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

Journal Name :	Journal of Applied Biology and Biotechnology
Manuscript ID :	125-1548207611
Manuscript Type :	Original Article

Abstract :	In our search into biological activity and chemical profiling of Indonesian medicinal plants, phytochemicals and biological of Hyptis capitata grown at two location in East Kalimantan, Indonesia, were investigated. 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 plant was evaluated its biological activities 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 leaves extract of H. capitata contain 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 DPPH (IC50 13.69 µg/ml) and superoxide (IC50 65.11 µg/ml) tests. Brine shrimp lethality test showed no toxicity of H. capitata leaves extracts. The results suggested that biological activities and phytochemicals of Hyptis capitata were affected by the growing location represented the diferrent environmental factors including light intensity, temperature, soil nutrition and predatory risks.

Keywords :	antimicrobial activity; antioxidant activity; Hyptis capitata; medicinal plants; phytochemicals

ABSTRACT

Hyptis capitata is a plant in the family of Lamiaceae used to prevent wound infection and as antidote of 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. The present work aims to investigate 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 plant was evaluated its biological activities 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 leaves extract of *H. capitata* contain 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 DPPH (IC_{50} 13.69 µg/ml) and superoxide (IC_{50} 65.11 µg/ml) tests. Brine shrimp lethality test showed no toxicity of *H. capitata* leaves extracts. The results displayed that *H. capitata* collected from two location showed good antibacterial and antioxidant activities. The results suggested the the biological activites and phytochemical of *H. capitata* were affected by the environment where the plant was collected. A study into deep analysis on the effect of light intensity, temperature, soil nutrition and predatory risks to the biological activity and phytochemicals of the plant is required.

Key words: antimicrobial activity; antioxidant; *Hyptis capitata;* invasive plant, medicinal plant; secondary metabolite.

INTRODUCTION

A medicinal plant is described as any plant with substances having therapeutic purposes in its organ and may contain precursor for the production of synthetic drugs [1]. It is reported that about 80% of world medicinal plants are existed in Indonesia forest. More than 28,000 plant species have been inventoried in Indonesia. Of these, 7,000 species are classified as medicinal plant [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 illness. The Bentian people in East Kalimantan use the leaves to prevent infection of external wounds and the roots as antidote of food poisoning. In other side of world, the Guyanese use the leaf extract as a sedative and calmative and to reduce heart while an infusion of leaves is used to treat black diarrhea [7].

In the frame to optimize utilization of plant as raw material for herbal products, the extract standardization is a key point. An important related with the plant extract is environmental effect to the growth the plant and its extract. However, research on the effect of environmental factors to 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 condition such as drought stress may stimulate the difference in polyphenols and volatile metabolites of grapevine [11]. 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 with the difference geographical location where the samples were taken [13]. Chemical composition of herbal medicines needs to be confirmed in order to assess the extract quality in relation with safety concern. However, plant contains complex mixtures of secondary metabolites that play also play significant role in plant efficacy [14]. Therefore, as a promising medicinal plant, it is important to analyze the chemical contents an biological activities of the leaves of *Hyptis capitata* and its potential source of bioactive compounds.

The objective of the present work was to analyze the phytochemicals and evaluate the antimicrobial and antioxidant activities of the leaves 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).

MATERIALS AND METHODS

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 a 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 speciment was kept at the Laboratory of Forest Products Chemistry (KK-1805-HC003 and KK-1805-HC004).

General

TLC analysis was done on a silica gel coated alumunium sheet (Merck, Darmstadt, Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Dimethyl sulfoxide, Folin-Ciocalteau reagen, sulfuric acid, hydrochloric acid, acetic anhydride, potassium iodide and peptone were purchased from Merck (Darmstadt, Germany). β-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, USA). Nutrient broth was obtained from Difco (Detroit, MI, USA). Other chemicals was obtained in highest purity commercially available.

Preparation of extracts

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

Microbial strains

Gram +ve (*Propionibacterium acnes*, *Staphylococcus aureus*, *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^{o}C for 24 h. The fungal strain was cultured in Saboraud Dextrose Agar and incubated at 26^{o}C for 48 h prior to antimicrobial assays.

Phytochemicals analysis

Phytochemical analysis was carried out by qualitative and quantitative determination to analyze the occurrences of phenolic, alkaloid, flavonoid, steroid, terpenoid, tannin, saponin, and carbohydrate in a standard manner.

Alkaloid test

Five milligrams of plant extract were added by 2 ml of hydrochloric acid and followed by 1 ml of Dragendorff solution. The appearance of orange to red color in solution indicates the presence of alkaloid [15].

Flavonoid test

The alkaline method was taken to evaluate the presence of flavonoid. One milligram of plant sample was soaked in 5 ml of hot water for 5 min. A few drop of 20% sodium hydroxide solution was added to the filtrate was added and the existence of flavonoid was recorded upon the appearance of yellow color in the mixture [15].

Steroid

One milliliter of plant extract in 5 ml chloroform was mixed with 6 ml of conc. sulfuric acid. The formation of red color upper layer and yellow to green sulfuric acid layer indicates the steroid content [16].

Terpenoid

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

Tannin

Ten milliliter of plant extract were added by 1% lead acetate solution. The appearance of yellow deposit on the bottom of the tube indicates the presence of tannin [17]

Saponin

The presence of saponin was evaluated by the frothing test. One milligram plant sample was soaked in hot water for 10 min. Two milliliter of the extract solution were diluted with 10 ml of water in a test tube. The mixture was shaken for 2 min to produce froth if the sample contains saponin [18].

Carbohydrate

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

Total phenolic content (TPC)

Total phenolic content of *H. capitata* extract was analyzed by means of Folin-Ciocalteu method measured on a spectrophotometer [18]. Calibration curve was set on the basis

of the mixture of gallic acid solution with Folin-Ciocalteu reagent and sodium carbonate (4 ml, mg/ml). The mixture solution was left for 60 min. The sample absorbances were measured at 765 nm on a UV-Vis spectrophotometer (Shimadzu UV-VIS 1240, Shimadzu Corp., Kyoto, Japan). The total phenolic content of extract in term of gallic acid equivalents (GAE) mg/g of the dry extract was calculated.

