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CHEMICAL COMPOSITION, ANTI-MICROBIAL, ANTI-INFLAMMATORY, AND CYTOTOXIC ACTIVITIES OF ESSENTIAL OILS FROM *MELICOPE PTELEIFOLIA* (CHAMP. EX BENTH.) T.G.HARTLEY LEAVES AND STEMS COLLECTED IN LAM DONG PROVINCE

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Summary

Chemical Composition, Anti-Microbial, Anti-Inflammatory, and Cytotoxic Activities of Essential Oils from *Melicope pteleifolia* (Champ. ex Benth.) T.G.Hartley Leaves and Stems Collected in Lam Dong Province

The essential oils extracted by hydrodistillation from the leaves and stems of *Melicope pteleifolia* (MP) collected in Bidoup-Nui Ba National Park, Lam Dong province, Vietnam were investigated for their chemical composition and bioactivity. 29 compounds were identified in the leaf essential oil (MPL), of which *trans*-α-bergamotene (41.1%), *trans*-γ-bisabolene (13.0%), linalool (7.6%), and β-atlantol (7.3%) were the most abundant. The stem oil (MPS) contained 21 identified constituents, mainly *trans*-α-bergamotene (63.7%), limonene (7.4%), and linalool (7.1%). MPL exhibited inhibitory effects on *S. aureus, B. subtilis,* and *L. fermentum*, while MPS significantly inhibited the growth of *S. aureus, B. subtilis, E. coli,* and *C. albicans* and showed minimal inhibition against three other bacteria. MPL inhibited NO production in macrophage RAW 264.7 cells (IC_{50} 19.86 ± 0.16 μg/mL). Both MPL and MPS showed moderate cytotoxic effects on SK-LU-1, MCF-7, and HepG2 cell lines with the IC_{50} values of 20.80 ± 1.42, 25.90 ± 1.47, and 23.42 ± 1.78 μg/mL (for MPL) and 25.36 ± 2.41, 46.46 ± 3.13, and 47.41 ± 2.91 μg/mL (for MPS), respectively. Their antifungal, anti-inflammatory, and cytotoxic activities were evaluated and reported for the first time.

Keywords: *Melicope pteleifolia, Essential oil, GC-MS, Anti-microbial activity, NO inhibition, Cytotoxicity.*

1. Introduction

Melicope pteleifolia (Champ. ex Benth.) T.G. Hartley (MP), synonym *Euodia lepta* (Spreng.) Merr. belongs to the family Rutaceae and is mainly found in tropical areas of Asia such as South China and Southeast Asia countries including Cambodia, Laos, Myanmar, Thailand, and Vietnam [1]. It is a perennial herbaceous shrub or tree, and parts of the plant have traditionally been used to treat rheumatism, sore throat, dermatitis, eczema, and insect and snake bites [1]. Compounds that have been isolated from MP include chromenes, dichromenes, flavonoids, coumarins, and alkaloids [2],[4]. Previous studies indicated that MP possessed antioxidant, anti-inflammatory, hepatoprotective, analgesic, antimicrobial, and anti-tumor effects [1]. The essential oil of its plant parts collected from China, Vietnam (Nghe An, Ba Ria - Vung Tau, Da Nang provinces) has been investigated for its chemical composition, antibacterial, and insecticidal activities [5],[6],[7],[8],[9]. This

species is widely distributed in evergreen forests of Bidoup-Nui Ba National Park at altitudes of 1200 to 1500 m, however, only its fruit essential oil has been studied for chemical composition and antibacterial activity [10]. Therefore, this study was carried out to elucidate and compare the essential oil composition of the leaves and stems of MP collected from the Bidoup - Nui Ba National Park, Lam Dong Province, Vietnam. Their antibacterial (against six bacterial strains), antifungal (against *Candida albican*), inhibition of NO production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells, and cytotoxic activity against three cancer cell lines (SK-LU-1, MCF-7, and HepG2) were evaluated for the first time.

