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# FORMULATION AND EVALUATION OF NOVEL HERBAL ANTI-AGEING FORMULATION (GEL-CREAM)

**Dr. Rashmi Shukla Srivastava[1]* and Khushbu Pravin Shah[2]**

[1]Head of Department in Pharmacognosy, Mumbai Education Trust's Institute of Pharmacy, MET Complex, Bandra Reclamation, Bandra (W), Mumbai 4000-50, India.

[2]M.PHARM Student, Mumbai Education Trust's Institute of Pharmacy, MET Complex, Bandra Reclamation, Bandra (W), Mumbai 4000-50, India.

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***Correspondence for Author**
**Dr. Rashmi Shukla Srivastava**
Head of Department in Pharmacognosy, Mumbai Education Trust's Institute of Pharmacy, MET Complex, Bandra Reclamation, Bandra (W), Mumbai 4000-50, India.

## ABSTRACT

In the present study, the aim was to formulate a herbal anti-ageing gel-cream which overcomes the lacunae of available anti-ageing formulations. The formulated herbal gel-cream contains a mixture of herbal extract (pomegranate extract), nourishing oils (almond oil, olive oil, wheat germ oil , carrot seed oil , grape seed oil and avocado oil ) , volatile oil (orange oil) and gelling agents (*Aloe vera* and carbomer). Initial studies were carried out on pomegranate extract and orange oil that proved to be efficacious. The herbal gel-cream (F5) which contains pomegranate extract (2%) and orange oil (1%) showed maximum anti-oxidant activity ($IC_{50}$ value 0.40 mg) and significant anti-bacterial activity in comparison with the marketed synthetic gel (Clindac gel).The pharmaceutical evaluation and stability studies concluded that the scientific approach adapted to formulate herbal gel-cream containing combination of herbal extract, volatile oil, nourishing oils and gelling agents is an effective contribution in the segment of anti-ageing formulations.

**KEYWORDS:** Gel-cream, anti-ageing, anti-oxidants and nourishing oils.

## INTRODUCTION

Ageing is defined as a progressive deterioration of physiological functions in organisms, eventually leading to senescence and death. Although each part of the body ages with the time, the skin is the most visible organ which makes us aware of the ageing process every minute. The signs of ageing include fine lines and wrinkles, alterations in skin pigmentation,

and a thinner appearance of the skin due to epidermal and dermal atrophy. The main cause of ageing is photo-ageing by UV-A and UV-B rays which causes production of free radicals that affect DNA production. Other reasons responsible for ageing include smoking, hormonal changes and life style.[1, 2, 3]

The topical formulations used to treat ageing are creams and transparent gels.[4]These preparations have several disadvantages like low moisturizing ability, poor spreadability, slower drug permeation and stability problems. To overcome these lacunae, a novel gel-cream formulation can be developed. A gel-cream consists of oily and aqueous phases together with a stabilizing, thickening and/or emulsifying agent. Gel-cream has a smooth texture, good spreadability, feel and provides good nourishment with mositurization. It has rapid drug permeation and is also thermodynamically stable. Herbal anti-oxidants are more advantageous than synthetic drugs because they fight against the free radical and provide nourishment to the skin causing rejuvenation at the same time.[1, 3, 4]

Pomegranate extract provides a wealth of wonderful antioxidant and free radical neutralizing ingredients, for example, ellagic acid, gallic acid, punicalins and punicalagins.[5] Grape seed oil contains flavonoids as antioxidants. These antioxidants contain vitamin E which is required to maintain healthy skin. The essential oils like orange oil boost up the metabolism rate and nourishing oils like almond oil, olive oil, wheat germ oil and avocado oil are rich in vitamin A, E, C and other fatty acids.[4, 5, 6]

The present work aims to formulate and evaluate a herbal anti-ageing gel-cream which is safe, highly stable and less toxic as compared to synthetic formulations.

**Table1: List of herbal anti-ageing agent used**

| Sr. No | Herbal anti-ageing agent | Biological source |
|---|---|---|
| 1 | Pomegranate peel | *Punica granatum,* |
| 2 | Orange peel | *Citrus sinensis* |
| 3 | Olive oil | *Olea europaea* |
| 4 | Almond oil | *Prunus dulcis* |
| 5 | Wheat germ oil | *Triticum aestivum* |
| 6 | Grape seed oil | *Vitis vinifera* |
| 7 | Carrot seed oil | *Daucus carota* |
| 8 | Avocado oil | *Persea americana* |

## MATERIALS AND METHODS

The nourishing oils were purchased from the market and analysed. The raw materials for the preparation of pomegranate extract (peels) and volatile oil from orange (peel) were procured form the local market. Eeuxyl 9010, *Aloe vera* mucilage 200X was procured as gift sample. Ascorbic acid, carbopol 940, DPPH (2, 2-Diphenyl-1 picryl hydrazy) were purchased from Merck Pvt. Ltd, Mumbai. All reagents used were of analytical grade.

## PREPARATION OF HERBAL EXTRACTS

### a. Pomegranate extract

The peels of ripened pomegranates (***Punica granatum***) were manually separated, sun-dried and powdered in a grinder to get 40-mesh size. The powder (50gm) was macerated using methanol as solvent (500ml) at room temperature for 24hours with continuous shaking using mechanical shaker. The extract was filtered and dried using a rotary evaporator (Rota vapor® R-215) at 40 °C. (% yield= 41w/w).

