The Metabolite Fingerprints, Antimalarial Activities and Toxicities of *Artocarpus champeden* Stembark from Various Regions in Indonesia

Imam Taufik¹, Ati Widyawaruyanti², Mochammad Yuwono³

1. National Agency of Drug and Food Control (BPOM RI), Ambon, 97116, Indonesia
2. Department of Pharmacognosy and Phytochemistry, Airlangga University, 60115, Indonesia
3. Department of Pharmaceutical Chemistry, Airlangga University, Surabaya, 60115, Indonesia

**ABSTRACT**

In Indonesia, cempedak (*Artocarpus champeden* Spreng) stembark from family of moraceae had been traditionally used for malarial treatment. Difference in the location of growth could cause the difference of metabolite fingerprints. As a result, there might be different toxicity and antimalarial activity in the same plants. The goal of this study was to obtain the fingerprints of the metabolites found in *A. champeden* stembark from different parts of Indonesia in order to authenticate and control the extract's quality. Fingerprints were performed using the HPTLC-Densitometry technique, *in vitro* toxicity and antimalarial activity were also determined using MTT assay and HRP2 assay. The correlation between metabolite fingerprints, toxicity and antimalarial activity was analysed using chemometrics tools: Principle Component Analysis (PCA), Partial Least Square (PLS) and Hierarchical Clustering Analysis (HCA). As a result, there is significant difference between fingerprints and toxicity profiles of *A. champeden* (p<0.05), whereas for antimalarial profiles, there is no significant difference between of them (p>0.05). Meanwhile, the nutrients (copper, zinc and manganese) are suspected to be responsible for the metabolite content. Besides morachalcone-A, compounds with Rf values of 0.66 and 0.63 can be proposed as additional markers because they have responsibility for antimalarial activity and toxicity. In conclusion, *A. champeden* from Maluku with the highest antimalarial activity (60.41+5.67 μg/mL) and safety at a therapeutic dose of 10 ppm (97.45+5.76) is recommended as a source of material for the development of herbal medicine.

**Keywords:** *Artocarpus champeden*, metabolite fingerprints, antimalarial, chemometrics

**INTRODUCTION**

*Artocarpus champeden* Spreng (Moraceae) also known as “cempedak” in Indonesia, was originally used to treat malaria, fever, and diarrhea (Hafid et al., 2012). Malaria is the most common parasite infection, with an estimated 219 million new cases and 435,000 fatalities per year. In Indonesia, the number of malaria cases was 600,000 cases and the mortality rate was 1% (WHO, 2020). Currently, there have were many multi-drug resistant malaria cases, including in Indonesia (Elyazar et al., 2011). For this reason, this research has been carried out on medicinal plants as an alternative to antimalarial drugs (Noronha et al., 2020). *A. champeden* was spread widely throughout the subtropical and tropical zone of South-East Asia (Lopes et al., 2018). *A. champeden* contained several prenyl-flavonoids isolated from stembark of this plant as *in vitro* antimalarial against *P. falciparum* 3D7 strain and *in vivo* against *P. berghei* (Widyawaruyanti et al., 2011). The cytotoxicity of *Artocarpus sp* against *P. falciparum* strain K1 and 3D7 also had been reported (Musthapa et al., 2010). Morachalcone-A has also been described as an antimalarial active marker molecule of *A. champeden* (Hafid et al., 2012).

The development of *A. champeden* extract as a phytopharmaca had been carried out (Widyawaruyanti, 2013). However, the development herbal medicine met the problem caused by the variation of metabolites of plants.
Internal and environmental factors influenced the secondary metabolite content (Verma, 2015). Environmental influences may have a qualitative and quantitative impact on secondary metabolites, resulting in a range of bioactivities (Ningsih et al., 2015). As a result, herbal plant metabolite fingerprinting investigations are critical for guaranteeing their safety and efficacy (Kim et al., 2011).

