Analgesic Activity Test of the Ethanol Extract Combination of Kersen Leaves (Muntingia calabura L.) and Lamtoro Leaves (Leucaena leucocephala (Lam) de Wit) on Acetic Acid-Induced Mice

Rifda Naufa Lina[1]*, Bagus Riyanto[2], Amsiati[3]

[1,2,3]Institut Teknologi Kesehatan Cendekia Utama Kudus, Indonesia

*Corresponding Author: Naufalinarifda@gmail.com

Abstract. Analgesics are drugs that selectively reduce pain that act in the central nervous system or suppress the sensitivity of pain receptors to mechanical stimuli. The presence of dangerous side effects caused by chemical drugs, the use of natural ingredients as drugs tends to increase. Kersen leaves (Muntingia calabura L.) and leaf lamtoro (Leucaena leucocephala (Lam) de Wit) have the same content, namely flavonoids which have analgesic activity. Flavonoids can function as analgesics because they can inhibit the formation of prostaglandin as a mediator of pain sensations. Flavonoids act as analgesics whose mechanism of action can inhibit the work of the enzyme cyclooxygenase which catalyzes the change of arachidonic acid into prostaglandins so as to reduce pain (Ishak, 2017). The purpose of this study was to determine the analgesic activity in kersen leaf extract, lamtoro leaves and a combination of both. This study is an experimental study using stretching method. The test animals used were male mice (Mus Musculus) types of Swiss mice. This study used 40 mice and divided into 8 groups: healthy mice group, negative control positive control, dose group ethanol extract of kersen leaf 50 gram / BB, dose of ethanol extract leaf lamtoro 50 gram / BB, dose combination ethanol extract kersen leaves and lamtoro (1: 2, 1: 1, 2: 1). Data were obtained from observing the amount of stretching of mice by giving each test solution as much as 1mL orally the ethanol extract stock solution of kersen leaves and lamtoro leaves. After 30 minutes all groups of animals treated were intraperitonially injected with 3% v / v acetic acid solution. The data were then analyzed statistically using the SPSS application using the One Way ANOVA method. The results showed that the ethanol extract of leaves of kersen dose of 50 grams / BB, leaves of lamtoro dose of 50 grams / BB, dose combination of etano extract of kersen leaves and leaves of lamtoro 1: 2, 1: 1, 2: 1 had analgesic activity. The combination dose of ethanol extract of kersen leaves and lamtoro leaves is effective as an analgesic, namely a combination dose of 2: 1.

Key words: [Analgetic, Ethanol Extract of Kersen Leaf and Leucaena Leaves, Mice.]

INTRODUCTION

Nyeri menurut Tjay dan Rahardja (2015) adalah perasaan sensoris dan emosional yang tidak nyaman. Keadaan psikis sangat mempengaruhi nyeri, misalnya emosi dapat menimbulkan sakit (kepala) atau memperhebatnya. Batas nyeri untuk suhu konstan, yaitu pada 44-45°C. Analgetik atau obat penghalang nyeri adalah zat-zat yang mengurangi atau menghalau rasa nyeri tanpa menghilangkan kesadaran (Meisyayati dkk., 2017).

Suboptimal pain management will increase patient morbidity. High morbidity rates lead to prolonged healing time, extended hospital stays, and increased hospitalization costs. Therefore, optimal pain management is not only an effort to reduce patient suffering but also to enhance their quality of life. It has been proven that without optimal pain management, patients will experience physiological and psychological disturbances, ultimately increasing both morbidity and mortality rates (Chandra et al., 2016).

