Antioxidant and Antibacterial Properties Derived from *Horsfieldia Spicata* (Roxb.) J. Sinclair Stem Bark Extract and Its Active Fraction

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Abstract. *Horsfieldia spicata* (Roxb.) J. Sinclair is a species of plant from the *Myristicaceae* family which has more than 100 species and is widely distributed in South Asia, from India to the Philippines and Papua New Guinea. Numerous species of this family are utilized for wood, and other species are reported to contain several groups of active compounds such as alkaloids, polyphenols, flavonoids and saponins. Various active compounds from this family are known to have various bioactivities including antioxidant, antibacterial, analgesic, and cytotoxic. This study was conducted to determine the value of antioxidant and antibacterial activities from the stem bark extract of *H. spicata* and its active fractions. Of note we used some bacterial tested of the gram negative, *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (FNCC-0195), as well as gram positive, namely *Bacillus subtilis* (FNCC-0059), and *Staphylococcus aureus* (FNCC-0047). The stems of *H. spicata* were extracted using methanol as a solvent to obtain a crude extract. Further, the extract was then fractionated to obtain n-hexane, ethyl acetate and butanol fractions. The extracts and its fractions were then tested for antioxidants using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The sample was then assayed for antibacterial using the disc diffusion and Minimum Inhibitory Concentration (MIC) methods. The results of the antioxidant activity test showed that the ethyl acetate fraction had the strongest activity with an IC_{50} value of 23.53 μg/mL, followed by the butanol fraction, methanol extract, and the n-hexane fraction with an IC_{50} value of 63.36, 75.37 and 114.17 μg/mL, respectively. As for the antibacterial test results showed that the methanol extract had the best ability to inhibit the growth of *B. subtilis* and *P. aeruginosa* with clear zone values of 13.33 ± 2.36 and 15.33 ± 4.50 mm, respectively. On the other hand, the hexane fraction showed the best antibacterial activity against *E. coli* and *S. aureus* with clear zone values of 12.00 ± 1.41 and 12.33 ± 0.47 mm, respectively. MIC analysis showed that n-hexane fraction had the strongest activity value against *P. aeruginosa*, *E. coli* and *B. subtilis* with MIC value of 500, 125 and 500 μg/mL, respectively. These results indicate that the extract and active fractions from the stem bark of the *H. spicata* have the potential to be developed as an alternative raw material for pharmaceutical uses.

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1 Introduction

During the last decades there has been an increasing interest in the study of medicinal plants and their benefit values in different parts around the world. Compounds derived from medicinal plants have been studied due to their promising pharmacological activities, economic viability, and low toxicity [1]. This revival of interest in medicinal plant derived active compounds leads to drugs is mainly due to the recent widespread belief that green medicine is safer and more reliable than the costly general synthetic drugs, some of which might have diverse side effects [2]. This development could lead to new drugs discovery or advance investigation to the use of indigenous herbal medicines.

Resistance in microbial due to overuse of antibiotics has become a global concern in health care systems [3]. The discovery and development of newly active compounds against superbugs microbial and to overcome antimicrobial resistance is very crucial issues. In addition to health problems with resistance in microbial, the preference for searching new natural antioxidants is also increasing to counteract the problem of non-infectious or degenerative diseases. Reactive oxygen species (ROS) are by-products of biological processes that might promote oxidative stress and damage to cellular functions [4]. To counteract the negative effects of exposure ROS in the cells, exogenous antioxidants are required. The primary characteristics of antioxidant agents are their capability to stabilize and capture free radicals, along with inhibit the occurrence of free radical reactions [5]. These corresponding mechanisms are crucial in the prevention of some diseases including coronary heart disease, and cancer [6].

