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Short Communication: Antioxidant and antibacterial properties of tree fern *Cyathea contaminans*

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Abstract. *Faizal A, Taufik I, Rchman AF, Azar AWP. 2020. Short Communication: Antioxidant and antibacterial properties of tree fern* Cyathea contaminans. *Biodiversitas 21: 2201-2205. Cyathea contaminans* (Wall. ex Hook) Copel is a tree fern used in traditional practices as herbal remedies to treat different kinds of diseases. To explore its further medicinal uses, we designed a study to determine the antioxidant and antibacterial activities of polar and non-polar extracts of this fern. The fern leaves (mature fronds), young fronds, and hairs were collected from Tangkuban Perahu Nature Park, West Java, Indonesia, and were extracted by methanol 80% or n-hexane. Antioxidant activity was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, whereas antibacterial activity was measured by growth inhibition of *Escherichia coli* and *Staphylococcus aureus*. Results showed that methanol extract from mature leaves (fronds) exhibits weak to very strong antioxidant activities (IC_{50} 37.13-225.19 µg/mL), whilst hairs and young fronds showed weak activities, i.e. IC_{50} 179.50-255.49 µg/mL and IC_{50} 544.27->2000 µg/mL, respectively. Hexane fraction from fronds was active against *E. coli* and *S. aureus* (43.92% and 46.8%), and from hairs against *E. coli* (48.1%) in concentration of 250 µg/mL. Gas Chromatography-Mass Spectrometry (GC-MS) analysis indicated that active compounds from fronds extract were dominated by 2H-tetrazole, 5-(thiophen-2yl) methyl (14,29 %), 2-thiophene acetic acid, 2-methyl phenyl ester (14,54%), and phenol, 2,6-bis(1,1-dimethyl ethyl)-4-methyl (10.54%). This study concluded that mature leaves (fronds) of *C. contaminans* is the potential to be used as antioxidant and antibacterial agents.

Keywords: Antibacterial, antioxidant, *Cyathea contaminans*, tree fern

INTRODUCTION

Many researchers have performed screening on medicinal plants to unravel bioactive substances for pharmaceutical purposes. However, these findings were mostly reported from flowering plants (Ahmad et al. 2019; Majolo et al. 2019; Thakur and Pathak 2018; Xu et al. 2017). Hitherto, ferns or pteridophytes are less explored for human ailments compared to those from angiosperms. Interestingly, many ferns have been explored for nutraceutical due to their high content in vitamins, minerals, essential amino acids, and protein. Comprehensive phytochemical studies also reported that ferns produce a plethora of specialized metabolites such as phenols, flavonoids, terpenes, and fatty acid derivates which are potential toward development of ferns as traditional herbal drugs as well as industrial healthcare products (Ho et al. 2010; Xavier-ravi et al. 2019).

Ferns are a group of nonflowering vascular plants consist of more than 12,000 species in the world. About 25% of these ferns are found in a humid region or in tropical montane areas in Indonesia (Karger et al. 2014; Kusmana and Hikmat 2015). Thoroughly screening of plant-derived extracts as a part of drug discovery program showed that ferns have interesting biological properties such as anti-inflammatory, antibacterial, antiviral, antitumor hepatoprotective, cytotoxic, antihyperglycemic, antinociceptive, and immunomodulatory activities (Greeshma and Sridhar 2019; Hendra et al. 2019; Ho et al. 2010).

Cyathea contaminans (Wall. ex Hook) Copel belongs to family Cyatheaceae, a family of tree fern which is the second-largest fern group among the pteridophytes. *C. contaminans* is easily recognized by its single, erect trunk which reaches heights up to 10 m or more. It has large fronds (leaves) reaching 3-4 m in length, with stout, thorny, and purplish leaf stalk. *Cyathea* genus is considered economically important as it has been used as ornamental part of building decoration, flowerpot, substrate for orchids, as well as for traditional medicine (Hartini 2006; Ho et al. 2010). Several bioactivity studies of *Cyathea* have also been reported. Ethanolic extracts of *C. nilgirensis*, *C. gigantea*, and *C. crinita* indicated to have antioxidant and free radical scavenging activities (Janakiraman and Johnson 2016). Furthermore, *C. dregei* was reported to show antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* (Adamu et al. 2014).

