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Antioxidant Activity Study of Zingiber Zerumbet Rhizome Extract and Fraction

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ABSTRACT

The Zingiber zerumbet was identified as a member of the Zingiberaceae family with pharmacological potential. This study aims to characterise the phytochemical compound and evaluate the antioxidant potential of the methanol extract, ethyl acetate, and n-hexane- fractions of Zingiber zerumbet rhizome. Maceration with methanol was used for extraction, and the extract was then fractionated with n-hexane, ethyl acetate, and water. The phytochemical identification was conducted for alkaloids, saponins, flavonoids, polyphenols, and steroids/triterpenoids. Antioxidant activity was determined based on DPPH absorbance measurements using UV-Vis spectrophotometry. The methanolic extract contained alkaloids, flavonoids, polyphenols, and steroids/triterpenoids. The ethyl acetate fraction contained flavonoids and polyphenols, and the n-hexane fraction contained alkaloids and flavonoids. The free radical scavenging activities of ascorbic acid, methanol extract, ethyl acetate, and the n-hexane soluble fraction were 7.79; 100,956; 69,476, and 77,780 µg/mL, respectively. The findings indicated that the methanolic extract, ethyl acetate, and n-hexane soluble fraction contained antioxidant compounds.

Keywords: DPPH scavenging, natural product, Zingiberaceae

INTRODUCTION

Zingiber zerumbet is a plant of the Zingiberaceae family that grows in tropical climates with humid conditions. The characteristic of this plant is that its beautiful flowers are green and turn pale yellow to red over time. Flowers emerge from the leaf axils and have slime with a more liquid consistency between the petals. The leaves are thin, oblong, and purplish, with the abaxial part being downy. The rhizome is yellowish with a fragrant aroma and bitter taste with a slightly spicy taste (Kemenkes RI, 2017). Z. zerumbet plant is dormant. The stems wilt and die in the dry season and will grow in the rainy season. The best time for harvesting is when the leaves begin to wither or about 7–10 months after planting.

The rhizome of *Z. zerumbet* is commonly used as a carminative to cure stomach pain, asthma, diarrhea, and worms (Evans, 2009). According to an empirical study by Hafizah and Fitmawati (2016), *Z. zerumbet* rhizome is the main ingredient of the herb for postpartum, fertility therapy, and eradicating dysmenorrhea (Hafizah & Fitmawati, 2021). Various pharmacological activities of the *Z. zerumbet* rhizome can be attributed to the antioxidant activity of its active substance

content. Koga et al. (2016) reported that *Z. zerumbet* contains various chemical compounds, such as polyphenols, terpenes, and zerumbone, which are the main bioactive compounds (Koga et al., 2016).

Research on identifying phytochemicals and assessing their antioxidant activity was carried out to obtain scientific data regarding the antioxidant activity of *Zingiber zerumbet* rhizoma. The findings of this study will likely serve as the starting point for the discovery and development of new antioxidant compounds based on natural ingredients.

MATERIAL AND METHODS

Materials and Instrument

The sample is Zingiber zerumbet rhizome (determined by the Plant Determination Unit of the Pharmacognosy-Phytochemical Laboratory, Faculty of Pharmacy, Universitas Muslim Indonesia, No.007/C/UD-FF/UMI/X/2022). All chemicals for phytochemical identification and activity testing were analytical grade: HCl (2N) (Merck), ascorbic acid (Merck), acetic acid (Merck), H_2SO_4 (Merck), Bouchardate reagent (Iodine, KI, water), DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Sigma Aldrich), $FeCl_3$(Merck), chloroform (Merck), Mg powder (Merck). Technical grades such as methanol, ethyl acetate, n-hexane, and aqua distillate are used for extraction and fractionation purposes. Instrument: UV-Vis Spectrophotometer (Genesys 10S UV-Vis, Thermo Scientific).

