Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

Free radical scavenging and anti-acne activities of mangosteen fruit rind extracts prepared by different extraction methods

Werayut Pothitirat, Mullika Traidej Chomnawang, Roongtawan Supabphol & Wandee Gritsanapan

To cite this article: Werayut Pothitirat, Mullika Traidej Chomnawang, Roongtawan Supabphol & Wandee Gritsanapan (2010) Free radical scavenging and anti-acne activities of mangosteen fruit rind extracts prepared by different extraction methods, Pharmaceutical Biology, 48:2, 182-186, DOI: 10.3109/13880200903062671

To link to this article: https://doi.org/10.3109/13880200903062671

Published online: 06 Oct 2009.

Pharmaceutical Biology, 2010; 48(2): 182–186

informa
healthcare

RESEARCH ARTICLE

Free radical scavenging and anti-acne activities of mangosteen fruit rind extracts prepared by different extraction methods

Werayut Pothitirat[1], Mullika Traidej Chomnawang[2], Roongtawan Supabphol[3], and Wandee Gritsanapan[1]

[1]*Department of Pharmacognosy,* [2]*Department of Microbiology, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand, and* [3]*Department of Physiology, Faculty of Medicine, Srinakarintarawirot University, Bangkok, Thailand*

Abstract

The ethanol extracts of mangosteen fruit rinds prepared by several extraction methods were examined for their contents of bioactive compounds, DPPH-scavenging activity, and anti-acne producing bacteria against *Propionibacterium acnes* and *Staphylococcus epidermidis*. The dried powder of the fruit rind was extracted with 95% ethanol by maceration, percolation, Soxhlet extraction, ultrasonic extraction, and extraction using a magnetic stirrer. Soxhlet extraction promoted the maximum contents of crude extract (26.60% dry weight) and α-mangostin (13.51%, w/w of crude extract), and also gave the highest anti-acne activity with MIC 7.81 and 15.63 µg/mL and MBC 15.53 and 31.25 µg/mL against *P. acnes* and *S. epidermidis*, respectively. Ethanol 70% and 50% (v/v) were also compared in Soxhlet extraction. Ethanol 50% promoted the extract with maximum amounts of total phenolic compounds (26.96 g gallic acid equivalents/100 g extract) and total tannins (46.83 g tannic acid equivalents/100 g extract), and also exhibited the most effective DPPH-scavenging activity (EC_{50} 12.84 µg/mL). Considering various factors involved in the process, Soxhlet extraction carried a low cost in terms of reagents and extraction time. It appears to be the recommended extraction method for mangosteen fruit rind. Ethanol 50% should be the appropriate solvent for extracting free radical-scavenging components, phenolic compounds, and tannins, while 95% ethanol is recommended for extraction of α-mangostin, a major anti-acne component from this plant.

Keywords: *Acne vulgaris; anti-acne; free radical scavenging activity;* Garcinia mangostana*; mangostin; tannin*

Introduction

Extraction is important for the quality and quantity of bioactive components in medicinal plant extracts. It separates compounds from the cellular matrix, and exhaustive extraction should be performed. Ideally, the extraction method should be simple, rapid, and inexpensive for routine analysis (Benthin et al., 1999; Ong, 2004).

Mangosteen (*Garcinia mangostana* Linn.) is a tropical plant in the family Guttiferae. The fruit of this plant is known as the "queen of fruits" due to its delicious flavor. It is not commonly grown in the Tropics, but is gaining in popularity because of its high economic value. The fruit rind of mangosteen has been used as a dyeing agent, and traditional medicine for antidiarrhea, antidysentery, and treatment of wounds (Gritsanapan & Chulasiri, 1983). It is reported to contain several groups of phenolic compounds such as tannins, flavonoids, and xanthones, supporting its traditional uses (Fransworth & Bunyapraphatsara, 1992; Yu et al., 2007). A major xanthone in the fruit rind is α-mangostin (Walker, 2007).

