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Studies on the Free Radical Scavenging Activity and Transition Metal Ion Chelation Ability of the Extracts from *Osmanthus fragrans* Seeds

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Green and aubergine *Osmanthus fragrans* seeds were extracted with ethanol using ultrasound-assisted extraction. The free radical scavenging activity (FRSA) and transition metal ion chelation ability of green *Osmanthus fragrans* seeds extract (GE) and aubergine *Osmanthus fragrans* seeds extract (AE) were evaluated. Both of GE and AE showed good free radical scavenging activity and GE displayed stronger free radical scavenging activity than the synthetic antioxidant butylated hydroxytoluene (BHT) in ABTS$^{\bullet}$ assay, with the IC_{50} 8.06 μg mL^{-1}. The binding constants K_a (and fluorescence quenching constants K_{sv}) of GE and AE, binded with Fe(II), Cu(II) and Cu(I) ion, were in the range of 1.04×10^3-4.00×10^4 M^{-1} (2.04×10^2-5.15×10^3 M^{-1}), indicating that both GE and AE were good ion chelator and hence may play a vital role in modulating the body's transition metal ion homeostasis.

Key Words: *Osmanthus fragrans*, Free radical scavenging activity, Transition metal ion chelation ability, Binding constant, Quenching constants.

INTRODUCTION

Age-related diseases, such as atherosclerosis, Alzheimer's disease, cancers and other degenerative diseases are characteristically associated with free radicals and transition metal ion catalyzed oxidative damages to DNA, lipids, carbohydrates and proteins[1-3]. The free radicals and transition metal ion homeostasis modulation is maintained by a balanced system of antioxidant defences, including enzymes and antioxidant compounds[1,2,4]. But only endogenous antioxidant defenses are not completely efficient. Dietary antioxidants are required to not only diminish the cumulative effects of free radicals and oxidative damages, but also to maintain the transition metal ion homeostasis modulation that related to the Fenton reaction[3,5]. However, some synthetic antioxidants such as butylated hydroxyanisole (BHA) and BHT have been reported to be carcinogenic and have also some other side effects[6,7]. Therefore, the development and isolation of natural antioxidants from natural plants has been become the focus of the research of antioxidant, since what may be learned from nature could help to overcome the toxicity issues of synthetic antioxidant compounds and to develop new antioxidants[4,8-10].

Osmanthus fragrans, widespread throughout southern China, is one of the most important commercial osmanthus[11]. It is of great value and used in the most expensive perfumes[9]. It has been also reported that *Osmanthus fragrans* has antioxidation, antitussive, antiinflammation, nitric oxide scavenging and nitric oxide-suppressing, neuroprotection and aromatherapy properties[12]. In previous work, the isolation, characterization and antioxidant activity of melanin and novel red pigment from *Osmanthus fragrans*' seeds were reported[9,10]. Both of the melanin and red pigment from *Osmanthus fragrans*' seeds has been found to get good antioxidant activity[9,10]. So it aroused our interest to take more effort on the antioxidant activity of the extracts of *Osmanthus fragrans* seeds.

The aim of this study is to investigate the free radical scavenging activity and transition metal ion binding of ethanolic extracts that were extracted under ultrasound from green and aubergine *Osmanthus fragrans* seeds, respectively.

EXPERIMENTAL

Fresh berry of *Osmanthus fragrans* were collected from Guilin city of Guangxi Province (China) in May, 2007. Under ultrasound, the green (50 g) and aubergine fresh berry (50 g) were refluxed with 80 % ethanol aqueous solution (100 mL) at 80 ºC for 2 h and filtered through Whatman No. 4 filter paper, respectively. Then the two ethanol aqueous solutions were vacuum evaporated at 50 ºC to dryness to give GE and AE with 36 and 30 % yields, respectively.

The free radical scavenging activity of GE and AE were estimated using the previous methods[8-10]. The values of IC_{50} for extracts, the effective concentration at which 50 % of DPPH and ABTS free radicals were scavenged, were tested to evaluate the radical activity. The IC_{50} of BHT was also determined for comparison.

RESULTS AND DISCUSSION

Both of GE and AE exhibited good potent inhibition of DPPH and ABTS free radical, since their IC_{50} were smaller than the standard value[13] 10 mg/mL (Table-1). GE was shown to be almost as potent as BHT, with the DPPH$^•$ and ABTS$^•$ IC_{50} of 23.58 and 8.06 μM, respectively and its DPPH and ABTS free radical scavenging activity was better than that of AE, while its ABTS free radical scavenging activity was stronger than BHT. In addition, the solvent was found to have important effects on the DPPH free radical scavenging activity and DMF was found to the best solvent for the DPPH free radical scavenging activity. It was thus hypothesized that aprotic solvent was of great significance to the DPPH free radical scavenging activity of the GE and AE.

TABLE-1
IC_{50} OF THE GE AND AE

Samples	DPPH$^•$ IC_{50} (μg mL^{-1})			ABTS$^•$ IC_{50} (μg mL^{-1})
	In water	In ethanol	In DMF	
GE	33.33	53.96	23.56	8.06
AE	109.45	99.60	91.48	23.00
BHT	–	14.50	–	8.15

In order to probe whether they exert their antioxidant effect through metal ion chelation, the Fe(II), Cu(II) and Cu(I) ion chelating ability of GE and AE were measured by UV-VIS spectroscopy (Fig. 1(A)). In the UV-vis spectroscopy, the absorbance at a fixed wavelength 216 nm was ascribed to the phenol compounds, which may be attributed to the antioxidant activity of the extracts[14]. The binding constants (K_a) were estimated (Table-2) from the UV/vis spectral data using Benesi-Hildebrand plots, involving plots of the inverse of metal ion concentrations against the inverse of changes in their respective absorbances ($1/\Delta A$ *versus* $1/[M^{n+}]$) (Fig. 1(B))[3].

