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Antioxidant Effect of Flavonoids Isolated from the Root of *Clematis trichotoma* Nakai.

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ABSTRACT : The antioxidant effect of methanol extract (ME) and water extract (WE) from *Clematis trichotoma* was evaluated as primary study to scavenge stable 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH), inhibited iron-induce lipid peroxidation in linoleic acid emulsion, peroxidation of liposome induced by Fe^{3+}/H_2O_2/ascorbic acid, and on Fe^{2+}/H_2O_2 induced the mitochondrial lipid peroxidation. In secondary study, five flavonoids as luteolin (1), quercetin (2), apigenin (3), hirsutrin (4), kaempferol-3-*O*-glucoside were isolated (5). Among them, compounds 1 and 2 showed good activities in all the model systems. Compound 3 exhibited moderate antioxidant activities in both radical scavenging and these lipid peroxidation systems tested. Compound 4 showed significant inhibitions in liposome peroxidation and compound 5 displayed weak inhibition in all four tested systems. All the results presented herein indicate that products of *C. trichotoma* maybe useful in inhibiting membrane lipid peroxidation and preventing free radical-linked diseases.

Key words : *Clematis trichotoma*, flavonoids, antioxidant, lipid peroxidation, free radical.

INTRODUCTION

Plants are potential sources of natural antioxidants, they absorb the sun's radiation and generate high levels of oxygen as secondary metabolites of photosynthesis (Zin *et al.*, 2002). Ultra violet (UV) radiation and heat from the sunlight can easily activate oxygen to produce reactive oxygen species (ROS). Oxidation in living organisms are essential for acquirement of energy to proceed biological processes. However, oxygen-centred free radicals and other ROS, which are continuously, produced *in vivo* result in cell death and tissue damage (Chyau *et al.*, 2002). Oxidative damage caused by free radical, peroxidation of unsaturated lipids in living organisms maybe related to aging and diseases, such as atherosclerosis, diabetes, cardiovascular, cancer and cirrhosis (Halliwell and Gutteridge, 1999). Thus, identifying antioxidants are important inhibitors which can block the generation of free radical and lipid peroxidation chain reactions.

There are about 250 species belonging to the genus *Clematis* (Ranunculaceae) in the world. Among them, 18 species, 2 varieties and 1 forma are distributed in Korea (Lee, 1996). Several plants such as *C. apiifolia*, *C. brevicaudatav*, *C. florida*, *C. heracleifolia*··· have long been used as an analgesic, a diuretic, an antitumor, and an anti-inflammatory agent in Korean traditional medicine (Kim, 1998, Bae, 2000). In the course of searching for biological agents from medicinal plants we found that *Clematis trichotoma* was also a potential source of natural antioxidants. Although previous investigation has revealed the presence of rutin, kaempferol 3-*O*-neohesperidoside, adenosine, adenine, hirsutrin, caffeic acid 4-*β*-glucoside, 3-methoxyarbutin and uridine in *C. trichotoma* (Ham *et al.*, 1999), no more study has been given to chemical and biological activity of this plant. Thus, the purpose of the present study was to evaluate the antioxidant activity of methanol extract (ME), water extract (WE) and active constituents using DPPH radical scavenger, liposome model system, linoleic acid emulsion system, and lipid peroxidation on mitochondrial.

MATERIALS AND METHODS

Plant material

Clematis trichotoma Nakai was collected from Kangwon province, and identified by one of the authors, KiHwan Bae. Voucher specimen (CNU-0712) was deposited at the herbarium of the College of Pharmacy, Chungnam National University, Daejeon, Korea.

Instruments and reagents

Linoleic acid, ferric chloride, ammonium thiocyanate, iron (II) sulfate, ascorbic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), trichloroacetic acid (TCA), α-tocopherol, thiobarbituric acid (TBA) were obtained from

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Sigma Chemical Co. (St. Louis, MO). Soybean lecithin was purchased from Wako Pure Chemicals Co. (Osaka, Japan). Polyoxyethylenesorbitan monolaurate (Tween-20), 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from E. Merck Co. (Germany).

