

# Biological activities and phytochemicals of *Hyptis capitata* grown in East Kalimantan, Indonesia

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## ABSTRACT

*Hyptis capitata* is a plant in the family of Lamiaceae used to prevent wound infection and as an antidote for food poisoning by the Bentian people, a local tribe in East Kalimantan, Indonesia. Of the plants in the Lamiaceae family, the biological activities and phytochemical studies of *H. capitata* are less reported. This work aims to investigate the phytochemicals and biological activities of *H. capitata* grown at two locations in East Kalimantan, Indonesia. Leaves of *H. capitata* collected from location 1, Samarinda (coded by HSM), and location 2, West Kutai areas (coded by HWK), were macerated using ethanol. The biological activities of the plant were evaluated in terms of antimicrobial and antioxidant activity, and cytotoxicity against *Artemia salina*, while phytochemicals were analyzed by qualitative and quantitative tests. The results showed that leaf extract of *H. capitata* contains alkaloids, flavonoids, tannins, carbohydrates, and coumarins. HSM possessed higher phenolic and flavonoid content than that of HWK. HWK displayed more antimicrobial activity against the test microorganisms particularly *P. acnes* (29%), *S. sobrinus* (49%), *S. aureus* (51%), and *E. coli* (45%). HSM showed more radical scavenging activity in 1,1-diphenyl-2-picrylhydrazyl (IC<sub>50</sub> 13.69 µg/ml) and superoxide (IC<sub>50</sub> 65.11 µg/ml) tests. Brine shrimp lethality test showed no toxicity of *H. capitata* leaf extracts. The results displayed that *H. capitata* collected from the two locations showed good antibacterial and antioxidant activities. The results suggested that the biological activities and phytochemicals of *H. capitata* were affected by the environment where the plant was collected. A study into deep analysis of the effect of light intensity, temperature, soil nutrition, and predatory risks to the biological activity and phytochemicals of the plant is required.

## 1. INTRODUCTION

A medicinal plant is described as any plant with substances having therapeutic benefits in its organ and may contain precursors for the production of synthetic drugs [1]. It is reported that about 80% of world medicinal plants exist in the forests of Indonesia. More than 28,000 plant species have been discovered in Indonesia. Of these, 7,000 species are classified as medicinal plants [2].

Lamiaceae consists of more than 400 species widely distributed in tropical and subtropical locations, including *Hyptis* genus. Several species in the genus of *Hyptis* were found to have potential biological activities such as anti-influenza and constipation,

trypanocide, cytotoxic, and anti-candida activities, stomach and intestinal disorders, and as bactericidal, and to relieve fever [3–5]. Of the *Hyptis* species, *H. capitata* was less investigated. The plant has spread widely as a weed and is reported to be invasive in many areas. In our previous report, *H. capitata* root was proved to have potential antioxidant and antimicrobial activities [6]. This plant is traditionally used by local people in Kalimantan to treat various illnesses. The Bentian people in East Kalimantan use the leaves to prevent infection of external wounds and the roots as an antidote for food poisoning. On the other side of the world, the Guyanese use the leaf extract as a sedative and calmative and to reduce heartbeat, whereas an infusion of leaves is used to treat black diarrhea [7].

In the frame to optimize utilization of plants as raw material for herbal products, extract standardization is a key point. An important aspect related to the plant extract is the environmental

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effect on the growth of the plant and its extract. However, research on the effect of environmental factors on secondary metabolites, including from *H. Capitata*, is limited.

