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Isoflavanones from *Uraria picta* and their antimicrobial activity

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Abstract

Two isoflavanones, 5,7-dihydroxy-2′-methoxy-3′,4′-methylenedioxyisoflavanone (**2**) and 4′,5-dihydroxy-2′,3′-dimethoxy-7-(5-hydroxyoxychromen-7yl)-isoflavanone (**4**) along with six known compounds including isoflavanones, triterpenes and steroids were isolated from the roots of *Uraria picta*. The structures of these compounds were established unambiguously by UV, IR, MS and a series of 1D and 2D NMR analyses. The minimum inhibitory concentrations (MIC) for these compounds were found to be in the range of 12.5–200 μg/ml against bacteria (both Gram positive and Gram negative) and fungi.

Keywords: *Uraria picta*; Papilionaceae; Isoflavanones; 5,7-Dihydroxy-2′-methoxy-3′,4′-methylenedioxyisoflavanone; 4′,5-Dihydroxy-2′,3′-dimethoxy-7-(5-hydroxyoxy chromen-7yl)-isoflavanone; Antimicrobial

1. Introduction

Uraria picta Desv. (Syn. *Doodia picta* Roxb; Fam. Papilionaceae), a suffruticose sparingly branched perennial herb having a height of 0.9–1.8 m, is distributed throughout Bangladesh, India, Sri Lanka, Tropical Africa, Malay Islands and the Philippines (Hooker, 1879; Kirtikar et al., 1993; Yusuf et al., 1994). Traditionally, the plant is used as an antidote to the venom of a dangerous Indian snake, *Echis carinata* (Kirtikar et al., 1993). Its leaves are a good antiseptic and are used against gonorrhoea. The fruits and pods are effective against oral sores in children and the roots have use against cough, chills and fever (Kirtikar et al., 1993; Yusuf et al., 1994). Whilst this species has not been investigated before, *U. lagopoides* has been reported for its analgesic and anti-inflammatory activity (Hamid et al., 2004) and *U. critina* for nitric oxide-scavenging and antioxidant effects (Yen et al., 2001). As part of our research project focussing on Bangladeshi medicinal plants, we here report the isolation of two new isoflavanones (**2** and **4**) together with six known compounds including isoflavanones, triterpenes and steroids from the roots of *U. picta* as well as the antimicrobial activities of compounds **1**–**7** against bacteria (both Gram positive and Gram negative) and fungi.

2. Results and discussion

The root bark of *U. picta* was extracted sequentially with petroleum ether (60–80 °C), chloroform and methanol. Vacuum–liquid chromatography (VLC) fractionation of the petroleum ether extract followed by preparative TLC or recrystalisation yielded stigmasta-4,22-diene-3-one (Kojima et al., 1990), β-sitosterol (Kojima et al., 1990) and lupeol (Parsons, 1991) which were identified by direct comparison of the spectral data to those published in the literature.

VLC fractionation of the chloroform extract followed by gel filtration over Sephadex LH-20 and preparative TLC led to the isolation of a triterpene (**1**) and isoflavanones (**2**–**5**). By comparing the spectral data to those reported before, the triterpene (**1**) was identified as 12-ole-

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anene-3β,22β-diol (Kinjo et al., 1985) which is the first report from the genus *Uraria*.

The molecular formula of **2** was assigned as $C_{17}H_{14}O_7$ from the molecular ion peak at *m/z* 330.0745 in the high-resolution EIMS. Spectral data revealed the general features of an isoflavanone nucleus (Mabry et al., 1970; Markham and Chari, 1982). Absorption bands at 289 and 335 nm (UV), an absorbance at 1634 cm^{-1} (IR) and an ABX pattern with chemical shifts (^{1}H NMR) at 4.71 (*t*, *J* = 11.1 Hz), 4.52 (*dd*, *J* = 10.7, 5.6 Hz) and 4.45 (*dd*, *J* = 11.1, 5.6 Hz) were typical of H-2 (2 × H) and H-3 protons of an isoflavanone. The ^{1}H NMR spectrum (400 MHz, C_5D_5N, and Table 1) also exhibited an H-bonded C-5 hydroxyl at *δ* 12.98, *meta*-coupled (*J* = 2.1 Hz) aromatic protons at 6.55 and 6.44 attributable to H-6 and H-8 of ring A and *ortho*-coupled (*J* = 8.4 Hz) aromatic protons of ring-B (*δ* 6.65 and 6.81) of an isoflavanoid. Besides these, the presence of a methoxyl and a methylenedioxy group within the molecule was deduced from the chemical shifts at 3.90 and 5.92, respectively. The *J*-modulated ^{13}C NMR spectrum (100 MHz, C_5D_5N, Table 1) showed a total of 17 carbons including a carbonyl group. The assignment of all carbons and the placement of a methoxyl and a methylenedioxy group within the molecule were achieved by 2D experiments. In the HMBC experiment (Table 2), the C-5 hydroxyl group showed ^3J correlation to a methine at 97.9 and a quaternary carbon at 103.5 ppm which were thereby, assigned as C-6 and C-10, respectively. A ^2J correlation by both H-6 and H-8 to 169.0 confirmed its identity as C-7. A ^3J connectivity by the C-2 protons and a ^2J connectivity by H-8 with the carbon at 164.7 ppm favoured its assignment as C-9. The remaining carbon in ring A was identified as C-8 at