Unfortunately, a comprehensive study of biological activity for *C. contaminans* has not been explored. In point of fact, particularly the caudex apical, rhizomes, hairs, and leaves of *C. contaminans* have been used as herbal remedies in India and the Philippines to treat headaches and external wounds (Aya-ay 2016; Shil et al. 2014). Therefore, this study was designed to uncover the biological activity of *C. contaminans* by assessing its antioxidant and antibacterial activities using two different

polarities of solvents. The extracts were also analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The result of this study could provide important information for further development of *C. contaminans* as plant-derived therapeutic agents.

MATERIALS AND METHODS

Collection of plant material

The mature fronds, young fronds, and hairs of *C. contaminans* were collected from three different individual plants in different locations in Tangkuban Parahu Nature Park, West Java, Indonesia. The location and coordinate of each collected plants were 06º 46' 08,1" S & 1070 37' 43,3" E at 1668 m asl (1), 06º 46' 15,5" S & 1070 37' 50,8" E at 1620 m asl (2), and 06º 46' 15,3" S & 1070 37' 50,9" E at 1613 m asl (3). Confirmation and identification of plant species were done in Herbarium Bogoriense LIPI, Cibinong, Indonesia. Each sample was air-dried in the room temperature for further analysis.

Sample extraction

Mature fronds, young fronds, and hairs were extracted by 80% methanol and n-hexane: water with ratio 1: 3 (w/v). Samples were extracted by sonication for 60 min at 35 ºC. Supernatant was filtered by Whatman filter paper No. 1. Samples extracted in methanol and n-hexane were evaporated using a rotary evaporator for mature fronds and by air-drying for young fronds and hairs. Crude extracts were weighed and then stored at-20 ºC for further analysis.

Determination of total phenolic content

Total phenolic content was measured according to Folin-Ciocalteu method (Ainsworth and Gillespie 2007). About 0.5 mL methanolic extract was added by 2.5 mL of 0.2 N Folin-Ciocalteu reagent, homogenized, and incubated for 5 min. The solution was then added by 2 mL of 7.5% Na_2CO_3 and incubated for 60 min. Absorbance was determined at 765 nm using a spectrophotometer. Quantitative measurement was obtained based on gallic acid standard with concentration of 25, 50, 100, 200, and 400 µg/mL.

Antioxidant activity assay

Antioxidant activity of fern extract was determined based on the ability to reduce free radical using a stable free radical DPPH (1,1-Diphenyl-2-Picrylhydrazyl) (Kedare and Singh 2011). About 1 mL of sample in methanol extract was added by 1 mL of 0.1 mM DPPH and incubated for 30 min. Scavenging of free radical was measured according to the absorbance at 571 nm using spectrophotometry. Ascorbic acid was used as positive control. Scavenging activity (%) was calculated based on formula: (|A0-A1| / A0) x 100%, where A0 is ascorbic acid absorbance and A1 is sample absorbance. The antioxidant activity (µg/mL) showed as the amount of concentration to reduce DPPH as much as 50% (IC_{50}). The activity is classified into four groups: very strong (<50 µg/mL), strong (50-100 µg/mL), moderate (101-250 µg/mL), and weak (251-500 µg/mL), and very weak (> 500 µg/mL) (Jun et al. 2003).

Antibacterial activity assay

Cultures of *E. coli* and *S. aureus* were activated in Luria-Bertani (LB) liquid medium for 24 h and were inoculated as much as 10^8 cfu/mL (20 µL) in 96 well sterile microtiter plate. Samples were dissolved in 2.5% Dimethyl sulfoxide (DMSO) in following concentration 0.24, 0.98, 3.91, 15.63, 62.5, and 250 µg/mL. About 180 µL of samples were then added to each well of microtiter plate. LB medium without bacterial culture and sample was used as control. Culture in microtiter plate was then incubated for 20-24 h at 37 ºC. The optical density (OD_{595}) was measured for initial and final incubation time to measure antibacterial activity of each sample. The sample with highest potential for antibacterial activity was used for further analysis using GC-MS.