Horsfieldia spicata (Roxb.) J. Sinclair is a hardwood tree belonging to the *Myristicaceae* family, widely distributed in New Guinea and other southeast Asian regions including Indonesia (mainly from Sulawesi Island). Traditionally, this plant used for wood, which very limited are showed potential pharmacological benefits [7]. Interestingly, in previous studies, extract from *H. spicata* was showed potential activity against ring stage erythrocytic parasites, therefore could inhibit the trophozoite and schizont stages [8]. Moreover, procyanidins derivatives namely *Myristicyclins* A and B have been isolated from the mix of *H. spicata* wood, leaves, and twigs, which has promising antimalaria agents [9]. Despite reports on the phytochemicals and antimalaria activity of *H. spicata* extracts, various aspects related to these phytochemicals including antioxidant and antibacterial activity focusing to the stem bark parts are remain unexplored. Therefore, in this study, we aimed to determine the antibacterial and antioxidant activities derived from *H. spicata* stem bark extract and its active fractions.

2 Materials and Method

2.1 Materials and Chemicals

The materials used in this study were dried stem bark simplicial from the stems of *H. spicata*. Of note this plant was collected from Bogor botanic garden, Bogor, Indonesia (GPS

location: 6°35'53.4"S 106°47'58.0"E). This plant was determined by a botanist in the Bogor Botanic Gardens (Center for Plant Conservation), National Research and Innovation Agency (BRIN), Indonesia, which its voucher specimen was deposited in the Bogor Botanic Gardens (the collection number of IV.H.135a). As for bacterial tested including *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (FNCC-0195), *Bacillus subtilis* (FNCC-0059), and *Staphylococcus aureus* (FNCC-0047) were purchased from Food Nutrition Cultur Collection of Gadjah Mada University, Indonesia. Other material used in this current study are Nutrient agar (NA), Mueller Hinton Broth (MHB), 1,1-diphenyl-2-picrylhydrazyl (DPPH), dimethylsulfoxide (DMSO), methanol, n-hexane, ethylacetate, butanol, aqudest, quercetin (Sigma Aldrich Q4951 -10G), streptomycin (Sigma), Circular printed Whatman filter paper with a diameter of 6 mm.

2.2 Extraction Processes

Dried stem bark simplicial from *H. spicata* as much as 1500 g. The extraction stage starts from drying with a blower oven at a temperature of 50ºC, then mashed by cutting into small pieces and roughly blending. Subsequently, maceration was conducted by soaking the simplicial with 1500 mL of methanol solvent for 24 h. Further, 3 repetitions of all substances in the sample are extracted. The filtrate was filtered and dried using a rotary evaporator to obtain methanol extract.

2.3 Fractionation

A total of 110 g of methanol extract of the stem of *H. spicata* was separated using n-hexane: water in a ratio (1:1), ethyl acetate: water (1:1) and butanol: water (1:1). The filtrate was separated and dried using a rotary evaporator to obtain n-hexane, ethyl acetate, butanol, and water fractions.

2.4 Antioxidant Activity

The antioxidant activity of the extract and stem fraction was performed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method developed by Megawati et al. [10]. Quercetin standard was dissolved in methanol with an initial concentration of 1000 μg/mL and the final concentration was made at 1, 2.5, 5, 10 μg/mL after adding 500 μL 1,1-diphenyl-2-picrylhydrazyl (DPPH) 1 mM solution in methanol with the total volume of 2.500 μL, while the sample solution is made with an initial concentration of 1000 μg/mL and the final concentration of 10, 25, 50, 100 and 200 μg/mL after adding 500 μL of 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution 1 mM in methanol and the total volume of the mixture is 2500 μL. The solution without sample or blank was filled with 2000 μL of methanol solution plus 500 μL of 1,1-diphenyl-2-picrylhydrazyl (DPPH) 1 mM solution in methanol. The standard solutions, test samples and blanks were then incubated at 37 °C for 30 min, the absorption was measured using a UV-Vis spectrophotometer at a wavelength of 515 nm. The amount of DPPH radical absorption inhibition (% inhibition) determined the presence of antioxidant activity in the sample which can be calculated using following equation:

$$\%\ Inhibition = ((Blank\ Absorbance - Sample\ absorbance)/(Blank\ absorbance)) \times 100\%$$

The ability of standard and test samples to inhibit DPPH free radicals by 50% was determined by the concentration value at 50% inhibitory concentration (IC_{50}). Antioxidant activity data described in the form of IC_{50} values were obtained based on the regression value equation between concentration (X axis) and % inhibition (Y axis).

