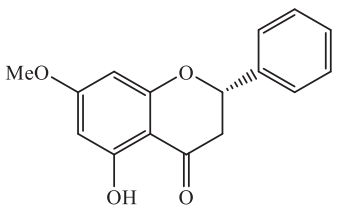
Discovery of bioactive phytoconstituents from medicinal plants collected in Myanmar

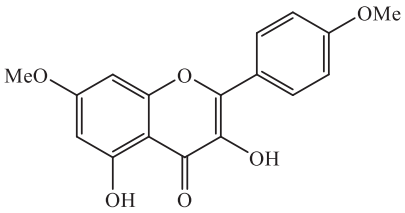
Name of dispatched researcher （派遣研究者名）	Prema	Demonstrator
Instructor （指導教官，研究指導者）	Hla Ngwe	Professor and Head of Department of Chemistry
Affiliation of Instructor （所属）	Department of Chemistry, University of Yangon,Myanmar	
Host Collaborator （受入研究者）	Hiroyuki Morita	教授

Abstract

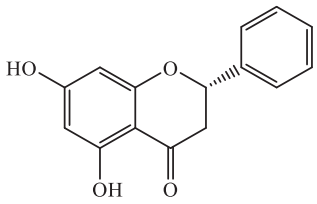
Phytochemical investigation of chloroform extract of rhizomes of the *Boesenbergia rotunda* led to the isolation of seven known compounds such as pinostrobin (**1**), 4', 7-dimethyl kaempferol (**2**), pinocembrin (**3**), tectochrysin (**4**), galanal A (**5**), galanal B (**6**), and quercentin 3,7,4'-trimethylether (**7**). Their structures were established on the basis of NMR spectroscopic analysis and by comparing with the reported data. In addition, methanol and chloroform crude extracts and four isolates (**1**, **2**, **5**, **6**) were tested for their cytotoxicity against lung cancer (LK-2, A549), stomach cancer (ECC4), breast cancer (MCF7), cervix cancer (HeLa), prostate



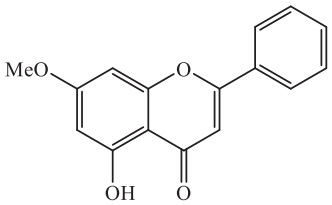
Pinostrobin (**1**)



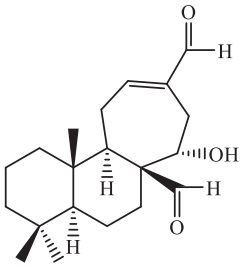
4',7-Dimethoxy kaempferol (**2**)



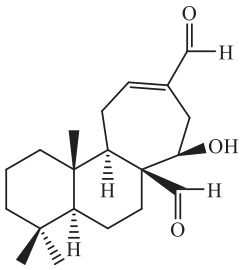
Pinocembrin (**3**)



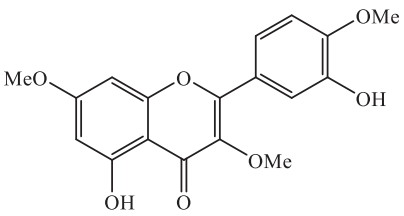
Tectochrysin (**4**)



Galanal A (**5**)



Galanal B (**6**)



Quercetin 3,7,4′trimethyl ether (**7**)

Fig. 1. Structures of isolated compounds (**1**–**7**) from *Boesenbergia rotunda.*

cancer (DU145), and normal human fibroblast (WI-38) cell lines. Among the tested compounds, galanal A (**5**) and B (**6**) exhibited cytotoxicity with IC_{50} values ranging between 5.98 ~38.25 μg/mL.

Keywords: Boesenbergia rotunda, diterpenoids, galanal A, galanal B, flavonoids, cytotoxicity.

