ISSN: 2621-4709 E-ISSN: 2621-2528
Journal of Applied Agricultural Science and Technology
Vol. 8 No. 4 (2024): 450-460

Phytochemical, Antioxidant, and Antibacterial Activity of Essential Oil *Hyptis capitata* Using Solvent-Free Microwave Extraction

Yuni Susanti [a,*], Ayu Qurota A'yun [a]

[a] Departement of Chemical Engineering, Institut Teknologi dan Bisnis Muhammadiyah Banyuwangi, Banyuwangi, Indonesia

Abstract. *Knobweed (Hyptis capitata) is a member of the genus Hyptis and the medicinal properties remain underexplored in scientific research. In this context, the use of Hyptis capitata essential oil as a medicinal plant depends on phytochemical content. Hyptis capitata is easily found in rice fields or agricultural areas such as Banyuwangi. Therefore, this research aimed to describe phytochemical compounds, antioxidants, and antibacterial activity of essential oil Hyptis capitata in Banyuwangi using Solvent-Free Microwave Extraction (SFME) method. Essential oil from leaves and inflorescence of the species was obtained with a yield of 0.273% and 0.282%, respectively. The results of Gas Chromatography-Mass Spectrometry (GC-MS) analysis showed that inflorescence and leaves of Hyptis capitata consisted of diterpenes, triterpenes, sesquiterpenes, esters, alcohols, ketones, and other groups of compounds. SFME method for the extraction affected both essential oil yield and phytochemical composition. Meanwhile, there were variations in phytochemical compounds of the plant parts used and from several previous research. The optimum % DPPH inhibition value of leaves extract and inflorescence were 19.187±0,06% and 18.784±0,06%, respectively. Antibacterial activity against Staphylococcus aureus and Eschericia coli reported no inhibition of plant extract. Phytochemical analysis showed several compounds with antibacterial potential, suggesting the need for further research of antibacterial activity against other pathogenic bacteria and exploration of additional biological activities.*

Keywords: *antibacterial; antioxidant; GC-MS; Hyptis capitata; Solvent-Free Microwave Extraction.*

Type of the Paper: Regular Article.

1. Introduction

Indonesia is a country with the second largest biodiversity after Brazil. This country has a rich variety of plants that offer significant benefits in the health sector, particularly as medicinal resources. Approximately 7000 species are classified as medicinal plants. An important genera functioning as a medicinal plant is *Hyptis* in the *Lamiaceae* family found within tropical and subtropical regions, such as Indonesia. The characteristics of these plants are trichome glands containing essential oil and strong aroma [1,2]. The number of species reported is about 775, which are found in Americas. Some of the pharmacological uses of *Hyptis* include treating respiratory disorders, nasal congestion, fever, liver disease, open wounds, coughs, digestive disorders, skin infections [3], and human immunodeficiency virus (HIV) [4]. *Hyptis suaveolens* was the first

https://doi.org/10.55043/jaast.v8i4.275
Received January 7, 2024; Received in revised form August 21, 2024; Accepted August 22, 2024; Published November 24, 2024
* First corresponding author
Email: ysusanti@itbmb.ac.id

species from the genus *Hyptis*, which was analyzed in 1952. This research reported the chemical composition contained in essential oil of *Hyptis suaveolens*. Phytochemical analysis showed that several bioactive compounds are contained in the genus *Hyptis* [3]. However, only 10% of the members have been researched in depth. In this context, further analyses are needed on other members of the genus to determine the composition of phytochemical compounds and their health benefits. The species of the genus *Hyptis* widely reported include *H. suaveolens*, *H. mutabilis*, *H. fructicosa*, *H. martiussi*, and *H. pectinata* [5].

Hyptis capitata known as knobweed originates from Central American countries and naturally grows in tropical areas [6]. *Hyptis capitata* is a type of invasive plant, namely a species of flora with the capacity to live and develop outside the natural habitat. In addition, this species has high-speed growth without natural enemies, dominates a new area or ecosystem, and conquers the growth of native plants, threatening biodiversity [7]. *Hyptis capitata* has different names according to the country or region of origin. In Spain and Vietnam, the species is known as biojo and botonesan, while in Indonesia, Toraja tribe, Banggai tribe, and Banyuwangi Regency name the plant as narang-narang or sualang, Pago-pago grass [8], and sontoloyoweed, respectively.

