Indonesian Journal of Pharmaceutical Science and Technology
Journal Homepage : http://jurnal.unpad.ac.id/ijpst/
Research Article

Antioxidant Activity of Extract Combination From *Averrhoa bilimbi* L. Leaves and Stingless Bee Honey

Bunga P. Sari*[1], Paula M. Kustiawan[1]
[1]Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Samarinda, East Kalimantan, Indonesia

Submitted 22 March 2023; Revised 05 May 2023; Accepted 30 May 2023; Published 31 July 2023
*Corresponding author: bungaputrisari7@gmail.com

Abstract
Averrhoa bilimbi L. leaves and stingless bee honey contains phenolic compounds that can act as antioxidants. This study aims to find out the antioxidant activity of extract combination from *Averrhoa bilimbi L.* leaves and stingless bee honey (*Trigona spp.*). This study uses the maceration method to obtain extracts and uses the DPPH (2,2-diphenyl-1-picrilhydrazyl) method to see the antioxidant activity of the extract combination from *Averrhoa bilimbi L.* leaves and stingless bee honey (*Trigona spp.*). Then the extract combination of kelulut honey bee (*Trigona spp.*) and *Averrhoa bilimbi L.* leaves was measured for its antioxidant activity using UV-VIS spectrophotometry. The results showed the greatest IC_{50} of combination extract was 2:1 ratio *Averrhoa bilimbi L.* leaves extract and stingless bee honey was 90.36 ppm included classified as strong antioxidants activity. This activity was influenced by the synergistic effect between the combination of secondary metabolites contained.

Keywords: antioxidants, stingless bee, honey, *Averrhoa bilimbi L.*

Aktivitas Antioksidan Kombinasi dari Ekstrak Daun *Averrhoa bilimbi* L. dan Madu Lebah Kelulut

Abstrak
Daun belimbing wuluh (*Averrhoa bilimbi L.*) dan madu lebah kelulut mengandung senyawa fenolik yang dapat berperan sebagai antioksidan. Penelitian ini bertujuan untuk mengetahui aktivitas antioksidan dari kombinasi ekstrak madu lebah kelulut (*Trigona spp.*) dan daun belimbing wuluh (*Averrhoa bilimbi L.*). Penelitian ini menggunakan metode maserasi untuk mendapatkan hasil ekstrak dan menggunakan metode DPPH (*2,2-diphenyl-1-picrilhidrazyl*) untuk melihat aktivitas antioksidan dari kombinasi ekstrak madu lebah kelulut (*Trigona spp.*) dan daun belimbing wuluh (*Averrhoa bilimbi L.*). Kemudian kombinasi ekstrak madu lebah kelulut (*Trigona spp.*) dan daun belimbing wuluh (*Averrhoa bilimbi L.*) diukur aktivitas antioksidannya dengan menggunakan spektrofotometri *UV-VIS*. Hasil menunjukan IC_{50} terbesar dari kombinasi rasio 2:1 ektrak daun *Averrhoa bilimbi L.* dan madu lebah kelulut adalah 90,36 ppm termasuk tergolong dalam aktivitas antioksidan yang kuat. Aktivitas ini dipengaruhi oleh efek sinergis antara kombinasi metabolit sekunder yang terkandung.

Kata Kunci: antioksidan, lebah kelulut, madu, *Averrhoa bilimbi L.*

1. Introduction

Indonesia is a tropical country characterized by high temperatures and ultraviolet radiation[1]. Excessive exposure to UV rays can lead to the formation of free radicals in the body that can cause a number of skin problems, including skin redness, pigmentation, and long-term cancer risk[2]. Human health will be affected by skin damage so maintaining and protecting the skin is necessary for health. Therefore, it is necessary to defend against the dangers of free radicals and premature aging that can harm the skin[3]. Premature aging can be prevented through 2 ways, namely internally and externally. Internal prevention comes from antioxidants produced by the body itself. Examples of such internal antioxidants are superoxide dismutase (SOD), glutathione peroxidase (GSH Px), catalase, and nonenzymes which are small protein compounds of glutathione[3]. While externally, consumption of fruits and vegetables that are high in antioxidant. And one of them is by using cosmetics that have active substances as antioxidants[1]. Antioxidants are very beneficial for health because they can prevent premature aging and degenerative disorders[4]. Antioxidants can fight free radicals found in the body produced by the body's metabolism, air pollution, contaminated food, and sunlight[5].