1. Introduction

Cancer is a leading cause of disease worldwide. In the anticancer drug discovery, natural products characterized from medicinal plants play an important role. Vincristine, irinotecan, etoposide, and paclitaxel are examples of plant-derived compounds that are being employed in cancer treatment.[1] According to GLOBOCAN 2012 report, the five most frequent cancers in Myanmar were lung, breast, cervix uteri, stomach, and liver. Many of Myanmar medicinal plants have been used to prevent alleviate and cure human disease by Myanmar people for time immemorial. In our ongoing research for the discovery of anticancer agents from Myanmar medicinal plants, we have screened crude extracts of Myanmar medicinal plants against seven different human cancer cell lines, including lung (LK-2, A549), stomach (ECC4), breast (MCF7), cervix (HeLa), prostate (DU145) human cancer, and normal human fibroblast (WI-38) cell lines. Among the crude extracts we have screened, $CHCl_3$-soluble extract of *Boesenbergia rotunda* exhibited potent cytotoxicity. *B. rotunda* is a perennial plant belongs to the Zingiberaceae family. In Myanmar, it is locally known as Seik-phoo-chin. The appearance of rhizomes of *B. rotunda* looks similar to *B. pandurata*. However, the taste is a little bit different from that of *B. pandurata*. *B. rotunda* is distributed in tropical Asia including Myanmar, Bangladesh, Thailand, Cambodia, Vietnam, Malaysia, and Indonesia.[2] The rhizomes are considered to be anthelmintic and depurative. They are used to treat a range of conditions including colic, asthma, cough, obesity, and rheumatism. They are used externally to treat skin conditions such as scurvy and itchy skins. They have been suggested as an application for treating mental derangement. In Myanmar, Indonesia and Thailand, rhizomes are the chief ingredient of a decoction given to women after childbirth to accelerate the lochia. Furthermore, the rhizome is popular for the treatment of stomach cancer. The reported major constituents were cyclohexenyl chalcones and flavonoids. However, our study on this plant suggested that the rhizome was lack of those reported compounds. The major constituents recently we obtained from this rhizome were labdane diterpenoids and flavonoids.

2. Materials and Methods

2.1 Plant material

The rhizomes of *B. rotunda* (Seik-phoo-chin) were collected from Kyauk Tan Village, Bago Region during September, 2016 and were identified and authenticated at Department of Botany, University of Yangon. The samples were dried under the shade for a week, cut into very small pieces and made powder.

2.2 General experimental procedures

The NMR spectra were recorded at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR), respectively, on a JEOL ECA500II spectrometer. Chemical shift values were expressed in δ (ppm) downfield from TMS as internal standard. Open column chromatography was performed with normal-phase silica gel 60N, spherical, neutral, 40−50 μm, (Kanto Chemical Co., Inc., Japan) and Cosmosil 75C18-OPN (Nacalai Tesque Inc., Kyoto, Japan). Analytical TLC was carried out on precoated silica gel $60F_{254}$ and RP-18 F_{254} plates (0.25 or 0.50 mm thickness,Merck). The cell lines LK-2, A549, HeLa, ECC 4 MCF 7, DU 145, and WI-38 were available and maintained in our laboratory. Cell culture flasks and 96-well plates were from Corning (NY, USA).

2.3 Preparation of Crude Extracts

The dried rhizome powder of *B. rotunda* (ca. 200 g) was extracted with 70% ethanol (1 L) under sonication for one hour and filtered. This procedure was repeated five times. The combined filtrate was concentrated under vacuum rotatory evaporator to obtain 22.5.g of 70% ethanol crude extract. The ethanol crudeextract was then extracted with chloroform by sonication to effort the condensed chloroform extract (5.11 g), which was kept for isolation of phytoconstituents.

