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Comparison of Chemical Constituents and Antioxidant Activity of *Achillea alpina L.* and *Achillea wilsoniana L.*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Achillea species have been widely used as herbal medicine for a time. *Achillea millefolium L*. has one of the broadest applications in herbal medicine because of its antioxidant activity. As congeneric subspecies of the *A. millefolium L*., the *A. alpina L*. and *A. wilsoniana L*. also have many medical properties. This paper explored the essential oils of these two plants by gas chromatography-mass spectrometry (GC-MS) and the differences in their antioxidant activity. The result showed that the major components of essential oil from *A. wilsoniana L*. were (+)-2-bornanone (8.51%), (-)-β-bisabolene (6.7%), chamazulene (6.4), neointermedeol (6.12%). And the major components of essential oil from *A. alpina L*. were chamazulene (6.53%), (1S)-(1)-beta-pinene (5.19%), nerolidol (3.6%), and esquisabinen (2.7%). The results indicated that *A. alpina L*. had the highest composition of chamazulene compared with the other two. Due to the variety of compounds in the two essential oils, their antioxidant activities were different on DPPH and ABTS assays. The antioxidant activity of *A. wilsoniana L*. was better than *A. alpina L*. but lower than the *A. millefolium L*.

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Keywords: Achillea alpina L.; Achillea wilsoniana L.; GC-MS; antioxidant activity.

1. INTRODUCTION

Herbal medicine is a typical and natural medical treatment used in China for a long time. As herbal ingredients, essential oils have a wide range of applications in the beauty and medical industries, including skin care [1], anti-inflammatory, and treating respiratory diseases [2]Moreover, its excellent antioxidant activity can reduce Reactive oxygen species (ROS), thus reducing melanin to achieve a whitening effect. However, essential oils also have some side effects, so it is vital to figure out their constituents before use.

Achillea millefolium L. is one of the medical plants widely grown in Asia, Africa, Europe, and America [3,4]. Its practical application in food, medicine, skincare, and even agriculture due to its antioxidant compounds such asFlavonoid, Saponin, and another particular component --- blue essential oil.

As congeneric subspecies of the *Achillea millefolium L*., the *Achillea alpina L*. and *Achillea wilsoniana L*. also have many medical records in ancient Chinese books like Yunnan Medicine Journal, Compendium of Materia Medica. *Achillea alpina L*. and *Achillea wilsoniana L*. are used as local medicine to cure external illnesses for a long history, such as sedative, pain relief, skincare, *etc.* However, fewer systematic papers about their essential oil applications play some role in whitening, antioxidant, and antibacterial. So it is especially critical to figure out the active ingredients and functions in those plants.

This paper focused on the essential oils of these two plants to provide a theoretical basis for the future development and application of these two local medicinal herbs.

2 EXPERIMENTAL SECTION

2.1 Plant Material and Reagents

The plant materials were collected from Mianyang, Sichuan province, in April 2022. *Achillea alpina L.* and *Achillea wilsoniana L.* were confirmed by one authors, Zhiqiang Zhang from Sichuan College of Traditional Chinese Medicine. The voucher specimens have been deposited in the School of Life Science and Engineering, Southwest University of Science and Technology. These materials were dried at room temperature, crushed into granules, passed through a 40 mesh sieve, and stored at 4℃ for later use.

DPPH (1,1-diphenyl-2-trinitrophenylhydrazine), ABTS (2,2'-diazo-bis-3-ethylbenzothiazoline-6-sulfonic acid), BHA (butylated hydroxyanisole), and Vc (ascorbic acid) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Sodium sulfate (Na_2SO_4), potassium persulphate ($K_2S_2O_8$) and other reagents were purchased from Chengdu Kelong Chemical Co., Ltd. Ultrapure water was made by the laboratory (Resistivity was 18.3M).

2.2 Essential Oil Extraction by Hydro-distillation

Essential oils were extracted by hydro-distillation for three hours of 100g-150g granules using a clevenger-type apparatus, according to methods used by Chinese Pharmacopoeia [5]. The obtained essential oils were dried for one hour by Na_2SO_4 and stored in sealed dark vials at 4°C.

