RASĀYAN *J. Chem.*
Vol. 16 | No. 2 |1042-1049| April - June | 2023
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

RED BETEL LEAF *(Piper cf. arcuatum* BLUME) ULTRASOUND-ASSISTED EXTRACTION OPTIMIZATION AND THEIR POTENTIAL ANTI-URIC ACID AND ANTIOXIDANT ACTIVITY

Candra Irawan[1], Hendra[3], Lany Nurhayati[3], Nurlela[3], Arum Widyasmara[2,✉] and Imalia Dwi Putri[1]

[1]Departement of Food Nanotechnology, Politeknik AKA Bogor, Bogor-16154, Indonesia
[2]Departement of Analytical Chemistry, Politeknik AKA Bogor, Bogor-16154, Indonesia
[3]Departement of Chemistry, Universitas Nusa Bangsa,Bogor, Indonesia
✉Corresponding Author: arumwidyasmara@gmail.com

ABSTRACT

Piper cf. arcuatum Blume is a plant that has long been used by people in Indonesia as a traditional medicine. Based on previous studies, *Piper cf. arcuatum* Blume has antioxidant activities and a potential reduction of uric acid. This study aims to ascertain the ideal amplitude and time to produce a large yield value using the ultrasound-assisted extraction (UAE) method and the anti-uric acid potential affected by antioxidant activity (IC_{50}) in red betel leaf using the CUPRAC (Cupric reducing antioxidant capacity) method. This study indicates that the outcome of the phytochemical test of red betel leaf extract using the UAE contained compounds of phenol, flavonoid, and alkaloid that had strong antioxidant activity and a high potential reduction of uric acid. In the UAE, the extraction time and optimum amplitudes were 35 minutes and 65%, respectively, with an IC_{50} value for antioxidant activity of 15.46 ± 0.04 mg/L and a 54.91% decrease in uric acid.

Keywords: *Piper cf. arcuatum Blume*, UAE, Antioxidant, Anti-Uric Acid, CUPRAC

RASĀYAN *J. Chem.*, Vol.16, No. 2, 2023

INTRODUCTION

Piper is the genus of the family Piperaceae, which has high economic value as traditional medicine, the complement of traditional ceremonies, and for culinary uses. Red betel (*Piper cf. arcuatum*) and green betel (*Piper betel* L) are the members of *Piper* that are commonly used in Indonesia as traditional medicine. Red betel leaf is effective as an antioxidant against hydroxyl (OH), 1,1-diphenyl-2-picrylhydrazyl (DPPH), anion superoxide ($O2^-$), and lipid peroxidation assay radicals. It was also discovered to be a potent OH radicals catcher.[1] Several studies have found that extracts of red betel leaf have anticancer[2], antioxidant[3], hepatoprotective properties[4], anti-inflammatory[5], antifertility[6], antidermatophytic[7], and anti-cariogenic.[8] Flavonoids, tannins, alkaloids, polyphenolics, quinones, saponins, steroids, and essential oils have all been reported to be present in red betel.[9] The extraction of the plant is an empirical process because different solvents are utilized under various extraction circumstances, such as time and temperature.[10] The extraction processes available may result in the loss of certain chemicals, inefficient extraction, and energy-and time-intensive procedures. These flaws have led to the adoption of new sustainable "green" extraction methods, such as UAE, microwave, and supercritical fluid extraction, which often consume less energy and solvent.[11] The research about the pomegranate peel's antioxidant yield, activities, and extraction kinetics using UAE in continuous (CUAE) and pulsed modes (PUAE) compared with conventional extraction showed higher extraction rates as compared to a conventional extraction process.[12] Antioxidants, which include reactive oxygen species and free radicals, are health-promoting substances that counteract an excess of oxidants. CUPRAC (Cupric reducing antioxidant capacity) is an electron transfer-based test for determining the total amount of antioxidants.[13] Previous studies on antioxidant activity in red betel leaf/RBL (*Piper cf. arcuatum Blume*) have been widely published, but no studies on antioxidant activity using the CUPRAC method in chemicals extracted using the UAE method have been published. In this study, we'll look at the amplitude and duration optimum for producing a high yield value using the ultrasound-assisted extraction method, as

http://doi.org/10.31788/RJC.2023.1628175

well as the anti-uric acid potential in red betel leaf as a function of antioxidant activity (IC_{50}) utilizing the CUPRAC method.