### b. Orange oil

The fresh peels of Orange (***Citrus sinensis***) were cut into pieces smaller than 2 x 2 cm. 400gm of peels were kept in round bottom flask of Clevenger's apparatus 600ml of water was added the assembly was fixed and the extraction was done for 8 hours. The volume of essential oil was determined by graduated tube. The essential oil in the distillate was dried over anhydrous $Na_2SO_4$ and was stored in air tight glass container (% yield=1.4 w/v).

## PREPARATION OF HERBAL ANTI-AGEING GEL-CREAM

### Step 1: Preparation of oil phase

The oil phase ingredients were weighed mixed with continuous stirring using mechanical stirrer at 1000rpm (Remi motor RQT-127 HP1/8) at the temperature 80$^{o}$C to form uniform liquid.

### Step2: Preparation of water phase

The water phase ingredients were weighed mixed with continuous stirring using mechanical stirrer at 1000rpm (Remi motor RQT-127 HP1/8) at the temperature 80$^{o}$C to form uniform liquid.

### Step3: Preparation of Gel-cream base

The oil phase was incorporated in the water phase at 80$^{o}$C with continuous stirring using mechanical stirrer at 1000rpm (Remi motor RQT-127 HP1/8) for 30 mins. Euxyl P.E. 9010 was added as preservative.

**Step4: Preparation of Gel-cream formulation**

Different concentrations of pomegranate peel extract and orange oil were added with continuous stirring using mechanical stirrer at 1000rpm (Remi motor RQT-127 HP1/8) to gel-cream bases till the uniform dispersion of the ingredients was achieved (Table-2).

All these batches were allowed to equilibrate for 24 hours at room temperature. The prepared gel-cream was filled and stored in a wide mouth polypropylene container. The formulation was further evaluated.

**EVALUATION OF HERBAL ANTI-AGEING GEL-CREAM**

**1) Physical parameters**

**a) Color[7]**

All the formulated gel-creams were tested for color by visual inspection. They were checked against white background.

**b) Odor[7]**

The odors of all formulated gel-creams were checked by mixing the gel-cream in water.

**c) Consistency[8]**

The consistency was checked by applying on skin.

**d) Greasiness[8]**

The greasiness was assessed by the application on the skin.

**e) Homogeneity[8]**

All developed gel-creams were tested for homogeneity by visual inspection. They were checked for their appearance and presence of any aggregates.

**f) Grittiness[8]**

All the formulations were evaluated for the particulate matters under light microscope.

**g) Water washability[8]**

All the formulations were applied on the skin the ease and extent of washing with water were checked manually.

**h) Percentage Moisture Content**

Percentage moisture loss for the different batches was determined. The accurately weighed quantity (2gm) of formulation was kept in a desiccators containing 50gm anhydrous calcium chloride. After three days, the formulation was weighed and the percentage moisture loss was calculated using the formula [7]

$$\text{Percentage moisture loss} = \frac{\text{Initial weight - Final weight}}{\text{Final weight}} \times 100$$

**2) Pharmaceutical Parameters**

**a) pH determination**

The pH of the prepared gel-cream was determined using digital pH meter. 1% solution of the formulation was prepared using distilled water. The pH of each formulation was done in triplicate and average values were calculated.[9]

**b) Rheological Study/ Viscosity**

The viscosity of formulations was studied using viscometer (Brookfield digital viscometer RVT). The spindle (62) was rotated at 0.5 rpm. Samples of the gel-cream were allowed to settle over 30 min at the temperature (25 ±/1$^{o}$C) before the measurements were taken. Viscosity was reported in (cP).[10, 11]

**c) Spreadability**

Spreading coefficient was determined by apparatus suggested by Mutimer *et al* (1956). It consists of wooden block, which is attached to a pulley at one end. Spreading coefficient was measured on the basis of 'Slip' and 'Drag' characteristics of gel. A ground glass slide was fixed on the wooden block. An excess of gel-cream (about 2 gm) under study was placed on this ground slide. Gel-cream preparation was then sandwiched between two slides of which the second glass slide has same dimension as that of the fixed ground slide. The second slide is provided with the hook, the pan is attached to the pulley with the help of hook. Weight of 1 g was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of gel-cream between the two slides. Known weight was placed in the pan attached to the pulley with the help of hook. The time (in sec) required by the top slide to separate from

ground slide was noted. A shorter interval indicates better spreading coefficient. It is calculated by using the formula: [10, 11]

$$S = M. L / T$$

Where S is spreadability in g.cm/sec, M is the mass in grams, L is length and T is time in sec.

**d) Drug content**

To ensure uniformity in the formulation, samples were taken in triplicate and assayed for the drug content. Studies were performed by dissolving an accurately weighed quantity of gel-cream (1gm) in 100 ml of phosphate buffer (pH 6.8). These solutions were quantitatively transferred to volumetric flasks and appropriate dilutions were made. The resulting solutions were then filtered and subjected to spectrophotometric analysis at 260 nm for pomegranate extract.[11]

**3) *In-vitro* diffusion study and kinetic analysis**

**a) *In-vitro* diffusion study**

The release of drug from the gel was determined using Keshary-Chein diffusion cell for 4 hrs. The diffusion medium was phosphate buffer pH 6.8, maintained at $37^0$C. The parchment paper was soaked in phosphate buffer pH 6.8 for 1hr and then air-dried. It was mounted between the donor and receptor compartment and gel was placed on it. Both the compartments were clamped together. The phosphate buffer pH 6.8 was filled in the receptor compartment (11ml capacity) and stirred using magnetic stirrer. The samples were withdrawn at different time intervals and replaced with an equal volume of buffer. The samples were analyzed spectrophotometrically after appropriate dilution at 260 nm pomegranate extract. The % cumulative drug releases were calculated.[12]

**b) Kinetic analysis of *in vitro* drug diffusion study**

In order to investigate the mode of release from the topical gel-cream the release data were analyzed with the following mathematical models:

Zero-order kinetic: $f_t = k_0 t$

First-order kinetic: $\ln Q_t = \ln Q_0 + k1_t$

Higuchi equation: $f_t = k_H t^{1/2}$

Hixson Crowell's cube root equation: $Wo^{1/3} - Wt^{1/3} = k_s t$

Korsmeyer peppas equation: $Q = k_p t^n$

In the above equations

$f_t$ = fraction of dose released at timet.