2. Materials and methods

2.1. Plant materials

The leaves (3 kg) and stems (1.5 kg) of *Melicope pteleifolia* (Champ. ex Benth.) T.G. Hartley of mature wild plants, 3-5 m in height, were collected on March 4th 2022 (the flowering season is usually in April - May) from Bidoup-Nui Ba National Park, Lam Dong province, Vietnam at the coordinate 120°10'50" N, 108°41'03" E. The plants were identified by our research team member Dr. Luong Van Dung, Center for Climate Change and Biodiversity Research, Dalat University, Vietnam. Voucher specimen (DL220301) has been deposited in the Herbarium of Dalat University.

2.2. Obtaining of essential oils

The fresh materials were cut and ground using an electric grinder. The hydrodistillation of essential oils was performed using a Clevenger apparatus according to the Vietnam Pharmacopoeia (2017), for three and a half hours. The obtained essential oils were filtered through sodium sulfate to remove water completely and stored in sealed glass vials at 4°C before being used for chemical analysis or bioassay. The essential oils from the leaves and stems of MP were designated as MPL and MPS, respectively.

2.3. Analysis of essential oils

The essential oils were analyzed by gas chromatography coupled with mass spectrometry on a SCION 456-GC/SCION SQ (SCION Instruments, USA), equipped with a fused silica capillary Rxi-5MS column (30 m × 0.25 mm, film thickness 0.25 µm; 5% diphenyl/95% dimethyl polysiloxane; Restek, USA). The carrier gas used was helium at a rate of 1.0 mL/min; the oven temperature program started at 60°C, then gradually increase to 246°C at 3°C/min and increase from 246°C to 280°C at 20°C/min; the injector operated at a 1:20 split ratio, injector temperature 250°C; the injected volume was 0.1 µL of 10% (v/v) solution of essential oil in GC grade *n*-hexane. Mass spectra were recorded in electronic impact (EI) mode at 70 eV, scanning the 50-500 m/z range. The ion source temperature was 250°C with a 1 scan/sec cycle time. Linear retention indices (RIs) of the compounds were determined in relation to the homolog series of *n*-alkanes (C_9-C_{28}) ran under the same operating conditions. The identification of the compounds was based on the comparison of their mass spectra to those from the NIST mass spectra library (version 2.4, 2020), and the literature [11], as well as by the comparison of their RIs with those from the literature [11],[12]. Relative percentages of the compounds were calculated based on the peak areas by GC software.

2.4. Antibacterial and antifungal assay

The antibacterial activity of the oils was evaluated against the Gram-positive bacteria: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 13709), *Lactobacillus fermentum* (ATCC 9338), as well as the Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (13076). The antifungal activity of the essential oils was tested against *Candida albicans* (ATCC 10231). The culture media for bacteria were MHB (Mueller-Hinton Broth), MHA (Mueller-Hinton Agar), TSB (Tryptic Soy Broth), and TSA (Tryptic Soy Agar), while for fungi were SDB (Sabourand-2% dextrose broth) and SA (Sabourand- 4% dextrose agar).

The assay was performed in 96-well microtiter plates. Each well containing 10 µL of essential oil dissolved in DMSO was diluted with 190 µL of microorganism suspension (5×10^5 CFU/mL), then incubated at 37°C for 24 hours. The microbial growth/viability was assessed fluorimetrically after the addition of 10 µL resazurin per well using a TECAN GENIOUS microplate reader at 600 nm. The results were expressed as a % reduction of microorganism growth/viability compared with the control well, and IC_{50} values were determined from the dose-response curve.

