Variation in Chemical Composition and Antibacterial Activities of Essential Oils from Two Species of *Houttuynia* THUNB.

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***Houttuynia* THUNB. (Saururaceae) has been used for dozens of years in China for the treatment of cough, leucorrhea and ureteritis. The essential oils from the two species: *Houttuynia emeiensis* and *Houttuynia cordata* sold in China under one trade name 'Yuxingcao', obtained by hydrodistillation, were analyzed by GC-MS. The results show that fifty-five components were identified and methyl nonyl ketone (2.10—40.36%), bornyl acetate (0.4—8.61%) and β-myrcene (2.58—18.47%) were the most abundant components in oil, but the percentage of most of compounds in different species and parts varied greatly. The two fold broth dilution and agar dilution method were used to study essential oil of two *Houttuynia* THUNB. species for their antibacterial properties against microorganisms, *Staphylococcus aureus* and *Sarcina ureae*. The two fold dilution method was allowed to determine the minimum inhibitory concentration (MIC) of essential oil from different parts and species. Results showed that all essential oils possessed antibacterial effect, with MIC values in the range of 0.0625×10^{-3} to 4.0×10^{-3} ml/ml. However, essential oil from different parts and species differed clearly in their antibacterial activities. The essential oil from the aboveground part of the cultivated *Houttuynia emeiensis* exhibited higher activity than both parts of the wild and cultivated *Houttuynia cordata* when used on *Staphylococcus aureus* (MIC=0.25×10^{-3} ml/ml) and *Sarcina ureae* (MIC=0.0625×10^{-3} ml/ml), and had the same activity as the positive control ampicillin sodium.**

Key words *Houttuynia*; Saururaceae; essential oil; antibacterial; GC-MS; chemical component

Houttuynia THUNB. (Saururaceae) had a long history of use in the Chinese indigenous systems of medicine and was considered antiseptic, febrifuge, diuretic and deobstruan. It was frequently used in many traditional medicines for their antimicrobial, antiviral and anti-inflammatory properties.[1—4] The occurrence of the essential oil in this plant was associated with a possible role in HIV and SARS.[5,6]

According to Florae Reipublicae Popularis Sinicae and Flora Sichuanica, *Houttuynia* THUNB. had only one species *Houttuynia cordata* THUNB. (Yuxingcao in Chinese),[7,8] which was distributed throughout China and could be used as an edible and medicial plant. Recently, a new species *Houttuynia emeiensis* (locally called white Yuxingcao) was reported in China.[9] During our market surveillance, those two species have been sold in China under one trade name 'Yuxingcao', and almost all the commercial samples, either comprised *Houttuynia cordata* and *Houttuynia emeiensis* or mixture of *Houttuynia cordata* and *Houttuynia emeiensis*, either wild and cultivated or mixture of wild and cultivated, and either belowgound part and aboveground part or the whole plant were used as traditional medicines or material producing preparation. As a fact, those factors would influence the chemical composition and therefore the pharmacological activity of a herbal medicine, however, up to now, no work on pharmacological activity studies on those factors of *Houttuynia* THUNB. has been on record. This condition encouraged us to focus the studies on components and antibacterial properties of two species and different parts of wild and cultivated *Houttuynia* THUNB. The aim is to provide some help for drug using, plant resource protecting and cultivating.

Experimental

Plant Material The cultivated *Houttuynia cordata* and *Houttuynia emeiensis* was collected from a research plantation at Huaihua (central China), and the wild were collected in Huaihua in July, 2004. The plant was identified by Dr. Xianjin Wu. The aboveground and belowground parts were collected from the same batch of plant material. The voucher specimens have been deposited in the Department of Biology, Huaihua College.

Essential Oil Extraction The fresh plants (1000 g) were cut into small segments and subjected to hydrodistillation for 3 h. After dried over anhydrous sodium sulphate, it was stored at approximately 4 °C until tested and chemically analyzed. The essential oil was subjected to GC-MS analysis and antibacterial testing.

GC-MS Analysis GC-MS analysis was performed on a Shimadzu GC-2010 gas chromatography instrument coupled to a Shimadzu QP2010 mass spectrometer (Compaq-Pro Linear data system, class5k software), equipped with a OV-1 capillary column (30 m×0.25 mm I.D., film thickness 0.25 μm). The column was maintained at 50 °C for 6 min, and programmed to 230 °C at a rate of 10 °C/min, then held for 16 min. The temperature of the injection port and interface was set at 280 °C. Helium was used as the carrier gas with a flow rate of 0.7 ml/min. One microlitre of the samples was injected in the 1 : 10 split mode. The mass spectrometer was operated under electron impact (EI) mode at ionization energy of 70 eV and the scan rate was 5 scan/s. The mass spectrometer was operated with a scan mass range of 20 to 450 atomic mass units. The ionization source temperature was 280 °C. The analytes were identified using the NIST Mass Spectral Database. The relative responses of the individual components are expressed as percent peak area relative to total peak area.

