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# BIFLAVONOIDS FROM FLOWERS OF *BUTEA MONOSPERMA* (LAM.) TAUB.

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**Abstract** – A new aurone glucoside (**1**) and three new biflavonoids (**12** - **14**), together with fourteen known compounds, were isolated from the flowers of *Butea monosperma* (Lam.) Taub. The structures of the new compounds were established by 1D, 2D NMR, MS and CD analyses. The isolated compounds were evaluated for their influenza A neuraminidase inhibitory activity and DPPH free-radical scavenging activity.

## INTRODUCTION

*Butea monosperma* (Lam.) Taub. (Fabaceae), which is commonly known as 'Flame of forest' or 'Palash', is a small to medium-sized deciduous tree widely distributed in tropical southern Asia up to an altitude of 4,000 ft. This plant species has been used as a traditional herbal medicine in Ayurveda and Unani. It's roots have been used for elephatiasis and several eyesight defects, while the bark decoction has been used for the treatment of goiter, ulcer, dysentery, liver disorder and tumors.[1] Flowers have been used as an emmenagogue, diuretic, depurative and tonic, as well as for the treatment of leprosy, gout and skin diseases,[2] spermatorrhoea and leucorrhoea.[3] The flowers of this plant are also used commercially as a natural dyeing due to their bright orange-red color. Several biological activities for the flowers of *B. monosperma* have been reported including antidiabetic,[4] anticonvulsive,[5] antiesterogenic,[6] antioxidative,[7] hepatoprotective,[8] chemopreventive[9] and mast cell inactivation[10] activities, and some of which validated the traditional use of this plants. However, their active components have not been clarified. By contrast, previous phytochemical analysis for the flowers of *B. monosperma* revealed the presence of flavone,[11] flavanones,[12-14] isoflavones,[14] chalcones,[12-14] and aurones.[12,13,15] As part of our chemical studies of

medicinal plants aimed at searching for biologically active compounds, we have examined the flowers of *Butea monosperma*, which has resulted in the isolation of a new aurone glucoside and three new biflavonoids together with fourteen known compounds. The influenza A neuraminidase inhibitory activity and DPPH free-radical scavenging activity for the isolated compounds were also evaluated. The present paper articulates the structure elucidation of new compounds and evaluation of biological activities for the isolated compounds.

## RESULTS AND DISCUSSION

The flowers of *B. monosperma* were extracted with MeOH at room temperature. The MeOH extract was partitioned between EtOAc and water. The EtOAc-soluble fraction was further partitioned between hexane and 90% MeOH. Repeated chromatography of the 90% MeOH-soluble fraction over Sephadex LH-20, MCI gel CHP20P, YMC ODS-A, silica gel and purification by HPLC to afford a new aurone glucoside (**1**) and three new biflavonoids (**12 - 14**), together with fourteen known compounds. The known compounds were identified as sulfurein (**2**),[16] butein (**3**),[17] monospermoside (**4**),[13] isobutrin (**5**),[18] coreopsin (**6**),[19] butin (**7**),[20] isomonospermoside (**8**),[13] formononetin (**9**),[21] aformosin (**10**),[22] kaempferide (**11**),[23] umbelliferon,[24] 7,8-dihydroxychromone,[25] methyl 3,4,5-trihydroxybenzoate[26] and 2,4-dihydroxybenzoic acid[27] by comparison of their physical and spectral data with those reported in the literature. 7,8-Dihydroxychromone was isolated for the first time as a natural product.

Compound **1** gave an $[M–H]^-$ ion peak at *m/z* 431.0978 in the HRESIMS, indicating the molecular formula $C_{21}H_{20}O_{10}$. The $^1$H NMR spectrum of **1** revealed, together with an olefinic proton signal [$\delta_H$ 7.05 (s)], two sets of ABX-type aromatic signals [$\delta_H$ 6.93 (1H, dd, $J$ = 8.4, 1.8 Hz), 7.13 (1H, d, $J$ = 1.8 Hz), and 7.83 (1H, d, $J$ = 8.4 Hz); 7.24 (1H, d, $J$ = 8.3 Hz), 7.67 (1H, dd, $J$ = 8.3, 1.8 Hz), and 8.28 (1H, d, $J$ = 1.8 Hz)], suggesting the presence of two 1,2,4-trisubstituted aromatic rings. It also showed an anomeric proton signal at $\delta_H$ 5.62 (d, $J$ = 7.8 Hz) suggestive of a glycosidic nature of **1**. The $^{13}$C-NMR spectrum indicated the presence of 21 carbons, including 15 $sp^2$ carbons and six $sp^3$ carbons arising from a hexosyl moiety, whose chemical shifts were in good agreement with the occurrence of a glucosyl moiety. These spectral data were closely related to those of sulfurein (**2**), but differed the $^{13}$C chemical shifts for the aromatic signals, suggesting the position of the glucosyl moiety was different. The 2D-NMR analyses indicated the aglycone moiety of **1** to be sulphuretin, which was confirmed by acid hydrolysis of **1** with 5% HCl, which afforded an aglycone, identical with (*Z*)-6,3',4'-trihydroxyaurone,[28] together with glucose. The HMBC correlation of the anomeric proton signal with C-3' indicated the location of the glucosyl moiety to be at C-3'. The β-linkage of the glucosyl moiety was concluded from the coupling constant value (7.8 Hz) of the anomeric proton signal. Thus, the structure of **1** was assigned as sulphuretin 3'-*O*-β-glucopyranoside.

