

Characterization of Nano Bamboo Charcoal Drug Delivery System for *Eucommia ulmoides* Extract and Its Anticancer Effect *In vitro*

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ABSTRACT

Background: Nano bamboo charcoal is being widely used as sustained release carrier for chemicals for its high specific surface area, sound biocompatibility, and nontoxicity; however, there have been no reports on nano bamboo charcoal as sustained release carrier for traditional Chinese medicine (TCM). **Objective:** To study the effect of nano bamboo charcoal in adsorbing and sustained releasing *Eucommia ulmoides* extract (EUE) and to verify the *in vitro* anticancer effect of the sustained release liquid, so as to provide a theoretical basis for the development and utilization of nano bamboo charcoal as TCM sustained-release preparation.

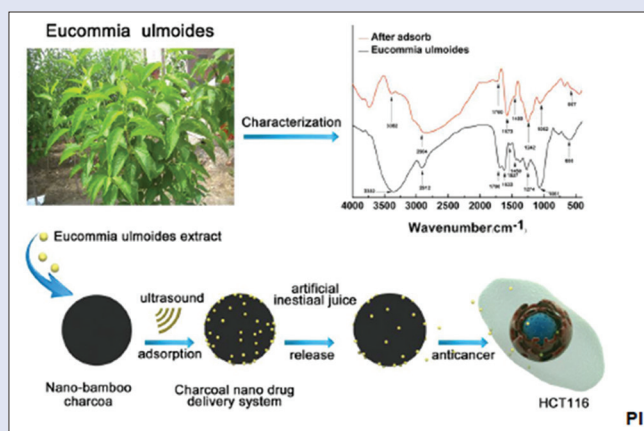
Materials and Methods: The adsorption capacity for the nano bamboo charcoal on EUE was measured by Langmuir model, and the release experiment was carried out under intestinal fluid condition. Characteristic changes for the nano bamboo charcoal nano-drug delivery system with and without adsorption of *E. ulmoides* were evaluated by scanning electron microscopy, Fourier transform infrared spectroscopy, thermogravimetric analysis, and specific surface area. In addition, the anticancer effect from this novel bamboo charcoal *E. ulmoides* delivery system was evaluated against a human colon cancer cell line (HCT116). **Results:** It was found that nano bamboo charcoal exhibits good adsorption capacity (up to 462.96 mg/g at 37°C). The cumulative release rate for EUE from this nano bamboo charcoal delivery system was 70.67%, and specific surface area for the nano bamboo charcoal decreased from 820.32 m²/g to 443.80 m²/g after EUE was loaded. An *in vitro* anticancer study showed that the inhibition rate for *E. ulmoides* against HCT116 cancer cells was 23.07%, for this novel bamboo charcoal nano-drug delivery system. **Conclusion:** This study provides a novel strategy for the delivery of traditional Chinese medicine using bamboo charcoal nano-drug delivery system.

Key words: Adsorption, anticancer effect, *Eucommia ulmoides* extract, nano bamboo charcoal, sustained release

SUMMARY

- The adsorption equilibrium was reached after 30 min of ultrasonic treatment
- The saturated adsorption capacity of *Eucommia ulmoides* extract by nano bamboo charcoal under ultrasonic condition was 462.96 mg/g

- The cumulative release rate of *E. ulmoides* extract from the nano bamboo charcoal delivery system in artificial intestinal juice was 70.67%
- The inhibition ratio of HCT116 cancer cells by sustained release liquid was 23.07%.



Abbreviation used: EUE: *Eucommia ulmoides* extract.

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INTRODUCTION

Eucommia ulmoides Oliv. (From *Eucommiaceae* family) is the sole species of the genus *Eucommia* that has been used as a rare tonic Chinese herbal medicine for nearly 2000 years.^[1] Multiple pharmacological activities of *E. ulmoides* have been reported including lowering of blood pressure and improvement of immunity, as well as anti-inflammatory, antiviral, anticancer, hepatic protection, and diuretic effects. In addition, antioxidation and antiaging effects of *E. ulmoides* have also been observed.^[2-5] *E. ulmoides* has also been processed into *Eucommia* tea and *Eucommia* vinegar beverages.

