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ASSESSMENT OF ANTIOXIDANT ACTIVITY, TOTAL PHENOLICS, AND FLAVONOIDS OF DIFFERENT SOLVENT FRACTIONS OF *Eriobotrya japonica* Lindl. FRUIT EXTRACT

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ABSTRACT

The purpose of the study was to determine the antioxidant potential, total phenol, and total flavonoid content in the n-hexane fraction, ethyl acetate fraction, and water fraction of *Eriobotrya japonica* fruit. The research method for testing the antioxidant activity of the fruit of Eriobotrya Japonica was the DPPH (1.1-diphenyl-2-picryhydrazil) method by UV-VIS spectrophotometry, while phenol and flavonoids contents were carried out by colorimetric method UV-Vis spectrophotometry. The determination of total phenol with Folin-Ciocalteu reagent and gallic acid as the standard, and the determination of total flavonoid content with AlCl3 reagent and quercetin as the standard. The results revealed that the IC50 values for the antioxidant activity of the n-hexane fraction, ethyl acetate fraction, and water fraction are 170.21, 68.33, and 351.95 g/mL, respectively. The total phenol concentration in the n-hexane fraction, ethyl acetate fraction, and water fraction, respectively, was 13.08, 95.34, and 15.24 mg GAE/g of material. The n-hexane fraction, ethyl acetate fraction, and water fraction contained 0.88, 12.65, and 2.6 mg QE per gram of material, respectively. Compared to the n-hexane and water fractions, the ethyl acetate fraction of Eriobotrya Japonica fruit had the highest quantities of total phenol and total flavonoid.

Keywords: Antioxidants, Phenols, Flavonoids, Fractions, *Eriobotrya japonica* Lindl.

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INTRODUCTION

The fruit of *Eriobotrya japonica* has a high nutritional value, the flesh of the *Eriobotrya japonica* contains malic acid, tartaric acid, citric acid, tannin, beta-carotene, vitamins A, B, and C. The leaves and seeds contain amygladin.[1] In addition, according to traditional Chinese medicine, the leaves of Eriobotrya Japonica work on the respiratory and digestive systems, lung, and stomach passages.[2] Extraction of antioxidant chemicals from plants often employs solvents with varying degrees of polarity, such as ethanol, ethyl acetate, n-hexane a, and water. The type of solvent based on the level of polarity is very useful for obtaining extracts with a higher yield, and it is also intended that the compound group with the highest antioxidant activity can be extracted.[3] Various studies reported that there were large amounts of flavonoid and phenolic compounds in the fruit and leaves of Eriobotrya Japonica, and both the methanol extract of the leaves of Eriobotrya Japonica and their individual.[4] Large numbers of secondary plant metabolites are phenol molecules. To date, over 8000 dietary phenolics have been discovered, the distribution and accumulation patterns of which are affected by genetic and environmental variables. The fruit of *Eriobotrya japonica* has a delightful flavor and is a good source of food phenolics.[5] The potential of phenolic compounds to function as biologically active molecules plays a significant role in human interests, resulting in their widespread use today. One of these uses is as an antioxidant for preventing and treating degenerative diseases, cancer, premature aging, and immune system issues. Medicinal plants containing flavonoids have antioxidant, antibacterial, antiviral, anti-inflammatory, and anticancer properties. Almost every component of a plant contains flavonoids, including fruit, roots, leaves, and stem bark. The higher the phenolic and flavonoid content in a plant sample, the greater the antioxidant activity.[6] In this study, fractionation was performed to separate the content of primary metabolites in crude extracts that can affect the reaction results so that specific secondary metabolite compounds can be obtained according to their respective polarities.

This study was undertaken to investigate the antioxidant potential, total phenol content, and total flavonoids of Eriobotrya Japonica n-hexane fraction, ethyl acetate fraction, and fruit water fraction.

