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Research Paper

Chemical composition, antimicrobial activity against *Staphylococcus aureus* and a pro-apoptotic effect in SGC-7901 of the essential oil from *Toona sinensis* (A. Juss.) Roem. leaves

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**A B S T R A C T**

Ethnopharmacological relevance: Leaves of *Toona sinensis* (A. Juss.) Roem. (TSL), a popular vegetable in China, have anti-inflammatory, antidoting, and worm-killing effects and are used in folk medicine for the treatment of enteritis, dysentery, carbuncles, boils, and especially abdominal tumors. Our aim was to investigate the in vitro antimicrobial activity against *Staphylococcus aureus* and anticancer property of the essential oil from TSL (TSL-EO), especially the pro-apoptotic effect in SGC-7901.

Materials and methods: TSL-EO obtained by hydrodistillation was analyzed by GC/MS and was tested in vitro against twenty clinically isolated strains of *Staphylococcus aureus* (SA 1–20), which were either methicillin-sensitive *Staphylococcus aureus* (MSSA) or methicillin-resistant *Staphylococcus aureus* (MRSA) and two standard strains viz. ATCC 25923 and ATCC 43300. The anticancer activity of TSL-EO was evaluated in vitro against HepG2, SGC7901, and HT29 through MTT assay. Moreover, the apoptosis-inducing activity of TSL-EO in SGC7901 cells was determined by Hoechst 33324 staining and flow cytometry methods. Also, the apoptosis-related proteins viz. Bax, Bcl-2 and caspase-3 were detected by western-blotting.

Results: GC–MS analysis showed that TSL-EO contained a high amount of sesquiterpenes (84.64%), including copaene (8.27%), β-caryophyllene (10.16%), caryophyllene (13.18%) and β-eudesmene (5.06%). TSL-EO inhibited the growth of both MSSA and MRSA, with the lowest MIC values of 0.125 and 1 mg/ml, respectively. Treatment with TSL-EO for 24 h could significantly suppress the viability of three different cancer cell lines (P < 0.05). Furthermore, the apoptosis-inducing activity of TSL-EO in SGC7901 cells increased in a dose-dependent manner, potentially resulting from the up-regulated expression of Bax, caspase-3 and down-regulated expression of Bcl-2.

Conclusions: TSL-EO possessed antibacterial activity against *Staphylococcus aureus* and significant cytotoxicity against cancer cells and particularly prominent pro-apoptotic activity in SGC7901 cells. These bioactivities were probably due to the high content of sesquiterpenes. Our results suggested that TSL-EO possessed potential health benefits and could serve as a promising natural food additive.

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1. Introduction

Essential oils (EOs), volatile secondary metabolites from aromatic plants, have recently gained wild popularity and scientific interests (Yang et al., 2010a). There is mounting evidence showing that EOs possess significant antimicrobial activity against different plant and human pathogenic micro-organisms (Horváth et al., 2010), and they have wide applications in the food flavoring and preservation industries (Bajpai et al., 2012). Additionally, they have been shown to exhibit various interesting bioactivities including antifungal, antiviral, antiparasitic, insecticidal, antispasmodic (Pavlović et al., 2012) and anticancer activities (Yu et al., 2011). Moreover, EOs have the potential of offering several advantages over synthetic drugs as they are made up of naturally occurring chemical constituents that do not cause long-term genotoxic risk.
Toona sinensis (A. Juss.) Roem. (Meliaceae), commonly known as Chinese mahogany cedar or Chinese Toona, is a perennial deciduous arbor widely distributed in China. The tender leaves of Toona sinensis (TSL) are used as a nutritious vegetable and flavoring of food products in China. And they possess anti-inflammatory, antidepressant, and worm-killing effects and are used in folk medicine for the treatment of enteritis, dysentery, carbuncles, boils, dermatitis, scabies, tinea blanca (Chang et al., 2006), and especially abdominal tumors (Chang et al., 2002). Currently, pharmacognostic and phytochemical studies have shown the presence of several types of phytochemicals in this plant, including flavonoids, limonoids, phytols and coumarins in this plant (Cheng et al., 2009). It has also been reported that TSL possesses a wide range of biological/pharmacological activities such as anti-cancer (Chang et al., 2002, 2006; Chen et al., 2009; Chia et al., 2010; Yang et al., 2010b), anti-inflammation (Hseu et al., 2011), anti-diabetes (Hsieh et al., 2012) and antioxidant activities (Hseu et al., 2008); it also inhibits leydig cell steroidogenesis (Poon et al., 2005) and SARS coronavirus replication (Chen et al., 2008). However, these studies mainly focus on the high or mid-high polar extracts of TSL. Recently, the volatile compounds of TSL obtained through microwave-assisted extraction and headspace solid-phase micro-extraction have been determined by Mu et al. (2007). Additionally, the non-polar TSL extract prepared by supercritical-CO2 fluid has been shown to prevent the progression of diabetes and hepatotoxicosis (Hsieh et al., 2012). Although the chemical composition and DPPH scavenging activity of TSL-EO, separated by simultaneous distillation solvent extraction, was studied by Chen et al. (2013), little is still known about the bioactivities of TSL-EO such as antibacterial and anticancer activities.