2.4 Separation and Isolation of Secondary Metabolites

The $CHCl_3$ extract (5 g) was separated by column chromatography over silica gel with a *n*-hexane:EtOAc gradient system, (90:10 to 0:100, v/v) followed by MeOH elution to obtain twelve mainfractions. Fraction 3 (564.8 mg) was rechromatographed on silica gel with *n*-hexane:CH_2Cl_2:EtOAc solvent system to give seven subfractions. Subfraction $F_{3\text{-}1}$ was subjected to normal-phase preparative TLC with C_6H_6:CH_2Cl_2 (2:1 v/v) to give compound **1** (15.8 mg) as yellow amorphous and compound **2** (15 mg) as yellow needle crystals. Fraction 4 (324.5 mg) was rechromatographed on silica gel open column eluted with the same solvent system to obtain six further subfractions. Compound **3** (4.2 mg) as colourless crystal was isolated from subfraction $F_{4\text{-}5}$ using normal-phase preparative TLC with *n*-hexane:CH_2Cl_2:EtOAc (20:20:1). Fraction 6 (1.35 g) was rechromatographed on cosmosil 75C18-OPN with MeOH:H_2O (15:1 v/v) to give 8 subfractions. Subfraction $F_{6\text{-}3}$ was separated by normal-phase preparative TLC with *n*-hexane:CH_2Cl_2:EtOAc (20:20:1) to give compound **4** (3 mg). Subfraction $F_{6\text{-}8}$ was purified by Sephadex LH 20 with MeOH followed by normal-phase preparative TLC with n-hexane:CH_2Cl_2:EtOAc (2:2:1 v/v) to afford compound **5** (25.7 mg) and compound **6** (50 mg), and Fraction 7 (2.43 g) was rechromatographed on cosmosil 75C18-OPN with MeOH:H_2O (15:1 v/v) to give 3 subfractions. Subfraction $F_{7\text{-}2}$ was separated by RP silica gel with MeOH: H_2O (5:1 v/v) to obtain compound **7** (3.1 mg) as pale yellow amorphous solid, which was recrystallized by $CHCl_3$: MeOH (5:1).

Pinostrobin (**1**). Yellow amorphous solid, ^{1}H NMR, $CDCl_3$, δ ppm: 2.85 (1H, dd, J = 17.0, 3.0 Hz, H-3a), 3.12 (1H, t, H-3b), 3.81 (3H, s, 7-OCH_3), 5.44 (1H, dd, J = 13.0, 3.0 Hz, H-2), 6.08 (2H, d, J = 2.3 Hz, H-6, 8), 7.47 (5H, m, H-2', 3', 4', 5', 6'), 12.02 (1H, s, 5-OH). The ^{13}C NMR, $CDCl_3$, δ ppm: 43.3 (C-3), 55.1 (7-OCH_3), 79.2 (C-2), 94.2 (C-8), 95.1 (C-6), 103.1 (C-10), 126.1 (C-2', C-6'), 128.8 (C-3', C-4', C-5'), 138.3 (C-1'), 162.7 (C-5), 164.1 (C-9), 167.9 (C-7), 195.4 (C-4).

4',7-Dimethyl kaempferol (**2**). Yellow needle, ^{1}H NMR, $CDCl_3$, δ ppm: 3.89 (6H, s, 4', 7-OCH_3), 6.38 (1H, d, J = 2.2 Hz, H-6), 6.49 (1H, d, J = 2.2 Hz, H-8), 7.04 (2H, d, J = 9.1 Hz, H-3', 5'), 8.19 (2H, d, J = 9.1 Hz,H-2', 6'), 11.72 (1H, s, 5-OH). The ^{13}C NMR, $CDCl_3$, δ ppm: 55.5 (4'-OCH3), 55.9 (7-OCH3), 92.2 (C-8), 97.9 (C-6), 104.0 (C-10), 114.2 (C-2', C-6'), 123.2 (C-1'), 129.4 (C-3', C-5'), 135.7 (C-3), 145.7 (C-2), 156.9 (C-9), 160.8 (C-5), 165.8 (C-7), 175.2 (C-4) .

Pinocembrin (3). Colourless crystal, ^{1}H NMR, CD_3OD, δ ppm: 2.69 (1H, dd, J=17, 3.0 Hz, H-3_{cis}), 3.02 (1H, dd, J =13, 3.0 Hz, H-3_{trans}), 5.37 (1H, d, J = 13, 3.0 Hz, H-2), 5.79 (1H, d, J = 2.0 Hz, H-8), 5.83 (1H, d, J =2.0 Hz, H-6), 7.24-7.40 (5H, m, H-2', 3', 4', 5', 6'). The ^{13}C NMR, $CDCl_3$, δ ppm: 44.5 (C-3), 80.4 (C-2), 96.3 (C-8), 97.2 (C-6), 103.3 (C-10), 127.4 (C-2', C-6'), 129.6 (C-3', C-5'), 140.4 (C-1'), 156.9 (C-9), 164.7 (C-5), 165.5 (C-7), 197.3 (C-4).

