African Journal of Biotechnology Vol. 11(7), pp. 1789-1795, 24 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2947
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Chemical composition and anti-bacterial activity of essential oil from *Cedrela sinensis* (A. Juss.) Roem. seed

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Accepted 13 December, 2011

This work assesses the chemical compositions and *in vitro* anti-bacterial activities of seed essential oil from *Cedrela sinesis* (A. Juss.) Roem. seed. which has abundant mineral elements such as K, Ca, Fe. The fatty acid profiles of seed essential oil are characterized by considerable unsaturated fatty acids (90.39%) including linoleic acid (56.81%), linolenic acid (17.63%) and oleic acid (15.95%). Gas chromatography-mass spectrophotometry (GC-MS) analyses show that major components comprising 62.7% of the oil are 1,6-cyclodecadiene (42.64%), 9,17-octadecadienal (13.72%), and Z,E-2,13-octadecadien-1-ol (6.32%). Squalene (1.94%) is detected in this seed oil for the first time. The inhibition zone diameters and minimal inhibition concentrations (MIC) against four tested bacteria are in the ranges of 17 to 23 mm and 192 to 390 μg/mL, respectively. The effects of pH and temperature on the anti-bacterial activities of essential oil show no significant difference.

Key words: *Cedrela sinesis* (A. Juss.) Roem. seed, chemical composition, essential oil, GC-MS, anti-bacterial activity.

INTRODUCTION

The genus *Toona* were confined to two species, *T. ciliate* Roem. and *T. sinensis* Roem. (Fang et al., 2010). The latter is also called as *Cedrela sinensis* (A. Juss.) Roem., which is commonly known as Chinese mahogany cedar or Chinese *Toona*. *C. sinensis.* It belongs to a perennial deciduous tree of the family Meliaceae (Figure 1) (Lee et al., 2010; Wang et al., 2009). *C. sinensis*, a tranditional Chinese medicine, widely distributes in north and southeast China, especially in Shandong, Hebei and Henan provinces. Many researches on the chemical constituents and medical function of the *C. sinensis*, including leaves, bark, root, and petioles, have been comprehensively reported (Chen et al., 2009; Feng et al., 2009; Liao et al., 2009; Mu et al., 2007). However, so far there are no reports on the chemical constituents and anti-bacterial activities of the essential oil of *C. sinensis* seed, which is oval in shape with wooden wings (Fig. 1-B). As we know, essential oils and/or their components are very important in medical and plant pathology as well as in the food industry for the control of pathogenic microorganisms to consumers and/or responsible for food spoilage (Cantore et al., 2009; Patil et al., 2010; Viuda-Martos et al., 2010).

Therefore, this work firstly assessed the chemical compositions and *in vitro* anti-bacterial activities of seed essential oil from *C. sinesis* (A. Juss.) Roem. seed. The objectives of this work were mainly focused on three aspects: (1) to investigate the nutrition constituents of *C. sinensis* seed such as protein, carbohydrate, lipids, ash, moisture and mineral elements; (2) to analyze the fatty acid profiles and compositions of seed essential oil; and (3) to evaluate the *in vitro* anti-bacterial activities of essential oil from *C. sinensis* seed.

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Figure 1. Leaves, seeds and seed essential oil of *C. sinensis* (A. Juss.) Roem. (**A,** leaves; **B,** seeds; **C,** seed essential oil).

MATERIALS AND METHODS

Materials

C. sinensis seed was collected in May 2007 in Huaiyang county, Henan province, China. Tested microorganisms such as *Staphylococcus aureus* ATCC 25925, *Bacillus subtilis* ATCC 9372, *Pseudomonas aeruginosa* ATCC 25916, *Escherichia coli* ATCC 25922, *Aspergillus niger* ATCC16404, *Aspergillus flavus,* and *Aspergillus fumigatus* were gifts from Pharmaceutical College of South-Central University for Nationalities, China. The $CDCl_3$ [99.8% D with 0.03% (v/v) tetramethylsilane (TMS)] used as the a proton nuclear magnetic resonance (^{1}H-NMR) solvent was purchased from J & K Chemical Ltd. (Shanghai, China). Penicillin (for G^+ bacteria) and streptomycin (for G^- bacteria) as positive controls were bought from J & K Chemical Ltd (Shanghai, China). Hexane with analytic grade was obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other reagents used in this work were of analytic grade and obtained in locate chemical reagent company.

