Phenolic Compounds and Flavonoids as Plant Growth Regulators from Fruit and Leaf of *Vitex rotundifolia*

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Five phenolic compounds, 4-hydroxybenzoic acid methyl ester (**1**), vanillic acid methyl ester (**2**), 4-hydroxy benzaldehyde (**3**), 4-hydroxybenzoic acid (**4**) and ferulic acid (**5**), and four flavonoids, 5,5′-dihydroxy-4′,6,7-trimethoxyflavanone (**6**), luteolin (**7**), vitexicarpin (**8**) and artemetin (**9**), were isolated from fruits and leaves of *Vitex rotundifolia* L. The biological activities of these nine compounds have been examined using a bioassay with lettuce seedlings.

Key words: Phenolic Compounds, Flavonoids, *Vitex rotundifolia*

Introduction

Tottori sand dune runs about 16 km from east to west and 2 km from north to south and is the largest dune in Japan. The vegetation of Tottori sand dune consists of 12 species of trees and shrubs, 10 species of annual and biennial herbs, and 12 species of perennial herbs (Fujiki *et al.*, 2001). *Vitex rotundifolia* L. (Verbenaceae), a rapidly spreading shrub, is widely distributed at the Asian and Oceanian coast. This *Vitex* can be found on Tottori sand dune and dominates over this semi-fixed dune the forming of extensive colonies (Fujiki *et al.*, 2001). Its fruit is used as a folk medicine for headaches (Ono *et al.*, 1997, 2002). VR-I (10-*o*-vanilloyl aucubin) a component of this fruit was found to show strong antioxidative activity (Okuyama *et al.*, 1995). However, plant growth and allelopathic activities of the chemical constituents of this plant have not been previously studied. In the course of our search for plant growth regulators from plant material suitable for developing new herbicides and suitable for the study of chemical ecology, we found the presence of plant growth regulators in fruit and leaf using a bioassay method with lettuce seedlings. In this report, we describe the isolation, structural identification and biological activities of the active compounds.

Materials and Methods

General

The IR spectra were recorded on a JASCO FT IR-7000 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded with a JEOL JNM-ESP 500 NMR spectrometer at 500 and 125 MHz, respectively. Vanillic acid, quercetin and fisetin were purchased from Wako Pure Chemical Industry, Osaka, Japan.

Plant material

Fresh leaves and fruits of *Vitex rotundifolia* L., growing wild on Tottori sand dune, were collected in September and November 2001 and dried at room temperature.

Extraction and isolation of compounds

Dried fruits of *V. rotundifolia* L. (5 kg) were extracted with MeOH (15 l) at room temperature and the solvent was removed under reduced pressure to afford a brown syrup. This syrup was redissolved in water and adjusted to pH 2.0 with 2 N HCl, before being extracted twice with EtOAc. The EtOAc-soluble acidic phases were combined and partitioned twice between a saturated sodium hydrogen carbonate solution. The EtOAc-soluble neutral phases were combined, and concentrated *in vacuo*. The resulting residue (12 g) was first fractionated by column chromatography on silica gel (*n*-hexane/EtOAc).

1) Fraction 4 (482 mg), obtained by elution with 30% EtOAc, was further purified by preparative TLC (*n*-hexane/EtOAc, 7:3, v/v) developing three times to afford 15 mg of **1** and 20 mg of **2**.

2) Fraction 5 (1019 mg), obtained by elution with 40% EtOAc, was chromatographed on a silica gel column (*n*-hexane/EtOAc). Fraction 3 (680 mg), obtained by elution with 30% EtOAc, was further chromatographed on a silica gel column ($CHCl_3$/MeOH). Fraction 1 (312 mg), obtained by elution with $CHCl_3$, was purified by preparative TLC ($CHCl_3$/MeOH, 98:2, v/v) and the solid was recrystallized from acetone/*n*-hexane to afford 11 mg of **6**. Fraction 3 (17 mg), obtained by elution with 2% MeOH, was purified by preparative TLC ($CHCl_3$/MeOH, 99:1, v/v) and the solid was recrystallized from EtOAc/*n*-hexane to afford 6 mg of **3**. Fraction 4 (17 mg), obtained by elution with 40% EtOAc, was purified by preparative TLC (*n*-hexane/acetone, 1:1, v/v) and the solid was recrystallized from EtOAc/*n*-hexane to afford 10 mg of **4**.