Indonesia is rich in natural resources that can be utilized as a source of natural antioxidants, one of which is *Averrhoa bilimbi L*. and stingless bee honey. Stingless bee honey and *Averrhoa bilimbi L*. leaves have been widely used as traditional medicine, not only traditional medicine, the efficacy of *Averrhoa bilimbi L*. leaves and stingless bee honey has been scientifically tested[6]. These plants contain secondary metabolite compounds such as flavonoids and phenolics and *Averrhoa bilimbi L*. leaves have activities as antibacterial, antioxidant, antimicrobial and anti-inflammatory (reduce and suppress inflammation)[7,8,9]. Stingless bees are characterized by having no sting and being small in size[10]. Meanwhile, stingless bee honey has pharmacological effects as an antidiabetic, antioxidant, and antibacterial[11,12]. Flavonoid components have been widely reported for their role as antioxidants. The aim of this study was to determine whether the combination of two types of antioxidants might result in a higher potential total antioxidant activity, known as the synergism effect.

2. Method

2.1. Materials

The materials used for this study include extract combination from stingless bee honey (*Trigona spp*.) taken from Samarinda (Arista Avimaro Bee Farm) and *Averrhoa bilimbi L*. leaves taken from Anggur Street, Samarinda with the characteristics of leaves that are not too old and not too young (the texture is not too slippery and not too thick, but also not hard, and there are no yellow spots that indicate the leaves have begun to age), DPPH (Tokyo Chemical Industry, Japan), etanol 95% (Medika, Sukoharjo), methanol (Emsure Merck, Germany), and aquadest (OneLab, Bandung).

2.2. Instruments

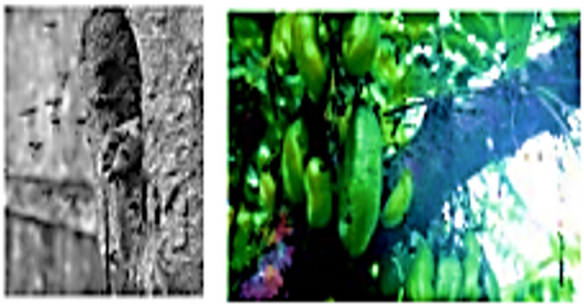
The tools used extraction include a set of Rotary evaporator (Buchi Interface I-100 from China), spektrofotometer UV-Vis (Genesys 10s UV-Vis from Germany), Volumetric flask (Iwaki), vortex (Scilogex MX-S from North America), Measuring cup (Iwaki), Spatula, Micropipettes (Scilogex from North America), and Pasteur pipette.

2.3. Procedure

2.3.1. Preparation Extract from *Averrhoa bilimbi L.* Leaves

The obtained *Averrhoa bilimbi L.* leaves were dried in an oven and mashed, then 100 grams of *Averrhoa bilimbi L.* leaves were macerated with 1000 ml of 95% ethanol solvent. The resulting macerate was concentrated using a rotary evaporator at 50°C with a speed of 200 rpm[13]. The resulting extract was then made into a comparator as shown in table 1. Each comparator was tested for antioxidant activity.

2.3.2. Preparation of 0.1 mM DPPH Solution



(a) (b)

Figure 1. (a) Stinglees bee honey (b) *Averrhoa bilimbi L.*

DPPH powder weighed as much as 1.98 mg was dissolved using methanol p.a and put into a 50 mL volumetric flask until the volume was sufficient with methanol p.a to the limit mark, then the volumetric flask was given aluminum foil and placed in a dark room[14].

2.3.3. Antioxidant Test Combination of Stinglees Bee Honey and Extract from *Averrhoa bilimbi L.*

Extract from *Averrhoa bilimbi L.* leaves and stingless bee honey and their combination were weighed as much as 100 mg, then dissolved with methanol in a 10 mL volumetric flask. Then dilution with methanol was carried out so that several concentrations of test solutions were obtained at 10; 30; 50; 70; 90 ppm. The results were homogenized using a vortex and added 0.1 mM DPPH as much as 3 mL and incubated for 30 minutes at room temperature under dark conditions, then measured the absorbance at a wavelength of 517 nm. The IC_{50} value was calculated using a linear regression equation with the relationship between concentration and % inhibition[15].