Metabolite fingerprinting approaches using HPTLC has several advantages like: the analysis time is shorter and the fingerprint can be tailored to certain target molecules and costs (Adhami et al., 2013). For testing in vitro antimalarial activity of A. champeden, the HRP2 method, a simple enzyme-linked immune sorbent assay (ELISA) technique can be used and the advantages were more sensitive, faster, easier and could be applied on many samples (Widyawaruyanti et al., 2014). For testing in vitro toxicity of A. champeden, the MTT assay as one of the tools used to compare and evaluate can be used (Scherlie, 2011). This in vitro cytotoxic technique has advantages like reduced cost, speed and potentially to be automated with other instruments (Aslanturk, 2018).

Until now, there has never been a paper made on A. champeden metabolite fingerprinting, antimalarial activity or toxicity from diverse areas in Indonesia. In this work, HPTLC, MTT and HRP2 technique were used to determine the active and negative markers of A. champeden extract for standardization purpose of herbal medicine raw materials.

**MATERIAL AND METHODS**

**Plant materials and extractions**

Twenty-five samples of A. champeden Spreng stem bark were collected from 5 different regions in Indonesia: Papua, Maluku, Kalimantan, Sumatera and Java (Table 1). The plants should have age 20-40 years old and 200 g stem bark were harvested at 2:00-4:00 pm LT in dry season. Plant identification and authentication were carried out at the Bogorienese Herbarium, Indonesian Research Centre for Biology, West Java (Rahajoe, 2016). All samples were dried on oven 40°C for 5 days and ground until be powder. 20g of powders were weight and extracted by maceration technique assisted ultrasonic using 80 percent ethanol for two to three times replication. Then, using rotavapor, the ethanol extracts were filtered, pooled, and dried. The extracts also could be dried using oven at 40°C to help be dried extracts. All of them were kept in vials and stored in 4°C refrigerator. Next, they could be used to determine in antimalarial assay and metabolite fingerprinting (Widyawaruyanti et al., 2014).

**Mobile phase optimization**

Standard solution for HPTLC analysis: morachalcone-A standard solution (100 ppm) was dissolved in methanol. Sample solution for HPTLC analysis: each of 25 dried extracts of A. champeden (10,000 ppm) were dissolved in methanol. Optimization of mobile phase on Thin Layer Chromatography: (1) Ethyl acetic: H2O: formic acid: CH3COOH = 100:26:11:11 v/v/v/v; (2) Chloroform: methanol = 90:10 v/v; (3) Hexane: ethyl acetic: acetic acid = 20:10:1 v/v/v; (4) Ethyl acetic: hexane = 8:2 v/v (Pudumo et al., 2018; Reich & Schibli, 2011).

**Method validation**

Two parameters in the validation method for metabolite fingerprinting were stability and precision tests on a plate of HPTLC. Stability tests and precision were determined by applying 5μL of samples to a plate (concentrations of 10,000 and 20,000ppm) at 0, 15, 30, 45, 60, 75 and 90 minutes over three times. Stability test (on the plate) and precision test (inter day) were analyzed using PCA (Mutiah et al., 2019; Ningsih et al., 2015).

**Metabolite fingerprinting test**

HPTLC analysis condition: sample and standard solutions were applied on the HPTLC
plate SG 60 F254 20x10 cm. Each of samples was applied 2 µL. For 10 minutes, the plates were pre-conditioned with vapor of the developing solvents chloroform: methanol (95:5 v/v) before being developed up to 8 cm in the saturated solution for 20 minutes. After development and 5 min of automatic drying quantitative evaluation of the plates was performed by TLC Scanner-4. The plates were sprayed with sulfuric acid 10% and 100 µL of the parasite culture were mixed into the 96 well micro plates with a density of 2.3 x 10⁴ cells/well as much as 100 µL/well. The plates were then incubated for 24 hours and Huh7it cell culture would grow and stick to the bottom of the well microplate (Aty Widyawaruyanti et al., 2015).