3) Fraction 7 (1.2 g), obtained by elution with 60% EtOAc, was chromatographed on a silica gel column (*n*-hexane/EtOAc). Fraction 3 (371 mg), obtained by elution with 50% EtOAc, was purified by preparative TLC ($CHCl_3$/MeOH, 95:5, v/v). One solid was recrystallized from EtOAc to afford 23 mg of **7**, and another solid was recrystallized from acetone/*n*-hexane to afford 18 mg of **8**.

On the other hand, the EtOAc-soluble neutral phase from dried leaves of *V. rotundifolia* L. (7 kg) was prepared by the procedure mentioned above, and concentrated *in vacuo*. The resulting residue (67 g) was first fractionated by column chromatography on silica gel (*n*-hexane/EtOAc).

4) Fraction 5 (2.1 g), obtained by elution with 40% EtOAc, was chromatographed on a silica gel column ($CHCl_3$/MeOH). Fraction 3 (300 mg), obtained by elution with 2% MeOH, was further chromatographed on a silica gel column (*n*-hexane/acetone, 3:2, v/v) and the solid was recrystallized from EtOAc/*n*-hexane to afford 92 mg of **5**.

5) Fraction 6 (2.8 g), obtained by elution with 50% EtOAc, was chromatographed on a silica gel column (*n*-hexane/EtOAc, 3:2, v/v). Fractions 15–29 (870 mg) were further chromatographed on a LH-20 column eluted with MeOH. Fractions 6–13 (380 mg) were recrystallized from acetone/*n*-hexane to afford 227 mg of **8**. Mother liquor of **8** was purified by preparative TLC ($CHCl_3$/MeOH, 99:1, v/v) and the solid was recrystallized from MeOH to afford 10 mg of **9**.

4-Hydroxybenzoic acid methyl ester (**1**): IR (KBr): ν = 3265 (OH), 1689 (O–C=O), 1610 (C=C), 1518 (C=C), 1286 (OCH_3), 1103 (OH), 771 (C=C) cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 3.90 (s, 3H, 7-OCH_3), 6.88 (d, J = 8.7 Hz, 2H, 3-H and 5-H), 7.93 (d, J = 8.7 Hz, 2H, 2-H and 6-H). – $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ = 52.1 (q, OCH_3-7), 115.3 (d, C-3 and C-5), 122.3 (s, C-1), 132.0 (d, C-2 and C-6), 160.3 (s, C-4), 167.4 (s, C-7).

Vanilic acid methyl ester (**2**): IR (KBr): ν = 3381 (OH), 2941 (CH_3), 1682 (O–C=O), 1606 (C=C), 1446 (OCH_3), 1103 (OH) cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 3.89 (s, 3H, 3-OCH_3), 3.96 (s, 3H, 7-OCH_3), 6.93 (d, J = 8.7 Hz, 1H, 5-H), 7.55 (d, J = 1.8 Hz, 1H, 2-H), 7.63 (dd, J = 8.7, 1.8 Hz, 1H, 6-H). – $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ = 51.9 (q, OCH_3-7), 56.1 (q, OCH_3-3), 111.7 (d, C-2), 114.0 (d, C-5), 122.2 (s, C-1), 124.1 (d, C-6), 146.1 (s, C-4), 149.9 (s, C-3), 166.5 (s, C-7).

4-Hydroxy benzaldehyde (**3**): IR (KBr): ν = 3408 (OH), 1680 (H–C=O), 1597 (C=C), 1508 (C=C), 1248 (C=C), 821 (C=C) cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 6.89 (d, J = 8.9 Hz, 2H, 3-H and 5-H), 7.86 (d, J = 8.7 Hz, 2H, 2-H and 6-H), 9.57 (s, 1H, 7-H). – $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ = 115.9 (d, C-3 and C-5), 124.7 (s, C-1), 132.9 (d, C-2 and C-6), 163.0 (s, C-4), 194.3 (s, C-7).

