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ANTI-INFLAMMATORY ACTIVITY OF FRUIT EXTRACTS FROM *PHYLLANTHUS EMBLICA* AND *ZANTHOXYLUM LIMONELLA*

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KEYWORDS: *P. emblica*, *Z. limonella*, Mix herbal extract, Anti-inflammation

INTRODUCTION

Inflammatory response is the organism's protective reaction to irritation, injury or infection. It is characterized by redness, heat, swelling, loss of function and pain. Inflammation starts with dilation of the arterioles and the opening of new capillaries and venular beds in the area. This causes an accelerated flow of blood, accounting for the signs of heat and redness. Next follows increased permeability of the microcirculation, which permits leakage of protein-rich fluid out of small blood vessels and into the extra vascular fluid compartment, accounting for the inflammatory edema. There are reports that a great number of plant-derived constituents, especially flavonoids, show promising anti-inflammatory activity by interacting with important cellular targets involved in the inflammatory process, namely arachidonic acid metabolite pathways, nitric oxide and NF- κ B^{1,2}.

The aim of this research was to study the anti-inflammatory activity of crude ethanol fruits extracts from *Phyllanthus emblica* L. (EUPHORBIACEAE) and *Zanthoxylum limonella* Alston (RUTACEAE) to consider their potential benefit to be used in cosmeceutical products.

P. emblica, or "Ma-Kham Pom" in Thailand, is native to the tropics of South and Southeast Asia. Its edible fruits have been used as anti-oxidant, anti-inflammatory and anti-microbial agent in herbal medicines. The fruit is non toxic, and non-mutagenic. Its constituents are vitamin C, tannin, polyphenols, alkaloids, flavonoids etc^{3,4,5}.

Z. limonella, or "Ma-khwaen" in Thai, is found in the northern of Thailand. It is a deciduous tree, up to 18 m tall. The bark of young trees is green with spines, becoming grey with woody prickles of 2 cm-3 cm on older trees. The extraction of roots, stem-barks, stems and fruits are widely used for antibacterial, anti-inflammatory and anesthetic properties. The fruit is non toxic, edible and used as food flavors. Its constituents are alkaloids, amides, coumarins, sterols and phenylpropanoid-ligans⁶.

Crude ethanol extracts from fruits of *P. emblica*, *Z. limonella* and their patented anti-acne mix extract were subjected to anti-inflammatory assay using croton oil-induced rat ear edema. Chief constituent of croton oil is tetraacetylphorbol acetate responsible for the inflammatory induction mechanism involving phospholipase A₂ activation, which in turn leads to the release of arachidonic acid and subsequent biosynthesis of arachidonic acid metabolites as leukotrienes and prostaglandins. The arachidonic acid metabolites have been firmly shown to act as mediators of the inflammatory response via cyclooxygenase and lipoxygenase activity, and have therefore been a target for the development of therapeutic agents^{7,8}.



(1a)

(1b)

Figure 1 (1a) Emblica (*Phyllanthus emblica*) and (1b) Ma-khwaen (*Zanthoxylum limonella*).

MATERIALS AND METHODS

Plant materials: Dried fruits powder of Emblica (*P. emblica*) and Ma-khwaen (*Z. limonella*) were provided by the Agricultural Technology Department, Thailand Institute of Scientific and Technological Research (TISTR).

Plant extracts preparation: The dry powder 500 g was separately macerated with different patented ratios of ethanol-water based on the anti-acne effectiveness. The ethanolic extracts were filtered and the solvent was removed using a rotary evaporator at 45°C.

Preparation of mix extracts: The crude ethanol fruits extract of Emblica (*P. emblica*) and Ma-khwaen (*Z. limonella*) were weighed and mixed in a patented appropriate ratio to be active against acne-causing microbial. Small amount of 80% ethanol was added, stirred thoroughly, removed the solvent at 45°C, and then kept at 5-10°C for further investigation.

Animal: Male Wistar rats of 80-100 g BW were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. The animals were housed in animal care facility at Thailand Institute of Scientific and Technological Research for 1 week before experimentation.

Anti-inflammatory assay: The **croton oil-induced rat ear edema** method was modified from Choi *et al.*⁹⁾. Male Wistar rats were divided into groups of six animals. Rat ears were measured by a pocket thickness gage before and after the croton oil applied. Edema was induced by 20 µl croton oil 16% (v/v) diluted in acetone applied to the inner and outer surfaces of each rat ear for 2 hours prior to the extracts application. The control group was applied with vehicle: a solution of acetone/water (1:1). Diclofenac dose 5 mg/ear was used as the positive control. The crude extracts in vehicle solutions were applied to rat ears of each group at 20 mg/ear dose. The ear thickness was measured at 1, 2, 3 and 4 hrs after test samples application, and calculated in term of percentage of swelling as: % Swelling = $(T_1 - T_0 \times 100) / T_0$. While T_0 is the thickness of ear rat at initial; T_1 is the thickness of ear rat at 1, 2, 3 and 4 hr.

Statistical analyses: Data of research were analyzed as means and standard deviation (Means ± SD). The statistical analysis was performed using analysis of variance (One way ANOVA) at the 0.05 significance levels.

