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Full Length Research Paper

Composition, *in vitro* antioxidant and antimicrobial activity of the essential oils from *Artemisia lavandulaefolia* DC.

Jiwen Zhang[#], Baojun Shi[#], Peng Gao, Junru Wang* and Wenjun Wu

Institute of Pesticide Science and College of Sciences Northwest A&F University, Yangling, Shaanxi 712100, China.

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The essential oils from the aerial parts of *Artemisia lavandulaefolia* DC., a traditional medicinal plant growing in China, were obtained by hydro distillation and analyzed by gas chromatograph and mass spectrometer (GC-MS). Fifteen monoterpenes, six sesquiterpenes, three monoterpenoid acetates with two aromatic compounds and two others were identified among twenty-eight compounds, representing 99.69% of the total oils. Eucalyptol (20.62%), Borneol (15.32%), Eudesm-7(11)-en-4-ol (13.81%), Sabinol (9.75%), β-Terpineol (6.86%), trans-Pinocarvyl acetate (6.13%) and α-Terpineol (5.35%) were the major seven compounds in the essential oils. The essential oils displayed significant *in vitro* antimicrobial activities against *Bacillus subtilus*, *Pseudomonas aeruginosa, Bacillus cereus1* and *Staphylococcus aureus* with minimum inhibitory concentration values range of 125 to 250 µg/ml. The *in vitro* antioxidant activities were assessed by DPPH radical scavenging and inhibition of lipid peroxidation methods. The oils showed a remarkable free radical scavenging activities, as evidenced by the low IC_{50} value for DPPH radical (275.7 µg/ml) and inhibition of lipid peroxidation (induced by $FeSO_4$, H_2O_2 and CCl_4) with IC_{50} values of 393.6, 562.8 and 297.2 µg/ml, respectively.

Key words: *Artemisia lavandulaefolia* DC., antioxidant activities, antimicrobial activities, essential oils.

INTRODUCTION

The Duanwu Festival (端午节) also known as Dragon Boat Festival is a traditional and statutory holiday associated with Chinese cultures. It is celebrated not only in China but also in other Asian countries, such as Japan, Korea, Singapore, Malaysia and Vietnam. Hanging *Artemisia lavandulaefolia* DC. near the doors and windows is popular along with eating *ZongZi*, (large rice wraps) and racing dragon boats on the Duanwu Festival. Chinese people believe that *A. lavandulaefolia* DC. was a very supernatural plant that can exorcise and help people keep in good health, drive mosquito and flies away, and keep the environment clean. According to the literature this herb was used widely in Chinese traditional medicine and it can clear away heat, detoxify and relieve coughs and inflammation (Jiang et al., 2008; Xu et al., 2010; Wang et al., 2011).

In this paper we described the chemical composition of the essential oils isolated from the aerial parts of *A. lavandulaefolia* DC. and its *in vitro* antioxidant and antimicrobial activities were evaluated.

MATERIALS AND METHODS

Plant material

The aerial parts of *A. lavandulaefolia* DC. were collected from Yangling, Shaanxi Province (China) on the Duanwu Festival (May 28th) 2009 and identified by Dr. Wenquan Yang of College of Life Science Northwest A & F University. The voucher specimen was deposited at the College of Life Science, Northwest A & F University (Figure 1). The aerial parts were dried in the shade (at room temperature).

Extraction of oils

The air-dried aerial parts of the plants (100 g) were powdered and the volatile fraction was isolated by hydro distillation to give a

*Corresponding author. E-mail: nwzjw@yahoo.com.cn.

# These authors contributed equally to this work.

yellow-green essential oils (0.95 g). The oils were stored in sealed vial at 4°C for further testing.

Gas chromatography/mass spectrometry (GC/MS) analysis

The essential oils were analyzed by GC and GC/MS. GC-MS was performed with a Finnigan Trace DSQ GC-MS spectrometer (Thermo Company, US) employing the electron impact (EI) mode (ionizing potential 70 eV) and a capillary column (30 m × 0.25 mm, film thickness 0.25 µm) packed with 5% phenyldimethylsilicone on HP-5 (Hewlett-Packard, Palo Alto, CA). Ion source temperature was 280°C. The GC settings were as follows: the initial column temperature was set at 40°C and held isothermal for 1 min; the temperature was programmed from 40 to 220°C at a rate of 3°C/min, and was kept 220°C for 25 min, then improved to 280°C at 5°C/min and hold for 10 min. The oven temperature was 280°C. Helium was used as the carrier gas, flow rate 1 ml/min. Split ratio 1:50.

Identification of components

Identification of individual compounds was based on comparison of their retention indices (RI) and mass fragmentation patterns with those on the stored NIST2001 mass spectral library. The relative proportions of the essential oils constituents were expressed as percentages obtained by peak area normalization, all relative response factors being taken as one.

