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Flavonoids from *Friesodielsia discolor*

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ABSTRACT

Phytochemical investigation of the fresh leaves of *Friesodielsia discolor* (Craib) D. Das led to the isolation of four new flavonoids, 3′-formyl-2′,4′-dihydroxy-6′-methoxychalcone (**1**), 8-formyl-7-hydroxy-5-methoxyflavanone (**2**), 8-formyl-5,7-dihydroxyflavanone (**3**) and 5,3′-dihydroxy-7-methoxyflavone (**6**), together with two known compounds, lawinal (**4**) and tectochrysin (**5**). The structures of the compounds were elucidated by spectroscopic analysis, mainly 1D and 2D NMR techniques (^{1}H, ^{13}C, COSY, HMQC and HMBC), as well as comparison with literature data. The isolates were tested for antiplasmodial, antimycobacterial and cytotoxic activities. Compounds **1**, **2**, **5** and **6** exhibited cytotoxicity against human tumor cell lines, KB and MCF-7 with the IC_{50} values in the range of 3.45–14.82 μg/ml. Compounds **1**, **2**, and **5** also showed significant antiplasmodial activity with respective IC_{50} values of 2.75, 2.78 and 2.08 μg/ml.

1. Introduction

Friesodielsia is a genus of Annonaceae with about 60 species found in Africa and Asia (Le Thomas, 1969), with five species occurred in Thailand (Chalermglin, 2001). Until 1948, species belonging to this genus were placed under *Oxymitra* (Perry, 1980). Only a few species of the genus have been chemically investigated. Hexahydroxanthenic derivatives and a flavanone have been isolated from *F. kingii* (syn. *O. kingii*) (Richomme et al., 1990), alkaloids, flavonoids, phenylpropanoids and terpenoids were found in the leaves and twigs of *F. velutina* (syn. *O. velutina*) (Achenbach and Hemrich, 1991), benzyl benzoates, bisabolene sesquiterpenes and flavonoides were found in the stem bark of *F. enghiana* (Fleischer et al., 1997), and a diformylchalcone, a dimethylchalcone, a formylflavanone, a dimethylflavanone, an alkaloid (−)-crotepoxide and benzyl benzoate were isolated from the stem and root bark of *F. obovata* and were tested for antiplasmodial activity (Joseph et al., 2007). In the course of our continuing search for bioactive constituents from Thai medicinal plants, preliminary screening of the ethyl acetate extract of the leaves of *Friesodielsia discolor* displayed cytotoxicity against human tumor cell lines, KB (epidermoid carcinoma in the mouth) and MCF-7 (human breast adenocarcinoma) with IC_{50} values of 11.87 and 6.13 μg/ml, respectively, and also showed antimycobacteria activity against *Mycobacterium tuberculosis* with an MIC value of 200 μg/ml. Chemical and biological studies on this species have not been reported previously. This paper reports the isolation, structure elucidation and biological activities of four new flavonoids, 3′-formyl-2′,4′-dihydroxy-6′-methoxychalcone (**1**), 8-formyl-7-hydroxy-5-methoxyflavanone (**2**), 8-formyl-5,7-dihydroxyflavanone (**3**) and 5,3′-dihydroxy-7-methoxyflavone (**6**), and two known compounds, lawinal (**4**) and tectochrysin (**5**) (Fig. 1), found in this plant.

2. Results and discussion

The fresh leaves of *F. discolor* were extracted with EtOAc and the extract was partitioned between CH_2Cl_2 and water. Chromatographic separation of the CH_2Cl_2 fraction on silica gel led to the isolation of compounds **1**–**6** (Fig. 1)

Compound **1** was obtained as yellow needles and had the molecular formula $C_{17}H_{14}O_5$ (*m*/*z* 299.0916, $[M+H]^+$, calcd for $C_{17}H_{15}O_5^+$ 299.0919) by HR-TOF-MS. The UV spectrum showed absorption bands at λ_{max} 292 and 341 nm and the IR spectrum indicated hydroxyl (ν_{max} 3136 cm^{-1}), aldehyde (ν_{max} 2849, 2766 cm^{-1}) and carbonyl (ν_{max} 1626 cm^{-1}) functional groups. The ^{1}H NMR spectrum of **1** (Table 1) showed a three-proton multiplet at δ_H 7.59–7.64 (3H) and a two-proton doublet of doublet at δ_H 7.61 (J = 7.4, 2.1 Hz) (2H), assigned to a monosubstituted phenyl ring (ring B) (Ye et al., 2004). A pair of AB system doublets at δ_H 7.80 and 7.86 (J = 15.6 Hz) indicated the presence of an α,β-unsaturated ketone moiety. The above data, suggested a chalcone

