



Original article

Flavonoid glycosides from leaves and straw of *Oryza sativa* and their effects of cytotoxicity on a macrophage cell line and allelopathic on weed germination

Ill-Min Chung^a, Sung-Kyu Park^a, Mohd Ali^b, Mayakrishnan Prabakaran^a, Young-Tek Oh^a, Seung-Hyun Kim^a, Nasir Ali Siddiqui^c, Ateeque Ahmad^{d,e,*}

^a Department of Applied Bioscience, College of Life and Environmental Science, Konkuk University, Seoul 05029, South Korea

^b Department of Pharmacognosy and Phytochemistry, Hamdard University, New Delhi 110062, India

^c Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, P.O. Box 2457, Saudi Arabia

^d Process Chemistry and Technology Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow 226015, India

^e Department of Applied Bioscience, Konkuk University, Seoul 143-701, South Korea

ARTICLE INFO

Article history:

Received 30 November 2017

Accepted 9 January 2018

Available online 12 January 2018

Keywords:

Oryza sativa L.

Gramineae

Rice leaves and straw

New chemical constituents

Cytotoxicity test

Allelopathic effect

Barnyardgrass

Pigweed

ABSTRACT

Five new flavonoids namely, 5-hydroxy-6-isoprenyl-7,4'-dimethoxyflavonol-3-O-β-D-arabinofuranoside (**1**), 5,7-dihydroxy-4'-methoxyflavone-7-O-β-D-arabinopyranosyl-2''-n-decan-1'''-oate (**2**), 3-butanoyl-5,6,8-trihydroxy-7,4'-dimethoxyflavonol--5-O-β-D-glucopyranoside (**3**), 7, 4'-dimethoxy-5-hydroxyflavone-5-O-α-D-arabinopyranosyl-(2'' → 1''')-O-α-D-arabinopyranoside (**4**), and 5,6-dihydroxy-7, 4'-dimethoxyflavone-5-O-α-D-glucopyranoside (**5**), together with two known compounds, were isolated from the methanol extract of *Oryza sativa* leaves and straw. Their structures of new compounds were elucidated by 1D and 2D NMR spectral methods, viz: COSY, HMBC and HSQC aided by mass techniques and IR spectroscopy. The cytotoxicity of these compounds (**1–7**) were assessed by using (RAW 264.7) mouse macrophages cell line, and allelopathic effects of compounds (**1–7**) on the germination characteristics of barnyardgrass (*Echinochloa oryzicola*) and pigweed (*Chenopodium album* L.) were also evaluated. The compounds **1**, **6** and **7** showed cytotoxicity and compounds **1–7** exhibited significant inhibitory activity on the seed germination of two weed species.

© 2018 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Rice (*Oryza sativa* L.) is the principal cereal food in Asia and the major staple of the majority of the population. It generally occurs as two types, with white and colored hulls, although the white hulled variety is more common (85%). The germination of rice seed is of great agricultural importance, and it has long been known to be influenced by compounds present in the seed coat (hull) (Dutta, 1973).

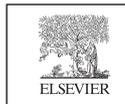
Naturally occurring diterpenes, momilactones derived from rice have exhibited significant biological activities including as growth and germination inhibitors, herbicidal, algicidal, as well as potent inhibitory effects on several weeds and other activity (Kato et al., 1973; Kato et al., 1977; Kato-Naguchi et al., 2002; Kato-Naguchi and Ino, 2003). Earlier phytochemical investigation of rice husks, straw and leaves have led to the discovery of many classes of compounds and biological activities have been reported (Chung et al., 2005a,b; Chung et al., 2006a,b; Chung et al., 2007a,b; Ahmad et al., 2013; Chung et al., 2017).

This paper deals with the isolation and structure elucidation of five new flavonoid glycosides, (**1–5**) on the basis of ¹H and ¹³C NMR spectroscopic studies, including 2D-NMR COSY, HSQC, HMBC and chemical reactions from *O. sativa*. This is the first report of isolation of flavonoid glycosides (**1–5**; Fig. 1) along with two known compounds (**6–7**, Fig. 2; Meyer et al., 2006). The cytotoxicity of the new and known compounds (**1–7**) were evaluated in a macrophage cell line RAW 264.7 by using an MTT assay and evaluated for their allelopathic effect on barnyardgrass (*Echinochloa oryzicola*) and pigweed (*Chenopodium album*), and characterization of weed seed

* Corresponding author at: Process Chemistry and Technology Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow 226015, India.

E-mail address: a.ahmad@cimap.res.in (A. Ahmad).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

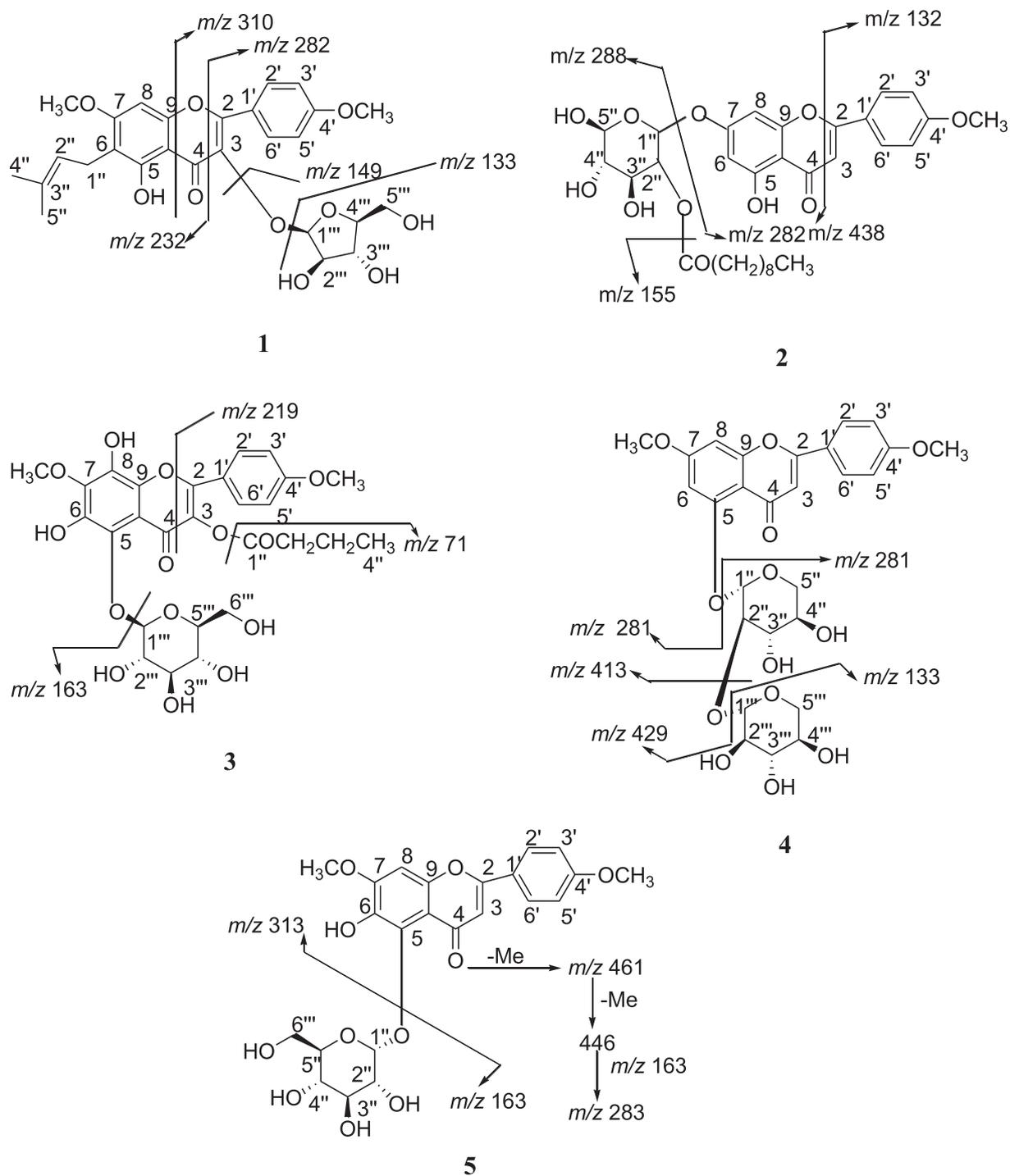


Fig. 1. Structures of compounds 1–5.

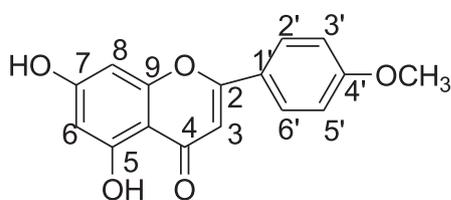
germination and morphology was accomplished by treatment with different concentrations of the purified natural products are discussed. The objective of the present investigation was to report some of the new findings in the form of natural products and biological activities of compounds (1–7) from leaves and straw of *O. sativa*.

2. Experimental

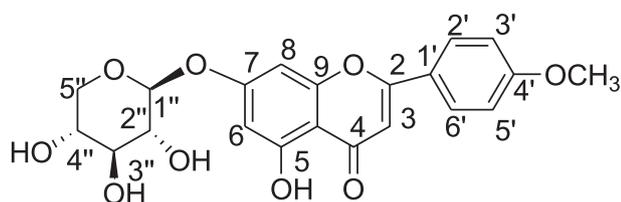
2.1. General experimental procedures

Melting points of the compounds were determined using a model IA9100 melting point apparatus (Electrochemical Engineer-

ing, Seoul, South Korea). Optical rotations were measured on a model AA-10 polarimeter (Instrument Ltd., Seoul, Korea). Ultraviolet (UV) spectra were collected on a TU-1800_{PC} UV-vis spectrophotometer (Instrument Ltd., Seoul, Korea). Infrared (IR) spectra were recorded on a Thermo Scientific FT-IR model Nicolet 6700 spectrophotometer (Waltham, MA, USA). Both nuclear magnetic resonance (NMR) spectra were measured on a Bruker Avance-600 spectrometer (Billerica Massachusetts (MA) using deuterated solvents. NMR spectra were recorded in deuterated chloroform, pyridine-*d*₅, and methanol-*d*₄ using tetramethylsilane (TMS) as an internal standard, with chemical shifts expressed in parts per million (δ) and coupling constants (*J*) in Hertz. High-resolution



6



7

Fig. 2. Structures of compounds 6–7.

electrospray ionization Fourier transform (ESI/FT) mass spectra were recorded on a Thermo-Finnigan LTQ-Orbitrap instrument (Thermo Scientific, Bartlesville, OK, USA) equipped with a Dionex U 3000 HPLC system. All chemicals were of analytical grade. *n*-Hexane, ethyl acetate (EtOAc), methanol, ethanol, sulfuric acid (H₂SO₄), and vanillin were purchased from Daejung Chemicals

and Metals (Seoul, South Korea). Normal thin layer chromatography (TLC) was performed on precoated silica gel 60 F₂₅₄ plates (Merck). TLC plates were visualized using a 5% H₂SO₄ in ethanol spray reagent. Column chromatography (CC) was performed using silica gel (70–230 mesh from Merck) and LiChroprep RP-18 [40–63 μm; octadecyl silica (ODS) gel, Merck]. The authentic standards of chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Plant material

The rice plant (*O. sativa*, leaves and straw) used in the present study was collected after the harvesting of rice cereal at Konkuk University Experimental Farm, Yeosu, South Korea, in September/October 2013. The collected samples were dried in the laboratory at a temperature range of 30–35 °C for 3 weeks, with some modifications to the procedure performed in a previous study. A voucher specimen (reference code ILPUM variety) was dried and deposited in the herbarium of the Department of Applied Life Science, Konkuk University, Seoul, South Korea.

