ISSN 2745-455X (Online)
Indonesian Journal of Pharmacology and Therapy

Antiplasmodial activity and cytotoxicity of kapur (*Harmsiopanax aculeatus*) leaf extracts traditionally used for the treatment of malaria in Maluku

Rachel Turalely[1,2], Mahardika Agus Wijayanti[3], Triana Hertiani[4], Mustofa[5]

[1]Chemistry Education Study Program, Faculty of Teacher Training and Education Science, Pattimura University, Ambon, Maluku, [2]Doctoral Program of Biotechnology, Graduate School, [3]Department of Parasitology, Faculty of Medicine-Public Health and Nursing, [4]Department of Biological Pharmacy, Faculty of Pharmacy, [5]Department of Pharmacology and Therapy, Faculty of Medicine-Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.

https://doi.org/10.22146/ijpther.512

Submitted: 20/07/2020
Accepted : 30/08/2020

Keywords:
cytotoxicity,
antiplasmodial activity,
Vero cells,
T47D cells,
HeLa cells,

ABSTRACT

Harmsiopanax aculeatus leaf, locally name *kapur*, has been use traditionally to treat malaria in Maluku, Indonesia. However, the scientific evidences that support its use are still limited. This study aimed to investigate antiplasmodial activity of *H. aculeatus* leaf extract and its cytotoxicity on cancer cells line. Three extracts i.e. methanolic, *n*-hexanic and ethyl acetate extracts were evaluated for their *in vitro* activity against *Plasmodium falciparum* FCR3 strain using microscopic method. Cytotoxicity of the extracts on T47D, HeLa and Vero cells lines were determined using MTT assay method. The inhibitory concentration 50% (IC50) against *P. falciparum* or the cells lines growth was determined using probit analysis. Furthermore, their selectivity index (SI) were determined. The results showed that the methanolic extract was the most active extract with an IC_{50}value of 13.82 µg/mL and the most selective with a SI value of 172.84. The three extracts tested exhibited weak or no cytotoxicity against the cells lines used with IC_{50} values ranged 101-2388.69 µg/mL. Further study will be conducted to isolate active antiplasmodial compounds from the methanol extract.

ABSTRAK

Daun *Harmsiopanax aculeatus*, secara local dinamkan tanaman kapur, telah digunakan secara tradisional digunakan untuk mengobati malaria di Maluku, Indonesia. Namun bukti ilmiah yang mendukung penggunaannya masih terbatas. Penelitian ini bertujuan mengkaji aktivitas antiplasmodial ekstrak daun *H. aculeatus* dan sitotoksiknya pada kanker sel. Tiga ekstrak daun kapur yaitu ekstrak methanol, *n*-heksan dan etilasetat aktivitasnya secara *in vitro* terhadap *Plasmodium falciparum* strain FCR3 menggunakan metode mikroskopi. Sitotoksisitas ekstak selanjutnya diuji pada kultur sel kanker payudara T47D, sel kanker servik HeLa dan sel normal Vero menggunakan metode uji MTT. Kadar penghambatan 50% (IC_{50}) terhadap pertumbuhan *P. falciparum* atau kultur sel tersebut ditetapkan dengan analisis probit. Selanjutnya indeks sel ektivitasnya (IS) ditetapkan. Hasil penelitian menunjukkan bahwa ekstrak methanol merupakan ekstrak paling aktif dengan nilai IC_{50} sebesar 13,82 µg/mL dan paling selektif dengan nilai IS sebesar 172,84. Ketiga ekstrak uji menunjukkan sitotoksik lemah atau tidak toksik terhadap kultur sel yang digunakan dengan nilai IC50 bervariasi 101-2388.69 µg/mL. Penelitian lanjutan akan dilakukan untuk mengisolasi senyawa aktif anti plasmodial dalam ekstrak metanol.