The composition of secondary metabolites among plants may differ on the basis of the difference of growing location and environmental factors such as sunlight, temperature, rainfall, and humidity as well as the amount of soil nutrients [8,9]. In line with this condition, Bertolucci *et al.* [10] reported that environment with different intensity of shade caused the difference of phytochemical composition of *Mikania laevigata* and *M. glomerata*. Furthermore, certain conditions such as drought stress may stimulate the difference in polyphenols and volatile metabolites of grapevine [11]. The substantial difference in total phenolic, total flavonoid and total saponin contents and antioxidant activity of *Strobilanthes crispus*, a medicinal plant in South East Asia collected from three different locations in Malaysia, has been reported [12]. Furthermore, *Nigella sativa* seed extracts displayed variability in cytotoxicity in relation to the different geographical locations where the samples were taken [13]. The chemical composition of herbal medicines needs to be confirmed in order to assess the extract quality in relation to safety concerns. However, the plant contains complex mixtures of secondary metabolites that also play a significant role in plant efficacy [14]. Therefore, as a promising medicinal plant, it is important to analyze the chemical contents and biological activities of the leaves of *Hyptis capitata* and its potential source of bioactive compounds.

The objective of the present work is to analyze the phytochemicals and evaluate the antimicrobial and antioxidant activities of the leaf extracts of *H. capitata* collected from two locations in East Kalimantan, namely, Samarinda (location 1, the plant was coded by HSM), and West Kutai (location 2, the plant was coded by HWK).

## 2. MATERIALS AND METHODS

### 2.1. Plant Material

Plant materials of *H. capitata* were selected on the basis of our previous investigation. The materials were collected from Samarinda and West Kutai areas in East Kalimantan, Indonesia, representing typically different environments, in May 2018. The samples were identified and authenticated by a taxonomy at the Laboratory of Forest Ecology and Wildlife Biodiversity, Faculty of Forestry, Mulawarman University. The voucher specimen was kept at the Laboratory of Forest Products Chemistry (KK-1805-HC003 and KK-1805-HC004).

### 2.2. General

Thin-layer chromatography (TLC) analysis was done on a silica gel coated aluminum sheet (Merck, Darmstadt, Germany);

1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Dimethyl sulfoxide, Folin-Ciocalteu reagent, sulfuric acid, hydrochloric acid, acetic anhydride, potassium iodide, and peptone were purchased from Merck (Darmstadt, Germany).  $\beta$ -Nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), ascorbic acid, 1-naphtol, bismuth (III) nitrate, gallic acid, and catechin were purchased from Sigma (St. Louis, MO). The nutrient broth was obtained from Difco (Detroit, MI). Other chemicals were obtained in the highest purity commercially available.

### 2.3. Preparation of Extracts

The dried parts of the leaves were pulverized through an electric blender. Every 100 g of plant extract was treated with methanol at room temperature followed by mechanical shaking (7400 Tübingen; Edmun Buchler, Germany) for 48 hours which was repeated twice. Filtration of the sample was done by using a filter paper. The extract solution was subjected to a rotary evaporator at 40°C and under reduced pressure to yield gummy extract. Upon drying of the extract using a vacuum oven, the yield percentage of the dry extract was calculated as shown in Table 1.

### 2.4. Microbial Strains

Gram +ve (*Propionibacterium acnes*, *Staphylococcus aureus*, and *Streptococcus sobrinus*) and Gram -ve (*Escherichia coli*) bacterial strains and yeast-like fungi (*Candida albicans*) were used in antimicrobial assays. Microbial cultures were obtained from the collection of our laboratory. The bacterial strains were cultured in nutrient agar and incubated at 37°C for 24 hours. The fungal strain was cultured in sabouraud dextrose agar and incubated at 26°C for 48 hours prior to antimicrobial assays.

### 2.5. Phytochemicals Analysis

Phytochemical analysis was carried out qualitatively and quantitatively to analyze the occurrences of phenolic, alkaloid, flavonoid, steroid, terpenoid, tannin, saponin, and carbohydrate contents in a standard manner.

### 2.6. Alkaloid Test

Five milligrams of plant extract was added by 2 ml of hydrochloric acid and followed by 1 ml of Dragendorff solution. The color change of the solution from orange to red indicates the presence of alkaloids [15].