$Q_0$ = the amount of drug released at zero time.

Qt = drug amount s remaining to be released at time t.

$k_0$, $k_1$ and $k_H$ are the coefficients of the zero order, first order and higuchi order equation respectively.

$k_P$ = constant incorporating structural and geometric characteristics of the release device. n is the release exponent indicative of the mechanism of release.[13]

The value of n indicates the drug release mechanism which is depicted in Table 3

**Table 2: Interpretation of diffusion release mechanisms from gel**

| Release exponent (n ) | Mechanism |
|---|---|
| 0.5 | Fickian diffusion |
| 0.45 < n= 0.89 | Non-Fickian transport |
| 0.89 | Case-II transport |
| > 0.89 | Super case-II transport |

**4) Biological activity**

**a) Anti-microbial activity-** was carried out by Paper disc diffusion method. [14]

**b) Anti-oxidant activity (*in vitro*)**

❖ **PREPARATIONS**

**a) 0.3mM DPPH solution**

0.012gm of DPPH was weighed and dissolved in 90ml of methanol and volume was made up to 100ml.

**b) Standard preparation**

10mg of ascorbic acid was weighed and dissolved in 100ml of distilled water in an amber colored volumetric flask. The solution was vortexed on a cyclomixer and used as standard.

**c) Sample preparation**

The stock solution of 1gm of sample in 10ml of methanol was prepared. The solution was sonicated for 10 mins and vortexed. The clear methanolic solution obtained was used for assay.

❖ **PROCEDURE**

i) Different concentrations of test samples were prepared in methanol. 2.5ml of each test sample was mixed with 1ml of 0.3mM DPPH solution. These samples were kept in dark for incubation at room temperature for 30mins.

ii) Absorbance was measured at 516nm using UV- visible spectrophotometer (*Jasco V-630*).

iii) Blank was prepared by combining 1ml of methanol with 2.5ml of test sample. Control sample was prepared by adding 1ml of 0.3mM DPPH solution to 2.5ml of methanol. Ascorbic acid was used as reference standard. All readings were taken in triplicates.

iv) The results were expressed as % Inhibition of DPPH radical induced by both the tests and standard samples. $IC_{50}$ values were calculated and compared with that of the standard.

v) The inhibition of DPPH radicals by the samples was calculated according to the following equation:

DPPH-scavenging activity (%): **$[1-(A_1-A_2) / A_0] \times 100$**,

Where,

**$A_0$**=absorbance of control.

**$A_1$**=absorbance of the sample.

**$A_2$**= absorbance of blank

One month trial version of Graph Pad Prim 6.5 software was used to plot graphs and for the calculation of $IC_{50}$ values by point to point curve method.[15]

**5) Phytochemical assay of optimized gel-cream.**

**a) Total phenolic content[16,17]**

**b) Total flavonoid content[18.19]**

**c) Total tannin content[20]**

**6) Stability study**

The optimized herbal gel-cream formulation (F5) was tested for stability under two conditions for a period of three months. The gel-cream was kept in polypropylene air tight wide mouth containers and stored in stability chamber maintained at $40^0$C/75% RH and room temperature. Formulation was evaluated for their physical characteristics, *in vitro* drug release, content of active ingredient and anti-oxidant activity at the end of 30 days, 60 days and 90 days of storage period.[21]

**Table 3: Composition of anti-ageing herbal gel-cream**

| Sr. No | Ingredients (%) | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|---|
| **1. OIL PHASE** | | | | | | | |
| 1 | Bees wax | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | Stearic acid | 3 | 3 | 3 | 3 | 3 | 3 |
| 3 | Cetyl alcohol | 5 | 5 | 5 | 5 | 5 | 5 |
| 4 | Liquid paraffin | 10 | 10 | 10 | 10 | 10 | 10 |
| 5 | Olive oil | 2 | 2 | 2 | 2 | 2 | 2 |
| 6 | Almond oil | 2 | 2 | 2 | 2 | 2 | 2 |
| 7 | Wheat germ oil | 2 | 2 | 2 | 2 | 2 | 2 |
| 8 | Grape seed oil | 2 | 2 | 2 | 2 | 2 | 2 |
| 9 | Carrot seed oil | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| 10 | Avocado oil | 1 | 1 | 1 | 1 | 1 | 1 |
| **2. Water phase** | | | | | | | |
| 1 | *Aleo vera* mucilage extract | 8 | 8 | 8 | 8 | 8 | 8 |
| 2 | Carbopol 940 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| 3 | Triethanolamine | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| 4 | Glyceral Monosterate | 2 | 2 | 2 | 2 | 2 | 2 |
| 5 | Euxyl 9010 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| **3. Formulation of Gel-cream** | | | | | | | |
| 1) | Pomegranate peel extract | 0.25 | 0.50 | 1 | 1.5 | 2 | 2.5 |
| 2) | Orange oil | 0.2 | 0.4 | 0.6 | 0.8 | 1 | 1.2 |
| 3) | Gel-cream base | 100 | 100 | 100 | 100 | 100 | 100 |

## RESULTS

### 1) Physical parameters

All the developed formulations were found to be homogenous, non-greasy, non-gritty, light pink in color with characteristic odor. The studies showed that F5 formulation complies with requirements of physical parameters and found to be the best amongst all the batches.