EXPERIMENTAL

Plant Collections

The *Eriobotrya japonica* plant was identified by the Herbarium Medanense (MEDA) Faculty of Mathematics and Natural Sciences, University of Sumatera Utara, Medan with authentication number 4506/MEDA/2019. Plant samples were collected in September 2020 from the Simalem Farm Brastagi in North Sumatra, Indonesia. All chemicals used are pro-quality analysis.

Instrument and Materials

The instrument used in this research was analytical balance (Mettler Toledo), hotplate (Hanna), Oven (Memmert), micropipette (Eppendorf; 10-100 L), micropipette (Eppendorf; 100-1000 L), volume pipette (Herma; 1 ml), volume pipette (Herma; 2 ml), volume pipette (Herma; 5 ml), Rotary evaporator (Haake D), spectrophotometer UV-Vis (Shimadzu). The High-grade reagents used in this study were Gallic acid (E-Merck), aluminum (III) chloride, Folin-Ciocalteau reagent, 96% ethanol, ethyl acetate, quercetin (Sigma), n-hexane, 1,1-diphenyl-2-picrylhydrazyl (Himedia).

Sample Preparation and Extraction

Fresh fruit from Eriobotrya japonica was selected, cleaned of any debris still attached, rinsed under running water, drained, split in half, and the seeds were extracted. The fruit is then measured as wet weight. In a drying cabinet, the fruit of the *Eriobotrya japonica* is dried. The fruit has been dried till it is brittle, weighed as dry weight, and then mashed with a blender. Put one-part dry powder and ten parts solvent into a glass container. The dried powder was kept at room temperature in a firmly closed plastic container with silica gel. Stirring occasionally, soak for the first six hours, then let stand for 18 hours, then separate the macerate. At least twice more, repeat the search process. Gather all the macerate, then use a rotary evaporator to evaporate the solvent.[7]

Qualitative Phytochemical Identification

Phytochemical screening of n-hexane, ethyl acetate, and water fractions includes an examination of alkaloids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids.[8,9]

Fractions of *Eriobotrya japonica*

Using n-hexane and ethyl acetate as solvents, liquid-liquid extraction was used to obtain the n-hexane and ethyl acetate fractions. As much 10 g ethanol extract was combined with 40 ml of ethanol and 100 ml of distilled water, homogenized, placed in a separating funnel, and extracted with 50 ml of n-hexane until the fraction did not yield a positive result when tested with Liebermann-Burchard reagent. The water fraction was extracted with 50 mL ethyl acetate until it no longer yielded a positive result when tested with $FeCl_3$. Using a water bath, the ethyl acetate fraction and the water fraction were concentrated.[10]

Antioxidant Assays

Determination of antioxidant potential of different fruit fractions of *Eriobotrya japonica* using a calibration curve in which the n-hexane fraction was given concentrations of 50, 100, 150, and 200 µg/mL, the ethyl acetate fraction was given concentrations of 25, 50, 75, and 100 µg/mL, and the water fraction was given concentrations of 100, 200, 300, and 400 µg/mL. To each concentration, 1 All of the flasks were left alone for 30 minutes, and then a UV-Vis spectrophotometer was used to measure the absorbance of each solution concentration at the maximum wavelength. The IC_{50} (Inhibitory Concentration) value is used to figure out how well a compound can get rid of free radicals. This value shows how much of the test compound is needed to get rid of 50% of the free radicals. The results of the calculations are put into the regression equation, with the sample concentration (g/ml) on the x-axis and the percentage of inhibition on the y-axis (Molyneux, 2004). In particular, a compound is a very strong antioxidant if its IC_{50} value is less than 50 g/ml. If its IC_{50} value is between 50 and 100 g/ml, it is a strong antioxidant. If its IC_{50} value is between 100 and 150 µg/mL, it is a weak antioxidant.[11]

