Natural Product Sciences
20(1) : 22-28 (2014)

Antioxidant Constituents from the Stem of *Tetrastigma erusbescense* Planch. (Vitaceae)

Phan Thi Anh Dao[1], Tran Le Quan[2], and Nguyen Thi Thanh Mai[2,*]

[1]Faculty of Chemistry and Food, University of Technical Education Hochiminh City, 01 Vo Van Ngan, Hochiminh City, Vietnam
[2]Faculty of Chemistry, University of Science, National University Hochiminh City, 227 Nguyen Van Cu, Hochiminh City, VietNam.

Abstract – A new natural product, tetrastigmol A (**1**), and several known compounds as flavonoids (**2 - 8**), steroids (**9 - 10**), bergenin and its derivative (**11 - 12**), stilbens (**13 - 15**), lignan (**16**), benzenecarboxylic derivative (**17**) and two norisoprenoid (**18 - 19**) were isolated from the stem of *Tetrastigma erubescens* Planch. (Vitateae). Their structures were determined on the basis of NMR spectroscopic data. This is the first report on chemical constituents of this plant. Compounds **1, 6 - 8** and **12 - 15** showed strong antioxidant activity using two methods including DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging and lipid peroxidation inhibitory assays.
Keywords – *Tetrastigma erubescens*, Vitateae, Antioxidant activity, DPPH, Lipid peroxidation.

Introduction

Free radicals are well documented for playing a dual role in our body as both deleterious and beneficial species. In low/moderate concentrations free radicals are involved in normal physiological functions but excess production of free radicals or decrease in antioxidant level leads to oxidative stress.[1] It is a harmful process that can be mediates damage to cell structures, including lipids, proteins, RNA and DNA which leads to number of serious diseases such as heart disease, macular degeneration, cancer, diabetes, and more. Therefore, antioxidant substances are required for the protection against the oxidizing agents. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, which have stimulated the interest of many investigators to search natural antioxidant.[2]

Tetrastigma erubescens Planch. (Vitaceae) grows commonly at the Bay Nui area in An Giang province. The plant is used in Vietnamese folk medicine to treat inflammatory, fever, stomachache, and hypertension diseases.[3] In previous study, we found that the methanolic extract prepared from the stem of *T. erubescens* showed strongest antioxidant activity among 90 medicinal plants using two methods including DPPH free radical scavenging and lipid peroxidation inhibition assays.[4] Until now, there are no scientific report on chemical constituents and biological activities of this plant. Therefore, this study was carried out to determine phytochemical compositions of the stem of *T. erubescens* and their antioxidant activity.

*Author for correspondence
Nguyen Thi Thanh Mai, University of Science, VietNam National University-HoChiMinh City, 227 Nguyen Van Cu Street, Distrist 5, Ho Chi Minh City, Vietnam
Tel: +84-907-426-331; E-mail: nttmai@hcmus.edu.vn

Experimental

General – UV measurements were obtained on a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). Aluminum sheets (4.5 × 10 cm), coated with silica gel 60 F254, were used for analytical TLC, and compounds were visualized under UV light with a Vilber Lourmat lamp and subsequently detected after spraying with chemical reagents and heating. NMR experiments were performed on a Bruker Avance 500 MHz spectrometer. Low resolution electrospray ionization (ESI) MS was recorded on a Bruker Esquire 3000 Plus spectrometer in the positive or negative mode. HR-ESI-MS were conducted on a LTQ Orbitrap mass spectrometer (micro OTOF-Q 10187T).

Plant materials – The stem of *T. erubescens* used in this study was collected at the Bay Nui area in An Giang province on August 2009. The plants were identified by Professor Hoang Viet, Faculty of Biology, University of Science, Hochiminh city National University (VNU). The voucher specimen (1085) is preserved at Department of Analytical Chemistry, Faculty of Chemistry, University of Science, VNU.

Extraction and isolation -The dried stem (4.5 kg) of *T.*

erubescens was extracted with methanol (reflux, 3 h, x 3), to yield a methanolic extract (1 kg) which suspended in water and partitioned successively with *n*-hexane, ethyl acetate to yield *n*-hexane (2 g), ethyl acetate (250 g) and water (700 g) fractions, respectively. The ethyl acetate fraction was applied to silica gel column chromatography eluted with chloroform:methanol to give six fractions: fr. 1 (5.4 g), fr. 2 (14.9 g), fr. 3 (12 g), fr. 4 (20 g), fr. 5 (30 g), fr. 6 (50 g). Fraction 1 was separated by preparative TLC with *n*-hexane: acetone to give **9** (7.5 mg) and **10** (6.3 mg). Fraction 2 was separated by preparative TLC with *n*-hexane:acetone to give **2** (3.5 mg), **3** (3.7 mg), **4** (4.2 mg), **5** (3.9 mg), **17** (5.2 mg), **18** (2.5 mg), and **19** (2.5 mg). Fraction 3 was separated by preparative TLC with chloroform : methanol to give **16** (20 mg), and **13** (20.2 mg). Fraction 4 was applied to silica gel column chromatography eluted with chloroform : methanol to give **11** (12 g). Fraction 5 was separated by preparative TLC with chloroform:methanol to give **6** (20.5 mg), **7** (57.3 mg), (**8**, 5.5 mg), **1** (8.8 mg), **12** (150.0 mg), **14** (5.0 mg), and **15** (43.3 mg).

Tetrastigmol A (1) – Yellow powder. ^{1}H and ^{13}C NMR (CD_3OD) see Table 1. The HR-ESI-MS showed a molecular ion peak at *m/z* 559.1143 $[M + Na]^+$ corresponding to the molecular formula of $C_{30}H_{24}NaO_{12}$ (cal for $C_{30}H_{24}NaO_{12}$, 559.1160).

