

**CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITIES OF
ESSENTIAL OILS FROM FRUITS OF *Melicope pteleifolia* (Champ. Ex Benth.)
T.G. Hartley GROWN IN LAM DONG PROVINCE, VIETNAM**

**Hoang Thi Binh^{1,*}, Tran Thi Bao Tram¹, Do Ngoc Dai²,
Vuong Thuy Tien³, Le Minh Tam⁴, Nguyen Van Ngoc¹**

¹Faculty of Biology, Dalat University, Vietnam

²Faculty of Agriculture, Forestry and Fisheries, Nghe An Economic University, Vietnam

³Thang Long Highschool for the gifted, Lam Dong Province, Vietnam

⁴Vaccine Company Limited of Dalat Pasteur

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ABSTRACT

In the present study, chemical composition and antibacterial properties of essential oil obtained from the aerial parts of the *Melicope pteleifolia* (Champ. ex Benth.) T.G Hartley in Dalat were evaluated. Essential oil was isolated through hydro-distillation. Twenty-nine constituents comprising 100% of the essential oil were characterized by gas chromatography/mass spectrometry (GC-MS) techniques. The major compounds in the essential oil were (+)-Sabinene (34.73%), Cis- α -bergamotene (13.15%), Z- α -trans-bergamotol (5.28%), β -mycrene (4.98%), and 1,3,6-octatriene, 3,7-dimethyl-(4.71%). Antibacterial activities of *Melicope pteleifolia* essential oil were investigated against Gram-positive and-negative bacteria. Results showed significant activities against *Streptococcus pyogenes* and *Escherichia coli* using an agar well diffusion method. The application of this essential oil in preventing and eliminating bacteria could be useful in fields as medicine and cosmetics.

Keywords: *Melicope pteleifolia*, antibacterial activity, essential oil.

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*Corresponding author email: binhht@dlu.edu.vn

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INTRODUCTION

Melicope J.R. Forst. & G. Forst., one of the largest genera in the family Rutaceae (Kubitzki et al., 2011) with 235 species, is widely distributed in the Hawaiian Islands, tropical Asia, Australia and New Zealand (Hartley, 2001). It has been divided into four sections comprising *Lepta* (with 102 species), *Melicope* (with 38 species), *Pelea* (with 85 species), and *Vitiflorae* (with 8 species) (Hartley, 2001). *Euodia* J.R. Forst. & G. Forst., the genus with the fewest number of species among the Rutaceae family, was merged with the genus *Melicope* by Hartley, 2001.

According to Ho (2003), *Euodia* species were recorded in Vietnam including *E. bodinieri* (Dode.), *E. calophylla* (Guill.), *E. crassifolia* (Merr.), *E. lepta* (Spreng.) Merr., *E. meliaefolia* (Benrth.), *E. oreophila* (Guill.), *E. pasteuriana* (A. Chev. Ex Guill.), *E. rutaecarpa* (A. Juss.) Benth., *E. simplicifolia* (Ridl.), and *E. sutchuenensis* (Dode.), in which *E. lepta* is widely distributed in evergreen forests throughout Vietnam (Ho, 2003). The Plant List (2013) currently treated *Euodia lepta* (Spreng.) Merr. as a synonym of *Melicope pteleifolia* (Champ. ex Benth.) T.G. Hartley. Recently, we recorded several populations of *M. pteleifolia* in the evergreen forests of Bidoup-Nui Ba National Park and the nearby areas.

In ethnomedicine, diseases such as arthritis, fever, chickenpox, epidemic influenza, meningitis and infective hepatitis have been treated using roots and leaves of *M. pteleifolia* (Duke & Ayensu, 1985). The vegetative parts as well as the flowers and fruits of *M. pteleifolia* are also extensively used in both the ethnic communities and the traditional health care system in Vietnam (Bich et al., 2004). Phytochemical studies on parts of *M. pteleifolia* reported several biologically active compounds including leptol A (Li et al., 2003), chromenes (Li et al., 1997a; Li et al., 1997b; Li & Zhu., 1998), chromans (Li et al., 1998) and benzopyrane

derivatives (Thang et al., 2007). The chemical compositions of essential oil of leaves, stems and flowers of *M. Lepta*, obtained by hydrodistillation, were reported by Thang et al. (2015). According to their report, (E)- β -ocimene (24.4%), α -pinene (9.8%), (Z)- β -ocimene (6.3%) and δ -cadinene (5.2%) were the main compounds of leaves oil of *M. lepta*, while the stems oils contained spathulenol (26.0%), (E)- β -ocimene (9.9%) and (Z)-9-octadecenamide (7.7%) and the flowers oil comprised of cis-carane (19.2%), α -cadinol (10.8%), α -pinene (10.5%) and (E)- β -ocimene (9.0%) (Thang et al., 2015). However, until now, there has been no report on the volatile constituents of essential oil derived from fruits of *M. pteleifolia* and the antibacterial activities of the essential oil of this species. The purpose of this study is to identify the chemical compositions and evaluate the antibacterial activity of essential oil from fruits of *M. pteleifolia*.

