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Chemical Constituents, Antibacterial and Coagulation Activity of the Essential Oil from the Stem of *Artemisia argyle* H. Lév.

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Abstract: *Artemisia argyle* H. Lév. (*A. argyle*) is a plant of historical importance known as the "mother of herbs" in the Middle Ages. As the leaves of *A. argyle* have been studied more, but the stems has not been reported. This paper explores the chemical constituents, antibacterial and coagulation activities of the Essential Oil from the Stems of *A. argyle* (EOSAA) for better exploitation and utilization of resources. EOSAA was extracted by hydrodistillation and its chemical constituents were determined by GC-MS. *Escherichia coli, Staphylococcus aureus and Bacillus pumilus* were used as test microbial strains to evaluate the antimicrobial potential of EOSAA. Three indicators of Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT) and Thrombin Time (TT) were used to evaluate the coagulation activity. The extraction rate was 0.20% and 36 compounds were identified, accounting for 93.75% of the total content. Among the identified compounds, 41.72% belong to oxiterpenes, 21.31% belong to sesquiterpenes and terpenoids are extremely abundant. The main constituents were as follows: 3-Biphenylmethanol (16.01%), 2,6-Dimethyl-8-(tetrahydropyran-2-yloxy)-octa-2,6-dien-1-ol (9.22%), Germacrene D (7.22%), α-Bisabolol (5.85%), L(-)-Borneol (5.75%), Eucalyptol (5.71%) and so on. EOSAA exhibited certain inhibitory effects against *Escherichia coli, Staphylococcus aureus* and *Bacillus pumilus*, the minimum inhibitory concentrations were 40, 42 and 48 mg/mL, respectively. Compared with Yunnan Baiyao, APTT, PT and TT time of high concentration (60 mg/mL) EOSAA were shortened by 28.83, 42.86 and 68.99%, respectively. The experimental results show that EOSAA is a natural coagulant with antibacterial effect and has a broad application prospect.

Keywords: *Artemisia argyi H.* Lév, Essential Oil, Chemical Constituents, Antibacterial Activity, Coagulation Activity

Introduction

Artemisia argyle H. Lév. (*A. argyle*) is a plant of the genus Artemisia in the compositae family which appears perennial herbs or slightly subshrub forms with strong fragrance. It widely distributes around the world (Zhang *et al*., 2013). *A. argyi* has been received considerable attention due to its abundant bioactive substances. The leaves of *A. argyle* smoke have obvious antibacterial effect on the affected area, reducing the number of bacterial colonies in the air and completely inhibiting the growth of pyogenic bacteria (Zhang *et al*., 2014). The leaves of *A. argyle* are one of the common gynecological drugs, which were recorded as "hemostatic drugs" in medical records of past dynasties (Tan *et al*., 1992; Zheng *et al*., 2004), regulating the meridians and protecting the fetus, etc. (Adams *et al*., 2012). Pharmacological studies show that *A. argyle* has the effect of anti-fibrinolysis hemostasis by reducing capillary permeability (Yu *et al*., 2012). *A. argyle* is a traditional Chinese medicine and the whole grass can be used as medicine (Li *et al*., 2008).

The leaves of *A. argyle* contain essential oil, flavonoids, glycosides, terpenoids and other active compounds, among which the essential oil is the most important chemical component. For example, the Essential Oil of *A. argyi* Leaves (EOLAA) showed anti-histaminic effects and antifungal activity (Huang *et al*., 2012). Moreover, EOLAA had strong antibacterial effects against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enteritidis* (Smith-Palmer *et al*., 2001). The chemical constituents of the EOLAA are extensive and thorough, mainly including eucalyptol, thujone, alcanfor and borneol (Xiang *et al*., 2018; Junjie *et al*., 2016). According to traditional knowledge and medical records, the roots, stems, leaves, buds and flowers of fresh or dried plants all contain some active ingredients that can cure some diseases (Lae *et al*., 2019). For *A. argyle*, in addition to the leaves, the stems are also rich in chemical constituents. In the current study, usually only the leaves are fully utilized, while the stems are discarded as waste, which not only causes environmental pollution but also wastes resources.

At present, there are numerous reports about the leaves of *A. argyle*, but few on stems. Herewith, the chemical constituents and its antibacterial and coagulation activity of Essential Oil from the Stems of *A. argyle* (EOSAA) obtained by hydrodistillation were done in this study, which has a broad application prospect as a kind of natural coagulant with antibacterial effect.

