Antimicrobial and quorum sensing inhibitory activities of the pericarp of *Garcinia mangostana*

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Abstract: A prenylated xanthone, α-mangostin was separated from the alcoholic extract of *Garcinia mangostana* pericarp. Its structure was established by different spectroscopic analysis. The total methanolic extract (TME) and different fractions of *G. mangostana* pericarp as well as α-mangostin were assessed for their antimicrobial and quorum sensing inhibitory effects (QSI). The TME, $CHCl_3$ fraction, and α-mangostin exhibited strong activity against all tested strains. While, EtOAc, *n*-BuOH, and aqueous fractions showed moderate activity against some of the tested organisms. In addition TME, $CHCl_3$, EtOAc, and α-mangostin showed promising QSI, while *n*-BuOH and aqueous fractions showed moderate activity. Minimal inhibitory concentration (MIC) for TME, $CHCl_3$ fractions, and α-mangostin was also assessed.

Keywords: *Garcinia mangostina*, α-mangostin, antimicrobial, antiquorum sensing

INTRODUCTION

Diseases, which caused by bacteria, viruses, fungi, and parasites are an important cause of mortality and morbidity, in all regions of the world particularly in the developing countries (Teka *et al*., 2015). Bacteria and fungi resistance to antimicrobial agents has grown in the last decades (Otimenyin *et al*., 2008). The increased prevalence of their resistant is due to extensive use and misuse of antimicrobial agents in treatment of infectious diseases (Okeke *et al*., 2005). Thus, the current obtainable antimicrobial agents inadquate to control the infection of microbes (Cowan, 1999) and create major public health problems (Bax and Mullan, 2000; Alade and Irobi, 1993). Thus, many researchers have focused on the investigation of natural products as a source of antimicrobial agents (Barbour *et al*., 2004; Recio and Rios, 1989; Silver and Bostian, 1993). *Garcinia mangostana* L. (mangosteen, Clusiaceae) is tropical tree with dark purple fruits and leathery glabrous leaves (Mohamed *et al*., 2014; Liandhajani *et al*., 2013). It is mostly spread in Myanmar, India, Thailand, and Sri Lanka. Its fruits are eaten with a slightly acid, sweet taste and delightful aroma (Liandhajani *et al*., 2013). About 80 xanthones have been isolated from *G. mangostana* (Mohamed *et al*., 2014; Chin *et al*., 2008). The pericarps have been used as a remedy for various aliments as diarrhea, urinary bladder infections, gonorrhea, and skin rashes for more than one hundred years (Gutierrez-Orozco and Failla, 2013). A variety of biologically active compounds, such as prenylated xanthones, benzophenones, bioflavonoids, triterpenes, anthocyanins, tannins, and phenols were reported from *G. mangostana* pericarp (Mohamed *et al*., 2014; Al-Massarani *et al*., 2013; Shan *et al*., 2011; Nilar Nguyen *et al*., 2005; Williams *et al*., 2003). In the present work, a prenylated xanthone, α-mangostin was separated from *G. mangostana* pericarp. The antibacterial and antifungal activities of the TME, different fractions and α-mangostin have been evaluated against different pathogenic bacteria and fungi. In addition, their antiquorum sensing activity towards *Chromobacterium violaceum* CV026 was tested.

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MATERIALS AND METHODS

General experimental procedures

The UV spectrum was estimated by the double beam Perkin Elmer spectrophotometer (Model 550S, Perkin-Elmer, MA, USA). Bruker Ultrashield spectrometer was utilized for measuring NMR spectra (BioSpin GmbH 400 and 100 MHz, Bruker BioSpin, Massachusetts, USA). The chromatographic isolation and TLC were done on SiO_2 60 (0.04-0.063mm) and TLC pre-coated plates (silica gel 60 F_{254}, 0.2mm), respectively (Merck, Darmstadt, Germany). Ciprofloxacin, clotrimazole, and (+) catechin were obtained from Sigma-Aldrich.

