FORMULATION AND EVALUATION OF CREAM CONTAINING CURCUMIN AND LYCOPENE FOR THE ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY.

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ABSTRACT
Objective: The present study was to prepare and evaluate cream containing Curcumin obtained from Curcuma longa L. and Lycopene obtained from Lycopersiconesculentum M. Both Curcumin and Lycopene have sufficient partition co-efficients (c log p values) to being formulated in the form of cream. Methods: Different o/w emulsions were formulated (F1-F7) by adding different quantities of stearic acid, cetyl alcohol and coconut oil. All formulations were evaluated for different quality control parameters like pH, viscosity and acid value. Results: Formulations F5 and F6 showed good spreadability, they produced non-greasy smear and were found easy to be removed by washing under tap water. All formulations were found to be non-irritant and safe for applications on skin. Conclusion: This study suggested that formulations (F5 and F6) are stable, safe and may gain better patient compliance.

KEYWORDS: Carotenoids, topical formulation, DPPH radical scavenging activity, Well-diffusion method.

INTRODUCTION
Creams are semisolid dosage forms intended mainly for external use and commonly consist of two immiscible phases, an oily internal phase and an aqueous external phase. Due to emulsified nature of skin surface, drugs formulated as cream more effectively interact with skin and more readily penetrate through biological membranes.

The skin is a protective covering of the body. On average, it covers a surface area of 2 square meters. In its intact state, the skin is a strong barrier, impenetrable to life threatening microorganisms and resistant to chemicals and harmful UV rays.1,2 Drugs are applied topically to the skin mainly for their local actions. Although the topical route can also be used for systemic drug delivery, percutaneous or trans-dermal absorption of drug is generally poor and erratic.

Topical drug absorption takes place through sweat glands, hair follicles, sebaceous gland and the stratum corneum.

Skin exposure to ionizing and UV radiation generates free radicals in excessive quantities that quickly overwhelm tissue antioxidants and other oxidant-degrading pathways.3 Uncontrolled release of these free radicals are involved in the pathogenesis of a number of human skin disorders including sunburn, dermatitis etc. Therefore the cosmetic products, which are used for these conditions, must be having enough potential to quench the free radicals.4

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, commonly known as diferuloyl methane is a hydrophobic, a yellow coloured bioactive natural product, obtained from Curcuma longa L. belonging to family Zingiberaceae. Structurally, it has of two phenolic rings, each substituted with a methoxy ether functionality in the ortho-position (Figure 1). The two phenolic rings are joined via an aliphatic unsaturated heptene linker in the para-position that also contains α, β-diketonic functionality on carbon-3 and -5. Various
studies have indicated that the diketone functionality can undergo reversible tautomerization between enolic- and ketonic-forms.\cite{5} Tautomerization of curcumin occurs in a pH-dependent manner, with the bis-keto form predominating in acidic and neutral solutions, and the enol-form in alkaline solutions.\cite{6} While in the bis-keto form, carbon-4 of the heptene linker can function as an extremely powerful proton donor, while the enol form functions mainly as an electron donor, chemical activity that bestows upon curcumin its antioxidant properties.\cite{7}

In fact, the antioxidant properties of curcumin are several times more potent than those exhibited by vitamin E.\cite{8} Curcumin also functions as a pro-oxidant under certain conditions most likely as a result of electron transfer to molecular oxygen to generate reactive oxygen species (ROS).\cite{9}

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.\cite{10}

Characterization of Curcumin
Isolated compound was characterized by Thin Layer Chromatographic technique using Silica gel G as stationary phase and chloroform: methanol (98:2) as mobile phase. Rf value was then calculated on detection of spot under light in visible region (380nm – 800nm).\cite{10}

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, 1/3 volume 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 crystallized out on drop-wise addition of 1/2 ml of boiling methanol.\cite{10}

Characterization of Lycopene
Isolated compound was characterized by Thin Layer Chromatographic technique using Silica gel G as stationary phase and petroleum ether: dichloromethane (9: 1) as mobile phase. Rf value was then calculated on detection of spot under light in visible region (380nm – 800nm).\cite{10}