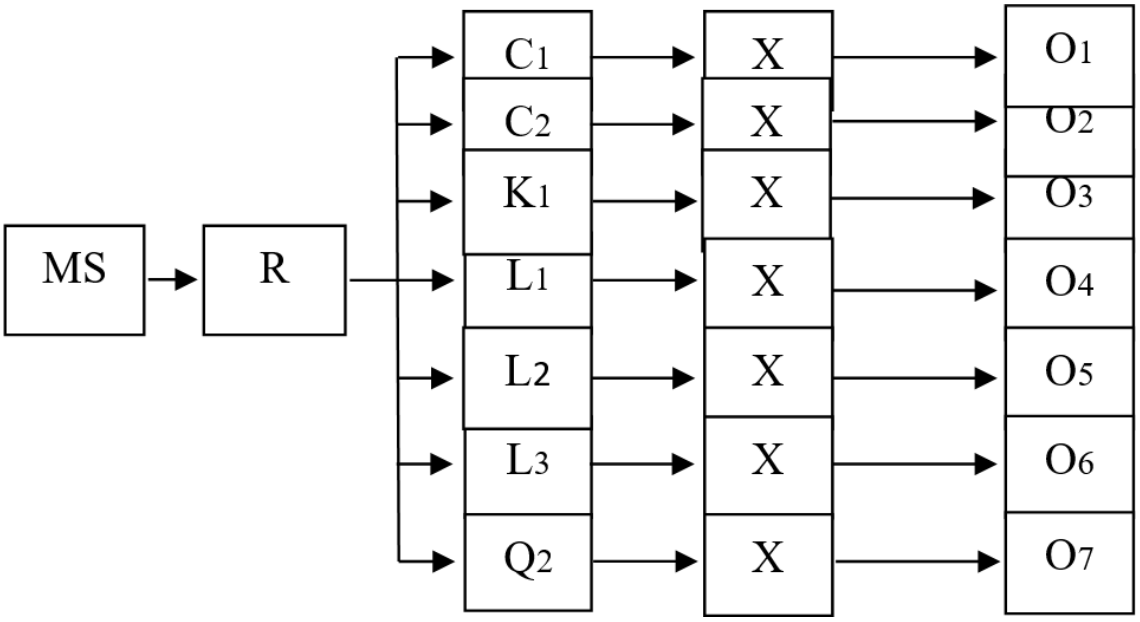
According to the Indonesian Health Profile in 2008, the national prevalence of illness was 33.24%. Of this total, 65.5% opted for self-medication using both modern and traditional medicines. The remaining 34.41% sought outpatient care at community health centers, doctor's practices, and other healthcare facilities. This indicates a considerable interest in traditional medicine among the public (Kamasturyani, 2018). The advantages of using natural remedies include their effective efficacy, good tolerance, and minimal side effects and allergies (Sasongko et al., 2016).

According to Danugroho and Widyaningrum, extracts from the leaves of the kersen tree (Muntingia calabura L.) have analgesic properties, as demonstrated in male white mice. Kersen leaves contain bioactive compounds, namely flavonoids. Lamtoro leaves (Leucaena leucocephala (Lam) de Wit) contain secondary metabolites such as alkaloids, saponins, flavonoids, and tannins. Flavonoids act as analgesics by inhibiting the action of the cyclooxygenase enzyme, which catalyzes the conversion of arachidonic acid into prostaglandins, thereby reducing pain sensation (Ishak, 2017).

METHODS

The Type and Design of the Study.

This study is a true experimental research with male mice as subjects.



Picture 1. Design of the Study

- R = Randomization

- C1 = Negative control induced with acetic acid.
- C2 = Positive control, mice with induced pain given paracetamol.
- K1 = Treatment group, mice with induced pain given ethanol extract of kersen leaves at a dose of 50 g / 10 ml.
- L1 = Treatment group, mice with induced pain given ethanol extract of kersen leaves: ethanol extract of lamtoro leaves (1:2 ratio).
- L2 = Treatment group, mice with induced pain given ethanol extract of kersen leaves: ethanol extract of lamtoro leaves (1:1 ratio).
- L3 = Treatment group, mice with induced pain given ethanol extract of kersen leaves: ethanol extract of lamtoro leaves (2:1 ratio).
- Q2 = Treatment group, mice given ethanol extract of lamtoro leaves at a dose of 50 g / 10 ml.
- X = Treatment group, mice induced with 3% acetic acid.
- O2-O8 = Observation of the number of writhing movements of mice.

The design used in this study is a post-test only control group design because the examination of variables cannot be performed before treatment, but is conducted after treatment (post-test).

Research Location and Time

This research was conducted at the Microbiology and Pharmacology Laboratory of the School of Health Sciences at Cendekia Utama Kudus. The research took place from January to April 2019.

Population and Sample of the Study

The population used in this research consisted of male white mice. The sample used in this study was male white mice (*Mus musculus*) obtained from the microbiology and pharmacology laboratory of UNISSULA Semarang. The selected mice had an average weight of 20-30 grams, aged 2-3 months, and were healthy and free from infection.

Sample Size
This study used 35 male BABL/c white mice divided into 7 groups, with each group comprising 5 mice.