Hyptis capitata (Fig. 1a) is often found growing wild in Banyuwangi area but is only considered a weed. The plant can overgrow in empty land close to water sources, agricultural land, and the side of the road. Several research have shown that *Hyptis capitata* as a medicinal plant depends on phytochemical compositions. The chemical compounds in this species include monoterpene, sesquiterpene, diterpene, alkaloid, alcohol, ester, lignin polyphenol compounds, and flavonoids [5,9,10]. The existence of genetic variations, plant phenology, geographical conditions [11], climatic factors [12], temperature, rainfall, type of material (fresh or dry), and extraction methods influence the yield and variability in phytochemical composition of essential oil [13,14]. Several research have shown potential antibacterial [15,16], antioxidant [5,10,15,16], antiviral, and anticancer [17] activities of *Hyptis capitata*. However, this has not been explored more widely. In Indonesia, research on *Hyptis capitata* is limited to a few places, namely Southeast Sulawesi [17], Central Sulawesi [18], South Sulawesi [9], Jambi [14], and East Kalimantan [15]. In response to the perception of *Hyptis capitata* as a weed by Banyuwangi farmers, phytochemical compounds and biological activity of essential oil should be investigated. The essential oil is obtained by maceration with ethanol [9,14,18] or methanol [10,15], soxhletation with methanol [19], and hydrodistillation [5]. This conventional method has a smaller yield, requires a relatively long time, and has an enormous cost. Therefore, methods such as Solvent-Free Microwave Extraction (SFME) should be developed for extracting essential oil. SFME has advantages compared to the earlier methods, including having a faster extraction rate, shorter extraction time, yield, and higher extract purity. This method can be categorized as green technology because the concept does not

add solvents during extraction and reduces the energy requirement per ml of essential oil extraction [20,21].

Extraction of essential oil using SFME has not been reported previously. Limited research have shown phytochemicals and biological activity of *Hyptis capitata* essential oil. Therefore, this research aims to analyze phytochemical composition, antioxidant activity, and antibacterial activity of essential oil of *Hyptis capitata* grown in Banyuwangi Regency using SFME.

2. Materials and methods

2.1. Plant Materials

Fresh leaves and inflorescence of *Hyptis capitata* were gathered from Sarimulyo Village, Banyuwangi Regency (8.2192° S, 114.3692° E), Indonesia. The plant was identified and taxonomically by UPT Laboratorium Herbal Materia Medica Batu. In addition, the plant parts are collected and directly used for extraction.

2.2. Preparations of Extracts

Approximately 250 g fresh leaves or inflorescences were ground using a blender. Ground plant samples were extracted in SFME Apparatus (Electrolux EMM20M38GW) (Fig. 1b) for 3 h with a power of 700 watts. Essential oil from the extraction process was taken by adding 10 mL of n-hexane (Merck), dehydrated using sodium sulfate anhydrous (Merck), and stored for further analysis. The percentage yield of essential oil was determined using Equation (1) [22].

$$\%\ Yield = \frac{weight\ of\ essential\ oils\ produced\ (g)}{weight\ of\ the\ plant\ materials\ used\ (g)\ x\ (1 - water\ content\ (\%))}\ x\ 100 \qquad (1)$$

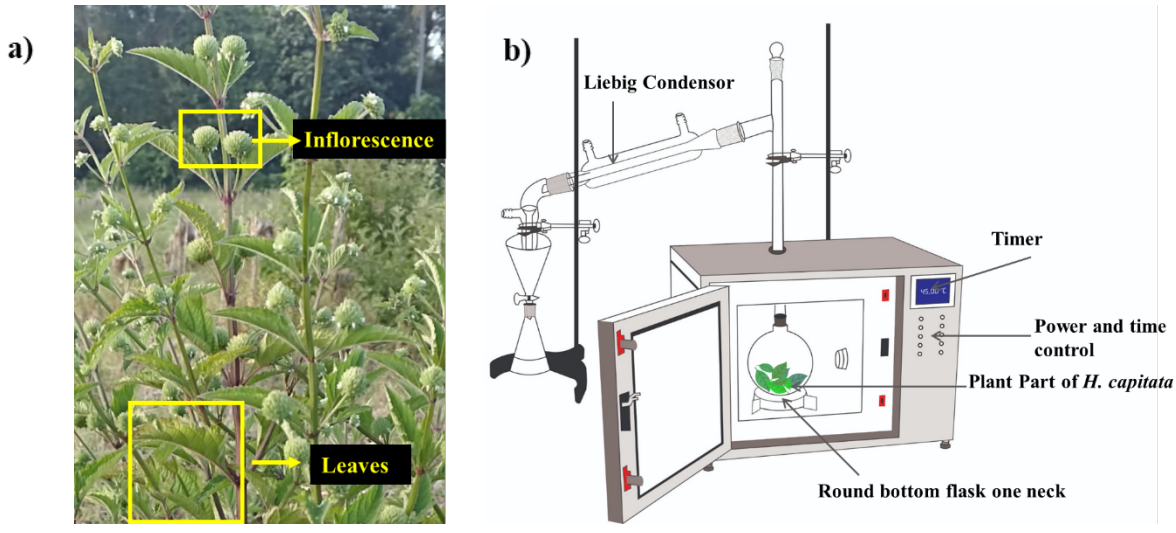

Fig. 1. a) Plant part of *Hyptis capitata*, b) SFME Apparatus

2.3. Gas Chromatography-Mass Spectrometry (GC-MS)

Essential oil of *Hyptis capitata* were subjected to GC-MS analysis (Shimadzu QP 2020 NX). The oven had an initial temperature of 80ºC maintained for 2 minutes, which increased to 280 ºC

for 2 minutes at the rate of 50°C for 6 minutes. The samples were injected in split mode, and the m/z range was scanned from 50 to 500. Identification of the components was compared with the spectra of WILEY 7 Library [19].