2.3 Gas Chromatography-mass Spectrometry Analysis

The two essential oils were analyzed by Gas chromatography-mass spectrometry(SHIMADZU GC-MS QP2010SE). The analysis was carried out on fused SH-Rxi-5Sil MS (30 m × 0.32 mm i.d., film thickness 0.25 μm). The column temperature started at 40℃, raised to 140℃ at the rate of 25℃/min, and rose continually to 240℃ at the rate of 20℃/min. At last, the column temperature increased to 270℃ at 10℃/min. The injector temperatures and the GC/MS interface were kept at 290°C. The transmission line temperature was 280℃. The carrier gas was He, and its flow was 1.0mL/min. The shunting ratio was 100:1. And the injection volume was 1μL.

MS conditions: EI source. Electron energy was 70eV. The ion source temperature was 230℃. Quadrupole temperature was 150℃. The scanning quality range was 35-500U. The solvent delay was 3min.

2.4 Antioxidant Activity

Two standard methods (ABTS+, DPPH·) were used to evaluate essential oils *in vitro* antioxidant capacity. These methods target different

oxidation groups and can be used together to provide a more comprehensive assessment of the antioxidant capacity of the two essential oils.

2.4.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

Referring to the method by El-Kalamouni [6], DPPH is widely used to evaluate antioxidant activity that can provide stable free radicals. When the free radical is scavenging, its Maximum UV absorption at 519nm will decrease, so it could be an excellent model to evaluate the antioxidant activity of two kinds of essential oils. Vitamin C was a positive control at the same concentrations and conditions.

1mg DPPH was dissolved in 24ml anhydrous ethanol, sonicated for five minutes, mixed well and diluted to absorbance between 0.6-1.0. Mixed DPPH with the sample 1:1 and keep the reaction away from light for half an hour. Inhibition percent was obtained by Equation (1).

$$\text{Inhibition}(\%)=[1-(A_i-A_j)/A_0]\times 100\% \quad (1)$$

A_i: absorption of a sample
A_0: absorption of blank
A_j: absorption of sample basis

2.4.2 ABTS+ radical scavenging activity

The ABTS method directly generates the ABTS+ chromophore through the reaction between ABTS and $K_2S_2O_8$ [7]. The ABTS+ free radical has a maximum absorption value of 734nm. As the color changes from green to light, the absorption value decreases. Butylhydroxyanisol (BHA) was used as a positive control at the same concentrations and conditions. The radical ABTS•+ was obtained by mixing an aqueous ABTS solution (7 mM) with an aqueous potassium persulfate solution (2.45 mM) [7], with a ratio of 2:1. The mixture, was then stored for 16 h in darkness at room temperature. Inhibition percent was obtained by Equation (1)

$$\text{Inhibition}(\%)=[1-(A_i-A_j)/A_0]\times 100\% \quad (2)$$

A_i: absorption of the sample
A_0: absorption of blank
A_j: absorption of the sample base

3. RESULTS AND DISCUSSION

3.1 Gas Chromatography-mass Analytical Results

Referring to Figs. 1, 2 and Tables 1, 2, it could know that the major components of *Achillea wilsoniana L.* were (+)-2-bornanone(8.51%),(-)-β-bisabolene(6.7%), chamazulene(6.4), neointermedeol(6.12%), and the significant components of *Achillea alpina L*. were Chamazulene (6.53%), (1S)-(1)-beta-Pinene (5.19%), Nerolidol (3.6%), Sesquisabinen (2.7%). The components of *Achillea millefolium L* mentioned in the paper of El-Kalamouni [7] were composed of camphor (12.8%), trans-chrysantenyl acetate (6.6%), terpinen-4-ol (4.70%), (E)-p-mentha-2,8-dien-1-ol (4.5%), and 1,8-cineole (4.0%), it was clear that all three of them are made of Olefins and terpenes. They all have a significant component of Nerolidol, chamazulene, 1,8-Cineole, Phytol, Cyclohexene, D-Camphene, sesquisabinene, Caryophyleneoxide, Pellitorine, Sabinene hydrate, Spinacene. However, there were significant differences in the composition. The main reason for the difference in the composition may be due to the difference in their genes and the growth environment (climate, altitude, soil, sunshine).

