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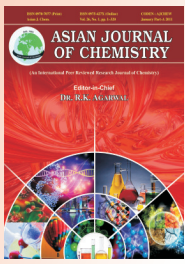

Chemical Composition and Antimicrobial Activities of Essential Oil of Baisu, named *Perilla frutescens* (L.) Britt.

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The essential oil was isolated by hydro-distillation from the *Perilla frutescens* (L.) Britt. The content and composition were analyzed by GC-MS. Thirty two different components were identified constituting approximately > 99 %. The major components were 1-(2-furyl)-1-hexanone (25.79 %), amylphenol (20.24 %), apiol (10.13 %) and *o*-xylene (9.33 %) *etc*. Among which, the apiol was detected from essential oil of *Perilla frutescens* leaves for the first time. The antifungal activity of the essential oil was tested by poisoned food technique against seven plant pathogenic fungi *viz Verticillium dahliae*, *Gibberella zeae*, *Glomerella cingulata*, *Thanatephorus cucumeris*, *Botrytis cinerea*, *Phytophthora capsici* and *Pyricularia oryzae*. The antibacterial activity of essential oil was tested by Oxford cup method against four bacteria *viz Staphyloccocus aureus, Salmonella typhimurium, Bacillus subtilis* and *Bacillus anthracis*. In general, the inhibiting ability of *Perilla frutescens* essential oil on plant pathogenic fungi was better than that on bacteria. Under the test concentration of 0.25 mg/mL, the inhibition rates of essential oil were above 50 % against all the seven fungi.

Keywords: Chemical components, Essential oil, Plant pathogens, *Perilla frutescens*.

INTRODUCTION

Perilla frutescens (L.) Britt. is an annual herb in the Perilla of Labiatae family[1]. It's a native plant of China and is called Baisu in Chinese, now grows in all the northeast provinces of China, as well as Hebei, Shanxi, Jiangsu, Anhui, Hubei, Sichuan, Fujian, Yunnan, Guizhou, *etc*. It also exists in Japan, North Korea and the northern part of India. *Perilla frutescens* has one variety named *Perilla frutescens* (L.) Britt. Var. *arguta* (Benth.) Hand-Maxx and called Zisu in China. Zisu seeds can be extracted for oil and used as medicine in combination with the stem together widely, which has been recorded by Pharmacopoeia of People's Republic of China[2]. For a long time, people applied Zisu as one medicinal herb and did deeply researches on it. Zisu have many active ingredients, in which the flavones has antipyretic, antimicrobial, antitumor, anticoagulant, hemostatic, antiinflammatory and antiallergic activities, as well as strong antioxidation[3-5].

The Baisu seeds contain rich amino acids and various mineral elements and the seeds and seed oil have good nutritional values as well as medicinal healthcare efficacy[6,7]. In addition, Baisu also has the effects of antimicrobial, antivirus and free radicals scavenging and inhibiting; therefore, it may replace the medicinal effects of Zisu in some degree[8,9]. Liu *et al*[9]. found that the aqueous and alcohol extracts of Baisu leaves had better fungal growth inhibiting activity than Zisu extract. But the antimicrobial activity of the Baisu essential oil has not been studied. In order to make use of the Baisu resource fully and provide a scientific basis for its application in antimicrobial, this study analyzed the chemical composition in the essential oil of Baisu leaves as well as determined its antimicrobial activities on seven phytopathogens and four bacteria.

EXPERIMENTAL

Plant material and extraction of essential oil: Baisu leaves were collected from Hubei Province, China, in September 2010. The leaves was dried at room temperature and stored in a refrigerator at 4 °C. 200 g of Baisu leaves was treated by supersonic for 20 min, then subjected to hydrodistillation for 4 h with 5000 mL distilled water. The oil-water mixture obtained was saturated with NaCl and then extracted with ethyl ether for three times. Solvent was evaporated by rotary evaporator machine to obtain essential oil. The oil yields were calculated on the basis of the dry plant weight from three replicates.

Microorganisms: The phytopathogens for the test included *Verticillium dahliae*, *Gibberella zeae*, *Glomerella cingulataa*, *Thanatephorus cucumeris*, *Botrytis cinerea*, *Phytophthora capsici* and *Pyricularia oryzae*. The strains were maintained on potato dextrose agar (PDA) medium slants in a refrigerator at 4 °C and were subcultured in Petri dishes prior at 25 °C for testing.

Test bacteria included *Staphyloccocus aureus*, *Salmonella typhimurium*, *Bacillus subtilis* and *Bacillus anthracis*. The strains were maintained on beef extract peptone medium (LB) medium slants in a refrigerator at 4 °C and were subcultured in Erlenmeyer flask prior at 37 °C for testing.