Formulation of cream
Oil in water (o/w) emulsion-based cream was formulated. The lycopene and other oil soluble components were dissolved in oil phase and heated to 75°C. The curcumin and 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. The formulae for the creams are given in Table 1.
Table: 1. Composition of cream formulae (F1 to F7)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1(gm)</th>
<th>F2(gm)</th>
<th>F3(gm)</th>
<th>F4(gm)</th>
<th>F5(gm)</th>
<th>F6(gm)</th>
<th>F7(gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curcumin</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
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<tr>
<td>Stearic acid</td>
<td>2.1</td>
<td>2.1</td>
<td>2.0</td>
<td>2.0</td>
<td>1.8</td>
<td>1.5</td>
<td>1.5</td>
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<tr>
<td>Cetyl alcohol</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Aqueous phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
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<td>0.5</td>
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<tr>
<td>Ascorbic acid</td>
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<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
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<tr>
<td>Tri-ethanolamine</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>4.5</td>
<td>5.0</td>
<td>5.0</td>
<td>4.5</td>
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<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Water</td>
<td>Qs</td>
<td>Qs</td>
<td>Qs</td>
<td>Qs</td>
<td>Qs</td>
<td>Qs</td>
<td>Qs</td>
</tr>
</tbody>
</table>

Evaluation of cream
All the formulated creams were evaluated for following the parameters.

pH of cream
About 0.5 g of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured on pH meter, previously calibrated using standard buffer solution.

Viscosity
Viscosity of the formulation was determined by Brookfield Viscometer at 100 rpm, using spindle no.7.

Dye test
A water soluble dye (Methylene blue)\(^{[17,18]}\) was added to small quantity of cream and observed under microscope.

Appearance
Appearance of the cream was examined by its colour, pearlescence and roughness.

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.

Acid value
Accurately weighed 10 g of cream was dissolved in 50.0 ml mixture of equal volumes of alcohol and solvent ether and refluxed until sample was dissolved completely. It was then titrated with 0.1 N NaOH using Phenolphthalein as an indicator upto persistence of fair pink colour. Volume (v) of 0.1 N NaOH was noted and Acid value was calculated using formula:

\[
\text{Acid value} = v \times \frac{5.6}{10}
\]

Irritancy test
The cream was applied on specified area of 1sq.cm on dorsal surface of left hand.

Antioxidant activity of formulated cream
The DPPH radical scavenging activity was performed according to the standard protocols reported earlier [23]. About 2 ml of formulatons F5 and F6 (10, 20, 40 µg/ml in mixture of solvents containing n-hexane, pet. ether and ethanol in proportion of 1:1:1) was mixed with 1 ml of DPPH solution (0.2 mM/ml in methanol) thoroughly. The mixture was incubated in dark at 20º C for 40 min. Absorbance was measured at 517 nm using UV-Vis spectrophotometer. The percentage scavenging of DPPH by the formulatations was calculated according to the following formula:

\[
\% \text{ DPPH Radical scavenging} = \left(\frac{A_c - A_t}{A_c}\right) \times 100
\]

Where,
Ac is the absorbance of the control (DPPH)
At is the absorbance of test sample.

SCREENING OF ANTIMICROBIAL ACTIVITY OF FORMULATED CREAM

Culture and Maintenance of microorganisms
Pure cultures of bacteria, \textit{E. coli} and \textit{S. aureus} were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The pure bacterial cultures were maintained on nutrient agar medium at 4ºC.

Antimicrobial activity
The antimicrobial activity of the formulated creams (F5 and F6) were screened by well diffusion method in petri plates containing nutrient agar inoculated with test cultures and incubated at 37ºC for 24 h. The next day, the wells (6 mm diameter) were made with help of 6 mm diameter cork borer and the wells were loaded with formulations F5 and F6 along with positive control, ciprofloxacin. After 24 hrs of incubation, the test determines the efficacy of the product in terms of zone of inhibition of the organism.

RESULTS

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
pH of cream
The pH of cream was found to be in the range of 5.6 to 6.8 which is suitable for skin pH.

Viscosity
The viscosity was in the range of 28008 to 27019 cps which indicates that cream is easily spreadable by applying a little shear. Formulations F5 and F6 showed good spreadability than that of others.

