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Original Research Article

Characterization and DPPH Radical Scavenging Activity of Gallic Acid-Lecithin Complex

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Abstract

Purpose: *To investigate the physicochemical properties and DPPH radical scavenging activity of gallic acid–lecithin complex*
Methods: *The complex of gallic acid with lecithin was prepared by solvent method. The physicochemical properties of the complex were investigated by ultraviolet-visible spectrometry (UV), infrared spectrometry (IR), scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and x-ray diffractometry (XRD). Antioxidant activity was examined by 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay.*
Results: *The UV and IR spectra of the complex showed an additive effect of gallic acid and lecithin, in which the characteristic absorption peaks of gallic acid and lecithin were retained. SEM suggests that gallic acid was dispersed into lecithin while DSC and XRD results for the complex mainly showed the presence of lecithin with the characteristic peaks for gallic acid absent. At all concentrations of the complex (0.2 - 1.0 mg/mL), the DPPH radical scavenging activity of the complex was higher than that of the reference antioxidant, butylated hydroxytoluene (BHT).*
Conclusion: *Gallic acid and lecithin in the complex are combined by a non-covalent bond, and did not form a new compound. The complex is an effective scavenger of DPPH radicals.*

Keywords: *Gallic acid, Lecithin, Complex, Antioxidant*

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INTRODUCTION

Gallic acid, 3, 4, 5-trihydroxybenzoic acid can be found in gallnuts, sumac, witch hazel, tea leaves, oak bark, etc. Gallic acid possesses antioxidant, antifungal, antiviral, anticancer activities [1-3]. Due to its poor lipophilic property, the application of gallic acid in functional food and medicine is restricted. The low hydrophobicity of gallic acid can result in its poor permeation across the intestinal epithelial cells and minimize gastrointestinal tract absorption, leading to decreased oral bioavailability. Lecithin is a group of yellow-brownish fatty substances occurring in animal and plant tissues, and in egg yolk [4], which can be used as a matrix to improve lipophilic property and targeted delivery of bioactive compounds [5,6]. It is expected that gallic acid combined with lecithin might result in the improvement of lipophilic property of gallic acid. In this study, the complex of gallic acid and lecithin was prepared, and the physicochemical properties and DPPH radical scavenging activity of the complex were investigated.

EXPERIMENTAL

Materials and reagents

Gallic acid was obtained from Aladdin (Shanghai, China) while 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Chemicals Co (USA). Lecithin from soya bean was a product of Sangon (Shanghai, China). Other chemicals used were of analytical grade.

Preparation of gallic acid-lecithin complex

Gallic acid (500 mg) and lecithin (1000 mg) were dissolved in 60 mL of tetrahydrofuran and stirred for 4 h. After tetrahydrofuran was removed, the residue was collected and ground. The resultant yellow power was taken as gallic acid-lecithin complex.

Ultraviolet-visible (UV) and infrared spectroscopy (IR)

UV analysis was performed on a TU-1810PC UV-visible spectrophotometer (Purkinje, China) and while IR analysis was carried out on a Tensor 27 infrared spectrophotometer (Bruker, Germany) by the KBr method.

Scanning electron microscopy (SEM)

Examination with scanning electron micrographs (SEM) was performed with a Quanta 200 environmental scanning electron microscope (FEI, USA). The sample was evenly distributed on SEM specimen stubs with double adhesive tape. The micrographs were obtained at an accelerating potential of 15 kV under low vacuum.

X-ray diffractometry (XRD)

Monochromatic Cu Ka radiation (wavelength = 1.54056 A˚) was produced by a D8 X-ray diffractometer (Bruker, Germany). The powders of samples were packed tightly in a rectangular aluminum cell. The samples were exposed to the X-ray beam from an X-ray generator. The scanning region of the diffraction angle, 2θ, was 5 – 80˚. Duplicate measurements were made at ambient temperature. Radiation was detected with a proportional detector.

