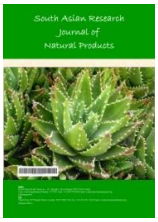

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Antioxidant and Antibacterial Activity of Essential Oil from *Artemisia argyi* Leaf during Different Collecting Stages

Huan-Yang Li[1,2], Wen-Shang Yan[2] and Jian-Guo Xu[1,2] *

[1]Modern College of Humanities and Sciences of Shanxi Normal University, Linfen 041004, China.
[2]School of Food Sciences, Shanxi Normal University, Linfen 041004, China.

Authors' contributions

This work was carried out in collaboration among all authors. Author HYL designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors JGX and WSY managed the analyses of the study. Author WSY managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Artemisia argyi essential oil is a volatile aromatic substance with antioxidant and antibacterial activities. Here, we investigated the composition, antioxidant and antibacterial activities of essential oil from *A. argyi* in different collecting stages. The results showed that the essential oil extracted from *A. argyi* collected on June 7 (Dragon Boat Festival) had the highest extraction rate of 0.88% in five different collection periods. According to GC-MS analysis, there are 29, 29, 31, 28 and 31 different compounds detected in the essential oil of *A. argyi* at different collection stages, among which there are 19 kinds of the same compounds, and the components with high content include 1,8-cineole, β-thujone, 4-p-menth-1-en-8-ol, caryophyllene, caryphylleneoxide, etc. Furthermore, the results of functional activity analysis showed that the essential oil extracted from *A. argyi* collected on the day of Dragon Boat Festival has higher antioxidant and antibacterial activities.

Keywords: Artemisia argyi; essential oil; antibacterial activity.

**Corresponding author: Email: xjg71@163.com;*

1. INTRODUCTION

Artemisia argyi is widely distributed throughout the country, mainly produced in Shandong, Anhui, Hubei, Hebei and other provinces [1]. It is a widespreadly perennial herb, 45-120 cm high, pinnatifid leaves, dark green above, gray green below, with off-white hairs [2]. *A. argyi* has a long history of homologous application in medicine and food as well as a high medicinal value in China, which has been described and recorded in the herbal works and ancient medical books. Modern studies have shown that *A. argyi* contained active ingredients such as coumarin, glycosides, flavones, sterols, terpenes, and volatile oils. Because of the complexity of the active components in *A. argyi*, its pharmacological effects are diversified, with anti-ulcer [3], promoting blood circulation [4], antioxidation [5], antimutagenesis, anti-inflammatory [6], anticancer, antibacterial, immune regulation [7] and other effects.

As the main active ingredient extracted from *A. argyi*, essential oil can become a cheap natural germicidal antiseptic and has high research and application value in the preservation of fruits and vegetables [8]. It has been reported that the volatile oil of *A. argyi* not only has the effect of sun protection, antioxidant [9] and antibacterial [10], but also has neuroregulatory function, which can treat epilepsy, depression, insomnia, anxiety and other mental diseases [11]. Specifically, *A. argyi* essential oil has a special aroma, so it is favored by many people in the fields of food, medicine, chemical industry, cosmetics and other fields. Some literatures have reported that there are some differences in the main components and contents of *A. argyi* essential oil, which may be related to the region, environment and climate of plant growth. Researchers measured the volatile oil from *A. argyi* in Turkey [12], Kashan region [13] and northwestern Iran [14], and the yields were 0.4%, 0.25% and 1.4%, respectively. The main components of *A. argyi* in Turkey are platyclone, carpinene and cineole; the main components of *A. argyi* in Kasan region of Iran are trans-carpinene, cineole and carsage; the main components of *A. argyi* in northwest Iran are pinene, menthol and eucalyptol. Jin et al. [15] found that the constituents and contents of essential oil in *A. argyi* from five different habitats were significantly different. Although many literatures have studied the chemical composition and antibacterial property of the essential oil of *A. argyi*, due to different environment and climatic conditions, the chemical composition of the essential oil from different areas is complex and there are obvious differences in components. At present, there have been many literatures on the essential oil of *A. argyi*, but most of them are extracted from *A. argyi* produced in Shandong, Hubei and other places, while the essential oil extracted from *A. argyi* produced in Shanxi has not been further reported.

