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SPECTROSCOPIC ANALYSIS ON THE PHENOLIC CONSTITUENTS FROM Garcinia mangostana

(Analisis Spektroskopi Terhadap Sebatian Fenolik dari *Garcinia mangostana*)

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Abstract

The *Garcinia mangostana* was belong to Clusiaceae family and usually found in subtropical countries. This plant was rich with phenolic compounds that showed good biological activities such as anti-tumor, anti-malarial, anti-inflammatory, anti-viral and anti-oxidant. Study on the *G. mangostana* has been done in order to isolate these phenolic compounds. The bark and pericarp of *G. mangostana* were extracted with hexane and ethyl acetate for 48 hours by using cold extraction methods. Then the isolation part was done by subjected the extracts to a series of column chromatography. The pure isolated compounds confirmed their structures by spectroscopic analysis such as 1 H and 13 C-NMR, HMBC, HMQC, DEPT and MS and comparison with literature data. Four prenylated xanthones, α -mangostin, β -mangostin, garcinone D (pericarp) and 9-hydroxycalabaxanthone (bark) were isolated from the *G. mangostana*.

Keywords: Garcinia mangostana, nuclear magnetic resonance, phenolic, xanthones

Abstrak

Garcinia mangostana adalah dari famili Clusiaceae dan biasanya dijumpai di negara-negara subtropika. Pokok ini kaya dengan sebatian fenolik yang menunjukkan aktiviti biologikal yang baik seperti anti-tumor, anti-malaria, anti keradangan, anti-viral dan anti-oksida. Kajian terhadap *G. mangostana* telah dijalankan untuk memisahkan sebatian-sebatian fenolik ini. Kulit buah dan perikap dari *G. mangostana* diekstrak dengan heksana dan etil asetat selama 48 jam dengan menggunakan kaedah pengekstrakan sejuk. Bahagian pemisahan dilakukan dengan meletakkan ekstrak ke dalam beberapa siri turus kromatografi. Sebatian yang telah diasingkan disahkan strukturnya dengan analisis spektroskopi seperti ¹H dan ¹³C-NMR, HMBC, HMQC, DEPT dan MS dan perbandingan dengan data terdahulu. Empat isoprenil xanthon, α-mangostin, β-mangostin, garcinon D (perikap) dan 9-hidroksicalabaxanthon (kulit) telah berjaya diasingkan dari pokok *G. mangostana*.

Kata kunci: Garcinia mangostana, resonan magnetic nukleus, fenolik, xanthon

Introduction

Garcinia mangostana L. which also known as mangosteen was belongs to Clusiaceae family is a slow-growing tree that usually can be found in tropical and subtropical countries of South East Asia, West and East Africa, and Central and South America [1, 2]. This plant has leathery glabrous leaves and dark purple to red-purple fruits with white, soft and juicy edible pulp with a slightly acid and sweet flavor and a pleasant aroma [3]. Some part of this tree has

been used in traditional remedy. Previous work showed the pericarp of this species is used for the treatment of skin infections, diarrhea, dysentery, gonorrhea and chronic ulcer [4-7]. Previous research on the phytochemical of G. mangostana had shown its rich source of secondary metabolites such as oxygenated and prenylated xanthones, triterpenoids and benzophenones [8, 9]. Some of these compounds gave significant biological activities such as antimicrobial, anti-oxidant, anti-tumor, anti-malarial and anti-inflammatory [10, 11]. In our present work on the pericarp and bark of G. mangostana collected from Segamat, Johor Bahru, afforded four prenylated xanthones, α -mangostin (1), β -mangostin (2), garcinone D (3) and 9-hydroxycalabaxanthone (4). All these compounds were identified by spectroscopic analysis and comparison with the previous data from literature.

Material and Methods

Plant material

The G. mangostana Linn. (Voucher specimen - RJ 078) pericarps and bark were collected from Segamat, Johor Malaysia.

The general experimental procedure

¹H and ¹³C Nuclear Magnetic Resonance (NMR) were recorded on JEOL FTNMR 400 MHz transformed spectrometer in CDCl₃, or acetone-D₆ and using tetramethylsilane (TMS) as the internal standard at 400 MHz for ¹H NMR and 100 MHz for ¹³C respectively. Melting point was determined by using OptiMelt Automated Melting Point System. Mass spectra were recorded on Agilent Technologies GCMS 7890B spectrometer and Agilent Technologies 5977A for mass spectrometer detector. An infrared spectrum was determined in ATM on a Perkin – Elmer SPECTRUM 100 FTIR – Spectrometer. Ultra violet spectra were recorded on Shidmadzu UV – 1800 UV-spectrometer.