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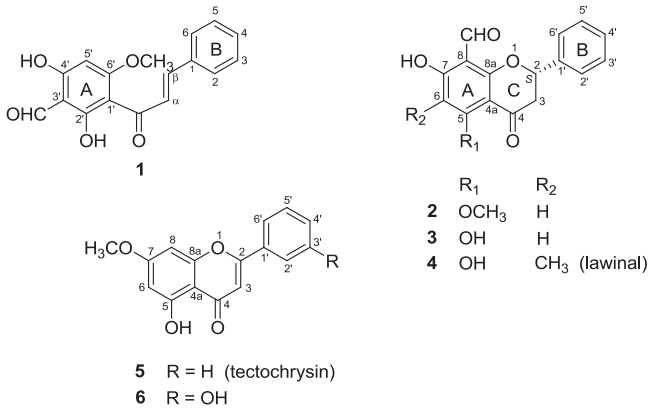

Fig. 1. Structures of compounds **1**–**6**.

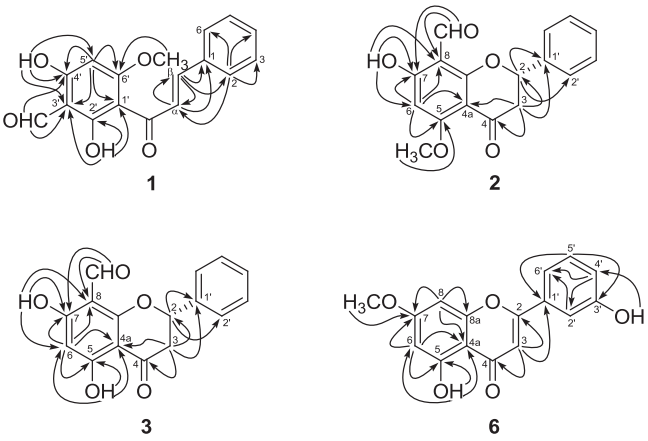

Fig. 2. HMBC correlations of **1**–**3** and **6**.

backbone for **1** (Itokawa et al., 1981). The ^{1}H NMR spectrum of **1** also showed signals for one methoxy group at δ_H 4.02 (s, 6′-OCH$_3$), one aldehyde proton at δ_H 10.24 (s, 3′-CHO), one pentasubstituted aromatic ring proton at δ_H 5.96 (s, H-5′) and two hydrogen bonded phenolic hydroxyl groups at δ_H 15.87 (s, 2′-OH) and 13.08 (s, 4′-OH).

The ^{13}C NMR spectrum of **1** (Table 1) exhibited signals of one monosubstituted aromatic ring (ring B) at δ_C 135.1 (C-1), 128.5 (C-2), 129.0 (C-3), 130.6 (C-4), 129.0 (C-5) and 128.5 (C-6), one pentasubstituted aromatic ring (ring A) at δ_C 104.6 (C-1′), 172.3 (C-2′), 105.4 (C-3′), 170.4 (C-4′), 91.1 (C-5′) and 168.2 (C-6′), one methoxy carbon at δ_C 56.5 (6′-OCH$_3$), one aldehyde carbon at δ_C 192.7 (3′-CHO), two olefinic carbons at δ_C 126.4 (C-α) and 144.0 (C-β) and one carbonyl carbon at δ_C 192.6. The substitution pattern on the A ring was determined to be 3′-formyl-2′,4′-6′-methoxy from the ^{1}H → ^{13}C HMQC and HMBC correlations between 2′-OH (δ_H 15.87)/C-1′ (δ_C 165.3), C-2′ (δ_C 165.3), and C-3′ (δ_C 165.3); 4′-OH (δ_H 13.08)/C-3′ (δ_C 105.4), C-4′ (δ_C 170.4), and C-5′ (δ_C 91.1); 3′-CHO (δ_H 10.24/C-3′ (δ_C 105.4), C-4′ (δ_C 170.4) and 6′-OCH$_3$/C-6 (δ_C 168.2) (Fig. 2). Compound **1** was thus assigned as 3′-formyl-2′,4′-dihydroxy-6′-methoxychalcone.