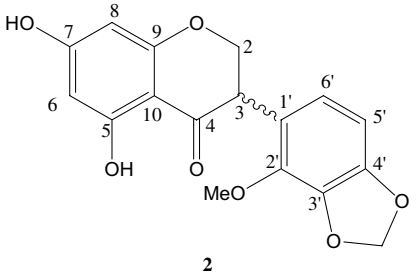
Table 2
HMBC data (400 MHz, C_5D_5N) of **2** and **4**

Protons	2 (H → C)		4 (H → C)	
	^2J	^3J	^2J	^3J
H-2	C-3	C-9, C-4, C-1′	–	C-9, C-4, C-1′
H-3	C-2, C-4, C-1′	C-2′, C-6′	C-2, C-4	C-6′
H-6	C-5, C-7	C-10, C-8	C-5, C-7	C-10, C-8
H-8	C-7, C-9	C-6, C-10	C-7, C-9	C-6, C-10
H-5′	C-4′	C-1′, C-3′	–	C-1′, C-3′
H-6′	C-5′	C-3, C-2′, C-4′	–	C-2′
H-2″	–	–	C-3″	C-4″, C-9″
H-3″	–	–	C-2″	C-10″
H-6″	–	–	C-5″	C-8″, C-10″
H-8″	–	–	C-7″	C-6″, C-10″
HO-5	C-5	C-6, C-10	C-5	C-6, C-10,
HO-5″	–	–	C-5″	C-6″, C-10″
MeO-2′	–	C-2′	–	C-2′
MeO-3′	–	–	–	C-3′
–OCH_2O–	–	C-3′, C-4′	–	–

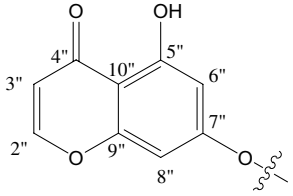
Table 1
^{1}H NMR (400 MHz) data of **2** and **4** and ^{13}C NMR (100 MHz) data of **2-4** in C_5D_5N

Position	^{1}H		^{13}C		
	2	**4**	**2**	**3**	**4**
2	4.71, *t*, *J* = 11.1 Hz 4.52, *dd*, *J* = 10.7, 5.6 Hz	4.73, *t*, *J* = 10.8 Hz 4.57, *dd*, *J* = 10.7, 5.5 Hz	71.3	71.8	71.8
3	4.45, *dd*, *J* = 11.1, 5.6 Hz	4.51, *dd*, *J* = 10.9, 5.5 Hz	48.7	48.1	48.1
4	–	–	197.9	198.2	198.3
5	–	–	166.1	166.1	166.1
6	6.55, *d*, *J* = 2.1 Hz	6.53, *d*, *J* = 2.1 Hz	97.9	97.9	97.9
7	–	–	169.0	169.2	168.9
8	6.44, *d*, *J* = 2.1 Hz	6.43, *d*, *J* = 2.1 Hz	96.6	96.2	96.6
9	–	–	164.7	164.2	164.7
10	–	–	103.5	103.6	103.6
1′	–	–	121.6	120.0	120.3
2′	–	–	142.7	153.3	153.3
3′	–	–	138.0	142.6	142.6
4′	–	–	150.0	153.3	153.3
5′	6.65, *d*, *J* = 8.4 Hz	7.00, *d*, *J* = 8.4 Hz	103.8	111.3	113.3
6′	6.81, *d*, *J* = 8.4 Hz	7.03, *d*, *J* = 8.4 Hz	125.3	125.9	125.9
2″	–	7.92, *d*, *J* = 6.0 Hz	–	–	157.1
3″	–	6.30, *d*, *J* = 6.0 Hz	–	–	111.7
4″	–	–	–	–	182.6
5″	–	–	–	–	163.7
6″	–	6.72, *d*, *J* = 2.0 Hz	–	–	100.7
7″	–	–	–	–	166.6
8″	–	6.63, *d*, *J* = 2.0 Hz	–	–	95.4
9″	–	–	–	–	159.3
10″	–	–	–	–	106.4
5-OH	12.98, *br. s*	13.01, *br. s*	–	–	–
5″-OH	–	13.37, *br. s*	–	–	–
2′-OMe	3.90, *s*	3.96, *s*	59.8	61.1	61.1
3′-OMe	–	3.88, *s*	–	60.7	60.7
–OCH_2O–	5.92, *s*	–	102.2	–	–

