RJC

RASĀYAN ***J. Chem.***
Vol. 12 | No. 4 |1822 - 1827| October - December | 2019
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

ISOLATION OF QUERCITRIN FROM *Dendrophthoe pentandra* (L.) Miq LEAVES AND IT'S ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

Rini Hardiyanti[1], Lamek Marpaung[1,*], I. Ketut Adnyana[2] and Partomuan Simanjuntak[3,4]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia.
[2]Department of Pharmacology, Faculty of Pharmacy, Institut Teknologi Bandung, Jl. Ganesha, Bandung 40132, Indonesia.
[3]Department of Natural Product Chemistry, Research Centre for Biotechnology, Indonesian Institute of Science, Jl. Raya Bogor Km 46, Cibinong 16911, Indonesia.
[4]Faculty of Pharmacy, Universitas Pancasila, Jl. Srengseng Sawah, Jagakarsa, Jakarta, 12640, Indonesia
[*]E-mail: toyamaikayakadai@yahoo.co.id

ABSTRACT

A flavonoid, quercitrin, has been isolated from *Dendrophthoepentandra* (L.) leaves and its bioactivities, including antioxidant and antibacterial, has been evaluated. The isolation process of quercitrin was performed using a common method, i.e. maceration, column chromatography and followed by TLC preparative to obtain the pure isolate. The presence of quercitrin was confirmed using UV-Vis spectrophotometry, FT-IR, ^{1}H-NMR,^{13}C-NMR and MS data. The antioxidant property of quercitrin was determined using DPPH method that showed IC_{50} of 3.59 ppm. The antibacterial property of quercitrin was evaluated to four bacterial species, i.e. *S. typhi*, *Pseudomonas sp.*, *E. coli* and *S. aeureus*. The diameter of the inhibition zone was linear with the applied concentration of quercitrin.

Keywords: *Dendrophthoepentandra* (L.) Miq, Flavonoid, Quercitrin, Antioxidant, Antibacterial

INTRODUCTION

Mistletoes are a parasitic plant that can grow on many different species of trees. In Indonesia, there are a lot of mistletoes species and it depends on their host tree, also it has different local name depends on the place where they occur. *Dendrophthoepentandra (L.)* is a species of mistletoe that can be found in duku, orange, cocoa and coffee. In many countries, including Indonesia, the leaves of mistletoes can be used as traditional medicine for treating several diseases, i.e. diabetes, hypertension, cancer, diuretic, smallpox, ulcer, skin infection and after child-birth.[1-4] Furthermore, mistletoes also have potency as antioxidant and antibacterial.

Based on phytochemical screening in several studies, the leaves of *Dendrophthoepentandra (L.)* showed the positive result to the presence of flavonoid, terpenoid and steroidal compounds. And supporting with other previous studies, flavonoid commonly isolated from any species of mistletoes, i.e. catechin, epicathecin, quercetin, quercitrin, rutin, etc. As a common secondary metabolite, flavonoid can be found in more than 4000 species of plant that distributed in a broad range as fruits, vegetables and commercial product, such as tea, wine and cocoa[5]–[7]. Flavonoid is a common name for the secondary metabolite which has C_6-C_3-C_6 pattern in their chemical structure and they were classified into several subclasses such as flavonols, flavones, flavanols, flavanones, anthocyanidins and isoflavonoids.[6,7] In many references, the flavonoid showed a lot of biological and pharmacological activities, i.e. anticancer, antiviral, anti-inflammatory, antiallergy, and antitumor that has been proved through *in vitro* and *in vivo* tests.[5,8,9]

http://dx.doi.org/10.31788/RJC.2019.1235353

CrossMark

The existing ethnobotanical studies covering traditional uses of *Dendrophthoe*species do not provide scientific proof of their effectiveness. Published studies revealed that antioxidant and antibacterial properties of the active compound of Deku's mistletoe leaves (*Dendrophthoepentandra* (L.) Miq)have not been investigated so far, in our previous study revealed that the total flavonoid extract showed the highest antioxidant activity.[10] The primary objective of this study was to isolate the bioactive compound from the leaves of *Dendrophthoepentandra* (L.) Miq and investigate the antioxidant and antibacterial activities to find the scientific proof of folkloric use of *Dendrophthoepentandra* (L.) Miq.

