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FORMULATION AND *INVITRO*EVALUATION OF POLY HERBAL ANTI AGING FACE CREAM

Sai Lakshmi Jyothirmai Kala* and Supriya Palaparthi

Department of Pharmaceutics, Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur.

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***Corresponding Author**
Sai Lakshmi Jyothirmai Kala
Department of Pharmaceutics, Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur.

ABSTRACT

Cosmetics are the substances intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, and altering the appearance without affecting the body's structure or functions. 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. Herbal Cream is a polyherbal formulation that consists of extracts of Aloe barbadensis, Ocimum sanctum, Azadirachta indica, Curcuma longa, Cedro oil, Myristica fragrans, Olium rosae (Rose Oil), Orange Oil, Prunus dulcis, Ocimum sanctum. Herbs such as aloevera leaf, turmeric, banana, neem, papaya, cucumber and other plants are used as herbs in cosmetics. In the present research article the polyherbal face cream was prepared using the following ingredients extract of curcuma longa, solanum lycoperisum, carica papya, olive oil, almond oil, strawberry essence and distilled water. Solanum lycoperisum is used as an anti oxidant and prevents skin aging. Olive oil moisturese sthe skin, papya nourishes the skin, curcumin acts as an antimicrobial agent. The skin aging is mainly caused by destruction of collagen layer in the skin which can be prevented by using almond oil The prepared polyherbal cream was evaluated for pH, spreadability, homegenity, viscosity, extrudability, invitrodiffusion studies, physical appearance, dye test, removal test, skin irritation, antimicrobial studies, HPLC study for curcumin. Among all the prepared creams F2,F3 were found to be the best formulations. The cumulative %drug release from the prepared cream was found to be 91%.

KEYWORDS: Curcuma longa, solanum lycoperisum, carcica papaya, almond oil, olive oil, distilled water.

INTRODUCTION

Skin care is the age old necessity of mankind. Smooth, supple skin is a sign of youthfulness, but a structural protein called collagen is behind this flawless look. Collagen forms an even layer under the skin and acts as a good padding that keeps the skin taut, yet supple. Fine wrinkles start to appear as we age because of thinning of the skin and unevenness in the collagen padding underneath. It is an inevitable part of the natural aging process, but premature aging can result from excess exposure to sun, tobacco smoke, and dehydration. With proper protective measure and skin care, the aging process can be slowed down to some extent. Oxidative stress is one main reason for the deterioration of collagen layer. This necessity lead to the continuous modification and invention of more and more skin care cosmetic preparations. Skin care preparations are not new and dates back to earliest antiquity. The use of slaves and unguents for preserving and beautifying the skin is a very old process. There were prepared, mainly, by digesting roots, flowers, gums, aromatic resins with fats and oils.

The first notable change was made by the Greek physician Galen during second century A.D., by addition of water to his sales. That preparation was considered to be the foundation of modern days cleansing creams and cold creams Though over the centuries a continuous modification took place, but the basic concept remained unchanged. I t has become so important that plenty of efforts and money are being continuously spent on research dealing with effect of various materials and preparations on the skin and to design better skin care preparations.

With the availability of wide spectrum additives like emulsifying agents., etc and development of various techniques, preparation of cream has become very simple. Mostly, the creams are emulsion type and consistency can vary from a liquid to a spreadable solid.

All the skin care creams can be classified on different basis:-

1. According to function EX:-.cleansing, foundation, a massage, etc.
2. According to characteristic properties EX:-cold creams, vanishing creams, etc.
3. According to the nature or type of emulsion.

The most widely accepted classification is based on fuction.

According to the function the cream can be classified as follows-

1. Cleansing and cold creams.
2. Foundation and vanishing creams.

3. Night and massage creams.
4. Hand and body creams.
5. All purpose and general creams.

1. Cleansing creams and cold creams:- Cleansing cream or lotion is required for removal of facial make up, surface grime oil and water and oil soluble soil efficiently, mainly from the face and throat. A good and properly formulated cleansing cream should be able to remove quickly and efficiently, applied cosmetics as face powder, rouge, foundation bases, cake make –up, lipstick. Cleansing cream overcomes the dis advantage of the normal soap such that it makes the skin look soft where as the soap makes the skin to look dry. The cleansing cream has the following mechanism, Such that it can readily remove the chemical substances of the facial make –up by dissolving or lifting away the greasy binding materials holding pigments or grime on the skin.