Seed essential oil extraction

C. sinensis seed with woody wing was ground using a mechanical grinder and dried at 105°C for 2 h before oil extraction. The essential oil was extracted from the *C. sinensis* seed powder with *n*-hexane (boiling point of 40 to 60°C) according to the soxhlet extraction method. Ten grams of the crushed dry seed were refluxed using 150 ml hexane in a soxhlet apparatus. The hexane solvent was recovered using rotary evaporator apparatus at 40°C and - 0.1 MPa until solvent free residue. The obtained essential oil was then dried over anhydrous sodium sulfate and, after filtration, stored in a tightly closed, dark vial at 4°C for subsequent analyses. The essential oil yield and density was estimated to be 31.29% (v/w) and 0.934 mg/ml, respectively. The seed essential oil is brown in color and has a sharp odor.

Assay of basic chemical constituents of *C. sinensis* seed

AOCS methods with a few modifications were used to determine the moisture, ash, and lipids contents of *C. sinensis* seed powder (AOAC, 1998). The contents of moisture and ash were calculated by mass balance. The lipids content was estimated by volume weight ratio. The measurement of inorganic elements such as N, C, H, S, O was performed on a Vario Micro Cube Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The content of mineral elements such as Na, K, Ca, Fe, Cu was determined by ELAN DRC-e Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (PerkinElmer Inc., Califonia, USA). The primary plant metabolites such as protein, lipids and carbohydrate in *C. sinensis* seed were qualitatively characterized by VERTEX 70 Fourier transform-infrared (FT-IR) spectroscopy (Bruker Co. Ltd., Karlsruhe, Germany) according to the method reported in our previous work (Liu et al., 2009). The FT-IR conditions were: 4 cm^{-1} spectral resolution, 20 kHz scan speed, 128 scan co-additions, and scanning range 400 to 4000 cm^{-1}. A factor of 6.25 was adopted for estimating the protein content. The carbohydrate content was obtained by difference method.

Measurement of fatty acid compositions of seed essential oil by ^{1}H-NMR

The fatty acid (FA) compositions of seed essential oil were determined by ^{1}H-NMR spectroscopy according to the method by Guillen and Ruiz (2003). For ^{1}H-NMR, 20 - 25 mg of the essential oil sample was dissolved in 0.6 mL of $CDCl_3$ and the spectrum was recorded at 25°C on a Bruker AV-400 300 MHz spectrometer (Bruker Co. Ltd, Switzerland); 30 scans for each sample were taken during the measurement. A standard 4 mm quadronuclei (1H) probe (QNP) was used. An acquisition time of 3.9 s, relaxation delay of 1 s, flip angle of 30°, and sweep width of 4.139 kHz were employed in the spectral measurements. TMS with the concentration of 0.03% (v/v) was used as internal standard. Each FA content measured by ^{1}H-NMR spectroscopy was estimated by equations 1 to 4:

$$\text{Linolenic (\%)} = 100\,[B/(A + B)] \quad (1)$$

$$\text{Linoleic (\%)} = 100\,[(E/D) - 2(B/(A + B)] \quad (2)$$

$$\text{Oleic (\%)} = 100\,[(C/2D) - (E/D) + (B/A + B)] \quad (3)$$

$$\text{Saturated FA (\%)} = 100\,[1 - (C/2D)] \quad (4)$$

where, A is the terminal methyl protons of saturated, oleic and linoleic acyl chains; B is the terminal methyl protons of linolenic acyl chains; C is the allyl methylene protons; D is the six α-methylene protons adjacent to carbonyl carbon; E is the divinyl methylene protons.