Total flavonoid content (TFC)

Total flavonoid content was measured by a colorimetry method with reference to previous report [20]. A hundred µl of each extract and catechin standard solution were added with 100 µl of 5% (w/v) sodium nitrite solution in a test tube. The mixture solution was added with 100 µl of 10% aluminium chloride solution. The total volume was made up to 1 mL using distilled water. Total flavonoid contents were calculated as mg of catechin equivalents per gram of extracts (mg CE/g). The experiments were done in triplicate.

Antimicrobial activity assay

The antimicrobial activity of the extracts were carried out by agar well diffusion method using nutrient agar or saboraud dextrose agar [21] with slight modification. Sterilized media solution (20 ml) were poured into Petri dishes and left until molten. Twenty milliliters of microbial suspension were spread on the surface of the plates. Twenty microliters of acetone solution containing 31.25-500 µg extracts were dropped in7 mm well made with sterile cork borer on the media. Standard antibiotic, chloramphenicol at the concentration of 10 µg/20 µl was applied positive control. The culture plates were incubated under the absence of light at 37^{o}C for 24 hours. 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 test sample divided by the mean inhibition zone for the chloramphenicol.

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 a 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 concentration were added to 0,5 ml of freshly prepared DPPH solution in ethanol. The mixture solution was made up with ethanol to a final volume of 1 ml. The reaction mixture was left for 20 min in dark and absorbance was measured at 517 nm using UV-Vis spectrofotometer. The test were run in triplicate with ascorbic acid as a positive control. 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_{50}, a concentration (µg/ml) of extract that inhibits the formulation of DPPH radicals by 50%.

Superoxide radical scavenging activity assay

Superoxide radical scavenging activity assay was performed by mechanism of the reduction of nitroblue tetrazolium as reported by Babu *et al*. [23]. One ml of nitroblue tetrazolium (NBT) solution (1 M NADH in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of *H. capitata* leaves extracts and a control, ascorbic acid (50 mM phosphate buffer, pH 7.4) were mixed. The reaction was started by addition of 100 µl of phenazine metosulphate (PMS)

solution (60 µM 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:

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

Where A_0 is the absorbance of the control; A_1 is the absorbance of the sample extract/standard.

Brine shrimp lethality assay

Brine shrimp lethality test was applied the evaluate the acute toxicity of the plant extracts as previously reported with slight modification [24]. The egg of *Artemia salina* were hatched in a flask containing sea water for 48 h. Extract samples in a serial concentration of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL incubated with brine shrimps in seawater in triplicates. The number of survivor were counted and used to determine the toxicity of the samples. The experiment was performed in triplicate. Lethal concentration 50 (LC_{50}) values were determined by probit analysis on a Finney computer program.

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* leaves extracts collected from two different locations in East Kalimantan, Indonesia.

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* leaves extracts showed no difference between to growing locations. Phytochemicals are plant chemicals without nutritive function and can be found in various parts of plants such as leaves, fruit, root, flower, bark, and so on. Most phytochemicals have protective or disease preventive properties [25]. The occurence of phytochemicals can be a marker to identify a plant species or genus and to differentiate plant growing at different location.

Total phenolic and flavonoid contents

The phenolic content found in the extracts were determined using the linear regression equation using gallic acid (for total phenolic content) or catechin (for total flavonoid content) as standard. The analysis of total phenolic and total flavonoid contents were displayed in Table 2. Phenolic compounds are secondary metabolites occur abundantly in plants. The phenolic and flavonoid compounds were reported to play important roles in antioxidant activity of plant. Total phenolic (TPC) and total flavonoid contents (TFC) of *H. capitata* were measured. The results obtained in this study showed that *H. capitata* collected from location 1 (Samarinda area) possessed higher total phenolic. Furthermore, the content of flavonoids as one class of secondary plant metabolites mostly used in plants to produce yellow and other pigments was determined using aluminium chloride colorimetric. 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 secondary metabolites, including in 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 significant role in the bioactivity of plants. In the scavenging mechanism against free radicals, phenolic and flavonoids are two class of chemicals belong to highly active scavengers. Flavonoids have been reported to play significant roles in antioxidant activity of plant. Furthermore, phenolics increase plant tolerance to oxidative stress [26, 27].

Thin-layer chromatography analysis

The profile of chemicals exist 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 were presented in Figure 1. TLC chromatogram developed by two solvent systems of *H. capitata* leaves 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) showed more compounds, particularly the red spot (Rf 0.68 at the solvent system 1 and 0.56 at solvent system 2). Thin layer chromatography is a fast, reliable, cheap and powerful tool as initial analysis of the phytochemical profile. This method allow the differentiation of chemical composition as displayed on the silica gel plate under UV illumination. Our analysis showed that there was clear difference between the phytochemicals of *H. capitata* grew in Samarinda (HSM) and that of grew at West Kutai (HWK). This condition may affect the biological activities of *H. capitata* as evaluated in the present study.

Antimicrobial activity

The leaves extracts of *H. capitata* collected from two growing location were assayed their antimicrobial activity against 5 human pathogen microbial strains, *Propionibacterium acnes*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus sobrinus*, and *Candida albicans*. The results were recorded in Table 3. *H. 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.

Antioxidant activity

The leaves 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_{50} values to scavenge DPPH and superoxide radicals. As depicted in Table 4, growing location caused a significant difference of DPPH and superoxide free radical scavenging activity of *H. capitata.* HSM demonstrated the highest scavenging activity (lowest IC_{50}; 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_{50} values represent stronger free radical scavenging activity, as strong free-radical scavengers are active at low concentrations. Previous reports explained that 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 and flavonoids and phenolic acids was observed in *H. capitata* from location 1 (Samarinda area) as presented in Table 5. This could also be related with variation in climatic conditions, soil nutrients, and water quality (hydrogen potential, electrical conductivity) of two growing locations and subsequently, influence the production of phytochemicals and the antioxidant activities.