EXPERIMENTAL

Material

Mistletoe leaves on *Lansiumdomesticum*Cor.r's were collected from Medan Johor village, North Sumatera, Indonesia, in Mei 2016. The plant was identified at Herbarium Bogoriensis, LIPI, Cibinong-Indonesia.

Extraction and Purification

The extraction, fractionation and purification process were conducted using the following method.[10] The fractions which showed positive phytochemical testing of flavonoid were continued to the purification process using preparative thin-layer chromatography (TLC) to separate the impurity from the desired compound. The pure compound was indicated with the presence of a single spot on the TLC.[11,12]

Identification of Pure Compound

The isolate was elucidated using UV-Vis, FT-IR, ^{1}H-NMR,^{13}C-NMR and MS data to determine the chemical structure of that compound.

Antioxidant Activity

The antioxidant activity of the extract was determined by the DPPH method.About 7.9 mL of DPPH radical solution (50 mL in methanol) was added to 1 mL each of various concentrations of sample from 5, 10, 25, 50 to 100µg/mL and standard ascorbic acid (concentrations 3, 6, 9, 12, 15µg/mL). The reaction mixtures with some concentration were incubated in a 37^{o}C water bath for 30 min. the absorbance of all the resulting solutions was measured at 515 nm using a UV-visible spectrophotometer. Percentage inhibition was calculated by the following equation.[13,14]

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control solution} - \text{Absorbance of sample})}{\text{Absorbance of control}} \text{ x } 100\%$$

Antibacterial Activity

The antibacterial activity was performed with the agar diffusion method. A total 0.1mL of each inoculum was applied by using a cotton bud into an agar nutrient medium. The disc paper was immersed in various concentration test solutions. Then placed on a medium that had been rubbed by bacteria to be incubated in an incubator at 35±2^{o}C for 18-24 hours and measured that drag line diameter.[10,15]

RESULTS AND DISCUSSION

Quercitrin was successfully isolated from the *Dendrophthoepentandra* (L.) Miq leaves and obtained as a yellowish powder. The chemical structure of quercitrin was determined using the UV-Vis,FT-IR, ^{1}H-NMR,^{13}C-NMR, and MS data.

UV-Vis spectrum (Fig.-1) of the isolated quercitrin showed the presence of two maximum wavelength peaks at 250 and 350 nm indicated the existence of the C=C double bond of aromatic structure.[16]The FT-IR spectrum (Fig.-2) showed the presence of many specific peaks that leads to quercitrin. Peak at 1731 cm^{-1} indicated the presence of vibration of C=O stretching from carboxylate acid. The common group, hydroxyl, in quercitrin structure can be found at 3556 cm^{-1}. The presence of C-H stretching of aromatic structure and vibration of C-H outside of the ring was indicated with a peak at 1228 and 823 cm^{-1}, respectively. The other peaks at 1452 and 1603 cm^{-1} also show the presence of C-C and C=C of aromatic.[17]