*corresponding author: mustofafk@ugm.ac.id

INTRODUCTION

Althoughthe malaria prevalence and incidence malaria were significantly reduced globally, malaria remains to be one of the most public health problems worldwide including in Indonesia. In 2018, an estimated 228 million cases and 405,000 deaths from malaria was reported worlwide.[1] In Indonesia, the most endemic areas are observed in the eastern regions of Indonesia including Maluku Province. In 20015, Maluku Province ranked fourthof high endemicity of malaria with annual parasite incidence (API) of 5.81% although new cases tended to decrease in last few years.[2]

Plasmodium resistance to antimalaria drugs available in clinic is the major problems in the treatment of malaria. Resistance to chloroquine in *Plasmodium falciparum* has led to adopt artemisinin combination therapy (ACT) as the first-line drug for the treatment of malaria in most endemic countries.[3] However, *P. falciparum* resistant to artemisinin has been reported in the several endemic countries.[4] Therefore, discover new chemical compounds with antimalarial activities is urgently needed. Medicinal plants traditionally used to treat malaria have been subjected to identify new antimalarial compounds.[5]

Medicinal plants have been used traditionally to treat malaria in various regions in Indonesia including in Maluku. One of the medicinal plants is *Harmsiopanax aculeatus* (Blume) Warb. Ex Boerl from Araliacease family which locally well known as *kapur* plant. It is used to treat malaria by drip of the young leaves sap on the patient's eyes. In order to search of active compounds from medicinal plants, *in vivo* antiplasmodial activity of *H. aculeatus* leaf extracts has been evaluated against *P. berghei* infected Swiss mice.[6] In this further study we reported *in vitro* antiplasmodial activity and cytotoxicity of kapur (*H. aculeatus*) leaf extracts.

MATERIALS AND METHODS

Plant collection and authentication

Kapur leaf samples were collected from Amahai Village, Central Maluku Regency, Maluku, Indonesia and determined in the Taxonomy Laboratory, Faculty of Biology, Gadjah Mada University, Indonesia as *H. aculeatus* (Blume) Warb. Ex Boerl (Araliaceae, Voucher number 1 HaA).

Preparation of plant extracts

A total of 2 kg of *kapur* leaf powder were extracted using multilevel maceration techniques using *n*-hexane (3x24 h), ethyl acetate (4x24 h) and methanol (4x24 h) respectively. The solvents in each obtained mass were evaporated, then the crude extract got was weighed to determine the rendemen of each extract. The compound profiles in the three extracts were identified using TLC both in visible light and under UV lamp.

In vitro antiplasmodial activity

The chloroquine-resistant *P. falciparum* strain (FCR3) was used in this study and cultures using a candle jar method according to Trager and Jensen after modification.[7,8] One hundred µL of the Plasmodium culture in ring stage, after synchronized with 5% sorbitol, in a final 2% haematocrit and 0.5% parasitemia was added into the wells of 96-well microtiter plate. Serial five-fold dilutions were prepared using the culture medium from stock solutions of the *kapur* leaf extracts to obtain working concentrations of 200 to1 $\mu g.mL^{-1}$, depend on each extract. One hundred µL of the extract solution was then added in the wells in triplicate. The wells containing culture medium without the extracts were used as negative control. The plates containing the mixture of the Plasmodium culture and the extracts solution were placed in a candle jar and incubated at 37°C for 72 h in a CO_2 incubator. Followed after incubation,

a thin blood smear of each wells was prepared and then Giemsa staining was conducted. Parasitemia of each the Giemsa stained thin blood smears was observed visually using a light microscope to calculate the Plasmodium growth. The antiplasmodial activity was expressed by inhibitory concentration 50% (IC_{50}) or concentration that inhibit 50% Plasmodium growth which determined using probit analysis with SPSS 16 for windows.