Nobiletin (**2**) – Pale-yellow powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.56 (1H, dd, J = 8.5, 2.0 Hz, H-6'), 7.41 (1H, d, J = 2.0 Hz, H-2'), 7.01 (1H, d, J = 8.5 Hz, H-5'), 6.60 (1H, s, H-3), 4.10 (3H, s),4.02 (3H, s), 3.98 (3H, s), 3.96 (3H, s), 3.95 (3H x 2, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 161.0 (C-2), 106.9 (C-3), 177.2 (C-4), 114.9 (C-4a), 148.5 (C-5), 144.1 (C-6), 151.4 (C-7), 138.1 (C-8), 152.1 (C-8a), 124.4 (C-1'), 109.0 (C-2'), 149.6 (C-3'), 152.2 (C-4'), 111.6 (C-5'), 119.8 (C-6'), 62.2 (OCH_3), 61.9 (OCH_3), 61.7 (OCH_3), 61.6 (OCH_3), 56.1 (OCH_3), 56.0 (OCH_3).

Tangeretin (3) – Pale-yellow powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.87 (2H, d, J = 9.0 Hz, H-2', 3'), 7.02 (2H, d, J = 9.0 Hz, H-5', 6'), 6.60 (1H, s, H-3), 4.09 (3H, s), 4.02 (3H, s), 3.94 (3H x 2, s), 3.88 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz,) δ: 161.4 (C-2), 106.9 (C-3), 177.5 (C-4), 115.1 (C-4a), 148.6 (C-5), 144.3 (C-6), 151.6 (C-7), 138.3 (C-8), 147.9 (C-8a), 124.2 (C-1'), 127.9 (C-2', 6'), 114.7 (C-3'), 162.5 (C-4'), 114.7 (C-5'), 55.6 (OCH_3), 61.9 (OCH_3), 62.2 (OCH_3), 62.4 (OCH_3), 61.8 (OCH_3).

6-Demethoxytangeretin (4) – Pale-yellow powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.88 (2H, d, J = 9.0 Hz, H-2', 3'), 7.02 (2H, d, J = 9.0 Hz, H-5', 6'), 6.60 (1H, s, H-3), 6.44 (1H, s, H-6), 3.99 (3H, s), 3.97 (3H, s), 3.94 (3H, s), 3.87 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.9 (C-2), 107.4 (C-3), 178.0 (C-4), 109.6 (C-4a), 157.6 (C-5), 93.1 (C-6), 157.6 (C-7), 131.1 (C-8), 152.2 (C-8a), 124.4 (C-1'), 127.9 (C-2', 6'), 114.8 (C-3'), 162.4 (C-4'), 114.8 (C-5'), 55.6 (OCH_3), 56.5 (OCH_3), 56.7 (OCH_3), 61.7 (OCH_3).

6-Demethoxynobiletin (**5**) – Pale-yellow powder. ^{1}HNMR ($CDCl_3$, 500 MHz) δ: 7.58 (1H, dd, J = 8.5, 2.0 Hz, H-6'), 7.43 (1H, d, J = 2.0 Hz, H-2'), 6.99 (1H, d, J = 8.5 Hz, H-5'), 6.61 (1H, s, H-3), 6.44 (1H, s, H-6), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.96 (3H x 2, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.7 (C-2), 107.4 (C-3), 177.9 (C-4), 109.4 (C-4a), 156.7 (C-5), 93.1 (C-6), 156.5 (C-7), 131.2 (C-8), 152.1 (C-8a), 124.4 (C-1'), 109.0 (C-2'), 149.6 (C-3'), 152.2 (C-4'), 111.6 (C-5'), 119.8 (C-6'), 56.2 (OCH_3), 56.3 (OCH_3), 56.8 (OCH_3), 61.6 (OCH_3), 56.5 (OCH_3).

Catechin (**6**) – Pale-yellow powder. ^{1}HNMR ($CDCl_3$, 500 MHz) δ: 4.56 (1H, d, J = 8.0 Hz, H-2), 3.97 (1H, ddd, J = 8.0, 8.0, 4.8 Hz, H-3), 2.50 (1H, dd, J = 8.5, 16.0 Hz, H-4ax), 2.85 (1H, dd, J = 5.0, 16.0 Hz, H-4ex), 5.86 (1H, d, J = 2.0 Hz, H-6), 5.93 (1H, d, J = 2.5Hz, H-8), 6.84 (1H, d, J = 2.0 Hz, H-2'), 6.76 (1H, d, J = 8.0 Hz; H-5'), 6.72 (1H, dd, J = 2.0, 8.5 Hz, H-6'); ^{13}C NMR (125MHz, CD_3OD) δ: 82.8 (C-2), 68.7 (C-3), 28.4 (C-4), 115.2 (C-4a), 156.8 (C-5), 95.5 (C-6), 157.5 (C-7), 96.3 (C-8), 157.8 (C-8a), 132.2 (C-1'), 115.2 (C-2'), 146.2 (C-3'), 146.2 (C-4'), 116.0 (C-5'), 120.0 (C-6').