2.5 Antibacterial Activity by Disc Diffusion Method

Testing the antibacterial activity of extracts and fraction using the disc method developed by Bashiti et al. [11]. The test sample was dissolved with 1% dimethylsulfoxide (DMSO) with a sample concentration of 4000 μg/mL. Bacterial strains were recultured with nutrient agar (NA) media in an incubator at 37 °C for 18–24 h. Antibacterial activity was carried out by agar diffusion by adding 100 μL of bacterial solution into a sterile petri dish and 10 mL of sterile nutrient solution while stirring until homogeneous and allowed to stand for 15 min. After the agar solidified, a disk with a diameter of 6 mm was added. Next, 10 μL of the sample was dripped onto the disk and incubated for 18 h. The antibacterial activity would be indicated by a transparent and clear zone around the disc. We employed 1% DMSO and tetracycline (200 μg/mL) as negative and positive controls, respectively.

2.6 Determination of MIC and MBC Values

MIC value was determined using a standard dilution technique [12]. In short, each of 96 well plate was filled with 100 uL MHB medium. Next, 4 mg/mL samples in 1% DMSO was serially diluted on each well with each volume of 100 μL. Each concentration of extract was mixed with bacterial culture in equal volume to make a total volume of 200 μL. Each bacterial cells were set up in 0.85% NaCl sterile and adjusted to McFarland standard 0.5 (equivalent to 1×10^8 CFU mL^{-1}) and then filled to the each well with the dilution to achieve the cells number of 5×10^7 CFU mL^{-1}. 1% DMSO and tetracycline were applied for negative and positive control, respectively. All treatments were incubated at 30 °C overnight. The MIC of sample was determined by observing the clear visual of the well. As for MBC value was measures to be the lowest concentration of the extract that could suppress 100% the bacterial growth observing on NA plate medium.

2.7 Statistical Analyses

All data were collected as mean ± SEMs (3 replications). Analysis was carried out using one-way Analysis of Variance (ANOVA) followed by multiple Duncan test range. A p-value of less than 0.05 was considered as statistically difference.

Table 1. Yield of extract and fractions from *H. spicata* stem bark.

Samples	Yield (%)
Methanol extract	7.72
n-Hexane fraction	8.47
Ethyl acetate fraction	3.39
Butanol fraction	9.77
Water fraction	23.42

3 Results

3.1 Yield of H. Spicata Stem Bark Samples

Based on the results of the extraction and fractionation processes, we obtained that the largest yield of *H. spicata* stem bark was the water fraction of 23.42%. On the other hand, the smallest yield was the ethyl acetate fraction of 3.39% (Table 1).

3.2 Antioxidant Activities of H. Spicata Stem Bark Samples

The results for the DPPH free radical scavenging of all samples and known antioxidants were presented in Fig. 1. Of note, the lowest IC_{50} value indicates stronger activity. The results of one-way ANOVA test indicated that there was a significant difference of mean percentage scavenging between all the tested samples. The results showed that ethyl acetate fraction exhibited the greatest DPPH free radical scavenging activity among all samples with an IC_{50} value of 23.53 μg/mL. On the contrarily, n-hexane fractions showed the higher IC_{50} value represent the lowest antioxidant activity than others.

3.3 Antibacterial Properties of H. Spicata Stem Bark Samples

Antibacterial activity by disc diffusion method results of *H. spicata* stem bark methanol extracts and its fractions were given in Table 2. As for the most active sample was showed in Fig. 2. In general, the mean zone of inhibition produced by the commercial antibiotic, tetracycline, and streptomycin, was between 18.67 to 31.33 mm and was larger than those produced by all samples which was between 7.67 to 15.33 mm. Based on these results, methanol extract showed the highest zone of inhibition against *P. aeruginosa* and *B. subtilis* with clear diameter zone of 15.33 and 13.33 mm, respectively. As for the best activity against *E. coli* and *S. aureus* was found in h-Hexane fraction with clear diameter zone of 12.00 and 12.33 mm, respectively. On the other hand, 1% DMSO (negative control) did not exhibit any effect on all the tested bacteria.

