Anti-Acne-Inducing Bacterial Activity of Mangosteen Fruit Rind Extracts

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Key Words

Acne vulgaris • Antiacne • Mangosteen • Mangostin • Propionibacterium acnes • Staphylococcus epidermidis

Abstract

Objective: The aims of this study were to determine the most effective solvent extract of mangosteen, anti-acne-inducing bacterial activity and the amount of α-mangostin, a major active component in each mangosteen fruit rind extract, using high-performance liquid chromatography (HPLC). Materials and Methods: The fruit rinds of mangosteen were extracted with hexane, dichloromethane, ethanol and water. The extracts were tested for antibacterial activity against bacteria that induce acne, including \textit{Propionibacterium acnes} and \textit{Staphylococcus epidermidis}. Thin-layer chromatographic autobiography against these bacteria was also performed for each extract, while the α-mangostin content was analyzed using a validated HPLC method. Results: The dichloromethane extract exhibited the strongest antibacterial effect with minimum inhibitory concentration values for both bacterial species at 3.91 \textmu g/ml, while the minimum bactericidal concentration values against \textit{P. acnes} and \textit{S. epidermidis} were 3.91 and 15.63 \textmu g/ml, respectively. Thin-layer chromatographic autobiography indicated that α-mangostin was present in all extracts, except the water extract, and is a major active component against both \textit{P. acnes} and \textit{S. epidermidis}. Using HPLC, the dichloromethane extract yielded the highest content (46.21% w/w) of α-mangostin followed by the ethanol extract (18.03% w/w), the hexane extract (17.21% w/w) and the water extract (0.54% w/w). Conclusions: Dichloromethane extract exhibited the strongest anti-acne-inducing bacterial effect and this extract yielded the highest amount of α-mangostin.

Introduction

Acne vulgaris is a cutaneous pleomorphic disorder of the pilosebaceous unit involving abnormalities in sebum production and is characterized by both inflammatory and noninflammatory lesions. It has painful social and psychological effects on patients. The affected areas contain the largest oil glands, including the face, back and trunk. It is characterized by comedones, papules, pustules, cysts, nodules and often scars [1]. The pathogenesis of acne is multifactorial, namely hormonal imbalance, bacterial infection, stress, food or cosmetic application. \textit{Propionibacterium acnes} and \textit{Staphylococcus epidermidis}, which are bacteria isolated from the skin surface, are often involved in the development of acne [2]. \textit{P. acnes} are obligate anaerobic bacteria and act as an immunostimulator that produces a variety of biologically active mole-
cules and enzymes, such as lipases, proteases, hyaluronidase and chemotactic factors, which influence the development of inflammatory acne [3]. S. epidermidis, an aerobic organism, is usually involved in superficial infections within the sebaceous unit [4]. The common therapy for acne vulgaris includes oral and topical treatment using comedolytics and antibiotics [5]. However, these therapies can produce a number of potential side effects, including the development of resistance to frequently used antibiotics. Thus, the development of new antimicrobial agents for resistant organisms is becoming critically important. Plants produce many secondary metabolites with pharmacological activities that can be sources of pharmacologically active agents against pathogenic micro-organisms.

The fruit of *Garcinia mangostana* Linn. or mango-steen, of the family Guttiferae, is known as ‘the queen of fruit’ due to its delicious taste and pleasant aroma. The origin of this plant is in Southeast Asia [6, 7] and it is mainly distributed in Thailand, India, Sri Lanka, Myanmar, Indonesia, Malaysia, Philippines, China and other tropical countries. The fruit rind of *G. mangostana* has been used in Asian traditional medicines for the treatment of skin infections, wounds, diarrhea, dysentery, suppuration, leucorrhea, chronic ulcer and gonorrhea. It contains high amounts of xanthones, such as α-mangostin, which is a major component and other bioactive substances including tannins, flavonoids and polyphenolics compounds [8]. α-Mangostin is soluble in alcohol, ether, acetone, chloroform and ethyl acetate, while flavonoids and polyphenolic compounds are soluble in water and other polar solvents [9, 10]. Recent reports have shown that extracts from *G. mangostana* fruit rind have several medicinal properties, such as antioxidant [11–14], anti-inflammatory [15] and also inhibition of HIV [16]. In addition, it has been known to promote high antimicrobial activity against bacteria frequently involved in acne inflammation, *P. acnes* and *S. epidermidis* [17]. This extract is popularly used as a raw material in herbal cosmetics and herbal drug preparations to prevent or treat acne. Therefore, it is necessary to study the appropriate extraction solvent promoting high antiacne activity.

