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Fitoterapia 78 (2007) 401 – 408

FITOTERAPIA

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Effect *of Garcinia mangostana* on inflammation caused by *Propionibacterium acnes*

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Received 7 September 2006; accepted 6 February 2007
Available online 2 June 2007

Abstract

The present study was aimed to investigate the activity of Thai medicinal plants on inflammation caused by *Propionibacterium acnes* in terms of free radical scavenging and cytokine reducing properties. *P. acnes* have been recognized as pus-forming bacteria triggering an inflammation in acne. Antioxidant activity was determined by DPPH scavenging and NBT reduction assay. The result showed that *Garcinia mangostana* possessed the most significant antioxidant activity and reduced reactive oxygen species production. *Houttuynia cordata, Eupatorium odoratum*, and *Senna alata* had a moderate antioxidant effect. In addition, *Garcinia mangostana* extracts could reduce the TNF-α production as determined by ELISA. *Garcinia mangostana* was highly effective in scavenging free radicals and was able to suppress the production of pro-inflammatory cytokines. This study has identified the promising source of anti-inflammatory agent which could be useful in treatment of acne vulgaris.

Keywords: *Garcinia mangostana*; Acne; *Propionibacterium acnes*; Anti-inflammation; Reactive oxygen species; Cytokines

1. Introduction

Propionibacterium acnes has been recognized as an obligate anaerobic organism which is usually found as a normal skin commensal. This organism has been implicated over other cutaneous microflora in contributing to the inflammatory response of acne. It acts as an immunostimulator which can produce a variety of enzymes and biologically active molecules, which are involved in the development of inflammatory acnes. These products include lipases, proteases, hyaluronidases, and chemotactic factors [1]. The main components of pilosebaceous unit on the skin, such as keratinocytes and sebocytes can be activated by *P. acnes* leading to the production of pro-inflammatory cytokines [2]. It has been reported that a secreted peptidoglycan of *P. acnes* can stimulate the production of the proinflammatory cytokines such as IL-1, IL-8, and tumor necrosis factor-alpha (TNF-α) by human monocytic cell lines and freshly isolated peripheral blood mononuclear cells from acne patients [3].

Moreover, *P. acnes* significantly induces the interleukin-8 (IL-8) mRNA expression and selectively stimulates the expression of human beta-defensin-2 mRNA in keratinocytes [4]. Previous findings suggest that *P. acnes* has a major

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doi:10.1016/j.fitote.2007.02.019

role in the inflammation of acne vulgaris by both antigenic and mitogenic reactions [5]. Additionally, *P. acnes* can evoke mild local inflammation by producing neutrophil chemotactic factors. As a consequence, neutrophils which are attracted to the acne lesion constantly release inflammatory mediators such as reactive oxygen species (ROS) [6]. The ROS including superoxide radical anion, hydrogen peroxide and hydroxyl radical generated play a critical role in irritation and disruption of the integrity of the follicular epithelium and are responsible for the progression of inflammatory acne [7]. This toxic ROS can also act as second messengers in the induction of several biological responses such as NF-kB and AP-1, the generation of cytokines. Removal of the ROS can significantly reduce cell damage that may occur during acne inflammation [8].

Current treatment for acne is mostly based on antibiotics such as clindamycin and tetracycline derivatives. However, antibiotic-resistant *P. acnes* are widely spread and become a critical problem worldwide [9]. Therefore, an alternative treatment of acne must be studied and developed. This has led our interest in the possible effects of natural substances on anti-inflammation in acne lesions. Natural substances have been extensively studied in their biological activities especially during this decade. In the present study, Thai medicinal plants were examined for biological properties against inflammatory acne induced by *P. acnes* in terms of free-radical scavenging and cytokine reducing activities.

2. Experimental

2.1. Plants

Plants, collected from various locations in Thailand, were authenticated in the Herbariums in the Bangkok Herbarium, Botanical Section Botany and Weed Science Division, Department of Agriculture, Bangkok, Thailand. The specimens were deposited in the Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

2.2. Preparation of extracts

Parts of the 19 plants were used in this study according to their usages in traditional medicines. Plant materials dried, moderately powdered and macerated in EtOH were filtered. The filtrate evaporated in vacuum rotary evaporator gave the extracts reported in Table 1.

2.3. Microorganisms

P. acnes (ATCC 6919) was purchased from the American Type Culture Collection, USA.

2.4. DPPH radical scavenging activity

The free radical scavenging activity of the extract was first screened by the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The reduction of the DPPH was determined on TLC plate spotted with 1 mg/ml of sample and developed with $CHCl_3$: EtOAc (1:4). After drying, the plate was sprayed with a 0.2% solution of DPPH (Fluka, BioChemika, Switzerland) in MeOH and left at r.t. for 8 h. Activated products appeared as yellow spots against a purple background [10].