Extraction

The dried and powdered pericarps (300 g) and bark of *G. mangostana* (400 g) were extracted with hexane and ethyl acetate, respectively by cold soaking method for 48 hours at room temperature. These extracts were then concentrated by using rotary evaporator to give 2% of hexane crude extract from pericarps and 1.25% of ethyl acetate crude extract from bark of *Garcinia mangostana*, respectively.

Isolation and purification of compounds

Approximately 4.0 g of hexane crude extract from the pericarps was subjected to a series of various column chromatographic techniques. The isocratic mobile phase of hexane-ethyl acetate (9:1) was performed and gave 20 fractions. Fraction 3 gave a yellow amorphous powder of α - mangostin (20 mg) (1). Meanwhile, fraction 5 and 6 were pooled together and rechromatographed by using 100% chloroform to give β - mangostin (20 mg) (2) as yellow needle crystals. Further purification on the fraction 15 *via* Sephadex LH-20 with methanol as eluent yielded another prenylated xanthone, garcinone D (10 mg) (3).

Three (3.0) g of the ethyl acetate bark extract was separated using gravity column chromatography with petroleum ether: ethyl acetate (5:5) and chloroform: methanol (9:1) as eluents which afforded 20 crude fractions. Crude fractions 4 to 9 were pooled together and re-subjected to a mini column chromatography by using hexane: ethyl acetate (1:1) as eluting solvents to give another 10 fractions. Fractions 3 was gave a single spot on the thin layer chromatography plate, thus confirmed the isolation of another xanthone, 9 - hydroxycalabaxanthone (15 mg) (4) as a yellow gum.

Results and Discussion

 α -mangostin (1) was isolated from the pericarp of *G. mangostana* as a yellow amorphous powder with melting point of 180 – 182 °C [12]. The Electron Impact Mass Spectroscopy (EIMS) analysis gave a molecular ion peak at m/z 410 which correspond to the molecular formula $C_{24}H_{26}O_6$. The Infrared (IR) spectrum showed strong absorption at 3422 cm⁻¹ indicating the presence of a phenolic group in (1). On the other hand, absorptions at 1610 and 1282 cm⁻¹ were due to the presence of a chelated carbonyl group in the middle ring and a methoxyl group, respectively. Maximum absorptions at 214.5, 243.0 and 317.0 nm in the ultra violet analysis suggested compound (1) has a xanthone skeleton [13]. The existence of phenolic nature on this compound was confirmed when it gave positive result with alcoholic ferric chloride solution. The ¹H NMR spectrum of (1) showed singlet signal of one proton at δ

6.25 and δ 6.72 which assigned to the two isolated aromatic protons at positions C - 4 and C - 5, respectively. The singlet which resonates at δ 3.78 in the ¹H-NMR spectrum was assigned to the methoxyl group at C-7. The presences of two 3-methylbut-2-enyl groups were validated by the following characteristic signals. Two doublet signals of two protons at δ 3.37 (J = 7.3 Hz) and δ 4.10 (J = 7.3 Hz) were assigned to benzylic methylene groups at C - 16 and C - 11. Meanwhile, a triplet at δ 5.26 which integrated to two protons was attributed the vinylic protons at C - 12 and C - 17. Four singlet signals at δ 1.68, 1.83, 1.71 and 1.82, were assigned to H - 14, H - 15, H - 19 and H-20, respectively. Finally, the singlet signal at δ 13.72 in ¹H NMR indicating the presence of hydroxyl moiety in (1).

The 13 C NMR analysis showed a total of twenty four carbon signals whereas the very downfield signal at δ 181.8 was assigned to carbonyl carbon in (1). Meanwhile, the methoxy carbon was found to resonate at δ 61.2. In the Heteronuclear Multiple Bond Correlation (HMBC) spectrum analysis, the position for the methoxy group was assigned to be at C-7 (δ 142.7). Meanwhile, two prenyl moieties for (1) were confirmed to be at C-2 and C-8 positions. This is shown by the correlations between the methylene signal at δ 4.10 (C-11, δ 26.3) and with C-7 at δ 142.7, C-8 at δ 137.2, C-8a at δ 111.7, C-12 at δ 122.1 and C-13 at δ 131.7. Meanwhile, the signal at δ 3.37 (C-16, δ 21.3) was correlated to C-1 at δ 160.2, C-2 at δ 109.7, C-17 at δ 123.4 and C-18 at δ 132.6. Based on the comparison with the previous work that have been done by Arunrattiyakorn et al. [14] compound (1) was identified as 1,3,6-trihydroxy-7- methoxy-2,8-di (3-methylbut-2-enyl)-xanthone or also known as α -mangostin.