Biological activity studies have shown that *G. mangostana* promotes antiinflammatory, anticancer, antimicrobial, and antioxidant properties (Iinuma et al., 1996; Nakatani et al., 2002; Moongkarndi et al., 2004; Chomnawang et al., 2005). Mangosteen fruit

Address for Correspondence: Wandee Gritsanapan, Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudhaya Rd., Ratchathewi, Bangkok 10400, Thailand. Tel: +66 2644 8677-89, ext 1500, 5530. Fax: +66 2644 8701. E-mail: pywgs@mahidol.ac.th

(Received 24 October 2008; revised 22 January 2009; accepted 06 March 2009)

ISSN 1388-0209 print/ISSN 1744-5116 online © 2010 Informa UK Ltd
DOI: 10.3109/13880200903062671

extract is popularly used as a food supplement due to its antioxidant activity, while the extract from the fruit rind, promoting antibacterial properties, has been recommended for the treatment of acne vulgaris (Kumar et al., 2008). Recently, *G. mangostana* fruit rind extract has become popular, used as a dietary supplement, herbal medicine, and cosmetic combinant. The biological quality of mangosteen-derived products is based on the contents of α-mangostin, tannins, and other phenolic compounds. It is important to determine the appropriate extraction method producing the extract with a high yield of these compounds and promoting high biological activities. Thus, this work compared free radical-scavenging and anti-acne activities, and contents of total phenolic compounds, tannins, and α-mangostin in the ethanol extracts of mangosteen fruit rinds were prepared using several extraction methods, including maceration, percolation, Soxhlet extraction, ultrasonic extraction, and extraction using a magnetic stirrer. The appropriate method can then be selected for the extraction of *G. mangostana* fruit rind for further standardization and use in commercial production.

Materials and methods

Chemicals

α-Mangostin was purchased from ChromaDex Inc. (Santa Ana, CA). 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical was obtained from Sigma-Aldrich (St. Louis, MO, USA). Folin–Ciocalteu reagent, bovine serum albumin, and ferric chloride were obtained from Fluka Biochemika (Steinheim, Germany). Sodium dodecylsulfate, sodium chloride, and sodium bicarbonate were purchased from Ajax Finechem (Molecule Co. Ltd., Thailand). Gallic acid was purchased from Merck (Darmstadt, Germany). Tannic acid was purchased from May and Baker Chemical Laboratory (Dagenham, England). The other chemicals and solvents were analytical grade or high performance liquid chromatography (HPLC) grade, and were purchased from Labscan Asia (Bangkok, Thailand), except for 95% ethanol which was obtained from the Excise Department, Bangkok, Thailand and was distilled before use.

Microorganisms and media

The test organisms used in this study were *Propionibacterium acnes* (ATCC 6919) and *Staphylococcus epidermidis* (ATCC 14990). These bacteria were obtained from the American Type Culture Collection, USA. Brain–heart infusion (BHI) and tryptic soy broth (TSB) were purchased from Difco (Detroit, USA).

Plant material

The ripe fruits of *G. mangostana* were purchased from a local market in Bangkok, Thailand in June 2006. The samples were identified by Dr. W. Gritsanapan at the Faculty of Pharmacy, Mahidol University (Bangkok, Thailand). Voucher specimens (WGM1406) were 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 oven at 50°C for 72 h. The dried samples were ground into powder, and passed through a sieve (20 mesh). The powdered samples were kept in airtight containers and protected from light until used.

Extraction methods

Several extraction methods were done using 95% (v/v) ethanol as a solvent, except for Soxhlet extraction in which 95, 70, and 50% (v/v) ethanol were used. Each method was performed in triplicate.

Maceration (ME 95)
The dried powder (10 g) was macerated with 95% ethanol (100 mL) at room temperature for 10 days with occasional shaking. The mixture was filtered through a Whatman No. 1 filter paper. Other portions of solvent were added to the marc and the extraction was repeated until exhaustion (100 mL × 10) (the last extract was colorless and no spot was detected on thin layer chromatography (TLC)).

Percolation (PE 95)
The dried powder (10 g) was mixed with 95% ethanol (10 mL) and the mixture was allowed to stand for 1 h. The mixture was transferred to a percolator and 95% ethanol was added (3.6 L). The extraction was done at room temperature with flow rate 3 mL/min until the percolate was exhausted (20 h).

Extraction with magnetic stirrer (EMS 95)
The powder (10 g) was extracted at room temperature (28–30°C) with 95% ethanol (100 mL each) in a 250 mL flask for 1 h using a magnetic stirrer with speed of 500 rpm. The extraction was repeated (10 times) until exhaustion. The total extraction time was 10 h (1 h × 10).

Ultrasonic extraction (UE 95)
This was carried out by extracting 10 g of the dried fruit rind powder with 95% ethanol (100 mL) in an ultrasonic apparatus. The temperature of the water in the water bath was controlled at 30°C by changing the water every 30 min until the extraction was exhausted. The total extraction time was 10 h (1 h × 10).

Soxhlet extraction (SE 95, SE 70, SE 50)
The sample (10 g) was placed into a thimble and was separately extracted with 400 mL of 50, 70, and 95% ethanol. Extraction was carried out at five cycles/h until exhaustion (15 h).