Parasite culture
Parasite culture of this antimalarial assay was *Plasmodium falciparum* 3D7 strain. They were originally from the Malaria Laboratory of Eijkman Institute for Molecular Laboratory and maintained in the Malaria Laboratory of the Centre of Natural Product Medicine Research and Development, Institute Tropical Disease, Indonesia at 5% hematocrit. Culture was incubated using modified candle jar method at 37°C. Culture was diluted with medium and red blood cell to get 1.5% hematocrit and 0.05% parasitemia. Finally, this parasite culture was ready to be used for antimalarial assay (Hidayati et al., 2020).

Antimalarial assay and analysis
Antimalarial assay had been performed using HRP2 technique on single concentration. Twenty-five extracts of *A. champeden* were diluted until 10 µg/ml concentration. 100 µL of this extract and 100 µL of the parasite culture were mixed into the 96 wells-plate then incubated at 37°C for 72 hours. The optical density was read with an ELISA reader at 630 nm as maximum wavelength (Aty Widyawaruyanti et al., 2014). Inhibition percentage was calculated using formula:

\[ A = \frac{B - C}{B} \times 100\% \]

A= % Inhibition; B= optical density of control well; C= optical density of sample well

Cell culture
Huh7it cell culture was grown on DMEM media on 96 well micro plates with a density of 2.3 x 10⁴ cells/well as much as 100 µL/well. Incubation of 5% CO₂ 37°C for 24 hours and Huh7it cell culture would grow and stick to the bottom of the well microplate (Aty Widyawaruyanti et al., 2015).

Toxicity assay and analysis
MTT cell proliferation assay was utilized in the toxicity test for two concentrations 10 µg/mL (for safety profile) and 100 µg/mL (for toxicity profile) of *A. champeden* ethanol extract. Huh7it cells were treated with repeated dilutions of the samples or a control in 96 well plates. At 720 nm and 650 nm wavelengths, prepared wells were examined using an ELISA reader (Aty Widyawaruyanti et al., 2015).

\[ A = \frac{B}{C} \times 100\% \]

A= % Viability; B=tical density of treatment cell; C= optical density of control cell.

Soil nutrient analysis
The soil on which it was grown from five different locations in Indonesia was analyzed for its mineral content. The parameters tested included nine minerals. Phosphorus was tested by the Bray I method, calcium magnesium potassium sodium was tested by the percolation method of ammonium acetate and iron, copper, manganese and zinc were tested by AAS (Joy & R., 2017).

Statistical analyses
The results of metabolite fingerprinting were presented as visualization of PCA and PLS like score plot and loading plot. The HCA was presented as histogram. PCA, PLS and HCA were processed by Multi base v2015 (Lazar et al., 2015) add ins Microsoft Excel Professional Plus 2016. The results of antimalarial bioassay were expressed as mean ± SD. One-way analysis of variance was used to compare different mean values statistically (ANOVA). p<0.05 was used to identify a statistically significant difference. SPSS v16.0 was used to conduct the analysis (Widyawaruyanti et al., 2020).

