Australian Journal of Crop Science
AJCS
AJCS 7(12):1809-1813 (2013) ISSN:1835-2707

Optimization of supercritical CO_2 extraction and characterization of antifungal activity of essential oils in *Cuminum cyminum* L.

Lin-Feng Hu[1,2], Jun He[1], Jun-Tao Feng[1*], Xing Zhang[1]

[1]Research and Development Center of Biorational Pesticide/Shaanxi Province Technology and Engineering Center of Biopesticide, Yangling, Shaanxi Province, China
[2]School of Chemistry and Chemical Engineering, Henan Institute of Science and Technology, Xinxiang, Henan, China

***Correspondence: fengjt67@sohu.com**

Abstract

Supercritical CO_2 extraction of essential oils from *Cuminum cyminum* seeds was optimized by the adjustment of pressure, particle size, CO_2 flux and extraction temperature. An orthogonal test of $L_{16}(4^5)$ was designed to obtain an optimized combination of these four factors. Result showed that extractions performed at 34.5 MPa and 30°C using a particle size 60-80 mesh and a CO_2 flux of 80 mL, the extraction ratio for *C. cyminum* seed essential oil was 21.59%, which was higher than the ratio for other common extraction methods. Furthermore, antifungal tests using 100 mg/mL of essential oil obtained using the optimized extraction method, showed that the oil can completely inhibit the mycelial growth of *Sclerotinia sclerotiorum*, and that the extraction ratio was positively correlated with the inhibitory effect of the oil against *S. sclerotiorum* (R^2 = 0.9777). The results of GC-MS analysis showed that the main component of the essential oil with the highest activity was 4-(1-methylethyl)-benzaldehyde (30.56%).

Introduction

Cuminum cyminum L. is commonly known as cumin. Cumin seeds have a strong aromatherapeutic scent and are commonly used as a cooking spice. In addition, cumin seed have been used to treat hypopepsia, stomach colds and abdominal pain (The CAS committee of the flora of China, 1985; China National Corporation of Traditional & Herbal Medicine, 1994). Previous research has shown that cumin seed essential oil has antifungal, insecticidal and antioxidant activities (Hu et al., 2005). A tracking antifungal method was applied to isolate antifungal ingredients from cumin seed extract (Hu et al., 2007; Hu et al., 2008). The results showed that the main ingredient of cumin seed oil, 4-(1-methylethyl)-benzaldehyde, was one of the main antifungal ingredients, and that cumin seed oil may also be used as a botanical pesticide for plant pathogen control (Hu et al., 2007; Hu et al., 2008). The aim of this study was to explore the relationship between extraction technique and antifungal activity. At present, the most common methods for the extraction of cumin seed essential oil include hydrodistillation (Jiang and Lou, 1993; Liu et al., 2002; Yan et al., 2002; Gachkar et al., 2007), super critical carbon dioxide (CO_2) extraction (Eikani et al., 1999; Liu, 2000; Heikes et al., 2001), microwave extraction (Liu et al., 2002; Wang et al., 2006), Likens-Nickerson extraction (Jalali-Heravi et al., 2007), Clevenger extraction (Jalali-Heravi et al., 2007) and superheated steam extraction (Eikani et al., 2007). Of these extraction methods, supercritical CO_2 extraction method is the most successful as it gives the highest extraction ratio and ensures that the chemical components of the essential oil are preserved (Liu, 2000). The supercritical CO_2 extraction method does not leave organic solvent residues, and is safe and effective for food processing. Factors such as pressure, temperature and CO_2 flux are important when optimizing the supercritical CO_2 extraction process to maximize the extraction ratio. Zhang and Yu (Zhang and Yu, 2001) and Han (Han, 2002) have carried out preliminary research on the supercritical CO_2 extraction of essential oil from cumin seeds. The optimized extraction conditions and relationship between the extraction factors were different for the two studies. Zhang and Yu (Zhang and Yu, 2001) research showed that the relative importance of factors on essential oil extraction ratio in decreasing order of importance was: extraction pressure > CO_2 flux > particle size > extraction temperature (Zhang and Yu, 2001). In contrast, Han (2002) results ranked the factors as: extraction pressure > particle size > CO_2 flux > extraction temperature (Han, 2002). In this study, these four factors were further optimized to ensure maximal extraction ratio. The antifungal activity of cumin essential oil against *Sclerotinia sclerotiorum* at different extraction levels was also determined.

