

The Antioxidant Activity of Red Fruit Juice (*Pandanus conoideus* Lam.) Using The FRAP Method

Mufti Ainunnisa¹, Muammar Fawwaz^{1,2*}, Mamat Pratama¹

¹ Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, Indonesia

² Molecular Probes Discovery Group (MoleGro), Universitas Muslim Indonesia, Makassar, Indonesia

* Corresponding Author. E-mail: muammar.fawwaz@umi.ac.id

Received: 05 Mei 2025 / Revised: 08 Mei 2025 / Accepted: 14 Mei 2025

ABSTRACT: Red fruit is a member of the *Pandanaceae* family which is a type of pandan. Empirically, red fruit (*Pandanus conoideus* Lam.) has been proven to cure various degenerative diseases such as cancer, heart disease, tuberculosis, respiratory tract disorders, external antiseptic and eye and skin diseases. The aim of this research was to determine the antioxidant activity of red fruit juice using the ferric reducing antioxidant power (FRAP) method. The juice was tested qualitatively and quantitatively using the FRAP method. The qualitative test results after adding the FRAP reagent were positive for antioxidant activity because the color changed to blue. In quantitative determination of absorbance, antioxidant activity was measured using the UV-Vis spectrophotometric method. Absorbance was measured at a maximum wavelength of 701 nm using ascorbic acid as a reference solution. The equation obtained is $y = 0.0103x + 0.3801$ with a value of $r = 0.9978$. The total antioxidant activity value was calculated based on absorption data. The calculation results for the total antioxidant activity value were 173.68 mgAAE/L sample. Therefore, we can conclude that red fruit can be used as a natural antioxidant.

KEYWORDS: Absorbance; antioxidant; ascorbic acid; FRAP; spektrofotometer UV-Vis.

1. INTRODUCTION

Red fruit (*Pandanus conoideus* Lam.) is one of the *Pandanaceae* family whose distribution can be found almost throughout the Papuan plains (Ponglabba et al., 2020). This red fruit grows three times every year. This plant can grow in the lowlands up to an altitude of 2,500 m above sea level (asl), with low soil fertility, acidic to slightly acidic (pH 4.30-5.30). The red fruit plant will produce fruit for the first time after more than three years since planting. However, in the lowlands, plants produce fruit when they are two years old and are ready to be harvested (Sangkala et al, 2014). The fruit of this plant physically comes out following the rotation of the leaves, is located at the top of the stem and is usually protected by a covering sheath consisting of pith to which the seeds are attached which are very hard and wrapped in red flesh. The seeds attached to the pith are neatly arranged, about 1 cm long and 0.2 cm in diameter, and at first glance their shape resembles jackfruit skin (Sangkala et al, 2014).

The fruit of this plant physically comes out following the rotation of the leaves, is located at the top of the stem and is usually protected by a covering sheath consisting of pith to which the seeds are attached which are very hard and wrapped in red flesh. The seeds attached to the pith are neatly arranged, about 1 cm long and 0.2 cm in diameter, and at first glance their shape resembles jackfruit skin (Sangkala et al, 2014).

As a local resource, red fruit is very important for the Papuan people for several reasons, namely red fruit oil is used as edible oil and a basic ingredient for medicine. Red fruit oil has been analyzed for its chemical content because of its potential as a medicinal base, and the results show that it contains fatty and proximate acids. Pieces of red fruit boiled with sweet potato leaves are used as pig feed (Wawo et al., 2019). Red fruit pulp is an intermediate product that can be further processed to make sauce, jam, dodol and various types of cakes such as sponge cake (Ponglabba et al., 2020).

It is reported that red fruit flesh contains protein levels of 1.86-2.66%, fat 50.8-55.58%, carbohydrates 36.78-46.3%, and ash 2.7-5.03%, with levels total carotenoids of 552-1592 ppm. With a high content of active carotenoid and tocopherol components, red fruit is a source of natural antioxidants and has the potential to be processed as a functional food (Ponglabba et al., 2020). Red fruit (*Pandanus conoideus* Lam.) has been empirically proven to cure various degenerative diseases including cancer, heart disease, tuberculosis, respiratory tract disorders, and is an external antiseptic, as well as eye and skin diseases (Saa et al., 2022).