**Table 4: Physical parameters of herbal gel-cream**

| Physical | FORMULATION CODES | | | | | |
|---|---|---|---|---|---|---|
| Parameters | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** |
| Color | Light pink | Light pink | Light pink | Light pink | Light pink | Light pink |
| Odor | Characteristic | Characteristic | Characteristic | Characteristic | Characteristic | Characteristic |
| Consistency | + + | + | + | + + | + + + | + + |
| Homogeneity | + | + | + | + | + | + |
| Greasiness | - | - | - | - | - | - |
| Grittiness | - | - | - | - | - | - |
| Percentage Moisture Content | 95.81% ± 0.005 | 95.84% ± 0.005 | 98.01% ± 0.005 | 95.84% ± 0.005 | 95.86% ± 0.005 | 95.82% ± 0.005 |
| Water wash ability | + | + | + | + | + | + |

**Consistency=Excellent +++, Good ++ and Satisfactory +, Homogeneity = Homogenous + Grittiness = No grittiness -, Greasiness = Non greasy – and Washablitiy = Washable**

### 2) Pharmaceutical parameters

All the developed formulations were found to be within the limits of pharmaceutical parameters. The drug content of F5 formulation was found to be highest. F5 formulation complies with requirements of pharmaceutical parameters.

**Table 5: Pharmaceutical parameters of herbal gel-cream**

| Sr. no | Formulation Code | pH± S.D. ( n=3) | Viscosity (cP) ± S.D. | Spreadability (g.cm/sec) ± S.D. ( n=3) | Drug content %± S.D. ( n=3) |
|---|---|---|---|---|---|
| 1) | F1 | 6.38 ±0.0057 | 9416±0.57 | 7.75±0.57 | 98.26±0.16 |
| 2) | F2 | 6.40 ±0.0005 | 9450±0.57 | 7.95±0.57 | 98.21±0.16 |
| 3) | F3 | 6.42±0.0057 | 9389±0.57 | 8.03±0.57 | 98.21±0.28 |
| 4) | F4 | 6.42 ±0.0015 | 9410±0.57 | 7.03±0.57 | 98.28±0.15 |
| 5) | **F5** | **6.40±0.0057** | **9445±0.57** | **6.08±0.57** | **98.33±0.57** |
| 6) | F6 | 6.51±0.0057 | 9416±0.57 | 5.94±0.57 | 98.13±0.12 |

### 3) Diffusion study and release kinetics

Diffusion study was carried out for calculating percentage cumulative release of pomegranate extract in herbal gel-cream formulations through parchment paper (Table 6).The maximum cumulative drug release of the herbal extract was found in F5 formulation.

**Table 6: % CDR data of pomegranate extract in gel-cream formulation**

| Time (mins) | %CDR of Pomegranate extract (n=3) (%CDR ± S.D.) | | | | | |
|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 26.81± 0.29 | 26.81± 0.29 | 26.64± 0.29 | 26.64± 0.29 | 27.32±0.97 | 26.64± 0.29 |
| 30 | 39.68 ± 0.32 | 39.68 ± 0.32 | 39.49 ± 0.32 | 34.40 ± 0.32 | 39.74±0.20 | 39.49 ± 0.32 |
| 45 | 46.77 ± 0.23 | 46.77 ± 0.23 | 46.91 ± 0.23 | 44.36± 0.23 | 46.84±0.35 | 46.91 ± 0.23 |
| 60 | 60.95 ± 0.26 | 60.95 ± 0.26 | 61.15 ± 0.26 | 54.80 ± 0.26 | 62.59±0.38 | 61.15 ± 0.26 |
| 120 | 74.20 ± 0.29 | 75.32± 0.29 | 72.16 ± 0.29 | 67.80 ± 0.29 | 75.44±0.41 | 72.16 ± 0.29 |
| 180 | 84.65 ± 0.32 | 84.65 ± 0.32 | 86.49 ± 0.32 | 78.27 ± 0.32 | 89.90±0.52 | 85.47 ± 0.32 |
| 240 | 94.55 ± 0.35 | 98.45 ± 0.05 | 95.9 ± 0.05 | 90.95 ± 0.05 | 98.76±0.15 | 95.12 ± 0.32 |