Extraction and isolation

Ten g of dry sample was extracted with boiled MeOH (500 mℓ) or water (500 mℓ), respectively for 2 hours. The extracts were filtered through Whatman No. 2 filter paper, and then freeze-dried at 50 ℃. Both extracts were stored at −4 ℃ until used. For isolation of active components, 700 g of dry sample was extracted with boiled MeOH (3 times) under reflux and obtained the MeOH extract (110 g). The extract was suspended in H_2O and then successively partitioned with *n*-hexane, EtOAc and BuOH, and then each layer was concentrated to obtain hexane fraction (45 g), EtOAc fraction (8 g) and BuOH fraction (25 g). The bioactive EtOAc fraction was subjected on a silica gel column using stepwise gradient elution with the solvents $CHCl_3$-MeOH to give ten fractions (E.1~E.10). Fraction E.7 (1.2 g) was chromatographed over a silica gel column (3×30 ㎝), eluted with mixtures of $CHCl_3$-MeOH-H_2O (4 : 1 : 0.1), to give six subfractions (E.7-1~7-6). Fr. 7-2 (280 ㎎) was subjected to MPLC on ODS-S-50A column (11×300 ㎝, Yamazen), eluted with gradient from 30 to 70% acetonitril in H_2O (flow rate: 2 mℓ/min, detection at 254 and 360 ㎚), to afford 1 (7.5 ㎎, t_R = 28.6 min), 2 (9 ㎎, t_R = 20.5 min) and 3 (10 ㎎, t_R = 15.5 min), respectively. Fr. 7-6 (316 ㎎) was subjected to MPLC on ODS-S-50A column (11×300 ㎜, Yamazen), eluted with gradient from 50 to 70% methanol in H_2O (flow rate: 2 mℓ/min, detection at 254 and 360 ㎚), to afford 4 (8 ㎎, t_R = 16.5 min) and 5 (7 ㎎, t_R = 21.5 min), respectively.

Compound 1

Positive FAB-MS: *m/z* 287.1 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz) *δ*: 6.64 (1H, s, H-3), 6.42 (1H, d, J = 2.0 Hz, H-8), 6.16 (1H, d, J = 2.0 Hz, H-6), 7.37 (1H, d, J = 2.0 Hz, H-2'), 6.87 (1H, d, J = 8.0 Hz, H-5'), 7.40 (1H, dd, J = 8.0, 2.0 Hz, H-6'); ^{13}C-NMR (DMSO-d6, 75 MHz) δ: 164.5 (C-2), 103.4 (C-3), 182.2 (C-4), 162.1 (C-5), 99.5 (C- 6), 165.0 (C-7), 94.5 (C-8), 158.0 (C-9), 104.2 (C-10), 121.9 (C-1'), 113.9 (C-2'), 146.4 (C-3'), 150.6 (C-4'), 116.6 (C-5'), 119.6 (C-6').

Compound 2

MS negative ES-MS, *m/z* 301 $[M-H]^-$; ^{1}H-NMR (DMSO-d_6, 300 MHz) *δ*: 12.54 (1H, s, OH-5), 9.39 (3H, br, OH-3, H-1', H-4'), 7.68 (1H, d, J = 8.5 Hz, H-2'), 7.54 (1H, dd, J = 2.2 Hz, H-6'), 6.88 (1H, d, J = 8.5 Hz, H-5'), 6.40 (1H, d, J = 2.0 Hz, H-8), 6.19 (1H, d, J = 2.0 Hz, H-6); ^{13}C-NMR (DMSO-d_6, 75 MHz) *δ*: 167.7 (C-2), 135.7 (C-3), 175.8 (C-4), 163.9 (C-5), 98.2 (C-6), 160.7 (C-7), 93.3 (C-8), 147.7 (C-9), 103.0 (C-10), 120.0 (C-1'), 115.0 (C-2'), 145.0 (C-3'), 146.8 (C-4'), 115.6 (C-5'), 121.9 (C-6').

