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SKIN IRRITATION STUDY OF TWO PATENTED MIX EXTRACTS FROM EMBLICA, MA-KHWAEN AND KHANUN

Sareeya Reungpatthanaphong¹, Tuanta Sematong¹, Buppachart Potduang^{*1}, Phanukit Kunhachan¹ and Cholticha Niwaspragrit²

¹ Pharmaceuticals 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

² Agricultural Technology Department, Thailand Institute of Scientific and Technological Research, Technopolis, 35 Mu 3, Thanon Liab Klong 5, Klong Luang, Pathumthani 12120, Thailand.

KEYWORDS: *P. emblica*, *Z. limonella*, *A. heterophyllus*, mix extract, 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 [1]. 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}.

Zanthoxylum limonella Alston; in Thai “Ma-Khwaen”, is found in the Northern part of Thailand. It has 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 activity⁵.

Khanun (*Artocarpus heterophyllus* Lamk) or Jackfruit is a well known economic tree in Southeast Asia. The seeds and leaves have been used as an herbal remedy in Chinese traditional medicine. It has been reported that the ash of its leaf can heal the skin wounds and ulcer^{6, 7}. We have found that 40% ethanol leaves extracts of different varieties of *A. heterophyllus* was economically beneficial to be a source of tyrosinase inhibitor, with ranging IC₅₀ 0.07 – 2.3 mg/ml⁸. Their leaves extracts are not skin irritate⁹.

The patented mix extracts of Emblica fruits+Ma-Khwaen fruits and Khanun leaves+Ma-Khwaen fruits were investigated for skin irritation or contact dermatitis. The skin irritation test was conducted following the Test Guideline No.404 of the OECD Guidelines for testing of chemical⁵. We found that the mix extracts have potential to be used as active extracts in cosmeceutical products with anti-oxidation, anti-tyrosinase, anti-acne and anti-inflammatory benefit.

MATERIALS AND METHODS

Plant material: Dry powder of Emblica fruits, Ma-Khwaen fruits and Khanun leaves were provided by the Agricultural Technology Department of TISTR.

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

Preparation of mix extracts: The crude ethanol extracts were weighed and mixed in appropriate ratios to be active against acne-causing microbial. Small amount of 80% ethanol was added and mixed thoroughly by stirring, and then evaporated to get rid of the solvent at 45°C to provide the patented mix extracts of Emblica+Ma-Khwaen and Khanun+Ma-Khwaen to be subjected to skin irritation test.

Animals: The healthy adult albino rabbits of New Zealand white hybrid strain were purchased from the Department of Animal Science, Faculty of Agriculture, Kasetsart University. Their body weight ranges were 2-3 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 dermal irritation test: One day before experimentation, an area of skin approximately 10 cm × 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 × 2.5 cm were selected. The 0.5 g of sample on a 2.5 cm × 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. Its entire trunk was wrapped with elastic cloth to avoid dislocation of the patches. At the end of the 4 hrs exposure period, all patches were removed and gently wiped the treated skin 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 hr, 24, 48 and 72 hrs after removal of the patches. The skin reactions were independently scored by two inspectors using the numerical scoring system as follows⁵.

<u>Skin reactions</u>	<u>Score</u>
<u>Erythema</u> (Most severely affected area graded):	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation)	4
 <u>Oedema formation</u> (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 of all rabbits treated with distilled water (control area) which were scored as zero. The scores of rabbits for the mix extract samples are shown in Table 1.

It was found that both mix extracts showed no oedema with very slight erythema on 1/3 rabbits at 1 hr after removal of the patches. The recovery of this skin reaction was found within 48 hrs of the observation period for Emblica+Ma-Khwaen mix extract, and within 24 hrs for Khanun+Ma-Khwaen mix extract. Thus the mix extracts could be categorized as 'non- or slight irritant'.

Table 1. The skin irritation scores of the rabbits treated with the patented mix extracts of Emblica+Ma-Khwaen and Khanun+Ma-Khwaen

Treatment	Rabbit No.	Scoring time (hr)							
		1		24		48		72	
		Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
Distilled water	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
Emblica + Ma-Khwaen	1	1	0	1	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
Khanun + Ma-Khwaen	1	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0

CONCLUSION

The patented mix extract of Emblica fruits+Ma-Khwaen fruits and mix extract of Khanun leaves+Ma-Khwaen fruits showed slightly erythema on 1 in 3 rabbits at 1 hr, which recovered within 48 hrs and 24 hrs, respectively. The mix extracts could be categorized as 'non- or slight irritant'. Further toxicity studies should be conducted as safety assessment on cosmeceutical products containing the mix extracts as active constituents.

REFERENCES

1. Verma, R.C., Gupta, A., 2004. Effect of pre-treatments on quality of solar-dried amla. Journal of Food Engineering 65, 397-402.
2. Liu, X., M. Zhao, J. Wang, W. Luo, B. Yang and Y. Jiang. 2008. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. Journal of Food Composition and Analysis. 21: 219-228.

3. Zhang, Y.J., Tanaka, T., Iwamoto, Y., Yang, C.R., Kouno, I., 2000. Phyllaemblic acid, a novel highly oxygenated norbisabolane from the roots of *Phyllanthus emblica*. *Tetrahedron Letters* 41, 1781–1784.
4. Zhang, Y.J., Nagao, T., Tanaka, T., Yang, C.R., Okabe, H., Kouno, I., 2004. Antiproliferative activity of the main constituents from *Phyllanthus emblica*. *Biological and Pharmaceutical Bulletin* 27, 251–255.
5. Organization for Economic Co-operation and Development (2002). OECD Guidelines for Testing of Chemicals, Volume 2, Section 4: Health Effects. Acute Dermal Irritation / Corrosion. Test Guideline No. 404.
6. Jackfruit. Available at <http://www.allallergy.net>. Accessed September 15, 2012.
7. Jackfruit, *Artocarpus heterophyllus*. Available at http://www.ahort.purdue.edu/newcrop/mortion/jackfruit_ars.html. Accessed September 15, 2012.
8. Pradabphan, P., Potduang, B., Phasuk, S., Takolpuckdee, P., Rapan, N., Krasaithong, M., Ketmanee, N., Intarangsie, A., Ngamnon, Y., Kongsombat, B., Chindachia, R. and Tanpanich, S. (2012). Tyrosinase inhibition of leaf extracts from different varieties of *Artocarpus heterophyllus*. *The Thai Journal of Pharmaceutical Sciences Vol. 35* Suppl. Issue January, p.50-51.
9. Reungpatthanaphong, S., Potduang, B., Sematong, T., Ngamnon, Y., Rapan, N., Pradabphan, P., Krasaithong, M., Ketmanee, N., Intarangsie, A., Khoeynok, V. and Tanpanich, S. (2012). Skin irritation study of the leaves extracts from different varieties of *Artocarpus heterophyllus*. *The Thai Journal of Pharmaceutical Sciences Vol. 35* Suppl. Issue January, p.32-33.