Antibacterial Testing Two microorganisms tested in this experiment were selected on the basis that they cause a lot of infections in humans. Gram-positive bacterial species *Staphylococcus aureus* and *Sarcina ureae* were selected as test microorganisms. The microorganisms were obtained from the Department of Pharmacy at the Central South University.

The test microorganisms were performed using 24 h culture growth at 37 °C and adjusted to approximately 10^5 CFU/ml. Stock essential oil solution was prepared by dissolving 0.24 ml of essential oil into 60 ml of sterile water, with a small quantity of surface active agent Tween 80 (Sigma 0.5%, v/v). Stock essential oil solution was diluted with sterile water to produce the following concentrations: 8.0×10^{-3}, 4.0×10^{-3}, 2.0×10^{-3}, 1.0×10^{-3}, 0.5×10^{-3}, 0.25×10^{-3}, 0.125×10^{-3}, 0.0625×10^{-3} and 0.0313×10^{-3} (ml/ml). MIC evaluations of the essential oil from *Houttuynia* THUNB. were performed by two fold broth dilution and two fold agar dilution methods.[10] MH substrate was used for the dilution and incubation.

In two fold broth dilution method, each 0.1 ml of the bacterial suspensions was added to 0.5 ml of each serial two-fold dilution of the test material in tube and then was mixed. The bacteria were incubated at 37 °C for 16 h.

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In two fold agar dilution method, agar was melted in a steam bath set at 30 °C to prevent solidification. Five Petri dishes were pre-inoculated with the bacteria in the following manner. 0.1 ml of the bacterial suspension was pipetted into the appropriately labeled Petri dish to which 25 ml of molten MH agar was then added followed by thorough mixing of the bacteria and molten MH agar. The agar was allowed to set for 1 h. The essential oil of a specific concentration was introduced into an appropriately labeled Petri dish using a sterile micropipette. The dishes were then incubated at 37 °C for 24 h.

Ampicillin sodium ($C_{16}H_{18}N_3NaO_4S$), used as positive control, was obtained from North China Pharmaceutical Group Aino Co. It was kept at 4 °C prior to use; it was then dissolved and finally diluted at concentrations ranging from 0.0975×10^{-3} to 1.56×10^{-3} mg/ml.

The growth or no-growth of the bacteria was assessed by the naked eye. The minimum inhibitory concentration (MIC) was defined as the lowest concentration able to inhibit any visible bacterial growth. MIC was detected by lack of visual turbidity.

Results and Discussion

Chemical Composition of the Essential Oil Hydrodistillation of *Houttuynia* THUNB. produced a clear, colourless to pale yellow oil with a yield of 0.03—0.06%. The relative contents of the oils were in accordance with previous report.[2] Fifty five components could be identified, representing 96.5—99.6% of the total oils, which are listed in Table 1.

Methyl nonyl ketone (2.10—40.36%), bornyl acetate (0.40—8.61%) and β-myrcene (2.58—18.47%) were the most abundant components of the essential oils. The following were α-pinene (tr—10.62%), β-pinene (tr—24.65%), D-limonene (0.23—11.14%), acetic acid geraniol ester (0.54—4.08%), 4-tridecanone (0.25—3.14%), 4-terpineol (tr—5.65), α-terpineol (tr—0.68), *n*-decanoic acid (tr—4.43%) and caryophyllene (0.35—2.68%) so on. It was noted that the monoterpene fraction was present in relatively high amounts. This result differed from the result of previous reports,[11,12] which attributed to the difference from the samples, extraction method and analysis method.

The studied essential oil displayed different chemical profile from those observed from *Houttuynia* THUNB. plants of different species and parts (Table 1). The essential oil from the belowground parts of both species were dominant by α-pinene (3.21—10.62%), camphene (0.32—1.64%), sabinene (0.85—7.26%) and β-pinene (12.62—24.65%), while essential oil from four aboveground parts were characterized by high *n*-decanal contents (3.37—59.96%), high decanol content (1.16—3.70%), high *n*-decanoic acid content (2.19—4.43%) and high lauraldehyde content (0.21—16.92%). Additionally, some components were present in a certain sample that not present in the other samples. Zeng also repoted this phenomena that eleven components were present in the sample from the aboveground part of *Houttuynia cordata* but not in the sample from the belowground part, while seven components were present in the sample from the belowground part of *Houttuynia cordata* that were not present in the sample from the aboveground part.[12]

Antibacterial Activity of *Houttuynia* THUNB. Essential Oil The preliminary antibacterial screening of essential oil afforded the *Staphylococcus aureus* and *Sarcina ureae* as the most sensitive strains. As a result, *Staphylococcus aureus* and *Sarcina ureae* were used to evaluate the antibacterial activity. The MICs of eight essential oil samples were assayed and the results shown in Tables 2 and 3. As can be noticed from Tables 2 and 3, the essential oil exhibited antibacterial activity against both bacterial species tested.

