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New xanthones and cytotoxic constituents from *Garcinia mangostana* fruit hulls against human hepatocellular, breast, and colorectal cancer cell lines

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ABSTRACT

Ethnopharmacological relevance: Cancer has proceeded to surpass one of the most chronic illnesses to be the major cause of mortality in both the developing and developed world. *Garcinia mangostana* L. (mangosteen, family Guttiferae) known as the queen of fruits, is one of the most popular tropical fruits. It is cultivated in Southeast Asian countries: Malaysia, Indonesia, Sri Lanka, Burma, Thailand, and Philippines. Traditionally, numerous parts of *G. mangostana* have been utilized to treat various ailments such as abdominal pain, haemorrhoids, food allergies, arthritis, leucorrhoea, gonorrhea, diarrhea, dysentery, wound infection, suppuration, and chronic ulcer.

Aim of study: Although anticancer activity has been reported for the plant, the goal of the study was designed to isolate and characterize the active metabolites from *G. mangostana* and measure their cytotoxic properties. In this research, the mechanism of antiproliferative/cytotoxic effects of the tested compounds was investigated.

Materials and methods: The $CHCl_3$ fraction of the air-dried fruit hulls was repeatedly chromatographed on SiO_2, RP_{18}, Diaion HP-20, and polyamide columns to furnish fourteen compounds. The structures of these metabolites were proven by UV, IR, 1D, and 2D NMR measurements and HRESIMS. Additionally, the cytotoxic potential of all compounds was assessed against MCF-7, HCT-116, and HepG2 cell lines using SRB-U assay. Antiproliferative and cell cycle interference effects of potentially potent compounds were tested using DNA content flow cytometry. The mechanism of cell death induction was also studied using annexin-V/PI differential staining coupled with flow cytometry.

Results: The $CHCl_3$ soluble fraction afforded two new xanthones: mangostanaxanthones V (**1**) and VI (**2**), along with twelve known compounds: mangostanaxanthone IV (**3**), *β*-mangostin (**4**), garcinone E (**5**), *α*-mangostin (**6**), nor-mangostin (**7**), garcimangosone D (**8**), aromadendrin-8-C-*β*-D-glucopyranoside (**9**), 1,2,4,5-tetrahydroxybenzene (**10**), 2,4,3`-trihydroxybenzophenone-6-O-*β*-glucopyranoside (**11**), maclurin-6-O-*β*-D-glucopyranoside (rhodanthenone) (**12**), epicatechin (**13**), and 2,4,6,3`,5`-pentahydroxybenzophenone (**14**). Only compound **5** showed considerable antiproliferative/cytotoxic effects with IC_{50}'s ranging from 15.8 to 16.7 μM. Compounds **3**, **4**, and **6** showed moderate to weak cytotoxic effects (IC_{50}'s ranged from 45.7 to 116.4 μM). Using DNA content flow cytometry, it was found that only **5** induced significant cell cycle arrest at G_0/G_1-phase which is indicative of its antiproliferative properties. Additionally, by using annexin V-FITC/PI differential staining, **5** induced cells killing effect via the induction of apoptosis and necrosis in both $HepG_2$ and HCT116 cells. Compound **3** produce necrosis and apoptosis only in HCT116 cells. On contrary, **6** induced

Abbreviations: Akt1, serine-threonine protein kinase; Bax/Bcl-2, Bcl-2-associated X protein/B-cell lymphoma 2; DLD-1, colon cancer cell; DMEM, Dulbecco's Modified Eagle's Medium; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetate; ESIMS, electrospray ionization mass spectrometry; GIT, gastrointestinal tract; HCT-116, colorectal adenocarcinoma; HepG2, human hepatocellular carcinoma; HMBC, heteronuclear multiple bond correlation; HRESIMS, high resolution electron spray ionization mass spectroscopy; HSQC, heteronuclear single quantum coherence; IR, infra-red; K562, human erythromyeloblastoid leukemia; NMR, nuclear magnetic resonance; MCF-7, human breast adenocarcinoma; PBS, phosphate-buffered saline; RNAase A, Ribonuclease A; RP_{18}, reversed phase-18; Raji, human Burkitt's lymphoma; RPMI-1640, Roswell Park Memorial Institute 1640; SKBR3, human breast cancer cell; SK-MEL-28, human malignant melanoma; SiO_2, silica gel; SRB-U, sulforhodamine B; TCA, trichloroacetic acid; TLC, thin layer chromatography; UV, ultraviolet; VLC, vacuum liquid chromatography