2.3. Extraction and isolation

Dried rice plant (2.4 kg, powdered leaves and straw) was immersed in methanol (10 L × 3) for 1 week at room temperature (25–30 °C). Then, the supernatant was concentrated under vacuum to yield 190 g of extract. This freeze-dried extract was again dissolved in methanol to remove fat and kept refrigerated for 3 h. The fat was crystallized and filtered through a sintered funnel. The filtrate was then concentrated to obtain 132 g of extract.

Table 1

¹H NMR spectroscopic data of 1, 2, 3, 5 and 5 (in methanol-*d*₄).^a

Position	1	2	3	4	5
2	–	–	–	–	–
3	–	6.54 s	–	6.48 s	6.54 s
4	–	–	–	–	–
5	–	–	–	–	–
6	–	6.11 s	–	6.46 s	–
7	–	–	–	–	–
8	6.95 s	6.35 s	–	6.68 s	6.79 s
9	–	–	–	–	–
10	–	–	–	–	–
1'	–	–	–	–	–
2'	7.42 d (8.0)	7.01 d (8.5)	7.04 d (8.0)	7.36 d (9.0)	7.01 d (8.5)
3'	6.77 d (8.0)	6.81 d (8.5)	6.70 d (8.0)	6.70 d (9.0)	6.71 d (8.5)
4'	–	–	–	–	–
5'	7.16 d (8.5)	6.98 d (9.0)	6.74 d (8.5)	6.78 d (9.0)	6.75 d (8.5)
6'	7.37 d (8.5)	6.98 d (9.0)	6.90 d (8.5)	7.34 d (9.0)	7.17 d (8.5)
1''	2.93 d (7.0), 2.90 d (7.5)	5.01 d (7.0)	–	5.40 d (4.5)	5.01 d (4.5)
2''	6.65 d (7.5)	4.21 m	2.59 t (8.5)	4.63 m	3.80 m
3''	–	3.87 m	1.22 m	4.28 m	3.71 m
4''	2.05 br s	3.82 m	0.95 t (6.3)	3.64 m	3.67 m
5''	2.01 br s	3.78 d (4.5)	–	3.29 m	4.20 m
		3.76 d (5.0)			
6''	–	–	–	–	3.29 br s
1'''	6.40 d (7.5)	–	4.80 d (7.0)	5.13 d (4.5)	–
2'''	3.88 m	2.24 t (7.0)	3.77 m	4.36 m	–
3'''	3.85 m	2.08 m	3.57 m	4.13 m	–
4'''	4.56 m	1.55 m	3.46 m	3.47 m	–
5'''	3.56 d (7.0), 3.54 d (7.0)	1.30 m	3.86 m	3.17 m	–
6'''	–	1.27 br s	3.50 d (7.0)	–	–
			3.48 (7.0)		
7'''	–	1.27 br s	–	–	–
8'''	–	1.27 br s	–	–	–
9'''	–	1.27 br s	–	–	–
10'''	–	0.94 t (7.5)	–	–	–
(OMe)	3.30 s	–	3.82 s	3.87 s	3.85 s
(OMe)	3.29 s	3.32 s	3.75 s	3.86 s	3.77 s

^a Coupling constants in parenthesis are given in hertz.

Table 2
¹³C NMR spectroscopic data of 1, 2, 3, 5 and 5 (in methanol-d₄)^a.

Position	1	2	3	4	5
2	147.9	164.8	147.0	164.7	164.0
3	133.1	104.9	136.7	134.3	104.9
4	179.2	183.5	175.1	183.3	180.2
5	160.9	163.0	150.4	163.0	163.1
6	118.6	100.2	132.6	102.3	149.6
7	160.4	165.9	162.8	165.4	164.8
8	90.0	95.2	131.3	96.0	96.3
9	151.1	159.1	148.9	158.4	160.1
10	103.5	105.7	104.9	107.1	107.2
1'	124.2	127.5	129.4	121.0	127.8
2'	130.9	130.7	128.9	127.9	129.5
3'	116.5	111.8	115.2	116.5	115.6
4'	145.9	144.6	146.2	144.4	144.8
5'	112.5	115.8	115.3	112.3	114.6
6'	128.3	129.7	128.8	128.9	128.8
1''	41.4	105.4	172.1	105.3	105.2
2''	123.2	87.5	38.6	89.6	77.3
3''	141.6	74.5	28.2	78.6	74.4
4''	26.4	74.0	18.2	74.3	71.2
5''	26.4	62.8	–	62.7	78.6
6''	–	–	–	–	62.6
1'''	116.6	178.2	103.1	101.3	–
2'''	74.0	38.2	74.9	79.7	–
3'''	72.3	35.5	71.3	75.2	–
4'''	79.0	30.4	66.1	71.6	–
5'''	69.8	30.3	78.1	62.4	–
6'''	–	30.1	62.5	–	–
7'''	–	30.1	–	–	–
8'''	–	26.1	–	–	–
9'''	–	22.6	–	–	–
10'''	–	14.1	–	–	–
(OMe-7)	56.5	–	56.8	57.0	57.0
(OMe-4')	56.4	56.6	56.4	56.3	56.4

The methanol extract (132 g) was subjected to normal-phase CC on silica gel (70–230 mesh, 1.2 kg, 1500 × 45 mm), yielding 50 fractions (each fraction 500 mL) with the following eluents: fraction 1–5 in hexane, fractions 6–10 in hexane:EtOAc (H:Et; 8:2), fractions 11–15 in H:Et (6:4), fractions 16–20 in H:Et (4:6), fractions 21–25 in H:Et (8:2), fractions 26–30 in EtOAc, fractions 31–35 in EtOAc:MeOH (Et:M; 9.5:0.5), and fractions 36–40 in Et:M (9:1), fractions 41–45 in Et:M (8.5:1.5), and fractions 45–50 in Et:M (8:2). Fractions 6–9 were crystallized and yielded β -sitosterol (23 mg) after purification by CC. Compound **6** was obtained (34 mg) from fraction 10 after CC in small column with chloroform and collecting 5 fractions amount (25 mL each), fraction 3–5 are in pure form and its identity was confirmed by comparison with an authentic sample from Sigma and with the spectra of a previously isolated compound. After mixing fractions 31–35 of main column and rechromatographed over on silica gel with chloroform and methanol were obtained 10 fractions. Fractions 9–10 obtained after CC of fractions 31–35 and were subjected to CC over LiChroprep RP-18 (ODS column) and eluted sequentially with methanol containing 80, 60, 40, 20, 10, and 0% water to yield three new compounds: **1** (52 mg), **2** (49 mg), and **3** (39 mg). Fractions 36–40 were combined after chromatography on a silica gel column with chloroform and methanol, rechromatographed over LiChroprep RP-18, and eluted sequentially with methanol containing 80, 60, 40, 20, 10, and 0% water to yield **4** (39 mg), **5** (49 mg), **7** (54 mg), and β -sitosterol- β -D-glucoside (29 mg).

2.3.1. 5-Hydroxy-6-isoprenyl-7, 4'-dimethoxyflavonol-3-O- β -D-arabinofuranoside (**1**)

Dark yellow semi-solid; R_f: 0.52 (CHCl₃/MeOH; 9:1); [α]_D²¹ –37.2 (c 0.1, MeOH); UV (MeOH) λ_{\max} : 270, 310, 328 nm; IR (KBr) ν_{\max} : 3415, 3369, 2932, 2837, 1663, 1614, 1590, 1501, 1454, 1358, 1260, 1165, 1123, 1029, 835 cm⁻¹; ESI/MS *m/z* (rel. int.): 515 [M + H]⁺ (C₂₇H₃₁O₁₀) (9.8), 310 (15.6), 282 (2.8), 232 (4.8), 177 (73.1), 149 (18.4), 133 (9.9); HRESI/FTMS, *m/z* 515.1898 (calcd for C₂₇H₃₁O₁₀, 515.1917). For ¹H and ¹³C NMR data. See Tables 1 and 2.

int.): 515 [M + H]⁺ (C₂₇H₃₁O₁₀) (9.8), 310 (15.6), 282 (2.8), 232 (4.8), 177 (73.1), 149 (18.4), 133 (9.9); HRESI/FTMS, *m/z* 515.1898 (calcd for C₂₇H₃₁O₁₀, 515.1917). For ¹H and ¹³C NMR data. See Tables 1 and 2.

2.3.2. 5,7-Dihydroxy-4'-methoxyflavone-7-O- β -D-arabinopyranosyl-2''-n-decan-1'''-oate (**2**)

Yellow solid; R_f: 0.48 ((CHCl₃/MeOH; 9:1); [α]_D²¹ –31.0 (c 0.1, MeOH); UV (MeOH) λ_{\max} : 266, 309, 338 nm; IR (KBr) ν_{\max} : 3415, 3364, 2940, 2838, 1721, 1699, 1655, 1592, 1513, 1466, 1359, 1237, 1165, 1122, 1021, 833, 752 cm⁻¹; ESI/MS *m/z* (rel. int.): 571 [M + H]⁺ (C₃₁H₃₉O₁₀) (3.1), 155 (26.8), 151 (12.6), 132 (8.8), 123 (16.2), 310 (10.8), 288 (15.1); HRESI/FTMS, *m/z* 571.2492 (calcd for C₃₁H₃₉O₁₀, 571.2499). For ¹H and ¹³C NMR data. See Tables 1 and 2.

2.3.3. 3-Butanoyl-5,6,8-trihydroxy-7,4'-dimethoxyflavonol-5-O- β -D-glucopyranoside (**3**)

Yellow semi-solid; R_f: 0.48 (CHCl₃/MeOH; 9:1); [α]_D²¹ –32.2 (c 0.1, MeOH); UV (MeOH) λ_{\max} : 277, 312, 339 nm; IR (KBr) ν_{\max} : 3515, 3450, 3361, 3212, 2941, 2836, 1722, 1680, 1655, 1590, 1513, 1421, 1355, 1235, 1162, 1122, 1021, 833, 725 cm⁻¹; ESI/MS *m/z* (rel. int.): 579 [M + H]⁺ (C₂₇H₃₁O₁₄) (4.8), 550 (5.8), 415 (3.6), 344 (13.2), 212 (3.5), 163 (5.1), 131 (21.6); HRESI/FTMS, *m/z* 579.1661 (calcd for C₂₇H₃₁O₁₄, 579.1669). For ¹H and ¹³C NMR data. See Tables 1 and 2.

2.3.4. 7,4'-Dimethoxy-5-hydroxyflavone-5-O- α -D-arabinopyranosyl-(2'' → 1''')-O- α -D-arabinopyranoside (**4**)

Yellow solid; R_f: 0.38 (CHCl₃/MeOH; 9:1); [α]_D²¹ –29.2 (c 0.1, MeOH); UV (MeOH) λ_{\max} : 269, 309, 329 nm; IR (KBr) ν_{\max} : 3415, 3371, 3262, 2937, 2841, 1663, 1610, 1495, 1457, 1344, 1257, 1175, 1138, 1073, 1028, 836 cm⁻¹; HRESI/FTMS, *m/z* 563.1711 (calcd for C₂₇H₃₁O₁₃, 563.1720). For ¹H and ¹³C NMR data. See Tables 1 and 2.

