

1-1-2013

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Recommended Citation

Giwanon., Rattanasiri; Thisayakom, Krittiva; Limsiriwong, Pongsatom; Ruengsri, Saowaluck; Kheunok, Vichein; Sriyam, Kanchana; Srisom, Marudech; Pahusee, Darunee; Nakakaew, Sawai; Ketmanee, Natthachest; Peungsumrong, Sontaya; and Banchonglikitkul, Chulerattana (2013) "PILOT STUDY OF ANTIBACTERIAL AND ANTIINFLAMMATORY ACTIVITIES OF INDIAN GOOSEBERRY (PHYLLANTHUS EMBLICA L. EXTRACT) AGAINST BACTERIA ASSOCIATED WITH PITTED KERATOLYSIS," *The Thai Journal of Pharmaceutical Sciences*: Vol. 38: Iss. 0, Article 21.

Available at: <https://digital.car.chula.ac.th/tjps/vol38/iss0/21>

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**PILOT STUDY OF ANTIBACTERIAL AND ANTIINFLAMMATORY ACTIVITIES
OF INDIAN GOOSEBERRY (*PHYLLANTHUS EMBLICA* L. EXTRACT)
AGAINST BACTERIA ASSOCIATED WITH PITTED KERATOLYSIS**

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KEYWORDS: *P. emblica* L., *K. sedentarius*, pitted keratolysis, antibacterial, antiinflammatory

INTRODUCTION

Pitted keratolysis is superficial bacterial infection on the skin, especially the foot. This skin disorder is characterized by malodour and erosions on the soles of the foot. The malodour is caused by the sulfur byproducts of the bacteria. The causative bacteria is *Kytococcus sedentarius*. *Phyllanthus emblica* L., also known as Indian gooseberry or Makham-pom in Thai is rich sources of bioactive constituents such as polyphenols, gallic acids, alkaloids, tannin, saponin, flavonoids, essential oils and ellagic acids. The ethanolic fruit extract shows pharmacological activities such as antimicrobial, anti-inflammatory, antioxidative and antimutagenic activities. Some previous studies reported the potential antibacterial activity of *P. emblica* L. against *Micrococcus* species. The study on antibacterial efficacies against pitted keratolysis caused pathogens and antiinflammatory efficacies from *P. emblica* L. to use as an anti-foot odor hygienic product should be accomplished. Therefore, the purpose of this study is to investigate antibacterial potencies against bacterial associated with pitted keratolysis and antiinflammatory potencies from *P. emblica* L. extract.

MATERIALS AND METHODS

Plant material and Preparation of extracts 2 parts (seeds (MS) and pulp (MP)) of *P. emblica* L. were collected from Nakhon Ratchasima province, Thailand. The pulps were separated from the seed and cut into small pieces. Both parts were dried and were pulverized into fine powder by a grinder. All of these were extracted with 95 % ethanol (w/v) for 48 h 3 times and were then evaporated. The yields of the 2 extracts (MS and MP) were 8.94 % (w/w) and 55.32 % (w/w), respectively.

Antibacterial method The antibacterial activities of the 2 extracts against 3 bacterial strains relevant to pitted keratolysis (*K. sedentarius* ATCC 27573, *K. sedentarius* ATCC 27574 and *K. sedentarius* ATCC 27575) which provided from ATCC Culture Collection, were performed by agar diffusion and agar dilution method.

The agar diffusion testing According to the Clinical and Laboratory Standards Institute (CLSI; Document M2-A8, 2006), the diameters of inhibition zones (mm) of the 2 extracts against the 3 pitted keratolysis associated pathogens were evaluated by agar diffusion method (20 mg/disc) using Muller Hinton Agar (MHA). All experiments were carried out in duplicate. Student's t-Test was used for comparison between the 2 extracts. Statistical significance was defined as $p < 0.05$.

The agar dilution testing According to CLSI standards (Document M7-A7, 2006), the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) of the selected extract were determined by agar dilution using MHA. No growth of the 3 dermatogens were observed as MICs and MBCs. All experiments were performed in duplicate.

Antiinflammatory method (Preliminary study) The preliminary study of topical antiinflammatory activities of the selected extract were assessed by ethyl phenylpropionate (EPP)-induced rat ear edema (Brattsand, et al., 1982).

Animals 15 male Wistar rats weighing 105 ± 10 g, obtaining from the National Laboratory Animal Center, Thailand were housed under standard environmental conditions of temperature at 24 ± 10 °C under

a 12 h dark-light cycle, and allowed free access for food and water. All animals were deprived of food except water at least 16 h prior the experiments. The Animal Ethics Committee of TISTR approved all experimental protocols.

Test drugs The inflammogen (EPP (Fluka Chemika,Switzerland)) was dissolved in 5 % (v/v) of acetone (Merck, Germany). Phenylbutazone (Sigma, USA) was used as a standard anti-inflammatory drug.