Brine shrimp lethality test

Safety information of plant extract as a medicinal plant is essential for development of further products. As a quick reference and suitable tools, brine shrimp lethality assay was conducted to assess the cytotoxicity. The result of cytotoxicity assay of *H. capitata* extract was presented in Table 5. The results of 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 toxic when the LC_{50} values are less than 1000 µg/mL. Concerning the concentration tested without any mortality to the 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 with the environmental condition, since the plants have unique adaptation to the 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 has been proved to cause the difference of special metabolites production [32].

In the present research, *H. capitata* plants collected from two growing regions displayed different composition of phytochemical, in term of phenolic and flavonoid contents. Activities of the leaves extracts as antioxidant and antibacterial substances were also different. Sample 1 of *H. capitata* (HSM) was collected from the outskirt of Samarinda city, with typical humid and under shaded environment. Sample 2 of *H. capitata* was collected from drier and relatively open area. In line with our results, biosynthesis of flavonoid and phenolic compounds were reported to be different under different growing location, 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.

CONCLUSIONS

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

ACKNOWLEDGEMENTS

The present work was funded by the Indonesian Ministry of Research, Technology and Higher Education under the Competitive-Based Research Grant (Grant number:119/UN17.41/KL/2018). The authors thank to Mr. Murdiyanto (West Kutai) for providing plant materials. We sincerely express our thanks to the Lab Head of Forest Products Chemistry, Mulawarman University and the members for technical support.

CONFLICT OF INTEREST

There is no conflicts of interest reported by each author.

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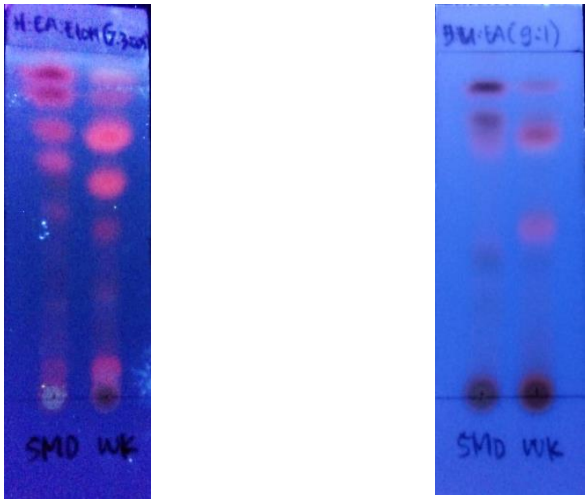

(a) (b)

Fig 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 1. Phytochemical analysis of *Hyptis 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

Table 2. Total phenolic content in leaves extracts of *Hyptis 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

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

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

Remarks: Relative growth inhibition was calculated by the inhibition zone of test sample divided by 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* = *Propionibcaterium acnes*; *S. sobrinus* = *Streptococcus sobrinus*; *S. aureus* = *Staphylococcus aureus*; *E. coli* = *Escherichia coli*; *C. albicans* = *Candida albicans*; NI = no inhibition.

Table 4. IC_{50} of *Hyptis capitata* for DPPH dan superoxide scavenging activity assays.

Sample	IC_{50} (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)

Tabel 5. Brine shrimp lethality bioassay of *Hyptis capitata* extracts

Sample	Concentration (µg/mL)	Mortality (%)	Lethal Concentration (LC_{50}, 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)

ABSTRACT

In our search into biological activity and chemical profiling of Indonesian medicinal plants, phytochemicals and biological of *Hyptis capitata* grown at two location in East Kalimantan, Indonesia, were investigated. 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 plant was evaluated its biological activities 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 leaves extract of *H. capitata* contain 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 DPPH (IC_{50} 13.69 µg/ml) and superoxide (IC_{50} 65.11 µg/ml) tests. Brine shrimp lethality test showed no toxicity of *H. capitata* leaves extracts. The results suggested that biological activities and phytochemicals of *Hyptis capitata* were affected by the growing location represented the diferrent environmental factors including light intensity, temperature, soil nutrition and predatory risks.

Key words: antimicrobial activity; antioxidant activity; *Hyptis capitata;* medicinal plants; phytochemicals.

Comment [what is t1]: separate background from aim and make sure that background is properly justify

Comment [what is t2]: no conclusion and recommendation

INTRODUCTION

Medicinal plant is a class of plants having therapy or pharmacological uses either for human or animal and has been implemented by community in healthcare treatmen. Indonesia covers 110 million hectares with abundance species of medicinal plants. About

Comment [what is t3]: please contact a botanist to advice on the suitability of this statement

80% of world medicinal plants are reported to be existed in Indonesia forest. More than 28.000 plant species are present in Indonesia in which 7.000 species are medicinal plants. Of these, 1.000 species have been applied as traditional medicine by local community [1].

A genus of medicinal plant having interesting biological activity is Hyptis, from the family of Lamiaceae. It consists of more than 400 species widely distributed in tropical and subtropical locations. 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 [2-4]. Of the Hyptis species, *H. capitata* is 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 [5]. This plant is traditionally used by local people in Kalimantan to treat various illness. The Bentian people in East Kalimantan use the leaves to prevent infection of external wounds and the roots as antidote of food poisoning. In other side of world, the Guyanese use the leaf extract as a sedative and calmative and to reduce heart while an infusion of leaves is used to treat black diarrhea [6].

Comment [what is t4]: contradicting

In order to optimize the medicinal plant benefit, investigation into the optimization of extract quantity of plants has been conducted intensively, whether using extraction or culture bioproduction optimization. However, research on the effect of environmental factors to secondary metabolites, including from *H. capitata* is limited.

The difference of growing location and environmental factors such as temperature, rainfall, humidity and solar radiation as well as the amount of soil macro- and micronutrients may effect plant secondary metabolite composition [7,8]. Bertolucci *et al.* [9] reported that the composition of coumarin and diterpenes in *Mikania laevigata* and *M. glomerata* were different under different shade levels. Furthermore, polyphenols and volatile metabolites of

grapevine leaves were reported to be varied under drought stress [10]. Therefore, it is important to analyze the chemical contents, antimicrobial and antioxidant potential of the leaves of *Hyptis capitata* and its potential source of bioactive compounds.