Extraction and Fractionation

The rhizomes of Z. zerumbet are harvested in the Maros Regency of South Sulawesi Province, Indonesia. Soil and other impurities are removed from the rhizomes, washed with water and dried. The rhizomes were cut into small pieces and then dried in a simplisia drying cabinet. 500 g of dried rhizomes were macerated with 1500 mL of methanol at room temperature for 3 x 24 hours with periodic stirring. The methanol filtrate was evaporated by a vacuum rotary evaporator, yielding a thick brown methanolic extract. Partition and fractionation were conducted according to (Nuari et al., 2017) with a minor adjustments. The thick methanolic extract (20 g) was then mixed in 200 mL water. This aqueous mixture was partitioned with n-hexane (10 x 50 mL) and ethyl acetate (10 x 50 mL), respectively. The fraction was condensed with a rotary evaporator and ready for further analysis.

Phytochemical Identification

Alkaloids

In an Erlenmeyer flask, 500 mg of the extract was added along with 1 ml of 2N HCl and 10 ml of distilled water. It was then cooled, filtered, and heated in a water bath for 15 minutes. Two drops of Bouchard's reagent were added to the watch glass solution. A brown-to-black precipitate indicates positive results (Le Thi et al., 2021; Utami, 2020)

Flavonoids

One gram (1 g) of extract was dissolved in methanol (50%, 1-2 mL) by heating, then magnesium metal and 5-6 drops of concentrated HCl were added. When it is red, the solution indicates flavonols and orange for flavones (Auwal et al., 2014; Shaikh & Patil, 2020)

Steroids

A few drops of the sample were added to a test tube containing 2 mL chloroform. Subsequently, 10 drops of anhydrous acetate and 3 drops of sulfuric acid were added to the test tube. The positive results can be seen from the color change of the sample: initially, the color will turn red; afterwards, it will change to blue or green. The red color indicates the presence of terpenoids, while the green color indicates the presence of steroids (Yuniati et al., 2020).

Saponins

In a test tube, ten ml of the test solution was shaken vertically for 10 seconds before being allowed to stand for 10 seconds. The presence of saponins is indicated by the formation of foam up to 1-10 cm in height that is stable for at least 10 minutes. The foam does not disappear when 1 drop of 2N HCl is added (Rasyid, 2012).

Phenolic

Two milliliters (2 mL) of the extract solution is added with a few drops of 10% Ferric chloride solution (light yellow). The appearance of a blue or green-black color indicates the presence of phenolic compound (Akerina & Sangaji, 2019).

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity assay

This experiment was carried out using the method previously described by (Shekhar & Anju, 2014) with slight modifications. A total of 4 mL of a solution of the fraction in methanol (20, 40, 60, 80, and 100 µg/mL) was mixed with 1 mL of DPPH solution (0.45 mM). The reaction mixture was incubated at room temperature and in a dark place for 30 minutes. The maximum wavelength of the DPPH solution was determined to be 517 nm. The standard compound is vitamin C (2, 4, 6, 8, and 10 µg/mL). The absorbance of the sample was measured at 517 nm, and the percentage of free radical scavenging was calculated. Each measurement was repeated three times. The percentage of DPPH radical scavenging is calculated using the formula below.

$$Scavengging\ effect = [\frac{A_{DPPH} - A_S}{A_{DPPH}}]\ x\ 100$$

Where As is the absorbance of the sample solution and ADPPH is the absorbance of the DPPH solution (blank). After processing the sample absorbance data, a linear equation will be obtained, which is used to calculate the IC50 value of the sample's antioxidant activity.

RESULTS AND DISCUSSION

Identification of the phytochemical content of the methanolic extract, ethyl acetate, n-hexane, and fractions was carried out using chemical reactions that have been commonly applied so far. From the qualitative analysis conducted, it was found that methanol extract of *Z.zerumbet* rhizome contained alkaloid, flavonoids, polyphenols, and triterpenoid and steroids. The phytochemical compounds are then divided into the n-hexane fraction (low polarity) and the ethyl acetate fraction (higher polarity). The n-hexane fraction contained alkaloids and flavonoids, while the ethyl acetate soluble fraction contained phytochemicals belonging to the polyphenol group. The results of identifying chemical groups are shown in Table 1 below.