RESULT AND DISCUSSION
Method validation
From the results of the stability test and precision test (intraday), it can be seen that the standard solution of Morachalcone-A in methanol during the test is at the 0; 15;30; 45 and 60 minutes and analyzed by PCA showed that the standard solution and sample were well separated in clusters (Figure 1.b). The total number of variations described by the three main components (PC1, PC2 and PC3) were 100.0%.
Figure 1 (a). Up: HPTLC metabolite fingerprints of ethanol extract of *A. champeden* at 366 nm using sulphuric acid 10% solution. Track M1-M5 (Maluku), P1-P5 (Papua), K1-K5 (Kalimantan), S1-S5 (Sumatera), J1-J5 (Java). Down: HPTLC densitogram using TLC Scanner-4 (Camag) at 366 nm. (b) PCA Profile for Stability Test (on HPTLC plate) and Precision Test (interday) Morachalcone-A Sample and Standard at 0 minute; 15; 30; 45 and 60 min After Spotting, PC1 vs PC2 (Up), PC1 vs PC3 (Middle), PC2 vs PC3 (Down)
Figure 2. (a) Principal Component Analysis (PCA) of *A. champeden* ethanol extract, left: loading plots, right: score plots, (up) PC1 vs PC2; (middle) PC1 vs PC3; (down) PC2 vs PC3. (b) Histogram of Hierarchical Clustering Analysis (HCA) of *A. champeden* from Maluku, Papua, Kalimantan, Sumatera and Java.
The stability test (on HPTLC plate for 60 min) and precision test showed that the RSD results for the standard, sample solutions of 10,000 ppm and 20,000 ppm were 0.03%; 0.09% and 0.11% (Requirement <2.0%). These results were confirmed by PCA analysis results which also showed the presence of cluster density. This shows that the sample and the spotted standard were stable for 60 min and showed good precision (Indrayanto, et al., 2019; Jayachandran et al., 2017; Ningsih et al., 2015).

**Metabolite fingerprints of A. champeden extract**

HPTLC fingerprinting (Figure 1.a) showed the similarity fingerprinting pattern of ethanol extract of *A. champeden* stembark from Maluku, Papua, Kalimantan, Sumatera, Java. The fingerprinting pattern indicated ethanol extract of *A. champeden*...
The Metabolite Fingerprints

Volume 32 Issue 4 (2021) 509

stembark contained polar flavonoid (lower retention factor) and nonpolar flavonoid (higher retention factor) (Reich & Schibli, 2011; Widyawaruyanti et al., 2011). HPTLC densitogram detected 5-10 metabolites of *A. champeden* from Maluku, 4-9 metabolites from Papua, 6-9 metabolites from Kalimantan, 4-12 metabolites from Sumatera and 6-10 metabolites from Java. Data also showed that not all of *A. champeden* stembarks were detected containing morachalcone-A. These results indicated that *A. champeden* of which might contain morachalcone-A in a smaller content. The age of *A. champeden* plants also affected secondary metabolites contained (Verma, 2015). Retention factor (Rf) data and chromatogram profile data explained that all of tracks had three secondary metabolites in Rf 0.63, Rf 0.66 and Rf 0.82. These results gave information about three candidate compounds that can be proposed as markers. These metabolites might be nonpolar flavonoid compounds (Aty Widyawaruyanti et al., 2011)

Principal Component Analysis (PCA)

PCA of pair Retention factor (Rf) and area percentage of all tracks in triplicate (Figure 2) showed a definite discrimination of samples didn’t have a good separation. The total number of variations described by the three main components (PC1, PC2 and PC3) were 28.5%. In order to visualize the significant metabolites affected to metabolite fingerprinting of *A. champeden* from five different locations, the score plots and the loading plots of PCA were used. The score plots reflected the significant samples whilst the loading plots reflected the significant variable (Bro & Smilde, 2014; Sidou & Borges, 2020). There were three significant metabolites affected in loading plots i.e. Rf 0.82; Rf 0.63; and Rf 0.66 (PC1, PC2, PC3). It indicated that there were similarities among *A. champeden* plants growth in Maluku, Papua, Kalimantan, Sumatera and Java with the altitude 18.0-419.0 m and 20-40 years old of plant.

Figure 4. PLS between variable importance (VIP) as secondary metabolites and antimalarial activity (up), toxicity (down)
Hierarchical Clustering Analysis (HCA)

HCA was clustering technique to make groups of samples based on the similarity or difference to easy analyze (Pinteaux et al., 2020; Zhang et al., 2017). Data from all samples' histograms Figure 2 shows that samples were not properly categorized due to metabolite commonality across all samples. In spite of that, histogram also showed there was the similarity between A. champeden from Maluku and Java, also A. champeden from Kalimantan-Sumatera whilst A. champeden from Papua was different from others.

