ISSN 0974-3618 (Print)
0974-360X (Online)
www.rjptonline.org

RJPT
Research Journal of Pharmacy and Technology

REVIEW ARTICLE

Antioxidant Assay with Scavenging DPPH Radical of *Artocarpus anisophyllus* Miq Stem bark extracts and Chemical compositions and Toxisity Evaluation for the Most Active Fraction

Erwin[1]*, Djihan Ryn Pratiwi[2], Iwan Saputra[1], Alimuddin[3]
[1]Organic Chemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Jalan Barong Tongkok, Kampus Gn. Kelua, Samarinda, East Kalimantan, Indonesia.
[2]Biochemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Jalan Barong Tongkok, Kampus Gn. Kelua, Samarinda, East Kalimantan, Indonesia.
[3]Analytical Chemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Jalan Barong Tongkok, Kampus Gn. Kelua, Samarinda, East Kalimantan, Indonesia.
*Corresponding Author E-mail: **winulica@yahoo.co.id**

ABSTRACT:
The purpose of this study was to determine the antioxidant activity of crude extract and their fractions, the content of secondary metabolite types of crude extract, and the chemical content and toxicity of the most active fractions as antioxidants from stem bark of *Artocarpus anisophyllus* Miq. (Mentawa). The methods used are phytochemical screening, toxicity tests against *Artemia salina* by the Brine Shrimp Lethality Test (BSLT), antioxidant activity assay with DPPH radical reduction method, and determination of chemical compound by GC-MS analysis. Phytochemical test results showed that crude extract containing alkaloids, triterpenoids, phenolics and flavonoids. The results of the antioxidant activity of the n-hexane, ethyl acetate and methanol fractions obtained IC_{50} values of 127.69, 28.65 and 79.43ppm, respectively. Ethyl acetate (as the most active fraction) was then fractionated using a vacuum chromatography column and the fractions obtained were E1 (268.8mg), E2 (337.1), E3 (234.3mg) and E4 (431.2mg). The antioxidant activity test showed that E2 was the most active compared to other fractions with an IC_{50} value of 37.24ppm. While the toxicity test results showed that E2 was very active with an LC_{50} value of 6.23ppm indicating that E2 was also potentially developed as an anticancer drug. Based on GC-MS spectrum analysis, of the several main compounds, four of which are aromatic compounds that have the potential to be developed as antioxidants, namely 2- (Benzyloxy) phenol (phenolic compound) (3.96%) (51),), 1, 2- Benzenedicarboxylic acid, dinonyl ester (2.44%) (52), Linderazulene (2.43%) (30), p-Nonylphenol (2.36%). Some other aromatic minor compounds can also be active as antioxidants.

KEYWORDS: *Artocarpus anisophyllus* Miq., Secondary Metabolites, Mentawa, Toxicity, Antioxidant, DPPH.

INTRODUCTION:
Plants are actually very important for human life, one of which is an important source of compounds as medicinal ingredients. Empirically, medicinal plants are still used to cure various types of diseases as an alternative treatment[1]. Indonesia consists of seven bioregions and it has the second largest biodiversity after Brazil[2].

Artocarpus, one of the main genus of Moraceae that is found in tropical forests of Indonesia and spread on various islands, such as Kalimantan, Sulawesi, Sumatra, Java and others. Some species of *Artocarpus* have been used in traditional medicines which have effects such as Antibacterial, anticancer, antidiabetic, fever medication, worms, antiviral, anti-inflammatory, diuretic, and anti-hypertensive. This pharmacological effect may be due to the presence of aromatic compounds such as flavonoids, stilbenoids, xantones and arylbenzofurans[3,4,5,6]