EXPERIMENTAL

General Experimental Procedure

This research was conducted at the Food Nanotechnology Laboratory of the Politeknik AKA Bogor. This research was carried out in several stages. They were sample preparation, sample extraction, phytochemical screening, antioxidant activity assay using the CUPRAC method, and anti-uric acid potential activity test.

Chemical and Reagent

Red betel leaves/RBL (*Piper cf. Arcuatum Blume*), ethanol (purify 90%), $CuCl_2.2H_2O$ (0.01M), neucuproine (0.0075M), ammonium acetate buffer pH (7.1 M), uric acid FS-TBHBA reagent kit (2,4,6-tribromo-3-hydroxybenzoic acid), standard uric acid (0.48 g/mL), allopurinol, and phytochemical test reagents.

Instrumentation

Ultrasonic extraction machines (Vibra-Cell 505), a spectrophotometer UV-Vis (Specord 200), a digital analytic balance, an oven, and laboratory glassware.

Sample Preparation

Red betel leaves/RBL (*Piper cf. Arcuatum Blume*) were harvested from Bandar Lampung. Before the drying process, the leaves were washed under running water to get the dirt off. The leaves were fragmented and allowed to dry at room temperature for three weeks before being baked at 40 °C to preserve the organic compounds in the leaves. Following that, the leaf pieces were mashed with a blender until the sample was in the form of powder. The sample will be stored in a clean, tightly closed container for future testing.

Sample Extraction Using the UAE Method

The UAE method was used to prepare samples with 90% ethanol as the solvent. The dried RBL powder (*Piper cf. arcuatum Blume*) weighed roughly 16 grams and was applied four times to a 250 mL beaker. After that, ethanol (90%) was added, and the dried RBL powder was well combined while being agitated. The samples were then sonicated using a vibrating ultrasonic probe for various times (minutes) and amplitudes (%) (35-45, 35-65, 60-45, and 60-65).[14]

Determination of Extraction Yield

The extraction results were filtered into a beaker (250 mL), which had been weighed to separate the liquid extract from the residue. The yield of RBL was then obtained by evaporating the filtrate in an oven set at 40°C until all of the ethanol had evaporated. Without containing a solvent, the weight of the extract can be measured, and the yield value can be determined.

Using the following equation, the crude extract's yield from RBL powder was obtained:

$$Yield = \frac{Wd}{Wa}\ x\ 100\%$$

Where
Wd = the crude extract's weight (g)
Wa = the raw sample's weight (g)

Phytochemical screening

The identification of chemical compounds was carried out on the crude extract of RBL with each time (minutes) and amplitude (%) variation of UAE extraction. The assay included the identification of saponins, phenol, tannins, steroids, triterpenoid sterols, flavonoids, and alkaloids using standard reagents.[15,16,17]

Antioxidant Activity Assay Using the CUPRAC Method

The dried RBL powder (*Piper cf. arcuatum Blume*) was weighed as much as 0.01 grams and was dissolved in 90% ethanol up to 10 mL, producing a sample solution with a concentration of 1000 mg/L. The solution was pipetted into a measuring flask, and 1 mL of CUPRAC solution and 90% ethanol was added until the sample solution has a concentration of 4, 32, and 64 mg/L, respectively. The solution was incubated for 30 minutes before measuring its absorbance at 459 nm.[14] Butylhydroxytoluene (BHT) was used as a standard

antioxidant. The same work is done for BHT until the resulting sample concentration is 1.5; 2.0; 2.5; and 3 mg/L, respectively. The following equation can be used to calculate reducing activity:

$$\%\ Reduction\ activity = \frac{A_{sample} - A_{blank}}{A_{sample}}\ x\ 100\%$$