Therefore, our present study was conducted to investigate the chemical composition of TSL-EO by a GC–MS method, the antibacterial activity against several strains of Staphylococcus aureus, including two standard strains viz. ATCC 25923 and ATCC 43300, and 20 clinically isolated strains (SA 1–20) of Staphylococcus aureus, as well as cytotoxicity against three cancer cell lines, namely HepG2, SGC7901, and HT29. Moreover, the apoptosis-inducing activity in SGC7901 was tested by Hoechst 33324 staining and flow cytometry methods. Also, the expression of pro-apoptotic Bax, caspase-3 and anti-apoptotic Bcl-2 protein was detected by Western blotting.

2. Materials and methods

2.1. Plant material and chemicals

Fresh leaves of Toona sinensis were collected in October 2010 from Fuzhou, Fujian Province, China. The plant was identified by Associate Professor Cheng Zi Yang of the Pharmaceutical College, Fujian University of Traditional Chinese Medicine. A voucher specimen was deposited at Academy of Integrative Medicine, Fujian University of Traditional Chinese Medicine.

Anhydrous sodium sulfate and n-hexane were purchased from Sinopharm Chemical Reagent, Co., Ltd. (Shanghai, China). Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT), paraformaldehyde (PFA) and the antibiotic oxacillin (OKA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS), Dulbecco’s Modified Eagle Medium (DMEM), trypsin, and penicillin–streptomycin were obtained from Hyclone Laboratories, INC. (Logan, UT, US). Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit was purchased from Nanjing KeyGen Biotech CO., Ltd. (Nanjing, China). Hoechst 33324 staining kit was from Beyotime Institute of Biotechnology (Beijing, China). Caspase-3 antibody was from GeneTex Inc. (Irvine, CA, USA). Bcl-2, Bax and β-actin antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). n-Alkanes C8-C40 were purchased from Accu-Standard, Inc. (New Haven, CT, USA). All other chemicals were of the highest purity available in China.

2.2. Chemical preparation

TSL-EO was extracted from air-dried leaves by hydro-distillation in an all-glass Clevenger’s apparatus. The leaves (300 g) were boiled with distilled water (3000 ml) for 4 h in a 5 l round bottom flask fitted with a condenser. The obtained distillate was extracted with n-hexane, then dried with anhydrous sodium sulfate and filtered. The solvent was evaporated using a rotary vacuum evaporator. The yield was determined by weighing the remaining oil on an analytical balance. The resulting TSL-EO was stored at 4 °C prior to further analyses.

2.3. GC–MS analysis

The essential oil was analyzed using an Agilent-Technologies Agilent 7890 N/5973 N Network GC system equipped with a flame ionization detector and HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm. Agilent-Technologies, Little Falls, CA, USA). The injector and detector temperatures were set at 300 °C and 290 °C, respectively. The oven temperature program started at 60 °C for 2 min and then increased at a rate of 5 °C/min up to 135 °C, retained at this temperature for 10 min and increased again at a rate of 20 °C/min up to 260 °C, where it remained for 4 min. The flow rate of the carrier gas (helium) was 1.0 ml/min. TSL-EO was dissolved in diethyl ether, a sample of 1.0 µl was injected using split mode (split ratio, 5:1). The ionizing energy was 70 eV. Electron multiplier voltage was obtained from autotune. All data were obtained by collecting the full-scan mass spectra within the scan range of 40 to 650 amu. The composition was reported as a relative percentage of the total peak area. The identification of the essential oil constituents was based on a comparison of their retention times to n-alkanes (C8-C40). Compounds were further identified and authenticated using their mass spectra compared to data in the NIST Standard Reference Database.