2.3.5. 5, 6-Dihydroxy-7,4'-dimethoxyflavone-5-O- α -D-glucopyranoside (**5**)

Yellow crystalline solid; R_f: 0.43 (CHCl₃/MeOH; 9:1); mp 221–22 °C; [α]_D²¹ –31.1 (c 0.1, MeOH); UV (MeOH) λ_{\max} : 279, 313, 334 nm; IR (KBr) ν_{\max} : 3415, 3383, 3281, 2936, 2842, 1701, 1602, 1513, 1424, 1340, 1263, 1222, 1160, 1115, 1070, 1025, 834 cm⁻¹; ESI/MS *m/z* (rel. int.): 477 [M + H]⁺ (C₂₃H₂₅O₁₁) (5.3), 461 (3.8), 446 (2.6), 313 (4.5), 283 (6.8), 163 (3.8); HRESI/FTMS, *m/z* 477.1347 (calcd for C₂₃H₂₅O₁₁, 477.1352). For ¹H and ¹³C NMR data. See Tables 1 and 2.

2.3.6. 4'-Methoxyapigenin (**6**)

Yellow solid; IR (KBr) ν_{\max} : 3415, 3371, 2931, 2842, 1662, 1614, 1560, 1499, 1454, 1357, 1260, 1166, 1224, 1029, 836 cm⁻¹; ¹H NMR (methanol-d₄, 500 MHz): δ 7.12 (1H, d, *J* = 7.5 Hz, H-6'), 7.09 (1H, d, *J* = 9.5 Hz, H-2'), 6.89 (1H, d, *J* = 9.5 Hz, H-3'), 6.76 (1H, d, *J* = 7.5 Hz, H-5'), 6.58 (1H, d, *J* = 1.8 Hz, H-8), 6.37 (1H, s, H-3), 6.13 (1H, d, *J* = 1.8 Hz, H-6), 3.87 (3H, br s, OMe); ¹³C NMR (methanol-d₄, 125 MHz): δ 164.9 (C-2), 104.9 (C-3), 183.6 (C-4), 163.1 (C-5), 100.2 (C-6), 166.0 (C-7), 95.1 (C-8), 159.2 (C-9), 105.3 (C-10), 120.9 (C-1'), 127.8 (C-2'), 115.8 (C-3'), 154.7 (C-4'), 111.8 (C-5'), 128.6 (C-6'), 56.9 (OMe); ESI MS *m/z* (rel. int.): 285 [M + H]⁺ (C₁₆H₁₂O₅) (6.1), 152 (94.2), 132 (11.5), 132 (15.3); HRESI/FTMS, *m/z* 285.0712 (calcd for C₁₆H₁₂O₅, 285.0718).

2.3.7. 5,7-Dihydroxy-4-methoxyflavone-7-O- β -D-arabinopyranoside (**7**)

Yellow semi-solid; IR (KBr) ν_{\max} : 3415, 3395, 3274, 2941, 2837, 1665, 1633, 1589, 1496, 1464, 1425, 1366, 1242, 1163, 1121, 1026, 832 cm⁻¹; ¹H NMR (methanol-d₄; 500 MHz): δ 7.05 (1H, d, *J* = 8.5

Hz, H-2'), 7.01 (1H, d, $J = 8.0$ Hz, H-6'), 6.75 (1H, d, $J = 8.5$ Hz, H-3'), 6.73 (1H, d, $J = 8.0$ Hz, H-5'), 6.51 (1H, s, H-3), 6.32 (1H, d, $J = 2.0$ Hz, H-8), 6.09 (1H, d, $J = 2.0$ Hz, H-6), 5.01 (1H, d, $J = 7.0$ Hz, H-1'), 4.38 (1H, m, H-2'), 4.23 (1H, m, H-3'), 3.91 (1H, m, H-4'), 3.30 (1H, d, $J = 3.0$ Hz, H₂-5'a), 3.29 (1H, d, $J = 3.0$ Hz, H₂-5'b), 3.34 (3H, brs, OMe); ¹³C NMR (methanol-*d*₄, 125 MHz): δ 164.6 (C-2), 104.8 (C-3), 183.5 (C-4), 163.0 (C-5), 100.2 (C-6), 165.9 (C-7), 95.1 (C-8), 159.1 (C-9), 105.6 (C-10), 120.7 (C-1'), 129.4 (C-2'), 115.8 (C-3'), 154.5 (C-4'), 111.8 (C-5'), 127.6 (C-6'), 104.7 (C-1''), 87.6 (C-2''), 74.5 (C-3''), 74.2 (C-4''), 62.0 (C-5''), 56.8 (OMe); ESIMS *m/z* (rel. int.): 417 [M + H]⁺ (C₂₁H₂₁O₉) (2.1); HRESIFTMS, *m/z* 417.1179 (calcd for C₂₁H₂₁O₉, 417.1186).

2.3.8. Acid hydrolysis of **2** and **3**

Compounds **2** and **3** (10 mg each) were heated at 70–80 °C with 2 M hydrochloric acid (2.5 mL) diluted in 70% aqueous ethanol (3 mL) for 30 min. The reaction mixture was dried under vacuum, neutralized with aqueous sodium bicarbonate, and extracted with chloroform (3 × 5 mL) to separate the flavonoid moiety. The chloroform extract was washed with water (3 × 5 mL), dried over anhydrous sodium sulfate, and evaporated to produce the aglycone moiety. The mother liquor obtained after separation of the flavonoid was treated with dilute 2 M hydrochloric acid to liberate the free fatty acids, which were isolated by re-extraction with chloroform (3 × 5 mL).

2.3.9. Acid hydrolysis of **1**, **4**, and **5**

Compounds **1**, **4**, and **5** (10 mg) were refluxed with 2 mL of 1 M hydrochloric acid/dioxane (1:1, v/v) in a water bath for 4 h. The reaction mixture was evaporated to dryness, mixed with water (10 mL) and extracted three times with chloroform (5 mL). The chloroform extract contained the aglycone portion, whereas the water extract contained sugar part.

2.4. Biological activities

2.4.1. Cell culture

RAW 264.7 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and grown in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. All cells were maintained at 37 °C in a humidified atmosphere of 5% CO₂ throughout the study and were routinely grown in 25-cm² culture flasks. After attaining confluence, the cells were harvested by trypsinization. Meanwhile, some differences were considered for the trypsinization procedure of semi-adherent (RAW 264.7) cells and two other adherent cells. After centrifugation (1300 ×g for 7 min), the cells were resuspended in the culture medium and used for the following study.

2.4.2. Cytotoxicity test–MTT assay

To evaluate the cytotoxic effect of the seven chemical constituents isolated from *O. sativa* (straw and leaves) in dimethyl sulfoxide (DMSO) on RAW 264.7 cells, viability tests were performed using an MTT colorimetric assay. Briefly, all cells were seeded in 96-well plates at a density of 2 × 10⁵ cells per well and then incubated at 37 °C in 5% CO₂ for cell attachment. The medium was removed and replaced with fresh medium containing various concentrations of the seven chemical constituents (0, 100, 500, and 1000 μM). After a 24-h treatment, 100 μL of MTT (1 mg/mL) was added to each well, and the plate was further incubated.

After 4 h, the supernatants were removed, and 100 μL of DMSO was added to each well to dissolve the resulting formazan crystals. Finally, the absorbance at 570 nm was read using a multilabel plate reader (VICTOR™X3, Perkin Elmer, Waltham, MA, USA). The cell viability percentage was calculated using the equation: [mean optical density (OD) of treated cells/mean OD of control cells] × 100.

2.4.3. Weed collection and dormancy breakage

The seeds of two prominent weeds (*Echinochloa oryzicola* and *Chenopodium album* L.) found in the rice paddies of Korea were collected in 1997 and 1998. The seeds were dried (12.04% water content based on fresh weight) and stored at 5 °C in the dark. Before the start of the experiment, the seeds were scarified with H₂SO₄ (98%) for 30 min to render the seed coat permeable to water. Pigweed seeds were rinsed several times with distilled water to remove traces of H₂SO₄. The seeds of barnyardgrass were soaked in distilled water for one day to remove the germination inhibiting compound in the seed coat (water priming, WP). Their germination was determined and was >80% in all cases.

2.4.4. Seed germination inhibition assay

A bioassay based on seed germination was used to assess the inhibitory activity of the isolated compounds on seed germination. Test solutions at concentrations of 0, 100, 500, and 1000 μM were prepared by serial dilution of stock solutions of the constituents (1 M in DMSO). Test solutions of pyributicarb (PBC), an inhibitor of germination, were also prepared by serial dilution in the same solvent. Prior the seeds being sown in petri dishes (9 cm in diameter), the barnyardgrass and lamb's quarters seeds were surface sterilized with distilled water, then with 95% ethanol for 15 s, then rinsed with distilled water. Ten seeds were sown in each petri dish. Each test solution (7 mL) was poured on double-layered filter paper placed in the petri dishes. Distilled water and a DMSO solution were taken as the controls. All treatments were replicated three times. The germination tests were performed in a germinator with 70% relative humidity at 25 °C under 16-h light and 8-h dark lighting conditions. To measure the germination indices (GIs), the germinated seeds were counted daily. At the end of the last day of germination, the seedling growth and indices, including the final germination percentage (FGP), GI, the coefficient of velocity of germination (CVG), germination speed (GS), and the mean germination time (MGT), were calculated using the following formulas:

$$(1) \text{ FGP:}$$

$$\text{FGP} = \text{Ng/Nt} \times 100$$

$$\text{Ng} = \text{Total number of seeds germinated}$$

$$\text{Nt} = \text{Total number of seeds evaluated}$$

$$(2) \text{ GI:}$$

$$\text{GI} = (13 \times \text{N}_1) + (12 \times \text{N}_2) + \dots + (1 \times \text{N}_{13})$$

N₁, N₂ and ... are the number of germinated seeds on the first day, second, and other days, and the numbers 9, 10 and ... are the weights imposed on the number of seeds germinated at first day, second, and other days, respectively.

$$(3) \text{ CVG:}$$

$$\text{CVG} = 100 \times \frac{\sum \text{Ni}}{\sum \text{NiTi}}$$

$$\text{Ni} = \text{Number of germinated seeds per day}$$

$$\text{Ti} = \text{Days from the start of the experiment}$$

$$(4) \text{ GS was calculated according to the method of Magour:}$$

$$\text{Gs GS} = \sum \text{Si/Di}$$

$$\text{Si} = \text{Number of seeds germinated on the } i^{\text{th}} \text{ day}$$