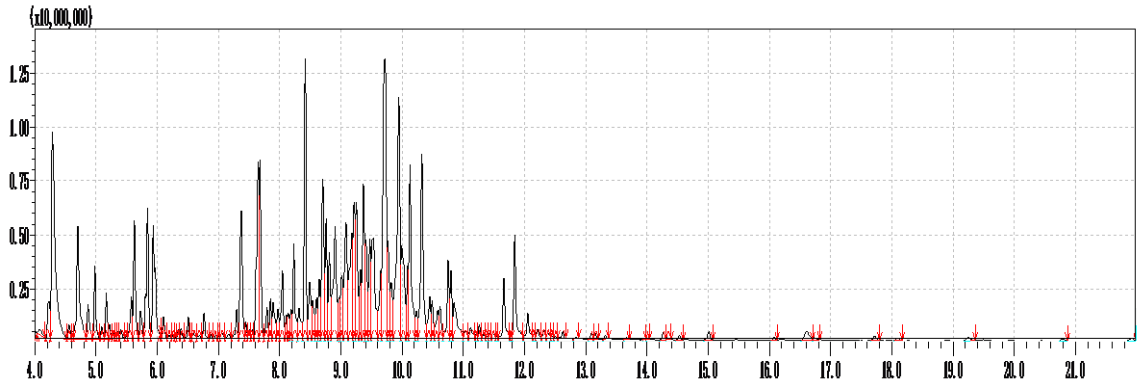


Fig. 1. GC-MS ion flow chromatograms of *Achillea alpina L*

Table 1. Components analysis of *Achillea alpina L*

No.	Peak area (%)	Components
1	6.53	Chamazulene
2	5.19	(1s)-(1)-beta-pinene
3	3.6	Nerolidol
4	2.7	γ-cis-himachalane
5	2.58	Spathulenol
6	2.46	4(15),5,10(14)-germacratrien-1-ol
7	2.3	Bicyclo[7.2.0]undecan-5-ol, 10,10-dimethyl-2,6-bis(methylene)-, (1s,5r,9r)-
8	2.22	L-.alpha.-terpineol
9	2.21	1,8-cineole
10	2.18	Hexadecanal
11	2.07	B-bourbonene
12	2.03	Sesquisabinene
13	1.95	L-4-terpineol
14	1.61	Phytol
15	1.48	D-camphor
16	1.45	Caryophyleneoxide
17	1.28	Beta-funebrene
18	1.19	Ylangenol
19	1.14	Pellitorine
20	1.08	Salvial-4(14)-en-1-one
21	1.01	.beta.-copaene
22	1	Pentadecanoic acid
23	0.94	4-(6-methylhept-5-en-2-yl)cyclohex-2-en-1-one
24	0.93	N-nonadecanol-1
25	0.92	Spinacene
26	0.86	Sabinene hydrate
27	0.82	Neophytadiene
28	0.77	4(15),5,10(14)-germacratrien-1-ol
29	0.7	(9z)-9,17-octadecadienal
30	0.64	Sabinen
31	0.62	Alpha.-humulene
32	0.59	(e)-pinocarveol
33	0.59	Sesquirosefuran
34	0.55	Gamma.-terpinen
35	0.55	Nonanal
36	0.52	Γ-e-bisabolene
Total	59.26	

