Journal of Chemistry, Vol. 45 (6), P. 768 - 771, 2007

PROTECTIVE ACTION OF *AMPELOPSIS CANTONIENSIS* AND ITS MAJOR CONSTITUENT – MYRICETIN AGAINST LDL OXIDATION

Received 16 January 2007

DO THI HA[1, 2], PHUONG THIEN THUONG[1], NGUYEN DUY THUAN[2]

[1] *College of Pharmacy, Chungnam National University, Daejeon 305–764, South Korea*

[2] *Department of Phytochemistry, National Institute of Medicinal Materials*

SUMMARY

It is widely accepted that oxidative modification of lowdensity lipoprotein (LDL) plays a pivotal role in the initiation and development of atherosclerosis. In the present study, we found that the MeOH and H_2O extracts of the plant Ampelopsis cantoniensis, and its main constituent, myricetin, possessed significant protective effects on LDL oxidation induced by either a metal ion (Cu^{2+}) or a free radical (AAPH). All of these (MeOH ex., H_2O ex., and myricetin) exhibited higher antioxidant activity than that of α-tocopherol in a dose dependent manner, and especially, myricetin disclosed stronger inhibitory effect than that of (+)-catechin, a major component of green tea. The result suggests that the decoction of the medicinal plant "che day" could be used beneficially as a remedy to prevent the LDL oxidation involved in the atherosclerotic lesion.

I - INTRODUCTION

Atherosclerosis, a disease of arteries characterized by a local thickening of vessel wall that develops in the inner coat (tunica intima), is the leading cause of death in the industrial world. It has been recognized that there are many risk factors that may cause atherosclerosis in human [1]. Among them, the oxidative modification of LDL in artery wall is generally accepted to play a key role in the initiation and development of atherosclerosis [2]. Hence, inhibitory action of LDL oxidation by supplemental antioxidants is considered as an attractive therapeutic strategy to prevent atherosclerosis and other related diseases [3].

A. cantoniensis (Hook. & Arn.) Planch (Vitaceae) is distributed in China, India, Japan, Vietnam, normally called Canton ampelopsis. This is a wild plant used as a herbal to treat inflammatory diseases such as rheumatic-arthritis, hepatitis, dermatitis, pyelitis, gastritis, acute tonsillitis, acute bronchitis and tracheitis, and eczema in Vietnam [4, 5]. Previous studies have reported that total extracted flavonoids revealed the anti-ulcer effect and good antioxidant activity of this plant [5]. Recently, Thuong *et al.* reported the free radical scavenging and antioxidant properties of the MeOH and H_2O extracts of this plant [4]. In the current study, we further investigated that MeOH and H_2O extracts, and its main constituent, myricetin, show significant antioxidant effects on LDL oxidation mediated by either a metal ion Cu^{2+} or a free radical AAPH. This paper describes the isolation of myricetin, and the antioxidant properties of this compound, MeOH and H_2O extracts from the title plant.

II - MATERIALS AND METHODS

Plant material: The leaves of *Ampelopsis cantoniensis* Planch were collected in Lao Cai

province, Vietnam, in April 2004. The sample was identified by Mr. Ngo Van Trai, National Institute of Medicinal Materials, Hanoi, Vietnam.

Extraction and Isolation of main compound: The leaves of *A. cantoniensis* (0.2 kg) were extracted with MeOH for 1 h (3 L x 3 times). The MeOH extracts were combined, filled, and exhaustively concentrated to give a MeOH extract (29.3 g). This crude extract was suspended in water (300 mL) and partitioned successively with hexane, EtOAc (each 3 time x 300 mL), and then evaporated to yield a hexane fraction (9.5 g), and an EtOAc fraction (6.5 g), respectively. The EtOAc fr. was subjected to silica gel column and eluted with Hx–EtOAc (20:1, 19:1 $\rightarrow$ 0:1) and separated into 10 fractions. Fraction 9 (0.3 g) was chromatographed over an RP-18 column using MeOH–H_2O (1:2) as an eluting solvent to yield compound **1** (32 mg).