Comment [what is t5]: contact more literature in other to justify extensively the essence of the research.

statement of problem need to be revisit

The aim of this study was to analyze the phytochemicals and biological activities of the leaves 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).

MATERIALS AND METHODS

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 a typically different environments, in May 2018. The samples were identified and authenticated by a taxonomy at the Laboratory of Dendrology and Forest Ecology, Faculty of Forestry, Mulawarman University. The voucher speciment was deposited at the Laboratory of Forest Products Chemistry under the accession code KK-1805-HC003 and KK-1805-HC004 .

General

TLC analysis was done on a silica gel coated alumunium sheet (Merck, Darmstadt, Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). DMSO (dimethyl sulfoxide), Folin-Ciocalteau reagen, sulfuric acid, hydrochloric acid, acetic anhydride, potassium iodide and peptone were purchased from Merck (Darmstadt, Germany).β-Nicotinamide adenine dinucleotide (NADH), nitroblue

tetrazolium (NBT), ascorbic acid, 1-naphtol, bismuth (III) nitrate, gallic acid, and catechin were obtained from Sigma (St. Louis, MO, USA). Nutrient broth was obtained from Difco (Detroit, MI, USA). Other chemicals was obtained in highest purity commercially available.

Preparation of extracts

The dried parts of the leaves were pulverized through a electric blender. Each 100 gram of plant meals was extracted with methanol at room temperature followed by mechanical shaking (7400 Tübingen; Edmun Buchler, Germany) for 48 h. Following filtration of the suspension through filter paper, the crude methanolic extracts were rotoevaporated at 40ºC and put in a vacuum oven to near dryness to yield the plant extract as listed in Table 1.

Microorganisms and culture media

Comment [what is t6]: contact a microbiologist to help out because this would create so many doubts

Microorganisms were obtained from the culture collection of Laboratory of Forest Product Chemistry, Forestry Faculty, Mulawarman University. In-vitro antimicrobial studies were carried out against five microbial strains, *Propionibacterium acnes*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus sobrinus*, and *Candida albicans*. Bacterial cultures were maintained on Nutrient Agar (NA), while *Candida albicans* was maintained on Sabouraud Dextrose Agar (SDA).

Phytochemicals analysis

Comment [what is t7]: quantitative determination needs to be included

Phytochemical analysis was carried out by qualitative determination which included alkaloid, flavonoid, steroid, terpenoid, tannin, saponin, and carbohydrate in a standard manner. The procedures to analyze each bioactive phytochemical were described by Harborne [11] and Kokate [12].

Determination of total phenolic content (TPC)

Total phenolic content was analyzed by means of Folin-Ciocalteu method measured on a spectrophotometer according to a previous work [13]. Calibration curve was set on the basis of the mixture of gallic acid solution with Folin-Ciocalteu reagent and sodium carbonate (4 ml, mg/ml). The mixture was left for 60 min and then the absorbance was measured at 765 nm on a UV-Vis spectrophotometer (Shimadzu UV-VIS 1240, Shimadzu Corp., Kyoto, Japan). All determinations were carried out in triplicate. The total content of phenolic in extract was expressed as gallic acid equivalents (GAE) mg/g of the dry extract.

Determination of total flavonoid content (TFC)

Total flavonoid content was determined by colorimetry method with reference to previous report [14]. Briefly, 100 µl of each extract and catechin standard solution was mixed with 100 µl of 5% (w/v) sodium nitrite solution in a test tube, followed by addition of 100 µl of 10% aluminium chloride solution and the total volume was made up to 1 mL using distilled water. Total flavonoid contents were expressed as mg of catechin equivalents per gram of extracts (mg CE/g). The experiments were done in triplicate.

Antimicrobial activity assay

The antimicrobial activity of the extracts were carried out by agar well diffusion method using nutrient agar or saboraud dextrose agar as previously reported [15] with slight modification. Twenty-milliliter of sterile media were poured into Petri dishes and left for several minutes. On the surface of the plates, 20 µl of test microbial suspension was spread uniformly. A seven-mm well were cut using a sterile cork borer and 20 µl acetone solution containing 31.25-500 µg extracts were added to the well. Chloramphenicol was used as a

positive control at the concentration of 10 µg/20 µl in each well. The plates were incubated in the dark at 37^{o}C for 24 hours. Zones of inhibition around the well were measured in mm. Percent inhibition was calculated as the mean inhibition zone for test sample divided by the mean inhibition zone for the standard drug, chloramphenicol.

DPPH radical scavenging test

The DPPH radical scavenging method was performed as previously described by Shimizu *et al*. (2001). The sample was first dissolved in DMSO and used at a 30 times dilution for the actual experiment. UV absorption was measured on a Shimadzu UV-VIS 1240 spectrophotometer [16]. Briefly, 33 µL of extract and control at different concentration were added to 0,5 ml of freshly prepared DPPH solution in ethanol and made up with ethanol to a final volume of 1 ml. The reaction mixture was incubated for 20 min in dark and absorbance was measured at 517 nm using UV-Vis spectrofotometer. The test were run in triplicate with ascorbic acid as a positive control. 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_{50}, a concentration (µg/ml) of extract that inhibits the formulation of DPPH radicals by 50%.

Superoxide radical scavenging activity assay

Superoxide scavenging activity assay was conducted by means of the reduction of nitroblue tetrazolium as previously reported by Babu *et al*. [17]. One ml of nitroblue

tetrazolium (NBT) solution (1 M NADH in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of *H. capitata* leaves extracts and a control, ascorbic acid (50 mM phosphate buffer, pH 7.4) was mixed. The reaction was started by addition of 100 µl of phenazine metosulphate (PMS) solution (60 µM 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:

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

Where A_0 is the absorbance of the control; A_1 is the absorbance of the sample extract/standard.

Brine shrimp lethality assay

A brine shrimp lethality test was applied the evaluate the acute toxicity of the plant extracts as previously reported with slight modification[18]. The egg of *Artemia salina* were hatched in a flask containing sea water for 48 h. A serial concentration of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL of each extract were incubated with brine shrimps in seawater. After 24 h of incubation the number of survivor were counted. The experiment was performed in triplicate. Analysis of the data was performed by probit analysis on a Finney computer program to determine LC_{50}.