Antimicrobial activity

Microorganisms tested including *Bacillus cereus1* 1846, *Bacillus subtilus* 1.88, *Staphylococcus aureus* 1.89 and *Pseudomonas aurginosa* 1.203.1 were obtained from General Microbiological Culture Collection Center, Beijing, China (CGMCC), all the strains were maintained on nutrient agar at 4°C and were subcultured every month in our laboratory.

Antibacterial activities of the essential oils were tested by minimum inhibitory concentration (MIC). The MIC of the oils was determined by a dilution method in 96-well culture plates using Mueller-Hinton broth (MHB), according to the Standard of National Committee for Clinical Laboratory. The oils were two-fold serially diluted with dimethylsulfoxide (DMSO)-water solution (1%, v/v) to obtain concentrations from 10 to 0.625 mg/ml. 10 µl of the serially diluted essential oil was added to 80 µl nutrient broth in each well. 10 µl of 10^6 bacterial suspensions were added to the well. The 96-well culture plates were incubated at 37°C for 24 h. The lowest concentrations of the essential oils that inhibited the bacterial growth after 24 h were recorded as the minimum inhibitory concentration (MIC). In order to ensure that the solvent had no effect on bacterial growth, a control test was also performed containing broth supplemented with dimethyl sulfoxide (DMSO)–water solution (1%, v/v) at the same dilution as that used in the assay. Each experiment was performed in triplicate (Bozin et al., 2006).

DPPH radical scavenging assay

The antioxidant activities were determined by a modification of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method of Yamaguchi et al. (1998). Each sample (20 µl) was mixed with 900 µl of 100 mM Tris-HCl buffer (pH 7.4) and added to a mixture of 30 µl of ethanol and 50 µl of 0.5% (w/w) Tween 80 solution. This mixture was then added to 1 ml of 0.5 mM DPPH in ethanol (250 µM in the reaction mixture). Tween 80 was used as an oil in water emulsifier. The mixture was shaken with a mechanical shaker and left to stand for 30 min at room temperature in the dark. DPPH is a stable free radical and has a dark violet color. The absorbance was measured at 517 nm against the corresponding blank, the synthetic antioxidant reagent tert-Butylhydroquinone (TBHQ) was used as a positive control and all tests were carried out in triplicate.

Lipid peroxidation inhibition (LPO)

Two adult Sprague dawley male rats were fasted overnight and sacrificed by cervical dislocation, dissected and the abdominal cavity per fused with 0.9% saline. Whole liver was removed and a weighed amount processed to obtain a 10% homogenate in cold phosphate buffered saline (pH 7.4) (PBS). The degree of lipid peroxidation was assayed by estimating the thiobarbituric acid reactive substances.

LPO induced in $FeSO_4$ system

lipid peroxidation (LPO) was induced by addition of 100 µl of 0.5 mM $FeSO_4$ in the reaction mixture containing 400 mg of the chopped liver tissues in 3.9 ml phosphate buffered saline (PBS), and one of the concentrations (78.2, 156.3, 312.5, 625 and 1250 µg/ml) of the essential oils dissolved in distilled water (DW). After incubating at 37°C for 2 h, homogenate of chopped liver was centrifuged at 1200 g and the supernatant was used to measure LPO by the thiobarbituric acid (TBA) reaction method, as stated earlier. A control set was run in which all materials other than either $FeSO_4$ or essential oils were added. Five concentrations (2.5, 5, 10, 20 and 40 µg/ml of final volume) of TBHQ were used as a standard antioxidant for comparison.

LPO induced in H_2O_2 and CCl_4 system

A somewhat similar procedure to the aforementioned was repeated with H_2O_2 and CCl_4. In the H_2O_2 system, the reaction mixture contained 400 mg of the chopped liver tissues in 3.9 ml of PBS, 100 µl of 800 mM H_2O_2 and the test essential oils in 1 of the 5 concentrations (78.2, 156.3, 312.5, 625.0 and 1250.0 µg/ml, in triplicate). The mixture was incubated at 37°C for 2 h. Following the addition of trichloroacetic acid (TCA) and TBA, the optical density (OD) was measured at 532 nm. In the case of the CCl_4 system, the reaction mixture also contained 400 mg of the chopped liver tissues in 3.9 ml of PBS, 100 µl CCl_4 (1:4 in DMSO, v/v) and the test essential oils of equivalent concentration. Following the incubation at 37°C and the addition of TCA and TBA, OD was measured at 532 nm. The observed effects were compared with TBHQ.

Statistical analysis

Data were expressed as mean ± SE. Statistical analysis utilized analysis of variance (ANOVA) followed by student's t-test; *P* values of 5% and less were considered to be significant. Correlation between antioxidant activity was carried out using correlation and regression in the EXCEL program.

RESULTS AND DISCUSSION

The essential oils displayed significant *in vitro* antimicrobial activities against *B. subtilus*, *P. aeruginosa,*

Table 1. The essential oils components from *A. lavandulaefolia* DC.