Further purification through a series of mini column chromatography on the hexane extract of pericarp of G. mangostana has led to the isolation of compound (2) as a yellow needle crystals with a melting point of 170 - 173 °C [12]. The EIMS spectrum gave a molecular ion $[M]^+$ at 424 which validated to the molecular formula, $C_{25}H_{28}O_6$. The Infrared (IR) spectrum gave strong absorption at 1648 and 3400 cm⁻¹ were due to a highly chelated carbonyl and hydroxyl group. The maximum UV absorptions at 213.5, 245.0, 259.0 and 315.5 nm indicated compound (2) has a xanthone structure [13]. The phenolic nature of compound (2) was confirmed when it gave a blue greyish onto the Thin layer Chromatography (TLC) that have been stained with ferum (III) chloride solution. The ¹H NMR spectrum showed the presence of one chelated hydroxyl group at δ 13.42 as a singlet signal. Moreover two doublets of two protons at δ 3.35 (J = 7.3 Hz, H - 11) and δ 4.10 (J = 7.3 Hz, H - 16), a triplet with integrated of two protons at δ 5.25 (J = 7.3 Hz, H - 12 and H - 17) and four singlets at δ 1.69 (3H, H - 14), 1.80 (3H, H - 15), 1.69 (3H, H - 19) and 1.83 (3H, H - 20) were attributed to the presence of two isoprene moieties in (2). On the other hand, two singlet signals of one proton that resonating at δ 6.34 and 6.82 were assigned to H - 4 and H - 5, respectively. Meanwhile, the presence of two methoxy groups were confirmed with two singlet signals at δ 3.80 (3H, 3-OMe) and 3.90 (3H, 7-OMe). The presence of another one more methoxy group in (2) makes it different with α -mangostin (1).

The 13 C NMR spectrum showed a total of twenty five resonating carbon signals which correspond to molecular formula, $C_{25}H_{28}O_6$. A very downfield signal at δ 181.9 was duly to carbonyl group in the middle ring in (2). Meanwhile, two methoxy carbon signals were exhibited at δ 55.8 and δ 62.1, respectively. Furthermore, Distortionless Enhancement Polarization Transfer (DEPT) experiments indicating this compound consisted of six methyls, two methylenes and four methines and thirteen quaternary carbons. Based on the spectral data above and comparison with previous work done by Yates and Bhat [12] compound (2) was named as β -mangostin [1,6-dihydroxy-3,7-dimethoxy-2,8-di(3-methylbut-2-enyl)-xanthone].

Compound (3) was obtained as a yellow gum from the polar fraction of hexane crude extract of G. mangostana pericarp. This compound reacted positively with alcoholic FeCl₃ solution, hence suggested this compound have at least one phenolic group. The UV spectrum of (3) exhibited characteristic absorption bands of an oxygenated xanthone at 244.5 and 318.0 nm [13]. The IR spectrum showed absorptions at 3210 and 1646 cm⁻¹ which indicated the presence of a chelated hydroxyl and conjugated carbonyl group in this compound. The molecular formula for this compound, $C_{24}H_{28}O_7$ was confirmed when EIMS spectrum showed molecular ion peak at m/z 428. The fragment ion peak at m/z 339 was due to the losses of one 3-hydroxy-3-methylbutanyl side chain and methoxy group, respectively. The ¹H NMR spectrum showed two singlet signals that resonated at δ 13.82 (OH - 1) and 3.86 (s, 3H) duly to the presence of chelated hydroxyl and methoxyl moieties in (41). Meanwhile, two singlets at δ 1.79

(3H, s, H - 14) and δ 1.65 (3H, s, H - 15), a doublet at δ 3.32 (2H, J = 7.2 Hz, H - 11) and a triplet at δ 5.28 (1H, J = 7.2 Hz, H - 12) exhibited the presence of a 3,3-dimethylallyl substituent in (3). Furthermore, the presence of 3-hydroxy-3-methylbutyl side chain was confirmed by a singlet signal of six protons at δ 1.34 (6H, s, H-19, H - 20) and a pair of multiplets of two protons at δ 1.75 (2H, m, H - 17) and δ 3.46 (2H, m, H - 16). The remaining two singlet signals at δ 6.42 and 6.82 were assigned to two aromatic protons H-4 and H-5, respectively.