3.4 MIC and MBC Values of H. Spicata Stem Bark Samples

Furthermore, we carried out other antibacterial test using the MIC and MBC methods. Notably, the smaller the value of MIC and MBC, the more active the sample as an

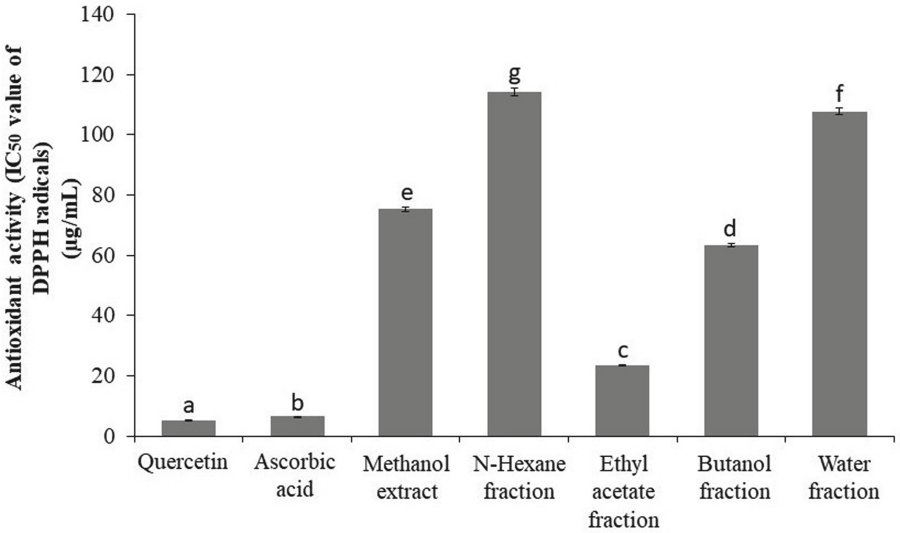

Fig. 1. Antioxidant activity using DPPH radical of *H. spicata* stem bark extract and fractions. The different letters of superscript of a-g indicate the mean percentage scavenging (IC_{50} value) between the tested populations is significantly different based on statistical ANOVA analysis.

antibacterial compound. Interestingly, the n-hexane fraction had the strongest activity value against *P. aeruginosa*, *E. coli* and *B. subtilis* with MIC values of 500, 125 and 500 µg/mL, respectively. As for the smallest MIC value against *S. aureus* was found in methanol extract with MIC value of 31.25 µg/mL. However, all the activities of the best samples were still lower than the positive control of tetracycline and streptomycin (Table 3).

4 Discussion

The solvent used for the extraction of medicinal plants is crucial step in the process for investigation the potential pharmaceutical properties of plants compounds. The choice of solvent depends on the type of plant, nature of the bioactive compounds, part of plant to be extracted, and the availability of solvent. In general, polar solvents such as water, methanol, and ethanol are used in extraction of polar compound, whereas nonpolar solvents such as hexane and dichloromethane are used in extraction of nonpolar compounds [13]. Based on several references, methanol is one of the best solvents that can be used to extract active compounds from plants with better yields and diversity of compounds so that they have the potential to have diverse promising bioactivity. Dhanani et al. [14] reported that the extraction of plant *Withania somnifera* using methanol as a solvent resulted high yields with high antioxidant activity. In addition, methanol was also used to extract compounds from *Severinia buxifolia* with a yield of 33.2% and good antioxidant, anti-bacterial and anti-inflammatory activities from the extract [15]. Therefore, we also used methanol to extract the stems bark of *H. spicata* and proven obtaining high yields. Interestingly, the water fraction is known to have the highest yield