2.5. Bioassay for NO inhibition and cytotoxicity

Materials

Lipopolysaccharide (LPS) from *Escherichia coli* was purchased from Sigma Chemical Co. (St.. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Life Technologies, Inc. (Gaithersburg, MD, USA). Sodium nitrite, sulfanilamide, dimethyl sulphoxide (DMSO), N-1-napthylethylenediamine dihydrochloride,

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), sulforhodamine B (SRB), trichloroacetic acid (TCA), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma Chemical Co. (USA). RAW 264.7 cell lines were a kind gift from Prof. Dr. Domenico Delfino, University of Perugia, Perugia, Italy. The cancer cell lines HepG2 (human hepatocellular carcinoma), MCF-7 (human breast carcinoma), and SK-LU-1 (human lung carcinoma) were provided by Dr. Jeanette Maier, University of Milan, Italy, and Dr. J. M. Pezzuto, University of Long Island, USA.

Cell culture

All cell lines were cultured in DMEM medium with 1 mM sodium pyruvate, 2 mM L-glutamine, 10 mM HEPES, and 10% FBS in an incubator at 37°C, 5% CO_2. The cells were subcultured every three days at a 1:3 ratio.

NO inhibitory assay

RAW 264.7 cells were seeded into a 96-well plate at a concentration of 2×10^5 cells/well. After incubation for 24 h, the cultured medium was refreshed with FBS-free DMEM. Then, the cells were treated with samples at different concentrations in the presence of LPS (1 μg/mL) for 24 h. Dexamethasone (Sigma) was used as a positive control, and the cells treated with a diluted solution of DMSO (1.0%) were used as the negative control. Nitrite, an indicator of NO production, was determined by the Griess reagent system (Promega Corporation, WI, USA). Protocols supplied with the assay kit were used for the application of the assay procedure. Briefly, 100 μL of cell culture medium with an equal volume of 100 μL Griess reagent (50 μL of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid, and 50 μL 0.1% (w/v) N-1-naphthyl ethylenediamine dihydrochloride) was incubated in a 96-well plate at room temperature for 10 min. At that point, the absorbance was measured at 540nm in a microplate reader (Bio-Rad, California, USA). The amount of nitrite in the media was calculated from the sodium nitrite ($NaNO_2$) standard curve. The ability to inhibit the nitric oxide (NO) was measured at doses of 100, 20, 4, and 0.8 μg/mL respectively, and estimated as a half maximal inhibitory concentration (IC_{50}), which was calculated by the program Table Curve Version 4.0 (Systat Software Inc., San Jose, CA, USA) [13].

MTT cell viability assay

RAW 264.7 cells were seeded in 96-well plates in the presence of various concentrations of essential oil similar to those of the NO experiment. After 24 h of incubation, MTT was added to a final concentration of 0.5 mg/mL, and the cells were incubated for 4 h at 37°C and 5% CO_2. Finally, the supernatant was removed and the formazan crystals were dissolved in dimethyl sulfoxide (DMSO). The absorbance was measured at 540 nm. The percentage of dead cells was determined relative to the control group.

Cytotoxicity assay

SRB assay was used for determining the cytotoxic activity against three cancer cell lines (HepG2, MCF-7, and SK-LU-1) of the essential oils in a 96-well plate according to the method of Skehan et al. [14]. The test evaluated the cell density based on the measurement of the total cellular protein content stained with sulforhodamine B (SRB). Briefly, trypsinized cells were seeded in a 96-well plate and incubated with the sample for 48 h. The cell-containing wells treated with a diluted solution served as the negative control. After a period of incubation time, the cells were fixed with TCA for 1 h and then stained with SRB for 30 min at 37°C. The plates were washed three times with acetic acid to remove all nonstaining dye and allowed to dry at room temperature. SRB staining protein in cells was dissolved in 10 mM unbuffered Tris base and the plate was gently shaken for 10 min at room temperature. The optical density (OD) at the wavelength of 540 nm was measured by using an ELISA plate reader (BioTek, Winooski, VT, USA). Experiments were triplicated for the accuracy of data. The inhibition percentage of cell growth in the presence of the treated sample was determined by the following formula: (%) inhibition = 100% - $[(OD_{sample} - OD_{day\ 0}) / (OD_{blank\ control} - OD_{day\ 0})] \times 100$. Ellipticine was used as a positive control. The IC_{50} value was determined by using the TableCurve 2Dv4 computer software.