2.4. DPPH Radical Scavenging Test

Antioxidant activity of a sample is determined by measuring the mechanism of radical scavenging activity against DPPH (2,2-Diphenyl-1-picrylhydrazyl) with a Shimadzu UV-VIS 1240 spectrophotometer [23]. A total of 50 µl test samples with various concentrations were added with 1.0 ml of 0.4 mM DPPH and 3.950 ml ethanol. Subsequently, the mixture was vortexed and left for 30 minutes. The solution was measured for absorbance at a length of 517 nm against a blank which consisted of 50 µl and 4,950 ml of extract and ethanol, respectively. The control absorbance measurement comprises 1.0 ml DPPH and 4.0 ml ethanol, with ascorbic acid as a standard, and % inhibition was determined using Equation (2) [5].

$$\%\ inhibition = 1 - \frac{(sample\ absorbance - blank\ absorbance)}{control\ absorbance}\ x\ 100 \qquad (2)$$

2.5. Antibacterial Activity Assay

Essential oil of *Hyptis capitata* was assayed for antibacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Eschericia coli*) bacteria using the agar well disk diffusion method [24]. This assay used nutrient agar (NA) as media, which was poured into Petri dishes. A cotton swab was dipped in a bacterial suspension and applied to the surface of NA media. In addition, a well was made on the media using a sterile cork borer about 6-8 mm and sample solutions (50 and 100 µg/mL) were dropped into the well. Chloramphenicol and n-hexane were applied as positive and negative controls, respectively. The agar plates were incubated at 37°C for 24 h and samples with antibacterial activity inhibited bacterial growth to produce a clear zone.

3. Results and Discussion

3.1. GC-MS Analysis and Identification Phytochemicals

The effect of microwave irradiation causes higher internal pressure, which ruptures the trichomes' exterior cuticle (membrane) and causes essential oil to be extracted. Essential oil from leaves and inflorescence of *Hyptis capitata* was obtained with a yield of 0.273% and 0.282%, respectively. The oils were colourless with fragrant smell and six peaks indicated the presence of phytochemical compounds (Fig. 2). Identification of phytochemical compounds based on retention time and the area is given in Table 1.

Based on Table 1, phytochemical compounds contained in essential oil of *Hyptis capitata* leaves are dominated by diterpene (66.33%), triterpene (19.67%), ketone (10.28%), ester (3.73%) and other compounds (0.31%). This is interesting because the content of phytochemical

compounds is slightly different. Previous research showed that the compositions of *Hyptis capitata* leaves were dominated by triterpene (68.78%), diterpene (16.51%), monoterpene (3.88%), sesquiterpene (0.86%), and other compounds (9.97%) [19]. Other results also reported that there was sesquiterpene (38.92%), aliphatic alcohol (34.08%), and ester (19.77%) obtained in essential oil [5]. Therefore, the differences in extraction methods [13,14] and geographical conditions [3,11] resulted in different compound compositions.