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* leaves extracts collected from two different locations in East Kalimantan, Indonesia.

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* leaves extracts showed no difference between to growing locations. Phytochemicals are plant chemicals without nutritive function and can be found in various parts of plants such as leaves, fruit, root, flower, bark, and so on. Most phytochemicals have protective or disease preventive properties [19]. The occurence of phytochemicals can be a marker to identify a plant species or genus and to differentiate plant growing at different location.

Total phenolic and flavonoid contents

The phenolic content found in the extracts were determined using the linear regression equation using gallic acid (for total phenolic content) or catechin (for total flavonoid content) as standard. The analysis of total phenolic and total flavonoid contents were displayed in Table 2. Phenolic compounds are secondary metabolites occur abundantly in plants. Antioxidant activity of plants is likely to be related with phenolic content. Total phenolic (TPC) and total flavonoid contents (TFC) of *H. capitata* were measured. The results obtained in this study showed that *H. capitata* collected from location 1 (Samarinda area) possessed higher total phenolic. Furthermore, the content of flavonoids as one class of secondary plant metabolites mostly used in plants to produce yellow and other pigments was determined using aluminium chloride colorimetric. 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 secondary metabolites, including in the mechanism of phenolic and flavonoid biosynthesis. The differences that this study has found between the sampled locations in total phenolic and total flavonoid compounds could be related to environmental conditions. Phenolics and flavonoids play significant role in the bioactivity of plants. In the scavenging mechanism against free radicals, phenolic and flavonoids are two class of chemicals belong to highly active scavengers. Flavonoids have been reported to play significant roles in antioxidant activity of plant. Furthermore, phenolics increase plant tolerance to oxidative stress [20, 21].

Thin-layer chromatography analysis

The profile of chemicals exist 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 were presented in Figure 1. TLC chromatogram developed by two solvent systems of *H. capitata* leaves 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) showed more compounds, particularly the red spot (Rf 0.68 at the solvent system 1 and 0.56 at solvent system 2). Thin layer chromatography is a fast, reliable, cheap and powerful tool as initial analysis of the phytochemical profile. This method allow the differentiation of chemical composition as displayed on the silica gel plate under UV illumination. Our analysis showed that there was clear difference between the phytochemicals of *H. capitata* grew in Samarinda (HSM) and

that of grew at West Kutai (HWK). This condition may affect the biological activities of *H. capitata* as evaluated in the present study.

Antimicrobial activity

The leaves extracts of *H. capitata* collected from two growing location were assayed their antimicrobial activity against 5 human pathogen microbial strains, *Propionibacterium acnes*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus sobrinus*, and *Candida albicans*. The results were recorded in Table 3. *H. 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.

Antioxidant activity

The leaves 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_{50} values to scavenge DPPH and superoxide radicals. As depicted in Table 4, growing location caused a significant difference of DPPH and superoxide free radical scavenging activity of *H. capitata.* HSM demonstrated the highest scavenging activity (lowest IC_{50}; 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_{50} values represent stronger free radical scavenging activity, as strong free-radical scavengers are active at low concentrations. Previous reports explained that antioxidant activity of plants is considerably associated with their phytochemical contents, especially that of flavonoids and phenolic acids

[22, 23]. In this study, the highest antioxidant activity as well as the highest content of phenolics and flavonoids and phenolic acids was observed in *H. capitata* from location 1 (Samarinda area) as presented in Table 5. This could also be related with variation in climatic conditions, soil nutrients, and water quality (hydrogen potential, electrical conductivity) of two growing locations and subsequently, influence the production of phytochemicals and the antioxidant activities.

Brine shrimp lethality test

Safety information of plant extract as a medicinal plant is essential for development of further products. As a quick reference and suitable tools, brine shrimp lethality assay was conducted to assess the cytotoxicity. The result of cytotoxicity assay of *H. capitata* extract was presented in Table 5. The results of cytotoxicity assay of *H. capitata* at different concentrations showed no toxicity of the plant extracts. According to Meyer *et al.* [24] the plant extract is classified toxic when the LC_{50} values are less than 1000 µg/mL. Concerning the concentration tested without any mortality to the 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 with the environmental condition, since the plants have unique adaptation to the environmental change. Many of the special metabolites are directly involved in the mechanisms that allow the adaptation of plants to their habitat [25]. Biological activities of plants may also be affected by many environmental factors that has been proved to cause the difference of special metabolites production [26].

In the present research, *H. capitata* plants collected from two growing regions displayed different composition of phytochemical, in term of phenolic and flavonoid contents.

Activities of the leaves extracts as antioxidant and antibacterial substances were also different. Sample 1 of *H. capitata* (HSM) was collected from the outskirt of Samarinda city, with typical humid and under shaded environment. Sample 2 of *H. capitata* was collected from drier and relatively open area. In line with our results, biosynthesis of flavonoid and phenolic compounds were reported to be different under different growing location, possibly caused by weather, temperature, latitude, soil nutrition, and sunlight [27, 8].

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.

CONCLUSIONS

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

ACKNOWLEDGEMENTS

The present work was funded by the Indonesian Ministry of Research, Technology and Higher Education under the Competitive-Based Research Grant (Grant number:119/UN17.41/KL/2018). The authors thank to Mr. Murdiyanto (West Kutai) for providing plant materials. We sincerely express our thanks to the Lab Head of Forest Products Chemistry, Mulawarman University and the members for technical support.

CONFLICT OF INTEREST

There is no conflicts of interest reported by each author.