Materials and Methods

Materials

The samples were collected in June 2019 in Longtan District, Jilin City, Jilin Province. The voucher specimen (20190612) was preserved in the herbarium of Jilin Engineering Research Center for Agricultural Resources and Comprehensive Utilization, Jilin Institute of Chemical Technology, Jilin, China. All chemicals and reagents were of analytical grade and microbial strains were purchased from Beijing Zhongke Zesheng Biotechnology Co., Ltd. (Beijing, China). Assay kits used for the determination of Prothrombin Time (PT), Thrombin Time (TT) and Activated Partial Thromboplastin Time (APTT) content were provided by SINNOWA Medical Science and Technology Co., Ltd (Nanjing, Jiangsu, China). The fresh plasma kits were purchased from Dade Behring Marburg Gmbh (Marburg, Hesse, Germany).

Methods

Extraction of Essential Oil

Fresh stems of *A. argyle* (60 g) were placed into a 1000 mL two-necked flask equipped with 600 mL of distilled water and connected with a Clevenger-type device, then heated together for 3 h. The essential oil was collected, dried with anhydrous Na_2SO_4 and hermetically stored at 0℃ for GC-MS analysis (Sparkman, 2005).

Component Analysis

GC-MS Analysis

The chemical composition of EOSAA was analyzed by GCMS-QP2010 instrument (Shimadzu, Kyoto, Japan) and the column was Rxi-5sil columnn (30 m, 0.25 mm, film thickness 0.25 μm). The carrier gas was nitrogen and flow rate was 1 ml/min. The collision energy for Mass Spectrometry (MS) detection was 70 eV and data were recorded within 40-450 amu. The vaporizer temperature and ion-source temperature were respectively adjusted to 280 and 230℃. Each chemical composition was identified by comparing the retention index obtained from a database (NIST05) with the retention indexes calculated on the basis of n-alkanes (C_9-C_{46}) (Rehman *et al*., 2021; Chzhu *et al*., 2020).

An essential oil sample dissolved in diethyl ether (60 mg/ml) was injected automatically into a vaporizer at 250℃ with a split ratio of 1:30, the conditions of the column temperature were as follows: Starting at 60℃ and maintaining it for 6min; then increasing 60 to 300℃ at 3℃/min; finally keeping 300℃ for 10 min (Padalia *et al*., 2016).

Antibacterial Activity

Escherichia coli ATCC 33456, *Staphylococcus aureus* ATCC 49775 and *Bacillus pumilus* ATCC 700814 were used as test microbial strains to evaluate the antimicrobial potential of EOSAA. Using 50% Dimethyl Sulfoxide (DMSO) as solvent, then the EOSAA solution was successively diluted to 100~10 mg/ml by 2 folds dilution method. Adding 50 microliters solution to 96-well plate and 150 μL microorganism liquid which was prepared by fresh nutrient medium containing 10^7~10^8 CFU/ml microbial strains were added into each hole, then it was placed in an incubator at 37℃ for 24 h, after that the absorbance was measured at 600 nm. Chloramphenicol solution (100~1 mg/ml) was used as the positive control and 50% DMSO as the negative control (Zheng *et al*., 2019; Toledo *et al*., 2020).

Coagulation Activity

Taking 0.50% Yunnan Baiyao (a well-known hemostatic on the market, positive control) and 0.90% NaCl (blank control) as reference, the coagulation test was carried out using CL-2000BV coagulometer (Jiangsu Xenova medical Technology Co., Ltd, China) to determine the coagulation activity of EOSAA. Three indicators of Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT) and Prothrombin Time (PT) were used to evaluate the coagulation activity.