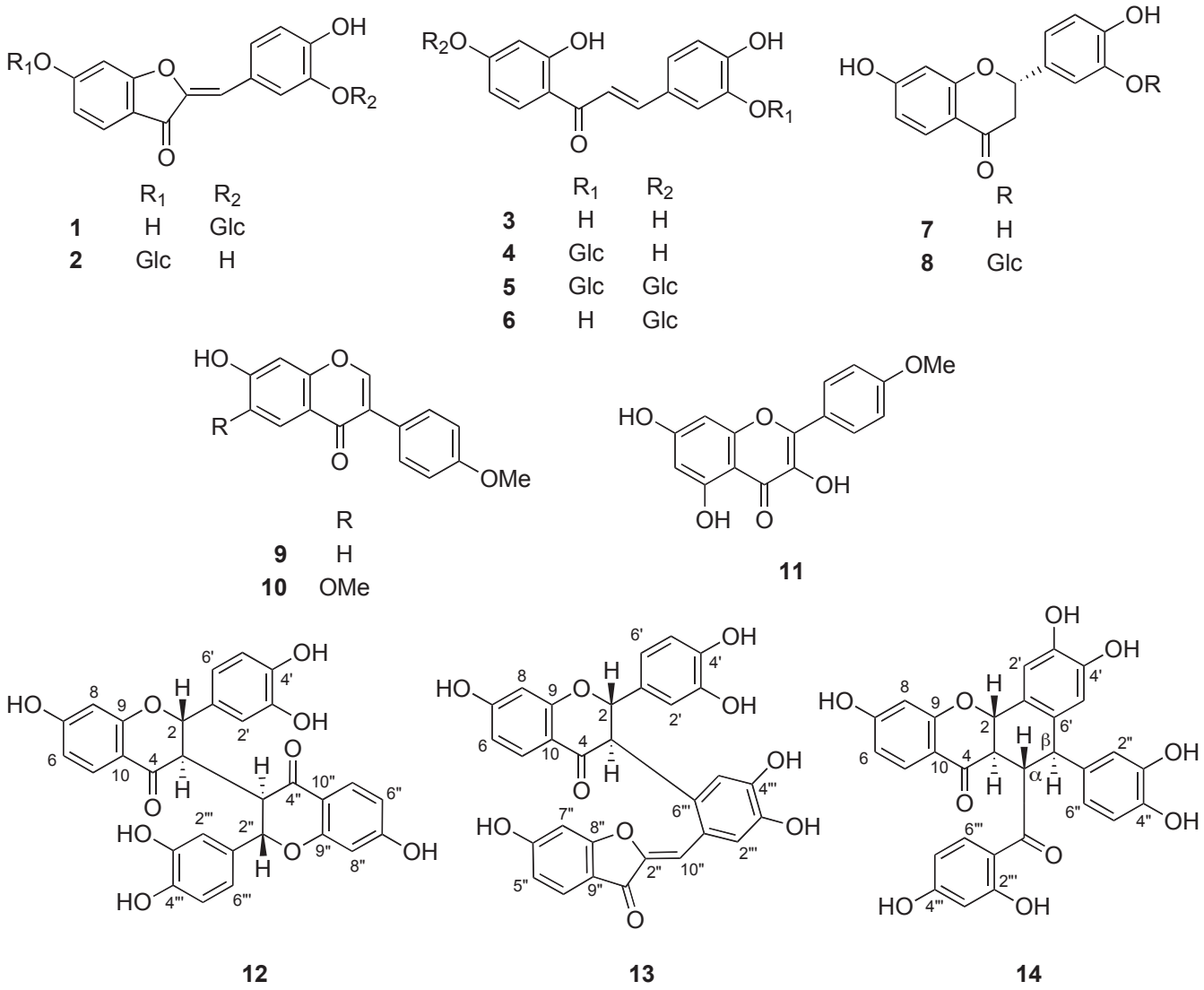


**Figure 1**. The structures of flavonoids isolated from the flowers of *B. monosperma*

An $[M–H]^-$ ion peak at *m/z* 541.1115 (calcd for 541.1135) was observed in the HRESIMS of compound **12**, indicating the molecular formula $C_{30}H_{22}O_{10}$. The $^1$H NMR spectrum showed two sets of ABX-type aromatic signals [$\delta_H$ 8.14 (d, *J* = 8.7 Hz), 6.82 (dd, *J* = 8.7, 2.2 Hz) and 6.66 (d, *J* = 2.2 Hz); 7.31 (d, *J* = 8.1 Hz), 6.99 (dd, *J* = 8.1, 2.0 Hz) and 7.43 (d, *J* = 2.0 Hz)] and a pair of doublets [$\delta_H$ 6.61 and 3.46 (each d, *J* = 11.9 Hz)], while the $^{13}$C NMR spectrum of **12** revealed 15 carbon signals, including a carbonyl carbon ($\delta_C$ 191.6), six sp$^2$ quaternary carbon ($\delta_C$ 114.2, 128.7, 146.8, 147.7, 163.7, and 165.8), six sp$^2$ methine ($\delta_C$ 103.1, 111.0, 116.1, 116.1, 119.6, and 129.2), an oxygen-bearing sp$^3$ methine ($\delta_C$ 84.9) and sp$^3$ methine ($\delta_C$ 51.3). Taking its molecular formula into account, compound **12** was indicated to be a symmetrical biflavanone with a C-3/C-3" linkage. The positions of hydroxyl groups were assigned to be at C-7/C-7″, C-3′/C-3″′ and C-4′/C-4″′ from the HMBC correlations of H-5/H-5" with C-4/C-4", and of H-2'/H-2"' and H-6'/H-6"' with C-2/C-2". The *trans* configuration of H-2/H-3 (H-2″/H-3″) was elucidated from their *J* values (11.9 Hz). The absolute stereochemistry was assigned as 2*S*,3*R* by the CD spectral