Compound **2** was isolated as colorless needles. The HR-TOF-MS of **2** exhibited a pseudo molecular ion at *m*/*z* 299.0916 [M+H]$^+$ (calcd for $C_{17}H_{15}O_5^+$ 299.0919), corresponding to the molecular formula $C_{17}H_{14}O_5$. The UV spectrum of **2** exhibited absorption bands at λ_{max} 265, 295 and 327 nm and the IR spectrum showed strong absorption bands for hydroxyl (ν_{max} 3033 cm^{-1}), aldehyde (ν_{max} 2846, 2760 cm^{-1}) and carbonyl (ν_{max} 1674, 1626 cm^{-1}) functional groups. The ^{1}H NMR spectrum of **2** (Table 2) showed a multiplet of five protons at δ_H 7.38–7.50, characteristic of a monosubstituted phenyl ring (ring B), and an ABX system at δ_H 2.88 (dd, *J* = 16.6, 3.1 Hz, Ha-3), 3.07 (dd, *J* = 16.6, 13.1 Hz, Hb-3) and 5.56 (dd, *J* = 13.1, 3.1 Hz, H-2) (ring C), suggesting the flavanone with unsubstituted in ring B (Ye et al., 2004). The ^{1}H NMR spectrum of **2** also showed an aromatic methine proton at δ_H 6.09 (s, H-6), an aldehyde proton at δ_H 10.14 (s, 8-CHO), one aromatic methoxy group at δ_H 3.97 (s, 5-OCH$_3$) and one hydrogen bonded phenolic hydroxyl group at δ_H 12.91 (s, 7-OH). The ^{13}C NMR spectrum of **2** (Table 3) exhibited signals of one monosubstituted aromatic ring (ring B) at δ_C 137.6 (C-1′), 126.0 (C-2′), 129.0 (C-3′), 129.1 (C-4′), 129.0 (C-5′), 126.0 (C-6′), one pentasubstituted aromatic ring (ring A) at δ_C 104.9 (C-4a), 167.9 (C-5), 93.5 (C-6), 169.8 (C-7), 104.9 (C-8), 167.1 (C-8a), one methoxy carbon at δ_C 56.8 (5-OCH$_3$), one aldehyde carbon at δ_C 192.1 (8-CHO), one oxygenated methine carbon at δ_C 80.2 (C-2), one methylene carbon at δ_C 45.0 (C-3) and one carbonyl carbon at δ_C 187.7 (C-4). The position of the aldehyde group at C-8 (δ_C 104.9) and the chelated hydroxyl group at C-7 (δ_C 169.8) on the A ring was established through HMBC correlations between 8-CHO (δ_H 10.14)/C-7 (δ_C 169.8) and C-8 (δ_C 104.9) and 7-OH (δ_H 12.91)/C-6 (δ_C 93.5), C-7 (δ_C 169.8), and C-8 (δ_C 104.9). The attachment of the methoxy group to C-5 on the A ring was also determined by an HMBC correlation

Table 1
^{1}H and ^{13}C NMR spectroscopic data of compound **1** in CDCl$_3$ (δ_H in ppm, mult., *J* in Hz).

Position	δ_H	δ_C
α	7.80 *d* (15.6)	126.4
β	7.86 *d* (15.6)	144.0
CO		192.6
1		135.1
2	7.61 *dd* (7.4, 2.1)	128.5
3	7.59–7.64 *m*	129.0
4	7.59–7.64 *m*	130.6
5	7.59–7.64 *m*	129.0
6	7.61 *dd* (7.4, 2.1)	128.5
1′		104.6
2′		172.3
3′		105.4
4′		170.4
5′	5.96 *s*	91.1
6′		168.2
2′-OH	15.87 *s*	
4′-OH	13.08 *s*	
3′-CHO	10.24 *s*	192.7
6′-OCH$_3$	4.02 *s*	56.5

Table 2
^{1}H NMR spectroscopic data of compounds **2**, **3** and **6** in CDCl$_3$ (δ_H in ppm, mult., *J* in Hz).