4-Hydroxybenzoic acid (**4**): IR (KBr): ν = 3400 (OH), 2920 (C=C), 2800–2100 (COOH), 1670 (O–C=O), 1590 (C=C), 855 (C=C) cm^{-1}. – ^1H NMR (500 MHz, CD_3OD): δ = 6.81 (d, J = 8.7 Hz, 2H, 3-H and 5-H), 7.87 (d, J = 8.7 Hz, 2H, 2-H and 6-H). – $^{13}C\{^1H\}$ NMR (125 MHz, CD_3OD): δ = 115.7 (d, C-3 and C-5), 122.7 (s, C-1), 132.7 (d, C-2 and C-6), 163.0 (s, C-4), 170.0 (s, C-7).

Ferulic acid (**5**): IR (KBr): ν = 3479 (OH), 3000–2500 (COOH), 1682 (O–C=O), 1606 (C=C), 1446 (OCH_3), 1365 (OCH_3), 1103 (OH) cm^{-1}. – ^1H NMR (500 MHz, CD_3OD): δ = 3.64 (s, 3H, 3-OCH_3), 6.15 (d, J = 15.6 Hz, 1H, 8-H), 6.67 (d, J = 8.3 Hz, 1H, 5-H), 6.83 (dd, J = 8.3, 2.1 Hz, 1H, 6-H), 6.93 (d, J = 2.1 Hz, 1H, 2-H), 7.44 (d, J = 15.6 Hz, 1H, 7-H). – $^{13}C\{^1H\}$ NMR (125 MHz, CD_3OD): δ = 51.9 (q, OCH_3-3), 114.8 (d, C-8), 115.1 (d, C-2), 116.4 (d, C-5), 122.7 (d, C-6), 127.6 (s, C-1), 146.6 (s, C-4), 146.7 (s, C-7), 149.3 (s, C-3), 169.5 (s, C-9).

5,5′-Dihydroxy-4′,6,7-trimethoxyflavanone (**6**): IR (KBr): ν = 3447 (OH), 2941 (OCH_3), 1641 (C=O), 1574 (C=C), 1514 (C=C), 1454 (OCH_3), 1290 (OCH_3), 798 (C=C) cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 2.71 (dd, J = 17.2, 2.8 Hz, 1H, 3-H), 3.00 (dd, J = 17.2, 13.1 Hz, 1H, 3-H), 3.89 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 5.25 (dd, J = 13.1, 2.8 Hz, 1H, 2-H), 6.06 (s, 1H, 8-H), 6.81 (d, J = 8.3 Hz, 1H, 3′-H), 6.85 (dd, J = 8.3, 2.1 Hz, 1H, 2′-H), 6.98 (d, J = 2.1 Hz, 1H, 6′-H). – $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ = 43.1 (t, C-3), 56.0 (q, OCH_3), 56.1 (q, OCH_3), 60.8 (q, OCH_3), 79.6 (d, C-2), 91.6 (d, C-8), 103.1 (s, C-10), 110.6 (d, C-3′), 112.6 (d, C-6′), 118.1 (d, C-2′), 130.4 (s, C-6), 131.3 (s, C-1′), 145.9 (s, C-5′), 147.0 (s, C-4′), 154.9 (s, C-5), 158.6 (s, C-9), 160.8 (s, C-7), 196.5 (s, C-4).

Luteolin (**7**): IR (KBr): ν = 3387 (OH), 1657 (C=O), 1605 (C=C), 1512 (C=C), 1259 (OH), 837 (C=C) cm^{-1}. – ^1H NMR (500 MHz, CD_3OD): δ = 6.09 (d, J = 2.1 Hz, 1H, 6-H), 6.32 (d, J = 2.1 Hz, 1H, 8-H), 6.42 (s, 1H, 3-H), 6.87 (d, J = 8.9 Hz, 1H, 3′-H), 7.26 (br.s, 1H, 6′-H), 7.26 (dd, J = 8.9, 2.1 Hz, 1H, 2′-H). – $^{13}C\{^1H\}$ NMR (125 MHz, CD_3OD): δ = 95.1 (d, C-6), 100.3 (d, C-8), 103.8 (d, C-3), 105.2 (s, C-10), 114.1 (d, C-6′), 116.8 (d, C-3′), 120.3 (d, C-2′), 123.6 (s, C-1′), 147.1 (s, C-4′), 151.1 (s, C-5′), 159.5 (s, C-5), 163.2 (s, C-9), 166.3 (s, C-2), 166.4 (s, C-7), 183.8 (s, C-4).