Table 1. The Result of Phytochemical Identification

Phytochemicals	Extract		
	Methanol	n- hexane	E. acetate
Alkaloid	+	+	-
Saponin	-	-	-
Flavonoid	+	+	+
Polyphenol	+	-	+
Triterpenoid and Steroid	+	-	-

Identification of alkaloid groups using Bouchardat reagent (potassium iodide-iodine solution), known as the James method (Van Haga, 1954a, 1954b). According to the same authors, this approach is non-specific and requires additional supporting methods. Other researchers state that this reaction is based on nitrogen in the alkaloid structure, which will react with KI-I2 to produce a brown precipitate (Parbuntari et al., 2018)' as shown in **Figure 1**.

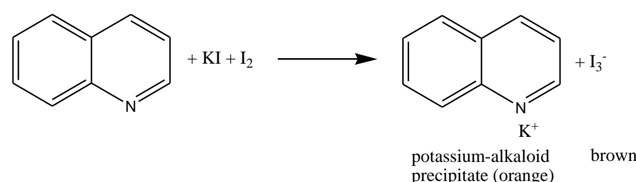

Figure 1. The reaction of Alkaloid- Bouchardat's reagent (Parbuntari et al., 2018)

Identification of flavonoid compounds was carried out using magnesium powder and concentrated HCl. This approach is known as the Wilstater reaction(Syarifah et al., 2019a), and it is carried out to determine the presence of flavonoid compounds with γ-benzopyrone groups, such as flavones, flavonols, and isoflavones. In addition, the magenta color change indicates that flavonoids have a C=O group at the C-4 position and an-OH group at C-5, C-3 position, and adjacent dihydroxyl groups in ring B (ortho positions at 3' and 4' or 4' and 5') (Syarifah et al., 2019b) as shown in **figure 2.**

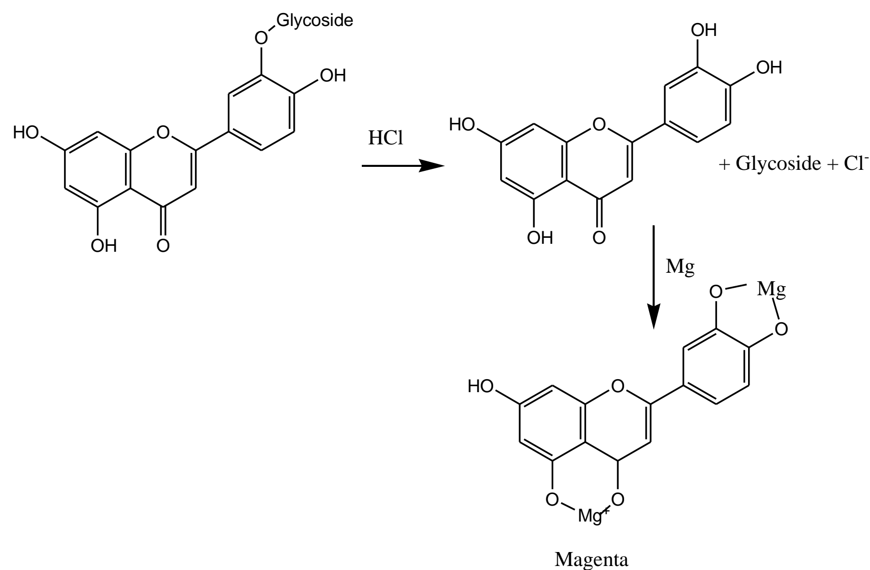

Figure 2. Flavonoid reaction with HCl-Mg reagent (Wilstater Reaction)(Syarifah et al., 2019a)

Reaction with $FeCl_3$ is a simple and fast technique for identifying phenolic compounds and tannins. A positive test is revealed by the appearance of blue color in the presence of hydrolysable tannins and greenish blue in the presence of condensed tannins(Boufellous et al., 2017; Haida et al., 2021). Phenolic compounds containing catechol and ortho-hydroxy benzoyl groups develop the most intense colors (Klangmanee & Athipornchai, 2019)· as shown in **Figure 3.**