Calculation of Total Phenolic Content

25 mg of n-hexane, ethyl acetate, and water fractions were each diluted in 10 mL of methanol to reach a concentration of 2500 µg/mL. Each fraction was then taken with 0.5 mL of Folin-Ciocalteu reagent, 1.5 mL of sodium carbonate (20%), and 7.9 mL of aqua distillate and thoroughly mixed. The reaction mixture was incubated for 85 minutes at 37 °C, and the absorbance at 750.4 nm was determined. The total phenol concentration was computed using a gallic acid-prepared standard curve.[12]

Calculation of Total Flavonoid Content

25 mg each of fraction n-hexane, ethyl acetate, and water were dissolved in 5 mL of methanol to achieve a concentration of 5000 µg/mL. To each fraction, 1.5 ml methanol pa, 0.1 ml of 10% aluminum chloride solution, 0.1 ml of 1M sodium acetate, and 2.8 ml of distilled water were added. After 32 minutes of incubation at room temperature, the absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 429.2 nm. Rate The collected flavonoids were represented as mg equivalent of quercetin per gram of extract.[13]

Data Analysis

Data analysis was carried out for the study after the determination of antioxidant potential, total phenolic content, and total flavonoids using linear regression equations calculated by Microsoft Excel program in order to obtain a standard linear equation of the absorbance curve (y) vs concentration (x). The concentration of scavenging free radicals (IC_{50}), phenol, and flavonoids from the sample solution was calculated based on a standard linear equation.[14]

RESULTS AND DISCUSSION

Qualitative Phytochemical Identification Result

The results of the phytochemical screening for the n fraction of hexane, ethyl acetate, and fruit juice of *Eriobotrya japonica* can be seen in Table-1.

Table-1: The Results of Phytochemical Screening of n-hexane, Ethyl Acetate, and Fruit Juice Fractions of *Eriobotrya japonica*

No.	Compounds	Fraction n-hexane	Ethyl Acetate Fraction	Water Fraction
1.	Alkaloids	-	-	-
2.	Flavonoids	-	+	+
3.	Glycosides	-	-	-
4.	Saponins	-	+	-
5.	Tannins	-	+	+
6.	Triterpenoids/steroids	+	-	-

Determination of Antioxidant Activity

The percentage of DPPH captured increased in direct proportion to the increasing concentration of the test sample solution in various solvents, as shown in Table-2. The larger the percentage of attenuation, the greater the activity of a drug in trapping radicals, as measured by the IC_{50}, and the greater its antioxidant potential. Calculating the regression equation by plotting the concentration of the test solution and the percent reduction of DPPH as a measure of antioxidant activity, with sample concentration (g/mL) as the abscissa (X axis) and percent inhibition value as the ordinate (Y axis), yielded the IC_{50} (Y axis). Table-3 displays the results of the linear regression equation and the analysis of the IC_{50} values obtained from the test sample and vitamin C.

Table-2: Results of Total Phenolic and Flavonoid Content

Test Solution	Concentration (µg/mL)	Mean Absorbance Value	Mean Value % Damping
n-Hexane Fraction	0	0.9221	0
	50	0.7811	15.2917
	100	0.6459	29.9545
	150	0.5073	44.9787
	200	0.5893	57.7832
Ethyl Acetate Fraction	0	0.9285	0
	25	0.7472	19.5254

	50	0.5806	37.4749
	75	0.4151	55.2915
	100	0.2612	71.8732
Water Fraction	0	0.9498	0
	100	0.8071	15.0277
	200	0.6716	29.2939
	300	0.5405	43.0898
	400	0.4173	56.0679
Vitamin C	0	0.9368	0
	0.5	0.7758	17.1797
	1	0.6222	33.5837
	1.5	0.4536	51.5746
	2	0.2987	68.1173