Vitexicarpin (**8**): IR (KBr): ν = 3435 (OH), 2924 (OCH_3), 2852 (OCH_3), 1660 (C=O), 1604 (C=C), 1464 (OCH_3), 1361 (OCH_3), 1270 (OCH_3), 806 (C=C) cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 3.87 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 6.52 (s, 1H, 8-H), 6.96 (d, J = 8.6 Hz, 1H, 3′-H), 7.68 (d, J = 1.9 Hz, 1H, 6′-H), 7.71 (dd, J = 8.6, 1.9 Hz, 1H, 2′-H). – $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ = 56.1 (q, OCH_3), 56.3 (q, OCH_3), 60.2 (q, OCH_3), 60.9 (q, OCH_3), 90.4 (d, C-8), 106.6 (s, C-10), 110.4 (d, C-3′), 114.3 (d, C-2′), 121.6 (s, C-1′), 123.6 (d, C-6′), 132.2 (s, C-6), 139.0 (s, C-3), 145.6 (s, C-5′), 148.8 (s, C-4′), 152.3 (s, C-9), 152.7 (s, C-5), 155.7 (s, C-2), 158.8 (s, C-7), 179.0 (s, C-4).

Artemetin (**9**): IR (KBr): ν = 3427 (OH), 2937 (OCH_3), 1655 (C=O), 1591 (C=C), 1512 (C=C), 1473 (OCH_3), 1358 (OCH_3), 1270 (OCH_3), 804 (C=C) cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 3.86 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 6.51 (s, 1H, 8-H), 6.99 (d, J = 8.5 Hz, 1H, 3′-H), 7.69 (d, J = 2.1 Hz, 1H, 6′-H), 7.74 (dd, J = 8.5, 2.1 Hz, 1H, 2′-H). – $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ = 55.9 (q, OCH_3), 56.0 (q, OCH_3), 56.3 (q, OCH_3), 60.1 (q, OCH_3), 60.8 (q, OCH_3), 90.3 (d, C-8), 106.5 (s, C-10), 110.8 (d, C-3′), 111.1 (d, C-2′), 122.1 (s, C-1′), 122.8 (d, C-6′), 132.2 (s, C-6), 138.8 (s, C-3), 148.7 (s, C-5′), 151.3 (s, C-4′), 152.3 (s, C-9), 152.7 (s, C-5), 155.8 (s, C-2), 158.7 (s, C-7), 178.8 (s, C-4).

Methylation of quercetin (**11**) *and fisetin* (**13**)

11 (5 mg) was methylated with ethereal diazomethane (1 ml) and MeOH (0.5 ml) for 24 h at room temperature. Purification by preparative TLC in $CHCl_3$/MeOH (96:4, v/v) afforded quercetin tetramethyl ether (**12**) (3.7 mg).

Quercetin (**11**): IR (KBr): ν = 3408 (OH), 1660 (C=O), 1612 (C=C), 1560 (C=O), 1521 (C=C), 1199 (OH), 825 (C=C) cm^{-1}. – ^1H NMR (500 MHz, acetone-d_6): δ = 2.83 (br.s, 4H, OH), 6.25 (br.s, 1H), 6.51 (br.s, 1H), 7.01 (d, J = 8.5 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.81 (br.s, 1H), 12.16 (s, 1H, OH).

Quercetin tetramethyl ether (**12**): IR (KBr): ν = 2945 (OCH_3), 1662 (C=O), 1593 (C=C), 1514 (C=C), 1458 (OCH_3), 1383 (OCH_3), 810 (C=C) cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 6.36 (d, J = 2.1 Hz, 1H), 6.45 (d, J = 2.1 Hz, 1H), 7.00 (d, J = 8.6 Hz, 1H), 7.69 (d, J = 2.1 Hz, 1H), 7.74 (dd, J = 8.6, 2.1 Hz, 1H), 12.65 (s, 1H, OH).