Where:
A_{sample} = Absorbance of sample
A_{blank} = Absorbance without sample

Antioxidant activity using the CUPRAC method was determined based on plotting the concentration of the sample and the % value of reduction activity on the x and y axes, respectively in the linear regression equation (Y=bX + a). The IC_{50} value can be calculated using its calibration curve and obtained from the calculation when the % reduction is 50%.[18]

$$IC_{50} = \frac{50 - a}{b}\ x\ 100\%$$

Anti-Uric Acid Potential Activity Test
Each extract was weighed at as much as 5 mg and dissolved in 90% ethanol up to 5 mL, yielding a sample solution with a concentration of 1,000 mg/L. Pipetting 40 µL of the solution into a 5 mL volumetric flask, adding 40 µL of uric acid standard (6 mg/dL), and allowing it to stand for 5 minutes. 0.25 mL of reagent 1 was added to the solution, and it was allowed to stand for 5 minutes. Then added to 62.5 mL of reagent 2, and allowed to stand for 30 minutes at a temperature of 20–25 °C. Using a visible spectrophotometer with a 513 nm wavelength, the solution was measured. The uric acid standard, the positive control allopurinol, and the blank received the same treatment.[19]
The reduction of uric acid (%) can be calculated using the following equation:

$$Uric\ acid\ concentration\ (after\ incubated) = \frac{A_{sample}}{A_{uric\ acid\ standard}}\ x\ C_{uric\ acid\ standard}$$

$$The\ reduction\ of\ Uric\ Acid\ (\%) = \frac{C_{uric\ acid\ standard} - C_{uric\ acid\ after\ incubated}}{C_{uric\ acid\ standard}}\ x\ 100\%$$

Where
A = Absorbance
C = Concentration

RESULTS AND DISCUSSION

Determination of Extraction Yield Using Ultrasound-Assisted Extraction (UAE) Method
Using an ultrasonic probe and 90% ethanol as a solvent, the dried RBL powder (*Piper cf. arcuatum Blume*) was extracted under various extraction conditions of time (minutes) and amplitude (%) (35-45, 35-65, 60-45, and 60-65). As a result of its reputation as a non-hazardous, eco-friendly substance, and secure solvent, ethanol was chosen as the solvent for this research.[20] Table-1 shows the crude ethanol extract and the yield (%) of crude extract at various times and amplitudes from the UAE Process.

Table-1: The Crude Extract's Yield using the UAE Method

Sample	T (minutes) – A (%)	Mass of Sample (g)	Mass of Crude Extract (g)	Yield (%)
A	35-45	15.2164	1.7723	11.65
B	35-65	15.2272	1.9085	12.53
C	60-45	15.2414	1.9188	12.59
D	60-65	152375	1.8798	12.34

The results of the optimization of RBL extraction showed that the enhancement of the extraction time from 35 minutes to 60 minutes causes an increase in the yield. This is because the longer the reaction time between the material and the solvent, the better the penetration process into the material cell, causing more compounds to diffuse out of the cell. According to a prior study on the relationship between extraction time and yield, under UAE conditions, the yield increased dramatically as the extraction duration increased. This

could be explained by the result of cavitation bubbles on the product surface, which facilitated the diffusing extract and caused a difference in osmotic pressure between the interior and exterior of the cell.[21] The highest yield was obtained at 60 minutes of extraction time with 45% of amplitude (C), which was 12.59%. This result is similar to the 35 minutes extraction time with 65% of amplitude (B), which was 12.53%. The yield was 12.34% at 60 minutes of extraction time with 65% of amplitude (D). It showed there was a decrease in extraction yield. Earlier studies showed that increased amplitude resulted in increases in extraction yield. This is caused by how amplitudes affect extract release, which enhances solvent penetration into cells by promoting wall cell disintegration. However, due to their ability to scavenge newly formed free radicals, excessively high amplitudes can result in a decrease in extraction yield.[22] Based on the description, the length of time required to extract RBL is directly related to the increase in yield. However, the use of a longer extraction time causes more energy and power to be used, which increases the cost of the extraction process and also the degradation of bioactive compounds. Therefore, it can be stated that RBL extraction with UAE is optimal at 35 minutes with an amplitude of 65% (B).