The combined extract of each extraction method was filtered through a Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressured at 50°C using a rotary vacuum evaporator. The crude extract was then evaporated on a boiling water bath until a constant weight was obtained.

Determination of total phenolic compounds content

The content of total phenolic compounds was determined using the method adapted by Kim et al. (2006). Each sample (1 mg/mL) of 0.2 mL was mixed with 0.5 mL of Folin–Ciocalteu reagent (diluted 1:10 with deionized water) and 0.8 mL of sodium bicarbonate solution (7.5%, w/v). The mixture was allowed to stand at room temperature for 30 min with intermittent shaking. The absorbance of the mixture was measured at 765 nm using an ultraviolet (UV)-visible spectrophotometer (PerkinElmer, USA). Different concentrations of standard gallic acid (10–100 μg/mL) were used to obtain a standard curve. The total phenolic content was expressed as gallic acid equivalents (GAE) in grams per 100 g of sample.

Determination of total tannins content

The method for determination of total tannins content was adapted from Hagerman and Butler (1978). Bovine serum albumin (2 mL) was put into a centrifuge tube; the sample solution (1 mL) was added and mixed immediately.The reaction mixture was kept for 20 min at room temperature, then centrifuged (Kubota, Japan) for 15 min at 3500 rpm and the supernatant rinsed off. The remaining part was dissolved with 4 mL of sodium dodecylsulfate and triethanolamine.Ferric chloride (1 mL) was added and the mixture was shaken vigorously. The mixture was then incubated again for 15 min. The absorbance of the mixture was measured at 510 nm. The total tannins content was determined using a standard curve of tannic acid (200–900 μg/mL). The content was then calculated as mean ± SD (n = 3) and expressed as grams of tannic acid equivalents (TAE)/100 g of extract.

Determination of α-mangostin content by HPLC method

The HPLC method was performed on a Shimadzu SCL-10A HPLC system, equipped with a model LC-10AD pump, UV-vis detector SPD-10A, and Rheodyne injector fitted with a 20 μL loop and auto injector SIL-10A. A Hypersil® BDS C18 column (250 × 4. 6 mm, 5 μm size) with a C18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1 mL/min at ambient temperature. The mobile phase consisted of 0.1% (v/v) *ortho*-phosphoric acid (A) and acetonitrile (B). The mobile phase was prepared daily, filtered through a 0.45 μm pore size filter, and sonicated before use. Total running time was 37 min and 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 post-running for reconditioning. The sample injection volume was 10 μL. The wavelength of the UV-vis detector was set at 320 nm. Quantitative determination was performed by the CLASS-VP software program using the external calibration method.

A stock solution of α-mangostin was prepared in methanol at 1000 μg/mL. Standard solutions were prepared by diluting the stock solution with methanol to give the concentration range of 10–200 μg/mL.

For sample preparation, 25 mg of dried extract was dissolved in methanol and the volume was adjusted to 25 mL in a volumetric flask (concentration 1 mg/mL). An aliquot of this solution (2.5 mL) was diluted with methanol to make a final concentration of 250 μg/mL. Prior to analysis, the solutions were filtered through a 0.45 μm membrane filter. Mangostin in the sample was quantitatively analyzed by HPLC using the previous mentioned conditions.

Determination of scavenging activity using DPPH-scavenging assay

A stock solution of the sample (5 mg/mL) was diluted to make a two-fold dilution series. The DPPH-scavenging reaction was performed when DPPH solution (152 μM) was added to the sample solution in the same volume (750 μL). An aliquot of the mixture was measured for absorbance at 517 nm after 30 min of the reaction by a UV-visible spectrophotometer (PerkinElmer, USA). The corresponding blank readings were also taken, and percent inhibition was then calculated as follows:

$$\%\,\text{Inhibition} = \left[A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}} \right] \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. The EC_{50} value, the concentration of sample required for 50% scavenging of the DPPH free radical, was determined from the curve of percent scavenging plotted against concentration. Each determination was carried out in triplicate, and the average EC_{50} value was then calculated.

Antibacterial susceptibility test

P. acnes was incubated in BHI medium for 72 h at 37°C under anaerobic conditions, while *S. epidermidis* was incubated in TSB for 24 h at 37°C under aerobic conditions, and adjusted to yield approximately 10^8 CFU/mL.