2.6. Statistical analysis

Data were expressed as the mean of three replicate determinations ± standard deviation (SD). Statistical comparisons were made with Student's test, p value < 0.05 was considered as significant difference.

3. Results and discussion

3.1. Chemical composition of MP leaf and stem essential oils

The yields of MPL and MPS obtained from leaves and stems of MP by hydrodistillation were 0.094 ± 0.002% and 0.020 ± 0.002% (w/w), calculated on fresh weight, respectively. The chemical composition of the investigated essential oils is presented in Table 1. 36 Compounds were identified in the two

investigated essential oils, including 29 and 21 compounds in MPL and MPS, accounting for 99.2% and 94.4% of the oils, respectively. Terpene compounds were predominant in the two essential oils with a total amount of 97.7% in the leaf and 93.4% in the stem oils. The main constituent of the leaf essential oil was *trans*-α-bergamotene (41.1%), followed by *trans*-γ-bisabolene (13.0%), linalool (7.6%), and β-atlantol (7.3%). In the stem oil, *trans*-α-bergamotene (63.7%) and linalool (7.1%) were also the major components, whereas there was an absence of *trans*-γ-bisabolene and limonene was present at 7.4%. Compared to those published data on the chemical composition of the essential oil of MP leaves and stems, these results have in common that the essential oil belongs to the terpene type. However, the material collected from different locations contains different amounts of major components. For example, the main components of the leaf essential oil collected in Nghe An were (*E*)-β-ocimene (24.4%), α-pinene (9.8%), and (*Z*)-β-ocimene (6. 3%) [6], collected in Da Nang were β-citronellal (16.73%), β-citronellol (13.93%), D-limonene (12.12%) [8], in Ba Ria-Vung Tau were 1,9-decadiyne ((32.59%) and patchoulane (10.04%) [7], collected in China were α-thujene (25.17-83.27%) and ψ-limonene (38.95%) [9]. The situation is similar for essential oil from stems, where spathulenol (26.0%), (*E*)-β-ocimene (9.9%), and (*Z*)-9-octadecenamide (7.7%) were the main substances from samples in Nghe An [6]. These differences may be due to differences in geographical location, soil conditions, and climate that affect the accumulation of secondary plant metabolites.

Table 1. Composition of the essential oils from leaves and stems of MP

No	RI[a]	RI[b]	MS Match	Compound name	Relative percentage (%)	
					MPL	MPS
1	1028	1024	896	Limonene		**7.4**
2	1099	1095	888	Linalool	**7.6**	**7.1**
3	1374	1374	900	α-Copaene	2.1	0.8
4	1388	1387	829	β-Cubebene	0.3	
5	1390	1389	889	β-Elemene	0.5	
6	1403	1403	906	Methyleugenol	0.6	
7	1420	1416	893	α-Santalene	1.7	2.0
8	1438	1432	928	*trans*-α-Bergamotene	**41.1**	**63.7**
9	1454	1452	886	α-Humulene	0.2	
10	1456	1454	860	(*E*)-β-Farnesene	0.8	
11	1461	1457	863	β-Santalene	0.7	0.7
12	1482	1479	804	α-Curcumene	0.6	0.4
13	1484	1481	858	γ-Curcumene	0.8	0.9
14	1515	1513	811	γ-Cadinene	0.5	
15	1523	1522	861	δ-Cadinene	1.1	0.8
16	1527	1526	816	*ar*-Macrocarpene		2.5
17	1529	1529	880	*trans*-γ-Bisabolene	**13.0**	
18	1543	1542	820	*cis*-Sesquisabinene hydrate	0.8	
19	1546	1547	821	Italicene epoxide	0.8	
20	1553	1550	835	Lippifoli-1(6)-en-5-one	0.4	
21	1557	1555	843	Elemicin	0.9	0.4
22	1564	1561	852	(*E*)-Nerolidol	4.7	0.4
23	1578	1577	842	Spathulenol	2.0	0.4
24	1583	1582	819	Caryophyllene oxide	0.8	0.5
25	1600	1601	813	1,3,5-Bisabolatrien-7-ol	1.2	
26	1603	1604	851	Khusimone	0.3	
27	1609	1608	867	β-Atlantol	**7.3**	1.0
28	1629	1630	810	Muurola-4,10(14)-dien-1-β-ol	1.6	
29	1652	1646	871	Ledene oxide-(II)	0.4	
30	1655	1652	877	Himachalol	0.3	0.4
31	1670	1668	838	14-Hydroxy-9-epi-(E)-caryophyllene		2.3
32	1671	1670	873	Bulnesol	3.2	
33	1690	1690	856	(*Z*)-α-*trans*-Bergamotol		0.5
34	1727	1728	824	γ-(*Z*)-Curcumen-12-ol		0.6
35	1887	1889	832	8*S*,14-Cedranediol		1.1
36	1961	1959	839	*n*-Hexadecanoic acid		0.6
				Monoterpene hydrocarbons		7.4
				Oxygenated monoterpenes	7.6	7.1
				Sesquiterpene hydrocarbons	66.3	71.7