Simultaneously, gas chromatography (GC) was also used to monitor the FA profiles of seed essential oil as a control. The essential oil was fully esterified before it was analyzed by GC, which was detailed in our previous work (Liu et al., 2010). The conditions of GC were: GC-9790 gas chromatography instrument (Fuli Analytical Instrument Co. Ltd., Wenlin City, Zhejiang Province, China) equipped with an Agilent INNOWAX capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, J & W Scientific, Folsom, CA, USA) was used. The column temperature of each run was started at 200°C for 2 min, then raised to 235°C at 3°C/min and maintained at 235°C for 1 min. The temperatures of the injector and the flame ionization detector (FID) were 240 and 280°C, respectively.

Composition identification of seed essential oil analyzed by GC-MS

Composition identification of seed essential oil was analyzed with an Agilent 7890 / 5975C GC-MS (American Agilent Technologies Co. Ltd. , CA, USA) with an Agilent 19091S-433 capillary column (30 m × 0.25 mm i. d. × 0.25 μm film thickness, American Agilent Technologies Co. Ltd., CA, USA). GC conditions were: carrier gas, high purity helium; flow rate, 1 mL/min; split ratio, 1:20; injection volume, 1 μL; injector temperature, 250°C; interface temperature, 240°C. The column temperature of each run was started at 120°C for 0 min, then raised to 240°C at 5°C/min and maintained at 240°C for 10 min. MS conditions were: Ion source, EI (70 eV); ion source temperature, 230°C; quadrupole temperature, 150°C; scanning range, 50 - 500 amu; electronic multiplier voltage, 1600 V.

The constituents of seed essential oil were identified by calculation of their retention indices (RI) relative to *n*-alkanes (C_6-C_{24}) on a DB-1 HT MS column. Individual compound was identified by comparison of its mass spectrum with those of the internal reference mass spectral library or with authentic compounds and confirmed by comparison of its RI with the NIST 2005 GC/MS library data of the GC-MS system and Adams-spectral library (2001). For the quantification, relative area percentages obtained by FID were used without the use of correction factors.

Evaluation of anti-bacterial activities of seed essential oil

Agar disk diffusion assay

The agar disc diffusion method (Luangtongkum et al., 2007) was employed to determine the diameters of inhibition zones of seed essential oil against the seven tested microorganisms, *viz., S. aureus* ATCC 25925, *B. subtilis* ATCC 9372, *P. aeruginosa* ATCC 25916, *E. coli* ATCC 25922, *A. niger* ATCC16404, *A.flavus,* and *A. fumigatus*. Inoculums (1 mL) of the tested bacteria (10^6 - 10^7 colony forming units, CFU/ml) were poured and spread uniformly on the Mueller–Hinton (MH) broth agar solid medium (Microorganism agent Co. Ltd., Hangzhou, China) plates. Filter paper discs (6 mm diameter) were impregnated with the essential oil (3 mg/disk) dissolved in 1% Tween 80. Penicillin (for G^+ bacteria) and streptomycin (for G^- bacteria) (1 mg/disk) dissolved in 1% sterile deionized water were used as positive control, and 1% Tween 80 was used as negative control. The discs were applied on the agar surface, and the plates were incubated at 37°C for 24 h. The diameters of inhibition zones were measured in mm using a Vernier caliper. Results are expressed as mean ± standard errors (SE) in triplicates.

Micro broth dilution assay

Minimum inhibitory concentrations (MICs), defined as the lowest concentrations of seed essential oil that result in a complete inhibition of visible growth of microorganisms in the broth, were determined according to the method of the NCCLS (2001). All tests were performed in MH broth. The essential oil was dissolved in 1% Tween 80 and serial doubling dilutions of each essential oil were prepared in a 96 micro titer plate to get the final concentration ranging from 500 to 192 μg/mL.

Effect of pH on anti-bacterial activities of seed essential oil

The pH of MH medium was adjusted to 6.0, 7.0 and 8.0 with 0.5 M NaOH and citric acid. The effect of different pH on anti-bacterial activities of essential oil was investigated with the determination of inhibition zone diameters (agar disk diffusion assay).

