

## Immunomodulatory and Antioxidant Activity of Green Grass Jelly Leaf Extract (*Cyclea barbata* Miers.) In Vitro

Rendi Mahadi<sup>1\*</sup>, Mustafid Rasyiid<sup>1</sup>, Krisnanda Surya Dharma<sup>1</sup>, Lindia Anggraini<sup>1</sup>,  
Rahma Nurdiyanti<sup>1</sup>, Tri Rini Nuringtyas<sup>1</sup>

<sup>1</sup>Faculty of Biology, Universitas Gadjah Mada, Jl. Teknik Selatan, Sekip Utara, Yogyakarta, Indonesia, 55281

\*Corresponding author, tel.: +6282389013198, email: [rendi.mahadi.11@gmail.com](mailto:rendi.mahadi.11@gmail.com)

### ARTICLE INFO

#### Article history:

Received 24/02/2018

Received in revised form 22/07/2018

Accepted 02/12/2018

#### Keywords:

*Cyclea barbata* Miers.

green grass jelly

Immunomodulator

antioxidant

DPPH

DOI: 10.22146/jtbb.33441

©2018 JTBB

### ABSTRACT

Green grass jelly (*Cyclea barbata* Miers.) is known for its benefit to human health especially in supporting body's immune system and wellness. This research aimed to determine immunomodulatory and antioxidant activity of green grass jelly leaf extracts in vitro. Old leaves were collected as sample then dried and ground to powder. The extraction was done with soxhletation using three different solvents, chloroform, ethyl acetate, and ethanol. The immunomodulatory activity was evaluated by treating the crude extracts at concentrations of 50, 100, and 500 mg/mL on macrophages of rat in vitro. Macrophage cells separated from peritoneal fluid used RPMI medium. Phagocytosis activity and phagocytosis capacity of macrophages were performed in vitro using latex beads that suspended in phosphate buffered saline (PBS). The antioxidant activity was measured by spectrophotometry technique with 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution. All treatments were done three replicates. Detection of the bioactive groups of the extracts was done by Thin Layer Chromatography (TLC). The results showed that ethyl acetate extract has the highest phagocytosis activity followed by chloroform extract and ethanol extract, respectively. Optimum concentration was reached at 100 mg/mL of ethyl acetate extract. The ethyl acetate extract was also the highest antioxidant activity index 7.7 followed by both extracts of chloroform and ethanol similar index value of 6.25 and 6.3, respectively. The ethyl acetate extract has a high immunomodulatory activity and antioxidant activity which contained phenolics, flavonoids, tannins, and terpenoids.

### 1. Introduction

A decrease in the body's immune system can be caused by increase dense and stressful lifestyle, low environmental quality, and erratic of weather change. These all may exert the susceptibility of the body to disease and at the end can reduce the quality of human life. Improving the body's immune system can be obtained in various ways, one of them is immunomodulators or antioxidant supplements. Antioxidants are compounds that can defeat the negative effects of oxidants in the body to donate one of its electrons to the oxidant compounds that inhibited the activity of the oxidant. Oxidant compounds presenting in the form of free radicals or other reactive compounds can cause intensive damages in the body due to the low antioxidant condition that makes the body unable to compensate for the oxidative reactivity of the compound (Louis *et al.*, in Joel, *et*

*al.* 2007). The decline of antioxidants in the body is directly proportional to the increase diseases. Hence, supplementation with antioxidants may slow the progression of the disease (Nursalam and Kurniati, 2007).

Immunomodulatory or immunostimulant, is a substance capable of stimulating leukocyte cells, especially macrophages to form the body's immune system and acts as a suppressant of the overactive immune reaction and return it back to the normal body condition. Immunostimulant provides independently a response to the modifying nonspecific defense system or when combined with other substances. The nonspecific defense system is very important for the prevention of pathogenic microorganisms in multicellular animals (Paulsen, 2000).

Both immunomodulatory compounds and antioxidant can be derived from raw materials such as synthetic or

natural immunostimulant. One of Indonesia's plants *Cyclea barbata* Miers. or green grass jelly plant has been widely used locally to support the fitness of the body and increase immunity. This indicates the potential of green grass jelly leaves as an immunomodulator and natural antioxidants.