GC-MS analysis

Based on the result from antibacterial activity assay, we only proceed with hexane extracts of mature fronds for GC-MS analysis. GC-MS was performed using GC-17A (Shimadzu) gas chromatography equipped with capillary column HP-1 (30 m x 0.25 mm x 0.25 µm), using Helium gas as gas carrier at 1 mL/min flowrate. Initial temperature used was 60 ºC and final temperature was 200 ºC. The compounds within the samples were detected based on their retention time and identified using Wiley MS libraries 2008.

Statistical analysis

All the experiments involved three different individual plants from different elevations. The samples were then divided into mature fronds, young fronds, and hairs with at least three replicates. Data were reported as means ± standard deviation (SD) and were analyzed using one-way ANOVA. Mean comparison from each result was contrasted using Duncan's multiple range test. All statistical analyses were performed at $P < 0.05$ using an IBM SPSS Statistics 20 Package. The Pearson correlation test was used to determine the correlation between extract concentration and antibacterial activity.

RESULTS AND DISCUSSION

Analysis of total phenolic content

Different parts of *C. contaminans* ferns contain varying total phenolic contents, with mature fronds having the highest amount (47.35-208.58 mg GAE/g sample) compared to young fronds (2.02-3.63 GAE/g sample) or hairs (0.95-2.98 GAE/g sample) (Figure 1). The young fronds and hairs exhibited low phenolic content and significantly different with that of mature fronds. The hairs of *C. contaminans* has the lowest total phenolic content.

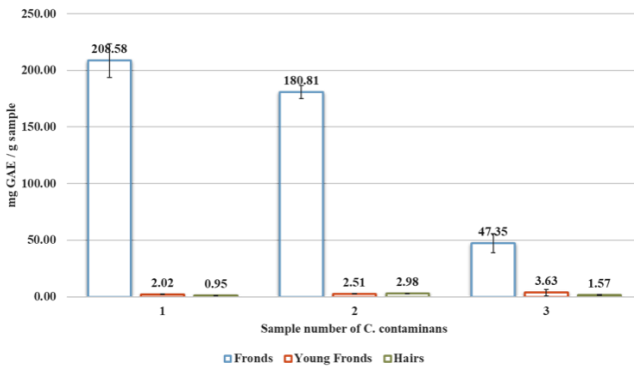

Figure 1. Total phenolic content of different *Cyathea contaminans* parts

Free radical scavenging activity

Inhibition concentration which showed concentration of the extracts to reduce free radical reagent (i.e. DPPH) was also known as antioxidant activity. IC_{50} is the concentration needed to reduce 50% or the free radical reagent. The results showed that the mature fronds of *C. contaminans* have a very strong to moderate antioxidant activity, meanwhile, young fronds and the hairs have a moderate to very weak antioxidant activity (Table 1).

Antibacterial activity

Antibacterial activity is represented by growth inhibition of *E. coli* and *S. aureus* (Figure 2). The highest antibacterial activity against *E. coli* was found from hexane mature fronds-and hair-extracts (48.1% and 43.9%) at 250 µg/mL concentration. Similar concentration of methanol extracts from mature fronds, young fronds, and hair exhibit growth inhibition of *E. coli* by 20.39%, 25.15%, and 23.63%. In addition, hexane extracts of mature fronds have 46.8% inhibition of *S. aureus.* Statistical analysis of aforementioned result showed that concentration of extracts correlated positively with growth inhibition of *E. coli* (Table 2). Higher concentration of extract would result in higher growth inhibition.

GC-MS analysis

As hexane extracts of *C. contaminans* have high antibacterial activity, with the highest from that extracted from the mature fronds, we only proceed for phytochemical analysis using this fraction. GC-MS analysis detected that compounds found in fronds extract were dominated by fatty acids (Table 3). The highest percentage (%) of compounds detected in fronds extract were 2-thiophene acetic acid, 2-methyl phenyl ester, and 2H-tetrazole-, 5-(thiophen-2yl)-methyl, which are sulfur-containing compounds, and phenol, 2,6-bis(1,1-dimethyl ethyl)-4-methyl.