Effect of temperature on anti-bacterial activities of seed essential oil

The essential oil was placed at 40, 80 and 121°C for 15 min, and then experiments of anti-bacterial activities were carried out to evaluate the thermal stability of essential oil by determining inhibition zone diameters (agar disk diffusion assay).

RESULTS AND DISCUSSION

Basic nutritional constituents of *C. sinensis* seed

The basic nutritional constituents such as protein, lipids, carbohydrate, moisture and ash of *C. sinensis* seed were measured by AOCS methods. The contents of mineral and inorganic elements were determined using ICP-MS and Elemental Analyzer, respectively. The results are shown in Table 1.

Table 1 shows that the content of protein, lipids and carbohydrate in *C. sinensis* seed is 30.99, 31.29 and 26.85%, respectively. Analyses of mineral elements show *C. sinensis* seed is very abundant in K, Ca and Fe. K is very important as diuretic and it takes part in ionic balance of the human body and maintains tissue excitability. Ca imparts strength and rigidity to bones and teeth. Ca is also needed in neuromuscular transmission, in excitability of nerves for normal excitability of heart, in clotting of blood and promoting muscular contraction. Fe is a blood agent element. It was revealed that *C. sinensis* seed is a promising bioresource of mineral nutrition element.

FT-IR spectroscopy has been found to be an extremely useful technique for non-destructive analysis of the intact fresh plant tissue without the necessity to perform any sample clean-up steps (Schulz and Baranska, 2007). Therefore, FT-IR was employed to qualitatively characterize the primary plant metabolites such as protein, lipids and carbohydrate in *C. sinensis* seed.

Table 1. The chemical constituents of *C. sinensis* seeds.

Nutritional component	Protein	Lipid	Moisture	Ash	Carbohydrate[a]
Values/%	30.99 ± 0.81	31.29 ± 0.06	5.00±0.34	6.09 ± 0.29	26.85 ± 0.71
Mineral elements	**Na**	**K**	**Ca**	**Fe**	**Cu**
Values/ppm	61.11 ± 2.11	1,297.69 ± 3.84	1,212.47±2.97	664.94 ± 1.46	9.15 ± 0.11
Inorganic elements	**C**	**H**	**O**	**S**	**N**
Values / %	492.23 ± 3.26	69.71 ± 0.25	308.95±0.69	4.43 ± 0.21	49.33 ± 1.25

[a]Carbohydrates% = 1- (protein% + lipid% + moisture% + ash%).

