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EFEKTIVITAS TEH DAUN KERSEN (*Muntingia calabura linn.*) SEBAGAI MINUMAN FUNGSIONAL PENURUN KADAR GLUKOSA DARAH

The Effectiveness Of Kersen (Muntingia calabura linn.) Tea As Functional Drinks To Decrease Blood Glucose Level.

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ABSTRACT

The aim of this research was to find the best treatment in order to achieve kersen tea with the highest flavonoid content and was expected to decrease blood sugar levels. This research was conducted in three stages. Stage I was qualitative anylisis of flavonoids, the leaf position, different flavonoids extraction condition whether it was extracted in daylight or dark condition, and the sample:solvent ratio that gives the highest flavonoids contents. Stage II was to find out the effect of temperature and drying time on total flavonoids, antioxidant activity, and hedonic test for color, taste and aroma. Stage III was in-vivo test to determine the ability of kersen leaf tea to reduce blood sugar levels in hyperglycemic mice. Kersen leaves positively contained flavonoids after tested in quantitative and qualitative ways. Sample from middle leaves that was dried at 60oC for 60 minutes and was extracted in daylight condition with the sample:solvents ratio of 1:10 gives the highest flavonoids contents (7,75 mg/g extract), highest antioxidant activity (IC50 of 54,82 ppm), and tea color that was preferred by panelist. Kersen tea was able to decrease high blood sugar level of hyperglycemic BALB/c mice from 272.1 mg/dL to 250.2 mg/dL, or by 8.04%.

Keywords : *kersen (Muntingia calabura Linn.), hyperglicemic, flavonoids, antioxidant, blood sugar level*

ABSTRAK

Penelitian ini dilakukan dengan tujuan untuk mengetahui efektivitas teh daun kersen (Muntingia calabura Linn.) sebagai minuman fungsional penurun kadar gula darah. Penelitian dilakukan dalam tiga tahapan diantaranya penelitian tahap I yaitu uji kualitatif flavonoid, pengaruh posisi daun, kondisi perendaman dan rasio bahan:pelarut terhadap total flavonoid daun kersen segar, penelitian tahap II yaitu pengaruh suhu dan lama pengeringan terhadap total flavonoid, aktivitas antioksidan, dan uji hedonik terhadap warna, rasa dan aroma. Penelitian tahap III yaitu uji in-vivo kemampuan air seduhan teh daun kersen dalam menurunkan kadar gula darah mencit putih hiperglikemik. Hasil yang diperoleh adalah daun kersen positif mengandung flavonoid setelah diuji secara kualitatif dan kuantitatif. Daun bagian tengah yang dikeringkan pada suhu 60oC selama 60 menit lalu diekstraksi dalam kondisi terang dengan rasio bahan:pelarut 1:10 memiliki total flavonoid tertinggi sebesar 7,75 mg/g ekstrak, memiliki nilai IC50 sebesar 54,82 ppm yang menandakan tingkat antioksidan kuat, serta warna yang disukai. Kemampuan teh daun kersen untuk menurunkan kadar gula darah diuji secara in-vivo mampu menurunkan kadar gula mencit galur BALB/c hiperglikemik dari 272,1 mg/dL menjadi 250,2 mg/dL atau sebesar 8,04%.

Kata kunci : kersen (Muntingia calabura Linn.), hiperglikemik, flavonoid, antioksidan, kadar gula darah

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INTRODUCTION

Diabetes Mellitus (DM) is a disease caused by the deficiency of insulin due to pancreas are not capable of producing insulin consistent with the number of insulin needed by the body. DM can be controlled with insulin and anti diabetic drugs that are given orally. The high cost of synthetic drugs, their limited availability and the increasing understanding about the benefits of drugs derived from natural material resulted in the increasing demand of various kinds of herbal plants that can treat various kinds of diseases, including DM. In Indonesia, one of the plants that have been used to treat DM is kersen (Muntingia calabura Linn.). Apriyanti (2016) said 0,25 g/kg body mass of ethanoic kersen leaves extract lowers the high blood sugar level of mouse induced with alloxan. Santoso (2014) also said kersen leaves extract of 100 mg/kg body mass significantly reduce high blood sugar level in mouse by 21%. Kersen were able to decrease blood sugar level because it contains flavonoids that can act as antioxidant (Puspita, 2010). Although many research has been conducted about the ability of kersen leaves extract to reduce blood sugar level, a more specific research was needed to find out the potency of kersen leaves to be made into functional drinks such as tea that is effective, ready to use, and efficient in decreasing blood sugar level.