Dye test
Dye test confirmed that all the cream formulations were o/w emulsions.

Table: 2. Acid values of formulations (F1 to F7)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Value</td>
<td>6.8</td>
<td>6.5</td>
<td>6.5</td>
<td>6.4</td>
<td>6.2</td>
<td>5.8</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Irritancy test
Formulations F5 and F6 showed no redness, erythema, inflammation, and irritation during irritancy studies. These formulations were found to be safe for applications on skin.

DPHP Radical scavenging activity of formulations
Curcumin, lycopene and other phytocompounds when formulated O/W cream, exhibited ability to scavenge DPPH radicals. The results of DPHP radical scavenging activity of formulations F5 and F6 are reported in Figure 1. The formulation F5 and F6 exhibited high DPHP radical scavenging activity with an IC50 value 22.34 and 24.51 µg/ml, respectively.

Figure 1: DPPH radical scavenging activity of formulations, F5 and F6.

Antimicrobial activity
The antimicrobial activity was determined by measuring the diameter of zone of inhibition. The results obtained in the evaluation of the antibacterial activity of both formulations were depicted in table no.3. Formulations F6 showed zone of inhibition comparable to that of control. However, formulation F5 has maximum activity against E. coli (Fig.2A) and formulation F6 has maximum activity against S. aureus (Fig.2B).

Table: 3 Antimicrobial sensitivity result of the formulations, F5 and F6.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Test micro-organism</th>
<th>Zone of inhibition(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F5</td>
</tr>
<tr>
<td>1.</td>
<td>E. Coli.</td>
<td>10.13</td>
</tr>
<tr>
<td>2.</td>
<td>S. aureus</td>
<td>8.54</td>
</tr>
</tbody>
</table>
DISCUSSION
Curcuma longa L. (Zingiberaceae) and Lycopersicon esculentum M. (Solanaceae) are well known for their pharmacological activities and thereby cosmeceutical value, even in traditional system of medicine. In the present work it was decided to isolate Curcumin from Curcuma longa L. (Zingiberaceae) and Lycopene from Lycopersicon esculentum M (Solanaceae.) and formulate them in cream.

Both Curcumin and lycopene act as scavengers of oxygen free radicals. In vitro, Curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H₂O₂ and nitrite radical generation by activated macrophages, which play an important role in inflammation.[19,20] Curcumin has very effective wound healing activity, examined in rats and guinea pigs. In situ hybridization and PCR analysis shows an increase in the mRNA transcripts of transforming growth factor beta 1 (TGFβ1) and fibronectin in Curcumin treated wounds. Transforming growth factor beta 1 enhance wound healing, therefore it is possible that Curcumin modulates TGF β1 activity.[21] Lycopene, because of its high number of conjugated double bonds, exhibits higher singlet oxygen quenching ability compared to β-carotene or α-tocopherol.

The carotenoids, curcumin and lycopene were chosen to formulate the cream because of their antioxidant and antibacterial activities, exhibited in-vitro. The dye test confirmed that the formulated creams were o/w type of emulsion cream. Our study indicated that formulations F5 and F6 found to be more stable as compare to other formulations. Stable formulations were safe with respect to skin irritation and allergic sensitization. Efficacy of both the compounds can be increase by synergism. In this regards we formulated cream containing Curcumin and Lycopene to improve and synergize pharmacological properties of formulation.

CONCLUSION
The turmeric (Curcuma longa L.) and tomato (Lycopersicon esculentum M.) are widely used for its medicinal value in the traditional system of medicine. The antioxidant and antibacterial creams are widely used today as they appear to be an interesting way to safeguard the skin against oxidative stress caused by various extrinsic sources and bacterial infection. Here creams were formulated from curcumin and lycopene; formulations were optimized; and evaluated for antioxidant activity by DPPH radical scavenging model and antibacterial potential by well diffusion method. Formulation F5 and F6 were found to posses good antioxidant and antibacterial activities.

ACKNOWLEDGEMENTS
We, authors are thankful to Dr. (Mrs.) Anagha M Joshi, Principal, SCES’s Indira College of Pharmacy, Tathawade, Pune for her support regarding provision of facilities required for carrying out this research work.

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