Sriwijaya Journal of Dentistry

Sriwijaya Journal of Dentistry (SJD)
Volume 4 Issue 1 2023 : 1-10
https://sjd-fk.ejournal.unsri.ac.id/index.php/sjd/index

Antibacterial Activity of Suruhan Leaf Extract *(Peperomia pellucida L)* Against *Staphylococcus aureus*

Nadyah Maharani[1], Galuh Anggraini[2], Valentino Haksadjiwo[1], Bambang Nuryadi[1], Rosada Sintya Dwi[1*]

[1]Dentistry Program, Faculty of Medicine, Universitas Sriwijaya, Palembang, South Sumatera , Indonesia
[2]Dr.Mohammad Hoesin Central General Hospital, Palembang,South Sumatera , Indonesia
[*]Correspondence author email: rosadasintyadwi@fk.unsri.ac.id

Abstract

Introduction*: Staphylococcus aureus* is one of the opportunistic bacteria in the oral cavity that can cause various diseases, including periapical abscesses. Microbial infection treatment must pay attention to selected antibiotics. Traditional medicine was very popular with the community because it had very minimal side effects and the availability that is easy to obtain. Suruhan leaves could be utilized as an antibacterial due to their content of tannins, saponins, flavonoids, and alkaloids, which inhibit bacterial growth. **Purpose***:* This study aimed to determine the effectiveness of *the antibacterial extract of suruhan leaves (Peperomia pellucida L) against Staphylococcus aureus.* ***Methods****:* This study was an in vitro laboratory experimental study. The test group used an extract of suruhan leaf with concentrations of 20%, 40%, and 60% obtained by the soxhlation method. Clindamycin was used as a positive control, and distilled water as a negative control. The antibacterial potency was tested using the disc diffusion method to determine the value of the inhibition zone, and the dilution method to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The inhibition zone values were then analyzed statistically using one-way ANOVA and post-hoc Tukey tests. **Results***:* This study revealed that the 60% concentration of extract suruhan leaf had an average inhibition zone of 18.27 mm, but smaller than clindamycin. The MIC test results of extract suruhan leaf were determined at a concentration of 20% and MBC at 40%. **Conclusion***: Suruhan leaf extract (Peperomia pellucida L.) had antibacterial activity against Staphylococcus aureus.*

Keywords: *antibacterial; Peperomia Pellucida L; suruhan leaves; Staphylococcus aureus*

Introduction

Staphylococcus aureus is a normal microflora in the oral cavity that can be opportunistic to cause infection if it is influenced by predisposing factors such as a decrease in the host's immune system and an unbalanced number of microorganisms.[1] This infection can occur because *Staphylococcus aureus* produces toxins or direct invasion that can damage tissue.[2] Staphylococcus aureus bacteria are gram-positive bacteria, shaped like purple cocci with gram staining, and tend to form clusters (groups) that resemble grapes.[1]

Staphylococcus aureus is the etiology of many oral diseases, such as abscesses, gingivitis, angular cheilitis, parotitis, staphylococcal mucositis, and denture stomatitis.[3] The most common oral abscess is a periapical abscess caused by bacterial invasion of the periapical area due to infection of the pulp, periodontal or pericoronal tissues.[4]

Periapical infection can be treated by eliminating the source of infection by drainage, intracanal medication, and administration of antibiotics.[4] Amoxicillin is a beta-lactam antibiotic that can be selected as an additional therapy, but *Staphylococcus aureus* strains have a significant resistance rate to antibiotics of 30%-70%, especially a class of beta-lactam antibiotics called Methicillin-resistant *Staphylococcus aureus* (MRSA).[5] Clindamycin has long been an option for treating both methicillin sensitive *Staphylococcus aureus* (MSSA) and methicillin resistant S. aureus (MRSA) infections.[6] However recent studies have shown higher resistance of S. aureus to Clindamycin, including MRSA strains.[7]

This has motivated many researchers to look for alternative therapies that are safer and more effective, especially by utilizing organic components such as suruhan leaf extract. Suruhan leaves (*Peperomia pellucida L.)* are known for their various pharmacological properties, and several researchers have shown that bioactivities such as analgesic, cytotoxic, anti-inflammatory, and antimicrobial.[8] This is because suruhan leaves have active substances such as steroids, tannins, flavonoids, alkaloids, and saponins.[9]

