FOODSCITECH
Food Science and Technology Journal

Evaluation of Phytochemicals And Antioxidant Activity (IC50) of Bintaro Fruit Ethanol Extract (*Cerberaodollam L.)*

Sutrisno Adi Prayitno[1*], Lilla Puji Lestari[2], Dwi Retnaningtyas Utami[1], Nadhifah Salsabila[1],

[1 *]Department of Food Technology, University of Muhammadiyah Gresik, East Java Indonesia
[2]Health Faculty, University of Maarif Hasyim Latif Sidoarjo, East Java Indonesia
Corres.Author Email [*]: sutrisnoadi2007@umg.ac.id

ABSTRACT

The phytochemical compounds in bintaro fruit can be used as herbs to maintain health. The compounds that play a role are flavonoids and phenols. These two compounds can be used as a reference for measuring the level of antioxidant activity in natural materials. There is a correlation between total phenols, total flavonoids, and antioxidants (IC50) found in natural ingredients. The purpose of this study was to determine the total phenol content, flavonoids, and antioxidant activity in the ethanol extract of Bintaro fruit. Bintaro fruit was extracted using ethanol solvent by maceration method which was then evaporated with a rotary evaporator on the obtained macerate. The extract obtained was then analyzed for levels of total flavonoids, total phenols, and antioxidants (IC50). Ethanol extract of bintaro fruit in the study produced total phenol content of 73.04 mg GAE/g extract, flavonoids 41.61 mg QE/g extract and had antioxidant activity (IC50=275.06 ppm). Recommended for in vivo or in vitro assay for certain diseases using these extracts.Further research is needed with the use of other solvent to see optimall in the screening of active compounds.

Keywords:*Bintaro fruit; Extraction; Phenol; Flavonoids; Antioxidants*

INTRODUCTION

Bintaro plant *(Cerbera odollam* L.)is a type of plant that has potential as herbs in health that are rarely used by humans. This plant has active compounds that can be used as a therapy for certain diseases. Can be used as an antiproliferative, anticancer, antiestrogenic, antimicrobial, antinociceptive, and sedative effect of different dosages no matter in vitro or in vivo (Ahmed et al., 2006; Ahmed et al., 2008). In another study, bintaro fruit was reported to be used in treating diarrhea, abdominal pain, colds, bronchitis, cough, asthma, and urethritis (Das et al., 2014). The active compounds contained in Bintaro fruit are flavonoids, tannins, phenols, saponins, alkaloids, anthraquinones, cardenolides, beta-sitosterol, stigmasterol, apigenin, naphthoquinone, iridoid glycosides, 3-hydroxyoctanol glycosides. Besides, there are also flavonoid compounds-quercetin. Quercetin can be used in anti-cancer therapy (Baghel et al., 2012). Flavonoids act as an antioxidant system to ward off free radicals. Meanwhile, alkaloid compounds act as anti-inflammatory agents (Ejelonu et al., 2011). However, this plant has the potential for naturally occurring toxic compounds in plant parts, such as those found in the branches, roots.

Extraction is a method that can be used to obtain compounds in Bintaro fruit which usually use organic solvents with different polarity. The ethanol extract of Bintaro fruit is reported to be able to inhibit free radicals which are associated as a result of the positive correlation between phenolic compounds and the antioxidant activity present in the fruit. Antioxidant compounds can be used in the protection of body cells due to the presence of ROS (Reactive oxygen species) superoxide anion ($O^{2\bullet-}$), hydroxyl radicals (OH•), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2) (Das et al., 2014). Another study stated that extraction of Bintaro leaves can identify active compounds that play an important role in the health of the human body such as apigenin, which is a flavonoid class of the flavone class (Sholahuddin et al., 2018).