Compound 3

Positive FAB-MS: *m/z* 271.2 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz) *δ*: 6.73 (1H, s, H-3), 6.41 (1H, d, J = 2.1 Hz, H-8), 6.14 (1H, d, J = 2.1 Hz, H-6), 7.90 (2H, d, J = 8.7 Hz, H-2', 6'), 6.91 (2H, d, J = 8.7 Hz, H-3', 5'); ^{13}C-NMR (DMSO-d_6, 75 MHz) *δ*: 164.1 (C-2), 102.5 (C-3), 180.8 (C-4), 161.5 (C-5), 99.1 (C-6), 164.7 (C-7), 94.2 (C-8), 157.6 (C-9), 103.3 (C-10), 121.3 (C-1'), 128.5 (C-2', 6'), 116.0 (C-3', 5'), 161.1 (C-4').

Compound 4

Positive FAB-MS: *m/z* 645.2 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz) *δ*: 3.08-3.60 (m, sugar-H), 5.46 (d, J = 7.5 Hz, H-1"), 6.20 (d, J = 2.0 Hz, H-6), 6.40 (d, J = 2.0 Hz, H-8), 6.84 (d, J = 9.0 Hz, H-5'), 7.57 (m, H-2', 6'); ^{13}C-NMR (DMSO-d_6, 75 MHz) *δ*: 156.4 (C-2), 133.5 (C-3), 177.6 (C-4), 161.4 (C-5), 98.8 (C-6), 164.2 (C-7), 93.6 (C-8), 156.5 (C-9), 104.1 (C-10), 121.7 (C-1'), 115.3 (C-2'), 144.9 (C-3'), 148.6 (C-4'), 116.3 (C-5'), 121.3 (C-6'), 101.0 (C-1"), 74.2 (C-2"), 77.7 (C-3"), 70.1 (C-4"), 76.7 (C-5"), 61.1 (C-6").

Compound 5

Positive FAB-MS: *m/z* 453.1 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz) *δ*: 3.08-3.66 (m, sugar-H), 5.45 (d, J = 7.2 Hz, H-1"), 6.19 (d, J = 1.9 Hz, H-6), 6.42 (d, J = 1.8 Hz, H-8), 6.88 (d, J = 5.8 Hz, H-3', 5'), 8.04 (d, J = 5.9 Hz, H-2', 6'), 12.60 (s, OH-5); ^{13}C-NMR (DMSO-d_6, 75 MHz) *δ*: 156.1 (C-2), 133.1 (C-3), 177.3 (C-4), 161.1 (C-5), 98.7 (C-6), 164.5 (C-7), 93.6 (C-8), 156.4 (C-9), 103.8 (C-10), 120.8 (C-1'), 130.8 (C-2', 6'), 115.0 (C-3', 5'), 159.9 (C-4'), 100.8 (C-1"), 74.1 (C-2"), 77.4 (C-3"), 69.8 (C-4"), 76.3 (C-5"), 60.8 (C-6").

DPPH radical scavenging activity

The DPPH radical-scavenging assay was carried out according to the procedure described previously (Taco *et al.*, 1994) with a slight modification. Briefly, the DPPH radical-scavenging activity was measured in a reaction mixture containing 190 μℓ DPPH radical solutions (150 μM in ethanol) and 10 μℓ of sample solution in the 96 well plates. The solution was mixed for 30 min at room temperature in the dark and scavenging capacity was decrease in absorbance at 517 ㎚. The antioxidant activities of each extracts and compounds were expressed as IC_{50}, which was defined of the formation of DPPH radicals by 50%. BHT, ascorbic acid and α-tocopherol were used as

the positive controls.

