ORIGINAL ARTICLE

Synthesis, characterization and free radical scavenging activity of apigenin with or without magnesium(II)

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Key Words
Apigenin; DPPH•; Magnesium; FTIR

Abstract

Objective: Many flavonoids are natural chelators and flavonoid-metal complexes have shown to possess significant cytotoxic activity. Apigenin (4',5,7-trihydroxyflavone) is one of the active flavonoids found naturally in a variety of fruits and vegetables. In this study, a complex of apigenin with magnesium (Mg)(II) was synthesized and its radical-scavenging and antioxidant capacity was determined.

Methods: The complex of apigenin with magnesium (Mg)(II) has been synthesized by reflux with stirring. It was was characterized by fourier transform infrared spectroscopy (FTIR) in potassium bromide (KBr). Apigenin is coordinated with Mg(II) through oxygen atoms of -OH and 4-C=O groups. Kinetic studies of scavenging DPPH• free radical with apigenin and Mg(II) complex were done at room temperature.

Results: The complex of apigenin with Mg(II) was more reactive than free apigenin in means of scavenging DPPH• free radical.

Conclusion: Apigenin complex with Mg(II) ion may be benefit to further development as promising potential metal-based drug.

INTRODUCTION

Flavones derivatives serve as ingredients for biochemical and pharmacological products used as human dietary supplements. Due to their natural yellow color, unique chemical properties and protective effects, flavonoids are recognized as potential drug candidates to be used in the treatment of diseases such as cancer, atherosclerosis, cardiovascular, coronary heart diseases, antioxidant, anti-inflammatory, anti-carcinogenic and lipid-lowering activities [1-3], as well as neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, and other age-related diseases [4]. Most flavones derivatives have been obtained from plants [5] and many of these flavonoids exist there as sugar derivatives (glycosides) [6]. They are able to modulate cell signaling pathways and gene expression. Flavonoids are also involved in cell death, carcinogenesis and mutagenesis [7-11].

Apigenin (4',5,7-trihydroxyflavone; Fig.1) is one of the active ingredients found naturally in a variety of fruits and vegetables, including parsley, onions, orange, tea, wheat sprouts and so on [7, 12]. As an important food functional factor and potential therapeutic drug for some diseases, it has been widely investigated, including anti-inflammatory effects [13], free radical scavenging effects [14], growth inhibitory properties in several cancer lines including breast [15], colon [16], skin [17], myeloid leukemia cells [18] and pancreas [19]. Moreover, recent clinical

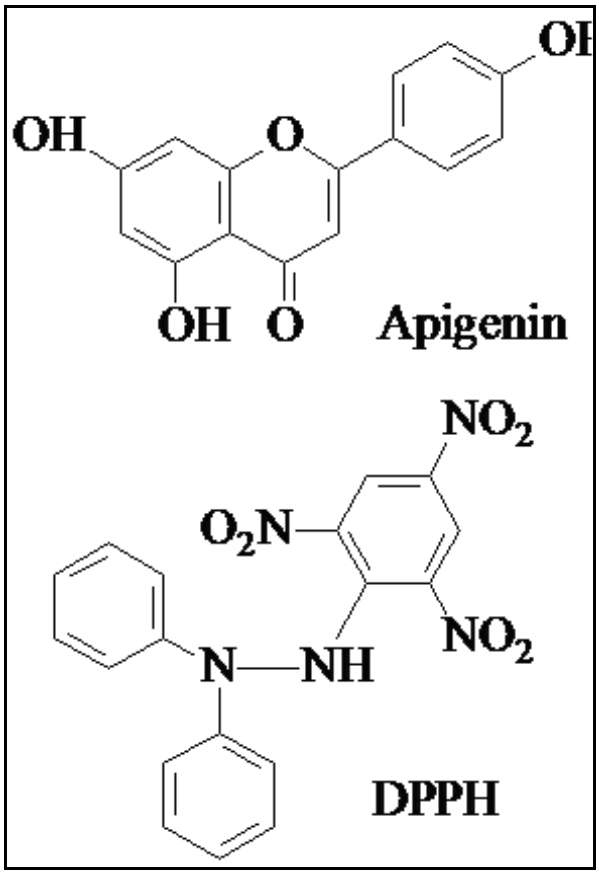

Figure 1. The chemical structures of apigenin and DPPH•.

studies demonstrated that apigenin intake was associated with a suggestive decrease in woman's risk of ovarian cancer [20, 21], which indicated apigenin could be a very promising drug candidate for future drug development.