### 2.7. Flavonoid Test

The alkaline method was used to evaluate the presence of flavonoids. One milligram of plant sample was soaked in 5 ml of hot water for

**Table 1:** Phytochemical analysis of *H. capitata*.

Sample	Phytochemical							
	Alkaloid	Flavonoid	Triterpenoid	Tannin	Carotenoid	Steroid	Carbohydrate	Coumarin
HSM	+	+	-	+	-	-	+	+
HWK	+	+	-	+	-	-	+	+

Remark: HSM = *H. capitata* sample from location 1 (Samarinda); HWK = *H. capitata* sample from location 2 (West Kutai) (+) = presence; (-) = absence.

5 minutes. A few drops of 20% sodium hydroxide solution were added to the filtrate and the existence of flavonoid was recorded upon the appearance of yellow color in the mixture [15].

### 2.8. Steroid

One milliliter of plant extract along with 5 ml chloroform was mixed with 6 ml of concentrated sulfuric acid. The formation of a red-colored upper layer and yellow-to-green sulfuric acid layer indicates the steroid content [16].

### 2.9. Terpenoid

One milliliter of plant extract was mixed with 0.5 ml of chloroform followed by 1.5 ml of concentrated sulfuric acid. The reddish-brown color between the two layers indicates the occurrence of terpenoid [16].

### 2.10. Tannin

Ten milliliters of plant extract was added into 1% lead acetate solution. The appearance of a yellow deposit on the bottom of the tube indicates the presence of tannin [17].

### 2.11. Saponin

The presence of saponin was evaluated by the frothing test: 1 mg plant sample was soaked in hot water for 10 minutes, 2 ml of the extract solution was diluted with 10 ml of water in a test tube, and the mixture was shaken for 2 minutes. If the sample contained saponin, then it would produce froth [18].

### 2.12. Carbohydrate

In 1 ml of plant extract, few drops of Molisch's reagent were added and followed by 1 ml of concentrated sulfuric acid at the side of the tubes. The mixture solution was kept to stand for 3 minutes. The presence of carbohydrates was indicated by the formation of a violet ring between two layers [16].

### 2.13. Total Phenolic Content (TPC)

The TPC of *H. capitata* extract was analyzed by means of the Folin-Ciocalteu method measured on a spectrophotometer [18,19]. The calibration curve was set on the basis of the mixture of the gallic acid solution with Folin-Ciocalteu reagent and sodium carbonate (4 ml, mg/ml). The mixture solution was left for 60 minutes. The absorbances of the sample were measured at 765 nm on a UV-VIS spectrophotometer (Shimadzu UV-VIS 1240, Shimadzu Corp., Kyoto, Japan). The TPC of the extract in terms of gallic acid equivalents (GAEs) mg/g of the dry extract was calculated.

### 2.14. Total Flavonoid Content (TFC)

TFC was measured by a colorimetry method with reference to a previous report [20]. A mixture of 100  $\mu$ l extract and catechin standard solution was added with 100  $\mu$ l of 5% (w/v) sodium nitrite solution in a test tube. The mixture solution was added with 100  $\mu$ l of 10% aluminum chloride solution. The total volume was made up to 1 ml using distilled water. TFCs were calculated as mg of catechin equivalents per gram of extracts (mg CE/g). The experiments were done in triplicate.

### 2.15. Antimicrobial Activity Assay

The antimicrobial activity of the extracts was carried out by agar well diffusion method using nutrient agar or sabouraud dextrose agar [21] with slight modification. Sterilized media solution (20 ml) was poured into Petri dishes and left until molten. Twenty milliliters of microbial suspension was spread on the surface of the plates. Twenty microliters of an acetone solution containing 31.25–500  $\mu$ g extracts were dropped in 7 mm well made with sterile cork borer on the media. Standard antibiotic, chloramphenicol at the concentration of 10  $\mu$ g/20  $\mu$ l was applied positive control. The culture plates were incubated under the absence of light at 37°C for 24 hours. The clear zone around the well was measured in mm and defined as the growth inhibitory activity of the extract. Relative growth inhibition was determined as the mean inhibition zone for the test sample divided by the mean inhibition zone for the chloramphenicol.