Epicatechin-3-*O*-gallate (7) – Off-white amorphous powder. ^{1}H NMR (CD_3COCD_3, 500 MHz) δ: 5.13 (1H, br, H-2), 5.53 (1H, m, H-3), 3.05 (1H, dd, J=17.5, 4.5, H-4ex), 2.93 (1H, dd, J = 18.0, 2.5 Hz, H-4ax), 6.07 (1H, d, J = 2.0 Hz, H-6), 6.00 (1H, d, J = 2.0 Hz, H-8), 7.09 (1H, d, J = 2.0 Hz, H-2'), 6. 78 (1H, d, J = 8.0 Hz, H-5'), 6.87 (1H, dd, J = 2.0, 8.0 Hz, H-6'), 7.04 (2H, s, H-2", 6"); ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 78.2 (C-2), 69.5 (C-3), 26.6 (C-4), 99.1 (C-4a), 155.04 (C-5), 96.7 (C-6), 157.30 (C-7), 95.9 (C-8), 156.32 (C-8a), 131.5 (C-1'), 115.0 (C-2'), 145.6 (C-3'), 145.7 (C-4'), 115.7 (C-5'), 119.2 (C-6'), 110.1 (C-2",6"), 121.9 (C-4"), 146.0 (C-3",5"), 166.2 (C=O, C-7").

Phlorizin (**8**) – Pale-yellow powder. ^{1}H NMR (CD_3COCD_3, 500 MHz) δ: 2.89 (2H, t, J = 7.4 Hz, β-CH_2), 3.4-4.0 (8H, m, sugar-H, α-CH_2), 5.08 (1H, d, J = 7.6 Hz, H-1"), 6.00 (1H, d, J = 2.2Hz, H-5'), 6.32 (1H, d, J = 2.2 Hz, H-3'), 6.74 (2H, d, J= 8.4 Hz, H-3, 5), 7.10 (2H, d, J = 8.4 Hz, H-2,6); ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 30.2 (β-CH_2), 46.3 (α-CH_2), 133.2 (C-1), 130.2 (C-2,6), 156.3 (C-4), 115.9 (C-3, 5), 106.3 (C-1'), 162.1 (C-2'), 98.1 (C-3'), 167.6 (C-4'), 95.4 (C-5'), 165.4 (C-6'), 102.2 (C-1"), 74.3 (C-2"), 78.4 (C-3"), 71.3 (C-4"), 78.1 (C-5"), 62.7 (C-6"), 206.0 (C=O).

Table 1. NMR spectral data of compound **1** (CD_3OD) (δ in ppm, *J* in Hz)

Position	**1**		
	δ_C	δ_H	HMBC ($^1H \rightarrow {}^{13}C$)
2C	79.6	3.98 (1H, d, *J* = 6.5Hz)	8aA, 1'B, 3C
3C	66.9	3.98 (1H, m)	4aA, 4C, 2C
4C	27.8	2.52 (1H, dd, *J* = 7.5, 16.5 Hz, H - 4axC 2.94 (1H, dd, *J* = 5.5, 16.5 Hz, H-4exC)	4aA, 3C, 2C
4aA	100.5	–	–
5A	156.4	–	–
6A	97.1	5.91 (1H, s)	5A, 7A, 4aA
7A	157.7	–	–
8A	95.9	5.54 (1H, s)	5A, 7A, 4Aa, 8aA
8aA	158.0	–	–
1'B	89.9	–	–
2'B	45.2	2.67 (1H, d, *J* = 11.5Hz) 2.49 (1H, d, *J* = 11.5)	1'B, 3'B
3'B	95.3	–	–
4'B	194.0	–	–
5'B	112.8	6.43 (1H, s)	3'B, 4'B, 6'B, 8D
6'B	164.4	–	–
2F	83.5	4.92 (1H, d, *J* = 6.5 Hz)	3F, 4F, 8aD, 1'E
3F	67.9	4.10 (1H, m)	2F, 1'E, 4F, 4aD
4F	28.3	2.60 (1H, dd, *J* = 7.5, 16.5Hz, H-4axF) 2.85 (1H, dd, *J* = 5.0, 16.5Hz, H-4exF)	2F, 3F, 4aD, 8aD
4aD	104.0	–	–
5D	166.3	–	–
6D	91.0	6.12 (1H, s)	7D, 8D, 4aD
7D	168.3	–	–
8D	105.6	–	–
8aD	155.1	–	–
1'E	131.3	–	–
2'E	114.9	6.85 (1H, d, J =2.0 Hz)	1'E, 3'E, 4'E, 6'E
3'E	145.4	–	–
4'E	146.6	–	–
5'E	116.4	6.79 (1H, d, *J* = 8.0 Hz)	1'E, 3'E, 4'E, 6'E
6'E	119.7	6.74 (1H, dd, *J* = 2.0, 8.0 Hz)	1'E, 2'E, 4'E, 5'E

3β-hydroxystigmast-5-en-7-one (**9**) – White powder. ^{1}HNMR ($CDCl_3$, 500 MHz) δ: 3.69 (1H, m, H-3α), 5.70 (1H, d, *J* = 1.7 Hz, H-6), 0.69 (3H, s, Me-18), 1.20 (3H, s, Me-19), 0.93 (3H, d, *J* = 6.5 Hz, Me-21), 0.84 (3H, d, *J* = 6.5 Hz, Me-26), 0.82 (3H, d, *J* = 6.7 Hz, Me-27), 0.85 (3H, t, *J* = 7.1 Hz, Me-29); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 36.6 (C-1), 31.2 (C-2), 70.5 (C-3), 41.8 (C-4), 169.4 (C-5), 126.2 (C-6), 204.2 (C-7), 45.4 (C-8), 50.0 (C-9), 38.2 (C-10), 21.2 (C-11), 39.7 (C-12), 41.8 (C-13), 49.9 (C-14), 26.3 (C-15), 28.5 (C-16), 54.7 (C-17), 11.9 (C-18), 17.3 (C-19), 36.1 (C-20), 18.9 (C-21), 33.9 (C-22), 26.1 (C-23), 45.8 (C-24), 29.1 (C-25), 19.8 (C-26), 19.0 (C-27), 23.04 (C-28), 11.9 (C-29).

