Indonesian Journal of Chemical Research

http://ojs3.unpatti.ac.id/index.php/ijcr Indo. J. Chem. Res., 11(1), 43-48, 2023

Antioxidant Activity of Ethanol and n-hexane Extracts of Javanese Bark *(Lannea coromandelica)* Using the DPPH Method

Nuramaniyah Taufiq*, Sulfiani

Department of DIII Medical Laboratory Technology, Faculty of Health Technology, Megarezky University, Jalan Antang Raya, Kecamatan. Manggala, Makassar City, South Sulawesi, 90234, Indonesia

**Corresponding Author: nuramaniyah53@gmail.com*

Received: February 2023
Received in revised: March 2023
Accepted: April 2023
Available online: May 2023

Abstract

Javanese bark (Lannea coromandelica) contains secondary metabolites of saponins, tannins, phenolics, and flavonoids. Flavonoids include aromatic compounds that are antioxidants. Antioxidants can inhibit the oxidation process that arises due to free radical reactions to form unreactive compounds. The active flavonoid compounds in counteracting free radicals are determined by the presence of the –OH (hydroxy) functional group. Flavonoid compounds that have antioxidant properties include catechins, flavones, flavanones, flavonols, chalcones, and isoflavones. This study aims to determine the antioxidant activity of ethanol extract and n-hexane bark of Javan bark (Lannea coromandelica) using the DPPH method. The method used is DPPH using UV-VIS spectrophotometry. Ethanol extract has an IC_{50} of 3.996 mg/L and has a strong antioxidant activity while the antioxidant activity of N-Hexan is obtained IC_{50} 2193.043 mg/L. has weak antioxidants weak antioxidants.

Keywords: Antioxidants, Lannea coromandelica, DPPH Method, flavonoid, IC_{50}

INTRODUCTION

Antioxidants are electron-donating compounds that can inhibit oxidation reactions by binding free radicals with highly reactive molecules (Najihudin et al., 2017). Free radicals are unstable and very reactive in achieving stability. Radicals with very high reactivity start a chain reaction in achieving stability, causing abnormal compounds and starting chain reactions that can damage important cells in the body (Tristantini *et al.*, 2016). Antioxidant compounds have an important role in the health of living things. Various scientific evidence shows that antioxidant compounds can prevent various diseases such as cancer and coronary heart disease. The main ability of antioxidant compounds is to capture free radicals. The use of antioxidant compounds is growing in both food and health. The use of natural ingredients is highly developed as an antioxidant (Sari *et al*., 2019).

Free radicals damage cell-forming macromolecules such as proteins, fats, carbohydrates, and DNA. The behavior of these free radicals is destructive to other molecules whose electrons are taken. Taking electrons by free radicals causes a reaction, forming more free radicals. The effects of reactions from free radical compounds can cause various health problems such as inflammation, aging, and cancer. Therefore, to avoid damage caused by free radicals, the body needs important substances, namely antioxidants, which can protect the body from free radical attacks (Mahardika & Fajri, 2023)

The behavior of these free radicals is destructive to other molecules whose electrons are taken. Taking electrons by free radicals causes a reaction, forming more free radicals. The effects of reactions from free radical compounds can cause various health problems such as inflammation, aging, and cancer. Therefore, to avoid damage caused by free radicals, the body needs important substances, namely antioxidants, which can protect the body from free radical attacks (Mahardika, R. G., & Fajri, 2023)

The use of natural ingredients is not enough just to be used as traditional medicine and experience passed down from generation to generation, but must be scientifically proven that contains active substances that can cure various diseases (Najihudin *et al*., 2017). Plants as the main raw material as a source of antioxidants, Antioxidants play a role in counteracting free radicals in the body so that they can fight oxidative damage and also inhibit the process of fat/oil oxidation so that they have a function as a preservative. Various plant studies can be used, based on research conducted (Manongko *et al*., 2020). The test results for the total phenolic content of ethanol extract have a value of 60.270 mg GAE/g. The antioxidant power is determined by the IC_{50} value which is based on the percent immersion of

free radicals by the test sample. The ethanol extract has an IC_{50} of 82.152 g/mL. Based on the results obtained, the ethanolic extract of the fracture plant has a strong potential as an antioxidant.