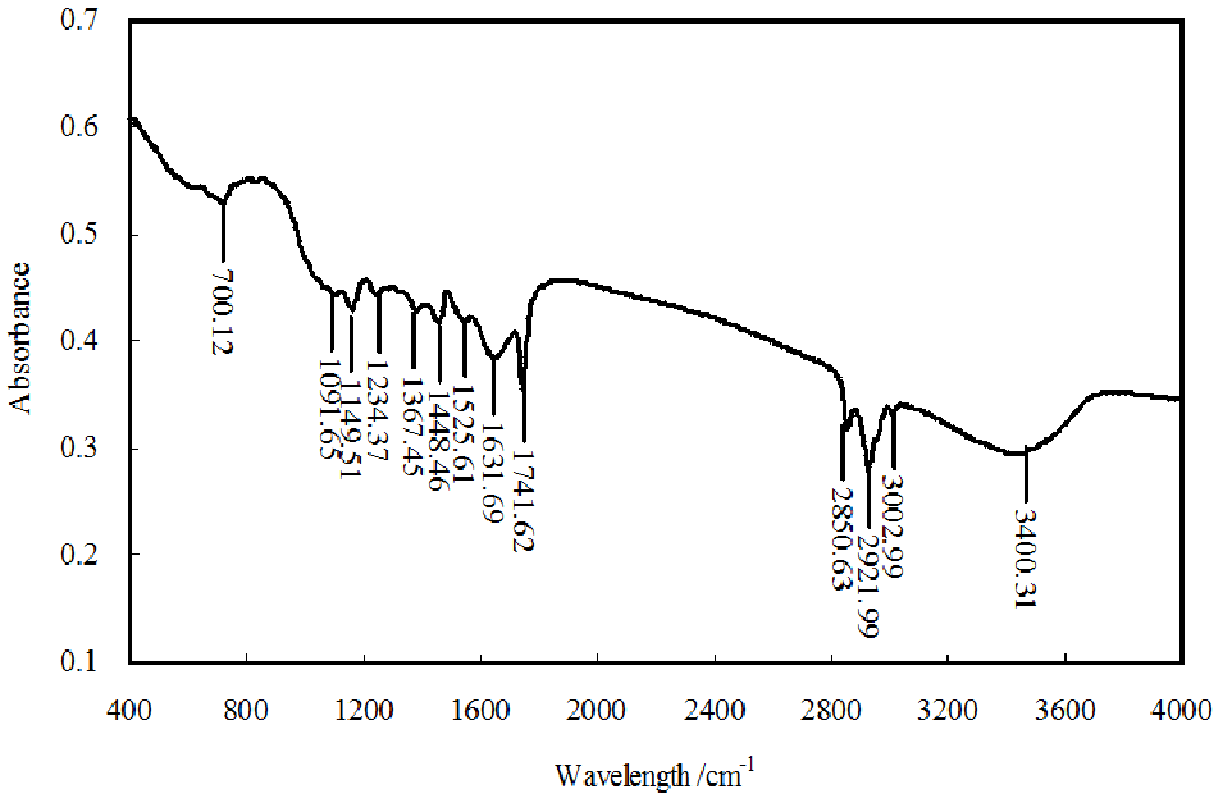

Figure 2. The FT-IR spectrum of *C. sinesis* seed (Conditions: 4 cm^{-1} spectral resolution, 20 kHz scan speed, 128 scan co-additions, and scanning range 400 - 4000 cm^{-1}).

Figure 2 provides the general information on FT-IR spectra of *C. sinensis* seed.

Figure 2 shows four characteristics zones of the wave number interval peaks: The first zone was 4000 - 1800 cm^{-1} area. In this area, 3400 cm^{-1} as the center was a strong broad peak, which is assigned to hydroxyl stretching vibration peak. Medium intensity peaks 2921 cm^{-1} and its acromial 3002 and 2850 cm^{-1} are -CH_2, -CH_3 stretching vibrations. The second zone was the carbonyl and -C=C- vibration zones of 1800-1500 cm^{-1}. Strong peaks of 1631 and 1525 cm^{-1} are assigned to protein amide I and II peaks. Strong peak of 1741 cm^{-1} is the carbonyl stretching vibration. The third district was 1500 - 1200 cm^{-1}. The peaks in this area were mixture vibration peaks of protein, fatty acids and polysaccharides. The characteristic peaks in this region were 1448, 1367 and 1234 cm^{-1}. The fourth region was 1200-700 cm^{-1}. The peaks in this region are mainly assigned to polysaccharide peaks, which are characterized by 1149 and 1091 cm^{-1}. 1149 cm^{-1} peak in this region was the strongest peak, which is assigned to -C-O- stretching vibration of carbohydrates. So, we can find the information of protein, lipids and carbohydrate in *C. sinensis* seed from FT-IR spectrum.

Table 2. The fatty acid profiles of seed essential oil measured by NMR and GC.

Profile	$C_{16:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Measured by GC /%	6.61 ± 0.22	25.95 ± 1.23	46.81 ± 1.47	17.63 ± 0.98
Measured by NMR /%	11.07 ± 0.26 [a]	25.72 ± 1.42	43.24 ± 1.54	19.97 ± 1.01

[a]The total content of saturated fatty acid, C16:0+C18:0.

Analyses of *C. sinensis* seed essential oil

Fatty acid profiles of seed essential oil by ^{1}H-NMR and GC

FA profiles of the important characteristic of seed essential oil, were simultaneously measured by ^{1}H-NMR and GC. The results are shown in Table 2.

