Sb(III)－Quercetin Complex：Synthesis, Characterization And Antioxidant Activity

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Abstract：Sb(III)–quercetin complex was synthesized by the raw material of quercetin and antimony ion at room temperature. The structure of the complex was characterized by UV-vis., IR, ^{1}H NMR, HPIC and thermal analysis. The antioxidant activity of the complex was evaluated by the method of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The results showed that the antioxidant activity of quercetin was better than the complex.

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1. Introduction

Antimony pollution in the environment is a serious problem nowadays [1]. Human and animal can be influenced by antimony pollution through the water, food, air and other route [2–3]. Antimony is a kind of poisonous heavy metal element. The plants, animals and human were influenced by contaminated antimony in the environment [4]. Atimony contamination of terrestrial environment has been significant issue for people more recently.

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a naturally occurring flavonoid, which exists widely in vegetables [5], fruits [6], herbs [7] and many drinks (tea, coffee, red wine and fruit juice) [8]. Quercetin has a wide range of biological activity, such as anti-cancer [9], anti-inflammatory [10], anti-virus [11], antibacterial [12], control of obesity [13]. It also can eliminate sputum, cough, wheezing, lower blood pressure, reduce blood capillary brittleness, blood fat and expansion of coronary artery, increase coronary blood flow [14]. As an excellent natural scavenging agent of free radical [15] and chelating agent of metal ion, quercetin has aroused wide concern [16]. Many studies showed that the biological activity and pharmacological effect of quercetin complex could be improved. [17].

Chelation therapy is the preferred method for reducing the toxicity caused by heavy metal poisoning. Heavy metal ions in the body can react with chelating agents, so that it can be excreted. And chelating agents can be used as an antidote to poison. Quercetin is a natural heavy metal chelator [18], it can chelate with many metal ions to form stable complexes. In this research, the Sb(III)–quercetin complex was synthesised easily from the raw material of quercetin and antimony ion at room temperature. Therefore, supplementing diet which was rich in quercetin and other flavonoids such as apples, onions, tea, ginkgo, buckwheat, may be contributed to removing free radicals and heavy metal ions in the body. The purpose of this paper is to introduce a method of synthesizing Sb(III)–quercetin complex and characterize its structure and evaluate its antioxidant capacity.

2. Material and Methods

2.1 Reagents and Materials

All reagent and solvents are of analytical reagent grade. Quercetin and DPPH were purchased from Shanghai Jingchun Scientific Co., Ltd. $SbCl_3 \cdot H_2O$ and other chemicals were obtained from Sinopharm Chemical Reagent Co., Ltd.

2.2 Physical measurements

UV–vis spectra, in MeOH, is performed using a Shimadzu UV-2550 spectrophotometer with standard 1.00cm quartz cuvette. IR spectra is recorded in the spectral range 4000–400nm on a Nicolet iS10 FT-IR spectrometer using KBr pellets. ^{1}H NMR in DMSO-d6, is obtained on a Bruker 400MHz spectrometer. Ic-1500-type high performance ion chromatography (U.S. Dionex Corporation). FLASH -EA-1112-type elemental analyzer (U.S. Thermo Electron Corporation).

RE-52AA-type rotary evaporator (the Shanghai Yarong Biochemical Instrument Factory).

2.3 Synthesis of the complex

The Sb(III)–quercetin complex was synthesized according to Literature [19]. Quercetin (604mg, 2mmol) and methanol (25ml) were added to a 100ml three-necked round-bottomed flask provide with electromagnetic stirrer and stirred until solid quercetin was completely dissolved. $SbCl_3 \cdot H_2O$ (230mg,1mmol) dissolved in methanol (25ml) was dropped in slowly at

room temperature for 0.5h. The solution was adjusted pH to 8.5 by adding sodium methoxide, which the deprotonation of the OH groups favours the solubility of quercetin. Then, the reaction was stirred for 6h at room temperature and monitored by TLC. Filtered, and the filtrate was evaporated to dryness in a rotary evaporator to get a brownish yellow solid, washed three times with methanol, and dried in a vacuum desiccator. A dark brown yellow product Sb(III)–quercetin complex was obtained in 63% yield. The Sb(III)–quercetin complex was soluble in MeOH, EtOH and dimethyl sulphoxide (DMSO), whereas insoluble in acetone and ethyl acetate.