Nano bamboo charcoal is widely used as a multifunctional material because of its high mechanical strength, well-developed pore structure, and excellent adsorptive property.^[6] The nano bamboo charcoal has

been recently developed and used as a sustained release carrier in the pharmaceutical area.^[7,8] The nano bamboo charcoal has a large specific surface area and high adsorptive capacity when compared with other drug delivery carriers. It also has the capacity to adsorb a wide variety of

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drugs without affecting their molecular properties; hence, it is regarded as an ideal drug delivery carrier.^[9-11] There are currently some concerns that large amount of drugs may be unexpectedly released from drug delivery systems in a very short period of time, which may rapidly increase the *in vivo* blood concentration as a result, possibly leading to adverse reactions. There is, therefore, an urgent need for developing the drug carrier system with sustained-releasing effect.

In the current study, *E. ulmoides* extract (EUE) was loaded into the nano bamboo charcoal which was used as the drug delivery carrier. The characteristics for the prepared nano bamboo charcoal delivery system were also evaluated in this study, including adsorption and release of *Eucommia* extract from the delivery system. The adsorption capacity for the nano bamboo charcoal to adsorb EUE was characterized by a combination of methods including scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy, thermogravimetric (TG) analysis, and specific surface area (Brunauer-Emmett-Teller [BET]). We also investigated the *in vitro*^[12,13] anticancer effect of nano bamboo charcoal as the sustained release carrier for EUE.

MATERIALS AND METHODS

Materials

Nano bamboo charcoal (with a mean diameter of 100 nm) was purchased from Shanghai Hairuo Charcoal Industry Co. Ltd. (Shanghai, China). EUE was purchased from Hunan Yuanhang Biological Technology Co. Ltd. (Hunan, China).

Reagents

Fetal bovine serum and 0.25% trypsin were purchased from GIBCO (California, USA). Mylicin double antibody was purchased from CHINO Pharmaceutical Co. Ltd. (Shijiazhuang, China). Modified RPMI-1640 culture medium was obtained from Hyclone (Los Angeles, America). Phosphate-buffered saline was purchased from Hyclone. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide were purchased from Sigma (USA) and American Ameresco, respectively. HCT116 cell line was generously donated by Prof. Ying-Liang from National Engineering Laboratory for Further Processing of Grains of Central South University of Forestry and Technology. All other chemicals and solvents were of analytical or chromatographic grade.

Characterization of nano bamboo charcoal drug delivery system for adsorption and release

Wavelength selection

EUE solutions were prepared using distilled water (pH 7.4) at 0.05 mg/L and 0.1 mg/L concentrations, followed then by their scanning at 200–600 nm wavelength range, with distilled water being used as a blank. Results showed that EUE exhibited a maximum adsorption at the 280 nm and 326 nm wavelengths. Three hundred and twenty-six nanometers was chosen as detection wavelength because of the observed stable adsorption.

Standard curve for *Eucommia ulmoides* extract

EUE was dissolved in distilled water at the following standard concentrations 40, 50, 60, 80, 100, and 120 mg/L. The ultraviolet (UV) absorbance for standard solutions with different concentrations was determined at 326 nm wavelength using the distilled water as a blank. Linear regression for the concentration (C) against the absorbance (D) was plotted.

Determination of adsorption equilibrium time

A volume of 600 mg/L EUE solution was added into 0.1 g nano bamboo charcoal, and the suspension was then ultrasonically oscillated for 6,

12, 18, 24, 30, and 36 min. The supernatant absorbance at appropriate dilution time was then measured at 326 nm. The concentration of EUE in the supernatant was then calculated using the regression equation for the standard curve. The adsorption capacity (X) for the nano bamboo charcoal was determined as follows:

$$X = \frac{(C_0 - C)}{m} \nu \quad \text{Eq. (1)}$$

where C_0 represented the initial concentration of EUE; C represented the supernatant concentration for EUE after centrifugation; m represented the amount of added nano bamboo charcoal; and ν represented the solvent volume.

