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Assesment of Antioxidant Activity Test of Kersen Leaf (*Muntingia calabura* L.) and Epiphyte with DPPH(2.2-Diphenyl-1-Picrylhidrazyl)

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ABSTRACT

Antioxidant is very important to give protection against free radical activity and highly reactive molecules that could lead in slowing the progre ssion of de ge nerative disease. In case of de ge nerative disease, internal antioxidant cannot neutralize the increasing concentration of free radical. Because of that, human needs external antioxidant. Kersen (Muntingia calabura L.) is a plant that is known for its antioxidant content. Plants containing antioxidant experience is kersen (Muntingia calabura L.). Research study to determine the antioxidant activity of Kersen plant and knows the difference of antioxidant activity, based on the process of extract and infusion. Research was done by experimental study which was oriented in testing antioxidant activity in (Morinda citrifolia L.) extract and infusion. Extraction was done by using 96% ethanol as solvent, meanwhile infusion was made by using aquadest. Extract and infusion was divided into group of concentration and antioxidant activity was tested by DPPH(2,2-Diphenyl-1-Picrylhidrazyl) method by measuring the absorbance using spectrophotometer at 520 nm wavelength. Percentage of DPPH inhibition and IC50 then analyze d using linear re gre ssion analysis. Ethanolic extract of kersen leaf and epiphyte had IC50 value of 113,801 ppm and 98,7802 ppm, respectively. Kersen leaf infusion showed 191,7624 ppm IC50 values, besides its epiphyte had 131,6750 ppm . Antioxidant activity of Muntingia calabura L. in the order from kersen leaf an epiphyte, and epiphyte extract has a higher antioxidant content than others.

1. Introduction

Indonesia is one of the countries which has a high level biodiversity in the world, 3rd ranked after Brazil and Zaire. The biodiversity includes plants of flora and fauna which are spread throughout Indonesia.[1] There are 40,000 species of flora that grow in the world, 30.000 species found in Indonesia with 1.845 species of plants have the potential as traditional medicine.[2] According to the POM Agency (2006), there are 283 types of plants that have been registered for the use of traditional medicines. Only 13 of the 283 types of medicinal plants that have been cultivated. Traditional medicine is widely used to treat several diseases such urolithiasis, diabetes, high blood pressure and others.

The ability of a plant as a drug is caused by the content of chemical compounds or active compounds that have the working power of treatment. One of the chemical constituents that has the working power of treatment is antioxidant.[3] The emergence of free radicals (hydroxyl) in biochemical mechanisms in the body are the causes of degenerative diseases.[4] Degenerative diseases, such as osteoporosis, cardiovascular, cancer, diabetes mellitus and others can be reduced by consuming antioxidants. This is related to the work system of antioxidants which can inhibit oxidation reactions, by binding free radicals and molecules which are very reactive.[5]

One potential source of natural antioxidants is plants because they contain flavonoid compounds, chlorophyll and tannin.[6] Kersen (*Muntingia calabura* L.) is a plant that has the potential to be a natural antioxidant.[7] Antioxidants kersen (*Muntingia calabura* L.) are found in all parts of flowers, fruit and leaves, and the highest activity on the part of the leaf. Various studies show that kersen leaves contain active components of saponins, flavonoids and tannins, when extracted using methanol and ethanol solvents.[8]

The administration of the ethanol extract of the leaves has an effect on B.*carambolae* fruit fly. The higher the concentration of extracts, the lower the number of pupae and imago fruit fly that appear (Putri, 2016) and the greater the total flavonoid content, the higher the antibacterial activity.[9,10] Kersen leaves are believed to have the ability as antibacterial to *Streptococcus mutans* which have glucosyltransferase enzymes (GTF).[11]

Another study stated that the antioxidant activity of extract of noni leaf was smaller with IC_{50} value of 98.68 µg/mL than noni leaf infusion with IC_{50} value of 75.65 µg/mL.[12] There were differences in the results of antioxidant activity using cold (extraction) and heat (infusa) on medicinal plants. As we know, people use medicinal plants by boiling the plants. Even though the effects of infusion or heating can damage the secondary metabolites in the plant.[13]

The epiphyte has a chemical compound similar to the host plant it occupies. In another study, ethanol extract of kepel parasite leaves has higher antioxidant activity (IC_{50} value 6.43 µg/mL) than ethanol extract of leaves of kepel (IC_{50} value 12.57 ± 0.7 µg/mL).[14,15] It is also expected that the associated epiphyte will contain antioxidant activity. In general, Kersen leaf and epiphyte of the truth contain flavonoids which have antioxidant power.

2. Research Methods

This study is an experimental analytic study with post-test only control group design to determine the ratio of antioxidant levels to leaves and epiphyte kersen (*Muntingia calabura* L.). The study was conducted from October to November 2018 in the Laboratory.

Biochemistry, Faculty of Medicine, Sriwijaya University. The object of this researchis a green plant (*Muntingia calabura* L.) which will be extracted in cold (extraction) and hot (infusa). The parts of the plant to be sampled are kersen leaves and epiphyte. The criteria for this research object are fresh, perfectly shaped and clean dark green leaves and parasitic leaves.

Data analysis was performed using the Statistical Package for Social Science (SPSS) program and Linear Regression Test to determine the direction and relationship between the independent variables and the dependent variable and to predict the value of the dependent variable if it increases or decreases.

3. Results

Table 1 below presents the absorbance values and percent inhibition of kersen leaf extract. From the 6 concentrations of the kersen leaves, the highest inhibition was obtained at 49.17279% at a concentration of 100 ppm and the lowest was 43.060666% at a concentration of 10 ppm.

Table 2 below shows the absorbance and percent inhibition values of the parasite leaf extract. From the 6 concentrations of parasitic leaves, the highest percentage inhibition was 49.17279% at a concentration of 100 ppm and the lowest was 39.52206% at a concentration of 10 ppm.

Table 3 below shows the absorbance value and the percentage of inhibition in the infusion of kersen leaves. From the 6 concentrations of the kersen leaves, the highest inhibition was obtained at 47.61029% at a concentration of 100 ppm and the lowest was 45.18382% at a concentration of 10 ppm.

Table 4 below shows the absorbance value and percent inhibition of epiphyte infusion. From the 6 concentrations of epiphyte leaf, the highest inhibition was obtained at 48.71324% at a concentration of 100 ppm and the lowest was 44.71507% at a concentration of 10 ppm.

The measurement of antioxidant activity was carried out using linear regression analysis in SPSS. Linear regression analysis was used to see how much

influence x (concentration) has on y (% inhibition) so that the results of linear regression equation can be seen the value of x as IC_{50} (Inhibitory Consentration 50) by replacing the y value to 50. In table 5 below shows that leaf extract cherry leaf has a moderate antioxidant activity with an IC_{50} value of 113.801 ppm.

In **table 6** below, it shows that epiphyte extract has a strong antioxidant activity with IC50 value of 98.7802 ppm.

In **table 7** below, it shows that the infusion of kersen leaves has a weak antioxidant activity, that is, with an IC_{50} value of 191.7624 ppm.

In **table 8** below, it shows that the parasite leaf infusion has a moderate antioxidant activity with an IC_{50} value of 98.7802 ppm.

In **table 9** below, the results of antioxidant extracts of ethanol extract of leaves and epiphyte plants (*Muntingia calabura* L.) are presented. From the sample, the highest antioxidant activity was found in the epiphyte extract with an IC_{50} value of 98,7802 ppm, compared to kersen leaf extract with an IC_{50} value of 113.801 ppm.

In **table 10** below, the results of antioxidant activity of leaf infusion and epiphyte are obtained from plants (Muntingia calabura L.). From the sample, the highest antioxidant activity was obtained in epiphyte infusion with IC50 value 131.6750 ppm, compared to kersen leaf infusion with IC50 value of 191.7624 ppm.