Artocarpus anisophyllus Miq (Mentawa) is one of the forest plants that produce edible fruit that is commonly found on the island of Borneo. A mixture of Mentawa leaves ash with coconut oil can be used to treat burns by applying it to the skin of burns[7]. The plant leaves are also used by people to treat boils and itching[8]. Crude extracts of Mentawa stem have strong antioxidant activity and it contains secondary metabolite compounds such as flavonoids, phenolics and triterpenoids[9], root extracts exhibited toxicity against *Artemia salina*[10]. While the crude extracts of Mentawa leaves have antibacterial and antioxidant activities[11] and from the leaves and heartwood this plant have been isolated prenylated flavonoid compounds which are antioxidant and tyrosinase inhibitory activities.[12]

As a continuation of our research on *A. anisophyllus* Miq, in this article we will report the antioxidant properties of total extracts and their fractions from Mentawa bark and the chemical composition of the most active fractions as antioxidants.

METHODOLOGY:

Sample Collection and Preparation:

Mentawa (*Artocarpus anisophyllus*) was collected from Tanah Merah village, Samarinda, East Kalimantan. The plant was identified in the Plant Anatomy and Systematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda. The sample (stem bark of *A. anisophyllus*) was cleaned, dried and mashed by a smoothing machine to obtain dry powder samples.

Extraction and fractionation:

1.082gram dried powder samples were extracted using maceration method in methanol for 2x 24 hours. The filtrate obtained was concentrated by a rotary evaporator to give crude brown extract (10grams). Crude extract was further partitioned with n-hexane and ethyl acetate respectively. The most active fraction as an antioxidant will be fractionated using vacuum column chromatography.

Phytochemical Test:

The phytochemical test was conducted as a preliminary test to find out the types of secondary metabolite compounds in crude extracts which covered the test of alkaloid, steroid and triterpenoid, flavonoid, phenolic and saponin compounds. The research procedure refers to previous studies[13,14].

Toxicity Test with the Brine Shrimp Lethalily Test (BSLT) Method:

Fraction most active as antioxidant obtained from vacuum column chromatography will be further tested for toxicity against *Artemia salina* larvae. Sample was made in various concentrations, then *A. salina* larvae were added (500ppm; 250ppm; 125ppm; 62.5ppm; 31.2 ppm; 15.6ppm and 7.8ppm). Sample was tested three times (triplo). The number of dead shrimp larvae was counted after 24 hours of treatment. The larval mortality data obtained were analyzed using the SAS probit analysis program to determine the LC_{50} value.[15,16,17]

Antioxidant Activity Test:

Samples were made in concentrations of 20ppm, 40 ppm, 60ppm and 80ppm. Each sample was put 4mL into a cuvette and then added 1 ml of a 0.024mg/mL DPPH solution, homogenized and then incubated for 30 minutes. Then the absorbance samples were measured at a wavelength of 517nm using a UV-Vis spectrophotometer. The IC_{50} calculation refers to the previous research procedure[18,19,20,21,22,23].

GC-MS analysis:

GC-MS used is Thermo Scientific Trace 1310 Gas Chromatoraph-High Resolution Mass Spectrometry (Thermo Scientific ISQLT Single Quadropole Mass Spectrometer). The specifications of this instrument is covering auto injector TriPlus type RSH, UHP helium used as carrier gas, and use the software Chromeleon own library for easy knowing that compound was detected when there are no standards in accordance with the tested compounds.

RESULTS AND DISCUSSION:

Extraction and partitioning results obtained crude extracts, fractions of n-hexane; ethyl acetate and methanol were 10, 0.98, 4.21, and 2.49 grams, respectively. The phytochemical test exhibited that crude extracts containing alkaloids, triterpenoids, phenolics and flavonoids. Based on the results of antioxidant tests using DPPH radical reduction showed that the ethyl acetate fraction had the lowest IC_{50} value compared to the n-hexane and methanol fractions with IC50 values of 28.57, 127.67 and 79.43ppm, respectively. Furthermore, fractionation is carried out of the ethyl acetate fraction (the most active as an antioxidant) using liquid column chromatography by increasing the eluent polarity gradually to give E1 (268.8mg), E2 (337.1), E3 (234.3 mg) and E4 (431.2mg). The IC_{50} of the four fractions (E1-E4) are showed in table 1. E2 has the highest antioxidant properties compared to other fractions with an IC_{50} value of 37.24ppm.