Position	**2**	**3**	**6**
2	5.56 *dd* (13.1, 3.1)	5.57 *dd* (13.1, 3.2)	
3	2.88 *dd* (16.6, 3.1)	2.90 *dd* (17.3, 3.2)	6.63 *s*
	3.07 *dd* (16.6, 13.1)	3.15 *dd* (17.3, 3.2)	
6	6.09 *s*	6.02 *s*	6.37 *d* (2.2)
8			6.49 *d* (2.2)
2′	7.38–7.50 *m*	7.37–7.50 *m*	7.37 *dd* (2.4, 1.3)
3′	7.38–7.50 *m*	7.37–7.50 *m*	
4′	7.38–7.50 *m*	7.37–7.50 *m*	7.38 *dt* (7.9, 1.3)
5′	7.38–7.50 *m*	7.37–7.50 *m*	7.33 *t* (7.8)
6′	7.38–7.50 *m*	7.37–7.50 *m*	7.03 *ddd* (7.8, 2.4, 1.3)
5-OH		12.54 *s*	12.73 *s*
7-OH	12.91 *s*	12.73 *s*	
5-OCH$_3$	3.97 *s*		
7-OCH$_3$			3.87 *s*
6-CH$_3$			
8-CHO	10.14 *s*	10.10 *s*	
3′-OH			8.23 *s*

Table 3
^{13}C NMR spectroscopic data of compounds **2**, **3** and **6** in $CDCl_3$ (δ_C in ppm).

Position	2	3	6
2	80.2	80.5	164.1
3	45.0	42.7	105.9
4	187.7	195.1	182.6
4a	104.9	101.8	105.7
5	167.9	169.3	162.1
6	93.5	97.5	98.1
7	169.8	170.8	165.6
8	104.9	104.5	92.7
8a	167.1	166.6	157.8
1′	137.6	137.0	132.6
2′	126.0	126.1	113.3
3′	129.0	129.1	157.5
4′	129.1	129.5	117.8
5′	129.0	129.1	130.1
6′	126.0	126.1	119.2
5-OCH_3	56.8		
7-OCH_3			55.8
6-CH_3			
8-CHO	192.1	191.3	

between 5-OCH_3 (δ_H 3.97) and oxygenated aromatic carbon C-5 (δ_C 167.9) (Fig. 2) (Carcache-Blanco et al., 2003). The levorotatory nature of **2** indicated the normal flavanone *S* absolute configuration at C-2 by comparing the optical rotation value with literature data for 6-geranyl-5,7-dihydroxy-3′,4′-dimethoxyflavanone (Asai et al., 2008) and (2*S*)-8-formyl-5,7-dihydroxy-6-methylflavanone (lawinal) (Joshi and Gawad, 1974). Compound **2** was therefore elucidated as (2*S*)-8-formyl-7-hydroxy-5-methoxyflavanone.

Compound **3** was isolated as colorless needles and had the molecular formula $C_{16}H_{12}O_5$ (*m*/*z* 285.0753 $[M+H]^+$, calcd for $C_{16}H_{13}O_5^+$ 258.0763) by HR-TOF-MS. The structure of **3** was closely related to **2** based on ^1H NMR, ^{13}C NMR, IR and UV spectroscopic data (Tables 2 and 3, Experimental). However, **3** had one carbon and two protons less than **2**. The appearance of a sharp singlet at δ_H 12.54 in the ^1H NMR spectrum of **3** and the absence of the signals of a methoxy group and a methoxy carbon in the ^1H and ^{13}C NMR spectra of **3**, respectively, suggested a hydrogen bonded phenolic hydroxyl group at C-5. The HMBC experiment (Fig. 2) of **3** showed correlations between 5-OH (δ_H 12.54)/C-5 (δ_C 169.3), C-4a (δ_C 101.8) and C-6 (δ_C 97.5), confirming the substitution of the phenolic hydroxyl group at C-5. The configuration of C-2 was inferred to be the same in **2**. Compound **3** was thus identified as (2*S*)-8-formyl-5,7-dihydroxyflavanone.

Compounds **4** and **5** were identified as 8-formyl-5,7-dihydroxy-6-methylflavanone (lawinal) and 5-hydroxy-7-methoxyflavone (tectochrysin), respectively, by comparing their spectroscopic data with literature values (Joshi and Gawad, 1974; Ji-Hong et al., 2012 and Lojanapiwatna et al., 1981; Park et al., 2007).