Types of cleansing creams

1. White, emulsified cold creams(bees wax-borax type)

2. Translucent, liquefying type, anhydrous in character and consisting of a mixture of hydrocarbon oils and waxes.

2. Foundation and vanishing creams

a. Vanishing creams: Vanishing and foundation creams are widely used for various purposes. The names have been derived according to the functions. Vanishing creams are vanishing creams because they seemed to be disappear when rubbed into the skin these preparations are stearic acid based and part of the stearic acid is saponified with an alkali and rest of the stearic acid is emulsified with the soap in a large quantity of water. After application the cream leaves a dry but tacky residual film which also has a drying effect on the skin, beacuse of this reason the stearic acid soap based creams are still favoured for use with greasy skin conditions.

b. Foundation creams: Foundation creams are applied to the skin to provide a smooth emolient base or foundation before the application of face powder and other make –up preparations, they help the powder to adhere to the skin due to possession of good holding power, they should spread well and should be non greasy and should leave non –occlusive film on the face. The humectant present in the preparation helps in the retention of the powder.

3. Night and massage creams: Skin nourishment is important and required to preserve the normal characters of skin or as a treatment for dry skin. To supplement foods for skin and to treat the dry skin various creams containing different ingredients are used. The common feature of all the creams is that they are generally applied on the skin and left for several hours, say over night and all of them assist in the repair of skin which has been surface damaged by exposure to various elements or exposure solution or soap. Night creams are applied are night and are normally assigned to skin preservation and feeding.

Emolient action can be achieved by two mechanisms.

1. Prevention of water loss from the skin and thus building up of water content from within.
2. Supplementing the water content of the skin by attracting the water from atmosphere by means of humectant material. The mechanism of attracting water from atmosphere and thus maintaining the water content of the skin is called moisturising which is the main advantage of night and massage creams.

4. Head and body creams: Hand and other body parts skin may be exposed to water, water soap, detergents causes removal of lipids and others secretions from the skin. Skin dry, scaly, infections due to microbes can leads to dermatitis, so to control all these hand body creams are applied.

Main function of hand and body creams are:-

- Provide an oily film to protect the skin.
- Keep the skin smooth but not greasy.
- Easy to apply.

5. All purpose creams: In recent times may has been tremendous increase in consumption of preparations which are normally known as all purpose creams. They are well known as sports creams. As they were used by sports men in skilling and outdoor activities. They are some what oily but non greasy type and can spread easily on the skin to give a protective film. They can also function, when applied exclusively as a skin food or nourishing cream or night cream or protective cream for prevention or elevation of sunburn or for the treatment of roughened skin areas. Also when applied sparingly they function as hand creams or foundation creams. Thus they are called as all purpose creams. So the composition of these creams is such that it can act

1. As a foundation cream to provide a foundation base for makeup
2. As a hand cream and should have an emollient character.

3. As a protective cream and should form a continuous non occlusive cream.
4. As a cream to smooth the rough surface of skin.

The aim of the present study was to formulate and evaluate polyherbal cream containing the extract of curcuma longa, carcica papaya, solanum lycoperisum, alomond oil, olive oil, isopropyl myristrate, propyl paraben, distilled water.

Solanum lycoperisum contains carotenoids, lycopene and β-caroten; as well as other natural antioxidant compounds such as: vitamin C and vitamin E. Lycopene is a carotenoid pigment that gives the red color in tomatoes ripe. Moreover, lycopene is a powerful antioxidant that has a high potential for inhibiting free radicals. The antioxidant power of lycopene as a catcher singlet oxygen is twice compared to β-carotene and ten times compared to α-tocopherol. Tomato juice works as an excellent astringent. Tomato extract removes blackheads, remove tan, improves the fairness of skin.