Differential scanning calorimetry (DSC)

DSC analysis was performed on a Q200 differential calorimeter (TA, USA). The sample (approx. 2 mg), sealed in a crimped aluminum cell, was heated at a speed of 10 °C/min from 50 to 300 °C in an atmosphere of nitrogen. The data were recorded and processed by Universal Analysis 2000 software (TA, USA).

DPPH radical scavenging assay

DPPH radical scavenging assay was done according to a published method [7]. Briefly, 2 mL of DPPH solution (0.2 mmol/L, in ethanol) was incubated with different concentrations of the sample. The reaction mixture was shaken and incubated in the dark for 30 min, at room temperature. And the absorbance was read at 517 nm against ethanol. Controls containing ethanol instead of the antioxidant solution, and blanks containing ethanol instead of DPPH solution were also made.

Statistical analysis

Statistical comparisons were made with Student's t-test. $P < 0.05$ was considered statistically significant.

RESULTS

UV and IR spectra

The UV spectra of gallic acid, lecithin, their physical mixture and the complex are shown in Figure 1. There was no difference between the physical mixture and the complex. The characteristic absorption peaks of gallic acid were still present at 228 and 263 nm.

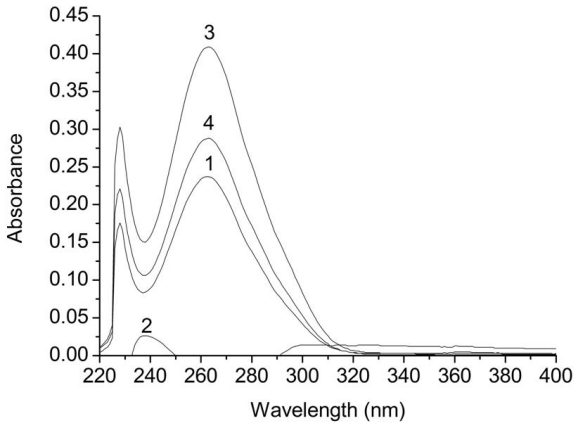

Figure 1: UV spectra of gallic acid (1), lecithin (2), physical mixture of gallic acid and lecithin (3) and their complex (4)

The infrared spectra of gallic acid, lecithin, their physical mixture and the complex are shown in Figure 2. There was no significant difference between the physical mixture and the complex. The spectra of the physical mixture and the complex showed an additive effect of gallic acid and lecithin, in which the characteristic

absorption peaks of gallic acid and lecithin could be still found.

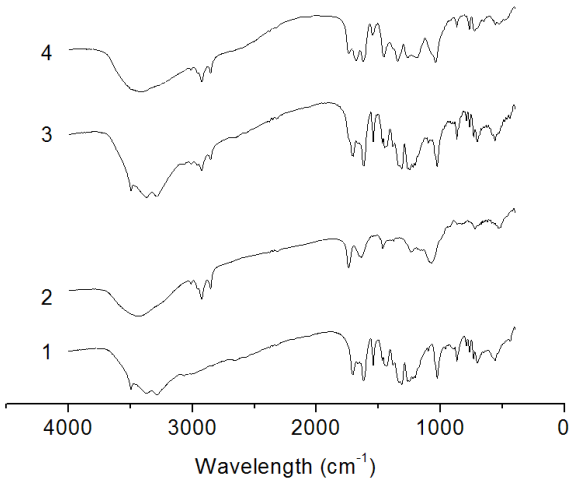

Figure 2: IR spectra of gallic acid (1), lecithin (2), physical mixture of gallic acid and lecithin (3) and their complex (4)

Particle morphology

The surface morphology of the complex as examined by SEM was shown in Figure 3. It could be found that gallic acid did not exit on the appearance of crystal but dispersed in lecithin.