Stigmast-4-ene-3β, 6β-diol (**10**) – Colorless needles. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.54 (1H, br s, H-4), 4.23 (1H, t, *J* = 3.0 Hz, H-6), 4.18 (1H, br t, *J* = 8.0 Hz, H-3), 1.26 (3H, s, H-19), 0.92 (3H, d, *J* = 6.5 Hz, H-21), 0.91 (3H, s, H-18), 0.85 (3H, t, *J* = 4.1 Hz, H-29), 0.83 (3H, d, *J* = 10.8 Hz, H-26), 0.81 (3H, d, *J* = 10.8 Hz, H-27); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 36.8 (C-1), 29.4 (C-2), 74.4 (C-3), 128.8 (C-4), 147.8 (C-5), 68.1 (C-6), 38.9 (C-7), 30.3(C-8), 54.3 (C-9), 36.9 (C-10), 21.0 (C-11), 39.3 (C-12), 42.6 (C-13), 56.2 (C-14), 24.2 (C-15), 28.2 (C-16), 56.3 (C-17), 12.0 (C-18), 21.5 (C-19), 36.3 (C-20), 18.8

(C-21), 34.0 (C-22), 26.3 (C-23), 46.0 (C-24), 29.7 (C-25), 19.8 (C-26), 19.1 (C-27), 23.2 (C-28), 12.1 (C-29).

Bergenin (11) – White powder. ^{1}H NMR (CD_3OD, 500 MHz) δ: 7.06 (1H, s, H-5'), 4.93 (1H, d, J = 10.5, H-1), 3.67 (1H, m, H-5), 3.45 (1H, dd, J = 9.5, 9.0, H-4), 3.82 (1H, dd, J = 9.5, 8.5Hz, H-3); 4.06 (1H, dd, J = 9.5, 10.0Hz, H-2), 3.90 (3H, s, OCH_3), 3.67 (1H, m, H-6), 4.03 (1H, dd, J = 10.5, 2.5 Hz, H-6); ^{13}C NMR (125MHz, CD_3OD) δ: 74.4 (C-1), 81.5 (C-2), 75.7 (C-3), 71.9 (C-4), 83.0 (C-5), 62.7 (C-6), 117.4 (C-1'), 149.5 (C-2'), 142.4 (C-3'), 152.4 (C-4'), 111.1 (C-5'), 119.5 (C-6'), 164.6 (C-7'), 60.9 (OCH_3).

3-*O*-Galloybergenin (**12**) – Pale-yellow powder. ^{1}H NMR (CD_3OD, 500 MHz) δ: 7.06 (1H, *s*, H-5'), 5.14 (1H, d, J = 10.5 Hz, H-1), 4.42 (1H, dd, J = 9.5, 10.0 Hz, H-2), 5.56 (1H, t, J = 8.5, H-3), 3.76 (1H, dd, J = 8.5, 1.5, H-4), 3.79 (1H, m, H-5), 4.05 (1H, dd, J = 12.0, 1.5 Hz, H-6), 3.75 (1H, dd, J = 10.0, 1.5 Hz, H-6), 7.14 (2H, s, H-2', 6'), 3.92 (3H, s, OCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ: 74.3 (C-1), 79.2 (C-2); 76.3 (C-3); 70.2 (C-4); 83.2 (C-5); 62.5 (C-6); 117.1 (C-1'); 149.4 (C-2'); 142.2 (C-3'); 152.8 (C-4'); 111.3 (C-5'); 110.3 (C-6'); 165.2 (C-7'), 121.3 (C-1"); 110.5 (C-2",6"); 146.5 (C-3",5"), 140.4 (C-4"), 167.8 (C-7"), 60.0 (OCH_3).

(*E*)-Resveratrol (13) – White powder. ^{1}H NMR (CD_3 OD, 500 MHz) δ: 7.35 (2H, d, J = 8.5 Hz, H-2',6'), 6.95 (1H, d, J = 17.0 Hz, H-7'), 6.80 (1H, d, J = 17.0 Hz, H-7), 6.76 (2H, d, J = 8.5 Hz, H-3',5'), 6.45 (2H, d, J = 2.0 Hz, H-2, 6), 6.17 (1H, t, J = 2.0 Hz, H-4); ^{13}C NMR (125 MHz, CD_3OD) δ: 141.0 (C-1), 105.7 (C-2, 6), 159.3 (C-3, 5), 102.4 (C-4), 126.8 (C-7), 130.2 (C-1'), 128.5 (C-2', C-6'), 116.3 (C-3', 5'), 158.0 (C-4'), 129.1 (C-7').

(*E*) 2,3,5,4'-Tetrahydroxystilbene-2-*O*-*β*-D-glucoside (**14**) – Brown powder. ^{1}H NMR (CD_3COCD_3, 500 MHz) δ: 7.80 (1H, d, J = 16.5 Hz, H-7'); 7.49 (2H, d, J = 8.5 Hz, H-2',6'); 6.96 (1H. d, J = 16.5 Hz, H-7); 6.83 (2H, d, J = 8.5 Hz, H-3',5'); 6.67 (2H, d, J = 3.0 Hz, H-4); 6.29 (1H, d, J = 3.0 Hz, H-6); 4.55 (1H, d, J = 7.5 Hz, H-1"), 3.79 (2H, m, H-6"), 3.54 (1H, m, H-4"), 3.57 (1H, m, H-2"), 3.51 (1H, m, H-5"), 3.39 (1H, m, H-3"); ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 128.7 (C-1), 137.8 (C-2), 152.2 (C-3), 101.3 (C-4), 155.9 (C-5), 106.9 (C-6), 121.9 (C-7), 130.5 (C-1'), 129.1 (C-2',6'), 116.3 (C-3',5'), 158.2 (C-4'), 133.4 (C-7'), 107.9 (C-1"), 62.7 (C-6"), 78.0 (C-3"), 77.9 (C-5"), 75.4 (C-2"), 71.4 (C-4").