Therefore, in this paper, the chemical content and composition of *A. argyi* essential oil collected at different stages in Shanxi Province were compared by gas chromatography-mass spectrometry (GC-MS), and the antioxidant and antibacterial properties of *A. argyi* essential oil were also detected. The purpose of this study is to confirm the optimum period of *A. argyi* harvesting and to provide a theoretical basis for the extraction, exploitation and utilization of *A. argyi* essential oil.

2. MATERIALS AND METHODS

2.1 Plant Materials

A. argyi, produced in Shanxi Province, were collected on May 24, May 31, June 7 (Dragon Boat Festival), June 14, and June 21, 2019, respectively.

2.2 Bacterial Strains and Culture Conditions

The antibacterial activity of essential oil against six different microorganisms was determined. Four Gram-positive strains were *Staphylococcus epidermis*, *Staphylococcus aureus*, *Bacillus subtilis* and *Listeria monocytogenes,* and two Gram-negative strains were *Escherichia coli* and *Salmonella typhimurium*. These strains were provided by the College of Life Science, Shanxi Normal University, and stored with liquid paraffin wax at 4°C. All strains were cultured at 37°C on Nutrient agar (NA) or nutrient broth (NB) mediums.

2.3 Determination of Essential Oil Content

According to the Pharmacopoeia of the People's Republic of China Reference [16], *A. argyi* in five different collection periods were successively numbered as EO-1 ~ EO-5. After crushing, 100 g of each was weighed and placed in a round-bottom flask with 800 mL distilled water. Then

reflux extraction for 3 h with constant pressure drop funnel and reflux condensing tube. After separation, anhydrous sodium sulfate was used for drying and filtration. Weight and calculate the content of *A. argyi* essential oil according to the following formula. *A. argyi* was extracted three times at different collection stages and the average value was taken.

A. argyi essential oil content = *A. argyi* essential oil weight / *A. argyi* weight × 100%.

2.4 GC-MS analysis

The analysis of the essential oil was performed using a HP-5M, equipped with an elastic quartz capillary column (30 m x 0.25 mm; film thickness, 0.25 μm) and a HP 5972 mass selective detector for the separation. The oven temperature was programmed from 50°C for 5 min, raised to 260°C at a rate of 4°C/min, and isotherm at 260°C for 10 min. Nitrogen was carrier gas. A sample of 1 μL of essential oil was injected manually (in split mode 2:1). The mass selective detector was operated in electron-impact ionization (EI) mode with a mass scan range from 50 to 550 m/z at 70 eV. The components with high content were identified by mass spectrometry retrieval standard library: NIST library and manual map analysis, and the normalized content of each component in essential oil was measured quantitatively by peak area normalization method [17].

2.5 Analysis of Antioxidant Activity

Referring to Xu et al. [18], 2.5 mL DPPH ethanol solution (60 μmol/L) and 0.5 mL *A. argyi* essential oil of different concentrations was mixed evenly in a test tube. After the reaction of 30 min, the absorbance value of the system was determined at 517 nm. The scavenging rate of DPPH radical was calculated according to the absorbance value.

The formula is: DPPH radical clearance rate (%) = $(1 - A_1 / A_0) \times 100$,

A_0 is the absorbance value of 0.5 mL ethanol +2.5 mL DPPH, and A_1 is the absorbance value of 0.5 mL *A. argyi* essential oil +2.5 mL DPPH. The experiment was repeated three times and the average value was taken. The DPPH free radical scavenging ability of *A. argyi* essential oil was expressed by IC50 (concentration value of the corresponding drug when free radical scavenging rate was 50%).

2.6 Determination of Antibacterial Activity

The essential oil was dissolved in DMSO and sterilized by filtration through 0.22 μm Millipore filters. Antimicrobial tests were then carried out by the Oxford cup method [19]. Briefly, oxford cups (6 mm in diameter) were placed on the inoculated agar, and then 100 μL of essential oil was added with a micropipette. The diameter of inhibition zone (DIZ) was measured after 24 h of incubation at 37°C, and DMSO was used as a negative control. Tests were performed in triplicate.