Table 1. Absorbance and Percent Inhibition of Kersen Leaf Extract

Extract	Concentration (ppm)	Absorbance	Absorbance Of DPPH	% Inhibition
Kersen Leaf	10	0.619	1.088	43.06066
	20	0.609	1.088	44.02574
	30	0.598	1.088	44.99081
	50	0.588	1.088	45.95588
	70	0.577	1.088	46.92096
	100	0.553	1.088	49.17279

Table 2. Absorbance and Percent Inhibition of Epiphyte Extract

Extract	Concentration (ppm)	Absorbance	Absorbance DPPH	% Inhibition
Epiphyte	10	0.658	1.088	39.52206
	20	0.6335	1.088	41.77390
	30	0.623	1.088	42.73897
	50	0.595	1.088	45.31250
	70	0.567	1.088	47.88603
	100	0.553	1.088	49.17279

Table 3. Absorbance and Percent Inhibit Kersen Leaf Infusion

Infusion	Concentration (ppm)	Absorbance Sample	Absorbance DPPH	% Inhibition
Kersen Leaf	10	0.5964	1.088	45.18382
	20	0.5928	1.088	45.51471
	30	0.5892	1.088	45.84559
	50	0.5832	1.088	46.39706

	70	0.5796	1.088	46.72794
	100	0.57	1.088	47.61029

Table 4. Absorbance and Percent of inhibition of Epiphyte Infusa

Infusion	Concentration (ppm)	Absorbance Sample	Absorbance DPPH	% Inhibition
Epiphyte	10	0.6015	1.088	44.71507
	20	0.5895	1.088	45.81801
	30	0.585	1.088	46.23162
	50	0.5805	1.088	46.64522
	70	0.5715	1.088	47.47243
	100	0.558	1.088	48.71324

Table 5. Antioxidant Activity of Kersen Leaf Extract

Sample	Regression Value	Equation IC50	Antioxidant Activity
Kersen Leaf Extract	Y = 0.0642 x + 42.649	113.801 ppm	Moderate

Table 6. Antioxidant Activity of Epiphyte Extract

Sample	Regression Value	Equation IC50	Antioxidant Activity
Epiphyte Extract	Y = 0.107 x + 39.391	98.7802 ppm	Strong

Table 7. Antioxidant Activity of Kersen Leaf Infusion

Sample	Regression Value	Equation IC50	Antioxidant Activity
Kersen Leaf Infusion	Y = 0.0261 x + 44.995	191.7624 ppm	Weak

Table 8. Antioxidant Activity of Epiphyte Infusa

Sample	Value Regression	Equation IC50	Antioxidant Activity
Epiphyte Infusion	Y = 0.04 x + 44.733	131.6754 ppm	Moderate

Table 9. Antioxidant Activity of Ethanol Extract of Kersen Plant (Muntingia calabura L.)

Sample	IC50 Value
Kersen Leaf Extract	113.801 ppm
Epiphyte Leaf Extract	98.7802 ppm

Table 10. Antioxidant Activity of Kersen Infusion (Muntingia calabura L.)

Sample	IC50 Value
Kersen Leaf Infusion	191.7624 ppm
Epiphyte Leaf Infusion	131.6750ppm

4. Discussion

An increase in% inhibition of ethanol extract indicated that an increase in extract concentration would affect the ability of extracts to soak free radicals.

This result is supported by research that states the percentage inhibition (percent inhibition) of free radical activity will also increase along with concentration increasing.[16]

The results of the existence of ethanol extract of kersen leaves have an IC_{50} value of 113.801 ppm, classified as a moderate antioxidant (100-150 ppm). While the ethanol extract of the epiphyte has an IC_{50} value of 98.7802 ppm, classified as a very strong antioxidant (50-100 ppm). This shows that the leaves and epiphyte in kersen plants have antioxidant activity that is classified as strong with the highest antioxidant activity found in epiphyte, because the smaller the IC_{50} value the greater the antioxidant activity.

The results of this study are in accordance with previous studies using different objects namely kepel plants, where ethanol extract of kepel parasite leaves had higher antioxidant activity (IC_{50} value 6.43 ppm) than ethanol extract of kepel leaves (IC_{50} value 12.57 ± 0.7 ppm).[14,17]

Another theory which supports ethanol extract of the epiphyte has antioxidant activity that is higher than the leaves of the stem cell because the epiphyte contain more antioxidant compounds than the stem cell. Antioxidant compounds found in epiphyte are amino acids, carbohydrates, alkaloids, saponins, flavonoids, alkaloids, terpenoids and tannins.[18,19]While the antioxidant compounds found in kersen leaves are active components of saponins, flavonoids and tannins.[20]

In this study, infusion (heating) was carried out by soaking the plants with 500 ml of water to boil so that the kersen leaves infusion were obtained with an IC_{50} value of 191.7624 ppm, classified as a weak antioxidant (150-200 ppm). While the parasite leaf infusion has an IC_{50} value of 131.675 ppm, classified as a moderate antioxidant (100-150 ppm).

The results of this study are in accordance with previous studies using different objects, namely on the soursop plant, where the infusion of leaves of soursop parasites has a higher antioxidant activity (IC_{50} value 33.5 ppm) than the infusion of soursop leaves (IC50 value 45 ppm).[15]

When compared between ethanol extract and infusion from each part of the plant, the results showed that the preparation of ethanol extract had a stronger antioxidant activity than infusion. The results of this study are in accordance with previous studies that kersen leaf infusion has a higher antioxidant activity of 196.80 ppm, whereas in ethanol extract, kersen leaves have antioxidant activity of 164.12 ppm.[21,22]

The heating process of the infusa which can cause a reduction or damage to the content of antioxidant compounds found in the leaves and leaves of the parasite, the plant seeds (*Muntingia calabura* L.). Cersen plant has phenolic compounds. Phenolic compounds is one of the constituents of green plants which act as antioxidants. Extraction at temperatures above 60 ^{o}C can reduce phenolic compounds found in plants[23] and in this study it eas heated at temperature of 100^{o}C. The use of heating at a high enough temperature will damage the active compounds contained in simplicia, especially antioxidant compounds.[24] In addition, compounds flavonoids and tannins found in plants are heat resistant.[25] Antioxidant activity decreases with increasing temperature and length of heating time.[26] This is because the longer the oven drying process (heating), the more free water content evaporates so that the mass of the dry material produced will also decrease.[27]

5. Conclusion

Chrysanthemum plants (*Muntingia calabura* L.) have antioxidant activity in the leaves and epiphyte. Epiphyte extract has stronger antioxidant activity than kersen leaf extract. Epiphyte extract has stronger antioxidant activity than the kersen leaves. The extract of the extract has a higher antioxidant activity than infusion.

6. Thanks You Note

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Submission acknowledgement

Dear author(s),

Nurlutfiyyah Aini, Nita Parisa*, Fatmawati has submitted the manuscript “Assesment of Antioxidant Activity Test of Kersen Leaf (*Muntingia calabura L.*) and Epiphyte with DPPH (2.2-Diphenyl-1-Picrylhidrazyl)“ to Archives of The Medicine and Case Reports. The paper will be screened by editor and reviewed by peer review.

Cordially,

Prof. Paula Magnano, PhD

Editor HM Publisher

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Peer Review Results

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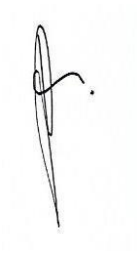
Cordially,



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Reviewer 1: Revision required

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ABSTRACT→3

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Another study stated that the antioxidant activity of extract of noni leaf was smaller with IC_{50} value of 98.68 µg/mL than noni leaf infusion with IC_{50} value of 75.65 µg/mL.[12] There were differences in the results of antioxidant activity using cold (extraction) and heat (infusa) on medicinal plants. As we know, people use medicinal plants by boiling the plants. Even though the effects of infusion or heating can damage the secondary metabolites in the plant.[13]

The epiphyte has a chemical compound similar to the host plant it occupies. In another study, ethanol extract of kepel parasite leaves has higher antioxidant activity (IC_{50} value 6.43 µg/mL) than ethanol extract of leaves of kepel (IC_{50} value 12.57 ± 0.7 µg/mL).[14,15] It is also expected that the associated epiphyte will contain antioxidant activity. In general, Kersen leaf and epiphyte of the truth contain flavonoids which have antioxidant power.

