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Analysis of Essential Oil of *Salix babylonica* Collected in Vietnam: Phytochemical Components and Antibacterial and Anticancer Activity

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Abstract: This study investigated the chemical compositions and inhibitory activities of essential oils (EOs) of *Salix babylonica* from Vietnam. The gas chromatography–mass spectrometry (GC/MS) system was used to analyze the chemical compositions of *Salix babylonica* essential oils. A total of twenty-eight and thirty-one compounds were identified in essential oils of the leaves and bark, among which many chemical compositions were identified for the first time in this plant. *Salix babylonica* essential oils demonstrated antibacterial activities against Gram-negative strains such as *Pseudomonas aeruginosa* (PA) and *Escherichia coli* and Gram-positive strains such as *Staphylococcus aureus* (SA), and demonstrated anti-cancer activities against three cancer cell lines (HepG2, MCF-7 cell, and A549). The evaluation of the ability to inhibit three strains of microorganisms and inhibit the growth of three cancer cell lines was first conducted using essential oils extracted from the plant species *S. babylonica* collected in Asia, which will be the basis for using essential oils of this plant in medicine.

Keywords: antibacterial activity; anti-cancer activity; essential oil; *Salix babylonica*; volatile oil

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1. Introduction

The *Salix* genus (Salicaceae family, *Salix* genus), commonly known as weeping willow, is a perennial shrub plant widely growth in Africa, Asia, Europe, and North America [1]. *Salix* plants are used as an ornamental plant and also as herbs in folk medicine for the treatment of thorn pain, gingivitis, antipyretic, delirium, blindness, loess, and hemoptysis [1]. Its flowers and fruits are used to treat overturning and radiation, and *Salix* plants are also used for rheumatism, neuralgia, deworming, as an antiseptic, and as an anthelmintic drug [1,2].

Many works have reported the biological and chemical compositions of *Salix* plants such as flavonoids, organic acids groups, phenolics groups and their derivatives, terpenes, sterols, lignans, and volatiles [1–19]. Flavonoids of the *Salix* plant constitute typical chemical compositions such as flavones, flavan-3-ols, chalcones, dihydrochalcones, anthocyanins, and other derivatives [1,3–20]. In the *Salix babylonica* (L.) plant, major flavonoids such as apigenin glycoside (apigenin-7-*O*-galactoside [15]) and chrysoeriol [20]; luteolin [20,21] and its glycosides such as luteolin-6-*C*-*β*-D-glucopyranoside (iso-orientin) [21], luteolin-4′-*O*-glucoside, luteolin-7-*O*-*β*-D-glucopyranoside [15], and kaempferol-7-*O*-glucoside [3]; and flavan-3-ols such as (epi)catechin gallate and (epi)gallocatechin gallate [19] have been reported. For phenolics and its derivatives of the *Salix* plant, more than 90 compounds have been reported. 4-(Hydroxymethyl)phenyl *β*-D-glucopyranoside [3], salicin [15], salidroside [8], tremuloidin [21], triandrin [18], trichocarpin, 2′-*O*-acetyltrichocarpin [15], and vimalin [8] of *Salix babylonica* (L.) were isolated as major chemical compositions, and this is

also the main group of compounds found in plants of the genus *Salix*. The phenolic acids in *Salix* plants are either benzoic or cinnamic acid or hydroxycinnamic acid derivatives. *Salix* plants are rich resources of phenolic acids, among which *S. purpurea* L. and *S. alba* L. bark have been identified with the highest number of phenolic acids [22]. Terpenes, volatile terpenes, and lignans have been detected in many species of the *Salix* genus. *S. cheilophila* C. K. Schneid. twigs [23]; *S. tetrasperma* Roxb. leaves, bark, and flowers [2,24]; *S. subserrata* Willd. [25]; *S. babylonica* L. [3,26,27]; *S. caprea* L. [28]; *S. egyptiaca* L. [29]; and *S. alba* L. [30] have been found to contain more terpenes, volatile terpenes, and lignans. In *Salix babylonica* leaves collected in Egypt, depending on the comparisons of retention time or mass spectral data, Salem et al. [26] discovered 59 compounds in their sample. The main compositions were tritetracontane (an aliphatic hydrocarbon: 15.2%), 9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E, atrioleoylglycerol: 11.1%), hexadecanoic acid-methylester (a saturated fatty acid: 10.5%), 1,3-dioxane-4-(hexadecyloxy)-2-pentadecyl (a heterocyclic organic compound: 10.3%), phytol (3,7,11,15-tetramethy-2-hexadecen-1-ol: 9.7%), and aliphatic hydrocarbons such as nonadecane (1.2%) and hexatriacontane (0.8%) [26].