Fruit juice is the result of pressing or extracting filtered fruit. Fruit juice may contain crushed fruit and appear cloudy or clear. The principle of making fruit juice is different for each type of fruit, but the main aim is to extend the shelf life and usefulness of the fruit (Hermawan et al., 2016).

Antioxidants are compounds that have the ability to prevent and slow down damage caused by free radicals through inhibiting oxidative mechanisms. Antioxidants can help protect the human body against damage caused by reactive oxygen compounds (ROS; reactive oxygen species) and other free radicals (Pratama et al., 2016).

The FRAP method or Ferric Reducing Antioxidant Power is a method for determining antioxidant content spectrophotometrically which is based on the reduction of the ferrioxal analogue, Fe^{3+} complex from tripyridyltriazine $Fe(TPTZ)^{3+}$ to Fe^{2+} complex, $Fe(TPTZ)^{2+}$ which is intensely blue in color by antioxidants in sour atmosphere. Determination of the TAC (Total Antioxidant Capacity) value of the sample is carried out by mixing the FRAP reagent with the sample extract. FRAP reagent is a colorless Fe^{3+} -TPTZ complex compound (different from the Fe^{2+} complex

which is blue). The Fe^{3+} -TPTZ compound represents an oxidizing compound that may be present in the body and can damage body cells, while the sample extract contains antioxidants which can then reduce Fe^{3+} -TPTZ to Fe^{2+} -TPTZ so that the Fe^{3+} -TPTZ compound will not carry out reactions that damage cells. body cells. The greater the concentration of Fe^{3+} -TPTZ that the sample reduces to Fe^{2+} -TPTZ, the greater the antioxidant activity of the sample (Yefrida et al., 2015). Based on the description above, researchers are interested in conducting research on the antioxidant activity of red fruit (*Pandanus conoideus* Lam.) ethanol extract using the Ferric Reducing Antioxidant Power (FRAP) method.

2. EXPERIMENTAL SECTION

2.1. Sample collection

The red fruit (*Pandanus conoideus* Lam.) that has been obtained is washed thoroughly first, then the sample is separated from the pith. A total of 400 grams of red fruit seeds were put into a juicer to separate the pulp and juice, then filtered and put into a brown bottle. Store in the refrigerator. spectrophotometry method in quantitative analysis.

2.2. Qualitative analysis

Pipette 1 mL of the sample solution using a micropipette and put it in a test tube, then add 3 mL of FRAP reagent. The mixture was homogenized and incubated at 37 °C for 30 minutes. Observe the change in color of the solution that occurs. If the sample solution turns blue, then the sample has antioxidant activity.

2.3. Standard solution

A 1000 ppm stock solution was prepared by dissolving 25 mg of ascorbic acid dissolved in 1% oxalic acid to the limit of a 25 mL volumetric flask. Next, 4.0 was taken from the stock solution of 1000 ppm; 4.1; 4.2; 4.3 and 4.4 mL and placed in different 10 mL volumetric flasks and diluted with 1% oxalic acid to 10 mL and homogenized. The concentration of a standard solution of 1000 ppm ascorbic acid is 400, 410, 420, 430 and 440 ppm.

2.4. Quantitative analysis

2.4.1. Determination of maximum wavelength

The maximum wavelength was obtained by measuring the absorbance of a standard solution of ascorbic acid at a concentration of 400 $\mu\text{g/mL}$. 4 mL of this solution was taken and then mixed with 0.5 mL of 0.2 M phosphate buffer (pH 6.6) and 0.5 mL of 1% potassium ferricyanide, the mixture was incubated at 50 °C for 20 minutes. After incubation, 0.5 mL of trichloroacetic acid solution was added, then centrifuged at 3000 rpm for 10 minutes. The top layer of the solution was pipetted as much as 1 mL plus 0.5 mL of distilled water and 0.5 mL of 0.1% FeCl_3 . Leave the solution for 10-15 minutes and measure the absorbance at each wavelength in the range of 500-800 nm using a UV-Vis spectrophotometer.