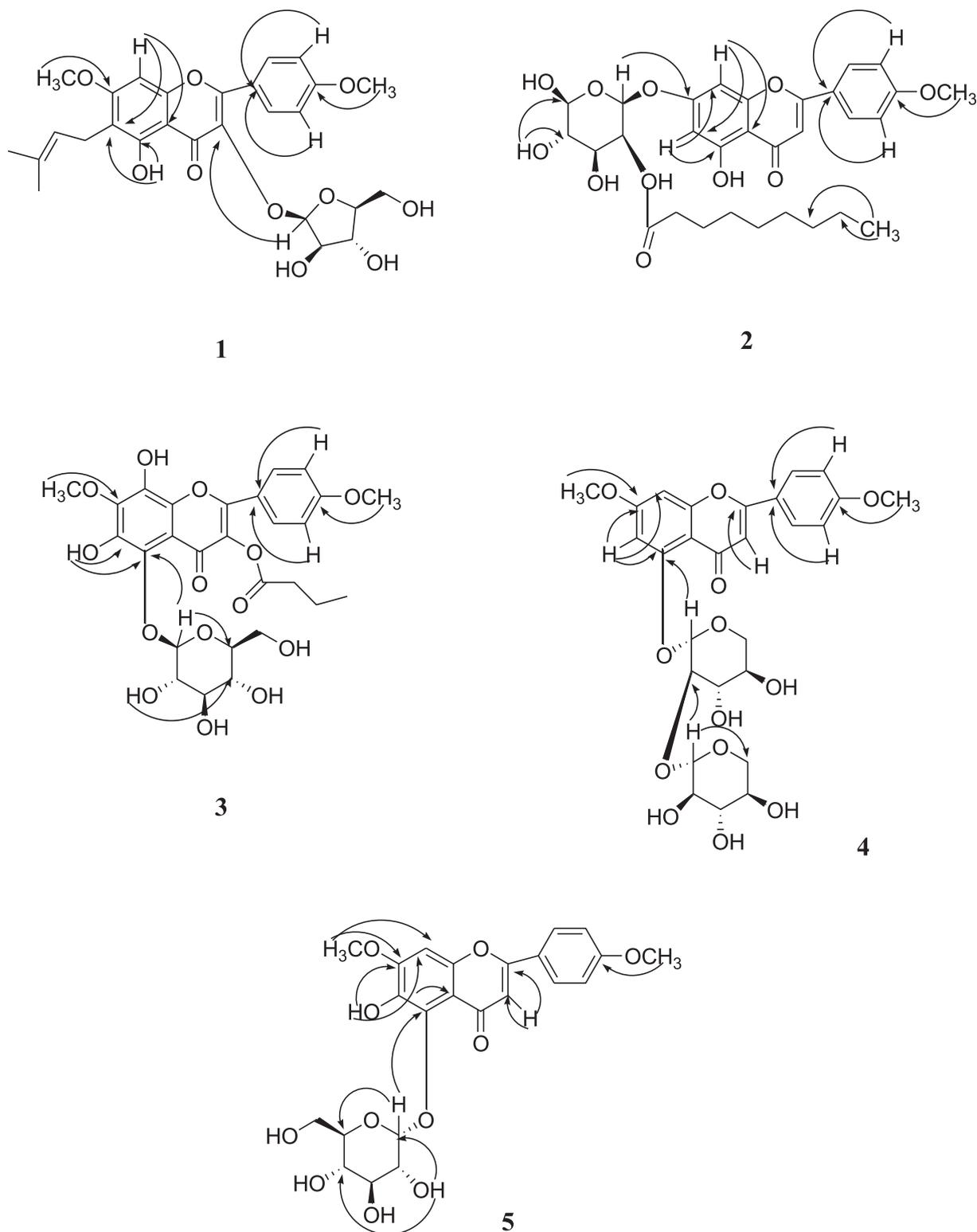


Fig. 3. HMBC correlation of compounds 1–5.

Di = Number of days to counting nth

(5) MGT:

$$\text{MGT} = \frac{\sum \text{NiTi}}{\sum \text{Ni}} = 100/\text{CVG}$$

Ni = Number of seeds germinated per day

Ti = Days from the beginning of the experiment

3. Results and discussion

The methanol extract of leaves and straw of *Oryza sativa* was column chromatographed and obtained seven compounds (1–7). Their structures were elucidated on the basis of spectroscopic data.

Compound 1 was isolated as a yellow semi-solid, $[\alpha]_D^{21} -37.2$ (c 0.1, MeOH). The molecular formula C₂₇H₃₁O₁₀ was established by ¹³C NMR and HRESI/FTMS data (*m/z* 515.1898 [M + H]⁺ calcd

for 515.1917), suggesting 13 indices of hydrogen deficiency. The UV absorption maxima at 270, 310 and 328 nm were characteristic of a flavonoid (Harborne and Williams, 1975; Markham, 1982; Chung et al., 2009; Mabry et al., 1970). The IR spectrum displayed characteristic absorption bands for hydroxy groups (3415 and 3369 cm^{-1}), carbonyl groups (1662 cm^{-1}) and aromatic rings (1614, 1590 cm^{-1}). The mass fragmentation patterns of compound **1** are shown in Fig. 1.

The ^1H NMR spectrum of **1** indicated a flavonoid moiety, as it displayed four one-proton meta-coupled doublets at δ_{H} 7.42 ($J = 8.0$ Hz), 6.77 ($J = 8.0$ Hz), 7.16 ($J = 8.5$ Hz), and 7.37 ($J = 8.5$ Hz) assigned to the H-2', H-3', H-5', and H-6' protons. Two one-proton doublets at δ_{H} 3.56 ($J = 7.0$ Hz) and 3.54 ($J = 7.0$ Hz) assigned to the H₂-5a'' and H₂-5b'' sugar protons, respectively. Two three-proton broad singlets at δ_{H} 3.29 and 3.30 and another two broad singlets at δ_{H} 2.01 and 2.05 were assigned to the methoxy protons at C-7 and C-4' in flavone moiety and Me-4'' and Me-5'' protons, respectively. The methylene and methine protons in isoprenyl moiety attached to flavone moiety appeared at δ_{H} 2.90 ($J = 7.5$ Hz), 2.93 ($J = 7.0$ Hz), and 6.65 ($J = 7.5$ Hz) and were assigned to the H-1''a, H-1''b, and H-2'' protons. The methine protons in the sugar that appeared as one doublet δ_{H} 6.40 ($J = 7.5$ Hz) and three multiplets δ_{H} 3.88, 3.85, and 4.56 were assigned to the H-1''' and H-2''', H-3''', H-4''' protons. The sugar unit in **1** was identified as β -arabinofuranoside by analyzing the coupling constant of the anomeric proton signal, which is evident as a one-proton doublet at δ_{H} 6.40 ($J = 7.5$ Hz). The ^{13}C NMR spectrum of **1** showed signals for the C-4 flavone carbonyl carbon at δ_{C} 179.2, for the other flavone carbons between δ_{C} 160.9 and 90.0, for the anomeric carbon at δ_{C} 116.6, for methoxy carbons at δ_{C} 56.5 and 56.4, for the other sugar carbons between δ_{C} 72.3 and 79.0, for vinylic carbons at δ_{C} 123.2 (C-2'') and 141.6 (C-3''), and for the other isoprenyl carbons at δ_{C} 41.4 (C-1''), 26.4 (C-4'') and 26.4 (C-5''). The ^1H and ^{13}C NMR shifts of the flavone moieties were compared with those described for similar compounds in the literature (Agrawal, 1989; Waffo et al., 2006). The ^1H - ^1H COSY spectrum of **1** showed correlations of H-2' with H-3' and H-6'; H-5' with H-6'; H-1'' with H-2'' and H-1'' with H-2'''. The appearance of the C-3 signal at δ_{H} 133.1 in the ^{13}C NMR spectrum supported the attachment of the sugar moiety at this carbon (Mariani et al., 2008; Schliemann et al., 2006; Saleem et al., 2006). The HMBC spectrum of **1** exhibited correlations of H-1''' with C-3; H-5 with C-5, C-6; H-8 with C-6, C-10; CH₃O with C-7, H-3', H-5' with C-1' and CH₃O with C-4' (Fig. 3). The HSQC correlations were used to assign all protons and carbons atoms in the molecule; some common correlations are H-1'' with C-1''. Acid hydrolysis of **1** yielded sugar part (see Section 2). According to the analysis of the spectroscopic data given above and the 2D-NMR data (COSY, HSQC, and HMBC), as well as the results from the chemical reaction, the structure of **1** has been established as 5-hydroxy-6-isoprenyl-7,4'-dimethoxyflavonol-3-O- β -D-arabinofuranoside, which is a new flavone arabinofuranoside as shown in Fig. 1.

Compound **2** was isolated as a yellow solid, $[\alpha]_{\text{D}}^{21} -31.0$ (c 0.1, MeOH). The molecular formula C₃₁H₃₉O₁₀ was established by ^{13}C NMR and HR-ESI/FTMS data (m/z 571.2492 [M + H]⁺ calcd for 571.2499), suggesting 13 indices of hydrogen deficiency. The UV absorption maxima at 266, 309 and 338 nm were characteristic of a flavonoid (Harborne and Williams, 1975; Markham, 1982; Chung et al., 2009; Mabry et al., 1970). The IR spectrum displayed characteristic absorption bands for hydroxy groups (3415 and 3364 cm^{-1}), ester functionalities (1721 cm^{-1}), carbonyl groups (1699 and 1655 cm^{-1}). The mass fragmentation patterns of compound **2** are shown in Fig. 1.

The ^1H NMR spectrum of **2** indicated a flavone moiety, as it displayed two one-proton meta-coupled singlets at δ_{H} 6.11 and 6.35 and ortho-coupled doublets at δ_{H} 7.01 ($J = 8.5$ Hz), 6.81 ($J = 8.5$

Hz), 6.98 ($J = 9.0$ Hz), and 6.78 ($J = 9.0$ Hz) assigned to the H-6, H-8, H-2', H-3', H-5' and H-6' protons, suggesting a 4'-oxygenated substitution pattern in ring B, a meta-coupled AX system corresponding to the H-6 and H-8 protons in ring A, and ortho-coupled protons characteristic of an AA'XX' spin system of a para-substituted phenyl ring in ring B. The sugar unit in **2** was identified as β -arabinofuranosyl by analyzing the coupling constant of the anomeric proton signal, which is evident as a one-proton doublet at δ_{H} 5.01 ($J = 7.0$ Hz). The methine protons H-2'', H-3'' and H-4'' in sugar appeared as multiplets at δ_{H} 4.21–3.82, and methylene proton in sugar H₂-5'' appeared as double doublets at δ_{H} 3.78 ($J = 4.5$ Hz) and 3.76 ($J = 5.0$ Hz). A three-proton triplet at δ_{H} 0.94 ($J = 7.5$ Hz) was attributed to the C-10''' primary methyl protons. Methylene protons resonated between δ_{H} 2.24 and 1.27, and a three-proton broad signal at δ_{H} 3.32 was assigned to the C-4' methoxy protons. The ^{13}C NMR spectrum of **2** showed signals for the C-4 flavone carbonyl carbon at δ_{C} 183.5, for the other flavone carbons between δ_{C} 165.9 and 95.2, for the anomeric carbon at δ_{C} 105.7 (C-1'') and ester carbon at δ_{C} 178.2 (C-1'''), for an aliphatic chain methyl carbon at δ_{C} 14.1 (C-8'''), for a methoxy carbon at δ_{C} 56.6, and for the other sugar carbons resonated between δ_{C} 87.5 and 62.8. The ^1H NMR signals in the deshielded region at δ_{H} 4.21 (H-2'') as well as the corresponding carbon signals at δ_{C} 87.5 (C-2'') suggested a (2 → 1) glycosidic linkage and confirmed the attachment of the ester group at C-2''. The appearance of the C-7 signal at δ_{C} 165.9 in the ^{13}C NMR spectrum was compared with literature data (Chung et al., 2009). The ^1H - ^1H COSY spectrum of **2** showed correlations of H-6 with H-8; H-2' with H-3' and H-6'; H-2'' with H-1'' and H-3''. The HMBC spectrum of **2** exhibited correlations of H-4'' with C-4'', C-5''; H-1'' with C-7; H-6 with C-5, 8, H-8 with C-6, C-10; H-3', H-5' with C-1'; CH₃O with C-4' (Fig. 3). The HSQC correlations were used to assign all protons and carbons atoms in the molecule and some important correlations are H-1'' with C-1'' and C-4'' interacted with H-5''. The ^1H and ^{13}C NMR signals of the flavone moieties were compared with those described for similar compounds in the literature (Agrawal, 1989). Acid hydrolysis of **2** yielded sugar part (see Section 2). According to the analysis of the spectroscopic data given above and the 2D NMR data (COSY, HSQC and HMBC) as well as the results from the chemical reaction tests, the structure of **2** has been established as 5,7-dihydroxy-4'-methoxyflavone-7-O- β -D-arabinopyranosyl-2'-*n*-decan-1-oate, which is a new compound as shown in Fig. 1.