**Materials and Methods**

This study was to determine and compare anti-acne-inducing bacterial activity by broth microdilution method and the content of a major active component, α-mangostin, in mangosteen fruit rind extracts prepared using different solvents including hexane, dichloromethane, ethanol and water. Thin-layer chromatographic (TLC) autobiography for antibacterial activity against *P. acnes* and *S. epidermidis* of each extract was also performed. The extract with high anti-acne activity should be recommended as the appropriate extract for further development of antiacne preparation.

**Preparation of *G. mangostana* Fruit Rind Extract Plant Material**

The ripe fruits of *G. mangostana* were collected from Lang Suan District, Chumphon Province, in the South of Thailand in June 2006. The samples were identified by Dr. Wanadee Gritsanapan at the Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. The voucher specimen (WGM0806) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

The fresh fruits were cleaned and the edible aril parts were removed. The fruit rinds were cut into small pieces and dried in a hot air oven at 50°C for 72 h. The dried samples were ground into powder and passed through a sieve (20 mesh). The powdered sample was kept in an air-tight container and protected from light until used.

**Extraction of *G. mangostana* Fruit Rind Extract**

Powdered rinds (10 g) were extracted separately with 1,000 ml of hexane, dichloromethane and 95% ethanol using a Soxhlet apparatus. Each extract was filtered through a Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure at 50°C using a rotary vacuum evaporator. To prepare a water extract, the powdered *G. mangostana* fruit rind (10 g) was boiled with 200 ml of water for 1 h and filtered through a Whatman No. 1 filter paper. This method was repeated 5 times for exhaustive extraction and the filtrates were combined and evaporated on a boiling water bath. Each extraction was done in triplicate and yields of the dried extracts were recorded.

**Isolation of α-Mangostin**

α-Mangostin was separated from the dichloromethane extract of *G. mangostana* fruit rind by column chromatography eluted with hexane, hexane/ethyl acetate and then ethyl acetate with increasing polarity. After purification by recrystallization, α-mangostin was identified by comparison of 1H-NMR spectra with reference data of α-mangostin [18]. The purity of isolated α-mangostin was determined by high-performance liquid chromatography (HPLC), which was used as a standard compound in this study.

**Anti-Acne-Inducing Bacterial Susceptibility Testing**

The test organisms used in this study were *P. acnes* (ATCC 6919) and *S. epidermidis* (ATCC 14990), which were obtained from the American Type Culture Collection, USA. Brain-heart infusion and tryptic soy broth were purchased from DIFCO (Detroit, Mich., USA).

*P. acnes* was incubated in brain-heart infusion for 72 h at 37°C under anaerobic conditions, while *S. epidermidis* was incubated in tryptic soy broth for 24 h at 37°C, and they were adjusted to approximately 10⁸ CFU/ml.

The minimal inhibitory concentration (MIC) values were determined by 2-fold serial microdilution assay [17]. The extracts were incorporated into media to obtain a concentration of serial dilution from 500 to 0.24 µg/ml. A sample of 10 µl standardized
suspension of each tested organism was transferred to each well. The broth cultures of S. epidermidis and P. acnes were incubated for 24 and 72 h, respectively. The MIC defined as the lowest concentration of the compound that can inhibit the micro-organisms were determined. The minimal bactericidal concentration (MBC) values of the extracts were recorded as the lowest concentration that showed no visible growth after subculture of each clear well onto a new plate containing suitable media.