There are several studies related to grass jelly plants. There are 3 types of grass jelly plants are often used by humans both for health and as a beverage. Green grass jelly (*Cyclea barbata* Miers.), black grass jelly (*Mesona palustris* B.) and grass jelly shrubs (*Premna parasitica* Blume.). According to Handayani *et al.* (2017), black grass jelly (*Mesona palustris* B.) has benefits as Anti-Dyslipidemia in high cholesterol Diet-Fed rats and antioxidant activity. Shrubs grass jelly (*Premna parasitica* Blume.) is known to have benefits as anticancer and human diets (Nurdin *et al.*, 2017).

According to some studies, green grass jelly has various benefits such as antioxidants, anti-inflammatory, antimalarial and antibacterial (Shodiq, 2012; Santi *et al.*, 2017). According to (Angelina *et al.*, 2008; Kuswardiyani *et al.*, 2014; Shodiq, 2012) green grass jelly (*Cyclea barbata* Miers) has a variety of bioactive compounds such as chlorophyll, alkaloids, polyphenols, saponins, flavonoids, and others. The known root of green grass jelly contains bisbenzylisoquinoline alkaloids compounds that can act as anti-malarial (Lin *et al.*, 1993). The previous study reported that the leaves contain active chemical compounds including chlorophyll, alkaloids, dimethyl tetrandin, polyphenols, saponins, and flavonoids (Shodiq, 2012).

This study tries to explore the potential of green grass jelly leaves as immunostimulants in daily life that can be developed in the future as a source of immunomodulators and natural antioxidants. As so far, there have been rare studies related to immunomodulatory activity testing in vitro using experimental animal lymphocytes. Then the data obtained is associated with supporting data in the form of antioxidant activity and some biochemical compounds contained in green grass jelly leaves.

In this regards, it is necessary to do research on immunomodulatory and antioxidant activity in leaf extracts of green grass jelly, as an effort to increase the body's immune system as well as natural antioxidants. The leaves are green grass jelly can be processed into nutritious drinks, natural supplements, or herbal medicines which can play a role in increasing endurance and body health.

This research aims to determine the immunomodulatory and antioxidant activity of leaf extracts of green grass jelly (*Cyclea barbata* Miers.) in vitro. In this study investigated phagocytosis activity, phagocytosis

capacity, and antioxidant activity index analysis, as well as the content of bioactive compounds in green grass jelly leaf extract.

## 2. Materials and Methods

### 2.1. Sample preparation

Samples of green grass jelly plants taken in Banguntapan, Bantul, Yogyakarta, plants aged about 2 - 3 years, taken a sample of leaves that are old. Leaves green grass jelly (*Cyclea barbata* Miers.) were collected and washed with water, sorted, and air dried. To complete the drying, the leaves were put in the oven at temperature of 40-50 °C until reached constant weight and powdered using blender.

### 2.2. Extraction

Extraction was done by a serial soxhletation method using three different solvents including chloroform, ethyl acetate, and ethanol. An amount of 10 g dried powder was wrapped filter paper, then placed into extractor tube that has been assembled into a soxhlet device. The extraction considers complete when the solvent turned to clear. The serial soxhletation did from the most non-polar solvent, chloroform followed by ethyl acetate and ethanol, respectively. The crude extracts were dried using rotary evaporator at 40 °C.

### 2.3. Macrophage cell suspension preparation

Rat was euthanized using ketamine and further received a neck dislocation, in this research required one rat. Rat placed in the supine position and the abdomen subsequently cleaned using alcohol 70 %. Surgery was performed by opening the skin on the abdomen. 15 ml of cold RPMI (Roswell Park Memorial Institute) 1640 medium was injected to the intraperitoneal using syringe and left for 3 min with occasional shaking using fingers and helped the peritoneal macrophage cells separated from the tissue. Peritoneal fluid was aspirated using the syringe followed by centrifugation at 1200 rpm at 4 °C for 10 min. The supernatant was discarded and replaced with 1 ml complete RPMI medium (containing 10 % fetal bovine serum (FBS)) in pellets. A mixture containing 50 µL giemsa and 10 µL cell suspension were dropped into hemocytometer and subjected for cell density determination. The cell suspension was diluted to reach a density of  $2,5 \times 10^6$  /mL using complete RPMI medium. Subsequently, the cell suspension was cultured in 24-well microplate which previously mounted with coverslips. Each well contained 200 µL (equal to  $5 \times 10^5$  cells) of the cell suspension. The plates were incubated at 37 °

C for 30 min, with 5 % CO<sub>2</sub>. Medium was replaced after overnight incubation (Wijayanti *et al.*,1999).