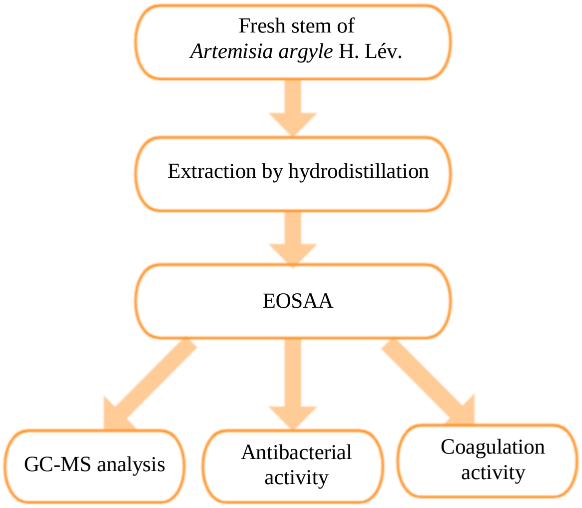

Fig. 1: The flow chart of the experiment methods

Plasma pretreatment: 0.109 mol/L sodium citrate was accurately mixed with fresh plasma kits (Dade Behring Marburg Gmbh) at a volume ratio of 1:9 and centrifuged at 3000 r/min for 20 min. The supernatant of platelet-poor plasma was collected, sealed with plastic tubes and took the refrigerated preservation. Before the experiment, the plasma preheating was carried out at 37°C. Note that all experiments in this process were carried out at 37°C and should be completed within 2 h (Dore *et al*., 2013).

APTT Assay

20 μL of five different concentrations of EOSAA (20, 30, 40, 50 and 60 mg/mL) and 80 μL of plasma were incubated for 1 min, then added 100 μL APTT reagent and continue to incubate for 1 min, at last 25 mmol/L $CaCl_2$ (100 μL) was added (Martinichen-Herrero *et al*., 2005).

PT Assay

20 μL of five different concentrations of EOSAA (20, 30, 40, 50 and 60 mg/mL) and same plasma were incubated for 30 s, then 200 μL of PT reagent was added to the above mixture (Sun *et al*., 2018).

TT Assay

20 μL of five different concentrations of EOSAA (20, 30, 40, 50 and 60 mg/mL) were incubated with same plasma for 30 s, then 0.1 mL of preheated TT reagent was added to the above mixture (Wang *et al*., 2013).

In a word, the flow chart of the experiment methods is shown in Fig. 1.

Results and Discussion

GC-MS Analysis

The EOSAA was extracted by hydrodistillation and the blackish green oil was obtained with the extraction rate of 0.20%. 36 compounds were identified by gas chromatography-mass spectrometer (GC-MS) with total contents of 93.75% (Table 1). The results were as follows: 3-Biphenylmethanol (16.01%), 2,6-Dimethyl-8-(tetrahydropyran-2-yloxy)-octa-2,6-dien-1-ol (9.22%), Germacrene D (7.22%), α-Bisabolol (5.85%), L(-)-Borneol (5.75%), Eucalyptol (5.71%), (R)-camphor (2.05%), (+)-3-Thujone (1.82%) and so on. In the identified compounds, where 41.72% of the compounds belonged to oxyterpenes terpenes, followed by sesquiterpenes with content of 21.31%, this indicates that terpenoids are extremely abundant.

The EOLAA was extracted by hydrodistillation and the extraction rate was 0.50% (Guan *et al*., 2019). The main chemical components of the EOLAA were identified by GC-MS as monoterpenes and their derivatives, sesquiterpenes and their derivatives, ketones (aldehydes) and so on (Pan *et al*., 2012), the main active compounds are eucalyptol (14.67%), camphor (6.87%), borneol (6.482%), α-Thujone (7.989%), caryophyllene oxide and so on (Dhanapal *et al*., 2016; Guan *et al*., 2019). Eucalyptol (5.71%), camphor (2.05%) and borneol (5.75%) were found in the EOSAA, but the content was lower than that of the EOLAA. Because the constituents of the essential oil of *A. Argyi* is different due to the different parts, the content is also different, but both the main parts are generally eucalyptol, camphor, borneol, platycladone, caryophyllene, oxidative caryophyllene and so on (Hu *et al*., 2020).

Antibacterial Activity

The Minimum Inhibitory Concentration (MIC) values of EOSAA against *Escherichia coli*, *Staphylococcus aureus and Bacillus pumilus* were shown in Table 2. EOSAA has a certain inhibitory effect on *Escherichia coli*, *Staphylococcus aureus and Bacillus pumilus*, which may be related to its a large amount of terpenoids containing oxygen, such as, α-Bisabolol, L(-)-Borneol, Eucalyptol and so on (Popović *et al*., 2010; de Morais *et al*., 2016). Borneol is a valuable medicinal ingredient, advanced flavor and chemical used in food and folk medicine in China and India for its anti-inflammatory and neuroprotective properties (Asadollahi *et al*., 2019). Eucalyptol has antibacterial, anti-inflammatory and anti-oxidation effects (Jiang *et al*., 2019). So antibacterial activity of EOSAA may be related to terpenoids, terpenoids have antimicrobial and bactericidal activities, which can destroy the permeability of cell membranes (Yang *et al*., 2019), lead to the leakage of nucleic acid and other macromolecular substances and interfere with the synthesis and accumulation of cell bacterial proteins. In addition, terpene interactions can induce changes in cellular respiration, leading to the subsequent decoupling of microbial oxidative phosphorylation (Zengin and Baysal, 2014), which has synergistic inhibitory effects on drug-resistant bacteria. (Zacchino *et al*., 2017).