2.4 Antioxidant activity of the complex by DPPH method

The antioxidant activity of quercetin and the Sb(III)–quercetin complex was evaluated by a modified method previously reported in literature [15] by UV-vis spectrophotometer using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method. Ethanol solution(1ml) containing different concentration of standards (20, 40, 60, 80, 100mg/L) was added to 3 ml of freshly prepared (1×10^{-4}mol/L) DPPH in ethanol. The reduction of the DPPH was followed by monitoring the decrease in absorbance at 517nm (Ay). The blank solution of DPPH was screened to estimate DPPH decomposition during the time of measurement (As). DPPH free radicals was calculated according to the following equation.

DPPH scavenging rate（%）= (As-Ay)/As×100%

3. Results and discussions

3.1 UV–vis spectra

Quercetin like most flavones and flavonols, shows two major absorption bands in the UV–Vis region. Band I (cinnamoyl) located in the wavelength range of 300–400 nm is related to conjugated system between ring B and carbonyl of ring C and belongs to π → π* (B ring) electronic transitions. Band II (benzoyl) located in the wavelength range of 240–300 nm is related to conjugated system between ring A and carbonyl of ring C and belongs to the n →π* (A ring) electronic transition.

Comparison of quercetin and Sb(III)–quercetin complex ultraviolet spectrum found that band I shifted from 375nm to 391nm and band II shifted from 255 nm to 265 nm. The band of the Sb(III)–quercetin complex is shifted to the long-wavelength region as shown in Fig.1, which is just the characteristic of the formation of a complex.

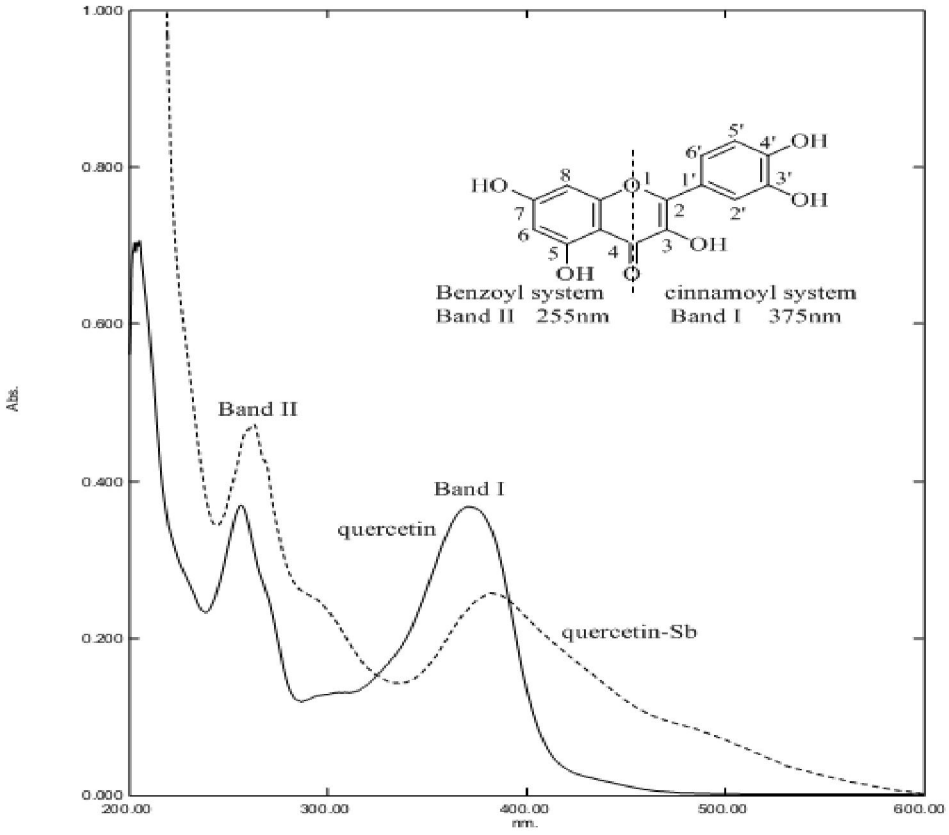

Fig.1. UV of quercetin and Sb(III)–quercetin complex

Such bathochromic shift can be explained by the extension of the conjugated system with the complexation [20]. The catechol structure of quercetin is an important chelating site in the complexation process, which is also the reason that band I has a bigger displacement. This result agrees well with the evidences that the catechol-type B ring is the most active antioxidant moiety in flavonoids.

