

Comparison research of chemical composition and antioxidant activity

of *Achillea alpina* L. and *Achillea wilsoniana* L.

Comment [U1]: Unless I am mistaken, the last sentence of the abstract indicates that the comparison of antioxidant power concerns three plants of the same genus and not two as the title of the article clearly shows. Am I to assume that the authors were wrong or that the title was written with an error or is simply poorly written?

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.

Comment [U2]: The abstract was written in accordance with the relevant requirements. However, after its review, I suggest small modifications to the text as can be seen with the parts written in bold and with a red colour.

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 **due to its 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 revealed 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.

Comment [U3]: In the part of the text reproduced below, the first sentence seems to me devoid of meaning and deserves to be rewritten or restructured to give it more meaning. In addition, I suggested a change to the sentence that talks about the antioxidant capacity of essential oils (see the parts of the text written in bold, red and underlined. Moreover, the authors use the abbreviation ROS without having previously explained it.

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

Comment [U4]: I recommend keeping only [2] as reference

because of its antioxidant and plentiful ingredients, including Flavonoid, Saponin, and another particular component --- blue essential oil.

Comment [U5]: The phrase reproduced below is poorly structured and therefore the idea shared by the authors is difficult to understand.

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.

Comment [U6]: This part of the sentence deserves to be rewritten for more clarity of the ideas that authors want to share with readers

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.

Comment [U7]: This sentence is in contradiction with the last sentence of the abstract which does not align with the field circumscribed by the title of the article

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°C for later use.

Comment [U8]: this does not constitute sufficient evidence to certify the origin or authenticity of the plant material used in this research. I suggest you look for a non-partisan reference to do so

DPPH (1,1-diphenyl-2-trinitrophenylhydrazine), ABTS (2,2'-dialoxy-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 ($\text{K}_2\text{S}_2\text{O}_8$) and other reagents were purchased from Chengdu Kelong Chemical Co., Ltd. Ultrapure water was made by the laboratory (Resistivity was 18.3M).

Comment [U9]: change suggested: **Ultrapure water was prepared at 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°C, raised to 140°C at the rate of 25°C/min, and rose continually to 240°C at the rate of 20°C/min. At last, the column temperature increased to 270°C at 10°C/min. The injector temperatures and the GC/MS interface were kept at 290 °C. The transmission line temperature was 280°C. 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°C. Quadrupole temperature was 150°C. 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).

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

A_i: absorption of a sample

A₀: absorption of blank

A_j: absorption of sample basis

2.4.2 ABTS+ radical scavenging activity

Comment [U10]: I suggest writing this sentence as:

The two essential oils were analyzed by Gas chromatography coupled with the mass spectrometry, using a SHIMADZU GC-MS QP2010SE-type apparatus.

Comment [U11]: I recommend that authors write this part of the text in a more comprehensible form, as they did for the other parts of the text that contain or describe the experimentation procedure used.

The ABTS method directly generates the ABTS^{•+} chromophore through the reaction between ABTS and K₂S₂O₈ [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

A₀: 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, Caryophylleneoxide, 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).

Comment [U12]: I recommend that authors use **mL instead of ml** and write the units of time as **16 hours instead of 16 h**. Also, ensure that units are well separated from numbers, for example, 734 nm instead of **734nm**.

Comment [U13]: I suggest bringing some modification to the text as:
Referring to Figures 1, 2 and Table 1, 2, **it is obvious** that the major components of *Achillea wilsoniana L.* **are** (+)-2-bornanone(8.51%),(-)-β-bisabolene(6.7%), chamazulene(6.4), neointermedeol(6.12%), and the significant components of *Achillea alpina L.* **are** 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, Caryophylleneoxide, Pellitorine, Sabinene hydrate, Spinacene. However, there are significant differences in the specific composition content. The main reason for **the difference in a composition may be due to their genes and the growth environment** (climate, altitude, soil, sunshine).

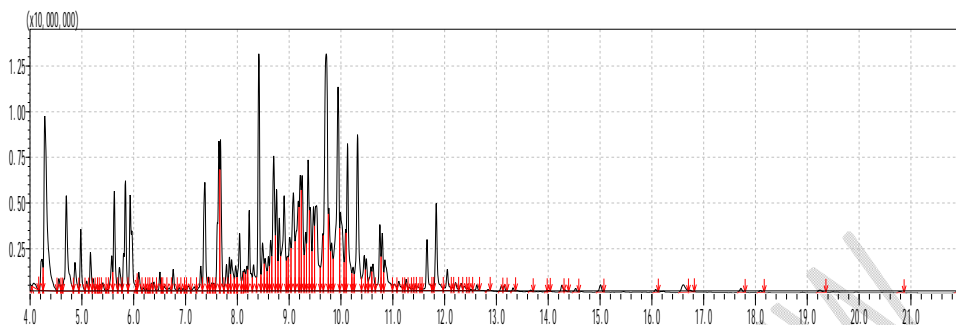


Figure 1. GC-MS ion flow chromatograms of *Achillea alpina* L.