Tectochrysin (**4**). White amorphous solid, ^{1}H NMR, $CDCl_3$, δ ppm: 3.84 (3H, s, 7- OCH_3), 5.51 (1H, d, J = 2.5 Hz, H-6), 5.95 (1H, d, J = 2.5 Hz, H- 8), 6.60 (1H, s, H-3), 7.38 (3H, m, H-3', H-4', H-5'), 7.53 (2H, m, H-2', H-6'). The ^{13}C NMR, $CDCl_3$, δ ppm: 56.0 (-OCH_3), 92.9 (C-8), 98.4(C-6), 105.9 (C-10), 106.1 (C-3), 126.5 (C-2', C-6'), 129.3 (C-3', C-5'), 131.6 (C-1'), 132.0 (C-4'), 158.1 (C-9), 162.5 (C-5), 164.3 (C-2), 165.9 (C-7),

182.5 (C-4).

Galanal A (**5**). White amorphous solid, ^{1}H NMR, $CDCl_3$, δ ppm: 0.77 (3H, s, 20-CH_3), 0.87 (1H, m, H-1a), 0.89 (3H, s, 19-CH_3), 0.93 (1H, m, H-5a), 0.93 (3H, s, 18-CH_3), 1.16 (1H, dd, J = 13.4, 4.1 Hz, H-3a), 1.40 (1H, m, H-3b), 1.43 (1H, m, H-6b), 1.45 (1H, m, H-2a), 1.56 (1H, m, H-2b), 1.61 (1H, m, H-9), 1.67 (1H, m, H-5b), 1.78 (1H, m, H-1b), 1.78 (1H, m, H-6a), 2.28 (1H, m, H-7), 2.50 (1H, dd, J = 18.5, 8.3 Hz, H-11a), 2.65 (1H, m, H-14), 2.79 (1H, m, H-11b), 4.07 (1H, dd, J = 8.9, 3.1 Hz, H-5), 6.89 (1H, dd, J = 8.3, 1.6 Hz, H-12), 9.38 (1H, s, 16-CHO), 10.11 (1H, s, 17-CHO). The ^{13}C NMR, $CDCl_3$, δ ppm: 16.5 (C-20), 18.3 (C-2), 18.5 (C-6), 21.1 (C-19), 23.5 (C-11), 27.5 (C-7), 28.4 (C-14), 33.1 (C-18), 33.3 (C-4), 38.4 (C-10), 38.5 (C-1), 41.6 (C-3), 53.6 (C-9), 55.4 (C-8), 55.6 (C-5), 71.2 (C-15), 142.2 (C-13), 156.1 (C-12), 193.3 (C-16), 206.5 (C-17).

Galanal B (**6**). White amorphous solid, ^{1}H NMR, $CDCl_3$, δ ppm: 0.76 (3H, s, 20-CH3), 0.77 (3H, s, 19-CH3), 0.82 (1H, m, H-1a), 0.82 (1H, m, H-5a), 0.85 (3H, s, 18-CH_3), 1.10 (1H, m, H-3a), 1.10 (1H, m, H-6b), 1.26 (1H, m, H-5b), 1.38 (1H, m, H-3b), 1.48 (1H, m, H-9), 1.54 (1H, m, H-2a), 1.57 (1H, m, H-2b), 1.64 (1H, m, H-6a), 1.85 (1H, dd, J = 12.6, 2.5 Hz, H-1b), 2.56 (1H, m, H-7), 2.63 (1H, dd, J = 16.4, 1.0 Hz, H-14b), 2.91 (1H, dd, J = 16.4, 8.8 Hz, H-14a), 3.12 (1H, m, H-11b), 3.55 (1H, dd, J = 8.8, 1.0 Hz, H-15), 7.03 (1H, dd, J = 8.4, 3.9 Hz, H-12), 9.38 (1H, s, 16-CHO), 10.19 (1H, s, 17-CHO). The ^{13}C NMR, $CDCl_3$, δ ppm: 15.7 (C-20), 18.5 (C-2), 18.9 (C-6), 21.2 (C-19), 24.0 (C-11), 28.4 (C-14), 33.2 (C-18), 33.3 (C-4), 34.1 (C-7), 38.7 (C-1), 38.7 (C-10), 41.5 (C-3), 55.1 (C-8), 55.3 (C-5), 55.4 (C-9), 78.5 (C-15), 140.5 (C-13), 157.9 (C-12), 193.6 (C-16), 208.2 (C-17).