Tools and Materials
The tools used in this study included mouse cages, mouse drinking bottles, wire mesh cage covers, 1 cc syringes, mouse gavage needles, glass beakers, 10 ml and 100 ml measuring cups, 100 mL volumetric flasks, glass funnels, brown glass bottles, analytical balances, stirring rods, gloves, porcelain dishes, stopwatch, rotary evaporator, pipettes, water baths, spirit flasks, reaction tube clamps, and reaction tubes. The materials used in this study included kersen leaf extract, lamtoro leaf extract, 70% ethanol, distilled water, stock solutions (0.5% CMC, 3% acetic acid, 1% paracetamol), 45 male white mice, concentrated H2SO4, and 10% NaOH.

Procedure
The study used male white mice weighing 20-30 grams obtained from STIFAR Semarang. Seven mouse cages and their covers were cleaned and prepared. Thirty-five healthy male white mice with weights around 20-30 grams were acclimatized for a week and provided with food and water. The mice were then divided into 7 groups: the first group received oral administration of 0.5% CMC solution as a negative control, the second group received 1% paracetamol orally as a positive control, the third group received ethanol extract of kersen leaves at a dose of 50 g / 10 ml, the fourth group received ethanol extract of lamtoro leaves at a dose of 50 g / 10 ml, the fifth group received a mixture of ethanol extract of kersen leaves and lamtoro leaves (1:2 ratio), the sixth group received a mixture of ethanol extract of kersen leaves and lamtoro leaves (1:1 ratio), and the seventh group received a mixture of ethanol extract of kersen leaves and lamtoro leaves (2:1 ratio). After 30 minutes, all groups were intraperitoneally injected with 3% acetic acid to induce pain. The mice's writhing movements were observed for 30 minutes with a 5-minute interval.

Data Analysis
Statistical analysis of the data involved testing for normality and homogeneity. Data were considered normally distributed and homogenous if the p-value was > 0.05. The normality test showed that the significance value for each group was > 0.05, indicating that the data were normally distributed. One-way ANOVA test was then conducted, and a significant value of < 0.05 was obtained. After the one-way ANOVA test, the Bonferroni test was performed to determine the most effective dose among the treatment groups.

RESULTS AND DISCUSSION

Table 1. The Relationship Between Average Twitches and Average Analgesic Power

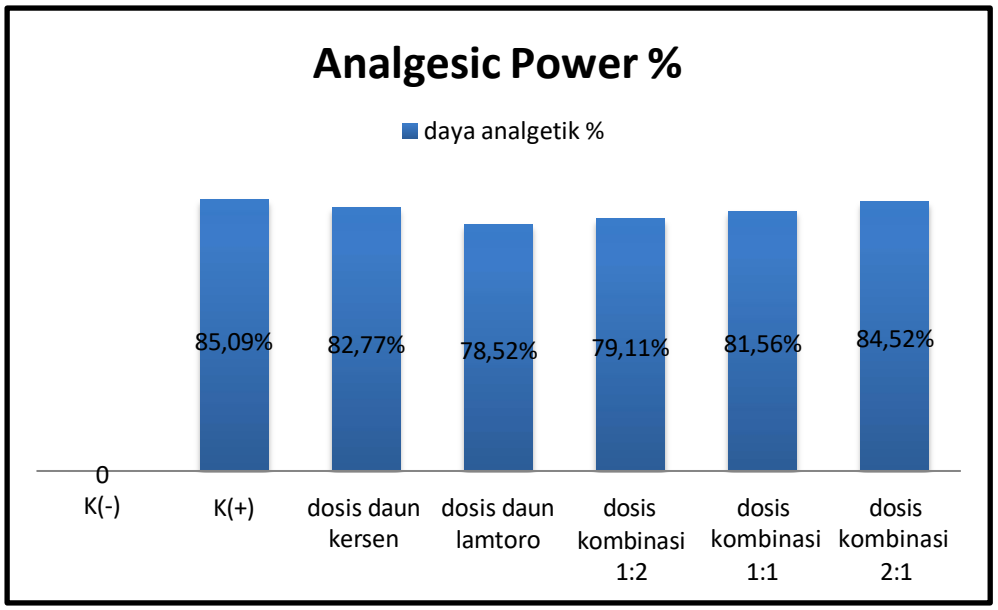
No	Treatment	Number of Twitches Over 30´	Average Analgesic Power %
1	K (-)	33,6 ± 1,34	-
2	K(+)	5 ± 1,00	85.09%
3	DK	5,8 ± 0,84	82.77%
4	DL	7,6 ± 0,55	78.52%
5	DK:DL 1:2	7 ± 0,71	79.11%
6	DK:DL 1:1	6,2 ± 0,84	81.56
7	DK:DL 2:1	5,2 ± 0,84	84.52