Determination of adsorption capacity of nano bamboo charcoal on *Eucommia ulmoides* extract

However, assessing the adsorption capacity of nano bamboo charcoal loaded with EUE is critical. Prepared solutions of EUE at 500, 550, 600, 650, 700, and 800 mg/L concentrations were added into 1% (m/v) nano bamboo charcoal. The adsorption equilibrium was completed after ultrasonic oscillation for 30 min. The suspensions were then centrifuged at 7800 g for 5 min, and the UV adsorbance of EUE in the diluted supernatant was measured at 326 nm. The Langmuir adsorption calibration curve for C/X versus C was then plotted as follows;

$$C/X = 1/K \times X_m + C/X_m \quad \text{Eq. (2)}$$

where C represented free EUE concentration after equilibrium; X represented the adsorptive capacity for the nano bamboo charcoal on EUE; K represented the Langmuir equilibrium parameter; and X_m represented the maximum adsorption capacity.

Evaluation of retention release for prepared nano bamboo charcoal drug delivery system

The release of EUE was carried out as follows: 500 mg/g (weight of EUE [mg/L]/weight of nano bamboo charcoal [g]) nano bamboo charcoal formulation was prepared as previously described. Briefly, 500 mg of EUE was added into 1 g of nano bamboo charcoal, and the mixture was then ultrasonically oscillated to reach adsorption equilibrium. A dried solid dispersion was obtained after removal of the supernatant, and 0.1 g of the above solid dispersion was then dispensed by stirring in 200 mL of simulated intestinal fluid at 37°C. Five milliliters of the suspension was then drawn and filtered through 0.45 μ m membrane filter. A total volume of 200 mL was kept constant by adding 5 mL of blank simulated intestinal fluid after each sampling. EUE in the released solution was determined by UV-visible. The percent release capacity Q (%) was calculated as follows;

$$Q(\%) = \frac{\left(C_n \times V_0 + V_i \sum_{i=1}^{n-1} C_i \right) M}{m} \quad \text{Eq. (3)}$$

where Q (%) is the cumulative release rate of EUE, C_n (mmol/L) is the concentration of EUE after the n-th sampling, V_0 (mL) is the total volume of EUE solution, V_i (mL) is the sampling volume, C_i (mmol/L) is the concentration of EUE at the i-th sampling, whereas m(g) means the mass of solid dispersion. The cumulative release rate was then calculated, and the drug release curve drawn using Higuchi regression.

Physical and structural characterization of nano bamboo charcoal drug delivery system

Morphological changes in the nano bamboo charcoal before and after adsorption were observed using a SEM (FEI Quanta 450, USA). Images were taken without prior treatment to ensure the acquisition of accurate images. The surface area and the pore size distribution were calculated according to BET and Barrett-Joyner-Halenda method (Kurt SA3100, USA). The

weight loss of the nano bamboo charcoal before and after adsorption was determined by Pyris 1 TG/derivative thermogram (TG/DTG) analyzer using N_2 as protective gas, 40 mL/min flow rate, and 10°C/min temperature rising speed. The initial and terminal temperatures were set at 25°C and 800°C, respectively, and the sample weight was about 8 mg. The functional groups of the nano bamboo charcoal before and after adsorption were investigated with a FT-IR spectroscopy (IRAffinity-1, Shimadzu Corp., Japan).

Cytotoxicity effect of nano bamboo charcoal drug delivery system on colon cancer cell line HCT116

The cytotoxicity of the released solutions from the prepared nano bamboo charcoal drug delivery system at different time points against the colon cancer cell line HCT116 was evaluated using MTT assay.

RESULTS AND DISCUSSION

Nano bamboo charcoal adsorption performance

A standard curve for EUE was made as depicted in Figure 1a. A good correlation ($D = 0.00718C - 0.05207$; $R^2 = 0.9997$, $P < 0.0001$) between the measured UV adsorption at 326 nm (D_{326}) and EUE concentration (C) was observed from 40 to 120 mg/L range. Next adsorption equilibrium time was explored. From Figure 1b, it can be observed that the adsorption capacity X_m for EUE increases with increasing ultrasonic time of EUE. This can be attributed to the accelerated diffusion of EUE molecules onto nano bamboo charcoal by the increase ultrasonic time of EUE. Respectively, the adsorbed amounts of EUE were (326.47 ± 8.62) mg/g, (369.82 ± 7.21) mg/g, (412.83 ± 6.24) mg/g, (441.61 ± 9.86) mg/g, (454.63 ± 10.11) mg/g, and (454.88 ± 10.86) mg/g at 6, 12, 18, 24, 30, and 36 min. The maximum adsorption capacity of EUE is 454.88 mg/g around 36 min, which indicated that the adsorption equilibrium was reached after 30 min of ultrasonic treatment.