Quercetin 3, 7, 4'-trimethyl ether (**7**). Pale yellow amorphous solid, ^{1}H NMR, $CDCl_3$, δ ppm: 3.88 (6H, s,3, 7-OCH_3), 3.39 (3H, s, 4'-OCH_3), 6.36 (1H, d, J = 2.2 Hz, H-6), 6.45 (1H, d, J = 2.2 Hz, H-8), 6.98 (1H, d, J = 8.5 Hz, H-5'), 7.70 (1H, d, J = 2.2 Hz, H-2'), 7.73 (1H, dd, J = 8.5, 2.2 Hz, H-6'), 12.64 (1H, s, 5-OH). The ^{13}C NMR, $CDCl_3$, δ ppm: 55.9 (4'-OCH_3), 56.1 (7-OCH_3), 60.2 (3-OCH3), 92.2 (C-8), 97.9 (C-6), 106.2 (C-10), 110.5 (C-5'), 114.5 (C-2'), 120.3 (C-6'), 121.7 (C-1'), 138.6 (C-3), 145.6 (C-2), 150.2 (C-3'), 155.7 (C-4'), 156.8 (C-9), 162.1 (C-5), 165.5 (C-7), 176.5 (C-4).

2.5 In Vitro Cytotoxic Activity

Cytotoxic activity of *B. rotunda* rhizome crude extracts and some isolated compounds were studied *in vitro* using cancer cell lines such as LK-2, A549 (human lung cancer), ECC4 (human stomach cancer), MCF7 (human breast cancer), Hela (human cervix cancer), DU145 (human prostate cancer), and WI-38 (normal human fibroblast). Briefly, each cell line was seeded in 96-well plates (2×10^3 per well) and cultured either in α-MEM or DMEM at 37 °C under a 5% CO_2 and 95% air atmosphere for 24 h. After the cells were washed with PBS (Nissui Pharmaceuticals), serial dilutions of the samples were added. After 72 h incubation, the cells were washed with PBS, and 100 μL of a-MEM or DMEM, containing 10% WST-8 cell counting kit solution (Dojindo; Kumamoto, Japan), was added to the wells. After 2 h incubation, the absorbance at 450 nm was measured. The different concentrations of the serial dilutions of the tested samples were 100-1 μg/mL for the crude extract, 100-1 μM for the isolated compounds, and 100-1 μM for the positive control, respectively. Cell viability was calculated from the mean values of data from three wells by using the following equation, and the cytotoxicity was expressed as the IC_{50} (50% inhibitory concentration) value.

(%) Cell viability = 100 ´ [{$Abs_{(test\ samples)}$ - $Abs_{(blank)}$}/{$Abs_{(control)}$ - $Abs_{(blank)}$}]

Results and Discussion

Compound 1 was isolated as a yellow amorphous solid. The ^{1}H NMR spectrum showed singlet of one

hydroxy proton at δ_H 12.02, one methoxy singal at δ_H 3.81 and a doublet aromatic proton at δ_H 6.08 (d, J = 2.3 Hz) attributable to meta coupling protons H-6 and H-8 of ring A. The multiplets of five aromatic protons at δ_H 7.47 confirmed the non-substitution of the B-ring. A doublet of doublet methine proton at δ_H 5.44 (J = 13, 3 Hz), and one triplet and one doublet of doublet methylene protons at δ_H 3.12 and 2.85 were attributable to H-3 (*cis*) and H-3 (trans) of the C-ring. The ^{1}H NMR spectrum shows the typical characteristics of flavanone. The ^{13}C NMR spectrum showed 16 carbon signals, including one carbonyl carbon, five quaternary carbons, eight methine carbons, one methylene carbon, and one methoxy carbon. The ^{1}H NMR and ^{13}C NMR spectral data of **1** were consisted with those of reported pinostrobin.[3]