In order to measure the degree of antioxidant activity, DPPH free radical scavenging assay was carried out according to the procedure described by Sanchez et al. [11]. Briefly, aliquots of the sample in MeOH solution (1 mg/ml) were added to 3 ml of a 0.0004% MeOH solution of DPPH and the reaction mixture was shaken vigorously. The reaction involved a color change from violet to yellow which could be measured by the decrease in an absorbance at 515 nm after 10 min. The radical scavenging activity was obtained from the following equation:

$$\text{Inhibition Activity}(\%) = \frac{(\text{OD control} - \text{OD sample})}{\text{OD control}} \times 100$$

OD control represents the absorbance without extract
OD sample represents the absorbance with extract

Table 1
Usage of the Thai medicinal plants extracts

Botanical name	Part extracted	% yield (w/w)	Activity
Andrographis paniculata Nees (Acanthaceae)	leaves	8.17	Excellent for the upper respiratory tract infections, anti-inflammatory, diabetes, reducing blood pressure, immune enrichment, antiviral, antioxidant [24,25]
Azadirachta indica A. Juss (Meliaceae)	leaves	8.35	Inflammation-related diseases, antimicrobial activity [26,27]
Barleria lupulina Lindl. (Acanthaceae)	leaves	6.43	Anti-inflammatory [28]
Carthamus tinctorius L (Asteraceae)	flowers	47.7	Treats fever, night sweat, and dizziness, anti-inflammatory, anti-allergic [29,30]
Centella asiatica (L.) Urban (Apiaceae)	whole plants	2.95	Reduces inflammation, swelling, and wound healing [31]
Clinacanthus nutans Lindau (Acanthaceae)	leaves and branches	5.91	Relieves nettle rash, dysentery, fever, heals burns , scalds, insect stings, cures oral inflammatory symptoms [24]
Cymbopogon citrates Stapf (Graminae)	whole plants	2.77	Refreshes and natures the voice, reduces fever from colds, relieves cough, sore throat, nasal congestion and runny nose [24]
Eupatorium odoratum L. (Asteraceae)	leaves	5.72	Antibacterial activity [32]
Garcinia mangostana L. (Guttiferae)	pericarps	15.63	Reduces inflammation, pain and fever [22]
Hibiscus sabdariffa L. (Malvaceae)	flowers	3.79	Antiseptic, aphrodisiac, astringent, use for medicinal and nutritional purposes [33]
Houttuynia cordata Thunb (Sauruaceae)	leaves	2.86	Antiviral, anti-leukemic, antioxidative and antimutagenic effects [10,34]
Lawsonia inermis L. (Lythraceae)	leaves and branches	10.04	Antiseptic, wound healing, antimicrobial, antifungal [35,36]
Lycopersicon esculentum Mill. (Solanaceae)	fruits	1.19	Enhances the immune system, inhibits the cancer cell growth and protects against virulent bacteria [37]
Murdannia loriformis Hassk Commelinaceae)	leaves	0.96	Excellent for the upper respiratory tract infections, anti-inflammatory, diabetes, reduces blood pressure, immune enrichment, antiviral [24,25]
Psidium guajava L. (Myrtaceae)	leaves	7.11	Leaf, root and bark extracts are used for treatment of diarrhea, external ulcer, and skin diseases, antibacterial , anticough [25,38]
Senna alata L. (Fabaceae)	leaves	4.73	Laxative, diuretic effects. When used outside, reduces pus and inflammatory wounds [38]
Senna occidentalis L. (Fabaceae)	leaves	4.27	Analgesic, antibacterial, antifungal, antihepatotoxic, anti-inflammatory [39,40]
Senna siamea Lam. (Fabaceae)	leaves	13.29	Mild transquilizer, reduces anxiety, promotes better sleep, improves well-being, rich in vitamin C and A [24]
Tagetes erecta L. (Asteraceae)	flowers	5.01	Carotenoids used as anticancer and antiaging effect, flavonoids having pharmacological properties, essential oils use as antibacterial and insecticidal properties [41–43]

Antioxidant activity of the extract was expressed as the IC_{50}. The IC_{50} value was defined as the concentration (μg/ml) of extracts that inhibited the formation of DPPH radicals by 50%.

