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Geranyl chalcone derivatives with antifungal and radical scavenging properties from the leaves of *Artocarpus nobilis*

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Abstract

Antifungal activity guided fractionation of the *n*-butanol extract from the methanol extract of the leaves of *Artocarpus nobilis* furnished 2′,4′,4-trihydroxy-3′-geranylchalcone (**1**), 2′,4′,4-trihydroxy-3′-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]chalcone (**2**), 2′,4′,4-trihydroxy-3′-[2-hydroxy-7-methyl-3-methylene-6-octaenyl]chalcone (**3**), 2′,3,4,4′-tetrahydroxy-3′-geranylchalcone (**4**), 2′,3,4,4′-tetrahydroxy-3′-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]chalcone (**5**). The chalcones **3** and **5** are new natural products whereas **1** and **2** are reported first time from the family Moraceae. All these compounds showed good fungicidal activity against *Cladosporium cladosporioides* and high radical scavenging activity towards the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical in TLC bio-autography method.

Keywords: *Artocarpus nobilis*; Moraceae; Chalcones; Antifungal; *Cladosporium cladosporioides*; Radical scavenging; DPPH

1. Introduction

In a continuation of our research work on search for bioactive compounds from Sri Lankan plants the present investigation was carried out on *Artocarpus nobilis* Thw. of the family Moraceae. *A. nobilis* is a tree of moderate size and the only endemic species of the genus *Artocarpus* found in Sri Lanka. Several phenolic compounds have been reported from the stem bark of the plant (Pavanasasivam and Sultanbawa, 1973; Pavanasasivam et al., 1974; Kumar et al., 1977; Sultanbawa and Surendrakumar, 1989). In this paper, we report the isolation and structure elucidation of five chalcone derivatives including two new with antifungal activity against *Cladosporium cladosporioides* and radical scavenging properties towards 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical from the leaves of *A. nobilis*.

2. Results and discussion

The leaves of *A. nobilis* were defatted with *n*-hexane and extracted with methanol. The preliminary investigation of the methanol extract and the *n*-butanol extract from the methanol extract of the leaves of *A. nobilis* showed positive response in antifungal bioassay against *Cladosporium cladosporioides* by TLC bio-autography method (Homans and Fuchs, 1970). Antifungal activity guided fractionation of the *n*-butanol extract by a combination of chromatography over silica gel, RP-18 silica gel, sephadex LH-20 and reversed phase HPLC afforded compounds **1**, **2**, **3**, **4** and **5** (Fig. 1).

The absorption maxima observed at 208, 230, 373 nm in the UV spectra of compound **1**–**5** gave the first clue that all these compounds belongs to the same group of chalcones. Compound **1** was assigned the molecular formula $C_{25}H_{28}O_4$ [FABMS(−): *m*/*z* 391[M–H]$^-$. Detailed analysis of ^{1}H and ^{13}C NMR spectral data of **1** including DEPT, H–H COSY, HMQC and HMBC furnished evidence that the compound **1** to be 2′,4,4′-tetrahydroxy-3′-geranylchalcone. Compound **2** was