Javanese wood is a tropical plant with a height of 10-15 m and is spread in various tropical countries, one of which is Indonesia. In addition, Javanese wood (*Lannea coromandelica*) is used by bark as a wound medicine by applying it to the body to be treated (Husain, Halimah. Sudding, 2019). In general, Javanese wood plants (*Lannea coromandelica*) contain secondary metabolites such as alkaloids, steroids, triterpenes, glycosides, flavonoids, tannins and saponins, and polyphenolic compounds (Majdiyah & Salempa, 2021). Based on research (Calsum *et al*.,2018) Javanese bark *(Lannea coromandelica*) contains secondary metabolites of saponins, tannins, phenolics, and flavonoids. Flavonoids include aromatic compounds that are antioxidants. Antioxidants can inhibit the oxidation process that arises due to free radical reactions to form unreactive compounds. The active flavonoid compounds in counteracting free radicals are determined by the presence of –OH (hydroxy) functional group. Flavonoid compounds that have antioxidant properties include catechins, flavones, flavanones, flavonols, chalcones, and isoflavones (Calsum *et al*., 2018). The Javanese bark *(Lannea coromandelica* can be shown at Figure 1.

Figure 1. Javanese bark *(Lannea coromandelica*)

Fractionation is the process of separating mixed components of an extract based on differences in polarity properties. The fractionation method used in this research is the liquid-liquid partition. Liquid-liquid partitioning is done by adding n-hexane as a solvent in the extract which has been dissolved in ethanol so that two phases are formed. The components of bioactive compounds in the extract will dissolve between the two phases according to their polarity. The process of dissolving substances in this solvent is by the principle of like dissolves, i.e. the solubility of a compound between two phases will depend on the similarity of the polarity of the compound with the liquid solvent. This process causes the weight of crude obtained from the liquid-liquid partition extraction of Dengen leaves ethanol extract in ethanol solvent to be heavier than in n-hexane (Nasriadi *et al*., 2022)

Research on the use of ethanol extract in bark has been carried out (Septiana & Simanjuntak, 2018) the combination of ethanol extract of Bintangur bark has antioxidant activity, phenol, and total flavonoid levels are higher than the single extract. The combination has the best antioxidant activity is the ethanol extract of the bark of the Bintangur stem *C. pulcherrimum*+ *C. soulattri*+*C. teysmannii* with a ratio of 1:1:1, while the ethanolic extract of the stem bark of C. pulcherrimum has the lowest antioxidant activity. Research (Manurung *et al*., 2020) the antioxidant activity test obtained an IC_{50} value of 20.94 ppm crude extract, 36.29 ppm ethanol fraction, 83.28 ppm ethyl acetate fraction, and 39.13 ppm n-hexane fraction. The research result shows the crude extract, ethanol fraction, and n-Hexane fraction are categorized as very strong antioxidants, and ethyl acetate fraction is categorized as strong antioxidants. This study aims to determine the antioxidant activity of ethanol extract and n-hexane bark of Javan (*Lannea coromandelica*) using the DPPH method.

METHODOLOGY

Instrumental and Materials

The tools used were maceration vessels, porcelain dishes, beakers, 10 ml, 25 ml, 50 ml, and 100 ml volumetric flasks, beakers, vials, analytical balances, and UV-VIS spectrophotometers. The ingredients used are Javanese bark (*Lannea coromandelica*), ethanol 96%, n-hexane 96%, methanol, DPPH, and vitamin C

Preparation and extraction

Javanese bark (*Lannea coromandelica*) is dried by aerating then dry sorting and then pureeing. The sample was weighed as much as 250 grams for the solvent ethanol and 250 grams for the solvent n-hexane, macerated for 3 x 24 hours with 3 times of maceration. The liquid extract obtained was collected and evaporated to obtain a thick extract.