2.3.4. Preparation of Vitamin C Solution

Vitamin C weighed as much as 10 mg was put into a 50 mL volumetric flask and dissolved with methanol to obtain a concentration of 200 ppm. This solution was further diluted with methanol until the concentration of vitamin C solution was obtained, namely 2; 3; 4; 5; 6 ppm. The results of vitamin C dilution were added with 3 mL of 0.1 mM DPPH and incubated for 30 minutes at room temperature under dark conditions, then measured the absorbance at a wavelength of 517 nm. The IC_{50} value was calculated using a linear regression equation with the relationship between concentration and % inhibition[16].

Figure 2. Preparation of Antioxidant Test Combination of Stinglees Bee Honey and Extract from *Averrhoa bilimbi L.*

3. Result

The result of thick extract *Averrhoa bilimbi L.* leaves in the study was 13 grams. Maceration results are said to be good if the yield is> 10%13, in the results of the study, the yield of the extract was 13% so that these results met the standard requirements. Then extract combination from *Averrhoa bilimbi L.* leaves and stingless bee honey was made in a ratio of 1:0, 2:1, 1:1, 1:2, and 0:1. Then tested for antiokxidant activity of extract combination from *Averrhoa bilimbi L.* leaves and stingless bee honey which has the highest antioxidant activity in the 2:1 ratio with an IC_{50} value of 90.36 ppm. The measurement results of antioxidant activity of the combination of 1:1, the combination 1:2, and stingless bee honey are (IC_{50} 106.18 ppm), (IC_{50} 102.10 ppm), (IC_{50} 132,76 ppm) respectively. That the highest activity is possessed by *Averrhoa bilimbi L.* leaves extract 0:1 with an IC_{50} value of 18,05 ppm.

4. Discussion

4.1. Extract Yield Results

This study uses the maceration method which uses 95% ethanol solvent. Ethanol 95% is preferred because it can extract more antioxidant compounds than water in producing antioxidant compounds[17,18]. The result of thick extract in the study was 13 grams. Maceration results are said to be good

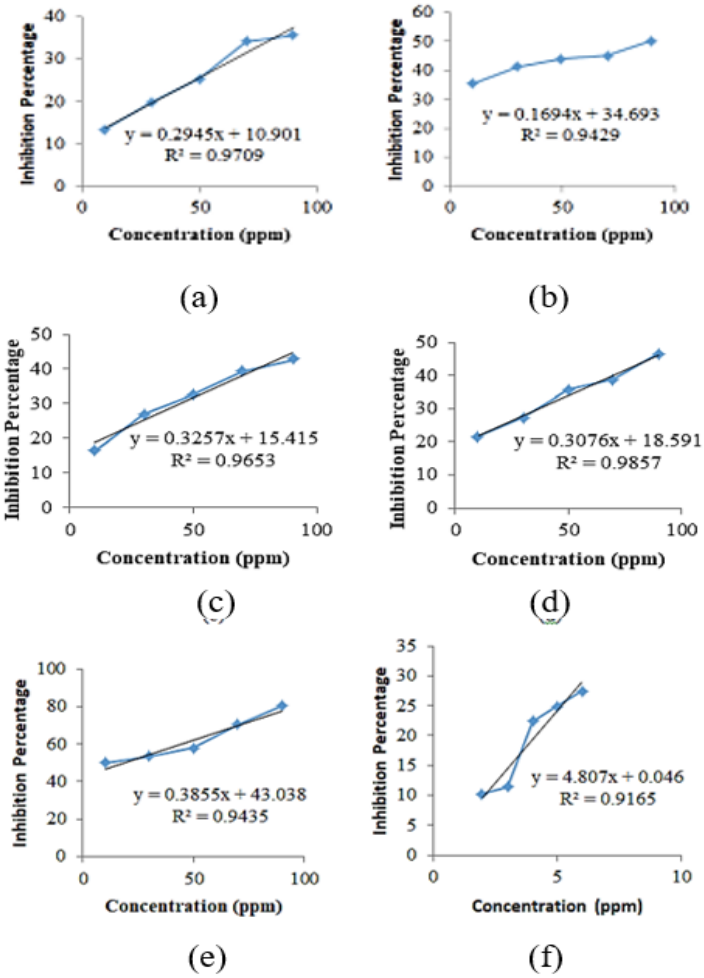


Figure 3. (a) Antioxidant Activity Ratio 1:0 (b) Antioxidant Activity Ratio 2:1 (c) Antioxidant Activity Ratio 1:1 (d) Antioxidant Activity Ratio 1:2 (e) Antioxidant Activity Ratio 0:1 (f) Antioxidant Activity Vitamin C

if the yield is> 10%13, in the results of the study, the yield of the extract was 13% so that these results met the standard requirements.