Compound **1**: Bright yellow-green powder; mp 357 - 359°C; $FeCl_3$ reaction: positive; R_f = 0.48 [MeOH–H_2O (2:1); C_{18}–Merck]; UV λ_{max} nm (log ε): 262 (4.1), 345 (4.0); IR ν_{max}^{KBr} cm^{-1} 3320 (OH), 1650 (C=O), 1615, 1515, 1450 (aromatic C=C), 1360, 1315, 1210, 1160, 1025; ^{1}H-NMR (300 MHz, CD_3OD) δ: 7.37 (2H, s, H-2', 6'), 6.40 (1H, d, *J* = 2.1 Hz, H-8), 6.21 (1H, d, *J* = 2.1 Hz, H-6); ^{13}C-NMR (75 MHz, CD_3OD) □: 147.2 (C-2), 135.9 (C-3), 176.3 (C-4), 161.5 (C-5), 98.2 (C-6), 164.6 (C-7), 93.4 (C-8), 157.2 (C-9), 103.5 (C-10), 122.1 (C-1'), 107.5 (C-2', 6'), 145.7 (C-3', 5'), 136.3 (C-4').

LDL Preparation: Blood was drawn from healthy normolipidemic volunteers and human LDL was prepared from plasma by sequential flotation ultracentrifugation as described previously [6]. For Cu^{2+}- mediated oxidation experiments, LDL was dialyzed for 20 h at 4°C against EDTA-free, phosphate buffered saline (PBS) to remove EDTA [6, 7]. For azo-initiated oxidation experiments, LDL was dialyzed overnight against the same PBS containing 1 mM EDTA [6, 7]. The purity of LDL evaluated by agarose gel electrophoresis was > 97%. The LDL protein was determined by the bicinchoninic acid method using bovine serum albumin as a standard [6, 7].

Cu^{2+}- Mediated LDL Oxidation: The oxidation of LDL induced by copper ion was measured as described previously [6, 7]. Briefly, LDL (100 μg/ml) in PBS (pH 7.4, final volume of 1 ml) was pre-incubated with samples, and then 5 μM $CuSO_4$ was added to initiate the oxidation at 37°C. The reaction was terminated by the addition of 1 àM EDTA and cooled at 4°C. The oxidation of LDL was monitored by measuring the production of thiobarbituric acid reactive substances (TBARS) assay after 3 hrs incubation, measured at 532 nm [6, 7].

Azo-Initiated LDL Oxidation: The oxidation of LDL mediated by an azo compound was determined as previously described [6, 7]. Briefly, LDL (100 mg/ml) in PBS (pH 7.4, final volume of 1 ml) was pre-incubated with samples, and then 5 mM of an aqueous AAPH was added to initiate the oxidation at 37°C for 3 hrs. The reaction was then stopped by addition of 500 mM BHT and stored at 4°C. The oxidation of LDL was quantified by the generation of TBARS [6, 7].

The inhibitory effects (IE, %) on LDL oxidation of the test samples in two these assays were calculated as follow:

$$IE\ (\%) = 100 \times (Ac–Ab)/(As–Ab)$$

Where Ac was the absorbance of the control, As was absorbance of the sample, and Ab was the absorbance of the blank.

III - RESULTS AND DISCUSSION

The isolated compound (**1**) was obtained as a yellow-green powder with a mp 357 - 359°C. It showed a phenolic reaction with $FeCl_3$, which was supported by the absorbance band at 3320 cm^{-1} of the IR spectrum. Moreover, the UV spectrum of **1** presented two maximum band at 262 and 345 nm. These observations were suggestive of a flavone skeleton for **1**, evidenced by the 15 carbon signals in ^{13}C-NMR. Hence, compound **1** might be a major flavonoid of the studied plant. Further comparisons of ^{1}H- and ^{13}C-NMR with reported values led to the

identification that **1** was myricitin [8], a major flavonoid of *A. cantoniensis* (Fig. 1).