### 2.16. DPPH Radical Scavenging Test

Antioxidant activity was measured in the mechanism of radical scavenging activity against DPPH with reference to the method described by Shimizu *et al.* [22]. The sample was first dissolved in dimethyl sulfoxide and used at 30 times dilution for the actual experiment. Sample absorbance was measured on a Shimadzu UV-VIS 1240 spectrophotometer. Thirty-three microliters of extract and control at different concentrations were added to 0.5 ml of freshly prepared DPPH solution in ethanol. The mixture solution was made up of ethanol to a final volume of 1 ml. The reaction mixture was left for 20 minutes in a dark environment and absorbance was measured at 517 nm using the UV-VIS spectrophotometer. The test was run in triplicate with ascorbic acid as a positive control. The percentage of inhibition of DPPH was calculated using the following equation.

$$\% \text{ Inhibition of DPPH activity} = \frac{\text{Control absorbance} - \text{test absorbance}}{\text{Control absorbance}} \times 100$$

The antioxidant activity of the extract was expressed as IC<sub>50</sub>, a concentration ( $\mu$ g/ml) of extract that inhibits the formulation of DPPH radicals by 50%.

### 2.17. Superoxide Radical Scavenging Activity Assay

Superoxide radical scavenging activity assay was performed by the mechanism of the reduction of NBT as reported by Babu *et al.* [23]. One ml of NBT solution (1 M NADH in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of *H. capitata* leaf extracts and control, ascorbic acid (50 mM phosphate buffer, pH 7.4) were mixed. The reaction was started by the addition of 100  $\mu$ l of phenazine methosulfate (PMS) solution (60  $\mu$ M PMS in 100 mM phosphate buffer, pH 7.4) in the mixture. The abilities to scavenge the superoxide radicals were calculated by using the following formula:

$$\% \text{ scavenging} = \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100$$

where A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the sample extract/standard.

### 2.18. Brine Shrimp Lethality Assay

Brine shrimp lethality test was applied to evaluate the acute toxicity of the plant extracts with slight modification as previously reported [24]. The eggs of *Artemia salina* were hatched in a flask containing seawater for 48 hours. Extract samples in a serial concentration of 1,000, 500, 250, 125, 62.5, and 31.25 µg/ml were incubated with brine shrimps in seawater in triplicates. The number of survivors was counted and used to determine the toxicity of the samples. The experiment was performed in triplicate. Lethal concentration 50 (LC<sub>50</sub>) values were determined by probit analysis on a Finney computer program.

## 3. RESULTS AND DISCUSSION

In the continuation of our search into the scientific basis of *H. capitata* utilization, the present study is limited to the analysis of the phytochemicals and biological activities of *H. capitata* leaf extracts collected from two different locations in East Kalimantan, Indonesia.

### 3.1. Phytochemical Analysis

Phytochemical screening of *H. capitata* extract collected from two locations in East Kalimantan, Indonesia, was conducted using various chemical assays to identify either the presence or absence of secondary metabolites such as alkaloids, flavonoids, triterpenoids, tannins, carotenoids, steroids, carbohydrates, and coumarins. Table 1 showed secondary metabolites present in all extracts assayed. Alkaloids, flavonoids, tannins, carbohydrates, and coumarins were found in all samples. The samples did not reveal any terpenoids, carotenoids, and steroids. Qualitatively, phytochemicals analysis of *H. capitata* leaf extracts showed no difference between growing locations. Phytochemicals are plant chemicals without nutritive function and can be found in various parts of plants such as leaves, fruit, root, flower, and barks. Most phytochemicals have protective or disease preventive properties [25]. The occurrence of phytochemicals can be a marker to identify a plant species or genus and to differentiate plants growing at different locations.