Cytotoxicity assay

Cytotoxicity of the hexane, ethyl acetate and methanol extract of *H. aculeatus* leaves were evaluated by using the MTT assay method as previously conducted.[8] Three cell lines i.e. Vero, T47D and HeLa cell lines were cultured in M199 cell culture medium and used in this study. Serial five-fold dilutions were prepared using the culture medium from stock solutions of the *kapur* leaf extracts to obtain working concentrations of 4000 to 12.5 $\mu g.mL^{-1}$, depend on each extract. One hundred µL of the extract solution was then added in the wells in triplicate. The wells containing culture medium without the extracts were used as negative control. The plate containing the mixture of the cells culture and the extracts solution was incubated at 37°C for 24 h in a CO_2 incubator. Followed after incubation period, the culture medium was removed from the wells and 25 µL of the MTT solution (2 mg/mL in PBS) was added to each well. The plates were incubated for 1.5 h at 37 °C, and 125 µL of DMSO was added to each well to dissolve the for mazan crystals. The plates were placed on a shaker for 15 min and the optical density was measured at a wavelength of 595 nm using ELISA Reader. The cytotoxicity was expressed by inhibitory concentration 50% (IC_{50}) or concentration that reduce 50% cell viability which determined using probit analysis with SPSS 16 for windows.

Thin layer chromatography (TLC)

The extracts were subjected to TLC analysis. The chromatograms were developed and dried on the silica gel TLC plates and the spots visually observed directly or under UV366 to characterize various chemical substances. The developing solvents used were the mixtures of n-hexane:chloroform (1:9), chloroform:ethyl acetate (8:2), andchloroform:ethyl acetate (9:1).

UV-Vis DADanalysis

The methanol extract was then subjected to UV-Vis DAD analysis using Dionex UV-Vis System. The sample was dissolved in a solvent and then as much as 20 uL injected in a Dionex device with the following conditions: Program Chromeleon version 6.3, Pump; Dionex P580A LPG, Detector; Dionex, UVD 340S Photodiode Array Detector; Column: Knauer, 5.0 mmID; packing material 5 mm and Eurospher-100 C-18). Isocratic column equilibration using methanol: acid nanopure water (pH 2 using phosphoric acid) with a ratio of 10: 9 for 5 min then the solvent was changed gradient to 100% methanol within 30 min later followed by 100% methanol for 40 min. Compounds that have a chromophore are detected using UV-Vis DAD at 235, 254, 280, and 340 nm.

The protocol of this study was approved by the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia (Ref: KE/FK/1152/EC/2016).

Dataanalysis

Data are presented as means ± standard deviation (SD). Selectivity index (SI) toward *P. falciparum* orT47D or HeLa cells lines versus Vero cell line were calculated from the following formula: SI = IC_{50} against Vero cell/IC_{50} against *P. falciparum* or T47D or HeLa cells lines.

RESULTS

***In vitro*antiplasmodialactivity**

Among three *H. aculeatus* leaf extracts tested, the methanolic extract with IC_{50} value of 13.82 µg/mL exhibited the most active antiplasmodial activity compared tothe extracts of *n*-hexane and ethyl acetate (TABEL 1).

TABLE 1. *In vitro* antiplasmodial activity of *H.aculeatus*leaf extracts

Extract	Concentration (µg/mL)	Growth inhibition (mean ± SD %)	IC_{50} (µg/mL)
Hexane	200	80.45±4.02	33.52
	50	52.78±3.85	
	25	39.12±4.12	
	5	25.97 ± 4.87	
	1	14.91± 3.76	
Ethyl acetate	200	64.67 ± 2.48	35.45
	50	50.02 ±3.42	
	25	45.12 ± 5.64	
	5	40.76 ± 2.56	
	1	22.18 ± 3.52	
Methanol	200	89.73 ± 2.68	13.82
	50	53.89± 3.54	
	25	50.06 ±3.29	
	5	41.76 ± 4.26	
	1	24.15± 3.28	

Cytotoxicity on T47D and HeLa and Vero cell lines

The cytotoxicity of the three *H. aculeatus* leaf extracts tested are presented on TABLE 2. All three extracts tested might not exhibit weak or no cytotoxicity against T47D, HeLa and Vero cell lines. The IC_{50} values of all the extracts tested ranged 101-2388.69 µg/mL.