13 (5 mg) was methylated with ethereal diazomethane (1 ml) and MeOH (0.5 ml) for 24 h at room temperature. Purification by preparative TLC in $CHCl_3$/MeOH (96:4, v/v) afforded fisetin tetramethyl ether (**14**) (3.8 mg).

Fisetin (**13**): IR (KBr): ν = 3350 (OH), 1640 (C=O), 1606 (C=C), 1568 (C=O), 1523 (C=C), 1205 (OH), 852 (C=C) cm^{-1}. – ^1H NMR (500 MHz, acetone-d_6): δ = 2.85 (br.s, 4H, OH), 6.98 (d, J = 8.5 Hz, 2H), 7.01 (br.s, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.83 (br.s, 1H), 7.99 (d, J = 8.5 Hz, 1H).

Fisetin tetramethyl ether (**14**): IR (KBr): ν = 2943 (OCH_3), 2839 (OCH_3), 1620 (C=O), 1602 (C=C), 1516 (C=C), 1448 (OCH_3), 1383 (OCH_3), 835 (C=C) cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 3.88 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.97 (s, 6H, OCH_3), 6.91 (d, J = 2.3 Hz, 1H), 6.97 (dd, J =

8.6, 2.3 Hz, 1H), 7.00 (d, J = 8.6 Hz, 1H), 7.72 (br.s, 1H), 7.74 (dd, J = 8.6, 2.3 Hz, 1H), 8.16 (d, J = 8.6 Hz, 1H).

Bioassay with lettuce seedlings

Lettuce seeds (*Lactuca sativa* cv. Kingcisco) were purchased from Takii Nursery, Kyoto, Japan and sown in a Petri dish (150 mm × 25 mm) lined with a filter paper containing deionized water. After one day under light at 24 °C, seedlings were selected for uniformity (radicles; 2 mm) and transferred into a mini-Petri dish (35 mm × 15 mm) lined with a filter paper containing 1 ml of deionized water and a defined amount of the test compound. The Petri dishes were kept at 24 °C for 4 d under continuous light (100 μE/m^2 s). Hypocotyls and roots of untreated seedlings grew at the rate of about 1 mm and 4 mm a day, respectively. The length of the hypocotyls and roots treated with the compounds was measured and the mean value of the length was compared with an untreated control (Kimura *et al.*, 2002).

Results and Discussion

The EtOAc-soluble neutral fraction (12 g) from the MeOH extract of dried fruits (5 kg) of *Vitex rotundifolia* L. was purified by silica gel column chromatography and preparative TLC to afford seven known compounds four of which were phenolic compounds (compounds **1**, **2**, **3**, and **4**) and three were flavonoids (compounds **6**, **7**, and **8**). Similarly, the EtOAc-soluble neutral fraction (67 g) of the MeOH extract of the dried leaves (7 kg) was purified by silica gel and Sephadex LH20 column chromatography and preparative TLC to afford three known compounds one of which was a phenolic compound (compound **5**) and two were flavonoids (compounds **8** and **9**). Compound **8** was isolated from both fruits and leaves, but compounds **1**, **2**, **3**, **4**, **6**, and **7** were isolated from fruits and compounds **5** and **9** were isolated from leaves.

Compounds **1**, **2**, **3**, **4** and **5** were identified as 4-hydroxybenzoic acid methyl ester, vanillic acid methyl ester, 4-hydroxy benzaldehyde, 4-hydroxybenzoic acid and ferulic acid, respectively, comparing the physicochemical properties with those reported (Fig. 1) (Pouchert and Behnke, 1993; Pouchert, 1993). Compounds **6**, **7**, **8** and **9** were identified as 5,5′-dihydroxy-4′,6,7-trimethoxyflavanone, luteolin, vitexicarpin and artemetin, respectively, comparing the physicochemical properties with those reported (Fig. 1) (Ono *et al.*, 2002).