The study on medicinal plants has increased in recent years due to their potential as alternative source of therapeutic agents to encounter the adverse effects of synthetic drugs. This approach combines both traditional and advanced techniques such as biological, ethnopharmacological, molecular, phytochemistry, and metabolic engineering (Chakraborty 2018; Süntar 2019). Therefore, as a part of drug discovery program using natural products, we did a screening on medicinal ferns, which are potential candidates for antioxidant and antibacterial agents. We selected the tree fern *C. contaminans* as this species is abundantly found in montane regions in West Java.

Table 1. IC_{50} from different parts of *Cyathea contaminans*

Sample	Sample number	IC_{50} (µg/mL)	Antioxidant activity classification
Mature fronds	1	83.67 ± 1.93	Strong
	2	37.13 ± 2.85	Very strong
	3	225.19 ± 15.76	Moderate
Young fronds	1	>2000	Very weak
	2	1008.58 ± 25.97	Very weak
	3	544.27 ± 75.24	Very weak
Hairs	1	229.57 ± 23.53	Moderate
	2	179.5 ± 21.04	Moderate
	3	255.49 ± 81.5	Weak

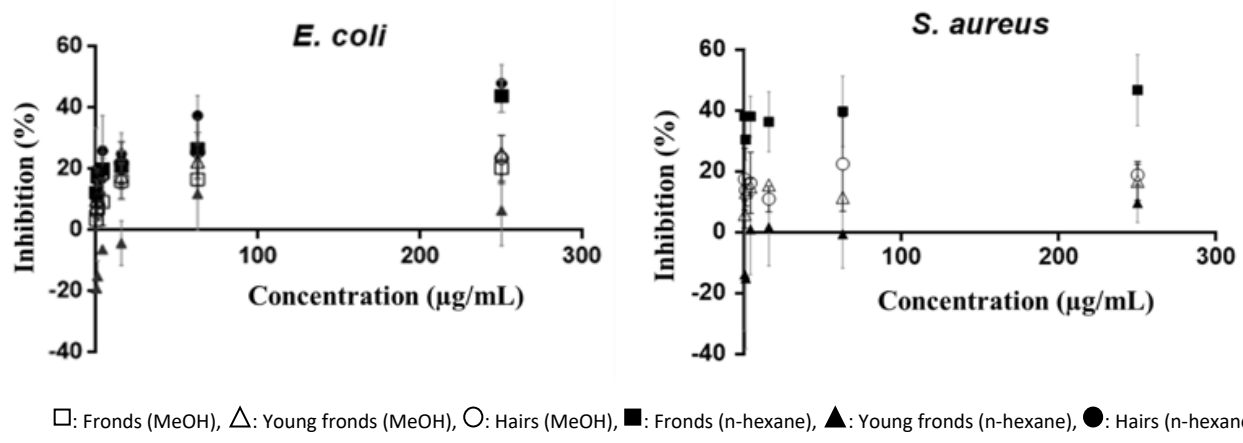

Figure 2. Antibacterial activity of *Cyathea contaminans* in methanol and hexane extracts against *Escherichia coli* and *Staphylococcus aureus*

Table 2. The Pearson correlation between concentration of the extracts and antibacterial activity

Solvent	Bacteria	Correlation (r^2)		
		Fronds	Young fronds	Hairs
Methanol	*E. coli*	0.97	0.93	0.87
	S. aureus	0.56	0.47	0.13
Hexane	*E. coli*	0.93	0.88	0.82
	S. aureus	0.71	0.79	0.37

Table 3. Chemical profiles from hexane fraction of frond samples detected by GC-MS

Retention Time (RT)	Relative abundance (%)	Compound
10.801	1.69	Benzene, 1,3-bis(1,1-dimethyl ethyl)
12.713	2.49	1-Tetradecane
14.109	7.79	Butylated hydroxytoluene
14.119	10.54	Phenol, 2,6-bis(1,1-dimethyl ethyl)-4-methyl
15.313	1.2	Hexadecane
16.434	4.41	Heptadecane
16.683	14.54	2-Thiophene acetic acid, 2-methyl phenyl ester
16.683	14.29	2H-Tetrazole-, 5-(thiophen-2yl)-methyl
17.427	8.95	1-Octadecene
21.227	4.24	Z-5-Nonadecene
21.259	3.97	1-Nonadecene
22.932	2.73	Cyclotetracosane