Plant material

G. mangostana fruits were bought from a local market at Jeddah city in 2015. It was confirmed by Dr. Nahed Morad, Faculty of Science, King Abdulaziz University, Saudi Arabia. A voucher specimen (GM-11-2015) was archived at King Abdulaziz University, Department of Natural Products and Alternative Medicine herbarium.

Extraction and isolation

The powdered pericarp (120g) was extracted with methanol and concentrated to give a dark brown solid (13g). The residue was fractionated on vacuum chromatography using *n*-hexane, $CHCl_3$, EtOAc, *n*-BuOH, and H_2O. The fractions were evaporated

separately under vaccuum to yield *n*-hexane (0.8g), $CHCl_3$ (2.4g), EtOAc (1.6g), *n*-BuOH (3.1g), and aqueous (4.2g) fractions. The $CHCl_3$ fraction (2.4g) was subjected to normal phase vacuum liquid chromatography (VLC) using *n*-hexane:EtOAc as an eluent to get five subfractions: GM-C1 to GM-C5. Subfraction GM-C3 (270mg) was chromatographed over silica gel column (35g, 50 × 2cm) using *n*-hexane: EtOAc (95:5 to 75:25) to afford α-mangostin (21mg, yellow amorphous powder).

Microbial strains

Gram-positive bacteria: *Bacillus cereus* (AUMC No. B-5) and *Staphylococcus aureus* (AUMC No. B-54), Gram-negative bacteria*: Escherichia coli* (AUMC No. B-53), *Pseudomonas aeurginosa* (AUMC No. B-73), and *Serratia marscescens* (AUMC No. B-55) and fungi: *Trichophyton rubrum* (AUMC No. 1804), *Fusarium oxysporum* (AUMC No. 5119), *Candida albicans* (AUMC No. 418), *Geotrichium candidum* (AUMC No. 226), *Aspergillus flavus* (AUMC No. 1276), and *Scopulariopsis brevicaulis* (AUMC No. 729) were provided by the Mycology Center, Assiut University, Egypt (http://www.aun.edu.eg/aumc/Catalog.htm). *Chromobacterium violaceum* CV026 was kindly provided by the School of Pharmacy, Mississippi University, Mississippi, USA.

Culture media

All strains of bacteria were grown in Luriae Bertani (LB) media (Tryptone 1%, 0.5% Yeast extract (Bacto-agar, BD Difco), and 1.0% NaCl) and hardened with 1.5% agar. In the antifungal assay Saboured's media (BD Difco) was used against *C. albicans*.

Evaluation of the antibacterial activity

LB agar (20mL) at 50ºC was seeded with 20µL of 1 X 10^6 CFU/mL of 18 h culture of the tested strains. The agar was poured into plates (10cm in diameter) and left to solidify. Cork borer was utilized to make wells in agar. Tested extracts and isolated compound were dissolved in DMSO to give 10mg/mL and 1mg/mL, respectively. Aliquots (100µL) of pure organic extracts and compound were put in the wells. DMSO (negative control) and ciprofloxacin (antibacterial agent, 1mg/mL) were included. The compounds were permitted to diffuse for 2 h and incubated at 37ºC for 24h (Ibrahim *et al*., 2016; Bonev *et al*., 2008; Pearson *et al*., 1980). Then, Vernier caliper was used to measure the inhibition zones (table 1).

Evaluation of the antifungal activity

Melted Saboured`s agar (20mL) at 50ºC was seeded with 20 µL of 1 X 10^6 CFU/mL of 24 h culture of *C. albicans*. The extracts and α-mangos tin were resolved in DMSO to give 10mg/mL and 1mg/mL, respectively. 100µL of the organic extracts and the isolated compound were put in the wells (Mohamed *et al*., 2014, Holt, 1975). In addition, clotrimazole (1mg/mL) was used as antifungal drug. Plates were incubated at 37ºC for 48h. Then, the diameter of the inhibition zone was determined (table 1).