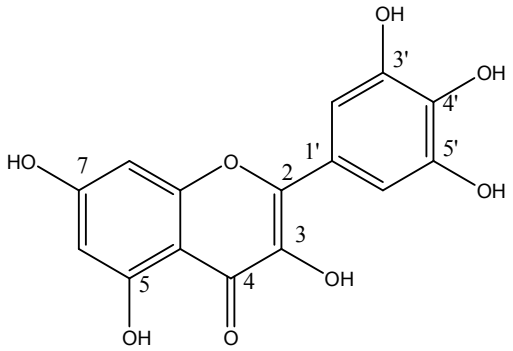

Fig. 1: Chemical structure of myricetin

The MeOH, H_2O extracts, and myricetin were evaluated for their protective actions against LDL-oxidation induced by Cu^{2+}. It was interesting to found that all these samples exhibited remarkably inhibitory effects on all these assays. As shown in the Fig. 2, the MeOH, H_2O extracts, and **1** showed the inhibition in a dose dependent manner, higher than that of α-tocopherol. All these samples absolutely inhibited the oxidation (IE = 100%) at the concentration of 10 μg/ml. It should be emphasized that myricetin was found to be stronger than (+)-catechin, a major composition of tea, at physiological concentrations. Both flavonoids, myricetin and (+)-catechin, displayed significant inhibitory effects, presenting IE = 73.4 and 64.4% at 1 μg/ml, respectively, whereas, *α*-tocopherol was found negative at this concentration.

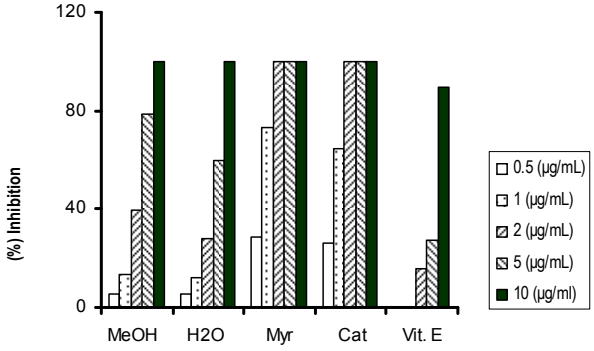

Fig. 2: Effects of MeOH and H_2O extracts of *A. cantoniensis* and myricetin on Cu^{2+}–mediated LDL oxidation

LDL (100 μg protein/ml) was incubated with 5 μM Cu^{2+} at 37 °C in PBS in the presence or absence of samples at various concentrations for 3 h. The inhibition action of LDL oxidation was monitored by TBA reaction.

In the next assay, when LDL was oxidized by a free radical AAPH, an interesting result was observed, depicted in Fig. 3. Two extracts showed equivalent remarkable inhibitory effects on LDL oxidation (IC_{50} = 3.6 and 4.1 μg/ml, respectively), while a well-known antioxidant, *α*-tocopherol, was completely inactive. Similar to the above experiment, two flavonoids exhibited significant protective effects in this assay. Nevertheless, it is noteworthy that myricetin (IC_{50} = 1.9 μg/ml) was also more efficient than (+)-catechin (IC_{50} = 2.6 μg/ml) on AAPH-induced LDL oxidation.

Accordingly, the protective effects of the MeOH and H_2O extracts of *A. cantoniensis* on oxidative modification of LDL due to the antioxidant action of its principles. Previous studies demonstrated that two flavonoids, myricetin and dihydromyricetin, are the major constituents of the leaf of this plant [8]. Therefore, the MeOH and H_2O extracts contained these antioxidant principles, and they consequently played as antioxidants in our experiments. The mechanisms by which flavonoids inhibited the oxidation of LDL might be: (1) free radical scavenging activity [1], and (2) chelating the metal ion (Cu^{2+}) [1, 2], The first, when flavonoids quench the free radicals which cause lipid peroxidation, such as hydroxy radical ($OH^{\bullet}$), lipoxy radical ($LOO^{\bullet}$), this may

help terminating the propagation phase of lipid peroxidation. So, the lipid peroxidation could be stopped [9, 10]. In the second mechanism, the substances bearing catechol group can chelated Cu^{2+} and therefore reduced the initial action of this ion [9, 10]. This markedly contributed to the anti-lipid peroxidation property of these flavonoids.