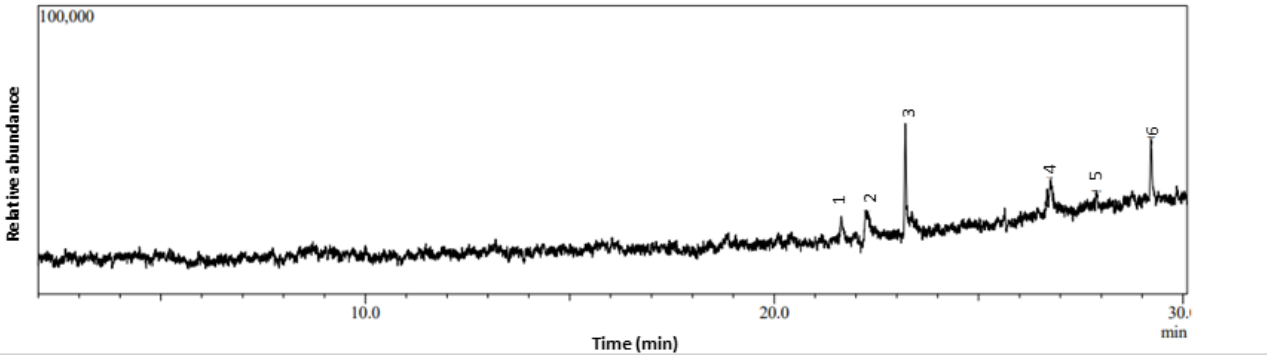

Fig. 2. GC-MS Chromatogram of Leaves Extract of *Hyptis capitata*

Table 1. Phytochemicals of Essential Oil of Leaves of *Hyptis capitata*

No.	Retention Time	Name of the Compounds	Molecular Formula	Nature of the Compound	% Peak Area
1	21.639	13-hexyloxacyclotridec-10-en-2-one	$C_{18}H_{32}O_2$	alkene, ketone	10.28
2	22.245	t-Phytol	$C_{20}H_{40}O$	hydrogenated diterpene alcohol	29.41
3	23.209	neophytadiene	$C_{20}H_{38}$	alkene, diterpene	36.92
4	26.750	ethyl linoleate	$C_{20}H_{36}O_2$	unsaturated fatty acid ethyl ester	3.73
5	27.872	2,4,6(1H,3H,5H)-Pyrimidinetrione	$C_{17}H_{34}N_2O_3Si_2$	-	0.31
6	29.211	Squalene	$C_{30}H_{50}$	triterpenoid	19.27
Class Compositions					
Diterpene					**66.33**
Triterpene					**19.67**
Keton					**10.28**
Ester					**3.73**
Other compounds					**0.31**

GC-MS data from *Hyptis capitata* inflorescence extract reported that 23 peaks indicated the presence of phytochemical compounds (Fig. 3). Identification of phytochemical compounds based on retention time and area is given in Table 2.

Based on Table 2, phytochemical compounds compositions in essential oil of *Hyptis capitata* inflorescence are dominated by ester compounds (36.72%), ketones (24.3%), alkanes (17.23%), sesquiterpenes (11.95%), diterpenes (4.5%), cyclo alcohols (2.61%), oxygenated hydrocarbons (1.93%), and triterpenes (0.76%). Based on Tables 1 and 2, there are variations in the composition of the plant parts used. The neophytadiene compound, a diterpene group, has a greater amount in

leaves than in inflorescence parts. Meanwhile, ester and ketone group compounds are found more in inflorescence than in leaves. Based on previous research, sesquiterpenes and monoterpenes are the dominant content in inflorescences part of *Hyptis capitata* [5,8]. *H. suaveolens* species also contains sesquiterpenes, diterpenes, and triterpene. The sesquiterpene δ-cadinene is a common compound in the genus *Hyptis* [25]. In the species *H. floribunda* and *H. glomerata*, cadinene is the main compound [5,25]. The production of essential oil from secondary metabolites is related to environmental conditions, habitat, and climate [26]. In addition, extraction methods can affect essential oil yield and phytochemical compositions [27–29]. Some phytochemical compounds in *Hyptis capitata* essential oil found in inflorescence and leaves have the potential as antioxidant and antibacterial. Some compounds include Germacrene D [30], δ-cadinene [31], and neophytadiene [32].

Table 2. Phytochemicals of Essential Oil of Inflorescence of *Hyptis capitata*

No.	Retention Time	Name of the Compounds	Molecular Formula	Nature of the Compound	% Peak Area
1	13.566	ɣ-muurolene	$C_{15}H_{24}$	bicylic sesquiterpenes	2.71
2	13.630	α-Amorphene	$C_{15}H_{24}$	bicylic sesquiterpenes	1.01
3	13.954	α-muurolene	$C_{15}H_{24}$	bicylic sesquiterpenes	1.75
4	14.190	Germacrene-D	$C_{15}H_{24}$	bicylic sesquiterpenes	2.04
5	14.317	δ-cadinene	$C_{15}H_{24}$	bicylic sesquiterpenes	4.44
6	15.108	heptyl hexanoate	$C_{13}H_{26}O_2$	ester	1.67
7	19.782	Farnesyl acetone	$C_{18}H_{30}O$	ketone	3.18
8	21.411	Bycyclo[5,3,1]undecan-11-one	$C_{11}H_{18}O$	ketone	21.12
9	21.893	methyl oleate	$C_{19}H_{36}O_2$	ester	0.87
10	22.920	tetratriacontane	$C_{34}H_70$	Oxygenated Hydrocarbon	1.93
11	23.188	neophytadiene	$C_{20}H_{38}$	diterpene	4.5
12	23.993	n-nonadecane	$C_{19}H_{40}$	Alkane Hydrocarbons	0.74
13	25.022	n-nonacosane	$C_{29}H_{60}$	Alkane Hydrocarbons	0.82
14	25.881	4-trimethylsilyloxycyclohexan-1-ol	$C_9H_{20}O_2$	Cyclic alcohol	2.61
15	26.008	eicosane	$C_{20}H_{42}$	Alkane Hydrocarbons	1.71
16	26.959	n-pentacosane	$C_{25}H_{52}$	Alkane Hydrocarbons	1.14
17	27.138	erythrodiol	$C_{30}H_{50}O_2$	triterpene	0.76
18	27.576	3,3-dichloro-1,1,2,2-tetramethyl-cyclopropane	$C_7H_{12}Cl_2$	alkane Hydrocarbons	6.72
19	27.785	methyl-2-cyclohexyl-2-methyl pentanoate	$C_{13}H_{24}O_2$	ester	0.89
20	27.875	hexacosane	$C_{26}H_{54}$	Alkane Hydrocarbons	2.94
21	28.963	methyl linoleate	$C_{19}H_{34}O_2$	ester	27.88
22	29.700	n-docosyl n-heptanoate	$C_{29}H_{58}O_2$	ester	5.41
23	29.823	heneicosane	$C_{21}H_{44}$	Alkane Hydrocarbons	3.16
		Class compositions			
		Sesquiterpene		**11.95**	
		Diterpene		**4.5**	
		Triterpene		**0.76**	
		Cyclic alcohol		**2.61**	
		Esters		**36.72**	
		Alkane Hydrocarbons		**17.23**	
		Ketone		**24.3**	
		Oxygenated Hydrocarbon		**1.93**	