A large group of compounds in natural materials can be in the form of flavonoids and phenols (phytochemical compounds). Phytochemicals are compounds that are naturally present in plants and distributed to every part of the plant and have functions as plant biological

effects, antioxidants, free radical scavengers, anti-inflammatory, anti-cancer, antibacterial, anti-fungal, and so on (Sahoo & Marar, 2018). Flavonoids act as enzymatic and non-enzymatic oxidation inhibitors. Is a polyphenol group that can capture free radicals, inhibit hydrolysis and oxidative enzymes, and works as an anti-inflammatory (Pourmorad et al., 2006). Whereas phenol is an active compound with an aromatic ring of one or more hydroxyl (OH) groups, which can inhibit lipid oxidation. Phenol is derived from simple molecules of phenolic compounds.

Antioxidants are molecules that can inhibit the oxidation process caused by free radicals. While free radicals are atoms that number one or more electrons and do not have a partner in their outer orbit (Molyneux, 2004). Antioxidant compounds can be indicated by the large number of phenolic and flavonoid compounds that are present in natural substances. Bintaro fruit is known to have levels of active compounds in the form of phytochemical compounds, one of which acts as an antioxidant compound. Besides, Bintaro fruit is also safe from natural toxic compounds in the form of cyanide acid or often referred to as HCN (Ogbuagu, 2008).

In this study was using ethanol as a solvent for its extraction. The use of ethanol solvent in this extraction with ethanol base is a safe solvent and is included in the list of solvents referred to as Generally Recognized As Safe (GRAS), since toxicological and medical studies show no adverse effects on human health over their use in food over a long period (Molino et al., 2018). In a study conducted by Molino et al (2018[a]) mentioned that ethanol is the GRAS solvents that is more promising in terms of environmental sustainability. It was also mentioned that ethanol is excellent in the analysis of HepG2, MDAMB-231, MCF-7 and VNBCRA1 cells in vitro (Nguyen et al., 2020)

MATERIASLS AND METHODS

The main research ingredient is Bintaro fruit. While the chemicals used include aquadest, 96% ethanol, methanol (Sigma), DPPH, quercetin (Merck), folinciocalteu (Merck), Na2CO3 (Merck), gallic acid, NaNO2 (Merck), AlCl3 (Merck), NaOH (Merck). The tools used are distillation ash, glass funnel, volume pipette (iwaky-pyrex), suction ball, volumetric flask (iwaky-pyrex), glass beaker (iwaky-pyrex), stainless steel blade, back cooler, spatula, erlenmeyer (iwaky-pyrex), plastic funnel, filter paper, aluminum foil, plastic, refrigerator, shaker(ShackerMax^{Q}2000, Barnstead I-Lab-Line),vortex (Velpscientifica), rotary evaporator (IKA HB 10 basic), electric stove, thermometer, analytical balance, 5 ml cuvette, spectrophotometer(Spectro 20 D Plus), and glass bottles.

Making Bintaro Fruit Extract (Maceration)

Bintaro fruit was peeled and small (dice size of 1 cm). Then weighed 50 grams and put in a beaker glass and added 500 ml of 96% ethanol. Then macerated (with a shaker) for 24 hours. The obtanined macerate was evaporated with a rotary evaporator to obtain bintarofruit extract for approximately two hours using a temperature of 60 ^{0}C.

Determination of phenol levels (Som et al., 2019)

Determination of the standard curve of gallic acid

A solution of gallic acid 100 mg / L was prepared. Diluted several concentrations (20, 40,60, 80, and 100%). Taken 100 µl of each concentration of a gallic acid solution, put it in a test tube and add 1 ml of 7.5% Na2CO3 solution and 2 ml of 10% FolinCiocalteau reagent then homogenized. After being homogeneous, it was incubated for 30 minutes at room temperature, then 2 ml of the pipette was taken and put in a cuvette to measure its absorbance at a wavelength of 765 nm. A standard curve of gallic acid was made with the equation y = bx + a, where: y = absorbance and x = concentration of gallic acid solution. Then calculate the regression equation and determine R2.