2.4.2. Determination of the standard curve for ascorbic acid

For the preparation of standard solutions, from a stock solution of 1000 $\mu\text{g/mL}$, working standards are made with concentrations of 400, 410, 420, 430, and 440 $\mu\text{g/mL}$. Each working standard concentration was pipetted at 4.0; 4.1; 4.2; 4.3; and 4.4 mL then added 0.5 mL of phosphate buffer pH 6.6 and 0.5 mL of 1% potassium ferricyanide. Then incubated at 50 °C for 20 minutes, then added 0.5 mL of 10% TCA. Next, it was centrifuged at 3000 rpm for 10 minutes, the top layer of 1 mL of the solution was mixed with 0.5 mL distilled water and 0.5 mL of 0.1% FeCl_3 , after which it was incubated at room temperature for 5 minutes. Absorbance was measured at a wavelength of 701 nm on a UV-Vis Spectrophotometer.

2.4.3. Determination of antioxidants

A total of 1 mL of red fruit juice was diluted with oxalate solution in a 10 mL volumetric flask. Pipet 1 mL then add 0.5 mL of 0.2 M phosphate buffer (pH 6.6) and 0.5 mL of 1% $\text{K}_3\text{Fe}(\text{CN})_6$ after that, incubate for 20 minutes at 50 °C. After incubation, 0.5 mL of TCA was added and centrifuged at 3000 rpm for 10 minutes. After centrifuging, pipet 1 mL of the top layer into a test tube, and add 0.5 mL of distilled water and 0.5 mL of 0.1% FeCl_3 . The solution was left for 10 minutes and the absorbance was measured at 701 nm. As a blank, a mixture of oxalate solution was used.

2.6. Data analysis

The absorbance value of the test substance using a spectrophotometer will be compared with the standard solution at each different concentration by linear regression method following equation: $Y = bx + a$, where: Y = Peak area; b = Slope; x = Concentration; a = Intercept. From the linear regression equation, the concentration of the sample is calculated by plotting the absorption results against the regression equation.

3. RESULTS AND DISCUSSION

In this research, red fruit seeds (*Pandanus conoideus* Lam.) were used which were obtained from Central Papua Province, Kab. Mimika, Timika City. The reason red fruit was chosen as a sample was because red fruit is a plant that has strong antioxidant power. Previous research on the extraction of red fruit showed the presence of high antioxidants using the DPPH method (Sangkala et al, 2014) and using the FRAP method (Wabula et al., 2019).

This research aims to determine the antioxidant capacity of red fruit juice samples. In this study, antioxidant activity was measured via the FRAP test. This FRAP test was chosen because the procedure is simple, the method is cheap, fast and the reagents used are quite simple and do not use special equipment to calculate total antioxidants. A preliminary test was carried out, namely a qualitative analysis of red fruit juice, which can be seen in **Table 1** below.

Table 1. Results of qualitative analysis of antioxidant activity using the FRAP method

| Sample | Reactor | Initial color | Discoloration | Note |
|-----------------|--------------|---------------|---------------|------|
| Red fruit juice | FRAP reagent | colorless | Blue | + |

From table 1 above shows the positive results of the sample that red fruit juice has activity as an antioxidant which is indicated by the formation of a blue color. The formation of green to blue colors is caused by complex compounds by the FRAP reagent, where fruit juice samples containing antioxidants can then reduce colorless Fe^{3+} -TPTZ to blue Fe^{2+} -TPTZ. The comparison solution used was ascorbic acid. Ascorbic acid is a commonly used solution because it has a free hydroxyl group which functions as a free radical scavenger and has a polyhydroxyl group which increases antioxidant activity.

In the maximum wavelength (λ max) study, a standard solution of ascorbic acid was prepared. Where 25 mg of ascorbic acid was weighed, then dissolved with 1% oxalic acid and the volume was increased to 25 mL to obtain a standard solution of ascorbic acid with a concentration of 1000 $\mu\text{g}/\text{mL}$ as stock. From a standard solution of 1000 $\mu\text{g}/\text{mL}$, pipette 4 mL and then fill the volume with 1% oxalic acid to 10 mL, then obtain a standard solution of ascorbic acid with a concentration of 400 $\mu\text{g}/\text{mL}$. 1 mL of the 400 $\mu\text{g}/\text{mL}$ standard solution was pipetted, added 0.5 mL of pH 6.6 phosphate buffer and 0.5 mL of potassium ferricyanide, then incubated at 50 °C for 20 minutes with the aim of speeding up the reaction. Add 0.5 mL of 10% TCA solution with the aim that the potassium ferricyanide complex precipitates. Then centrifuge for 10 minutes at 3000 rpm to speed up the settling process. Pipet 1 mL of the supernatant obtained, add 0.5 mL of distilled water and also add 0.5 mL of FeCl_3 to form the complex compound Fe^{3+} and slow down the reduction reaction of Fe^{3+} to Fe^{2+} which occurs very quickly by light. Then running wavelengths from 500-800 nm were carried out. The results of measuring the maximum wavelength of the standard ascorbic acid solution can be seen in **Table 2** below.