Results

Optimization of supercritical CO_2 extraction

The supercritical CO_2 extraction method of cumin seeds was optimized by orthogonal test (Table 1). The extraction ratios of various combinations are shown in Table 2. Extraction condition 1 had the lowest extraction ratio (2.42 %), while extraction condition 15 had the highest extraction ratio (21.38 %). K value analysis of four factors showed that pressure had the greatest influence on extraction ratio of cumin seed oil, while CO_2 flux and temperature had the least influence, which is consistent with the results of Zhang and Yu (2001). According to the range value analysis, the optimal conditions for supercritical CO_2 extraction of cumin seeds oil

Table 1. Factors and levels for optimization of the supercritical CO_2 extraction of *Cuminum cyminum* seed essential oil.

No. of level	Factor			
	Pressure (MPa) (A)	Particle size (mesh) (B)	CO_2 flux (ml) (C)	Temperature (°C) (D)
1	13.8	20 - 40	20	30
2	20.7	40 - 60	40	40
3	27.6	60 - 80	60	50
4	34.5	80 - 100	80	60

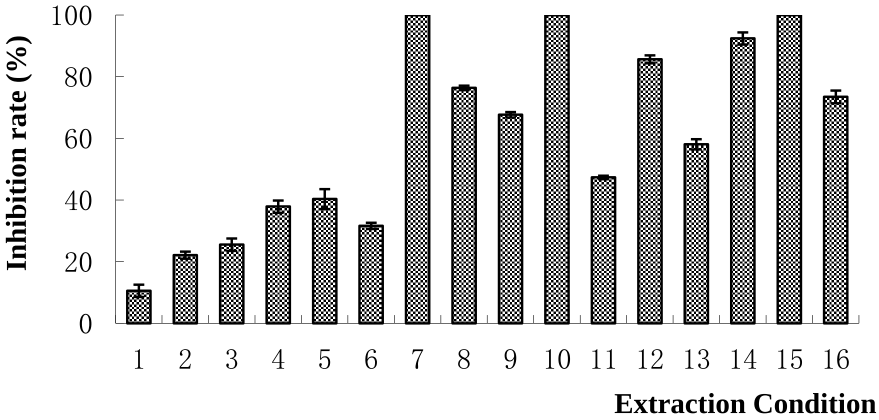

Fig 1. Antifungal inhibition of essential oil (100 mg/mL) extracted from *Cuminum cyminum* seeds against *Sclerotinia sclerotiorum*. The supercritical CO_2 extraction conditions are detailed in Table 2, Error bars indicate ± SD.

are as following: pressure 34.5 MPa, particle size 60-80 mesh, CO_2 flux 80 mL and temperature 30°C ($A_4B_3C_4D_1$). Under these optimal conditions, the extraction ratio of cumin seed oil was better than Zhang, Yu (2001) and Han (2002). ANOVA analysis of the extraction ratio showed that pressure (A) has a very significant influence ($p < 0.01$) on extraction ratio, while particle size (B) and CO_2 flux (C) have significant influences ($p < 0.05$) (Table 3). The optimized extracting conditions were applied to extract cumin seed essential oil in triplicate. The extraction ratios were 21.86%, 21.33% and 21.56% respectively, and the average extraction ratio of triplicate extractions was 21.59%. There was no significant difference in ratio among the three replicates, which indicating that the optimized conditions are repeatable and could be feasibly used for routine extraction of cumin seed oil.

Main components of cumin seed oil

The main components of *C. cyminum* essential oil extracted using the optimized supercritical CO_2 extraction method were determined by GC-MS and identified by searching the NIST mass-spectrogram automatically. The total ion current (TIC) diagram of cumin oil is shown in Figure 2. A total of 60 components, comprising 98.11% of all chemicals detected, were identified. The main components (relative content > 1%) are listed in Table 4. 4-(1-methylethyl)-benzaldehyde (30.56%) and 4-(1-methylethyl)-p-cymen-7-ol (23.59%) were the two most abundant components.