Plant growth activities of **1**–**9** together with vanillic acid (**10**), quercetin (**11**), quercetin tetramethyl ether (**12**), fisetin (**13**) and fisetin tetramethyl ether (**14**) were examined using a bioassay with lettuce seedlings (Table I). All compounds showed no effect on hypocotyl elongation at a concentration of 10^{-3} M. **1**, **2** and **3** showed no effect on root growth at a concentration of 10^{-3} M, but **4**, **5** and **10** inhibited the root growth to 34%, 43% and 29% of control at the same concentration, respectively. **1** and **2**, which are the methyl esters of **4** and **10**, respectively, showed less inhibitory activities than **4** and **10**. These results suggest that the methylation of the carboxy group in the molecule of phenolic acids reduces the inhibitory activity against root growth. **7**, **8** and **9** accelerated the root growth to 134%, 195% and 184% of control at a concentration of 10^{-3} M, respectively, but **6** did not show any accelerating effect on the growth at the same concentration. **12** and **14** accelerated the root growth to 138% and 169% of control at a concentration of 10^{-3} M, respectively, but **11** and **13** did not show any accelerating effect on growth at the same concentration. **8** and **9** showed higher accelerating activity than **7**. Similarly, **12** and **14** showed higher accelerating activity than **11** and **13**, respectively. These results suggest that the methylation of the hydroxy group in the

Table I. Effects of phenolic compounds (**1**–**5** and **10**) and flavonoids (**6**–**9** and **11**–**14**) on the root growth of lettuce seedlings.

Compound	Root growth activity (% of control)	
	10^{-4} M	10^{-3} M
1	95 ± 1.1	78 ± 1.0
2	95 ± 1.1	78 ± 1.0
3	95 ± 1.1	78 ± 1.0
4	89 ± 1.5	34 ± 0.7
5	74 ± 1.1	43 ± 0.7
10	53 ± 0.5	29 ± 0.9
6	120 ± 1.9	118 ± 1.0
7	119 ± 2.1	134 ± 1.9
8	114 ± 1.9	195 ± 2.4
9	125 ± 2.7	184 ± 2.1
11	112 ± 1.0	92 ± 1.2
12	127 ± 1.3	138 ± 1.5
13	112 ± 1.1	112 ± 1.0
14	138 ± 1.2	169 ± 1.6

Values are the means ± SD of three assays.