TLC Analysis

The TLC chromatograms of each extract and of a reference α-mangostin were performed on a precoated aluminium plate of silica gel 60F254 (Merck KGaA, Darmstadt, Germany) using dichloromethane:methanol (96:4) as a mobile phase: T1 = hexane extract; T2 = dichloromethane extract; T3 = α-mangostin reference standard; T4 = ethanol extract; T5 = water extract. Arrows indicate clear zone.

Table 1. Yields of crude extracts, contents of α-mangostin in G. mangostana fruit rind extracts prepared using different solvents and their MIC and MBC values against P. acnes and S. epidermidis

| Solvent | Yields of crude extracts % dry weight | α-mangostin content % w/w of extract | Susceptibility of bacteria to various extracts, µg/ml | P. acnes | S. epidermidis |
|---------|------------------------------------|-------------------------------------|-----------------------------------------------|--------|--------------|
|         |                                    |                                     | MIC | MBC | MIC | MBC |
| HEX     | 0.97 ± 0.03 (7.54)                  | 17.21 ± 0.50                       | 7.81 | 7.81 | 15.63 | 31.25 |
| DCM     | 8.01 ± 0.21 (1.39)                  | 46.21 ± 2.46                       | 3.91 | 3.91 | 3.91 | 15.63 |
| EtOH    | 20.20 ± 0.36 (0.34)                | 18.03 ± 0.71                       | 7.81 | 15.63 | 7.81 | 62.50 |
| H2O     | 27.50 ± 0.43 (0.02)                | 0.54 ± 0.01                        | 500  | >500 | 500  | >500  |

HEX = Hexane extract; DCM = dichloromethane extract; EtOH = ethanol; H2O = water extracts. Figures in parentheses indicate price in USD per gram of each extract.

The developed TLC plates were carefully overlaid with nutrient agar containing an aliquot of an overnight culture. The cultures were incubated at 37°C for 24 h under anaerobic condition for P. acnes and 24 h for S. epidermidis. The plates were sprayed with 1% of 2,3,5-triphenyl-tetrazolium chloride solution. The areas of inhibition were indicated by clear zones on the chromatogram and were compared with the Rf values of the related spots on the reference TLC plate.

Determination of α-Mangostin Content in Each Extract by HPLC Method

Standard solutions of α-mangostin (purity = 99.63%) were prepared by diluting the stock solution (1,000 µg/ml) with methanol to give the concentration range of 10–200 µg/ml. For sample preparations, 10 mg of each dried extract was dissolved in methanol and the volume was adjusted to 10 ml in a volumetric flask (concentration = 1 mg/ml). Aliquot of this solution (1.5 ml) was digital camera. The plates were run in duplicate; one set was used as the reference chromatogram and the other for bioautography.
diluted with methanol to make a final concentration of 150 μg/ml. The sample solution was filtered through a 0.45-μm membrane filter before injection.

A validated HPLC method [19] was performed on a Shimadzu SCL-10A HPLC system, equipped with a UV-vis detector SPD-10A. The separation was carried out using a Hypersil® BDS C18 column (250 × 4.6 mm, 5 μm size) with a C18 guard column. The mobile phase consisted of 0.1% v/v orthophosphoric acid (A) and acetonitrile (B). The elution was performed with gradient solvent systems at a flow rate of 1 ml/min and monitoring at 320 nm. The gradient program was as follows: 70% B for 0–15 min, 70–75% B for 3 min, 75–80% B for 1 min, constant at 80% B for 6 min, 80–70% B for 1 min and 11 min for postrunning for reconditioning. The quantitative determination was analyzed with a CLASS VP software program using the external calibration method.