TABLE 2. Cytotoxicity (IC_{50} in µg/mL) against T47D, HeLa and Vero cell lines

Extract	T47D	HeLa	Vero
Hexane	>200	101-200	667.74
Ethyl acetate	>200	101-200	262.99
Methanol	>200	101-200	2388.69

Selectivity Index (SI)

Selectivity index (SI) toward *P. falciparum* or cancer cells lines are presented in TABLE 3. All three extracts tested exhibited more selective toward the *P. falciparum* compared to the cancer cells lines. The SI values of all the extracts tested ranged <1.31-172.84.

TABLE 3. Selectivity index (SI) of *H. aculeatus* leaf extracts

Extract	*P. falciparum*	HeLa	T47D
Hexane	19.92	3.34 – 6.61	<3.34
Ethyl acetate	7.41	1.31 – 2.60	<1.31
Methanol	172.84	11.94 – 23.65	<11.94

Phytochemical analysis

The TLC chromatogram of *H. aculeatus* leaf extracts with visually and under UV 366 nm observation is presented in FIGURE 1. Some spots both in *n*-hexanic and methanolic extracts were observed in the ethyl acetate extract. Furthermore, some spots with blue and pink colors were observed in three extracts obtained that indicated the present of flavonoid in the extracts.

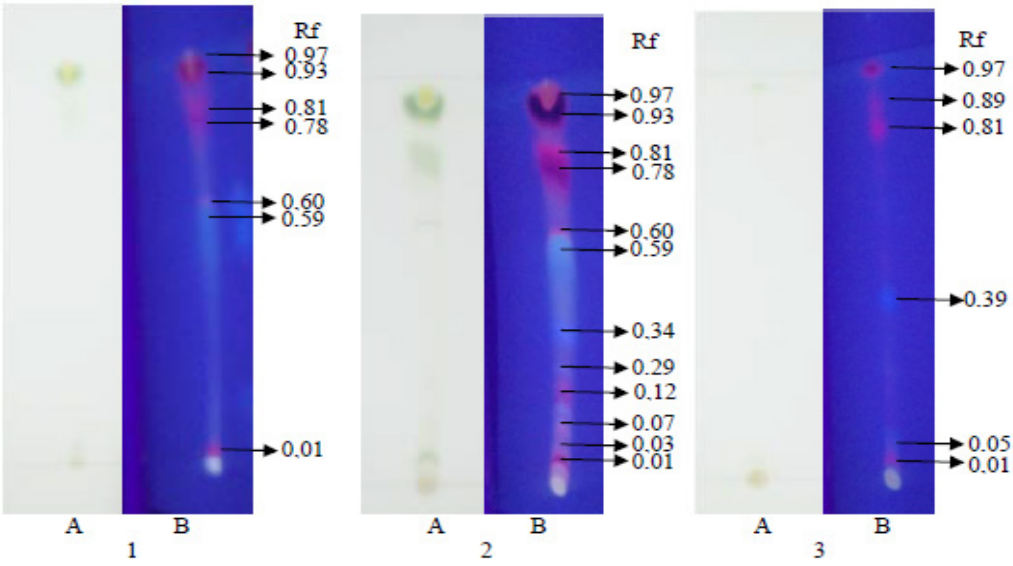

FIGURE 1. TLC profile of *H. aculeatus* leaf extracts. 1. *n*-Hexanic extractelucidatedusing n-hexane: chloroform (1:9); 2. Ethy acetate extract elucidated using chloroform : ethyl acetate (8:2); 3 Methanolic extract elucidated using chloroform : ethyl acetate (9:1); A=photo in visible light and B = photo in UV 366 nm.