Evaluation of Antiquorum sensing activity

C. violaceum CV026 was grown in Luria-Bertani (LB) broth for 24-48 h at 28ºC. Cultures were justified to 0.5 McFarland standard (Ca. 1 X 10^6 CFU/mL). *C. violaceum* (50µL/plate) was inoculated in 20mL LB agar. The samples were solublized in DMSO to give 10mg/mL (extracts) and 1mg/mL (compound). 100µL of test solution were put in the wells. (+)-catechin (1mg/mL) and DMSO were included as positive and negative controls, respectively. The plates were incubated for 48 h at 28 ºC. The pigment inhibition around the wells was checked. A halo clear zone around the disk would be resulted due to bacterial growth inhibition. However, a turbid halo of pigmentless *C. violaceum* reporter strain indicated the quorum sensing inhibitory potential (Mohamed *et al*., 2014, McClean *et al*., 1997). The radii r1 and r2 in mm refered to the growth inhibition and both growth and pigment inhibition, respectively (table 1). The QS inhibition in mm was calculated using the following equation:

QS inhibition = r2 - r1

Determination of minimal inhibitory concentrations (MICs)

The MICs of the active fractions and α-mangostin were assessed using agar dilution assay for *B. cereus*, *S. aureus*, *C. albicans* and *E. coli*. Two fold serial dilutions of the tested samples were mixed in 0.5mL LB broth. 100 µL tested culture were added in the wells of agar and incubated at 37°C for 18 hr. MIC values were recorded as the anti-Log concentration at which there is no observed growth of the microorganism (table 2) (Mohamed *et al*., 2014).

RESULTS

The $CHCl_3$ fraction of the pericarp of *G. mangostana* was subjected to different chromatographic techniques to yield α-mangostin, which was identified using different spectroscopic techniques: UV, IR, ^1H and ^{13}C NMR experiments together with ESIMS data as well as comparing of the spectral data with literature (fig. 1) (Mohamed *et al*., 2014; Liandhajani *et al*., 2013). α-Mangostin was the major compound isolated from *G. mangostana*, enabling QSI activity and antimicrobial evaluation. Results of anti-bacterial and QSI activities are presented in table 1 for TME, *n*-hexane, $CHCl_3$, EtOAc, *n*-BuOH, aqueous fractions and α-mangostin against the tested bacterial and fungal strains. The $CHCl_3$ fraction exhibited erratic effect on the tested bacterial strains. The zone of inhibition produced by it on different bacterial strains ranging from 10 to 22mm. Moreover, TME showed strong activity towards *S. aureus, E. coli, B. cereus, A. flavus* and *E. Coli* with inhibition zone diameters of 14.2, 17.9, 15.8, 26.8 and 17.9, respectively. The EtOAc fraction showed promising activity towards *C. albicans, B. cereus, E. coli,* and *S. aureus* with inhibition

zone diameters of 15, 13, 11 and 12mm, respectively. The *n*-BuOH fraction displayed moderate activity against *B. cereus*, *E. coli* and *C. albicans* and no activity towards other tested microorganisms. While, the aqueous fraction showed activity towards *S. aureus* with inhibition zone diameter of 14mm and moderate activity towards *C. albicans* with inhibition zone diameter of 10mm. α-Mangostin showed potential antifungal and antibacterial activities against all the tested microorganisms with inhibition zone diameter ranging from 7 to 21 mm. The *n*-hexane fraction was not effective against most of tested bacterial strains.

The TME, $CHCl_3$ fractions and α-mangostin depicted versatile potential towards most of the tested pathogenic fungal and bacterial strains. So, they were selected for MIC values against *S. aureus*, *B. subtilis*, *E. coli*, and *C. albicans* (table 2). In general, the MIC values of the TME against the tested bacteria ranged from 280 to 535µg/mL and for $CHCl_3$ fraction from 510 to 270µg/mL, respectively. Moreover, α-mangostin showed potent activity against *S. aureus*, *B. subtilis*, *E. Coli* and *C. albicans* with MIC vaues from 315 to 170μg/mL.