Antioxidant Activity Test

Preparation of the ethanol extract sample as much as 1% made stock solution with a concentration of 1000 ppm so that the obtained concentration of

0.1% stock solution 1000 ppm with a solution concentration of 1.5, 2.5, 5, and 7.5 while n-hexane sample as much as 1% was made with a stock solution with a concentration of 1000 ppm to obtain a concentration of 250, 500, 750, and 1000 ppm. Preparation of a comparison solution of vitamin C was made with as much as 1% stock solution, with a concentration of 1000 ppm to obtain a concentration of 0.1%. A stock solution of 1000 ppm then used to make another solution there are 2, 4, 6, and 7 ppm. Preparation of the DPPH solution, DPPH crystals are dissolved in methanol in a concentration of 0.01% as much as 100 ml of DPPH solution.

RESULTS AND DISCUSSION

The results of the research that has been carried out are the sample used in this study, namely Java wood *(Lannea coromandelica*) which is a plant originating from Makassar. Lannea coromandelica is a plant belonging to the Anacardiaceae family and the Lennea genus. The part of the Javanese wood plant that is taken is the bark of the Javanese wood. The bark of Java wood which has been separated from other parts of Java wood is dried without direct sunlight. Java bark drying aims to remove the water content in the bark of Java wood to facilitate the extraction process on the bark of Java wood. The dried samples were first mashed to speed up the release of organic compounds contained in the bark of the Javanese wood. The mashed sample was then weighed as much as 250 grams for ethanol solvent and 250 grams for n-hexane solvent and extraction was carried out using the maceration method with ethanol solvent and n-hexane solvent. Natural extracts may also include components that can interfere with the electron transfer reaction between antioxidants and free radicals, such as weak acid groups. The effect of the solvent on the extraction process can also affect the antioxidant activity

The maceration method is used because it is one of the methods that use simple equipment. This is done by immersing the sample and then stirring. Stirring aims to produce collisions between particles that can penetrate the cell walls of the particles and bind secondary metabolites in the sample. The maceration method does not require heating so it does not damage the structure of the compound due to heating or which is not resistant to heating. Maceration was carried out by immersing the sample using ethanol solvent for 3×24 hours and n-hexane solvent for 3 x 24 hours with 3 repetitions of the maceration method then the extract was filtered using whatman 40 paper to produce brown ethanol extract and slightly green n-hexane brownish. Furthermore, evaporation is carried out which aims to separate the solvent from secondary metabolites, evaporation is carried out at a temperature lower than 40 ˚C to avoid the decomposition of the nutritious substances contained in the extract A thick extract of Javanese bark was obtained after evaporation process.

Ethanol and n-hexane extracts of Javanese bark (*Lannea coromandelica*) were tested for antioxidants. Antioxidants are compounds that can Inhibit oxidation reactions by binding to free radicals and highly reactive molecules compound that have potential as antioxidants are generally flavonoids, phenolics, and alkaloids. Flavonoid and phenolic compounds are antioxidants, antidiabetic, anticancer, antiseptic, and anti-inflammatory, while alkaloids are antineoplastic which are also effective in inhibiting the growth of cancer cells (Amin *et al*., 2016).

Table 1. The antioxidant activity ethanol extract of Java (*Lannea coromandelica*) bark

Concentration (mg/L)	Control Absorbance	Sample absorbance	(C-D)/C	Percentage of H
1.5	0.336	0.253	0.25	24.70
2.5	0.336	0.215	0.36	36.01
5	0.336	0.132	0.61	60.71
7.5	0.336	0.055	0.84	83.63

Note: C: Control, D: Sample, H: Inhibition

In the antioxidant test, the DPPH method is used. The DPPH method is a method that can be used to determine the antioxidant activity in the sample to be tested by looking at its ability to ward off DPPH free radicals. The advantages of the DPPH method are that it is simple, easy, fast, sensitive, and requires very small samples. The DPPH test is an effective method for determining free radical activity. The DPPH radical is an unstable nitrogen-containing organic compound with a strong absorbance at length 2019) It is easy to apply because the DPPH radical compound is stable and reactive compared to other methods. The principle of this method is the donation of hydrogen atoms (H^+) from the tested substance to the DPPH radical into a non-radical compound diphenyl picryl hydrazine which will be indicated by a color change.