Compound **3** was isolated as a yellow solid, $[\alpha]_{\text{D}}^{21} -31.0$ (c 0.1, MeOH). The molecular formula C₂₇H₃₁O₁₄ was established by ^{13}C NMR and HR-ESI/FTMS data (m/z 579.1661 [M + H]⁺ calcd for 579.1669), suggesting 13 indices of hydrogen deficiency. The UV absorption maxima at 277, 312, and 339 nm were characteristic of a flavonoid (Harborne and Williams, 1975; Markham, 1982; Chung et al., 2009; Mabry et al., 1970). The IR spectrum displayed characteristic absorption bands for hydroxy groups (3515, 3450 and 3361 cm^{-1}), ester functionalities (1722 cm^{-1}), carbonyl groups (1680 and 1655 cm^{-1}). The mass fragmentation patterns of compound **3** are shown in Fig. 1.

The ^1H NMR spectrum of **3** indicated a flavone moiety, as it displayed four ortho-coupled double doublets at δ_{H} 7.04 ($J = 8.0$ Hz), 6.70 ($J = 8.0$ Hz), 6.74 ($J = 8.5$ Hz) and δ_{H} 6.90 ($J = 8.5$ Hz) assigned to H-2', H-3', H-5', and H-6' protons, suggesting a 4'-oxygenated substitution pattern in ring B and ortho-coupled protons characteristic of an AA'XX' spin system of a para-substituted phenyl ring in ring B. The sugar unit in **3** was identified as β -arabinopyranoside by analyzing the coupling constant of the anomeric proton signal, which is evident as a one-proton doublet at δ_{H} 4.80 ($J = 7.0$ Hz). The remaining H-2''', H-3''', and H-4''' sugar protons appeared as multiplets at δ_{H} 3.86–3.57. A three-proton triplet at δ_{H} 0.95 ($J = 6.3$ Hz) was attributed to C-4'' primary methyl protons, methylene protons resonated between δ_{H} 2.59–1.22, and a three-proton two

broad signals at δ_H 3.75 and 3.82 were assigned to methoxy protons of C-7 and C-4'.

The ^{13}C NMR spectrum of **3** showed signals for the C-4 flavone carbonyl carbon at δ_C 175.1, for the other flavone carbons resonated between δ_C 162.8 and 104.9, for the anomeric carbon at δ_C 103.1 (C-1'''), for the ester carbon at δ_C 172.1 (C-1'''), for an aliphatic chain methyl carbon at δ_C 18.2 (C-4''), for a methoxy carbons at δ_C 56.4, 56.8, and for the other sugar carbons resonated between δ_C 78.1 and 62.5. The ^1H and ^{13}C NMR shifts of the flavone moieties were compared with those described for similar compounds in the literature (Agrawal, 1989). The ^1H - ^1H COSY spectrum of **3** showed correlations of H-2'' with H-3' and H-6'; H-2'' with H-3''' and H-1''' with H-2'''. The glucopyranosyl residue was located at the C-5 position of flavones skeleton according to long-range HMBC correlations between C-5 at δ_C 150.4 and the anomeric H-1''' at δ_H 4.80 was compared with literature data (Zahir et al., 1999). The HMBC spectrum of **3** exhibited correlations of CH_3O with C-7; H-6 with C-5, C-6; H-1''' with C-5, C-5''; H-2'' with C-4''; H-3', 5' with C-1' and H_3CO with C-4' (Fig. 3). The HSQC correlations were used to assign all protons and carbons atoms in the molecule and some important correlations are H-1''' with C-1''' and H-4''' interacted with C-4''. Acid hydrolysis of **3** yielded sugar part (see Section 2). According to the analysis of the spectroscopic data given above and the 2D NMR data (COSY, HSQC, and HMBC) as well as the results from the chemical reaction tests, the structure of **3** has been established as 3-butanoyl-5,6,8-trihydroxy-7,4'-dimethoxyflavonol-5-O- β -D-glucopyranoside, which is a new compound.

Compound **4** was isolated as a yellow solid, $[\alpha]_D^{21} -29.2$ (c 0.1, MeOH). The molecular formula $\text{C}_{27}\text{H}_{31}\text{O}_{13}$ was established by ^{13}C NMR and HR-ESI/FTMS data (m/z 563.1711 $[\text{M} + \text{H}]^+$ calcd for 563.1720), suggesting 13 indices of hydrogen deficiency. The UV absorption maxima at 269, 309, and 329 nm were characteristic of a flavonoid (Harborne and Williams, 1975; Markham, 1982; Chung et al., 2009; Mabry et al., 1970). The IR spectrum displayed characteristic absorption bands for hydroxy groups (3415 , 3371 and 3262 cm^{-1}), carbonyl groups (1663 cm^{-1}). The mass fragmentation patterns of compound **4** are shown in Fig. 1.

The ^1H NMR spectrum of **4** indicated a flavone moiety, as it displayed two one-proton meta-coupled singlets at δ_H 6.46 and 6.68 and ortho-coupled doublets at δ_H 7.36 ($J = 9.0\text{ Hz}$), 6.70 ($J = 9.0\text{ Hz}$), 6.78 ($J = 9.0\text{ Hz}$) and 7.34 ($J = 9.0\text{ Hz}$) assigned to H-2', H-3', H-5', and H-6' protons, suggesting a 4'-oxygenated substitution pattern in ring B, a meta-coupled AX system corresponding to

the H-6 and H-8 protons in ring A, and ortho-coupled protons characteristic of an AA'XX' spin system of a para-substituted phenyl ring in ring B. The sugar unit in **4** was identified as α -arabinopyranosyl by analyzing the coupling constant of the anomeric proton signals, which are evidence as two one-proton doublets at δ_H 5.40 ($J = 4.5\text{ Hz}$) and 5.13 ($J = 4.5\text{ Hz}$). The sugar protons H-2'', H-2'''; H-3'', H-3''' and H-4'', H-4''' appeared as multiplets between at δ_H 4.63 – 3.47, and the methylene sugar protons H₂-5'' and H₂-5''' appeared as multiplets at δ_H 3.29 and 3.17.

The ^{13}C NMR spectrum of **4** showed signals for the C-4 flavone carbonyl carbon at δ_C 183.3, for the other flavone carbons between δ_C 164.7 and 96.0, for the anomeric carbons at δ_C 105.3 (C-1'') and 101.3 (C-1'''), for methoxy carbons at δ_C 57.0 and 56.3, and for the other sugar carbons between δ_C 89.6 and 62.43. The ^1H NMR signals in the deshielded region at δ_H 4.63 (H-2'') as well as the corresponding carbon signals at δ_C 89.6 suggested a (2 \rightarrow 1) glycosidic linkage. The ^1H and ^{13}C NMR values of the flavone moiety were compared with those described for similar compounds in the literature (Agrawal, 1989). The arabinopyranosyl residue was located at the C-5 position of flavones skeleton according to long-range HMBC correlations between C-5 at δ_C 163.0 and the anomeric H-1'' at δ_H 5.40 as well as H-6 at δ_H 6.46; these results were compared to the literature data (Zahir et al., 1999).

The ^1H - ^1H COSY spectrum of **4** showed correlations of H-6 with H-8 and OMe (ring A); H-3' with OMe (ring B); H-2' with H-3'; H-5' with H-6'; H-2'' with H-1''. The HMBC spectrum of **4** exhibited correlations of CH_3O with C-7; H-6 with C-5, C-7 and C-8; H-3 with C-2; H-3', H-5' with C-1'; CH_3O with C-4'; H-1'' with C-5 and H-1''' with C-2'' (Fig. 3). The HSQC correlations were used to assign all protons and carbons atoms in the molecule and some important correlations are H-1'' with C-1'; and H-1''' with C-1''. Acid hydrolysis of **4** yielded sugar part (see Section 2). According to the analysis of the spectroscopic data given above and the 2D-NMR data (COSY, HSQC and HMBC) as well as the results from the chemical reaction tests, the structure of **4** has been established as 7,4'-dime thoxy-5-hydroxyflavone-5-O- α -D-arabinopyranosyl-(2'' \rightarrow 1''')-O- α -D-arabinopyranoside, which is a new compound (Fig. 1).

Compound **5** was isolated as a yellow solid, $[\alpha]_D^{21} -39.2$ (c 0.1, MeOH). The molecular formula $\text{C}_{23}\text{H}_{25}\text{O}_{11}$ was established by ^{13}C NMR and HRESIFMS data (m/z 477.1347) $[\text{M} + \text{H}]^+$ calcd for 477.1352), suggesting 12 indices of hydrogen deficiency. The UV absorption maxima at 279, 313, and 334 nm were characteristic of a flavonoid (Harborne and Williams, 1975; Markham, 1982;

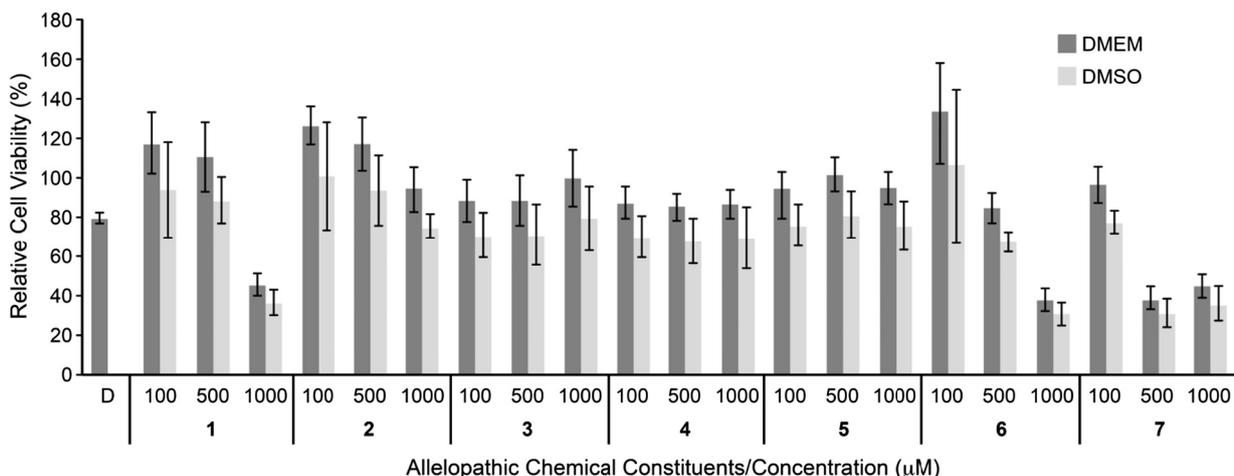


Fig. 4. Percentage viability of RAW 264.7 cells exposed to the isolated chemical constituents from *Oryza sativa* (straw and leaves). DMSO (Dimethyl sulfoxide). Data are presented for 24 h and calculated from the absorbance values obtained from the MTT assay. The cell viability percentage was the mean absorbance of the seven allelopathic chemical constituents at different concentrations (100, 500, 1000 μM) divided by that of the corresponding control group. The bars represent the mean \pm SD obtained from three independent experiments.