FOODSCITECH, Vol. 4 No. 1 July 2021
ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.v4i1.3686

Determination of total phenol

Take the sample to be measured with a pipette as much as 100 µl. Add 1 ml of Na2CO3 7.5 solution and 2 ml of 10% FolinCiocalteau regain and homogenize. Then incubation for 30 minutes at room temperature. 2 ml of extract was taken and put in a cuvette to measure the absorbance at a wavelength of 765 nm. Then it was calibrated with a standard curve of gallic acid to determine the total phenol content in units of µg GAE / ml. Calculated the total phenol content in µg GAE / ml with the equation: C = (CGAE x V) / G, where C = total phenol content (µg / g), CGAE = total phenol content in the form of gallic acid equivalent (µg / ml), V = volume of extract produced (ml) and G = mass of material (g)

Determination of flavonoid levels (Atanassova et al., 2011)

Quercetin standard curve determination

A standard solution of stock quercetin as much as 100 mg / L was prepared. Diluted to obtain quercetin solutions of 20, 40,60, 80, and 100%. Add 4 ml of distilled water and add a 5% solution of sodium nitrite (NaNO2) as much as 0.3 ml then homogenize and incubate for 5 minutes. When finished, add 0.3 ml of 10% AlCl3 solution and incubate for 6 minutes then add as much as 2 ml 1M NaOH and distilled water 2.4 ml and homogenize. Then the absorbance was measured at a wavelength of 510 nm. Quercetin standard curve was made with x = concentration of standard solution; y = absorbance then calculated the regression equation and R2.

Analysis of Flavonoid Levels

The sample was taken 1 ml into a test tube and added 4 ml of distilled water. Then 0.3 ml of 5% sodium nitrite (NaNO2) solution was added and homogenized then incubated for 5 minutes. After that, add 0.3 ml of 10% AlCl3 solution and incubate for 6 minutes (room temperature) then add 2.4 ml of distilled water and homogenize it. Measure the absorbance at a wavelength of 510 nm, then calibrate it with a standard quercetin curve.

AntioxidantActivity Assay (Sharma & Bhat, 2009)

Make a series of dilutions of the extracted sample to be tested (0, 20, 40,60, 80, and 100%). Take 2 ml of various dilution extract samples and put them in a test tube. DPPH (1,1-Diphenyl-2Picrythydrazyl) as much as 200 µM 1 ml was made in 1 ml of methanol, then 1 ml of this solution was put into a test tube containing 2 ml of the sample solution and standard solution separately then homogenized. The mixture of the two solutions was incubated in a dark room for 30 minutes at 30oC, then the absorbance was measured at a wavelength of 517 nm. A mixture of 1 ml methanol with 200 µM 1 ml DPPH solution was used as the blank. The antioxidant activity was calculated as the percentage reduction in DPPH color using the equation:

$$\%\ Inhibiton = \frac{A - B}{A}\ x\ 100\%$$

A : Blank absorbance
B : Sample absorbance

RESULT AND DISCUSSIONS

Bintaro Fruit Ethanol Extract

In extraction using the maceration method. The fruit of the bintaro that has been cut is put in ethanol solvent. The extract yield obtained was 10 grams with the yield is 20%. In maceration, it provides the advantage of the results obtained in large quantities, and the compounds in the material are slightly damaged because they do not use high temperatures. Alcohol is a universal solvent that is good for extracting all classes of secondary metabolite compounds present in natural materials, especially those with polar properties.

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.v4i1.3686

The yield of Bintaro fruit ethanol extract (20%) is greater than the results of research by Das et al., (2014), that the yield of Bintaro leaf ethanol extract was 9.5% and stem bark extract was 8.5%. These results indicate that many chemical compounds are polar in Bintaro fruit. This is supported by the results of research by Ejelonu et al., (2011), who reported that the results of phytochemical screening of the ethanol extract of Bintaro fruit contained phenolic compounds, flavonoids, alkaloids, tannins, saponins, anthraquinones, and cardenolides. From other research results, it is also stated that methanol has a polar group that is stronger than a nonpolar group, this is evident from the chemical structure of methanol which has a hydroxyl group (polar) and a carbon group (nonpolar).