Table-3: Results of Linear Regression Equations and IC_{50}

Test Solution	Regression Equation	IC_{50} (µg/mL)
n-Hexane Fraction	Y = 0.2905X + 0.5509	170.2165
Ethyl Acetate Fraction	Y = 0.7181X + 0.9305	68.3371
Water Fraction	Y = 0.1402X + 0.6563	351.9575
Vitamin C	Y = 34.1259X - 0.0349	1.4662

The n-hexane fraction displayed weak antioxidant activity, the ethyl acetate fraction exhibited strong antioxidant activity, and the water fraction demonstrated very weak antioxidant activity, with respective IC_{50} values of 170.21 g/mL, 68.33 g/mL, 351.95 g/mL, and 1.4662 g/mL for vitamin C. When graded from strongest to weakest, the ethyl acetate fraction, the n-hexane fraction, and the fruit water fraction of Eriobotrya japonica possessed the most antioxidant potential. When the IC_{50} is less than 200 µg/mL, a substance has antioxidant properties.[15]

Total Phenolic Content

The total phenol concentration was determined using gallic acid as a standard solution. Gallic acid is reacted with Folin-Ciocalteu reagent to generate a yellow hue, indicating the presence of phenol, and then Na_2CO_3 solution is added to produce a blue color. Phenolic compounds only react with the Folin-Ciocalteu reagent in alkaline conditions, causing proton dissociation into phenolic ions; hence, a solution of Na_2CO_3 is added. At a wavelength of 750.4 nm, various concentrations of 50, 100, 150, 200, and 250 µg/mL of gallic acid standard solution were detected. The absorbance value of the standard solution of gallic acid at various concentrations determined from earlier measurements is used to calculate the total phenol content of the sample using a straight-line equation. Figure-1 demonstrates the gallic acid calibration curve.

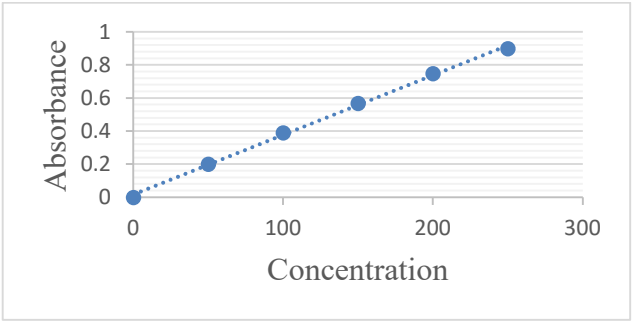

Fig.-1: Calibration Curves for Various Concentrations of Gallic Acid Standard Solution

The regression equation y = 0.0036x + 0.0164 is obtained in order to determine the standard calibration curve for gallic acid, and a linear relationship between absorbance and concentration is obtained with a

correlation coefficient (r) of 0.9991. The close proximity of r to 1 suggests that the regression equation is linear. At a wavelength of 750.4 nm, the absorbance value of the ethanol extract, n-hexane fraction, and ethyl acetate fraction of Eriobotrya Japonica fruit was measured 85 minutes after the addition of distilled water, Folin Ciocalteu reagent, and 20% sodium carbonate. Each test sample was measured three times to ensure proper data collection. The resulting absorbance value is then put into the gallic acid regression equation in order to calculate the total phenol concentration. Each sample's total phenol content was expressed in GAE (Gallic Acid Equivalent.[16] Table-4 displays the results of determining the total phenol content of the ethanol extract and various fractions of the fruit of Eriobotrya Japonica.