UV-Vis DAD analysis

TABLE 4 presents the compounds identified in methanolic extract of *H. aculeatus* by UV-Vis DAD and database from the Institute of Biology of Pharmacy and Biotechnology, Heinrich Heine University, Duesseldorf, Germany. Twenty nine compounds were identified in the λ 235nm (11 compounds), λ 254nm (6 compounds), λ 280 nm (5 compounds) and λ 340 nm (7 compounds).

TABLE 4. DAD UV-Vis data and tentative structure assignmentof compounds separated from methanolic extract of *H. aculeatus*leaf.

	λ=235 nm	λ=254 nm		λ=280 nm		λ=340 nm	
t_R	Tentative Compound	t_R	Tentative Compound	t_R	Tentative Compound	t_R	Tentative Compound
18.28	Luteolin-7-glucoside (998.98)	3.160	nf	12.473	nf	16.193	nf
19.35	Apigenin monoglycoside (998.47)	12.473	nf	16.903	nf	17.443	nf
19.99	Hyperoside (=quersetin-3-galactoside; 998.64)	15.190	nf	19.357	nf	18.227	nf

21.35	Kaempferol-3-rutinoside (998.62)	18.789	nf	19.993	nf	19.357	nf
26.54	Nf	19.353	nf	21.350	nf	19.993	nf
30.97	Aureonitol (998.20)	19.993	nf			21.350	nf
31.78	nf					24.257	nf
32.52	Aurenitol (985.99)						
33.12	Cerebroside (996.09)						
36.05	Naamine (985.35)						
46.30	nf						

Note.Database was obtained from the Institute of Biology of Pharmacy and Biotechnology, Heinrich Heine University, Duesseldorf, Germany; t_R: retention time; nf: not found.

DISCUSSION

In this study, the methanolic extract exhibited the most active antiplasmodial activity (IC_{50}: 13.82 µg/mL). Previous studies reported that the antiplasmodial activity of a natural product or compound can be categorized as high activity or very active if the IC_{50}< 5 µg/mL, promising activity or active if IC_{50} between 5-15 µg/mL, moderate activity or weakly active if IC_{50} between 15-50 µg/mL and not active if IC_{50}> 50 µg/mL.[9,10] Based on these previous studies therefore, the methanolic extract of *H. aculeatus* leaf could be categorized as active extract.

Cytotoxicity assay is widely used for cytotoxicity tests of compounds and for anticancer screening. Vero cells was isolated from kidney epithelial cells of an African green monkey. It was used as normal cells to evaluate cytotoxicity of the tested extracts, while T47D (breast cancer cell line) and HeLa (cervical cancer cell line) were used to evaluate anticancer activity of the tested extracts. Based on the IC_{50} values obtained, an extract can be labeled as highly active if the IC_{50} value ≤ 20 μg/mL or moderate active if the IC_{50} value between 21-200 μg/mL or weakly active if the IC_{50} value between 201-500 μg/mL or inactive if the IC_{50} value >500 μg/mL.[11,12] Based on this criteria therefore, the methanolic extract exhibited non active or noncytotoxic, whereas the hexane and ethyl acetate extracts exhibited weakly active or weakly cytotoxic against Vero cells line. Furthermore, the three extracts tested exhibited weakly active against T47D (IC_{50}> 200 μg/mL) and HeLa (IC_{50} 201-500 μg/mL) cells lines.

Prayong *et al.*[13] proposed that extracts or samples with an SI greater than three are considered to have a high selectivity. Based on these criteria, the three extracts tested exhibited high selectivity toward *P. falciparum*. The methanolic extract exhibited the most selective with SI value of 172.84 (TABLE 3). Among three tested extracts, the methanolic extract also exhibited the most selective toward the T47D and HeLa cells lines, although it was weakly active.