#### 2.4. Preparation of test extracts

Ten mg of each extract was weighed of each extract was dissolved in 1000 µL of dimethyl sulfoxide (DMSO) and vortex vigorously. This extract was diluted in the Laminar Air Flow (LAF) in order to reach a final concentration of 50 µg, 100 µg, and 500 µg. The complete medium was used to replace extract as the negative controls while Imboost® was used as an immunomodulator standardized supplement.

#### 2.5. Immunomodulators test

Phagocytosis activity and phagocytosis capacity were performed in vitro using latex beads that suspended in Phosphate Buffered Saline (PBS). Each well containing overnight cultured macrophages was added with 200 µL of latex suspension and incubated again at 37 °C for 60 min with 5 % CO<sub>2</sub>. After finished the incubation, the cells were washed with PBS for three times to remove the left latex, followed by drying and fixation with 100 µL abs methanol for 30 s, for 10 min. At the end of incubation, 50 µL of giemsa was added. Then, prior to coverslip removal, the cells were washed with distilled water three times. The coverslip then was observed under light microscope with 400 x magnification.

#### 2.6. Determination of phagocytic activity and phagocytic capacity

Both of phagocytosis capacity and phagocytosis activity was calculated using these following equations (Jensch-Junior *et al.*, 2006):

**Phagocytosis activity** = (amount of active macrophages / total of 100 macrophages counted) x 100%

**Phagocytosis capacity** = amount of latex macrophages / amount of 50 active macrophages

#### 2.7. DPPH assay

The 0.1 mM DPPH solution was prepared in methanol. The standard curve was prepared using Vitamin C in a serial concentration of 0.2, 0.4, 0.8, 1.6, and 3.2 mg/ml. The stock solutions of each crude extracts were prepared by dissolving 50 mg of dried crude extract in 50 ml methanol. These stock were used for a serial concentration from 2.5-160 ppm. The measurement was done by mixing 2 ml of DPPH solution with 1 ml sample extract in the cuvettes and incubated for 30 min in the dark. The absorbance of the solution was recorded at 517 nm using spectrophotometer. The antioxidant activity

index was calculated using this equation (Ghosal and Mandal, 2012):

**Antioxidant Activity Index (AAI)** = DPPH concentration (ppm) / IC50 value (ppm)

#### 2.8. Secondary metabolites detection.

Identification of the group of bioactive compounds was done using Thin Layer Chromatography (TLC) for the separation of the compounds from crude extract and followed by spraying detection reagents (Table 1). Identification of the compounds used different mobile phase. For the chloroform and the ethyl acetate extracts used a mixture of solvents n-hexane : ethyl acetate (3 : 7). While the ethanol extract with chloroform eluent : ethyl acetate : toluene (2 : 6 : 2).

**Table 1.** Detection reagents for identification of bioactive compounds (Harborne, 1987).

No	Detection reagent	Compound groups	Positive color
1	Anisaldehyde sulfuric acid	Terpenoids	Purplish red
2	Citroborat	Flavonoids	Yellow
3	FeCl <sub>3</sub>	Phenolics	Dark green-black
4	FeCl <sub>3</sub>	Tannins	Green-black
5	Dragendorf	Alkaloids	Orange

### 3. Results and Discussion

The crude extracts obtained from the three different solvents had comparable weight as much as 12.9 mg for ethanol extract, ethyl acetate extract 13.84 mg, and 12.18 mg extract of chloroform. The observed ethyl acetate extract relative has a larger portion than others indicating that the leaves of green grass jelly contain many compounds portion are semipolar.