analyses, in which the first positive [εΔ CD +11.9 (334 nm)] and the second negative [εΔ CD –17.5 (304 nm)] Cotton effects were similar to those of the related compound.[29] Therefore, the structure of **12** was elucidated as shown.

The molecular formula of compound **13** was assigned as $C_{30}H_{20}O_{10}$ by HRESIMS (*m/z* 539.0940 $[M–H]^-$), suggesting that **13** was also a biflavonoid. The $^1$H NMR spectrum, together with signals closely correlated with those of **12**, showed ABX-type aromatic signals [$\delta_H$ 6.60 (1H, brs), 6.87 (1H, brd, $J$ = 8.6 Hz) and 7.75 (1H, d, $J$ = 8.6 Hz)] and three singlet signals [$\delta_H$ 7.42, 7.52 and 8.29 (each s)] in the aromatic region. The $^{13}$C-NMR spectrum of **13** displayed the presence of 30 carbons, in which 15 signals were closely correlated with those found in **12**. The rest of 15 $^{13}$C resonances were closely correlated with those of sulphuretin, except for the signals of the B-ring moiety, suggesting that **13** is an auronoflavanone-type biflavonoid. Detailed interpretation of the $^1$H-$^1$H COSY, HSQC, and HMBC data indicated that **13** is a biflavonoid, consisting from a butin and a sulphuretin units. The linkage of these units was elucidate to be C-3–C-6''' from the HMBC correlation of H-3 with C-6''' and of H-5''' with C-3. The *Z*-configuration of the aurone moiety was assigned from the $^{13}$C resonance of C-10''.[30] By contrast, the 2,3-*trans* configuration of the butin unit was assigned from the *J*-value of H-2 ($J$ = 11.0 Hz). In addition, the CD spectrum of **13** showed positive (εΔ +2.2 at 332 nm) and negative (εΔ –1.3 at 304 nm) Cotton effects, which were similar to those seen in 3-aryl-flavanone derivatives with a 2*S*,3*R*-configuration.[31] Accordingly, the structure of **13** was elucidated as shown.

A bioflavonoid nature of **14** was revealed by the HRESIMS, which gave an $[M–H]^-$ ion peak at *m/z* 541.1135 (calcd for 541.1135), indicating the molecular formula $C_{30}H_{22}O_{10}$. The $^1$H NMR spectrum revealed, along with three sets of ABX-type aromatic signals and two aromatic signals, four methine signals [$\delta_H$ 5.47 (d, $J$ = 13.0 Hz), 4.03 (d, $J$ = 11.0 Hz), 3.94 (brt, $J$ = 11.0 Hz) and 3.57 (dd, $J$ = 13.0, 11.0

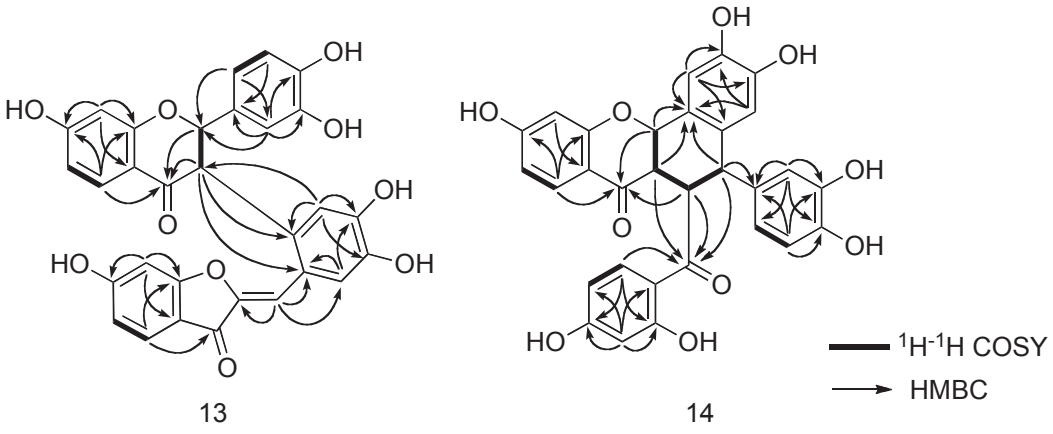


**Figure 2**. Selected 2D NMR correlations for compounds **13** and **14**

Hz)]. The $^{13}$C NMR spectrum exhibited the presence of two carbonyl carbons, six sp$^2$ quaternary carbons, six sp$^2$ methine carbons, one-oxygen-bearing sp$^3$ methine, and three sp$^3$ methines (Table 1). Detailed analyses of the $^1$H-$^1$H COSY, HSQC, and HMBC data indicated the presence of a butin unit and a 3,4,2',4'-tetrahydroxy-dihydrochalcone unit in **14**. In addition, the HMBC correlations of H-3 with C-α, C-β, and CO, and of H-β with C-1' and C-6' indicated the connectivity of these units at C-3–C-α and C-6'–C-β. The 2,3-*trans* configuration of the butin unit was elucidated from the *J*-value of H-2 (*J* = 11.0 Hz). The coupling pattern and *J*-values of H-α (brt, *J* = 11.0 Hz), together with the NOE correlation of H-α with H-2, indicated *trans* configurations of H-3/H-α and H-α/H-β. Compound **14** was considered to