**In vitro antimalarial of A. champeden extract**

Bar graphic data in Figure 3 (up) explained the highest antimalarial activity was M2 (65.65±2.38) from Maluku and the lowest antimalarial activity was K5 (34.35±0.34) from Kalimantan. Data explained that the highest antimalarial activity was from Maluku (60.41±5.67), followed from Sumatera and Papua (56.46±6.72 and 54.63±7.28) whilst the lowest antimalarial activity were from Kalimantan and Java with the antimalarial value 51.50±10.25 and 51.84±7.51 respectively. One-way ANOVA result data showed that there was no significant difference of antimalarial activity profile among A. champeden from Maluku, Papua, Kalimantan, Sumatera and Java (p>0.05).

In vitro toxicity of A. champeden extract

In the results of the safety profile with an effective concentration of 10 ppm Figure 3.a (middle), the average of % viability ranged from 97.45±5.76% (Maluku) to 100.00±2.47% (Sumatra). This indicates that the use of A. champeden extract at an effective concentration of 10 ppm is not toxic to Huh7it cells. While on the results of the the most toxic is the A. champeden sample from Java (J1) with a value of 49.25±3.50% toxicity test with a concentration of 100 ppm (Figure 3.a) (down), the lowest % viability or and the highest % viability or less toxic samples were A. champeden from Papua (P5) and Sumatra (S1 and S4) 100.00±0.07%. From the results of the probit analysis (CC50), the extract that had the highest toxicity was A. champeden extract from Maluku (M2) with a CC50 value of 125.50±0.13 ppm followed by Sumatra (S2), Java (J4) and Kalimantan (K1) successively with a CC50 value of 222.82±0.14 ppm; 295.22±0.18 ppm; 353.15±0.20 ppm and the least toxic is from Papua (P5) with a CC50 value of 1364.00±0.34 ppm. Referring to the results of antimalarial activity and cytotoxicity results in Huh7it, it is recommended to develop raw material for A. champeden extract from Maluku. This is based on its highest antimalarial activity (60.41±5.67) at a dose of 10 ppm and its cytotoxicity is also safe (97.45±5.76) at a dose of 10 ppm (Figure 3.b).
Correlation between metabolite fingerprint and in vitro antimalarial activity

PLS in Figure 4 (up) was used in order to make correlation between significant secondary metabolites and their antimalarial activity in percentage of inhibition. PLS analysis generated the positive correlation with R values were 0.8244. Data showed three significant metabolites had high antimalarial activity i.e morachalcone-A, followed metabolite with Rf 0.66 and Rf 0.63 respectively.

Correlation between metabolite fingerprint and in vitro toxicity

PLS in Figure 4 (down) was used in order to make correlation between significant secondary metabolites and their toxicity in percentage of viability. PLS analysis generated the positive correlation and R values were 0.7412. Data showed three significant metabolites had high toxicity. They were Morachalcone-A, followed metabolites with Rf 0.66 and Rf 0.63 respectively.

Correlation between metabolite fingerprint and soil nutrient content

The results of the two-way annova test showed that the Cu, Mn and Zn contents were significantly different (p<0.05) in the soil content of the growing places of Maluku, Java, Sumatra, Kalimantan and Papua. In Figure 5, the mineral content is correlated with the clustering metabolite profile in Figure 2 which shows a positive correlation. Meanwhile, the content of Ca, P, Mg, K, Na, Fe did not show a positive correlation. Copper has an influence on protein metabolism by increasing the flavonoid content in plants through the reaction mechanism of the tricarboxylic acid cycle (Mamat et al., 2015). In addition, manganese can also increase the total flavonol content through flavonoid biosynthesis (Chen et al., 2020). Likewise with zinc which can also increase the flavonoid content in plants (Vojodi Mehrabani et al., 2017). The content of flavonoids in plants, especially prenylated flavonoids can increase antimalarial activity (Atilaw et al., 2020)