Minimal inhibitory concentration (MIC) values were determined by two-fold serial microdilution assay (NCCLS, 2008). The extracts were incorporated into media to obtain a concentration of 1000 μg/mL and serially diluted to achieve 500, 250, 125, 62.50, 31.25, 15.63, 7.81, 3.91, 1.95, 0.98, 0.49, and 0.24 μg/mL, respectively. A standardized suspension (10 μL) 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 compound that inhibits the microorganism, was determined. The minimal bactericidal concentration (MBC), defined as the lowest concentration of compound that kills the microorganism, was recorded. This gave the lowest concentration of compound that showed no visible growth after subculture of each clear well onto a new plate containing suitable media.

Statistical analysis

Each experiment was done in triplicate. The results are expressed as mean and standard deviation (SD). The average data for the content of each bioactive compound and EC_{50} of each extract prepared by the different extraction methods were statistically analyzed using one-way analysis of variance (ANOVA) with least significant difference (LSD) by SPSS 11.5 program. A statistical probability (p value) less than 0.05 indicated a statistically significant difference between groups.

Results and discussion

The yields of crude extracts of *G. mangostana* fruit rind prepared by different extraction methods, along with the contents of α-mangostin, total phenolic compounds, and total tannins, and free radical-scavenging and antibacterial activities, are shown in Table 1. Results showed that ME and SE extracts exhibited the strongest DPPH-scavenging activity (EC_{50}: 14.88–12.84 μg/mL). Among them, SE 70 and SE 50, which contained high amounts of total tannins and phenolic compounds, promoted better scavenging activity (13.39 and 12.84 μg/mL) than the 95% ethanol extract (14.88 μg/mL), surely due to the well-known, good antioxidant properties of their components (Goli et al., 2005; Gu et al., 2008). Interestingly, SE 50, which displayed the best activity, gave the highest yield of crude extract (31.52%).

In turn, SE 95 showed the strongest bacteriostatic and bactericidal activity against *P. acnes* (7.81/15.63 μg/mL). This action could be attributed at first glance to the higher content of α-mangostin of SE 95 (13.51 ± 0.03%). Nevertheless, it is clear that the amount of this compound is similar to that of ME 95 (13.32 ± 0.15%), which displayed a lower activity (15.63/31.25 μg/mL) against *P. acnes*. So, the activity of ME 95 can hardly be attributed to this xanthone derivative, but it is possible that SE 95, as a whole, possesses compounds in the appropriate ratios to display the best activity. Mangostin was reported previously as the compound responsible for the activity of *G. mangostana* against *P. acnes* (Chomnawang et al., 2005). Nevertheless, results are not easily comparable, since the part of the plant and the solvent used were not reported from the previous study.

Table 1. Contents of bioactive compounds in *G. mangostana* fruit rind extracts prepared by different extraction methods and their free radical-scavenging and antibacterial activities against *P. acne* and *S. epidermidis*.

Extraction method	Time (h)/ amount of solvent (L)	Yield of extract* (% dry weight)	α-Mangostin content in extract* (% w/w)	Total phenolics* (g GAE/100 g extract)	Total tannins* (g TAE/100 g extract)	DPPH-scavenging activity*, EC_{50} (μg/mL)	Susceptibility of bacteria to *G. mangostana* fruit rind extracts*, MIC/MBC (μg/mL)	
							P. acnes	*S. epidermidis*
ME 95	240/1.0	24.04 ± 1.35^{d}	13.32 ± 0.15^{a}	24.31 ± 0.08^{b}	39.52 ± 0.35^{d}	14.24 ± 0.69^{b}	15.63/31.25	15.63/31.25
PE 95	20/3.6	24.81 ± 0.04^{cd}	12.71 ± 0.22^{b}	22.44 ± 0.01^{c}	36.64 ± 0.35^{e}	15.07 ± 0.08^{c}	15.63/31.25	15.63/31.25
EMS 95	10/1.0	24.24 ± 0.48^{d}	11.47 ± 0.27^{c}	19.90 ± 0.04^{d}	27.30 ± 0.41^{g}	19.55 ± 0.06^{d}	15.63/15.63	15.63/31.25
UE 95	10/1.0	25.75 ± 0.30^{cd}	10.14 ± 0.15^{d}	20.72 ± 0.02^{d}	34.23 ± 0.79^{f}	19.25 ± 0.01^{d}	15.63/15.63	15.63/31.25
SE 95	15/0.4	26.60 ± 1.05^{bc}	13.51 ± 0.03^{a}	24.83 ± 0.01^{b}	41.94 ± 0.44^{c}	14.88 ± 0.06^{bc}	7.81/15.63	15.63/31.25
SE 70	15/0.4	27.90 ± 1.18^{b}	10.15 ± 0.74^{d}	26.88 ± 1.14^{a}	43.12 ± 0.21^{b}	13.39 ± 0.55^{a}	15.63/15.63	15.63/125.00
SE 50	15/0.4	31.52 ± 0.76^{a}	9.02 ± 0.11^{e}	26.96 ± 0.91^{a}	46.83 ± 0.24^{a}	12.84 ± 0.08^{a}	15.63/15.63	15.63/125.00

Different letters (a, b, c,...) in the same column indicate significantly different at $p < 0.05$ using one-way ANOVA; *each experiment was done in triplicate.