Carcia Papaya is one the best fruit for incorporating in your skin care regime because it has an enzyme called papain that is responsible for skin whitening, reducing unwanted hair, exfoliating dead skin, repairing ageing skin etc. This papain enzyme is present in maximum concentration just under the peel of unripe papaya. Papaya is also a rich source of vitamin C, vitamin A, vitamin E, pantothenic acid, folate, magnesium and potassium.

Almond oil is a rich source of skin-replenishing ingredients including triglycerides and several fatty acids (oleic, linoleic, and myristic among them). Makes Skin Healthier. It is used for Smooth, flawless Skin for Deep Cleansing Of Skin, to Remove Dark Circles & Tan, To Relieve Eczema & Psoriasis, To Treat Skin Rashes, To Reduce Fine Lines & Other Signs Of Aging.

Olive oil is rich in vitamins**.** Olive oil does have some reported benefits to the skin. According to the International Olive Council, olive oil has many vitamins, including A, D, and K, as well as vitamin E. It has antioxidant properties**.** Olive oil is also an antioxidant, so it might help prevent or reverse damage from cancer-causing ultraviolet radiation. It has a very high concentration of an ingredient called squalene as compared to the other types of fats and oils that humans normally eat. The squalene is what gives olive oil the extra antioxidant boost. It moisturizes and fights bacteria. Olive oil is also known to moisturize and hydrate your skin.

Curcuma longa is Used as a key ingredient for making curries, turmeric (*Curcuma longa*) is a plant native to southwest India and a member of the ginger family. Curcumin – one of the most important active compounds found in turmeric – is an anti-inflammatory, antioxidant, antibacterial, antifungal and antiviral powerhouse. **Curcumin** has the following benefits it Hasten Healing, Correct Oily Skin **(**turmeric contains fatty acids and phytosterols, which have been observed in other studies to reduce excess skin oils). Sun Damage Protection, Anti-Aging Treatment, Skin Cancer Prevention**(**Curcumin has also been recognized as an impressive anti-cancer treatment, able to selectively kill tumor cells while leaving the normal cells intact), Treat Chronic Skin Conditions**.** curcumin helps increase connective tissue formation and promotes blood flow.

Preparation and isolation of herbal extracts

1. **Isolation of Curcumin:** Accurately weighed quantity of turmeric powder was macerated with n-hexane for 2 hrs. Then its marc was extracted with acetone for 2 hrs. From the extract so obtained, acetone was re-collected by distillation. Finally, Curcumin was obtained and re-crystallized using hot ethanol.
2. **Isolation of Lycopene:** To accurately weighed quantity of tomato paste, sufficient quantity of methanol was added and shaken vigorously. After 3 hrs, yellow filtrate was discarded and equal quantities of methanol and carbon tetrachloride were added to red mass. Upper phase of methanolic layer was then separated by filtration after vigorous shaking for few minutes. To methanolic layer, 1/3rdvolume of water was added to produce white emulsion. Again methanolic layer was separated and anhydrous sodium sulfate was added to it. Mixture was then filtered and filtrate was evaporated slowly on water bath. Dark oily residue so obtained was dissolved in small quantity of benzene and warmed for few minutes. Finally Lycopene was crystalled out on drop-wise addition of1/2 ml of boiling methanol.
3. **Isolation of papain:** Riped Papaya (*Carica papaya*) fruits were used to make the aqueous extract. Riped papaya fruit weighing 25g were thoroughly washed in distilled water, dried, cut into fine pieces and were crushed into 100 ml sterile distilled water and filtered through Whatman No.1 filter paper (pore size 25 µm).The filtrate was further filtered through 0.6 µm sized filters.

Formulation of cream

Oil in water (o/w) emulsion-based cream was formulated. The lycopene and curcumin extract other oil soluble components were dissolved in oil phase and heated to 75°C. The papaya extract other water soluble components were dissolves in water and heated to 75°C. After heating, water phase was added slowly to oil phase with continuous stirring until cooling of emulsion took place.