Antioxidant effect on liposome peroxidation

Liposomes were prepared from soybean lecithin (300 ㎎) and 30 ㎖ of 10 mM sodium phosphate buffer (pH 7.4), sonicated in an ultrasonic cleaner for 2 h. The mixture containing 1 ㎖ of sonicated solution (10 ㎎ liposome/㎖), 0.6 mL of sodium phosphate buffer, 0.05 ㎖ of 25 mM $FeCl_3$, 0.05 ㎖ of 25 mM ascorbic acid, and 0.25 ㎖ of test sample (various concentrations), was incubated for 1 h at 37 ℃. After incubation, the reaction was stopped by adding 0.6 ㎖ TCA (15 % in 0.2 M HCl) and 2 ㎖ TBA (0.4% in 0.2 M HCl), heated at 95 ℃ for 15 min, cooled in ice, centrifuged 2000 rpm for 5 min and absorbance of the supernatant was measured at 532 ㎚ against a blank containing all the reagents except lipid, α-tocopherol and BHA were used as the positive controls. The ability to inhibit MDA formation was calculated by the following equation: inhibitory effects (%) = [(absorbance of control-absorbance of sample)/absorbance of control]×100 (Duh and Yen, 1997).

Antioxidant activity in linoleic acid emulsion

The antioxidant activity was determined using the thiocyanate method (Mitsuda *et al.*, 1966) with some modifications. Each sample (10~100 ㎍/㎖) in 0.5 ㎖ of deionized water was mixed with linoleic acid emulsion (2.5 mL, 0.02 M, pH 7.0) and phosphate buffer (2 ㎖, 0.2 M, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2800 g of linoleic acid, 0.2800 g of Tween-20 as emulsifier, and 50 ㎖ phosphate buffer, and then the mixture was homogenized. The reaction mixture was incubated at 37 ℃. Aliquots of 0.1 ㎖ were taken at different intervals during incubation. The degree of oxidation was measured according to the thiocyanate method by sequentially adding ethanol (4.7 ㎖, 75%), an ammonium thiocyanate (0.1 ㎖, 30%), sample solution (0.1 ㎖), and ferrous chloride (0.1 ㎖, 0.02 M in 3.5% HCl). After the mixture had stood for 3 min, the peroxide value was determined by reading the absorbance at 500 ㎚ using a spectrophotometer (Shimadzu UV-1240). A control was performed with linoleic acid but without the sample. Ascorbic acid was used as positive control.

Mitochondrial preparation and oxidation

Mitochondria were prepared according to the method of Ham and Liebler (1995) with slight modification. Mitochondria were isolated from male Sprague-Dawley rats weighing ~200-220 g as follows: rat livers were minced and then homogenized in 9 volumes of homogenization buffer (0.25 M sucrose in 5 mM Tris-HCl buffer, pH 7.4). The homogenate was centrifuged at 600 g for 10 min, and the supernatant was centrifuged at 90000 g for 15 min at 4 ℃. The resulting pellet was washed three times with homogenization buffer, and the final pellet was resuspended in 0.15 M KCl in 20 mM Tris-HCl buffer (pH 7.4). The protein concentration in mitochondria was determined according to the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. Oxidation of rat liver mitochondria was carried out in a reaction mixture containing 200 ㎕ of mitochondria (equivalent to 0.5 ㎎ of protein), 10 ㎕ of test sample (1 ㎎/㎖), 25 μ of 5 mM $FeSO_4.7H_2O$, 10 ㎕ of 10 mM ascorbic acid, and 255 ㎕ of potassium phosphate buffer (20 mM, pH 7.4). The reaction mixtures were incubated at 37 ℃ for 2 h in the dark. At the end of incubation, 250 ㎕ of TCA (20%) was added to the mixture to stop the oxidation reaction. The oxidation of mitochondria was assessed by TBARS measurement with adding 250 ㎕ of TBA (0.67% in 0.2 M NaOH). Finally, the mixture was boiled at 95-100 ℃ for 15 min, cooled in ice, centrifuged at 5000 rpm for 5 min. The absorbance of the supernatant was measured spectrophotometrically at 532 ㎚ (Shimadzu UV-1240) against blank containing with replacing sample, $FeSO_4$ and ascorbic acid by similar volume of potassium phosphate buffer. Ascorbic acid was used as a positive control.