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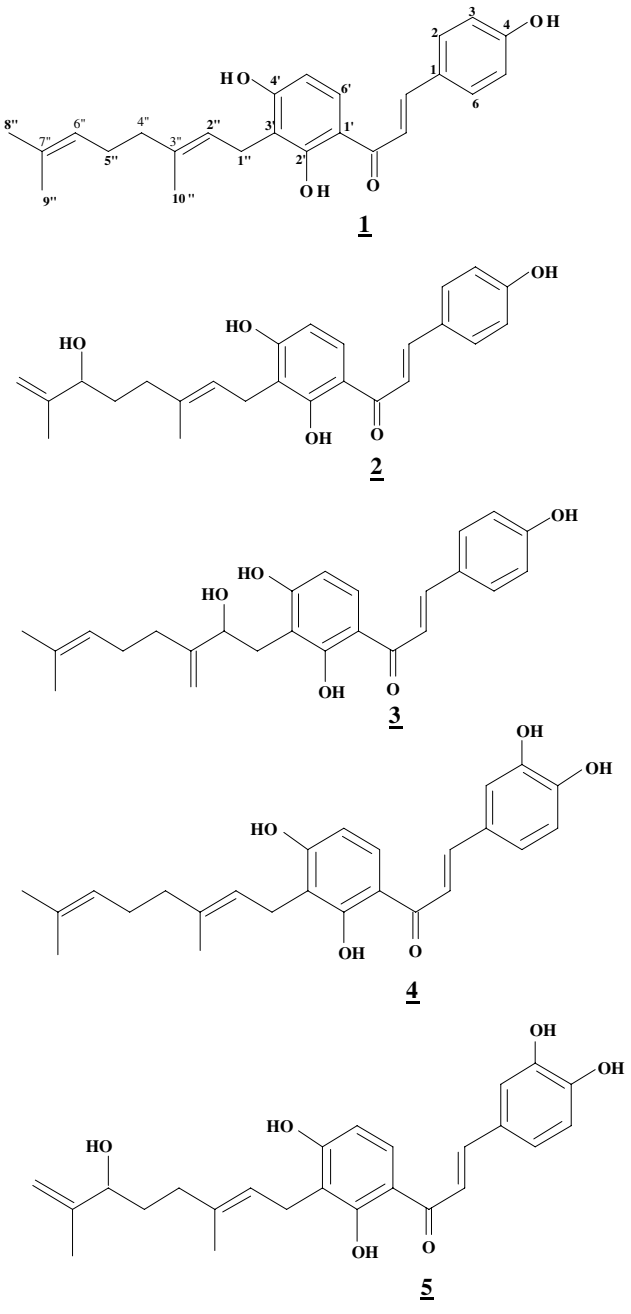

Fig. 1. Structures of compounds **1**–**5**.

assigned the molecular formula $C_{25}H_{28}O_5$ (EIMS: *m/z* 408), 16 mass units more than compound **1**. Comparison of the ^1H and ^{13}C NMR data of **2** with those of **1** indicated that **2** has the same 2′,4,4′-trihydroxychalcone core as **1**, but the C-3′ geranyl side chain is modified. Further analysis of ^1H NMR and EIMS indicated that the compound **2** to be 2′,4,4′-trihydroxy-3′-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]chalcone (**2**). The identity of both **1** and **2** were further confirmed by the comparison of reported spectral data for xanthoangelol and xanthoangelol B, respectively, from *Angelica keiskei* of the family Umbelliferae (Baba et al., 1990).

Compound **3** was assigned the same molecular formula $C_{25}H_{28}O_5$ (EIMS: *m/z* 408) as for **2**. Comparison of the ^1H, ^{13}C and EIMS spectral data of **3** with that of **2** indicated that the presence of the same 2′,4′,4-trihydroxychalcone core in the molecule **3** as for **2**, with some changes in the side chain attached to C-3′. Detailed analysis of the ^1H NMR spectrum of **3** indicated that the presence of exomethylene group [δ 5.11 and 4.92], oxymethine group [δ 4.43] in addition to the dimethylallyl group [δ 1.63(3H, *s*), 1.70(3H, *s*) and 5.16(1H, *m*)]. The benzylic methylene protons appeared at δ 2.83 (*dd*, $J = 14.9$, 8.5 Hz) and 3.25 (*dd*, $J = 14.9$, 1.9 Hz) which were coupled with the oxymethine proton. The peaks appeared in the ^{13}C NMR of **3** at δ 151.0, 109.2 (exomethylene) and δ 77.0 (oxymethine) also further supported for these assignments. These observations led us to place the exomethylene group at C-3″ and the hydroxyl group at C-2″. Hence the structure of **3** was established as a new natural product 2′,4′,4-trihydroxy-3′-[2-hydroxy-7-methyl-3-methylene-6-octaenyl]chalcone.