2.4. Antibacterial activity against Staphylococcus aureus

2.4.1. Microbial strains

Strains of Staphylococcus aureus were provided by Municipal Center for Disease Control and Prevention, Fuzhou, China. Twenty clinically isolated strains of Staphylococcus aureus (SA 1–20), which were either methicillin-sensitive Staphylococcus aureus (MSSA) or methicillin-resistant Staphylococcus aureus (MRSA) and two standard strains viz. ATCC 25923 (MSSA) and ATCC 43300 (MRSA) were used.

2.4.2. Determination of the minimum inhibitory concentrations (MIC)

The antibacterial activities of the TSL-EO were determined on a number of Staphylococcus aureus strains. Antibacterial minimum inhibitory concentrations (MIC) were determined in Mueller-Hinton broth (MH) using the broth dilution method as previously described (Peng et al., 2011). Dilutions of the TSL-EO were prepared as follows: 4, 2, 1, 0.5, 0.25, 0.125 and 0.0625 mg/ml. Oxacillin (OKA) was used as positive control and prepared as follows: 1024, 512, 256, 128, 64, 32, 16, 8, 4, 2, 1 and 0.5 µg/ml. The sterile broth employed for sample dilution was supplemented with 1% DMSO to enhance solubility. MIC values were taken as the...
lowest concentration of TSL-EO that prevented any visible bacterial growth after 24 h of incubation at 37 °C. Each experiment was repeated three times.

2.4. Dynamic bacterial growth assay

Dynamic bacterial growth assay was performed in order to investigate the inhibitory effect of TSL-EO on bacterial growth as described by Wu et al. (2013). According to the results of MIC test, the concentrations used in the study of TSL-EO and OXA were a quarter of their MIC. The TSL-EO and OXA were added to bacterial suspension (1.0 × 10^6 CFU/ml) and incubated aerobically at 37 °C in a slow, shaking rock bed for 24 h. Growth rate was calculated by determining the absorbance at the wavelength of 600 nm (OD_600) using an ELISA reader (BioTek, Model ELX800, USA) at regular intervals. Each experiment was repeated in triplicate.

2.5. MTT assay

Human liver carcinoma cell lines HepG2, human gastric carcinoma cell lines SGC7901, human colon carcinoma cell lines HT29 and human umbilical vein endothelial cells (HUVECs) were obtained from the American type culture collection (ATCC, Manassas, VA, USA), and they were cultured in DMEM supplemented with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin in a 37 °C humidified incubator with 5% CO₂ atmosphere. The growth inhibitory effect of TSL-EO was measured using the standard MTT assay (Yu et al., 2011). Briefly, cells with a density of 1 × 10^3 cells/ml were plated and permitted to adhere in 96-well plates for 24 h, and then treated with various concentrations of TSL-EO for a further 24 or 48 h, while treatment with 0.5% DMSO was used as vehicle control. At the end of the treatment, 20 μl MTT (5 mg/ml in PBS) was added to each well, and the samples were incubated for an additional 4 h at 37 °C. The purple-blue MTT formazan precipitate was dissolved in 100 μl DMSO after removal of the medium. The absorbance was measured at the wavelength of 570 nm using an ELISA reader (BioTek, Model ELX800, USA). Cell viability was expressed as a percentage relative to the untreated controls.

2.6. Nuclear staining with Hoechst 33324

SGC7901 cells from exponential phase cultures (1 × 10^6 cells/ml) were treated with TSL-EO at the concentrations of 0, 50, 100 or 200 μg/ml for 24 h. After washing twice with ice-cold PBS buffer (0.01 mol/l, pH 7.4) and fixed with 3.7% PFA in PBS for 10 min at room temperature, the fixed cells were washed with PBS and stained with DNA-binding fluorescent dye, Hoechst 33324 (5 μg/ml), for 15 min at room temperature. The stained cells were then observed under an inverted fluorescence microscope (Olympus IX70, Tokyo, Japan).

2.7. Detection of apoptosis by flow cytometry analysis

After incubation with different concentrations of TSL-EO (0, 50, 100 and 200 μg/ml) for 12 h, apoptosis of SGC7901 cells was determined by flow cytometry analysis using a fluorescence activated cell sorting (FACS) caliber (Becton Dickinson, CA, USA) and Annexin V-FITC/PI kit. Staining was performed according to the manufacturer’s instructions. The percentage of cells in early apoptosis was calculated by Annexin V-positivity and PI-negativity, while the percentage of cells in late apoptosis was calculated by Annexin V-positivity and PI-positivity.

