Comparison research of chemical composition and antioxidant activity of *Achillea alpina L.* and *Achillea wilsoniana L.*

Abstract: Achillea species have been widely used as herbal medicine for a long history. *Achillea millefolium L*. is one of the broadest applications in herbal medicine by its essential antioxidant activity. As congeneric subspecies of the *Achillea millefolium L*., the *Achillea alpina L*. and *Achillea wilsoniana L*. also have many medical treatments. This paper explored the significant component of these two plants' essential oil by gas chromatography-mass spectrometry (GC-MS) and the variation of their antioxidant activities. The result showed that the major components of essential oil from *Achillea 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 *Achillea alpina L*. were chamazulene (6.53%), (1S)-(1)-beta-pinene (5.19%), nerolidol (3.6%), and esquisabinen (2.7%). The results noticed that *Achillea 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 behaved differently on DPPH and ABTS assays. The antioxidant activity of *Achillea wilsoniana L*. was better than *Achillea alpina L*. but weaker than the *Achillea millefolium L.*

Keywords: *Achillea alpina L*.; *Achillea wilsoniana L*.; GC-MS; Antioxidant Activity;

1 Introduction

With the development of science concepts in people, natural medicine and food are gradually expected by more people, especially in the beauty and health industry. 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, respiratory diseases treatment [2], *et al.* Moreover, its excellent antioxidant capacity can reduce 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 compositions to use them fully.

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 because of its antioxidant and plentiful ingredients, including Flavonoid, 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 sedation, 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 oil 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 author of this article, 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 over 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 oil were analyzed by Gas chromatography-mass spectrometry, carried out on a 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 oil. Vitamin C was a positive control at the same concentrations and conditions.

Took 1mg DPPH, 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).

$$\text{Inhibition}(\%)=[1-(A_i-A_j)/A_0]\times100\% \quad (1)$$

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)

$$\text{Inhibition}(\%)=[1-(A_i-A_j)/A_0]\times100\% \qquad (2)$$

A_i: absorption of the sample

A_0: absorption of blank

A_j: absorption of the sample base

3 Result and Discussion

3.1 Gas chromatography-mass spectrometry analysis

Referring to Figures 1, 2 and Table 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 specific composition content. The main reason for the difference in a composition may be the difference in their genes and the growth environment (climate, altitude, soil, sunshine).