Tabke 1:- Formulation table of plyherbal cream.

S.NO	INGREDIENTS	F1	F2	F3	F4
oil phase					
1	Curcuma longa extract(g)	0.1	0.5	0.75	0.25
2.	Solanum lycoperisum extract(g)	0.1	0.5	0.75	0.25
2	Cetosteryl alcohol(g)	1.50	2.75	2.50	1.00
3	White soft paraffin(g)	2.75	3.75	3.50	2.05
4	Almond oil(ml)	1	2	4	1
5	Olive oil(ml)	1	2	4	1
Aqueous phase					
6.	Carcica papaya extract(g)	0.5	0.4	0.7	0.8
7.	Methyl paraben(g)	0.001	0.001	0.001	0.001
8.	Distilled water	q.s	q.s	q.s	q.s

Evaluation of creams

1. **Physical Properties**- The Cream was observed for color, odour and appearance.

2. **pH:**- The pH values of prepared cream was checked by using a digital pH meter.

3. **Spreadability:-** Two sets of glass slides of standard dimensions were taken. The herbal cream formulation was placed over one of the slides. The other slide was placed on the top of the cream, such that the cream was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slide. 100gweight was placed upon the upper slides so that the cream between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of cream adhering to the slides were scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip off freely by the force of

weight tied to it. A 20g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated by three times and the mean time taken for calculation. Spreadability was calculated by using the following formula.

$$S = \frac{m \times l}{t}$$

S – Spreadability,

m – Weight tied to the upper slide (20g)l - Length of the glass (7.5 cm)

4. **Extrudability:-** A closed collapsible tube containing above 20g of cream was pressed firmly at the crimped end and a clamp was applied to prevent any rollback. The cap was removed and the cream extrudes until the pressure was dissipated.

5. **Viscosity:-** Viscosity of the creams was determined by using Brookfield viscometer. Spindle type, at 10 rpm. 100g of the cream was taken in a beaker and the spindle was dipped in it for about 5 minutes and then the reading was taken.

6. **Skin irritation test:-** Test for irritation was performed on human volunteers. For each cream, five volunteers were selected and 1.0g of formulated cream was applied on an area of 2 square inch to the back of hand. The volunteers were observed for lesions or irritation.

7. **Homogeneity:-** All developed creams were tested for homogeneity by visual inspection after the creams have been set in the container. They were tested for their appearance and presence of any aggregates.

8. **In vitro release testing methods:-** The Franz diffusion cell was used to determine the amount of the drug(curcumin) diffused from different formulations Franz diffusion cells with a receiver compartment volume of 10 mL and effective diffusion area of2.84 cm2 were used to evaluate drug delivery characteristics from the eight selected compositions. A dialysis membrane (0.65 μm) was used. The receptor phase (ethanol 50 %, w/w) was continuously stirred and kept at a temperature of 32 ± 0.5°C during the experiments. One gram of formulation was placed in the donor compartment. At appropriate time, 1 mL of the sample was withdrawn from the receiver compartment and the same amount of fresh

solution was added to keep the volume constant. Each experiment was run in four independent cells.

PREPARATION OF EGG MEMBRANE

9. **In vitro release:-** In vitro release of the drug can be performed by diffusion flask method. Here egg membrane is used as a biological membrane.

10. **Preparation of egg membrane**

EGG MEMBRANE:- Egg membrane is prepared by a small hole was made on egg and separate the egg yolk. Egg membrane was separated out by placing the egg shell in conc.Hcl till the membrane was separated from shell. Then the separated egg membrane was continuously washed with purified water to make it free from Conc.Hcl and finally cleaned or washed in alcohol then experiment is carried out. Glass tube with two ends open were taken. At one end of the test tube egg membrane was tied and fitted to a burette stand such that surface of the membrane touches the buffer taken in a beaker which was placed on a magnetic stirrer before placing in buffer.