Total Phenol Content

Total phenol testing is the basis for testing antioxidant activity because it is known that phenolic compounds play a role in preventing oxidation events. The total phenol test aims to determine the total phenolic compounds contained in the sample, so it is assumed that if the phenolic compound levels in the sample are high, the antioxidant activity will be high. The results showed that the total phenol content of Bintaro fruit ethanol extract was 73.04 mg GAE / g extract. The total phenol content in the ethanol extract of Bintaro fruit was smaller when compared to the ethanol extract of Bintaro leaves with ethyl acetate fraction (371.23 ± 15.77 mg GAE / g extract) and Bintaro stem bark with ethyl acetate fraction (326.75 ± 4.659 mg GAE / g extract) (Das et al., 2014). This was also revealed by the research of Ukhty (2011).

Also, (Deore et al., 2009) stated that total phenol testing is highly dependent on its chemical structure. Phenolic compounds that have many hydroxyl functional groups or in free conditions (aglycones) will produce high levels of total phenol. Flavonoid aglycones or polyphenols are phenolic compounds with a chemical structure consisting of a benzene group (nonpolar) and a hydroxyl group (polar). Polyphenols have more benzene (nonpolar) groups so that the nonpolar groups in phenol compounds are stronger than the polar groups. This proves that the crude extract of ethyl acetate has more hydroxyl or aglycone, functional groups because ethyl acetate solvent has the same chemical properties as flavonoid aglycone compounds, which have nonpolar groups that are stronger than polar groups.

Based on the research results, it is suspected that there is a positive correlation between low levels of total phenol and low antioxidant activity in Bintaro fruit. As the results of research by Das et al., (2014), who reported that there was a correlation between polyphenol levels with antioxidant activity and phenolic compounds that were the most dominant contributor to the antioxidant activity of the ethanol extract of Bintaro leaves. The results of research by Ejelonu et al., (2011) reported that the ethanol extract of Bintaro fruit contains phenolic compounds, flavonoids, alkaloids, tannins, saponins, anthraquinones, and cardenolides, which are compounds that have high antioxidant properties.

Levels of Flavonoids

Generally, the compounds responsible for antioxidant activity are phenol and flavonoid group compounds. Therefore, it is important to quantitatively analyze the total levels of flavonoids and phenols in the ethanol extract of Bintaro fruit which were determined by the AlCl3 colorimetric method. The results showed that the flavonoid levels of Bintaro fruit ethanol extract were 41.6 mg QE / g extract. This amount is smaller than the flavonoid levels in the ethanol extract of Bintaro leaves, ethyl acetate fraction (144.64 ± 5.82 mg QE / g extract), and the bark extract (82.7 ± 9.03 mg QE / g extract) (Das et al., 2014). This is because flavonoids are the largest phenol group in nature, so that according to the levels of flavonoids and the total phenol.

Flavonoid compounds in plants generally bind to sugars called flavonoids glycones and do not bind to sugars called flavonoid aglycones. Glycone flavonoids tend to be more soluble in polar solvents whereas, for aglycone compounds, flavonoids are more soluble in semipolar solvents. Several compounds that are classified as flavonoid glycones include flavanonols, catechins, leukoantocyanidins, anthocyanidins, chalcones, and aurons while compounds are classified as flavonoid aglycones are flavonoids, flavones, flavanones, and isoflavones. This is following the results of the study, the total levels of flavonoids from the ethanol extract of

Bintaro fruit and the results of research on the ethyl acetate extract of Bintaro (Das et al., 2014), because ethyl acetate is a semi-polar solvent so that there are more total flavonoids in ethyl acetate extract.