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Comment [what is t8]: need to read more literature to help improve discussion

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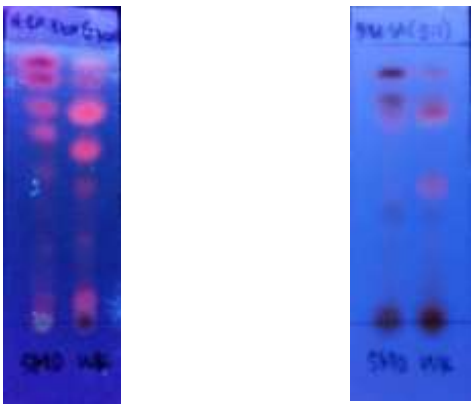

(a) (b)

Fig 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 1. Phytochemical analysis of *Hyptis 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

Table 2. Total phenolic content in leaves extracts of *Hyptis 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

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

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

Remarks: Relative growth inhibition was calculated by the inhibition zone of test sample divided by 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* = *Propionibcaterium acnes*; *S. sobrinus* = *Streptococcus sobrinus*; *S. aureus* = *Staphylococcus aureus*; *E. coli* = *Escherichia coli*; *C. albicans* = *Candida albicans*; NI = no inhibition.

Table 4. IC_{50} of *Hyptis capitata* for DPPH dan superoxide scavenging activity assays.

Sample	IC_{50} (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)

Tabel 5. Brine shrimp lethality bioassay of *Hyptis capitata* extracts

Sample	Concentration (µg/mL)	Mortality (%)	Lethal Concentration (LC_{50}, 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)

Revision letter

[6/11/2019]

To
The Editor
Journal of Applied Biology and Biotechnology

Regarding the reviewer comments on our manuscript, ID 125-1548207611, entitled "**Biological activities and phytochemicals of Hyptis capitata grown in two different locations in East Kalimantan, Indonesia**", we herewith sent you the revision letter with some following notes on the suggested points:

1. The background and aim on the introduction section have been separated and the background section has been improved;
2. The conclusion and recommendation on the abstract section have been added;
3. The definition of medicinal plant in the introduction section has been revised with reference to the botanic term;
4. A contradicting term at the introduction section has been clarified;
5. More literatures have been added to the introduction section. The problem statement has been improved;
6. The definition of microbial strain on the method section has been improved;
7. A quantitative phytochemical method on the method section has been added;
8. More literatures have been added to improve the discussion on the results and discussion section;

Thank you for kind attention.

Sincerely,
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ABSTRACT

Hyptis capitata is a plant in the family of Lamiaceae used to prevent wound infection and as antidote of 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. The present work aims to investigate 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 plant was evaluated its biological activities 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 leaves extract of *H. capitata* contain 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 DPPH (IC_{50} 13.69 µg/ml) and superoxide (IC_{50} 65.11 µg/ml) tests. Brine shrimp lethality test showed no toxicity of *H. capitata* leaves extracts. The results displayed that *H. capitata* collected from two location showed good antibacterial and antioxidant activities. The results suggested the the biological activites and phytochemical of *H. capitata* were affected by the environment where the plant was collected. A study into deep analysis on the effect of light intensity, temperature, soil nutrition and predatory risks to the biological activity and phytochemicals of the plant is required.

Key words: antimicrobial activity; antioxidant; *Hyptis capitata;* invasive plant, medicinal plant; secondary metabolite.

INTRODUCTION

A medicinal plant is described as any plant with substances having therapeutic purposes in its organ and may contain precursor for the production of synthetic drugs [1]. It is reported that about 80% of world medicinal plants are existed in Indonesia forest. More than 28,000 plant species have been inventoried in Indonesia. Of these, 7,000 species are classified as medicinal plant [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 illness. The Bentian people in East Kalimantan use the leaves to prevent infection of external wounds and the roots as antidote of food poisoning. In other side of world, the Guyanese use the leaf extract as a sedative and calmative and to reduce heart while an infusion of leaves is used to treat black diarrhea [7].

In the frame to optimize utilization of plant as raw material for herbal products, the extract standardization is a key point. An important related with the plant extract is environmental effect to the growth the plant and its extract. However, research on the effect of environmental factors to 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 condition such as drought stress may stimulate the difference in polyphenols and volatile metabolites of grapevine [11]. 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 with the difference geographical location where the samples were taken [13]. Chemical composition of herbal medicines needs to be confirmed in order to assess the extract quality in relation with safety concern. However, plant contains complex mixtures of secondary metabolites that play also play significant role in plant efficacy [14]. Therefore, as a promising medicinal plant, it is important to analyze the chemical contents an biological activities of the leaves of *Hyptis capitata* and its potential source of bioactive compounds.

The objective of the present work was to analyze the phytochemicals and evaluate the antimicrobial and antioxidant activities of the leaves 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).

MATERIALS AND METHODS

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 a 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 speciment was kept at the Laboratory of Forest Products Chemistry (KK-1805-HC003 and KK-1805-HC004).

General

TLC analysis was done on a silica gel coated alumunium sheet (Merck, Darmstadt, Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Dimethyl sulfoxide, Folin-Ciocalteau reagen, sulfuric acid, hydrochloric acid, acetic anhydride, potassium iodide and peptone were purchased from Merck (Darmstadt, Germany). β-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, USA). Nutrient broth was obtained from Difco (Detroit, MI, USA). Other chemicals was obtained in highest purity commercially available.

Preparation of extracts

The dried parts of the leaves were pulverized through a electric blender. Each 100 gram of plant meals was extracted with methanol at room temperature followed by mechanical shaking (7400 Tübingen; Edmun Buchler, Germany) for 48 h repeated twice. Filtration of the sample was done by using filter paper. The extract solution was subjected to a rotary evaporator at 40^{o}C and under reduced pressure to yield gummy extract. Upon the drying of the extract using a vacuum oven, the yield percentage of the dry extract was calculated as presented in Table 1.

Microbial strains

Gram +ve (*Propionibacterium acnes*, *Staphylococcus aureus*, *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^{o}C for 24 h. The fungal strain was cultured in Saboraud Dextrose Agar and incubated at 26^{o}C for 48 h prior to antimicrobial assays.

Phytochemicals analysis

Phytochemical analysis was carried out by qualitative and quantitative determination to analyze the occurrences of phenolic, alkaloid, flavonoid, steroid, terpenoid, tannin, saponin, and carbohydrate in a standard manner.