CONCLUSION

A. champeden from five different regions in Indonesia showed different in toxicity profile, antimalarial profile and metabolite fingerprints. Three metabolites were presented as analytical marker candidates, namely those with Rf 0.63, 0.66, and 0.82 as the greatest content. Two metabolites with Rf 0.63 and 0.66 could be used as antimalarial positive and negative indicators, respectively. A. champeden from Maluku had the highest activity (60.41±5.67 μg/ml) and from Papua had the lowest CC50 (1364.00±0.34 μg/ml). Based on the highest antimalarial activity (60.41±5.67) at a dose of 10 ppm and the cytotoxicity in HuH7it cells was also safe (97.45±5.76) at a dose of 10 ppm, so A. champeden from Maluku was recommended as a source of raw materials for antimalarial herbal medicines.

ACKNOWLEDGEMENT

The authors state that the publishing of this paper does not include any conflicts of interest. The authors confirmed that the paper was plagiarism-free. The authors would like to acknowledge to Indonesia Endowment Fund for Education (LPDP) for Master Program Scholarship funding.

REFERENCES

Adhami, H. R., Scherer, U., Kaehlig, H., Hettich, T., Schlotterbeck, G., Reich, E., & Krenn, L. (2013). Combination of bioautography with HPTLC–MS/NMR: a fast identification of acetylcholinesterase inhibitors from galbanum. Phytochemical Analysis, 24(4), 395-400.

Aslanturk, O. S. (2018). In vitro Cytotoxicity and Cell Viability Assays: Principles, Advantages, and Disadvantages. Genotoxicity - A Predictable Risk to Our Actual World, 1–19, https://doi.org/10.5772/17923

Atilaw, Y., Muiva-Mutisya, L., Bogaerts, J., Duffy, S., Valkonen, A., Heydenreich, M., Avery, V. M., Rissanan, K., Erdelyi, M., & Yenesew, A. (2020). Prenylated Flavonoids from the Roots of Tephrosia rhodesica. Journal of Natural Products, 83(8), 2390–2398. https://doi.org/10.1021/acs.jnatprod.0c00245

Bro, R., & Smilde, A. K. (2014). Principal component analysis. Analytical Methods, 6, 2812-2831, https://doi.org/10.1039/c3ay41907j

Chen, H., Yang, J., Deng, X., Lei, Y., Xie, S., Guo, S., Ren, R., Li, J., Zhang, Z., & Xu, T. (2020). Foliar-sprayed manganese sulfate improves flavonoid content in grape berry skin of Cabernet Sauvignon (Vitis vinifera L.) growing on alkaline soil and wine chromatic characteristics. Food Chemistry, 314, 126182. https://doi.org/10.1016/j.foodchem.2020.126182