**Table 1.** $^1$H and $^{13}$C NMR spectroscopic data for compounds **12-14**

| | **12**[a] | | **13**[a] | | **14**[b] | |
|---|---|---|---|---|---|---|
| Position | $^1$H[c] | $^{13}$C[d] | $^1$H[c] | $^{13}$C[d] | $^1$H[c] | $^{13}$C[d] |
| 2 | 6.61 (d, 11.9) | 84.9 | 6.00 (d, 11.0) | 85.3 | 5.47 (d, 13.0) | 79.6 |
| 3 | 3.46 (d, 11.9) | 51.3 | 5.15 (d, 11.0) | 54.5 | 3.57 (dd, 13.0, 11.0) | 51.9 |
| 4 | | 191.6 | | 191.1 | | 193.3 |
| 5 | 8.14 (d, 8.7) | 129.2 | 8.18 (d, 8.7) | 129.4 | 7.54 (d, 8.6) | 129.9 |
| 6 | 6.82 (dd, 8.7, 2.2) | 111.0 | 6.87 (brd, 8.7) | 111.1 | 6.45 (brd, 8.6) | 111.8 |
| 7 | | 165.8 | | 165.8 | | 166.5 |
| 8 | 6.66 (d, 2.2) | 103.1 | 6.78 (brs) | 103.1 | 6.46 (d, 1.9) | 103.7 |
| 9 | | 163.7 | | 163.5 | | 165.8 |
| 10 | | 114.2 | | 114.2 | | 114.7 |
| 1′ | | 128.7 | | 129.6 | | 126.3 |
| 2′ | 7.43 (d, 2.0) | 116.1 | 7.52 (bs) | 115.3 | 7.14 (s) | 112.9 |
| 3′ | | 146.8 | | 146.4 | | 145.0 |
| 4′ | | 147.7 | | 146.5 | | 145.4 |
| 5′ | 7.31 (d, 8.1) | 116.1 | 7.07 (d, 8.0) | 115.5 | 6.23 (s) | 116.8 |
| 6′ | 6.99 (dd, 8.1, 2.0) | 119.6 | 7.12 (brd, 8.0) | 119.2 | | 131.9 |
| 1″ | | | | | | 136.1 |
| 2″ | | | | 146.9 | 6.44 (d, 1.7) | 117.2 |
| 3″ | | | | 181.7 | | 145.4 |
| 4″ | | | 7.75 (d, 8.6) | 125.4 | | 145.0 |
| 5″ | | | 6.87 (brd, 8.6) | 112.7 | 6.52 (d, 8.1) | 116.2 |
| 6″ | | | | 167.6 | 6.34 (dd, 8.1, 1.7) | 121.9 |
| 7″ | | | 6.60 (brs) | 98.6 | | |
| 8″ | | | | 166.9 | | |
| 9″ | | | | 113.7 | | |
| 10″ | | | 7.52 (brs) | 108.6 | | |
| 1‴ | | | | 130.6 | | 115.9 |
| 2‴ | | | 8.29 (s) | 118.2 | | 165.4 |
| 3‴ | | | | 148.8 | 6.13 (d, 2.3) | 103.0 |
| 4‴ | | | | 145.7 | | 165.8 |
| 5‴ | | | 7.42 (s) | 118.2 | 6.04 (dd, 8.9, 2.3) | 108.3 |
| 6‴ | | | | 123.3 | 7.21 (d, 8.9) | 134.4 |
| α | | | | | 3.94 (brt, 11.0) | 47.6 |
| β | | | | | 4.03 (d, 11.0) | 51.3 |
| CO | | | | | | 207.7 |

[a] Measured in pyridine-$d_5$. [b] Measured in $CD_3OD$. [c] δ ppm (mult., *J* in Hz), 500 MHz. [d] δ ppm, 125 MHz.

be racemic from its small optical rotation value and CD spectrum, in which obvious Cotton effect was not observed. Consequently, the structure of **14** was characterized as shown.

Compounds **13** and **14** were an auronoflavanone-type and a chalconoflavanone-type biflavonoid, respectively, with new types of linkage.

Influenza A neuraminidase inhibitory activity and DHPP free-radical scavenging activity for the isolated compounds were evaluated. In an influenza A neuraminidase inhibitory assay, compound **3** displayed relatively potent inhibitory activity with an $IC_{50}$ value of 5.4 μgmL. Compounds **1**, **4**, and **6** showed moderate influenza A neuraminidase inhibitory activity with $IC_{50}$ values ranging from 28.5 to 34.9 μg/mL. By contrast, compound **14** showed potent activity in a DPPH free-radical scavenging assay with an $IC_{50}$ value of 1.7 μg/mL. Compounds **3**, **7**, **13** and 7,8-dihydroxychromone also showed relatively potent activity with $IC_{50}$ values ranging from 3.5 to 9.8 μg/mL.