The parameters of nano bamboo charcoal were extracted from the effect of initial EUE concentration on adsorption capacity [Figure 1c]. The data were obtained by fitting Langmuir. The Langmuir model assumes that the binding sites are homogeneously distributed over the adsorbent surface with monolayer coverage and uniform energies.^[14] A correlation ($Y = 0.00216X + 0.0216$; $R^2 = 0.9994$, $P < 0.0001$) between the free EUE concentration after equilibrium (C) and adsorptive capacity of nano bamboo charcoal. According to the Correlation coefficient (R^2), it can be concluded that the Langmuir model fits the equilibrium data. Indicating that adsorption is highly favorable for nano bamboo charcoal. According to Langmuir model, X_m was determined as 462.96 mg/g, indicating that 1 g of nano bamboo charcoal was able to adsorb 462.96 mg of EUE after reaching the adsorption equilibrium. In other words, the maximum mass ratio between the adsorbed EUE and nano bamboo charcoal was 1:2. These results indicate that the high adsorptive capacity for the nano bamboo charcoal as the drug carrier.

Nano bamboo charcoal release performance

Figure 1d shows the cumulative release rate for nano bamboo charcoal drug delivery system. From this Figure 1d, we can see that nearly 71% of EUE adsorbed by nano bamboo charcoal is released. A fast release of EUE can be observed within the initial 60 min, followed by a slow increase of concentration before reaching the plateau at 3 h. The drug releasing rate is relatively higher during the initial releasing period (<1 h), which indicates the occurrence of initial burst release. Furthermore, EUE near the entrance and on the surface of the nano bamboo charcoal diffuse into dissolution medium easily. In addition, EUE was loaded by physical adsorption which is a reversible process. Release will occur more easily in the dissolution medium. Moreover, the release *in vitro* of nano bamboo charcoal drug delivery system complied with Higuchi model. It shows that the release of EUE from artificial intestinal juice is controlled by a diffusion mechanism.

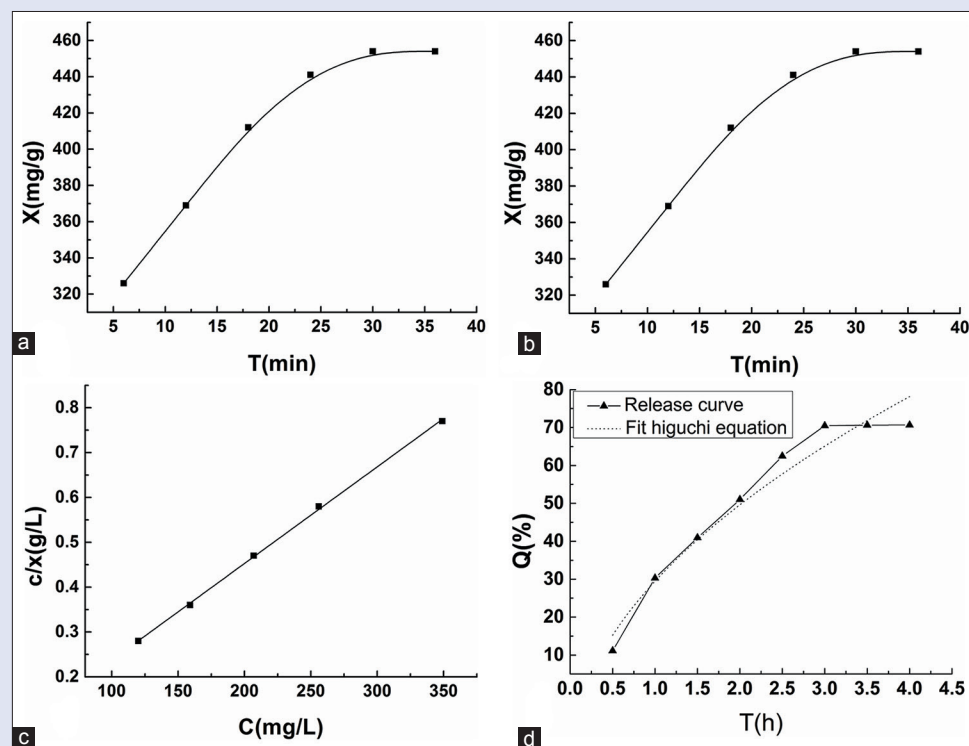


Figure 1: (a and b) Adsorption of *Eucommia ulmoides* extract (X [mg/g]) by nano bamboo charcoal at different ultrasonic time intervals (T [min]); (c) Adsorption isotherm curve for nano bamboo charcoal drug delivery system. The isotherm data fit the Langmuir and model ($Y = 0.00216X + 0.0216$, $r = 0.9996$, $P < 0.0001$); (d) Cumulative release rate of nano bamboo charcoal drug delivery system. Release *in vitro* of nano bamboo charcoal drug delivery system accorded with Higuchi model