The major FAs of *C. sinensis* seed essential oil are oleic, linoleic, and linolenic FA. Linoleic acid shows the highest percentage of 56.81%, followed by linolenic acid with 17.63% and oleic acid with 15.95%. Thus, *C. sinensis* seed essential oil should be typically classified as high content of polyunsaturated oil, which is helpful to health. Table 2 indicates that quantization of FA compositions by ^{1}H-NMR spectroscopy is in good agreement with that obtained by GC. Although GC and ^{1}H-NMR are both sensitive techniques, the latter is more rapid and easy to manipulate than the former because a chemical modification of the sample is not necessary for ^{1}H-NMR analysis. The areas of the ^{1}H-NMR peaks depend strongly on the numbers of protons and not on their response factors (Vinicius et al., 2008). Therefore, ^{1}H-NMR spectroscopy is a rapid and useful tool to measure the FA compositions of feedstock oil.

Composition identification of seed essential oil by GC-MS

C. sinensis seed essential oil was obtained by soxhlet extraction. The composition identification of seed essential oil was analyzed by GC-MS. The cumulative results are shown in Table 3.

As can be seen in Table 3, GC-MS analyses of *C. sinensis* seed essential oil result in the identification of 30 compounds representing 96.01% of the oil. The main components were identified as 1,6-cyclodecadiene (42.64%), 9,17-octadecadienal (13.72%), Z,E-2,13-Octadecadien-1-ol (6.32%), 2,6,10-dodecatrien-1-ol, 3,7,11- trimethyl-(Z,E)-(3.76%) and anthracene (2.64%). The compositions of *C. sinensis* seed essential oil in this work were quite different with the compositions of essential oil from *C. sinensis* leaves and barks (Chen et al., 2009; Mu et al., 2007). Squalene (1.94%) was firstly detected in *C. sinensis* seed essential oil. Squalene is a carbohydrate of the triterpene type containing six isoprene units with a pleasant, bland taste. It is a key intermediate in the biosynthetic pathway to steroids in both plants and animals. Thus, it can be considered as almost ubiquitous in most plant and animal cells at enormously different levels (Newmark, 1997).

Anti-bacterial activities of seed essential oil

The anti-microbial activities of *C. sinensis* seed essential oil against the microorganisms in this work were qualitatively and quantitatively assessed by evaluating the inhibition zones diameters (ID) and MIC values. The results are shown in Table 4.

Table 4 reveals that the *C. sinensis* seed essential oil showed anti-bacterial activities against all four tested bacterial strains and no anti-microbial activities against molds of *A. niger* ATCC16404, *A. flavus* and *A. fumigatus*. The maximal ID and MIC values for the four tested bacterial strains, sensitive to *C. sinensis* seed essential oil, were in the ranges of 17 to 23 mm and 192 to 390 μg/ml, respectively. Compared to the positive control, 1% penicillin and streptomycin and *C. sinensis* seed essential oil had moderate antibacterial activity. The reasonable explanation might be attributed to the presence of the main components in the essential oil: 1,6-cyclodecadiene, 9,17-octadecadienal, Z,E-2,13-Octadecadien-1-ol, 2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl and anthracene. To understand the nature of the aforesaid anti-bacterial activity, a comprehensive study is necessary, including pure compounds isolated from the essential oil in particular.

Effect of pH and temperature on the anti-bacterial activities of seed essential oil

The effects of pH and temperature on anti-bacterial activities of seed essential oil were investigated. The influence of pH on anti-bacterial activity of seed essential oil was not significant in the ranges of pH 6 to 8. The inhibition zones diameters against *S. aureus* ATCC 25925, *B. subtilis* ATCC 9372, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 25916 were from 13 to 23 mm in the ranges of pH 6 to 8.

C. sinensis seed essential oil was treated at 40, 80 and 121°C for 15 min, and temperature showed no significant effect on anti-bacterial activities against four tested bacteria. Even if the essential oil was treated at 121°C for 15 min, diameters of inhibition zones of ca. 9.5 mm were obviously detected. It is indicated that the antibacterial

Table 3. The compositions of *C. sinensis* seed essential oil.