Table 2. Formalin contamination in tofu circulated in Maros City – Indonesia.

| Sample | Maximum wavelength (λ) | Absorbance |
|--|----------------------------------|------------|
| Ascorbic acid 400 $\mu\text{g}/\text{mL}$ | 701 nm | 0.491 |

The purpose of determining the maximum wavelength is to determine the absorption area that can be produced in the form of an absorbance value from a standard solution of ascorbic acid. The absorbance results measured on a UV-Vis spectrophotometer were 0.491 with a wavelength of 701 nm. This maximum wavelength provides the greatest sensitivity.

Standard solution preparation is carried out by making a working standard solution from ascorbic acid with 5 concentration variants, 400, 410, 420, 430 and 440 $\mu\text{g}/\text{mL}$. Then pipet 1 mL of each concentration, add 0.5 mL of pH 6.6 phosphate buffer and 0.5 mL of potassium ferricyanide, then incubate at 50 °C for 20 minutes. Add 0.5 mL of 10% TCA, then centrifuge at 3000 rpm for 10 minutes. Pipette 1 mL of the supernatant obtained, add 0.5 mL of distilled water and 0.5 mL of FeCl_3 . Then the absorption was measured sequentially with a wavelength of 701 nm. The results of measuring the absorbance of the ascorbic acid comparison solution can be seen in **Table 3** and **Figure 1** below.

Table 3. Results of measuring the absorbance of the reference solution of ascorbic acid at λ max 701 nm

| Concentration ($\mu\text{g}/\text{mL}$) | Absorbance (701 nm) |
|--|------------------------|
| 400 | 0.304 |
| 410 | 0.411 |
| 420 | 0.542 |
| 430 | 0.617 |
| 440 | 0.713 |

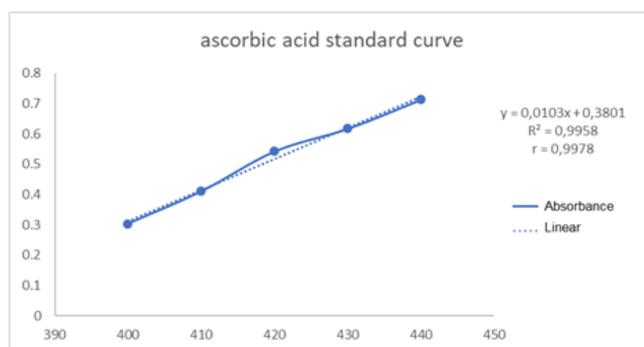


Figure 1. Results of measuring the absorbance of the reference solution of ascorbic acid at λ max 701 nm

After that, the total antioxidant capacity was measured using the FRAP test, by pipetting a 1 mL sample and then diluting it with oxalate solution in a 10 mL volumetric flask. Pipet 1 mL then add 0.5 mL of pH 6.6 phosphate buffer with the aim of facilitating the Fe^{3+} reduction process and adding 0.5 mL of potassium ferricyanide. Then incubated at 50 °C for 20 minutes with the aim of speeding up the reaction. Add 0.5 mL of 10% TCA solution with the aim that the potassium ferricyanide complex precipitates. Then centrifuged at 3000 rpm for 10 minutes to speed up the settling process. Pipette 1 mL of the supernatant obtained, then add 0.5 mL of distilled water and 0.5 mL of FeCl_3 . Next, the absorbance was measured at a maximum wavelength of 701 nm using a UV-Vis spectrophotometer. The same work was carried out for the 2nd and 3rd replications. The results of absorbance measurements from the three replications can be seen in **Table 4** below.