The results of bioactive compounds detection indicated that the chloroform and the ethyl acetate extracts contained a potential immunomodulator and antioxidant compounds such as terpenoids, flavonoids, tannins, and phenolics. While the ethanol extract contains two bioactive compounds are terpenoids and flavonoids (Table 2). This proved by the presence of spots that appear on the TLC plate. In this study, no alkaloid compound was detected. Because alkaloids are very heterogeneous chemically, they cannot be identified from plant extracts using a single chromatographic criterion. In general, it is difficult to identify alkaloids from new plant sources without knowing what type alkaloids may be found there. Alkaloid has a wide range of solubility and other properties of alkaloids, general screening procedures for alkaloids in plants may fail to detect certain compounds. In addition, another factor that influences the identification

**Table 2.** Bioactive compound of chloroform, ethyl acetate and ethanol extracts of Green grass jelly (*Cyclea barbata* Miers.) leaves

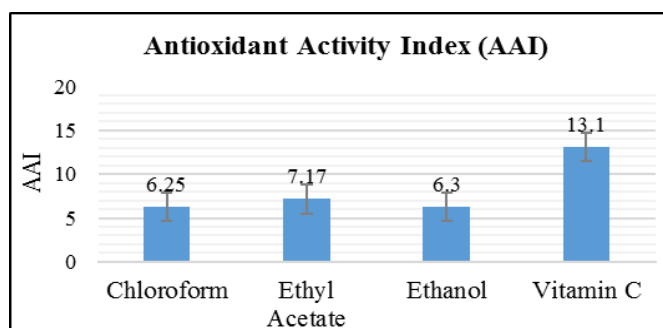
Extract	Bioactive Compound									
	Terpenoids		Flavonoids		Tannins		Phenolics		Alkaloids	
	n spot	Rf	n spot	Rf	n spot	Rf	n spot	Rf	n spot	Rf
Chloroform	1	0,925	1	0,425	1	0,762	1	0,850	-	-
Ethyl acetate	1	0,925	1	0,412	1	0,700	1	0,775	-	-
Ethanol	1	0,925	1	0,750	-	-	-	-	-	-

of alkaloids is finding the appropriate eluent system and mobile phase composition, so that the compound separation process in TLC works well (Harborne, 1987).

Compounds such as terpenoids, flavonoids, tannins, and phenolics are secondary metabolites that are widely present in plants. The plant secondary metabolites with known pharmacological significance are more appropriately called bioactive compounds (Swabha, 2018). Phenolics play several important functions in plants as immunomodulator and antioxidant. They provide resistance against pathogens and predators, besides that phenolics protect cellular membranes and tissues from lipid peroxidation, and repair DNA by electron transfer reactions (Shankar *et al.*, 2007). Flavonoids also offer disease resistance, these are induced in response to UV-B radiation and provide organ protection by absorbing in the wavelength range of 280-350 nm. They also function as reactive oxygen species (ROS) scavengers, regulate auxin transport and cell growth processes (Santos and Moreno, 2013). Tannins also serve as defenses against microorganisms and herbivores (Cai *et al.*, 2004). Terpenoids act as feeding deterrents, pollination attractants, defense compounds photoprotectants, free-radical scavengers, and signaling molecules. They are exploited for their aromatic qualities and pharmacological properties such as antibacterial, anti-inflammatory (Perez-Sanchez *et al.*, 2012 ; Zhang *et al.*, 2011).

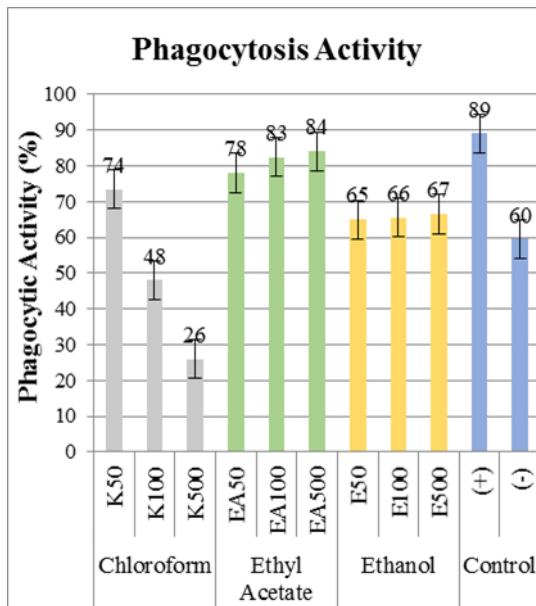
activity if the AAI 0.5-1, strong if the value AAI 1-2, and if the value of AAI > 2 that includes a very strong antioxidant (Vasic *et al.*, 2012). In Figure 1. was known that the AAI each extract greater than 2 so that it can be classified as a very strong antioxidant. The highest antioxidant activity of leaf green grass jelly was the ethyl acetate extract with value of 7.17, followed AAI ethanol extract 6.3, and chloroform extract 6.25, while the vitamin C were used as positive control (+), has a value of 13.1 AAI. According to another study conducted by Vasic *et al.* (2012), the AAI value of *Passiflora alata* ethanol extract was 0.10, while the acetone extract 0.07, and ethyl acetate extract 0.07. So the value of antioxidant activity index *Passiflora alata* is still categorized as low activity (< 0.5). In addition to other plant studies, the AAI value of *Acacia auriculiformis* leaves of ethanol extract is 0.09 and also considered low category (Sari and Putra, 2018). Meanwhile, according to Ulfah (2016), in her research using rambutan leaves (*Nephelium lappacheum* Linn.) has AAI value classified as very strong category (2.14). When compared with green grass (*Cyclea barbata* Miers.) the AAI value is larger than *Passiflora alata*. This means that (*Cyclea barbata* Miers.) has a high antioxidant activity compared to other plants.