Alkaloid test

Five milligrams of plant extract were added by 2 ml of hydrochloric acid and followed by 1 ml of Dragendorff solution. The appearance of orange to red color in solution indicates the presence of alkaloid [15].

Flavonoid test

The alkaline method was taken to evaluate the presence of flavonoid. One milligram of plant sample was soaked in 5 ml of hot water for 5 min. A few drop of 20% sodium hydroxide solution was added to the filtrate was added and the existence of flavonoid was recorded upon the appearance of yellow color in the mixture [15].

Steroid

One milliliter of plant extract in 5 ml chloroform was mixed with 6 ml of conc. sulfuric acid. The formation of red color upper layer and yellow to green sulfuric acid layer indicates the steroid content [16].

Terpenoid

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

Tannin

Ten milliliter of plant extract were added by 1% lead acetate solution. The appearance of yellow deposit on the bottom of the tube indicates the presence of tannin [17]

Saponin

The presence of saponin was evaluated by the frothing test. One milligram plant sample was soaked in hot water for 10 min. Two milliliter of the extract solution were diluted with 10 ml of water in a test tube. The mixture was shaken for 2 min to produce froth if the sample contains saponin [18].

Carbohydrate

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

Total phenolic content (TPC)

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

Total flavonoid content (TFC)

Total flavonoid content was measured by a colorimetry method with reference to previous report [20]. A hundred µl of each extract and catechin standard solution were added with 100 µl of 5% (w/v) sodium nitrite solution in a test tube. The mixture solution was added with 100 µl of 10% aluminium chloride solution. The total volume was made up to 1 mL using distilled water. Total flavonoid contents were calculated as mg of catechin equivalents per gram of extracts (mg CE/g). The experiments were done in triplicate.

Antimicrobial activity assay

The antimicrobial activity of the extracts were carried out by agar well diffusion method using nutrient agar or saboraud dextrose agar [21] with slight modification. Sterilized media solution (20 ml) were poured into Petri dishes and left until molten. Twenty milliliters of microbial suspension were spread on the surface of the plates. Twenty microliters of acetone solution containing 31.25-500 µg extracts were dropped in7 mm well made with sterile cork borer on the media. Standard antibiotic, chloramphenicol at the concentration of 10 µg/20 µl was applied positive control. The culture plates were incubated under the absence

of light at 37^{o}C for 24 hours. 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 test sample divided by the mean inhibition zone for the chloramphenicol.

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 a 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 concentration were added to 0,5 ml of freshly prepared DPPH solution in ethanol. The mixture solution was made up with ethanol to a final volume of 1 ml. The reaction mixture was left for 20 min in dark and absorbance was measured at 517 nm using UV-Vis spectrofotometer. The test were run in triplicate with ascorbic acid as a positive control. 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_{50}, a concentration (µg/ml) of extract that inhibits the formulation of DPPH radicals by 50%.

Superoxide radical scavenging activity assay

Superoxide radical scavenging activity assay was performed by mechanism of the reduction of nitroblue tetrazolium as reported by Babu *et al*. [23]. One ml of nitroblue tetrazolium (NBT) solution (1 M NADH in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of *H. capitata* leaves extracts and a control, ascorbic acid (50 mM phosphate buffer, pH 7.4) were mixed. The reaction was started by addition of 100 µl of phenazine metosulphate (PMS) solution (60 µM 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:

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

Where A_0 is the absorbance of the control; A_1 is the absorbance of the sample extract/standard.

Brine shrimp lethality assay

Brine shrimp lethality test was applied the evaluate the acute toxicity of the plant extracts as previously reported with slight modification [24]. The egg of *Artemia salina* were hatched in a flask containing sea water for 48 h. Extract samples in a serial concentration of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL incubated with brine shrimps in seawater in triplicates. The number of survivor were counted and used to determine the toxicity of the samples. The experiment was performed in triplicate. Lethal concentration 50 (LC_{50}) values were determined by probit analysis on a Finney computer program.

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* leaves extracts collected from two different locations in East Kalimantan, Indonesia.

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* leaves extracts showed no difference between to growing locations. Phytochemicals are plant chemicals without nutritive function and can be found in various parts of plants such as leaves, fruit, root, flower, bark, and so on. Most phytochemicals have protective or disease preventive properties [25]. The occurence of phytochemicals can be a marker to identify a plant species or genus and to differentiate plant growing at different location.

Total phenolic and flavonoid contents

The phenolic content found in the extracts were determined using the linear regression equation using gallic acid (for total phenolic content) or catechin (for total flavonoid content) as standard. The analysis of total phenolic and total flavonoid contents were displayed in Table 2. Phenolic compounds are secondary metabolites occur abundantly in plants. The phenolic and flavonoid compounds were reported to play important roles in antioxidant activity of plant. Total phenolic (TPC) and total flavonoid contents (TFC) of *H. capitata* were measured. The results obtained in this study showed that *H. capitata* collected from location 1 (Samarinda area) possessed higher total phenolic. Furthermore, the content of flavonoids as one class of secondary plant metabolites mostly used in plants to produce

yellow and other pigments was determined using aluminium chloride colorimetric. 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 secondary metabolites, including in 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 significant role in the bioactivity of plants. In the scavenging mechanism against free radicals, phenolic and flavonoids are two class of chemicals belong to highly active scavengers. Flavonoids have been reported to play significant roles in antioxidant activity of plant. Furthermore, phenolics increase plant tolerance to oxidative stress [26, 27].

Thin-layer chromatography analysis

The profile of chemicals exist 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 were presented in Figure 1. TLC chromatogram developed by two solvent systems of *H. capitata* leaves 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) showed more compounds, particularly the red spot (Rf 0.68 at the solvent system 1 and 0.56 at solvent system 2). Thin layer chromatography is a fast, reliable, cheap and powerful tool as initial analysis of the phytochemical profile. This method allow the differentiation of chemical composition as displayed on the silica gel plate under UV illumination. Our analysis showed that there was clear difference between the phytochemicals of *H. capitata* grew in Samarinda (HSM) and

that of grew at West Kutai (HWK). This condition may affect the biological activities of *H. capitata* as evaluated in the present study.