**Table 2.** Influenza A neuraminidase inhibitory activity and DPPH free-radical scavenging activity of compounds isolated from the flowers of *B. monosperma*

| | Anti-influenza neuraminidase activity $IC_{50}$ (μg/mL)[a] | DPPH free-radical scavenging activity $IC_{50}$ (μg/mL)[a] |
|---|---|---|
| **1** | 32.0 ± 2.7 | > 50 |
| **2** | > 100 | 14.9 ± 0.4 |
| **3** | 5.4 ±0.3 | 5.4 ± 0.3 |
| **4** | 28.5±2.8 | 18.9 ± 0.3 |
| **5** | > 100 | > 50 |
| **6** | 34.9 ±4.2 | 26.2 ± 0.3 |
| **7** | 89.9±4.3 | 3.5 ± 0.2 |
| **8** | > 100 | > 50 |
| **9** | > 10 | > 50 |
| **10** | > 10 | > 50 |
| **12** | 67.8 ±2.9 | 12.5 ± 0.4 |
| **13** | > 100 | 9.8± 0.2 |
| **14** | > 100 | 1.7 ± 0.1 |
| 7,8-dihydroxychromone | > 100 | 7.0 ± 0.1 |
| oseltamivir carboxylate | 0.002 ±0.0002 | |
| ascorbic acid | | 7.3 ± 0.4 |

[a] Data are mean ± SE from three or four experiments.
[b] Owing to the high fluorescence intensity from the sample, assay was performed at the highest concentration of 10 μg/mL.

## EXPERIMENTAL

### General experimental procedures

Optical rotations were measured on a JASCO P-2200 polarimeter. NMR spectra were recorded on a Bruker AVANCE-500 instrument ($^1$H NMR: 500 MHz, $^{13}$C NMR: 125 MHz) using TMS as an internal standard. HRESIMS were obtained on a Waters LCT Premier. Column chromatography was performed with silica gel 60N (63-210 μm, Kanto Kagaku), Sephadex LH-20 (25 – 100 μm, GE Health Care), Toyo pearl HW-40C (TOSOH Corporation), MCI gel CHP20P (75-150 μm; Mitsubishi Chemical), YMC-pack ODS-A (S-50 μm, YMC Co., Ltd). HPLC was performed on a JASCO apparatus consisting of a PU-980 prep pump, UV-970UV/VIS (at the wavelength of 280nm) and RI-930 refractive index detectors, and a Mightysil RP-18 GP (250 × 20 mm; 5 μm, Kanto Chemical) or a COSMOSIL πNAP (5 μm, ϕ20×250 mm, nacalai tesque) column. TLC was conducted on precoated silica gel 60 $F_{254}$ (0.20 mm, Merck) and spots were detected by UV illumination and by spraying 10% sulfuric acid reagent followed by heating.

### Plant Material

The flowers of *Butea monosperma* (Lam.) Taub. were collected in July, 2007 at the Jahangirnagar University campus, Savar, Dhaka. Identification was carried out by Mr. Sardar Nasir Uddin (Bangladesh National Herbarium, Dhaka, Bangladesh). A voucher specimen (DACB acc. no. 34193) was deposited at the Graduate School of Pharmaceutical Sciences, University of Tokushima.

### Extraction and Isolation

The dried flowers of *B. monosperma* (2.6 kg) were extracted three times with MeOH (5 L x 3) at room temperature. The MeOH extract was concentrated under reduced pressure to give a residue (450 g), which was partitioned with EtOAc and $H_2O$. The EtOAc layer, after removal of the solvent by evaporation, was further partitioned with *n*-hexane and 90% aqueous MeOH to give a hexane-soluble fraction (51.6 g) and a 90% MeOH-soluble fraction (93.8 g). The 90% MeOH-soluble fraction was subjected to chromatography over Sephadex LH-20 with $H_2O$ containing increasing amounts of MeOH to give twelve fractions (frs. 1.1–1.12). Fr. 1.3 (30.7 g) was subjected to MCI gel CHP20P [$H_2O$–MeOH (1:0→0:1)] to furnish 12 fractions (frs. 1.3.1–1.3.12). Fr. 1.3.4 (2.8 g) was further fractionated by YMC ODS-A [$H_2O$–MeOH (0:1→1:0)] chromatography into nine fractions (frs. 1.3.4.1–1.3.4.9). Sephadex LH-20 chromatography [$H_2O$–MeOH (1:0→1:4)] of fr. 1.3.4.4 (392 mg) followed by crystallization gave 7,8-dihydroxychromone (12 mg) and methyl 3,4,5-trihydroxybenzoate (25 mg). 2,4-Dihydroxybenzoic acid (10 mg) was obtained by repeated chromatography of fr. 1.3.4.7 (163 mg) over Sephadex LH-20 [$H_2O$–MeOH (1:0→4:1)] and silica gel [$CHCl_3$–MeOH–$H_2O$ (10:1:0.1)]. Chromatography of fr. 1.3.4.6 (1.5 g) with an ODS-A column [$H_2O$–MeOH (4:1→0:1)] followed by silica gel chromatography