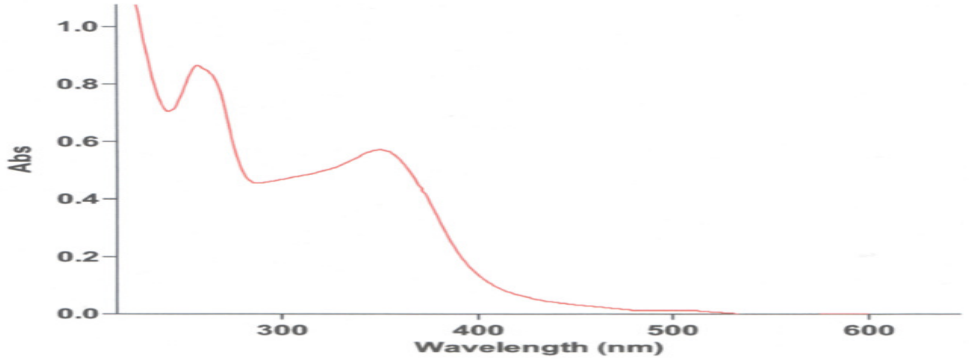

Fig.-1: UV-Vis Spectrum of Isolated Flavonoid

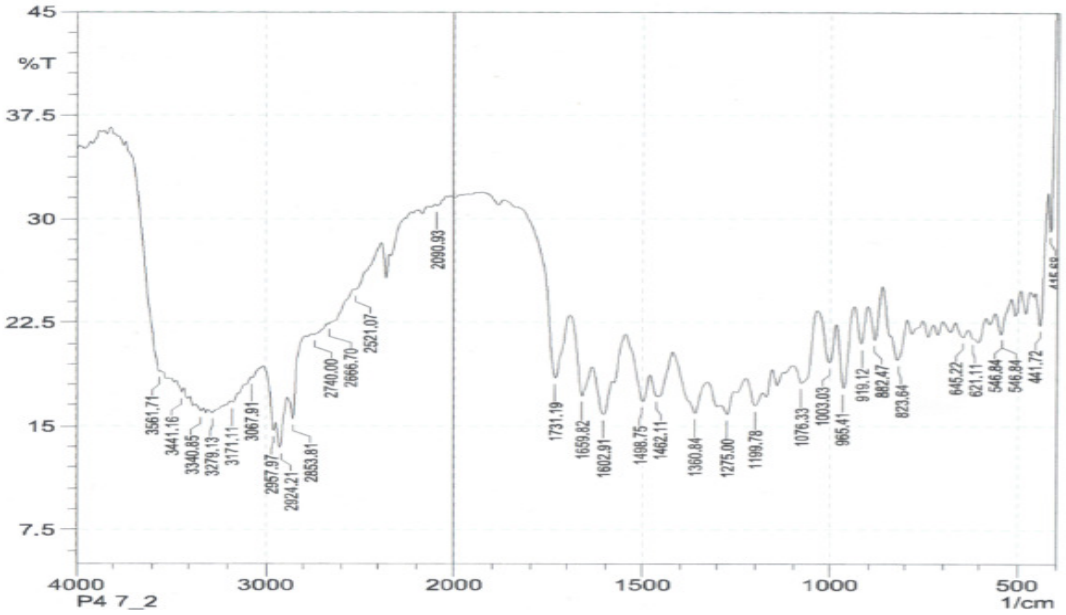

Fig.-2: FT-IR Spectrum of Isolated Flavonoid

Table-1: ^{1}H-NMR and ^{13}C-NMR Data of Compound

Position	Experimental		Reference Hasan, *et al* (2016)	
	^{13}C-NMR (CD_3OD)	^{1}H-NMR (CD_3OD)	^{13}C-NMR (CD_3OD)	^{1}H-NMR (CD_3OD)
2	159.3		159.3	
3	136.2		136.2	
4	179.6		179.6	
5	163.2		163.2	
6	99.8	6.20	99.8	6.19
7	165.8		165.8	
8	94.7	6.37	94.7	6.35
9	158.5		158.5	
10	105.9		105.9	
1’	122.9		123.0	
2’	116.9	7.32	116.9	7.33
3’	146.4		146.4	
4’	149.8		149.8	
5’	116.4	6.91	116.4	6.90
6’	122.9	7.30	122.9	7.29
Rhm				
1	103.5	5.35	103.5	5.34
2	71.9	4.22	71.9	4.22
3	72.0	3.75	72.0	3.75