Compound **2** was isolated as a yellow needles crystal. The ^{1}H NMR spectrum showed signals for flavonol substituted at H-6 (δ_H 6.38) and H-8 (δ_H 6.49) on the ring A and at H-3'/H-5' and H-2'/H-6' (δ_H 7.04 and 8.19) on the ring B, respectively. In addition, a signal at δ_H 3.89 for two methoxy groups and a signal at δ_H 11.72 for a hydroxyl group in hydrogen bond were observed. The ^{13}C NMR spectral data displayed signals for fifteen carbon atoms, which chemical shifts are compatible with C-3 (δ_C135.7) on the ring C, C-6 (δ_C 97.9) and C-8 (δ_C 92.2) on the ring A, and C-3'/5'and C-2'/6' (δ_C 129.4 and 114.2) on the ring B, as well as one carbonyl (δ_C 175.2) and two methoxy groups (δ_C 55.5 and 55.9) of a flavonol. All of the above mentioned ^{1}H NMR and ^{13}C NMR spectral data of **2** were consisted with those of reported 4',7-dimethyl kaempferol.[4]

Compound **3** was isolated and re-crystallized as a colourless crystalline from ethyl acetate. In the ^{1}H NMR spectrum, two doublets at δ_H 5.79 (J = 2.0 Hz) and δ_H 5.83 (J = 2.0 Hz) were attributable to *meta* coupled H-6 and H-8. A five protons multiplet at δ_H 7.24-7.40 confirmed the non-substitution of the B-ring. The ^{1}H NMR spectroscopic data suggested that the absence of H-3 proton and the presence of an ABX system of the typical flavanone. The ^{13}C NMR spectrum showed 15 carbon signals, including one methylene, eight methine, and six quarternary carbons. The six carbon signals at δ_C 140.4, 127.4, 129.6, and 131.9 were assignable to C-1', C-2'/C-6', C-3'/C-4', and C-5' of the mono-substituted aromatic ring (ring B), respectively. The six carbon signals at δ_C 103.3, 164.7, 97.2, 165.5, 96.3, and 197.3 were assigned to be C-4a, C-5, C-6, C-7, C-8, and C-4 on the ring A, and two carbon signals, δ_C 80.4 and 44.5, were located at C-2 and C-3, respectively. The ^{1}H NMR and ^{13}C NMR completed the elucidation of the structure pinocembrin.[5]

Compound **4** was isolated as a white amorphous solid. The ^{1}H NMR showed two doublet protons at δ_H 5.51 (1H, d, J = 2.5 Hz, H-6) and 5.95 (1H, d, J = 2.5 Hz, H-8), suggesting the presence of *meta* coupled aromatic protons. A singlet at δ_H 6.60 was assigned to H-3, while two multiplets at δ_H 7.38 (3H, m, H-3', H-4', H-5') and δ_H 7.53 (2H, m, H-2', H-6') belonged to the protons of the monosubstituted ring B. A singlet proton signal at δ_H 3.84 was due to the presence of a methoxy group. Moreover, the ^{13}C NMR spectrum showed the corresponded signals including a methoxy carbon signal at δ_C 56.0, and a signal at a very low region (δ_C 182.5, C-4), which was definitive of carbonyl carbon of the flavone structure. Four oxygenated aromatic carbon signals were observed at δ_C 165.9 (C-7), 164.3 (C-2), 162.5(C-5) and 158.1 (C-9). The rest aromatic carbon signals were observed from δ_C 140.0 to 92.9. ^{1}H NMR and ^{13}C NMR spectra data of isolated compound **4** were found to be consistent with those of tectrochrysin. Therefore, compound **4** was identified as tectrochrysin.[6]