2. Research Methods→5

This study is an experimental analytic study with post-test only control group design to determine the ratio of antioxidant levels to leaves and epiphyte kersen (*Muntingia calabura* L.). The study was conducted from October to November 2018 in the Laboratory.

Biochemistry, Faculty of Medicine, Sriwijaya University. The object of this researchis a green plant (*Muntingia calabura* L.) which will be extracted in cold (extraction) and hot (infusa). The parts of the plant to be sampled are kersen leaves and epiphyte. The criteria for this research object are fresh, perfectly shaped and clean dark green leaves and parasitic leaves.

Data analysis was performed using the Statistical Package for Social Science (SPSS) program and Linear Regression Test to determine the direction and relationship between the independent variables and the dependent variable and to predict the value of the dependent variable if it increases or decreases.

3. Results→6

Table 1 below presents the absorbance values and percent inhibition of kersen leaf extract. From the 6 concentrations of the kersen leaves, the highest inhibition was obtained at 49.17279% at a concentration of 100 ppm and the lowest was 43.060666% at a concentration of 10 ppm.

Table 2 below shows the absorbance and percent inhibition values of the parasite leaf extract. From the 6 concentrations of parasitic leaves, the highest percentage inhibition was 49.17279% at a concentration of 100 ppm and the lowest was 39.52206% at a concentration of 10 ppm.

Table 3 below shows the absorbance value and the percentage of inhibition in the infusion of kersen leaves. From the 6 concentrations of the kersen leaves, the highest inhibition was obtained at 47.61029% at a concentration of 100 ppm and the lowest was 45.18382% at a concentration of 10 ppm.

Table 4 below shows the absorbance value and percent inhibition of epiphyte infusion. From the 6 concentrations of epiphyte leaf, the highest inhibition was obtained at 48.71324% at a concentration of 100 ppm and the lowest was 44.71507% at a concentration of 10 ppm.

The measurement of antioxidant activity was carried out using linear regression analysis in SPSS. Linear regression analysis was used to see how much

influence x (concentration) has on y (% inhibition) so that the results of linear regression equation can be seen the value of x as IC_{50} (Inhibitory Consentration 50) by replacing the y value to 50. In table 5 below shows that leaf extract cherry leaf has a moderate antioxidant activity with an IC_{50} value of 113.801 ppm.

In **table 6** below, it shows that epiphyte extract has a strong antioxidant activity with IC50 value of 98.7802 ppm.

In **table 7** below, it shows that the infusion of kersen leaves has a weak antioxidant activity, that is, with an IC_{50} value of 191.7624 ppm.

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In **table 9** below, the results of antioxidant extracts of ethanol extract of leaves and epiphyte plants (*Muntingia calabura* L.) are presented. From the sample, the highest antioxidant activity was found in the epiphyte extract with an IC_{50} value of 98,7802 ppm, compared to kersen leaf extract with an IC_{50} value of 113.801 ppm.

In **table 10** below, the results of antioxidant activity of leaf infusion and epiphyte are obtained from plants (Muntingia calabura L.). From the sample, the highest antioxidant activity was obtained in epiphyte infusion with IC50 value 131.6750 ppm, compared to kersen leaf infusion with IC50 value of 191.7624 ppm.

Table 1. Absorbance and Percent Inhibition of Kersen Leaf Extract

Extract	Concentration (ppm)	Absorbance	Absorbance Of DPPH	% Inhibition
Kersen Leaf	10	0.619	1.088	43.06066
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Table 3. Absorbance and Percent Inhibit Kersen Leaf Infusion

Infusion	Concentration (ppm)	Absorbance Sample	Absorbance DPPH	% Inhibition
Kersen Leaf	10	0.5964	1.088	45.18382
	20	0.5928	1.088	45.51471
	30	0.5892	1.088	45.84559
	50	0.5832	1.088	46.39706

	70	0.5796	1.088	46.72794
	100	0.57	1.088	47.61029

Table 4. Absorbance and Percent of inhibition of Epiphyte Infusa

Infusion	Concentration (ppm)	Absorbance Sample	Absorbance DPPH	% Inhibition
Epiphyte	10	0.6015	1.088	44.71507
	20	0.5895	1.088	45.81801
	30	0.585	1.088	46.23162
	50	0.5805	1.088	46.64522
	70	0.5715	1.088	47.47243
	100	0.558	1.088	48.71324

Table 5. Antioxidant Activity of Kersen Leaf Extract

Sample	Regression Value	Equation IC50	Antioxidant Activity
Kersen Leaf Extract	Y = 0.0642 x + 42.649	113.801 ppm	Moderate

Table 6. Antioxidant Activity of Epiphyte Extract

Sample	Regression Value	Equation IC50	Antioxidant Activity
Epiphyte Extract	Y = 0.107 x + 39.391	98.7802 ppm	Strong

Table 7. Antioxidant Activity of Kersen Leaf Infusion

Sample	Regression Value	Equation IC50	Antioxidant Activity
Kersen Leaf Infusion	Y = 0.0261 x + 44.995	191.7624 ppm	Weak

Table 8. Antioxidant Activity of Epiphyte Infusa

Sample	Value Regression	Equation IC50	Antioxidant Activity
Epiphyte Infusion	Y = 0.04 x + 44.733	131.6754 ppm	Moderate

Table 9. Antioxidant Activity of Ethanol Extract of Kersen Plant (Muntingia calabura L.)

Sample	IC50 Value
Kersen Leaf Extract	113.801 ppm
Epiphyte Leaf Extract	98.7802 ppm

Table 10. Antioxidant Activity of Kersen Infusion (Muntingia calabura L.)

Sample	IC50 Value
Kersen Leaf Infusion	191.7624 ppm
Epiphyte Leaf Infusion	131.6750ppm

4. Discussion→7

An increase in% inhibition of ethanol extract indicated that an increase in extract concentration would affect the ability of extracts to soak free radicals.

This result is supported by research that states the percentage inhibition (percent inhibition) of free radical activity will also increase along with concentration increasing.[16]

The results of the existence of ethanol extract of kersen leaves have an IC_{50} value of 113.801 ppm, classified as a moderate antioxidant (100-150 ppm). While the ethanol extract of the epiphyte has an IC_{50} value of 98.7802 ppm, classified as a very strong antioxidant (50-100 ppm). This shows that the leaves and epiphyte in kersen plants have antioxidant activity that is classified as strong with the highest antioxidant activity found in epiphyte, because the smaller the IC_{50} value the greater the antioxidant activity.

The results of this study are in accordance with previous studies using different objects namely kepel plants, where ethanol extract of kepel parasite leaves had higher antioxidant activity (IC_{50} value 6.43 ppm) than ethanol extract of kepel leaves (IC_{50} value 12.57 ± 0.7 ppm).[14,17]

Another theory which supports ethanol extract of the epiphyte has antioxidant activity that is higher than the leaves of the stem cell because the epiphyte contain more antioxidant compounds than the stem cell. Antioxidant compounds found in epiphyte are amino acids, carbohydrates, alkaloids, saponins, flavonoids, alkaloids, terpenoids and tannins.[18,19]While the antioxidant compounds found in kersen leaves are active components of saponins, flavonoids and tannins.[20]

In this study, infusion (heating) was carried out by soaking the plants with 500 ml of water to boil so that the kersen leaves infusion were obtained with an IC_{50} value of 191.7624 ppm, classified as a weak antioxidant (150-200 ppm). While the parasite leaf infusion has an IC_{50} value of 131.675 ppm, classified as a moderate antioxidant (100-150 ppm).