11. **Permeability studies:-** The invitro diffusion studies of the cream were performed using egg membrane. The membrane was soaked in methanol for 6-8 hr& was clamped carefully to one end of the hollow glass tube. Methanol was used for in vitro release as a receptor medium. The cream sample was applied on the membrane and then fixed in between donor and receptor compartment of glass tube. The receptor compartment contained methanol (100ml) of pH 6.8. The temperature of diffusion medium was thermostatically controlled at 37º ± 1º by surrounding water in jacket and the medium was stirred by magnetic stirrer at 500rpm. The samples were withdrawn at predetermined intervals and were replaced by equal volume of fresh fluid. The samples withdrawn were spectrophotometrically estimated at 421 nm against blank.

12. **Test for microbial growth in formulated creams-** The formulated creams were inoculated on the plates of agar media by cup plate method and a control was marketed himalya polyherbal cream. The plates were placed in to the incubator and are incubated at 37^0C for 24 hours. After the incubation period, plates were taken out and check the microbial growth by comparing it with the control.

13. **Determination of marker ingredient(curcumin) by HPLC:-** The extracts and formulated creams were tested for the presence of curcumin by HPLC.

Chromatographic conditions

Column: Agilent-TC C18 column (250×4.6 mm; 5µ) Flow rate:1ml/min.

Wavelength: UV-420 nm

Column temperature: Ambient

Injection volume: 20 µL

Run time: 10 minutes.

Mobile phase: Mobile phase used was methanol, acetonitrile, and 5% acetic acid (35: 50:15, v/v).

Standard stock preparation: The stock solutions were prepared by dissolving 50 mg of Curcumin was dissolved in 50 ml methanol to get a concentration of 1000 µg/ml. Analytical standard solutions for linearity were prepared by diluting the stocksolution with methanol immediately prior to use. All the preparations were made in borosilicate glass wares.

Estimation of Curcumin in polyherbal cream

Equalent to 100 mg prepared polyherbal skin cream was accurately weighted and transferred to a 100 ml volumetric flask directly with butter paper. 5 ml methanol was added to it and sonicated for 15 minutes to extract the curcumin from the cream. Then the volume was made up to the mark and centrifuged for 2 minutes at 2000 rpm. The centrifuged solution was filtered with 0.45 µm syringe filter (nylon) and 20 µl was injected.

14. **Dye test:-**A water soluble dye (Methylene blue)was added to small quantity of cream and observed under microscope.

15. **Removal**:-The ease of removal of the cream applied was examined by washing the applied part with tap water.

RESULTS AND DISCUSSION

Characterization of Curcumin: -Curcumin was characterized as yellow spot under light in visible region (380-800 nm).

Characterization of Lycopene:- Lycopene was characterized as red spot under light in visible region (380-800 nm).

Evaluation of cream

1. **pH of cream**:- The pH of cream was found to be in the range of 6.5to 6.8 which is suitable for skin pH.
2. **Viscosity:-** The viscosity was in the range of 9500 to 20000 cps which indicates that cream is easily spreadable by applying a little shear. Formulations F2 and F3 showed good spreadability and extrubaility than that of others.
3. **Dye test**:- Dye test confirmed that all the cream formulations were o/w emulsions
4. **Homogeneity:** All formulations produce uniform distribution of extracts in cream. This was confirmed by visual appearance and by touch
5. **Irritancy test**:- All formulation shows no redness, edema, inflammation and irritation during irritancy studies. These formulations are safe to use for skin
6. **Removal**:- The formulations F2 and F3applied on skin were easily removed by simply washing under tap water.
7. **Invitro drug release studies:-** The drug (curcumin)from the formulation was found to be in the range of 19-91%.
8. **Antimicrobial studies:-** The antimicrobial studies revealed that the formulation was sterile and no zone of inhibition was observed.

Table 2:- $P^H$of the cream.

S.NO	pH
F1	6.5
F2	6.6
F3	6.7
F4	6.8

Table 2:- $P^H$of the cream.