No	RI[a]	RI[b]	MS Match	Compound name	Relative percentage (%)	
					MPL	MPS
				Oxygenated sesquiterpenes	23.8	7.1
				Phenylpropanoid	1.5	0.4
				Others		0.6
				Total	99.2	94.4

Note: RI[a]: Experimental linear retention indices on Rxi-5MS column relative to *n*-alkanes; RI[b] : Linear retention indices in the literature [11],[12].

3.2. Antimicrobial activity of MPL and MPS

The antimicrobial activities of MPL and MPS were evaluated against six bacterial strains (*S. aureus, B. subtilis, L. fermentum, S. enterica, E. coli, P. aeruginosa*) and one fungal strain (*C. albicans*). The results are summarized in Table 2. MPL showed inhibitory effects against *S. aureus, B. subtilis*, and *L. fermentum*, being most effective against *B. subtilis* with IC_{50} values of 7.1 ± 0.1 µg/mL and MIC value of 64.0 ± 1.2 µg/mL. In contrast, it showed minimal inhibitory activity against *S. enterica, E. coli, P. aeruginosa,* and *C. albicans*. MPS significantly inhibited the growth of *S. aureus, B. subtilis, E. coli*, and *C. albicans*, but showed only a weak inhibitory effect on *L. fermentum, S. enterica*, and *P. aeruginosa*. These results add to the knowledge of the antimicrobial activity of MP, which has been previously reported for the essential oils of the fruit and root [1,10], the ethanol extract of the stem, and the leaf extracts [1]. The present results on the MPL and MPS further elucidate the role of essential oil as a contributor of antibacterial and antifungal agents in the leaves and the stems. Furthermore, with the results obtained herein, it is known for the first time that the leaf and stem oil of this species also have antibacterial and antifungal activities. It shows that different parts of this plant have the potential to be used as antimicrobial agents.

Table 2. Half-maximal inhibitory concentration (µg/mL) and minimal inhibitory concentration (µg/mL) of MPL and MPS against bacterial and fungal strains