Guided by DPPH assay, we noticed the potential of antioxidant fraction of *C. contaminans*. DPPH is commonly employed for antioxidant assay due to its simple application with high sensitivity. Scavenging activity of DPPH is caused by the ability to transfer hydrogen atom or electron mainly by phenolic compounds such as polyphenols or flavonoids (Hidayati et al. 2017; Tohma et al. 2017). This also indicates that the respective phenolic content has a positive correlation with their antioxidant activities. Our result showed that each different parts of *C. contaminans* contain different accumulation phenolic compounds, thus varied in their antioxidant activities. Phenolics are a class of plant-derived substances reported as the main free radical scavenger due to their unique structure consisting of a number of hydroxyl groups. Phenolics are dominantly detected in fronds compared to young fronds and hairs, indicated that accumulation of these compounds is correlated with plant physiological and developmental ages. Young fronds would develop into fronds and during this period, their metabolite composition would undergo changes (Li et al. 2020). Similar studies also showed that total phenolic contents of *Ilex paraguensis*, *Psidium guajava*, and *Moringa oleifera* increases with the age of the plants (Blum-Silva et al. 2015; Evi et al. 2018; Nantitanon et al. 2010).

Three different individuals showed altitudinal variation of phenolic contents. Total phenolic content increased in mature fronds at higher elevation, while this was not significantly different in both young fronds and hairs. Notably, mature frond at the highest altitude recorded phenolic content 208.58 mg/g compared to the subsequent lower elevation of 180.81 and 47.35 mg/g, respectively. It was reported that production of plant secondary metabolites, including phenolic acid and flavonoid, are affected by environmental factors such as light intensity, air temperature, nutrient, and stress which are closely related to altitudinal gradient of a specific location (Li et al. 2020). Similarly, high elevation can also be associated with higher and longer exposure to solar irradiation, which harmful to the plants due to excessive ultraviolet (UV) radiation. Therefore, mature fronds of *C. contaminans* accumulate high phenolic as a defense response and lessening the negative impacts of UV irradiation.

Phytochemical analysis of several *Cyathea* species such as *C. nilgirensis*, *C. gigantea*, and *C. crinite* exhibited that this genus contains several types of tannin, flavonoids and other phenolic compounds (Naidoo et al. 2014; Narayanan and Marimuthu 2016). Our phytochemical analysis using GC-MS detected the presence of butylated hydroxytoluene (BHT) and phenol, 2,6-bis(1,1-dimethyl ethyl)-4-methyl which were accounted for strong antioxidant activity in methanol fraction of *C. contaminans* mature fronds. BHT is well known for strong antioxidant activity and has been used as a reference chemical for antioxidant assay together with classical antioxidant vitamin C, while phenol, 2,6-bis(1,1-dimethyl ethyl)-4-methyl was also proved a strong antioxidant despite that it is less effective than BHT (Tait et al. 1996; Yunfeng et al. 2018).

Development of resistant bacterial strain has emerged as one of the reasons why a number of antibiotics lost their beneficial effects. Therefore, there is a continuous need for antibacterial source which are effective, yet risk-free for the users. Therefore, we continued the screening of extracts from *C. contaminans* to assess their antibacterial potency. Notably, mature fronds extract of *C. contaminans* exhibited highest antibacterial activity toward *E. coli* and *S. aureus*. Phytochemical analysis revealed the primary constituents of the extracts were 2-thiophene acetic acid, 2-methyl phenyl ester and 2H-tetrazole-, 5-(thiophen-2yl) methyl, benzene, 1,3-bis(1,1-dimethyl ethyl), 1-tetradecane, Z-5-nonadecene, and cyclotetracosane which have been reported as antibacterial agents (Kim 2008; Rarassari et al. 2016).

Overall, the results of this study indicated that the extract from mature fronds of *C. contaminans* showed excellent antioxidant and antibacterial property. Further steps should be initiated to extend this research and develop new plant-based antioxidants and antibiotics for medicinal uses.