(*E*)-Resveratrol 3-*O*-*β*-D-glucopyranoside (**15**) – White powder. ^{1}H NMR (CD_3COCD_3, 500 MHz) δ: 7.41 (2H, d, J = 9.0 Hz, H-2',6'), 7.08 (1H, d, J = 16.5 Hz, H-7'), 6.90 (1H, d, J = 16.5 Hz, H-7), 6.84 (2H, d, J = 9.0 Hz, H-3',5'), 6.81 (1H, s, H-2), 6.67 (1H, s, H-6), 6.48 (1H, t, J = 2.0 Hz, H-4), 4.93 (1H, d, J = 7.5 Hz, H-1"), 3.92 (1H, dd, J = 2.0&11.5 Hz, H-6"), 3.71 (1H, dd, J = 5.5& 11.5 Hz, H-6"), 3.45-3.52 (m, 4H, H-2", 3", 4", 5"); ^{13}C NMR (125MHz, CD_3COCD_3) δ: 140.7 (C-1), 103.9 (C-2), 160.3 (C-3), 106.7 (C-4), 159.4 (C-5), 108.3 (C- 6), 126.6 (C-7), 129.9 (C-1'), 128.8 (C-2',6'), 116.5 (C-3',5'), 158.5 (C-4'), 129.7 (C-7'), 102.2 (C-1"), 74.8 (C-2"), 77.9 (C-3"), 71.6 (C-4"), 78.2 (C-5"), 62.9 (C-6").

(+)-Lyoniresinol (16) – White powder. $[\alpha]_D^{25}$: +43.5 (c 0.1, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ: 6.59 (1H, s, H-2'), 6.38 (2H, s, H-2, H-6), 4.31 (1H, d, J = 5.5 Hz, H-7), 3.88 (3H, s, 3'-OCH_3), 3.74 (6H, s, 3, 5-OCH_3), 3.61 (1H, dd, J = 11.0,5.5 Hz, H-9'a), 3.50 (1H, H-9'b, overlap), 3.50 (2H, d, J = 5.0 Hz, H-9), 3.38 (3H, s, 5'-OCH_3), 2.72 (1H, dd, J = 15.0, 5.0 Hz, H-7'a), 2.58 (1H, dd, J = 15.0,11.0 Hz, H-7'b), 1.97 (1H, br m, H-8), 1.62 (1H, brm, H-8'); ^{13}C NMR (125 MHz, CD_3OD) δ: 147.6 (C-3, 5),147.3 (C-3'), 146.4 (C-5'), 137.9 (C-1), 137.5 (C-4'), 133.3 (C-4), 128.8 (C-1'), 124.9 (C-6'), 106.3 (C-2'), 105.7 (C-2, 6), 65.5 (C-9'), 62.9 (C-9), 58.8 (C-5'-OCH_3), 55.5 (C-3, 5-OCH_3), 55.3 (C-3'-OCH_3), 47.8 (C-8), 40.9 (C-7), 39.6 (C-8'), 32.2 (C-7').

Vanillic acid (**17**) – White powder. ^{1}H NMR(CD_3OD, 500 MHz) δ: 7.56 (1H, d, J = 2.0 Hz, H-2), 6.83 (1H, d, J = 8.5 Hz, H-5), 7.56 (1H, dd, J = 8.5, 2.0 Hz, H-6), 3.89 (3H, s, OCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ: 123.9 (C-1), 116.1 (C-2), 148.9 (C-3), 152.5 (C-4), 114.0 (C-5), 125.3 (C-6), 56.6 (OCH_3).

Ioliolide (**18**) – White powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.97 (1H, dd, J = 14.5, 2.5 Hz, H-2a), 1.53 (1H, dd, J = 14.5, 3.5 Hz, H-2b), 4.33 (1H, m, H-3), 2.45 (1H, dt, J = 14.0, 2.5 Hz, H-4a), 1.79 (1H, dd, J = 13.5, 4.0 Hz, H-4b), 5.69 (1H, s, H-7), 1.28 (3H, s, H-9), 1.47 (3H, s, H-10), 1.78 (3H, s, H-11); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 36.0 (C-1), 47.5 (C-2), 66.9 (C-3), 45.8 (C-4), 86.8 (C-5), 182.6 (C-6), 113.0 (C-7), 172.1 (C-8), 30.8 (C-9), 26.3 (C-10), 27.1 (C-11).

(+)-Dehydrovomifoliol (**19**) – White powder. $[\alpha]_D^{25}$: +134.8 (c 0.15, MeOH); ^{1}H NMR (CD_3COCD_3, 500 MHz) δ: 2.58 (1H, d, J = 17.0 Hz, H-2a), 2.22 (1H, d, J = 17.0 Hz, H-2b), 5.86 (1H, br. s, H-4), 7.02 (1H, d, J = 16.0 Hz, H-7), 6.41 (1H, d, J =16.0 Hz, H-8), 2.28 (3H, s,H-10), 1.07 (3H, s, H-11), 1.03 (3H, s,H-12), 1.89 (3H, d, J =1.5 Hz, H-13); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ: 42.1 (C-1), 50.3 (C-2), 198.0 (C-3), 127.9 (C-4), 161.7 (C-5), 80.1 (C-6), 146.9 (C-7), 131.6 (C-8), 197.1 (C-9), 27.5 (C-10), 23.5 (C-11), 24.7 (C-12), 18.9 (C-13).