Among 29 compounds identified using DAD UV-Vis analysis, 8 compounds had been identified at λ 235 nm asluteolin-7-galactoside, apigenin monoglycoside, hyperoside (quersetin-3-gluctoside), kampferol-3-rutinoside, aureonitol (998.20), aurenitol(985.99), cerebroside and naamine (TABLE 4). These compounds have been isolated from another plants and evaluated for their biological activities.

Luteolin-7-glucoside is a glucoside flavanoid group found in burr parsley (*Caucalisplatycarpos* L).[14] It is also found in edible plants and medicinal plants traditionally used to treat various illness. Luteolin exhibits various biological activities includinganti-inflammatory activities, antioxidants, antimicrobials, cancer chemotherapy, cancer chemopreventive, cardioprotective, antihypertension, anticholesterol, antidiabetic, neuroprotective and

antiallergic.[15] In addition, rich diets of luteolin-7-glucoside may be useful in the control of both nonalcoholic fatty liver disease and cardiovascular diseases.[16]

Apigenin monoglycosides are compounds belonging to the flavanoid aglycone. Apigenin is known as 4',5,7, -trihydroxyflavone, which is a flavonoid class of flavonoids. Apigenin is also an aglycone of several glycosides that occur naturally with the molecular formula $C_{15}H_{10}O_5$ with a molecular weight of 270.24.[17] Apigenin is a yellow crystalline solid that has used to dye wool. Flavone compounds and their derivatives are well known to have several biological activities including antioxidants, anti-inflammatory, antitumor, antigenotoxic, anti-allergic, neuroprotective, cardioprotective, and antimicrobial.[17,18] Apigenin it self is known to have activities including antioxidants, antigenotoxic, anticarcinogenic, antiangiogenic, antihepatotoxic, antinephrotoxic. Apigenin may be useful to treat pulmonary fibrosis, cardiovascular disease, autoimmune diseases, Alzheimer's, Parkinson's, HIV, arthritis, HIV, arthritis, hemostasis, multiple sclerosis, multidrug and immunosuppressive resistance.[19]

Quercetin 3-galactoside is compound belonging to flavonoid-3-glycoside. It contains a flavonoid moiety which is O-glycosidically linked to carbohydrate moiety at the C3-position. Quercetin 3-galactoside is proven to have various biological activities including antinociceptive, antidepressant, neuroprotective, cardioprotective, antidiabetic, anticancer, antiviral, hepatoprotective, antioxidant and immunomodulator.[20]

Kaempferol-3-rutinoside is a class of flavonoids. It is proven to have anticancer, antiinflammatory, antioxidant, neuroprotective, cardioprotective, antiobesity, antiulcer, antidiabetic, and antiosteoporostic.[20,21] Where as aurenitolis a tetrahydrofuran derivative which is a hydrogenated analogue compound from the furan aromatic compound. It exhibits *in vitro* antiviral activity against influenza virus replication.[23,24] Cerebrosides are glycosphingolipids composed of hydrophobic ceramide linked to one sugar unit.[25] It can induce apoptosis in human colon cell lines.[26] Naamine is a group of alkaloids that have activity against plant viruses and phytopathogenic fungi.[27] It Naamine also has mild cytotoxic activity against MCF-7, A549, HeLa, and PC9 cells line.[28]

This UV-Vis DAD analysis showed that the methanolic extract of the *H. aculeatus* leaves contains compounds that have the potential to treat various diseases. However, the compounds of the methanolic extract showing antiplasmodial activity have not been reported, yet. Further study will be focused to isolate and determine antiplasmodial active compounds from the methanolic extract.

CONCLUSION

In conclusion, the methanolic extract of *H. aculeatus* leaf exhibits the most potential *in vitro* antiplasmodial activity compared to that *n*-hexanicand ethyl acetate extracts. Furthermore, the three extracts testedexhibit weak or no cytotoxicity against T47D, and HeLa cells line.