RESULTS AND DISCUSSION

It is well known that free radicals are able to induce lipid peroxidation. To elucidate the relationship between free radicals and extracts of *C. trichotoma*, 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used as the substrate in the present work. Table 1 shows the IC_{50} values after 30 min of scavenging activity of the extracts compared to the positive controls BHT, ascorbic acid and α-tocopherol. ME and WE exhibited a significant inhibition in a concentration-dependent manner with IC_{50} values of 13.3 ± 0.5 ㎍/㎖ and 22.5 ± 1.2 ㎍/㎖, respectively. Whereas the IC_{50} value for BHT was 26.4 ± 1.2 ㎍/㎖,

Table 1. Antioxidant activities of ME and WE extract of *C. trichotoma* toward free radical and lipid peroxidation of liposomes system

Extract/antioxidant	DPPH (IC_{50}, ㎍/㎖)[1]	LS(IC_{50}, ㎍/㎖)[2]
ME	22.5±1.2	56.1±2.2
WE	13.3±0.5	40.3±3.5
α-tocopherol	19.7±0.2	97.4±4.1
ascorbic acid	4.5±0.1	ND
BHT	26.4±1.2	ND
BHA	ND	15.5±3.3

[1] DPPH: free radical scavenging.
[2] LS: lipid peroxidation of liposomes system.
Results are presented as means±SD (n = 3).
ND, not detectable.

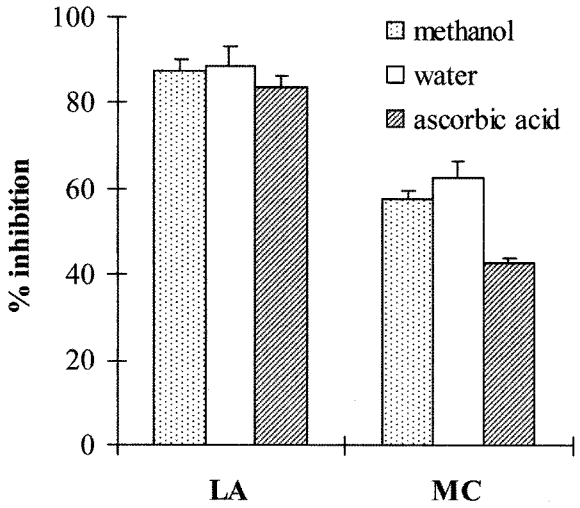


Fig. 1. Antioxidant activities of methanol and water extract for linoleic acid emulsion and lipid peroxidation of mitochondrial.
LA: capacity to inhibit the peroxide formation in linoleic acid measured by the thiocyanate method after incubation for 60 h of linoleic acid emulsion (%).
MC: effect on the lipid peroxidation of mitochondrial induced by $Fe^{2}+/H_2O_2$, inhibition of TBARS formation (%)
Inhibition (%) calculated by: [1−(absorbance of sample)/(absorbance of control)]x100.
Results are presented as means ± SD (n = 3).

ascorbic acid was 4.5 ± 0.1 ㎍/㎖ and α-tocopherol was 19.7 ± 0.2 ㎍/㎖.

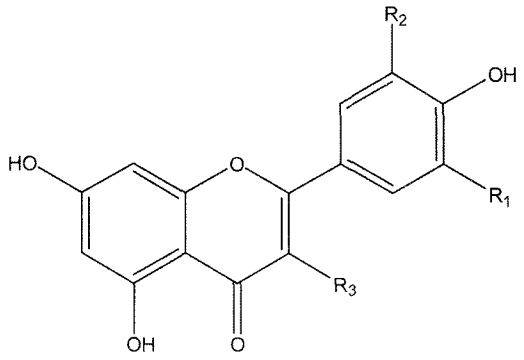
The inhibitory effect of ME and WE on TBARS formation in liposome model system is shown in Table 1. At the range concentration of 0.2~5.0 ㎎/㎖, those extracts exhibited concentration-dependent inhibitory activities on $FeCl_3$/ascorbate-induce lipid peroxidation. The IC_{50} value for ME (56.1 ± 2.2 ㎍/㎖) and WE (40.3 ± 3.5 ㎍/㎖), compare to pure bioactive compounds, α-tocopherol (IC_{50} 97.4 ± 4.1 ㎍/㎖) and BHA (15.5 ± 3.3 ㎍/㎖).