The presence of steroids compounds, tannins, flavonoids, alkaloids, and saponins in the leaves of suruhan (*Peperomia pellucida L.)*, which can be used as natural antibacterial alternatives and the minimum inhibitory concentration value has not been obtained in previous studies. This study aims to do further research on the potential antibacterial activity of extract of suruhan leaves (*Peperomia pellucida L.)* with different concentrations of 20%, 40%, and 60% against *Staphylococcus aureus* bacteria. The selection of this concentration is based on previous research by Asiyah IJ and Wulandari D.[10]

Methods

This is an in vitro laboratory experimental study to determine the antibacterial activity of suruhan leaf extract (*Peperomia Pellucida L.)* with concentrations of 20%, 40%, and 60% against the growth of *Staphylococcus aureus*. Leaf extraction was carried out at the Laboratory of the Sriwijaya Polytechnic Palembang, and the antibacterial tests against *S. aureus* were

obtained by the Research Center Laboratory of the Faculty of Dentistry, Airlangga University, Surabaya.

Suruhan leaves were obtained from Sindang Marga Village, Bayung Lencir District, Banyuasin Regency, South Sumatra Province. The leaves were dried in the sun then grind using a blender, and simplicia was filtered using a sieve. Simplisia was extracted using the soxhlation method by wrapping 240g of simplicia in filter paper and putting it in a soxhlation tube (thimble). The extraction process was performed at 70°C until the circulating droplets became colorless. After that, the extract was evaporated with a rotary evaporator for two days to get 100% viscous leaf extract.[2]

The disc diffusion method was used to determine the inhibition zone of the suruhan leaves against *S. aureus*. Mueller Hinton Agar (MHA) media were inoculated with *S. aureus* ATCC 25923 using a sterile cotton bud. The extract with each concentration (20, 40, and 60%) is saturated into the disc, then the disc paper were placed on the surface of the media. Then, the press was incu bator for 1 x 24 hours. The zone of inhibition is marked with a clear ar sensitivity of the bacteria to the antibacterial agent used. The formed in sured for vertical and horizontal diameters in units (mm) using a caliper

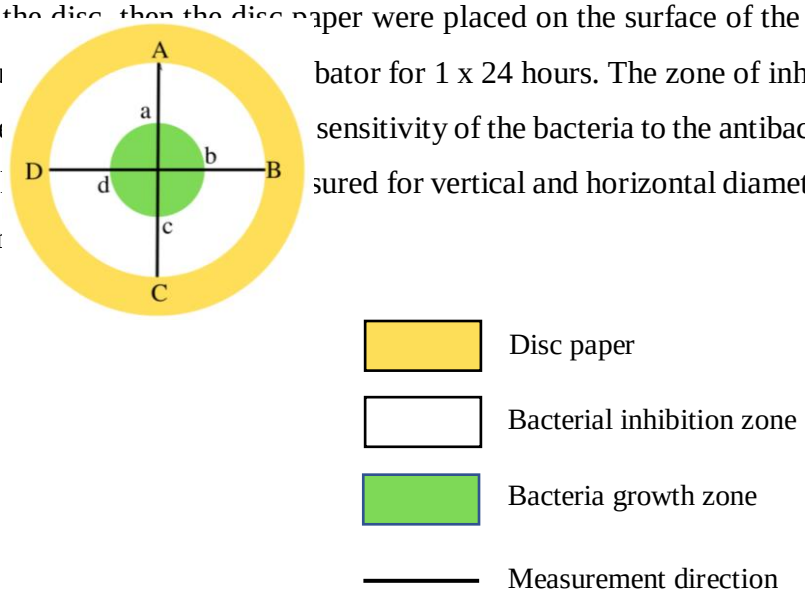

Figure 1. Illustration of Inhibition Zone Diameter Measurement

Determination of the value of the minimum inhibitory concentration (MIC) and minimum killing concentration (MBC) was carried out by the reliable dilution method using Mueller Hinton Agar (MHA) media. Suruhan leaf extract was added as much as 1 mL into a petri dish containing 9 mL of liquid MHA media and waited until it was solid. The bacterial suspension was mixed and homogenized in a petri dish using the spread palate method and incubated at 35°C for 18-24 hours. Read the results of the MIC by looking at the lowest concentration that can inhibit the growth of *S. aureus*. The MIB value was be determined by the lowest

concentration where there is no growth of bacteria colonies on the streaks marked with clear media after incubation.[11]

Data were processed and analyzed using SPSS. The distribution of data is normal, determined by the Shapiro-Wilk test. Levene's test was used to assess homogeneity. To determine whether there is an effect of different concentrations of *Peperomia Pellucida L.* on *S.aureus*, the two variables analyzed (independent and dependent variables) were examined using the One-Way Anova test to assess the validity of the data collected. Tukey's post-hoc test ($p<0.05$) was used to see if there was a significant difference between the control group and the concentration of the extract of the.