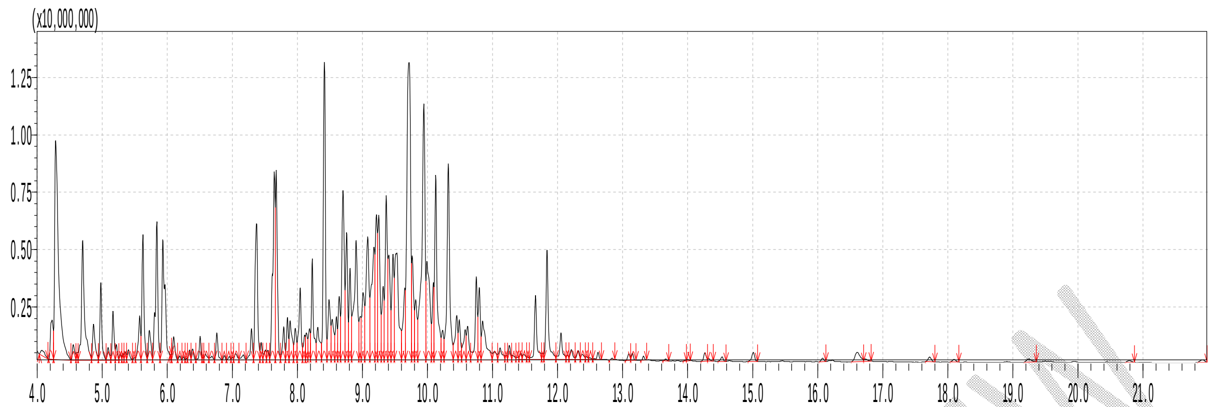


Figure 1. GC-MS ion flow chromatograms of *Achillea alpina L.*

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	

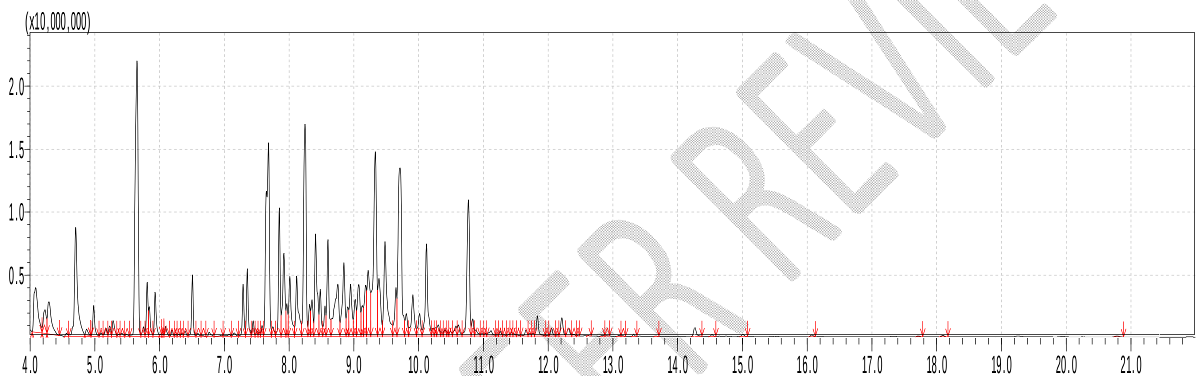


Figure 2. GC-MS ion flow chromatograms of *Achillea wilsoniana L.*

Table 2. Components analysis of *Achillea wilsoniana L.*

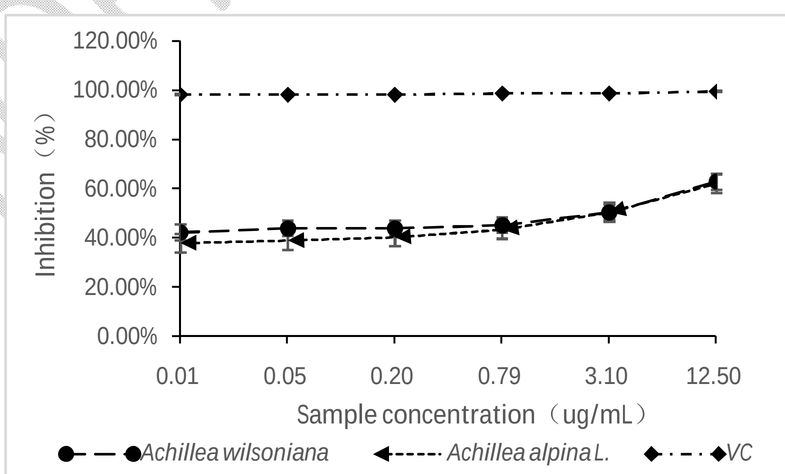
No.	Peak area (%)	Components
1	8.51	D-camphor
2	6.7	(-)-β-bisabolene
3	6.4	Chamazulene
4	6.12	Neointermedeol
5	5.61	Sesquisabinene
6	3.75	1,8-cineole
7	3.72	Pellitorine
8	3.3	Cuparenal
9	2.55	Nerolidol
10	2.52	Caryophyllene oxide
11	2.38	Fitone
12	2.37	Oleyl alcohol
13	2.09	(r)-1-isopropyl-3-methylene-cyclohexane
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	

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% (Figure 3).

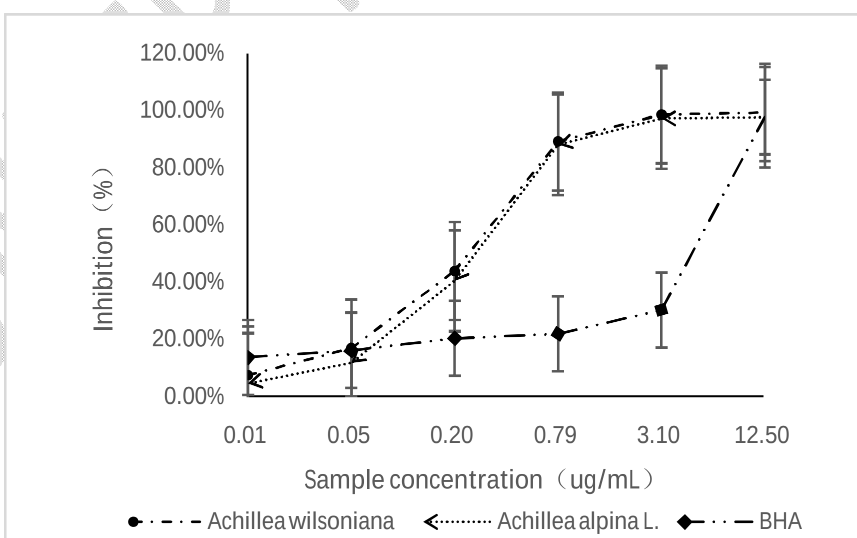


Samples	Content (μg/mL)					
	0.01	0.05	0.20	0.79	3.10	12.50
Achillea wilsoniana L.	42.57%	44.29%	44.15%	45.53%	50.68%	63.12%
Achillea alpina L.	38.20%	39.18%	40.68%	43.78%	50.75%	62.44%
VC	98.54%	98.79%	98.84%	98.99%	98.99%	99.94%

Figure 3 DPPH of samples

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*(Figure 4.). The chamazulene is why the essential oil is blue 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 all 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.



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%

Figure 4 ABTS+ radical scavenging activity

4 Conclusions

The three plants have subtle differences in habits and appearance characteristics and significant differences in biological activity and composition, Although the three plants belong to the same congeneric subspecies. Especially *Achillea alpina L.* has the highest content of chamazulene, an ingredient that could apply to treat migraine, indolent ulcers of the leg, and asthma [8-10]. It means that *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 following related studies of it like 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 Compositions, the antioxidant activities behave differently. Because essential oil has a unique and pleasant smell, it may have a considerable capacity to apply to the beauty, food, or medical industries. It is the first time we focus 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.

NOTE:

The study highlights the efficacy of "traditional medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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