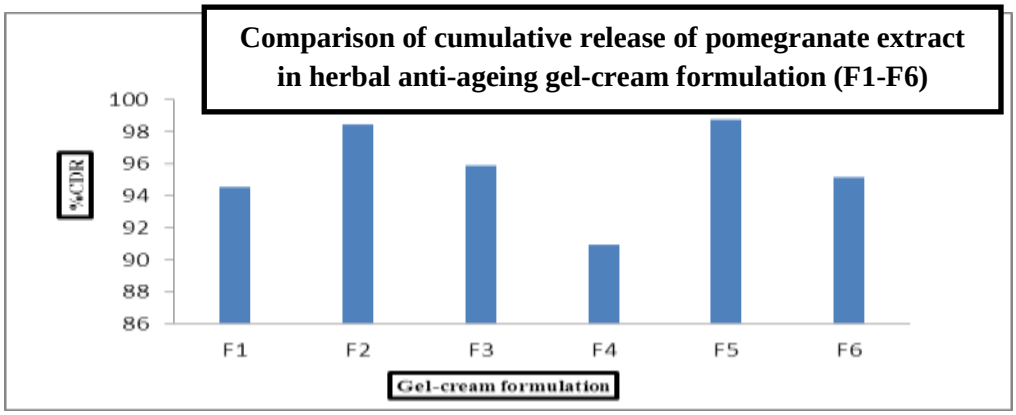


**Fig.1: Graphical comparison of drug release of gel-cream**

**Kinetic analysis of *in vitro* release data**

Table 7 gives the release mechanisms of the gel-cream formulations (F1-F6) of pomegranate extract. The interpretation of release kinetics data was based on the value of the resulting coefficients of determination. The pomegranate extract followed first order release. To understand the mechanism of release from the gel-cream; the extract release data was fitted into Korsmeyer -Peppas, Hixson Crowell Cube Root and Higuchi model and it showed the highest $r^2$ value for Hixson Crowell model closely followed by Higuchi model, indicating diffusion to be predominantly by the non-fickian type as the 'n' values lie between 0.24-0.45.

**Table 7: Kinetic analysis data of *in vitro* release data**

| Formulation code | Coefficient of determination ($r^2$) | | | | | n release exponent | Best fit model |
|---|---|---|---|---|---|---|---|
| | Zero order | First order | Higuchi square root | Hixson Crowell Cube Root | Korsmeyer plot | | |
| F1 | 0.83 | 0.98 | 0.979 | 0.978 | 0.96 | 0.4 | Higuchi model |
| F2 | 0.84 | 0.93 | 0.982 | 0.987 | 0.97 | 0.4 | Hixson crowell cube root |
| F3 | 0.84 | 0.97 | 0.98 | 0.97 | 0.93 | 0.45 | Higuchi model |
| F4 | 0.86 | 0.97 | 0.9921 | 0.9928 | 0.9652 | 0.44 | Hixson crowell cube root |
| F5 | 0.85 | 0.62 | 0.984 | 0.988 | 0.78 | 0.24 | Hixson crowell cube root |
| F6 | 0.84 | 0.62 | 0.981 | 0.988 | 0.78 | 0.24 | Hixson crowell cube root |

**3) Biological parameter of gel -cream**

**a) Anti-microbial activity**

The anti-microbial activity of the herbal gel-cream formulations showed significant and promising activity against *E. coli*, *S. aureus* and *C. albicans* in comparison with the marketed synthetic gel (Clindac gel). F5 formulations showed highest inhibition activity

**Table 8: Anti-microbial activity of gel-cream formulation (*in vitro)***

| Sr. No. | Microbial species | Zone of inhibition of Formulations (mm) ± S.D. (n=3) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 | F5 | F6 | Marketed |
| 1 | *E. coli* | 23±0.05 | 24±0.05 | 22±0.05 | 23±0.05 | 24±0.05 | 23±0.05 | 25±0.05 |
| 2 | *S. aureus* | 23±0.05 | 23±0.05 | 23±0.05 | 22±0.05 | 23±0.05 | 21±0.05 | 24±0.05 |
| 3 | *C. albicans* | 25±0.05 | 26±0.05 | 25±0.05 | 25±0.05 | 26±0.05 | 24±0.05 | 27±0.05 |

