HPTLC PROFILE OF EVEFRESH CREAM

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ABSTRACT

Aim: The present study discusses about the HPTLC confirmation of the herbs used in the Siddha drug – Evefresh Cream. Method: HPTLC analysis of oil extracts of individual herbs of Evefresh Cream. Results: The study findings clearly confirm that all the herbs are well preserved in the cream base without any disintegration or denaturation. Assuming the therapeutic value of all the herbs used the well preserved status of these herbs in Evefresh Cream base validate their drug efficacy. Findings are discussed in the paper.

Keywords: Curcuma, Aloe Vera, Evefresh, Hyperpigmentation

INTRODUCTION

From time immemorial, various herbal preparations are being used for in India as medicaments for different diseases/problems such as enhancing hair growth, treating dandruff, alleviating pain, skin dryness etc1-7. The traditional Indian system of medicine has rich mention of various herbs that are used for treating wide variety of diseases including Psoriasis and Vitiligo3,4. Most of the herbal drugs are prepared either with single herb or with multiple herbs.

When such medicinal plants are used in the siddha drug preparations with the assumption that these herbs would impart therapeutic benefits the presence of the herbs in the final formulation needs to be ascertain in order to validate the above assumption. The fingerprints of the individual herbs should be detectable in the finished product.

The cream is composed of two important herbs that are well documented in the Siddha system of medicine viz., Curcuma zedoaria (Kasturi manjal) and Aloe vera (Kumari). The company has done intensive and extensive research on Eve fresh both at the laboratory level and in clinical practice to ascertain its efficacy scientifically5,6,7,8 & 9.

In the present paper we report the confirmation and fingerprint matching of different herbs in the proprietary Siddha medicine-Evefresh Cream. The findings are discussed in the paper.

MATERIALS AND METHODS

Description
Evefresh Cream is a licensed proprietary Siddha medicine of Dr. JRK’s Research and Pharmaceuticals Pvt Ltd Chennai.

Composition of Evefresh Cream
Aloe vera: 2%
Curcuma zedoaria: 0.2%
Cream base: Q.S

Collection of the plant materials
The above two herbs were procured from the approved medicinal plant supplier. In brief the pulp/rhizome whichever is applicable of the above plants were procured and their quality was then ascertained. The above plant materials were then shade dried to reduce the moisture content to 5 %, wherever applicable.

Extraction process
Forty percentage extract of Aloe vera and Curcuma zedoaria were prepared individually in oil. In brief 40 kg of the herbal materials were treated separately with Cryo - fluid (liquid nitrogen) and stirred to coagulate the herbal materials. Then 50 kg of oil was added and stirred thoroughly. Subsequently the balance quantity of 50 kg of oil was added and once again the...
mixture was treated with Cryo – fluid. The entire mixture was incubated for 5 days with regular stirring. After incubation, the oil was filtered and used.

**Preparation of Evefresh cream**

Cream base was prepared. To the prepared Cream base *Aloe Vera* were incorporated at 2 % level and *Curcuma zedoaria* were incorporated at 0.2 %.

**HPTLC analysis of oil extracts of individual herbs of Evefresh cream**

All the above oil extracts were treated with methanol at 1:1 ratio. 5 microliter of the methanolic fraction of each oil extract was loaded separately on HPTLC plate- silica gel 60 F 254 of E. MERCK KGaA and was allowed to run using a mobile phase composed of Hexane: Ethyl acetate at 8: 2 ratio.

The plates were scanned through TLC scanner (CAMAG TLC Scanner 3 "Scanner3_150607" S/N 150607 (1.14.28) at 254 and the profile was subjected to derivatization using p-anisaldehyde sulphuric acid stain and the derivatized TLC plate was scanned at 366 nm. The profile of each herbal extract was recorded.

**RESULTS**

The HPTLC chromatogram has revealed the distinct signature fingerprints of all the six herbs present in the Evefresh Cream such as *Curcuma zedoaria, Aloe vera* (Table 1) when scanned at 254 nm and after derivatization at 366 nm. The individual profiles of each herb could be seen in Evefresh Cream as well.

**Table- 1 HPTLC profile of various herbal extracts Evefresh Cream scanned at 254nm**

<table>
<thead>
<tr>
<th>Details of the herbs and Rf Value</th>
<th>C.zedoaria</th>
<th>A.vera</th>
<th>Evefresh Cream</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.13</td>
<td>0.11</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>0.24</td>
<td>0.24</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>0.39</td>
<td>0.58</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>0.46</td>
<td>0.7</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>0.59</td>
<td>0.74</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>0.71</td>
<td>0.79</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>0.77</td>
<td>0.98</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

The signature fingerprint of the individual herb could be distinctly identified in Evefresh cream through chromatogram. (Table 2)

**Table- 2 Derivatization of various herbal Extracts of Evefresh Cream scanned at 366 nm**

<table>
<thead>
<tr>
<th>Details of the herbs and Rf Value</th>
<th>C.zedoaria</th>
<th>A.vera</th>
<th>Evefresh Cream</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
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<td>0.25</td>
<td></td>
</tr>
<tr>
<td>0.39</td>
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</tr>
<tr>
<td>0.46</td>
<td>0.54</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>0.54</td>
<td>0.59</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>0.75</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>0.64</td>
<td>0.81</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>0.71</td>
<td>0.92</td>
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<td></td>
</tr>
<tr>
<td>0.76</td>
<td>0.93</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>0.81</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**Fig-3** & **Fig-4** HPTLC finger print of herbs used in Evefresh Cream -254 nm & 366 nm

**Fig- 5** HPTLC finger print of herbs used in Evefresh Cream after derivatization

*Lane 1- Curcuma zedoaria; Lane 2- Aloe Vera; Lane 3– Evefresh Cream*
DISCUSSION

Despite the use of herbs with proven efficacy at laboratory level, when traditional medicine are made with such proven herbs, the clinical efficacy remains doubted and questioned. The question on the adequate presence of individual herbs in the finished product is the mandatory prerequisite for the therapeutic benefits. Whether this fundamental tenet of drug making is followed in traditional systems of medicine is unclear. The use of various herbs and by regressive following the traditional process may not need to a drug with a proven efficacy. To annul the above disconnect and to establish the credence between the product and its promise, we have evaluated the HPTLC chromatogram of Evefresh Cream a proprietary Siddha drug.

Findings of the study have clearly shown that Evefresh Cream is effective purely because it has followed all the essentials of drug development. The herbs used in the formulation are equally effective at laboratory test as well as in the product because the presence and concentration of each herb could be established in the finished product.

The presence of unique fingerprint of each herb in Evefresh Cream clearly suggests the least interaction between different herbs in Evefresh Cream resulting in either their modification or degradation. This proves that each herb individually and collectively exhibit their best therapeutic effect thus making Evefresh Cream the most effective anti-hyperpigmentation from siddha system of medicine.

Evefresh cream is very effective in decreasing the melanin synthesis when tested in murine melanoma cells. The potent skin lightening effect what we established for the combination of the extracts of Curcuma zedoaria and Aloe vera assumes great importance as both plants are widely used for various skin benefits. [5 & 6]. The distinct characteristics of all the herbs appeared to be well preserved in Evefresh Cream, thus resulting in its superior therapeutics.

Nevertheless the findings of the present study highlights the integration of an advanced science and scientific techniques by Dr. JRK’s Research and Pharmaceuticals in developing Siddha drugs and revitalizing the Siddha system of medicine.

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