GC-MS analysis: The component analysis of the essential oil was carried out on a DSQ II GC-MS instrument (Thermo Inc.), using a TR-5MS capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness). Helium was used as carrier gas at the flow rate of 1 mL/min. The injection volume was 1 μL and the split ratio was 1:50. The initial column temperature was set as 40 °C for 1 min; then the temperature was raised to 100 °C at the rate of 3 °C/min and maintained for 10 min. After that the temperature was raised to 200 °C at the rate of 5 °C/min and maintained for 10 min. Finally the temperature was raised to 250 °C with the rate of 5 °C/min and maintained for 15 min. The interface temperature of the mass spectrometer was 280 °C and EI ionization was adopted. Mass spectra were recorded in the range 40-500 amu, operating at 70 eV and the ion source temperature was maintained at 280 °C. The constituents of the essential oil were identified by automatically retrieving through the NIST mass spectrogram library stored in the GC-MS database.

Antimicrobial assay: The antifungal activity of the essential oil was determined by the poisoned food technique[10]. The essential oil was prepared and got the serial required concentrations by using water as solvent and Tween-40 as emulsifier. The oil and melted PDA culture medium were mixed at the proportion of 1:19 and then poured into a plate to form the culture plate with oil. The final concentration of emulsifier was 0.01 % (m/m). When the medium in the plates solidified, each mycelia agar plugs (4 mm diameter) of test fungi cut from earlier subcultured Petri dishes was inoculated at the center of the solidified plate and the mycelia touched the medium directly. The control was treated with addition of equivalent emulsifier. All experiments were performed in triplicates and cultured in an incubator at 25 ± 2 °C with the lid of Petri dishes upside down. The antifungal activity was evaluated by measuring the diameter of fungi colonies after 72 h incubation and the inhibitory effect was calculated according to the following formula.

$$\text{Mycelial inhibition (\%)} = \frac{dc - dt}{dc} \times 100\ \%$$

where, dc, average diameter of fungal colony in control sets; dt, average diameter of fungal colony in treatment sets.

The antibacterial activity of the essential oil was determined by the Oxford cup method[11]. The essential oil of Baisu leaves was prepared and got the serial required concentrations of 2, 10 and 50 mg/mL by using water as solvent and Tween-40 as emulsifier. The Tween-40 concentration in the solutions was 0.2 % (m/m). Warm LB medium mixed 0.1 mL of diluted inoculum (105 CFU/mL) microorganism suspensions was poured into Petri dishes, then allowed to solidify. Sterilized Oxford cups (inner diameter 6 mm, outer diameter 8 mm, height 10 mm) was then placed on the solidified medium. Each Oxford cup was filled with 0.2 mL of essential oil solvent and equivalent amounts of solvent was set as control. The plates were incubated for 24 h at 37 °C. The antibacterial activity was evaluated by measuring the zone of inhibition against test microorganism. The experiments were repeated three times and the data were calculated as means ± SD.

RESULTS AND DISCUSSION

On the basis of the dry plant weight by triplicates, the oil yield of Zisu leaves was 0.5525 ± 0.0032 % (w/w). The major components in the essential oil of Baisu leaves were presented in Table-1. Thirty-two compounds were separated and identified, accounting for 99.8 % of the total amount of the compounds. The main compounds of Baisu leaves were 1-(2-furyl)-

TABLE-1
MAIN CHEMICAL COMPONENTS OF ESSENTIAL OIL FROM BAISU LEAVES

Peak number	RT	Name	m.f.	m.w.	Area (%)
1	7.52	4-Hydroxy-4-methyl-2-Pentanone	$C_6H_{12}O_2$	116	1.88
2	7.90	Ethylbenzene	C_8H_{10}	106	3.54
3	8.31	*o*-Xylene	C_8H_{10}	106	9.33
4	9.20	1,3-Dimethyl-Benzene	C_8H_{10}	106	2.31
5	15.16	Phenol	C_6H_6O	94	0.26
6	19.05	Linalool	$C_{10}H_{18}O$	154	0.74
7	25.10	Valeric acid, pent-2-en-4-ynyl ester	$C_{10}H_{14}O2$	166	0.60
8	26.59	4-(1,1-Dimethylpropyl)-phenol	$C_{11}H_{16}O$	164	0.42
9	29.27	1-(2-Furyl)-1-hexanone	$C_{10}H_{14}O_2$	166	25.79
10	29.37	3-(But-3-enyl)-cyclohexanone	$C_{10}H_{16}O$	152	0.29
11	32.88	Eugenol	$C_{10}H_{12}O_2$	164	1.00
12	33.82	Amylphenol	$C_{12}H_{20}$	164	20.24
13	38.61	Borneol	$C_{10}H_{18}O$	154	0.15
14	43.40	5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-Benzofuranone	$C_{11}H_{16}O_2$	180	0.20
15	43.85	Nerolidol	$C_{15}H_{26}O$	222	0.29
16	44.57	Caryophyllene oxide	$C_{15}H_{24}O$	220	2.64
17	45.78	Apiol	$C_{12}H_{14}O_4$	222	10.13
18	49.51	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	0.29
19	49.62	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	0.18
20	53.73	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	4.68
21	64.83	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	1.11
22	69.47	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	1.28
23	69.73	2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-Phenol	$C_{23}H_{32}O_2$	340	0.33
24	72.19	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	5.96
25	72.49	(3á,5à, 6á,7à)- Cholestane-3,6,7-triol	$C_{27}H_{48}O_3$	420	0.23
26	75.28	Nonacosane	$C_{29}H_{60}$	408	0.28
27	76.80	17-Pentatriacontene	$C_{35}H_{70}$	490	0.31
28	77.90	Hexacosanoic acid, methyl ester	$C_{27}H_{54}O_2$	410	0.17
29	78.70	1-Heptatriacotanol	$C_{37}H_{76}O$	536	0.22
30	78.76	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$	424	0.14
31	81.59	13,27-Cycloursan-3-one	$C_{30}H_{48}O$	424	0.18
32	83.76	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	$C_{31}H_{48}O_3$	468	4.63