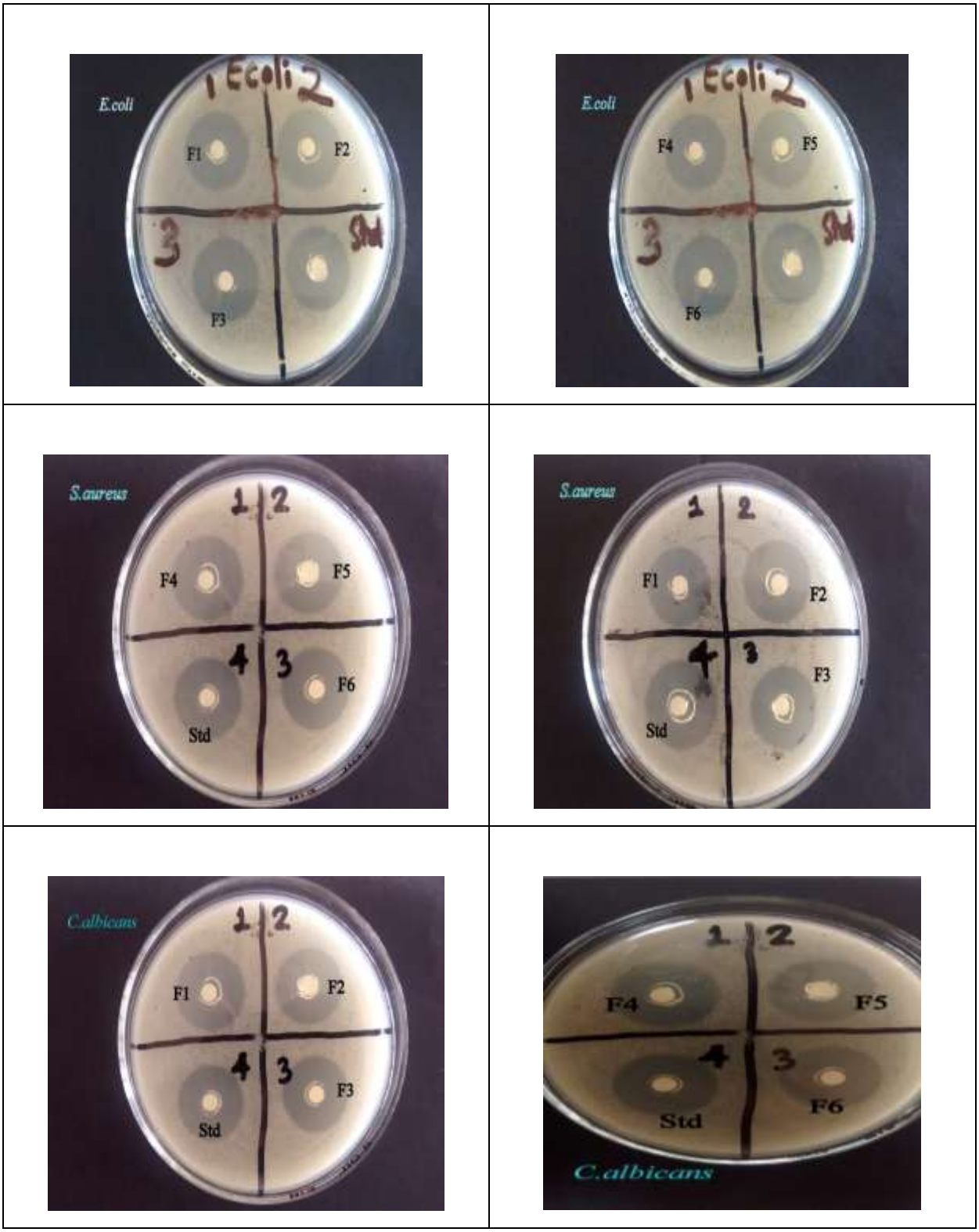


**Fig. 2: Anti-bacterial activity of herbal gel-cream**

**b) Anti-oxidant activity**

The herbal gel-cream formulations showed significant and promising anti-oxidantactivity. F5 formulations showed highest anti-oxidant activity.

### 1) Anti-oxidant activity of ascorbic acid

**Table 9: Anti-oxidant activity data of ascorbic acid**

| Concentration (mcg/ml) | % Inhibition of DPPH radical | Standard deviation |
|---|---|---|
| 0 | 0 | 0 |
| 10 | 11.234 | 0.58845 |
| 20 | 23.833 | 0.422383 |
| 30 | 36.83 | 0.29964 |
| 40 | 50.16 | 0.541516 |
| 50 | 71.55 | 0.277978 |
| 60 | 81.92 | 0.305054 |
| 70 | 87.38 | 1.036101 |
| 80 | 94.69 | 0.925993 |
| 90 | 95.49 | 0.981949 |

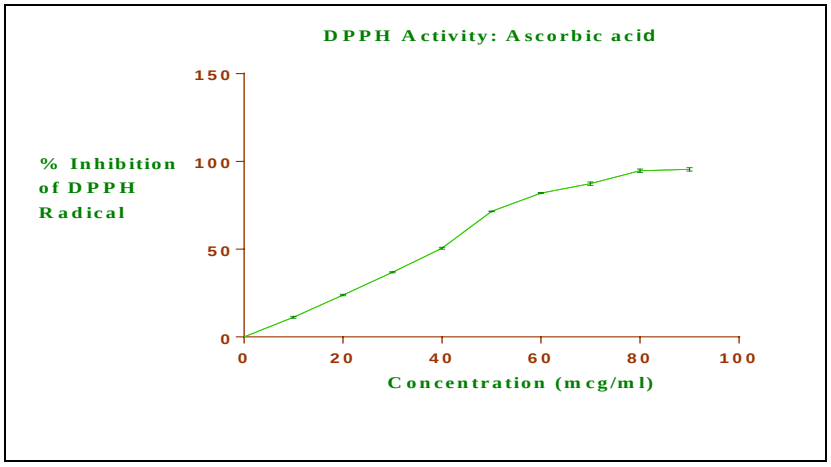


**Fig. 3: Plot of % inhibition of DPPH v/s concentration of ascorbic acid.**

### 2) Anti-oxidant activity of Herbal extracts

**Table 10: Anti-oxidant activity of herbal actives**

| Concentration (mg/ml) | % Inhibition of Pomegranate extract ±S.D. | % Inhibition of Orange oil ±S.D. |
|---|---|---|
| 0 | 0 | 0 |
| 0.5 | 40.33±0.94 | 38.48±0.90 |
| 1 | 73.71±1.02 | 60.27±1.00 |
| 1.5 | 81.68±0.94 | 66.74±1.11 |
| 2 | 82.82±0.94 | 68.30±0.90 |
| 2.5 | 84.05±0.92 | 69.06±0.93 |