4.2. Antioxidant Activity Measurement

The antioxidant activity of stingless bee honey is produced by high phenolic and flavonoid compounds in honey[19,20,21,22], while *Averrhoa bilimbi L.* leaves are produced by high flavonoid, phenol, alkaloid, tannin, and coumarin compounds in the leaves, so a combination from *Averrhoa bilimbi L.*[23]. leaves and stingless bee honey is carried out in order to produce higher total antioxidant activity, known as the synergism effect.

The results in Table 3 show that the single extract, the extract combination from *Averrhoa bilimbi L.* leaves and stingless bee honey 2:1; 1:1; 1:2 ratio, and the comparator have different antioxidant activities from one another. The difference in IC_{50} values in each extract or combination of extracts is due to the distribution of the type and number of secondary metabolite compounds that are antioxidants based on the solvent polarity used[24].

Based on figure 3 and table 2, it shows that the highest activity is possessed by *Averrhoa bilimbi L.* leaves extract with the a linear regression equation y= 0.3855x + 43.038 and R^2 = 0.9435 which shows the IC_{50} value of 18,05 ppm, while of the three extract combination from *Averrhoa bilimbi L.* leaves and stingless bee honey which has the highest antioxidant activity in the 2:1 ratio with the a linear regression equation y = 0,1694x + 34,693 and R^2 = 0,9429 which shows the IC_{50} value of 90,36 ppm. The measurement results of antioxidant activity of the combination of 1:1 with the a linear regression equation y = 0,3257x + 15,415 and R^2 = 0,9653 which shows the IC_{50} value of 106,18 ppm, the

Table 1. Comparison of antioxidant test of the combination of stingless bee honey and *Averrhoa bilimbi L.* leaves extract[15]

Ratio	Quantity of antioxidant test material	
	Stingless bee honey (mg)	*Averrhoa bilimbi L.* leaves extract (mg)
1 : 0	100	0
2 : 1	50	25
1 : 1	50	50
1 : 2	25	50
0 : 1	0	100

combination 1:2 with the a linear regression equation y = 0,3076x + 18,591 and R^2 = 0,9857 which shows the IC_{50} value of 102,10 ppm, and 1:0 with persamaan regresi linear madu lebah kelulut yaitu y = 0,2945x + 10,901 dan R2 = 0,9709 which shows the IC_{50} value of 132,76 ppm. The IC_{50} value of the three extract combination from *Averrhoa bilimbi L.* leaves and stingless bee honey has moderate antioxidant activity.

In extract combination from *Averrhoa bilimbi L.* leaves and stingless bee honey gives a decreasing effect on its antioxidant activity in reducing DPPH free radicals compared to single extracts, this occurs allegedly due to interactions between chemical compounds

Table 2. Antioxidant Activity

Ratio	Concentration (ppm)	Absorbance	Inhibition Percentage	IC_{50} (ppm)
1:0	10	0.696	13.32 %	132.76 ppm > 50 ppm
	30	0.644	19.80 %	(moderate)
	50	0.600	25.28 %	
	70	0.529	34.12 %	
	90	0.517	35.61 %	
2:1	10	0.520	35.24 %	90.36 ppm >50 ppm
	30	0.472	41.22 %	(strong)
	50	0.449	44.08 %	
	70	0.442	44.95 %	
	90	0.399	50.31 %	
1:1	10	0.671	16.43 %	106.18 ppm > 50 ppm
	30	0.587	26.89 %	(moderate)
	50	0.538	33.00 %	
	70	0.486	39.47 %	
	90	0.460	42.71 %	
1:2	10	0.630	21.54%	102.10 ppm > 50 ppm
	30	0.584	27.27%	(moderate)
	50	0.516	35.74%	
	70	0.492	38.72%	
	90	0.429	46.57%	
0:1	10	0.402	49.93%	18.05 ppm < 50 ppm
	30	0.374	53.42%	(very strong)
	50	0.338	57.90%	
	70	0.239	70.23%	
	90	0.160	80.07%	
Vitamin C	2	0.721	10.21%	10.39 ppm < 50 ppm
	3	0.711	11.45%	(very strong)
	4	0.624	22.29%	
	5	0.603	24.90%	
	6	0.584	27.52%	

contained in each extract. In theory, in medicinal plants, in addition to the active substance as the main component that is most influential, there are still other side compounds, which may affect the expected response[24].