Description:
1. Negative control : Negative control induced with acetic acid.
2. Positive control : Pain-induced mice given 1% paracetamol (1 gram / 100 mL).
3. DK Dose : Pain-induced mice in the treatment group given cherry leaf extract at a dose of 50 g / 10 mL.
4. DL Dose : Pain-induced mice in the treatment group given leucaena leaf extract at a dose of 50 g / 10 mL.

5. DK Dose : DL. 1:2 : Pain-induced mice in the treatment group given cherry leaf extract and leucaena leaf extract in a ratio of 1:2.
6. DK Dose : DL. 1:1 : Pain-induced mice in the treatment group given cherry leaf extract and leucaena leaf extract in a ratio of 1:1.
7. DK Dose : DL. 2:1 : Pain-induced mice in the treatment group given cherry leaf extract and leucaena leaf extract in a ratio of 2:1.

In the treatment groups given ethanol extracts of cherry leaves and leucaena leaves, each demonstrated analgesic activity. Among the five dosage groups, the most effective analgesic activity was observed in group 7 with a combination dose of 2:1 (50:25). This dosage is the most effective ratio as an analgesic from the combination of cherry leaf extract (Muntingia calabura L.) and leucaena leaf extract (Leucaena leucocephala (Lam) de Wit) in male white mice (Mus musculus).

Table 1 shows the test results of cherry leaves and leucaena leaves, indicating the number of writhes over 30 minutes and the analgesic effect. In the negative control group, the average number of writhes in the mice was 33.6, with an analgesic effect of 0%. The average number of writhes indicates that the writhing produced by the test animals was quite high after being induced with 3% acetic acid pain, and also shows that the negative control (10% CMC Na) had no pharmacological activity to reduce pain induced by 3% acetic acid (Wulandari and Hendra, 2011).



Picture 2. Graph of the Analgesic Power of Ethanol Extracts from Kersen Leaves and Lamtoro Leaves

The percentages of analgesic efficacy obtained are as follows: positive control: 85.09%, kresen leaf dose: 82.77%, lamtoro leaf dose: 78.52%, combination dose 1:2: 79.11%, combination dose 1:1: 81.56%, combination dose 2:1: 84.52%. These values are almost the same as the analgesic efficacy of the positive control, paracetamol. The ability of cherry leaves and leucaena leaves to alleviate pain is due to the presence of flavonoids, which inhibit the action of the cyclooxygenase enzyme, thereby inhibiting prostaglandin formation and reducing pain (Syamsul et al., 2016).

The data obtained were then processed using SPSS software to test for normality and to determine data homogeneity. The main test conducted was the normality test, which showed a significance value > 0.05, indicating that the data were normally distributed. The data homogeneity test followed, which yielded a significance value > 0.05 (0.201 > 0.05). Since the significance value was > 0.05, it was concluded that all groups of mice writhing data were homogeneous and had no differences or the same variance. After testing for normality and homogeneity, the data were found to be normally distributed, and a one-way ANOVA test was conducted, which yielded a significant value of 0.000 < 0.05. This indicates that there are significant differences between treatments. In the Post Hoc test, significance values can be seen. If the significance value is > 0.05, the data are considered to have no difference, while a significance value < 0.05 indicates a difference, marked by an asterisk (*) to show the differences. The lack of differences among the treatment groups suggests that the various doses of cherry leaf, leucaena leaf, combination doses, and the positive control group all have analgesic activity and thus can reduce pain.

CONCLUSION AND SUGGESTIONS

CONCLUSION

Based on the results of the study on the analgesic activity of the ethanol extract combination of kersen leaves (Muntingia calabura L.) and lamtoro leaves (Leucaena leucocephala (Lam.) de Wit) in male white mice (Mus musculus), it can be concluded that:

1. The ethanol extract combination of kersen leaves (Muntingia calabura L.) and lamtoro leaves (Leucaena leucocephala (Lam.) de Wit) has analgesic activity.
2. The most effective ratio for use as an analgesic is the 2:1 combination dosage.