DISCUSSION

Many pharmaceutical and health allied communities are focusing towards medicinal properties of plants, as the herbal formulations are usually safe with minimal undesirable effects when used in the appropriate therapeutic dosages (Verpoorte, 2012; Vashishtha, 2010). Unremitting development of resistance to existing and newer antibiotics is also responsible to spotlight over traditional claims of medicinal plants (Verpoorte, 2012; Vashishtha; 2010).

The present work was done to evaluate the antimicrobial activity and QSI effect of TME and different fractions of *G. mangostana* pericarp as well as α-mangostin against different pathogens. All the tested samples showed

Table 1: Results of antimicrobial and anti-quorum sensing activities.

Inhibition zone diameter (mm/Sample)										
Strains	TME	*n*-Hexane	$CHCl_3$	EtOAc	*n*-BuOH	Aqueous	*α*-Mangostin	Cipro.[a]	Clot.[b]	(+)-Catechin
S. aureus	17.5±0.09	5±0.02	19±0.21	12±0.07	3±0.00	14±0.45	18±0.26	24±0.33	-	-
B. Cereus	14.2±0.41	-	16±0.11	13±0.11	13±0.14	9±0.07	14±0.11	22±0.14	-	-
E. Coli	15.8±0.22	-	17±0.30	11±0.03	10±0.22	7±0.03	17±0.15	20±0.17	-	-
P. aeurginosa	-	-	11±0.19	3±0.00	-	-	10±0.22	18±0.11	-	-
S. marscescens	-	-	10±0.09	5±0.01	-	-	-	30±0.46	-	-
C. albicans	17.9±0.13	2±0.00	15±0.15	15±0.35	12±0.21	10±0.11	15±0.41	-	21±0.13	-
G. candidum	3.2±0.10	-	11±0.09	-	9±0.07	-	11±0.03	-	23±0.11	-
F. oxysporum	7.5±0.16	-	13±0. 19	-	-	-	7±0.07	-	18±0.09	-
A. flavus	26.8±0.29	-	22±0. 41	5±0.06	11±0.	-	21±0.31	-	29±0.57	-
S. brevicaulis	9.1±0.08	-	18±0.14	-	-	-	-	-	25±0.44	-
T. rubrum	2.4±0.00	-	10±0.09	-	-	-	14±0.21	-	26±0.19	-
C. violaceum	7±0.10	-	11±0.12	10±0.33	2±0.02	3±0.00	6±0.11	-	-	4.9±0.04

[a] Ciprofloxacin as antibacterial standard.[b] Clotrimazole as antifungal standard. Results are calculated after subtraction of DMSO activity. Not active (- inhibition zone\2 mm); weak activity (2-5 mm); moderate activity (9-10 mm); strong activity (>10 mm).