Table 1: Chemical constituents of the EOSAA by hydrodistillation and analyzed by GC-MS

NO.	Compounds	RI[a]	RI[b]	Molecular formula	%
1	α-Phellandrene	969	969	$C_{10}H_{16}$	0.84
2	Eucalyptol	1012	1031	$C_{10}H_{18}O$	5.71
3	(+)-3-Thujone	1062	1109	$C_{10}H_{16}O$	1.82
4	(R)-camphor	1121	1156	$C_{10}H_{16}O$	2.05
5	L(-)-Borneol	1138	1186	$C_{10}H_{18}O$	5.75
6	cis-β-Terpineol	1158	1201	$C_{10}H_{18}O$	1.15
7	γ-Terpineol	1191	1221	$C_{10}H_{18}O$	2.38
8	Nerol	1228	1231	$C_{10}H_{18}O$	1.26
9	L-alloaromadendrene	1386	1422	$C_{15}H_{24}$	3.81
10	β-sesquiphellandrene	1446	1457	$C_{15}H_{24}$	4.88
11	1,4,6-Trimethyl-5,8-dihydronaphthalene	1465	1495	$C_{13}H_{16}$	0.85
12	β-selinene	1469	1505	$C_{15}H_{24}$	0.77
13	(-)-Isocaryophyllene	1494	1511	$C_{15}H_{24}$	3
14	Germacrene D	1515	1512	$C_{15}H_{24}$	7.22
15	α-Bisabolene	1518	1586	$C_{15}H_{24}$	0.83
16	Viridiflorol	1530	1589	$C_{15}H_{26}O$	0.8
17	Linalyl valerate	1570	1593	$C_{15}H_{26}O_2$	1.35
18	β-Humulene	1574	1614	$C_{15}H_{24}$	0.8
19	α-Cadinol	1580	1616	$C_{15}H_{26}O$	1.44
20	Guaiol	1614	1647	$C_{15}H_{26}O$	1.46
21	α-Bisabolol	1625	1660	$C_{15}H_{26}O$	5.85
22	Octahydroanthracene	1652	1686	$C_{14}H_{18}$	1.13
23	1,2,3,4-tetrahydroanthracene	1717	1697	$C_{14}H_{14}$	1.06
24	3-Biphenylmethanol	1723	1770	$C_{13}H_{12}O$	16.01
25	2,6-Dimethyl-8-(tetrahydropyran-2-yloxy)-octa-2,6-dien-1-ol	1953	2024	$C_{15}H_{26}O_3$	9.22
26	Mono-2-ethylhexyl phthalate	2162	2167	$C_{16}H_{22}O_4$	1.25
27	Thunbergol	2211	2281	$C_{20}H_{34}O$	1.22
28	Pentacosane	2506	2568	$C_{25}H_{52}$	0.77
29	Hexanedioic acid, dioctyl ester	2543	2571	$C_{22}H_{42}O_4$	1.13
30	Triacontane	3003	3111	$C_{30}H_{62}$	1.46
31	Pentatriacontane	3500	3503	$C_{35}H_{72}$	0.96
32	17-Pentatriacontene	3508	3570	$C_{35}H_{70}$	1.3
33	Tetracontane	3997	4003	$C_{40}H_{82}$	0.79
34	2,3-bis[(3,7,11,15-tetramethylhexadecyl)oxy]propan-1-ol	4113	4183	$C_{43}H_{88}O_3$	1.75
35	Tetratetracontane	4395	4247	$C_{44}H_{90}$	1.68
	Oxygen terpene				43.4
	Sesquiterpenes				25.65
	lignans				16.01
	Others				10.37

RI[a] Retention indices relative to C_8-C_{46} n-alkanes on a HP-5MS column
RI[b] is based and calculated on retention time relative to C_9-C_{46}

