

# Research and Preliminary Evaluation on Inclusion Preparation of volatile oil in *Toona sinensis* fruit and $\beta$ -CD

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**Abstract.** The grinding-based inclusion preparation of volatile oil in *Toona sinensis* fruit and  $\beta$ -CD was optimized and the prepared inclusion compound was evaluated preliminary. **Methods:** The yield rate of inclusion compound and inclusion rate were used as evaluation indexes to investigate rate of charge, water addition and grinding time. The inclusion preparation of volatile oil  $\beta$ -CD was optimized through the orthogonal method. Inclusion compound was assessed by TLC method and microscope. **Results:** The optimized grinding technique is: 1:8 of mixing rate of volatile oil and  $\beta$ -CD, 4 times of water addition and 60min grinding. TLC method and microscope prove that composition of volatile oil in *Toona sinensis* fruit remains same before and after the inclusion. It is included by  $\beta$ -CD. **Conclusions:** The proposed grinding technique is simple in operation, reasonable and feasible. It can lay theoretical foundation for the development and application of volatile oil in *Toona sinensis* fruit.

## 1. Introduction

*Toona sinensis*, a kind of Meliaceae, is well known for its wide distribution, rich resources and edible. *Toona sinensis* fruit has many bioactivities, such as oxidation resistance, hyperglycemic, anticoagulation and cardio protection [1-2]. The volatile oil in *Toona sinensis* fruit is characteristic of low boiling point, easy volatilization, small solubility and easy oxidization [3-4]. Scholars attach high attentions to how to improve its stability, increase its solubility and reduce gastrointestinal stimulation. With unique molecular structure,  $\beta$ -CD can improve drug stability, reduce loss of volatile components, increase solubility of drugs and bioavailability, and decrease toxic and side effect as well as stimulation of drugs [5]. On this basis, it is necessary to make preliminary study and evaluation of inclusion process of volatile oil in *Toona sinensis* fruit and  $\beta$ -CD, aiming to provide references for further development and application of volatile oil of *Toona sinensis* fruit.

## 2. Materials

### 2.1. Drugs

*Toona sinensis* fruit (Jinan Shengke Technology Development Co., Ltd.),  $\beta$ -CD (Beijing Suolaibao Technology Co., Ltd), Rest reagents were analytical pure.



## 2.2. Equipments

Experimental equipments included electronic analytical balance (Mettler-Tolide Instrument Company), ZF-7A portable UV analyzer (Shanghai Jihui Scientific Analyzer Co., Ltd), optical microscope (Olympus Company) and UV-2000 ultraviolet and visible spectrophotometer (Unico (Shanghai) Instrument Co., Ltd).

## 3. Method

### 3.1. Test of volatile oil content

Volatile oil content was tested by A method in the volatile oil test method of Chinese Pharmacopoeia (Version I) (2015 version of appendix XD).

### 3.2. Extraction of volatile oil

Volatile oil was extracted from *Toona sinensis* fruit using petroleum ether (30 ~ 60 °C) according to Chinese Pharmacopoeia. The volatile oil was faint yellow oily liquid. It was dried by anhydrous sodium sulfate and stored in freezing conditions (4 °C).

### 3.3. Inclusion evaluation index [6]

Yield rate of inclusion compound and inclusion rate of volatile oil were used as evaluation indexes to optimize the grinding-based inclusion preparation of volatile oil in *Toona sinensis* fruit. The yield rate of inclusion compound = actual quantity of inclusion compound (g) / [dosage of  $\beta$ -CD (g) + dosage of volatile oil (mL)  $\times$  oil density]  $\times 100$  %. The inclusion rate of volatile oil = oil content in inclusion compound (mL) / [dosage of volatile oil (mL)]  $\times 100$  %.

### 3.4. Orthogonal optimization of inclusion preparation

**3.4.1. Grinding.** In this experiment, 8.00 g  $\beta$ -CD was weighted accurately in a mortar and 3 times of purified water was added. They were grinded by 10 min. Next, 1 mL volatile oil was dropped into the mortar and grinded by 1h. The solution was filtered and the filter cake was rinsed by water firstly and then rinsed by petroleum ether (10 mL per time) by several times. Later, it was dried by 40 °C, thus getting the  $\beta$ -CD inclusion compound.

**3.4.2. Orthogonal test design.** Based on preliminary experiment, dosages of volatile oil and  $\beta$ -CD, water addition and grinding time were chosen.  $L_9 (3^4)$  orthogonal design experiment was adopted to optimize technological conditions of grinding preparation (Table 1).

**Table 1.** The level of orthogonal factors

level	Factor		
	A oil: $\beta$ -CD (mL:g)	B $\beta$ -CD: water (g : mL)	C inclusion time (min)
1	1:6	1:3	20
2	1:8	1:4	40
3	1:10	1:5	60

### 3.5. Evaluation of inclusion compounds

**3.5.1. Thin layer chromatography (TLC).** In this study, four samples (1, 2, 3 and 4) were prepared by adding 2 mL absolute ethyl alcohol into 0.1g  $\beta$ -CD, adding 2 mL absolute ethyl alcohol into 0.1g inclusion compound, adding 2 mL absolute ethyl alcohol into 0.1 mL volatile oil, and adding 2 mL absolute ethyl alcohol into 0.1 mL volatile oil in inclusion compound.

Samples were dropped onto a thin layer silica gel plate. Petroleum ether-cyclohexane-ethyl acetate (8:1:1) was used as developing solvent and samples were developed in the saturate tank. Next, samples

were taken out and dried. Color developing conditions were 10% ethanol sulfate. Samples were observed under sunlight and ultraviolet (254 nm and 365 nm) before and after color developing.