In conclusion, mature fronds of *C. contaminans* has a strong to moderate antioxidant activity (IC50 37,13-225,19 µg/mL), the young frond has a weak antioxidant activity (IC50 37,13-225,19 µg/mL), and the hair of *C. contaminans* has a moderate to weak antioxidant activity

(IC50 179,50-255,49 µg/mL). Highest potential of antibacterial activity was found in hexane extract of fronds with inhibition percentage of *E. coli* is 43.92% and S. aureus is 46.8% at concentration 250 µg/mL. The active antibacterial compound detected in fronds extract are 2H-tetrazole, 5-(thiophen-2yl)-methyl (14,29 %) and 2-thiophene acetic, 2-methyl phenyl ester (14,54 %). These findings suggest that extract of mature fronds from *C. contaminans* have the potential for natural antioxidant and antibiotic.

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REFERENCES

Adamu M, Naidoo V, Eloff JN. 2014. The antibacterial activity, antioxidant activity and selectivity index of leaf extracts of thirteen South African tree species used in ethnoveterinary medicine to treat helminth infections. BMC Vet Res 10: 52 DOI: 10.1186/1746-6148-10-52.

Ahmad I, Ahmad MSA, Hussain M, Hameed M. 2019. Ethnobotanical aspects of some traditional medicinal plants. In: Egamberdieva D, Tiezzi A (eds.) Medically Important Plant Biomes: Source of Secondary Metabolites. Springer, Singapore.

Ainsworth EA, Gillespie KM. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Prot 2: 875-877.

Aya-ay A. 2016. Ethnobotany of ferns and ferns-allies in Mount Macabol, Marilog District, Davao City, Philippines. Ijaber 14: 1127-1137.

Blum-Silva CH, Chaves VC, Schenkel EP, Coelho GC, Reginatto FH. 2015. The influence of leaf age on methylxanthines, total phenolic content, and free radical scavenging capacity of *Ilex paraguariensis* aqueous extracts. Rev Bras Farmacogn 25: 1-6.

Chakraborty P. 2018. Herbal genomics as tools for dissecting new metabolic pathways of unexplored medicinal plants and drug discovery. Biochimie Open 6: 9-16.

Evi S, Muhammad Sulaiman Z, Nurafni Israyanti A, Syakila A, Ririen H, Risfah Y, Aliyah. 2018. Total phenolic, total flavonoid, quercetin content and antioxidant activity of standardized extract of *Moringa oleifera* leaf from regions with different elevations. Pharmacogn J 10: 104-108.

Greeshma AA, Sridhar KR. 2019. Nutraceutical and bioactive significance of ferns with emphasis on the medicinal fern *Diplazium.* In: Egamberdieva D, Tiezzi A (eds.) Medically Important Plant Biomes: Source of Secondary Metabolites. Springer, Singapore.

Hartini S. 2006. Ferns in Sago Malintang Nature Reserve, West Sumatera and their acclimatization in Bogor Botanical Garde. Biodiversitas 7: 230-236.

Hendra R, Khodijah R, Afham M, Fachira R, Sofiyanti N, Teruna HY. 2019. Tingkat toksisitas dari beberapa ekstrak tanaman paku kaki tupai (*Davalia denticulate*). Majalah Farmasetika 4: 46-49. [Indonesian]

Hidayati MD, Ersam T, Shimizu K, Fatmawati S. 2017. Antioxidant activity of *Syzygium polyanthum* extracts. Indon J Chem 17: 49-54.

Ho R, Teai T, Bianchini J-P, Lafont R, Raharivelomanana P (2010) Ferns: From traditional uses to pharmaceutical development, chemical identification of active principles. In: Kumar A, Fernández H, Revilla MA (eds.) Working with Ferns: Issues and Applications. Springer New York.

Janakiraman N, Johnson M. 2016. GC-MS analysis of ethanolic extracts of *Cyathea nilgirensis*, *C.gigantea*, and *C. crinita.* Egypt Pharmaceut J 15: 43-47.