The success of cisplatin and its derivatives as anticancer agents has stimulated the development of metal-based compounds [22-24]. Recently, interests possessed in copper (Cu)(II) complexes are increasing due to their possible medical uses as antitumor agents. And new bioactive ligands, involving natural product ligands [25, 26], have been applied for the design of Cu-coordination novel drugs; for that, naturally occurring compounds have served as a major source of drugs for centuries [27].

Many flavonoids are natural chelators and flavonoid metal complexes have shown significantly higher cytotoxic activity than those of the parent flavonoids, such as quercetin, morin, chrysin and so forth [28-30]. In this paper, a complex of apigenin with magnesium (Mg)(II) was synthesized and its radical-scavenging and antioxidant capacity was determined.

MATERIAL AND METHODS

Instrumentation and materials

UV-7504PC spectrophotometer (Shanghai Xinmao Instruments Co.); infrared spectra were obtained with FTIR-650 spectroscopy (Tianjin Gangdong Sci. & Tech. Development Co.) using KBr pelleting. The range of spectra was from 400 to 4000 cm^{-1}.

The stock solution of 1×10^{-4} mol/l apigenin (purity ≥ 98%; Nanjing Zelang Medical Technology Co.) was prepared by dissolving and diluting its crystals in ethanol. DPPH• was purchased from Shanghai Yuanye Bio-Technology Co. All other reagents were of analytical-reagent grade and were used without purification. Ultrapure water was used throughout. All experiments were carried out at room temperature. Stock solutions were stored at 4ºC and used no more than 4 days after preparation.

Synthesis of complexes

Alcohol solution (10 ml) of apigenin (0.2 mmol) was added to aqueous solution (20 ml) of magnesium acetate (0.1 mmol). The mixture was refluxed with stirring for 6 h at 60 ºC, then allowed to cool to room temperature and filtered. The solid was washed with water and ethanol, subsidences were filtered and vacuum drying was performed for 30 min at 40 ºC. The apigenin-Mg(II) complex was characterized by infrared (IR) spectra.

Scavenging activity of the stable radical DPPH•

The antioxidant activity was measured wherein the bleaching rate of a stable free radical, namely DPPH•, is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH• absorbs at 517-520 nm, but upon reduction by an antioxidant or a radical species its absorption decreases.

Stock solutions of DPPH• were prepared in methanol and ethanol (1×10^{-4} M), respectively. The sample bottles were wrapped with aluminum foil and used as the samples stored in the dark [31]. Spectrophotometric measurements were done at 517 nm.

The reaction was started by adding series of concentration of apigenin (1×10^{-4} M) or apigenin-Mg complex (1×10^{-4} M) and 4 ml of DPPH•. The mixed solution was diluted to the final volume with ethanol. After shaking up, the mixed solution was equilibrated for 30 min at room temperature.

The results were expressed as percentage of DPPH• elimination calculated according to following formula [32]:

$$\mathbf{I\ (\%) = [1- (A_{sample}-A_{blank})/A_{control}] \times 100}$$

-I; radical-scavenging activity,

-A_{sample}; absorbance of sample

-A_{blank}; absorbance of blank sample without DPPH•

-$A_{control}$; absorbance of DPPH• only

RESULTS

Infrared spectra studies

Fourier transform IR spectroscopy (FTIR) data in potassium bromide (KBr) of free ligand (apigenin) and Mg(II) complex was compared in Table 1, Fig.2. In brief, the absorption around 3200 cm^{-1} due to phenolic hydroxyl in the free ligand showed significantly spectral change in the complex form, indicating a chelate formation through hydroxyl group. The complex showed a medium broad band at 3428.81 cm^{-1} indicating existing coordinated water. The intense absorption bands due to 4-ν(C=O) of free ligand at 1654.62 cm^{-1} has also shifted to lower frequencies at 1634.37 cm^{-1}in the apigenin-Mg complex. Thus it suggests that apigenin is coordinated with Mg(II) through oxygen atoms of –OH and 4-C=O groups. This contention is further confirmed by the presence of ν(M–O) bands at about 609.40 cm^{-1} in the far IR frequency region.