Antimicrobial activity

The leaves extracts of *H. capitata* collected from two growing location were assayed their antimicrobial activity against 5 human pathogen microbial strains, *Propionibacterium acnes*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus sobrinus*, and *Candida albicans*. The results were recorded in Table 3. *H. 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.

Antioxidant activity

The leaves 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_{50} values to scavenge DPPH and superoxide radicals. As depicted in Table 4, growing location caused a significant difference of DPPH and superoxide free radical scavenging activity of *H. capitata.* HSM demonstrated the highest scavenging activity (lowest IC_{50}; 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_{50} values represent stronger free radical scavenging activity, as strong free-radical scavengers are active at low concentrations. Previous reports explained that 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 and flavonoids and phenolic acids was observed in *H. capitata* from location 1 (Samarinda area) as presented in Table 5. This could also be related with variation in climatic conditions, soil nutrients, and water quality (hydrogen potential, electrical conductivity) of two growing locations and subsequently, influence the production of phytochemicals and the antioxidant activities.

Brine shrimp lethality test

Safety information of plant extract as a medicinal plant is essential for development of further products. As a quick reference and suitable tools, brine shrimp lethality assay was conducted to assess the cytotoxicity. The result of cytotoxicity assay of *H. capitata* extract was presented in Table 5. The results of 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 toxic when the LC_{50} values are less than 1000 µg/mL. Concerning the concentration tested without any mortality to the 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 with the environmental condition, since the plants have unique adaptation to the 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 has been proved to cause the difference of special metabolites production [32].

In the present research, *H. capitata* plants collected from two growing regions displayed different composition of phytochemical, in term of phenolic and flavonoid contents.

Activities of the leaves extracts as antioxidant and antibacterial substances were also different. Sample 1 of *H. capitata* (HSM) was collected from the outskirt of Samarinda city, with typical humid and under shaded environment. Sample 2 of *H. capitata* was collected from drier and relatively open area. In line with our results, biosynthesis of flavonoid and phenolic compounds were reported to be different under different growing location, 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.

CONCLUSIONS

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

ACKNOWLEDGEMENTS

The present work was funded by the Indonesian Ministry of Research, Technology and Higher Education under the Competitive-Based Research Grant (Grant number:119/UN17.41/KL/2018). The authors thank to Mr. Murdiyanto (West Kutai) for providing plant materials. We sincerely express our thanks to the Lab Head of Forest Products Chemistry, Mulawarman University and the members for technical support.

CONFLICT OF INTEREST

There is no conflicts of interest reported by each author.

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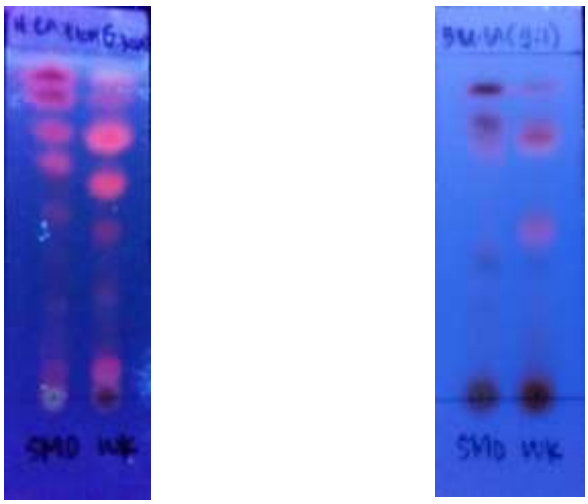

(a) (b)

Fig 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 1. Phytochemical analysis of *Hyptis 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

Table 2. Total phenolic content in leaves extracts of *Hyptis 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

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

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

Remarks: Relative growth inhibition was calculated by the inhibition zone of test sample divided by 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 = Propionibcaterium acnes*; *S. sobrinus = Streptococcus sobrinus*; *S. aureus = Staphylococcus aureus*; *E. coli = Escherichia coli*; *C. albicans = Candida albicans*; NI = no inhibition.

Table 4. IC_{50} of *Hyptis capitata* for DPPH dan superoxide scavenging activity assays.

Sample	**IC_{50} (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)

Tabel 5. Brine shrimp lethality bioassay of *Hyptis capitata* extracts

Sample	Concentration (µg/mL)	Mortality (%)	Lethal Concentration (LC_{50}, 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)

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Status of my Manuscripts under Review

Mns No	Submission Date	Title	Authors	Status
JABB-2019-01-014	2019-01-23	Initial Version (DOC): Initial Full Text (.docx) Initial Version (PDF): Biological activities and phytochemicals of Hyptis capitata grown in East Kalimantan, Indonesia **REVISIONS** Revised Version-1 (2019-11-06-16-03-28) : Revised Full Text (.docx) | Revised Full Text (.pdf)	Irawan Wijaya Kusuma, Rahmini Rahmini , Enos Tangke Arung, Arif Yudo Pramono, Erwin Erwin, Supomo Supomo,	Under Review

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Accepted Manuscripts

Title	Authors	Status
[Biological activities and phytochemicals of Hyptis capitata grown in East Kalimantan, Indonesia]	Irawan Wijaya Kusuma, Rahmini Rahmini , Enos Tangke Arung, Arif Yudo Pramono, Erwin Erwin, Supomo Supomo,	Acceptance letter in PDF

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['Biological activities and phytochemicals of Hyptis capitata grown in East Kalimantan, Indonesia'] Year:2020, Volume:8, Issue:2.000 Pages: 58-64	Irawan Wijaya Kusuma, Rahmini, Enos Tangke Arung, Arif Yudo Pramono, Erwin, Supomo	Acceptance letter in PDF

November 27, 2019

Dear Irawan Wijaya Kusuma

I am pleased to inform you that your manuscript titled as "Biological activities and phytochemicals of Hyptis capitata grown in East Kalimantan, Indonesia" (Manuscript Number: JABB-2019-01-014) was accepted for publication in the Journal of Applied Biology & Biotechnology. You are required to submit article processing charges within next three days of receiving this mail through Banking/Paypal.

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