With the combination of two types of extracts, each of which has secondary metabolite compounds, will interact with each other. Maybe the effect of potentiation at small concentrations, maybe also the opposite, namely mutually weakening. It should also be taken into consideration that at too high a concentration, excess antioxidants can turn into prooxidants. Therefore, at higher concentrations, the antioxidant response may not necessarily be better. This needs to be investigated further[2].

In the antioxidant test using the DPPH test using vitamin C as a comparison, because vitamin C is a natural antioxidant compound that can be used as a comparison in the antioxidant activity test[20]. The IC_{50} value of vitamin C in table 3 is used to compare the results of the IC_{50} value of extract combination from *Averrhoa bilimbi L*. leaves and stingless bee honey. The IC_{50} value of vitamin C is 10,39 ppm with the a linear regression equation y= 4.807x + 0.046 dan R^2 = 0.9165. The IC_{50} value of vitamin C shows that vitamin C has very strong antioxidant activity.

5. Conclusion

From the results of antioxidant tests with a extract combination from *Averrhoa bilimbi L.* leaves and stingless bee honey, which has the highest IC_{50} value, the combination 0:1 of 18.05 ppm but from the combination 0:1 only uses *Averrhoa bilimbi L.* leaves extract and there is no combination of stingless bee honey, it can be seen from the combination of 1:1; 2:1; 1:2 which has the largest IC_{50} value, the combination 2: 1 of 90.36 ppm. So that the combination of extract combination from *Averrhoa bilimbi L*. leaves and stingless bee honey has antioxidant activity that is included in the strong classification.

References

1. Wulansari, A. N. Alternatif cantigi ungu (Vaccinium varigiaefolium) sebagai Antioksidan. Farmaka. 2018;16(2).
2. Marianne, M., Patilaya, P., & Barus, B. T. Uji Aktivitas Antioksidan Kombinasi Ekstrak Etanol Rimpang Temu Giring (Curcuma Heyneana) dan Daun Pugun Tanoh(Curanga Fel-Terrae)Menggunakan Metode Diphenyl Picrylhydrazil (DPPH). In Talenta Conference Series: Tropical Medicine (TM). 2018;1(2):398-404.
3. Haerani, A., Chaerunisa, A. Y., & Subarnas, A. Artikel Tinjauan: Antioksidan Untuk Kulit. Farmaka. 2018;16(2):135-151.
4. Sari, AN. Anti alternatifoksidan untuk menangkal bahaya radikal bebas pada kulit. Elkawnie: Jurnal Sains dan Teknologi Islam. 2015;1(1):63-68.
5. Mulia, K., Hasan, A. E., & Suryani. Total Phenolic, Anticancer and Antioxidant Activity of Ethanol Extract of Piperretrofractum Vahl from Pamekasan and Karang Asem. Current Biochemistry. 2016;3(2):80-90.
6. Werdhasari, A. Peran Antioksidan Bagi Kesehatan. Jurnal Biotek Medisiana Indonesia. 2014;3(2):59-68.
7. Mariani, K. R. Uji Sitotoksik Ekstrak Alkaloid Daun Belimbing Wuluh (Averrhoa bilimbi L.) terhadap Larva Udang (Artemia salina). Jurnal Natural Scientiae, 1. 2021;(1):07-13.
8. Novika, D. S., Ahsanunnisa, R., & Yani, D. F. Uji Aktivitas Antiinflamasi Ekstrak Etanol Daun Belimbing Wuluh (Averrhoa bilimbi L.) Terhadap Penghambatan Denaturasi Protein. Jurnal Sains dan Terapan Kimia. 2021;3(1):16-22.
9. Fidrianny, I., Rahmawati, A., & Hartati, R. Comparison Profile of Different Extracts of Averrhoa Bilimbi L. In Antioxidant Properties and Phytochemical Content. Rasayan Journal of Chemistry. 2018;11(4):1628 – 1634.
10. Alipin, K., & Azizah, N. R. Morfologis Dan Berat Relatif Organ Hati Tikus Yang Diinduksi Karagenan Setelah Pemberian Ekstrak Kombinasi Rimpang Temulawak Dan Buah Belimbing Wuluh. Seminar Nasional Pendidikan Biologi dan Saintek (SNPBS) ke-VI. 2021:243-247.