Phytochemical Screening

The phytochemical screening results from the ethanolic extract of RBL powder (*Piper cf. arcuatum Blume*) can be seen in Table-2. They have a very strong reaction in flavonoid and phenol assays. It is well known that flavonoids and phenolic compounds exhibit antioxidant activity and impact human nutrition and health in significant ways. The processes that flavonoids use to exert their effects are scavenging or chelating.[23] Phenolic compounds are known as a type of antioxidants that act as free radical scavengers.[24] Although the reaction was small in both reagents, phytochemical screening of RBL extract on the alkaloid test yielded positive results. This demonstrates that the alkaloids found in red betel leaf powder are present in trace amounts. The formation of a brick-red precipitate indicated a positive result for Dragendorff's reagent, and the formation of a white precipitate indicated a positive result for the Mayer test.

Table-2: Phytochemical Screening of Crude Extract using the UAE Method

No	Phytochemical groups	Parameter			
		Time (minutes) – Amplitude (%)			
		35 – 45 (A)	35 – 65 (B)	60 – 45 (C)	60 – 65 (D)
1	Tannin	++	+++	+++	+
2	Phenols	++	+++	+++	+
3	Saponins	+	+	+	+
4	Steroid Glycoside	+++	+	++	++
5	Sterols	-	-	-	-
6	Flavonoids	+	++	+	+
7	Alkaloids				
	• Dragendrof's	+	+	+	+
	• Mayer	+	+	+	+

According to previous studies, the antioxidant activity of alkaloid extracts has been proven and showed strong antioxidant activity, especially a high radical scavenger capability.[25]

Antioxidant Activity Assay Using the CUPRAC Method

The CUPRAC assay for polyphenols and other antioxidant compounds employs the chromogenic oxidant Cu(II)-Nc. After 30 minutes of incubation, the antioxidant solutions were mixed with $CuCl_2$, neocuproine, and ammonium acetate solutions at pH 7. After that, the absorbance of the solution was measured. The hydroxyl group of a polyphenol (Ar-OH) was oxidized to a quinone, and Cu(II)-Nc was reduced to Cu(I)-Nc at 450 nm. As observed, the color shifts from blue to yellow.[26] The CUPRAC capacity was given as a percent reduction power, which was then linked to a set of standard and sample concentrations to make a curve with a regression equation. IC_{50} of CUPRAC capacity is the concentration of a standard or sample that can reduce 50% of CUPRAC capacity. Table-3 describes the regression equation and IC_{50} of CUPRAC capacities in various ethanolic extracts of RBL powder (*Piper cf. arcuatum Blume*).

Overall, BHT is more effective at reducing Cu^{2+} than red betel leaf extract, although its reduction power is classified as a very strong antioxidant because its IC_{50} value is less than 50 mg/L. Based on the results, it can be supposed that the lowest IC_{50} from the various red betel leaf extracts was extracted with 35 minutes

of extraction time and 65% of amplitude (B). The maximum antioxidant capacity is indicated by the lowest IC_{50}. Classification by Blois known that samples that had an IC_{50} 50 g/ml were very strong antioxidants, 50-100 g/ml were strong antioxidants, and 101-150 g/ml were medium antioxidants, whereas a weak antioxidant with an IC_{50} of >150 g/ml.[27]

The results for radiation amplitude showed a decrease in IC_{50} (higher antioxidant activity) between 45% and 65% amplitude. Due to the compression and rarefaction of the medium during the sonication process, which causes it to implode at high temperatures and pressures, cavities or microbubbles are created. Cavitation near the cell's surface disrupts the cell wall, allowing constituents from within to be released more easily. This not only promotes greater diffusion across cell walls but also improves the system's overall mass transfer, accelerating bioactive compound recovery.[20]

Table-3: The Antioxidant Activity Test Using CUPRAC Method

Sample	Concentration (mg/L)	% Reduction Power	Regression Equation	IC_{50} (mg/L)
BHT	1.5	38.01 ± 0.2	y = 47.812ln(x) + 18.199	0.33 ± 0.003
	2.0	51.36 ± 0.04		
	2.5	60.34 ± 0.03		
	3.0	71.95 ± 0.01		
A	8	25.37 ± 0.10	y = 25.024ln(x) -26.385	20.77 ± 0.02
	32	61.18 ± 0.05		
	64	77.13 ± 0.004		
B	8	39.50 ± 0.06	y = 16.621(x) + 4.4836	15.46 ± 0.04
	32	60.72 ± 0.02		
	64	74.52 ± 0.01		
C	8	29.54 ± 0.09	y = 22.923ln(x) – 17.773	19.04 ± 0.04
	32	62.72 ± 0.01		
	64	76.86 ± 0.002		
D	8	26.34 ± 0.1	y = 24.991ln(x) -25.777	20.40 ± 0.07
	32	60.38 ± 0.01		
	64	78.46 ± 0.01		