Eight essential oil samples showed different antibacterial activity. But, in general, the essential oil from the aboveground of *Houttuynia emeiensis* was more active than those from the belowground of both species and the aboveground of *Houttuynia cordata*. The essential oil from the aboveground of the cultivated *Houttuynia emeiensis* showed notable activity against *Sarcina ureae* (MIC=0.0625×10^{-3} ml/ml) and *Staphylococcus aureus* (MIC=0.25×10^{-3} ml/ml). It is noteworthy that the essential oil from the aboveground of the cultivated *Houttuynia emeiensis* was effective at the same concentration as ampicillin sodium against *Staphylococcus aureus* and *Sarcina ureae*. And others showed moderate activity compared with positive control.

These activities may be attributed to the presence of compounds found in *Houttuynia* THUNB. essential oil. High content component, methyl nonyl ketone produced moderate inhibition of the growth of *Escherichia coli*[13] and inhibited the growth of several wood-destroying fungi tested[14] and in a subsequent study the vapour inhibited the growth of four fungi tested.[15] β-Myrcene did not show observable antibacterial activity on its own. However, it provided enhanced activities when mixed with other components in essential oil. Interestingly enough, the antibacterial activities were not as effective when the constituent β-myrcene was taken out. This showed that the chemical components which make up an essential oil were complimentary to each other and actually perform better as a whole.[16] Caryophyllene and bornyl acetate have been claimed to also contain the antibacterial.[17—20] The α-pinene, β-pinene and limonene had a strong antibacterial activity[21—24] and exerted their toxic effects against these microorganisms through the disruption of bacteria or fungal membrane integrity[25—27] and the inhibition of respiration and ion transport processes. They also increased the membrane permeability in yeast cells and isolated mitochondria.[25,26] This is strongly supported by the study on the effects of different essential oil components on outer membrane permeability in Gram-negative bacteria.[28] Both 4-terpineol and α-terpineol has been reported to have antimicrobial properties[29—31] and antifungal activity.[32] Inhibition of *n*-decanoic acid on yeast growth was due to membrane rupture and loss of cytoplasm resulting in rapid cell death.[33] *n*-Decanal showed high fungicidal activity.[34]

Some low content components, for example, carvacrol was generally recognized as a safe food additive, carvacrol-containing essential oils were biostatic and/or biocidal against many bacteria.[35—38] The biocidal mode of action of carvacrol on bacteria was similar to that of other phenolic compounds and occurs *via* membrane damage resulting in an increase in membrane permeability to protons and potassium ions, depletion of the intracellular ATP pool and disruption of the proton-motive force.[28,39] Caryophyllene oxide has shown *in vitro* antifungal activity.[40]

From above references, most of constitutes in essential oils are active against microorganism. Significant difference in concentration of the components of essential oils, especially the variation in quantities of major and/or minor components might be responsible for the different antibacterial activity. However the reason of the aboveground of cultivated *Houttuynia emeiensis* oil's higher inhibitory effect on *Staphylococcus aureus* and *Sarcina ureae* can not be explained by

Table 1. Phytoconstituents of Essential Oils from *Houttuynia* THUNB.