3.2 IR spectra

IR spectra of quercetin and Sb(III)–quercetin complex are recorded respectively in Fig.2 and Fig.3.

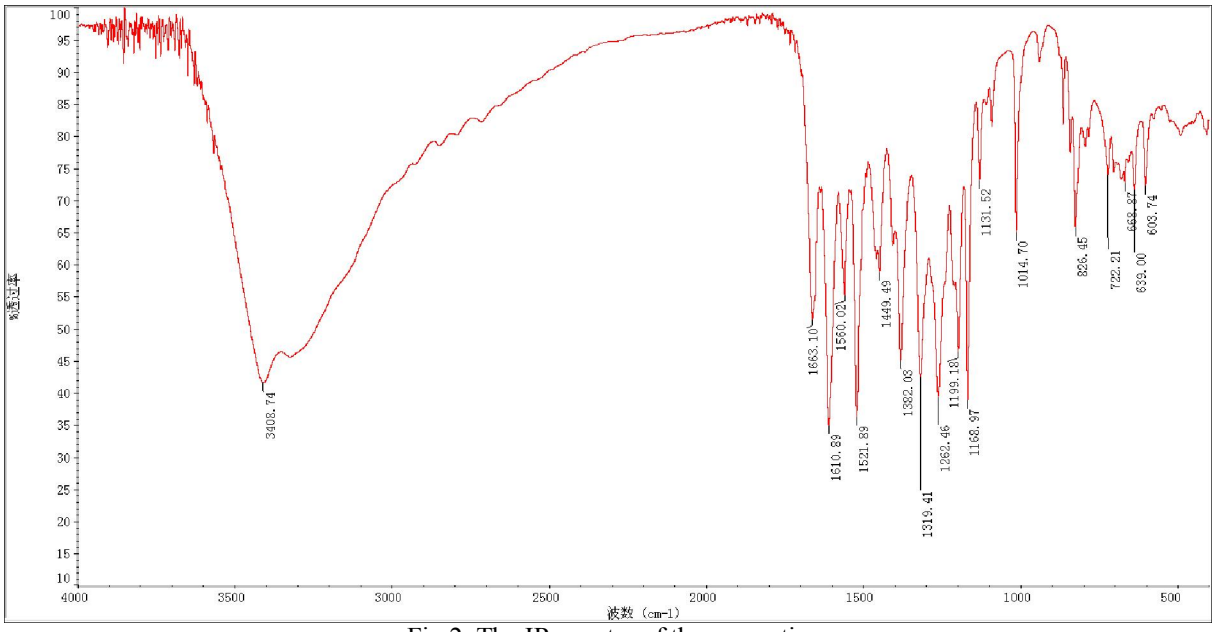

Fig.2. The IR-spectra of the quercetin

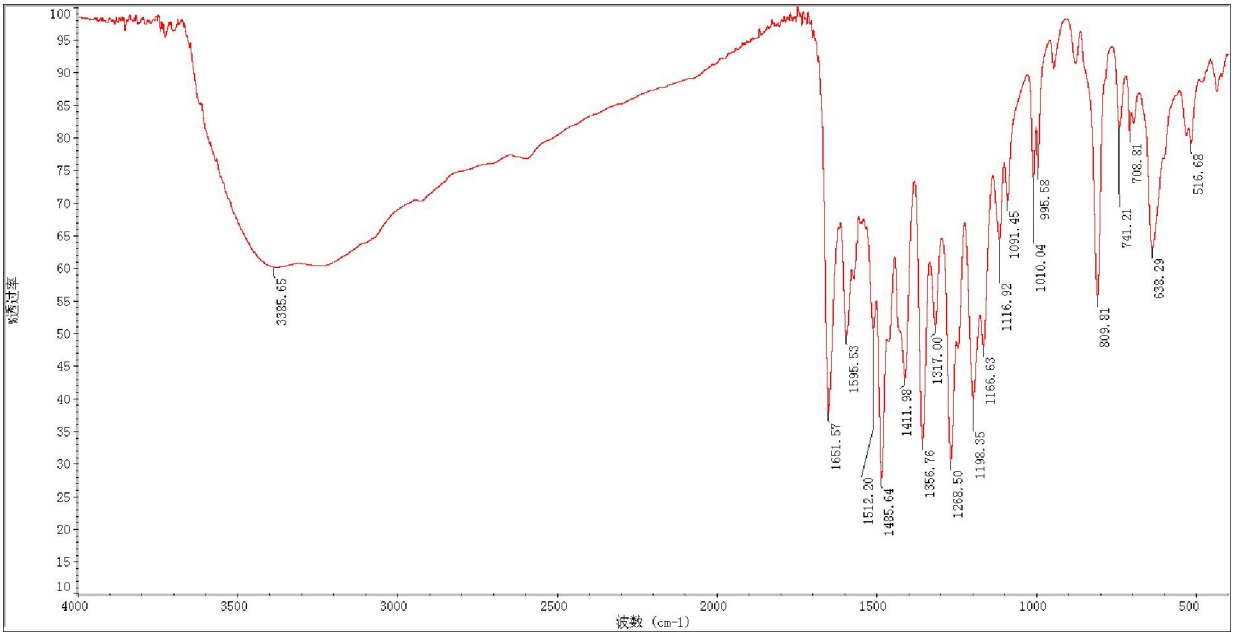

Fig.3. The IR-spectra of the Sb(III)–quercetin complex

The quercetin and Sb(III)–quercetin complex main IR values are listed in Table 1.

Table 1 Principal characteristics IR absorption frequency of quercetin and Sb(III)–quercetin complex (band position in cm^{-1}).

Compound	υ(O-H)	υ(C=O)	υ（C-O-C）	υ(C-O-H)	υ（Sb-O）
Quercetin	3408.74	1663.10	1262.46	1382.03	
Complex	3385.65	1651.57	1651.57	1356.76	638.29

The data in Table 1 are discussed below:

（1）υ(O-H) frequencies appear as broad bands (3690–2390 cm^{-1}) may be assigned for the presence of water. This agrees well with the structural of Sb(III)–quercetin complex.

（2）The characteristic stretching υ(C=O) mode of the quercetin occurs at 1663.10 cm^{-1}, while due to the formation of Sb(III)–quercetin complex this band appear at 1651.57 cm^{-1}. It can be suggested that the Sb(III) coordination occurs through the carbonyl oxygen atom and the 3-OH or 5-OH group of the quercetin [21].

（3）The bond related to the υ（C-O-C）at 1268.50 cm^{-1} is slightly shifted upon complexation indicating that the ring oxygen is not involved in the complexation.

（4）The υ(C-O-H) deformation mode observed at 1382.03 cm^{-1} in the quercetin is shifted to 1356.76 cm^{-1} in the Sb(III) complex, indicating an increase in bond order, which is normally observed when metal coordination involves with the ortho-phenolic υ(O-H) group on the quercetin B ring.