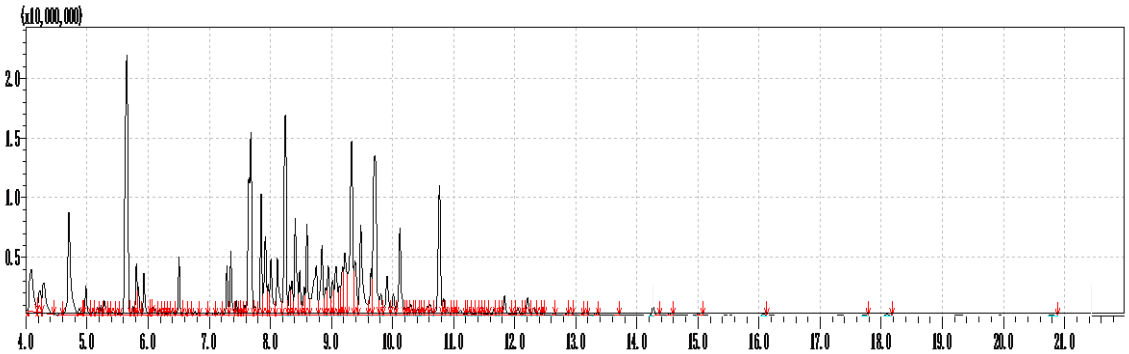
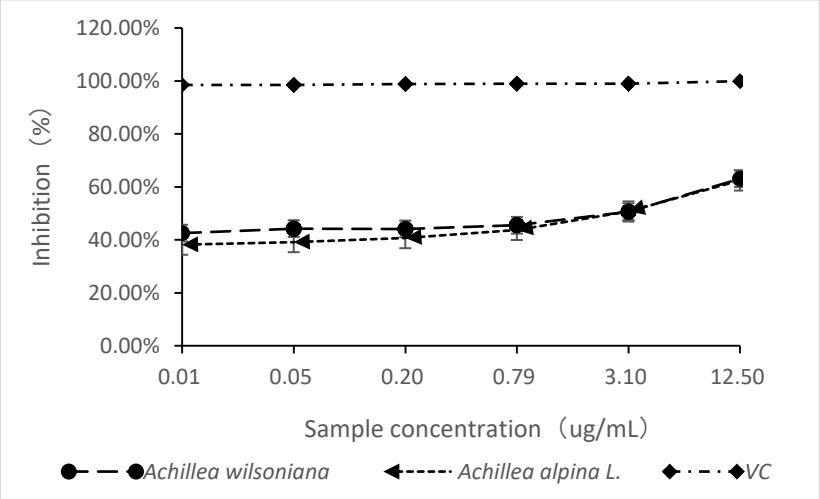


Fig. 2. GC-MS ion flow chromatograms of *Achillea wilsoniana L*

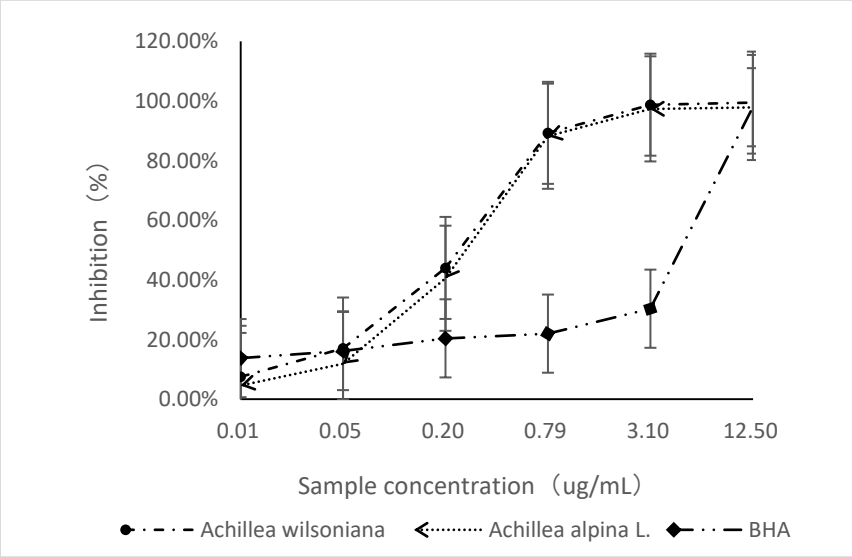
Table 2. Components analysis of *Achillea wilsoniana L*

No.	Peak area (%)	Components
1	8.51	D-camphor
2	6.7	(-)-β-bisabolene
3	6.4	Chamazulene
4	6.12	Neointermedeol
5	5.61	Sesquisabinene
6	3.75	1,8-cineole
7	3.72	Pellitorine
8	3.3	Cuparenal
9	2.55	Nerolidol
10	2.52	Caryophyllene oxide
11	2.38	Fitone
12	2.37	Oleyl alcohol
13	2.09	(r)-1-isopropyl-3-methylene-cyclohexane
14	2.07	Neointermedeol
15	2.03	Trans-sesquisabinene hydrate
16	2	D-camphene
17	1.76	(+)-b-cedrene
18	1.57	Trans-a-bergamotene
19	1.31	(1s)-(1)-beta-pinene
20	1.26	(-)-beta-elemene
21	1.24	B-bisabolol
22	1.02	L(-)-borneol
23	0.97	(1s,5s)-2-methyl-5-((r)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hex-2-ene
24	0.96	Cyclohexene
25	0.92	Spinacene
26	0.85	(+-)-cis-6,7-dihydro-farnesol
27	0.79	Nerolidol, trans
28	0.78	Sabinen
29	0.66	Dehydrochamazulene
30	0.64	4-thujanol
31	0.62	Carvyl angelate, cis-
32	0.57	Amorphadiene
33	0.57	9-isopropyl-1-methyl-2-methylene-5-oxatricyclo[5.4.0.0(3,8)]undecane
34	0.5	Phytol
Total	79.11	