**3.5.2. Microscope method.** Volatile oil (A),  $\beta$ -CD (B), physical compound of volatile oil and  $\beta$ -CD (C) and appropriate quantity of  $\beta$ -CD inclusion compound (D) were dropped on the glass slide, respectively. Water was added to disperse them and samples were observed under microscope.

## 4. Results

### 4.1. Orthogonal test results

Grinding-based inclusion preparation was studied according to orthogonal test design. Results are listed in Table 2 and Table 3. According to contributions of inclusion rate and yield rate to grinding process, their weight coefficients were 0.6 and 0.4, respectively. According to analysis results of variance, the inclusion preparation conditions were A2B2C3: 1:8 of volatile oil and  $\beta$ -CD, 4 times of water addition and 60min grinding time.

**Table 2.** Results of orthogonal design

No.	A Oil/ $\beta$ - CD	B $\beta$ - CD/water	C Inclusion time	D Envelopment rate (%)	E Production rate (%)	F Integration value (%)
1	1	1	1	58.98	73.53	64.80
2	1	2	2	67.84	73.67	70.17
3	1	3	3	73.35	73.42	73.38
4	2	1	2	80.30	82.67	81.25
5	2	2	3	88.09	82.62	85.90
6	2	3	1	78.53	77.05	77.94
7	3	1	3	66.51	85.68	74.18
8	3	2	1	54.90	82.23	65.83
9	3	3	2	52.40	79.13	63.09
k1	208.35	220.23	208.57	213.79		
k2	245.09	221.91	214.51	222.29		
k3	203.10	214.41	233.46	220.46		
R	14.00	2.50	8.30	2.83		

**Table 3.** Variance analysis of the envelopment rate

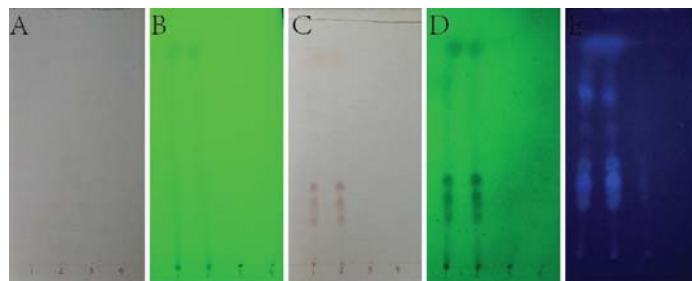
Source of variation	SS	V	MS	F	P
A	348.89	2	174.45	26.19	$p < 0.05$
B	10.32	2	5.16	0.77	
C	112.63	2	56.32	8.45	
D	13.32	2	6.66	1.00	
Error	13.32	2.00	6.66		
Total	485.17	8.00			

Note:  $F_{0.01}(1, 2) = 99$ ,  $F_{0.05}(1, 2) = 19$

### 4.2. Inclusion evaluation results

**4.2.1. TLC results.** It can be seen from Fig.1 that no evident spots have been observed on A and B, but there are obvious spots on C, D and E after color developing. Sample 1 and 2 have same color of spots at the same position of the thin layer plate, indicating that composition of volatile oil remains same

before and after the inclusion process. Samples 3 and 4 have no spots like those on sample 1 and 2, which reflects that volatile oil of *Toona sinensis* fruit has formed  $\beta$ -CD inclusion compound.

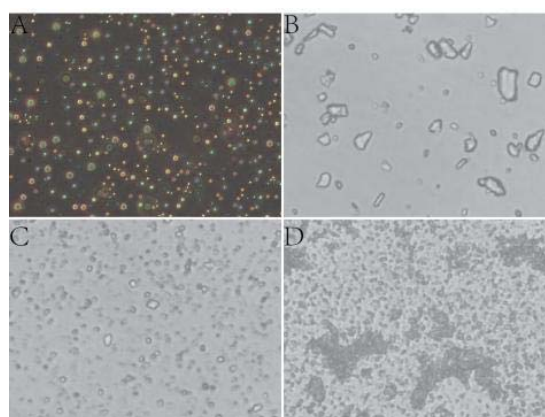


**Figure 1.** TLC verification of inclusion compound

1. Volatile oil 2. Volatile oil from the inclusion complex 3.  $\beta$ -CD 4. Inclusion complex

A. No coloration (Sunlight); B. No coloration (254 nm); C. Coloration (Sunlight); D. Coloration (254 nm); E. Coloration (365 nm)

**4.2.2. Microscope results.** In Fig.2, there are evident oily liquid drops in A, small bright irregular plate-shaped crystals in the middle of  $\beta$ -CD in B, small irregular plate-shaped crystal in C and oily liquid drops surrounding  $\beta$ -CD, as well as loosed inclusion compound in D, but no oily drops and small crystals. These reveal that volatile oil has been included in cyclodextrin cavities.



**Figure 2.** The results of microscope observation

A. Volatile oil B.  $\beta$ -CD C. Physical compound D. Inclusion complex

## 5. Conclusions

Based on inclusion rate and yield rate, the grinding conditions of inclusion compounds of volatile oil in *Toona sinensis* fruit are 1:8 of volatile oil and  $\beta$ -CD, 4 times of water addition and 60min grinding time. The inclusion compounds are proved by TLC method and microscope method. Results demonstrate that  $\beta$ -CD has satisfying inclusion effect to volatile oil of *Toona sinensis* fruit.

Inclusion compound powder from the inclusion and drying of volatile oil in *Toona sinensis* fruit by  $\beta$ -CD can prevent can prevent loss of volatile oil effectively during the production. It has important practical significance to increase stability of preparations containing volatile oil.

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