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Study on the Antioxidant Compounds Extracted from Longan (*Dimocarpus longan* Lour.) Shell

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Further study on the antioxidant activity of ethanol extracts of longan shell has been explored. Seven compounds have been separated from the main active fractions. The structures of these compounds were identified by nuclear magnetic resonance and mass spectroscopy. They were isovanillin (**1**), scopoletin (**2**), quercetin (**3**), hyperin (**4**), astragalin (**5**), β-phenylethyl alcohol (**6**) and piperine (**7**). The antioxidant activities of these compounds were measured and compared with the main active fractions. It was found that quercetin and hyperin performed excellent antioxidant activities and proved the antioxidant activity of longan shell extract.

Keywords: Longan shell, Antioxidant compounds, Separation, Antioxidant activity.

INTRODUCTION

Free radical is defined as any species capable of independent existence that contains one or more unpaired electrons[1]. The free radical produced in a living body in the form of superoxide anion ($^{\bullet}O^{2-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), lipid peroxides ($LO^{\bullet}$, $LOO^{\bullet}$) and nitric oxide ($NO^{\bullet}$) *etc.*, which named as reactive oxygen species (ROS). When the action of reactive oxygen species (ROS) is existed in a balanced system hold by the antioxidant, including antioxidant compounds and enzymes, for which the radical is benefit the living body[2]. However, if the ROS reach in a high level, it is will lead to a variety of biochemical and physiological uncomfortable even senium and cell death[3]. We have learned that almost creatures could produce and be protected by enzymes of superoxide dismutase (SOD), catalase (CAT) and peroxidase from ROS destroyed, but ageing, diseases and physiology function deterioration frequently cause damages because of shortage enzymes. Thus the living body needs external antioxidant to protect to work correctly[4]. In modern world, many synthetic and commercialized antioxidants such as butylated hydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ) and butylated hydroxytoluene (BHT) are often used to reduce these ROS which bring with damages, but these antioxidants are suspected to lead to the liver damage and cancer[5]. Therefore, the development of natural antioxidants from natural plant has become the focus of the research of antioxidant.

Longan (*Dimocarpus longan Lour.*) is a member of the Sapindaceae family which is a highly attractive subtropical fruit widely distributed in the south of China, and the previous study of its biochemical and physiological activities focus on longan pulp mostly[6]. The pulp is tasted fresh and nourishing and treated as traditional Chinese medicine. However, there are litter studies worked on the shell of longan before, the shell is discarded when the pulp is ate or processed. It is beneficial if we can find some materials which have some biochemistry activities from the shell, such as antioxidant, anticancer or sterilizing. Nowadays, people are paying more attention to the health and living qualities. Plants and food which have medicinal properties are popular with human being. Actually, plants contain a diverse group of phenolic compounds, including simple phenolics, phenolic acids, hydroxycinnamic acid derivatives and flavonoids[7]. All the phenolic classes have the structural requirements of free radical scavengers and have potential as food antioxidants. The compounds in plant with medicinal properties are distributed in all parts of the plants[8,9]. As a species of popular fruit with medical properties, we need pay further insight of the longan.

In the previous work, the antioxidant activities of the microwave-assisted extract longan (MEL) and Soxhlet extraction longan (SEL) shell were employed and found that the antioxidant capacity was in order: MEL > SEL > BHT[10]. Then the series of elution agent of ethanol crude extract were determined and found that some fractions have strong antioxidant activities[11]. It was indicated the direction to research the antioxidant

activity of longan shell. In the present work, we wish to isolate and obtain the pure active compounds and determined the antioxidant activity. The structures and properties of the compounds will be identified by nuclear magnetic resonance (NMR) and mass spectroscopy (MS). The aim of this study is to make an in-deep investigation of the antioxidant compounds of longan shell.

EXPERIMENTAL

ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-500 instrument with TMS as an internal standard; melting point was determined on Greatwall X-4 microscopic melting point instrument; Column chromatogragy (CC) was performed on silica gel (200-300 mesh), or on Silica gel H (10-40 mm, Qingdao Marine Chemical Inc., Qingdao, China), or on MCI resin (CHP20P, 75-150 μm) and Sephadex LH20 (Mitsubishi Chemical Industries Co., Tokyo, Japan). Other chemicals were purchased from China National Medicine Group Shanghai Corporation (Shanghai, China). All chemicals and solvents were used of analytical grade.

Longan was obtained from Guilin Pharmaceuticals Group of China (Zhongshan Road, Guilin City, China) and identified by Associate Professor Shouyang Liu of Guangxi Traditional Chinese Medical University. A voucher specimen was deposited at the Key Laboratory of Medicinal Chemical Resources and Molecular Engineering, Guangxi Normal University, China.