2.5. Reactive oxygen species production assay

2.5.1. Preparation of P. acnes culture supernatant

P. acnes was cultivated in BHI and glucose with and without plant extracts (100 μg/ml) for 72 h at 37 °C in anaerobic conditions. The supernatant was harvested, filtered through a 0.22 μm-pore size filter and stored at −20 °C until use.

2.5.2. Nitroblue tetrazolium dye (NBT) reduction test

The NBT test was performed according to the method of Park et al [12]. The test was aimed to determine ROS activity. Briefly, the 500 μl of venous blood of healthy volunteers, 50 μl of stimulants and positive or negative controls (Culture supernatant-*P. acnes* with and without extracts and zymosan and culture media) were added and the solutions

were incubated at 25 °C for 15 min. Then, 100-μl of NBT solution in 1 mg/ml of PBS was added to the above 100 μl primed blood and incubated at 37 °C for 30 min and then again at 25 °C for 20 min. Finally, smears were prepared and stained by Leishman stain (Sigma, St. Louis, MO, USA) for differential counting of formazan deposits in polymorphonuclear leukocytes (PMNLs).

2.6. Cytokine production assay

This study was investigated the anti-inflammatory properties of crude extracts, performed by the method of Jain and Basal [13]. The experiment was used an in vitro screening method based on inhibition of pro-inflammatory cytokines (TNF-α) producing by human peripheral blood mononuclear cells.

2.6.1. Preparation of P. acnes suspension

P. acnes was grown in 1% glucose BHI for 72 h at 37 °C in anaerobic atmosphere. The log phase bacterial culture was harvested, washed thrice in phosphate buffered saline (PBS, pH 7.2), and incubated at 80 °C for 30 min to heat-kill the bacteria. The heat-killed *P. acnes* suspension was stored at 4 °C until use.

2.6.2. Isolation of peripheral blood mononuclear cells (PBMCs)

Isolation of peripheral blood mononuclear cells was separated from venous blood of healthy volunteers. Blood was diluted 1:2 with phosphate-buffered saline (PBS, pH 7.2), layered on Histopaque (Sigma, Steinhem, Germany), washed thrice with PBS and resuspended in complete RPMI-1640 supplemented with 10% fetal calf serum (FCS). The cells were counted and resuspended at a concentration of 1×10^6 cells/ml in RPMI supplemented with 10% FCS. Cell viability was determined using the tryphan blue dye exclusion test. Viability of more than 95% was satisfactory.

2.6.3. Quantification of cytokines after treatment with plant extracts

A one-ml culture of PBMC (1×10^6 cells) was setup in 24-well tissue culture plates (Corning, USA) and stimulated with heat-killed *P. acnes* (10^8 organisms/ml) in the presence or absence of different crude extracts of plants at concentrations of 5 and 50 μg/ml. Cultures were incubated at 37 °C for 18 h in a humidified atmosphere containing 5% CO_2. Cultures without stimulants were set up as controls. The following day, cultures were transferred to microfuge tubes and centrifuged to collect cell-free supernatant containing secreted cytokines.

Cell-free supernatants were analyzed for TNF-α by commercially available sandwich ELISA (Biosource, USA). The ratio (%) of inhibition of the cytokine release was calculated using the following equation:

$$\text{Degree of inhibition}(\%) = 100 \times (1 - T/C)$$

T represents the concentration of cytokines in culture supernatant with the test compound.
C represents the concentration of cytokines in culture supernatant with the solvent.

3. Results and discussion

Nineteen Thai medicinal plants were selected to determine free radical scavenging activity using DPPH radical scavenging assay performed subsequently after TLC. After the preliminary screening, 7 of 19 plant extracts that possessed promising antioxidant effect were selected for further characterization. The interaction of antioxidants with

Table 2
Free radical scavenging activity of the Thai medicinal plants extracts

Plants	IC_{50} values (μg/ml)	% superoxide radical inhibition ratio ± SD
G. mangostana	6.13	77.80 ± 1.28
H. cordata	32.53	62.67 ± 0.75
E. odoratum	67.55	44.89 ± 1.38
S. alata	112.46	35.18 ± 2.66
B. lupulina	>150	10.74 ± 0.86
S. siamea	>150	67.60 ± 1.47
A. paniculata	>150	8.02 ± 2.11

The values represent the mean of triplicate measurements.