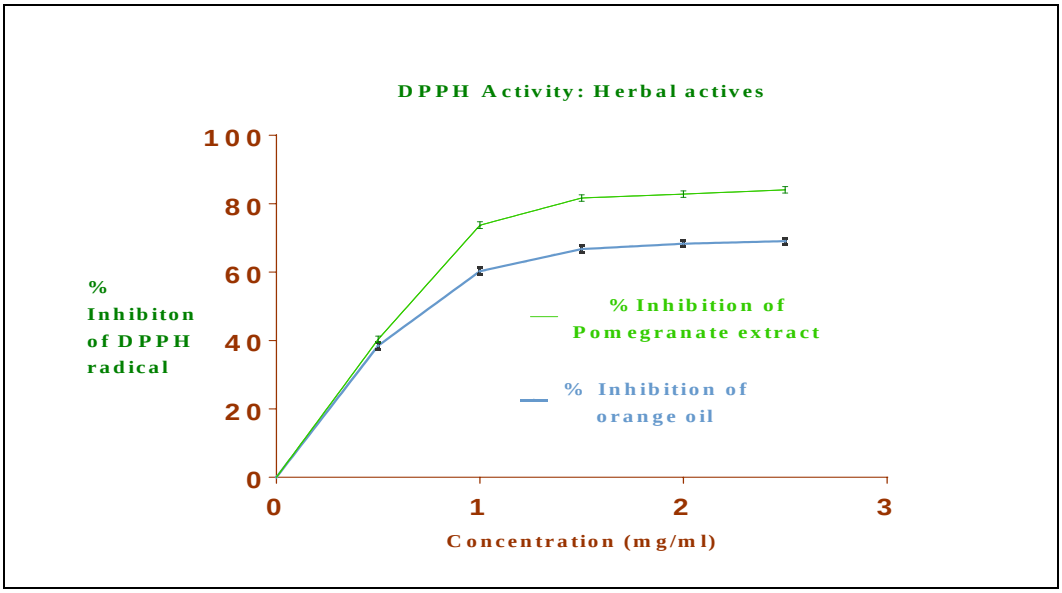


**Fig.4: Plot of % inhibition of DPPH v/s concentration of herbal active**

### 3) Anti-oxidant activity of herbal anti-ageing gel-cream

**Table 11: Anti-oxidant activity of herbal gel-cream formulation (*in vitro*)**

| Concentration (mg/ml) | % Inhibition of gel-cream formulation ±S.D. | | | | | |
|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 44.24±1.03 | 32.57±1.03 | 36.32±0.9 | 40.83±1.5 | 61.45±2.02 | 40.33±0.94 |
| 1 | 65.1±1.08 | 63.48±1.08 | 60.27±1.0 | 51.28±2.02 | 77.48±1.02 | 73.71±1.02 |
| 1.5 | 70.59±1.14 | 70.11±1.04 | 66.74±1.1 | 62.91±1.14 | 81.68±0.94 | 81.68±0.94 |
| 2 | 72.21±1.02 | 71.52±1.08 | 68.30±0.90 | 64.56±1.07 | 82.82±0.94 | 82.82±0.94 |
| 2.5 | 73.82±1.10 | 72.75±1.02 | 69.06±0.93 | 65.69±1.06 | 84.05±0.92 | 84.05±0.92 |

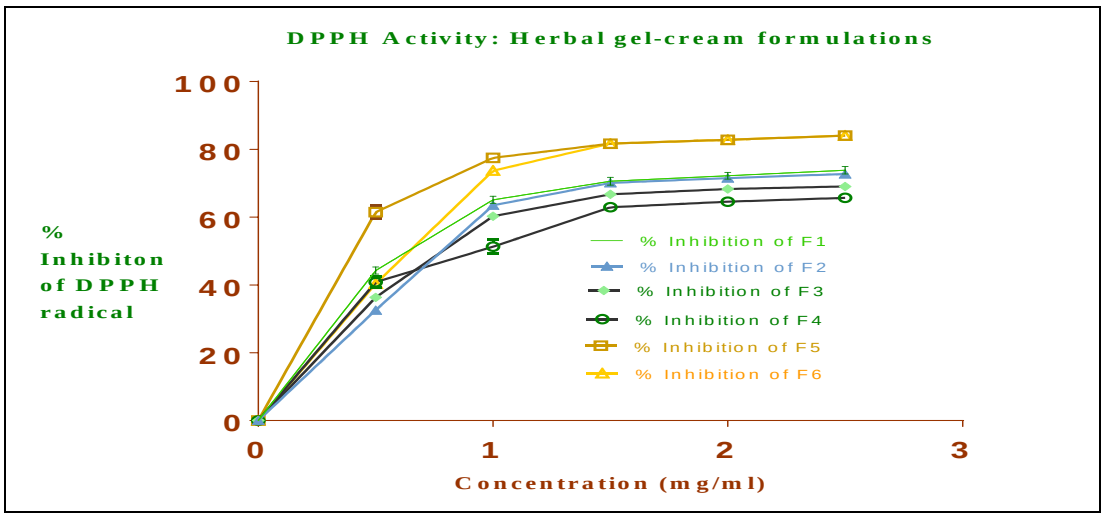


**Fig.5: Plot of % inhibition of DPPH v/s herbal gel-cream formulation**

From (Fig 4, 3, 5)

The $IC_{50}$ value of ascorbic acid was found to be 39.563 µg (0.039563 mg). (Reference standard).

The $IC_{50}$ value of pomegranate extract was found to be 0.645mg.

The $IC_{50}$ value of orange oil was found to be 0.764mg

The IC50 value of F1 gel-cream was found to be 0.63 mg

The IC50 value of F2 gel-cream was found to be 0.78 mg

The IC50 value of F3 gel-cream was found to be 0.78 mg

The IC50 value of F4gel-cream was found to be 0.93mg

The IC50 value of F5 gel-cream was found to be 0.40 mg.

The IC50 value of F6 gel-cream was found to be 0.62 mg

**5) Phytochemical assay of optimized gel-cream**

**Table 12: Quantitative phytochemical assay of F5 formulation**

| Sr. No. | Quantitative phytochemical assay | Results |
|---|---|---|
| 1 | Total phenolic content | 13.10± 0.09 mg of gallic acid equivalent /gram wt of extract |
| 2 | Total flavonoid content | 21.24± 0.176 mg of quercetin equivalent /gram wt of extract |
| 3 | Total tannin content | 27.00± 0.27 mg of tannic acid equivalent /gram wt of extract |

**6) Stability studies**

**a) Physical parameters of herbal gel-cream F5**

The physical parameters after 0, 30$^{th}$, 60$^{th}$ and 90$^{th}$ day were as mentioned in Table 13. All the physical parameters were in the acceptable limits which showed that optimised formulation was stable over the period of 90 days.