2.8. Western blotting analysis

The SGC7901 cells were harvested and washed twice with ice-cold PBS after treatment with different concentrations of TSL-EO (0, 100 and 200 μg/ml) for 24 h and then lysed with mammalian cell lysis buffer containing protease and phosphatase inhibitors. After centrifugation at 11000 rpm at 4 °C for 20 min, the supernatant was collected and the protein concentration was determined using Bradford assay. Equal amounts of denatured protein were separated on 12% SDS-PAGE gels and transferred onto PVDF membranes, which were blocked with 5% skimmed milk and probed with primary antibodies against Bcl-2, Bax, caspase-3 and β-actin (1:1000) over night at 4 °C, and then with appropriate horseradish peroxidase conjugated secondary antibody for at least 1 h followed by enhanced chemiluminescence detection using a ChemiDoc XRS imaging system (Bio-Rad Laboratories, Hercules, CA).

2.9. Statistical analysis

All data are presented as mean ± standard deviation of three determinations. Statistical comparison was performed via a one-way analysis of variance followed by Dunnett’s test by using SPSS software version 18.0 (SPSS, Chicago, IL, USA). A value of *P < 0.05 was considered to be statistically significant.

3. Results

3.1. Chemical compositions of the essential oil

TSL-EO was obtained by hydro-distillation with a yield of 0.975% (w/w) based on its dry weight. The composition of TSL-EO was determined by GC–MS, which is presented together with the retention indices in Table 1. The analyses of TSL-EO revealed the presence of 35 components, representing 90.95% of the total essential oil, of which the major components were caryophyllene (13.18%), β-caryophyllene (10.16%), copaene (8.27%), and β-eudesmen (5.06%). The components of TSL-EO were divided into four classes, sesquiterpene hydrocarbons (84.64%), oxygenated sesquiterpenes (1.33%), alcohols (2.99%) and esters (1.99%). Sesquiterpene hydrocarbons were found to be the main components of TSL-EO.

3.2. Antibacterial activity against Staphylococcus aureus

By using the standard broth microdilution method, the antimicrobial activity of TSL-EO was assessed against 20 clinically isolated strains of Staphylococcus aureus (SA 1–20), which were either methicillin-sensitive Staphylococcus aureus (MSSA) or methicillin-resistant Staphylococcus aureus (MRSA) and two standard strains viz. ATCC 25923 and ATCC 43300 were used. The minimum inhibitory concentrations (MIC) of TSL-EO for the tested Staphylococcus aureus are shown in Table 2. According to the results, TSL-EO showed antimicrobial activity against MSSA and MRSA strains with the lowest MIC value of 0.125 and 1 mg/ml, respectively, except for SA-17 with an MIC value > 4 mg/ml.

To confirm the antibacterial effect of TSL-EO, two standard Staphylococcus aureus viz. ATCC 25923 (MSSA) and ATCC 43300 (MRSA), were employed for observing dynamic bacterial growth. Fig. 1A and B shows the results of dynamic bacterial growth assay of TSL-EO against ATCC 25923 and ATCC 43300. In this test, the TSL-EO with concentrations of 0.125 and 1 mg/ml were used in the determination for the growth inhibitory effect against ATCC 25923 and ATCC 43300, respectively, according to the result of MIC test. The concentrations of positive control OXA used were
The results of our present study showed that TSL-EO presented antibacterial activity against *Staphylococcus aureus*.

### 3.3. Cell viability analysis

In order to evaluate the effect of TSL-EO on the cell viability of human cancer cell lines, MTT assay was conducted on three cancer cell lines, namely HepG2, SGC7901, and HT29. As shown in Fig. 2A, it was found that TSL-EO treatment resulted in a significant dose-dependent inhibition of the growth of all three cancer cells (the IC50 values of SGC7901, HepG2 and HT29 were 70.38, 82.2 and 99.94 μg/ml, respectively). And SGC7901 cells were shown to be more sensitive to TSL-EO than the other two cancer cell lines.