Total extracts and the isolated compounds of *Salix* species have been applied to treat headache, rheumatic diseases, menstrual cramps, and toothache in traditional medicine [31]. In recent research, extracts and the isolated compounds of *Salix* species confirmed many useful biological activities such as anticancer, anti-inflammatory, anti-microbial, anti-diabetic, anti-oxidant, neuroprotective, and hepatoprotective activities [27–34]. Antimicrobial activities of *Salix* plants have been observed as the most evident by limiting the activity of multidrug-resistant bacteria, which are high-risk species of bacteria, and these bacteria are used for research to provide a great opportunity for the treatment of disease and the discovery of new substances of natural resources that have the ability to inhibit bacteria, especially against multidrug-resistant bacteria [9,27,29–35]. The extracts of *S. babylonica* L. from leaves were experimented against bacterial strains by using twofold serial dilutions on Mueller–Hinton agar and using the Agar-gel diffusion method. The results showed that the average diameter of the inhibition zone was 13.38 $\pm$ 2.22 mm and the value of MIC_{50} was 70.4 $\pm$ 17.41 mg/mL against *E. coli* and *Salmonella enterica*, respectively [35]. The inhibitory activities of extract fractions from hydroalcoholic and subfractions of *S. babylonica* L. against two bacterial strains were determined by using the broth microdilution method to afford MIC values of *Listeria monocytogenes* and *S. aureus* of 0.78 mg/mL and 0.39 mg/mL, respectively [4]. In addition, through in vitro assay, *Salix* extracts were tested against cancer cell lines such as PC3 cells (prostate cancer cells), human acute lymphoblastic leukemia (ALL cells), Hep G2 cells (liver cancer cells), MCF7 (breast cancer cells), HCT116 (colorectal cancer cells), A549 (adenocarcinomic human alveolar basal epithelial cells), SW2 (small-cell lung cancer) cells, and H1299 (human lung cancer cell line) [34,36–38]. A fraction of *Salix* extracted by ether and chloroform was effective against AML cells (acute myeloid leukemia), while a fraction of extract from the young leaves of *S. safsaf* effectively reduced the tumor growth of cancer cell lines [37]. On other hand, the *Salix* plant also exhibited anti-HIV (human immunodeficiency virus) activity infection that causes acquired immunodeficiency syndrome (AIDS), which represents a major global health problem. In fact, chemical agents are usually used as an anti-retroviral therapeutic method for AIDS patients, but it has many side-effects and drug resistance for many of them. Recently, natural anti-retroviral factors have been discovered from natural resources to have the potential to replace synthetic drugs. Eftekhari et al. inspected the anti-retroviral effects of *S. egyptiaca* L. extract by using the cell proliferation kit II (XTT assay), which is a colorimetric assay for the nonradioactive quantification of cellular proliferation, viability, and cytotoxicity. As a result of that study and bioinformatics analyses, it was suggested that the *S. egyptiaca* L. had anti-HIV properties and could be a viable choice for AIDS patients [39]. Reactive oxygen species (ROS) have been identified as the cause of a few human infections, such as cardiovascular diseases, inflammation, viral infections, diabetes, and cancer [26–34]. The most important activities of *Salix* species such as good antioxidant activities were responsible for phenolic compounds. The extract of *Salix* and their antioxidant activities were mainly estimated