Compound **6** was obtained as yellow needles and had the molecular formula $C_{16}H_{12}O_5$ (*m*/*z* 285.0753 $[M+H]^+$, calcd for $C_{16}H_{13}O_5^+$ 285.0763) by HR-TOF-MS. The close similarity of the ^1H and ^{13}C NMR chemical shifts of **6** to those of **5** (Tables 2 and 3) indicated that the structures of the two compounds were closely related. The difference in 16 atomic mass units between **5** and **6** and the appearance of a one proton singlet at δ_H 8.23 in the ^1H NMR of **6**, suggested an additional phenolic hydroxyl group (Maurya et al., 2007). The positions of a methoxy group at C-7 (δ_C 165.6) on the A ring and the hydroxyl group at C-3′ on the B ring were further confirmed by HMBC correlations between 7-OCH_3 (δ_H 3.87)/C-7 (δ_C 165.6) and 3′-OH (δ_H 8.23)/C-4′ (δ_C 117.8) (Fig. 2). Compound **6** was therefore confirmed as 5,3′-dihydroxy-7-methoxyflavone.

The isolated flavonoids were evaluated for antimycobacterial activity against *Mycobacterium tuberculosis*, antiplasmodial activity against *Plasmodium falciparum* and cytotoxicity against two cancer cell lines (Table 4). Compound **5** exhibited better antiplasmodial activity against *P. falciparum* with IC_{50} value of 2.08 μg/ml than **1** and **2** with IC_{50} value of 2.75 and 2.78 μg/ml, respectively, while compound **1** showed only moderate antimycobacterial activity against *M. tuberculosis* (H37Ra strain) with the MIC value of 6.25 μg/ml. In addition, compound **1** showed potent cytotoxicity against both KB and MCF-7 cancer cell lines with the IC_{50} value of 6.50 and 4.13 μg/ml, respectively, whereas compound **6** also showed potent cytotoxicity against MCF-7 cancer cell line with the IC_{50} value of 3.45 μg/ml. It was suggested that a chalcone and a flavone skeletons could be essential for cytotoxicity.

Table 4
Antiplasmodial, antimycobacterial, and cytotoxic activities of flavonoids **1**–**6**.

Flavonoids	Activities against			
	P. falciparum	*M. tuberculosis*	KB[a]	MCF-7[b]
	(IC_{50}, μg/ml)	(MIC, μg/ml)	(IC_{50}, μg/ml)	
1	2.75	6.25	6.50	4.13
2	2.78	25.00	12.51	10.27
3	Inactive	100.00	Inactive	Inactive
4	Inactive	100.00	Inactive	Inactive
5	2.08	Inactive	14.82	4.49
6	Inactive	Inactive	Inactive	3.45
Rifampicin		0.003–0.012		
Isoniazid		0.023–0.046		
Dihydroartemisinin	0.005			
Ellipticin			0.338	0.695
Doxorubicin			0.142	0.318

[a] Human epidermoid carcinoma in the mouth.
[b] Human breast adenocarcinoma cell line.

3. Experimental

3.1. General experimental procedures

Melting points were determined by Buchi melting point B-540 apparatus and are reported without correction. Optical rotations $[\alpha]_D$ were measured in chloroform solution with sodium D line (590 nm) on JASCO DIP-370 digital polarimeter. UV spectra were recorded with a Shimadzu UV–VIS 2001S Spectrophotometer. IR spectra were recorded with a Perkin Elmer Spectrum One FT-IR Spectrophotometer using UATR technique. ^1H and ^{13}C NMR spectra were measured in $CDCl_3$ on a Bruker AVANCE 400 (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) spectrometer. Chemical shifts are given in δ (ppm) with tetramethylsilane an internal standard. Coupling constants (*J*) are given in Hz. The signals in the ^1H and ^{13}C NMR spectra were assigned unambiguously using 2D NMR techniques: COSY, HMQC and HMBC. Low resolution mass spectra were recorded on a Thermo Finnigan Polaris Q mass spectrometer at 70 eV (probe) for EIMS, Chulabhorn Research Institute. High resolution time-of-flight mass spectra (HR-TOF-MS) were recorded on a Bruker microTOF-LC mass spectrometer, equipped with an electrospray ionization (ESI) ion source, operating inpositive-ion mode. Instrument calibration was performed using a reference solution of sodium formate 0.1% in isopropanol/water (1:1) containing 5 mM sodium hydroxide. Typical mass accuracy was ±2 ppm. Column chromatography (CC) was carried out on silica gel 60 (Scharlau, 70–230 mesh or 230–400 mesh). TLC were performed on percolated silica gel 60 F_{254} plates (Merck); spots were detected by UV or spraying with 1% $Ce(SO_4)_2$ in 10% aq. H_2SO_4 followed by heating. All commercial grade solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements.