（5）Quercetin characteristic absorption peak of the benzene ring are 1610.89 cm^{-1}、1168.97 cm^{-1} and 1104.70 cm^{-1}.In addition, the benzene ring in the complex characterized absorption peaks are 1595.53 cm^{-1}、1166.63 cm^{-1} and 1010.04 cm^{-1}. Numerical substantially no change, indicating that the formation of complex, the benzene ring structure is not destroyed.

（6）The presence of υ（Sb-O）stretching vibration at 638.29 cm^{-1} indicates the formation of metal complex [22], while the quercetin exhibits no such band.

3.3 ^{1}H NMR spectra

^{1}H NMR spectra of free quercetin and Sb(III)–quercetin complex were obtained by using DMSO-d6 as a solvent and the main data are reported here:

Quercetin：^{1}H NMR (400 MHz, DMSO-d6) δ 12.48 (1H, 5-OH), 10.77 (1H, 7-OH), 9.58 (1H, 3-OH), 9.39 (1H, 4′-OH), 9.31 (1H, 3′-OH), 7.64 (1H, 2′-H), 7.53（1H, 6′-H）6.88 (1H, 5′-H), 6.39 (1H, 8-H), 6.13 (1H, 6-H) [23].

Sb(III)–quercetin complex：^{1}H NMR (400 MHz, DMSO-d6) δ 12.56 (1H,5-OH), 11.03 (1H, 7-OH), 9.35 (1H, 4′-OH), 9.30 (1H, 3′-OH), 7.68 (1H, 2′-H), 7.55 (1H, 6′-H), 6.67 (1H, 5′-H), 6.52 (1H, 8-H), 6.31 (1H, 6-H).

^{1}H NMR data of the complex formed between quercetin and antimony trichloride indicates that 3-OH group protons are not present in the complex; however four other hydroxyl group (7-OH, 5-OH, 3′-OH and 4′-OH) protons were remained after chelation. These data from ^{1}H NMR fulfil the data of UV–visible and IR spectroscopic studies.