Suggestions

It is suggested that future researchers investigate combinations with other plants that may have a better or more effective analgesic effect.

REFERENCES

Azis, T., Febrizky, S., & Mario, A. D. (2014). Pengaruh jenis pelarut terhadap persen yieldalkaloiddari daun salam india (Murraya Koenigii). Jurnal Teknik Kimia Universitas Sriwijaya, 20(2), 1–6.

Bahrudin, M. (2017). Patofisiologi nyeri (PAIN). Jurnal Kedokteran Universitas Muhammadiyah Malang, 13(1), 7–13.

Brunton, laurence L., Chabner, bruce A., & Knollmann, bjorn C. (2010). Universal Free E-Book Store Goodman & Gilman's The Pharmacological Basis Of Therapeutics 12 Th Edition. United States of America: Mc Graw Hill Professional.

Chandra, C., Tjitrosantoso, H., & Lolo, W. A. (2016). Studi penggunaan obat analgesik pada pasien cedera kepala (concussion) di rsup Prof . Dr . R . D . Kandou. Pharmacon jurnal Ilmiah Farmasi, vol. 5(2), p. 197–204.

Damanik, D. D. P., Surbakti, N., & Hasibuan, R. (2014). Ekstraksi katekin dari daun gambir (uncaria gambir roxb) dengan metode maserasi. Jurnal Teknik Kimia USU, vol. 3(2), p. 10–14.

Dewi, G. P., & Nugroho, T. E. (2016). Pengaruh pemberian analgesik kombinasi parasetamol dan tramadol terhadap kadar kreatinin serum tikus wistar. Jurnal Kedokteran Diponegoro, vol. 5(4), p. 917–925.

Febriyanti, R., Purba, A. V., & Simanjuntak, P. (2018). Uji aktifitas analgetik kombinasi ekstrak buah belimbing wuluh (Averrhoa Bilimbi L.) Dan daun seledri (Apium Graveolens L.) Terhadap mencit putih jantan (Mus musculus). Jurnal Para Pemikir, vol. 7(1), p. 197–201.

Goenarwo, E., Chodidjah, & Susanto, H. (2011). Uji Efektifitas Analgetik Madu Pada Tikus Dengan Metoda Geliat Asetat. Anatomi Fakultas Kedokteran Universitas Islam Sultan Agung, 3(1), 48–53.

Harborne, J.B. (1987). Metode Fitokimia : Penuntun cara modern menganalisa tumbuhan. Terbitan

Kedua. Terjemahan Kosasih Padmawinata dan Iwang Soediro. ITB : Bandung.

Hasanah, M., & Andriani, N. (2016). Perbandingan Aktivitas Antioksidan Ekstrak Etanol Daun Kersen (Muntingia calabura L .) Hasil Ekstraksi Maserasi Dan Refluks. Jurnal Scintea STIFI Bhakti Pertiwi, vol. 6(2), p. 84–90.

Hayati, Nur Intan. (2014). Pengaruh tehnik distraksi dan relaksasi terhadaptingkat nyeri pada pasien post operasi di rumah sakit immanuel bandung 1nur. Jurnal ilmu kesehatan Stikes Immanuel Bandung, vol. 8(2), p. 325–336.

Hindrawati, Sri., & Natalia, Hesty. (2011). Keunggulan Lamtoro Sebagai Pakan Ternak. Edisi Pertama. BPTU: Palembang.

Ilkafah. (2018). Daun kersen (Muntingia Calabura L .) Sebagai alternatif terapi. Pharmacy Medical Journal, vol. 1(1), p. 33-41.

Ishak, M., Bodhi, W., & Citraningtyas, G. (2017). Uji efek analgetik ekstrak etanol daun lamtoro (Leucaena leucocephala (Lam) de Wit) pada mencit putih jantan. Pharmacon Jurnal Ilmiah Farmasi Universitas Sam Ratulangi, vol. 6(4), p. 130-138.

Jamaluddin, Nasruddin, M. Y. B. (2018). Pengobatan dan obat tradisional suku sasak di lombok. Jurnal Biologi Tropis, vol. 18(1), p. 1-12.