3.2. Plant material

The leaves of *F. discolor* were collected in Narathiwat Province, Thailand in April 2003, and were identified by Dr. Piya Chalermglin,

Thailand Institute of Scientific and Technological Research, Pathum Thani, Thailand. A voucher specimen (PKRU2003004) was deposited at the Laboratory of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Phuket 83000, Thailand.

3.3. Extraction and isolation

The fresh leaves of *F. discolor* (4.0 kg) were extracted with EtOAc (3× 14 L for each solvent for one week) at room temperature. The EtOAc extract was partitioned between CH_2Cl_2 and water. The CH_2Cl_2 layer was evaporated to give a dark green residue (225.8 g). A portion (70.1 g) of the CH_2Cl_2 extract was separated on a column of silica gel 60 (Scharlau, 70–230 mesh, 1.57 kg) using hexane, gradient of hexane/EtOAc and EtOAc as the eluent to give 11 fractions.

Fraction 4 (356 mg) was chromatographed on a column of silica gel 60 (Scharlau, 70–230 mesh, 22 g) using hexane/CH_2Cl_2/EtOAc (20:2:1) as the eluent to give (2*S*)-8-formyl-5,7-dihydroxy-6-flavanone (lawinal) (**4**) as a colorless solid which was crystallized from hexane/CH_2Cl_2 as colorless needles (2 mg).

Fraction 5 (760 mg) was separated by CC using silica gel 60 (Scharlau, 70–230 mesh, 50 g). The column was eluted with hexane/CH_2Cl_2/EtOAc (20:2:1) to give compound **4** as a colorless solid which was crystallized from hexane/CH_2Cl_2 as colorless needles (4 mg).

Fraction 7 (14.63 g) was chromatographed on a column of silica gel 60 (Scharlau, 70–230 mesh, 1.53 kg) using hexane/CH_2Cl_2/EtOAc (5:4:1) as the eluent to give 3′-formyl-2′,4′-dihydroxy-6′-methoxychalcone (**1**) as a yellow solid, 5-hydroxy-7-methoxyflavone (tectochrysin) (**5**) as a pale yellow solid and (2*S*)-8-formyl-5,7-dihydroxyflavanone (**3**) as a colorless solid. Compounds **1**, **5** and **3** were crystallized from hexane/CH_2Cl_2 as yellow needles (1.55 g), pale yellow needles (173 mg) and colorless needles (9 mg), respectively.

Fraction 8 (6.45 g) was separated by CC using silica gel 60 (Scharlau, 230–400 mesh, 350 g). The column was eluted with hexane/CH_2Cl_2/EtOAc (3:7:1) to give 5,3′-dihydroxy-7-methoxyflavone (**6**) as a yellow solid which was crystallized from hexane/EtOAc/MeOH as yellow needles (156 mg).

Fraction 9 (5.29 g) was chromatographed on a column of silica gel 60 (Scharlau, 70–230 mesh, 440 g) using hexane/CH_2Cl_2/EtOAc (2:7:1) as the eluent to give (2*S*)-8-formyl-7-hydroxy-5-methoxyflavanone (**2**) as a colorless solid which was crystallized from hexane/CH_2Cl_2 as colorless needles (333 mg).

3.3.1. 3′-Formyl-2′,4′-dihydroxy-6′-methoxychalcone (**1**)

Yellow needles, m.p. 161 °C; UV (MeOH) λ_{max} (log ε): 341 (4.02), 292(4.09) nm; IR (UATR) ν_{max}: 3136, 3023, 2916, 2849, 2766, 1626, 1586, 1442, 1347,1203, 1133, 1064, 978, 959, 839, 823, 753, 655 cm^{-1}; ^1H and ^{13}C NMR (Table 1); EIMS *m*/*z* (rel. int.): 298 $[M]^+$ (81), 297 $[M-H]^+$ (40), 270 $[M-CO]^+$ (91), 269 $[M-CHO]^+$ (52), 221 $[M-C_6H_5]^+$ (53), 193 $[M-105]^+$ (100); positive-ion HR-TOF-MS *m*/*z*: 299.0916 $[M+H]^+$ (calcd for $C_{17}H_{15}O_5^+$ 299.0919).