Retention time (min)	Compound[a)]	Composition (%)[b)]							
		1	2	3	4	5	6	7	8
12.489	α-Pinene	1.41	tr	0.20	tr	10.62	3.21	10.2	9.21
12.827	Camphene	0.39	—	tr	0.17	1.41	0.32	1.55	1.64
13.367	Sabinene	1.78	0.45	—	tr	6.29	7.26	0.85	0.97
13.493	β-Pinene	1.94	0.35	0.28	tr	24.65	12.62	23.29	21.88
13.744	β-Myrcene	18.47	2.58	12.57	13.75	10.27	6.21	14.29	13.90
14.36	α-Terpinene	0.26	tr	—	—	0.33	0.56	tr	—
14.416	Cymene	0.69	0.3	tr	—	0.69	0.24	—	—
14.634	D-Limonene	1.01	0.23	0.51	0.24	9.55	4.5	11.14	10.51
14.688	*trans*-β-Ocimene	2.49	—	2.52	2.11	—	—	—	—
14.929	*cis*-β-Ocimene	0.26	—	tr	—	tr	tr	—	—
15.218	γ-Terpinene	0.80	0.59	—	—	0.74	1.49	tr	tr
15.624	2-Nonanone	—	—	tr	—	—	—	—	—
15.791	2-Methyl-6-methylene-oct-3,7-dien-2-ol	0.22	—	—	—	—	—	—	—
15.820	2,6-Dimethyl-3,5,7-octatriene-2-ol	0.22	—	0.52	0.43	—	—	—	—
15.841	4-Carene	0.26	—	—	—	—	—	—	—
15.850	(*Z*)-2-Nonen-1-ol	—	0.52	—	—	—	—	—	—
15.884	2-Carene	0.21	—	—	—	0.47	0.69	0.29	0.35
15.910	β-Linalool	—	—	0.61	0.78	—	—	—	—
15.957	Perillen	tr	—	tr	—	—	—	—	—
16.411	(*Z*)-β-Terpineol	—	—	—	—	tr	tr	—	—
16.544	4,4,6,6-Tetramethyl-bicyclo[3.1.0]hex-2-ene	0.77	—	0.73	0.63	—	—	—	—
16.909	2,6-Dimethyl-2,7-octadiene-1,6-diol	tr	—	tr	tr	—	—	—	—
17.187	Nonanol	0.56	0.30	0.77	0.62	tr	0.67	tr	tr
17.464	4-Terpineol	2.99	3.30	tr	tr	2.12	5.65	0.23	0.51
17.635	α-Terpineol	0.29	0.22	tr	0.21	0.47	0.68	0.29	0.43
17.761	*n*-Decanal	2.96	59.96	3.37	3.51	tr	0.31	—	—
18.743	*trans*-Pinocarvyl acetate	0.59	tr	0.94	0.82	—	—	—	—
18.937	Decanol	1.16	3.70	2.64	3.01	—	tr	—	—
19.157	Carvacrol	tr	—	—	—	tr	tr	tr	tr
19.298	Methyl nonyl ketone	30.10	2.11	36.07	34.96	23.40	40.36	23.96	25.02
19.301	Bornyl acetate	8.03	0.40	6.03	8.23	4.54	6.72	8.61	7.01
19.428	2-Decanol	0.22	—	tr	tr	—	tr	—	—
19.474	Dodecene	0.34	—	tr	tr	—	tr	—	—
19.55	Undecanal	—	1.74	0.42	0.38	—	—	—	—
19.719	α-Cyclogeraniol acetate	—	tr	0.24	0.29	0.29	0.29	tr	tr
19.831	Neodihydrocarveol	—	—	tr	tr	—	tr	tr	tr
20.367	*n*-Decanoic acid	4.43	2.19	4.20	3.99	tr	0.81	0.67	0.59
20.556	Acetic acid geraniol ester	4.08	1.01	6.22	6.75	0.59	1.42	0.72	0.54
20.621	3-Methylene-undecane	0.72	tr	1.63	1.57	0.62	0.45	0.40	0.42
20.813	2-Dodecanone	1.12	—	1.07	1.14	0.57	0.58	0.30	0.23
21.025	Lauraldehyde	0.35	16.92	0.21	0.23	—	tr	—	—
21.723	Caryophyllene	0.89	0.26	2.68	2.38	0.35	0.56	1.02	1.22
21.871	β-Farnesene	0.24	tr	0.23	tr	—	tr	—	—
21.983	Dodecanol	—	0.64	—	—	—	—	—	—
22.238	4-Tridecanone	2.54	0.25	3.14	3.13	0.73	2.13	1.64	1.93
22.340	Phenylcarbamic acid, 9-bromononyl ester	0.28	tr	0.27	0.28	—	—	—	—
22.443	Patchoulene	0.40	—	0.21	tr	0.22	—	—	—
22.556	α-Farnesene	0.29	—	tr	—	—	tr	—	—
22.739	7-Methoxy-3,7-dimethyl-octanal	0.23	—	tr	—	—	tr	—	0.26
23.057	Tetradecanoic acid	tr	tr	0.21	0.25	—	tr	—	—
23.285	*trans*-Nerolidol	0.35	—	0.79	0.77	—	tr	—	—
23.572	Ethyl tridecanoate	2.33	—	—	—	0.34	—	0.23	0.31
23.644	Docosanoic acid, ethyl ester	—	—	5.93	5.72	—	0.87	—	—
23.877	Tetradecanal	tr	0.76	—	—	—	—	—	—
23.909	Caryophyllene oxide	—	—	0.44	0.59	—	tr	—	—