de Oliveira Silva, E. de A. L. M. M. de S. K. O. (2018). Champedak—Artocarpus champeden. Exotic
Fruits, 121–127.
Elyazar, I. R., Hay, S. I., & Baird, J. K. (2011). Malaria distribution, prevalence, drug resistance and control in Indonesia. Advances in Parasitology, 74, 41–175.
Hafid, A. F., Ariantari, N. P., Tunewu, Lidy., Hidayati, A. R., Widyawaruwyanti, A. (2012). The active marker compound identification of Artocarpus champeden spreng. stem bark extract, morachalcone a as antimalarial. International Journal of Pharmacy and Pharmaceutical Sciences, 4(5), 246–249.
Hidayati, A. R., Widyawaruwyanti, A., Ilmi, H., Tanjung, M., Widiandani, T., Siswando, Syafrudin, D., & Hafid, A. F. (2020). Antimalarial Activity of Flavonoid Compound Isolated from Leaves of Artocarpus altillis. Pharmacognosy Journal, 12, 835–842. https://doi.org/10.5530/pj.2020.12.120
Indrayanto, G., Ayu, G., Ratih, M., Imawati, M. F., Nugroho, R. R., Purwanti, I., Wongs, S., & Prajogo, B. (2019). Phytochemicals of Gandarusa (Justicia gendarussa) and Its Preparations. Scientia, 14(6), 489-500. https://doi.org/10.1177/1934578X19851406
Jayachandran Nair CV, Ahamad S, Khan W, Anjum V, M. R. (2017). Development and validation of high-performance thin-layer chromatography method for simultaneous determination of polyphenolic compounds in medicinal plants. Pharmacognosy Research, 9, S67-73.
Joy, B. D. R. R., & R., S. (2017). Analisis Beberapa Sifat Kimia Tanah Terhadap Peningkatan Produksi Kelapa Sawit pada Tanah Pasir di Perkebunan Kelapa Sawit Selangkun. Jurnal Agrikultura, 28(1), 15–20
Kim, E. J., Kwon, J., Park, S. H., Park, C., SEO, Y. B., Shin, H. K., Kim, H. K., Lee, K. S., Choi, S. Y., Ryu, D. H., & Hwang, G. S. (2011). Metabolite profiling of Angelica gigas from different geographical origin using 1H NMR and UPLC-MS analyses. Journal of Agricultural and Food Chemistry, 59(16), 8806–8815. https://doi.org/10.1021/jf2016286
Lazar, G. A., Florina, R., Socaciu, M., & Socaciu, C. (2015). Bioinformatics Tools for Metabolomic Data Processing and Analysis Using Untargeted Liquid Chromatography Coupled With Mass Spectrometry. Animal Science and Biotechnologies, 72(2), 103–115. https://doi.org/10.15835/buasvmcn-11536
Mamat, D. D., C.-S. Chong, A. A. Samad, T.-T. C. and F. A. M. (2015). Effects of copper on total phenolics, flavonoids and mitochondrial properties of Orthosiphon stamineus callus culture. Int. J. Agric. Biol., 17, 1243–1248.
Musthapa, I., Hakim, E. H., Julawaty, L. D., Syah, Y. M., & Achmad, S. A. (2010). Preynel flavones from some Indonesian Artocarpus and their antimalarial properties. Medicinal Plants-International Journal of Phyto medicines and Related Industries, 2(2), 157–160.
Mutiah, R., Hadya, C. M., Burhan Ma’arif, Z. A., Bhagawan, W. S., Annisa, R., Indrawijaya, Y. Y. A., Huwaida, F. I., Ria Ramadhani, D. A., Susilowati, R., & Taufik, I. (2019). Metabolite fingerprinting of eleutherine palifolia (L) merr. By hptlc-densitometry and its correlation with anticancer activities and in vitro Toxicity. Indonesian Journal of Pharmacy, 30(3). https://doi.org/10.14499/indonesianjpharm. m30iss3pp157
Ningsih, I. Y., Intan Purwanti, D., Wongs, S., W Prajogo, B. E., & Indrayanto, G. (2015). Metabolite Profiling of Justicia gendarussa Burm. f. Leaves Using UPLC-UHR-QTOF-MS. Scientia Pharmaceutica, 83, 489–500. https://doi.org/10.3797/scipharm.1411-08
Noronha, M., Pawar, V., Prajapati, A., Subramanian, R. B. (2020). A literature review on traditional herbal medicines for malaria. South African Journal of Botany, 128, 292–303.
Pinteaux, E., Blasco-Fontecilla, H., Elahi, F. M., Wilcock, D. M., Winder, Z., Sidduth, T. L., Fardo, D., Cheng, Q., Goldstein, L. B., Nelson, P. T., Schmitt, F. A., & Jicha, G. A. (2020). Hierarchical Clustering Analyses of Plasma Proteins in Subjects With Cardiovascular Risk Factors Identify Informative Subsets Based on Differential Levels of Angiogenic and Inflammatory Biomarkers. Front. Neurosci., 14, 84. https://doi.org/10.3389/fnins.2020.00084
Pudumo, J., Chaudhary, S. K., Chen, W., Viljoen, A., Vermaak, I., & Veale, C. G. L. (2018). HPTLC fingerprinting of Croton gratissimus leaf extract with Preparative HPLC-MS-isolated marker compounds. South African Journal of Botany, 114, 32–36. https://doi.org/10.1016/j.sajb.2017.10.004
Rahajoe, J. S. (2016). Certificate of Determination, Authentication Number 2003/IPH.I.02/ If.07/IX/2016. Indonesian Institute of Science.