Scavenging of DPPH• free radical

The DPPH• radical scavenging activity of apigenin alone and apigenin-Mg complex was determined (Fig.3). The half maximal inhibitory concentration (IC_{50}) for apigenin and apigenin-Mg complex were 8.5 μM and 10.5 μM, respectively. DPPH•-scavenging activity is influenced by the polarity of the reaction medium, chemical structure of the radical scavenger and the pH of the reaction mixture [33, 34]. In addition, the antioxidant activity of phenolic compounds depends on the position and degree of hydroxylation, as well as the nature of the radicals' ring structure.

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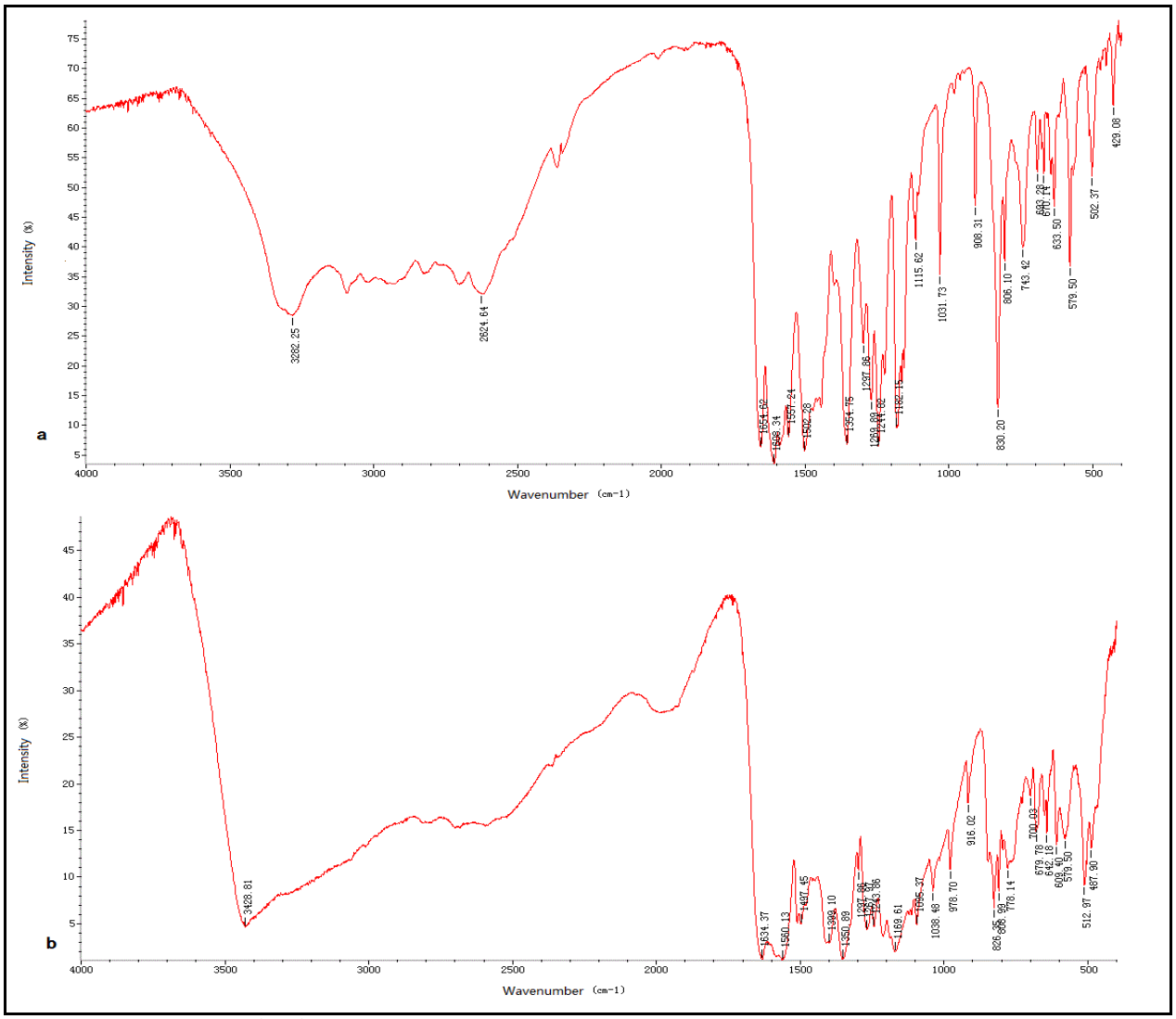