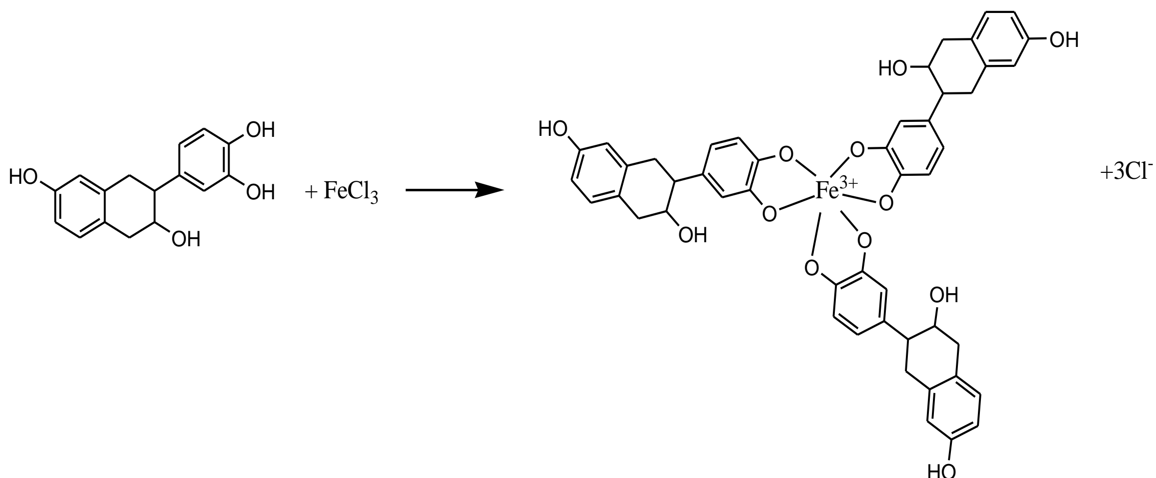

Figure 3. The reaction of polyphenolic with $FeCl_3$

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is the most commonly used antioxidant assay for plant extract (Nagarajan et al., 2017). This method has been widely used to predict antioxidant activity because it is a fast, inexpensive, and affordable approach (Baliyan et al., 2022; Halliwell, 2011). The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an electron

transfer-based antioxidant assay that yields a violet solution in ethanol or methanol (Garcia et al., 2012). In this assay, a molecule or antioxidant with weak A-H bonding will react with a stable free radical DPPH• (2,2-diphenyl-1-picrylhydrazyl, λmax=517 nm), causing discoloration of the molecule (Sridhar & Charles, 2019). The proposed reaction of DPPH radical with antioxidant compounds can be described in Figure 4.

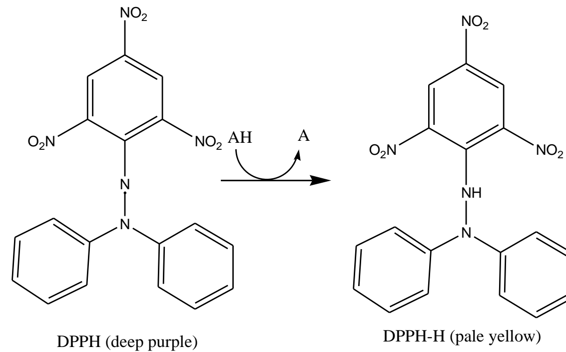

Figure 4. DPPH-antioxidant reaction

The IC_{50} of an antioxidant-containing substance is the concentration required to scavenge 50% of the initial DPPH radicals. The lower the IC_{50} value, the more effective the substance is at scavenging DPPH, implying higher antioxidant activity (Olugbami et al., 2014; Rivero-Cruz et al., 2020). The percentage of DPPH free radical reduction and the IC_{50} value of each fraction showed in Figure 5.

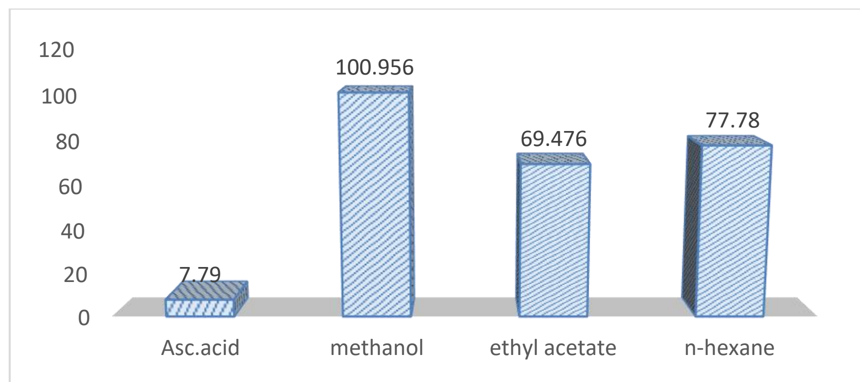

Figure 5. IC_{50} of DPPH Scavenging activity of ascorbic acid, methanolic extract, ethyl acetate, and n-hexane soluble fraction of *Z.zerumbet* Rhizome