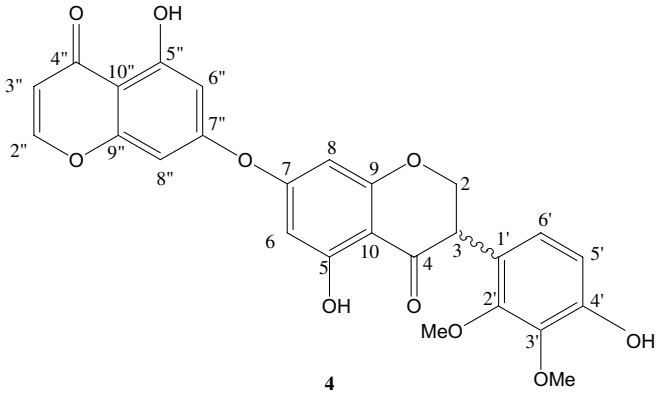
96.6 ppm from its ^3J correlation with H-6 and its direct correlation from an HMQC experiment. A ^3J correlation by H-3, H-6′ and the methoxyl protons with the carbon at 142.7 in the HMBC experiment proved its identity as C-2′ and thereby, confirming the placement of a methoxyl substituent at this carbon. The protons of methylenedioxy group exhibited ^3J correlation with δ_C 138.0 and 150.0. The ^3J correlations by H-5′ to 138.0 and H-6′ to 150.0 thereby placed the methylenedioxy at C-3′ and C-4′. The assignments of C-5′ and C-6′ at 103.8 and 125.3 respectively, were achieved from their direct coupling in the HMQC experiment. In ring C, a ^3J connectivity of H-6′ with a methine carbon at δ_C 48.7 proved its identity as C-3. Furthermore, C-2 was assigned to the resonance at 71.3 as it showed a ^2J correlation to H-3 in the HMBC spectrum and direct coupling with H-2 in HMQC spectrum. The COSY 90 experiment showed an expected coupling of H-3 with the two non-equivalent protons at C-2. The aryl substituent at C-3 is defined as equatorial due to the large axial coupling ($J = 11.1$ Hz) seen between H-3 and H-2 ax. On this basis, compound **2** was identified as 5,7-dihydroxy-2′-methoxy-3′,4′- methylenedioxyisoflavanone which is described here for the first time.



2



4a



4

The HREIMS of **3** showed the molecular ion peak at m/z 332.0898, corresponding to $C_{17}H_{16}O_7$, which was 2 a.m.u. more than **2**. The ^{1}H and ^{13}C NMR data of **3** were almost identical to those of **2** except for C-3′ and C-4′ in ring C. Instead of a methylenedioxy group at C-3′ and C-4′, the presence of methoxyl and hydroxyl were evident. The placement of this methoxyl was confirmed at C-3′ from its NOESY interaction with the methoxyl group at C-2′. Thus, **3** was identified as 5,7,4′-trihydroxy-2′,3′-dimethoxyisoflavanone, also known as parvisoflavanone, reported before only from *Poecilanthe parviflora* (Assumpcao, 1973). However, its ^{13}C NMR data (Table 1) confirmed by 2D HMBC is presented here for the first time.