through DPPH, FRAP, ABTS, the Folin–Ciocalteu method, total antioxidant capacity (TAC) assays, β-carotene bleaching, lipid peroxidation activities, linoleic acid anti-oxidation, alkyl radical scavenging assays, and superoxide anion radical scavenging [14–18,24–26,28]. A recent study showed that *S. tetrasperma* Roxb. extract exhibited antioxidant effects on neuropathic pain and its mechanism of action was useful in vitro and in vivo [24]. Furthermore, *S. atrocinerea* Brot., *S. fragilis* L., and *S. viminalis* L. displayed antioxidant activities from the polyphenolic compounds [40], and the extracts from *S. subserrata* Willd. leaf contained major compositions such as isorhamnetin-3-*O*-*β*-D-rutinoside, aromadendrin, gallocatechin, tremuloidin, triandrin, chrysoeriol-7-*O*-glucuronid, and salicin, also exhibiting antioxidant effects against the oxidative stress process in Caenorhabditis elegans [41]. In Vietnam, besides being used as an ornamental tree, the *S. babylonica* plant is also used in many traditional remedies such as healing painful tendon and bone injuries, treating internal anger, transferring heat pain to another location, or treating convulsions in limbs: boil and drink 40–60 g of Rhizophora leaves [42]; treating prince's pimples and allergies to chewing gum: boil 100–150 g of young leaves and branches in water, drink, and wash; curing boils in the breast: use jade talisman—it will feel hot at first, continue to build up, become normal, and then go away [42]; curing tooth decay: use rubber tree branches [42]; treating diseases and coughing up blood: crush dried capers and take 4 g each time; treating children with saw-toothed orange: use the stamens of the Rhizome flower (do not cut into ash), grind it with a little musk or ice, and rub it on the roots of the teeth [42].

Currently, in Vietnam, there are no studies on the chemical components and biological activity of the EOs of this plant, so in this study, we determine the phytochemical components and antibacterial and anticancer activities of the EOs extracted from the *S. babylonica* collected in the mountainous area of Northern Vietnam.

2. Materials and Methods

2.1. Materials

Amounts of 20 kg of fresh leaves and 10 kg of fresh bark of *S. babylonica* were collected in May 2021 from each Bac Giang and Thai Nguyen province, Vietnam. The samples were authenticated by Dr. S.D. Thuong, Faculty of Biology, Thai Nguyen University of Education. All solvents and reagents used in this work were purchased from Merck & Co., Inc., Rahway, NJ, USA.

2.2. Extraction of Essential Oils through Steam Distillation Extraction

The extraction of essential oils in 5 kg of fresh samples was carried out via steam distillation method for 6 h. Essential oils (50 mL from leaves, 27 mL from barks), which were extracted using a separatory funnel, had a light-yellow color and strong scent, and were dried by Na_2SO_4. Essential oils were preserved in a glass vial at 4–5 °C prior to the following analysis.

2.3. GC/FID and GC/MS Analysis

GC/MS analysis was conducted using a Hewlett Packard 5890 Series II w/HP 5971 MSD GC/MS System coupled with a quadrupole MS system. The system was equipped with an electron impact source operating at 200 °C. A fused silica-capillary column with an apolar stationary phase HP5-MS (30 m × 0.25 mm, 0.25 μm film thickness) was used. The gas chromatographic (GC) conditions used helium (0.9 mL/min) as a carrier gas for the separation of compounds in essential oils and oven heating rates around 3 to 5 °C/min, and compounds were identified via a flame ionization detector (FID) (GC-FID) [27]. The electron impact spectra were recorded at an ion voltage of 70 eV, covering a scan range of 30–600 uma [27]. The identification of compounds was accomplished by comparing their retention indices (Ris) on an HP-5MS column and cross-referencing them with the data available in the NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/ Accessed on 18–22 December 2022). Additionally, the mass spectra of the compounds were compared

with those stored in the Wiley NBS75K.L and NIST/EPA/NIH mass spectral libraries for further confirmation.

2.4. Antibacterial Activity

Gram-negative strains included *Pseudomonas aeruginosa* (PA) and *Escherichia coli*, and Gram-positive strains included *Staphylococcus aureus* (SA), which were provided by Dr. Nguyen Thi Ngoc Lan (Dean of Faculty of Biology, Thai Nguyen University of Education). The antibacterial action of the essential oil was determined using the disc agar diffusion method [43], and the diameter of the inhibition zones around the wells was measured to evaluate the oil's antibacterial effectiveness. Essential oils were diluted with DMSO solution at concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL. The studied bacterial strains were cultured on nutrient medium supplemented with agar at 30 °C for 24 h. Microbial suspensions were diluted with sterile distilled water to a concentration of about 10^8 CFU/mL. An amount of 0.1 mL of the microorganism suspension was spread onto a plate with nutrient agar, 5 wells were made in the agar (diameter 6 mm), and then 50 µL of essential oils in DMSO was added to each well for 1 h at 4 °C. The bacteria were nurtured at 37 °C for 24 h. After nurture, the results were observed by measuring the diameter of inhibition zones in centimeters. The negative control was DMSO without test material. The positive control was ampicillin 50 mg/mL. The experiments were performed in triplicate.