Compound **5** was isolated as a white amorphous solid. ^{1}H NMR suggested the presence of 30 protons. Three singlet signals (3H each) at δ_H 0.77, 0.89, and 0.93 indicated the presence of three CH_3 groups. The two singlet signals at δ_H 9.38 and 10.11 allow us to confirm the presence of two CHO groups. The doublet-doublet signal at δ_H 4.07 suggested the presence of a secondary carbinol proton (-CHO-) adjacent to a pair of diasterotopic methylene protons. This observation indicated that compound **5** contains the –CH_2-CHOH-C- moiety. The doublet-doublet signal at downfield, δ_H 6.89, was assigned due to the presence of an olefinic proton

of –C=CH-group, indicating that compound 5 possesses only an olefinic group. ^{1}H-^{1}H COSY spectrum revealed the connectivity between δ_H 2.50, 2.79, 2.65, 4.07, and 6.89. An olefinic methine proton at δ_H 6.89 (dd, J = 8.3, 1.6 Hz, H-12) was coupled with the methylene protons at δ_H 2.50 (dd, J =18.5, 3.1 Hz, H-11a) and δ_H 2.79 (m, Hb-11). Oxygenated methine proton at δ_H 4.07 (d, J = 8.9, 3.1 Hz, H-15) was correlated with methylene proton at δ_H 2.65 (H-14). One methylene proton at δ_H 2.79 (H-11b) was coupled with δ_H 2.50 (dd, J=18.5, 8.3, H-11) and 1.61 (C-9). Similarly, one methylne proton at δ 0.93 (H-5) was coupled with δ 1.78 (H_b-6). ^{13}C NMR spectrum showed 20 signals, including two aldehyde carbons (δ_C 193.3 and 206.5), two olefinic carbons (δ_C 142.2 and 156.1), and one carbinol carbon (δ_C 71.2). The remaining 15 carbon atoms were assigned as the sp^3 methyl, methylene, methane, and quarternary carbons. On the basis of ^{13}C NMR, ^{1}H NMR and COSY spectral data, compound **5** was assumed as galanal A.[7]

Compound **6** was obtained as a white amorphous solid. In ^{1}H NMR spectrum, the singlet proton signal at δ_H 10.22 indicated the presence of the CH-OH proton. The singlet proton signals at δ 9.40 and 10.22 indicated the presence of two aldehyde protons. The three quaternary singlet methyl signals at δ_H 0.76, 0.99, and 0.85 (3H each) were coincident with those of reported galanal B. ^{13}C NMR spectrum showed twenty carbon signals. The upfield one methyl group and the downfield one carbonyl signal were simultaneously observed at δ_C 15.7 (C-20) and δ_C 208.2 (C-17), respectively. ^{1}H NMR, ^{13}C NMR, and COSY spectral data were consisted with those ofreported galanal B.[7]

Compound **7** was isolated as a pale yellow amorphous solid. The ^{1}H NMR spectrum showed ABX system at δ_H 7.70 (d, J = 2.2 Hz, H-2'), 6.98 (d, J = 8.5 Hz, H-5'), and 7.73 (dd, J = 8.5, 2.2 Hz, H-6') and a pair of doublets (J = 2.2 Hz) in the aromatic region at δ_H 6.36 and 6.45, three methoxyl signals (δ_H 3.99, 3.88, and 3.88), and an OH-phenolic signal at δ_H 12.64. The ^{13}C NMR spectrum showed 18 carbon signals. Among them, the signals at δ_C138.6 and 176.5 were characteristic for the flavonol structure and six oxyaryl signals (δ_C 145.6, 150.2, 155.7, 156.8, 162.1, and 165.5) indicated that compound **7** is a quercetin derivative. The above mentioned ^{1}H NMR and ^{13}C NMR spectral data of compound **7** were similar to those of quercetin 3,7,4'-trimethyl ether.[8]

In the cytotoxicity assay (Table 2), the MeOH extract and the $CHCl_3$ extract of the *B.rotunda* rhizomes