ACKNOWLEDGEMENT

We would like to thank Mrs. Rumbiwati, Mr.Purwono, Mr.Wagimin, Mrs.Mosa for their valuable assistances during laboratory works.We also would like to thank Prof.Peter Prokschand and Dr. Rini Muharini from Universiteit Duesseldorf, Germany for providing LC-MS and UV-Vis DAD analysis.

REFERENCES

1. World Health Organization. World Malaria Report 2018. 2018
2. Oktaviani P, Hartono B, Ekasari. Trend of malaria cases in Maluku Province 2012-2016. Indian J Public Health Res Develop 2020; 11(1):1417-24. https://doi.org/10.37506/vll/ill/2020/ijphrd/194041

3. Torres REM, Banegas FI, Mendoza M, Diaz C, Becheli STM, Fontecha GA, *et al.* Efficacy of chloroquine for the treatment of uncomplicated *Plasmodium falciparum*malaria in Honduras. Am J Trop Med Hyg 2130; 88(5):850-4. https://doi.org/10.4269/ajtmh.12-0671
4. WHO. WHO Global Malaria Programme: Status report on artemisinin resistance. Geneva: World Health Organization; 2014. Available from : http://www.who.int/malaria/publications/atoz/status_rep_artemisinin_resistance_jan2014.pdf?ua=1. Accessed April 2020.
5. Aguiar ACC, da Roche EMM, de Souza NB, França TCC, Krettli AU. New approaches in antimalarial drug discovery and development- A review. Mem inst Oswaldo Cruz, Rio de Janeiro 2012; 107(7):831–45. https://doi.org/10.1590/S0074-02762012000700001
6. Turalely R, Susidarti RA, Wijayanti MA. *In vivo* antiplasmodial of the most active fraction and its compound of *kapur* leaves (*Harmsiopanax aculeatus* Harms) extract against *Plasmodium berghei*. Trop Med J 2011; 01(02):131-40. https://doi.org/10.22146/tmj.4575
7. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science 1976; 193(4254):673-5. https://doi.org/10.1126/science.781840
8. Turalely R, Mustofa, Wijayanti MA, Hertiani T. Activity of anti-plasmodial and cytotoxicity of kapur leaves (*Harmsiopanax aculeatus*, Harms) potential fraction (FG2, FG3 and FG4) traditionally used to treat malaria in Maluku Indonesia. JKPK 2018; 3(2):46-52. https://doi.org/10.20961/jkpk.v3i2.21068
9. Jonville MC, Kodja H, Humeau L, Fournel J, Mol P De, Cao M, *et al.* Screening of medicinal plants from Reunion Island for antimalarial and cytotoxic activity. J Ethnopharmacol 2008; 120(3):382-6. htpps://doi.org/10.1016/j.jep.2008.09.005
10. Adia M, Emami S, Byamukama R, Faye I, Borg-Karlson A-K. Antiplasmodial activity and phytochemical analysis of extracts from selected Ugandan medicinal plants. J Ethnopharmacol 2016; 186:14-9. https://doi.org/10.1016/j.jep.2016.03.047
11. Geran RI, Greenberg NH, MacDonald MM, Schumaker AM, Abbott BJ. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemother Rep 1972; 3:59-61.
12. Susanti S, Iwasaki H, Itokazu Y, Nago M, Taira N, Saitoh S, Oku H. Tumor specific cytotoxicity of arctigenin isolated from herbal plant *Arctium lappa* L. J Nat Med 2012; 66(4):614-21. https://doi.org/10.1007/s11418-012-0628-0
13. Prayong P, Barusrux S, Weerapreeyakul N. Cytotoxic activity screening of some indigenous Thai plants. Fitoterapia 2008; 79(7-8):598-601. https://doi.org/10.1016/j.fitote.2008.06.007
14. Plazonic A, Bucar F, Males Z, Mornar A, Nigove B, Kujundzic N. Identification and quantification of flavonoids and phenolic acids in burr parsley (Caucalis platycarpos L.), using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry. Molecules 2009; 14(7):2466-90. https://doi.org/10.3390/molecules14072466
15. López-lázaro M. Distribution and biological activities of the flavonoid luteolin. Med Chem 2009; 9(1):31-59. https://doi.org/10.2174/138955709787001712
16. Sá C, Oliveira AR, Machado C, Azevedo M, Pereira-Wilson C. Effects on liver lipid metabolism of the naturally occurring dietary flavone luteolin-7-glucoside. Evidence-Based Compl Alter Med 2015; (647832):1-10. https://doi.org/10.1155/2015/647832
17. Ali F, Naz F, Jyoti S, Siddique YH, Ali F, Naz F, *et al.* Health functionality of apigenin: a review. Int J Food Prop 2017; 20(6):1197–238.