The HREIMS of compound **4** showed a molecular ion peak at m/z 492.1036 which analyzed for $C_{26}H_{20}O_{10}$. The J-modulated ^{13}C NMR spectrum (100 MHz, C_5D_5N, Table 1) exhibited a total of 26 carbons including two carbonyls (198.3, 182.6), two methoxyls (60.7, 61.1), one oxymethylene (71.8), nine methines and the remaining twelve carbons as quaternary. The presence of an H-bonded hydroxyl (δ 13.01), *meta*-coupled ring-A protons (6.53, *d*, $J = 2.1$ Hz; 6.43, *d*, $J = 2.1$ Hz), *ortho*-coupled ring-B protons (7.03, *d*, $J = 8.4$ Hz; 7.00, *d*, $J = 8.4$ Hz), two methoxyls (3.88, *s*; 3.96, *s*) and an isoflavanone nucleus further proved the presence of **3** as a part of this molecule. The COSY-90 and HMBC correlations for this part were very similar to those in **3**. However, the ^{1}H NMR spectrum (400 MHz, C_5D_5N, Table 1) also showed another H-bonded hydroxyl (13.37), *meta*-coupled protons (6.72, *d*, $J = 2.0$ Hz; 6.63, *d*, $J = 2.0$ Hz) and an *ortho*-coupled AB quartet (7.92, *d*, $J = 6.0$ Hz; 6.30, *d*, $J = 6.0$ Hz). In the HMBC experiment (Table 2), H-2″ showed ^3J correlations with the carbonyl at 182.6 (C-4″) and an oxygenated quaternary carbon (159.3, C-9″) while H-3″, H-6″, H-8″ and HO-5″ showed a common ^3J correlation with C-10″ (106.4). The *meta*-coupled H-6″ and H-8″ were further connected to C-7″ (166.6) by a ^2J correlation. These spectral data constituted a 5,7-dihydroxychromene moiety (**4A**) as a part of the molecule. The joining of these two parts could be achieved through an ether bridge between C-7″ to C-7 or C-7″ to C-4′. A NOESY interaction between H-6 to H-6″ and H-8 to H-8″ confirmed the connectivity between an ether bridge at C-7. The aryl substituent at C-3 is defined as equatorial due to the large axial coupling ($J = 10.9$ Hz) seen between H-3 and H-2 ax. Therefore, compound **4** was identified as 4′,5,-dihydroxy-2′,3′-dimethoxy-7- (5-hydroxyoxychromen-7yl)-isoflavanone,a novel isoflavanone. By comparison of spectral data, compound **5** was identified as 4′,5,7-trihydroxy-2′-methoxyisoflavanone (isoferreirin) (Keen and Ingham, 1980).

VLC fractionation followed by size exclusion chromatography over Sephadex LH20 yielded two isoflavanones which were identified as 2′,4′,5,7-tetrahydroxy-6-(3-methylbut-2-enyl)isoflavanone (**6**) (Tsanuo et al., 2003) and 2′,4′,5,7-tetrahydroxyisoflavanone (**7**) (Krishnamurty and Sathyanarayana, 1986; Chang and Nair, 1995). Although the isoflavanones **5**–**7** were previously reported from

Table 3
Minimum inhibitory concentrations (MIC) of the constituents of *Uraria picta*

Compounds	*Staphylococcus aureus*		*Bacillus subtilis*		*Escherichia coli*		*Proteus vulgaris*		*Aspergillus niger*		*Candida albicans*	
	μg/ml	μmol	μg/ml	μmol	μg/ml	μmol	μg/ml	μmol	μg/ml	μmol	μg/ml	μmol
1	50	0.113	NT	NT	25	0.076	12.5	0.028	200	0.453	100	0.226
2	12.5	0.038	50	0.152	100	0.303	25	0.076	50	0.152	100	0.303
3	50	0.151	12.5	0.038	25	0.075	12.5	0.038	25	0.075	50	0.151
4	12.5	0.025	25	0.102	50	0.203	12.5	0.051	25	0.102	50	0.203
5	100	0.331	50	0.166	50	0.166	25	0.083	50	0.166	100	0.331
6	50	0.140	NT	NT	12.5	0.035	25	0.070	25	0.070	12.5	0.035
7	50	0.174	12.5	0.043	25	0.087	12.5	0.043	25	0.087	25	0.087
A	3.13	0.008	6.25	0.017	12.5	0.034	6.25	0.017	–	–	–	–
F	–	–	–	–	–	–	–	–	–	–	25	0.082

A, amoxycillin; F, fluconazole; NT, not tested.

legumes (Keen and Ingham, 1980; Tsanuo et al., 2003), this is the first time report of their isolation from the genus *Uraria* (ISIS database, 2007).