Reich, E., & Schibli, A. (2011). High-performance thin-layer chromatography for the analysis of medicinal plants. In Thiem. http://agris.fao.org/agris-search/search.do?recordID=US201300115573

Scherlie, R. (2011). The MTT assay as tool to evaluate and compare excipient toxicity in vitro on respiratory epithelial cells. International Journal of Pharmaceutics, 411(1–2), 98–105. https://doi.org/10.1016/j.ijpharm.2011.03.053

Sidou, L. F., & Borges, E. M. (2020). Teaching Principal Component Analysis Using a Free and Open Source Software Program and Exercises Applying PCA to Real-World Examples. Journal of Chemical Education, 97(6), 1666–1676. https://doi.org/10.1021/acs.jchedu.9b00924

Verma, S. S. (2015). Impact of various factors responsible for fluctuation in plant secondary metabolites. Journal of Applied Research on Medicinal and Aromatic Plants, 2(4), 105–113. https://www.sciencedirect.com/science/article/pii/S2214786115300152

Vojodi Mehrabani, L., Kamran, R. V., Hassanpourghadam, M. B., & Pessarakli, M. (2017). Zinc Sulfate Foliar Application Effects on Some Physiological Characteristics and Phenolic and Essential Oil Contents of Lavandula stoechas L. Under Sodium Chloride (NaCl) Salinity Conditions. Communications in Soil Science and Plant Analysis, 48(16), 1860–1867. https://doi.org/10.1080/00103624.2017.1406105

WHO. (2020). Malaria. World Malaria Report. https://www.who.int/data/gho/data/them/malaria

Widyawaruyanti, Aty; Zaini, N. S. (2011). Antimalarial Activity and Mechanism of Action of Flavonoid Compounds Isolated from Artocarpus champeden Spreng Stembark. Jurnal Biosains Pascasarjana, 13(2), 67–77.

Widyawaruyanti, A. (2013). Development of antimalarial phytomedicine of Artocarpus champeden: A model for antimalarial drugs discovery. The Open Conference Proceedings Journal, 4 (1), 10-10.

Widyawaruyanti, A, Harwiningtias, N., Tumewu, L., Hafid, A. F., & Soetjipto. (2020). Effect of Formulated Artocarpus champeden Extract on Parasite Growth and Immune Response of Plasmodium berghei-Infected Mice. Evidence-Based Complementary and Alternative Medicine : ECAM, 2020, 4678634. https://doi.org/10.1155/2020/4678634

Widyawaruyanti, Aty, Devi, A. P., Patria, N., Tumewu, L., Tantular, I. S., & Fuad Hafid, A. (2014). In vitro Antimalarial Activity Screening of Several Indonesian Plants Using HRP2 Assay. International Journal of Pharmacy and Pharmaceutical Science, 6, 125-128. http://repository.unair.ac.id/56542/

Widyawaruyanti, Aty, Khasanah, U., Tumewu, L., Ilmi, H., Fuad Hafid, A., & Tantular, I. S. (2015). Antimalarial Activity and Cytotoxicity Study of Ethanol Extract and Fraction from Alectryon Serratus Leaves. International Journal of Pharmacy and Pharmaceutical Sciences, 7, 250–253. http://repository.unair.ac.id/56571/

Zhang, Z., Murtagh, F., Van Poucke, S., Lin, S., & Lan, P. (2017). Hierarchical cluster analysis in clinical research with heterogeneous study population: highlighting its visualization with R. Annals of Translational Medicine, 5(4), 75. https://doi.org/10.21037/atm.2017.02.05