DPPH free radical scavenging assay – The stable free radical (DPPH) was used for determination of free radical-scavenging activity of the extracts.[5] Briefly, a 0.1

mM solution of DPPH in 90% ethanol was prepared and then 1.5 ml of this solution was mixed with 1.5 ml of each sample (crude extract) at concentrations of 100, 50, 25, 10 μg/ml in 90% ethanol. After 30 min incubation in the dark, the decrease in the solution absorbance was measured at 517 nm in a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). DPPH inhibitory activity was expressed as the percentage inhibition (I%) of DPPH in the above assay system, calculated as $(1-B/A) \times 100$, where A and B are the activities of the DPPH without and with test material. IC_{50} (inhibitory concentration, 50%) values were calculated from the mean values of data from three determinations. Trolox at various concentrations (1, 2.5, 5, 10 μM) was used as a positive control.

Lipid peroxidation inhibitory assay *in vitro* – TBA reacts with MDA to form a di-adduct, a red chromogen, which can be detected spectrophotometrically at 532 nm.[6] Normal rats (200 - 250 g) were used for the preparation of brain homogenate. Brain was excised and washed with 0.95 M NaCl solution. Brain homogenate was prepared with homogenizer at −5 ºC with 5 mM phosphate buffered saline (PBS) buffer (1 : 10) for 30 min. The homogenate was centrifuged for 15 min, and clear cell free supernatant was used for the study of *in vitro* lipid peroxidation. 0.1 ml of each extract at different concentrations (10 - 2000 μg/ml) in dimethyl sulfoxide was taken in test tube. 1.4 ml of 50 mM PBS buffer and 0.5 ml of rat brain homogenate were added to the test tubes. After incubation at 37 ºC for 15 min, the reaction was stopped by addition of 1 ml of 10% TCA and 1ml of 0.8% TBA. The mixture was then heated at 100 ºC for 15 min. The samples were cooled, centrifuged and the absorbance of the supernatants was measured at 532 nm. The percentage inhibition of lipid peroxidation is calculated as $(1 - B/A) \times 100$, where A and B are the activities of the MDA without and with test material. IC_{50} values were calculated from the mean values of data from three determinations. Trolox at various concentrations (10, 50, 100, 500, 1000 μM in 90% ethanol) was used as a standard.

Results and Discussion

The ethyl acetate extract of the stem of *T. erubescens* was subjected to silica gel column chromatography. Further separation and purification of the fractions with normal-phase preparative TLC led to the isolation of nobiletin (**2**),[7] tangeretin (**3**),[7] 6-demethoxytangeretin (**4**),[7] 6-demethoxynobiletin (**5** g),[7] catechin (**6**),[8] epicatechin-3-*O*-gallate (**7**),[8] phlorizin (**8**),[9] 3*β*-hydroxystigmast-5-en-7-one (**9**),[10] stigmast-4-ene-3*β*, 6*β*-diol (**10**),[11] bergenin (**11**),[12] 3-*O*-galloybergenin (**12**),[13] (*E*)-resveratrol (**13**),[14] (*E*) 2,3,5,4'-tetrahydroxystilbene-2-*O*-*β*-D-glucoside(**14**),[15] (*E*)-resveratrol 3-*O*-*β*-D-glucopyranoside (**15**),[16] (+)-lyoniresinol (**16**),[17] vanillic acid (**17**),[18] ioliolide (**18**),[19] and (+)-dehydrovomifoliol (**19**) (Fig. 1).[20] Their structures were determined on the basis of NMR spectroscopic data. Until now, this is the first report on these chemical constituents of this plant.

Tetrasigmol A (**1**) was isolated as a yellow powder. The possitive-ion HR-ESI-MS of **1** displayed a molecularion peak at m/z $[M + Na]^+$ 559.1143 together with its ^{13}C-NMR spectrum supported a molecular formula of $C_{30}H_{24}O_{12}$. The ^{13}C and DEPT-NMR of **1** exhibited a pair of catechin carbon signals,[8] especially in the high field region (δ_C 25 to 85 ppm) associating with the heterocyclic C and F rings, where the C-2, C-3 and C-4 carbon resonances showed up as twin peaks of comparable intensity. The major differences between the two catechins were found in the resonances for the B ring of one catechin unit (part A) and the D ring of the other catechin unit (part B), for instance the presence of one carbonyl signal at δ_C 194.0 ppm, one methylene group at δ_C 45.2 ppm, two quaternary carbons at δ_C 89.9 and 95.3 ppm, and two olefin carbons at δ_C 112.8 and 164.4 ppm from the B- ring, in place of an unsubstituted aromatic carbon of E ring. The ^1H NMR spectrum of **1** showed two sets of C-ring and F-ring signals similarly; however, only one set of E-ring signals [δ_H 6.85 (1H, d, J = 2.0 Hz, H-6'E)], [δ_H 6.79 (1H, d, J = 8.0 Hz; H-3'E)], and [δ_H 6.74 (1H, dd, J = 2.0, 8.0 Hz, H-2'E)]) were observed instead of the one set of B-ring signals; and two signals were observed for the A- ring at [δ_H 5.91 (1H, *s*, H-8A or H-6A)] and [δ_H 5.54 (1H, d, H-6A or H-8A)] instead of the expected one signal of D- ring at [δ_H 6.12 (1H, *s,* H-6D)]. Additionally, the HMBC spectrum of (**1**) show cross peaks between proton methylene H-2'B and [δ_C 95.3, C-3'B] and [δ_C 89.9 (C- 1'B)]. Moreover, the correlation between olefin proton H-5'B (δ_H 6.41) and C- 6'B (δ_C 164.4), carbonyl carbon C-4'B (δ_C194.0) of B ring and C-8D (δ_C105.6) of D ring indicated that the link between the part A and part B is C-6'B (part A) → C-8D (part B) (Table 1).