[$CHCl_3$–MeOH–$H_2O$ (10:1:0.1)] gave compound **6** (8 mg). Sephadex LH-20 chromatography [$H_2O$–MeOH (1:0→0:1)] of fr. 1.3.4.8 (163 mg) afforded seven fractions (frs. 1.3.4.8.1–1.3.4.8.7). Fr. 1.3.4.8.2 was repeatedly separated by ODS-A [$H_2O$–MeOH (1:0→3:7)] and silica gel [$CHCl_3$–MeOH–$H_2O$ (10:1:0.1)] chromatography to furnish compounds **5** (12 mg) and **8** (8.5 mg). Fr. 1.3.4.8.5 was purified by silica gel chromatography [$CHCl_3$–MeOH–$H_2O$ (10:1:0.1)] to furnished compound **2** (14 mg). Purification of fr. 1.3.4.9 by silica gel chromatography [$CHCl_3$–MeOH (10:1)] afforded compound **4** (6 mg). Fr 1.3.5 (14 g) was subjected to ODS-A chromatography [$H_2O$–MeOH (1:0→0:1)] to give eight fractions (fr. 1.3.5.1–1.3.5.8). Chromatography of fr. 1.3.5.5 (10 g) over Sephadex LH-20 with EtOH yielded five fractions (frs. 1.3.5.5.1–1.3.5.5.5). Purification of fr. 1.3.5.5.2 (65.5 mg) with a silica gel [$CHCl_3$–MeOH (8:2)] column gave umbelliferone (35.5 mg). Fr. 1.5 (15 g) was rechromatographed over MCI gel CHP20P [$H_2O$–MeOH (7:3→0:1)] to furnish ten fractions (frs.1.5.1–1.5.10). Fraction 1.5.4 (700 mg) was further fractionated by ODS-A chromatography [$H_2O$–MeOH (1:0→0:1)] into further seven fractions (frs. 1.5.4.1–1.5.4.7). Sephadex LH-20 chromatography [$H_2O$–MeOH (1:0→1:1)] of fr. 1.5.4.7 (185 mg), followed by crystallization (from EtOAc) gave compound **1** (7.9 mg). Fr. 1.5.5 (8.5 g) was chromatographed over ODS-A [$H_2O$–MeOH (3:2→0:1)] to give eight fractions (frs. 1.5.5.1–1.5.5.8). Crystallization of fraction 1.5.5.4 (from EtOAc) gave compound **7** (28 mg). Fr. 1.5.5.6 (1.6 g) was further chromatographed over Sephadex LH-20 [$H_2O$–MeOH (1:0→0:1)] to give six fractions (frs. 1.5.5.6.1–1.5.5.6.6). ODS-A chromatography [$H_2O$–MeOH (3:2→0:1)] of fr. 1.5.5.6.6 (50 mg) followed by crystallization (from $H_2O$) gave compound **3** (14.5 mg). Fr. 1.5.8 (500 mg) was fractionated by ODS-A chromatography [$H_2O$–MeOH (1:1→0:1)] to give six fractions (frs. 1.5.8.1–1.5.8.6). Fr. 1.5.8.6, consisted mainly of a mixture of compounds **9** and **10**, which was separated by HPLC on Mightysil RP-18 [MeOH–1% AcOH (1:2)] and pure samples were crystallized from $H_2O$ in each case; **9** (9.2 mg) and **10** (11.4 mg). Fr. 1.10 (5.3 g) was chromatographed over MCI gel CHP20P [$H_2O$–MeOH (1:0→0:1)] to afford eight further fractions (1.10.1–1.10.8). Fr. 1.10.4 (1.0 g) was separated by ODS-A chromatography [$H_2O$–MeOH (1:1→1:4)] to give three fractions (1.10.4.1–1.10.4.3). Further fractionation of fr. 1.10.4.2 (700 mg) by Sephadex LH-20 [$H_2O$–MeOH (1:0→0:1)] afforded seven fractions (1.10.4.2.1–1.10.4.2.6). Fr. 1.10.4.2.6 was crystallized from $H_2O$ giving compound **12** (39 mg). Sephadex LH-20 chromatography [$H_2O$–MeOH (4:1→0:1)]) of fr.1.10.4.2.4 (50 mg) followed by HPLC on Mightysil RP-18 [MeOH-1% AcOH (2:3)] gave compound **13** (6.1 mg). Fr. 1.10.4.3 (170 mg) was chromatographed over Sephadex LH-20 [$H_2O$–MeOH (4:1→0:1)] and then purified by ODS HPLC (Mightysil RP18) [MeOH–1%AcOH (2:3)] to yield compound **14** (19.7 mg). Fr. 1.10.7 (1.6 g) was repeatedly chromatographed over ODS-A [$H_2O$–MeOH (7:3→0:1)] and silica gel [$CHCl_3$–MeOH–$H_2O$ (10:1:0.1)] to furnish compound **11** (19 mg).