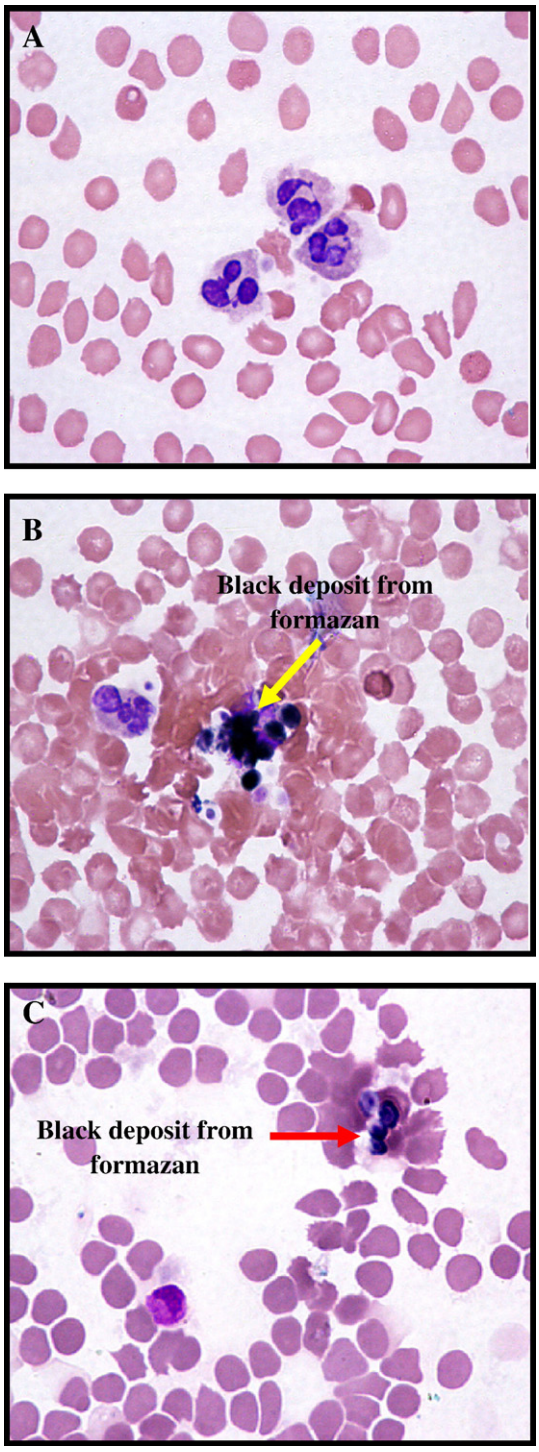

Fig. 1. The influence of *G. mangostana* extract on ROS level was determined by nitroblue tetrazolium dye reduction assay. A large black deposit in neutrophils was classified as a NBT positive cell. A) Neutrophils in normal condition B) Positive neutrophils induced by zymosan presenting large black deposits within the cells. C) Neutrophils induced by zymosan after incubation with *G. mangostana* presenting black deposits.

Table 3
Effect of the Thai medicinal plant extracts on TNF-α production [a]

Material	Concentration (μg/ml)	Concentration of TNF-a (pg/ml)	%inhibition
PBMC [b]	–	8.54	–
PBMC [b] + *P. acnes*	–	112.87	–
PBMC [b] + Zymosan [c]	–	78.71	–
G. mangostana	5	25.02	77.84
	50	6.1	94.59
E. odoratum	5	28.37	74.86
	50	23.19	79.46
S. alata	5	30.21	73.24
	50	31.12	72.43
H. cordata	5	22.27	80.27
	50	19.83	82.43
B. lupulina	5	21.05	81.35
	50	30.21	73.24
S. siamea	5	25.02	77.84
	50	14.64	87.03
A. paniculata	5	25.33	77.57
	50	31.73	71.89

The values represent the mean of triplicate measurements.
[a] The concentration of TNF-α produced by peripheral blood mononuclear cells was analyzed by ELISA. The concentration of TNF-α was calculated from the standard curve of commercial TNF-α at various concentrations.
[b] Peripheral blood mononuclear cells.
[c] Positive control.

DPPH resulted in either transfer electron or hydrogen atom to DPPH and neutralized its free radical character. The IC_{50} could be determined by the changes in the absorbance at different concentrations of each plant extract (Table 2). *G. mangostana* showed the best antioxidant activity and could inhibit 50% of free radicals at the concentration of 6.13 μg/ml (r^2 = 0.9992). *H. cordata* (r^2 = 0.9922), *E. odoratum* (r^2 = 0.9986) and *S. alata* (r^2 = 0.9946) had some antioxidant effects at the IC_{50} concentrations of 32.52, 67.55 and 112.46 μg/ml, respectively. The extracts of *B. lupulina, S. alata* and *A. paniculata* showed no significant activity in IC_{50} values.