Surface morphology of nano bamboo charcoal drug delivery system

The surface morphology of the nano bamboo charcoal before and after adsorption was observed using the SEM as shown in Figure 2. It can be seen that the surface of the nano bamboo charcoal was smooth and square [Figure 2a], which can realize the homogeneity of the drug distribution. Hence, it was extremely helpful for calculating accurately the drug loading and drug release. However, for the nano bamboo charcoal after adsorption [Figure 2b], the surface of it was covered by EUE. Moreover, a much lighter surface of the nano bamboo charcoal was observed after the adsorption, which may have been due to poor conductivity caused by the attachment of chlorogenic acid, geniposide, ginsipodic acid, and other low-conductivity components from EUE. These findings suggested that EUE was successfully loaded onto the nano bamboo charcoal.

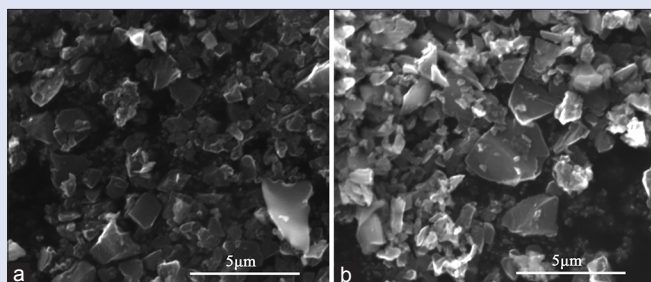


Figure 2: Scanning electron microscopy image of nano bamboo charcoal before (a) and after (b) adsorption

Chemical and structural properties of nano bamboo charcoal drug delivery system

Brunauer–Emmett–Teller and pore size distribution analysis

According to the IUPAC classification, as seen in Figure 3, the N_2 adsorption isotherms of the nano bamboo charcoal before [Figure 3a] and after [Figure 3b] adsorptions indicated a Type I isotherm. The detailed data about the nano bamboo charcoal before and after adsorptions resulting from the N_2 adsorption are summarized in Table 1. It can be seen that the nano bamboo charcoal had large BET surface area ($820.32 \text{ m}^2/\text{g}$) and pore volume (0.4833 mL/g) which was sufficiently large to load EUE molecules. With these extraordinary advantages, nano bamboo charcoal as a drug carrier can disperse EUE particles effectively and also prevent agglomeration. Furthermore, the BET surface area and pore volume were sharply reduced after nano bamboo charcoal had been loaded. The total specific surface area decreased to $443.80 \text{ m}^2/\text{g}$, and total pore volume also decreased to 0.2885 mL/g , suggesting that EUE was adsorbed onto the surface or inner structure of the charcoal and resulting in the decrease of both total surface area and total pore volume.

Thermogravimetric analysis

The TG/DTG result of the nano bamboo charcoal before [Figure 4a] and after [Figure 4b] adsorption was shown in Figure 4. A three-phase thermal decomposition was observed before adsorption, according to the TG/DTG diagram. The weight loss of the first stage (40°C – 100°C) is primarily due to water evaporation. The second stage involved 100°C – 400°C temperature range, with flat thermal decomposition curve indicating that there was no thermal decomposition and weight