Antifungal activity of essential oil extracted from Cuminum cyminum

The antifungal activity of essential oil extracted from *C. cyminum* against *S. sclerotiorum* is shown in Figure 1. Using 100 mg/L of extracted essential oil, the extraction ratio was positively correlated with antifungal activity on *S. sclerotiorum* ($R^2 = 0.9777$). At the same oil concentration (100 mg/L), when the extraction ratio was higher than 15%, the inhibition rate was higher than 70 %. When the extraction ratio was lower than 10 %, the inhibition rate was less than 50 %.

Discussion

Many bioactive compounds are obtained from plant material. Different extracts from *Cuminum cyminum* seeds have antifungal activities, where 4-(1-methylethyl)-benzaldehyde acts as the main antifungal component. Supercritical CO_2 extraction of *Cuminum cyminum* seeds oil was optimized by an orthogonal design. The optimized method yielded an essential oil with a higher concentration of 4-(1-methylethyl)-benzaldehyde and better antifungal activity than other extraction methods (Yan et al., 2002; Jiang and Lou, 1993; Liu et al., 2002; Gachkar et al., 2007; Liu, 2000; Heikes et al., 2001). By analyzing the extraction parameters, the relative importance of four factors on the extraction ratio was: pressure > CO_2 flux > particle size > temperature. Under the optimized conditions, the extraction ratio of essential oil was 21.59%, and the result was better than previously reported (Zhang and Yu, 2001 and Han, 2002). GC-MS analysis showed that the main components of cumin seed essential oil were 4-(1-methylethyl)-benzaldehyde (30.56%) and 4-(1-methylethyl)-p-cymen-7-ol (23.59%). The contents of both compounds were higher than with other extraction methods (Zhang and Yu, 2001; Han, 2002). The antifungal activity of essential oil obtained using the optimized method against *S. sclerotiorum* was measured *in vitro*. The results indicated that the essential oil extracted from cumin seeds has potential as a preventative or curative preparation for phytopathogen infections (The CAS committee of the flora of China, 1985; China National Corporation of Traditional & Herbal Medicine, 1994). Cumin seed essential oil is nontoxic and safe, listed as

Table 2. Orthogonal test of $L_{16}(4^5)$ for supercritical CO_2 extraction of *Cuminum cyminum* seed essential oil.

Extraction Condition	Pressure (psi) A	Particle size of seed powder (mesh) B	CO_2 Flux (mL) C	Temperature (°C) D	Extraction ratio (%)
1	13.8	20 - 40	20	30	2.42
2	13.8	40 - 60	40	40	4.68
3	13.8	60 - 80	60	50	6.66
4	13.8	80 - 100	80	60	8.41
5	20.7	20 - 40	40	50	7.60
6	20.7	40 - 60	20	60	4.53
7	20.7	60 - 80	80	30	20.45
8	20.7	80 - 100	60	40	17.06
9	27.6	20 - 40	60	60	13.89
10	27.6	40 - 60	80	50	20.58
11	27.6	60 - 80	20	40	9.93
12	27.6	80 - 100	40	30	17.51
13	34.5	20 - 40	80	40	13.05
14	34.5	40 - 60	60	30	17.35
15	34.5	60 - 80	40	60	21.38
16	34.5	80 - 100	20	50	15.39
K1	22.16	36.96	32.27	57.73	61.44
K2	49.64	47.14	51.17	44.73	54.41
K3	61.91	58.42	54.96	50.22	41.74
K4	67.17	58.36	62.48	48.21	43.29
k1	5.541	9.239	8.066	14.432	15.361
k2	12.410	11.784	12.793	11.182	13.603
k3	15.477	14.606	13.740	12.554	10.434
k4	16.791	14.591	15.621	12.052	10.822
Range	11.25	5.37	7.55	3.25	4.93

