Identification and determination of (+)-sesamin in *Semen Cuscutae* by capillary GC and GC-MS

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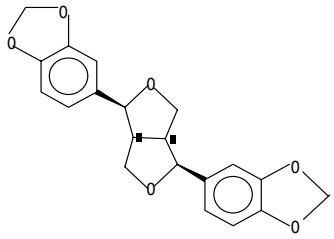
Abstract: (+)-Sesamin was found in *Semen Cuscutae* for the first time. A rapid and simple approach for the analysis of (+)–sesamin in different sources of *Semen Cuscutae* is proposed, which used GC-FID for the determination of (+)-sesamin and GC-MS for its identification. The result suggested that this approach could be used to identify *Semen Cuscutae* from various sources based on the different content of (+)-sesamin in them.

Keywords: Gas chromatography, gas chromatography-mass spectrometry, (+)–sesamin, *Semen Cuscutae*.

(+)-Sesamin has been found to have pharmacological effect which can improve activity, prevent aging and has the effect of antihypertension [1-2]. **Figure 1** shows its chemical structure. (+)-Sesamin has been known to be abundant in sesame originally, but our previous study shows that (+)-sesamin also presents in a Chinese herbal medicine *Semen Cuscutae*[3-4]. Gas chromatography (GC) and gas chromatography - mass spectrometry (GC-MS) have been applied for the determination and identification of (+)-sesamin in this plant. Furthermore, the result suggests that GC and GC-MS can be used to identify different species of *Semen Cuscutae*.

Figure 1 The chemical structure of (+)-sesamin



Experimental

A HP 6890 gas chromatography system (Hewlett-Packard, USA) with a flame ionization detector (FID) was used for the separation and determination of (+)-sesamin. Separation was performed on a HP-5 30m×0.25mm i.d.×0.25μm fused silica capillary column

(Hewlett-Packard, USA), with nitrogen as carrier gas at the flow velocity of 25cm/s. The temperature of the injector and FID were both set at 330℃. The column temperature was maintained at 300℃. GC-MS analysis were carried out using Finnigan MAT GCQ system (Finnigan, USA), with helium as the carrier gas at the rate of 35cm/s. Identification of (+)-sesamin was performed on a RTX-5MS 30m×0.25mm i.d.×0.25μm fused silica capillary column (Restek, USA). The ion source and transfer line temperature were maintained at 200℃ and 275℃, respectively. The mass spectrum was obtained by electron impact at 70eV. The other conditions were the same as in GC.

Six real samples of *Semen Cuscutae* as listed in **Table 1**, were collected and identified by one of the authors. Authentic samples are preserved in the herbarium in BUCM. Standard (+)-sesamin was extracted and purified from *Semen Cuscutae* by TLC and semi-preparative HPLC, the structure of which was elucidated by chemical and spectral methods such as MS, ^{1}H-NMR, ^{13}C-NMR and polarimetric analysis. (+)–

Sesamin obtained was a colorless crystal; $[\alpha]_{D589nm}^{25}$ =+63; EI–MS (*m/z*): 354, 204, 203, 161, 149 (100), 135, 122; ^{1}H–NMR (δ, $CDCl_3$): 6.829 (2H, S), 6.789 (2H, d, J=8.4 Hz), 6.755 (2H, d, J=8.4 Hz), 5.927 (4H, S), 4.692 (2H, d, J=4.5 Hz), 4.212 (2H, dd, J=6.7 Hz, 9 Hz), 3.845 (2H, dd, J=4 Hz, 9 Hz), 3.028 (2H, m). Spectroscopic data were in full agreement with the authentic data [5].

Table 1. The information for samples

Sample No.	Sample name	Source	Host	Sampling date	Sampling site
1	*Cuscuta chinensis* Lam.	stem	Hui cai	September, 97	Beijing
2	*Cuscuta australis* R. Br.	stem	Niu qi	September, 97	Beijing (cultivated)
3	*Cuscuta japonica* Choisy	stem	unknown	August, 98	Sichuan
4	*Cuscuta chinensis* Lam.	seed	--	September, 97	Hebei
5	*Cuscuta australis* R. Br.	seed	--	April, 99	Beijing
6	*Cuscuta japonica* Choisy	seed	--	June, 98	Sichuan

*-- represent with no host.

About 0.5 g dried stem or ripe seed of plant material was soaked with 5 ml chloroform over night and extracted by ultrasonic treatment. The extraction was repeated once with 4 ml chloroform, then the extracts were combined, filtered through a 0.45 μm membrane filter and diluted to 10.0 ml with chloroform. Standard (+)-sesamin was dissolved in chloroform and then diluted to give different concentrations for constructing the calibration curve. Each sample of the standard was analyzed for five times. And given amount of (+)-sesamin were added respectively to the sample **1** and **2** for recovery test.