The negative ion FABMS peak of **4** at *m/z* 407 [M–H]$^-$ gave evidence for the molecular formula $C_{25}H_{28}O_5$. The comparison of ^1H NMR spectrum of **4** with that of **1** clearly indicated that the presence of a geranyl group in **4** (Table 1). The ^1H NMR signals observed at δ 6.42 (H-5′) and δ 7.70 (H-6′) of **4** were super imposable with the H-5′ and the H-6′ of **1**. Further the signals observed at δ 7.13 (*dd*, $J = 8.2$, 1.7), 6.90(*d*, $J = 8.2$) and 7.18(*d*, $J = 1.7$) gave clear evidence for the presence of an *ortho*-dihydroxy group at C-3 and C-4 of the phenyl group attached to C-β. Hence the structure of **4** was established as 2′,3,4,4′-tetrahydroxy-3′-geranylchalcone. It has been previously reported from the leaves of *Artocarpus incisus* (Shimizu et al., 2000).

The EIMS of **5** indicated the molecular ion peak at *m/z* 424, consistent with the molecular formula $C_{25}H_{28}O_6$. Direct comparison of the ^1H NMR spectrum of **5** with that of **4** indicated the chalcone core of **5** is identical to that of **4**. Further the comparison of the same spectrum of **5** with that of **2** clearly indicated that the side chain at C-3′ of **2** is identical to that of **5**. Hence the structure of **5** was established as a new natural product 2′,3,4,4′-tetrahydroxy-3′-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]chalcone. The optical rotation of new compounds **3** and **5** were determined to be levorotatory and not reported because of the small amount of material used to measure the optical rotation.

All these compounds showed fungicidal activity at **1** (5 μg/spot), **2** (5 μg/spot), **3** (5 μg/spot), **4** (2 μg/spot) and **5** (15 μg/spot) against *Cladosporium cladosporioides* on TLC bio-autography method, wherein spore germinates as black zones and antifungal compounds appear as white zones (Homans and Fuchs, 1970). Benlate was used as positive control. Antioxidant properties of **1**–**5** were evaluated against the DPPH radical by TLC bio-

Table 1
^{1}H NMR data of **1** – **5** ($CDCl_3$, 500 MHz)

Carbon number	1	2	3	4	5
2	7.55 (*d*, 8.6)	7.56 (*d*, 8.6)	7.56 (*d*, 8.7)	7.18 (*d*, 1.7)	7.19 (*d*, 2.0)
3	6.87 (*d*, 8.6)	6.88 (*d*, 8.6)	6.88 (*d*, 7.8)	–	–
5	6.87 (*d*, 8.6)	6.88 (*d*, 8.6)	6.88 (*d*, 7.8)	6.90 (*d*, 8.2)	6.90 (*d*, 8.2)
6	7.55 (*d*, 8.6)	7.56 (*d*, 8.6)	7.56 (*d*, 8.7)	7.13 (*dd*, 8.2, 1.7)	7.13 (*dd*, 8.2, 2.0)
α	7.45 (*d*, 15.4)	7.45 (*d*, 15.4)	7.47(*d*, 15.4)	7.41 (*d*, 15.4)	7.41 (*d*, 15.4)
β	7.83 (*d*, 15.4)	7.84 (*d*, 15.4)	7.83 (*d*, 15.4)	7.76 (*d*, 15.4)	7.77(*d*, 15.4)
5′	6.42 (*d*, 8.9)	6.40 (*d*, 8.9)	6.53 (*d*, 8.9)	6.42 (*d*, 8.9)	6.40 (*d*, 8.9)
6′	7.71 (*d*, 8.9)	7.71 (*d*, 8.9)	7.75 (*d*, 8.9)	7.70 (*d*, 8.9)	7.70 (*d*, 8.9)
1″	3.49 (*d*, 7.2)	3.49 (t, 7.1)	2.83 (*dd*, 14.9, 8,5) 3.25 (*dd*, 14.9, 1.9)	3.49 (*d*, 7.1)	3.48 (*d*, 7.1)
2″	5.31 (t, 7.2)	5.34 (t, 7.0)	4.43 (*d*, 8.3)	5.30 (t, 6.8)	5.34 (*d*, 6.7)
4″	2.10 (m)	2.12 (m)	2.18 (bs)	2.10 (m)	2.12 (m)
5″	2.10 (m)	1.68 (m)	2.18 (bs)	2.10 (m)	1.68 (m)
6″	5.06 (t, 6.1)	4.05 (t, 6.2)	5.16 (m)	5.06 (t, 6.0)	4.05 (*d*, 6.5)
8″	1.67 (s)	4.83 (bs) 4.92 (bs)	1.70 (s)	1.67 (s)	4.83 (bs) 4.92 (bs)
9″	1.59 (s)	1.72 (s)	1.63 (s)	1.59 (s)	1.71 (s)
10″	1.83 (s)	1.85 (s)	4.92 (bs) 5.11 (bs)	1.83 (s)	1.85 (s)
2′ (OH)	13.8 (bs)	13.9 (bs)	13.9 (bs)	13.8 (bs)	13.8 (bs)