The 13 C NMR spectrum showed a total of 24 carbon signals whereas the very downfield signal at δ 182.0 (C - 9) belong to conjugated carbonyl carbon in (3). Moreover, this compound also consists of ten substituted aromatic carbons [δ 160.9 (C - 1), δ 110.2 (C - 8a), δ 162.2 (C - 3), δ 154.9 (C - 10a), δ 155.5 (C - 4a), δ 143.6 (C - 7), δ 139.2 (C - 8), δ 111.2 (C - 2), δ 102.8 (C - 9a) and δ 156.6 (C - 6)], of which six were oxygenated. Based on the data that reported earlier by Bennet et al. [15], compound (3) was identified as garcinone D [1,3,6-trihydroxy-8-(3-hydroxyl-3-methylbutyl)-7-methoxy-2-(3-methylbut-2-enyl)-xanthone].

Compound (4) was isolated as a yellow gum from the ethyl acetate extract of bark of G. mangostana. This compound reacted positively with alcoholic ferric chloride solution, thus confirmed its phenolic character. The EIMS spectrum gave a molecular ion of 408 which related to its molecular formula of $C_{24}H_{24}O_6$. The fragment ion at 295 in EIMS spectrum was due to losses of isoprene and methoxy moieties. The ultra violet (UV) absorptions at 319.6 and 284.8 nm show that (4) was an oxygenated xanthone [13]. The IR spectrum indicated strong bands for chelated hydroxyl and conjugated carbonyl group at 3234 cm⁻¹ and 1607 cm⁻¹, respectively. The ^{1}H NMR experiment showed a singlet signal at δ 13.7 (1H, s) which referred to hydroxyl group in (4). This hydroxyl group can form an intermolecular hydrogen bonding with oxygen from the carbonyl group, thus make it shifted more to downfield region in proton NMR spectrum.

Meanwhile, a pair of doublet of one proton at δ 6.74 (d, 1H, J = 10.1 Hz, H - 4) and 5.56 (d, 1H, J = 10.1 Hz, H - 3) and one singlet of six protons at δ 1.46 (s, 6H, H - 14 and H - 15) indicated the presence of a 2,2-dimethyl chromene ring substitution at the xanthone ring, with the chromene double bond ortho to the C-1 hydroxyl. Moreover, a triplet signals at δ 5.26 (t, 1H, J = 6.4 Hz, H - 17), a doublet at δ 4.10 (d, 2H, J = 6.4 Hz, H - 16), and two singlets at δ 1.83 (s, 3H, H - 19) and δ 1.69 (s, 3H, H - 20) were suggested to the existence of isoprene unit which was ortho to the C-9 carbonyl. Two isolated aromatic protons in (4) were assigned at δ 6.84 (s, 1H, H - 5) and δ 6.25 (s, 1H, H - 4), respectively. Finally, a singlet signal of three protons that resonates at δ 3.81 was attributed to the methoxy group in (4). The ¹³C NMR spectrum was provided a total of twenty four carbons which verified the molecular formula $C_{24}H_{24}O_6$ of (4). The very downfield signal at of δ 181.9 was assigned to the carbonyl group at C-9. Compound (4) consists of five methyls, one methylene, five methines and thirteen quaternary carbons. Ten out of thirteen quaternary carbons in (4) were aromatic carbons. In HMBC analysis, the methylene proton (H-16) gave cross peak to C-7 (δ 142.6) and C-8 (δ 136.9), thus suggesting the location of the prenyl moiety to be at C-8. On the other hand, the HMBC spectrum also showed that the methoxy group was attached to C-7. Comparison of these data with previous reported by Sen et al. [16] suggested that (4) was elucidate as 9-hydroxycalabaxanthone (1,6-dihydroxy-7-methoxy-8-(3-methylbut-2-enyl)- 6',6'-dimethylpyrano-[2'3':3,2]-xanthone).