11. Batistuta, M. A., Aulia, A., & Kustiawan, P. M. (2021). Review: Potensi Aktivitas Anti Virus dari Produk Alami Lebah Kelulut. Jurnal Farmasi Udayana. 2021;10(2):144-148.
12. Zahra, N. N., Muliasari, H., Andayani, Y., & Sudarma, I. M. Karakteristik Fisikokimia Ekstrak Madu Dan Propolis Trigona Sp. Asal Lombok Utara. Jurnal Agrotek Ummat. 2021;8(1).
13. Qadariah, L., Andrie, M., & Taurina, W. Uji Sifat Fisik Sediaan Salep Kombinasi Madu Kelulut (Heterotrigona itama), Ekstrak Sirih Hijau (Piper betle L.), Dan Minyak Cengkeh (Syzygium aromaticum L.). Jurnal Mahasiswa Farmasi Fakultas Kedokteran Universitas Tanjungpura. 2019;4(1).
14. Febriyanti, L., & Citra, A. Analisis Kuantitatif Fenol Total Dan Uji Aktivitas Antioksidan Ekstrak Air, Metanol, Dan N-Heksan Daun Pepaya Dengan Metode DPPH. Prosiding Seminar Nasional Kimia Dan Terapan 2021.
15. Haveni, D., Mastura, M., & Sari, RP. Ekstrak Etanol Kulit Buah Naga Super Merah (Hylocereus costaricensis) sebagai Anti Oksidan dengan Menggunakan Metode DPPH. Katalis: Jurnal Penelitian Kimia dan Pendidikan Kimia 2019;2(2):30-37.
16. Kristiningrum, N., Hernawati, S., Aulia, R. P., & Wardani, P. Studi Aktivitas Antioksidan Kombinasi Ektrak Etanol Daun ManggaBachang (Mangifera foetida Lour.) dan Kelopak Bunga Rosella (Hibiscu sabdariffa L.). Prosiding Seminar Nasional Pendidikan Biologi dan Saintek Ke-3. 2018.
17. Wimpy, W., & Harningsih, T. Uji Aktivitas Antioksidan Kombinasi Ekstrak Sarangsemut (Myrmecodia pendans) dan Ekstrak Keladi Tikus (Typhonium flagelliforme Lodd.) dengan Metode DPPH (1, 1-Dipheyl-2-Picrilhidrazil). Jurnal Kesehatan Kusuma Husada. 2017.
18. Basito, B. Sifat Fisik, Kimia, Dan Organoleptik Pada Pembuatan Dodol Yang Disubstitusi Dengan Wortel (Daucus carota, Linn). Jurnal Teknologi Hasil Pertanian. 2011;4(1):10-17.
19. Lung, J. K., & Destiani, D. P. Uji Aktivitas Antioksidan Vitamin A, C, E dengan metode DPPH. Farmaka. 2017;15(1):53-62.
20. Pramesti, R. Aktivitas antioksidan ekstrak rumput laut Caulerpa serrulata dengan metode DPPH (1, 1 difenil 2 pikrilhidrazil). Buletin Oseanografi Marina. 2013;2(2):7-15.
21. Yulianty, R., Murdifin, M., & Asma, N. Aktivitas antioksidan kombinasi ekstrak etanol Kayu Secang (Caesalpinia sappan L.) dan kelopak bunga Rosella (Hibiscus sabdariffa L.). In Proceeding of Mulawarman Pharmaceuticals Conferences. 2016;3:349-356.
22. Afriliah, N., Taurina, W., & Andrie, M. Karakterisasi Simplisia Madu Kelulut (Heterotrigona Itama) Sebagai Bahan Baku Sediaan Obat Penyembuhan Luka. Majalah Farmasi dan Farmakologi, 2022;26(3):104-110.
23. Valsan,A.,Raphael,R.K.Pharmacognostic profile of Averrhoa bilimbi Linn. Leaves. South Indian Journal of Biological Science. 2016;2(1):75-80.
24. Huliselan, Y., dan Defny S.W.Aktivitas Antioksidan Ekstrak Etanol, Etilasetat, dan n-Heksan dari Daun Sesewanua (Clerodendron Squamatum Vahl.). Pharmacon Jurnal Ilmiah Farmasi2015;4(3):155-163.