Extraction and isolation: The shell of Longan was mashed (max particle size 0.4 mm) after dried in oven at 60 °C. The dried, powdered shell (2 × 2.5 kg) of longan shell was extracted with 95 % ethanol (3 × 3 L, 4 h each), then filtered along with hot solution. After removing the solvent under reduced pressure, the extract was suspended in the water (1 L) and then extracted by ethyl acetate (EtOAc, 3 × 3 L). Removing the solvent and then extract P1 was obtained.

The extract P**1** was dissolved in ethanol (EtOH, 80 %), and the supernate liquid flow was taken to through the D101 macroporous (MCI) resin until the effluent liquid was colorless. Removing the solvent under reduced pressure and obtained extract P**2**. Then eluted the resin with EtOH (100 %) again and the extract P**3** was obtained. The P**2** was eluted stepwise by 10 % on a MCI column with EtOH-aqueous (30-100 %), and P**21**-P**23** were obtained. Fraction of 30 % ethanol P**21** (10.23 g) was separated on a silica gel (200-300 mesh) column with petroleum-EtOAc-methanol (2:1:1) eluent, and the same fractions (10-35 fractions, 50 mL per fraction) were merged. The fraction was eluted on a silica gel (300-400 mesh) column with petroleum-EtOAc (10:1, 8:1, 5:1, v/v) to obtain three subfractions (Fr. 21.1-Fr. 21.3). Fr. 21.1 and Fr. 21.2 were separated stepwise on a silica gel H with petroleum-acetone (8:1, 3:1, v/v) to gain compound **1** (5 mg) and compound **2** (63 mg). Fr. 21.3 (40-48 fractions) was separated further on a silica gel (300-400 mesh) with petroleum-EtOAc-methanol (10:1:1, v/v/v) and obtained three compounds (compound **3**, 4.5 mg; compound **4**, 10 mg; compound **5**, 3.2 mg). P**22** (5.61 g) was separated stepwise on a silica gel (200-300 mesh) column with petroleum-EtOAc (20:1-2:1, 50 mL per fraction) to give three fractions (Fr. 22.1-Fr. 22.3). The Fr. 22.1 was eluted on a silica gel (300-400 mesh) column with petroleum-EtOAc (10:1, v/v) and compound **2** (15 mg) was obtained. The other two fractions were separated on a gelatin sephadex LH20 column and compound **3** (7 mg) and compound **6** (4 mg) was obtained. P**23** (50 % ethanol fraction, 2.33 g) was subjected on a silica gel (200-300 mesh) column with petroleum-EtOAc (7:1, v/v) to obtain compound **7** (32 mg) which purified by crystallized in ethanol. All of the compounds **1-7** were analysised by ESI-MS and NMR spectral.

Radical scavenging assays: The DPPH and ABTS radical scavenging tests for the extracts were carried out using the reported procedure[12,13].

RESULTS AND DISCUSSION

Based on the spectral data and compared with literature values, seven known compounds (Fig. 1) had been separated from the longan shell. They were isovanillin (**1**)[14], scopoletin (**2**)[15], quercetin (**3**)[16], hyperin (**4**)[17], astragalin (**5**)[17], β-phenylethyl alcohol (**6**) and piperine (**7**)[18].

According the compounds and antioxidant activity test (Table-1), we recognized there were three flavonoids compounds and one coumarin, which had excellent antioxidant activities. The quercetin's derivative compound (**4**) hyperin has showed the strongest antioxidant activity on the radical scavenging test, the IC_{50} values on DPPH and ABTS radical scavenging were 6.54 and 4.01 μg/mL, respectively. It was lower than the ethanol extracts and positive control BHT. Compound (**3**) quercetin, according to the literature[19], just afforded little lower protection than BHA against ROS generation, lipid peroxidation in oxidized erythrocytes. However, cellular integrity and stability were better protected by quercetin owing to the hemolytic effect of BHA. In terms of radical scavenging activity, the IC_{50} values of quercetin on DPPH and ABTS could reach 8.55 and 6.25 μg/mL, respectively. It was the second important component which responsed to the antioxidant activity. Owing to less one hydroxide radical, astragalin (compound **5**) had poor activity than compounds **3-4** and P**21**-P**23**. We were also checked the antioxidant activity of scopoletin and found that the DPPH and ABTS radical scavenging IC_{50}

TABLE-1
DPPH AND ABTS RADICAL SCAVENGING TEST OF EXTRACTS[a]