Table 3
Germination Characteristics of Pigweed from Treatments with Different Concentrations of the Constituents **1–7** from rice.

Compounds	Concentration (μM)	FGP ^c (%)	MGT ^d (days)	GS ^e (days)	CVG ^f	GI ^g
CON ^a		76.67 \pm 12.55	2.86 \pm 0.58	7.67 \pm 0.66	51.11 \pm 12.86	1.53 \pm 0.33 ^h
DMSO ^b	5% in DW	43.21 \pm 13.26	3.33 \pm 0.51	4.33 \pm 1.51	32.04 \pm 12.53	0.87 \pm 0.12
PBC ^h	100	48.26 \pm 13.09	3.97 \pm 0.12	5.33 \pm 1.04	29.52 \pm 16.50	0.87 \pm 0.18
	500	49.27 \pm 11.55	5.00 \pm 0.58	5.00 \pm 1.58	29.26 \pm 8.49	1.00 \pm 0.29
	1000	43.33 \pm 12.36	5.33 \pm 0.51	4.33 \pm 1.51	22.04 \pm 12.53	0.87 \pm 0.09
1	100	43.33 \pm 13.09	2.17 \pm 0.75	4.33 \pm 1.51	25.13 \pm 8.88	0.87 \pm 0.020
	500	56.67 \pm 10.66	4.50 \pm 1.50	5.00 \pm 1.61	28.84 \pm 8.75	0.82 \pm 0.27
	1000	43.33 \pm 10.09	5.00 \pm 0.73	5.00 \pm 1.46	11.11 \pm 5.25	0.40 \pm 0.09
2	100	50.00 \pm 10.04	3.67 \pm 1.51	6.00 \pm 1.21	23.10 \pm 2.54	1.20 \pm 0.44
	500	43.33 \pm 9.54	4.00 \pm 1.29	4.00 \pm 1.29	20.00 \pm 6.46	0.80 \pm 0.06
	1000	56.67 \pm 10.09	3.89 \pm 1.08	4.67 \pm 1.16	19.44 \pm 1.35	0.63 \pm 0.03
3	100	43.33 \pm 9.09	2.67 \pm 0.85	6.33 \pm 1.31	16.67 \pm 2.87	1.27 \pm 0.46
	500	13.24 \pm 10.07	5.00 \pm 3.61	7.67 \pm 5.13	18.70 \pm 1.71	0.53 \pm 1.03
	1000	– ⁱ	–	–	–	–
4	100	50.45 \pm 8.48	2.44 \pm 0.98	4.16 \pm 0.78	50.17 \pm 8.21	0.84 \pm 0.01
	500	43.33 \pm 8.09	2.83 \pm 0.94	5.67 \pm 2.89	53.33 \pm 3.09	0.45 \pm 0.08
	1000	26.67 \pm 6.23	3.67 \pm 2.89	5.67 \pm 2.89	46.67 \pm 1.55	0.47 \pm 0.28
5	100	56.67 \pm 10.48	2.50 \pm 0.87	4.33 \pm 1.31	60.00 \pm 4.64	0.97 \pm 0.06
	500	36.67 \pm 9.54	3.33 \pm 0.58	6.33 \pm 0.58	32.06 \pm 7.74	0.97 \pm 0.12
	1000	33.33 \pm 8.75	5.83 \pm 1.46	5.00 \pm 0.20	56.06 \pm 1.24	1.00 \pm 0.04
6	100	63.78 \pm 11.21	3.33 \pm 0.08	5.00 \pm 1.30	68.33 \pm 14.81	1.00 \pm 0.06
	500	36.67 \pm 10.45	4.00 \pm 0.48	5.00 \pm 1.46	25.56 \pm 9.62	0.84 \pm 0.09
	1000	33.33 \pm 10.11	4.50 \pm 0.50	5.33 \pm 1.08	21.11 \pm 8.39	0.87 \pm 0.02
7	100	50.00 \pm 10.09	3.38 \pm 0.89	5.00 \pm 1.00	31.14 \pm 1.55	1.00 \pm 0.10
	500	43.33 \pm 12.14	3.32 \pm 1.53	6.33 \pm 1.53	24.44 \pm 5.92	0.77 \pm 0.31
	1000	43.33 \pm 11.16	3.67 \pm 1.21	6.67 \pm 1.21	12.22 \pm 5.72	0.73 \pm 0.34

^aData are presented as the means of three experiments performed in triplicate ($p < .01$).

^a CON; Control (germinated by distilled water).

^b DMSO; Dimethyl sulfoxide.

^c FGP; Final germination percentage.

^d MGT; Mean germination time.

^e GS; Germination speed.

^f CVG; Coefficient of velocity of germination.

^g GI; Germination index.

^h PBC; Pyributicarb.

ⁱ –; Not germinated.

Chung et al., 2009; Mabry et al., 1970). The IR spectrum displayed characteristic absorption bands for hydroxy groups (3415, 3383, and 3281 cm^{-1}), carbonyl groups (1701 cm^{-1}). The mass fragmentation patterns of compound **5** are shown in Fig. 1.

The ^1H NMR spectrum of **5** indicated a flavone moiety, as it displayed two one-proton singlets at δ_{H} 6.54 and 6.79 and ortho-coupled doublets at δ_{H} 7.01 ($J = 8.5$ Hz), 6.71 ($J = 8.5$ Hz), 6.75 ($J = 8.5$ Hz), and 7.17 ($J = 8.5$ Hz) assigned to the H-3, H-8, H-2', H-3', H-5', and H-6' protons, suggesting a 4'-oxygenated substitution pattern in ring B and ortho-coupled protons characteristic of an AA'XX' spin system of a para-substituted phenyl ring in ring B. The sugar unit in **5** was identified as α -glucopyranosyl by analyzing the coupling constant of the anomeric proton signal, which is evident as a one-proton doublet at δ_{H} 5.01 ($J = 4.5$ Hz). The other methine protons H-2'', H-3'', H-4'' and H-5'' appeared as multiplets between at δ_{H} 4.20–3.67, and methylene protons H₂-6'' appeared as a broad singlet at δ_{H} 3.29. The ^{13}C NMR spectrum of **5** showed signals for the C-4 flavone carbonyl carbon at δ_{C} 180.2; for the other flavone carbons at δ_{C} 164.0 (C-2), 104.9 (C-3), 163.1 (C-5), 149.6 (C-6), 164.8 (C-7), 96.3 (C-8), 160.1 (C-9), 107.2 (C-10), 127.8 (C-1'), 129.50 (C-2'), 115.69 (C-3'), 154.8 (C-4'), 114.64 (C-5'), and 120.8 (C-1''); for the anomeric carbon at δ_{C} 105.2 (C-1''); for methoxy carbons at δ_{C} 57.0 and 56.4, and for the other sugar carbons between δ_{C} 89.6 and 62.4. The ^1H and ^{13}C NMR values of the flavone moieties were compared with those described for similar compounds in the literature (Agrawal, 1989). Acid hydrolysis of **5** yielded flavones and sugar. The glucosyl residue was located at

the C-5 position of flavones skeleton according to long-range HMBC correlations between C-5 at δ_{C} 163.1 and the anomeric H-1''' at δ_{H} 3.67 was compared with literature data (Zahir et al., 1999).

The ^1H - ^1H COSY spectrum of **5** showed correlations of H-2' with H-3'; H-5' with H-6'; H-2'' with H-1'' and H-3''; H-8 with OMe (ring A); H-3' with OMe (ring B). The HMBC spectrum of **5** exhibited interactions of H-3 with C-2, C-3; H-6 with C-7, C-8, C-10; H₃CO with C-7, C-8; H₃CO with C-3' (Fig. 3). The HSQC correlations were used to assign all protons and carbons atoms in the molecule and some important correlations are H-1' with C-1'. Acid hydrolysis of **5** yielded sugar part (see Section 2). According to the analysis of spectroscopic data given above and the 2D NMR data (COSY, HSQC and HMBC) as well as the results from the chemical reaction tests, the structure of **5** has been established as 5, 6-dihydroxy-7, 4'-dimethoxyflavone-5-O- α -D-glucopyranoside, which is a new compound (Fig. 1).

4'-methoxyapigenin (**6**), 5,7-dihydroxy-4'-methoxyflavone-7-O- β -D-arabinopyranoside(**7**), β -sitosterol, β -sitosterol- β -D-glucoside were identified by comparison with literature data (Meyer et al., 2006). This is the first report of the isolation of compounds **6–7** from this plant.

The cytotoxicity of the constituents (**1–7**) on a (RAW 264.7) macrophage cell line were determined by the MTT assay, and the results are shown in Fig. 4. The data indicate that the cell viability rate decreased significantly ($p < .05$) compared to those of the control group as the concentrations of compounds **1**, **6** and **7** increased. However, the cytotoxicity increased dose dependently.