Formulations	Viscosity	(mPas)
	6 rpm	12 rpm
F1	15000±0.12	10000±0.23
F2	12000±0.34	9500±0.45
F3	18000±0.11	16000±0.35
F4	20000±0.21	18000±0.31

Table 3:- viscosity of polyherbal cream.

Formulations	Viscosity	(mPas)
	6 rpm	12 rpm
F1	15000±0.12	10000±0.23
F2	12000±0.34	9500±0.45
F3	18000±0.11	16000±0.35
F4	20000±0.21	18000±0.31

Table 4:- zone of inhibition of prepared polyhebal cream.

Formulation code	ZOI (cm)	Test organism
Marketed formulation	Nill	*staphylococcus aureus*
F1	Nill	*staphylococcus aureus*
F 2	Nill	*staphylococcus aureus*
F 3	Nill	*staphylococcus aureus*
F 4	Nill	*staphylococcus aureus*

Table 5:-cumulative %drug release of prepared polyherbal formulations.

CUMULATIVE %DRUG RELEASE				
TIME	**F1**	**F2**	**F3**	**F4**
0	0	0	0	0
1	29	39	24	19
2	38	41	42	23
3	67	63	59	37
4	71	86	71	41
5		91	88	56

Figure 1:- polyherbal cream containing all ingredients.