Samples	Content (μg/mL)					
	0.01	**0.05**	**0.20**	**0.79**	**3.10**	**12.50**
Achillea wilsoniana L.	42.57%	44.29%	44.15%	45.53%	50.68%	63.12%
Achillea alpina L.	38.20%	39.18%	40.68%	43.78%	50.75%	62.44%
VC	98.54%	98.79%	98.84%	98.99%	98.99%	99.94%

Fig. 3. DPPH of samples radical scavenging activity



Samples	Content (μg/mL)					
	0.01	**0.05**	**0.20**	**0.79**	**3.10**	**12.50**
Achillea wilsoniana	7.58%	17.06%	44.07%	89.34%	98.78%	99.47%
Achillea alpina L.	4.67%	11.92%	40.61%	88.21%	97.38%	97.84%
BHA	13.84%	16.21%	20.46%	22.05%	30.40%	97.92%

Fig. 4. ABTS+ radical scavenging activity

3.2 Antioxidant Activity

3.2.1 DPPH assay

Both essential oils showed some scavenging ability for DPPH, but the overall performance was average compared to the antioxidant Vc, with a scavenging rate of only about 60% (Fig. 3).

3.2.2 ABTS assay

As the essential oil concentration increased, the sample's ability to scavenge ABTS+ free radicals increased, with the antioxidant capacity decreasing significantly when the concentration was below 0.4‰. Their antioxidant activity was even more potent than the synthetic antioxidant BHA. However, compared with each other, *Achillea wilsoniana L*. was better than *Achillea aplpina L* (Fig. 4.). The chamazulene is has a charcteristicblue colour and has a particular smell. Furthermore, the antioxidant activity is due to the synergistic expression of various olefins, especially (+)-2-bornanone, 1,8-Cineole, and (-)-β-bisabolene. But *Achillea wilsoniana L*. has better antioxidant activity than *Achillea alpina L*. Furthermore, two plants have a better radical scavenging activity on ABTS+ because 1,8-Cineole, (+)-2-bornanone, β-bourbonene, and other Olefins have been reported in papers [7-9] about it. Compared with the antioxidant activity of *Achillea millefolium L*. in the article written by the El-Kalamouni [7], the *Achillea millefolium L*. essential oil has the highest activities of antioxidant.

4. CONCLUSIONS

The three plants have subtle differences in habits and morphological characteristics and significant differences in biological activity and composition. Especially *Achillea alpina L.* has the highest content of chamazulene, an ingredient that could be used to treat migraine, indolent ulcers of the leg, and asthma [8-10]. *Achillea alpina L*.has a broad medical future in the medical industry,

continuing to follow up on research into its antibacterial and anthelmintic activity and can be integrated with multiple areas of development. It can even be used as a precursor for specific chemical components.

Therefore, compared with *Achillea millefolium L.* and *Achillea wilsoniana L.*, Their primary ingredients overlap highly, but in terms of antioxidant activity, *Achillea millefolium L*.is better. But it can't be the only indicator to evaluate the application of the plant without studies on antimicrobial properties, anti-inflammatory, antibacterial and other activities. Above all, As congeneric subspecies of the *Achillea millefolium L,* the *Achillea alpina L.* and *Achillea wilsoniana L.* also have high antioxidant activities and variable compositions in their essential oil. Because of the variations in their chemical constituents, the antioxidant activities differ. Because essential oil has a unique and pleasant smell, it may be used in beauty products, food, or medical industries. It is the first time we focused on the essential oils of *Achillea wilsoniana L.* and *Achillea aplina L.* There will be many activities to explore in the future, including Anti-inflammatory, antibacterial, and melanin-eliminating activity to study and research. These two essential oils are expected to be a vital natural product resource.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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