Jun M, Fu H-Y, Hong J, Wan X, Yang CS, Ho C-T. 2003. Comparison of antioxidant activities of isoflavones from Kudzu Root (*Pueraria lobata* Ohwi). J Food Sci 68: 2117-2122.

Karger DN, Weigelt P, Amoroso VB, Darnaedi D, Hidayat A, Kreft H, Kessler M. 2014. Island biogeography from regional to local scales: evidence for a spatially scaled echo pattern of fern diversity in the Southeast Asian archipelago. J Biogeogr 41: 250-260.

Kedare S, Singh R. 2011. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol 48: 412-422

Kim J. 2008. Phytotoxic and antimicrobial activities and chemical analysis of leaf essential oil from *Agastache rugosa.* J Plant Biol 51: 276-283.

Kusmana C, Hikmat A. 2015. The biodiversity of flora in Indonesia. J Nat Res Environ Manag 5: 187-198.

Li Y, Kong D, Fu Y, Sussman MR, Wu H. 2020. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol Biochem 148: 80-89.

Majolo F, de Oliveira Becker Delwing LK, Marmitt DJ, Bustamante-Filho IC, Goettert MI. 2019 Medicinal plants and bioactive natural compounds for cancer treatment: Important advances for drug discovery. Phytochem Lett 31: 196-207.

Naidoo Y, Sadashiva CT, Kasim N, Nicholas A, Naidoo G. 2014. Chemical composition and antimicrobial activity of the essential oil of *Ocimum obovatum* E. Mey. Ex Benth. (Lamiaceae). J Essent Oil Bear Plants 17: 142-147.

Nantitanon W, Yotsawimonwat S, Okonogi S. 2010. Factors influencing antioxidant activities and total phenolic content of guava leaf extract. LWT-Food Sci Technol 43: 1095-1103.

Narayanan J, Marimuthu J. 2016. HPTLC fingerprint profile (phenolics) of selected *Cyathea* species from Western Ghats, South India. Chin J Biol 2016 DOI: 10.1155/2016/6420371

Rarassari MA, Darius D, Kartikaningsih H. 2016. Inhibition of *Euchema spinosum* extract with different concentration of *Bacillus cereus.* Samakia, Jurnal Ilmu Perikanan 7: 5-11.

Shil S, Dutta Choudhury M, Das S. 2014. Indigenous knowledge of medicinal plants used by the Reang tribe of Tripura state of India. J Ethnopharmacol 152: 135-141.

Süntar I. 2019. Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochem Rev. https//doi.org/10.1007/s11101-019-09629-9.

Tait A, Ganzerli S, Bella MD. 1996. Synthesis and free radical scavenging activity of 4-(2H-1,2,4-benzothiadiazine-1,1-dioxide-3-yl)-2,6-bis(1,1-dimethyl ethyl)phenols. Tetrahedron 52: 12587-12596.

Thakur A, Pathak SR. 2018. Introduction to medicinally important constituent from Chinese medicinal plants. In: Tewari A, Tiwari S (eds.) Synthesis of Medicinal Agents from Plants. Elsevier, Nederlands.

Tohma H, Gülçin İ, Bursal E, Gören AC, Alwasel SH, Köksal E. 2017. Antioxidant activity and phenolic compounds of ginger (*Zingiber officinale* Rosc.) determined by HPLC-MS/MS. J Food Meas Charact 11: 556-566.

Xavier-ravi B, Antony-varuvel Gv, Thangaraj P, Doulathabad M-R, Rajan K. 2019. Antioxidant, anti-inflammatory activities and HPLC quantification of flavonoids in *Pteris tripartita* Sw. a critically endangered medicinal fern from India. Biocatal Agric Biotechnol 21: 101304.

Xu D-P et al. 2017. Natural antioxidants in foods and medicinal plants: extraction, assessment and resources. Int J Mol Sci 18: 96

Yunfeng Z, Lin L, Lan S, Lidong Z, Yang X. 2018. In comparison with vitamin C and butylated hydroxytoluene, the antioxidant capacity of aqueous extracts from buds and flowers of *Lonicera japonica* Thunb. J Trad Chin Med 38: 373-379.