Microbial strains		C (µg/mL)					
		MPL		MPS		Positive control(*)	
		IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
Gram (+)	*Staphylococcus aureus*	**28.0 ± 0.9**	**256.0 ± 0.0**	**192.0 ± 3.5**	>256	0.02 ± 0.005	0.13 ± 0.0
	Bacillus subtilis	**7.1 ± 0.1**	**64.0 ± 1.2**	**39.4 ± 1.1**	**64.0 ± 0.0**	3.62 ± 0.15	32.0 ± 0.0
	Lactobacillus fermentum	**28.0 ± 1.1**	>256	>256	>256	1.03 ± 0.07	32.0 ± 0.0
Gram (-)	*Salmonella enterica*	>256	>256	>256	>256	0.43 ± 0.05	32.0 ± 0.0
	Escherichia coli	>256	>256	**173.1 ± 1.3**	>256	0.007 ± 0.002	0.5 ± 0.0
	Pseudomonas aeruginosa	>256	>256	>256	>256	4.34 ± 0.15	8.0 ± 0.0
Fungi	*Candida albican*	>256	>256	**52.0 ± 1.2**	**256.0 ± 0.0**	1.32 ± 0.05	8.0 ± 0.0

Note: *Positive controls were ampicillin (for Gram-positive bacteria), cefotaxime (for Gram-negative bacteria), and nystatin (for *C. albicans*).

3.3. Inhibitory activity on NO production of MPL and MPS

Nitric oxide (NO) is involved in different responses of host resistance to various pathogens and participates in regulating vascular integrity, maintaining hemostasis, or regulating neural activity [15]. Overproduction of NO causes pathological problems associated with acute and chronic inflammation, apoptosis and necrosis, or neurodegenerative diseases [16]. RAW 264.7 cells activated by LPS produce large amounts of NO. Therefore, LPS-induced NO production in murine macrophage RAW 264.7 cells was used to evaluate the anti-inflammatory potential of the essential oils. The results presented in Table 3 showed that MPL significantly inhibited NO production with an IC_{50} value of 19.86 ± 0.16 µg/mL, while the IC_{50} value of the positive control dexamethasone was 14.20 ± 0.54 µg/mL. At the same time, it showed a low level of toxicity to macrophage cells (76.23% cell survival at the concentration of 100 µg/mL). In contrast, MPS was toxic to macrophage cells with 44.43% cell survival at the concentration of 100 µg/mL, making it unsuitable as a potential anti-inflammatory agent. To the best of our knowledge, this is the first report on the inhibition of NO production of MPL and MPS essential oils.

Table 3. NO inhibitory activity of MPL and MPS

C	MPL		MPS		Dexamethasone	
(μg/mL)	PI (%)	CS (%)	PI (%)	CS (%)	PI (%)	CS (%)
100	98.65	76.23	82.83	44.43	89.54	86.51
20	50.69	97.49	24.1	99.68	52.5	93.71
4	12.44		11.45		39.59	
0.8	-1.38		-1.2		28.24	
IC_{50}	**19.86±0.16**		**NC**		**14.20 ± 0.54**	

Note: PI: Percentage of inhibition, CS: Cell survival, NC: not calculated.

3.4. Cytotoxic activity of MPL and MPS on cancer cell lines

The cytotoxicity of MPL and MPS on the human lung cancer cell line (SK-LU-1), human breast cancer cell line (MCF-7), and human hepatocellular cancer cell line (HepG2) was evaluated using SRB assay. As shown in Table 4, both essential oils showed moderate cytotoxic effects against three cancer cell lines with the IC_{50} value ranging from 20.80 ± 1.42 to 47.41 ± 2.91 μg/mL. MPL exhibited a better cytotoxic effect on all three cancer cell lines with IC_{50} values from 20.80 ± 1.42 to 25.90 ± 1.47 μg/mL. In previous studies, the *n*-hexane and ethyl acetate extracts of MP leaves demonstrated anti-proliferation, apoptosis induction and cancer cell cycle inhibition activities against four human cancer cell lines: breast (HCC1937, MDA-MB-231), colorectal (HCT116) and liver (HepG2) with IC_{50} values that were mostly below 100 μg/mL [17]. Our results are consistent with those studies because essential oils are a group of less polar substances that are present in the non-polar solvent extracts, which may be the active substances that contribute to the cytotoxicity against cancer cells.