The results of antimicrobial activities of compounds **1**–**7** by a newly developed microdilution technique (Drummond and Waigh, 2000) are presented in terms of minimum inhibitory concentrations in Table 3. From the table it is evident that both triterpene (**1**) and isoflavanones (**2**–**7**) showed significant activity against the test organisms. The triterpene (**1**) showed the highest activity (MIC = 12.5 μg/ml; 0.028 μmol) against the Gram-negative species *Proteus vulgaris*. The new isoflavanones (**2** and **4**) were found to be the most active against *Staphylococcus aureus*. In molar concentration, the order of activity against *S. aureus* was $4 > 2 > 1 > 6 > 3 > 7 > 5$. However, the 6-prenylisoflavanone (**6**) showed the highest activity against Gram negative *Escherichia coli* and the fungi, *Candida albicans*. The sequence of relative potencies against Gram-negative *E. coli* was $6 > 3 > 1 > 7 > 5 > 4 > 2$ and against *C. albicans* was $6 > 7 > 3 > 4 > 1 > 2 > 5$. The sequence was almost the same for *A. niger* except for the higher MIC of **1**.

Flavonoids and isoflavanoids are known to exhibit a range of activities including anti-inflammatory, antithrombotic, antiviral and hepatoprotection which may, in some measure, be due to their ability to scavenge free-radicals (Akdemir et al., 2001; Saija et al., 1995). Genistein and 2′-hydroxygenistein were reported to be potent inhibitors of indole-3-acetic acid oxidase activity (Ferrer et al., 1992). Genistein has also been reported to have strong lipid peroxidation inhibitory effects and cytotoxicity (Cos et al., 2001). When tested against oral microorganisms genistein's MIC was found to be 12.5 μg/ml against *Porphyrmonas gingivalis* and more than 50 μg/ml against *Lactobacillus casei*, *L. fermentum*, *Streptococcus mutans*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Actinobacillus actinomycetemicomitans*, *A. naeslandiim* and *Staphylococcus aureus* (Iinuma et al., 1994). Prenylated isoflavanones were reported to have antimicrobial activity (Monache et al., 1996). Thus, the present findings on the antimicrobial activities of the isoflavanones and triterpene isolated from *U. picta* have further strengthened the previous findings of effectiveness of certain flavonoids and isoflavanoids against microbial infections and make some sense of the folk medicinal uses of this plant for the treatment of oral sores and gonorrhoea (Kirtikar et al., 1993; Yusuf et al., 1994).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer Polarimeter 341. IR spectra were recorded as dry film on a Mattson Galaxy 5000 FT-IR spectrometer. UV spectra were obtained on a Unicam UV 4-100 UV/Vis spectrophotometer in MeOH. HREIMS were recorded on a JEOL JMS-AX505HA double-focusing instrument at 70 eV. FAB-MS was taken using a VG ZAB-E spectrophotometer with a glycerol matrix. NMR spectra (both 1D and 2D) were obtained on a Bruker AMX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer, using the residual solvent peaks as internal standard. *J*-modulated ^{13}C spectra were acquired with a relaxation time (d_1) of 4 s. HMBC spectra were optimized for a long range J_{H-C} of 7 Hz ($d_6 = 0.07$ s) and NOESY experiment was carried out with a mixing time of 0.5 s. Vacuum-liquid chromatography (VLC) was carried out using Merck Si gel 60 H. Gel filtration was performed using Sephadex LH-20 (Sigma). PTLC was carried out using Merck Si gel 60 PF_{254} on glass plates (20 cm × 20 cm) at a thickness of 0.5 mm. TLC was conducted on normal-phase Merck Si gel 60 PF_{254} on plates. Spots on TLC and PTLC plates were visualised under UV light (254 and 366 nm) and spraying with 1% vanillin–H_2SO_4 followed by heating at 110 °C for 5–10 min.

3.2. Plant material

The roots of *U. picta* were collected from Rajshahi University Botanical Garden, Bangladesh in August, 1999. A voucher specimen (DACB9948) has been deposited at the Bangladesh National Herbarium, Mirpur, Dhaka.