### 3.2. Total Phenolic and Flavonoid Contents

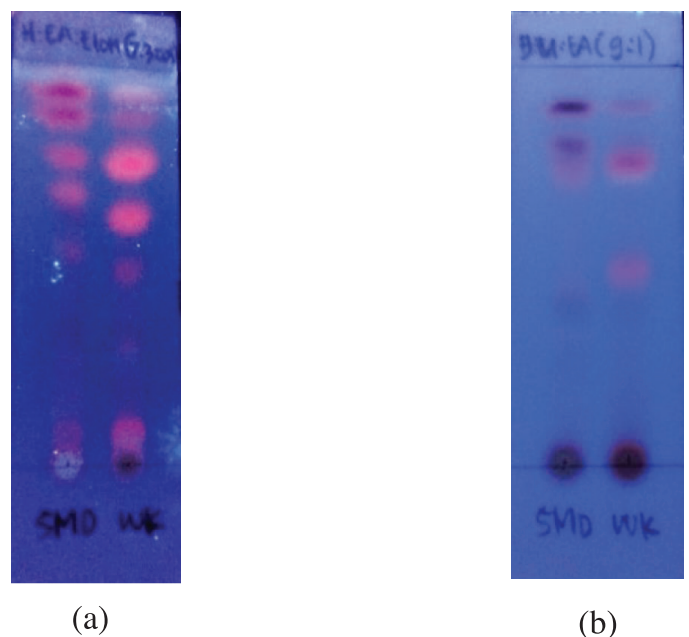
The phenolic content found in the extracts was determined by the linear regression equation using gallic acid (for TPC) or catechin (for TFC) as standard. The analysis of total phenolic and TFCs was displayed in Table 2. Phenolic compounds are secondary metabolites that occur abundantly in plants. The phenolic and flavonoid compounds were reported to play important roles in the antioxidant activity of plants. TPC and TFCs of *H. capitata* were measured. The results obtained in this study showed that *H.*

*capitata* collected from location 1 (Samarinda area) possessed a higher TPC. Furthermore, the content of flavonoids as one class of secondary plant metabolites mostly used in plants to produce yellow and other pigments was determined by the aluminum chloride colorimetry method. The result displayed that *H. capitata* collected from location 1 (Samarinda area) had higher flavonoid content than the one collected from location 2 (West Kutai).

The environmental condition such as light intensity, temperature, soil nutrition, and predator risk may affect the production of secondary metabolites, including the mechanism of phenolic and flavonoid biosynthesis. Based on the results of this study, the differences between the sampled locations in total phenolic and total flavonoid compounds could be related to environmental conditions. Phenolics and flavonoids play a significant role in the bioactivity of plants. In the scavenging mechanism against free radicals, phenolic and flavonoids are two classes of chemicals belonging to highly active scavengers. Flavonoids have been reported to play significant roles in the antioxidant activity of the plants. Furthermore, phenolics increase plant tolerance to oxidative stress [26,27].

### 3.3. Thin-Layer Chromatography Analysis

The profile of chemicals in *H. capitata* extracts obtained from the plant grown in two different locations was analyzed by normal phase thin-layer chromatography. The chromatograms of the plant extracts are presented in Figure 1. Thin-layer chromatography (TLC) chromatogram developed by two solvent systems of *H. capitata* leaf extracts displayed the difference of *H. capitata* collected from location 1 (HSM, left spot) and location 2 (HWK, right spot). *H. capitata* collected from location 2 (West Kutai)



**Figure 1:** Thin-layer chromatogram of *H. capitata* collected from location 1 (HSM, left spot) and location 2 (HWK, right spot) (a = solvent system 1, *n*-hexane:ethyl acetate:ethanol 7:3:0.1; b = solvent system 2, dichloromethane:ethyl acetate 9:1).