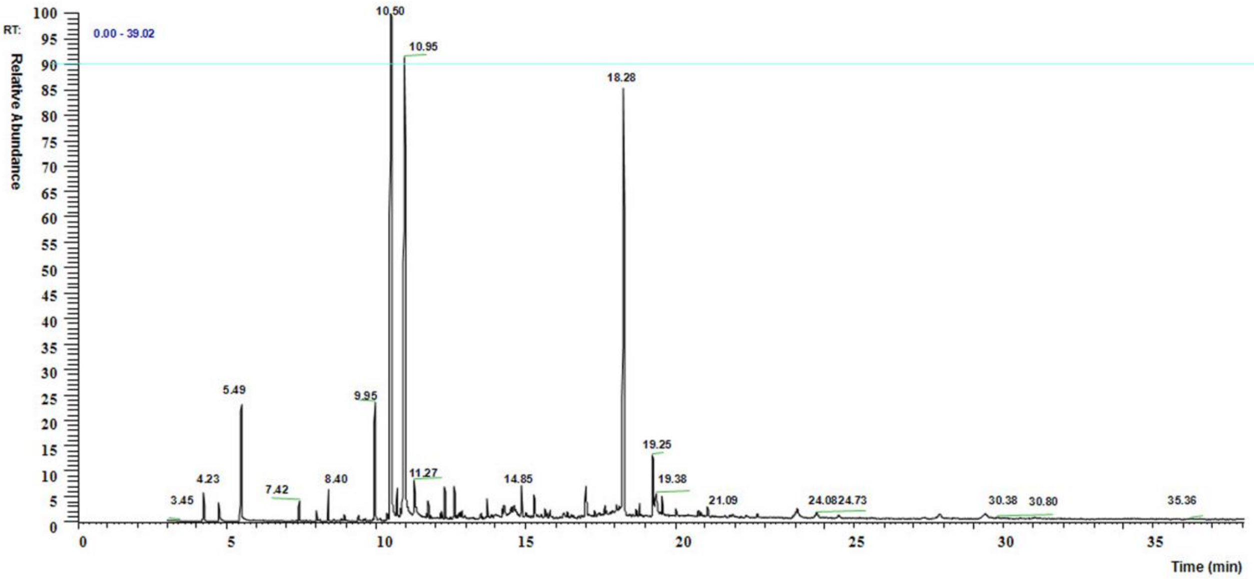

Fig 2. TIC diagram of *Cuminum cyminum* seed essential oil extracted under optimized supercritical CO_2 conditions.

"generally recognized as safe" (GRAS) by the US FDA (Hall and Oser, 1965) and is used as a cooking spice. Further studies of the antifungal activity of cumin seed essential oil, as well *in vivo* bioassays, are required to support potential use of the essential oil as an antifungal agent in agricultural applications. Optimization of the extraction ratio of 4-(1-methylethyl) -benzaldehyde is important as previous studies have shown that the compound has antifungal activity (Singh and Upadhyay, 1991). Using the 4-(1-methylethyl)-benzaldehyde content as a guide to optimize the extraction may result in cumin seed essential oil extracts with better antifungal activity. Another main components, 4-(1-methylethyl)-p-cymen-7-ol, is also worth further research on antifungal activity. This study showed that the antifungal activity of cumin seed essential oil was positively correlated with 4-(1-methylethyl)-benzaldehyde content. However, 4-(1-methylethyl)-p-cymen-7-ol was also found in the essential oil at high levels. Further research is necessary to determine if 4-(1-methylethyl)-p-cymen-7-ol also has antifungal activity.

Table 3. Variance analysis of the orthogonal test of $L_{16}(4^5)$ for the supercritical CO_2 extraction of *Cuminum cyminum* seed essential oil.

Variance	Degrees of freedom	Sum of squares (SS)	Mean of squares (MS)	F value	$F_{0.05}$	$F_{0.01}$
Pressure (MPa)	3	302.82	100.94	53.627**	9.28	29.46
Particle size (mesh size)	3	79.76	26.59	14.124*	9.28	29.46
CO_2 flux (mL)	3	124.04	41.35	21.966*	9.28	29.46
Temperature (°C)	3	22.65	7.55	n.s	9.28	29.46
Error	3	65.88	21.96			

Note: *, **, and ns – denote significant difference at P<0.05, P< 0.01 and not significant, respectively.

Table 4. Main components of *Cuminum cyminum* seed essential oil.