To determine the cytotoxicity of TSL-EO against normal cells, HUVECs were used as control. As shown in Fig. 2B, no significant cytotoxic effect was observed against HUVECs, when treated with no more than 100 μg/ml of TSL-EO for 24 h (P > 0.05). However, the viability of HUVECs was inhibited evidently by TSL-EO, when exposed to no less than 50 μg/ml of TSL-EO for 48 h (P < 0.01).

### 3.4. Pro-apoptotic effect of TSL-EO in SGC7901 cells

In order to determine whether the cytotoxic effect of TSL-EO was due to apoptosis, SGC7901 cells were stained with the cell-permeable DNA dye Hoechst 33342 to examine the nuclear morphological changes in response to TSL-EO treatment and

### Table 1
Chemical composition of TSL-EO detected by GC–MS.

| Component | RT/min | KRI  | Content/% |
|-----------|--------|------|-----------|
| δ-elemene | 17.788 | 1137 | 3.89      |
| α-cubebene | 18.291 | 1349 | 3.07      |
| Copaene   | 19.47  | 1377 | 8.27      |
| β-bourbonene | 19.808 | 1393 | 2.95      |
| elemene   | 20.166 | 1409 | 10.16     |
| Caryophyllene | 20.884 | 1421 | 13.18     |
| β-cubebene | 21.794 | 1442 | 0.95      |
| 1,5,9,9-tetramethyl-, Z,Z,Z-1,4,7-Cycloundecatriene | 22.476 | 1452 | 4.21      |
| (E)-b-farnesene | 23.145 | 1456 | 3.12      |
| γ-cadinene | 24.06  | 1475 | 1.33      |
| α-muurolene | 24.24  | 1479 | 0.57      |
| β-eudesmene | 24.531 | 1485 | 5.06      |
| γ-selinene | 24.972 | 1494 | 4.45      |
| α-muurolene | 25.221 | 1500 | 1.14      |
| cis-α-Bisabolene | 25.393 | 1503 | 0.93      |
| β-Bisabolene | 25.717 | 1508 | 4.81      |
| δ-cadinene | 26.4   | 1520 | 4.63      |
| Cadine-1,4-diene | 26.793 | 1527 | 0.52      |
| (E,E)-germacrene B | 28.027 | 1548 | 0.44      |
| Caryophyllene oxide | 29.592 | 1575 | 1.33      |
| α-elemene   | 32.654 | 1623 | 0.71      |
| Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene | 33.992 | 1643 | 1.47      |
| 1,2,4a,5,6,8a-hexahydro-3,6,11-dimethyl-1-(1-methylethyl)-cyclohexadiene | 35.074 | 1659 | 2.01      |
| β-vatirenene | 39.935 | 1828 | 0.32      |
| cis-nerolidol | 40.128 | 1847 | 1.05      |
| 8,9-dehydro-cyclosolongifolene | 40.28 | 1862 | 0.35      |
| Isolongifolene, 9,10-dehydro- | 40.901 | 1929 | 1.44      |
| Dibutyl phthalate | 42.183 | 2120 | 1.94      |
| α-Aromadendrene | 42.038 | 2094 | 0.36      |
| Hydroxyisovalerate | 45.203 | 2559 | 1.01      |

**Grouped components/%**

Sesquiterpene hydrocarbons: 84.64
Oxygenated sesquiterpenes: 1.33
Alcohols: 2.99
Esters: 1.99
Total: 90.95

### Table 2
Determination of the MIC of TSL-EO against *Staphylococcus aureus*.

| Strains | MIC TSL-EO (mg/ml) | MIC OXA (μg/ml) |
|---------|-------------------|-----------------|
| SA-1(S) | 0.5               | < 0.5           |
| SA-2(R) | 2                 | 64              |
| SA-3(S) | 0.25              | 1               |
| SA-4(S) | 0.25              | < 0.5           |
| SA-5(S) | 0.5               | 1               |
| SA-6(S) | 1                 | 2               |
| SA-7(S) | 0.25              | < 0.5           |
| SA-8(R) | 2                 | 128             |
| SA-9(S) | 0.5               | 1               |
| SA-10(R) | 2             | 256             |
| ATCC 25923(S) | 0.5     | 0.5           |

