Extraction, Chemical Composition and Antibacterial Activities of The Essential Oil of *Salix Babylonica* in Viet Nam

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Abstract: This study investigated the chemical compositions and the antibacterial activities of *Salix babylonica* essential oils from Viet Nam. The gas chromatography-mass spectrometry system was used to analyze the chemical compositions of essential oils. The antibacterial activities of ***S.** babylonica* Eos were determined by using diameter of inhibition zones. The result showed that ***S.** babylonica* EOs exhibited antibacterial activity against the tested bacteria in a concentration-dependent manner.

Keywords: Salix Babylonica, Leaves, Essential Oil, GC-MS, Antibacterial Activity.

1. INTRODUCTION

Salix babylonica (S. babylonica) (Salicaceae family), commonly known as weeping willowis perennial shrubs plant widely distributed in Africa, North America, Europe, and Asia. In Vietnam, S. babylonica is used as an ornamental plant. S. babylonica is also used as a herb in folk medicine for treatment the thorn pain, peeling gums, heat absorption, delirium, blindness, loess, inner heat Leaves. It's flowers and fruits are used to treat overturning, overturning, and radiation. The S. babylonica bark is also used for rheumatism, neuralgia, deworming, antiseptic, and as an anthelmintic drug (El-Shazly et al. 2012).

S. babylonica plant has many biological chemical compositions, such as flavonoids, terpenoids, lignans, and phenolic compounds (Wahab et al., 2018; Tawfeek et al., 2021; Hussain et al., 2012). Extraction of S. babylonica has shown that the the ability to antifungal, antibacterial (Mostafa et al., 2020), Anti-HIV Activity (Eftekhari et al., 2014), antioxidant (Gligorić et al., 2019; El-Sayed et al. 2015; Enayat et al., 2009), (Sobeh et al., 2019), anticancer (Hostanska et al., 2007; El-Shemy et al., 2007, 2003;).

Currently, in Vietnam, there have not been many studies on the chemical composition and biological activity, especially the chemical composition and biological activity of the essential oil of this plant, so in this study, we determine the chemical compositions and biological activity of the essential oil extracted from the S. babylonica collected in the mountainous area of Northern Vietnam.

2. MATERIALS AND METHODS

2.1.Plant material: Fresh leaves of S. babylonica were collected in Viet Nam in July, 2022. The samples were authenticated by Dr S.D. Thuong, Faculty of Biology, Thai Nguyen University of Education.

2.2.Extraction of essential oils by steam distillation extraction: The fresh leaves were distilled with 2000 ml sterile distilled water by steam distillation device for 6h. Essential oils which are extracted using a separatory funnel is light yellow-colored, strong scent. Essential oils stored in a glass vial at 4-5 ℃ prior to next analysis.

2.3.GC/MS analysis: The gas chromatography-mass spectrometry (GC/MS) analysis was conducted using a Hewlett Packard GC (HP5890 series II) coupled with a quadripole MS system (model HP MSD5971). The system was equipped with an electron impact source operating at 200°C. A fused silica-capillary column with an apolar stationary phase HP5MS (30 m x 0.25 mm, 0.25 µm film thickness) was used. The chromatographic conditions mirrored those of the GC analysis. The electron impact spectra were recorded at an ion voltage of 70 eV, covering a scan range of 30-600 uma.

2.4.Identification of essential oil constituents: The identification of compounds was accomplished by comparing their retention indices (RI) on an HP-5MS column and cross-referencing them with the data available in the NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/). Additionally, the mass spectra of the compounds were compared with those stored in the Wiley NBS75K.L and NIST/EPA/NIH (2002 and 2014 version) mass spectral libraries for further confirmation.

2.5.Antibacterial activity: Gram-positive strain includes Pseudomonas aeruginosa-PA and Staphylococcus aureus - SA, and gram-negative strains include Escherichia coli – E. coli, which were provided in lyophilized form by Faculty of Biology, Thai Nguyen University of Education. The antibacterial activity of the essential oil was assessed using the disc agar diffusion method (Hadacek & Greger, 2000) and the diameter of the antibacterial ring was measured to evaluate the oil's antibacterial effectiveness.

The studied bacterial strains were cultured on nutrient medium supplemented with agar at the condition of 30℃ for 24 h. Microbial suspensions were diluted with sterile distilled water to a concentration of about 10^8 CFU/ml. Spread 0.1 ml of the microorganism suspension onto a plate with nutrient agar and made 5 wells in agar (diameter 6mm) then 50 µL test solutions added each wells for 1h, 4ºC. The bacteria were incubated at 37 ℃ for 24 h. the test solutions are essential oils diluted with DMSO solution at concentrations of 25 g/mL, 50 g/mL and 100 g/mL. After incubation, antibacterial results were observed by measuring the diameter of inhibition zones in centimeter. A negative control is DMSO without test material. A positive control is Ampicilline 50mg/ml. The experiments were performed in triplicate.

3. RESULTS AND DISCUSSION

Willow essential oil obtained by steam distillation is a liquid, pale yellow, lighter than water. In this study, the chemical composition of the essential oil of willow collected in two provinces of Bac Giang and Thai Nguyen was determined and compared with each other. The results of chemical composition, molecular formula, RI and Relative content of willow essential oil of leaves and willow bark collected in 2 provinces was shown in Tables 1 and 2. The results of Tables 1 and 2 show that the composition of essential oils of willow bark and willow leaves are the same except for a few components.