4	73.2	3.34	73.2	3.35
5	72.1	3.36	72.1	3.38
6	17.6	0.94	17.6	0.94

The result in this work is compared to the ^{1}H-NMR(Fig.-3) and ^{13}C-NMR(Fig.-4) of quercitrin that isolated from *Dendrophtohoefalcata*[18] and based on flavonoid references. The chemical structure of quercitrin is based on quercetin that linked with a glycoside, rhamnose, in the position C3via C-O-C bonding (Fig.-5). The presence of rhamnose or the glycoside bond was confirmed due to the presence of chemical shift at 5.35 ppm and 136.2 ppm, in ^{1}H-NMR and ^{13}C-NMR, respectively. The elucidating process of isolated flavonoid from *Dendrophthoepentandra* (L.) Miq leaves that supported with UV-Vis, FT-IR, ^{1}H-NMR, ^{13}C-NMR and based on MSdata it was concluded as quercitrin (Fig.-5) that confirmed with the presenceof $[M+H]^{+}$ peak at m/z of 449.

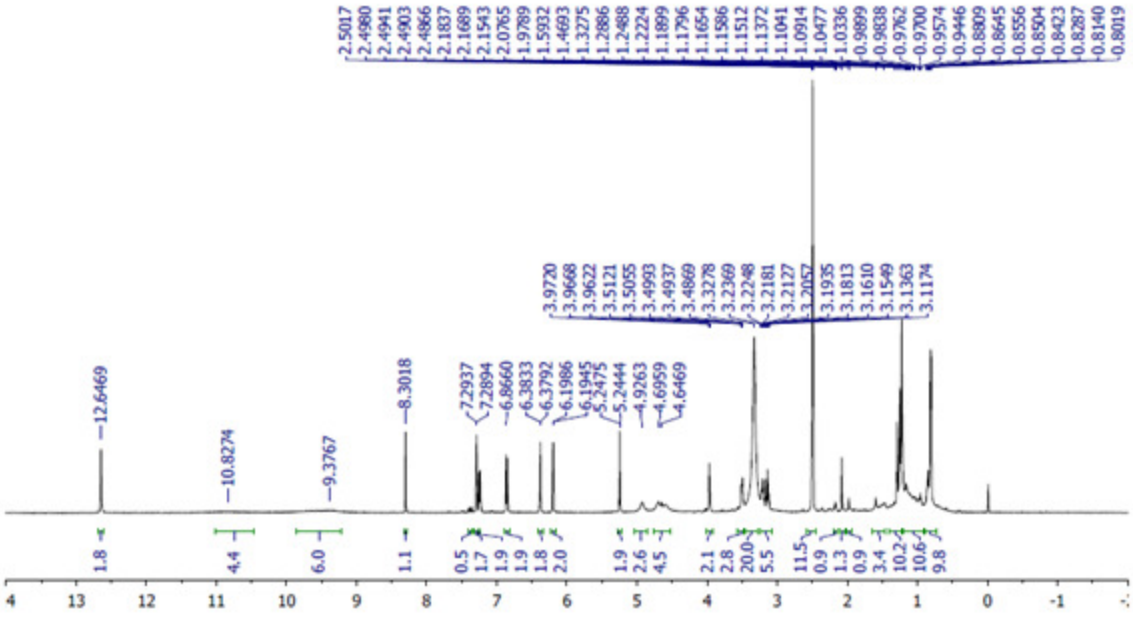

Fig.-3. ^{1}H-NMR Spectrum of Isolated Flavonoid

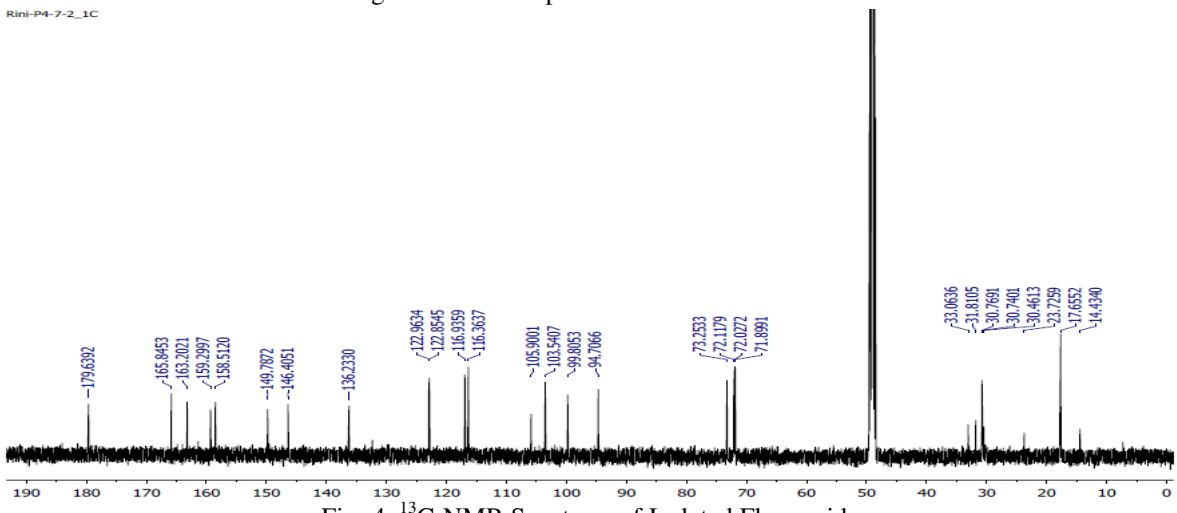

Fig.-4. ^{13}C-NMR Spectrum of Isolated Flavonoid

Based on literature reviews, quercitrin has a lot of potential to be utilized as antioxidant, antinociceptive and toxicity activity.[18] Furthermore, in the present work, the antioxidant and antibacterial properties of the isolated quercitrin was determined using DPPH method and disk paper test, respectively. Based on the bioactivities result of these two measurements, the further application of quercitrin can be decided.

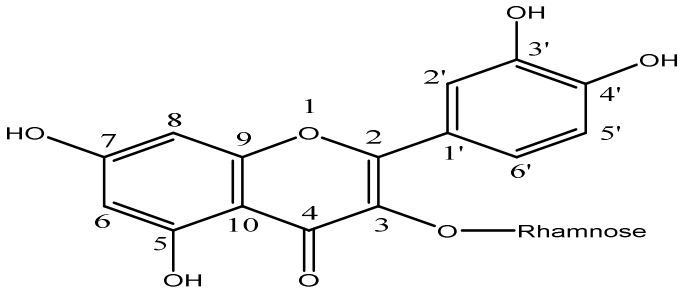

Fig.-5: Chemical Structure of Quercitrin (Quersetin 3-O-α-rhamnoside)

Antioxidant Activity

The antioxidant activity of quercitrin from *Dendrophthoepentandra* (L.Miq) is shown in Fig.-6.