*The Staphylococcus aureus strains were either methicillin-resistant Staphylococcus aureus (R: MRSA) or methicillin-sensitive Staphylococcus aureus (S: MSSA), including two standard strains viz. ATCC 25923 (S) and ATCC 43300 (R), and 20 clinically isolated strains (SA 1–20). Oxacillin (OXA) was used as the positive control. The concentration of the bacterial suspension was 1.0 × 10^8 CFU/ml.*
visualized by fluorescent microscopy (as shown in Fig. 3A). Faintly stained, round and intact nuclei were observed in the control group, indicating typical living cells. Meanwhile, the TSL-EO-treated groups (50, 100, 200 μg/ml) presented gradually increasing degree of apoptosis as well as the number of apoptotic cells with some evident morphological changes, including improved brightness, reduced cellular volume and condensed chromatin or fragmented nuclei, suggesting that TSL-EO exhibited significant apoptosis-inducing activity against SGC7901 cells in a dose-dependent manner.

To quantify the extent of apoptosis, SGC7901 cells that had been treated with indicated concentrations of TSL-EO for 12 h were stained with Annexin V-FITC/PI followed by flow cytometry analysis (Fig. 3B). In Fig. 3B, LL (lower left) represented the living cells, UR (upper right) referred to the early apoptosis cells and LR (lower right) indicated the late apoptosis. It was found that the number of cells in UR and LR gradually increased with the increase of TSL-EO concentration, particularly when high dosage (200 μg/ml) was used. The percentage of cells undergoing apoptosis following treatment with 0, 50, 100 and 200 μg/ml of TSL-EO for 12 h (including the early and late apoptotic cells) was 4.84, 7.75, 13.94 and 45.79%, respectively (Fig. 3B). Therefore, it was concluded that TSL-EO promoted SGC7901 cell apoptosis in a dose-dependent manner.

To confirm whether caspase-dependent apoptotic activity could be modulated by TSL-EO, SGC7901 cells were treated with increasing concentrations of TSL-EO for 24 h. The expressions of Bcl-2, Bax and caspase-3 were determined by western blot analysis. The result showed that the expressions of pro-apoptotic protein Bax and caspase-3 were up-regulated, whereas anti-apoptotic protein Bcl-2 was down-regulated. It was suggested that TSL-EO could elicit apoptosis in SGC7901 cells through caspase-3 pathway (Fig. 4A and B).

4. Discussion

Owing to its unique scents and flavors, TSL has been commonly used as a popular traditional spice in the Chinese cuisine. TSL-EO, as extracted by hydro-distillation and analyzed by GC–MS in the current study, was found to be rich in sesquiterpene hydrocarbons (84.64%), including caryophyllene (13.18%), β-caryophyllene (10.16%), copaene (8.27%), and β-eudesmene (5.06%) (see Table 1).
Recently, both Mu et al. (2007) and Chen et al. (2013) have reported that caryophyllene was the main component of the total volatile compound, representing 21.422% and 19.51%, respectively, a result that was partially in line with ours. And TSL-EO separated by Chen et al. was also found to be rich in sesquiterpenes. However, there were significant differences in certain components in Mu et al.’s study. For example, 2-Hexenal and 5-Ethyl thiazole were detected as the major constituents, representing 14.678% and 11.806% of the total volatile compound, respectively, but they were not detected in our work. These differences were probably due to differences in the original material, extraction process and detection conditions.

In recent years, food-borne disease, referred to as food poisoning, has attracted considerable attention (Vinayaka and Thakur, 2010). Staphylococcus aureus, representative of the important pathogenic bacteria, plays a crucial role in the cause of food contamination by producing toxin, thus resulting in increased number of cases of food poisoning or food-borne illness in many countries (Scott, 2003). In order to control food-borne pathogenic bacteria, natural antimicrobial substances such as EOs are
proposed to be applied in view of their relative safety (Bajpai et al., 2013). Our previous study was performed to determine the antibacterial activity of TSL-EO against a number of Staphylococcus aureus strains, including two standard strains (SA 1–20), 20 clinically isolated strains (SA 1–20). As shown in Table 2, the results indicate that TSL-EO could inhibit the growth of both MSSA and MRSA. According to the result of the MIC test, the growth inhibitory effects of TSL-EO were further demonstrated by dynamic bacterial growth assay (Fig. 1A and B). It was suggested that the high concentration of sesquiterpenes was probably due to the presence of the high content of sesquiterpene compounds in TSL-EO, such as α-caryophyllene (Azaz et al., 2002) and caryophyllene oxide (Magiatis et al., 2002).