There are 25 compounds in leaves essential oils were determined in the S. babylonica essential oils collected from Bac Kan and Dinh Hoa provinces in VietNam, including Camphene (7.5%), 2-(4-methyl-3-cyclohexen-1-yl)-2-propanol (4.5%), 3,7-dimethyl-1,6-octadien-3-ol (2.6%), Geranyl acetate (4.2%), α-Humulene (4.3%), Phytol (5.6%), Pentacosane (33.2%) are major chemical compositions in essential oils of S. babylonica leaves collected in Bac Kan province. 2-(4-methyl-3-cyclohexen-1-yl)-2-propanol (5.5%), 3,7-dimethyl-1,6-octadien-3-ol (5.5%), Methyl citronellate (4.6%), Trans - Caryophyllene (8.9 %), α-Humulene (15,6%), Farnesol (5.2%), and Pentacosane (14.3%) are major chemical compositions in essential oils of S. babylonica bark. According to Mu et al. (Mu et al., 2014) also determined the volatile compounds from Salix babylonica Pentacosane's derivativies, salicylic acid derivatives are major compositions. In S. babylonica bark essential oils, pentacosane has smaller concentrations in comparisson with its leaf essential oils.

Table 1. Chemical compositions of *S.* babylonica leaves essential oils

No	Compound	Molecular formula	RI	Relative content (%)	
				Bac Kan sample	Dinh Hoa sample
1.	• α-Pinene	$C_{10}H_{16}$	917	2.5	1.1
2.	Camphene	$C_{10}H_{16}$	947	7.5	0.3
3.	1 Nonan-4-ol	$C_9H_{20}O$	1078	2.5	0.1
4.	2 Citronellal	$C_{10}H_{18}O$	1158	0.4	0.2
5.	2-(4-methyl-3-cyclohexen-1-yl)-2-propanol	$C_{10}H_{18}O$	1190	0.7	5.5
6.	3,7-dimethyl-1,6-octadien-3-ol	$C_{11}H_{18}O_2$	1215.4	2.6	5.5
7.	Methyl citronellate	$C_{11}H_{20}O_2$	1262	2.8	4.6
8.	Trans-Carvone oxide	$C_{10}H_{14}O_2$	1279	1.1	0.4
9.	Salicylic acid	$C_7H_6O_3$	1297	2.4	0.2
10.	3 Dihydrocarvyl acetate	$C_{12}H_{20}O_2$	1304	0.8	2.5
11.	4 α-Terpinyl acetate	$C_{12}H_{20}O_2$	1367	1.4	0.5
12.	Geranyl acetate	$C_{12}H_{20}O_2$	1386	4.2	0.3
13.	5 Methyleugenol	$C_{11}H_{14}O_2$	1410	0.4	0.3
14.	6 Cedrene	$C_{15}H_{24}$	1422	0.3	2.2
15.	7 Trans-Caryophyllene	$C_{15}H_{24}$	1444	5.2	8.9
16.	α-Humulene	$C_{15}H_{24}$	1452	4.3	15.6
17.	α-Farnesene	$C_{15}H_{24}$	1507	1.0	3.1
18.	8 Nerolidol	$C_{15}H_{26}O$	1531	1.3	2.8
19.	9 Dodecanoic acid	$C_{12}H_{24}O_2$	1562	0.3	2.5
20.	10 Benzoic acid, hexyl ester	$C_{13}H_{18}O_2$	1576	0.2	2.1
21.	11 (-)-Spathulenol	$C_{15}H_{24}O$	1582	1.2	1.3
22.	12 Hexadecene	$C_{16}H_{32}$	1592	0.7	2.1
23.	13 Farnesyl acetate	$C_{17}H_{28}O_2$	1818	0.7	5.2
24.	14 Phytol	$C_{20}H_{40}O$	2122	5.6	4.3
25.	Pentacosane	$C_{25}H_{52}$	2500	33.2	14.3

After analyzing and comparing the chemical composition, we evaluated the antibacterial activity of the essential oils of willow bark and leaves using the disc agar diffusion method (Hadacek & Greger, 2000). The results are given in Table 2.

Table 2. DD value of antibacterial activity of the *S. babylonica* essential oil

Test sample	**Concentration**	***Escherichia coli***		***Pseudomonas aeruginosa***		***Staphylococcus aureus***	
		Leaves	Bark	Leaves	Bark	Leaves	Bark
Ampicilline	50 mg/ml	23		14		13	
DMSO	-	0	0	0	0	0	0
Bac kan sample	25 mg/ml	22	21	24	20	22	25
	50 mg/ml	31	31	37	35	35	33
	100 mg/ml	35	37	34	33	34	34
Dinh Hoa sample	25 mg/ml	24	23	24	27	28	29
	50 mg/ml	32	32	25	23	32	31
	100 mg/ml	33	26	32	32	34	33

Negative control (0): DMSO; positive control (+)

From the Table 2, The results indicate that both the essential oils derived from the leaves and bark of S. babylonica exhibit antibacterial activity against the tested bacteria. The inhibitory effects of the oils increase with higher concentrations of the samples. Furthermore, under the experimental conditions, the antibacterial activity of the *S.* babylonica essential oil surpasses that of antibiotics. The essential oils demonstrate stronger inhibitory activity against P. aeruginosa and S. aureus compared to E. coli.

CONCLUSION

In conclusion, we extracted essential oil and determined chemical compositions the *S.* babylonica essential oil from Bac Kan and Dinh Hoa provinces had many same components, but the relative content of them were different. The results from this study indicate that both essential oils have demonstrated antibacterial activity against the tested bacteria. However, further research is required to investigate the specific factors contributing to these differences.

Disclosure Statement

No potential conflict of interest was reported by the author(s).

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DOI: https://doi.org/10.15379/ijmst.v10i1.2879