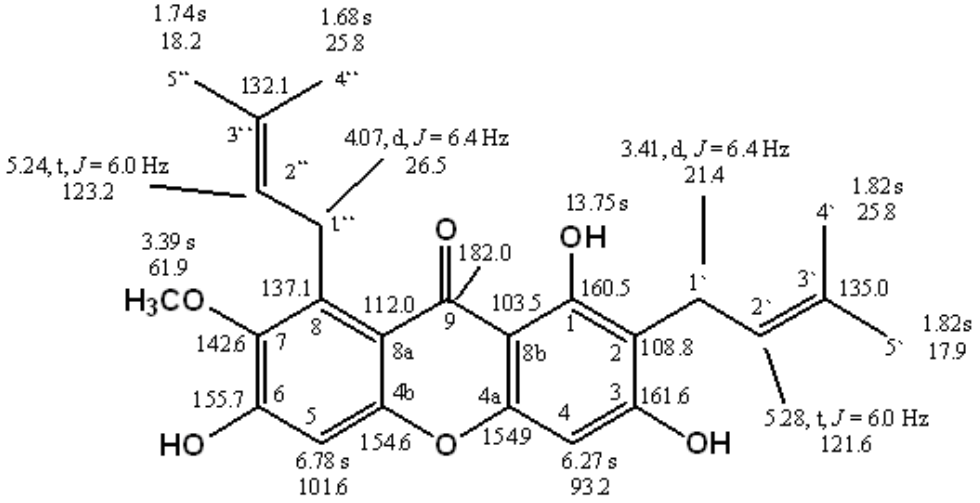

Fig.**1**: NMR data of α-mangostin in $CHCl_3$ (400 and 100 MHz).

varying degrees of activities on the tested microorganisms. It was evident that antimicrobial activity was more apparent in TME and $CHCl_3$ fraction.

The effect of the TME and $CHCl_3$ fraction of *G. mangostana* may be attributed to the presence of antibacterial phytochemicals such as xanthones, polyphenolics, and triterpenoids compounds (Mohamed *et al*., 2014; Al-Massarani *et al*., 2013; Shan *et al*., 2011; Nilar Nguyen *et al*., 2005; Williams *et al*., 2003). The production of such metabolites is one of the mechanisms of plants defense against microbial attacks. They act by perturbation of a microbe's cell membrane, an effect due to the polar groups on these compounds, which disrupt the phospholipid membrane (Chadwick *et al*., 2013; Cowan, 1999).

Table 2: Results of minimal inhibitory concentrations of TME, $CHCl_3$ and α-mangostin

Strains	Minimal Inhibitory Concentrations (µg/mL)		
	TME	$CHCl_3$	*α*-Mangostin
S. Aureus	490	355	315
B. Cereus	310	270	210
E. Coli	280	510	170
C. Albicans	535	325	260

Increasing extent of pathogenic resistance to drugs has encouraged the seeking for new anti-virulence drugs. Treatment with anti-quorum sensing could be a feasible way to weaken the virulence of bacteria without killing them. This may lessen the bacterial acquired-drug resistance (Adonizio *et al*., 2006). Here, we evaluated the QSI activity of the TME, different fractions, and isolated α-mangostin against reported strain *Chromobacterium violaceum* CV026 (McClean *et al*., 1997). The results showed that most of the tested fractions inhibited the production of violacein by *C. violaceum* (table 2). The TME and $CHCl_3$ fractions were the most QSI fractions against *C. violaceum*. On the other hand, α-mangostin exhibited strong QSI effect with disappearance of violet pigment.

In the present study, α-mangostin showed activity against *E. coli*, *P. aeruginosa*, and *C. albicans*. In contrast, Al-Massarani *et al*. (2013) indicated that *C. albicans*, *E. coli*, and *P. aeruginosa* were insusceptible to α-mangostin (Al-Massarani *et al*., 2013). Several researches have reported that α-mangostin in *G. mangostana* extract is effective towards methicillin-resistant *S. aureus* (MRSA) (Negi *et al*., 2008; Voravuthikunchai *et al*., 2005). MRSA is one of the most critical strains that are commonly found in places such as hospitals (Chomnawanga *et al*., 2009). Thus, the current results are significant, as pericarp extract could be developed as an alternative treatment for MRSA (Yin *et al*., 2013). The current results were in agreement with several reports in which *G. mangostana* exhibited strong activity against *S. aureus* and *B. cereus* due to the presence of xanthones (Yin *et al*., 2013; Lim *et al*., 2013; Geetha *et al*., 2011; Sundaram *et al*., 1983).

To the best of our knowledge, no data is avaliable in the literature regarding the QSI activity of *G. mangostana*. This study clearly illustrates that *G. mangostana* acts as a good source of antimicrobial agent against various bacterial pathogens tested and exhibited broad spectrum of antibacterial activity.

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