Table-4: Total Phenol Content of n-Hexane Fraction, Ethyl Acetate Fraction, and Fruit Water Fraction of *Eriobotrya japonica*

Sample	Total Phenol Level (mg GAE/g sample)	Average Phenol Levels Total (mg GAE/g sample)
n-hexane fraction	12.66	13.08
	11.74	
	14.84	
Ethyl acetate fraction	96.05	95.34
	94.30	
	95.66	
water fraction	16.41	15.24
	14.19	
	15,11	

The quantity of total phenol found in the n-hexane fraction was 13.08 3.5847; the amount found in the ethyl acetate fraction was 95.34 1.1936; and the quantity found in the water fraction was 15.24. Ethyl acetate fraction comes first, followed by n-hexane fraction, and then water fraction when it comes to overall phenol concentration. Since the majority of phenolic compounds are polar, n-hexane, a nonpolar solvent, cannot be used to extract phenolic compounds.[17] The presence of at least one aromatic ring with one or more hydroxyl groups on it distinguishes phenolic compounds from other types of chemicals.[18] Consequently, the n-hexane fraction has the lowest overall phenol content. The difference in the level of the polarity of the solvent determines the structure and types of phenolic compounds extracted so that certain compounds will be extracted specifically for each solvent used. Since ethyl acetate is a semi-polar solvent, the solubility of phenol compounds is highest in semi-polar solvents. The polyphenol group, which shares the same polarity as ethyl acetate solvents like xanthones and flavonoids, is assumed to be responsible for the high overall polyphenol content in ethyl acetate solvents.[19] Phenolic compounds have the potential to act as antioxidants due to the hydrogen atom of the phenolic OH group, which can be swiftly grabbed by free radicals as hydrogen proton donors.[20] Additionally, the core structure's heterocyclic ring's double bond and carbonyl group, which can boost antioxidant activity by stabilizing phenolic radicals through electron conjugation and delocalization, also have an impact on antioxidant activity.[21]

Total Flavonoid Content

Determination of total flavonoid content was carried out by comparing it with the standard quercetin. The implementation stage begins with determining the wavelength, and operating time Determining the maximum absorption wavelength of quercetin with a concentration of 45 g/mL is carried out at 30 minutes after the addition of 10% $AlCl_3$ reagent and 1M sodium acetate and aquades 2,8 mL using a UV-Vis spectrophotometer. The color of the resulting solution is yellow. The maximum absorption wavelength obtained is 429.2 nm. The operating time obtained is in the 32nd to 34th minute which is indicated by a stable absorbance value. Determination of the standard gallic acid calibration curve was carried out with a concentration variation of 50 µg/mL; 100 µg/mL; 150 µg/mL; 200 µg/mL; and 250 µg/mL and measured at a wavelength of 750.4 nm.

In determining the standard calibration curve for gallic acid, the regression equation $y = 0.01005x + 0.003$ and a linear relationship between absorbance and concentration were obtained with a correlation coefficient (r) of 0.9999. Determination of total flavonoid content was carried out by colorimetric method using $AlCl_3$ reagent. $AlCl_3$ solution is used to form colored complexes with flavonoids so that there is a shift in

wavelength towards the visible (visible) marked by the formation of a more yellow-colored solution. The addition of sodium acetate solution stabilizes and maintains wavelengths in the visible region. The principle used in the analysis of total flavonoids using $AlCl_3$ is the formation of complexes between $AlCl_3$ with the keto group on the C-4 atom and also with the hydroxy group on the neighboring C-3 or C-5 atom of flavones and flavonols.

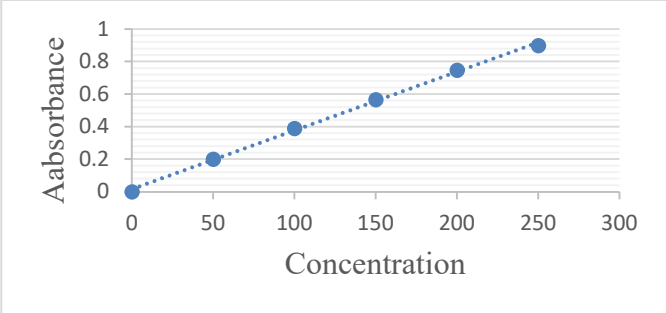

Fig.-2: Calibration Curves for Various Concentrations of Quercetin Standard Solution