3.4 HPIC study of the complex

The Sb(III)–quercetin complex 11.48 mg was dissolved in 100 ml distilled water, and it`s tested retention time is 4.693 min. Then, chlorine 44.92 mg was tested within 100ml distilled water, the measured retention time is 4.707 min. Both of them have the same retention time, it proved that there existed chloride ion in the Sb(III)–quercetin complex.

3.5 Thermal analysis

Compounds of physical and chemical changes caused by heating may be accompanied by the release and absorption of heat, as well as changes in the quality. By thermal gravimetric analysis, we can understand the stability and structure of the complex [24]. In static air atmosphere, heating rate of 10℃/min, and the differential thermal range for 100 μv, and the measurement temperature range from 20℃ to 710℃. Thermal study of the complex is recorded in Fig.4.

The DSC curves for the complex shows endothermic and exothermic peaks that agree with the mass losses observed in the TG. The endothermic peaks about 91.6℃ are due to dehydration, in agreement with the mass losses observed by the TG. The exothermic peak near the 279.3℃ corresponds to Sb(III)–quercetin complex the first step of decomposition, accompanying weight loss of 36.44%. The exothermic peak near the 515.3℃ corresponds to the oxidative decomposition of organic matter, accompanying weight loss of 40.11%. The last remaining residue is antimony oxide.

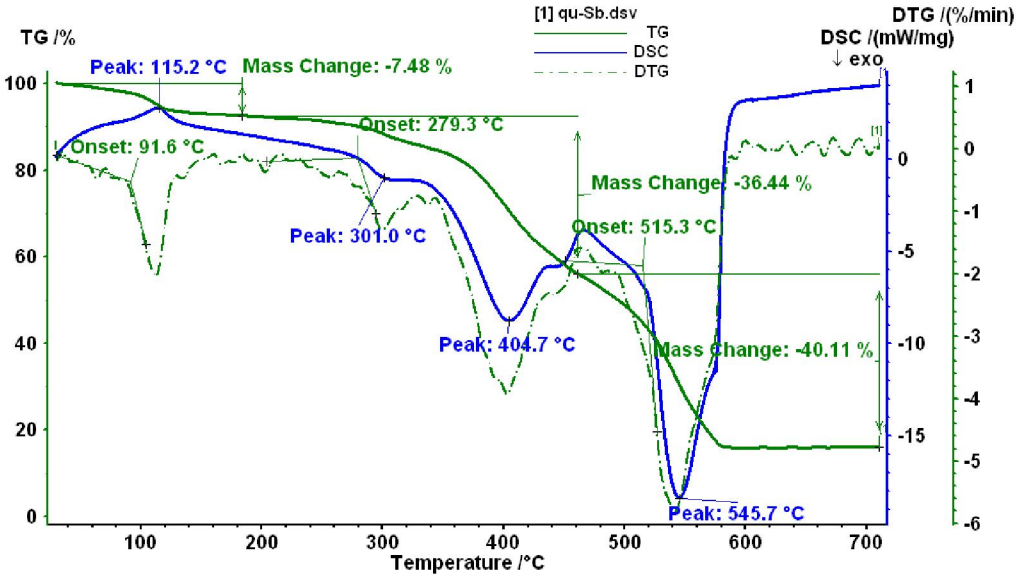

Fig.4. Thermogravimetric analysis of Sb(III)–quercetin complex

Table 2 The maximum temperature, T_{max} (◦C), and weight loss values of the decomposition stages for the Sb(III)–quercetin complex.

Compound	Mass(g/mol)	Decomposition T_{max}(℃)	Eliminated species	%mass loss	
				Found	calculated
$[Sb(L)_2(H_2O)_2]Cl^-$ $(H_2O)_6$ (905.2g/mol)	First step	91.6	4 H_2O	7.48	7.95
	Second step	279.3	4 H_2O，$C_{12}H_{12}O_4Cl$	36.44	36.18
	Third step	515.3	$C_{18}H_6O_9$	40.11	40.43
	Total loss Residue		Sb_2O_3	84.03 15.97	84.56 16.10

Content of Sb in Sb_2O_3: 2Sb/ Sb_2O_3 = 2×121.7/(121.7×2 +16×3)×100% = 83.5%
Content of Sb in Sb(III)–quercetin complex (experimental value): 15.97%×83.5%×100% = 13.33%

(15.97%: As can be seen from Table 2, the last of the remaining residue is Sb_2O_3, and its content is 15.97%. 83.5%: Content of Sb in Sb_2O_3）.