Comparison of the spectroscopic of **1** with literature values analyzed data,[21] **1** was identified as shown (Fig. 1) and given name tetrastigmol A. Even though this chemical structure has been reported by the oxidation product of (+)-catechin obtained in the presence of peroxidase,[21] this is the first isolated of **1** from natural sources.

The DPPH analysis is a quick and simple test; it guarantees reliable results and needs only a UV-Vis spectrophotometer to perform, which probably explains

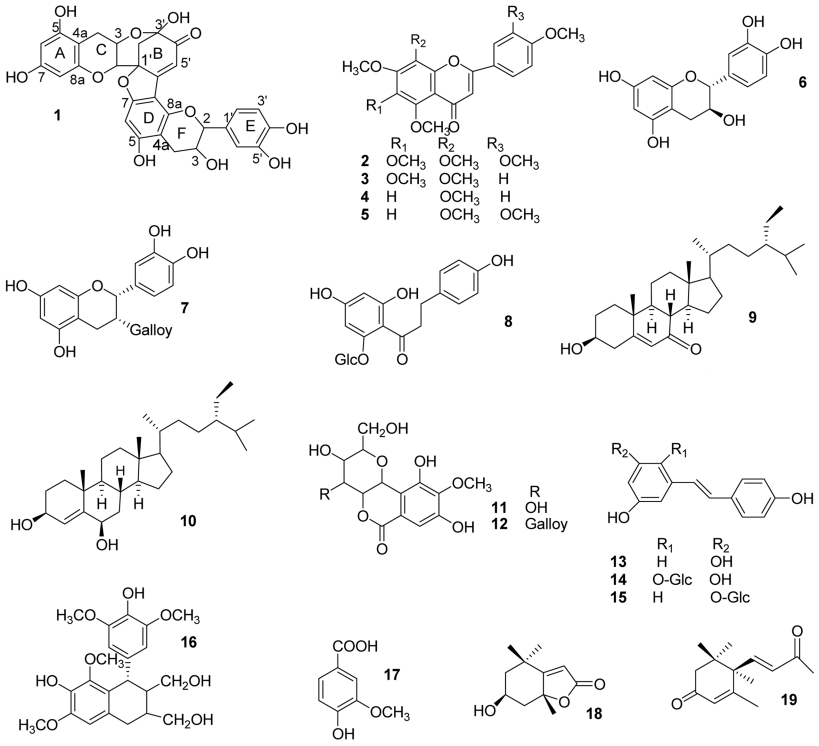

Fig. 1. Structures of the isolated compounds from the stem of *T. erusbescen.*

its widespread use in antioxidant screening.[22] The isolated compounds were tested for their antioxidant activities by DPPH assay (Table 2). Among them, compounds **1**, **6 - 8**, and **12 - 16** showed more potent activities, with IC_{50} values range from 1.8 to 60.4 μM. Compounds **6**, **7**, and **12** exhibited much more active than trolox (IC_{50}, 7.0 μM) with IC_{50} values of 5.4, 2.2, and 1.8 μM, respectively.

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. Therefore, the significant activity differences between compounds were likely due to the number of hydroxyls present in the aromatic ring,[23] though the series of compounds from **2 - 5**, **9 - 11**, and **17 - 19** tested were limited. Besides, the results of **1**, **6**, **7** and **12** were exhibitied that *ortho*-hydroxyl structures are crucial for the enhanced antioxidant activity because the *ortho*-quinone was easily formed.[24] Compounds **7** and **12** showed much strong free radical scavenging activities suggesting for the presence of *galloy* group in their structures.

Based on the results obtained from the DPPH assay, 9 isolated compounds which exhibited free scavenging activities were tested for lipid peroxidation inhibition by the thiobarbituric acid assay *in vitro* (Table 3). Compound **1**, **6 - 8**, and **12 - 15** showed stronger antioxidant activity than positive control, trolox (IC_{50}, 2265.2 μM).

As showed in Tables 2 and 3, compounds which

Table 2. Antioxidant activity of 19 isolated compounds from the stem of *T. erubescens* using DPPH assay

Compounds	IC_{50}, μM[a]
1	9.5
2	> 100
3	> 100
4	> 100
5	> 100
6	5.4
7	2.2
8	60.4
9	> 100
10	> 100
11	> 100
12	1.8
13	31.3
14	31.1
15	17.7
16	8.8
17	> 100
18	> 100
19	> 100
Trolox[b]	7.0

[a] Inhibitory concentration, 50%
[b] Possitive control

Table 3. Antioxidant activity of 9 isolated compounds from the stem of *T. erubescens* using TBA assay

Compounds	IC_{50}, μM[a]
1	87.7
6	379.2
7	52.1
8	364.7
12	60.9
13	607.5
14	157.3
15	69.9
16	> 2,000
Trolox[b]	2265.2

[a] Inhibitory concentration, 50%
[b] Possitive control

showed significant DPPH free radical scavenging activities exhibited more potent lipid peroxidation inhibition. Compounds **13 - 15** belong to stilbens which are strong antioxidants have been reported to be effective radical scavengers and inhibitors of lipid peroxidation.[6] Compounds **7** and **12** showed strong activities using two assays suggesting for the presence of *galloy* group in their structures. In the lipid peroxidation assay, it is reported that the compounds inhibit the formation of peroxides and hydroperoxides.[25] So, the active compounds are therefore able to slow the process of rancidity and greatly increase the shelf life of foods.