Flavonoids are found in almost all parts of the plant including fruit, roots, leaves, and outer bark (Prayitno & Rahim, 2020; Lumbessy et al., 2013). Flavonoids are good reducing compounds that inhibit many oxidation reactions, both enzymes, and non-enzymes, which are a group of polyphenolic compounds that are known to have properties as free radical scavengers, inhibitors of hydrolysis and oxidative enzymes, and works as an anti-inflammatory (Pourmorad et al., 2006). The antioxidant effect of compounds is caused by the scavenging of free radicals through the hydrogen atom donor of the flavonoid hydroxyl group. Flavonoids play a medicinal role in preventing cancer and cardiovascular disease (Neldawati & Gusnedi, 2013).

Antioxidant Activity (IC50)

Analysis of antioxidant activity using the DPPH method and obtained the IC50 value of Bintaro fruit ethanol extract of 275 ppm, this value is much higher than the positive control quercetin which has an IC50value of 9.87 ppm. This shows that the antioxidant activity of Bintaro fruit ethanol extract is still smaller when compared to the antioxidant activity of quercetin as a positive control which is a strong antioxidant. The IC50 value can be defined as the amount of concentration that can inhibit free radical activity, namely inhibiting DPPH free radical activity by as much as 50%. The smaller IC50 value indicates the greater the antioxidant activity of the material being tested (Molyneux, 2004).

Although the Bintaro fruit ethanol extract has a large IC50 value, the Bintaro fruit extract still has antioxidant properties and is a weak antioxidant. The opinion of Sulandi (2013) states that a substance has antioxidant properties if the IC50 value obtained is between 200-1000 µg / mL, then the substance is less active but still has the potential to be an antioxidant. The use of positive control in testing antioxidant activity is to determine how strong the antioxidant potential is in the extract when compared to quercetin which is classified as a very strong antioxidant. The results of the quercetin used in this study indicate a very strong antioxidant category. These antioxidant categories as according to Molyneux (2004),

The antioxidant activity of the Bintaro fruit ethanol extract is smaller than the results of the research by Das et al., (2014), who reported that the ethanol extract of Bintaro leaves with ethyl acetate fraction had an IC value of 8.78 ppm and was included in the very strong antioxidant category. This is thought to be due to a positive correlation between total phenol and flavonoid levels and antioxidant activity. The results showed that in addition to low antioxidant activity, the total phenol content (73.04 mg GAE / g extract) and total flavonoids (41.6 mg QE / g extract) of Bintaro fruit extract were also smaller when compared to the ethanol extract of Bintaro leaves, ethyl fraction. acetate (total phenol = 371.23 ± 15.77 mg GAE / g extract; total flavonoids = 144.64 ± 5.82 mg QE / g extract) (Das et al., 2014). The results of research by Widyawati et al., (2014) shows a positive correlation between total phenol and antioxidant activity, if in a substance has a high concentration of phenol compounds then the antioxidant activity in the substance is also high.

Usually, the compounds that have antioxidant activity are phenolic compounds that have hydroxy groups that are substituted in the ortho and para positions against the –OH and –OR groups. The antioxidant activity of phenolic compounds and tannins, both of these compounds are phenolic compounds, namely compounds with –OH groups attached to aromatic carbon rings. Phenolic compounds can donate hydrogen atoms so that DPPH radicals can be reduced to a more stable form. The free radical scavenging activity of phenolic compounds is influenced by the amount and position of phenolic hydrogen in the molecule. The greater the number of hydroxyl groups possessed by phenolic compounds, the greater the resulting antioxidant activity.

According to Wiyani et al., (2018) that semipolar ethyl acetate can extract polar glycone components and non-polar aglycone components, which causes ethyl acetate to extract more flavonoid components which are active as antioxidants. More hydroxyl group or aglycone molecules, the stronger the DPPH free radicals are captured because the ability to donate hydrogen atoms is getting bigger, besides that the aglycone structure is known to have higher

antioxidant activity compared to the glycoside structure. Aglycones are components of glycosides that do not bind to sugars or glycosides that are not carbohydrates. consisting of several chemical compounds such as phenolics, isothiocyanates, cyanogenetic nitriles, flavonoids, and steroids, where some of these chemical compounds are known to have antioxidant activity. The aglycone can only be extracted by ethyl acetate solvent because the chemical structure of the aglycone has the same properties as ethyl acetate, which has a non-polar group that is stronger than the polar group.