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)-germacatrien-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	Caryophylleneoxide
17	1.28	Beta-funebrene
18	1.19	Ylangenol
19	1.14	Pellitorine
20	1.08	Salvia-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)-germacatrien-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	

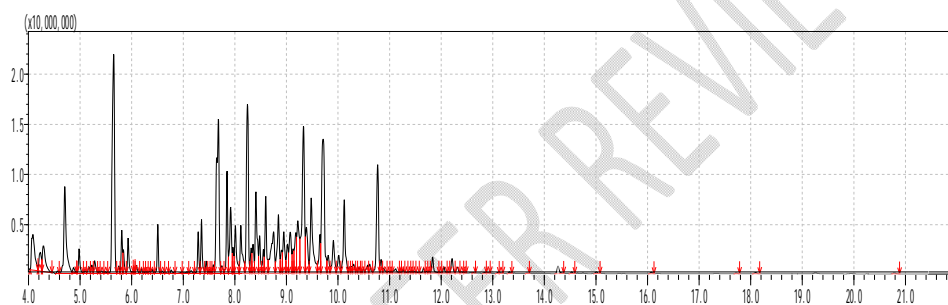


Figure 2. GC-MS ion flow chromatograms of *Achillea wilsoniana L.*

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

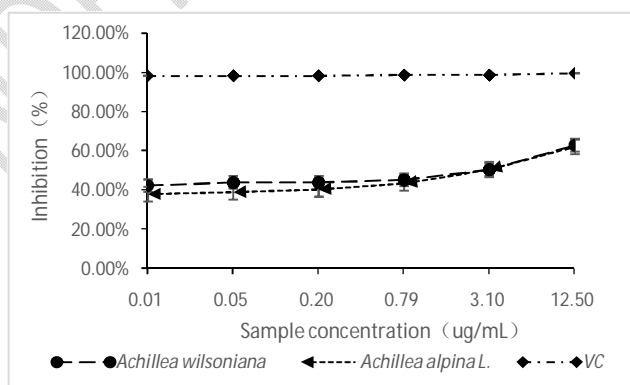
No.	Peak area (%)	Components
1	8.51	D-camphor
2	6.7	(-)-β-bisabolene
3	6.4	Chamazulene
4	6.12	Neointermedeol
5	5.61	Sesquisabinene
6	3.75	1,8-cineole
7	3.72	Pellitorine
8	3.3	Cuparenal
9	2.55	Nerolidol
10	2.52	Caryophyllene oxide
11	2.38	Fitone
12	2.37	Oleyl alcohol
13	2.09	(r)-1-isopropyl-3-methylene-cyclohexane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

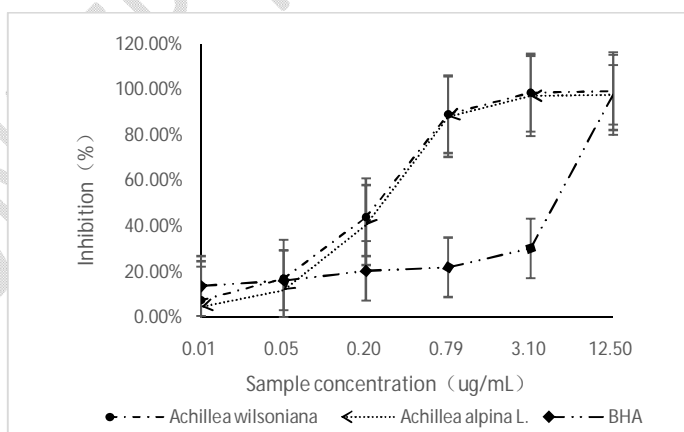


Samples	Content (µg/mL)					
	0.01	0.05	0.20	0.79	3.10	12.50
<i>Achillea wilsoniana L.</i>	42.57%	44.29%	44.15%	45.53%	50.68%	63.12%
<i>Achillea alpina L.</i>	38.20%	39.18%	40.68%	43.78%	50.75%	62.44%
VC	98.54%	98.79%	98.84%	98.99%	98.99%	99.94%

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 alpina 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

Comment [U14]: Suggestion: The sentence needs to be written as follows: Their antioxidant activity was **even greater** than the synthetic antioxidant BHA. However, compared with each other, *Achillea wilsoniana L.* was better than *Achillea alpina L.* (Figure 4.)

Comment [U15]: These are interesting statements, but you have to say it by falsifying evidence so that readers can believe you. Did you mention in the experimental part a procedure that allowed us to analyze odors? I think some of your assertions are more based on your impressions than on the results of experimentation.

Comment [U16]: You repeated this word twice. Is there any way to find anything else such as "however", for example?

<i>Achillea wilsoniana</i>	7.58%	17.06%	44.07%	89.34%	98.78%	99.47%
<i>Achillea alpina L.</i>	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 alpina 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.

Comment [U17]: I do not totally disagree with the authors regarding the quality of this conclusion, which seems to me to be going in all directions. I suggest that they write a conclusion that refers to the salient results or significant differences observed in the analyses that enabled them to compare the two vegetable species studied. In addition to see to what extent avoid to mention a third plant of the same kind at the end of the abstract so as not to have to make changes under the article.

Comment [U18]: In my opinion, this note is not necessary but that its content would allow the authors to support their arguments during the discussion of their results.

References:

1. Abdossi, V. and M. Kazemi, Bioactivities of *Achillea millefolium* Essential Oil and Its Main Terpenes from Iran. *International Journal of Food Properties*, 2016. 19(8): 1798-1808.
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9. Blazso, S., [Further results with chamazulene in the treatment of asthmatic diseases in infancy and childhood]. *Schweizerische medizinische Wochenschrift*, 1951. 81(5): 110-1.
10. Vargha, M., [Treatment of migraine with chamazulen]. *Psychiatrie, Neurologie, und medizinische Psychologie*, 1950. 2(4): 116-7.

Comment [U19]: For an article of this value, I am of the opinion that bibliographic entries are really insufficient, that is to say a small number. In addition, the references that can be considered recent date from 2014 to 2017 and represent 60%. This is a good thing. Authors should make efforts to have only about thirty bibliographic entries. This would reassure readers about the consistency of the literary subbasement of this article which led to quite relevant results.