The IC50 values of the methanol extract, ethyl acetate fraction, and n-hexane fraction of Z. zerumbet rhizoma were 100.956, 69.476, and 77.78 µg/mL. These values are still higher than the IC50 of vitamin C, which is 7.79 µg/mL. The difference in DPPH free radical scavenging activity is strongly influenced by the type and amount of compound contained in each extract and fraction. By comparing the three samples tested, it was found that the n-hexane fraction had a lower IC_{50} than the methanol extract and n-hexane fraction. This is due to the higher concentration of antioxidant

compounds in the n-hexane fraction, specifically alkaloids and flavonoids. Alkaloids are one of the primary antioxidants in natural products, and their antioxidant activity has been demonstrated in previous studies (Gan et al., 2017). Some researchers have stated that one type of antioxidant is phenolic-alkaloid (Jang et al., 2009). In addition, it can be assumed that the zerumbone compound, which is the main ingredient of *Z.zerumbet* rhizome, is attracted to the n-hexane (low polarity) fraction. This statement aligns with (Jiang et al., 2016) that most of the main bioactive compounds extracted from *Z. zerumbet* rhizomes are terpene compounds with long hydrocarbon tails, generally resulting in low polarity. The terpene compound referred to in the statement is most likely zerumbone, as stated by (Abuzahra et al., 2021) al,) that zerumbone is known to have significant antioxidant properties

This study has several areas for improvement, such as not specifically identifying the terpenoid content and only being limited to identifying triterpenoids and steroids. Terpenoids and steroids, initially detected in the methanol extract, apparently were not detected in the n-hexane or ethyl acetate fractions, so this requires a rational scientific explanation. Zerumbone, the main component previously reported (Koga et al., 2016), belongs to the terpenoid group, especially the sesquiterpenoids. However, in the phytochemical identification test conducted in this study, only the triterpenoid group was tested. In addition, it is necessary to conduct qualitative tests of antioxidant-active compounds with more specific techniques, such as LC/MS or GC/MS, in each *Z.zerumbet* fraction.

CONCLUSION

As a result of this study, the methanolic extract of *Z. zerumbet* rhizome contains alkaloids, flavonoids, polyphenols, triterpenoid and steroids. The n-hexane fraction contains alkaloids and flavonoids, and the ethyl acetate fraction contains flavonoids and polyphenols. The methanol extract, n-hexane fraction and ethyl acetate fraction showed antioxidant activity, proving the presence of different antioxidant compounds. These findings can be used as a basis for further research on the potential and types of antioxidant compounds contained in *Z.zerumbet* rhizome.

REFERENCES

Abuzahra, H. M., Rajendran, P., & Ismail, M. B. (2021). Zerumbone exhibit a protective effect against zearalenone induced toxicity via ameliorating inflammation and oxidative stress induced apoptosis. *Antioxidants*, *10*(10), 1–14. https://doi.org/10.3390/antiox10101593

Akerina, F. O., & Sangaji, J. (2019). Analisis Fitokimia dan Toksisitas serta Aktivitas Antioksidan Beberapa Jenis Teripang di Desa Kakara, Halmahera Utara. *Agrikan: Jurnal Agribisnis Perikanan*, *12*(2), 188–196. https://doi.org/10.29239/j.agrikan.12.2.188-196

Auwal, M. S., Saka, S., Mairiga, I. A., Sanda,

K. A., Shuaibu, A., & Ibrahim, A. (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa). *Veterinary Research Forum : An International Quarterly Journal*, *5*(2), 95–100. http://www.ncbi.nlm.nih.gov/pubmed/25568701%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4279630

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. *Molecules*, *27*(4). https://doi.org/10.3390/MOLECULES27041326

Boufellous, M., Berrani, A., Haoud, H. EL, Zaher, A., Bouhaddioui, B., Bengueddour, R., Moncef Boufellous, C., & Aicha Lrhorfi, L. (2017). Phytochemical screening of a medicinal plant: Lavandula stoechas (Lamiaceae). *~ 56 ~ Journal of Pharmacognosy and Phytochemistry*, *6*(2), 56–62.