Compounds	RI	Area (%)	Compounds	RI	Area (%)
Acetonyldimethylcarbinol	703	0.89	Germacrene D	1521	0.20
β-Phellandrene	1038	0.17	Sabinol	1542	9.75
Yomogi alcohol	1102	0.42	Caryophyllene oxide	1549	2.30
Eucalyptol	1229	20.62	allo-aromadendrene oxide	1563	0.46
Artemisia ketone	1297	1.60	Camphor	1568	3.61
β-Terpineol	1343	6.86	Eudesm-7(11)-en-4-ol	1569	13.81
4,9-Dodecanedione	1356	0.38	α-Cedrene	1573	0.62
Artemisia alcohol	1361	1.86	cis-Verbenol	1600	0.56
Trans-Pinocarvyl acetate	1378	6.13	Borneol	1634	15.32
Carvyl acetate	1414	0.23	Terpinen-4-ol	1654	2.33
Sabinene hydrate	1431	3.02	α-Terpineol	1690	5.35
Isobornyl acetate	1452	0.19	Carveol	1756	0.53
Chrysanthenone	1492	1.05	trans-Anethol	1945	0.62
Caryophyllene	1504	0.17	p-Allylanisole	1975	0.64

Results of the *in vitro* antimicrobial screening tests were shown in Table 2.

Table 2. The MIC (μg/ml) values of the essential oils.

S/N	Bacteria	Essential oils	Streptomycin
1	*Bacillus subtilus* 1.88	≤125	≤10
2	*Pseudomonas aeruginosa* 1.203.1	≤250	≤10
3	*Bacillus cereus1* 1846	≤125	≤10
4	*Staphylococcus aureus* 1.89	≤125	≤10

Table 3. Antioxidant activities of the essential oils.

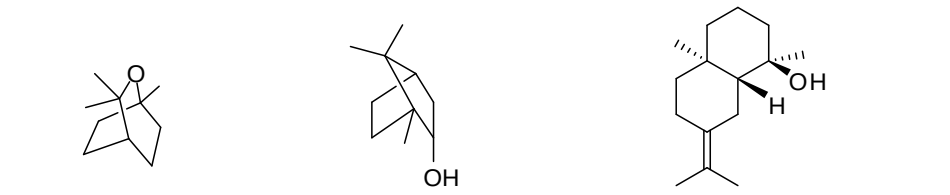
Samples	DPPH (IC_{50} μg/ml)	lipid peroxidation (IC_{50} μg/ml l)		
		$FeSO_4$	H_2O_2	CCl_4
Essential oils	275.7±25.8	393.6±86.9	562.8±210.9	297.2±60.5
TBHQ	1.94±0.19	3.4±1.2	18.6±5.2	12.1±4.2

Further, to assess the possible utilization of the essential oils from *A. lavandulaefolia* DC., its antioxidant activities were evaluated by comparing to the activities of known antioxidant TBHQ by the following four *in vitro* assays: inhibition of DPPH radical and lipid peroxides induced by $FeSO_4$, H_2O_2 and CCl_4. The results were tabulated in Table 3.

B. cereus1 and *S. aureus* with minimum inhibitory concentration values range of 125 to 250 μg/ml. Results of the *in vitro* antimicrobial screening tests were shown in Table 2. The *in vitro* antioxidant activities were assessed by comparing to the activities of known antioxidant TBHQ by DPPH radical scavenging and inhibition of lipid peroxides induced by $FeSO_4$, H_2O_2 and CCl_4. The oils showed a remarkable free radical scavenging activities, as evidenced by the low IC_{50} value for DPPH radical (275.7 μg/ml) and inhibition of lipid peroxidation (induced by $FeSO_4$, H_2O_2 and CCl_4) with IC_{50} values of 393.6, 562.8 and 297.2 μg/ml, respectively. The results were tabulated in Table 3. The chemical composition of the essential oils from *A. lavandulaefolia* DC. **(**Figure 2) is different from that which was reported earlier (Jiang et al., 2008; Cha et al., 2005;Gu et al., 1998) β-Caryophyllene (16.1%) is the main compound (Cha et al., 2005) on the other hand,Eucalyptol (20.62%) is the main compound in our plant.

Fifteen Monoterpenes, three Monoterpenoid acetates and six Sesquiterpenes with two aromatic compounds and two others among twenty-eight compounds, representing 99.69% of the total oils were identified (Table 1). The chemical composition of the essential oils from *A. lavandulaefolia* DC. is different from that which was reported earlier (Jiang et al., 2008; vCha et al., 2005;

Figure 1. Picture of *Artemisia lavandulaefolia* DC.



Eucalyptol 20.62% Borneol 15.32% Eudesm-7(11)-en-4-ol 13.81%

Figure 2. Structures of the main composition of the essential oils from *Artemisia lavandulaefolia* DC.

Gu et al., 1998) β-Caryophyllene (16.1%) is the main compound (Cha et al., 2005) on the other hand, Eucalyptol (20.62%) is the main compound in our plant.

Conclusion

The chemical composition of the essential oils from *A.*

lavandulaefolia DC. and its bioactivities is different depending on the place where the plant was grown.

ACKNOWLEDGEMENTS

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