Table 2: MIC values of EOSAA

	E. coli	*S. aureus*	*B. pumilus*
EOSAA (mg/mL)	40	42	48
Chloramphenicol (mg/mL)	＜0.8	＜0.8	＜0.8

Table 3: The coagulation activity of EOSAA

Samples	Concentration (mg/mL)	APTT(s)	PT(s)	TT(s)
EOSAA	60	$23.7\pm2.9^{***\#}$	$3.2\pm0.7^{****}$	$4.0\pm0.4^{***\#}$
	50	$25.6\pm2.4^{****\#}$	$8.2\pm1.4^{**\#}$	$4.9\pm0.8^{****}$
	40	$41.0\pm1.9^{***\#}$	$13.2\pm1.6^{***\#\#\#\#}$	$8.4\pm1.1^{**}$
	30	$45.5\pm3.3^{**\#\#}$	$14.7\pm1.4^{**\#\#\#}$	$12.6\pm2.3^{\#\#\#}$
	20	$50.2\pm3.9^{***\#\#\#}$	$15.6\pm0.8^{**\#\#\#}$	$16.2\pm2.0^{**\#\#\#}$
Yunnan Baiyao	0.5	$33.3\pm3.8^{****}$	$5.6\pm0.7^{****}$	$12.9\pm1.4^{***}$
0.90%NaCl		59.7 ± 5.4	19.3 ± 2.1	16.7 ± 3.6

****P<0.0001, or, ***P<0.001, or, **P<0.01 Vs. Control group; $^{\#\#\#\#}$P<0.0001, or, $^{\#\#\#}$P<0.001, or, $^{\#\#}$P<0.01, or, $^{\#}$P<0.05 Vs. Yunnan Baiyao

Coagulation Activity

Coagulation is a series of enzymatic reactions, activated by Pre correlation factors, ultimately produces thrombin and fibrin. APTT is a screening test to test whether the endogenous blood coagulation system is normal. PT is a screening test that reflects whether the exogenous coagulation pathway is normal. TT refers to the time required for blood clotting after thrombin is added to plasma. It is commonly used to test the function of blood coagulation, anticoagulation and fibrinolytic system (Jastrzebski *et al.*, 2014). As shown in Table 3, the coagulation activity was positively correlated with the sample concentration. Moreover, compared with Yunnan Baiyao, APTT, PT and TT time of high concentration EOSAA were shortened by 28.83, 42.86 and 68.99%, respectively. Since EOSAA affects APTT, PT and TT, it is indicated that it affects the coagulation function through the way of endogenous and exogenous coagulation or/and common. This is consistent with the application of *A. argyle* leaves to the treatment of menorrhagia, leakage, hemostasis and other blood syndromes (Dhanapal *et al.*, 2016). Since the coagulation activity of the EOSAA was reported for the first time and the pharmacological study of the essential oil from *A. Argyi* was mainly conducted in animal experiments, the coagulation mechanism of the specific components remains to be further studied.

Conclusion

EOSAA was extracted by hydrodistillation and the extraction rate was 0.20%. A total of 36 compounds were identified by GC-MS, accounting for 93.75% of the total coment, which its large amount of constituents is terpenoids such as eucalyptol, borneol, camphor and so on. The EOSAA has a certain inhibitory effect on *Escherichia coli*, *Staphylococcus aureus* and *Bacillus pumilus*. The time of APTT, PT and TT were shortened, indicating that EOSAA had a certain coagulation effect. Therefore, EOSAA is a kind of coagulant with antibacterial effect, which has potential application prospects in cosmetics and medicine. This research provides value for the further development and utilization of the stem of *A. argyle*.

Nomenclature

EOSAA: The essential oil from the stem of *Artemisia argyle* H. Lév.
EOLAA: The essential oil from the leaves of *Artemisia argyle* H. Lév.
A. argyle: *Artemisia argyle* H. Lév.
GC-MS: Gas Chromatography-Mass Spectrometer.
DMSO: Dimethyl sulfoxide.
APTT: Activated partial thromboplastin time.
TT: Thrombin time.
PT: Prothrombin time.
MIC: Minimum Inhibitory Concentration.

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Author's Contributions

Jiale Shan: The chemical composition, antibacterial and coagulation activity of the EOSAA were studied and analyzed in detail.

Hongli Zhou: Design experimental of this manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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