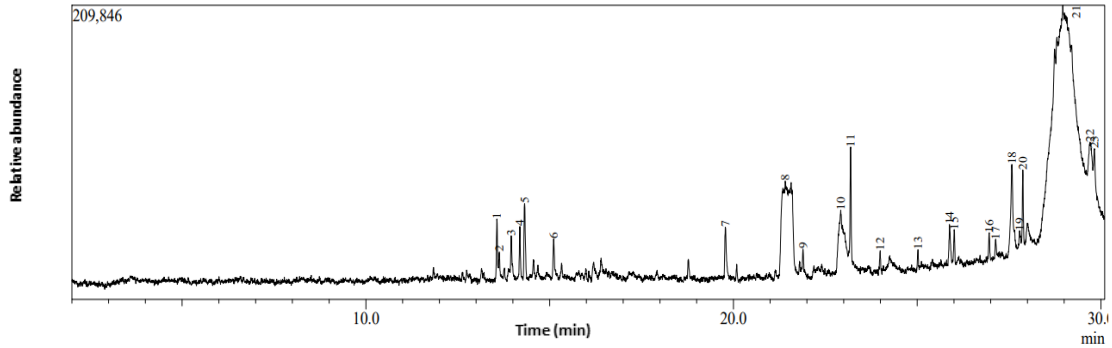

Fig. 3. GC-MS Chromatogram of Inflorescence Extract of *Hyptis capitata*

3.2. Antioxidant Activity

Antioxidant activities of leaves and inflorescence extract were calculated by DPPH radical scavenging using the spectrophotometry method, as reported in Fig. 4. The result showed that there was a direct relationship between sample concentration and % DPPH inhibition. DPPH scavenging ability of leaves extract is higher than inflorescence extract. The optimum % inhibition and concentration values of the extract were 19.187±0.06% and 65.731 mg/mL, respectively. The optimum % inhibition and concentration values of inflorescence extract were 18.784±0,06% and 67.366 mg/mL, respectively.

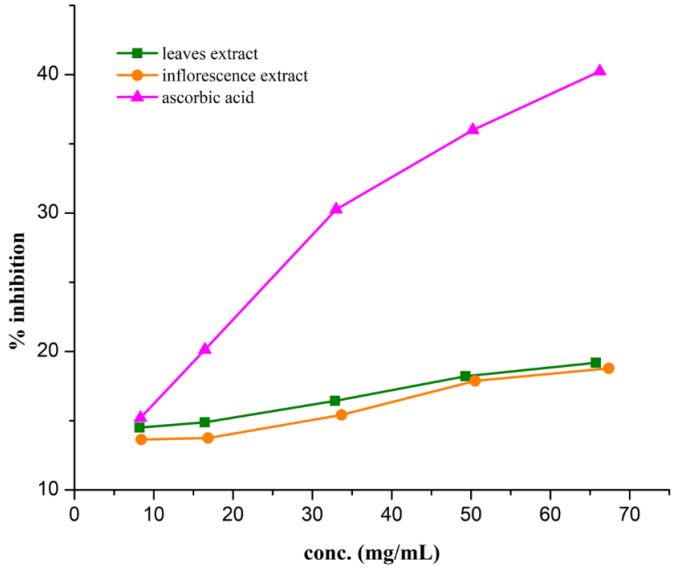

Fig. 4. DPPH radical scavenging activity of essential oil from *Hyptis capitata*

Table 3. Antibacterial activity of essential oil *Hyptis capitata*

Tested microorganisms	Sample	Inhibition Zone (mm)/extract tested (µg) 50	100
S. aureus	Leave Extract	NI	NI
	Inflorescence Extract	NI	NI
	Chloramphenicol	25.28±0.67	26.20±0.47
	n-hexane	NI	NI
E. coli	Leave Extract	NI	NI
	Inflorescence Extract	NI	NI
	Chloramphenicol	25.28±0.67	26.20±0.47
	n-hexane	NI	NI