Fig. 2. HPLC chromatograms of the fruit rind extracts of G. mangostana: α-mangostin standard (a), hexane extract (b), dichloromethane extract (c), ethanol extract (d) and water extract (e).
Results

The TLC autobiographs of all the extracts of *G. mangostana* fruit rinds except that of water (fig. 1) promoted main inhibition zones at the same R_f value (R_f = 0.4). The activity of α-mangostin against *P. acnes* and *S. epidermidis* had the same MIC but different MBC values against *P. acnes* at 1.95 μg/ml and *S. epidermidis* at 3.91 μg/ml. The dichloromethane extract of *G. mangostana* fruit rind showed the strongest antibacterial activities against *P. acnes* and *S. epidermidis* at the same MIC values of 3.91 μg/ml but MBC values at 3.91 and 15.63 μg/ml against *P. acnes* and *S. epidermidis*, respectively. The hexane and ethanol extracts promoted moderate activity with the same MIC at 7.81 μg/ml and MBC at 7.81 and 15.63 μg/ml, respectively, against *P. acnes*. For *S. epidermidis*, the hexane extract promoted the MIC at 15.63 μg/ml and MBC at 31.25 μg/ml, while the ethanol extract gave the MIC and MBC values at 7.81 and 62.50 μg/ml, respectively. On the other hand, the water extract showed the lowest activity (MIC = 500 μg/ml, MBC >500 μg/ml for both of the tested bacteria; table 1).

The content of α-mangostin in each extract was in the following order: dichloromethane extract (46.21% w/w), ethanol extract (18.03% w/w), hexane extract (17.21% w/w) and water (0.54% w/w), respectively. The purity of isolated α-mangostin extract was 99.6%.

Discussion

The results showed that hexane, dichloromethane and ethanol extracts of mangosteen fruit rind exhibited higher activity against *P. acnes* than *S. epidermidis*, thereby implying that the polarity of extractant had influence upon antibacterial activity. Using HPLC analysis, the highest content of α-mangostin, the main xanthone found in *G. mangostana* fruit rinds, was found in the dichloromethane extract followed by ethanol, hexane and water extracts, respectively. These results show that because of the moderate polarity of the chemical structure of α-mangostin, it is more soluble in a moderate polarity solvent (dichloromethane) than a nonpolar solvent (hexane) or polar solvents (ethanol or water) [9]. HPLC and TLC chromatograms of all extracts showed different patterns, but α-mangostin was found to be a major component in all extracts, supporting the previous report [20, 21] (fig. 1, 2). This compound also promotes the strong antibacterial activities against *P. acnes* and *S. epidermidis*. The results indicate that the antiacne activity of the extracts might depend on the α-mangostin content.

Based on the current findings, dichloromethane seemed to be the appropriate solvent to extract *G. mangostana* fruit rind for antiacne purposes because it promoted the highest α-mangostin content and anti-acne-inducing bacterial activity. However, dichloromethane could be toxic to humans [22]. On the other hand, ethanol was found to be a less toxic [23] and cheaper solvent that also provided a high antiacne property. Thus, ethanol extract of *G. mangostana* fruit rind might be an alternative choice for this purpose in pharmaceutical production.

Conclusions

Dichloromethane provided the extract of *G. mangostana* fruit rind with high content of α-mangostin and high anti-acne-inducing bacterial activity. Considering various factors, i.e. safety, cost and antiacne activity, ethanol extract might be used as an alternative natural source in antiacne preparations instead of antibiotic drugs. However, toxicology and clinical trials in animal models have to be studied before use in humans.