MATERIALS AND METHODS

Plants materials

The aerial parts of *Melicope pteleifolia* (Champ. ex Benth.) T.G. Hartley) were collected from Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam, between June and August 2018 at an altitude of 1,867 m. The plant was identified by Dr. Nguyen Van Ngoc, a researcher at the Faculty of Biology, Dalat University, Vietnam. The specimens were deposited in the herbaria DLU of Dalat University, Vietnam.

Bacteria

The two bacteria for testing, *Streptococcus pyogenes* (*Streptococcus* β -hemolytic type A; gram (+); *S. pyogenes*) and *Escherichia coli* (gram (-); *E. coli*), were provided by the General Hospital of Lam Dong Province, Vietnam and were grown in nutrient agar (NA) at 30 °C for 24 hours.

Isolation of the essential oil

Essential oil was extracted from fruits of *M. pteleifolia* using hydro-distillation. 500 g

of fresh fruits were placed in a distillation apparatus with 10 L of water and hydro-distilled for 2.5 h. After that, sodium sulphate was used to dry the anhydrous essential oil, which was then kept at 4 °C until use for GC-MS analyses.

Gas chromatography-Mass spectrometry (GC-MS)

Essential oil from fruits of *M. pteleifolia* fruits was analyzed using GC-MS method on an Agilent Technologies HP 6890N Plus Chromatograph connected to a mass spectrometer HP 5973 MSD. The analytical conditions were; Column: Agilent DB-5MS; Length: 30 m, Film: 0.25 µm, diam: 0.25 mm; MS transfer line temperature: 220 °C; Ion source temperature: 200 °C; Injector temperature: 220 °C; Temperature program: from 70 °C (15 min) up to 250 °C with increments of 10 °C/min; Flow: 1.2 ml/min; Mass range (m/z): 50–450.

Identification of the constituents

The constituents of essential oil were identified based on the retention times (RT) of the co-injected standard terpenes. Further identification was carried out by comparing their mass spectra with values from NIST 08 and Wiley 275 libraries or with mass spectra from the literature (Adams, 2007).

Measuring antibacterial activities using the agar well diffusion method

The agar well diffusion method (Devillers et al., 1989; Valgas et al., 2007) was used to test for antibacterial activity of the essential oil from fruits of *M. pteleifolia*. A broth culture (1%, containing 10⁶–10⁸ CFU/ml) of the respective bacterial strains was poured over base plates containing 7 ml nutrient agar at 4 °C in sterile 9 cm Petri dishes.

Different concentrations of the essential oil, including undiluted and four dilutions (50, 25, 12.5 and 5%), were used to evaluate antimicrobial activity. Sterile dimethyl

sulphoxide (DMSO) was used to dilute the essential oil of *M. pteleifolia*. DMSO was used as a negative control while chloramphenicol 250 mg (Vidipha Central Pharmaceutical Joint Stock Company, Vietnam) was used as a positive control. Plates were incubated at 30 °C for 24 hours. After that, the growth inhibition zones were measured in millimeters. Each test was performed in triplicate, from which the size of the growth inhibition zone was averaged.

Statistical analysis

All data analyses were performed using Microsoft excel 2017. Mean ± standard deviation was used to present data calculated from triplicate determinations. Statistical differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical compositions of the essential oil from fruits of *M. pteleifolia*

The analysis of hydro-distilled essential oil from *M. pteleifolia* fruits using GC-MS method identified 29 compounds (Table 1).

The main components identified were (+)-sabinene (34.7%), cis- α -bergamotene (13.2%), Z- α -trans-bergamotol (5.3%), β -mycrene (5.0%) and 1,3,6-octatriene,3,7-dimethyl- (4.7%). Among those, sabinene was the most abundant, although this compound was not found in the essential oils from leaves, stems and flowers of *M. leptota* (Thang et al. 2015). Moreover, Thang et al., (2015) reported that (E)- β -ocimene (24.4%), spathulenol (26.0%) and cis-carane (19.2%) were the dominant constituent of essential oils derived from leaves, stems and flowers of *M. leptota*. The different parts of this plant species contain quantitatively and qualitatively different compound compositions. In addition, geographic differences also affect the constituents of essential oils of plant species (Saei-Dehkordi et al., 2010).

