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Antioxidant properties of natural components from *Salvia plebeia* on oxidative stability of ascidian oil

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Abstract

Six compounds hispidulin-7-glucuronide (**1**), β-sitosterol (**2**), conferyl aldehyde (**3**), 2′-hydroxyl-5′-methyoxybiochanin A (**4**), and hispidulin-7-*O*-D-glycoside (**5**), 6-methoxyluteolin-7-glycoside (**6**) were isolated from *Salvia plebeia* and identified by UV, IR, Mass, ^{1}H and ^{13}C NMR spectra. Their antioxidant activities were investigated individually and compared with α-tocopherol by the oxidative stability instrument (OSI) at 40 °C. The oils extracted from *Styela clava* and *Ciona intestinalis Linnaeus* with supercritical fluid (SCF) CO_2 and ethyl acetate, respectively, were used as substance oils, which were rich in polyunsaturated fatty acids (PUFA), especially in the long chain PUFA icosapentaenoic acid (EPA, 20:5$n-3$) and docosahexaenoic acid (DHA, 22:6$n-3$). β-Sitosterol, 2′-hydroxyl-5′-methyoxybiochanin A and 6-methoxyluteolin-7-glycoside exhibited strong antioxidant activities, which increased markedly with increasing concentration, and even stronger than α-tocopherol. The results indicate that the components extracted from *S. plebeia* should preserve the quality of ascidian oil from ascidian oxidative deterioration.

Keywords: *Salvia plebeia*; Ascidian; Oil; Antioxidant activity; Fatty acid

1. Introduction

Lipid peroxidation is one of the major factors that cause deterioration during processing and storage of food [1]. Oxidized polyunsaturated fatty acids may not only affect food's nutritional quality, wholesomeness, color, flavor and texture, but also induce aging, carcinogenesis, mutagenesis, cytotoxicity and atherosclerosis.

Antioxidants are defined as any substances that, when present at low concentrations compared with those oxidizable substrates, significantly delay or prevent oxidation of that ubstrate. Antioxidants work by inhibiting the formation of new free radical species, by converting existing free radicals into less harmful molecules and by preventing chain reactions.

In industrial processing, addition of highly effective antioxidants has become a popular and highly effective means to lengthen the shelf life of food and to reduce nutritional losses and harmful substances formed [2]. Synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and *tert*-butylhydro-quinone (TBHQ), previously widely used, are now doubted toxicologically [3,4]. Development and utilization of more effective and non-toxic antioxidants of natural origin are desired.

Many spices (fruits, vegetables, medicinal herbs, etc.) contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinines, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which possess antioxidant activity [5–7].

Weng et al. screened over 700 species of the most commonly used herbs using the Oxidative Stability Instrument (OSI), and 64 herbs were founds to be significantly active [8]. Among them, *Salvia plebeia* was identified to be a potent antioxidant plant. *S. plebeia* R. Br, a biannual grass, which distributes widely in many countries, contains flavones, lignars and diterpenoids [9], and was traditionally used in folk medicine for its hemogenetic, hemostatic, antioncotic and anti-inflammatory effects. The extracts from its flowers, leaves and stems were all as active as α-tocopherol at a concentration of 0.02% (w/w)

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[10]. So there might be some compounds possessing very strong antioxidant properties, which have not yet been isolated from this plant.

In recent years, interest has focused on $n-3$ polyunsaturated fatty acids (PUFAs). There are plenty of the long chain polyunsaturated fatty acids (LCPUFAs) icosapentaenoic acid (EPA, 20:5$n-3$) and docosahexaenoic acid (DHA, 22:6$n-3$) in fish oils. $n-3$ LCPUFAs have been reported to possess beneficial effects on cardiovascular functions. Recently, we found that ascidian, a primitive chordate, contains abundant PUFAs, especially EPA and DHA [11]. PUFAs are highly susceptible to lipid peroxidation and as such may contribute to an overall enhancement of peroxidative stress in the body [12–14]. A number of studies showed that enrichment of $n-3$ PUFAs in the diet, even in low amounts, may result in lower concentrations of endogenous antioxidants such as Vitamin E, and may indirectly affect other labile micronutrients such as retinol [15]. The susceptibility of $n-3$ fatty acids to oxidation may be modulated by concomitant administration of antioxidants such as Vitamin E [16,17].

This paper is concerned with the investigation of antioxidant activities of six compounds isolated from *S. plebeia* by chromatography on a silica gel column, and their effects on oxidative stability of ascidian oil have also been studied.

2. Materials and methods

2.1. Materials

Commercially available *S. plebeia* obtained from local drug vendors was dried with ventilation at ambient, and stored at 4 °C.

Styela clava and *Ciona intestinalis Linnaeus* collected from Yantai, Shandong Province, China, were dried with ventilation at ambient temperature and stored under 0 °C.

α-Tocopherol, AR grade quality, was purchased from a Guangzhou Chemical Company (China). Silica gel was obtained from Qingdao Ocean Chemical Factory (China). Ethanol, petroleum, chloroform, ethyl acetate (EtOAc), butanol and acetone were all of AR grade from local franchiser.