After 60 h incubation, antioxidants effects of ME and WE at the same concentration on peroxidation of linoleic acid emulsion are summarized in Fig. 1. The inhibitory potential with the percentages of inhibition was 88.9 ± 2.9% (WE), 87.3 ± 2.3% (ME), and positive control ascorbic acid (83.7 ± 1.1%), respectively.

The potency of those extracts at a concentration of 100 ㎍/㎖ was 57.8 ± 1.8% with ME and 62.4 ± 2.5% with water extract on TBARS formation of mitochondrial peroxidation. Meanwhile, ascorbic acid showed inhibition of 42.7 ± 2.4% at the same concentration (Fig. 1). These results express that almost ME and WE have potent inhibitor of mitochondrial lipid peroxidation. A little but in somewhat, the extract of *C. trichotoma* have pharmacological potential for preventing the onset of radical-induced neurodegenerative disorders by protecting the function and morphology of mitochondrial.

A single antioxidant assay would not be representative of the antioxidant potential of a mixture of compounds because those contained in crude natural extracts and different *in vivo* and in vitro tests could provide a more reliable approach to assess the antioxidant activity (Wu *et al.*, 1988, Moure *et al.*, 2000, Yagi *et al.*, 1987). Polar extracts as methanol and water extract have significant antioxidant activity; it is highly possible that several compounds may contribute to the antioxidant activity. Both those extracts have the ability to enhance the stability against primary oxidation, they may prevent the formation of toxic carbonyl compounds such as malondialdehyde in vitro and may play important roll in protecting against damage to membrane functions. Further studies were concentrated on the isolation active components. Further fractionations using several solvents were carrying out. The bioactive EtOAc fraction (data not showed) was subjected on a silica gel column using stepwise gradient elution with the solvents $CHCl_3$-MeOH to give ten sub-fractions (E1~E10). Fr. 7, which exhibited strong antioxidant activity, was fractionated to further chemical analysis with the expecting of flavonoids and phenolic derivative would be present. Five known flavonoids (1-5) were isolated as the active principles (Fig. 2). These compounds were readily identified by comparison of reported spectroscopic data as luteolin (Matsuda *et al.*, 2003), quercetin (Exarchou *et al.*, 2002), apigenin (Matsuda *et al.*, 2003), hirsutrin (Ham *et al.*, 1999), kaempferol-3-O-glucoside (Oh *et al*, 2004). The isolation of 1, 2, 4 and 5 are the first report of its occurrence in this plant.

The antioxidant activities results of isolated compounds were showed in Fig. 3 and Table 2. Compound 1 and 2 possessed good activities in all four model systems tested. Compound 3 also showed moderated antioxidant activities in radical scavenging (IC_{50} 48.8 ± 2.4 μM), liposome peroxidation (IC_{50} 66.5 ± 3.1 μM), and mitochondrial lipid peroxidation (IC_{50} 29.3 ± 2.6 μM), while in the linoleic acid emulsion, 3 showed 29.4% inhibition concentration of 100 ㎍/㎖. Compound 4 showed weak inhibitor in liposome peroxidation (IC_{50} > 100 μM), mitochondrial lipid peroxidation (IC_{50} 84.5 ± 3.7 μM), and compound 5 displayed 29.9% inhibition in linoleic acid emulsion and weak inhibition in all three remain tested systems. Accordance with the other research on the structure-antioxidant activity relationship of flavonoids (Rice-Evans *et al.*, 1997, Matsuda *et al.*, 2003; Lee *et al.*, 2003), the results suggest that the 3',4'-ortho functional group on B-ring of 1, 2, and 3 played an importance role for antioxidant activities. When the hydroxyl groups at the C-3 positions are changed by a glycoside in 4 and 5, their antioxidant abilities were decreased. The different of abilities antioxidant are dependent on their