Table 1. Chemical composition of the essential oil of *Melicope pteleifolia* fruits

No	Compounds	RSI	RT	Area (%)
1	2-Thujene	953	4.56	1.0
2	α -Pinene	901	4.79	1.8
3	(+)-Sabinene	961	6.00	34.7
4	β -Pinene	953	6.19	1.2
5	β -Mycerene	945	6.57	5.0
6	α -Terpinene	934	7.84	1.1
7	o-Cymene	928	8.20	1.4
8	D-Limonene	936	8.47	3.8
9	1,8-Cineole	916	8.62	0.3
10	Cis-ocimene	925	8.85	1.0
11	1,3,6-octatriene,3,7-dimethyl-	943	9.49	4.7
12	τ -Terpinene	954	10.22	2.2
13	p-Mentha-1,4(8)-diene	931	12.23	0.5
14	Linalool	955	13.64	3.7
15	Geijerene	939	16.78	2.1
16	6-octenal,3,7 dimethyl-	912	17.46	0.2
17	Levomenthol	924	18.40	0.3
18	Terpinen-4-ol	920	18.47	3.6
19	α -Terpineol	931	18.98	0.9
20	β -citral	936	20.23	0.5
21	Linalyl acetate	928	20.57	0.7
22	α -Citral	922	20.95	0.8
23	Pregeijerene	955	21.48	2.3
24	Copaene	916	23.04	0.9
25	Cis- α -bergamotene	953	23.94	13.2
26	Cadina-1(10),4-diene	905	25.16	1.8
27	Bergamotol,Z- α -trans-	838	25.24	5.3
28	Benzene,5-allyl-1,2,3-trimethoxy-	922	25.46	1.6
29	(\pm)-trans-nerolidol	956	25.68	3.5

Notes: RSI: Reversed Search Index; RT: Retention times.

Antibacterial activities of the essential oil derived from fruits of *M. Pteleifolia*

Antibacterial activities of essential oil derived from fruits of *M. pteleifolia* against

Escherichia coli and *Streptococcus pyogenes* were examined after 24 hours of culture. Diameter of inhibition were used to express the anti-bacteria levels (Table 2).

Table 2. Antibacterial activities of essential oil derived from fruits of *M. pteleifolia*

	Antibacterial activity (mm)						
	Chloramphenicol	DMSO	Concentration (% of essential oil and DMSO)				
			100%	50%	25%	12.5%	5%
<i>E. coli</i>	22.3 \pm 0.6	-	17.3 \pm 2.3	19.3 \pm 1.2	12.0 \pm 1.7	11.7 \pm 1.2	11.3 \pm 0.6
<i>S. pyogenes</i>	24.7 \pm 0.6	-	24.3 \pm 1.5	21.0 \pm 1.0	16.0 \pm 1.0	12.3 \pm 0.6	14.3 \pm 2.1

Notes: "-": No antibacterial activity.

The essential oil of *M. pteleifolia* fruits showed growth inhibition against both *E. coli* and *S. pyogenes* in a dose-dependent manner. Against *S. pyogenes*, pure (100%) essential oil produced inhibition zones 24.33 mm in diameter, comparable to those of chloramphenicol, a positive control. Against *E. coli*, the essential oil from fruits *M. pteleifolia* produced the best inhibitory activity at 50% concentration with an inhibition zone diameter of 19.33 mm. According to the classification of De Billerbeck (2007) on the susceptibility of bacteria against antibiotics based on the diameters of inhibitory zones (resistant: $D < 6$ mm; intermediate: $13 \text{ mm} > D > 6$ mm; sensitive: $D > 13$ mm), both tested bacteria strains were moderately sensitive to the essential oil from fruits of *M. pteleifolia*. In this study, the gram-negative bacteria (*E. coli*) are less susceptible than gram-positive bacteria (*S. pyogenes*) at every concentrations examined. This can be explained based on the outer membrane of bacteria. The outer membrane of gram-negative bacteria contains hydrophilic lipopolysaccharides (LPS) which creates a barrier against macromolecules and hydrophobic compounds, making gram-negative bacteria more tolerant to hydrophobic component of the essential oil (Trombetta et al., 2005).

Recently many reports showed that the antimicrobial effects of many essential oils is depend on the individual components and the combination of components within. Major components of essential oils, such as monoterpene or sesquiterpene hydrocarbons and their oxygenated derivatives, have potential antimicrobial activities (Diao et al., 2014). Essential oils rich in phenolic compounds have been reported to have antimicrobial activity (Aligiannis et al., 2001; Panizi et al., 1993; Sivropoulou et al., 1996).

CONCLUSION

Results of this study show that the essential oil from fruits of *M. pteleifolia* contains 29 components with the major compounds being (+)-sabinene (34.73%), cis- α -bergamotene (13.15%), Z- α -trans-

bergamotol (5.28%), β -myrcene (4.98%) and 1,3,6-octatriene, 3,7-dimethyl-(4.71%). The antibacterial properties of the essential oil from fruits of *M. pteleifolia* harvested in Dalat, Vietnam, have growth inhibiting activities against *E. coli* and *S. pyogenes* with different efficacy depending concentrations.

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