The use of standard quercetin as a standard solution is because quercetin is a flavonoid of the flavonol group which has a keto group at C-4 and has a hydroxyl group on the C-3 or C-5 atom which is neighboring of flavones and flavonols.[22] Measurement of the absorbance value of the n-hexane fraction, ethyl acetate fraction, and the water fraction of Eriobotrya Japonica fruit was carried out at 32 minutes after the addition of 10% AlCl3 reagent and 1M sodium acetate and distilled water at a wavelength of 429.2 nm. The absorbance value obtained is then substituted into the quercetin regression equation so that the total flavonoid content can be calculated. The total flavonoid content of each sample was expressed in QE (Quercetin Equivalent).[23] The results of the determination of the total flavonoid content of the ethanol extract and several fractions of the fruit of *Eriobotrya japonica* can be seen in Table-5.

Table-5: Total flavonoid Content of n-hexane Fraction, Ethyl Acetate Fraction, and Fruit Water Fraction of *Eriobotrya japonica*

Sample	Total Flavonoid Level (mg QE/g sample)	Average Flavonoid Level Total (mg QE/g sample)
n-hexane fraction	0.89	0.88
	0.72	
	1.04	
Ethyl acetate fraction	12.19	12.65
	12.89	
	12.86	
water fraction	2.88	2.6
	2.81	
	2.11	

The n-hexane fraction's total flavonoid content was 0.88±0.0363; the ethyl acetate fraction was 12.65±0.2215; and the water fraction was 2.6. Ethyl acetate fraction, ethanol extract, and n-hexane fraction are listed in order of their respective total flavonoid contents. The n-hexane fraction contained the least amount of total flavonoid concentration. Flavonoids are polyphenols with 15 carbon atoms that are made up of two aromatic rings joined by a bridge of three carbon atoms. Glycoside molecules are formed when sugars and flavonoids are bonded together. The polarity and solubility in water are both increased by the presence of hydroxyl groups and sugars.[24] Results indicated that n-hexane is a nonpolar solvent which unable to extract flavonoid compounds. While the ethyl acetate fraction has the highest overall flavonoid concentration because it was a semi-polar that can most of the polyphenols groups. Flavonoid groups that have low polarities, such as isoflavones, flavanones, methyl flavones, and flavonols, can bond during extraction when ethyl acetate is used as the solvent.[25,26]

CONCLUSION

The conclusion of this study is that the ethyl acetate fraction of *Eriobotrya Japonica* fruit has the highest antioxidant potential, total phenol, and flavonoid compared to the n-hexane and water fractions.

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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 numbers given below:

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REFERENCES

1. A. Dhiman, R. Suhag, D.Thakur, V. Gupta, and P.K. Prabhakar, *Food Reviews International*, **2021(1)**, 1(2021), https://doi.org/10.1080/87559129.2020.1866007
2. Mahomoodally, M. Fawzi, and L.D. Muthoorah, *Asian Pacific Journal of Tropical Biomedicine,* **4**, 399(2014), https://doi.org/10.12980/APJTB.4.2014C775
3. A. Wakeel, S.A. Jan, I. Ullah, ZK, Shinwari, M.Xu, *PeerJ*, **9(7)**, 7857(2019).
4. C. Zhou, C. Sun, K. Chen, X. Li. *International Journal of Molecular Sciences*, **12(5),** 2935(2011), https://doi.org/10.3390%2Fijms12052935
5. X. Li, C. Xu, K. Chen, Nutritional and Composition of Fruit Cultivars: Loquat (*Eriobotrya japonica* Lindl.), In Nutritional Composition of Fruit Cultivars, Academic Press, pp. 371-394(2016), http://dx.doi.org/10.1007/978-3-030-06120-3_10-1
6. S.M. Sinaga, Sudarmi, Iksen, K. Kevin, M.P. Sari, *Rasayan Journal of Chemistry*, **11(4)**, 1604(2018), http://dx.doi.org/10.31788/RJC.2018.1144067
7. R. I. Depkes, Indonesian Herbal Pharmacopeia, Ditjen POM, Jakarta, p.56 (2000).
8. Masfria, G.Haro, and V. Mierza, *Rasayan Journal of Chemistry*, **12(2)**, 1010(2019), http://dx.doi.org/10.31788/RJC.2019.1225153
9. P. Tiwari, B. Kumar, K. Mandeep, G. Kaur, and H. Kaur, *Internationale Pharmaceutica Sciencia*, **1(1)**, 98(2011).
10. D. Smink, S. R. A. Kersten, B. Schuur, *Separation and Purification Technology*, **235**, 116127(2020), https://doi.org/10.1016/j.seppur.2019.116127
11. S.B. Kedare, R. P. Singh, *Journal of Food Science and Technology*, **48(4)**, 412(2011), https://doi.org/10.1007%2Fs13197-011-0251-1
12. L.Y. Chen, C.W. Cheng, J.Y. Liang, *Food Chemistry*, **138(170)**, 10(2015), https://doi.org/10.1016/j.foodchem.2014.08.038
13. D. Mammen, M. Daniel, *Food Chemistry,* **135(3)**, 1365(2012), https://doi.org/10.1016/j.foodchem.2012.05.109
14. N. Saeed, M.R. Khan, M. Shabbir, *BMC Complementary and Alternative Medicine,* **12(1)**, 1(2012), https://doi.org/10.1186/1472-6882-12-221
15. P. Molyneux P. Songklanakarin, *Journal of Science and Technology*, **26(2)**,211(2004)
16. M.R.Rover, R.C. Brown, *Journal of Analytical and Applied Pyrolysis,* **104**, 366(2013), http://dx.doi.org/10.1016%2Fj.jaap.2013.06.011
17. S.C. Mahugo, F.Z. Sosa, T.P.M.Esther, *Molecules*, **14(1)**, 298(2009), https://doi.org/10.3390/molecules14010298

18. O. Abbas, G. Compère , Y. Larondelle, D. Pompeu, H. Rogez,V. Baeten, *Vibrational Spectroscopy*, **92**,111(2017), https://doi.org/10.1016/j.vibspec.2017.05.008
19. J. Torres-Vega , S. Gómez-Alonso, J. Pérez-Navarro, J. Alarcón-Enos, E. Pastene-Navarrete. *Molecules*, **26(8)**, 2192(2021), https://doi.org/10.3390%2Fmolecules26082192
20. A. Mittal, V. K. Vashistha, D. K. Das, *Free Radical Research,* **56(5-6)**, 378(2022), https://doi.org/10.1080/10715762.2022.2120396
21. M. Olszowy, *Plant Physiology and Biochemistry*, **144**, 135(2019), https://doi.org/10.1016/j.plaphy.2019.09.039
22. S. Salwa. *Journal of Nutraceuticals and Herbal Medicine,* **4(1)**, 31(2021), https://doi.org/10.23917/jnhm.v4i1.15642
23. R.O. Gurung. *International Journal of Pharmacy and Pharmaceutical Sciences,* **12**, 54(2020), http://dx.doi.org/10.22159/ijpps.2020v12i1.35914
24. J.Cramer, C.P. Sager, B. Ernst B. *Journal of Medicinal Chemistry,* **62(20)**, 8915(2019), https://doi.org/10.1021/acs.jmedchem.9b00179
25. A. Dalimunthe, P.A.Z. Hasibuan, H. Nufus, M. Muhammad, and D. Satria, *Rasayan Journal of Chemistry*, **15(2)**, 1553(2022), http://dx.doi.org/10.31788/RJC.2022.1526736
26. M. Xu, L. Ran, N. Chen, X. Fan, D. Ren, L. Yi. *Food Chemistry*, **297**, 124970(2019), https://doi.org/10.1016/j.foodchem.2019.124970

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