Extracts/ Compounds	IC_{50} values (μg/mL)	
	DPPH	ABTS
P21	7.53 ± 0.05	5.24 ± 0.03
P22	9.80 ± 0.05	3.24 ± 0.05
P23	10.48 ± 0.04	4.58 ± 0.05
1	>100[c]	>100
2	18.30 ± 0.05	12.96 ± 0.04
3	8.55 ± 0.02	6.25 ± 0.04
4	6.54 ± 0.05	4.01 ± 0.05
5	10.8 ± 0.03	8.12 ± 0.03
6	>100	>100
7	>100	>100
BHT[b]	12.50 ± 0.04	3.53 ± 0.02

Results are mean ± SD of three parallel measurements. $P < 0.05$
[a]All the test were performed in triplicate, [b]Positive control. [c]inactive

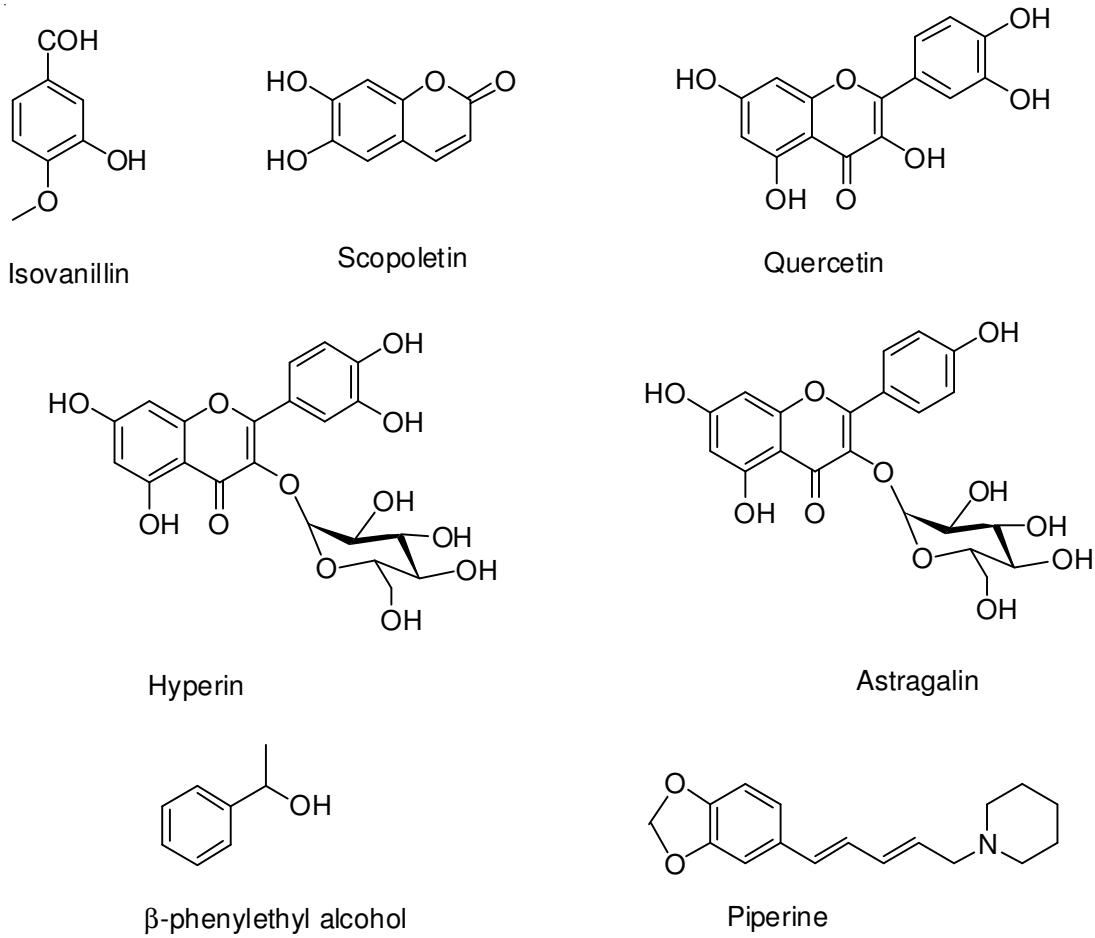

Fig. 1. Structures of the compounds **1-7**

values reached 18.30 and 12.96 μg/mL, respectively. The others extract were not appear to show good antioxidant activity. So we could declare that the antioxidant ability of longan shell was owing to containing abundant flavonoids and coumarin compounds. It was confirmed in our previous research and take a further study on longan shell.

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