2.5. Cytotoxicity Activities

The cytotoxicity of essential oils against HepG2 cells (human hepatocarcinoma), MCF-7 cells (human breast carcinoma), and A549 cells (adenocarcinomic human alveolar basal epithelial cells) was determined by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay at the Institute of Biotechnology, Vietnam Academy of Science and Technology. The MTT assay is a colorimetric assay and relies on the cellular reduction of tetrazolium salts to their formazan crystals. The MTT assay is one of main methods to measure cell metabolic activity, cell proliferation, and viability, and is the main standard for cytotoxicity testing in vitro [37].

The culture media for this experiment were DMEM (Dulbecco's Modified Eagle Medium) with L-glutamine, sodium pyruvate, $NaHCO_3$, penicillin/streptomycin, 10% FBS (Fetal Bovine Serum), and Trypsin-EDTA (0.05%). The detailed test steps were described by Khang et al. [44]. Optical density of plates was read on a microplate reader at a wavelength of 550 nm. IC_{50} values were calculated using the logarithm formula.

2.6. Statistical Analysis

All experiments were repeated three times, and data in this work were afforded from two-way ANOVA and are presented as mean ± standard deviation, with p-value < 0.05 being considered as a statistically significant difference.

3. Results and Discussions

S. babylonica essential oil obtained via steam distillation was a liquid, had a pale-yellow color, and was lighter than water. In this research, the chemical components of the EOs of *S. babylonica* collected in two provinces of Bac Giang and Thai Nguyen were determined and compared with each other. The results of the chemical composition, molecular formula, RI, and relative content of the *S. babylonica* essential oil of leaves and *S. babylonica* bark collected in two provinces are shown in Tables 1 and 2.

There were 28 compounds (in leaves' essential oils) and 31 compounds (in bark essential oils) that were determined in the *S. babylonica* essential oils collected from Bac Giang and Thai Nguyen provinces. Camphene (7.3%), 2-(4-methyl-3-cyclohexen-1-yl)-2-propanol (4.5%), 3,7-dimethyl-1,6-octadien-3-ol (5.4%), geranyl acetate (5.2%), α-humulene (14.5%), and pentacosane (32.2%) were the major chemical compositions in essential oils of *S. babylonica* leaves. Trans-carvone oxide (4.3%), thymol (4.5%), trans-caryophyllene (24.5%), α-humulene (30.2%), cadinol (5.5%), and farnesol (4.8%) were the major chemical composi-

tions in essential oils of *S. babylonica* bark. These results demonstrated the affordance of conditional growth of the plants on the essential oils' chemical composition.

Table 1. Chemical components of *S. babylonica* leaves' essential oils.