1-hexanone (25.79 %), amylphenol (20.24 %), apiol (10.13 %) and *o*-xylene (9.33 %); all the four compounds accounted for 65.49 % of the total amount of the essential oil. These main components obtained in this study were basically consistent with the study results obtained by Qiu *et al.*[12] and Zeng *et al.*[13]. However, the content values were different and the apiol was detected in the essential oil of Baisu leaves for the first time. Among the 32 compounds, the ester compounds accounted for 13.7 % and oxygen compounds accounted for 63.9 %.

Determination of antimicrobial activity: The inhibiting effects of the essential oil of Baisu leaves on seven phytopathogens were determined by using the poisoned food technique[13] and the inhibiting effect was illustrated in Fig. 1. Under the minimal test concentration of 0.0625 mg/mL, Baisu oil had the best inhibiting effect on *Thanatephorus cucumeris* and the inhibition rate was 61.90 % at 72 h, which was higher than that on the other 6 fungi. Under the same concentration, the inhibiting effect on *Pyricularia oryzae* was the worst (3.11 %). When the test concentration reached 0.25 mg/mL, the inhibition rate on *Pyricularia oryzae* was just 59.32 %, which was lower than that on the other fungi. All the inhibition rates of the essential oil on test fungi gradually improved along with the increase of the test concentration. When the concentration of the essential oil reached 0.5 mg/mL, the growth of all the seven test fungi was inhibited completely.

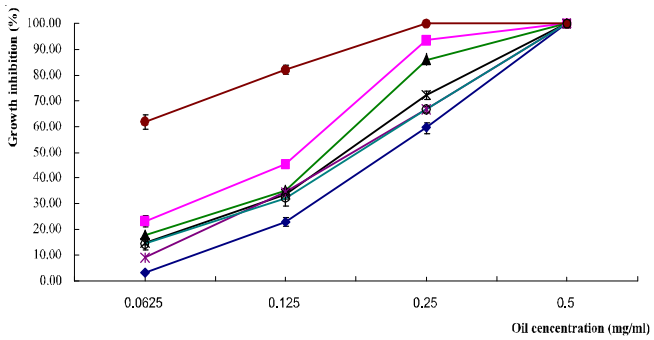

Fig. 1. Antifungal activity of essential oil from Baisu leaves against seven phytopathogens (significant difference at $p < 0.05$, ANOVA test); (□) *Pyricularia oryzae*, (■) *Botrytis cinerea*, (□) *Phytophthora capsicis*, (×) *Verticillium dahliae*, (·) *Glomerella cingulataa*, (•) *Thanatephorus cucumeris*, (o) *Gibberella zeae*. Error bars indicate the mean ± standard error

Inhibiting effects of the essential oil on the four bacteria were determined by using the oxford-cup method and the results were presented in Table-2. Under the three test concentrations, the essential oil of *Pyricularia oryzae* leaves had the best inhibiting effect on *Bacillus anthracis*, but there were no inhibiting effects on *Salmonella typhimurium* and *Staphylococcus aureus*. Under the test concentrations of 2 and 10 mg/mL, its inhibiting effect on *Bacillus subtilis* was not very good and the diameter of the inhibition zone was 0 mm-5 mm; when the test concentration was increased to 50 mg/mL, the diameter of the inhibition zone was still below 10 mm.

TABLE-2
ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL FROM BAISU LEAVES AGAINST FOUR BACTERIA

Concentration (mg/mL)	Inhibitory effect			
	S. aureus	*B. subtilis*	*B. anthraci*	*S. typhimurium*
2	-	+	+++	-
10	-	+	+++	-
50	-	++	+++	-

"-" means no inhibition zone; "+" means the diameter of inhibition zone is among 0 mm to 5 mm; "++" means the diameter of inhibition zone is among 5 mm to 10 mm; "+++" means the diameter of inhibition zone is larger than 10 mm

Conclusion

The results of this study showed that the essential oil of Baisu leaves had good inhibiting activities on both phytopathogens and some bacteria and may be used as an alternative of synthetic fungicides. Which was reported for the first tme. Chemical ingredients analysis indicated that 1-(2-furyl)-1-hexanone, amylphenol, apiol and *o*-xylene were the main components, which could be the antifungal ingredients. Identifying antimicrobial ingredients through further research and development will provide the necessary scientific basis for its application as a natural fungicide.

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