TABLE-2
BINDING AND QUENCHING CONSTANTS OF THE GE AND AE

Samples	Binding constants K_a (× 10^3 M^{-1})			Quenching constants K_{sv} (× 10^2 M^{-1})		
	Cu^{2+}	Cu^+	Fe^{2+}	Cu^{2+}	Cu^+	Fe^{2+}
GE	5.80	40.0	1.04	4.39	2.04	32.23
AE	5.21	38.2	1.16	3.56	3.17	51.45

As can be seen in Table-2, binding constants K_a were in the range of 1.04×10^3-4.00×10^4 M^{-1}. GE and AE showed good transition metal ions chelating abilities with Fe^{2+}, Cu^{2+} and Cu^+, with the binding constants of 1.04×10^3 (1.16×10^3), 5.80×10^3 (5.21×10^3) and 4.00×10^4 (3.82×10^4) M^{-1}, respectively. The above binding constant data suggested a strong binding between GE and AE with Fe^{2+}, Cu^{2+} and Cu^+, showing that GE and AE have good chelation ability with transition metal ions. It implied that chelating mechanism, which would lead to the inhibition of Fenton reaction, may be the medicinal effects of GE and AE.

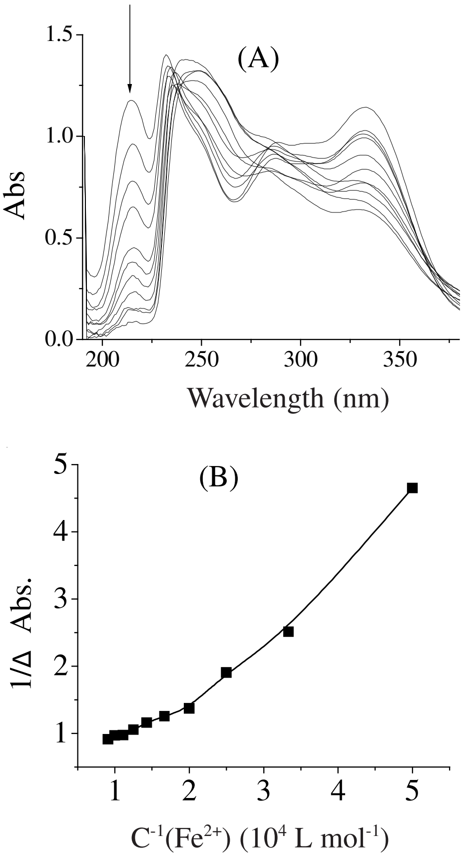
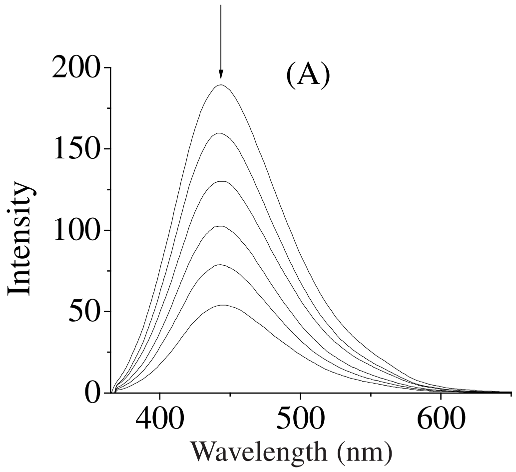


Fig. 1. UV-Vis absorbance (A) and Benesi-Hildebrand plot (B) of extract of AE-Fe(II) complex

Further studies on the chelation of transition metal ions by GE and AE were carried out by the fluorescence quenching spectroscopy. Upon addition of transition metal ions (Fe^{2+}, Cu^{2+} and Cu^+) into the aqueous solution of GE and AE, respectively, the fluorescence intensity decreased gradually as in Fig. 2(A). The fluorescence quenching data were described by the Stern-Volmer equation[15] and from the Stern-Volmer plot [Fig. 2(B)], the value of Stern-Volmer quenching constant (K_{sv}) for the metal ion can be determined (Table-2).



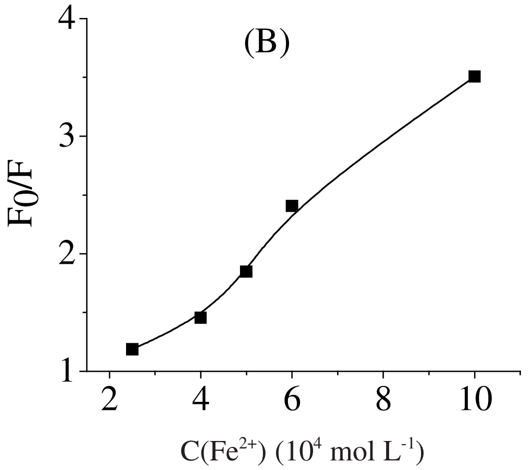


Fig. 2. Fluorescence equenching spectroscopy (A) and Stern-Volmer plot (B) of extract of green *Osmanthus fragrans*-Fe(II) complex

It can be seen from Table-2 that the quenching constants K_{sv} were in the range of 2.04×10^2-5.15×10^3 M^{-1}, confirming that the chelation of transition metal ions happened when GE and AE were treated with transition metal ions. It implied that GE and AE could prevent the Fenton reactions, which may inhibit the formation of a variety of toxic byproducts and so modulation of metal ion homeostasis and the inhibition of Fenton chemistry may be one of the possible mechanisms for the antioxidant activities of GE and AE.

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