autography method (Takao et al., 1994). In this assay antioxidants react with the free radical DPPH and produce colorless 2,2′-phenyl-1-picrylhydrazine. α-Tocopherol was used as the positive control. All these compounds **1**–**5** exhibited off-white spots in purple background at the level of 1 μg/spot.

3. Experimental

3.1. General

Mps were determined by Gallenkamp apparatus and are uncorrected. UV spectra were recorded on a UV-160 A spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker DRX500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer in $CDCl_3$ solution. Tetramethylsilane was used as an internal standard for ^{1}H shifts and $CDCl_3$ signal ($\delta = 77.0$) was used as a reference for ^{13}C shifts. EI-MS (70 eV) and FABMS were obtained on a JEOL JMS-AX505HA spectrometer. HPLC analyses were carried out on Shimadzu LC-6A apparatus equipped with UV detector under a reversed phase C_{18} column under isocratic solvent condition.

3.2. Plant material

Leaves of the *A. nobilis* Thw. were collected from the central province of Sri Lanka in August, 2002. A voucher specimen is deposited at the institute of Fundamental Studies, Kandy.

3.3. Extraction, isolation and antifungal bioassay

Dried ground leaves of *A. nobilis* (210 g) were defatted with *n*-hexane and extracted with methanol. Evaporation of methanol gave a dark brown solid (47.5 g). The dried methanol extract (47 g) was partitioned with *n*-butanol and water. Evaporation of *n*-butanol gave a dark brown solid (21 g). The *n*-butanol extract was subjected to antifungal activity test on TLC bioautography method against *Cladosporium cladosporioides*. A portion of *n*-butanol extract (8 g) was chromatographed over a column of silica gel (Merck Art 7734) with *n*-hexane–EtOAc–MeOH to give 10 major fractions (fr. 1–fr. 10). Only the fractions fr. 4 and fr. 5 showed antifungal activity. Each fraction was further purified by a combination of chromatography over column of silica gel (eluant: *n*-hexane–EtOAc–MeOH), sephadex LH-20 (eluant: MeOH), RP-18 silica gel (eluant : H_2O–MeOH) and reversed phase HPLC (STR Prep – ODS 20×250 mm column, 20–15% H_2O–MeOH; 5 ml/min, UV detection 254 nm) to give compounds **1** (500 mg), **2** (44 mg), **3** (14 mg) from fr. 4 and compounds **4** (304 mg) and **5** (15 mg) from fr. 5.

Compounds **1**–**5** were subjected to antifungal activity (Homans and Fuchs, 1970) and antioxidant activity (Takao et al., 1994) tests by TLC bioautography method.

3.3.1. 2′,4,4′-Trihydroxy-3′-geranylchalcone (*1*)

Mp. 141–143 °C; UV λ_{max}^{EtOH}: 208, 230, 373 nm; ^{1}H NMR ($CDCl_3$, 500 MHz): see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 2; FABMS(−): *m/z* 391[M–H]$^-$, [$C_{25}H_{28}O_4$].