2.7 Minimum Inhibitory Concentration (MIC) and Minimum Bactericide Concentration (MBC)

MIC and MBC were determined according to the method described by Kubo et al. [20] with some modifications. The stock solution of essential oil was prepared in DMSO. After being filtered by a 0.22 μm Millipore filters, the essential oil was diluted into a concentration of 0.125 to 10 mg/mL in aseptic NB medium for two consecutive times. To each tube, 50 μL of the inoculum containing approximately 1 × 10^7 CFU/mL microorganisms determined by blood count assay were added. The control group containing inoculated broth supplemented with only DMSO was also performed. The tubes were then incubated at 37°C and examined for evidence of the growth. The MIC was determined as the lowest concentration of the essential oil that demonstrated no visible growth for incubating for 24 h, while the MBC was the lowest concentration of the test essential oil that showed no visible growth in the culture incubating at 37°C for 48 h.

2.8 Statistical Analysis

One-way analysis of variance (ANOVA) and Duncan's multiple range tests were carried out to determine significant differences ($P < 0.05$) between the means by Data Processing System (DPS, version 7.05) and EXCEL program.

3. RESULTS AND DISCUSSION

3.1 Essential Oil Contents of *A. argyi* in Different Collection Stages

The content of essential oil from *A. argyi* in five different collecting stages was measured in this study. Under the same conditions, the *A. argyi* essential oil from light green to light yellow was

extracted by steam distillation. The extraction rates of the obtained essential oils are shown in Table 1. The extraction rate of essential oil increased gradually on May 24, solstice on June 7, and the extraction rate of *A. argyi* collected on June 7 was the highest, followed by a downward trend.

Therefore, due to the change of collection time, the content of *A. argyi* essential oil showed certain differences, but in general, EO-3 group was the highest (0.88%) in the contents of *A. argyi* essential oil picked during the Dragon Boat Festival. This is consistent with the tradition that *A. argyi* leaves are usually gathered on the Dragon Boat Festival. However, Hong et al. [21] concluded that the best time for collecting *A. argyi* was early June, not the Dragon Boat Festival, which might be caused by the different harvesting time, raw material producing area, climate and growing environment.

3.2 Composition Analysis of *A. argyi* Essential Oil

Through GC-MS analysis, we found that the content of *A. argyi* essential oil was different in different collection stages Table 2. Except for trace compounds and unlicensed compounds, 29, 29, 31, 28 and 31 different compounds were detected in *A. argyi* essential oils at different harvesting periods respectively. A total of 19 identical components were identified, and their content accounted for 81.6%, 82.59%, 79.44%, 56.42% and 82.82% of the essential oil quality. The components with the highest content in *A. argyi* essential oil at different collection stages are: 1,8-cineole, β-thujone, 4-p-menth-1-en-8-ol, caryophyllene, which was also the characteristic substances in *A. argyi* essential oil. Dai et al. [22] measured the chemical composition percentage of volatile oil in Hubei transplanting products in Shanxi Province as 88.26%, which was consistent with the measured results. However, relevant studies have shown that there are differences in composition and content of *A. argyi* essential oil in different regions, which may be directly affected by the growing environment, producing area, climate, and collection time.

3.3 Antioxidant Activity Analysis of *A. argyi* Essential Oil

DPPH free radical scavenging effect of *A. argyi* essential oil was used to demonstrate its antioxidant capacity. In this study, DPPH radical scavenging rate determination results of *A. argyi* essential oil in different collection stages are shown in Table 3. By comparing and analyzing the IC50 value of five groups, the group with the best DPPH free radical scavenging effect was EO-3 (IC50 = 0.41 mg/mL), which was significantly different from the other four groups ($p < 0.05$). It also can be found that different collection period will affect the free radical scavenging ability of *A. argyi* essential oil.

3.4 Determination of Antibacterial Activity of *A. argyi* Essential Oil

The antimicrobial activity of *A. argyi* essential oil was determined by the Oxford cup method. The inhibition activity of *A. argyi* essential oil in five different collection stages on six microorganisms including *S. pidermidis*, *S. aureus*, *B. subtilis*, *L. monocytogenes*, *E. coli* and *S. terphimurium* was detected. It can be found that *A. argyi* essential oil in different collection stages has inhibitory effect on various foodborne microorganisms Table 4. The DIZ values of all tested strains ranged from 14.0 to 29.5 mm, and it was found that the antibacterial effect of EO-3 group on five microorganisms was significantly different from the other four groups. However, there was no significant difference in the antibacterial activity against *S. typhimurium* between the EO-3 group and the EO-2 and EO-4 groups.