References

1. Kumar A, Baboota S, Agarwal SP, Ali J, Ahuja A: Treatment of acne with special emphasis on herbal remedies. Expert Rev Dermatol 2008;3:111-122.
2. Hamnerius N: Acne – etiology and pathogenesis. Treat Acne 1996;32:29–38.
3. Jeremy AHT, Holland DB, Robert SG, Thomson KF, Cunliffe WJ: Inflammatory events are involved in acne lesion initiation. J Invest Dermatol 2003;121:20–27.
4. Burkhart CG, Burkhart CN, Lehmann PF: Acne: a review of immunologic and microbiologic factors. J Postgrad Med 1999;75:328–331.
5. Clark C: Acne – general practice management. Practitioner 1993;237:160–164.
6. Martin FW: Durian and mangosteen; in Nagy S, Shaw PE (eds): Tropical and Subtropical Fruits. New York, AVI Publishing Inc, 1980, pp 401–414.
7. Kanchanapoom K, Kanchanapoom M: Mangosteen; in Shaw PE, Chan Jr HT, Nagy S (eds): Tropical and Subtropical Fruits. Auburndale, Agriscience Inc, 1998, pp 191–215.
Pedraza-Chaverri J, Cárdenas-Rodríguez N, Orozco-Ibarra M, Pérez-Rojas JM: Medicinal properties of mangosteen (*Garcinia mangostana*). Food Chem Toxicol 2008;46:3227–3239.

Budavari S: The Merck Index, ed 11. Whitehouse Station, Merck & Co, Inc, 1989.

Marston A, Hostettmann K: Separation and quantification of flavonoids. in Andersen OM, Markham KR (eds): Flavonoids: Chemistry, Biochemistry and Applications. Boca Raton, CRC Press, 2006, pp 1–32.

Weecharangsan W, Opanasopit P, Sukma M, Ngawhirunpat T, Sotanaphun U, Siripong P: Antioxidative and neuroprotective activities of extracts from the fruit hull of mangosteen (*Garcinia mangostana* Linn.). Med Princ Pract 2006;15:281–287.

Márquez-Valadez B, Lugo-Huirtrón R, Valdivia-Cerda V, Miranda-Ramírez LR, Pérez-De La Cruz V, González-Cuahutencos O, Rivero-Cruz I, Mata R, Santamaría R, Pedraza-Chaverri J: The natural xanthone α-mangostin reduces oxidative damage in rat brain tissue. Nutr Neurosci 2009;12:35–42.

Pedraza-Chaverri J, Reyes-Fermin LM, Nuñolascos-Amaya EG, Ibarra MO, Medina-Campos ON, González-Cuahutencos O, Rivero-Cruz I, Mata R: ROS scavenging capacity and neuroprotective effect of α-mangostin against 3-nitropropionic acid in cerebellar granule neurons. Exp Toxicol Pathol 2009;61:491–501.

Lin CN, Chung MI, Liou SJ, Lee TH, Wang JP: Synthesis and anti-inflammatory effects of xanthone derivatives. J Pharm Pharmacol 1996;48:532–538.

Chen SX, Wan M, Loh BN: Active constituents against HIV-1 protease from *Garcinia mangostana*. Planta Med 1996;62:381–382.

Chomnawang MT, Surassmo S, Nukoolkarn VS, Gritsanapan W: Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria. J Ethnopharmacol 2005;101:330–333.

Inuma M, Tosa H, Tanaka T, Asai F, Kobayashi Y, Shimano R, Miyaiuchi K: Antibacterial activity of xanthones from guttiferaceous plants against methicillin-resistant *Staphylococcus aureus*. J Pharm Pharmacol 1996;48:861–865.

Pothitirat W, Gritsanapan W: HPLC quantitative analysis method for the determination of α-mangostin in mangosteen fruit rind extract. Thai J Agric Sci 2009;42:7–12.

Pothitirat W, Gritsanapan W: Quantitative analysis of total mangostins in *Garcinia mangostana* fruit rind. J Health Res 2008;22:161–166.

Pothitirat W, Chomnawang MT, Supaphbol R, Gritsanapan W: Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the mangosteen fruit rind at two stages of maturity. Fitoterapia 2009;80:442–447.

Skrabalak SD, Babish GJ: Safety standards for occupational exposure to dichloromethane. Regul Toxicol Pharm 1983;3:139–143.

Greim H, Reuter U: Classification of carcinogenic chemicals in the work area by the German MAK Commission: current examples for the new categories. Toxicology 2001;166:11–23.