NI: no inhibition

Essential oil of *Hyptis capitata* has low antioxidant activity compared to ascorbic acid. This result was in line with the previous report, where there were no phenolic and flavonoid compounds in the oil [3,5]. Neophytadiene (diterpene), t-phytol (diterpene), and squalene (triterpene) were dominant compounds and would have contributed a significant percentage of antioxidant activity in the extract [32–35]. Terpenoids are one of secondary metabolites present in medical plants. These metabolites have various bioactivity such as antioxidant, antimicrobial, and larvacidal activity. Neophytadiene, which has the greatest amount detected in the extract, might influence greater antioxidant activity than extract inflorescence. Meanwhile, in inflorescence extract, synergistic compounds contributed to antioxidant properties such as esters, ketones, alkanes, sesquiterpenes, diterpenes, cyclo alcohols, and triterpenes. Some of the compounds observed, including Germacrene D [30], δ-cadinene [31], and neophytadiene [32] might influence antioxidant properties. Previous reports on other species of *Hyptis*, such as *H. suaveolens*, *H. rhombiodea*, and *H. brevipes*, showed impressive antioxidant activities. The phenolic and flavonoid compounds observed in essential oil contributed to the excellent antioxidant activity [16,36].

3.3. Antibacterial Activity

Essential oil of leaves and inflorescence were assayed for antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, as shown in Table 3. There are very restricted research on *Hyptis capitata*'s antibacterial activity. The extract and inflorescence did not show the ability to inhibit *S. aureus* and *E. coli* at a concentration of 50 and 100 µg/mL, respectively. The results were similar to previous research that plant extract of *Hyptis capitata* could not inhibit *S. aureus* and *E. coli* at a concentration below 100 µg/mL. Essential oil of *Hyptis capitata* showed antibacterial activity at a high concentration of 500 µg/mL. The inhibition zones were 29.64 mm, 48.99 mm, 51.40 mm, 45.10 mm, and 0 mm for *P. acnes*, *S. sobrinus*, *S. aureus*, *E. coli*, and *C. albicans*, respectively. However, the pathogen bacteria have no inhibition at a concentration below 100 µg/mL [37]. In this context, further investigation on antibacterial activity against the other pathogen microbial strains should be conducted. This is because GC-MS results showed several compounds with antibacterial potential. Some compounds in essential oil of *Hyptis capitata*, such as Germacrene D, cadinene derivatives, terpenoids, and t-phytol could play important roles as antibacterial agents [38].

4. Conclusions

In conclusion, this research was reported as the first detailed analysis to examine phytochemicals and bioactivity of essential oil *Hyptis capitata* using a new method. The results showed that SFME method influenced the different phytochemicals content of essential oil.

Moreover, there were variations in the composition of the plant parts used. Essential oil of leaves and inflorescence reported weak antioxidant activity. In this context, essential oil of leaves had higher antioxidant activity compared to inflorescence. Antibacterial activity test of essential oil *Hyptis capitata* showed no inhibition against *S. aureus* and *E. coli* at 50 and 100 µg/mL concentrations. GC-MS analysis would support the further development of this plant for testing antibacterial activity against other pathogenic microbial strains, as well as the potential for biological activities. In addition, the present results would be references as a source of medicines and pharmaceutical product improvement.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Yuni Susanti: Writing – Original Draft, Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation. Ayu Qurota A'yun: Investigation, Writing -review and editing.

Declaration of Competing Interest

The authors of this manuscript declare no conflict of interest or competing interest.

Acknowledgement

We would like to thanks Indonesian Ministry of Research, Technology and Higher Education under the Competitive-Based Research Grant for funding this research.

References

[1] Sá S de, Fiuza TS, Borges LL, Ferreira HD, Tresvenzol LMF, Ferri PH, et al. Chemical composition and seasonal variability of the essential oils of leaves and morphological analysis of Hyptis carpinifolia. Revista Brasileira de Farmacognosia 2016;26:688–93. https://doi.org/10.1016/j.bjp.2016.05.011.

[2] McNeil M, Facey P, Porter R. Essential Oils from the Hyptis genus- A Review (1909-2009). Nat Prod Commun 2011;6:1934578X1100601. https://doi.org/10.1177/1934578X1100601149.

[3] Sedano-Partida MD, dos Santos KP, Sala-Carvalho WR, Silva-Luz CL, Furlan CM. A review of the phytochemical profiling and biological activities of Hyptis Jacq.: a Brazilian native genus of Lamiaceae. Brazilian Journal of Botany 2020;43:213–28. https://doi.org/10.1007/s40415-020-00582-y.

[4] Kashiwada Y, Wang H-K, Nagao T, Kitanaka S, Yasuda I, Fujioka T, et al. Anti-AIDS Agents. 30. Anti-HIV Activity of Oleanolic Acid, Pomolic Acid, and Structurally Related Triterpenoids. J Nat Prod 1998;61:1090–5. https://doi.org/10.1021/np9800710.

[5] John R, Sabu KR, Manilal A. Chemical Composition, Antioxidant, and Mosquito Larvicidal Activity of Essential Oils from Hyptis capitata Jacq. J Exp Pharmacol 2022;Volume 14:195–204. https://doi.org/10.2147/JEP.S355280.