Conclusions

A new natural product (**1**) and eighteen compounds (**2 - 19**) were isolated and identified from the stem of *T. erubescens.* The studied on DPPH free radical scavenging and lipid peroxidation inhibitory activities. Compounds **1**, **6 - 8** and **12 - 15** showed strong antioxidant activity using two methods including DPPH free radical scavenging and lipid peroxidation inhibitory assays. Among them, compounds 6, **7**, and **12** showed higher antioxidant activities than the positive control, trolox. In addition, this investigation of antioxidant compounds suggested that the potency of these compounds could provide a chemical basis for some of the health benefits claimed for *T. erubescens* in foods and folk medicine.

References

(1) Saikat, S.; Raja, C.; Sridhar, C.; Reddy, Y. S. R.; Biplab, D. *Int. J. Pharm. Sci. Rev. Res.* **2010**, *3*, 91-100.
(2) Nagulendran, Kr.; Velavan, K.; Mahesh, R.; Hazeena, B. V. *J. Chem.* **2007**, *4*, 440-449.
(3) Vo, V. C. Dictionary of the Common Plant; Science Technology Publishing House, Ha Noi, 1996, pp 268.
(4) Phan, T. A. D.; Nguyen, X. H.; Nguyen, T. N.; Tran, L. Q, Nguyen, T. T. M. *Nat. Prod. Sci*. **2012**, *18*, 1-7.
(5) Molyneux, P. *Songklanakarin. J. Sci. Technol*. **2004**, *26*, 211-219.
(6) Mareike, K.; Claus, J.; Mario, D.; Marc, D. *Molecules*. **2010**, *15*, 7035-7074.
(7) Hiroyuki, N.; Naoki, O.; Akiko, O.; Osamu, N.; Takeshi, T.; Akihito, Y.; Yutaka, S.; Yoshihiro, M.; Tohru, Y,; Yasushi, O. *Biochem. Biophys. Res. Commun*. **2005**, *337*, 1330-1336.
(8) Adrienne, L. D.; Ya, C.; Alan P.; Davies.; Lewis, J. R. *Magn. Reson. Chem.* **1996**, *34*, 887-890.
(9) Yinrong, L.; Yeap, F. L. *Food. Chem*. **1998**, *61*, 29-33.
(10) Qu, W.; Liang, J. Y.; Jing, Y.; Li, M. R. *Chin. J. Nat. Med*. **2009**, *7*, 425-427.
(11) Parwaiz, A.; Mohd, A.; Maheesh, S. P.; Humaira, F.; Hami. *J. Phytology.* **2010**, *2,* 89-100.
(12) Jamal, A. K.; Yaacob, W. A.; Laily, B. D. *Eur. J. Sci. Res*. **2009**, *28*, 76-81.
(13) Takashi, Y.; Kaoru, S.; Yukiko, T.; Takuo, O. *Phytochemistry.* **1982**, *21*, 1180-l 182.
(14) Yu, Y. L.; Soon, H. K.; Ho, J. K.; Hyo, J. P.; Eun, J. Y.; Sung, K. K.; Young, H. Y.; Chung, G. K.; Jong, W. P.; Kyung, S. S. *J. Korean Soc. Appl. Biol*. **2009**, *52*, 40-44.
(15) Ming, L. X.; Ming, S. Z.; Yeon, K. L; Dong, C. M.; Chong, S. L.; Mi, H. W.; Byeong, S. J.; Eung, S. L.; Yungdong, J.; Hyeung, H. L.; Jong, K. S.; *Arch. Pharm. Res.* **2009**, *29*, 946-951.
(16) Heeyoung, S; Wanpyo, H.; Yonghyun, A. *Bull. Korean. Chem. Soc.* **2003**, *24*, 1680-1682.
(17) Hyun, B. P.; Kyu, H. L.; Ki, H. K.; Kyun, L.; Hyung, J. N.; Sang, U. C.; Kang, R. L. *Nat. Prod. Sci*. **2009**, *15*, 17-21.
(18) Alvarez-Suarez, M. J.; Tulipani, S.; Romandini, S.; Vidal, A.; Battino, M. *Curr. Anal. Chem*. **2009**, *5,* 293-302.
(19) Gilda, E. R.; Luis, M. P. R.; Olov, S. *J. Mex. Chem. Soc.* **2013**, *53*, 44-47.
(20) Yi, Y.; Mahinur, B.; Dongyu, G.; Haji, A. A. *J. Liquid. Chrom. Relat. Tech*. **2013**, *36*, 573-582.
(21) Sylvanin, G; Joseph, V.; Veronique, C. *Phytochemistry.* **1996**, *42*, 1279-1288.
(22) Zhang, Z.; Liao, L.; Moore, J.; Wua, T.; Wang, Z. *Food Chem*. **2009**, *113*, 160-165.
(23) Sroka, Z.; Cisowski, W. *Food Chemical. Toxicol*. **2003**, *41*, 753-758.
(24) Han, J.; Weng, X.; Bi, K. *Food Chem*. **2008**, *106*, 2-10.
(25) Singh, R.; Arora, S. *J. Chin. Clin. Med*. **2007**, *2*, 196-204.

Received October 7, 2013
Revised October 31, 2013
Accepted November 9, 2013