ME 95, maceration with 95% ethanol; PE 95, percolation with 95% ethanol; EMS 95, extraction with magnetic stirrer using 95% ethanol; UE 95, ultrasonic extraction with 95% ethanol; SE 95, Soxhlet extraction with 95% ethanol; SE 70, Soxhlet extraction with 70% ethanol; SE 50, Soxhlet extraction with 50% ethanol.

Conclusions

From this study, the Soxhlet extraction method of *G. mangostana* fruit rinds with 50% ethanol gave the highest yields of crude extract, total phenolic compounds, and total tannins. In addition, it promoted the highest free radical-scavenging activity with the DPPH radical. By the same extraction method, the 95% ethanol extract exhibited the strongest activity against *P. acnes*. Therefore, Soxhlet extraction with 50 and 95% ethanol is recommended as the extraction method for high antioxidant and anti-acne activities of *G. mangostana* fruit rinds, respectively.

Acknowledgement

This study is a part of PhD thesis of Mahidol University, Bangkok, Thailand and was granted by the University Research Fund.

References

Benthin B, Danz H, Hamburger M (1999): Pressurized liquid extraction of medicinal plants. *J Chromatogr A* 837: 211–219.

Chomnawang TM, Surassmo SV, Gritsanapan W (2005): Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria. *J Ethnopharmacol* 101: 330–333.

Fransworth NR, Bunyapraphatsara N (1992): *Thai Medicinal Plants, Recommended for Primary Health Care System*. Bangkok, Prachachon Company, pp. 160–162.

Goli AH, Barzegar M, Sahari MA (2005): Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. *Food Chem* 92: 521–525.

Gritsanapan W, Chulasiri M (1983): A preliminary study of antidiarrheal plants: I. Antibacterial activity. *Mahidol Univ J Pharm Sci* 10: 119–122.

Gu HF, Li CM, Xu YJ, Hu WF, Chen MH, Wan QH (2008): Structural features and antioxidant activity of tannin from persimmon pulp. *Food Res Int* 41: 208–217.

Hagerman AE, Butler LG (1978): Protein precipitation method for the quantitative determination of tannins. *J Agric Food Chem* 26: 809–812.

Iinuma M, Tosa H, Tanaka T, Asai F, Kobayashi Y, Shimano R, Miyauchi KI (1996): Antibacterial activity of xanthones from guttiferaeous plants against methicillin resistant *Staphylococcus aureus*. *J Pharm Pharmacol* 48: 861–865.

Kim KH, Tsao R, Yang R, Cui SW (2006): Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chem* 95: 466–473.

Kumar A, Baboota S, Agarwal SP, Ali J, Ahuja A (2008): Treatment of acne with special emphasis on herbal remedies. *Expert Rev Dermatol* 3: 111–122.

Moongkarndi P, Kosem N, Kaslungka S, Luanratana O, Pongpan N, Neungton N (2004): Antiproliferation, antioxidation and induction of apoptosis by *Garcinia mangostana* (mangosteen) on SKBR3 human breast cancer cell line. *J Ethnopharmacol* 90: 161–166.

Nakatani K, Atsumi M, Arakawa T, Oosawa K, Shimura S, Nakahata N, Ohizumi Y (2002): Inhibitions of histamine release and prostaglandin E2 synthesis by mangosteen, a Thai medicinal plant. *Biol Pharm Bull* 25: 1137–1141.

NCCLS (2008): *Performance Standards for Antimicrobial Susceptibility Testing*; Ninth Informational Supplement. NCCLS document M100-S9.Wayne, PA, National Committee for Clinical Laboratory Standards.

Ong ES (2004): Extraction methods and chemical standardization of botanicals and herbal preparations. *J Chromatogr B* 812: 23–33.

Walker EB (2007): HPLC analysis of selected xanthones in mangosteen fruit. *J Sep Sci* 30: 1229–1234.

Yu L, Zhao M, Yang B, Qiangzhong Z, Jiang Y (2007): Phenolics from hull of *Garcinia mangostana* fruit and their antioxidant activities. *Food Chem* 104: 176–181.