Evans, W. C. (2009). *Trease and Evans Pharmacognosy* (16th ed.). Saunders Elsevier.

Gan, J., Feng, Y., He, Z., Li, X., & Zhang, H. (2017). Correlations between Antioxidant Activity and Alkaloids and Phenols of Maca (Lepidium meyenii). *Journal of Food Quality*, *2017*. https://doi.org/10.1155/2017/3185945

Garcia, E. J., Cadorin Oldoni, T. L., de Alencar, S. M., Reis, A., Loguercio, A. D., & Miranda Grande, R. H. (2012). Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. *Brazilian Dental Journal*, *23*(1), 22–27. https://doi.org/10.1590/S0103-64402012000100004

Hafizah, N., & Fitmawati. (2021). Studi Etnobotani Famili Zingiberaceae Dalam Kehidupan Masyarakat Lokal Di Pulau Rupat Kabupaten Bengkalis, Riau. *Repository of University of Riau*, *1*, 1–14.

Haida, S., Bakkouche, K., Kribii, A. R., & Kribii, A. (2021). Chemical Composition of Essential Oil, Phenolic Compounds Content, and Antioxidant Activity of Cistus monspeliensis from Northern Morocco. *Biochemistry Research International*, *2021*. https://doi.org/10.1155/2021/6669877

Halliwell, B. (2011). Free radicals and antioxidants - quo vadis? *Trends in Pharmacological Sciences*, *32*(3), 125–130. https://doi.org/10.1016/J.TIPS.2010.12.002

Jang, M. H., Piao, X. L., Kim, J. M., Kwon, S. W., & Park, J. H. (2009). Phenolic Alkaloids as a New Class of Antioxidants in Portulaca oleracea. *Phytotherapy Research*, *23*, 1032–1035. https://doi.org/10.1002/ptr

Jiang, Z., Kempinski, C., & Chappell, J. (2016). Extraction and Analysis of Terpenes/Terpenoids. *Current Protocols in Plant Biology*, *1*(2), 345–358. https://doi.org/10.1002/CPPB.20024

Kemenkes RI. (2017). Farmakope Herbal Indonesia. In *Kementerian Kesehatan Republik Indonesia*. https://doi.org/10.1201/b12934-13

Klangmanee, K., & Athipornchai, A. (2019). A rapid phytochemical screening of the effective phenolic antioxidant agents using FeCl 3 reagent. *Journal of Pharmaceutical Sciences and Research*, *11*(10), 3475–3480.

Koga, A. Y., Beltrame, F. L., & Pereira, A. V. (2016). Several aspects of Zingiber zerumbet : a review. *Revista Brasileira de Farmacognosia*, *26*(3), 385–391. https://doi.org/10.1016/j.bjp.2016.01.006

Le Thi, V. A., Nguyen, N. L., Nguyen, Q. H., Van Dong, Q., Do, T. Y., & Nguyen, K. O. T. (2021). Phytochemical Screening and Potential Antibacterial Activity of Defatted and Nondefatted Methanolic Extracts of Xao Tam Phan (Paramignya trimera (Oliv.) Guillaum) Peels against Multidrug-Resistant Bacteria. *Scientifica*, *2021*. https://doi.org/10.1155/2021/4233615

Nagarajan, J., Ramanan, R. N., Raghunandan, M. E., Galanakis, C. M., & Krishnamurthy, N. P. (2017). Carotenoids. In *Nutraceutical and Functional Food Components: Effects of Innovative Processing Techniques*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-805257-0.00008-9