Table 1. The IC_{50} value of the fractionation results from ethyl acetate fraction (Average of three replicates performed for each concentration)

Fractions	Concentration (ppm)	Absorbance	% inhibition	Linear regression equation	IC_{50} (μg/mL)
E1	20	0.198	29.985	Y = 0.7188+ 17.003	45.91
	40	0.150	47.368		
	60	0.110	61.403		
	80	0.076	73.333		
E2	20	0.204	28.401	Y = 1.1348x + 7.737	37.24
	40	0.130	54.364		
	60	0.058	79.425		
	80	0.012	96.692		
E3	20	0.193	32.435	Y = 0.9149x + 11.505	42,08
	40	0.160	43.618		
	60	0.088	68.579		
	80	0.042	84.732		
E4	20	0.331	20.241	Y = 0.414x + 10.396	95.52
	40	0.309	25.542		
	60	0.276	33.494		
	80	0.201	45.232		
Vitamin C	2	0,547	18.358	Y = 12.291x + 9.4776	3.07
	4	0,153	77.164		
	6	0,045	93.284		
	8	0,034	94.925		

Table 2. The LC_{50} value of E2 (Average of three replicates performed for each concentration)

Sample	concentration	Log concentration	Total larvae	Dead larvae	% Mortality	Probit	LC_{50} (ppm)
E2	500	2.6989	9.67	9.67	100.00	8.09	6.23
	250	2.3979	9.33	8.67	92.92	6.48	
	125	2.0969	10.33	9.00	87.12	6.13	
	62.5	1.7959	10.67	8.67	81.26	5.88	
	31.25	1.4948	8.67	5,00	57.67	5.20	
	15.63	1.1938	10.33	7.00	67.76	5.47	
	7.81	0.8928	9.00	5.67	63.00	5.33	