	R_1	R_2	R_3
1	OH	H	H
2	H	OH	OH
3	H	H	H
4	H	OH	OGlc
5	H	H	OGlc

Fig. 2. Chemical structure of flavonoids isolated from *C. trichotoma*

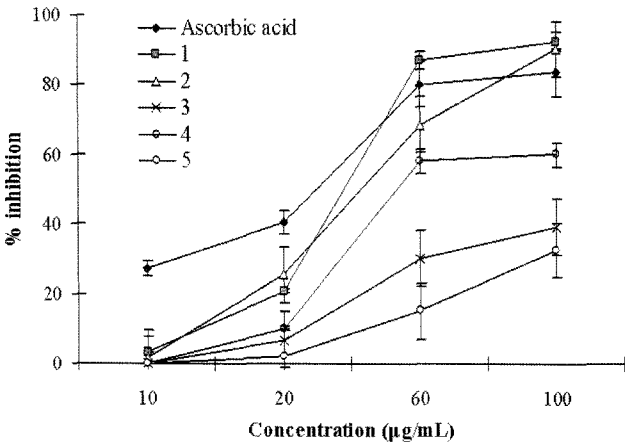


Fig. 3. Effects of flavonoids from *C. trichotoma* in linoleic acid emulsion
% inhibition as measured by thiocynate method after incubation for 60 h of linoleic acid emulsion and calculated by: [1-(absorbance of sample)/(absorbance of control)]×100.
Concentration was injection concentrations.
Results are presented as means±SD (n = 3).

basic structure, as well as the number, and arrangement of the functional group such as hydroxyl group, methoxy group and sugar units. The positions of function groups were important

Table 2. Antioxidant activities of flavonoids from *C. trichotoma*

Compounds	DPPH IC_{50} (μM) [1]	LS IC_{50} (μM) [2]	MC IC_{50} (μM) [3]
1	22.7±0.8	42.5±1.5	8.7±1.2
2	23.5±1.3	35.7±1.2	8.0±0.4
3	48.8±2.4	66.5±3.1	29.3±2.6
4	28.1 ±2.3	>100	84.5±3.7
5	>100	>100	>100
α-tocopherol	19.7±0.2	97.4±4.1	ND
ascorbic acid	4.5±0.1	ND	42.7±1.4
BHT	108.5±2.2	ND	ND
BHA	ND	15.5±3.3	ND

(1) DPPH: free radical scavenging.
(2) LS: liposome peroxidation.
(3) MC: Mitochondrial lipid peroxidation.
Results are presented as means±SD (n = 3).
ND, not detectable.

and they could be responsible for inhibitory activity to oxidation but experimental protocols and results were different in some cases, and the structure-antioxidant activity relationships of flavonoids are still interesting. Gordon and Roedig (1998) expressed quercetin was very effective at a concentration of 5 ×10-2 mol/mol phospholipids. Singh *et al*, (2004) showed that apigenin was expected to play a role in cancer chemoprevention and cancer chemotherapy. The effects of quercetin and its glycoside on mitochondrial were investigated on energy metabolism might be the cause of the reduction of the ATP levels in embryonic axes (Takahashi *et al*., 1998). In somewhat comparison, those test flavonoids of this study were not only suitable with the results of reported papers but also being to bear evidences of potential antioxidant source of *C. trichotoma* especially abilities in anti-lipid peroxidation.

Polyphenolic compounds are associated with antioxidant activity and play an important role in stabilizing lipid peroxidation (Cotton *et al*., 1993, Yen *et al*., 1993, Yen *et al*., 1998). Of our results above, those five flavonoids could be showed antioxidant capacities, however, to define the right for antioxidant activity of *C. trichotoma* toward radical scavenging and various lipid peroxodation, the investigation of further antioxidant principles and the biochemical mechanisms of active components remain should be elucidated in the future.

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