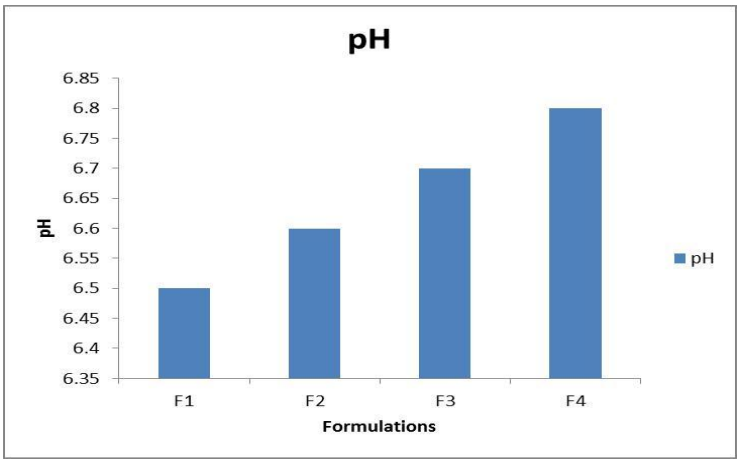

Figure 2:- p^{H} of polyherbal cream

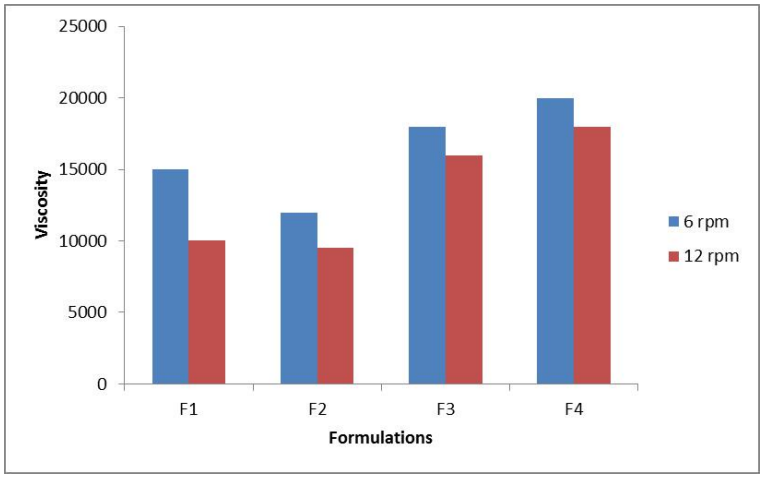

Figure 2:-viscosity of polyherbal cream

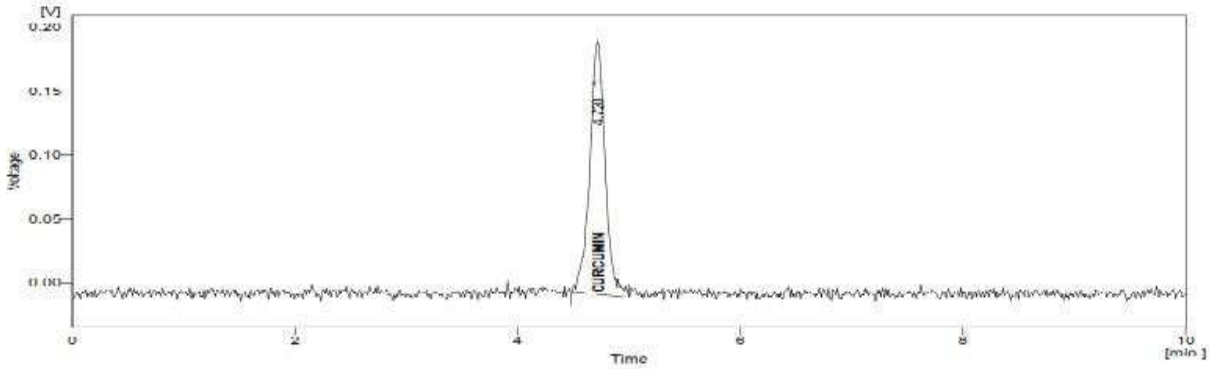

Figure 3:- HPLCchromatogram of standard pure curcumin.

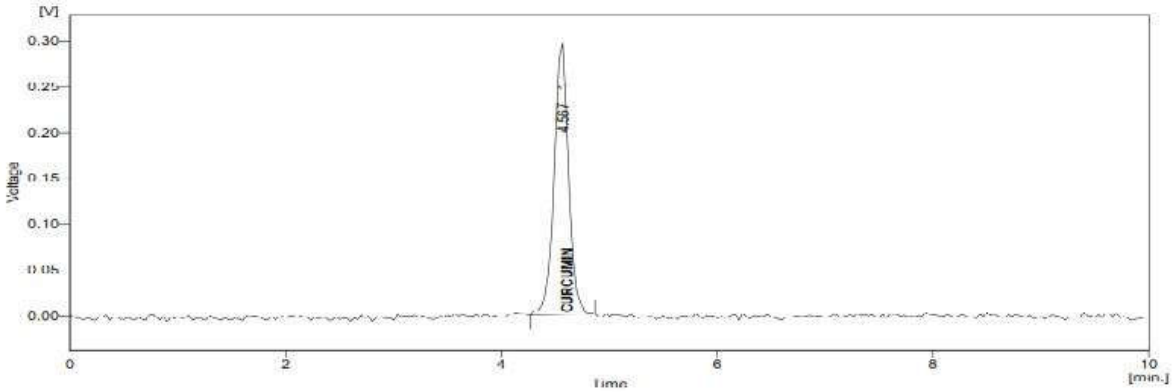

Figure 4:-HPLC chromatogram of curcumin loaded polyherbal cream.

Figure 5:- separated egg membrane soaked in methnol.