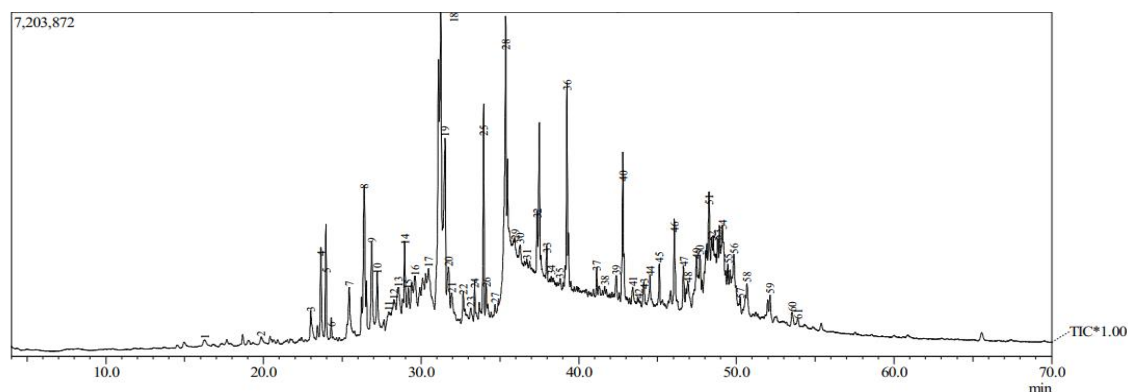

Fig. 1. GC-MS chromatogram of E2

Table 2. Chemical composition of E2

S. No	Peak	% Area	Molecular formula	Molecular weight	Compound
1	16.284	0.19	$C_4H_8O_3$	104	Propanoic acid, 2-hydroxy-2-methyl-
2	19.874	0.18	$C_{10}H_{22}O$	158	1-Octanol, 2,7-dimethyl-
3	23.020	0.59	$C_{12}H_{22}$	166	Hexane, 1-(isopropylidenecyclopropyl)-
4	23.656	1.31	$C_{15}H_{26}O$	222	Globulol
5	23.992	1.11	$C_{13}H_{20}O$	192	alpha.-Ionone
6	24.325	0.23	$C_{14}H_{22}O$	206	Phenol, 3,5-bis(1,1-dimethylethyl)-
7	25.452	1.26	$C_8H_{14}O$	126	2-Hexanone, 3-methyl-4-methylene-
8	26.402	3.44	$C_{11}H_{22}$	152	Bicyclo[2.2.1]heptane, 2-(1-methylpropyl)-
9	26.886	1.60	C_8H1_2O	124	3,5-Octadien-2-one, (E,E)-
10	27.230	1.19	$C_{10}H_{18}$	138	Cyclopentene, 1,4-dimethyl-5-(1-methylethyl)-
11	27.958	0.89	$C_{18}H_{26}O_2$	250	Sclarrolide
12	28.299	1.18	$C_{10}H_{12}O_3$	180	2,3-Epoxypropyl para-methoxyphenyl ether
13	28.571	1.40	$C_{10}H_{16}O_2$	168	2-Cyclohexen-1-one, 2-hydroxy-6-methyl-3-(1-methylethyl)-