Where:
BHT: Antioxidant standard
A: RBL extract with 35 minutes extraction time - 45% of amplitudes
B: RBL extract with 35 minutes extraction time - 65% of amplitudes
C: RBL extract with 60 minutes extraction time - 45% of amplitudes
D: RBL extract with 60 minutes extraction time - 65% of amplitudes

However, a longer extraction time, on the other hand, would reduce the yield. This discovery can be attributed to the catalytic action of carbohydrates on the release of bound phenolics during the early stages of maceration, followed by enzyme inactivation by bioactive inhibitors (phenolic compounds).[22] The antioxidant activities of red betel leaf extract were primarily attributed to phenolic, flavonoid, and alkaloid compounds. Some of these substances are important as antioxidants or play crucial roles in biological systems; they are possibly involved in electron transfer reactions.[28]

Potential Anti-Uric Acid Activity Test

The anti-uric acid activity of the RBL extract was examined in vitro using the reagent TBHBA and pure uric acid solution as a standard. After incubation with various RBL extracts and allopurinol, the reduction of uric acid (%) is shown in Table-4 and Fig.-1.

The in vitro anti-gout test is based on the uricase enzyme's oxidation of uric acid to allantoin and peroxide molecules. A spectrophotometer can be used to quantify quinone min, which is produced when the resultant peroxidase complex reacts with TBHBA.

The reduction of uric acid can be calculated by subtracting the concentrations of uric acid before and after the addition of allopurinol and various RBL extracts.[19] Based on the results, various RBL extracts have a potential activity for the reduction of uric acid better than allopurinol. The highest reduction occurred in RBL extract with an amplitude of 35 minutes-65% amplitudes (B). Similar to antioxidant activity, the

higher amplitude can increase the extract's potential activity to lower uric acid due to the increasing penetration into the cell. According to evidence from another study, red guava juice has the ability to prevent an increase in blood uric acid and creatinine levels due to its antioxidant content, which inhibits the development of xanthine oxidase and free radical chain breaker.[29] Polyphenols and flavonoids can lower levels of uric acid by working as antioxidants and blocking free radicals, along with a variety of enzymes, including xanthine oxidase, lipoxygenase, and cyclooxygenase.[30] Substrates for the xanthine oxidases that, in most cases, convert xanthine to uric acid are flavonoids and polyphenols, respectively. This substrate competition will reduce the production of uric acid since xanthine oxidase prefers to oxidize any polyphenols and flavonoids over xanthine.[31]

Table-4: The Uric Acid Reduction After Incubated with Allopurinol and RBL Extract

Sample	Absorbance	Uric Acid Concentration (mg/L)	Uric Acid Reduction (mg/L)	Uric Acid Reduction (%)
Uric Acid Standard (0.5 mg/L)	0.0601	0.5000		
	After incubated by			
Allopurinol (0.5 mg/L)	0.0586	0.4875	0.0125	2.5
35 minutes - 45% (A)	0.0475	0.4052	0.1048	20.96
35 minutes - 65% (B)	0.0271	0.2254	0.2745	54.91
60 minutes - 45%(C)	0.0273	0.2271	0.2729	54.58
60 minutes - 65% (D)	0.0412	0.3428	0.1572	31.45

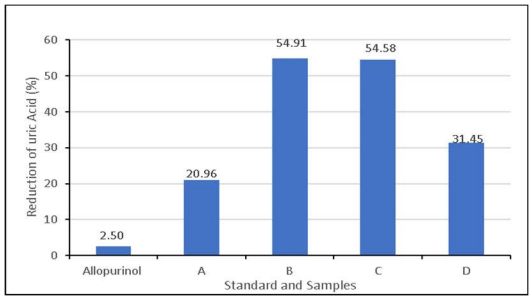

Fig.-1: Graph of the Uric Acid Reduction (%) after Incubation with Allopurinol and RBL Extract. Where: (A) RBL with 35 Minutes Extraction Time- 45% of Amplitude, (B) RBL Extract with 35 Minutes Extraction Time- 65% of Amplitude, (C) RBL Extract with 60 Minutes Extraction Time- 45% of Amplitude, (D) RBL Extract with 60 Minutes Extraction Time- 65% of Amplitude