Table 4. Half-maximal inhibitory concentration of MPL and MPS on three cancer cell lines SK-LU-1, MCF-7, and HepG2

Samples	IC_{50} (μg/mL)		
	SK-LU-1	MCF-7	HepG2
MPL	20.80 ± 1.42	25.90 ± 1.47	23.42 ± 1.78
MPS	25.36 ± 2.41	46.46 ± 3.13	47.41 ± 2.91
Ellipticine	0.42 ± 0.03	0.39 ± 0.02	0.35 ± 0.01

The compounds identified in the essential oil constituents such as linalool and limonene certainly contributed significantly to the above mentioned antibacterial, antifungal, anti-inflammatory and cytotoxic activities. Previous reports mentioned that linalool [18] and limonene [19] possessed a number of the related activities and trans-α-bergamotene is also a major constituent occured in several essential oils had antibacterial and insecticidal activities [20]. However, essential oils are a mixture of many substances, so the interaction between components to create the overall effect is still unknown and requires further research. In general, this study contributed to provide additional evidence that essential oil is an important group of substances that create the folk medicinal effects of MP in addition to other known substances such as dichromenes, flavonoids, coumarins and alkaloids [1].

4. Conclusions

Contents of essential oil of MPL and MPS obtained by hydrodistillation were 0.094 ± 0.002% and 0.020 ± 0.002% (w/w), respectively. Among 29 components found in MPL essential oil, *trans*-α-bergamotene (41.1%), *trans*-γ-bisabolene (13.0%), linalool (7.6%), and β-atlantol (7.3%) were major compounds. Besides, *trans*-α-bergamotene (63.7%), limonene (7.4%), and linalool (7.1%) were main compounds out of 21 components occurred in MPS essential oil.

MPL exhibited inhibitory effects on *S. aureus, B. subtilis,* and *L. fermentum*, while MPS significantly inhibited the growth of *S. aureus, B. subtilis, E. coli,* and *C. albicans*. MPL essential oil but not MPS showed inhibitory activity against NO production in macrophage RAW 264.7 cells. Both MPL and MPS showed moderate cytotoxic effects on SK-LU-1, MCF-7, and HepG2 cancer cell lines with the IC_{50} value ranging from 20.80 ± 1.42 to 47.41 ± 2.91 μg/mL. This is the first report on antimicrobial, inhibition of NO production, and cytotoxicity of MPL and MPS essential oils.

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IRIDOID GLYCOSIDES, CHLOROGENIC ACID, AND ITS ACYL DERIVATIVES FROM ROOTS OF *DIPSACUS ASPER* WALL EX DC. IN VIETNAM

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Summary

Iridoid Glycosides, Chlorogenic Acid, and its Acyl Derivatives from Roots of *Dipsacus asper* Wall ex DC. in Vietnam

From the ethyl acetate fraction of *Dipsacus asper* Wall. ex DC. (DA) roots, five compounds were isolated, including sweroside (**1**), 6'-*O*-acetylsweroside (**2**), chlorogenic acid (**3**), 4,5-di-*O*-caffeoyl quinic acid (**4**), and methyl 4,5-di-*O*-caffeoylquinate (**5**). Their structures were verified through the analysis of NMR and MS spectral data in comparison with those published in the literatures. Compound **2** was isolated for the first time from *D. asper* Wall.

Keywords: *Dipsacus asper*, *Tuc doan nhon*, *sweroside, Chlorogenic acid, Quinic acid.*

1. Introduction

Dipsacus asper Wall. ex DC. (synonym: *Dipsacus inermis* Wall.) (DA) is a perennial herb belonging to the Dipsacaceae family and is naturally distributed in mountainous regions of China, Korea, and Japan. In Vietnam, it grows naturally in the northwestern provinces such as Lai Chau, Lao Cai, and Ha Giang [1]. The root of this plant is commonly used in traditional medicine to treat various conditions such as back pain, knee fatigue, and pregnancy [2]. Modern pharmacological studies have also shown that DA root extracts exhibit diverse bioactivities, including anti-osteoporosis, neuroprotective, anti-