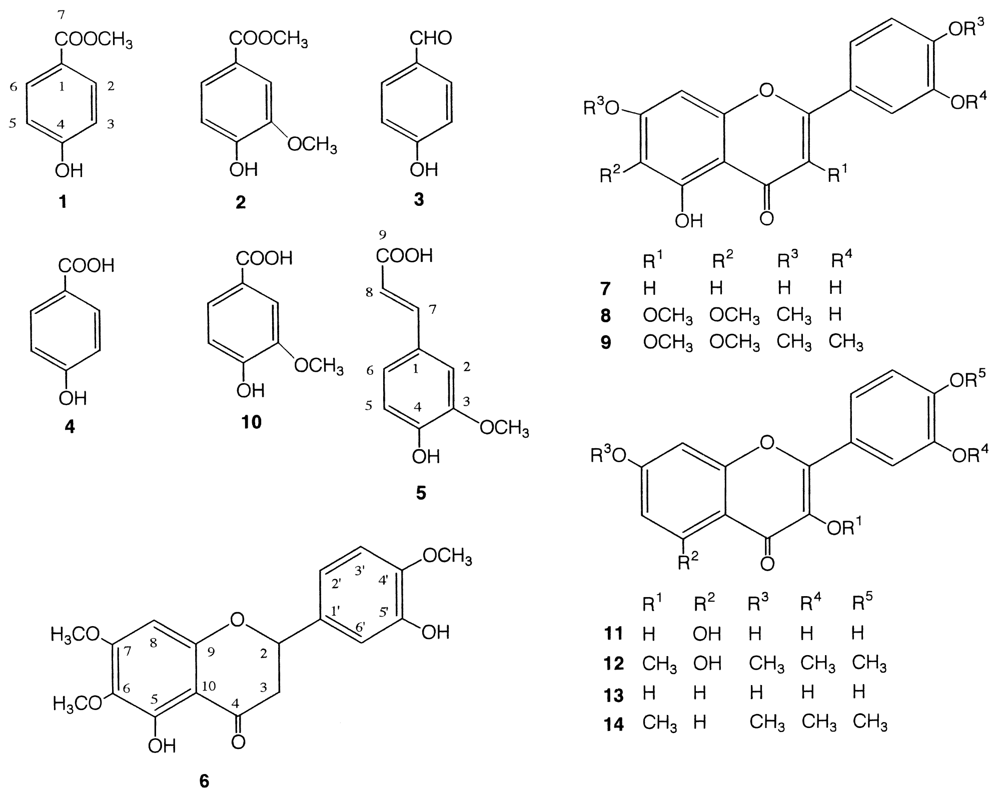

Fig. 1. Structures of 4-hydroxybenzoic acid methyl ester (**1**), vanillic acid methyl ester (**2**), 4-hydroxy benzaldehyde (**3**), 4-hydroxybenzoic acid (**4**), ferulic acid (**5**), 5,5′-dihydroxy-4′,6,7-trimethoxyflavanone (**6**), luteolin (**7**), vitexicarpin (**8**), artemetin (**9**), vanillic acid (**10**), quercetin (**11**), quercetin tetramethyl ether (**12**), fisetin (**13**), and fisetin tetramethyl ether (**14**). Compounds **10**–**14** were not isolated from fruit and leaf of *Vitex rotundifolia* but included for comparison.

flavone skeleton produced the accelerating activity against root growth.

Methylation of the chemical constituents of *V. rotundifolia* L. might play an important role in the survival of the fittest in the sand dune, since phenolic acids and flavonoids are known to act as allelochemicals (Correa *et al.*, 2000; Wu *et al.*, 2001, 2002).

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Correa J. F., Souza I. F., Ladeira A. M., Young M. C. M, and Aragushi M. (2000), Allelopathic potential of *Eupatorium maximiliani* Schrad. leaves. Allelopathy J. **7**, 225–234.

Fujiki D., Yamanaka N., and Tamai S. (2001), Relationship between vegetation types and seed banks on Tottori sand dune. J. Jpn. Soc. Reveget. Tech. **26**, 209–222 (in Japanese).

Kimura Y., Shimada A., Kusano M., Yoshii K., Morita A., Nishibe M., Fujioka S., and Kawano T. (2002), Myxostiolide, myxostiol, and clavatoic acid, plant growth regulators from the fungus *Myxotrichum stipitatum*. J. Nat. Prod. **65**, 621–623.

Okuyama E., Fujimori S., and Yamasaki M. (1995), Abstracts of papers. The 41st Annual Meeting of the Japanese Society of Pharmacognosy, p. 64 (in Japanese).

Ono M., Ito Y., Kubo S., and Nohara T. (1997), Two new iridoids from *Viticis trifoliae* Fructus (fruit of *Vitex rotundifolia* L.). Chem. Pharm. Bull. **45**, 1094–1096.

Ono M., Yanaka T., Yamamoto M., Ito Y., and Nohara T. (2002), New diterpenes and norditerpenes from the fruits of *Vitex rotundifolia*. J. Nat. Prod. **65**, 537–541.

Pouchert C. J. (1993), in: The Aldrich Library of Infrared Spectra (Pouchert C. J., ed.). Aldrich Chemical Company, Milwaukee, pp. 795–915.

Pouchert C. J. and Behnke J. (1993), in: The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra (Pouchert C. J. and Behnke J., eds.). Aldrich Chemical Company, Milwaukee, Vol. II, pp. 925–1326.

Wu T., Haig T., Pratley J., Lemerle D., and An M. (2001), Allelochemicals in wheat (*Triticum aestivum* L.): variation of phenolic acids in shoot tissues. J. Chem. Ecol. **27**, 125–135.

Wu T., Haig T., Pratley J., Lemerle D., and An M. (2002), Biochemical basis for wheat seedling allelopathy on the suppression of annual ryegrass (*Lolium rigidum*). J. Agric. Food Chem. **50**, 4567–4571.