Gan et al. [23] found that *A. argyi* essential oil can effectively inhibit *S. aureus*, *E. coli* and *Salmonella*, and its mechanism may be to destroy the permeability of cell membrane, thereby inhibiting the respiration of bacteria.

Table 1. The contents of *Artemisiae argyi* essential oil collected at 5 different growing periods

Number	Picking time	Character	Volatile oil mass fraction（%）
EO-1	May 24th	Pale green	0.51
EO-2	May 31st	Light green	0.63
EO-3	June 7th (Dragon Boat Festival)	Blue-green	0.88
EO-4	June 14th	Yellow green	0.71
EO-5	June 21st	Pale yellow	0.59

Table 2. The results of GC-MS analysis on chemical composition of the essential oil

Retention time/ min	Chemical composition	Matter content（%）				
		EO-1	EO-2	EO-3	EO-4	EO-5
6.81	α-pinene	0.63	0.55	1.61	—	0.33
7.23	camphene	—	—	0.79	0.42	0.21
7.95	4-methylene-1-(1-methylethyl)-bicyclo [3.1.0] hexa	—	0.57	0.82	—	0.26
8.11	1-octen-3-ol	1.63	1.74	1.95	0.86	1.02
8.52	1,2,3-trimethylbenzene	—	—	—	—	1.31
8.68	3,3,6-trimethyl-14-heptadien-6-ol	—	—	1.01	3.88	—
9.21	α-terpinene	0.47	0.53	1.08	0.48	0.36
9.46	2-isopropyltoluene	1.05	0.78	1.77	1.02	1.01
9.63	1-methyl-1-cyclohexene	0.19	0.21	0.82	0.25	—
9.74	1,8-cineole	14.18	9.36	18.31	15 .73	13.21
10.60	1,4-cyclohexadiene, 1-methyl-4-isopropyl	1.11	1.69	2.17	1.17	1.06
10.65	3-aminopyrazole	—	—	—	9.38	7.17
10.91	3-allyl-6-methoxyphenol	0.67	0.53	0.29	—	—
11.39	artemisia ketone	0.55	—	2.51	4.74	0.63
11.50	artemesia alcohol	—	—	—	—	0.74
11.62	1-methyl-4-isopropyl-1-cyclohexene	—	0.37	0.43	—	—
11.97	(1α,2β,5α)-2-methyl-5-(1-methylethyl) bicyclo[3.1.0]hexan-2-ol	1.22	—	—	—	—
12.26	β-thujone	27.15	38.44	20.71	15.68	26.57
12.53	alpha-(-)-thujone	2.68	4.66	3.70	2.06	5.18
12.74	isocarveol	0.48	0.53	0.74	0.31	0.52
12.91	diethyldimethyl-2-cyclohexen-1-on	—	—	—	0.13	0.69
13.29	1,3,4-trimethyl-3-cyclohexene-1-carboxaldehyde	—	—	—	—	0.85
13.50	camphor	1.26	0.95	3.98	6.71	2.03
13.77	benzyl alcohol	0.84	1.17	0.61	0.14	—
14.26	verbenol	5.82	1.49	3.11	—	—
14.39	borneol	1.77	2.13	6.17	5.83	3.65
14.63	4 -p-menth-1-en-8-ol	4.81	2.69	5.04	4.11	3.98
15.11	α-terpineol	2.31	1.07	2.19	2.53	1.35
16.02	2-methyl-5-(1-methylvinyl) cyclohex-2-en-1-yl	3.57	1.16	1.37	1.52	1.73
16.74	crithmene	0.64	0.13	—	—	0.52
17.81	isophorone	—	—	—	—	0.36
18.30	1,7,7-trimethyl-, acetate, endo-bicyclo[2.2.1]heptan-2-ol	—	0.16	0.51	0.43	0.32
20.62	eugenol	0.75	1.26	0.33	0.82	1.14
22.63	caryophyllene	11.62	10.31	6.15	9.11	14.03
23.85	α-epoxycaryophyllene	1.88	1.17	0.83	0.96	1.79
24.49	germacrene d	2.15	1.58	1.14	0.88	1.76
24.81	α-selinene	1.88	0.17	—	—	—
27.52	β-eudesmene	—	—	—	—	0.84
27.85	caryphylleneoxide	1.89	1.63	1.24	1.71	1.58
29.33	10,10-dimethyl-2,6-dimethylenebicyclo [7. 2. 0] undecan-5β-ol	0.84	0.91	0.57	0.66	0.85
29.67	β-panasinsene	2.93	—	6.03	5.11	—
		96.97	87.94	97.98	80.90	97.05