14	28.968	1.71	$C_{16}H_{34}$	226	Hexadecane
15	29.199	0.89	$C_{14}H_{22}O$	206	Phenol, 4-(2,2,3,3-tetramethylbutyl)
16	29.625	2.36	$C_{15}H_{24}$	220	p-Nonylphenol
17	30.486	5.34	$C_{15}H_{26}O$	222	Palustrol
18	31.253	9.52	$C_{11}H_{20}O$	168	3-Decen-2-one, 3-methyl
19	31.522	3.81	$C_{13}H_{18}O_3$	222	2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)
20	31.751	1.22	$C_{17}H_{34}O_2$	270	Isopropyl myristate
21	31.958	0.82	$C_8H_{14}O$	126	3-Hepten-2-one, 4-methyl-
22	32.689	0.72	$C_{16}H_{22}O_4$	278	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
23	33.158	0.22	$C_{12}H_{26}O$	186	1-Octanol, 2-butyl-
24	33.414	0.42	$C_{20}H_{42}$	282	Eicosane
25	33.980	2.29	$C_{17}H_{34}O_2$	270	Hexadecanoic acid, methyl ester
26	34.138	0.46	$C_{18}H_{28}$	292	Methyl-3-(3,5-diterbutyl-4-hydroxyphenyl) propionate
27	34.692	0.15	$C_{16}H_{22}O_4$	278	Dibutyl phthalate
28	35.380	9.58	$C_{18}H_{36}$	252	1-Octadecene
29	35.958	2.69	$C_{17}H_{34}O_2$	270	Isopropyl myristate
30	36.292	2.43	$C_{15}H_{14}O$	210	Linderazulene
31	36.725	3.70	$C_{13}H_{15}NO_3$	233	4-Oxazolecarboxylic acid
32	37.381	4.49	$C_{17}H_{30}O_2$	266	9,12-Hexadecadienoic acid, methyl ester
33	37.992	1.02	$C_{19}H_{38}O_2$	298	Octadecanoic acid, methyl ester
34	38.260	1.18	$C_{13}H_{28}$	184	Undecane, 4,5-dimethyl-
35	38.825	0.53	$C_{19}H_{40}$	268	Octadecane, 2-methyl
36	39.262	2.97	$C_{22}H_{44}$	308	1-Docosene
37	41.153	0.40	$C_{20}H_{42}$	282	Eicosane
38	41.657	0.17	$C_{16}H_{32}O_2$	256	Methyl 12-methyltetradecanoate
39	42.389	0.31	$C_{10}H_{21}Cl$	176	Decane, 1-chloro-
40	42.815	1.78	$C_{22}H_{44}$	308	1-Docosene
41	43.438	0.29	$C_{25}H_{42}O_2$	374	Octadecanoic acid, phenylmethyl ester
42	43.825	0.13	$C_{15}H_{32}$	212	Tetradecane, 4-methyl-
43	44.158	0.32	$C_{22}H_{46}O_3Si$	386	Hexadecanoic acid, 3- (trimethylsilyl)oxy propyl ester
44	44.535	0.47	$C_{20}H_{42}$	282	Eicosane
45	45.129	0.55	$C_{24}H_{38}O_4$	390	Di-n-octyl phthalate
46	46.085	1.53	$C_{27}H_{56}O$	396	1-Heptacosanol
47	46.660	0.60	$C_{13}H_{10}F_2O_2$	236	Phenol, 2-benzyloxy-3,6-difluoro
48	46.934	0.85	$C_{23}H_{44}O_4$	384	1,3-Dioxolane, 4- (2-methoxy-4-hexadecenyl)oxy methyl -2,2-dimethyl-
49	47.488	1.49	$C_{15}H_{14}O_3$	242	Benzaldehyde, 3-methoxy-4-(phenylmethoxy)-
50	47.692	0.98	$C_{19}H_{40}$	268	Nonadecane
51	48.292	3.96	$C_{13}H_{12}O_2$	200	2-(Benzyloxy)phenol
52	48.440	2.44	$C_{26}H_{42}O_4$	418	1,2-Benzenedicarboxylic acid, dinonyl ester
53	48.827	1.73	$C_{28}H_{46}O_4$	446	1,2-Benzenedicarboxylic acid
54	49.124	3.95	$C_{29}H_{60}O$	424	Nonacosanol
55	49.463	1.24	$C_{28}H_{46}O_4$	446	1,2-Benzenedicarboxylic acid, diisodecyl ester
56	49.852	1.69	$C_{14}H_{20}O_2$	300	Pentanoic acid, 3,3-dimethyl-5-[(phenyl methyl)seleno]-
57	50.258	0.23	$C_{28}H_{46}O_4$	446	1,2-Benzenedicarboxylic acid, bis(8-methylnonyl) ester
58	50.687	0.75	$C_{10}H_{12}O_2$	164	2-Methoxyphenylacetone
59	52.146	0.63	$C_{16}H_{18}O_2$	242	Benzene, 1,1'-(1,2-ethanediyl) bis[4-methoxy-
60	53.552	0.27	$C_{34}H_{48}O_2$	488	Cholesta-4,6-dien-3-ol, benzoate
61	53.925	0.14	$C_{14}H_{22}O$	206	Irone alpha

Toxicity test results showed that E2 was very toxic against to *Artemia salina* larvae with LC_{50} value of 6.23 ppm. Based on the results of previous studies indicate that the toxicity test against *A. salina* is not specific to anticancer but it can be used as a screening to determine the anticancer potential of the extract or fraction. In addition, this method is inexpensive, easy to do, and quickly known the results[15]

The results of the GC MS E2 analysis were showed in Table 2. The mayor chemical content of E2 (above 2%) consists of 1-Octadecene (28), 3-Decen-2-one, 3-methyl (18), Palustrol (17), 9,12-Hexadecadienoic acid, methyl ester (32), 2-(Benzyloxy) phenol (51), Nonacosanol (54), 2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl) (19), 4-Oxazole carboxylic acid (31), Bicyclo [2.2.1] heptane, 2-(1-methylpropyl) - (8), 1-Docosene (36), Isopropyl myristate (29), 1,2-Benzenedicarboxylic acid, dinonyl ester (52), Linderazulene (30), p-Nonylphenol (16) and Hexadecanoic acid, methyl ester (25). Four of them are aromatic compounds that have antioxidant potential, namely 2- (Benzyloxy) phenol (51), 1,2-Benzenedicarboxylic acid, dinonyl ester (52), Linderazulene (30), p-Nonylphenol (16).