The presence of flavonoids-quercetin, apigenin, naphtoquinones, tannins, and steroids in Bintaro fruit can contribute to antioxidant activity, because these compounds have been known to be antioxidants, where the involvement of free radicals, especially increased production can trigger cardiovascular diseases and cancer. Thus consumption of Bintaro can prevent oxidative stress associated with various chronic diseases.

CONCLUSION

The ethanol extract of Bintaro fruit in the study produced a total phenol content of 73.04 mg GAE / g extract, 41.61 mg QE / g flavonoids extract, and had antioxidant activity (IC50) 275.06 ppm. Recommended for in vivo or in vitro assay for certain diseases using these extracts. Further research is needed with the use of other solvent to see optimall in the screening of active compounds.

REFERENCES

Ahmed, F, Amin, R., Shahid, I., & Sobhani, M. (2008). Antibacterial, cytotoxic and neuropharmacological activities of Cerbera odollam seeds. *Oriental Pharmacy and Experimental Medicine*, *8*(4), 323–328. https://doi.org/10.3742/opem.2008.8.4.323

Ahmed, Firoj, Hossain, H. M., Rahman, Ayedur, A., & Shahid, Z. I. (2006). Antinociceptive and sedative effects of the bark of Cerbera odollam Gaertn. *Oriental Pharmacy and Experimental Medicine*, *6*(4), 344–348.

Atanassova, M., Georgieva, S., & Ivancheva, K. (2011). Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. *Journal of the University of Chemical Technology and Metallurgy*, *46*(1), 81–88.

Baghel, S. S., Shrivastava, N., Baghel, R. S., & Rajput, S. (2012). I S S N 2278 – 4357 a Review of Quercetin : Antioxidant and Anticancer Properties. *World Journal of Pharmacy and Pharmaceutical Sciences*, *1*(1), 146–160.

Das, N., Islam, M. E., Jahan, N., Islam, M. S., Khan, A., Islam, M. R., & Parvin, M. S. (2014). Antioxidant activities of ethanol extracts and fractions of Crescentia cujete leaves and stem bark and the involvement of phenolic compounds. *BMC Complementary and Alternative Medicine*, *14*. https://doi.org/10.1186/1472-6882-14-45

Deore, S. L., Khadabadi, S. S., Patel, Q. R., Deshmukh, S. P., Jaju, M. S., Junghare, N. R., … Jain, R. G. (2009). In vitro antioxidant activity and quantitative estimation of phenolic content of lagenaria siceraria. *Rasayan Journal of Chemistry*, *2*(1), 129–132.

Ejelonu, B. C., Lasisi, A. A., Olaremu, A. G., & Ejelonu, O. C. (2011). The chemical constituents of calabash (Crescentia cujete). *African Journal of Biotechnology*, *10*(84), 19631–19636. https://doi.org/10.5897/AJB11.1518

Lumbessy, M., Abidjulu, J., & Paendong, J. J. E. (2013). Uji Total Flavonoid Pada Beberapa Tanaman Obat Tradisonal Di Desa Waitina Kecamatan Mangoli Timur Kabupaten Kepulauan Sula Provinsi Maluku Utara. *Jurnal MIPA*, *2*(1), 50. https://doi.org/10.35799/jm.2.1.2013.766

Molino, A., Mehariya, S., Iovine, A., Larocca, V., Di Sanzo, G., Martino, M., … Musmarra, D. (2018). Extraction of Astaxanthin and Lutein from Microalga Haematococcus pluvialis in the Red Phase Using CO_2 Supercritical Fluid Extraction Technology with Ethanol as Co-Solvent. *Marine Drugs*, *16*(11). https://doi.org/10.3390/md16110432