The results showed that administration of three green grass jelly leaf extract in cell culture can increase the phagocytosis activity and phagocytosis capacity of macrophages. From Figure 2 and 3. known that the value ratio of the phagocytosis activity and phagocytosis capacity of the three extract approach positive control (+) treated Imboost®. The average concentration of the ethyl acetate extracts phagocytic capacity indicate even higher than the control value (+). Treatment with ethyl acetate extracts of green grass jelly leaf causes an increase in phagocytosis activity and phagocytosis capacity of macrophage cells highest compared to other extracts. Ethyl acetate extracts of green grass jelly leaves at a concentration of 100 µg/ml was optimal concentration in enhancing phagocytosis activity and the phagocytosis capacity of macrophage cells. Unknown ethyl acetate extract at concentration of 100 µg/ml had a value of 83 % phagocytosis activity and phagocytosis capacity 234 %. In general, the value of the phagocytosis activity and phagocytosis capacity highest in the ethyl acetate extract

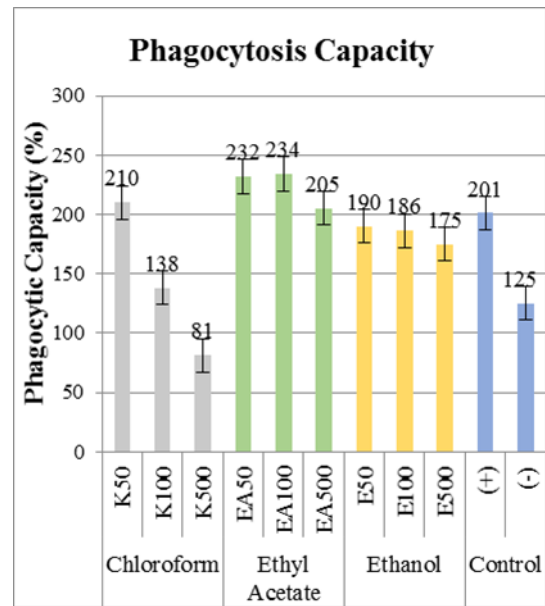


**Figure 1.** Antioxidant activity index values of extract and vitamin C (control).

Green grass jelly extract was known has very strong antioxidant activity, based on the classified value of Antioxidant Activity Index (AAI). The classified natural extract has low antioxidant activity if the value AAI < 0.5, medium



**Figure 2.** Phagocytosis activity three green grass jelly leaf extract, positive control imboost®.



**Figure 3.** Phagocytosis capacity three green grass jelly leaf extract, positive control imboost®.

followed ethanol extract, and then chloroform extract.

This study focuses on how an extract from green grass jelly plants can modulate immune cells, especially macrophage cells. Green grass jelly extract contains biochemical compounds of terpenoids, flavonoids, tannins, and phenolics synergistic effects in the role of immunomodulators and antioxidants. According to Inalci *et al.* (2005), some bioactive components derived from plants have a pleiotropic effect (having various physiological effects) and combinations of various bioactive components on some extract will have a synergistic effect.

These bioactive compounds can increase lymphocyte activity such as macrophage cells, especially compounds such as phenolics and derivatives. These bioactive compounds can lead to increased macrophage phagocyte activity (Zakaria and Rajab, 1999), induces interferon production, promotes lymphocyte cell proliferation such as macrophages and suppresses dead lymphocyte cells. The immunomodulatory activity of a compound can be determined by proliferation of lymphocytes or macrophage cells. Proliferation is the process of cell multiplication through cell division or mitosis in response to antigen or mitogen. Macrophage cell proliferation is a marker of the activation phase of the body's immune response (Zakaria *et al.*, 1996).