**Table 13: Physical parameters of herbal gel-cream were subjected to stability study.**

| Physical Parameter | Condition | 0 day | 30$^{th}$ day | 60$^{th}$ day | 90$^{th}$ day |
|---|---|---|---|---|---|
| Appearance | 40ºC/75% RH | No change | No change | No change | No change |
| | RT | No change | No change | No change | No change |
| pH | 40ºC/75% RH | 6.399±0.001 | 6.393±0.001 | 6.388±0.001 | 6.384±0.002 |
| | RT | 6.40±0.0005 | 6.395±0.001 | 6.393±0.001 | 6.389±0.002 |
| Drug content | 40ºC/75% RH | 98.33±0.5 | 98.28±0.15 | 98.26±0.16 | 98.13±0.12 |
| | RT | 98.33±0.5 | 98.3±0.13 | 98.28±0.15 | 98.26±0.16 |
| Viscosity (cP) | 40ºC/75% RH | 9442±1.52 | 9433±1 | 9429±1.52 | 9426±1 |
| | RT | 9445±0.57 | 9440±0.57 | 9437±1.52 | 9432±1.52 |

**(*RT: room temperature)**

The results of the physical parameters stability studies indicated that herbal gel-cream had reasonable stability.

**b) Drug release studies (*in vitro*)**

The drug release data obtained when the optimized formulation F5 was kept under the condition (**40ºC/75% RH)** is given in (Table 14) and (Fig. 6). The release of herbal extract was within the permissible limits.

**Table14: Stability study data of drug release from herbal gel**

| Days | Conditions | Percentage cumulative drug release (%) | | | | |
|---|---|---|---|---|---|---|
| | | 30min | 1hr | 2hr | 3hr | 4hr |
| 0 | 40ºC/75% RH | 39.68 ± 0.32 | 60.95 ± 0.26 | 75.32± 0.29 | 84.65 ± 0.32 | 98.45 ± 0.05 |
| | RT | 39.74±0.20 | 62.59±0.38 | 75.44±0.41 | 89.90±0.52 | 98.76±0.15 |
| 30 | 40ºC/75% RH | 39.68±0.3 | 62.59±0.3 | 75.79±0.7 | 85.38±0.30 | 98.21±0.05 |
| | RT | 39.68 ± 0.32 | 60.95 ± 0.26 | 75.32± 0.29 | 84.65 ± 0.32 | 98.45 ± 0.05 |
| 60 | 40ºC/75% RH | 29.19±0.5 | 62.40±0.3 | 74.94±0.20 | 83.97±0.22 | 97.55±0.3 |
| | RT | 39.30±0.3 | 62.59±0.3 | 75.79±0.7 | 85.38±0.3 | 98.21±0.05 |
| 90 | 40ºC/75% RH | 36.96±0.3 | 61.89±0.2 | 74.06±0.5 | 86.08±0.5 | 97.35±0.3 |
| | RT | 39.39±0.3 | 62.2±0.05 | 74.86±0.2 | 86.57±0.02 | 98.18±0.02 |

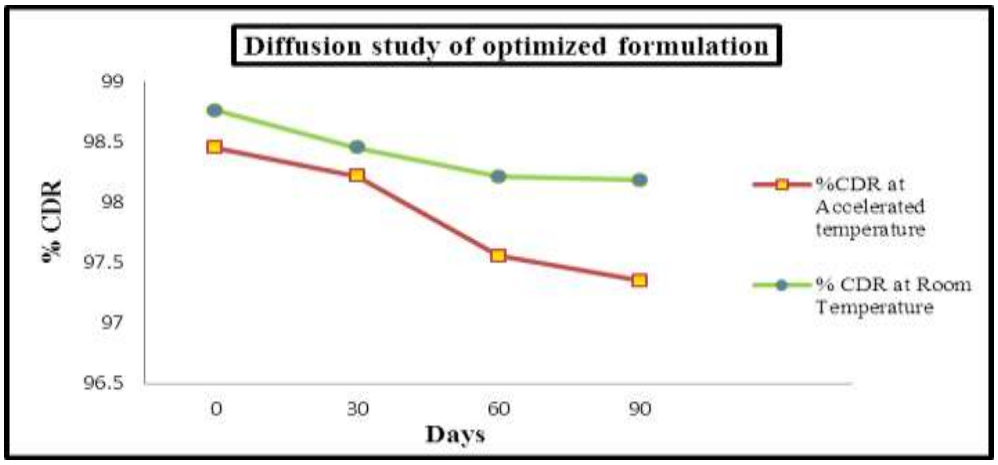


**Fig 6: *In vitro* drug release data of gel-cream**

**DISCUSSION**

The herbal gel-cream (F5) which contains pomegranate extract (2%) and orange oil (1%) showed maximum anti-oxidant activity ($IC_{50}$ value 0.40 mg) and significant anti-bacterial activity in comparison with the marketed synthetic gel (Clindac gel).The pharmaceutical evaluation and stability study concludes that the scientific approach taken to formulate herbal gel-cream that contains mixture of herbal extract, volatile oil, nourishing oils and gelling agents proved to be an effective contribution in the segment of anti-ageing formulations.

## CONCLUSION

In the present research work an attempt has been made to formulate a novel herbal anti-ageing gel-cream which overcomes the lacunae of available anti-ageing formulations. The studies conclude that all six herbal anti-ageing gel-cream formulations showed anti-oxidant and anti-microbial activity. Formulation F5 showed highest activity. An elaborate protocol for the clinical trials is needed to be designed and implemented to check the anti-ageing activity on human volunteers.

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