No.	RT/min	Component	Value (%)
1	5.334	Alpha-Cubebene	1.48
2	5.972	Cyclohexane	1.67
3	6.496	Caryophyllen	0.47
4	6.631	1,6-Cyclodecadiene	0.35
5	7.061	Alpha-Caryophyllene	0.38
6	7.585	1,6-Cyclodecadiene	42.64
7	7.793	Bicyclogermacrene	2.16
8	8.193	Naphthalene	0.60
9	9.225	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	0.32
10	9.287	(-)-Spathulenol	1.23
11	9.915	Isoaromadendrene epoxide	0.72
12	10.460	t-Muurolol	0.86
13	10.719	Alpha.-Cadinol	2.20
14	11.358	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen -2-ol	1.47
15	11.908	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-	3.76
16	13.194	Bicyclo[3.3.0]octan-2-one, 7-ethylidene-	0.49
17	14.191	2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl-,acetate, (E,E)-	1.05
18	14.289	Anthracene	2.64
19	14.704	Beta-Cedren-9-alpha-ol	1.34
20	15.176	Tricyclo[3.3.0.0(2,8)]octan-3-one, 4-methyl-4-(2-methyl-2-propenyl)-	0.68
21	15.586	Decahydro-4,4,8,9,10-pentamethylnaphthalene	0.53
22	16.084	2-Amino-6-methyl-5H-pyrrolo[3,4-c] pyridine-1,3,4-trione	0.85
23	16.546	n-Hexadecanoic acid	1.86
24	19.872	9,17-Octadecadienal	13.72
25	19.945	Z,E-2,13-Octadecadien-1-ol	6.32
26	20.604	2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	0.89
27	20.687	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	0.70
28	21.066	Squalene	1.94
29	22.591	Alpha.-Farnesene	2.07
30	22.985	Oxirane	0.62
Total			96.01

RT, Retention time.

Table 4. The diameters of inhibition zones and MIC of essential oil of *C. sinensis* seed.

Tested microorganism	Diameter of inhibition zone /mm	MIC (μg/ml)
S. aureus ATCC 25925	17 ± 0.5	200 ± 3.5
B. subtilis ATCC 9372	18 ± 1.0	192 ± 4.2
E. coli ATCC 25922	23 ± 1.5	390 ± 5.0
P. aeruginosa ATCC 25916	22 ± 1.0	390 ± 4.5
A. niger ATCC16404	-	/
A. flavus	-	/
A. fumigatus	-	/
Negative control [a]	-	/
Positive control [b]	24 - 26 ± 0.5	4 - 8

-, No inhibition zone; [a] negative contro, 1% Tween 80; [b] positive control, 1% penicillin for G^+ bacteria and 1% streptomycin for G^- bacteria. MIC, Minimal inhibition concentrations.

activities of *C. sinensis* seed essential oil show good thermal stability, which is very important in food processing application.

Conclusion

The essential oil obtained from *C. sinensis* seed was for the first time explored to evaluate its compositions and *in vitro* anti-bacterial activities. The nutritional constituents of *C. sinensis* seed was investigated by AOAC methods and qualitatively assessed using FT-IR. Elements analyses showed that *C. sinensis* seed is characterized with the richment in K, Ca, and Fe. The *C. sinensis* seed essential oil is abundant in polyunsaturated fatty acids and squalene is firstly detected in seed essential oil. The seed essential oil showed moderate anti-bacterial activities against *S. aureus* ATCC 25925, *B. subtilis* ATCC 9372, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 25916. In conclusion, *C. sinensis* seed is a potential promising bioresource with nutrition and anti-bacterial activities for food and cosmetics industries.

ACKNOWLEDGEMENTS

The authors are deeply indebted to Mr. Shangyong Wang to collect *C. sinensis* seed and the Analytical and Testing Center of Huazhong University of Science and Technology for NMR, FT-IR and ICP-MS analyses. The authors also acknowledge the financial support from National Science and Technology Support Program (2011BAD22B08).

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