Molyneux, P. (2004). The Use of the Stable Free Radical Diphenylpicryl-hydrazyl (DPPH) for Estimating Antioxidant Activity. *Songklanakarin Journal of Science and Technology*,

26(December 2003), 211–219. https://doi.org/10.1287/isre.6.2.144

Neldawati, R., & Gusnedi. (2013). Analisis Nilai Absorbansi dalam Penentuan Kadar Flavonoid untuk Berbagai Jenis Daun Tanaman Obat. *Pillar of Physics, 2*, 76–83.

Nguyen, S. T., Nguyen, H. T.-L., & Truong, K. D. (2020). Comparative cytotoxic effects of methanol, ethanol and DMSO on human cancer cell lines. *Biomedical Research and Therapy*, *7*(7), 3855–3859. https://doi.org/10.15419/bmrat.v7i7.614

Ogbuagu, M. N. (2008). Nutritive and Anti-Nutritive Composition of the Wild (In-Edible) Species of Dioscorea bulbifera (Potato Yam) and Dioscorea dumentorum (Bitter Yam). *The Pacific Journal of Science and Technology*, *6*(5), 224–226.

Pourmorad, F., Hosseinimehr, J, S., & Shahabimajd, N. (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology*, *5*(TS32.744), 1142–1145. Retrieved from http://www.3gpp.org/ftp/Specs/html-info/32744.htm

Prayitno, S. A., & Rahim, A. R. (2020). The Comparison of Extracts (Ethanol And Aquos Solvents) Muntingia calabura Leaves on Total Phenol, Flavonid And Antioxidant (Ic50) Properties. *Kontribusia (Research Dissemination for Community Development)*, *3*(2), 319. https://doi.org/10.30587/kontribusia.v3i2.1451

Sahoo, A., & Marar, T. (2018). Phytochemical analysis, antioxidant assay and antimicrobial activity in leaf extracts of cerbera odollam gaertn. *Pharmacognosy Journal*, *10*(2), 285–292. https://doi.org/10.5530/pj.2018.2.50

Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food Chemistry*, *113*(4), 1202–1205. https://doi.org/10.1016/j.foodchem.2008.08.008

Sholahuddin, A. H., Subchan, W., & Prihatin, J. (2018). Toxicity of Granules of Bintaro Leaf Extract (Cerbera odollam Gaertn.) on Armyworm (Spodoptera litura Fab.). *Bioedukasi*, *XVI*(1), 15–21. https://doi.org/10.19184/bioedu.v16i1.7717

Som, A. M., Ahmat, N., Abdul Hamid, H. A., & Azizuddin, N. M. (2019). A comparative study on foliage and peels of Hylocereus undatus (white dragon fruit) regarding their antioxidant activity and phenolic content. *Heliyon*, *5*(2), e01244. https://doi.org/10.1016/j.heliyon.2019.e01244

Sulandi, A. (2013). *AKTIVITAS ANTIOKSIDAN EKSTRAK KLOROFORM BUAH LAKUM (Cayratia trifolia) DENGAN METODE DPPH (2,2 DIFENIL-1-PIKRILHIDRAZIL*.

Widyawati, S. P., Budianta, T. D. W., Kusuma, F. A., & Wijaya, E. L. (2014). Difference of solvent polarity to phytochemical content and antioxidant activity of Pluchea indicia less leaves extracts. *International Journal of Pharmacognosy and Phytochemical Research*, *6*(4), 850–855.

Wiyani, L., Aladin, A., Yani, S., Mutmainnah, S. H. N., & Mandang, H. D. (2018). Effect of Sucrose and Citric Acid Addition in the Virgin Coconut Oil Emulsion. *IOP Conference Series: Earth and Environmental Science*, *175*(1). https://doi.org/10.1088/1755-1315/175/1/012024