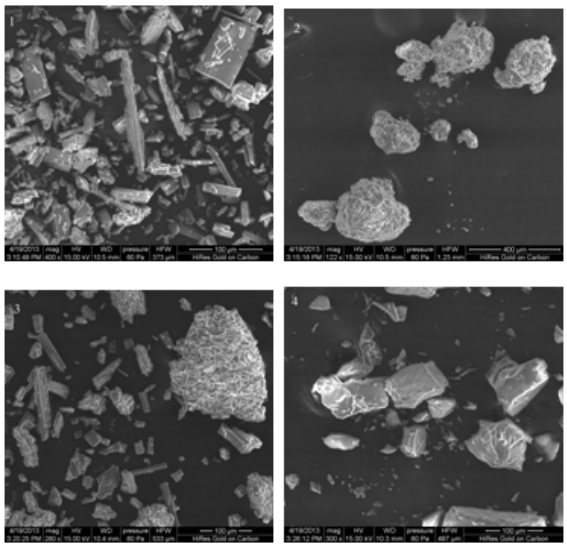

Figure 3: Scanning electron micrographs of gallic acid (1), lecithin (2), physical mixture of gallic acid and lecithin (3) and their complex (4)

Thermal analysis

Figure 4 shows the thermograms of gallic acid, lecithin, their physical mixture and the complex. The thermogram of gallic acid showed an endothermal peak with onset temperature at 258 °C, which was attributed to the melting of gallic acid while the thermogram of the physical mixture mainly shows the individual endotherms of gallic acid and lecithin. However, the thermogram of the complex mainly showed the endotherm for lecithin, while the characteristic endothermal peak for gallic acid was absent.

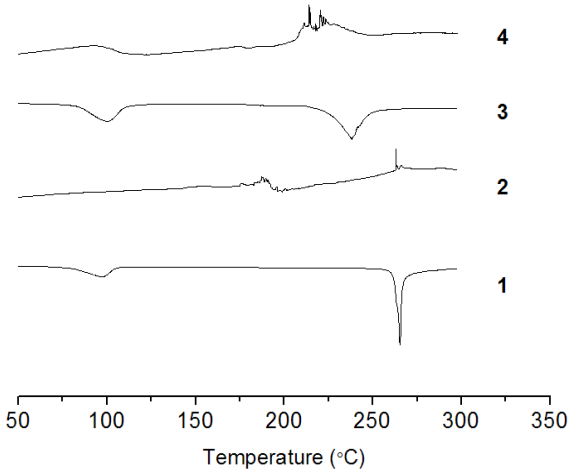

Figure 4: DSC thermograms of gallic acid (1), lecithin (2), physical mixture of gallic acid and lecithin (3) and their complex (4)

X-ray diffractograms

The powder x-ray diffraction patterns of gallic acid, lecithin, their physical mixture and the complex are shown in Figure 5. The powder diffraction pattern of gallic acid displayed sharp crystalline peaks, which is the characteristic of an organ molecule with crystallinity [8]. In contrast, lecithin showed amorphous lacking crystalline peaks. Compared with that of the physical mixture, the crystalline peaks had disappeared in the complex.

DPPH radical scavenging activity

PPH radical is a stable organic free radical with adsorption band at 517 nm. It loses this adsorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow [9]. In the present study, the high DPPH radical scavenging activity of the complex was observed in a concentration-dependent manner (Figure 6). At all the concentrations tested, the DPPH radical scavenging activity of the complex was higher than that of BHT, the reference antioxidant.