Kamasturyani, Y. (2018). Tanggung jawab kesehatan terhadap pengobatan tradisional dalam upaya peningkatan kesehatan bagi masyarakat studi kasus di kota cirebon. Jurnal Ilmiah Stikes Mahardika, vol. 3(5), p. 140-148.

Meisyayati, S., Immanuel, J., & Darwis, D. (2017). Efek analgetik kombinasi ekstrak daun pepaya (Carica papaya L.) Dan ekstrak daun asam jawa (Tamarindusindica L.) Pada mencit putih jantan. Jurnal Ilmiah Stifar Bhakti Pertiwi, vol. 2(1), p.63–70.

Muntaha, Akhmad., Haitami, N. H. (2015). Perbandingan penurunan kadar formalin pada tahu yang direbus dan direndam air panas. Medical Laboratory Technology Journal, vol. 1 (2), p. 84-90.

Mutschler, Ernst. (1999). Dinamika Obat Farmakologi Dan Toksikologi. Edisi Kelima. ITB: Bandung. Noor, Rasuane.,& Asih, Triana. (2018). Tumbuhan Obat. Edisi Pertama. IKAPI: Lampung.

Nuraeni, Y. (2015). Hama utama tanaman lamtoro (Leucaena leucocephala (Lam.) de Wit). Jurnal Ilmiah Kampus Balitbang Kehutanan, vol. 1(2), p. 13–18.

Puspitasari, A. D., & Proyogo2, L. S. (2013). Perbandingan metode ekstraksi maserasi dan sokletasi terhadap kadar fenolik total ekstrak etanol daun kersen (Muntingia calabura L.). Jurnal Ilmiah Cendekia Eksakta Universitas Wahid Hasyim, p. 1–8.

Rahman, S. (2017). Efek antiinflamasi ekstrak etanol daun kersen (Muntingia calabura L.) Pada mencit (Mus musculus). Pharmacon Jurnal Ilmiah FMIPA UNSRAT, vol. 09(01), p. 51–57.

Redha, A. (2010). Flavonoid: struktur, sifat antioksidatif dan peranannya dalam sistem biologis. Jurusan Teknologi Pertanian Politeknik, vol. 9(2), p. 196-202.

Rosandari, T., Thayib, M. H., Krisdiawati, N. 2011. Variasi penambahan gula dan lama inkubasi pada proses fermentasi Cider Kersen (Muntingina calabura L.). Program Studi Teknologi Industri Pertanian.

Sasongko, H., Farida, Y., Efendi, N. R., Pratiwi, D., & Dwi, A. (2016). Aktivitas analgesik ekstrak etanol daun karika (Carica pubescens) Secara In Vivo. Journal of Pharmaceutical Science And Clinical Research universitas sebelas maret, vol. 01, p. 83–89.

Sayuthi, M. I., & Kurniawati, P. (2017). Validasi metode analisis dan penetapan kadar parasetamol dalam sediaan tablet secara spektrofotometri uv-visible. Prosiding Seminar Nasional Universitas Islam Indonesia, p. 190–201.

Schoorl, 1988. Materi Pelengkap Kemurnian Cara Pemisahan Obat. Yogyakarta: Gajah Mada University Press.

Syamsul, E. S., Andani, F., & Soemarie, Y. B. (2016). Analgesic Activity Study Of Ethanolic Extract Of Callicarpa longifolia Lamk . IN MICE. Traditional Medicine Journal, vol. 21(2), p. 99–103.

Suryanto, E. 2012. Fitokimia Antioksidan. PMN, Surabaya.

Wahidmurni, D. M. P. (2017). Pemaparan Metode Penelitian Kuantitatif. Uin Maulana Malik Ibrahim, p. 1–16.

Yudiyanta, Khoirunnisa, N., & Novitasari, R. W. (2015). Assessment Nyeri. Universitas Gadjah Mada vol. 42(3), p. 214–234.

Yulianingtyas, A., & Kusmartono, B. (2016). Optimasi volume pelarut dan waktu maserasi pengambilan flavonoid daun belimbing wuluh (Averrhoa Bilimbi L.) Aning. Jurnal Ilmiah Fakultas Teknologi Industri, vol. 10(2), p. 58–64.

Zahara, M. (2018). Kajian morfologi dan review fitokimia tumbuhan kersen (Muntingia calabura L). Jurnal Ilmiah Pendidikan dan Pembelajaran Universitas Muhammadiyah, vol. 5(2), p. 69-74.