No	Compound	Molecular Formula	RI	Relative Content (%)	
				Thai Nguyen Sample	Bac Giang Sample
1.	α-Pinene	$C_{10}H_{16}$	917	3.5	0.1
2.	Camphene	$C_{10}H_{16}$	947	7.3	0.3
3.	Nonan-4-ol	$C_9H_{20}O$	1078	2.5	0.1
4.	Linalool	$C_{10}H_{18}O$	1104	0.6	0.1
5.	Citronellal	$C_{10}H_{18}O$	1158	0.4	0.2
6.	2-(4-methyl-3-cyclohexen-1-yl)-2-propanol	$C_{10}H_{18}O$	1190	0.5	4.5
7.	3,7-dimethyl-1,6-octadien-3-ol	$C_{11}H_{18}O_2$	1215.4	1.5	5.4
8.	Methyl citronellate	$C_{11}H_{20}O_2$	1262	2.8	4.6
9.	Trans-Carvone oxide	$C_{10}H_{14}O_2$	1279	1.1	0.4
10.	Salicylic acid	$C_7H_6O_3$	1297	2.8	0.2
11.	Dihydrocarvyl acetate	$C_{12}H_{20}O_2$	1304	0.4	2.7
12.	α-Terpinyl acetate	$C_{12}H_{20}O_2$	1367	1.4	0.5
13.	Geranyl acetate	$C_{12}H_{20}O_2$	1386	5.2	0.2
14.	Methyleugenol	$C_{11}H_{14}O_2$	1410	0.4	0.3
15.	Cedrene	$C_{15}H_{24}$	1422	0.3	2.2
16.	Trans-Caryophyllene	$C_{15}H_{24}$	1444	5.2	9.3
17.	α-Humulene	$C_{15}H_{24}$	1452	4.5	14.5
18.	α-Farnesene	$C_{15}H_{24}$	1507	1.0	3.1
19.	Gamma—Cadinene	$C_{15}H_{28}$	1518	1.1	0.2
20.	Tridecanal	$C_{13}H_{26}O$	1519	0.2	2.2
21.	Nerolidol	$C_{15}H_{26}O$	1531	1.3	2.8
22.	Dodecanoic acid	$C_{12}H_{24}O_2$	1562	0.3	2.5
23.	Benzoic acid, hexyl ester	$C_{13}H_{18}O_2$	1576	0.2	2.1
24.	(-)-Spathulenol	$C_{15}H_{24}O$	1582	0.2	2.3
25.	Hexadecene	$C_{16}H_{32}$	1592	0.1	2.1
26.	Farnesyl acetate	$C_{17}H_{28}O_2$	1818	0.3	5.3
27.	Phytol	$C_{20}H_{40}O$	2122	5.6	4.2
28.	Pentacosane	$C_{25}H_{52}$	2500	32.2	11.3

From the above results, we found that some main chemical components were new components discovered for the first time in *S. babylonica* species collected in Vietnam: α-humulene (14.5, 30.2%), pentacosane (32.2%), trans-carvone oxide (4.3%), and trans-caryophyllene (24.5%). Pentacosane's concentration in the bark was smaller than those of its leaves' essential oils. These compositions have been proven to have many good biological activities and have been of good value as a starting material for the synthesis of other substances to serve in medicine. From the Vietnam samples, we found that the main components in oil samples were different from those of Mu et al.' [27] and Salem et al.'s [26]. Salem et al. [26] utilized *S. babylonica* samples collected in Mexico and extracted using an

organic solvent system such as methanol/acetone/n-hexane at a ratio of 1/1/1 (*v*/*v*/*v*), while our study extracted essential oils via the steam distillation method [16]. According to Mu et al.'s work [27], they experimented with *S. babylonica* samples collected in China and determined pentacosane's derivatives, finding that salicylic acid derivatives are the major components in essential oils. Meanwhile, the research group of Zeid et al. [21] analyzed the composition of substances in essential oils extracted via steam distillation from *S. babylonica* samples collected in Egypt, and the results showed that many components were similar to samples collected in Vietnam, but the main composition of samples collected in Vietnam and Egypt was also different. For *S. babylonica* samples collected in Egypt, essential oils had *α*-pinene, *β*-cedrene, salicylaldehyde, *Cis*-4-hexen-1-ol, linalool, and 1,2-cyclohexanedione as the main components, while *S. babylonica* samples collected in Vietnam had camphene, 2-(4-methyl-3-cyclohexen-1-yl)-2-propanol, 3,7-dimethyl-1,6-octadien-3-ol, geranyl acetate, *α*-humulene, pentacosane, *trans*-carvone oxide, thymol, *trans*-caryophyllene, *α*-cadinol, and farnesol as the derivatives of the substances in the samples collected in Egypt as the main chemical components. This also demonstrates the influence of geological and growing conditions on the chemical components of the plant, especially the composition of substances contained in their essential oils. This is the basis to promote research on the chemical components of EOs to serve life.

Table 2. Chemical compositions of *S. babylonica* bark essential oils.