CONCLUSION

According to the findings of the research, it may be said that the result of a phytochemical test on red betel leaf extract using UAE contained compounds of phenol, flavonoid, and alkaloid that had strong antioxidant activity and potential for reduction of uric acid. In the UAE method, the optimum extraction time and amplitude were 35 minutes and 65%, respectively, with an IC_{50} value for antioxidant activity of 15.46 ± 0.04 mg/L and a 54.91% decrease in uric acid. The potential anti-uric acid and antioxidant activities of red betel leaf extract in UAE indicate that they can be employed as natural and beneficial sources of natural antioxidants for therapeutic and commercial purposes.

ACKNOWLEDGEMENTS

Both Politeknik AKA Bogor and Universitas Nusa Bangsa in Bogor, West Java, Indonesia, provided funding and facilities for this study.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

C. Irawan https://orcid.org/0000-0001-9870-6543
L. Nurhayati https://orcid.org/0009-0005-3609-7689
Nurlela https://orcid.org/0000-0003-4207-7906
Hendra https://orcid.org/0009-0006-8662-7788
A.Widyasmara https://orcid.org/0009-0006-7789-1579
I.Dwi Putri https://orcid.org/0009-0006-2119-1768

REFERENCES

1. I.N.E. Lister. C.N. Ginting, E. Gisrsang, A. Armansyah, H.H. Marpaung, A.P. F. Sinaga, R.A. S. Handayani, R. Rizal, *Journal of Natural Remedies*, **19,** 4(2019), https://doi.org/10.18311/jnr/2019/23633
2. S.R. Gundala, R. Aneja, *Cancer Prevention Research (Phila)*, **7,** 5(2014), https://doi.org/10.1158/1940-6207.CAPR-13-0355
3. M. Rahardjo, G. Mangalik, M. Sihombing, J.F. Costa, *Indonesian Journal of Agricultural Research*, **1,** 1(2018), https://doi.org/10.32734/injar.v1i1.173
4. S.M. Prabu, M. Muthumani, K. Shagirtha, *Saudi Journal of Biological Sciences,* **19,** 2(2012), https://doi.org/10.1016/j.sjbs.2012.01.005
5. D. Runtu, M.Shinjini, M. Kaustab, P. Pramathadip, P.S. Umesh, E.R. Banerjee, *Journal Nutrition and Food Science*, **5**, 5(2015), http://dx.doi.org/10.4172/2155-9600.1000415
6. S. Biswal, *Ancient Science of Life,* **34, 1** (2014), https://doi.org/10.4103/0257-7941.150770
7. N. Trakranrungsie, A. Chatacha-Wanchonteera, W. Khunkitti, *Research in Veterinary Science,* **84,** 1**(**2008), https://doi.org/10.1016/j.rvsc.2007.03.006
8. V.S.Varunkumar, M.G. Nair, Sam Joseph, *IOSR Journal of Dental and Medical Sciences*, **13,** 8(2014). https://doi.org/10.9790/0853-13814348
9. N. Wulandari, A. Meiftasari, H. Fadliyah, R.I. Jenie, *Indonesian Journal of Cancer Chemoprevention,* **9,** 1(2018), https://doi.org/10.14499/indonesianjcanchemoprev9iss1pp1-8
10. F. Chemat, N. Rombaut, A.G. Sicaire, A. Meullemiestre, A.S.F. Tixier, M.A. Vian, *Ultrasonis Sonochemistry*, **34**, 540(2017), https://doi.org/10.1016/j.ultsonch.2016.06.035
11. R. Rutkowska, J. Namiesnik, P. Koniecxka, *The Application of Green Solvents in Separation Processes,***2017**,301(2017), https://doi.org/10.1016/B978-0-12-805297-6.00010-3
12. Z. Pan, W. Qu, H. Ma, G.G. Atungulu, T.H. Mchugh, *Ultrasonic Sonochemistry*, **19(2)**, 365(2012), https://doi.org/10.1016/j.ultsonch.2011.05.015
13. M. Özyürek, K. Güçlü, E. Tütem, K.S. Başkan, E. Erçağ, E. Çelik, et. al, *Analytical Methods,* **3**, (2011), https://doi.org/10.1039/c1ay05320e
14. C. Irawan, B. Elya, M. Hanafi, F.C. Saputri, *Pharmacognosy Journal,* **113,** 5(2021), https://doi.org/10.5530/pj.2021.13.164
15. J.R. Shaikh and M.K. Patil, *International Journal of Chemical Studies*, **8,** 2(2020), https://doi.org/10.22271/chemi.2020.v8.i2i.8834
16. A. Marsoul, M. Ijjaali, F. Elhajjaji, M. Taleb, R. Salim, A. Boukir, *Material Today: Proceeding*, **27,** 4(2020), https://doi.org/10.1016/j.matpr.2020.04.202
17. S. Gurudeeban, T. Ramanathan, K. Satyavani, *Pharmaceutical Chemistry Journal*, **49,** 1(2015), https://doi.org/10.1007/s11094-015-1218-8
18. I. Fidrianny, E.N. Rezeki, K.Ruslan, R.Hartati, N.M.D.M.W. Nayaka, *Rasayan Journal of Chemistry,* **14,** 1(2021), https://doi.org/10.31788/RJC.2021.1415907
19. C. Irawan, A. Utami, E. Styani, I.D. Putri, R.K. Putri, A. Dewanta, A. Ramadhanti, *Pharmacognosy Journal*, **13**, 6(2021), https://doi.org/10.5530/pj.2021.13.168