.

Results

Qualitative phytochemical tests are used to prove the presence or absence of certain active compounds in suruhan leaves to determine their biological activity. In this study, the suruhan leaf extract (*Peperomia Pellucida L.*) was subjected to phytochemical tests at the Chemistry Laboratory of the Sriwijaya Polytechnic. The results showed that the suruhan leaf extract contained saponins, alkaloids, tannins, and flavonoids (Figure 2).

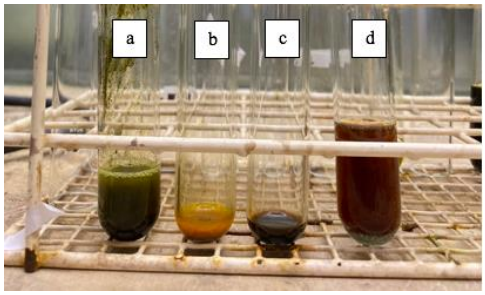

Figure 2. The results of the phytochemical test of suruhan leaf extract: (a) Saponins, (b) Alkaloids, (c) Tannins, (d) Flavonoids

The phytochemical test of *Peperomia Pellucida L.* on flavonoid compounds with alkaline reagent test showed a change in the color of the extract into brownish red. A color change characterizes alkaloid compounds with the Wagner test, and there is sediment. The presence of persistent foam describes saponin compounds with the foam test. Tannin compounds with the Braymer test indicated a dark green color change.

Inhibition Zone of Suruhan

Antibacterial activity tests of *Peperomia Pellucida L.* on the growth of *S. aureus* at concentrations of 20%, 40%, and 60%, as well as the positive and negative control group were carried out using the disc diffusion method. After 24 hours of incubation at 37°C, the diameter of the inhibition zone formed around the disc paper was measured using a caliper with five repetitions. The results showed in Table 1 and Figure 3.

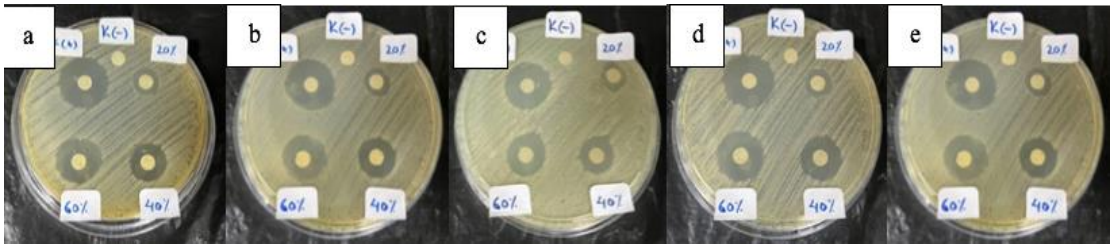

Figure 3. Antibacterial test results of suruhan leaf extract (*Peperomia Pellucida L.*) concentrations of 20%, 40%, and 60%, positive control, and negative control: (a) Repetition 1, (b) Repetition 2, (c) Repetition 3,(d) Repetition 4, (c) Repetition 5

Table 1. Diameter of Inhibition Zone of Suruhan Leaf Extract (*Peperomia Pellucida L.)* Against *S.aureus*

No.	Treatment	Diameter of Inhibition Zone (mm)					average
		Repetition					
		1	2	3	4	5	
1.	Suruhan leaf extract 20%	12,80	12,60	12,55	12,40	12,20	12,51
2.	Suruhan leaf extract 40%	16,05	15,60	15,80	15,95	16,00	15,88
3.	Suruhan leaf extract 60%	18,40	17,80	18,20	18,60	18,35	18,27
4.	Positive control (Clindamycin 30 µg)	23,00	23,20	23,55	24,05	23,05	23,37
5.	Negative control (Distilled water)	0	0	0	0	0	0

Minimum Inhibitory Concentration Test (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests of *Peperomia Pellucida L.* concentrations of 20%, 40%, and 60% against *S. aureus* were carried out using the solid dilution method and repeated five times for each test group. The results are shown in Figure 4, and the results of counting the number of *S.aureus* colonies are in Table 2.