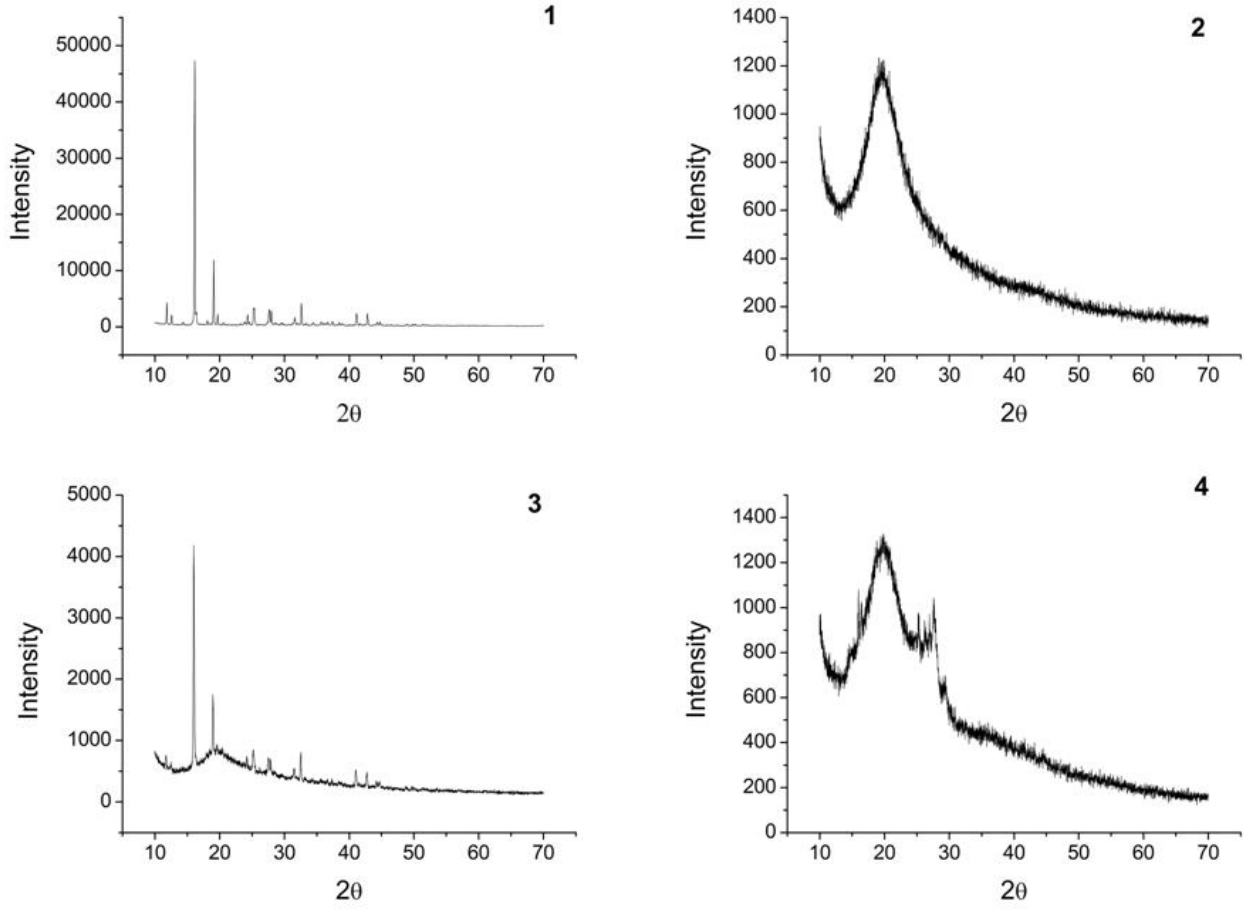

Figure 5: X-ray diffraction patterns of gallic acid (1), lecithin (2), physical mixture of gallic acid and lecithin (3) and their complex (4)