Table 2. Antiproliferative activity of crude extract of *B. rotunda* and isolated compounds (IC_{50} μg/mL or μM) against lung (LK-2, A549), stomach (ECC4), breast (MCF7), cervix (HeLa), prostate (DU145) human cancer, and normal human fibroblast (WI-38) cell lines

Samples	Cell line						
	LK-2	A549	ECC4	MCF7	HeLa	DU145	WI-38
Pinostrobin (**1**)	>100	78.74	>100	>100	>100	>100	>100
4', 7-Dimethyl kaempferol (**2**)	8.79	>100	35.18	>100	>100	91.57	40.71
Galanal A (**5**)	4.38	28.20	32	38.62	5.10	3.57	2.60
Galanal B (**6**)	5.75	8.02	5.26	6.79	5.73	7.73	5.68
$CHCl_3$-Extract	65.43	56.12	55.65	73.74	48.70	68.45	70.24
MeOH-Extract	70.02	57.42	60.32	73.06	70.07	70.75	75.88
[a]5-Fluorouracil	>10	9.0	>10	>10	7.11	8.05	>10

[a]Positive control

exhibited mild cytotoxicity against all the tested cancer cell lines. Among the tested compounds, galanal B (**6**) showed the strong cytotoxicity against all the tested cancer cell lines with IC_{50} values ranging from 5.26 to 8.02 μM. In contrast, galanal A (**5**) exhibited selectively cytotoxicity against LK-2 (IC_{50} 4.38 μM), HeLa (IC_{50} 5.10 μM), DU145 (IC_{50} 3.57 μM), and WI-38 (IC_{50} 2.60 μM) cells with IC_{50} values of 4.38 μM, 5.10 μM, 3.57 μM, and 2.60 μM, respectively. However, two flavonoid compounds, **1** and **2**, did not show any activity against all the tested cancer cells.

CONCLUSION

Pinostrobin (**1**), 4',7-dimethyl kaempferol (**2**), pinocembrin (**3**), tectochrysin (**4**), galanal A (**5**), galanal B (**6**), and quercetin 3,7,4'-trimethylether (**7**) were isolated from *B.rotunda* rhizomes collected in Myanmar. Furthermore, the cytotoxicity assay revealed that the crude extracts, galanal A (**5**), and galanal B (**6**) showed cytotoxicity against human cancer cell lines. These observations may suggest that the crude extracts and its phytoconstituents may be useful for the treatment and prevention of cancer.

References

1. Parkin, D.M.; Bray, F.; Ferlay, J.; Pisani, P.; *Int. J. Cancer*, **2001**, *94*, 153-156.
2. Morikawa, T.; Fnakoshi, K.; Ninomiya, K.; Yasuda, D.;*Chem. Pharm. Bull.*, **2008**, *56*, 956-962.
3. Ching, A. Y. L.; Wah, T.S.; Sukari, M. S.; Lian, G.E.C.; Rahmani, M.; Khalid, K.; *Malaysian Journal of Analytical Sciences*, **2007**, *11*, 154-159.
4. Oliveria, P.V.; Lemons, R.P.L.; Conserva, L.M.; *Revista Brasileria de Farmacognosia Brazilian Journal of Pharmacognosy*. **2012**, *22*, 451-454.
5. Nagwa, M.M.; Howaida, I.A.A.; Manal, A.H.; Samira, N.A.G.; Suhair, M.S.J.; *International Journal of Phytomedicine.* **2012**, *4*, 27-39.
6. Khanh, L.P.; True, H.K.; Kim, N.T.T.; Phung, N.K.P.; Tu, N.T.H.; *Science & Technology Development*, **2013**, *16*, 20-25.
7. Abe, M.; Ozawa, Y.; Ude, Y.; Yamada, Y.; Morimitus, Y.; Nakamura, Y., Osawa, T.; *Biosci. Biotechnol. Biochem*. **2002**, *66*, 2698-2700.
8. Kritanti, A.N.; Aminah, N.S.; Tanjung, M.; *Der. Pharmacia. Lettre*. **2015**, *7*, 149-152.