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apoptosis and necrosis in $HepG_2$ cells and moderate necrosis in HCT116 cells.
Conclusion: Fourteen compounds were isolated from chloroform fraction of *G. mangostana* fruit hulls. Cytotoxic properties exhibited by the isolated xanthones from *G. mangostana* reinforce the avail of it as a natural cytotoxic agent against various cancers. These evidences could provide relevant bases for the scientific rationale of using *G. mangostana* in anti-cancer treatment.

1. Introduction

Chemoprevention is an arising strategy to prohibit, delay, lessen, or reverse the incidence and succession of cancer by administration of one or more synthetic or natural compounds (Castrellon and Glück, 2008; Bishayee et al., 2010). Medicinal plants have been widely utilized since outdated times as medicines for the treatment of diverse diseases and will continue to be served as valuable and major resources for discovering drug candidates. They are considered as main sources for isolating bioactive compounds due to their chemical diversity (Frutuoso et al., 2007). Various bioactive constituents obtained from medicinal plants have been evaluated for their tolerability and effectiveness in treating different types of cancer with little or no negative effects or contraindication (Ahmed et al., 2013; Abdallah et al., 2013; Oluyemi et al., 2007). Fruits are abundant in antioxidant phenolic acids, flavonoids, vitamins as well as tannins, carotenoids, stilbenes, and lignans (Alwerdt et al., 2008; Szajdek and Borowska, 2008), all of which have shown to exhibit significant chemopreventive potential and variable health benefits (Stoner et al., 2008; Seeram, 2010). Family Clusiaceae (Guttiferae) involves around 40 genera and 1200 species (Santa-Cecília et al., 2011). In this family, *Garcinia* (Rheedia) is the most prevalent genus, including nearly 400 species, which are vastly spread in tropical Asia, Australia, Africa, New Caledonia, Brazil, and Polynesia (Magadula, 2010). It is known to be a prosperous source for production of various metabolites, such as xanthones, anthocyanins, oligomeric proanthocyanins, benzophenones, triterpenoids, depsidones, and phloroglucinols (Fu et al., 2007; Magadula, 2010; Bui et al., 2014). These molecules exhibited a wide extent of bioactivities, such as anti-HIV, antimicrobial, antitumor, anti-leishmanial, antimicrobial, anti-inflammatory, antimalarial, advanced glycation end-products inhibitory, anti-quorum sensing, antioxidant, antihypertensive, and cytotoxic activities (Siridechakorn et al., 2012; Abdallah et al., 2016, 2017; Mohamed et al., 2014; Auranwiwat et al., 2014; Mahamodo et al., 2014). *G. mangostana* L. (mangosteen, the queen of fruits) is known for its unique pleasant aroma and sweet taste. It is the most economical and popularly consumed tropical fruits in the Southeast Asian countries (Khumsupan and Gritsanapan, 2014). Its pericarp (peel, rind, and hull) or ripe fruit has been applied for hundreds of years in Ayurvedic and Chinese medicines for treating various ailments. In Thailand, India, and China, the dried-powdered fruit hull is utilized as antimicrobial agent and anti-parasitic for treating dysentery (Burkill, 1994; Saralamp et al., 1996a), also externally to heal chronic ulcers, wounds, and suppurations (Farnsworth and Bunyapraphatsara, 1992). Its leaves and barks are known to possess potent anti-inflammatory potentials. Thus, the ointment produced from them is applied for treating hyperkeratosis, eczema, and psoriasis (Watson and Zibadi, 2013; Upaganlawar and Badole, 2012). Mangosteen is also utilized to prevent loss of essential nutrients from gastrointestinal tract (GIT) and dehydration in diarrhea due to its astringent effects (Morton, 1987). The decoction of rind is used for relieving cystitis, diarrhea, gleet, and gonorrhea. In Malaysia, the dried-sliced rind is employed as astringent (Lim, 2012). Moreover, leaves infusion incorporated with a little benzoin and unripe banana is applied to the wound of circumcision and administration of the root decoction is helpful to treat menstrual disorders (Morton, 1987; Khumsupan and Gritsanapan, 2014). In Thai folk medicine, the hulls have been utilized for treating leucorrhoea, skin infections, wounds, gonorrhea, and chronic ulcer and for the relief of diarrhea (Perry and Metzger, 1980; Mahabusarakam et al., 1987; Saralamp et al., 1996b). In Malaya and Philippines, a tea made from the rind is used to reduce fever (febrifuge) and to treat diarrhea, thrush, dysentery, aphthae, and various disorders of urinary system (Perry and Metzger, 1980; Lim 2012; Williams, 2012). A decoction of root is taken by women with dysmenorrhea. Also, the bark extract is used for treating amoebic dysentery. In Latin America and Caribbean, fruits tea is commonly utilized as a tonic for low energy states and fatigue (Obolskiy et al., 2009). Similar tea has been utilized by Brazilians as aid for digestion. Poultices of the fruit rind are used to treat parasitic skin infections in Venezuela (Perry and Metzger, 1980; Lim 2012; Williams, 2012). Also, mangosteen has been used against diabetes, atherosclerosis, depression, aging, and cancer (Moongkarndi et al., 2004a; O'Mathuna and Larimore, 2010; Boone, 2013). Our previous study of *G. mangostana* revealed the isolation of flavonoids, xanthones, and phenolics (Mohamed et al., 2014; Abdallah et al., 2016, 2017). In our continuing search for bioactive and structurally unique compounds from *G. mangostana*, two new xanthones: mangostanaxanthones V (**1**) and VI (**2**), along with 12 known compounds (**3**–**14**) were obtained from the $CHCl_3$ fraction of the fruit hulls (Fig. 1). Moreover, their cytotoxic activity was evaluated towards human breast adenocarcinoma (MCF-7), colorectal adenocarcinoma (HCT-116), and human hepatocellular carcinoma (HepG2) cell lines using sulforhodamine B (SRB-U) assay. In addition, the mechanism of antiproliferative/cytotoxic effects of the tested compounds was investigated.