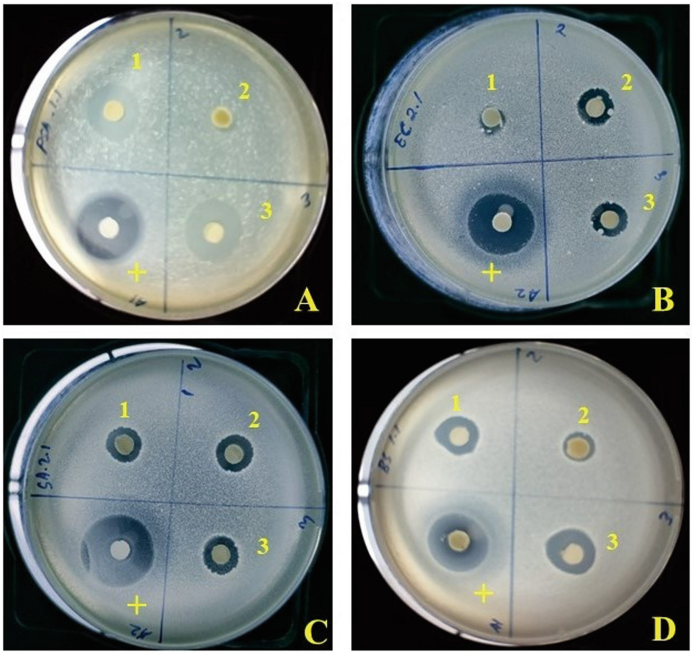

Fig. 2. Representation of antibacterial activity from extract and fraction from *H. spicata* stem bark by disc diffusion methods against some bacterial tested including A. methanol extract against *P. aeruginosa*; B. n-hexane fraction against *E. coli*; C. n-Hexane fraction against *S. aureus*; D. methanol extract against *B. subtilis*; each sample applied on 4 mg/mL; 1% DMSO and (+) 200 μg/mL tetracycline used for negative and positive control, respectively. 1,2, and 3 indicates repetition of samples.

Table 2. Antibacterial activity of extract and fraction from *H. spicata* stem bark by disc diffusion method

Samples	**Bacterial tested**			
	P. aeruginosa	***E. coli***	***S. aureus***	***B. subtilis***
	Diameter of clear zone (mm) ± SD			
Methanol extract	15.33 ± 4.50	10.67 ± 1.25	11.00 ± 0.82	13.33 ± 2.36
n-Hexane fraction	12.00 ± 3.56	12.00 ± 1.41	12.33 ± 0.47	11.67 ± 0.94
Ethyl acetate fraction	10.67 ± 2.62	9.00 ± 0.82	7.00 ± 0.82	9.00 ± 0.00
Butanol fraction	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
Water fraction	6.00 ± 0.00	7.67 ± 1.25	6.00 ± 0.00	6.00 ± 0.00
Tetracycline	24.00 ± 0.00	18.67 ± 0.94	27.67 ± 0.94	24.00 ± 2.94
Streptomycin	21.33 ± 2.05	28.67 ± 1.25	29.33 ± 0.94	31.33 ± 0.94

Table 3. MIC and MBC values of *H. spicata* stem bark extract and its fractions

Samples	Bacterial tested			
	P. aeruginosa	***E. coli***	***S. aureus***	***B. subtilis***
	MIC/MBC (μg/mL)			
Methanol extract	1000/>1000	1000/>1000	31.25/>62.5	1000/>1000
n-Hexane fraction	1000/>1000	1000/>1000	15.625/>31.25	1000/>1000
Ethyl acetate fraction	500/>1000	125/>250	125/>250	500/>1000
Butanol fraction	1000/>1000	1000/>1000	500>1000	1000/>1000
Water fraction	1000/>1000	1000/>1000	1000>1000	1000/>1000
Tetracycline	7.8125/>15.625	7.8125/>15.625	7.8125/>15.625	7.8125>15.625
Streptomycin	7.8125/>15.625	7.8125/>15.625	7.8125/>15.625	7.8125>15.625

fraction, this is presumably due to the dominant polarity of the compound so that it can bind nicely with water and produce high yields.