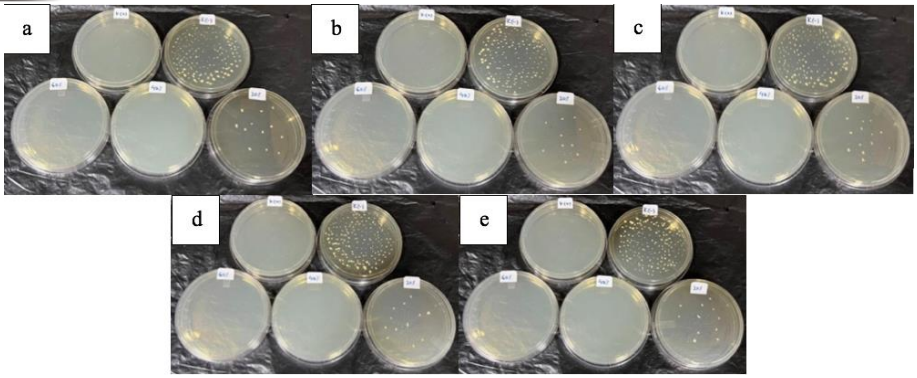

Figure 4. MIC and MBC test solid dilution results: a) Repetition 1, b) Repetition 2, c) Repetition 3, d) Repetition 4, e) Repetition 5

Table 2. Number of Colonies After 24 Hours of Incubation.

No.	Treatment	Calculation results of the number of bacterial colonies (CFU/mL)					Average
		Repetition					
		1	2	3	4	5	
1.	Suruhan leaf extract 20%	13	11	14	10	11	11,8
2.	Suruhan leaf extract 40%	0	0	0	0	0	0
3.	Suruhan leaf extract 60%	0	0	0	0	0	0
4.	Positive control (Clindamysin 30 µg)	0	0	0	0	0	0
5.	Negative control (Distilled water)	172	178	166	182	175	174,6

The results showed that the 20% treatment group had antibacterial power, marked by a visible zone of inhibition with less diameter. The concentration can be determined as the minimum inhibitory concentration of suruhan leaf extract against S. aureus.

The treatment groups at concentrations of 40%, 60%, and the positive control group were able to inhibit *Staphylococcus aureus* bacteria which was characterized by the absence of growth of bacterial colonies on agar media. Hence, the minimum bactericidal concentration of suruhan leaf extract in the current study was 40%.

Discussion

The antibacterial activity test by measuring the inhibition zone in this study showed that all concentrations of the suruhan leaf extract produced a clear zone around the disc paper. This proves that suruhan leaf extract has antibacterial activity against the growth of *S. aureus*. The results of this study indicate that the average value of the diameter of the inhibition zone

at concentrations of 20% and 40% was categorized as weak antibacterial activity, and the concentration of 60% was moderate.

The zone of inhibition in the positive control group was 23.7 mm, categorized as strong antibacterial power. Meanwhile, there was no clear zone in the negative control group, indicating no antibacterial activity. This is in line with Dandirwalu et al. that revealed the higher the concentration of plant extracts, the higher the diameter of the inhibition zone for the growth of Staphylococcus aureus bacteria. However, the study used a higher concentration of 75%.[12]

Table 2 shows that the extract concentration of 20% still contained bacterial growth with an average number of colonies of 11.8 CFU/m, while at concentrations of 40% and 60%, there was no longer any growth of *Staphylococcus aureus* bacteria on agar media, so the MIC values obtained on this study was 20% and the KBM value was a concentration of 40%. This study is similar to Aisyah et al. regarding the antibacterial activity of the ethanol extract of *Peperomia Pellucida L.* against *S. aureus* at concentrations of 20%, 40%, 60%, 80%, and 100% where the observations showed that at concentrations of 20 %, there is still growth of bacteria. At concentrations starting from 40%, there is no visible bacterial growth. So the concentration of 40% is determined as the MBC value.[13] Suryani et al. stated one factor that determined the antibacterial activity is extract concentration, where the higher the concentration, the greater the inhibition.[14]