[6] Sumitha V, Mini I. Pharmacognostical standardization of Hyptis capitata Jacq . Lamiaceae. J Pharmacogn Phytochem 2019;8:2791–6. https://www.phytojournal.com/archives/2019.v8.i3.8433/pharmacognostical-standardization-of-ltemgthyptis-capitataltemgt-jacq-lamiaceae

[7] Radosevich SR, Stubbs MM, Ghersa CM, Radosevich SR, Ghersa CM. Plant invasions — process and patterns. Weed Sci 2003;51:254–9. https://www.jstor.org/stable/4046728
[8] To'bungan N, Widyarini S, Nugroho LH, Pratiwi R. Ethnopharmacolgy of Hyptis capitata. Plant Science Today 2022. https://doi.org/10.14719/pst.1602.
[9] To'bungan N. Pemanfaatan dan Skrining Fitokimia Infusa Daun Rumput Knop (Hyptis capitata Jacq.). Biota : Jurnal Ilmiah Ilmu-Ilmu Hayati 2021:149–54. https://doi.org/10.24002/biota.v5i3.3520.
[10] Zuliani NE, Erwin, Kusuma IW. Uji Aktivitas Antioksidan (Metode DPPH) Ekstrak Metanol dan Fraksi - Fraksinya dari Daun Rumput Knop (Hyptis capitata Jacq.). Jurnal Atomik 2019;4:36–40. http://jurnal.kimia.fmipa.unmul.ac.id/index.php/JA/article/view/724/537
[11] Sampaio BL, Edrada-Ebel R, Da Costa FB. Effect of the environment on the secondary metabolic profile of Tithonia diversifolia: a model for environmental metabolomics of plants. Sci Rep 2016;6:29265. https://doi.org/10.1038/srep29265.
[12] Yang L, Zheng Z-S, Cheng F, Ruan X, Jiang D-A, Pan C-D, et al. Seasonal Dynamics of Metabolites in Needles of Taxus wallichiana var. mairei. Molecules 2016;21:1403. https://doi.org/10.3390/molecules21101403.
[13] Azevedo NR, Campos IFP, Ferreira HD, Portes TA, Santos SC, Seraphin JC, et al. Chemical variability in the essential oil of Hyptis suaveolens. Phytochemistry 2001;57:733–6. https://doi.org/10.1016/S0031-9422(01)00128-5.
[14] Rupa D, Sulistyaningsih YC, Dorly D, Ratnadewi D. IDENTIFICATION OF SECRETORY STRUCTURE, HISTOCHEMISTRY AND PHYTOCHEMICAL COMPOUNDS OF MEDICINAL PLANT Hyptis capitata Jacq. Biotropia (Bogor) 2017;24:94–103. https://doi.org/10.11598/btb.2017.24.2.499.
[15] Kusuma IW, Murdiyanto, Arung ET, Syafrizal, Kim Y. Antimicrobial and antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia. Food Science and Human Wellness 2014;3:191–6. https://doi.org/10.1016/j.fshw.2014.12.004.
[16] Xu D-H, Huang Y-S, Jiang D-Q, Yuan K. The essential oils chemical compositions and antimicrobial, antioxidant activities and toxicity of three Hyptis species. Pharm Biol 2013;51:1125–30. https://doi.org/10.3109/13880209.2013.781195.
[17] Haryanti S, Widiyastuti Y. Aktivitas Sitotoksik pada Sel MCF-7 dari Tumbuhan Indonesia untuk Pengobatan Tradisional Kanker Payudara. Media Penelitian Dan Pengembangan Kesehatan 2017;27. https://doi.org/10.22435/mpk.v27i4.5010.247-254.
[18] Ayu S, Harso W, Jannah M. Profil Toksikologis Ekstrak Daun Tumbuhan Baka-Baka (Hyptis capitata Jacq.) Pada Hati Tikus Putih (Rattus norvegicus). Biocelebes 2020;14:10–21. https://doi.org/10.22487/bioceb.v14i1.15082.
[19] Sumitha V, Mini S V, Nair LS. Larvicidal efficacy of Hyptis capitata Jacq. Against Culex quinquefasciatus mosquito (Culicidae). Int J Mosq Res 2021;8:42–6. https://www.dipterajournal.com/pdf/2021/vol8issue4/PartA/8-4-10-166.pdf
[20] Golmakani M, Moayyedi M. Comparison of heat and mass transfer of different microwave-assisted extraction methods of essential oil from Citrus limon (Lisbon variety) peel. Food Sci Nutr 2015;3:506–18. https://doi.org/10.1002/fsn3.240.
[21] Variyana Y, Susanti Y. Optimasi Ekstraksi dari capsicum frutescens L dengan Microwave-Assisted Soxhlet Extraction (MASE) Menggunakan Response Surface Methodology (RSM). JRST (Jurnal Riset Sains Dan Teknologi) 2022;5:131. https://doi.org/10.30595/jrst.v5i2.10677.
[22] Kusuma HS, Mahfud M. The extraction of essential oils from patchouli leaves (Pogostemon cablin Benth) using a microwave air-hydrodistillation method as a new green technique. RSC Adv 2017;7:1336–47. https://doi.org/10.1039/C6RA25894H.
[23] Marxen K, Vanselow KH, Lippemeier S, Hintze R, Ruser A, Hansen U-P. Determination of DPPH Radical Oxidation Caused by Linear Regression Analysis of Spectrophotometric Measurements. Sensors 2007;7:2080–95. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3864510/pdf/sensors-07-02080.pdf