No	Compound	Molecular Formula	RI	Relative Content (%)	
				Thai Nguyen Sample	Bac Giang Sample
1.	α-Pinene	$C_{10}H_{16}$	917	0.3	0.1
2.	Camphene	$C_{10}H_{16}$	947	0.4	0.2
3.	Nonan-4-ol	$C_9H_{20}O$	1078	0.1	0.2
4.	Linalool	$C_{10}H_{18}O$	1104	0.2	0.1
5.	Citronellal	$C_{10}H_{18}O$	1158	0.4	0.2
6.	2-(4-methyl-3-cyclohexen-1-yl)-2-propanol	$C_{10}H_{18}O$	1190	0.1	0.6
7.	3,7-dimethyl-1,6-octadien-3-ol	$C_{11}H_{18}O_2$	1215.4	1.7	1.8
8.	Methyl citronellate	$C_{11}H_{20}O_2$	1262	3.8	2.6
9.	Trans-Carvone oxide	$C_{10}H_{14}O_2$	1279	0.1	4.3
10.	Thymol	$C_{10}H_{14}O$	1297	0.3	4.5
11.	Dihydrocarvyl acetate	$C_{12}H_{20}O_2$	1304	0.4	0.7
12.	α-Terpinyl acetate	$C_{12}H_{20}O_2$	1367	1.0	1.2
13.	Geranyl acetate	$C_{12}H_{20}O_2$	1386	1.3	2.8
14.	Methyleugenol	$C_{11}H_{14}O_2$	1410	0.4	0.3
15.	Cedrene	$C_{15}H_{24}$	1422	0.3	0.5
16.	Trans—Caryophyllene	$C_{15}H_{24}$	1444	16.5	24.5
17.	α-Humulene	$C_{15}H_{24}$	1452	30.2	16.5
18.	Alpha-Farnesene	$C_{15}H_{24}$	1507	2.0	2.1
19.	Gamma—Cadinene	$C_{15}H_{28}$	1518	1.8	1.2
20.	Tridecanal	$C_{13}H_{26}O$	1519	1.2	0.5

Table 2. *Cont.*

No	Compound	Molecular Formula	RI	Relative Content (%)	
				Thai Nguyen Sample	Bac Giang Sample
21.	Nerolidol	$C_{15}H_{26}O$	1531	1.3	0.8
22.	Dodecanoic acid	$C_{12}H_{24}O_2$	1562	2.0	0.9
23.	Benzoic acid, hexyl ester	$C_{13}H_{18}O_2$	1576	1.8	0.7
24.	(-)-Spathulenol	$C_{15}H_{24}O$	1582	1.5	0.9
25.	Hexadecene	$C_{16}H_{32}$	1592	0.2	2.1
26.	trans-Muurolol	$C_{15}H_{26}O$	1652	0.2	4.5
27.	Cadinol	$C_{15}H_{26}O$	1679	0.2	5.5
28.	Farnesol	$C_{15}H_{26}O$	1695	0.3	4.8
29.	Farnesyl acetate	$C_{17}H_{28}O_2$	1818	6.6	1.3
30.	Phytol	$C_{20}H_{40}O$	2122	0.6	0.5
31.	Pentacosane	$C_{25}H_{52}$	2500	0.1	0.2

In addition, the antibacterial action of the EOs of *S. babylonica* bark and leaves was determined using the disc agar diffusion method [14]. The results are given in Table 3.

Table 3. Antibacterial action of the *S. babylonica* EOs.

Test Sample	Concentration	*Escherichia coli*		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
		Leaves	Bark	Leaves	Bark	Leaves	Bark
Ampicillin	50 mg/mL	22		17		14	
DMSO	-	0	0	0	0	0	0
Thai Nguyen sample	25 mg/mL	20	21	24	20	21	24
	50 mg/mL	31	30	38	34	34	33
	100 mg/mL	35	37	39	34	36	35
Bac Giang sample	25 mg/mL	24	23	25	27	28	29
	50 mg/mL	30	32	29	29	31	31
	100 mg/mL	32	26	32	32	34	34

Negative control (0): DMSO; positive control (+); Unit of antibacterial circle diameter in mm.

From Table 3, the results indicate that both essential oils derived from the leaves and bark of *S. babylonica* exhibit antibacterial action 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* EO surpasses that of antibiotics. The essential oils demonstrate stronger inhibitory activity against *P. aeruginosa* and *S. aureus* compared to *E. coli*. In conclusion, the essential oil from Thai Nguyen province exhibits a noticeably stronger antibacterial action than the EOs from Bac Giang province.