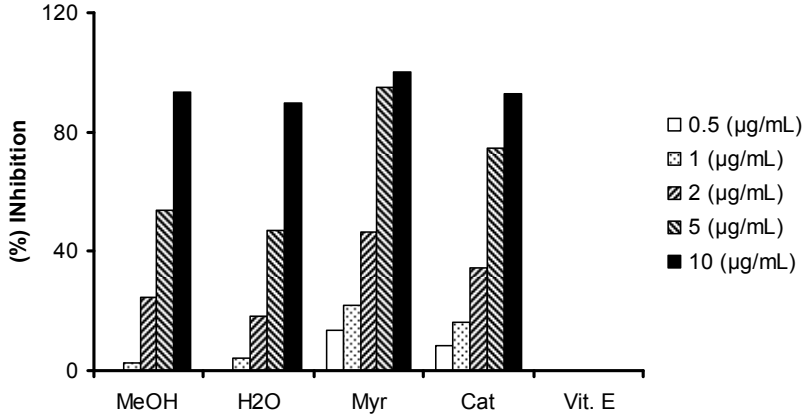

Fig. 3: Effects of MeOH and H_2O extracts of *A. cantoniensis* and myricetin on AAPH-initiated LDL oxidation

LDL (100 µg protein/ml) in PBS (pH 7.4) was incubated with 5 mM AAPH at 37°C in the present various concentrations of test samples for 3 h. The inhibitory action of LDL oxidation was monitored by TBA reaction.

Since the H_2O extract of *A. cantoniensis* was demonstrated to have significant antioxidant activities, it is suggested that this may be a promising traditionally therapeutic remedy. In this study, we further investigated that this extract remarkably inhibited LDL oxidation induced by either a metal ion (Cu^{2+}) or a free radical (AAPH) due to the presence of flavonoids in the leaf. This finding suggests that the decoction of the plant *A. cantoniensis* could be used beneficially as a remedy for oxidative-related diseases including atherosclerosis.

REFERENCES

1. B. Haliwell, and JMC. Gutteridge. Free Radicals in Biology and Medicine. London: Oxford University Press, 625 - 638 (2000).
2. JA. Berliner, JW. Heinecke. The role of oxidized lipoproteins in atherogenesis. Free Radic. Biol. Med., 20, 707 - 727 (1996).
3. J. Regnstrüm, G. Walldius, S. Nilson, LS. Elinder, J. Johansson, J. Mülgaard, I. Holme, AG. Olsson, J. Nillson. Atherosclerosis, 121, 217 - 219 (1996).
4. Thuong PT, Hung TM, Dang NH, Kiem PV, Nga CT, Ky PT. Tap chi Duoc hoc, 357, 27 - 30 (2006).
5. V. V. Chi. Dictionary of Vietnamese Medicinal Plant. Hanoi Medical Publishing House (1997).
6. Esterbauer H, Zollner H. Free Radic. Biol. Med., 7, 197 - 203 (1989).
7. Thuong PT, Na MK, Su ND, Seong RS, Lee YM, Sok DE, Bae K. Biol. Pharm. Bull., 28, 1095 - 1097 (2005).
8. Kinh CD, Ky PT, Vinh PT, Lingden B. Tap chi Duoc hoc, 4, 17 - 18 (1995).
9. Rice-Evans CA, Miller NJ, Paganga G. Free Radic. Biol. Med., 20, 933 - 956 (1996).
10. Pietta PG. Flavonoids as antioxidants. J. Nat. Prod., 63, 1035 - 1042 (2000).