[24] Hossain ML, Lim LY, Hammer K, Hettiarachchi D, Locher C. A Review of Commonly Used Methodologies for Assessing the Antibacterial Activity of Honey and Honey Products. Antibiotics 2022;11:975. https://doi.org/10.3390/antibiotics11070975.
[25] Li R, Tang G, Liu X, Li J, Wang D, Ji S. An ethnopharmacological review of Hyptis suaveolens (L.) Poit. Tropical Journal of Pharmaceutical Research 2020;19:1541–50. https://doi.org/10.4314/tjpr.v19i7.29.
[26] Fan G-J, Ndolo VU, Katundu M, Kerr RB, Arntfield S, Beta T. Comparison of Phytochemicals and Antioxidant Capacity in Three Bean Varieties Grown in Central Malawi. Plant Foods for Human Nutrition 2016;71:204–10. https://doi.org/10.1007/s11130-016-0548-3.
[27] Mohammadhosseini M. Chemical Composition of the Essential Oils and Volatile Fractions from Flowers, Stems and Roots of Salvia multicaulis Vahl. by Using MAHD, SFME and HS-SPME Methods. Journal of Essential Oil Bearing Plants 2015;18:1360–71. https://doi.org/10.1080/0972060X.2015.1024447.
[28] Mohammadhosseini M, Nekoei M. Chemical Compositions of the Essential Oils and Volatile Compounds from the Aerial Parts of Ferula ovina Using Hydrodistillation, MAHD, SFME and HS-SPME Methods. Journal of Essential Oil Bearing Plants 2014;17:747–57. https://doi.org/10.1080/0972060X.2014.884951.
[29] Mohanty S, Ray A, Naik PK, Sahoo A, Jena S, Das PK, et al. Variation in Yield, Chemical Composition and Biological Activities of Essential Oil of Three Curcuma Species: A Comparative Evaluation of Hydrodistillation and Solvent-Free Microwave Extraction Methods. Molecules 2023;28:4434. https://doi.org/10.3390/molecules28114434.
[30] Noge K, Becerra JX. Germacrene D, A Common Sesquiterpene in the Genus Bursera (Burseraceae). Molecules 2009;14:5289–97. https://doi.org/10.3390/molecules14125289.
[31] Babarinde SA, Olaniran OA, Ottun AT, Oderinde AE, Adeleye AD, Ajiboye O, et al. Chemical composition and repellent potentials of two essential oils against larger grain borer, Prostephanus truncatus (Horn.) (Coleoptera: Bostrichidae). Biocatal Agric Biotechnol 2021;32:101937. https://doi.org/10.1016/j.bcab.2021.101937.
[32] Pratama OA, TUNJUNG WAS, SUTIKNO S, DARYONO BS. Bioactive compound profile of melon leaf extract (Cucumis melo L. 'Hikapel') infected by downy mildew. Biodiversitas 2019;20. https://doi.org/10.13057/biodiv/d201143.
[33] Ibrahim N 'Izzah, Naina Mohamed I. Interdependence of Anti-Inflammatory and Antioxidant Properties of Squalene–Implication for Cardiovascular Health. Life 2021;11:103. https://doi.org/10.3390/life11020103.
[34] Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, et al. Phytol: A review of biomedical activities. Food and Chemical Toxicology 2018;121:82–94. https://doi.org/10.1016/j.fct.2018.08.032.
[35] Luhata LP, Hirao M, Mori N, Usuki T. Chemical composition and antioxidant activity of the hexane fraction from leaf extracts of Odontonema strictum. American Journal of Essential Oils and Natural Products 2023;11:12–6. https://www.essencejournal.com/pdf/2023/vol11issue1/PartA/7-2-5-261.pdf.
[36] Agarwal K, Varma R. Antioxidant activity and Phytochemical analysis of Hyptis suaveolens (L.) Poit. Journal of Advanced Pharmacy Education & Research 2013;3:541–9. https://japer.in/article/antioxidant-activity-and-phytochemical-analysis-of-hyptis-suaveolens-l-poit.
[37] Kusuma IW, Rahmini, Arung ET, Pramono AY, Erwin, Supomo. Biological activities and phytochemicals of Hyptis capitata grown in East Kalimantan, Indonesia. J Appl Biol Biotechnol 2020;8:58–64. https://doi.org/10.7324/JABB.2020.80210.
[38] Boudjedjou L, Ramdani M, Zeraib A, Benmeddour T, Fercha A. Chemical composition and biological activities of Algerian Santolina africana essential oil. Sci Afr 2019;4:e00090. https://doi.org/10.1016/j.sciaf.2019.e00090.