In the previous work, the cytotoxic activities of the *Salix* plant were mainly studied on ethanol and aqueous extracts, and essential oils [27–38]. The results showed that the aqueous extract of parts of the *Salix* plant had proliferative activity of all compounds in the rich flavonoids and proanthocyanidin fractions with 50% maximal growth inhibitory concentrations (GI(50)) between 33.3 and 103.3, and 50.0–243.0 microg/mL, respectively [36]. The extract from the leaves of *Salix safsaf* for action against human carcinoma cells in vitro, and in vivo in mice, decreased tumor growth [37] and reduced the majority of the blasts

of acute myeloid leukemia after 24 h of incubation [38]. Herein, the inhibition activity of EOs of the bark and leaves of *S. babylonica* were experimented against MCF-7, A549, and HepG2 cancer cell lines. The conclusion is presented in Table 4.

Table 4. Inhibition activities of the EOs of *S. babylonica* collected in Thai Nguyen, Vietnam.

Cancer Cell Lines	Essential Oil Samples	IC_{50} (μg/mL)
A549	Leave	45.4 ± 1.1
	Bark	65.3 ± 1.5
	Ellipticine	0.53 ± 0.05
MCF-7	Leave	86.1 ± 1.5
	Bark	95.3 ± 1.3
	Ellipticine	0.53 ± 0.05
HepG2	Leave	77.1 ± 1.4
	Bark	>100
	Ellipticine	0.53 ± 0.05

Table 4 presents the test results of the EOs of *S. babylonica* samples, which displayed medium inhibitory activity against the three tested cancer cell lines with IC_{50} values ranging from 45.4 to 95.3 μg/mL. Ellipticine was the positive control in the experiment. In addition, the essential oils of leaves exhibited inhibitory activities compared to its bark.

The bioactivity of essential oils commonly causes breakdown of the key chemical constituents present in that essential oil [7–11]. In the samples of the *S. babylonica* plant, which was collected in Vietnam, compounds with relatively large amounts (>3%) were found such as camphene, 2-(4-methyl-3-cyclohexen-1-yl)-2-propanol, 3,7-dimethyl-1,6-octadiene-3-ol, geranyl acetate, α-humulene, pentacosane, trans-carvone oxide, thymol, trans-caryophyllene, α-cadinol, and farnesol derivatives. Pentacosane is an n-alkane, which was the main component obtained in the leaf samples of *S. babylonica* that we collected in Vietnam. Recently, Pentacosane was found as the major component in the acetone extract from *Curcuma raktakanda* and *Malus domestica* [45]. Pentacosane exhibits anti-cancer activities against C-6, A549 (lung carcinoma), Hep-G2, KB, CHOK1 (ovarian), and THP-1 (acute monocytic leukemia) cells [45,46]. A plant rich in n-alkane, *Moringa oleifera* (including pentacosane), collected in Mozambique and Taiwan containing pentacosane and phytol, has major compositions similar to its plant species, which we used in this study, and it showed good inhibitory activity against two Gram-positive strains (*Bacillus cereus*, *Staphylococcus aureus*), two Gram-negative strains (*Escherichia coli*, *Pseudomonas aeruginosa*), and five fungal strains (*Penicillium aurantiogriseum*, *Penicillium expansum*, *Penicillium citrinum*, *Penicillium digitatum*, and *Aspergillus niger* spp.) of bacteria [46]. Meanwhile, β-caryophyllene (BCP) is a natural compound, in the bicyclic sesquiterpene group, and is one of the major components of essential oils extracted from medicinal and food plants, typically in the following plant genera: *Ocimum* spp., *Cinnamomum* spp., *Piper* spp., *Syzygium* spp., *Cannabis* spp., *Lavandula* spp., *Origanum* spp., and *Rosmarinus* spp. Its biological effects include anti-inflammatory [47–49], anticarcinogenic [50], antimicrobial [50], antioxidative [5], and anticancer activities [51,52]. Thus, the above content is analyzed and compared with previous studies to show that *S. babylonica* essential oil from Vietnam contains valuable biologically active compounds, which is the basis for the use of this species. This plant is used to make medicine to improve quality of life.

4. Conclusions

In conclusion, we extracted essential oil and determined that *S. babylonica* essential oil from Vietnam has many new chemical components discovered for the first time in this plant. The results from this research indicate that both essential oils demonstrated antibacterial

activity against the tested bacteria and anti-cancer activity against three cancer cell lines. The evaluation of the ability to inhibit three strains of microorganisms and to inhibit the growth of three cancer cell lines was first conducted with essential oils extracted from the plant species *S. babylonica* collected in Asia, which will be the basis for using essential oils of this plant in medicine.

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