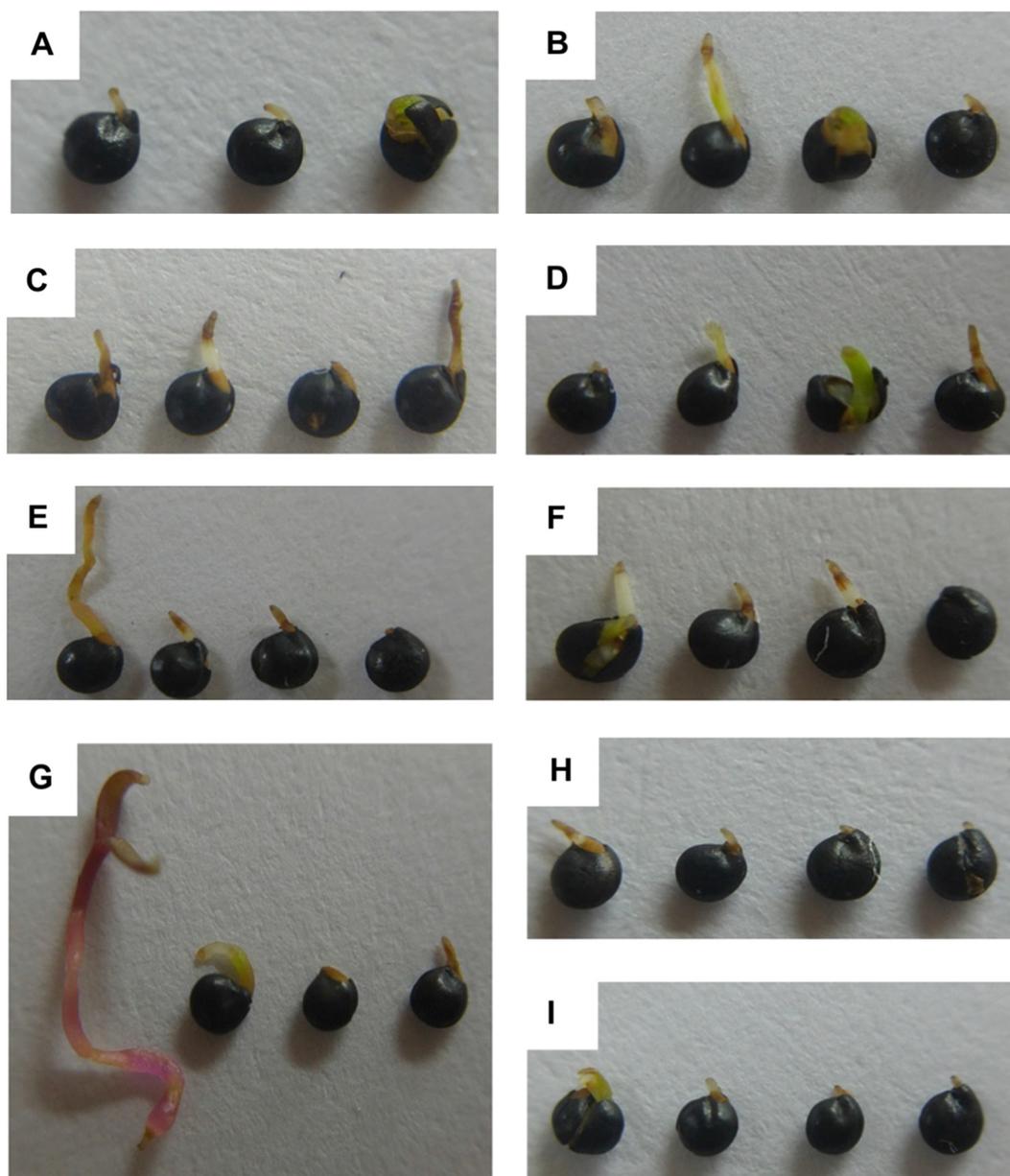


Fig. 5. Characterization of pigweed seed germination and morphology from treatments with different concentrations of the constituents isolated from rice (*Oryza sativa* L.). Left panel; pyributicarb, center panel; 0.5%DMSO treatment alone, right panel; distilled water treatment of control (A), pyributicarb (B), (C, compound 1), (D, compound 2), (E, compound 3), (F, compound 4) and (G, compound 5) as well as known compounds (H, compound 6) and (I, compound 7). The first panel was 0.5% DMSO treatment alone, followed by treatment of the isolated chemical constituents from rice at concentrations of 100, 500, and 1000 μM .

In each experiment in the current study, the absorbances among the three parallel experiments were similar. Overall, the results showed that the inhibitory effect of compounds **1**, **6**, and **7** at 100 μM were very nearly similar, with only slight differences. According to the results, 5% DMSO in distilled water displayed a higher cytotoxic effect (79.9%) than DMEM (100%). When the concentration of compounds **2**, **3**, **4**, and **5** in DMSO were 100 μM , the corresponding cell viability rate observed in RAW 264.7 cells was 101.1, 70.7, 69.9, and 75.8%, respectively. Compounds **3**, **4**, and **5** at 1000 μM had little or no toxicity, whereas compound **2** at higher concentrations inhibited cell growth. In addition, the viability rate of all chemical constituents did not exceed 69.4% at doses up to 1000 μM in Fig. 4.

The allelopathic effects of compounds **1–7** at different concentrations (100, 500, 1000 μM) on the tested germination parameters

final germination percentage (FGP), mean germination time (MGT), germination speed (GS), coefficient of velocity of germination (CVG) and germination index (GI)] are shown in Table 3. The FGP, MGT, GS, CVG, and GI values with distilled water were 76.67%, 2.86 days, 7.67 days, 51.1 1 day^{-1} , and 1.53, respectively. The corresponding values with DMSO were 43.21%, 3.33 days, 4.33 days, 32.04 day^{-1} , and 0.87.

Applying pyributicarb (PBC) had negligible inhibitory effects on pigweed germination (Fig. 6). FGP was slightly affected by compounds **2**, **5**, **6**, and **7**; however, the GS and CVG values showed greater variability than the other germination parameters. The GS and CVG ratios decreased as the concentration of the chemical constituents increased. According to this result, in the presence of PBC, compounds **2**, **5**, **6**, and **7** delayed the emergence of seeds. Germination is an important stage in plant growth, and these

Table 4

Germination characteristics of barnyard grass from treatments with different concentrations of the constituents 1–7 from rice.

Compounds	Concentration (μM)	FGP ^d (%)	MGT ^e (days)	GS ^f	CVG ^g	GI ^h
CON ^a		82.67 \pm 3.06	5.68 \pm 0.24	1.26 \pm 0.16	7.66 \pm 1.47	4.00 \pm 0.87 ⁱ
WP ^b		100.00 \pm 0.00	4.68 \pm 0.12	4.33 \pm 0.12	21.67 \pm 0.58	23.09 \pm 0.62
DMSO ^c	5% in DW	48.25 \pm 2.00	5.73 \pm 1.62	1.60 \pm 0.08	18.00 \pm 8.00	8.33 \pm 7.22
PBC ⁱ	100	26.14 \pm 1.55	7.00 \pm 1.20	1.60 \pm 0.04	3.00 \pm 5.20	3.70 \pm 6.42
	500	– ^j	–	–	–	–
	1000	–	–	–	–	–
1	100	13.23 \pm 3.09	2.00 \pm 0.46	1.39 \pm 0.02	6.93 \pm 2.00	3.75 \pm 0.44
	500	–	–	–	–	–
	1000	–	–	–	–	–
2	100	13.14 \pm 3.09	2.67 \pm 0.62	1.07 \pm 0.05	5.33 \pm 1.24	4.17 \pm 1.22
	500	13.16 \pm 2.14	2.83 \pm 0.91	1.13 \pm 0.06	5.67 \pm 1.81	3.92 \pm 1.79
	1000	–	–	–	–	–
3	100	20.25 \pm 1.25	5.67 \pm 0.93	1.73 \pm 0.40	8.67 \pm 0.02	7.87 \pm 1.85
	500	6.67 \pm 1.44	3.33 \pm 1.77	0.67 \pm 0.15	3.78 \pm 1.77	3.85 \pm 1.77
	1000	–	–	–	–	–
4	100	6.67 \pm 1.47	3.00 \pm 1.20	0.60 \pm 1.04	3.00 \pm 1.20	7.70 \pm 0.42
	500	3.33 \pm 0.55	5.78 \pm 1.01	1.87 \pm 0.85	1.43 \pm 0.90	3.70 \pm 0.69
	1000	–	–	–	–	–
5	100	–	–	–	–	–
	500	–	–	–	–	–
	1000	–	–	–	–	–
6	100	6.67 \pm 1.55	3.46 \pm 0.28	1.40 \pm 0.09	2.00 \pm 0.46	3.56 \pm 0.62
	500	–	–	–	–	–
	1000	–	–	–	–	–
7	100	20.00 \pm 4.64	2.78 \pm 0.81	1.67 \pm 0.89	8.33 \pm 1.43	4.00 \pm 0.93
	500	6.67 \pm 1.25	3.00 \pm 0.20	1.60 \pm 0.04	3.00 \pm 0.20	3.70 \pm 0.42
	1000	6.67 \pm 1.34	2.33 \pm 0.04	1.47 \pm 0.81	2.33 \pm 0.04	3.76 \pm 0.25

^aData are presented as the means of three experiments performed in triplicate ($p < .01$).

^a CON; Control (germinated by distilled water).

^b WP; Water priming by soaking seeds with distilled water for one day before the germination test for dormancy breakage.

^c DMSO; Dimethyl sulfoxide.

^d FGP; Final germination percentage.

^e MGT; Mean germination time.

^f GS; Germination speed.

^g CVG; Coefficient of velocity of germination.

^h GI; Germination index.

ⁱ PBC; Pyributicarb.

^j –; Not germinated.

chemical constituents can regulate these interactions, both within and between species in plant communities. Fernandez (Escudero et al., 2000) reported that seed germination includes several phases, including water imbibitions and catabolic and anabolic phases. Irregularities in the respiration rate reduced metabolic energy (ATP) and resulted in seed germination and growth reduction (Fernandez et al., 2008). The activity of enzymes such as proteinase, lipase, and α -amylase, which play an important role during germination, is inhibited under the influence of allelopathic chemicals. These compounds reduced and delayed germination (Escudero et al., 2000).

Clearly, compounds **3** and **4** drastically affected all germination parameters. Compound **3** inhibited FGP significantly more than the other chemical constituents. Compared to PBC and the other chemical constituents, compound **3** at 1000 μM had the strongest allelopathic effect on pigweed seed germination and growth (Fig. 5).

Barnyardgrass germination was significantly inhibited at low PBC concentrations (100 μM) of PBC and was completely inhibited at moderate (500 μM) and high (1000 μM) PBC concentrations (Table 4). In general, all the isolated chemical constituents from rice inhibited the FGP of barnyardgrass within the range of 3.33–20.25% compared to 48.25% FGP for DMSO and 100% for distilled water. Compared to an MGT of 4.68, a GS of 4.33, a CVG of 21.67 and a GI of 23.09 for distilled water. For the isolated chemical constituents from rice, the MGT ratios ranged from 1 to 5.78, GS ran-

ged from 1.87 to 0.60, CVG ranged from 8.33 to 1.43, and GI ranged from 7.87 to 3.56; by comparison, for distilled water, the MGT was 4.68, the GS was 4.33, the CVG was 21.67 and the GI was 23.09. The MGT value shows negligible changes compared to the other germination parameters. Conversely, the GS, CVG, and GI values differed from the aforementioned germination parameters. These parameters decreased as the concentrations of the isolated chemical constituents from rice decreased (Table 2), indicating that these chemical constituents decreased the intensity of germination induced by WP treatments and inhibited seed germination and seedling emergence. Many researchers have also reported that in weak or stored seeds, priming increased germination and emergence (Horii et al., 2007). Additionally, several reports have indicated that increasing seed age and decreasing seed vigor adversely affect the ability of seedlings to grow in the field (Parrish and Leopold, 1978).

The seedling growth (hypocotyl and radicle) of barnyard grass was very sensitive to the inhibition of hypocotyl elongation (Fig. 6). Seed germination was inhibited completely at high concentrations (1000 μM) of compounds **3** and **4**. Furthermore, all applied concentrations of compound **5** completely inhibited the germination and growth of barnyard grass.