Content of Sb in Sb(III)–quercetin complex (theoretical value)：

$$\frac{M(Sb)}{M(Sb\text{-}quercetin)}\times 100\% = \frac{121.7}{302\times 2+121.7+18\times 8+35.5}\times 100\% = \frac{121.7}{905.2}\times 100\% = 13.44\%$$

Sb(III)–quercetin complex thermal analysis shows that two kinds of different forms of cracking is dehydration and decomposition [25-26]. Below 91.6℃, the weight loss was 7.48% corresponding to a water molecule. When heated to 115.2℃, Sb(III)–quercetin complex begans to decompose.

3.6 The structure of Sb(III)–quercetin complex

According to the UV-visible, IR, ^{1}H NMR, HPIC, elemental analysis and thermal analysis we can infer that Sb(III)–quercetin complex may consist of two quercetin, an antimony atom, a chloride ion and eight water molecules (Fig.5).

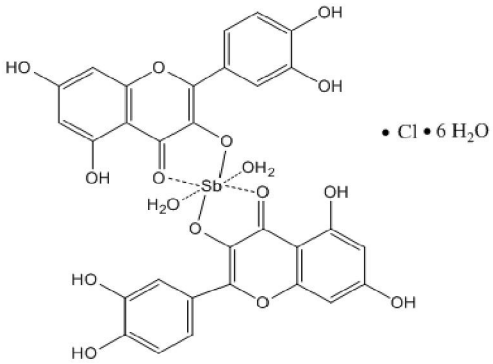

Fig.5. Structure of Sb(III)–quercetin complex

3.7 DPPH radical scavenging activity of quercetin and the Sb(III)–quercetin complex

DPPH is a stable free radical in organic solvent, its ethanol solution has the largest absorption at 517 nm place. The presence of free radical scavenge makes the absorbance decrease at 517 nm, because the DPPH radicals single electrons are paired. Therefore this method often used to evaluate the antioxidant capacity of sample [27].

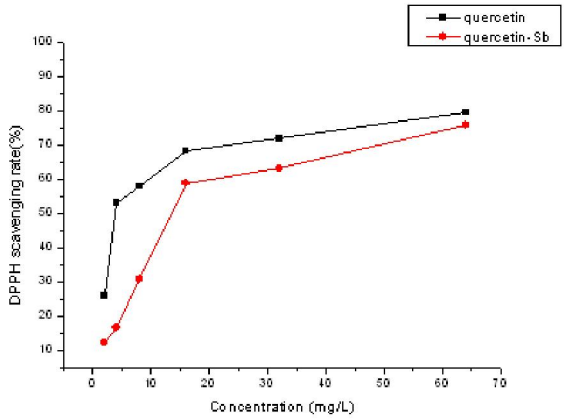

Fig.6. The scavenging effect on DPPH radical of quercetin and Sb(III)–quercetin complex

Fig.6 shows that with quercetin and Sb(III)–quercetin complex concentration increasing, DPPH radical scavenging rate also increases. But obviously quercetin is much more effective than the Sb(III)–quercetin complex in terms of antioxidant activity. According to the experimental results, the EC_{50} of quercetin and Sb(III)–quercetin complex are 3.78 and 13.46 mg/L, illustrating that the scavenging effect on DPPH radical of Sb(III)–quercetin complex is lower than quercetin. This may be due to the formation of the complexes, quercetin reduces ability of accepting protons.

4. Conclusion

The Sb(III)–quercetin complex was synthesized easily from the raw material of quercetin and antimony ion in alkalescence condition at room temperation. The pH of plasma is alkaline, so quercetin may be contributed to the removal of heavy metal ions in the plasma, through the formation of complexes with heavy metal ions. A probable structure for the investigated complex has been proposed based on the spectroscopic data of UV-visible, IR, ^{1}H NMR, HPIC and thermal analysis. Spectroscopic data suggests that quercetin molecule can chelate antimony ions from 3-hydroxy-carbonyl chelation site. The antioxidant activity of the complex was evaluated by the DPPH method. The experiment showed that quercetin is much more effective than the Sb(III)–quercetin complex in terms of antioxidant activity. So supplementing diet that rich in quercetin in daily life may be contributed to removing free radicals and heavy metal ions in the body.

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