The results of this study are in accordance with previous studies using different objects, namely on the soursop plant, where the infusion of leaves of soursop parasites has a higher antioxidant activity (IC_{50} value 33.5 ppm) than the infusion of soursop leaves (IC50 value 45 ppm).[15]

When compared between ethanol extract and infusion from each part of the plant, the results showed that the preparation of ethanol extract had a stronger antioxidant activity than infusion. The results of this study are in accordance with previous studies that kersen leafinfusion has a higher antioxidant activity of 196.80 ppm, whereas in ethanol extract, kersen leaves have antioxidant activity of 164.12 ppm.[21,22]

The heating process of the infusa which can cause a reduction or damage to the content of antioxidant compounds found in the leaves and leaves of the parasite, the plant seeds (*Muntingia calabura* L.). Cersen plant has phenolic compounds. Phenolic compounds is one of the constituents of green plants which act as antioxidants. Extraction at temperatures above 60 ^{o}C can reduce phenolic compounds found in plants[23] and in this study it eas heated at temperature of 100^{o}C. The use of heating at a high enough temperature will damage the active compounds contained in simplicia, especially antioxidant compounds.[24] In addition, compounds flavonoids and tannins found in plants are heat resistant.[25] Antioxidant activity decreases with increasing temperature and length of heating time.[26] This is because the longer the oven drying process (heating), the more free water content evaporates so that the mass of the dry material produced will also decrease.[27]

5. Conclusion→8

Chrysanthemum plants (*Muntingia calabura* L.) have antioxidant activity in the leaves and epiphyte. Epiphyte extract has stronger antioxidant activity than kersen leaf extract. Epiphyte extract has stronger antioxidant activity than the kersen leaves. The extract of the extract has a higher antioxidant activity than infusion.

6. Thanks You Note

The author would like to thank Ms. Fatmawati, S.Si, M.Sc, dr. Nita Parisa M.Bmd for guidance during this research. The researcher also thanked Mr. Drs. Sadakata Sinulingga, Apt, M.Kes., And dr. Veny Larasaty, M. Biomed for suggestions and input given.

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Reviewer Comment:

1→ Title of Manuscripts should be explained independent variable and dependent variable also subject of study.

2→ Keywords should be showed the main words of the study, the authors can use MeSH to develop keywords.

3→ Abstract should be showed the main of background, methods, results and conclusion of study.

- Background abstract should be showed the urgency of study and why the study important, in simple way.
- Conclusion should be wrote in simple way, specific to the main results. Conclusion in abstract should not showed statistic results.

4→Introduction should be showed the urgency of study (epidemiology data), biological plausibility concept, and lack of knowledge in the study.

- Paragraph 1→ need improvement in urgency of study and explain more about epidemiology data. Authors do not only show the data, but try to elaborate and make comparison about the data from year to year.
- Paragraph 2 and 3 need improvement to focus in biological plausibility concept.

5→ Methods should be showed more about how the study develop. Methods should be showed the design of study; population, sample and sample size of study; inclusion criteria; place of study; ethical clearence steatment; independent and dependent variable; data

analysis.

- Methods need to showed the design of study; population, sample and sample size of study; inclusion criteria; place of study; ethical clearence steatment; independent and dependent variable; data analysis, more specific but not to long.

6→ Results should be showed baseline characteristics subject of study, main results of study. Authors must be focused and try to make results no more table and figure.

7→ Discussion should be explored more biological plausibility, not only showed about statistical results.

8→ Conclusion should more specific and not more showed statistical results

9→ Authors must check the references for make update references. References should no more than 10 years.

Reviewer 2: Revision required

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Assesment of Antioxidant Activity Test of Kersen Leaf (*Muntingia calabura* L.) and Epiphyte with DPPH(2.2-Diphenyl-1-Picrylhidrazyl) →1

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ABSTRACT→3

Antioxidant is very important to give protection against free radical activity and highly reactive molecules that could lead in slowing the progre ssion of de ge nerative disease. In case of de ge nerative disease, internal antioxidant cannot neutralize the increasing concentration of free radical. Because of that, human needs external antioxidant. Kersen (Muntingia calabura L.) is a plant that is known for its antioxidant content. Plants containing antioxidant experience is kersen (Muntingia calabura L.). Research study to determine the antioxidant activity of Kersen plant and knows the difference of antioxidant activity, based on the process of extract and infusion. Research was done by experimental study which was oriented in testing antioxidant activity in (Morinda citrifolia L.) extract and infusion. Extraction was done by using 96% ethanol as solvent, meanwhile infusion was made by using aquadest. Extract and infusion was divided into group of concentration and antioxidant activity was tested by DPPH(2,2-Diphenyl-1-Picrylhidrazyl) method by measuring the absorbance using spectrophotometer at 520 nm wavelength. Percentage of DPPH inhibition and IC50 then analyze d using linear re gre ssion analysis. Ethanolic extract of kersen leaf and epiphyte had IC50 value of 113,801 ppm and 98,7802 ppm, respectively. Kersen leaf infusion showed 191,7624 ppm IC50 values, besides its epiphyte had 131,6750 ppm . Antioxidant activity of Muntingia calabura L. in the order from kersen leaf an epiphyte, and epiphyte extract has a higher antioxidant content than others.

1. Introduction→4

Indonesia is one of the countries which has a high level biodiversity in the world, 3rd ranked after Brazil and Zaire. The biodiversity includes plants of flora and fauna which are spread throughout Indonesia.[1] There are 40,000 species of flora that grow in the world, 30.000 species found in Indonesia with 1.845 species of plants have the potential as traditional medicine.[2] According to the POM Agency (2006), there are 283 types of plants that have been registered for the use of traditional medicines. Only 13 of the 283 types of medicinal plants that have been cultivated. Traditional medicine is widely used to treat several diseases such urolithiasis, diabetes, high blood pressure and others.

The ability of a plant as a drug is caused by the content of chemical compounds or active compounds that have the working power of treatment. One of the chemical constituents that has the working power of treatment is antioxidant.[3] The emergence of free radicals (hydroxyl) in biochemical mechanisms in the body are the causes of degenerative diseases.[4] Degenerative diseases, such as osteoporosis, cardiovascular, cancer, diabetes mellitus and others can be reduced by consuming antioxidants. This is related to the work system of antioxidants which can inhibit oxidation reactions, by binding free radicals and molecules which are very reactive.[5]

One potential source of natural antioxidants is plants because they contain flavonoid compounds, chlorophyll and tannin.[6] Kersen (*Muntingia calabura* L.) is a plant that has the potential to be a natural antioxidant.[7] Antioxidants kersen (*Muntingia calabura* L.) are found in all parts of flowers, fruit and leaves, and the highest activity on the part of the leaf. Various studies show that kersen leaves contain active components of saponins, flavonoids and tannins, when extracted using methanol and ethanol solvents.[8]

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	30	0.5892	1.088	45.84559
	50	0.5832	1.088	46.39706

	70	0.5796	1.088	46.72794
	100	0.57	1.088	47.61029

Table 4. Absorbance and Percent of inhibition of Epiphyte Infusa

Infusion	Concentration (ppm)	Absorbance Sample	Absorbance DPPH	% Inhibition
Epiphyte	10	0.6015	1.088	44.71507
	20	0.5895	1.088	45.81801
	30	0.585	1.088	46.23162
	50	0.5805	1.088	46.64522
	70	0.5715	1.088	47.47243
	100	0.558	1.088	48.71324

Table 5. Antioxidant Activity of Kersen Leaf Extract

Sample	Regression Value	Equation IC50	Antioxidant Activity
Kersen Leaf Extract	Y = 0.0642 x + 42.649	113.801 ppm	Moderate

Table 6. Antioxidant Activity of Epiphyte Extract

Sample	Regression Value	Equation IC50	Antioxidant Activity
Epiphyte Extract	Y = 0.107 x + 39.391	98.7802 ppm	Strong

Table 7. Antioxidant Activity of Kersen Leaf Infusion

Sample	Regression Value	Equation IC50	Antioxidant Activity
Kersen Leaf Infusion	Y = 0.0261 x + 44.995	191.7624 ppm	Weak

Table 8. Antioxidant Activity of Epiphyte Infusa

Sample	Value Regression	Equation IC50	Antioxidant Activity
Epiphyte Infusion	Y = 0.04 x + 44.733	131.6754 ppm	Moderate

Table 9. Antioxidant Activity of Ethanol Extract of Kersen Plant (Muntingia calabura L.)