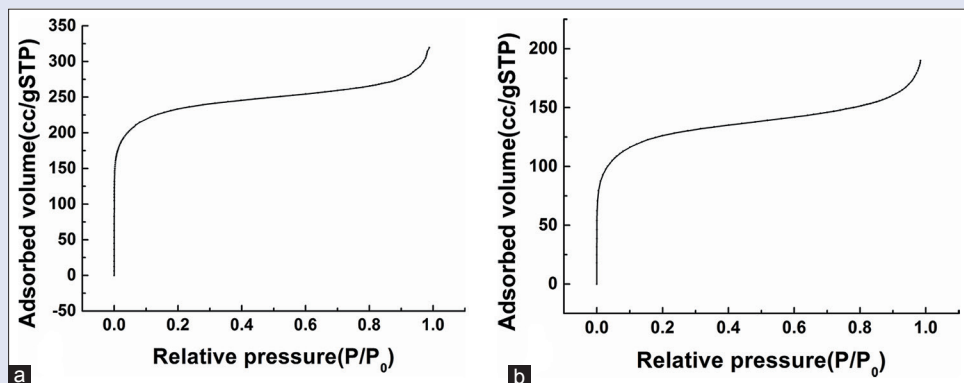


Figure 3: N_2 adsorption curve of nano bamboo charcoal before (a) and after (b) adsorption

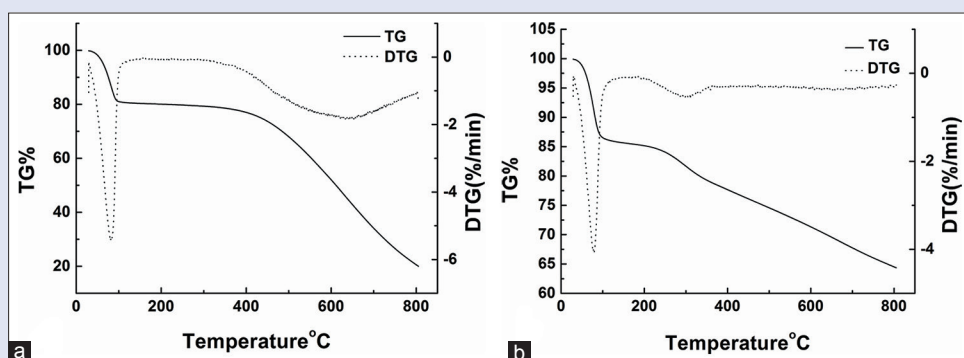


Figure 4: Derivative thermogram-thermogravimetric curves for nano bamboo charcoal before (a) and after (b) *Eucommia ulmoides* extract adsorption

loss for the nano bamboo charcoal in this phase. The weight of the nano bamboo charcoal fell from 77% to 20% in the third stage, from 400°C to 800°C.

Four phases of thermal decomposition for the nano bamboo charcoal were found after loading of EUE. Lower weight loss was observed in the first phase (from 40°C to 100°C) since the loaded EUE may also have broken down, leading to the slow decomposition rate as demonstrated by the DTG curve. The second phase was from 100°C to 200°C temperature range, in which no weight loss was observed. Significant thermal decomposition was observed at 200°C–380°C range in the third phase at which there was no decomposition for the nano bamboo charcoal before adsorption, and therefore, such unique decomposition was due to the adsorption of *E. ulmoides* components. Chlorogenic acid has been proven, by HPLC analysis, to be a major component of EUE whose thermal decomposition characteristics were reported to be in accordance with our present findings. The fourth stage was from 380°C to 800°C temperature range, and the gradual decomposition manner was consistent with those for the nano bamboo charcoal and chlorogenic acid. In summary, these observations further supported the findings that EUE was adsorbed by the nano bamboo charcoal.

Infrared analysis

The FT-IR spectrum, as shown in Figure 5, was collected to investigate the chemical bonds of the nano bamboo charcoal drug delivery system. Infrared analyses of nano bamboo charcoal before and after adsorption [Figure 5a] and EUE [Figure 5b]. The broad absorption band at 3600–3000 cm^{-1} with a maximum at about 3382 cm^{-1} is characteristic of the stretching vibration of hydrogen-bonded hydroxyl groups from carboxyls, phenols, or alcohols, and water adsorbed in the nano bamboo charcoal. The weak absorption peaks at 2904 cm^{-1} and 2837 cm^{-1} were characteristic for $-\text{CH}_3$. Peaks at 1573 cm^{-1} and 1242 cm^{-1} were specific carboxylic acid absorption peaks, whereas the peaks at 1066 cm^{-1} may have been caused by C-O stretching vibration of ether.^[15,16] The new absorption peaks at 3382 cm^{-1} and 667 cm^{-1} were observed during loading of EUE. Moreover, the peak absorptions at 1573 cm^{-1} , 1240 cm^{-1} , and 1066 cm^{-1} were enhanced after loading of EUE. In addition, the inverted peaks at 1450 cm^{-1} and 1108 cm^{-1} appeared in the prepared formulation. When compared with the IR spectra from the nano bamboo charcoal before the adsorption, the prepared formulation showed peaks at 1633 cm^{-1} , 1527 cm^{-1} , 1274 cm^{-1} , and 688 cm^{-1} , which are characteristic IR adsorption peaks for benzene derivatives in *Eucommia* extract, indicating that EUE was successfully loaded.