**Table 2:** TPC in leaf extracts of *H. capitata* from two locations.

Sample	Total phenolic compounds mg GAE/g extract	Total flavonoid compounds mg CE/g extract
HSM	59.60 ± 0.003	366.67 ± 0.006
HWK	40.40 ± 0.003	283.33 ± 0.006

Remarks: HSM = *H. capitata* sample from location 1 (Samarinda); HWK = *H. capitata* sample from location 2 (West Kutai); GAE= Gallic acid equivalent, CE = Catechin equivalent.

showed more compounds, particularly the red spot (Rf 0.68 at solvent system 1 and 0.56 at solvent system 2). Thin-layer chromatography is a fast, reliable, cheap, and powerful tool used for an initial analysis of the phytochemical profile. This method allows the differentiation of chemical composition as displayed on the silica gel plate under UV illumination. Our analysis showed that there was a clear difference between the phytochemicals of *H. capitata* grown in Samarinda (HSM) and that grown at West Kutai (HWK). This condition may affect the biological activities of *H. capitata* as evaluated in the present study.

### 3.4. Antimicrobial Activity

The leaf extracts of *H. capitata* collected from two growing locations were assayed their antimicrobial activity against five human pathogen microbial strains, *Propionibacterium acnes*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus sobrinus*, and *Candida albicans*. The results were recorded in Table 3. *Hyptis capitata* from West Kutai (HWK) was the most effective against all the tested microorganisms at concentration 500 µg/ml while *H. capitata* from Samarinda (HSM) was effective only against *S. aureus* and *S. sobrinus*. Both extracts had no ability to inhibit *C. albicans*. The antimicrobial activity of *H. capitata* extracts was considerably affected by the collecting location.

### 3.5. Antioxidant Activity

The leaf extracts of *H. capitata* collected from Samarinda area (HSM) and West Kutai area (HWK) were subjected to antioxidant assays by means of DPPH and superoxide radical scavenging activity. Antioxidant activity of *H. capitata* extracts was represented by IC<sub>50</sub> values to scavenge DPPH and superoxide radicals. As depicted in Table 4, growing location caused a significant difference in DPPH and superoxide free radical scavenging activity of *H. capitata*. HSM demonstrated the highest scavenging activity (lowest IC<sub>50</sub>: 13.69 µg/ml). However,

the radical scavenging activity of both *H. capitata* extracts was lower than that of positive standards (ascorbic acid). Lower IC<sub>50</sub> values represent stronger free radical scavenging activity, as strong free-radical scavengers are active at low concentrations. Previous reports explained that the antioxidant activity of plants is considerably associated with their phytochemical contents, especially that of flavonoids and phenolic acids [28,29]. In this study, the highest antioxidant activity, as well as the highest content of phenolics, flavonoids, and phenolic acids, was observed in *H. capitata* from location 1 (Samarinda area) as presented in Table 5. This could also be related to the variation in climatic conditions, soil nutrients, and water quality (hydrogen potential and electrical conductivity) of two growing locations, subsequently influencing the production of phytochemicals and the antioxidant activities.

### 3.6. Brine Shrimp Lethality Test

Safety information of plant extract as a medicinal plant is essential for the development of further products. As a quick reference and suitable tools, brine shrimp lethality assay was conducted to assess the cytotoxicity. The result of the cytotoxicity assay of *H. capitata* extract is presented in Table 5. The results of the cytotoxicity assay of *H. capitata* at different concentrations showed no toxicity of the plant extracts. According to Meyer *et al.* [30], the plant extract is classified as toxic when the LC<sub>50</sub> values are less than 1,000 µg/ml. Concerning the concentration tested without any mortality to the

**Table 4:** IC<sub>50</sub> of *H. capitata* for DPPH and superoxide scavenging activity assays.

Sample	IC <sub>50</sub> (µg/ml)	
	DPPH radical scavenging	Superoxide radical scavenging
HSM	13.69	65.11
HWK	23.93	>100
Ascorbic acid	3.12	26.24

Remarks: HSM = *H. capitata* sample from location 1 (Samarinda); HWK = *H. capitata* sample from 2 (West Kutai).