Sample	IC50 Value
Kersen Leaf Extract	113.801 ppm
Epiphyte Leaf Extract	98.7802 ppm

Table 10. Antioxidant Activity of Kersen Infusion (Muntingia calabura L.)

Sample	IC50 Value
Kersen Leaf Infusion	191.7624 ppm
Epiphyte Leaf Infusion	131.6750ppm

4. Discussion→7

An increase in% inhibition of ethanol extract indicated that an increase in extract concentration would affect the ability of extracts to soak free radicals.

This result is supported by research that states the percentage inhibition (percent inhibition) of free radical activity will also increase along with concentration increasing.[16]

The results of the existence of ethanol extract of kersen leaves have an IC_{50} value of 113.801 ppm, classified as a moderate antioxidant (100-150 ppm). While the ethanol extract of the epiphyte has an IC_{50} value of 98.7802 ppm, classified as a very strong antioxidant (50-100 ppm). This shows that the leaves and epiphyte in kersen plants have antioxidant activity that is classified as strong with the highest antioxidant activity found in epiphyte, because the smaller the IC_{50} value the greater the antioxidant activity.

The results of this study are in accordance with previous studies using different objects namely kepel plants, where ethanol extract of kepel parasite leaves had higher antioxidant activity (IC_{50} value 6.43 ppm) than ethanol extract of kepel leaves (IC_{50} value 12.57 ± 0.7 ppm).[14,17]

Another theory which supports ethanol extract of the epiphyte has antioxidant activity that is higher than the leaves of the stem cell because the epiphyte contain more antioxidant compounds than the stem cell. Antioxidant compounds found in epiphyte are amino acids, carbohydrates, alkaloids, saponins, flavonoids, alkaloids, terpenoids and tannins.[18,19]While the antioxidant compounds found in kersen leaves are active components of saponins, flavonoids and tannins.[20]

In this study, infusion (heating) was carried out by soaking the plants with 500 ml of water to boil so that the kersen leaves infusion were obtained with an IC_{50} value of 191.7624 ppm, classified as a weak antioxidant (150-200 ppm). While the parasite leaf infusion has an IC_{50} value of 131.675 ppm, classified as a moderate antioxidant (100-150 ppm).

The results of this study are in accordance with previous studies using different objects, namely on the soursop plant, where the infusion of leaves of soursop parasites has a higher antioxidant activity (IC_{50} value 33.5 ppm) than the infusion of soursop leaves (IC50 value 45 ppm).[15]

When compared between ethanol extract and infusion from each part of the plant, the results showed that the preparation of ethanol extract had a stronger antioxidant activity than infusion. The results of this study are in accordance with previous studies that kersen leaf infusion has a higher antioxidant activity of 196.80 ppm, whereas in ethanol extract, kersen leaves have antioxidant activity of 164.12 ppm.[21,22]

The heating process of the infusa which can cause a reduction or damage to the content of antioxidant compounds found in the leaves and leaves of the parasite, the plant seeds (*Muntingia calabura* L.). Cersen plant has phenolic compounds. Phenolic compounds is one of the constituents of green plants which act as antioxidants. Extraction at temperatures above 60 ^{o}C can reduce phenolic compounds found in plants[23] and in this study it eas heated at temperature of 100^{o}C. The use of heating at a high enough temperature will damage the active compounds contained in simplicia, especially antioxidant compounds.[24] In addition, compounds flavonoids and tannins found in plants are heat resistant.[25] Antioxidant activity decreases with increasing temperature and length of heating time.[26] This is because the longer the oven drying process (heating), the more free water content evaporates so that the mass of the dry material produced will also decrease.[27]

5. Conclusion→8

Chrysanthemum plants (*Muntingia calabura* L.) have antioxidant activity in the leaves and epiphyte. Epiphyte extract has stronger antioxidant activity than kersen leaf extract. Epiphyte extract has stronger antioxidant activity than the kersen leaves. The extract of the extract has a higher antioxidant activity than infusion.

6. Thanks You Note

The author would like to thank Ms. Fatmawati, S.Si, M.Sc, dr. Nita Parisa M.Bmd for guidance during this research. The researcher also thanked Mr. Drs. Sadakata Sinulingga, Apt, M.Kes., And dr. Veny Larasaty, M. Biomed for suggestions and input given.

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Reviewer Comment:

1→ Title of Manuscripts should be explained independent variable and dependent variable also subject of study.

2→ Keywords should be showed the main words of the study, the authors can use MeSH to develop keywords.

3→ Abstract should be showed the main of background, methods, results and conclusion of study.

- Background abstract should be showed the urgency of study and why the study important, in simple way.
- Conclusion should be wrote in simple way, specific to the main results. Conclusion in abstract should not showed statistic results.

4→Introduction should be showed the urgency of study (epidemiology data), biological plausibility concept, and lack of knowledge in the study.

- Paragraph 1→ need improvement in urgency of study and explain more about epidemiology data. Authors do not only show the data, but try to elaborate and make comparison about the data from year to year.
- Paragraph 2 and 3 need improvement to focus in biological plausibility concept.

5→ Methods should be showed more about how the study develop. Methods should be showed the design of study; population, sample and sample size of study; inclusion criteria; place of study; ethical clearence steatment; independent and dependent variable; data

analysis.

- Methods need to showed the design of study; population, sample and sample size of study; inclusion criteria; place of study; ethical clearence steatment; independent and dependent variable; data analysis, more specific but not to long.

6→ Results should be showed baseline characteristics subject of study, main results of study. Authors must be focused and try to make results no more table and figure.

7→ Discussion should be explored more biological plausibility, not only showed about statistical results.

8→ Conclusion should more specific and not more showed statistical results

9→ Authors must check the references for make update references. References should no more than 10 years.

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Assesment of Antioxidant Activity Test of Kersen Leaf (*Muntingia calabura* L.) and Epiphyte with DPPH(2.2-Diphenyl-1-Picrylhidrazyl)

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ABSTRACT

Antioxidant is very important to give protection against free radical activity and highly reactive molecules that could lead in slowing the progression of degenerative disease. In case of degenerative disease, internal antioxidant cannot neutralize the increasing concentration of free radical. Because of that, human needs external antioxidant. Kersen (Muntingia calabura L.) is a plant that is known for its antioxidant content. Plants containing antioxidant experience is kersen (Muntingia calabura L.). Research study to determine the antioxidant activity of Kersen plant and knows the difference of antioxidant activity, based on the process of extract and infusion. Research was done by experimental study which was oriented in testing antioxidant activity in (Morinda citrifolia L.) extract and infusion. Extraction was done by using 96% ethanol as solvent, meanwhile infusion was made by using aquadest. Extract and infusion was divided into group of concentration and antioxidant activity was tested by DPPH(2,2-Diphenyl-1-Picrylhidrazyl) method by measuring the absorbance using spectrophotometer at 520 nm wavelength. Percentage of DPPH inhibition and IC50 then analyzed using linear regression analysis. Ethanolic extract of kersen leaf and epiphyte had IC50 value of 113,801 ppm and 98,7802 ppm, respectively. Kersen leaf infusion showed 191,7624 ppm IC50 values, besides its epiphyte had 131,6750 ppm. Antioxidant activity of Muntingia calabura L. in the order from kersen leaf an epiphyte, and epiphyte extract has a higher antioxidant content than others.

1. Introduction

Indonesia is one of the countries which has a high level biodiversity in the world, 3rd ranked after Brazil and Zaire. The biodiversity includes plants of flora and fauna which are spread throughout Indonesia.[1] There are 40,000 species of flora that grow in the world, 30.000 species found in Indonesia with 1.845 species of plants have the potential as traditional medicine.[2] According to the POM Agency (2006), there are 283 types of plants that have been registered for the use of traditional medicines. Only 13 of the 283 types of medicinal plants that have been cultivated. Traditional medicine is widely used to treat several diseases such urolithiasis, diabetes, high blood pressure and others.