Figure 2. The FTIR spectrum of the free ligand apigenin **(a)** and apigenin-Mg(II) complex **(b)** in KBr.

Table 1. FT-IR spectrum data (cm^{-1}) for the free ligand apigenin and apigenin-Mg(II) complex

Compound	Apigenin	Apigenin-Mg(II)
***ν*(C=O)**	1654	1634
***ν*(C=C)**	1608	1560
***ν*(C–OH)**	1354	1350
***ν*(O–H)**	3282	3428
***ν*(M–O)**	-	609

Besides, our data on the comparative reaction of apigenin and apigenin-Mg(II) complex (Fig.4) indicates that the time course of inhibition also has to be determined. The radical scavenging reaction of apigenin and apigenin-Mg(II) complex with DPPH• are, essentially, instantaneous. On the other hand, the order of the radical scavenging reaction with DPPH• is complex > free AP, the absorbencies remain unchanged

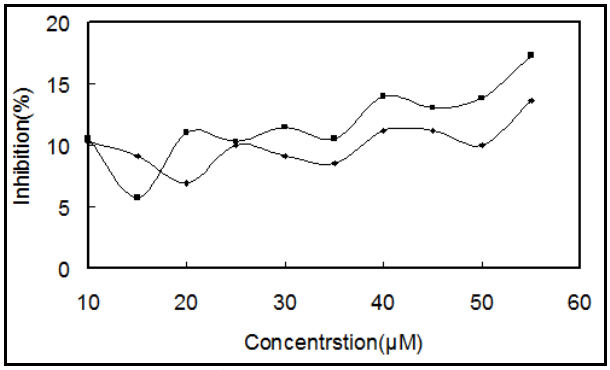

Figure 3. Scavenging of DPPH• radical by apigenin and apigenin-Mg(II) complex (from down to up) in ethanol solution at room temperature.

till a 90 min observation period. It is important to do a time course of radical scavenging activity while using DPPH• radical for the assay of antioxidant activity.

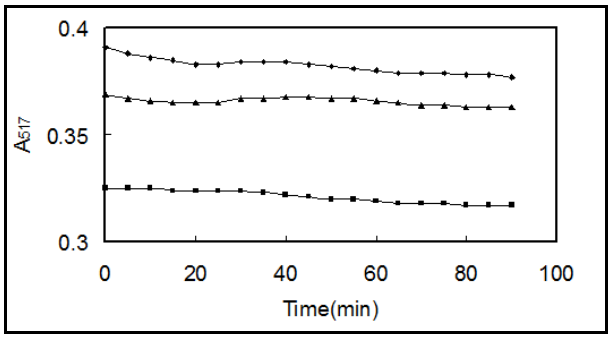

Figure 4. The time course of scavenging of DPPH• free radical (from up to down DPPH, apigenin and apigenin-Mg(II) complex).

DISCUSSION

In this study, the apigenin-Mg(II) complex was synthesized by reflux with stirring at 60ºC for 6 h and characterized by FTIR spectrum. From the FTIR spectrum, apigenin is coordinated with Mg(II) through oxygen atoms of –OH and 4-C=O groups. Besides, their antioxidant assays based on scavenging of DPPH• radical in ethanol were estimated against DPPH, and the complex showed higher activity than the free ligand against DPPH• radical; DPPH• radical scavenging activity is influenced by the polarity of the reaction medium, chemical structure of the radical scavenger and the pH of the reaction mixture.

The most important contribution of this research is that the synergistic enhancement effects of apigenin with Mg(II) ion may be benefit to further development as promising potential metal-based drug.

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