Cytotoxic effect of nano bamboo charcoal drug delivery system on colon cancer cell line HCT116

The inhibitory effect of the nano bamboo charcoal drug delivery system on the colon cancer cell line HCT116 is shown in Table 2. It was found that the inhibitory effect of the released EUE increased accordingly with increased release time. A maximum inhibitory effect of 23.07% on the HCT116 cancer cells was observed after 3 h release. These results showed that the prepared novel drug delivery system had an obvious inhibitory effect on the HCT116 cancer cells.

CONCLUSIONS

In summary, the current study successfully prepared a novel and sustained release nano bamboo charcoal drug delivery system for EUE. The adsorption experiment shows that nano bamboo charcoal exhibits an excellent adsorption capacity (up to 462.96 mg/g at 37°C). The nano bamboo charcoal drug delivery system can be well described by Langmuir and Higuchi model. Physical and chemical properties and structure analysis confirm that the specific surface area, surface morphology, and functional groups in nano bamboo charcoal drug delivery system, verifying their good adsorption and release behavior. Cell *in vitro* study demonstrated the significant anticancer effect of this novel drug delivery system. This is the first application of the nano bamboo charcoal drug delivery system to well-known traditional Chinese medicine (TCM) of *E. ulmoides*, which provides a new strategy

Table 1: Specific surface area and pore structure parameters of nano bamboo charcoal

	SBET (m^2/g)	Vtot (mL/g)
Nano bamboo charcoal before adsorption	820.32	0.4833
Nano bamboo charcoal after adsorption	443.80	0.2885

Table 2: Cytotoxic effect of nano bamboo charcoal drug delivery system on colon cancer cell line HCT116

Release time (h)	Adsorbance	Inhibition rate (%)
0.5	0.697±0.085	6.32
1.0	0.675±0.084	9.23
1.5	0.668±0.052	10.26
2.0	0.644±0.032	13.49
2.5	0.606±0.036	18.50
3.0	0.572±0.041	23.07

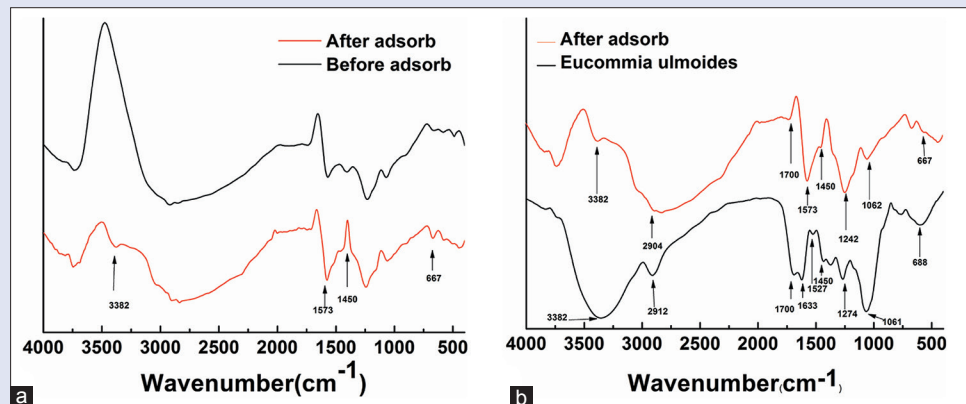


Figure 5: Infrared spectra from nano bamboo charcoal before (a) and after (b) adsorption of *Eucommia ulmoides* extract

of oral delivery of TCM. Therefore, nano bamboo charcoal would have enormous potential applications for drug delivery systems in the future. Nevertheless, future challenges remain, including improving specificity and stability and regulating bioavailability.

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Conflicts of interest

There are no conflicts of interest.

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