**Sulphuretin 3'-*O*-β-glucopyranoside (1)**: Yellowish brown amorphous powder; $[\alpha]^{20}_{D}$ –16.5 (*c* 0.11, MeOH); IR (KBr) $\nu_{max}$ 3400, 1628, 1513 $cm^{-1}$; $^{1}H$ NMR (500 MHz, pyridine-$d_5$): δ 4.13 (1H, m, H-5˝), 4.24 (1H, m, H-2˝), 4.32 (1H, m, H-3˝), 4.35 (1H, m, H-4˝), 4.42 (1H, dd, 12.0, 4.8 Hz, H-6˝ ), 4.55 (1H, dd, 12.0, 2.2 Hz, H-6˝), 5.62 (1H, d, *J* = 7.8 Hz, H-1˝), 6.93 (1H, dd, *J* = 8.4, 1.8 Hz, H-5), 7.05 (1H, s, H-10), 7.13 (1H, d, *J* = 1.8 Hz, H-7), 7.24 (1H, d, *J* = 8.3 Hz, H-5´), 7.67 (1H, dd, *J* = 8.3, 1.8 Hz, H-6´), 7.83 (1H, d, *J* = 8.4 Hz, H-4), 8.28 (1H, d, *J* = 1.8 Hz, H-2´); $^{13}C$ NMR: (125 MHz, pyridine-$d_5$): δ 61.4 (C-6˝), 70.3 (C-4˝), 74.1 (C-2˝), 77.6 (C-3˝), 78.3 (C-5˝), 98.8 (C-7), 103.5 (C-1˝), 111.0 (C-10), 112.9 (C-5), 113.4 (C-9), 116.9 (C-5´), 120.7 (C-2´), 124.0 (C-1´), 125.2 (C-4), 127.6 (C-6´), 146.1 (C-3´), 146.4 (C-2), 150.5 (C-4´), 166.9 (C-8), 168.0 (C-6), 181.5 (C-3); HRESIMS: *m/z* 431.0941$[M–H]^-$ (calcd for $C_{21}H_{19}O_{10}$, 431.0978).

**Acid hydrolysis of 1:** Compound **1** (2 mg) was hydrolyzed with 10% HCl for 3 h at reflux. The reaction mixture was diluted with $H_2O$, and extracted with EtOAc. The EtOAc-soluble fraction was purified by ODS on HPLC to afford an aglycone (0.8 mg), which was identified as (*Z*)-6,3',4'-trihydroxyaurone by spectral comparison. The aqueous layer was neutralized with Amberlite IRA-400 resin and was directly analyzed by TLC to detect glucose [$R_f$: 0.35, *n*-BuOH/Pyridine/$H_2O$ (6:4:3), Avicel SF cellulose].

**Compound 12:** Pale yellow granules; mp 222-228 °C; $[\alpha]^{20}_{D}$ +92.3 (*c* 0.18, MeOH); IR (KBr) $\nu_{max}$ 3380, 1649, 1604, 1531, 1462 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR data: Table 1; CD (MeOH; $1.84 \times 10^{-5}$ M, εΔ) $\lambda_{max}$ 334 (+11.9), 304 (–17.5) ; HRESIMS: *m/z* 541.1115 $[M–H]^-$ (calcd for $C_{30}H_{21}O_{10}$, 541.1135).

**Compound 13**: Pale orange amorphous powder; $[\alpha]^{20}_{D}$ +5.3 (*c* 0.07, MeOH); IR (KBr) $\nu_{max}$ 3430, 1630, 1500 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR: see Table 1; CD (MeOH; $1.84 \times 10^{-5}$ M, εΔ) $\lambda_{max}$ 332 (+2.2), 304 (-1.3); HRESIMS: *m/z* 539.0940 $[M–H]^-$ (calcd for $C_{30}H_{19}O_{10}$, 539.0978).

**Compound 14:** Yellow granules; mp 220-225 °C; $[\alpha]^{20}_{D}$ +5.6 (*c* 0.1, MeOH); IR (KBr) $\nu_{max}$ 3445, 1665, 1605, 1517 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR: see Table 1; HRESIMS: *m/z* 541.1135 $[M–H]^-$ (calcd for $C_{30}H_{21}O_{10}$, 541.1135).

**Viruses and cells**

Madin-Darby canine kidney (MDCK) cells were routinely passaged in the DMEM supplemented with 10% FBS and 100 μg/mL kanamycin. Influenza A/PR/8/34 (H1N1) was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Virus-infected cells were maintained in DMEM supplemented with 0.5% bovine serum albumin. Influenza viruses were propagated in MDCK cells.

**Influenza A neuraminidase inhibition assay**

A modified fluorometric assay using the fluorogenic substrate 2'-(4-methylumbellyferyl)-α-D-*N*-acetyl-neuraminic acid (MUNANA, Sigma) was used to determine the neuraminidase activity (NA) on the type A (H1N1) viruses.[32] All compounds were dissolved in DMSO and diluted to the corresponding concentrations in PBS. A 96-well plate containing a mixture of the diluted virus suspension ($1\times10^5$ PFU, 50 μL) and the different concentration of compound solution (50 μL) was incubated on ice for 1h. MUNANA substrate solution [0.2 mM in 0.1 M acetate buffer (pH 4.6), 25 mL] was added and the mixture was incubated for 30 min at 37 ˚C. The enzymatic reaction was quenched by adding 100 μL of glycine-NaOH buffer solution (pH 10.7). The fluorescence intensity of the product (4-methylumbellyferone) was measured in a spectrophotometer with excitation and emission wavelengths of 360 and 440 nm, respectively. Oseltamivir carboxylate was used as a positive control. The drug concentrations required to inhibit 50% of the NA ($IC_{50}$) were determined by plotting the percent inhibition of NA as a function of the drug concentrations.[33]

**Determination of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical**

This assay was performed using a previously reported method with some minor modifications.[34] The mixtures containing different concentration of test samples (in EtOH 100 μL) and DPPH ethanolic solution (60 μM, 100 μL) were allowed to react in a 96-well microplate. The plate was vigorously vortex mixed at room temperature for 30 min. Reduction of the DPPH free radical was measured by the absorbance at 540 nm using a UV-vis microplate reader. L-Ascorbic acid was used as a positive control. The scavenging activity was expressed in terms of the concentration of samples, which scavenged free radical by 50% ($IC_{50}$).