Peak number	RT (min)	Name	Molecular Formula	SI[a]	RSI[b]	Relative peak area (%)
3	5.48	4-hydroxy-4-methylpentan-2-one	$C_6H_{12}O_2$	893	894	4.52
11	9.95	(4-isopropylcyclohexa-1,3-dienyl)methanol	$C_{10}H_{16}O$	862	864	2.60
13	10.49	4-(1-methylethyl)-benz-aldehyde	$C_{10}H_{12}O$	937	944	30.56
16	10.95	4-(1-methylethyl)-p-cymen-7-ol	$C_{10}H_{14}O$	831	831	23.59
17	11.27	*p*-mentha-1,4-dien-7-ol	$C_{10}H_{16}O$	907	918	1.33
41	17.01	3-Ethyl-4,4-dimethyl-2-(2-methylpropenyl) cyclohex-2-enone	$C_{14}H_{22}O$	727	746	1.22
47	18.27	4a,7,7,10a-tetramethyl-dodecahydro-1H- benzo[f]chromen-3-ol	$C_{17}H_{30}O_2$	637	647	17.54
51	19.25	E,E,Z-1,3,12-Nonadeca-tri-ene-5,14-diol	$C_{19}H_{34}O_2$	726	734	2.53
52	19.37	Estra-1,3,5(10)-trien-17-ol	$C_{18}H_{24}O$	674	716	1.13

Note: [a] SI, Strength Index; [b] RSI, Relative Strength Index

Material and methods

Plant materials

Cuminum cyminum L. seeds were collected from Zhangye city in Gansu province in October, 2005. The cumin seed was ground and sieved to obtain samples of 20 - 40 mesh particle size, 40 - 60 mesh particle size, 60 - 80 mesh particle size and 80 - 100 mesh particle size. All samples were sealed and stored in refrigeration at 4°C until use.

Supercritical CO_2 extraction

Supercritical CO_2 extractions were carried out using an ISCO™ 2-10 supercritical CO_2 extraction system, 260D ram pump, pump control instrument and ISCO™ flow control tube (1 mL/min). Based on previous research, static extraction for 5 min followed by dynamic extraction was used in this study (Eikani et al., 2007; Jalali-Heravi et al., 2007). Pressure, particle size, CO_2 flux and temperature were selected as the four principle factors for optimization. Four levels were set for each factor. The design of factors and levels are listed in Table 1. An orthogonal design table $L_{16}(4^5)$ was used in this study. The extraction ratio of cumin seed essential oil was calculated using the following formula:

$$\text{Extraction ratio}(\%) = \frac{\text{Sample weight before extraction - Sample weight after extraction}}{\text{Sample weight before extraction}} \times 100\%$$

Gas Chromatography-Mass Spectrometry (GC-MS)

Analysis of the main components in the essential oil extract was carried out on a Finngan Trace DSQ GC-MS instrument (Thermo, Inc.) using an HP-1 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injection volume was 0.2 µL and the split ratio was 1:50. The initial column temperature was set at 40°C and maintained for 3 min; then, the temperature was increased to 320°C at a rate of 8°C/min and maintained for 1 min. The interface temperature of the mass spectrometer was 250°C. Electrospray ionization was used for sampling. Mass spectra were recorded in the range 40-550 amu operating at 70 eV, while the ion source temperature was maintained at 250°C. The chemical composition of the essential oil was identified by automatically searching the NIST mass-spectrogram library with an Xcalibur™ system.

Antifungal activity test

The antifungal activity of the essential oil was determined by the growth rate method (Mu, 1994; Fang, 1998). The essential oil was prepared and serially diluted with acetone to obtain the required concentrations. The oil and melted potato dextrose agar (PDA) culture medium were mixed at a ratio of 1:19, and the mixture was then poured into a Petri dish. The final acetone concentration was 0.05% (v/v). When the medium in the plates was solidified, mycelia agar plugs (4 mm diameter) of test fungi cut from previously sub-cultured Petri dishes were placed at the center of the PDA: oil plates. The Petri dishes were incubated with the lid upside down at 25 ± 2°C. The control dish was prepared by the addition of an equivalent amount of acetone without any essential oil. All experiments were performed in triplicate. The diameter of fungal colonies was measured after 72 hours incubation and the inhibition rate was calculated according to the following formula. The multiple comparisons of bioassay data was analyzed by SPSS 13.0 with Duncan's method.

$$\text{Inhibition rate}(\%) = \frac{\text{Mean colony diameter,control - Mean colony diameter,treatment group}}{\text{Mean colony diameter,control - 4}} \times 100\%$$

Conclusion

In this study, we optimized supercritical CO_2 extraction conditions to maximize the ratio of cumin seed essential oil. Oil extracted using the optimized method completely inhibited mycelial growth of *S. sclerotiorum*. Furthermore, GC-MS analysis indicated that the main active component of the essential oil is possibly 4-(1-methylethyl)-benzaldehyde.

