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See next page for additional authors

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FROM EMBLICA AND MA-KHWAEN

Authors
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Sareeva Reungpatthanaphong1, Tuanta Sematong1, Buppachart Potduang*, Phanukit Kunhachan1, Itsara Keeta1 and Cholticha Niwaspragrit2

1Pharmaceuticals and Natural Products Department, Thailand Institute of Scientific and Technological Research, Technopolis, 35 Mu 3, Thanon Liab Klong 5, Klong Luang, Pathumthani 12120, Thailand. E-mail: Buppachart@tistr.or.th
2Agricultural Technology Department, Thailand Institute of Scientific and Technological Research, Technopolis, 35 Mu 3, Thanon Liab Klong 5, Klong Luang, Pathumthani 12120, Thailand.

KEYWORDS: Phyllanthus emblica, Zanthoxylum limonella, Gel wash, Moisturizing gel, Skin irritation test

INTRODUCTION
Emblica (Phyllanthus emblica L.; in Thai “Ma-Kham-Pom”), a euphorbiaceous plant, is widely distributed in subtropical and tropical regions in China, India, Indonesia and Malaysia. Emblica fruit has plentiful amounts of vitamin C and superoxide dismutase. It has been widely used in many traditional medicines including Chinese, Tibetan and Ayurvedic medicine as anti-oxidant, anti-microbial, anti-cancer and anti-inflammatory agents 1 2 3 4. Ma-Khwaen (Zanthoxylum limonella Alston) is found in the Northern part of Thailand. Its fruits have been widely used in Indian traditional medicine for the treatment of cardiac, respiratory diseases, tooth infection and against stomach infection. It has been reported to reveal antimicrobial activity 5.

In this research, the facial & body gel wash and moisturizing gel products were formulated from a patented mixed extract of Emblica and Ma-Khwaen. The objective of this study was to evaluate and investigate for skin irritation or contact dermatitis of these products in animal models. The skin irritation test was conducted following the Test Guideline No.404 of the OECD Guidelines for testing of chemical 6.

MATERIALS AND METHODS
Plant material
Dry, powdered fruits of Emblica and Ma-Khwaen were provided by the Agricultural Technology Department of TISTR.

Preparation of plant extracts
Each dried powder (500 g) was macerated with different ratios of ethanol-water. The ethanol extracts were filtered and the solvent was removed using a rotary evaporator at 45°C.

Formulations and stability study
The gel products containing the patented mixed extract of Emblica and Ma-Khwaen were formulated and studied by the Pharmaceuticals and Natural Products Department of TISTR.

Animals
Healthy adult albino New Zealand rabbits (white hybrid strain) were purchased from the Department of Animal Science, Faculty of Agriculture, Kasetsart University. Their body weight range was from 2 to 3 kg. They were housed individually in stainless steel cages and were fed with foods and water ad libitum. Prior to starting the experiment, they were acclimatized to the animal room conditions for one week.

Acute skin irritation test
One day before experimentation, an area of skin approximately 10 cm x 10 cm on the dorso-lumbar region of each rabbit (3 rabbits/sample) was clipped free of hairs. Two areas of the shaven skin (approximately 2.5 cm x 2.5 cm) were selected. The 0.5 ml of sample on a 2.5 cm x 2.5 cm gauze patch was moistened with water to serve as a treated patch, while 0.5 ml of distilled water on another patch was served as a control patch. Entire trunk of the rabbit was wrapped with elastic cloth to avoid dislocation of the patches. At the end of the 4 hr exposure period, all patches were removed and the treated skin gently wiped with moistened cotton wool to remove any residual test material. The rabbits were assessed for the degree of erythema and oedema evidence on each site at 1, 24, 48 and 72 hrs after
The skin reactions were independently scored by two inspectors using the numerical scoring system as follows:

<table>
<thead>
<tr>
<th>Skin reactions</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Erythema</strong> (Most severely affected area graded):</td>
<td></td>
</tr>
<tr>
<td>No erythema</td>
<td>0</td>
</tr>
<tr>
<td>Very slight erythema (barely perceptible)</td>
<td>1</td>
</tr>
<tr>
<td>Well-defined erythema</td>
<td>2</td>
</tr>
<tr>
<td>Moderate to severe erythema</td>
<td>3</td>
</tr>
<tr>
<td>Severe erythema (beet red coloration to slight eschar formation)</td>
<td>4</td>
</tr>
</tbody>
</table>

| Oedema formation (Most severely affected area graded): |       |
| No oedema | 0 |
| Very slight oedema (barely perceptible) | 1 |
| Slight oedema (edges of area well defined by definite raising) | 2 |
| Moderate oedema (area raised approximately 1 mm) | 3 |
| Severe oedema (raised > 1 mm, and extending beyond the area of exposure) | 4 |

RESULTS AND DISCUSSION

After removal of the patches from the treated skin of each rabbit, skin reactions at 1, 24, 48 and 72 hrs were observed. There were no erythema and oedema reactions in all rabbits treated with distilled water (control area) which were scored as zero. The rabbit skin irritation scores for the facial & body gel wash and moisturizing gel products are shown in Table 1.

It was found that 50% diluted facial & body gel wash product produced slight erythema of three tested rabbits’ skin at 1 h after patch removal and the symptom disappeared within 48 h, indicating that the gel wash could cause only insignificant and mild rash at 1 h. However, at 4 h the rabbits with single application patch test of 100% moisturizing gel product exhibited no erythema or oedema reactions on their skin, which could indicate that the moisturizing gel was non-irritant.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Scoring time (hr)</th>
<th>Erythema</th>
<th>Oedema</th>
<th>Erythema</th>
<th>Oedema</th>
<th>Erythema</th>
<th>Oedema</th>
<th>Erythema</th>
<th>Oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Distilled water</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td>Facial &amp; body gel wash product</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MOISTURIZING GEL PRODUCTS</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1 The skin irritation scores of the rabbits treated with the facial & body gel wash and moisturizing gel products.

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CONCLUSION
Our results demonstrated that 50% diluted facial & body gel wash product from a mixed extract of Emblica and Ma-Khwaen caused slight erythema of all tested rabbits' skin at 1 h after patch removal and the symptom disappeared within 48 h. This indicated that the facial & body gel wash only caused insignificant and mild rash at 1 h. However, the 100% moisturizing gel product did not produce any skin reactions on the tested rabbits' skin, which could indicate that it was non-irritant. Further toxicity studies should be conducted as safety assessment on cosmeceutical products containing the mixed extract as active constituent.

REFERENCES