The next experiment was performed to determine the effect of plant materials on reactive oxygen species (ROS) production. The NBT dye reduction test was used to determine the superoxide radical production by measuring PMNLs containing formazan deposit (Fig. 1). The fresh human PMNLs were exposed to a stimulant alone or with plant extracts. The results showed that *G. mangostana* could significantly reduce ROS production with the inhibitory ratio at 77.80 ± 1.28%. *H. cordata* and *S. siamea* also showed high ability to inhibit ROS production. *E. odoratum* and *S. alata* showed some inhibitory activity whereas *A. paniculata* and *B. lupulina* had insignificant activity on the generation of ROS.

The next experiment was conducted to study the inhibitory effect of the plant extracts on pro-inflammatory cytokine synthesis (Table 3). The result showed that the concentration of TNF-α in culture supernatant without a stimulant was 8.54 pg/ml. There was an increase in the production of pro-inflammatory cytokines, TNF-α, up to 112.87 pg/ml when stimulated with heat-killed *P. acnes*, while the positive control, zymosan showed an increase in the production of TNF-α up to 78.71 pg/ml. The result implied that *P. acnes* could stimulate the pro-inflammatory cytokines and also played an important role in the pathogenesis of inflammatory acne.

Next, the inhibitory effect of the plant extracts on TNF-α synthesis was investigated. The highest inhibition of TNF-α was observed in the PBMC treated with 50 μg/ml of *G mangostana* extract (94.59%). The other plant extracts at the same concentration also showed high inhibitory activity against TNF-α production (more than 70% inhibition). In addition, the results also showed that the extracts of *G. mangostana, E. odoratum, H. cordata*, and *S. siamea* showed the potent inhibition on TNF-α in dose-dependent manner.

4. Conclusions

P. acnes has been reported to play a critical role in a chronic inflammatory disease of the pilosebaceous follicle or acne vulgaris by the capability to stimulate the production of pro-inflammatory cytokines [4,14]. In addition to the

cytokines, *P. acnes* can induce the release of reactive oxygen species which are highly toxic to the cells [7]. The excessive production of these inflammatory mediators results in a destructive phenomenon leading to scarring. In the present study, anti-inflammatory activity of Thai medicinal plants was investigated in terms of free radical scavenging and cytokine reducing activity. The results revealed that *G. mangostana* possesses the most significant antioxidant activity at IC_{50} of 6.13 μg/ml. *H. cordata, E. odoratum*, and *S. alata* had moderate antioxidant effect. The DPPH radical scavenging test is widely accepted for in vitro determination of antioxidant activity of natural products but it cannot actually generate free radicals from the living cells. To overcome this aspect, polymorphonuclear leukocytes were used to produce free radicals after stimulation. In this experiment, *G. mangostana* significantly reduced the ROS production with the highest inhibitory ratio at 77.80±1.28%. *H. cordata*, *S. siamea*, *E. odoratum*, and *S. alata* showed moderate inhibitory activity. Moreover, *G. mangostana* extract could significantly reduce the TNF-α production generated from PBMC by stimulating with *P. acnes*. Taken together, the results suggested that *G. mangostana* was effective in scavenging free radicals in all studies and was able to reduce the production of pro-inflammatory cytokines. Therefore, this plant may have a remarkable anti-inflammatory effect and possibly reduces cell damage.

G. mangostana, a plant in Guttiferae family, has been used as a folk medicine for a long history. The fruit rinds of *G. mangostana* have been claimed for effective therapy of skin infections, wounds, and diarrhea [15,16]. The pericarp of this plant is a concentrated source of xanthones. Xanthones in the pericarp are composed of mangostione, α-mangostin, β-mangostin, γ-mangostin, gartinin, and garcinone E [17–19]. Previously, we reported that *G. mangostana* and its xanthone compounds, α-mangostin, promoted strong antimicrobial activity against *P. acnes* and *S. epidermidis* which were the critical etiologic agents in acne [20]. The xanthone compounds have antibacterial activity against *S. aureus*, both penicillin-resistant and methicillin-resistant strains [16,21]. It also possesses antifungal activity against *F. oxysporum*, *A. tenuls*, and *D. oryzae* [22]. The prenylated xanthones also have an inhibitory effect on *M. tuberculosis* [23]. The antimicrobial and anti-inflammatory activities of *G. mangostana* are particularly noteworthy considering to solve antibiotic-resistant problem in acne treatment. Future work is still needed to identify the active components of *Garcinia mangostana* which will be developed as a preventive and therapeutic agent used in acne vulgaris.

Acknowledgments

This work was supported by the Thailand Research Fund, under the grant # MRG4680146. The authors wish to thank staffs at Department of Microbiology and Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University for their assistance.

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