The ability of a plant as a drug is caused by the content of chemical compounds or active compounds that have the working power of treatment. One of the chemical constituents that has the working power of treatment is antioxidant.[3] The emergence of free radicals (hydroxyl) in biochemical mechanisms in the body are the causes of degenerative diseases.[4] Degenerative diseases, such as osteoporosis, cardiovascular, cancer, diabetes mellitus and others can be reduced by consuming antioxidants. This is related to the work system of antioxidants which can inhibit oxidation reactions, by binding free radicals and molecules which are very reactive.[5]

One potential source of natural antioxidants is plants because they contain flavonoid compounds, chlorophyll and tannin.[6] Kersen (*Muntingia calabura* L.) is a plant that has the potential to be a natural antioxidant.[7] Antioxidants kersen (*Muntingia calabura* L.) are found in all parts of flowers, fruit and leaves, and the highest activity on the part of the leaf. Various studies show that kersen leaves contain active components of saponins, flavonoids and tannins, when extracted using methanol and ethanol solvents.[8]

The administration of the ethanol extract of the leaves has an effect on B.*carambolae* fruit fly. The higher the concentration of extracts, the lower the number of pupae and imago fruit fly that appear (Putri, 2016) and the greater the total flavonoid content, the higher the antibacterial activity.[9,10] Kersen leaves are believed to have the ability as antibacterial to *Streptococcus mutans* which have glucosyltransferase enzymes (GTF).[11]

Another study stated that the antioxidant activity of extract of noni leaf was smaller with IC_{50} value of 98.68 µg/mL than noni leaf infusion with IC_{50} value of 75.65 µg/mL.[12] There were differences in the results of antioxidant activity using cold (extraction) and heat (infusa) on medicinal plants. As we know, people use medicinal plants by boiling the plants. Even though the effects of infusion or heating can damage the secondary metabolites in the plant.[13]

The epiphyte has a chemical compound similar to the host plant it occupies. In another study, ethanol extract of kepel parasite leaves has higher antioxidant activity (IC_{50} value 6.43 µg/mL) than ethanol extract of leaves of kepel (IC_{50} value 12.57 ± 0.7 µg/mL).[14,15] It is also expected that the associated epiphyte will contain antioxidant activity. In general, Kersen leaf and epiphyte of the truth contain flavonoids which have antioxidant power.

2. Research Methods

This study is an experimental analytic study with post-test only control group design to determine the ratio of antioxidant levels to leaves and epiphyte kersen (*Muntingia calabura* L.). The study was conducted from October to November 2018 in the Laboratory.

Biochemistry, Faculty of Medicine, Sriwijaya University. The object of this research is a green plant (*Muntingia calabura* L.) which will be extracted in cold (extraction) and hot (infusa). The parts of the plant to be sampled are kersen leaves and epiphyte. The criteria for this research object are fresh, perfectly shaped and clean dark green leaves and parasitic leaves.

Data analysis was performed using the Statistical Package for Social Science (SPSS) program and Linear Regression Test to determine the direction and relationship between the independent variables and the dependent variable and to predict the value of the dependent variable if it increases or decreases.

3. Results

Table 1 below presents the absorbance values and percent inhibition of kersen leaf extract. From the 6 concentrations of the kersen leaves, the highest inhibition was obtained at 49.17279% at a concentration of 100 ppm and the lowest was 43.060666% at a concentration of 10 ppm.

Table 2 below shows the absorbance and percent inhibition values of the parasite leaf extract. From the 6 concentrations of parasitic leaves, the highest percentage inhibition was 49.17279% at a concentration of 100 ppm and the lowest was 39.52206% at a concentration of 10 ppm.

Table 3 below shows the absorbance value and the percentage of inhibition in the infusion of kersen leaves. From the 6 concentrations of the kersen leaves, the highest inhibition was obtained at 47.61029% at a concentration of 100 ppm and the lowest was 45.18382% at a concentration of 10 ppm.

Table 4 below shows the absorbance value and percent inhibition of epiphyte infusion. From the 6 concentrations of epiphyte leaf, the highest inhibition was obtained at 48.71324% at a concentration of 100 ppm and the lowest was 44.71507% at a concentration of 10 ppm.

The measurement of antioxidant activity was carried out using linear regression analysis in SPSS. Linear regression analysis was used to see how much

influence x (concentration) has on y (% inhibition) so that the results of linear regression equation can be seen the value of x as IC_{50} (Inhibitory Consentration 50) by replacing the y value to 50. In table 5 below shows that leaf extract cherry leaf has a moderate antioxidant activity with an IC_{50} value of 113.801 ppm.

In **table 6** below, it shows that epiphyte extract has a strong antioxidant activity with IC50 value of 98.7802 ppm.

In **table 7** below, it shows that the infusion of kersen leaves has a weak antioxidant activity, that is, with an IC_{50} value of 191.7624 ppm.

In **table 8** below, it shows that the parasite leaf infusion has a moderate antioxidant activity with an IC_{50} value of 98.7802 ppm.

In **table 9** below, the results of antioxidant extracts of ethanol extract of leaves and epiphyte plants (*Muntingia calabura* L.) are presented. From the sample, the highest antioxidant activity was found in the epiphyte extract with an IC_{50} value of 98,7802 ppm, compared to kersen leaf extract with an IC_{50} value of 113.801 ppm.

In **table 10** below, the results of antioxidant activity of leaf infusion and epiphyte are obtained from plants (Muntingia calabura L.). From the sample, the highest antioxidant activity was obtained in epiphyte infusion with IC50 value 131.6750 ppm, compared to kersen leaf infusion with IC50 value of 191.7624 ppm.

Table 1. Absorbance and Percent Inhibition of Kersen Leaf Extract

Extract	Concentration (ppm)	Absorbance	Absorbance Of DPPH	% Inhibition
Kersen Leaf	10	0.619	1.088	43.06066
	20	0.609	1.088	44.02574
	30	0.598	1.088	44.99081
	50	0.588	1.088	45.95588
	70	0.577	1.088	46.92096
	100	0.553	1.088	49.17279

Table 2. Absorbance and Percent Inhibition of Epiphyte Extract

Extract	Concentration (ppm)	Absorbance	Absorbance DPPH	% Inhibition
Epiphyte	10	0.658	1.088	39.52206
	20	0.6335	1.088	41.77390
	30	0.623	1.088	42.73897
	50	0.595	1.088	45.31250
	70	0.567	1.088	47.88603
	100	0.553	1.088	49.17279

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Infusion	Concentration (ppm)	Absorbance Sample	Absorbance DPPH	% Inhibition
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	30	0.5892	1.088	45.84559
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	70	0.5796	1.088	46.72794
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Table 4. Absorbance and Percent of inhibition of Epiphyte Infusa

Infusion	Concentration (ppm)	Absorbance Sample	Absorbance DPPH	% Inhibition
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Sample	Regression Value	Equation IC50	Antioxidant Activity
Kersen Leaf Extract	Y = 0.0642 x + 42.649	113.801 ppm	Moderate

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Sample	IC50 Value
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Table 10. Antioxidant Activity of Kersen Infusion (Muntingia calabura L.)

Sample	IC50 Value
Kersen Leaf Infusion	191.7624 ppm
Epiphyte Leaf Infusion	131.6750ppm

4. Discussion

An increase in% inhibition of ethanol extract indicated that an increase in extract concentration would affect the ability of extracts to soak free radicals.

This result is supported by research that states the percentage inhibition (percent inhibition) of free radical activity will also increase along with concentration increasing.[16]

The results of the existence of ethanol extract of kersen leaves have an IC_{50} value of 113.801 ppm, classified as a moderate antioxidant (100-150 ppm). While the ethanol extract of the epiphyte has an IC_{50} value of 98.7802 ppm, classified as a very strong antioxidant (50-100 ppm). This shows that the leaves and epiphyte in kersen plants have antioxidant activity that is classified as strong with the highest antioxidant activity found in epiphyte, because the smaller the IC_{50} value the greater the antioxidant activity.