2.2. Equipments

Melting points (MPs) were measured by Kofler-microscope (Reichert) uncorr, infrared radiation (IR) spectra were obtained using a JASCO IR-810 spectrometer (Tsukuba, Japan). Ultra violet (UV) spectra were determined with a Hitachi UV-3400 spectrophometer (Tokyo, Japan). Electron impact mass spectra (EIMS) were measured with a Shimadzu QP-100A mass spectrometer (Tokyo, Japan). Nuclear magnetic resonance (NMR) spaectra were run on a Bruker AM-300 spectrometer (Karlsruhe, German). The antioxidant activity was tested on the Oxidative Stability Instrument (OSI) (Omnion Inc., IL, USA). The oil of ascidian was extracted with a supercritical fluid (SCF) extractor (HA221-40-12, Jiangsu, China) and fat acids were analyzed by a Shimadzu GC-14C gas chromatography (Tokyo, Japan).

2.3. Extraction and isolation of antioxidants from S. plebeia

An amount of 1 kg dried and powdered herb of *S. plebeia* was exhaustively extracted with 95% ethanol for 1 week at room temperature. The extract was concentrated with a rotary evaporator under reduced pressure to give a residue (125 g), and then was suspended in distilled water and eluted with petroleum, chloroform, ethyl acetate and butanol, respectively.

An amount of 12 g of petroleum extract was obtained and chromatographed on a silica gel column (200–300 mesh, 120 g) with petroleum/EtOAc as developing solvent, which was monitored by thin-layer chromatography (TLC) analysis, a combination of appropriate fractions led to three fractions. From fraction 1 (pectroleum/EtOAc 20:1) a crude crystalline material was gained and purified by rechromatographing on a silica gel column (300–400 mesh) with petroleum/acetone (10:1) to isolate compounds **2** and **3**.

The $CHCl_3$ extract (9 g) was subjected to column chromatography (CC) on a silica gel column (200–300 mesh, 100 g) using petroleum/EtOAc gradient as mobile phase, and five fractions were isolated according to TLC analysis. Compound **4** was obtained from fraction 2 after repeated CC on silica gel column (300–400 mesh, benzene/acetone 15:1) and purified by preparing TLC followed by recrystallization.

The EtOAc extract (14 g) was chromatographed on a silica gel column (200–300 mesh, 150 g) with $CHCl_3$/MeOH to obtain five fractions. Fractions 2 and 3 were repeatedly chromatographed on silica gel (300–400 mesh, $CHCl_3$/acetone 15:1, 10:1) to gain compounds **5** and **6**.

The BuOH extract (12 g) was applied to a silica gel column (200–300 mesh, 120 g) and eluted with $CHCl_3$/MeOH to gain three fractions. Fraction 1 was chromatographed on silica gel (300–400 mesh, $CHCl_3$/MeOH 10:1) and compound **1** was yielded.

2.4. Oil extraction from ascidian

Supercritical fluid extraction: An amount of 3 kg dry powder of *S. clava* and *C. intestinalis Linnaeus* were loaded in the extraction cell, respectively. Pure CO_2 (99.995%) was passed into the cell as the supercritical fluid at a rate of 2 mL/min (20 MPa, 50 °C) for 2 h. The ratio of the weight of ascidian to the volume of EtOAc was 1:2. The extract was concentrated to dryness under reduced pressure.

Solvent extraction: Ascidian was extracted with triplication EtOAc at 50 °C for 4 h, and the extract was also concentrated to dryness under reduced pressure.

2.5. Fatty acid analyses

Gas chromatography (GC) analyses of fatty acids were performed with a Shimadzu GC-14C equipped with an cross-linked PEG-20 M silica capillary column (60 m × 0.32 mm × 0.25 μm), an FID, and a split:splitless injector. Analysis was carried out using nitrogen (99.99%) as the carrier gas, and the flow rate is 15 cm/s. The column temperature was programmed from 180 to 250 °C at 5 °C/min. The sample size was 4 μL, and the splitting ratio was 1:30. The injection port temperature was 250 °C. The fatty acids indices were determined by co-injection of the sample with a solution containing a homologous series of *n*-hydrocarbons (C9–C22) in a temperature-programmed run identical to that described above. The quantitative calculation was based upon the relative areas of the corresponding GC signals.

2.6. Antioxidant activity

Antioxidant properties of the six compounds isolated from *S. plebeia* were studied in 10 g oil extracted from ascidian with an Omnion OSI at 40 °C. The air flow rate was fixed at 4 L/h, and α-tocopherol was used as control sample. The induction period (IP) was determined with OSI and the antioxidant activity was expressed as protection factor (Pf) that was calculated according to Weng et al. [8]:

$$\mathrm{Pf} = \frac{\text{induction period (IP) of the oil containing antioxidants}}{\text{induction period (IP) of the oil without antioxidants}}$$

2.7. Statistical analyses

All determinations of antioxidant activities were conducted in quadruple and all results were calculated as mean ± standard deviation (S.D.) in this study. Statistic programs SPSS for Windows 10.0 (SPSS Inc.) were employed, and a probability of $P < 0.05$ was taken as an acceptable level of significance. Tukey's multiple comparison tests were used to detect differences between groups.