Accumulated evidence has shown that uncontrolled cell proliferation and insufficient apoptosis would eventually result in a net increase in the number of cancer cells, and thus are strongly associated with the development and progression of cancer (Mattern and Volm, 2004). Herein, inhibition of cell proliferation and/or induction of apoptosis in cancer cells to remove cells generated in excess are considered an effective means to fight cancer. To date, a number of anti-cancer agents, such as taxol, etoposide and ceramide, have been demonstrated to exert therapeutic effects by inducing or promoting apoptosis (Boldt et al., 2002). Apoptosis, which means programmed cell death, is a physiological process characterized by typical morphological and biochemical hallmarks, including cell shrinkage, nuclear DNA fragmentation and membrane blebbing (Fulda and Debatin, 2006). The mitochondrial pathway is documented as the most important apoptotic mechanism involving release of mitochondrial cytochrome-C, activation of caspase-9 and in turn caspase-3, and eventually causing apoptotic cell death (Lavrik et al., 2005). Caspase-3, one of the key protagonists of apoptosis, has been shown to partially/ completely account for the proteolytic cleavage of many key proteins (Kim et al., 2012). Bcl-2 family proteins, including Bax and Bcl-2, play a pivotal role in the induction of caspase-3 activation and regulation of apoptosis (Zhong et al., 2011). A higher Bcl-2/Bax ratio due to the over-expression of anti-apoptotic proteins such as Bcl-2 and/or the down-regulation or mutation of pro-apoptotic proteins such as Bax confers a survival advantage and resistance to apoptosis to cancer cells (Cai et al., 2012). With the purpose of examining the anticancer activity of TSL-EO, three cancer cell lines, namely HepG2, SGC7901 and HT29, were used in our study. As shown in Fig. 2A, the viability of all three cancer cell lines was markedly suppressed (P < 0.05), among them SGC7901 was found to be the most susceptible, with an IC50 value of 70.38 μg/ml for 24 h. Intriguingly, TSL-EO at a concentration of not more than 100 μg/ml did not present significant cytotoxicity against HUVECs (Fig. 2B). Based on above results, SGC7901 cells, the most sensitive cell lines, were used to confirm whether the cytotoxicity was attributable to apoptosis. Thus, a qualitative and quantitative evaluation of apoptosis was performed using Hoechst 33342 staining and flow cytometry technique, respectively, the mechanisms of both are based on the differential uptake of fluorescent dyes (Wijesinghe et al., 2013). Our results showed that treatment of SGC7901 cells with TSL-EO resulted in the appearance of the characteristic features of apoptosis, such as nuclear condensation and apoptotic body formation (Fig. 3A). The percentage of apoptotic cells in the experimental groups increased compared with the control group in a dose-dependent manner (Fig. 3B). Moreover, the apoptosis-inducing activity of TSL-EO was mediated through caspase-3 pathway by up-regulation of Bax, caspase-3, and down-regulation of Bcl-2 (Fig. 4). Recently, it was reported that sesquiterpenes such as caryophyllene and α-caryophyllene were shown to exhibit antiproliferative activity against cancer cells, whereas monoterpenes such as β-pinene, γ-terpinene and p-cymene were interesting inactive. Furthermore, EO with the higher content of sesquiterpene hydrocarbons was documented to possess stronger antiproliferative activity against M14 human melanoma cells (Cardile et al., 2009). Therefore, it was suggested that the high concentration of sesquiterpenes was probably responsible for the significant anticancer activity of TSL-EO.

Taken together, TSL-EO showed antibacterial activity, and significant cytotoxicity and apoptosis-inducing activity. The abundant sesquiterpene compounds in TSL-EO were mainly responsible for its bioactivities. From this point of view, TSL-EO possessed potential health benefits and could be used as a promising natural food additive.

5. Conclusion

Recently there has been considerable interest on the bioactive properties of essential oils due to their possible use as natural additives in replacement of synthetic drugs. And TSL has been serving as a special nutritious vegetable in China. In this respect, our recent work was performed to investigate the chemical composition, antimicrobial and anticancer activities of TSL-EO in vitro. The antibacterial activity of TSL-EO allowed its possible use in the prevention of Staphylococcus aureus infection. Furthermore, TSL-EO presented notable anticancer activity against three different cancer cells by suppressing cell viability and inducing apoptosis in SGC7901 cells. From this point of view, TSL-EO may be a potential natural food additive and can be put into pharmaceutical application for human health.

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