In addition, other minor aromatic compounds which have potential as antioxidants such as 1,2-Benzenedicarboxylic acid (53), Pentanoic acid, 3,3-dimethyl-5-[(phenyl methyl)seleno]- (56), Benzaldehyde, 3-methoxy-4-(phenylmethoxy)- (49), 1,2-Benzenedicarboxylic acid, diisodecyl ester (55), 2,3-Epoxypropyl para-methoxyphenyl ether (12), Phenol,4-(2,2,3,3-tetramethylbutyl) (15), 2-Methoxyphenylacetone (58), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (22), Phenol, 2-benzyloxy-3,6-difluoro (45), Benzene, 1,1'-(1,2-ethanediyl)bis[4-methoxy- (59), Di-n-octyl phthalate (45), Octadecanoic acid, phenylmethyl ester (41) Phenol, 3,5-bis(1,1-dimethylethyl)- (6), 1,2-Benzenedicarboxylic acid, bis(8-methylnonyl) ester (57) and Dibutyl phthalate (27).

CONCLUSION:

E2 obtained from fractionation by vacuum column chromatography of the ethyl acetate fraction of Mentawa stem bark was the most active fraction as an antioxidant against DPPH radicals with IC_{50} values of 37.24ppm. This fraction is also very toxic against to *Artemia salina* (LC_{50} value of 6.23ppm) which indicates anticancer potential. Several aromatic compounds have been identified from GC-MS data analysis which has potential as antioxidants.

ACKNOWLEGMENTS:

The authors appreciate and thank the Indonesian Ministry of Education and Culture for the assistance of the BOPTN Research Fund for the Faculty of Mathematics and Natural Sciences with a contract number: 1054 / UN17.7 / LT / 2018.

REFERENCES:

1. Kalita S, Kumar G, Karthik L, Rao KVB. Review on Medicinal Properties of Lantana camara Linn. Research J. Pharm. and Tech.; 2012; 5 (6): 711-715
2. Widjaja EA, Rahayuningsih Y, Rahajoe JS, Ubaidillah R, Maryanto I, Walujo EB, Semiadi G. Kekinian Keanekaragaman Hayati Indonenesia, LIPI Pres, Jakarta Indonesia; 2014, 5-11
3. Hamzah L, Soekanto NH, Firdaus. Phytocimia Test and Antibacterial Bioactivity of Extracts from Stem Bark of Artocarpus integer (Thunb.) Merr, Indonesia Chimica Acta; 2019; 12 (2): 84-90
4. Ee GCL, Teo SH, Rahmani M, Lim CK, Lim YM, Bong CFJ. Structure activity relationship of flavonoid derivatives from two Artocarpus species as inhibitors of leukemia and breast cancer cells, Journal of Science and Technology in the Tropics; 2011; 7: 121-125
5. Hakim EH, Achmad SA, Juliawaty LD, Makmur L, Syah YM, Aimi N, Kitajima M, Takayama H, Ghisalberti EL. Prenylated flavonoids and related compounds of the Indonesian Artocarpus (Moraceae). J. Nat. Med.; 2006; 60: 161–184
6. Nasution H, Rahmah M. Test for diphenilphikril hydrazyl (DPPH) free antiradical from acetate etil extract of nangka leaf (Artocarpus heterophyllus Lamk), J. Sains Dasar; 2014; 3 (2): 137 – 141
7. Setyowati FM, Riswan S, Susiarti S. Etnobotani Masyarakat Dayak Ngaju di Daerah Timpah Kalimantan Tengah, J. Tek. Ling. P3TL-BPPT; 2005; 6(3): 502-510.
8. Yaniv Z, Bachrach U. Handbook of Medicinal Plants. Food Product Press, Oxford; 2005; 10-12.
9. Pabita OT, Erwin, Kusuma IW. Phytochemical Screening and Antioxidant Activities, Of Methanol Extract of Mentawa Wood (Artocarpus anisophyllus Miq.), Prosiding Seminar Nasional Kimia; 2019; 57-61
10. Affriani N, Idiawati N, Alimuddin AH. Skrining Fitokimia dan Uji Toksisitas Ekstrak Akar Mentawa (Artocarpus anisophyllus) Terhadap Larva Artemia salina, JKK; 2016; 5 (1): 58-64.
11. Mulyani S, Ardiningsih P, Jayuska A. Aktivitas Antioksidan dan Antibakteri Ekstrak Daun Mentawa (Artocarpus anisophyllus), JKK; 2016; 5(1): 58-64.
12. Lathiff SM, Jemaon N, Abdullah SA, Jamil S. Flavonoids from Artocarpus anisophyllus and their bioactivities. Nat Prod Commun. 2015;10(3): 393-396
13. Kaur R and Kumar N. Composition and in vitro Antioxidant Activity of Leucas aspera Leaves Research J. Pharm. and Tech.; 2016; 9(12): 2217-2221
14. Sujatha J, Asokan S, Rajeshkumar S. Phytochemical analysis and antioxidant activity of chloroform extract of Cassis alata, Research J. Pharm. and Tec Research J. Pharm. and Tec.; 2018;11(2): 439-444.
15. Meyer BN. Ferrigni NR, Putnam JE, Jacobson LB, Nichols DE, McLaughlin JL. Brine Shrimp: a convenient general bioassay for active plant constituents. Planta Medica; 1982;45: 31-34.
16. Karolina A, Pratiwi DR, Erwin, Phytochemical and Toxicity Test of Merung Extracts (Coptosapelta tomentosa (Blume), Jurnal Atomik; 2018; 03 (2): 79-82
17. Erwin, Tonapa ZG, Alimuddin. 2020, Toxicity Assay of Baccaurea motleyana Mull. Arg. Wood Extracts (Rambai) and Chemical Compounds Evaluation for the Most Active Fraction, Research J. Pharm. and Tech.; 2020; 13 (11)
18. Jain A, Ojha V, Kumar G, Karthik L, Rao KVB, Phytochemical Composition and Antioxidant Activity of Methanolic Extract of Ficus benjamina (Moraceae) Leaves, Research J. Pharm. and Tech.; 2013; 6(11): 1184-1189.
19. Rao KVB, Munjal M, Patnayak A, Karthik L, Kumar G. Phytochemical Composition, Antioxidant, Antimicrobial and Cytotoxic Potential of Methanolic Extracts of Adhatoda vasica (Acanthaceae), Research J. Pharm. and Tech.; 2013; 6(9): 997-1002
20. Al-Azawi AH, Salih WY, Hassan ZH. Phytochemical and Antioxidant Activity of (Arachishypogaea l.) Skin Extract Scultiv Atedin Iraq, Research J. Pharm. and Tech.; 2017; 10 (11): 1994-2000.
21. Durgawale TP, Khanwelkar CC, Durgawale, PP. Phytochemical Screening using GC-MS and study of Anti-Oxidant Activity of two species of Portulaca, J. Pharm. and Tech.; 2018; 11(12): 5534-5540
22. Bohari, Karolina A, Pratiwi DR, Erwin, Rahmadi A. Toxicity test, antioxidant activity test and GC-MS profile of the active fraction of Coptosapelta tomentosa (Blume) root (Merung), EurAsian Journal of BioSciences; 2019; 13: 2403-2406
23. Supomo, Syamsul ES, Apriliana A, Saleh C, Erwin, Lestari D. Antioxidant Assay Of Dayak Onion (Eleutherine palmifolia) VIA DPPH (1,1-Difenil-2-Pikrilhidrazil) And BSLT Test For Its Active Fraction, Rasayan J. Chem.; 2019; 12(3): 1340-1346