Table 4. Results of absorbance measurements at λ max 701 nm and antioxidant activity values in red fruit juice samples

| Red fruit juice sample | Absorbance (701 nm) | Antioxidant activity (mgAAE/L sample) | Average antioxidant activity (mgAAE/L sample) |
|------------------------|---------------------|---------------------------------------|---|
| Replication 1 | 0.540 | 155.24 | 173.68 mgAAE/L sample |
| Replication 2 | 0.564 | 178.54 | |
| Replication 3 | 0.573 | 187.28 | |

The vitamin C content in each replication was expressed as ascorbic acid equivalent (AAE). AAE is a general reference for measuring the amount of vitamin C contained in a material. The results of measuring the absorbance and antioxidant activity values of red fruit juice are listed in the table above so that the average value obtained from samples of red fruit juice is 173.68 mgAAE/L sample, meaning that in every liter juice equivalent to 173.68 mg of ascorbic acid.

4. CONCLUSION

Based on the results of the research conducted, it can be concluded that: red fruit juice (*Pandanus conoideus* Lam.) has antioxidant activity based on qualitative testing using the FRAP method and the antioxidant activity value of red fruit juice using the FRAP method is an average of 173.68 mgAAE/L sample equivalent to ascorbic acid.

Acknowledgments: The authors are grateful to The Head of Pharmaceutical Chemistry Laboratory, Universitas Muslim Indonesia. The authors are thankful to The Dean of Faculty of Pharmacy Universitas Muslim Indonesia for the space to do this research.

Author contributions: All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Conflict of interest: The authors declared no conflict of interest.

Ethical Approval: Not applicable

REFERENCES

- AgroMedia, Redaksi. *Pro dan Kontra Buah Merah Pendapat Pakar dan Praktisi*. Jakarta: PT AgroMedia Pustaka. 2005.
- Dirjen Kefarmasian dan Alat Kesehatan. 2020. *Farmakope Indonesia Edisi VI*. Jakarta: Departemen Kesehatan Republik Indonesia
- Fawwaz, M., Pratama, P., Musafira., Wahab, I., Iriani, R., Aminah., Kusuma, A. T., Arsul, M. I. Evaluation of Antioxidant Activity of *Vernonia amygdalina* Leaves and Its Flavonoid-Phenolic Content. *Indonesian Journal of Pharmaceutical Science and Technology*, 2023; 10(2): 104-110.
- Fitria, E., Wulandari, N., Hariyadi, P., Wijaya, H. Identifikasi dan Fraksinasi Karotenoid pada Minyak Buah Merah (*Pandanus conoideus*). *Warta IHP/Journal of Agro-based Industry*, 2020; 37(1):7-19.
- Gandjar, I. G., Rohman, A. *Spektroskopi Molekuler untuk Analisis Farmasi*. Yogyakarta: UGM Press. 2018.