Table 2
^{13}C NMR data of compounds **1**–**4** in $CDCl_3$ and **5** in $CDCl_3$–CD_3OD (10:1) (125 MHz)

Carbon number	1	2	3	4	5
1	128.0	128.0	127.9	128.5	127.3
2	130.5	130.5	130.5	115.8	115.2
3	116.0	116.0	116.0	146.3	147.0
4	158.0	158.0	157.9	143.8	144.3
5	116.0	116.0	116.0	115.8	115.2
6	130.5	130.5	130.5	123.1	122.5
β	144.0	144.0	143.8	144.9	144.3
α	118.3	118.0	118.3	118.7	117.6
C=O	192.3	192.1	192.0	192.2	192.1
1′	114.1	114.0	113.7	114.1	113.4
2′	161.8	161.4	163.3	161.9	161.9
3′	114.1	114.2	113.7	114.1	113.4
4′	163.9	163.9	164.4	163.9	163.6
5′	107.9	107.7	109.0	107.9	107.1
6′	129.2	129.2	129.8	129.3	128.9
1″	21.8	21.7	32.1	21.8	21.5
2″	121.0	121.7	77.0	121.0	122.2
3″	139.8	135.1	151.0	139.9	135.3
4″	39.7	35.9	29.0	39.7	35.7
5″	26.4	32.8	26.5	26.4	32.6
6″	123.8	75.8	123.7	123.8	75.4
7″	132.1	147.2	132.2	132.1	147.5
8″	25.6	111.2	25.7	25.6	110.8
9″	17.7	17.7	17.7	17.7	17.3
10″	16.3	16.3	109.2	16.3	15.9

*3.3.2. 2′,4,4′-Trihydroxy-3′-[6-hydroxy-3,7-dimethyl-2(E),7-octadienyl]chalcone (**2**)*

Amorphous solid; UV λ_{max}^{EtOH}: 208, 230, 373 nm; ^1H NMR ($CDCl_3$, 500 MHz): see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 2; EIMS *m/z* (rel. int. %): 408[M^+](75), 390(50), 375(10), 347(30), 338(30), 322(75), 307(90), 281(85), 270(100), 255(20), 227(20), 215(30), 204(80), 187(90), 161(50), 147(80), 120(95), 107(40), 91(40), 77(20), 65(20).

*3.3.3. 2′,4′,4-Trihydroxy-3′-[2-hydroxy-7-methyl-3-methylene-6-octaenyl]chalcone (**3**)*

Amorphous solid; UV λ_{max}^{EtOH}: 208, 230, 373 nm; ^1H NMR ($CDCl_3$, 500 MHz): see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 2; EIMS *m/z* (rel. int. %): 408[M^+](25), 390(15), 375(5), 347(7), 339(10), 323(4), 288(25), 270(100), 253(5), 219(28), 201(5), 177(15), 164(15), 149(95), 137(10), 120(25), 107(10), 91(8), 69(15), 41(14); HREIMS *m/z*: 408.1932, $C_{25}H_{28}O_5$ requires 408.1937.

*3.3.4. 2′,3,4,4′-Tetrahydroxy-3′-geranylchalcone (**4**)*

Mp. 131–132 °C; UV λ_{max}^{EtOH}: 208, 230, 383 nm; ^1H NMR ($CDCl_3$, 500 MHz): see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 2; FABMS(−): *m/z* 407[M–H]$^-$.

*3.3.5. 2′,3,4,4′-Tetrahydroxy-3′-[6-hydroxy-3,7-dimethyl-2(E),7-octadienyl]chalcone (**5**)*

Amorphous solid; UV λ_{max}^{EtOH}: 209, 260, 373 nm; ^1H NMR ($CDCl_3$, 500 MHz): see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 2; EIMS *m/z* (rel. int. %): 424[M^+](5), 408(20), 390(15), 375(5), 347(10), 339(16), 307(10), 288(12), 270(80), 269(60), 227(10), 219(20), 187(12), 177(12), 149(100), 120(25), 107(15), 91(12), 69(25), 44(40), 28(35), 18(20); HREIMS *m/z*: 424.1906, $C_{25}H_{28}O_6$ requires 424.1886.

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