This research has been able to prove that green grass jelly leaf extract was able to increase the activity of the immunomodulatory and have high antioxidant activity. Ethyl acetate extract of green grass jelly leaf has a potential and could be a new alternative to improve the body's immune system by increasing the activity of immunomodulators and antioxidants in the body. The index value of antioxidant

activity, phagocytosis activity, and phagocytosis capacity ethyl acetate extract higher compared to ethanol extract and chloroform extract. Because the solvent ethyl acetate was semipolar that can bind bioactive compounds are polar, semipolar, or nonpolar thus having high content of bioactive compounds and can function as immunomodulators and antioxidants. Green grass jelly leaf extract has great potential to be developed into a standardized herbal supplements or drugs.

#### 4. Conclusions

The ethyl acetate extract of green grass jelly leaf extract (*Cyclea barbata* Miers.) has a high immunomodulatory activity and antioxidant activity. The ethyl acetate extract of green grass jelly leaves contained terpenoids, flavonoids, tannins, and phenolic. The ethyl acetate extract has antioxidant activity index value (AAI), phagocytosis activity, and the highest phagocytosis activity compared to other extracts with optimal concentration was 100 µg/ml. Further study should be prepared using in vivo approach to validate this report.

#### References

- Angelina, M., Hartati, S., Dewijanti, L. D., Banjarnahor, S. D. S., and Meilawati, L., 2008, Penetuan LD50 Daun Cinco (*Cyclea Barbata* Miers.) pada Mencit. *Makara Sains*. 12(1): 23-26.
- Cai, Y. Z., Luo Q., Sun, M., and Corke, H., 2004, Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci*, 74: 2157-2184.