a) Compounds listed in order of elution from a OV-1 column. *b*) 1, aboveground part of the cultivated *Houttuynia emeiensis*; 2, aboveground part of the wild *Houttuynia emeiensis*; 3, aboveground part of the cultivated *Houttuynia cordata*; 4, aboveground part of the wild *Houttuynia cordata*; 5, belowground part of the cultivated *Houttuynia emeiensis*; 6, belowground part of the wild *Houttuynia emeiensis*; 7, belowground part of the cultivated *Houttuynia cordata*; 8, belowground part of the wild *Houttuynia cordata*. tr: trace (<0.2). —: not detected.

simple linear correlation with the concentration of these components. According to Chinese medicine's theory, it may be in part due to the synergism or sum total of all the constituents, and the compound combination of the oil in the aboveground of cultivated *Houttuynia emeiensis* is better than that of the others.

Many researches have supported this hypothesis and showed for many plants that the whole plant or crude extract was more effective than isolated constituents. The reconstituted distillate which contained methyl nonyl ketone, lau-

Table 2. Antibacterial Activity against *Staphylococcus aureus*

Species	Cultivated or wild	Part of plant	MIC[a)]	
			Broth dilution method	Agar dilution method
Houttuynia cordata	Cultivated	Aboveground	0.5×10^{-3}	1.0×10^{-3}
		Belowground	0.5×10^{-3}	0.5×10^{-3}
	Wild	Aboveground	1.0×10^{-3}	1.0×10^{-3}
		Belowground	1.0×10^{-3}	1.0×10^{-3}
Houttuynia emeiensis	Cultivated	Aboveground	0.25×10^{-3}	0.25×10^{-3}
		Belowground	2.0×10^{-3}	1.0×10^{-3}
	Wild	Aboveground	1.0×10^{-3}	0.5×10^{-3}
		Belowground	4.0×10^{-3}	2.0×10^{-3}
Ampicillin sodium			0.195×10^{-3}	0.195×10^{-3}

a) Minimum inhibitory concentration, ml/ml for *Houttuynia* THUNB. and mg/ml for ampicillin sodium.

Table 3. Antibacterial Activity against *Sarcina ureae*

Species	Cultivated or wild	Part of plant	MIC[a)]	
			Broth dilution method	Agar dilution method
Houttuynia cordata	Cultivated	Aboveground	1.0×10^{-3}	0.5×10^{-3}
		Belowground	2.0×10^{-3}	1.0×10^{-3}
	Wild	Aboveground	0.5×10^{-3}	1.0×10^{-3}
		Belowground	0.5×10^{-3}	1.0×10^{-3}
Houttuynia emeiensis	Cultivated	Aboveground	0.0625×10^{-3}	0.0625×10^{-3}
		Belowground	1.0×10^{-3}	2.0×10^{-3}
	Wild	Aboveground	0.5×10^{-3}	0.5×10^{-3}
		Belowground	4.0×10^{-3}	1.0×10^{-3}
Ampicillin sodium			0.0975×10^{-3}	0.0975×10^{-3}

a) Minimum inhibitory concentration, ml/ml for *Houttuynia* THUNB. and mg/ml for ampicillin sodium.

raldehyde and capryl aldehyde in the ratio which is equivalent to that of the components found in distillate from *Houttuynia cordata* exhibited more effective activity against virus than three components used alone.[5)] The antibacterial activities of essential oil were not as effective when the constituent β-myrcene which did not show observable antibacterial activity on its own was taken out.[16)] Treatment of the juice with 1.25 mM carvacrol or *p*-cymene reduced the numbers of *E. coli* O157:H7 to undetectable levels within 1—2 d at both storage temperatures. The effective concentrations of carvacrol could be reduced even further by combining it at 0.5 mM with cymene at 0.25 mM.[41)] Synergism between carvacrol and *p*-cymene against *B. cereus in vitro* and in rice has been reported[42,43)] to be more antimicrobial than these used alone. Therefore, the antibacterial activity of essential oil from *Houttuynia* THUNB. can be attributed to its major components, and/or trace compound(s) and possible synergistic and antagonistic effect of compound(s) in the oils.

In conclusion, our observations confirm that essential oil from *Houttuynia* THUNB. possesses antibacterial activity *in vitro* though significant differences lie in the components of essential oil and antibacterial activity of different species and parts. The component combination in essential oil from the aboveground of cultivated *Houttuynia emeiensis* was considered to be optimal. This result provides some scientific basis for the utilization in folk medicine, species selection for planting of this plant and plant resource protecting.

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