Spectral data

α-mangostin (1) 180 - 182°C (Lit. 182 - 183°C, Yates, 1968); UV λ_{max} (EtOH) (log ε): 317.6 (1.359), 242.6 (1.964), 281.6 (0.529), 223.8 (1.351); IR v_{max} cm⁻¹: 3381.42 (broad OH), 2910.64 (C - H stretching), 1598.67 (aromatic C = O stretching), 1044.06 (C-O strong band); ¹H NMR (400 MHz, CD₃Cl): δ 13.72 (s, 1H, OH - 1), δ 6.72 (s, 1H, H-5), δ 6.25 (s, 1H, H - 4), δ 5.26 (t, J = 7.3 Hz, 2H, H - 12, H - 17), δ 4.10 (d, J = 7.3 Hz, 2H, H - 16), δ 3.78 (s, 3H, 7 - OMe), δ 3.37 (d, J = 7.3 Hz, 2H, H - 11), δ 1.83 (s, 3H, H - 15), δ 1.82 (s, 3H, H - 20), δ 1.71 (s, 3H, H - 19) δ 1.68 (s, 3H, H - 14); ¹³C NMR (100 MHz, CD₃Cl): δ 181.8 (C - 9), δ 161.6 (C - 3), δ 160.2 (C - 1), δ 155.4 (C - 6), δ 155.2 (C - 10a), δ 154.8 (C - 4a), δ 142.7 (C - 7), δ 137.2 (C - 8), δ 132.6 (C - 18), δ 131.7 (C - 13), δ 123.4 (C - 12), δ 122.1 (C - 17), δ 111.7 (C - 8a), δ 109.7 (C - 2), δ 103.1 (C - 9a), δ 101.6 (C - 5), δ 92.4 (C - 4), δ 61.2 (7 - OMe), δ 26.3 (C - 16), δ 25.7 (C - 19), δ 25.7 (C - 14), δ 21.3 (C - 11), δ 18.1 (C - 15), δ 17.7 (C - 20)

β-mangostin (2) 160 - 165°C (Lit. 162 - 163°C, [12]); UV λ_{max} (EtOH) (log ε): 316.8 (1.155), 243.6 (1.651), 281 (0.259), 224 (1.054); IR v_{max} cm⁻¹: 3241.54 (broad OH), 2912.13 (C - H stretching), 1607.23 (C = O stretching); ¹H NMR (400 MHz, CDCl₃): δ 13.42 (s, 1H, OH - 1), δ 6.82 (s, 1H, H - 5), δ 6.34 (s, 1H, H - 4), δ 5.25 (t, J = 7.3 Hz, 2H, H - 12, H - 17), δ 4.10 (d, J = 7.3 Hz, 2H, H - 11), δ 3.90 (s, 3H, 7 - OMe), δ 3.80 (s, 3H, 3 - OMe), δ 3.35 (d, J = 7.3 Hz, 2H, H - 16), δ 1.83 (s, 3H, H - 20), δ 1.80 (s, 3H, H - 15), δ 1.69 (s, 6H, H - 14, H - 19); ¹³C NMR (100 MHz, CDCl₃): δ 181.9 (C - 9), δ 163.4 (C - 3), δ 159.7 (C - 1), δ 155.6 (C - 6), δ 155.2 (C - 10a), δ 154.4 (C - 4a), δ 142.5 (C - 7), δ 137.0 (C - 8), δ 132.1 (C - 18), δ 131.7 (C - 13), δ 123.2 (C - 17), δ 122.3 (C - 12), δ 112.3 (C - 8a), δ 111.4 (C - 2), δ 103.8 (C - 9a), δ 101.4 (C - 5), δ 88.8 (C - 4), δ 62.1 (7 - OMe), δ 55.8 (3 - OMe), δ 26.5 (C - 11), δ 25.8 (C - 14), δ 25.8 (C - 19), δ 21.3 (C - 16), δ 18.2 (C - 20), δ 17.7 (C - 15)