Antiinflammatory testing (Preliminary study) Ear edema was induced in 15 male Wistar rats. The administration of 1 % and 7.5 % (w/v) of the MP per ear was applied onto inner (10 µl/ear) and outer (10 µl/ear) of the ear 30 min prior to EPP induction. The control group received vehicle only. Ear thickness was determined before and after treatment 30, 60 and 120 min using pocket thickness gauge after the treatment. The anti-inflammatory activities were calculated at each time point as percent inhibition of edema comparing with the vehicle-treated animals.

RESULTS

The 2 extracts of *P. emblica* L. revealed the antibacterial effects against all tested dermatogens. Both MP and MS possessed antibacterial activities against the 3 *K.sedentarius* strains with their inhibition zones ranging from 18.51-42.23 to 14.58-43.47 mm, respectively ($p < 0.05$). However, there was no significant differences between these 2 extracts. Therefore, the higher yield of extraction of the MP was an index for selecting to the further study (Table 1).

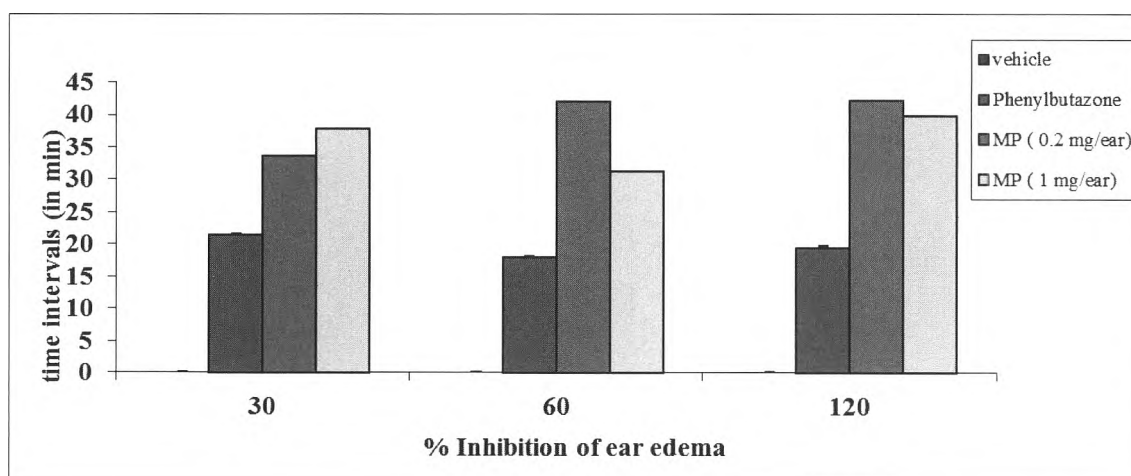
The MIC and the MBC value of the MP against the 3 *K. sedentarius* strains using agar dilution method was 10 mg/ml.

Both 1 % and 7.5 % (w/v) of the MP had tendency to inhibit the rat ear edema induced by EPP. (Figure 1).

Table 1 The comparison of inhibition zones between the 2 extracts from *P. emblica* L. (MP and MS) against the 3 *K. sedentarius* strains, pitted keratolysis associated pathogens, using agar diffusion method (20 mg/disc)

Bacterial strains	Inhibition zones (diameter, mm ± SD) of	
	MP	MS
<i>K. sedentarius</i> ATCC 27573	18.67 ± 0.22	14.72 ± 0.19
<i>K. sedentarius</i> ATCC 27574	42.37 ± 0.20	43.57 ± 0.14
<i>K. sedentarius</i> ATCC 27575	26.78 ± 0.16	24.54 ± 0.34

Figure 1 Preliminary study on anti-inflammatory activities of the MP by rat edema model



DISCUSSION

Among the tested extracts of *P. emblica* L, the MP possessed strong anti bacterial effect against all the 3 bacterial strains associated with pitted keratolysis. This result corresponded to some previous studies.

(Mayachiew, P and Devahastin, S., 2008, Dhale DA and Magle UP., 2011). The MP also had potent anti-inflammatory activity in EPP-induced rat ear edema. The pulp of *P. emblica* L. revealed abundant bioactive constituents such as alkaloids and phenolics. (Arunachalam M, et. al., 2011 and Rahman S, et. al., 2009). The result suggested that the MP may potentially to be an anti-pitted keratolysis agent. For further studies, its experimentally antiinflammatory study, active constituents identification and toxicological effect of the MP should be conducted.

CONCLUSIONS

The MP showed the tendency of antibacterial action against *K. sedentarius*, pitted keratolysis caused pathogens and antiinflammatory action in EPP-induced rat ear edema. These findings indicate the possibility to develop the MP as anti-pitted keratolysis agent from natural products.

ACKNOWLEDGEMENTS

We are grateful to Thailand Institute of Scientific and Technological Research (TISTR) for financial supporting.

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