- Intergrated Taxonomic Information System. Taxonomic Hierarchy: Pandanus conoideus Lam. <https://www.itis.gov/citation.html>
- Khaira, K. Menangkal Radikal Bebas dengan Anti-Oksidan. *Jurnal Saintek*, 2016; 2(2,): 183-187.
- Leo, R., Daulay, A, S. Penentuan Kadar Vitamin C Pada Minuman Bervitamin Yang Disimpan Pada Berbagai Waktu Dengan Metode Spektrofotometer UV. *Journal of Health and Medical Science*, 2022; 1(2):105-115.
- Maryam, S., Baits, M., Nadia, A. Pengukuran Aktivitas Antioksidan Ekstrak Etanol Daun Kelor (*Moringa oleifera Lam.*) Menggunakan Metode FRAP (*Ferric Reducing Antioxidant Power*). *Jurnal Fitofarmaka Indonesia*, 2016; 2(2): 115-118.
- Ningrum, D, M. *Buku Ajar Kimia Farmasi*. DKI Yogyakarta: Penerbit Samudra Biru. 2023.
- Noer, Z., Ritonga, S. *Alat-Alat Laboratorium Tingkat Universitas Kategori II*. Indonesia: Guepedia. 2021.
- Nurhayati, N., Qonita, F., Ahwan. Aktivitas Antioksidan Fraksi N-Heksan dan Fraksi Kloroform Ekstrak Etanol Daun Jeruk Purut (*Citrus hystrix D.C*) dengan Metode FRAP (*Ferric Reducing Antioxidant Power*). *Jurnal Ilmu Kefarmasian*, 2022; 3(1).
- Ponglabba, D., Sarungallo, Z., Santoso, B. Sifat Fisik dan Organoleptik Bubur Daging Buah Merah (*Pandanus conoideus Lamk*). *Warta Industri Hasil Pertanian*, 2020; 37(1): 58-65.
- Pratama, M., Baits, M., Yaqin, R. Uji Aktivitas Ekstark Etanol Daun Tomat Buah (*Lycopersicon esculentum Mill, var. pyriforme Alef*) dan Daun Tomat Sayur (*Lycopersicon esculentum Mill, var. commune Bailey*) Dengan Metode DPPH (*1,1-Diphenyl-2-Picryl Hydrazil*). *Jurnal Fitofarmaka Indonesia*, 2016; 2(1).
- Pratama, M., Muflihunna, A., Octaviani, N. Analisis Aktivitas Antioksidan Sediaan Propolis Yang Beredar Di Kota Makassar Dengan Metode FRAP (*Ferric Reducing Antioxidant Power*). *As-Syifaa*, 2018; 10(1): 11-18.
- Saa, S., Prabawati, R., Sirojuddin. Pengaruh Penggunaan Minyak Buah Merah (*Pandanus conoideus*) Sebagai Antiseptik Terhadap Proses Penyembuhan Luka Sayatan Kiri Pada Mencit (*Mus Musculus*). *Biolearning Journal*, 2022; 9(2).
- Safitri, A., Anna, R. *Biokimia Bahan Alam: Analisis dan Fungsi*. Malang: MNC Publishing. 2020.
- Sangkala, S, A., Jura, M, R., Tangkas, I Made. Uji Aktivitas Antioksidan Ekstrak Buah Merah (*Pandanus baccari L*) Di Daerah Poso Sulawesi Tengah. *Jurnal Akademika Kimia*, 2014; 3(4): 198-205.
- Sayuti, K., Yernina, R. *Antioksidan, Alami dan Sintetik*. Padang: Andalas University Press. 2015.
- Sembiring, T., Dayana, I., & Rianna, M. *Alat Penguji Material*. Bogor: Guepedia. 2019.
- Setiawan, F., Yunita, O., Kurniawan, A. Uji Aktivitas Antioksidan Ekstrak Etanol Kayu Secang (*Caesalpinia sappan*) Menggunakan Metode DPPH, ABTS, dan FRAP. *Media Pharmaceutica Indonesiana*, 2018; 2(2).
- Syarif, S., Kosman, R., Inayah, N. Uji Antioksidan Terong Belanda (*Solanum betaceum Cav.*) dengan Metode FRAP. *As-Syifaa*, 2015; 7(1): 26-33.
- Tahir, I. Arti Penting Kalibrasi Pada Proses Pengukuran Analitik: Aplikasi Pada Penggunaan pH Meter dan Spektrofotometer Uv-Vis. 2008.
- Tahir, M., H, Anda, C., Widiastuti, H. Uji Aktivitas Aantioksidan Buah Semangka (*Citrullus lanatus*) dengan Metode FRAP. *As-Syifaa*, 2016; 8(1): 31-38.
- Wabula, R., Seniwati., Widiastuti, H. Aktivitas Antioksidan Ekstrak Etanol Buah Merah (*Pandanus conoideus Lam.*) dengan Metode *Ferric Reducing Antioxidant Power* (FRAP). *Jurnal Kesehatan*, 2019; 2(4): 329-337.
- Wawo, A., Lestari, P., Setyowati, N. Buah Merah (*Pandanus conoideus Lamk*) Bioresources Pegunungan Tengan Papua: Keanekaragaman dan Upaya Konservasinya. *Jurnal Biologi Indonesia*, 2019; 15(1): 107-121.
- Winarsi, H. *Antioksidan dan Radikal Bebas*. Yogyakarta: Penerbit Kanisius. 2011.
- Yanlinastuti., Fatimah, S. Pengaruh Konsentrasi Pelarut Untuk Menentukan Kadar Zirkonium Dalam Paduan U-Zr dengan Menggunakan Metode Spektrofotometer UV-Vis. ISSN 1979-2409. 2016.
- Yefrida., Ashikin, N., Refilda. Validasi Metoda FRAP Modifikasi pada Penentuan Kandungan Antioksidan Total Dalam Sampel Mangga dan Rambutan. *Jurnal Riset Kimia*, 2015; 8(2).
- Yuslianti, E. *Pengantar Radikal Bebas dan Antioksidan*. Yogyakarta: Deepublish. 2018.