Nuari, S., Anam, S., & Khumaidi, S. (2017). Isolasi dan Identifikasi Senyawa Flavonoid Ekstrak Etanol Buah Naga Merah (Hylocereus polyrhizus (F.A.C.Weber)Briton & Rose). *Galenika Journal of Pharmacy)*, *2*(2), 118–125. https://doi.org/10.22487/j24428744.2017.v3.i2.8771

Olugbami, J. O., Gbadegesin, M. A., & Odunola, O. A. (2014). In vitro evaluation of the antioxidant potential, phenolic and flavonoid contents of the stem bark ethanol extract of Anogeissus leiocarpus. *African Journal of Medicine and Medical Sciences*, *43*(Suppl 1), 101. /pmc/articles/PMC4679201/

Parbuntari, H., Prestica, Y., Gunawan, R., Nurman, M. N., Adella, F., & Padang, N. (2018). Preliminary Phytochemical Screening (Qualitative Analysis) of Cacao Leaves (Theobroma cacao L.). *EKSAKTA: Berkala Ilmiah Bidang MIPA*, *19*(2), 40–45. https://doi.org/10.24036/EKSAKTA/VOL19-ISS2/142

Rasyid, A. (2012). Identification Of Secondary Metabolites Compounds, Antibacterial And Antioxidant Activities On The Methanol Extract Of Sea Cucumber Stichopus hermanii. *Jurnal Ilmu Dan Teknologi Kelautan Tropis*, *4*(2), 360–368. https://doi.org/10.29244/jitkt.v4i2.7799

Rivero-Cruz, J. F., Granados-Pineda, J., Pedraza-Chaverri, J., Pérez-Rojas, J. M., Kumar-Passari, A., Diaz-Ruiz, G., & Rivero-Cruz, B. E. (2020). Phytochemical Constituents, Antioxidant, Cytotoxic, and Antimicrobial Activities of the Ethanolic Extract of Mexican Brown Propolis. *Antioxidants (Basel, Switzerland)*, *9*(1). https://doi.org/10.3390/ANTIOX9010070

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Shekhar, T., & Anju, G. (2014). Antioxidant Activity by DPPH Radical Scavenging Method of Ageratum conyzoides Linn. Leaves. *Undefined*.

Sridhar, K., & Charles, A. L. (2019). In vitro antioxidant activity of Kyoho grape extracts in DPPH and ABTS assays: Estimation methods for EC50 using advanced statistical programs. *Food Chemistry*, *275*, 41–49. https://doi.org/10.1016/J.FOODCHEM.2018.09.040

Syarifah, A. L., Retnowati, R., & Soebiantoro, S. (2019a). Characterization of Secondary Metabolites Profile of Flavonoid from Salam Leaves (Eugenia polyantha) Using TLC and UVSpectrophotometry. *Pharmaceutical Sciences and Research*, *6*(3), 155–163. https://doi.org/10.7454/psr.v6i3.4219

Syarifah, A. L., Retnowati, R., & Soebiantoro, S. (2019b). Characterization of Secondary Metabolites Profile of Flavonoid from Salam Leaves (Eugenia polyantha) Using TLC and UVSpectrophotometry. *Pharmaceutical Sciences and Research*, *6*(3), 4. https://doi.org/10.7454/psr.v6i3.4219

Utami, N. F. (2020). *Potensi Antioksidan dari Biji Kopi Robusta 9 Daerah di Pulau Jawa* (A. K. Wardani (ed.)). Universitas Pakuan.

Van Haga, P. R. (1954a). Alkaloids in Germinating Seeds of Atropa belladonna. *Nature 1954 173:4406*, *173*(4406), 692–692. https://doi.org/10.1038/173692a0

Van Haga, P. R. (1954b). Alkaloids in germinating seeds of atropa belladonna [18]. *Nature*, *173*(4406), 692.

https://doi.org/10.1038/173692a0

Yuniati, R., Zainuri, M., & Kusumaningrum, H. (2020). Qualitative Tests of Secondary Metabolite Compounds in Ethanol Extract of Spirulina platensis from Karimun Jawa Sea. *Indonesia. Biosaintifika: Journal of Biology & Biology Education*, *12*(3), 343–349. http://dx.doi.org/10.15294/biosaintifika.v12i3.23153