RESULTS AND DISCUSSION

The crude ethanol fruit extracts from Emblica (*P. emblica*), Ma-khwaen (*Z. limonella*) and their patented anti-acne mix extract (1.25, 2.5 and 5.0 mg/ear) exhibited the anti-inflammatory activity by reducing %swelling of rat ear edema induced by croton oil as shown in Table 1.

The entire samples prominently reduced %swelling of rat ears at 1, 2, 3, 4 hr after applying the samples, in comparison with the control ($P < 0.05$). The extracts were as effective as Std. Diclofenac and the mix extract of 1.25 mg/ear was the most effective. The inflammatory reduction to 50% swelling was at 2 hr.

Comparative efficacy of 1.25 mg/ear samples at 2 hr exhibited as the anti-acne mix extract $> Z. limonella > P. emblica$ and Std. Diclofenac (5 mg/ear); %swelling at 2 hr was reduced to 18%, 19.23%, 29.43% and 24.83% respectively, in compared with 50.41% of control.

CONCLUSION

The patented anti-acne mix fruit extract of Emblica (*P. emblica*) and Ma-khwaen (*Z. limonella*) was proved to be a potent anti-inflammatory active extract. It was more potent than Std. Diclofenac and individual fruit extracts.

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Table 1 Anti-inflammatory effect on croton oil induced rat ear edema of the crude ethanol fruit extracts from *Embllica* (*P. emblica*), *Ma-khwaen* (*Z. limonella*) and their patented anti-acne mix extract

Group	% Swelling				
	After croton oil 2 hr	After sample 1 hr	After sample 2 hr	After sample 3 hr	After sample 4 hr
1. Control (60%ETOH)	53.81±6.14	53.22±6.01	50.41±6.20	47.01±5.28	44.16±4.09
2. Diclofenac 5 mg/ear	54.39±6.83	41.83±5.95*	24.83±6.22*	19.64±5.05*	17.35±4.80*
3. Emblica 1.25 mg/ear	55.89±8.10	41.75±2.30*	29.43±3.31*	23.28±2.77*	16.64±1.46*
4. Emblica 2.5 mg/ear	58.11±4.52	40.90±3.03*	22.66±1.36*	19.90±1.35*	18.77±0.93*
5. Emblica 5 mg/ear	52.27±3.08	40.19±5.01*	24.08±2.87*	23.64±2.53*	20.33±2.90*
6. Ma-khwaen 1.25 mg/ear	54.94±4.61	34.11±2.76*	19.23±2.87*	17.85±1.62*	14.98±2.10*
7. Ma-khwaen 2.5 mg/ear	52.83±4.97	31.83±3.95*	20.78±2.88*	18.69±1.87*	15.38±2.24*
8. Ma-khwaen 5 mg/ear	52.31±4.54	36.22±2.36*	18.32±1.37*	19.70±2.15*	18.65±1.95*
9. Mix extract 1.25 mg/ear	55.54±3.70	32.99±3.67*	18.00±1.94*	14.00±2.33*	12.31±2.03*
10. Mix extract 2.5 mg/ear	53.06±3.28	41.55±1.33*	26.54±1.41*	21.13±2.77*	17.26±3.90*
11. Mix extract 5 mg/ear	55.24±5.45	39.24±4.81*	25.61±4.24*	23.42±4.17*	19.62±3.42*

% swelling, presented as mean ± S.E.

*The data was significant differences ($P < 0.05$)

REFERENCES

- Calixto JB, Otuki M and Santos ARS. 2003. Anti-inflammatory compounds of plant origin. Part I. Action on arachidonic acid pathway, nitric oxide and nuclear factor κ B (NF- κ B). *Planta Med* 69: 973-983
- Inflammation. Available at <http://medical-dictionary.thefreedictionary.com/inflammation>, accessed 3 October 2012.
- Verma RC, Gupta A. Effect of pre-treatments on quality of solar-dried amla. 2004. *Journal of Food Engineering* 65: 397-402.
- Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS and Heinonen M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry* 47: 3954-3962.
- Lampronti I, Khan MTH, Bianchi, N, Borgatti M and Gambar R. 2004. Inhibitory effects of medicinal plant extracts on interactions between DNA and transcription factors involved in inflammation. *Minerva Biotechnological* 16: 93-99.
- Tangjitjaroenkun J, Supabphol R and Chavasiri W. 2012. Antioxidant effect of *Zanthoxylum limonella* Alston. *Journal of Medicinal Plants Research*. 1407-1414.
- Paula ACB, Hayashi LSS, and Freitas JC. 2003. Anti-inflammatory and antispasmodic activity of *Ipomoea imperati* (Vahl) Griseb (Convolvulaceae). *Braz J Med Bio Res* 36: 105-112.
- Silva DR, Baroni S, Svidzinski AE, Bersani-Amado CA and Cortez DAG. 2008. Anti-inflammatory activity of the extract, fractions and amides from the leaves of *Piper ovatum* Vahl (Piperaceae). *Journal of Ethnopharmacology* 116: 569-573.
- Choi HS, Kim HS, Min KR, Kim Y, Lim HK, Chang. YK, et al. 2000. Anti-inflammatory effects of fangchinoline and tetrandrine. *Journal of Ethnopharmacology* 69:173-179.