The color change that occurs is from purple to yellow, where the intensity of the color change of DPPH is proportional to direct with antioxidant activity to reduce these free radicals (Rahmawati et al., 2016). Measurement of antioxidant activity was

measured using an UV-VIS spectrophotometer with a wavelength of 520 nm. The antioxidant activity test of ethanol extract and n-hexane bark of Javan barks (*Lannea coromandelica*) was carried out using the DPPH method (Figure 2).

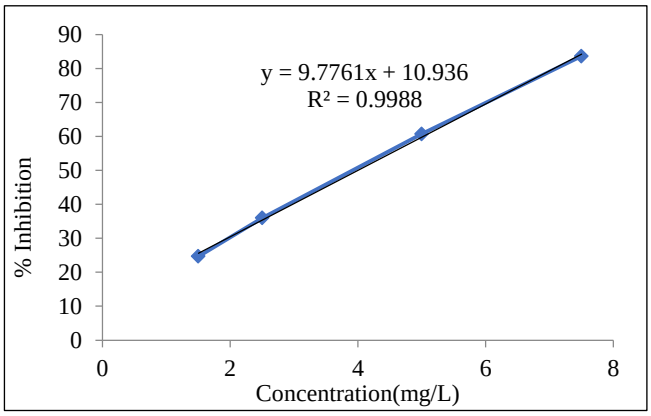

Figure 2. Graph of relationship between concentration and % IC_{50} inhibition ethanol extract of Javan (*Lannea coromandelica*) bark

The antioxidant activity of the ethanol extract from the bark of the Javanese wood *(Lannea coromandelica*) carried out by the DPPH method is presented in Table 1. IC_{50} (Inhibitory Concentration 50) is a number that indicates the sample concentration (ppm) capable of inhibiting free radicals by 50% (Figure 2). The smaller the IC_{50} value, the higher the antioxidant activity. Analysis of antioxidant activity is determined by the value of DPPH radical uptake by calculating the percentage of DPPH absorption inhibition (Fathiah Olpah Siara, Arsyik Ibrahim, Hanggara Arifian, 2016).

Table 2. The antioxidant activity n-hexane extract of Javan Bark *(Lannea coromandelica*)

Concetration (mg/L)	Control Absorbance	Sample Absorbance	(C-D) /C	Percentage of H
250	0.336	0.318	0.051	5.36
500	0.336	0.299	0.110	11.01
750	0.336	0.278	0.173	17.26
1000	0.336	0.26	0.23	22.62

Note: C: Control, D: Sample, H: Inhibition

Antioxidant activity of the ethanol extract from the bark of the Javan wood (*Lannea coromandelica*) using test concentrations of 1.5, 2.5, 5, and 7.5 ppm obtained IC_{50} 3.996 mg/L. has a very strong antioxidant activity Antioxidants are said to be very strong if they have an IC_{50} value of less than 50 ppm, a strong antioxidant has an IC_{50} value in the range of 50 ppm to 100 ppm (Purwanto *et al*., 2017). Based on research conducted (Mokoginta *et al.*, 2020) 96% ethanol solvent using the DPPH method showed antioxidant activity in the ethanol extract of Dayak onion bulb with an IC_{50} value of 41.46 mg/L and an IC_{50} value of 1.04 mg/L in vitamin C.