- Ghosal, M. and Mandal, P., 2012, Phytochemical screening and antioxidant activities of two selected “Bihi” fruits used as vegetables in Darjeeling Himalaya. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(2).
- Handayani D., Tri Dewanti W., Novita W., Mey E., and Hanifa H., 2017, Black Grass Jelly (*Mesona palustris* Bl) Effervescent Powder has Anti-Dyslipidemia in High Cholesterol Diet-Fed Rats and Antioxidant Activity, *Research Journal of Life Science*, 4(3): 159-167.
- Harborne, J. B., 1987. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, Chapman & Hall, London. pp 4-192.
- Inalci, M., Steward, W. P., and Gescher, A. J., 2005, Use of Cancer Chemopreventive Phytochemicals as Antineoplastic Agents, *Lanset Oncol*, 6: 899-904.
- Jensch-Junior, B. E., Pressinotil, N., Borges, J. C. S., & Cunha Da Silva, J. R. M., 2006, Characterization of Macrophage Phagocytosis of the Tropical Fish *Prochilodus scrofa* (Steindachner, 1881), *Aquaculture*, 251: 509-515.
- Kusmardiyani, S., Insanu, M., and Al Asyhar, M., 2014, Effect A Glycosidic Flavonol Isolated from Green Grass Jelly (*Cyclea barbata* Miers.) Leaves, *Procedia Chemistry*, 13: 194-197.
- Lin, L.Z., Shieh, H. L., Angerhofer, C. K., Pezzuto, J. M., Cordell, G. A., Xue, L., Johnson, M. E., and Ruangrunsi, N., 1993, Cytotoxic and antimalarial bisbenzylisoquinoline alkaloids from *Cyclea barbata*., *Journal of Natural Products*, 56(1): 22-29.
- Louis, S. G. and Alfred, G., Autakoid: Terapi Obat untuk Inflamasi. In: Joel G. Hardman, Lee E. Limbird, 2007, *Dasar Farmakologi Terapi*, Edisi 10, Alih bahasa oleh Amalia Hanif. Penerbit Buku Kedokteran EGC., Jakarta, p. 896.
- Nurdin, S. U., Le Leu, R. K., Young, G. P., Stangoulis, J. C. R., Christophersen, C. T., and Abbott, C. A., 2017, Analysis of the Anti-Cancer Effects of Cincau Extract (*Premna oblongifolia* Merr.) and Other Types of Non-Digestible Fibre Using Faecal Fermentation Supernatants and Caco-2 Cells as a Model of the Human Colon, *MDPI Journals: Nutrients*, 9(335).
- Nursalam and Kurniati, N. D., 2007, *Asuhan Keperawatan pada Pasien Terinfeksi HIV/AIDS*, Penerbit Salemba Medika, Jakarta, p. 136.
- Paulsen, B. S., 2000, *Bioactive Carbohydrate Polymers*, Kluwer Academic Publishers, Netherland, pp. 95-96.
- Perez-Sanchez, C., Ruiz-Limon, P., Aguirre, M. A., Bertolaccini, M. L., Khamashta M. A., and Rodriguez-Ariza, A., 2012, Mitochondrial dysfunction in antiphospholipid syndrome: implications in the pathogenesis of the disease and effects of coenzyme Q(10) treatment, *Blood*, 119: 5859–5870.
- Santi, I., Putra, P., and Wahyuni, S, 2017, Uji Efek Ekstrak Etanol Daun Cincau Hijau (*Cyclea barbata* Miers.) sebagai Antiinflamasi pada Tikus Putih yang Diinduksi Karagen, *As-Syifaa*, 9(1): 58-66.
- Santos, A. P. and Moreno, P. R. H., 2013, Alkaloids derived from histidine: Imidazole (pilocarpine, pilosine). In: Ramawat KG, Mérillon JM (eds.), *Natural Products*, Springer-Verlag, Berlin, Heidelberg. pp 861-882.
- Sari, N. K. Y. and Putra, M. W. A., 2018, Uji Aktivitas Antioksidan Ekstrak Daun Akasia (*Acacia auriculiformis*), *Jurnal Media Sains* 2, (1): 21 – 25.
- Shankar, S., Singh, G., and Srivastava R. K., 2007, Chemoprevention by Resveratrol: Molecular Mechanisms and Therapeutic Potential, *Front Biosci*, 12: 4839-4854.
- Shodiq, A. M., 2012, *Uji Aktivitas Antioksidan Ekstrak dan Fraksi Daun Cincau Hijau Rambat (Cyclea Barbata Miers.) dan Identifikasi Golongan Senyawa dari Fraksi Yang Paling Aktif*, Thesis, Fakultas Matematika dan Ilmu Pengetahuan Alam, Program Sarjana Farmasi, Universitas Indonesia, Depok.
- Swabha, T., 2018, Bioactive Compounds in Medicinal Plants: A Condensed Review, *SEJ Pharmacognosy*, (1)1.
- Ulfah, S., 2016, *Uji Aktivitas Antioksidan Ekstrak Daun Rambutan (Nephelium lappacheum Linn.) dengan Metode DPPH (diphenyl-2-picrylhydrazyl)*, Thesis, Fakultas Kedokteran dan Ilmu Kesehatan, Program Studi Farmasi, UIN Syarif Hidayatullah, Jakarta.
- Vasic, S. M., Stefanovic, O. D., Licina, B. Z., Radojevic, I. D., & Comic, L. R., 2012, Biological activities of extracts from cultivated Granadilla *Passiflora alata*, *EXCLI Journal*, 11: 208–218.
- Wijayanti, M. A., Supriyono, and Fitri L. K., 1999, Sekresi Tumor Necrosis Factor dan Reactive Oxygen Intermediate oleh Makrofag Peritoneum Mencit yang distimulasi dengan Antigen Terlarut *Plasmodium falciparum*, *Berkala Ilmiah Kedokteran*. 31: 23-27.

- Zakaria, F. R., Darsana. L., and Wijaya H., 1996, Immunity enhancement and cell protection activity of ginger buds and fresh ginger flesh on mouse spleen lymphocytes. *In Non-nutritive Health Factors for Future Foods. Proceedings IU FOST 1996 Regional Symposium Seoul Education and Culture Center, Seoul, Korea.*
- Zakaria, F. R., and Rajab, T. M., 1999, Pengaruh ekstrak jahe (*Zingiber officinale* Roscoe.) terhadap produksi radikal bebas makrofag men-cit sebagai indikator imunostimulan secara invitro, *Prosiding Seminar Nasional Teknologi Pangan*, pp 707-716.
- Zhang, Q., Zheng, Z., and Yu, D., 2011 Steroidal alkaloids from the bulbs of *Fritillaria unibracteata*, *J Asian Natural Pro Res*, 13: 1098-1103.