Acknowledgments

This research was supported by the National High Technology Research and Development Program of China "Demonstrate and popularize new botanical pesticides and relevant applied technology (No. 2011AA10A202-1).

References

The CAS committee of the flora of China (1985) The Flora of China, Science Press, Beijing.
China National Corporation of Traditional & Herbal Medicine (1994) Compendium of Chinese Medicinal Material Resources, Science Press, Beijing.
Eikani MH, Golmohammad F, Mirza M, Rowshanzamir S (2007) Extraction of volatile oil from cumin (*Cuminum cyminum* L.) with superheated water. J Food Process Eng 30: 255-266.
Eikani MH, Goodarznia I, Mirza M (1999) Supercritical carbon dioxide extraction of cumin seeds (*Cuminum cyminum* L.). Flavour Frag J 14: 29-31.
Fang Z (1998) Research method of plant pathology, 3rd edn. China Agriculture Press, Beijing.
Gachkar L, Yadegari D, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I (2007) Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Food Chem 102: 898-904.
Hall R L, Oser B L(1965) Recent progress in the consideration of flavoring ingredients under the food additives amendment. III. GRAS substances. Food Technology 19(2): 168.
Han J (2002) Priminary research on the fungicidal activity of botanical components. MS Thesis, Northwest Science and Technology University, pp 28.
Heikes DL, Scott B, Gorzovalitis NA (2001) Quantitation of volatile oils in ground cumin by supercritical fluid extraction and gas chromatography with flame ionization detection. J AOAC Int 84: 1130-1134.
Hu L, Chen C, Yi X, Feng J, Zhang X (2008) Inhibition of p-isopropyl Benzaldehyde and p-isopropyl Benzoic Acid extracted from *Cuminum cyminum* against Plant Pathogens. Acta Bot Boreal 28: 2349-2354.
Hu L, Feng J, Zhang X, Zhang Y (2007) Isolation and Structure Detection of Fungicidal Components from *Cuminum cyminum* Seed. Chinese J Pesticide Sci 94: 330-334.
Hu L, Li G, Li Y, Feng J, Zhang X (2005) Research Advances in Chemical Constituents and Their Bioactivities of *Cuminum cyminum*. Act Botanica Boreali-occidentalia Sinica 25: 1700.
Jalali-Heravi M, Zekavat B, Sereshti H (2007) Use of gas chromatography-mass spectrometry combined with resolution methods to characterize the essential oil components of Iranian cumin and caraway. J Chromatogr A 1143: 215-226.
Jiang Z, Lou Y (1993) Research on the chemical components and extract technique of essential oil from *Cuminum cyminum*. China Condiment 1: 11-13.
Liu Y (2000) The analysis of cumin oil of Xinjiang with super critical CO2 fluid extraction technique. J Wuhan Bot Res 18: 497-499.
Liu Z, Cui L, Lu J (2002) Microwave extract for naphtha in fruit of *Cuminum cyminum*. Lishizhen Medicine Materia Medica Res 13: 3-4.
Mu L (1994) Research method of plant chemical protection. China Agriculture Press, Beijing.
Singh G, Upadhyay R (1991) Fungitoxic activity of cumaldehyde, main constituent of the *Cuminum cyminum* oil. Fitoterapia 62: 86.
Wang Z, Ding L, Li T, Zhou X, Wang L, Zhang H, Liu L, Li Y, Liu Z, Wang H, Zeng H, He H (2006) Improved solvent-free microwave extraction of essential oil from dried *Cuminum cyminum* L. and *Zanthoxylum bungeanum* Maxim. J Chromatogr A 1102: 11-17.
Yan JH, Tang KW, Zhong M, Deng NH (2002) Determination of chemical components of volatile oil from *Cuminum cyminum* L. by gas chromatography-mass spectrometry. Se Pu. 20: 569-572.
Zhang Q, Yu M (2001) Study on technique of super critical CO_2 extraction of oleoresin cumin. Xinjiang Agric Sci 38: 273-274.