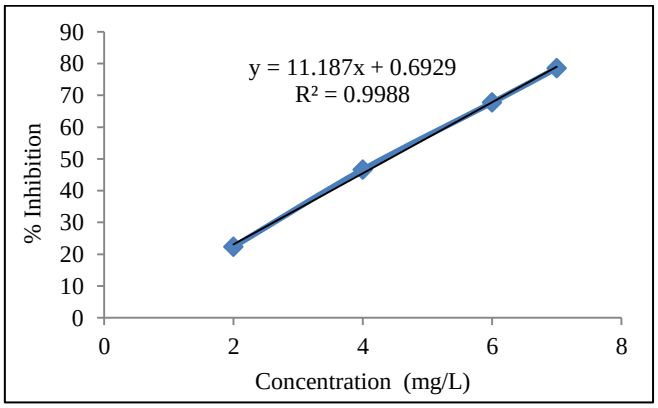

Figure 3. Graph of relationship between concentration and % IC_{50} Inhibition of n-hexane extract of Javan Bark (*Lannea coromandelica)*

Based on these results, it can be concluded that the ethanolic extract of Dayak onion bulbs has very strong antioxidant activity. The use of the polarity of the solvent greatly affects the antioxidant activity obtained. The highest antioxidant activity was obtained from extraction using a polar solvent which used ethanol *(*Purwanto *et al*., 2017). In addition, it contains secondary metabolites from Javanese wood plants *(Lannea coromandelica*) such as alcohol, steroids, triterpenoids, phenolics, flavonoids, tannins, and saponins. Flavonoid compounds are antioxidants that have the potential to prevent the formation of free radicals (Husain, Halimah. Sudding, 2019).

Antioxidant activity of n-hexane from Javan wood *(Lannea coromandelica*) using concentration tests of 250, 500, 750, and 1000 ppm obtained IC_{50} 2193.043 ppm (Table 2 and Figure 3). Weak antioxidants, weak antioxidants having a range of 150 ppm to 200 ppm, and IC_{50} values of more than 200 ppm are very weak antioxidants (Purwanto *et al*., 2017). Solvent n-hexane is a non-polar solvent n-hexane extract (nonpolar) that contains non-polar components including wax, fat, and essential oil. The antioxidant activity of the n-hexane fraction is weaker than that of the ethanol extract because the secondary metabolite content of n-hexane is only an alkaloid compound (Fathiah Olpah Siara, Arsyik Ibrahim, Hanggara Arifian, 2016).

In this study, vitamin C was used as a comparison of the antioxidant activity of ethanol extract and n-hexane extract (Table 3 and Figure 4). Vitamin C has a high polarity because it contains

many hydroxyl groups so it is easily absorbed by the body. Therefore, vitamin C can react with free radicals and can neutralize free radicals (Nurhasnawati et al., 2017). Vitamin C is used as the comparison or positive control and has much greater antioxidant activity. The IC_{50} value is inversely proportional to the antioxidant activity. The greater the antioxidant activity if thebsample has the lower the IC_{50} value. IC_{50} is a value indicating the ability to inhibit 50% of free radicals by a sample concentration (ppm) (Taba et al., 2019).

Table.3. Positive control (Vitamin C)

Concentration (mg/L)	Control Absorbance	Sample Absorbance	(C-D)/C	Percentage of H
2	0.313	0.243	0.224	22.36
4	0.313	0.167	0.467	46.65
6	0.313	0.101	0.677	67.73
7	0.313	0.067	0.786	78.59

Note: C: Control, D: Sample, H: Inhibition

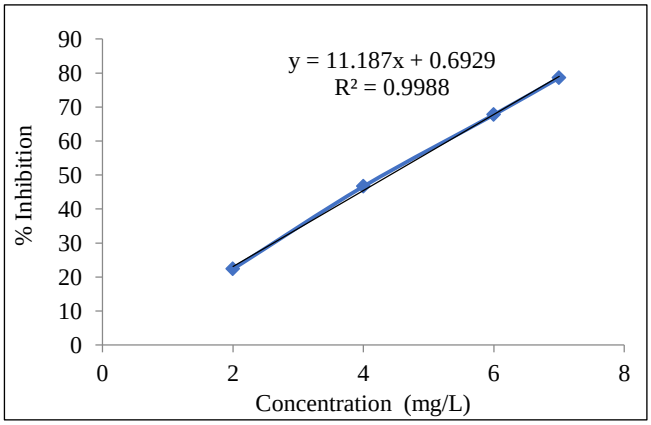

Figure 4. Graph of positive control (Vitamin C)