The ability of the extract to inhibit the growth of the tested bacteria depends on the type of test bacteria, the concentration level, and the length of contact time.[14] The contact time factor between the simplicia and the solvent used can also affect the extract's effectiveness. The longer the contact time between the simplicia and the solvent, the greater the results. This is due to the solvent's ability to attract compounds to the simplicia. Polar solvents such as 96% ethanol can easily penetrate the simple cell walls and are able to bind more chemical compounds when compared to methanol and water.[15]

The antibacterial activity of *Peperomia Pellucida L.* comes from active compounds such as alkaloids, tannins, saponins, and flavonoids, where these compounds have antibacterial functions. This is supported by Trianingsih R et al. in a research analysis of the chemical content of suruhan plants as herbal medicines explaining that suruhan extracts contain flavonoids, tannins, and alkaloids.[9] The active compounds may differ because several reasons, such as the extract method, geographical location, and growing environment.[16] Those

compounds can be efficacious for microbial infectious diseases.[18] They can inhibit the growth of bacteria with their respective mechanisms of action.

The antimicrobial mechanism of saponin compounds is by binding to lipopolysaccharides in the bacterial cell wall, which can increase the surface tension of the cell wall so that the cell will rupture or lysis. The mechanism of action of alkaloid compounds is by destroying the constituent components of peptidoglycan in bacterial cells, which can cause the bacterial cell wall layer not to form completely and only consist of the cell membrane.[16] Tannin compounds are antibacterial because they have astringent toxicity properties or substances that can shrink cell walls so that they can damage the bacterial cell membrane and disrupt cell permeability which results in cells being unable to function or bacterial death can occur. Other compounds with antibacterial activity are flavonoids that disrupt the function of the bacterial cell wall through the formation of complexes with extracellular proteins and inhibit bacterial mobility.[17]

The limitation of this research is the long processing time of drying the leaves, which is done naturally with the sun. The sun drying method is more economical, easy to do, and does not damage the active compounds, which are thermolabile. Therefore in future research, it is necessary to pay attention to the method of drying the leaves, which is more time effective without damaging the quality of the extract. Another alternative is using an oven that is considered more beneficial in reducing the water content in a shorter time by using drying temperatures for medicinal plants in the range of 40-60°C, and the moisture content of the simplicia produced ranges from 5-10%.[18]

Another limitation of this study was that the leaf extraction and antibacterial test were carried out in a different city. The extract delivery process takes three days to arrive at the destination. The extract is stored in a bottle, then packaged in a cool styrofoam box containing ice gel and sent using an AKR cold truck expedition to keep the temperature of the extracted cold until it reaches the destination city. Khotimah et al. stated that miana leaf extract stored at cool temperatures (8-15°C) for 7 days and 14 days experienced a decrease in total flavonoid levels on day 14. This showed that the storage period affected the flavonoid content from experiencing degradation due to the oxidation process of the active substance of the extract to oxygen.[19]

Conclusion

Suruhan leaf extract (*Peperomia Pellucida L.)* has antibacterial activity against the growth of *Staphylococcus aureus* derived from active compounds such as alkaloids, tannins, saponins, and flavonoids. *Peperomia Pellucida L.* can inhibit the growth of *Staphylococcus aureus* bacteria with a MIC value of 20% and a MIC value of 40%. The inhibition zone of each concentration shows differences in the antibacterial activity of *Peperomia Pellucida L.* Concentrations of 20% and 40% had antibacterial power in the weak category, and a concentration of 60% had moderate antibacterial power.