To evaluate the scavenging effect of the samples, DPPH reduction was investigated against positive controls (ascorbic acid and quercetin). The more antioxidants showed in the sample; the more DPPH reduction will happen. High reduction of DPPH is related to the high scavenging activity showed by sample. At a higher concentration, these samples may exhibit more significant free radical inhibition activity. Of note, IC_{50} value was calculated as amount of antioxidant present in samples in the term inhibition concentration of 50% [16]. The best IC_{50} value recorded in this study was stated on ethyl acetate fraction with an IC_{50} value of 23.53 μg/mL. Even though, it still lower than positive control, ascorbic acid, and quercetin. This is might due to the difference between sample that still on fraction which containing numerous compounds and positive control which belong to pure compounds with specific antioxidant activity. However, comparing with some previous reports, our fraction still has relatively high antioxidant activity. Study on antioxidant activity derived from *H. spicata* extracts remained underreported, indeed it comes from other *Horsfieldia* genus such as *H. glabra* seed extract with the DPPH IC_{50} value of 358 μg/mL [17]. Comparing with our potential fractions, *H. glabra* had ±23 lower antioxidant activity. It indicated that the ethyl acetate fraction shows slightly higher radical scavenging activity which may be attributed to its stronger proton-donating abilities.

Plant extract has long been a very crucial source of drug discovery with numerous therapeutic properties [18]. Therefore, we further evaluated the antimicrobial activity of *H. spicata* stem bark extract and its active fractions. In recent study, the antibacterial activity of the samples was evaluated by using standard disc diffusion and MIC along with MBC methods. The bacteria chosen to be studied were gram negative *E. coli*, and *P. aeruginosa* along with gram positive, *S. aureus* and *B. subtilis*. These bacteria were chosen to be studied as they are important model for pathogenic bacteria and due to rapidly developed antibiotic resistance as antibiotic use extremely increases [16]. In this study, the mean zone of inhibition produced by the commercial antibiotic, tetracycline,

and streptomycin, was larger than those produced by all samples. It may be attributed to the fact that the samples being in crude or fractions form contain a smaller concentration and diverse of active compounds [19]. In classifying the antibacterial capacity, it would be generally expected that much higher number would be active against gram positive than gram negative bacteria [20]. Thus, this is in accordance with our study which showed that the most active fraction was n-Hexane fraction against gram-positive bacteria, *S. aureus* with IC value of 15.625 μg/mL. Interestingly, as comparing with previous study like *H. glabra* seed extract has antibacterial activity against *S. aureus* with MIC value of 15.62 mg/mL [17]. Therefore, our prospective fraction has the anti- *S.aureus* with MIC value of ± 1000 fold higher than *H. glabra* extract. The variations in this activity might be due to the composition of the active compounds. In line with antimicrobial activity, previous study reported the cytotoxic properties derived from compound isolated from another genus of Horsfieldia namely *H. irya* with an IC_{50} value of 4.53 ± 0.05 and 4.53 ± 0.16 lg/mL, against HeLa and HCT116 cell lines, respectively [21].

5 Conclusion

In conclusion, we managed to get prospective active fractions, ethyl acetate and n-Hexane fractions, from *H. spicata* stem bark metabolites which has promising antioxidant and antibacterial properties. To the best of our knowledge, our study is the first to show a potential pharmaceutical activities of *H. spicata* stem bark. These interesting results remain to be deepened by further analyses to elucidate of primarily compounds correspond to its promising activity, but our findings should provide a new perspective for the studies and development of phytochemical analysis derived from the *H. spicata* stem bark.

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References

1. Prashant KR, Dolly J, Singh KR, Gupta KR, Watal G. Glycemic properties of *Trichosanthes dioica* leaves. Pharm. Biol. 2008; 46(12): 894-899.
2. Jigna P, Sumitra C. In-vitro antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). Afr. J. Biomed. Res. 2006; 9: 89-93.
3. Ferri M, Ranucci E, Romagnoli P, Giaccone V. Antimicrobial resistance: A global emerging threat to public health systems. Crit. Rev. Food Sci. Nutr. 2017; 57(13): 2857-2876.
4. Cui JL, Guo TT, Ren ZX, Zhang NS, Wang ML. Diversity, and antioxidant activity of culturable endophytic fungi from Alpine plants of *Rhodolacrenulata, R. angusta* and *R. schalinensin.* PLoS ONE 2015; 10(3): e0118204.
5. Prakash A, Rigelhof F, Miller E. Antioxidant activity. Eur. Rev. Med. Pharmacol. Sci. 2011; 15(4): 376–378.