https://doi.org/10.1080/10942912.2016.1207188
18. Singh P, Mishra SK, Noel S, Sharma S, RathSK. Acute exposure of apigenin induces hepatotoxicity in Swiss mice. PLoS One 2012; 7:e31964. https://doi.org/10.1371/journal.pone.0031964
19. Wilson T, Singh AP, Vorsa N. Cranberry quersetin-3-galactoside in postprandial human plasma. Int J Food Nutr Sci 2014; 1(1):17-9. https://doi.org/10.15436/2377-0619.14.008
20. Raza A, Xu X, Sun H, Tang J, Ouyang Z. Pharmacological activities and pharmacokinetic study of hyperoside: a short review. Trop J Pharm Res 2017; 16(2):483-9. http://doi.org/10.4314/tjpr.v.16i2.30
21. Riaz A, Rasul A, Hussain G, Zahoor MK, Jabeen F, Subhani Z, *et al.* Astragalin: a bioactive phytochemical with potential therapeutic activities. Adv Pharmacol Sci 2018; 2018:9794625. https://doi.org/10.1155/2018/9794625
22. Wang Y, Tang C, Zhang H. Hepatoprotective effects of kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside from Carthamus tinctorius L. on CCl_4-induced oxidative liver injury in mice. J Food Drug Anal 2015; 23(2):310-7. https://doi.org/10.1016/j.jfda.2014.10.002
23. Sacramento CQ, Marttorelli A, Fintelman-Rodrigues N, de Freitas CS, deMelo GR, Rocha MEN, *et al*. Correction: aureonitol, a fungi-derived tetrahydrofuran, inhibits influenza replicationby targeting its surface glycoprotein hemagglutinin. PLoS ONE, 10(10):1-17. https://doi.org/10.1371/journal.pone.0139236
24. Linnakoski A, Linnakoski R, Reshamwala D, Veteli P, Cortina-Escribano M. Antiviral agents from fungi: diversity, diversity, mechanisms and potential applications. Front Microbiol 2018; 9(2325):1-18. https://doi.org/10.3389/fmicb.2018.02325
25. Barreto-Bergter E, Pinto MR, Rodrigues M. Structure and biological functions of fungal cerebrosides. An Acad Bra Cien 2004; 76(1):67-84. http://doi.org/10.1590/S0001-37652004000100007
26. Aida K, Takakuwai N, Kinoshita M, Sugawara T, Imai H, Ono J, *et al.* Properties and physiological effects of plant cerebroside species as functional lipids. In: Murata N editor. Advance Research on Plant Lipids. Netherlands: Kluwer Academic Publishers 2003, pp. 233-6.
27. Guo P, Li G, Liu Y, Lu A, Wang Z, Wang Q. Naamines and naamidines as novel agents against a plant virus and phytopathogenic fungi. Mar Drugs 2018; 16(9):311. https://doi.org/10.3390/md16090311
28. Tang WZ, Yang ZZ, Sun F, Wang SP, Yang F, Lin HW. Leucanone A and naamine J, glycerol ether lipid and imidazole alkaloid from the marine sponge *Leucandra* sp. J Asian Nat Prod Res 2017; 19(7):691-6. https://doi.org/10.1080/10286020.2016.1240171