The results of this study are in accordance with previous studies using different objects namely kepel plants, where ethanol extract of kepel parasite leaves had higher antioxidant activity (IC_{50} value 6.43 ppm) than ethanol extract of kepel leaves (IC_{50} value 12.57 ± 0.7 ppm).[14,17]

Another theory which supports ethanol extract of the epiphyte has antioxidant activity that is higher than the leaves of the stem cell because the epiphyte contain more antioxidant compounds than the stem cell. Antioxidant compounds found in epiphyte are amino acids, carbohydrates, alkaloids, saponins, flavonoids, alkaloids, terpenoids and tannins.[18,19]While the antioxidant compounds found in kersen leaves are active components of saponins, flavonoids and tannins.[20]

In this study, infusion (heating) was carried out by soaking the plants with 500 ml of water to boil so that the kersen leaves infusion were obtained with an IC_{50} value of 191.7624 ppm, classified as a weak antioxidant (150-200 ppm). While the parasite leaf infusion has an IC_{50} value of 131.675 ppm, classified as a moderate antioxidant (100-150 ppm).

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When compared between ethanol extract and infusion from each part of the plant, the results showed that the preparation of ethanol extract had a stronger antioxidant activity than infusion. The results of this study are in accordance with previous studies that kersen leaf infusion has a higher antioxidant activity of 196.80 ppm, whereas in ethanol extract, kersen leaves have antioxidant activity of 164.12 ppm.[21,22]

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5. Conclusion

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Assesment of Antioxidant Activity Test of Kersen Leaf (*Muntingia calabura* L.) and Epiphyte with DPPH(2.2-Diphenyl-1-Picrylhidrazyl)

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ABSTRACT

Antioxidant is very important to give protection against free radical activity and highly reactive molecules that could lead in slowing the progre ssion of de ge nerative disease. In case of de ge nerative disease, internal antioxidant cannot neutralize the increasing concentration of free radical. Because of that, human needs external antioxidant. Kersen (Muntingia calabura L.) is a plant that is known for its antioxidant content. Plants containing antioxidant experience is kersen (Muntingia calabura L.). Research study to determine the antioxidant activity of Kersen plant and knows the difference of antioxidant activity, based on the process of extract and infusion. Research was done by experimental study which was oriented in testing antioxidant activity in (Morinda citrifolia L.) extract and infusion. Extraction was done by using 96% ethanol as solvent, meanwhile infusion was made by using aquadest. Extract and infusion was divided into group of concentration and antioxidant activity was tested by DPPH(2,2-Diphenyl-1-Picrylhidrazyl) method by measuring the absorbance using spectrophotometer at 520 nm wavelength. Percentage of DPPH inhibition and IC50 then analyze d using linear re gre ssion analysis. Ethanolic extract of kersen leaf and epiphyte had IC50 value of 113,801 ppm and 98,7802 ppm, respectively. Kersen leaf infusion showed 191,7624 ppm IC50 values, besides its epiphyte had 131,6750 ppm . Antioxidant activity of Muntingia calabura L. in the order from kersen leaf an epiphyte, and epiphyte extract has a higher antioxidant content than others.

1. Introduction

Indonesia is one of the countries which has a high level biodiversity in the world, 3rd ranked after Brazil and Zaire. The biodiversity includes plants of flora and fauna which are spread throughout Indonesia.[1] There are 40,000 species of flora that grow in the world, 30.000 species found in Indonesia with 1.845 species of plants have the potential as traditional medicine.[2] According to the POM Agency (2006), there are 283 types of plants that have been registered for the use of traditional medicines. Only 13 of the 283 types of medicinal plants that have been cultivated. Traditional medicine is widely used to treat several diseases such urolithiasis, diabetes, high blood pressure and others.

The ability of a plant as a drug is caused by the content of chemical compounds or active compounds that have the working power of treatment. One of the chemical constituents that has the working power of treatment is antioxidant.[3] The emergence of free radicals (hydroxyl) in biochemical mechanisms in the body are the causes of degenerative diseases.[4] Degenerative diseases, such as osteoporosis, cardiovascular, cancer, diabetes mellitus and others can be reduced by consuming antioxidants. This is related to the work system of antioxidants which can inhibit oxidation reactions, by binding free radicals and molecules which are very reactive.[5]

One potential source of natural antioxidants is plants because they contain flavonoid compounds, chlorophyll and tannin.[6] Kersen (*Muntingia calabura* L.) is a plant that has the potential to be a natural antioxidant.[7] Antioxidants kersen (*Muntingia calabura* L.) are found in all parts of flowers, fruit and leaves, and the highest activity on the part of the leaf. Various studies show that kersen leaves contain active components of saponins, flavonoids and tannins, when extracted using methanol and ethanol solvents.[8]

The administration of the ethanol extract of the leaves has an effect on B.*carambolae* fruit fly. The higher the concentration of extracts, the lower the number of pupae and imago fruit fly that appear (Putri, 2016) and the greater the total flavonoid content, the higher the antibacterial activity.[9,10] Kersen leaves are believed to have the ability as antibacterial to *Streptococcus mutans* which have glucosyltransferase enzymes (GTF).[11]

Another study stated that the antioxidant activity of extract of noni leaf was smaller with IC_{50} value of 98.68 µg/mL than noni leaf infusion with IC_{50} value of 75.65 µg/mL.[12] There were differences in the results of antioxidant activity using cold (extraction) and heat (infusa) on medicinal plants. As we know, people use medicinal plants by boiling the plants. Even though the effects of infusion or heating can damage the secondary metabolites in the plant.[13]

The epiphyte has a chemical compound similar to the host plant it occupies. In another study, ethanol extract of kepel parasite leaves has higher antioxidant activity (IC_{50} value 6.43 µg/mL) than ethanol extract of leaves of kepel (IC_{50} value 12.57 ± 0.7 µg/mL).[14,15] It is also expected that the associated epiphyte will contain antioxidant activity. In general, Kersen leaf and epiphyte of the truth contain flavonoids which have antioxidant power.

2. Research Methods

This study is an experimental analytic study with post-test only control group design to determine the ratio of antioxidant levels to leaves and epiphyte kersen (*Muntingia calabura* L.). The study was conducted from October to November 2018 in the Laboratory.

Biochemistry, Faculty of Medicine, Sriwijaya University. The object of this researchis a green plant (*Muntingia calabura* L.) which will be extracted in cold (extraction) and hot (infusa). The parts of the plant to be sampled are kersen leaves and epiphyte. The criteria for this research object are fresh, perfectly shaped and clean dark green leaves and parasitic leaves.

Data analysis was performed using the Statistical Package for Social Science (SPSS) program and Linear Regression Test to determine the direction and relationship between the independent variables and the dependent variable and to predict the value of the dependent variable if it increases or decreases.

3. Results

Table 1 below presents the absorbance values and percent inhibition of kersen leaf extract. From the 6 concentrations of the kersen leaves, the highest inhibition was obtained at 49.17279% at a concentration of 100 ppm and the lowest was 43.060666% at a concentration of 10 ppm.

Table 2 below shows the absorbance and percent inhibition values of the parasite leaf extract. From the 6 concentrations of parasitic leaves, the highest percentage inhibition was 49.17279% at a concentration of 100 ppm and the lowest was 39.52206% at a concentration of 10 ppm.

Table 3 below shows the absorbance value and the percentage of inhibition in the infusion of kersen leaves. From the 6 concentrations of the kersen leaves, the highest inhibition was obtained at 47.61029% at a concentration of 100 ppm and the lowest was 45.18382% at a concentration of 10 ppm.

Table 4 below shows the absorbance value and percent inhibition of epiphyte infusion. From the 6 concentrations of epiphyte leaf, the highest inhibition was obtained at 48.71324% at a concentration of 100 ppm and the lowest was 44.71507% at a concentration of 10 ppm.

The measurement of antioxidant activity was carried out using linear regression analysis in SPSS. Linear regression analysis was used to see how much

influence x (concentration) has on y (% inhibition) so that the results of linear regression equation can be seen the value of x as IC_{50} (Inhibitory Consentration 50) by replacing the y value to 50. In table 5 below shows that leaf extract cherry leaf has a moderate antioxidant activity with an IC_{50} value of 113.801 ppm.