Vitamin C as positive control also has very strong antioxidant activity. However, the antioxidant activity of the ethanol extract was greater than the antioxidant activity of vitamin C, which was stronger than that of the solvent n-hexane. This difference means that ethanol extract has weaker antioxidant activity than vitamin C. Vitamin C is an antioxidant that works as oxygen scavengers, which binds oxygen so it does not support oxidation reactions. In this case, vitamin C will react with oxygen in the system so that the amount of oxygen will decrease. In addition to vitamin C, compounds that work as oxygen scavengers include ascorbyl palmitate, erythorbic acid, and sulfites (Nurhasnawati et al., 2017).

CONCLUSION

The results of research on the antioxidant activity test of ethanol extract and n-hexane bark of Javan bark (*Lannea coromandelica*) using the DPPH method, it was concluded that the ethanol extract had an IC_{50} is 3.996 mg/L and it have strong antioxidant activity while the antioxidant activity of n-hexane was obtained IC_{50} is 2193.043 mg/L and it have properties as weak antioxidants.

ACKNOWLEDGMENT

The author would like to thank the Ministry of Research, Technology and Higher Education for Research Grant for Beginner Lecturers (PDP) and Megarezky University who have helped so that this research can be carried out and this paper can be published.

REFERENCES

Amin, Astuti, Wunas, J., & Anin, Y. M. (2016). Uji Aktivitas Antioksidan Ekstrak Etanol klika Faloak (*Sterculia Quadrifida* R.Br) Dengan Meode DPPH (2,2-Diphenyl-1-Picrylhydrazyl). *Jurnal Fitofarmaka Indonesia*, *22*(2), 111-114.

Calsum, U., Khumaidi, A., & Khaerati, K. (2018). Aktivitas Ekstrak Etanol Kulit Batang Kayu Jawa (*Lannea coromandelica*) terhadap Penyembuhan Luka Sayat pada Tikus Putih (*Rattus Norvegicus* L.). *Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal), 4(2)* 113-118.

Fathiah Olpah Siara, Arsyik Ibrahim, Hanggara Arifian, R. R. (2016). Aktivitas Antioksidan Ekstrak Kulit Batang Kersen (*Muntingia calabura* L.). *Proceeding of the 5th Mulawarman Pharmaceuticals Conferences, April 2017*, 1-23.

Hasanela, & Souhoka, F. A. (2022). Effect of Heating Coarse Extract of Brown Macroalgae (*Padina australis*) from Tial Waters, Salahutu District, Central Maluku Regency on Antioxidant Activity. *Indonesian Journal of Chemical Research, 10(2)*, 102-109

Husain, Halimah. Sudding, H. (2019). Isolasi dan Penentuan Struktur Senyawa Golongan Steroid Dari Kulit Kayu Jawa (*Lannea coromandelica*). *Prosiding Seminar Nasional LP2M UNM*, 685-688.

Mahardika, R. G., & Fajri, K. (2023). *Antioxidant Capacity Fraction of the Pelawan Stems (Tristaniopsis merguensis Griff)*. *10*(3), 143-148.

Majdiyah, R., & Salempa, P. (2021). Isolasi , Identifikasi, Dan Uji Bioaktivitas Senyawa

Metabolit Sekunder Ekstrak Metanol Daun Kayu Jawa *(Lannea Coromandelica)*. 36-44.

Manongko, P. S., Sangi, M. S., & Momuat, L. I. (2020). Uji Senyawa Fitokimia dan Aktivitas Antioksidan Tanaman Patah Tulang (*Euphorbia tirucalli* L.). *Jurnal MIPA*, *9*(2), 64-69

Manurung, D. P., Sundaryono, A., & Amir, H. (2020). Penentuan Potensi Ekstrak Kulit Batang Tumbuhan Sikkam (*Bischofia Javanica* Blume) Sebagai Antioksidan Dengan Metode Dpph Dan Itotoksik Dengan Metode Bslt. *Alotrop, 4(1),* 83-91.