References

1. Bhunia AK. Staphylococcus aureus. In: Foodborne Microbial Pathogens. Springer; 2018. p. 181–92.
2. Puspita S, Lutfialifta PO. The inhibitory effect of Kaffir Lime extract towards Staphylococcus aureus bacteria. J Int Dent Med Res. 2020;13(2):539–42.
3. Tuna MR. Uji daya hambat ekstrak daun sirsak (Annona muricata L.) terhadap pertumbuhan Staphylococcus aureus secara in vitro. Pharmacon. 2015;4(4).
4. Atmojo SPS, Sidiqa AN. Penanganan Abses Periapikal Kronis Palatal Anterior pada Gigi Insisif Lateral Rahang Atas. J Mater Kedokt Gigi. 2021;10(1):15–21.
5. Al-Akwa A, Zabara AQMQ, Al-Shamahy HA, Al-labani MA, Al-Ghaffari KM, Al-Mortada AM, et al. Prevalence of Staphylococcus aureus in dental infections and the occurrence of MRSA in isolates. Univers J Pharm Res. 2020;5(2):23–7.
6. Bhomi U, Rijal KR, Neupane B, Shrestha S, Chaudhary M, Acharya D, Thapa SU, Adhikari N, Ghimire P. Status of inducible clindamycin resistance among macrolide resistant Staphylococcus aureus. African Journal of Microbiology Research. 2016 Mar 7;10(9):280-4.7.
7. Assefa M. Inducible Clindamycin-Resistant Staphylococcus aureus Strains in Africa: A Systematic Review. International Journal of Microbiology. 2022 Apr 19;2022.8. Alves NSF, Setzer WN, da Silva JKR. The chemistry and biological activities of Peperomia pellucida (Piperaceae): A critical review. J Ethnopharmacol. 2019;232:90–102.
9. Trianingsih R, Achmad MA, Alibasyah LMP, Febriawan A. Analisis Kandungan Kimia Tumbuhan Suruhan (Peperomia pellucida) Sebagai Obat Herbal. J Biol Sci Educ. 2021;9(1):694–700.
10. Asiyah IJ, Wulandari D. Aktivitas Antibakteri Ekstrak Daun Suruhan (Peperomia pellucida L. Kunth) Terhadap Bakteri Staphylococcus aureus. J Farm Indones. 2019; 16 (2);98-105
11. Komala O, Sari BL, Sakinah N. Uji Efektivitas Ekstrak Etanol Buah Pare (Momordica charantia L.) Sebagai Antibakteri Salmonella typhi. Fitofarmaka J Ilm Farm. 2014;2(1):36–41.
12. Dandirwalu E, Watuguly TW. Uji Daya Hambat Ekstrak Etanol Suruhan (Piperumia pellucida LHB Kunth) Terhadap Pertumbuhan Bakteri Staphylococcus aureus Secara In-Vitro. BIOPENDIX J Biol Pendidik dan Terap. 2015;2(1):8–14.
13. Asiyah IJ, Wulandari D. Aktivitas Antibakteri Ekstrak Daun Suruhan (Peperomia pellucida L. Kunth) Terhadap Bakteri Staphylococcus aureus. J Farm Indones.

2019;16(2):98–105.
14. Suryani RD, Kusuma ARP, Putranto RR. Efektivitas Antibakteri Ekstrak Etanol Siwak (salvador persica) Berbagai Konsentrasi Dalam Menghambat Pertumbuhan dan Membunuh Actinomyces SPP (secara IN VITRO). Pros Konstelasi Ilm Mhs Unissula Klaster Kesehat. 2021;
15. Marfuah I, Dewi EN, Rianingsih L. Kajian potensi ekstrak anggur laut (Caulerpa racemosa) sebagai antibakteri terhadap bakteri Escherichia coli dan Staphylococcus aureus. J Pengolah dan Bioteknol Has Perikan. 2018;7(1):7–14.
16. Dwicahyani T, Sumardianto S, Rianingsih L. Uji Bioaktivitas ekstrak teripang keling Holothuria atra SEBAGAI ANTIBAKTERI Staphylococcus aureus dan Escherichia coli. J Pengolah dan Bioteknol Has Perikan. 2018;7(1):15–24.
17. Sadiah HH, Cahyadi AI, Windria S. Kajian Daun Sirih Hijau (Piper betle L) Sebagai Antibakteri. J Sain Vet. 2022;40(2):128–38.
18. Sembiring BB, Suhirman S. Pengaruh cara pengeringan dan teknik ekstraksi terhadap kualitas simplisia dan ekstrak meniran. In: Prosiding Seminar Nasional Pengembangan Teknologi Pertanian. 2014.
19. Khotimah H, Agustina R, Ardana M. Pengaruh Lama Penyimpanan Terhadap Aktivitas Antioksidan Ekstrak Daun Miana (Coleus atropurpureus L. Benth). Proc. Mul. Pharm. Conf. [Internet]. 2018 Dec. 31 [cited 2023 Jun. 18];8(1):1-7. Available from: http://prosiding.farmasi.unmul.ac.id/index.php/mpc/article/view/295