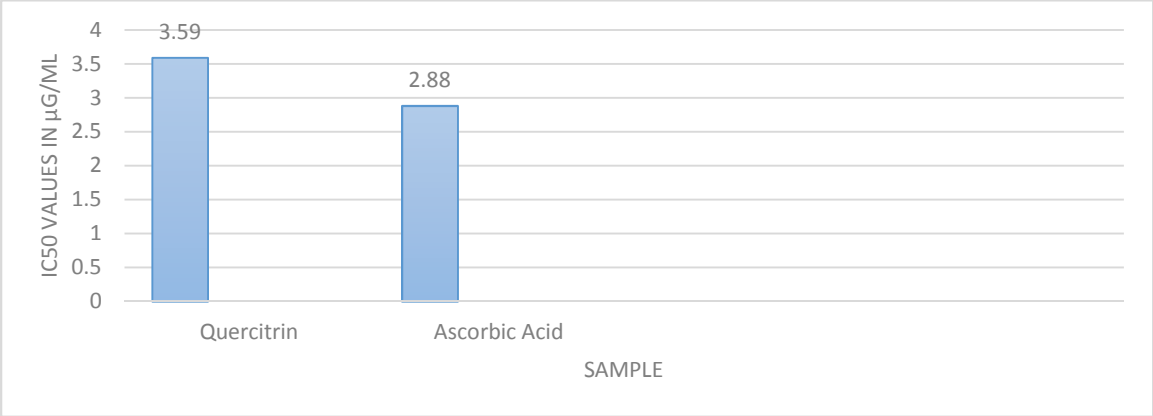

Fig.-6: The IC_{50} Values of Quercitrinand Ascorbic Acid on 1,1-diphenyl-2- picrylhydrazyl

The antioxidant activity of quercitrin that isolated from *Dendrophthoepentandra* (L.) Miqshowed as the strongest antioxidant than the standard, ascorbic acid solution (IC_{50} = 5.1 µg/mL) with an IC_{50} value of 3.59µg/mL.[18] This highest activity was assumed as the presence of OH bond homolytic dissociation phenomenon or ionization potential negatively affect the phenolic compound, i.e. quercitrin.[19] Also, the antioxidant molecule donates an electron or hydrogen atom to radical species to make them stable.[20]

Antibacterial Activity

The antibacterial activity of quercitrin of *Dendrophthoepentandra* (L.) Miq is shown in Table-2.

Table-2: Antibacterial Activity of the Obtained extract of *Dendrophthoepentandra* (L.) Miq

Name of Bacteria	Consentration (µg/mL)	Zone of Inhibition (mm)	
		Quercitrin	DMSO
Escherrichiacolli	10	6.74 ± 0.16	NZ*
	100	7.18 ± 0.08	NZ*
	1000	7.74 ± 0.15	NZ*
Salmonella typhi	10	5.49 ± 0.08	NZ*
	100	7.13 ± 0.18	NZ*
	1000	7.23 ± 0.11	NZ*
Staphylococcus aeureus	10	8.73 ± 0.17	NZ*
	100	9.39 ± 0.09	NZ*
	1000	9.54 ± 0.09	NZ*
Pseudomonas	10	6.63 ± 0.11	NZ*
	100	7.65 ± 0.19	NZ*
	1000	8.52 ± 0.09	NZ*

*NZ: No zone of inhibition, Results represent the means ± SD (standard deviation) from at least two separate experiments

Table-2 showed quercitrinhas good antibacterial properties against several pathogens bacterial that based on the inhibition zone value. The increase in the concentration of quercitrin that applied to the bacterial culture gave the linear value of inhibition zone.[21]

CONCLUSION

Quercitrinhas have been isolated from *Dendrophthoepentandra* (L.) Miq leaves. The structure elucidation of the isolatedquercitrin has been confirmed using UV-Vis, FT-IR, ^{1}H and ^{13}C-NMR. The bioactivity of the isolated quercitrin was evaluated for the antioxidant and antibacterial activities, which showed IC_{50} of 3.59 ppm for antioxidant activity against DPPH free radicals and a linear correlation between concentrations and their zone of inhibitions. At 1000 ppm of the concentration, against *E.coli*, *S.typhi*, *S. aeureus*, and *Pseudomonas sp.* quercitrin showed the inhibition zone of 7.74, 7.23, 9.54 and 8.52 mm, respectively.

ACKNOWLEDGMENT

Our thanks to the Ministry of Research Technology and Higher Education (KEMENRISTEK DIKTI) through PMDSU scholarship which has funded this research and Chemical Research Center LIPI which has facilitated a laboratory for this research.