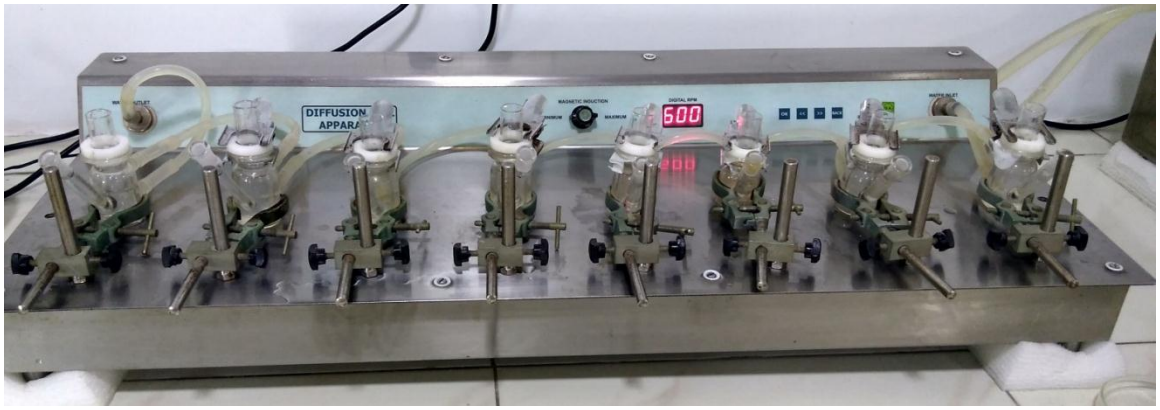

Figure 6:-franz diffusion studies of polyherbal cream.

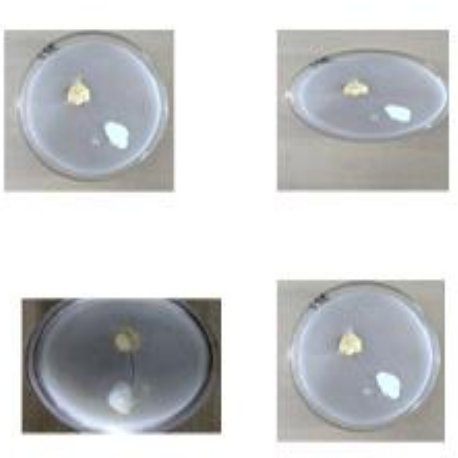

Figure 7:-antimicrobial studies of polyherbal cream.

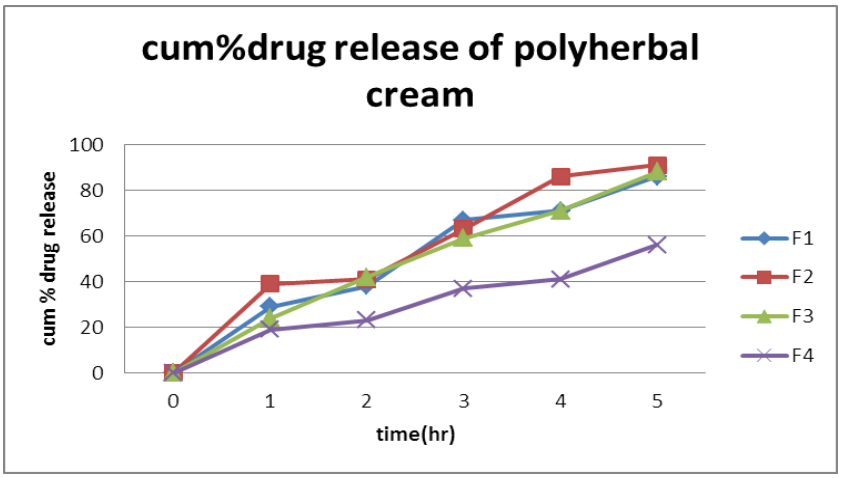

Figure 8:- cumulative %drug release of polyherbal cream.

CONCLUSION

From above discussion it is concluded that on combining the extracts of curcuma longa, solanum lycoperisum, carica papya, in different ratio to get multipurpose effect such as

whitening, antiwrinkle, antiaging and sunscreen effect on skin. As we know that it is not possible to increase the extent of efficiency of medicinal and cosmetic property of single plant extract, but by combining the different plant extracts it can be possible to increase the efficacy of extracts. In this regard, we mixed the extracts of curcuma longa, solanum lycoperisum, carica papya to improve as well synergize the cosmetic properties of prepared products compare to individual extracts. These studies suggest that composition of extracts and base of cream of F2 and F3 are more stable and safe.the solanum lycoperisumand almond oil provides the nourishment to skin and improves the brightening of the skin.among all the prepared formulations F2,F3 were found to be the best formulations.the preared cream was used as an anti aging cream and the cream was found to be stable, sterile, and the ingredients used were suitable for the cream preparation.

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