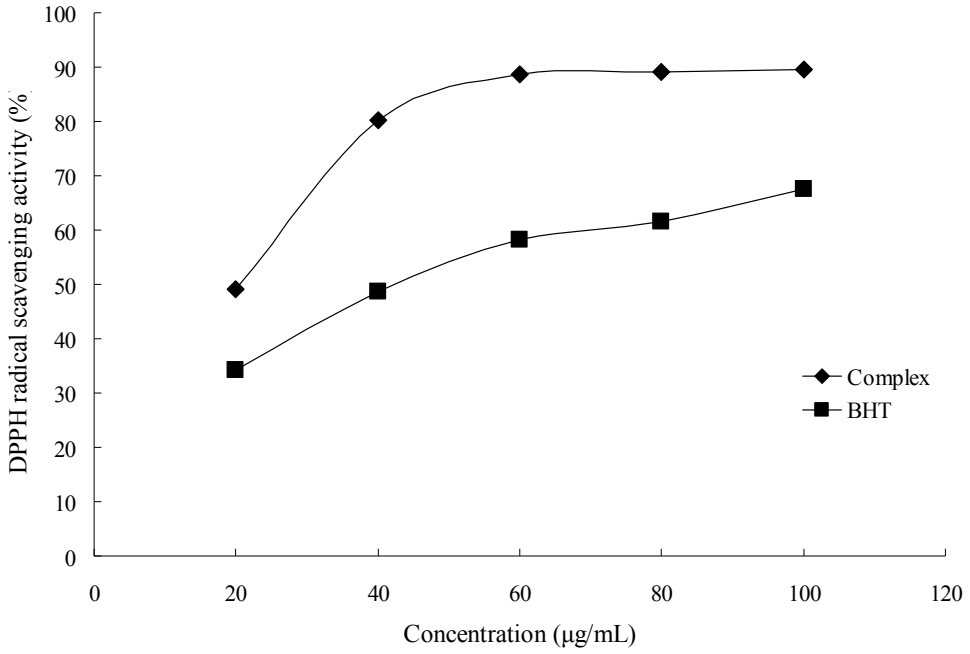

Figure 6: DPPH radical scavenging activity of gallic acid-lecithin complex and BHT

DISCUSSION

In this study, gallic acid-lecithin complex was prepared to improve the lipophilic property of gallic acid in varying ratios of lecithin and gallic acid. At a ratio < 2:1, the stability of the complex was decreased. For optimum complex quality using the smallest quantity of gallic acid, the gallic acid-lecithin complex was formed at a ratio of 2:1. And the obtained complex could be easily solved in oil, which suggested that the lipophilic property of gallic acid could be significantly improved by complexing with lecithin.

UV and IR spectroscopies are important tools for studying complexation. In UV and IR analysis, no new peaks were observed in the mixture and complex, which suggests that gallic acid and lecithin in the complex were combined by non-covalent bond, and some weak physical interactions between gallic acid and lecithin can take place during the formation of the complex.

SEM analysis demonstrated that when the powders of gallic acid and lecithin were simply mixed together, they formed no close association and continued to exist in their original individual forms, whereas their complex formed a close association, in which gallic acid no longer exist in the crystal state.

The characteristic peaks of gallic acid and lecithin coulwere present in the DSC thermograms of the physical mixture, indicating that there was no close association between the two molecules when the two powders are simply mixed together. In contrast, the DSC curve of the complex exhibited mainly the features of the lecithin thermogram while the characteristic endothermic peaks of gallic acid disappeared entirely. It seems that gallic acid had become completely dispersed in lecithin by some weak interactions. A similar observation of guest molecule losing its characteristic DSC peaks has also previously been reported for the complexation of dihydromyricetin with lecithin [4].

Powder x-ray diffractometry has been shown to be a method which can provide insight into the complexation between host and guest molecules. The formation of the complex between lecithin and a crystalline guest means that the latter has lost its crystalline nature and, consequently, the diffraction pattern of the complex would not be a simple superposition of those of the two components [10]. In this study, the XRD pattern of the physical mixture of the powder of gallic acid and lecithin showed essentially a superposition of the patterns of the two compounds, confirming that no complex was formed between them and that both retained their original physical characteristics. In contrast, the XRD pattern of the complex was virtually the same as that of the amorphous lecithin and exhibited none of the characteristic peaks of gallic acid, which suggests that gallic acid in the lecithin matrix was either molecularly dispersed or has become amorphous.

CONCLUSION

By forming complex with lecithin, the lipophilic solubility of gallic acid is significantly enhanced due to the transformation of gallic acid to an amorphous form. Physicochemical analysis shows that the complex was combined by non-covalent-bond, and thus a new compound was not formed. Gallic acid in the complex appears to be molecularly dispersed in the lecithin matrix. The complex formed is an effective scavenger of DPPH radical, which attributable to the presence of gallic acid.

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