3.3. Extraction and isolation

One hundred and twenty grams of dried, ground plant material was sequentially extracted with petroleum ether (b.p. 60–80°C), $CHCl_3$and methanol in a Soxhlet apparatus. VLC fractionation of the petroleum ether extract (1.8 g) on Si gel (5 g) was performed using a mobile phase (200 ml) of petroleum ether, EtOAc and MeOH in order of increasing polarity. Preparative TLC (5% EtOAc in toluene) on the VLC fraction eluted with 10% EtOAc in petroleum ether afforded 7.2 mg of stigmasta-4,22-diene-3-one. Recrystallisation of VLC fraction eluted with 15% EtOAc in petroleum ether yielded lupeol (3.3 mg) while β-sitosterol (4.5 mg) was recrystalised from subsequent VLC fractions (20–25% EtOAc in petroleum ether).

The $CHCl_3$ extract (3.5 g) was fractionated by VLC over Si gel 60H (5 g) using petroleum ether–EtOAc and EtOAc–MeOH mixtures (200 ml) of increasing polarity. The eluates were combined together on the basis of TLC analysis. VLC fractions eluted with 20–25% EtOAc in petroleum ether were further subjected to Sephadex column chromatography (20% petroleum ether in $CHCl_3$) to yield **1** (12.7 mg). Gel filtration over Sephadex LH20 (Petroleum ether:$CHCl_3$:MeOH = 2:5:1) on the VLC fractions eluting with 30–35% EtOAc in petroleum ether gave 5.4 mg of **2** and 3.5 mg of **4**. Compounds **3** (2.4 mg) and **5** (7.6 mg) were isolated from the VLC eluted with 50% EtOAc in petroleum ether followed by Sephadex column chromatography (5–10% MeOH in $CHCl_3$ to 100% MeOH) and preparative TLC (mobile phase, 35% EtOAc in petroleum ether).

The MeOH extract (4.5 g) was fractionated by VLC (Si gel 60H, 20 g) using combinations of $CHCl_3$ and MeOH of increasing polarity (200 ml). Gel filtration (100% MeOH) on VLC fraction eluted with 5–10% MeOH in $CHCl_3$ yielded **6** (10.0 mg) while **7** (4.5 mg) was obtained from the VLC fraction eluted with 15–20% MeOH in $CHCl_3$ followed by gel filtration (100% MeOH).

3.4. 5,7-Dihydroxy-2′-methoxy-3′,4′-methylenedioxyisoflavanone (2)

Yellow waxy amorphous solid. $[\alpha]_D^{20}$ − 23.03° (CH_3OH; c 0.14) UV λ_{max}^{MeOH}nm (log ε): 216 (4.11), 219 (4.14), 289 (4.00), 335 (sh) (3.27). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3296, 2914, 1634, 1470, 1388, 1359, 1258, 1229, 1160, 1100, 1068, 976, 926, 832, 754;. ^1H NMR, ^{13}C NMR and HMBC, see Tables 1 and 2. HREIMS *m/z* 330.0745 (calcd for $C_{17}H_{14}O_7$, 330.0740), EIMS 330 (32), 328 (4), 297 (2), 178 (100), 177 (4), 150 (3).

3.5. 4′,5,-Dihydroxy-2′,3′-dimethoxy-7-(5-hydroxyoxychromen-7yl)-isoflavanone (4)

Yellow gum. $[\alpha]_D^{20}$ −11.67° (CH_3OH; *c* 0.034). UV λ_{max}^{MeOH} nm (log ε): 219 (4.24), 259 (3.93), 289 (3.96), 336 (sh) (3.49). IR (KBr) *ν* cm^{-1}: 3350, 1637,1500, 1475, 1385, 1265, 1161, 1072, 1028, 959, 834, 754; ^1H NMR, ^{13}C NMR and HMBC, see Tables 1 and 2. HREIMS *m/z* 492.1036 (calcd for $C_{26}H_{20}O_{10}$, 492.1057), EIMS 492 (12), 462 (3), 332 (100), 331 (4).

3.6. Antimicrobial screening

The antimicrobial assay was performed by a newly developed microdilution titre technique (Drummond and Waigh, 2000; Rahman and Gray, 2005) using 96 well plates. Two Gram-positive bacteria (*Staphylococcus aureus* NCTC10788 and *Bacillus subtilis* NCTC8236), two Gram-negative bacteria (*E coli* NCTC9001 and *Proteus vulgaris* NCTC4175) and two fungi (*Aspergillus niger* NCPF3149 and *Candida albicans* IMI149007) were used as microorganisms in this assay.

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