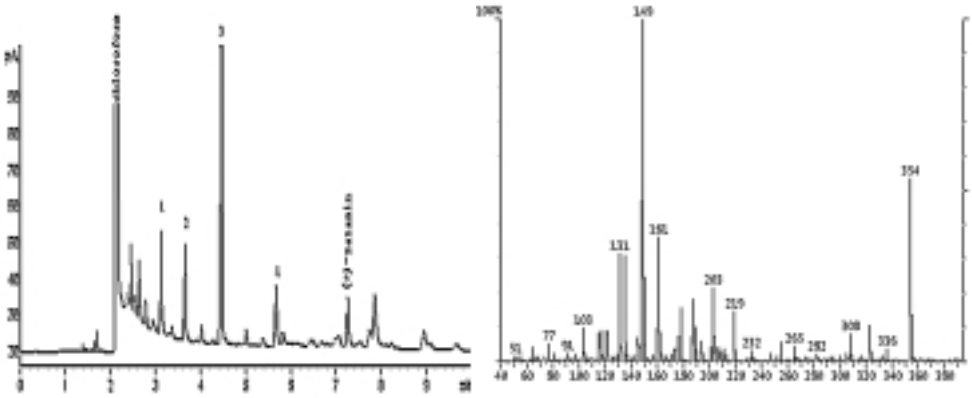
Result and Discussion

1. Identification of (+)-sesamin in Semen Cuscutae

In this study, GC capillary column was maintained at a constant high temperature 300℃. Our study shows that (+)-sesamin could be turned into gas and keep stable at this temperature. Furthermore, under this condition (+)-sesamin could be separated with interfering compounds and successfully determined within a short time (see **Figure 2**

(a)). (+)-Sesamin in *Semen Cuscutae* was identified by GC-MS based on comparison of the mass spectrum of the (+)-sesamin peak as shown in **Figure 2 (b)** with the standard mass spectrum. The match quality in the mass spectra of (+)-sesamin in real sample and the standard is as follows: Purity: 649, fit: 905, Rfit: 669. Sesamin was characterized by the presence of ions, *m/z*: 354=$[M]^{+\cdot}$, 204=$[M\text{-}ArCHO]^{+}$, 203=$[M\text{-}ArCHO\text{-}H]^{+}$, 161=$[ArCH{=}CHCH_2]^{+}$, 150=$[ArCHO]^{+}$, 149=$[ArCO]^{+}$, 135=$[ArCH_2]^{+}$, 122=$[Ar]^{+}$. The coincidence of retention time between the peak in real sample and that of the standard also verified the GC-MS result. Several unknown compounds (**1-4** in **Figure 2 (a)**) are identified to be odd number alkanes by GC-MS and retention indices in GC.

Figure 2 Chromatogram (a) and mass spectrum (b) of (+)-sesamin in real sample.



(a) (b)

Conditions see Experimental. t_R[(+)-sesamin]=7.259 min,
1=$C_{25}H_{52}$, 2=$C_{27}H_{56}$, 3=$C_{29}H_{60}$, 4=$C_{31}H_{64}$.

2. Determination of (+)-sesamin in Semen Cuscutae

The calibration curve was constructed in the concentration ranging from 0.05 to 1.1 mg/ml for (+)-sesamin. The linear regression equation and correlation coefficient were: y = 1008.6 x - 47.81 (r = 0.9992)**,** where y is the integrated peak area, x is the concentration (mg/ml). According to the regression equation, the amounts of (+)-sesamin in *Semen Cuscutae* can be determined in 7.5 min. The recovery was determined by the method of standard addition for (+)-sesamin in sample **1** and **2** (see **Table 2**).

3. Differentiation of Semen Cuscutae from various sources

Semen Cuscutae is originally specified as the dry ripe seed of *Cuscuta chinensis* Lam. in China Pharmacopoeia [6]. However, the result of an herb market investigation [7] shows that *Cuscuta australis* R. Br. is the main source of *Semen Cuscutae* and *Cuscuta japonica* Choisy. also acts as a source of this plant among the folks in some area. Because of the complexity of *Semen Cuscutae* in the herb market, an effective method for the quality

control of this plant is highly desirable. Although several methods have been applied for the differentiation of *Semen Cuscutae*, such as TLC, UV and IR [8], these methods have drawbacks of incomplete resolution and inconvenience. GC and GC-MS proposed give us a precise analysis with their simplicity, hence could play a very important role in the differentiation of Chinese herbal medicine. The results indicate that (+)-sesamin contents in the three species *Cuscuta chinensis* Lam., *Cuscuta australis* R. Br. and *Cuscuta japonica* Choisy, stems and ripe seeds, were dramatically different. For *Cuscuta chinensis* Lam., we found (+)-sesamin both in stem and seed, and the content of (+)-sesamin in the stem is the highest; for *Cuscuta australis* R. Br., (+)-sesamin was only found in the stem; while for *Cuscuta japonica* Choisy, there is no (+)-sesamin either in the stem or in the seed. We consider that *Semen Cuscutae,* especially the stem of *Cuscuta chinensis* Lam., could be a new plant resource of (+)-sesamin. GC could be an effective and rapid method in quality control of *Semen Cuscutae* by comparing the content of (+)-sesamin in different species.

Table 2 The content and recoveries of (+)-sesamin in *Semen Cuscutae* from different sources (n=5)

Sample No.	Concentration (mg/mL)	Content in percentage (%)	RSD (%)	Recovery (%)
1	0.10244	0.2049	2.08	97.10
2	0.08714	0.1743	2.99	96.06
3	ND	ND	ND	--
4	0.05784	0.1157	0.84	--
5	ND	ND	ND	--
6	ND	ND	ND	--

* ND = not detected; -- represent not tested.

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