Note: "—" indicates that it was not detected

Table 3. The results of antioxidant capacity of the essential oil

Essential oils	DPPH(mg/mL)
EO-1	0.64±0.04c
EO-2	0.53±0.02b
EO-3	0.41±0.02a
EO-4	0.62±0.04c
EO-5	0.77±0.04d

Table 4. Antimicrobial activities of the essential oil from *Artemisiae argyi*

Microorganisms	DIZ (mm)				
	EO-1	EO-2	EO-3	EO-4	EO-5
S. epidermidis	22.6±1.1c	25.1±0.6b	28.4±0.9a	23.6±0.6c	20.4±0.7d
S. aureus	21.9±0.8c	24.3±0.5b	27.5±0.7a	22.5±0.7c	19.8±0.5d
B. subtilis	18.7±0.5c	20.6±0.7b	24.1±0.4a	19.7±0.4b	16.9±0.3d
L. monocytogenes	17.2±0.3c	20.1±0.4b	23.9±0.3a	17.6±0.5c	15.3±0.4d
E. coli	16.7±0.4c	18.3±0.6b	20.2±0.5a	17.1±0.6bc	14.4±0.4d
S. typhimurium	17.8±0.2b	19.3±0.4a	19.6±0.3a	18.5±0.7ab	15.7±0.2c

Table 5. MIC and MBC of the essential oil from *Artemisiae argyi*

	EO-1		EO-2		EO-3		EO-4		EO-5	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. epidermidis	128	128	64	128	32	32	64	64	64	128
S. aureus	32	64	16	32	16	16	32	64	64	128
B. subtilis	64	128	64	64	32	64	64	128	128	256
L. monocytogenes	64	128	64	128	32	64	64	128	128	256
E. coli	64	64	32	64	32	32	32	64	64	128
S. typhimurium	64	128	64	64	32	64	64	128	128	256

Wu et al. [24] also showed that *A. argyi* essential oil had certain inhibitory effect on the growth of *S. aureus*, *E. coli* and *Salmonella*, which was consistent with our experimental results. Moreover, our experimental results also showed that *A. argyi* essential oil at different collection stages had a strong and effective inhibitory effect on all tested foodborne pathogenic bacteria (including Gram-positive and Gram-negative bacteria).

3.5 Determination of MIC and MBC of *A. argyi* essential oil

MIC and MBC values of *A. argyi* essential oil in different collection stages are shown in Table 5. The results showed that the MIC and MBC of the strains used in the test were between 16-128 μg/mL and 16-256 μg/mL respectively. Whether MIC or MBC, EO-3 group had the smallest values for the six test strains, which indicated that different collection periods had different effects on the antibacterial activity of *A. argyi* essential oil. By comparing the MIC and MBC of *A. argyi* essential oil against different experimental strains, it was found that *A. argyi* collected in any period had the best inhibitory effect on *S. aureus*, indicating that they were the most effective bacteriostatic agent and fungicide for *S. aureus*.

S. aureus was found to be the most sensitive microorganism with a large inhibitory circle and the lowest MIC and MBC values. Moreover, the MIC and MBC of EO-3 group were the lowest for different foodborne pathogenic bacteria, indicating that the different collection period had an impact on the antibacterial effect of *A. argyi* essential oil.

4. CONCLUSION

This study showed that *A. argyi* essential oil had obvious antioxidant activity, and good bacteriostasis. Besides, the functional activity of *A. argyi* essential oil was different in different harvest stages, which might be related to the composition content of *A. argyi* essential oil. Therefore, further research is needed to fully understand the mechanisms affecting the content

of major components. In this study, we found that the essential oil collected on June 7 had the largest total content of components and the strongest functional activity, which provided a basis for the commercial production of *A. argyi* essential oil.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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