**REFERENCES**

1. A. K. Sharma and N. Deshwal, *International Journal of PharmTech Research*, 2011, **3**, 864.
2. D. A. Burli and A. B. Khade, *Pharmacognosy Reviews*, 2007, **1**, 333.
3. M. V. Patil, S. Pawar, and D. A. Patil, *Nat. Prod. Rad.*, 2006, **5**, 323.
4. R. Somani, S. Kasture, and A. K. Singhai, *Fitoterapia*, 2006, **77**, 86.
5. V. S. Kasture, S. B. Kasture, and C. T. Chopde, *Pharmacol. Biochem. Behav*., 2002, **72**, 965.
6. S. Ramachandran, Y. Sridhar, S. K. G. Sam, M. Saravanan, J. T. Leonard, N. Anbalagan, and S. K. Sridhar, *Phytomedicine*, 2004, **11**, 165.
7. A. Sehrawat and S. Sultana, *Asian Pac. J. Cancer Prev.*, 2006, **7**, 140.
8. H. Wagner, B. Geyer, M. Fiebig, Y. Kiso, and H. Hikino, *Planta Med.*, 1986, **52**, 77.
9. T. Choedon, S. K. Shukla, and V. Kumar, *J. Ethnopahrmacol.*, 2010, **129**, 208.

10. Z. Rasheed, N. Akhtar, A. Khan, K. A. Khan, and T. M. Haqqi, *J. Pharmacol. Exp. Ther.*, 2010, **333**, 354.
11. R. N. Yadava and L. Tiwari, *J. Enzyme Inhib. Med. Chem*., 2007, **22**, 497.
12. B. Puri and T. R. Seshadri, *J. Chem. Soc.*, 1955, 1589.
13. S. R. Gupta, B. Ravindranath, and T. R. Seshadri, *Phytochemistry*, 1970, **9**, 2231.
14. R. Chokchaisiri, C. Suaisom, S. Sriphota, A. Chindaduang, T. Chuprajob, and A. Suksamrarn, *Chem. Pharm. Bull.*, 2009, **57**, 428.
15. S. Oberoi and L. Ledwani, *Arch. Appl. Sci. Res.*, 2010, **2**, 68.
16. J. Wang, N. Wang, X. Yao, and S. Kitanaka, *Asian Journal of Traditional Medicine*, 2007, **2**, 23.
17. Y. P. Chen, L. Liu, Y. H. Zhou, J. Wen, Y. Jiang, and P. F. Tu, *Journal of Chinese Pharmaceutical Sciences*, 2008, **17**, 82.
18. A. N. Kesari, R. K. Gupta, and G. Watal, *Phytochemistry*, 2004, **65**, 3125.
19. A. R. Jassbi, P. Singh, V. Krishna, P. K. Gupta, and S. Tahara, *Chem. Nat. Compd.*, 2004, **40**, 250.
20. K. M. Davies, S. J. Bloor, G. B. Spiller, and S. C. Deroles, *Plant J.*, 1998, **13**, 259.
21. W. Tolleson, D. R. Doerge, M. I. Churchwell, M. M. Marques, and D. W. Roberts, *J. Agric. Food Chem.*, **50**, 4783.
22. P. Lebreton, K. R. Markham, W. T. Swift, O. Boran, and T. J. Mabry, *Phytochemistry*, 1967, **6**, 1675.
23. J. Lu, Y. Jin, G. Liu, N. Zhu, M. Gui, A. Yu, and X. Li, *Chem. Nat. Compd.*, 2010, **46**, 205.
24. U. Afeka, J. Orensteina, S. Carmelib, V. Rodovc, and M. B. Joseph, *Phytochemistry*, 1999, **50**, 1129.
25. C. Christophe, T. Anne, and B. Herwig, European Patent, DE 10 2006004 327 ($A_1$), 2007.
26. C. J. M. Kane, J. H. Menna, and Y. C. Yeh, *Bioscience Reports*, 1988, **8**, 85.
27. K. N. Scott, *J. Am. Chem. Soc.*, 1972, **94**, 8564.
28. G. M. V. Júnior, C. M. de M. Sousa, A. J. Cavalheiro, J. H. G. Lago, and M. H. Chaves, *Helv. Chim. Acta*, 2008, **91**, 2159.
29. E. Nyandat, A. Hassanali, Y. D. Vicente, G. Multari, and C. Galeffi, *Phytochemistry*, 1990, **29**, 2361.
30. A. Pelter, R. S. Ward, and H. G. Heller, *J. Chem. Soc., Perkin Trans. 1*, 1979, 328.
31. R. Bekker, E. V. Brandt, and D. Ferreira, *J. Chem. Soc., Perkin Trans. 1*, 1996, **24**, 2535.
32. R. W. Myers, R. T. Lee, Y. C. Lee, G. H. Thomas, L. W. Reynolds, and Y. Uchida, *Anal. Biochem.*, 1980, **101**, 166.
33. L. Reed and H. Muench, *Am. J. Hygiene*, 1938, **27**, 493.
34. N. Rangkadilok, S. Sitthimonchai, L. Worasuttayangkurn, C. Mahidol, M. Ruchirawat, and J. Satayavivad, *Food. Chem. Toxicol.*, 2007, **45**, 328.