REFERENCES

1. H. Ishizu, T, Winarno, H, Tsujno, E, Morita, T & Shibuya, *Chem. Pharm. Bull*, **50**, (2002), **DOI**:10.1248/cpb.50.489
2. J. L. C. H. Valkenburg, (2003). *Dendrophthoe pentandra* (L.) Miq.In RHMJ Lemmens and N Bunyapraphatsara (Eds.) *Plant Resources of South East Asia (PROSEA) 12, Medicinal and Poisonous Plants 3*. (pp.159-158). Bogor, Indonesia: Prosea Foundation.
3. P. O. Osadebe, F. B. C. Okoye, P. F. Uzor, N. R. Nnamani, I. E. Adiele, and N. C. Obiano, *Asian Pac. J. Trop. Med.*, **5**, (2012), **DOI**:10.1016/S1995-7645(10)60008-9
4. N. Artanti, T. Firmansyah, A. Darmawan, and T. Selatan, (2012).
5. C. S.-B. M. Dueñas, F. Surco-Laos, S.González-Manzano, A.M. González-Paramás, *Eur. Food Res. Technol*, **232**, (2011), **DOI**:10.1007/s00217-010-1363-y
6. M. B. K. P.C.H. Hollman, *Food Chem. Toxicol*, **37**, (1999), **DOI:**10.1016/S0278-6915(99)00079-4
7. L. E. P. T. Guardia, A.E. Rotelli, A.O. Juarez, *Farm.*, **56**, (2001), **DOI:**10.1016/S0014-827X(01)01111-9
8. N. M. O. S.A. Aherne, *Nutrition*, **18**, (2002), **DOI:**10.1016/S0899-9007(01)00695-5
9. A. B. A.W. Boots, G.R.M.M. Haenen, *Eur. J. Pharmacol*, **585**, (2008), **DOI:**10.1016/j.ejphar.2008.03.008
10. R. Hardiyanti, L. Marpaung, I. K. Adnyana, and P. Simanjuntak, *Asian J. Pharm. Clin. Res.*, **11**, (2018), **DOI:**10.22159/ajpcr.2018.v11i12.29725
11. D. A. Nantenaina T, R. R. Zoarilala, R. David, D. Reine, R. Ramilison, M. Eric, *Am. J. Innov. Researc Appl. Sci.*, (2017).
12. N. F. Isabel LB, C. T. Juan, A. J. Ignacio, *Bio-protocol*, **6**, (2016), **DOI:**10.21769/BioProtoc.1867
13. R. Hardiyanti, L. Marpaung, I. K. Adnyana, and P. Simanjuntak, in *7th AIC-ICMR Heal. Life Sci. Annu. Int. Conf. 2017 Syiah Kuala Univ.*, (2017).
14. N. Nerdy and K. Manurung,*Rasayan J. Chem.*, **11**, (2018), **DOI:**10.31788/RJC.2018.1134018
15. J. A. O. Olugbuyiro, A. S. Banwo, A. O. Adeyemi, O. S. Taiwo, and O. A. Akintokun, *Rasayan J. Chem.*, **11**, (2018), **DOI:**10.31788/RJC.2018.1121823
16. G. S. Donald LP, M. L. Gary, in *Saunders Golden Sunburst Ser.*, (1979).
17. L. D. S. Yadav, in *Springer Sci. Media Dordr.*, (2005), **DOI:**10.1007/978-1-4020-2575-4
18. M. S. Hasan, M. I. Ahmed, S. Mondal, S. J. Uddin, M. M. Masud, S. K. Sadhu, and M. Ishibashi, *Orient. Pharm. Exp. Med.*, **6**, (2006), **DOI:**10.3742/OPEM.2006.6.4.355
19. B. D. Craft, A. L. Kerrihard, R. Amarowicz, and R. B. Pegg, (2012).
20. T. Okselni, A. Santoni, A. Dharma, and M. Efdi, *Rasayan J. Chem.*, **11**, (2018), **DOI:**10.31788/RJC.2018.1133058
21. K. Hassan, and M. E. Bagoury, *Rasayan J. Chem.*, **11**, (2018), **DOI:**10.7324/RJC.2018.1112019

[RJC-5353/2019]