3.3.2. (2S)-8-Formyl-7-hydroxy-5-methoxyflavanone (**2**)

Colorless needles, m.p. 167 °C; $[\alpha]_D^{27}$ −4.47° (*c*, 0.30, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 327sh (3.47), 295sh (3.99), 265 (4.48) nm; IR (UATR) ν_{max}: 3033, 2916, 2846, 2760, 1674, 1626, 1577, 1423, 1366, 1329, 1303, 1213, 1119, 812, 694 cm^{-1}; ^1H NMR (Table 2) and ^{13}C NMR (Table 3); EIMS *m*/*z* (rel. int.): 298 $[M]^+$ (100), 270 $[M-CO]^+$ (42), 221 $[M-C_6H_5]^+$ (14); positive-ion HR-TOF-MS *m*/*z*: 299.0916 $[M+H]^+$ (calcd for $C_{17}H_{15}O_5^+$ 299.0919).

3.3.3. (2S)-8-Formyl-5,7-dihydroxyflavanone (**3**)

Colorless needles, m.p. 169 °C, $[\alpha]_D^{27}$ −52.06° (*c*, 0.32, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 320 (2.57), 290 (4.21), 269 (4.66) nm; IR (UATR) ν_{max}: 3250, 2950, 2849, 2762, 1647, 1613, 1463, 1360, 1262, 1212, 1183, 1100, 1024, 800, 719 cm^{-1}. ^1H NMR (Table 2) and ^{13}C NMR (Table 3); EIMS *m*/*z* (rel. int.): 284 $[M]^+$ (100), 283 $[M-H]^+$ (41), 256 $[M-CO]^+$ (32), 207 $[M-C_6H_5]^+$; positive-ion HR-TOF-MS *m*/*z*: 285.0753 $[M+H]^+$ (calcd for $C_{16}H_{13}O_5^+$ 258.0763).

3.3.4. (2S)-8-Formyl-5,7-dihydroxy-6-methylflavanone (lawinal) (**4**)

Colorless needles, m.p. 214 °C (230 °C, Joshi and Gawad, 1974); $[\alpha]_D^{27}$ −15° (*c*, 0.20, $CHCl_3$); UV, IR and NMR data were in agreement with that of Joshi and Gawad (1974) and Ji-Hong et al. (2012).

3.3.5. 5-Hydroxy-7-methoxyflavone (tectochrysin) (**5**)

Pale yellow needles, m.p. 162 °C (163–164 °C, Lojanapiwatna et al., 1981); UV, IR and NMR data were in agreement with that of Lojanapiwatna et al. (1981) and Park et al. (2007).

3.3.6. 5,3′-Dihydroxy-7-methoxyflavone (**6**)

Yellow needles, m.p. 203 °C; UV (EtOH) λ_{max} (log ε): 316 (4.22), 270 (3.49), 245 (4.31) nm; IR (UATR) ν_{max}: 3244 (broad), 3080, 2919, 2846, 1663, 1610, 1590, 1507, 1434, 1375, 1297, 1267, 1207, 1160, 997, 934, 842, 819, 787, 767, 740, 700, 663 cm^{-1}; ^1H NMR (Table 2) and ^{13}C NMR (Table 3); EIMS *m*/*z* (rel. int.): 284 $[M]^+$ (100); positive-ion HR-TOF-MS *m*/*z*: 285.0753 $[M+H]^+$ (calcd for $C_{16}H_{13}O_5^+$ 285.0763).

3.4. Bioassays

3.4.1. Antimycobacterial assay

Antimycobacterial activity was assessed against *Mycobacterial tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau, 1997). Standard drugs, isoniazid and rifampicin, were used as the reference compounds (Table 4).

3.4.2. Antiplasmodial assay

Antiplasmodial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug resistant strain), using the method of Trager and Jensen (1976). Quantitative assessments of malarial activity *in vitro* were determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al. (1979). The inhibition concentration (IC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the *in vitro* incorporation of $[^3H]$-hypoxanthine by *P. falciparum*. The standard compound was dihydroartemisinin (Table 4).

3.4.3. Cytotoxicity assay

Cytotoxic assays against human epidermoid carcinoma (KB) and Human breast adenocarcinoma (MCF-7) cell lines were performed employing the colorimetric method described by Skehan et al. (1990). The reference substances were ellipticin and doxorubicin (Table 4).

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