Compounds **6** and **7** were originally isolated from rice leaf and straw as phytoalexins to participate in the defense of rice against pathogens, whereas, to the best of our knowledge, compounds **1**,

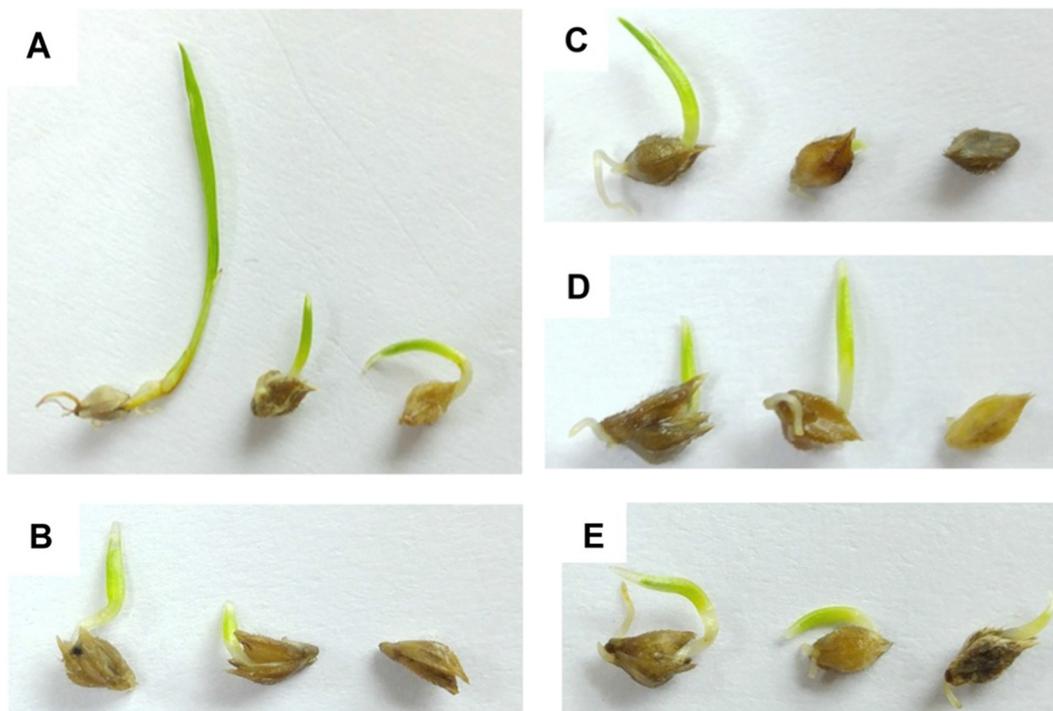


Fig. 6. Characterization of barnyardgrass seed germination and morphology from treatments with different concentrations of the constituents from rice (*Oryza sativa*). Left panel; distilled water, center panel; 0.5% DMSO treatment alone, right panel; pyributicarb treatment of the control (A), (B, compound 2), (C, compound 3), (D, compound 4), and (E, compound 7). The first panel shows treatment with the isolated chemical constituents from rice at 100, 500 and 1000 μM .

2, 3, 4, and 5 have not previously been reported in rice plants. Because they are substituted methoxy analogues of flavone, compounds **3** (5,6,8-trihydroxy-7,4'-dimethoxyflavanol-3-butanoyl-5-*O*- β -*D*-glucopyranoside) and **5** (5,6-dihydroxy-7,4'-dimethoxyflavone-5-*O*- α -*D*-glucopyranoside) could significantly inhibit the growth of pigweed and barnyardgrass. Biological trials were performed by method (Kong et al., 2004) showed the inhibitory activity of 5,7,4'-trihydroxy-3',5'-dimethoxyflavone against weeds of the species *E. crusgalli*, *Cyperus difformis*, and *Cyperus iria*.

Plant–plant interactions can be positive or negative and may depend on flavonoid concentrations (Chou, 1999). The negative interactions mainly involve inhibiting the germination of other plants' seedlings (Treutter, 2005). However, the precise mechanism by which flavonoids participate in allelopathy remains unknown. The potential ways in which these compounds can influence allelopathy may include inhibiting cell growth, disturbing ATP production, and hindering the proper functioning of auxins (Berhow and Vaughn, 1999). Flavanols were reported to provoke an abundance of reactive oxygen species, which activate the Ca^{2+} signal cascade and root system death (Bais et al., 2003).

These results suggested that the five new constituents and two known compounds isolated from rice might be used as natural herbicides in paddy fields. However, more experiments on the application rates or time of these compounds are needed to increase the efficacy of the isolated chemical constituents from rice. In this study, these isolated compounds were established as promising materials for the biological control of weeds such as pigweed and barnyardgrass. This method is safer for human health and the environment than herbicide application. However, further study is needed to verify the mechanism underlying the allelopathic characteristics of flavonoid and its substituted analogues. In addition, the extent to which dependence on synthetic herbicides can be reduced by this approach should be investigated.

4. Conclusions

To the best of our knowledge, there has been no prior report on the phytochemistry of straw and leaves on this paper isolation five new and two known compounds along with its biological activities including cytotoxicity and allelopathic activities on weed germination.

Notes

The authors declare that they have no conflicts of interest.

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2015R1A2A1A15051532) and also this paper was supported by Konkuk University Researcher Fund in 2017.

References

- Agrawal, P.K., 1989. Carbon-13 NMR of Flavonoids; Volume 39. Elsevier, New York.
- Ahmad, A., Kim, S.H., Ali, M., Park, I., Kim, J.S., Kim, E.H., Kim, J.J., Kim, S.K., Chung, I.M., 2013. New chemical constituents from *Oryza sativa* straw and their algicidal activities against blue-green algae. *J. Agric. Food Chem.* 61, 8039–8048.
- Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M., Vivanco, J.M., 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* 301, 1377–1380.
- Berhow, M.A., Vaughn, S.F., 1999. Principles and Practices in Plant Ecology. Allelochemical Interaction. CRC Press LLC, Florida, FL USA.
- Chou, C.H., 1999. Roles of allelopathy in plant biodiversity and sustainable agriculture. *Crit. Rev. Plant Sci.* 18, 609–636.
- Chung, I.M., Ahmad, A., Ali, M., Lee, O.K., Kim, M.Y., Kim, J.H., Yoon, D.Y., Peebles, C.A.M., San, K.Y., 2009. Flavonoid glucosides from the hairy roots of *Catharanthus roseus*. *J. Nat. Prod.* 72, 613–620.

- Chung, I.M., Ali, M., Ahmad, A., Chun, S.C., Kim, J.T., Sultana, S., Kim, J.S., Min, S.K., Seo, B.R., 2007a. Steroidal constituents of rice (*Oryza sativa*) hulls with algicidal and herbicidal activity against blue-green algae and duckweed. *Phytochem. Anal.* 18, 133–145.
- Chung, I.M., Ali, M., Ahmad, A., Lim, J.D., Yu, C.Y., Kim, J.S., 2006a. Chemical constituents of rice (*Oryza sativa*) hulls and their herbicidal activity against duckweed (*Lemna paucicostata* Hegelm 381). *Phytochem. Anal.* 17, 36–45.
- Chung, I.M., Ali, M., Chun, S.C., Jin, W.U., Cho, D.H., Hong, S.B., Ahmad, A., 2007b. New aliphatic alcohol and ester constituents from rice hulls of *Oryza sativa*. *Chin. J. Chem.* 25, 843–848.
- Chung, I.M., Ali, M., Hahn, S.J., Siddiqui, N.A., Lim, Y.H., Ahmad, A., 2005a. Chemical constituents from the hulls of *Oryza sativa* with cytotoxic activity. *Chem. Nat. Compds.* 41, 182–189.
- Chung, I.M., Hahn, S.J., Ahmad, A., 2005b. Confirmation of potential herbicidal agents in hulls of rice, *Oryza sativa*. *J. Chem. Ecol.* 31, 1339–1352.
- Chung, I.M., Kim, J.T., Kim, S.H., 2006b. Evaluation of allelopathic potential and quantification of momilactone A, B from rice hull extracts and assessment of inhibitory bioactivity on paddy field weeds. *J. Agric. Food Chem.* 54, 2527–2536.
- Chung, I.M., Kim, S.H., Oh, Y.T., Ali, M., Ahmad, A., 2017. New constituents from *Oryza sativa* L. straw and their algicidal activities against blue-green algae. *Allelopathy J.* 40, 47–62.
- Dutta, A.K., 1973. Germination and growth-inhibitors in relation to nonviability of rice seeds. *Ind. J. Agric. Sci.* 42, 894–900.
- Escudero, A., Albert, M.J., Pita, J.M., Pérez-García, F., 2000. Inhibitory effects of *Artemisia herba-alba* on the germination of the gypsophyte *Helianthemum squamatum*. *Plant Ecol.* 148, 71–80.
- Fernandez, C., Voiriot, S., Mévy, J.P., Vila, B., Ormeño, E., Dupouyet, S., Bousquet-Mélou, A., 2008. Regeneration failure of *Pinus halepensis* Mill.: The role of autotoxicity and some abiotic environmental parameters. *For. Ecol. Manage.* 255, 2928–2936.
- Harborne, J.B., Williams, C.A., 1975. *The Flavonoids*. Chapman and Hall, London, UK.
- Horii, A., McCue, P., Shetty, K., 2007. Enhancement of seed vigour following insecticide and phenolic elicitor treatment. *Bioresour. Technol.* 98, 623–632.
- Kato, T., Kabuto, C., Sasakin, N., Tsunagawa, M., Aizwa, H., Fujita, K., Kato, Y., Kitahara, Y., 1973. Momilactones, growth inhibitors from rice, *Oryza sativa* L. *Tetrahedron Lett.* 39, 3861–3864.
- Kato, T., Tsunagawa, M., Sasakin, N., Aizwa, H., Fujita, K., Kato, Y., Kitahara, Y., 1977. Growth and germination inhibitors in rice husks. *Phytochemistry* 16, 45–48.
- Kato-Naguchi, H., Ino, T., 2003. Rice seedlings release momilactone B into the environment. *Phytochemistry* 63, 551–554.
- Kato-Naguchi, H., Ino, T., Sata, N., Yamamura, S., 2002. Isolation and identification of a potent allelopathic substance in rice root exudates. *Physiol. Plant* 115, 401–405.
- Kong, C., Xu, X., Zhou, B., Hu, F., Zhang, C., Zhang, M., 2004. Two compounds from allelopathic rice accession and their inhibitory activity on weeds and fungal pathogens. *Phytochemistry* 65, 1123–1128.
- Mabry, T.J., Markham, K.R., Thomas, M.B., 1970. *The Systematic Identification of Flavonoids*. Springer-Verlag, New York.
- Mariani, C., Braca, A., Vitalini, S., De Tommasi, N., Visioli, F., Fico, G., 2008. Flavonoid characterization and in vitro antioxidant activity of *Aconitum anthora* L. (Ranunculaceae). *Phytochemistry* 69, 1220–1226.
- Markham, K.R., 1982. *Techniques of Flavonoid Identification*. Academic Press, London, UK.
- Meyer, H., Bolarinwa, A., Wolfram, G., Linseisen, J., 2006. Bioavailability of Apigenin from apiin-rich parsley in humans. *Ann. Nutr. Metab.* 50, 167–172.
- Parrish, D.J., Leopold, A.C., 1978. On the mechanism of aging in soybean seeds. *Plant Physiology* 61, 365–368.
- Saleem, M., Kim, H.J., Han, C.K., Jin, C., Lee, Y.S., 2006. Secondary metabolites from *Opuntia ficus-indica* var. *saboten*. *Phytochemistry* 67, 1390–1394.
- Schliemann, W., Schneider, B., Wray, V., Schmidt, J., Nimtz, M., Porzel, A., Böhm, H., 2006. Flavonols and an indole alkaloid skeleton bearing identical acylated glycosidic groups from yellow petals of *Papaver nudicaule*. *Phytochemistry* 67, 191–201.
- Treutter, D., 2005. Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Biology* 7, 581–591.
- Waffo, A.F.K., Coombes, P.H., Mulholland, D.A., Nkengfack, A.E., Fomum, Z.T., 2006. Flavones and isoflavones from the west african fabaceae *erythrina vogelii*. *Phytochemistry* 67, 459–463.
- Zahir, A., Jossang, A., Bodo, B., Provost, J., Cosson, J.P., Sevenet, T., 1999. Five new flavone 5-o-glycosides from *lethedon tannaensis*: lethedosides and lethediosides. *J. Nat. Prods.* 62, 241–243.