Garcinone D (3) yellow gum; UV λ_{max} (EtOH) (log ϵ): 244.5 (1.2), 318.0 (0.8); IR ν_{max} cm⁻¹: 3210 (broad OH), 2856 (C - H stretching), 1646 (C = O), 1610 (C = C); ¹H NMR (400 MHz, Acetone - D₆): δ 13.82 (s, 1H, OH - 1), δ 6.82 (s, 1H, H - 5), δ 6.42 (s, H, H - 4), δ 5.28 (bt, J = 7.2 Hz, 1H, H - 12), δ 3.86 (s, 3H, 7 - OMe), δ 3.46 (m, 2H, H - 16), δ 3.32 (d, J = 7.2 Hz, 2H, H - 11), δ 1.79 (s, 3H, H - 14), δ 1.75 (m, 2H, H - 17), δ 1.65 (s, 3H, H - 15), δ 1.34 (s, 6H, H - 19, H - 20); ¹³C NMR (100 MHz, Acetone - D₆): δ 182.0 (C - 9), δ 162.2 (C - 3), δ 160.9 (C - 1), δ 156.6 (C - 6), δ 155.5 (C - 4a), δ 154.9 (C - 10a), δ 143.6 (C - 7), δ 139.2 (C - 8), δ 130.5 (C - 13), δ 122.7 (C - 12), δ 111.2 (C - 8a), δ 110.2 (C - 2), δ 102.8 (C - 9a), δ 101.7 (C - 5), δ 92.3 (C - 4), δ 69.9 (C - 18), δ 60.8 (7 - OMe), δ 44.8 (C - 17), δ 29.3 (C - 19 & C - 20), δ 25.1 (C - 15), δ 22.4 (C - 16), δ 21.2 (C - 11), δ 17.1 (C - 14)

9-hydroxycalabaxanthone (4) yellow gum; UV λ_{max} (EtOH) (log ϵ):319.6 (2.570), 284.8 (0.993)IR v_{max} cm⁻¹:3234.06 (broad OH), 2913.50 (C - H stretching), 1607.01 (C = O stretching), 582.46 (aromatic C = C stretching); ¹H NMR (400 MHz, CDCl₃): δ 13.7 (s, 1H, OH - 5), δ 6.84 (s, 1H, H - 5), δ 6.74 (d, J = 10.1 Hz, 1H, H - 11), δ 6.25 (s, 1H, H - 4), δ 5.56 (d, J = 10.1 Hz, 1H, H - 12), δ 5.26 (t, J = 6.4 Hz, 1H, H - 17), δ 4.10 (d, J = 6.4 Hz, 2H, H - 16), δ 3.81 (s, 3H, 7 - OMe), δ 1.83 (s, 3H, H - 20), δ 1.69 (s, 3H, H - 19), δ 1.46 (s, 6H, H - 14 & H - 15); ¹³H NMR (100 MHz, CDCl₃): δ 181.9 (C - 9), δ 159.9 (C - 3), δ 157.9 (C - 1), δ 156.3 (C - 4a), δ 155.7 (C - 10a), δ 154.6 (C - 6), δ 142.6 (C - 7), δ 136.9 (C - 8), δ 132.2 (C - 18), δ 127.2 (C - 12), δ 123.1 (C - 17), δ 115.7 (C - 11), δ 112.2 (C - 8a), δ 104.5 (C - 2), δ 103.7 (C - 9a), δ 101.7 (C - 5), δ 94.1 (C - 4), δ 77.9 (C - 13), δ 62.0 (7 - OMe), δ 28.3 (C - 14), δ 28.3 (C - 15), δ 26.5 (C - 16), δ 25.8 (C - 19), δ 18.2 (C - 20).

Shaari et al: SPECTROSCOPIC ANALYSIS ON THE PHENOLIC CONSTITUENTS FROM Garcinia mangostana

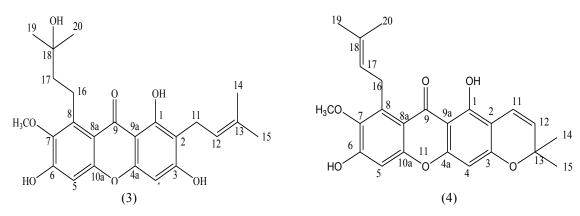


Figure 1. Isolated compounds from pericarps (1-3) and bark (4) of G. mangostana

Conclusion

The pure isolated compounds confirmed their structures by spectroscopic analysis such as 1 H and 13 C-NMR, HMBC, HMQC, DEPT and MS and comparison with literature data. Four prenylated xanthones such as α -mangostin, β -mangostin, garcinone D (pericarp) and 9-hydroxycalabaxanthone (bark) were isolated from the *G. mangostana*.

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