**Table 3:** Antimicrobial activity (percent of inhibition) of *H. capitata*.

Plant extract	Tested microorganisms	Relative growth inhibition (%)				
		500 µg	250 µg	125 µg	62.5 µg	31.25 µg
<b>HSM</b>						
	<i>P. acnes</i>	NI	NI	NI	NI	NI
	<i>S. sobrinus</i>	50.70 ± 1.89	40.14 ± 0.71	33.80 ± 0.00	NI	NI
	<i>S. aureus</i>	39 ± 1.26	NI	NI	NI	NI
	<i>E. coli</i>	NI	NI	NI	NI	NI
	<i>C. albicans</i>	NI	NI	NI	NI	NI
<b>HWK</b>						
	<i>P. acnes</i>	29.64 ± 0.33	NI	NI	NI	NI
	<i>S. sobrinus</i>	48.99 ± 0.35	39.86 ± 0.24	36.49 ± 0.47	NI	NI
	<i>S. aureus</i>	51.40 ± 1.39	43.02 ± 0.51	-	NI	NI
	<i>E. coli</i>	45.10 ± 0.19	38.24 ± 0.58	34.31 ± 0.19	NI	NI
	<i>C. albicans</i>	NI	NI	NI	NI	NI

Remarks: Relative growth inhibition was calculated by the inhibition zone of the test sample divided by the inhibition zone of a standard drug. Abbreviations used: HSM = *H. capitata* sample from location 1 (Samarinda); HWK = *H. capitata* sample from location 2 (West Kutai); *P. acnes* = *Propionibacterium acnes*; *S. sobrinus* = *Streptococcus sobrinus*; *S. aureus* = *Staphylococcus aureus*; *E. coli* = *Escherichia coli*; *C. albicans* = *Candida albicans*; NI = no inhibition.

**Table 5:** Brine shrimp lethality bioassay of *H. capitata* extracts.

Sample	Concentration (µg/ml)	Mortality (%)	Lethal concentration (LC <sub>50</sub> , µg/ml)
HSM	31.25	0	> 1000
	62.5	0	
	125	0	
	250	0	
	500	0	
	1000	0	
HWK	31.25	0	> 1000
	62.5	0	
	125	0	
	250	0	
	500	0	
	1000	0	

Remarks: HSM = *H. capitata* sample from location 1 (Samarinda); HWK = *H. capitata* sample from location 2 (West Kutai).

brine shrimp, this study showed the safe level for the use of *Hyptis capitata* as a herbal remedy.

Secondary metabolites play important roles in plants, especially as a natural defense of plants against the predator. It has been reported that the production of secondary metabolites is closely related to environmental conditions since the plants have a unique adaptation to environmental change. Many of the special metabolites are directly involved in the mechanisms that allow the adaptation of plants to their habitat [31]. Biological activities of plants may also be affected by many environmental factors that have been proved to cause the difference in special metabolites production [32].

In the present research, *H. capitata* plants collected from two growing regions displayed different compositions of phytochemicals, in terms of phenolic and flavonoid contents. Activities of the leaf extract as antioxidant and antibacterial substances were also different. Sample 1 of *H. capitata* (HSM) was collected from the outskirts of Samarinda city, with typical humid and under shaded environments. Sample 2 of *H. capitata* was collected from a dry and relatively open area. In line with our results, biosynthesis of flavonoid and phenolic compounds were reported to be different under different growing locations, possibly caused by weather, temperature, latitude, soil nutrition, and sunlight [33].

The present study displayed the difference of chemical composition and biological activities of *H. capitata* from two different locations, meaning that the uses of *H. capitata* as herbal raw materials should consider the effect of growing location to obtain standardized plant extracts.

#### 4. CONCLUSION

This study emphasized that the extracts of *H. capitata* collected from two different sampling locations had various differences in terms of phytochemicals and biological activities. The present results suggested that the environmental factors may play important roles in the characterization of phytochemicals and the biological activities of *H. capitata*.

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#### CONFLICT OF INTEREST

There is no conflicts of interest reported by each author.

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