2. Materials and methods

2.1. General

Ultraviolet (UV) spectra were obtained by a Hitachi-300 spectrophotometer (Kyoto, Japan). Infra-red (IR) spectra were assessed using a spectrophotometer Shimadzu Infrared-400. Electron spray ionization mass spectroscopy (ESIMS) spectra were performed on a LCQ-DECA spectrometer (Kyoto, Japan). LTQ Orbitrap was utilized to measure high resolution electron spray ionization mass spectroscopy (HRESIMS) (ThermoFinnigan, Bremen, Germany). Nuclear magnetic resonance (NMR) analyses were performed on Bruker Avance DRX-850 MHz (Bruker BioSpin, Billerica, MA, USA). For chromatographic separation, silica gel, Diaion HP-20, reversed phase-18 (RP_{18}), and polyamide 6 were utilized (Merck, Darmstadt, Germany). Thin layer chromatography (TLC) analysis was performed on pre-coated TLC plates with silica gel 60 F_{254} (Merck, Darmstadt, Germany). Compounds purification was achieved using LiChrolut RP_{18} tube (Merck, Darmstadt, Germany). All chemicals were obtained from Sigma-Aldrich (Taufkirchen, Germany).

2.2. Plant material

G. mangostana fruits were bought in December 2014 from a local market in Saudi Arabia. Its authentication was established by Dr. Emad Al-Sharif, Associate Professor of Plant Ecology, Faculty of Science & Arts, King Abdulaziz University and a voucher specimen (no. GM1424) was kept in the herbarium at Faculty of Pharmacy, KAU.

2.3. Extraction and isolation

The powdered fruit hulls (500 g) were extracted with MeOH (1 L ×