6. Leong LP, Shui G. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem. 2002; 76(1): 69-75.
7. Powell JM. In New Guinea Vegetation; Paijmans, K., Ed.; Commonwealth Scientific and Industrial Research Organization in Association with the Australian University Press: Canberra, 1976.
8. Grimberg BT, Erickson JJ, Sramkoski RM, Jacobberger JW, Zimmerman PA. Monitoring *Plasmodium falciparum* growth and development by UV flow cytometry using an optimized Hoechst-thiazole orange staining strategy. Cytometry A 2008; 73(6): 546-554.
9. Lu Z, Van Wagoner M, Pond CD, Pole AR, Jensen JB. et al. Myristicyclins A and B: Antimalarial procyanidins from *Horsfieldia spicata* from Papua New Guinea. Org. Lett. 2014; 16: 346-349.
10. Megawati, Saepudin E, Hanafi M, Darmawan A, Lotulung PDN. Identification and bioactivity studies of flavonoid compounds from *Macaranga hispida* (Blume) Mull. Arg. Makara J. Sci. 2015; 19: 96-100.
11. Luangnarumitchai S, Lamlertthon S, Tiyaboonchai W. Antimicrobial activity of essential oils against five strains of *Propionibacterium acnes*. Mahidol Univ J. Pharm. Sci. 2007; 34(104): 60-64.
12. Priyanto JA, Prastya ME, Sinarawadi GS, Datu'salamah W, Avelina TY, Yanuar AIA, Azizah E, Tachrim ZP, Mozef T. The antibacterial and antibiofilm potential of *Paederia foetida* Linn. leaves extract. J Appl Pharm Sci 2022; 12(10): 117-124.
13. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 2017; 6(42): 1-23.
14. Dhanani T, Shah S, Gajbhiye NA, Kumar S. Effect of extraction methods on yield, phytochemical constituents, and antioxidant activity of *Withania somnifera.* Arab J. Chem. 2017; 10: 1193-1199.
15. Truong D, Nguyen DH, Anh Ta NT, Bui AV, Do TH, Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-Inflammatory activities of *Severinia buxifolia.* J. Food Quality 2019; Article ID 8178294: 1-9.
16. Chew AL, Jessica JJA, Sasidharan S. Antioxidant, and antibacterial activity of different parts of *Leucas aspera.* Asian Pac. J. Trop. Biomed 2012; 2(3): 176-180.
17. Chaicana N. Nutrition composition and analysis of medicinal herb potential of *Horsfieldia glabra* Warb. Seeds. KMUTNB Int. J. Appl. Sci. Technol. 2016; 9(1): 61-69.
18. Rosy BA, Joseph H, Rosalie. Phytochemical, pharmacognostical, antimicrobial activity of *Indigofera spalathoids* Vahl., (Fabaceae). Int. J. Biol. Technol. 2010; 1: 12-15.
19. Zuraini Z, Yoga LL, Suryani S, Sasidharan S. Anti-*Candida albicans* activity of crude extract of the local plant, winged beans leaf. Plant Kuala Lumpur 2007; 80: 653-657.
20. Joshi B. Sah GP, Basnet BB, Bhatt MR, Sharma D, Subedi K. et al. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). J Microbiol Antimicrob 2011; 3(1): 1-7.
21. Suthiwong J, Sribuhom T, Wongphakham P, Senawong T, Yenjai C. Cytotoxicity of acylphloroglucinol derivatives from the fruits of *Horsfieldia irya.* Nat. Prod. Res. 2020; 35(23): 1-9.