In **table 6** below, it shows that epiphyte extract has a strong antioxidant activity with IC50 value of 98.7802 ppm.

In **table 7** below, it shows that the infusion of kersen leaves has a weak antioxidant activity, that is, with an IC_{50} value of 191.7624 ppm.

In **table 8** below, it shows that the parasite leaf infusion has a moderate antioxidant activity with an IC_{50} value of 98.7802 ppm.

In **table 9** below, the results of antioxidant extracts of ethanol extract of leaves and epiphyte plants (*Muntingia calabura* L.) are presented. From the sample, the highest antioxidant activity was found in the epiphyte extract with an IC_{50} value of 98,7802 ppm, compared to kersen leaf extract with an IC_{50} value of 113.801 ppm.

In **table 10** below, the results of antioxidant activity of leaf infusion and epiphyte are obtained from plants (Muntingia calabura L.). From the sample, the highest antioxidant activity was obtained in epiphyte infusion with IC50 value 131.6750 ppm, compared to kersen leaf infusion with IC50 value of 191.7624 ppm.

Table 1. Absorbance and Percent Inhibition of Kersen Leaf Extract

Extract	Concentration (ppm)	Absorbance	Absorbance Of DPPH	% Inhibition
Kersen Leaf	10	0.619	1.088	43.06066
	20	0.609	1.088	44.02574
	30	0.598	1.088	44.99081
	50	0.588	1.088	45.95588
	70	0.577	1.088	46.92096
	100	0.553	1.088	49.17279

Table 2. Absorbance and Percent Inhibition of Epiphyte Extract

Extract	Concentration (ppm)	Absorbance	Absorbance DPPH	% Inhibition
Epiphyte	10	0.658	1.088	39.52206
	20	0.6335	1.088	41.77390
	30	0.623	1.088	42.73897
	50	0.595	1.088	45.31250
	70	0.567	1.088	47.88603
	100	0.553	1.088	49.17279

Table 3. Absorbance and Percent Inhibit Kersen Leaf Infusion

Infusion	Concentration (ppm)	Absorbance Sample	Absorbance DPPH	% Inhibition
Kersen Leaf	10	0.5964	1.088	45.18382
	20	0.5928	1.088	45.51471
	30	0.5892	1.088	45.84559
	50	0.5832	1.088	46.39706

	70	0.5796	1.088	46.72794
	100	0.57	1.088	47.61029

Table 4. Absorbance and Percent of inhibition of Epiphyte Infusa

Infusion	Concentration (ppm)	Absorbance Sample	Absorbance DPPH	% Inhibition
Epiphyte	10	0.6015	1.088	44.71507
	20	0.5895	1.088	45.81801
	30	0.585	1.088	46.23162
	50	0.5805	1.088	46.64522
	70	0.5715	1.088	47.47243
	100	0.558	1.088	48.71324

Table 5. Antioxidant Activity of Kersen Leaf Extract

Sample	Regression Value	Equation IC50	Antioxidant Activity
Kersen Leaf Extract	Y = 0.0642 x + 42.649	113.801 ppm	Moderate

Table 6. Antioxidant Activity of Epiphyte Extract

Sample	Regression Value	Equation IC50	Antioxidant Activity
Epiphyte Extract	Y = 0.107 x + 39.391	98.7802 ppm	Strong

Table 7. Antioxidant Activity of Kersen Leaf Infusion

Sample	Regression Value	Equation IC50	Antioxidant Activity
Kersen Leaf Infusion	Y = 0.0261 x + 44.995	191.7624 ppm	Weak

Table 8. Antioxidant Activity of Epiphyte Infusa

Sample	Value Regression	Equation IC50	Antioxidant Activity
Epiphyte Infusion	Y = 0.04 x + 44.733	131.6754 ppm	Moderate

Table 9. Antioxidant Activity of Ethanol Extract of Kersen Plant (Muntingia calabura L.)

Sample	IC50 Value
Kersen Leaf Extract	113.801 ppm
Epiphyte Leaf Extract	98.7802 ppm

Table 10. Antioxidant Activity of Kersen Infusion (Muntingia calabura L.)

Sample	IC50 Value
Kersen Leaf Infusion	191.7624 ppm
Epiphyte Leaf Infusion	131.6750ppm

4. Discussion

An increase in% inhibition of ethanol extract indicated that an increase in extract concentration would affect the ability of extracts to soak free radicals.

This result is supported by research that states the percentage inhibition (percent inhibition) of free radical activity will also increase along with concentration increasing.[16]

The results of the existence of ethanol extract of kersen leaves have an IC_{50} value of 113.801 ppm, classified as a moderate antioxidant (100-150 ppm). While the ethanol extract of the epiphyte has an IC_{50} value of 98.7802 ppm, classified as a very strong antioxidant (50-100 ppm). This shows that the leaves and epiphyte in kersen plants have antioxidant activity that is classified as strong with the highest antioxidant activity found in epiphyte, because the smaller the IC_{50} value the greater the antioxidant activity.

The results of this study are in accordance with previous studies using different objects namely kepel plants, where ethanol extract of kepel parasite leaves had higher antioxidant activity (IC_{50} value 6.43 ppm) than ethanol extract of kepel leaves (IC_{50} value 12.57 ± 0.7 ppm).[14,17]

Another theory which supports ethanol extract of the epiphyte has antioxidant activity that is higher than the leaves of the stem cell because the epiphyte contain more antioxidant compounds than the stem cell. Antioxidant compounds found in epiphyte are amino acids, carbohydrates, alkaloids, saponins, flavonoids, alkaloids, terpenoids and tannins.[18,19]While the antioxidant compounds found in kersen leaves are active components of saponins, flavonoids and tannins.[20]

In this study, infusion (heating) was carried out by soaking the plants with 500 ml of water to boil so that the kersen leaves infusion were obtained with an IC_{50} value of 191.7624 ppm, classified as a weak antioxidant (150-200 ppm). While the parasite leaf infusion has an IC_{50} value of 131.675 ppm, classified as a moderate antioxidant (100-150 ppm).

The results of this study are in accordance with previous studies using different objects, namely on the soursop plant, where the infusion of leaves of soursop parasites has a higher antioxidant activity (IC_{50} value 33.5 ppm) than the infusion of soursop leaves (IC50 value 45 ppm).[15]

When compared between ethanol extract and infusion from each part of the plant, the results showed that the preparation of ethanol extract had a stronger antioxidant activity than infusion. The results of this study are in accordance with previous studies that kersen leaf infusion has a higher antioxidant activity of 196.80 ppm, whereas in ethanol extract, kersen leaves have antioxidant activity of 164.12 ppm.[21,22]

The heating process of the infusa which can cause a reduction or damage to the content of antioxidant compounds found in the leaves and leaves of the parasite, the plant seeds (*Muntingia calabura* L.). Cersen plant has phenolic compounds. Phenolic compounds is one of the constituents of green plants which act as antioxidants. Extraction at temperatures above 60 ^{o}C can reduce phenolic compounds found in plants[23] and in this study it eas heated at temperature of 100^{o}C. The use of heating at a high enough temperature will damage the active compounds contained in simplicia, especially antioxidant compounds.[24] In addition, compounds flavonoids and tannins found in plants are heat resistant.[25] Antioxidant activity decreases with increasing temperature and length of heating time.[26] This is because the longer the oven drying process (heating), the more free water content evaporates so that the mass of the dry material produced will also decrease.[27]

5. Conclusion

Chrysanthemum plants (*Muntingia calabura* L.) have antioxidant activity in the leaves and epiphyte. Epiphyte extract has stronger antioxidant activity than kersen leaf extract. Epiphyte extract has stronger antioxidant activity than the kersen leaves. The extract of the extract has a higher antioxidant activity than infusion.

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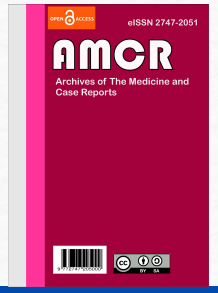

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