Mokoginta, R. V., Simbala, H. E. I., & Mansauda, K. L. (2020). Uji Aktivitas Antioksidan Ekstrak Etanol Bulbus Bawang Dayak (Eleutherine Americana Merr) Dengan Metode Dpph (1,1-Diphenyl-2-Picrylhydrazyl). *Pharmacon, 9(3),* 452-457.

Najihudin, A., Chaerunisaa, A., & Subarnas, A. (2017). Aktivitas Antioksidan Ekstrak Dan Fraksi Kulit Batang Trengguli (*Cassia Fistula* L) Dengan Metode DPPH. *Indonesian Journal of Pharmaceutical Science and Technology*, *4*(2), 70-78.

Nasriadi, Dali, S., Chairunnas, A., Amalia, H. A. M., & Puspitasari, S. A. A. (2022). *Extraction of The Chemical Components of Dengen Leaves (Dillenia serrata Thunb) by MAE Method and Activity Test as Antioxidant and Toxicity. Indo. J. Chem. Res.*, *10*(2), 74-82.

Nurhasnawati, H., Handayani, F., & Sukarmi. (2017). Sokletasi Terhadap Aktivitas Antioksidan Ekstrak Etanol Daun Jambu Bol (*Syzygium malaccense* L .). *Jurnal Ilmiah Manuntung*, *3*(1), 91-95.

Purwanto, D., Bahri, S., & Ridhay, A. (2017). Uji Aktivitas Antioksidan Ekstrak Buah Purnasjiwa (*Kopsia arborea* Blum). *Kovalen*, *3(1),* 24-32.

Rahmawati, R., Muflihunna, A., & Sarif, L. M. (2016). Analisis Aktivitas Antioksidan Produk Sirup Buah Mengkudu (*Morinda Citrifolia* L.) Dengan Metode Dpph. *Jurnal Fitofarmaka Indonesia*, *2*(2), 97-101.

Sari, P., Sugita, P., & Santoso, A. (2019). Aktivitas Antioksidan, Antibakteri, dan Toksisitas Ekstrak Kulit Batang Pohon Kesambi (S*chleichera oleosa* (Lour) Oken). *Jurnal Jamu Indonesia*, *4*(3), 112-118.

Septiana, E., & Simanjuntak, P. (2018). Aktivitas Antioksidan Ekstrak Etanol Kulit Batang *Calophyllum Antioxidant Activity of Stem Bark Ethanolic Extracts of Calophyllum pulcherrimum , C . soulattri , and C . 29*(2), 59-68.

Souhoka, F. A., Hattu, N., & Huliselan, M. (2019). Uji Aktivitas Antioksidan Ekstrak Metanol Biji Kesumba Keling (*Bixa orellana* L.) A*ntioxidant Activity Test of Methanol Extract of Kesumba Keling (Bixa orellana* L) *Seeds*. *Indo. J. Chem. Res.*, *7*(1), 25-31.

Taba, P., Parmitha, N. Y., & Kasim, S. (2019). Sintesis Nanopartikel Perak Menggunakan Ekstrak Daun Salam (*Syzygium polyanthum*) Sebagai Biokunduktor Dan Uji Aktivitasnya Synthesis of Silver Nanoparticles Using Syzygium polyanthum Extract as Bioreductor and the Application as Antioxidant. *Indo. J.Chem.Res*, *7*(1), 51-60.

Tristantini, D., Ismawati, A., Pradana, B. T., & Gabriel, J. (2016). Pengujian Aktivitas Antioksidan Menggunakan Metode DPPH pada Daun Tanjung *(Mimusops elengi* L*). Prosiding Seminar Nasional Teknik Kimia "*Kejuangan*,." *1-7.