20. A. Ali, X.Y. Lim, C.H. Chong, S.H. Mah, B.L. Chua, *Separation Science and Technology,* **53(14)**, 2192(2018), https://doi.org/10.1080/01496395.2018.1443137
21. Y. Sun, D. Liu, J. Chen, X. Ye, D. Yu, *Ultrasonics Sonochemistry*, **18(1)**, 243(2011), https://doi.org/10.1016/j.ultsonch.2010.05.014
22. J. Nishad, S. Saha, C. Kaur, *Journal of Food Processing and Preservation,* **43(8)**, e14046(2019), https://doi.org/10.1111/jfpp.14046
23. M. Kessler, G. Ubeaud, L. Jung, *Journal of Pharmacy and Pharmacology,* **55(1),** 131(2003), https://doi.org/10.1211/002235702559
24. F. Shahidi, P.K.J.P.D. Wanasundara, *Critical Review in Food Science and Nutrition,* **32(1)**, 67(1992), https://doi.org/10.1080/10408399209527581
25. I. Gulcin, R. Elias, A. Gepdiremen, A. Chea, F. Topal, *Journal of Enzyme Inhibition and Medical Chemistry,* **25,** 1**(**2009), https://doi.org/10.3109/14756360902932792
26. R. Apak, K. Güçlü, M. Ozyürek, S.E. Celik, *Critical Review in Food Science and Nutrition*, **160,** 413(2008), https://doi.org/10.1007/s00604-007-0777-0
27. M.S. Blois, *Nature*, **181**, (1958), https://doi.org/10.1038/1811199a0
28. S. Stenken And P. Neta, *Journal of Physical Chemistry*, **86,** 18(1982), https://doi.org/10.1021/j100215a033
29. F.W.D. Aprilinda, D. Paramasari, B. Wasita, *Bali Medical Journal*, **7(2)**(2018), https://doi.org/10.15562/bmj.v7i2.878
30. H. Zhu, D. Song, X Zhao, *Food Bioscience,* **43,** 101297(2021), https://doi.org/10.1016/j.fbio.2021.101297
31. F. Abdulhafiz, A. Mohammed, F. Kayat, M. Bhaskar, Z. Hamzah, S.K. Podapati, L.V. Reddy, *Molecules*, **25,** 11(2020), https://doi.org/10.3390/molecules25112658

[RJC-8175/2022]