This research was conducted to find out the best type of raw materials and processing treatments in order to achieve the best quality tea that contains the highest flavonoid content, highest antioxidant activity and was effective to decrease blood sugar level. Mu'nisa et al. (2012) said the maturity and the positioning of leaves could give significant effect on the polyphenol contents of plants. Felicia et al. (2017) said drying procedure could affect the end quality of tea. Based on the analysis mentioned above, it was important to conduct a research to find the type of leaves, sample:solvent ratio, temperature and drying time that could give an end product, kersen tea, with the highest flavonoid contents, antioxidant activity, and sensory attributes that was preferred by panelist.

MATERIALS AND METHODS

Sampling

Kersen (Muntingia calabura Linn.) leaves were handpicked at 3 maturity stages ie. leaves position (5 leaves on the buds, 5 middle leaves, and 5 leaves on the base of one leaf segment), and was collected from Mataram city in the morning at around 8.30 am on September, 2018. Kersen leaves were then rinsed, air-dried, sorted and was taken to laboratory for further treatments.

Qualitative Analysis

Qualitative analysis was conducted in order to find out whether the leaves samples contained flavonoids. Kersen leaves was shredded and then 2 g of the sample was miced with 5 ml of ethanol. The mixture was then heated in water bath for 5 minutes, and then 3 drops of concentrated HCl and 0,2 g of Mg was added. If the color of the mixture changes into red, then it is positively contains flavonoids (Harborne, 1987).

The Effect of Leaves Position and Extraction Condition to Flavonoid Contents of Fresh Kersen Extract

Kersen (Muntingia calabura Linn.) leaves were handpicked at 3 maturity stages ie. leaves position (5 leaves on the buds, 5 middle leaves, and 5 leaves on the base from one leaf segment). The leaves were shredded then immersed in hot water (95-98oC) for 24 hours in dark and light conditions. All treatments were repeated 3 times. The absorbance of the extract was then measured by Spectrophotometer at 420 nm. A standard line chart of Quercetin was made for the calculation of total flavonoids (Harborne, 1987). This experiment gave an end result of which leave

position contains the highest flavonoid contents, and then was used in the next experiment.

The Effect of Sample:Solvent Ratio to Flavonoid Contents of Fresh Kersen Extract.

3 types of sample:solvents ratio (1:5, 1:10 and 1:15) were examined to find out which ratio gave the highest flavonoid contents of fresh kersen extract. The 1:5 of sample:solvents ratio was made from 1 g of shredded kersen leaves (from the previous experiment that contained the highest favonoid content) was immersed in 5 ml of hot water (95-98oC) for 24 hours. All treatments were repeated 3 times. The absorbance of the extract was then measured with Spectrophotometer at 420 nm (Harnorne, 1987). This experiment gave an end result of which sample:solvent ratio gives the highest flavonoid contents during extraction, and then was used in the next experiment.

The Effect of Temperature and Drying Time to Flavonoids Contents of Dried Kersen Leaves Extract.

Kersen leaves from the previous experiment with the highest flavonoid contents were then dried in 3 different temperature and drying time. The temperature used was 50, 60 and 70oC, and was dried for 60, 120 and 180 minutes. The drying process is using by laboratory oven, and all treatments were repeated 3 times. After the leaves has dried, then 2 g of shredded dried leaves then immersed in hot water (95-98oC) for 24 hours. The absorbance of the extract measured using Spectrophotometer at 420 nm.

The Effect of Temperature and Drying Time to Antioxidant Activities of Dried Kersen Leaves Extract.

Kersen leaves from the previous experiment with the highest flavonoid contents were then dried in 3 different temperature and drying time. The temperature used was 50, 60 and 70oC, and was dried for 60, 120 and 180 minutes. The drying process is using by laboratory oven, and all treatments were repeated 3 times. After the leaves has dried, then 2 g of shredded dried leaves then immersed in ethanol 95% for 24 hours. Kersen extract was then made into 5 different concentrations by mixing kersen, DPPH and ethanol. DPPH solutions (made from 15 mg of DPPH mixed with 100 ml of ethanol) were then added into the mixture. The absorbance of the extract was then measured using Spectrophotometer at 515 nm (Guo et al., 2001). The absorbance dara was then used to find the percentage of DPPH Inhibition and IC50 number that clarifies the antioxidant activity.

The Effect of Temperature and Drying Time to Color, Taste and Aroma of Dried Kersen Leaves Extract.

Kersen leaves from the previous experiment with the highest flavonoid contents were then dried in 3 different temperature and drying time. The temperature used was 50, 60 and 70oC, and was dried for 60, 120 and 180 minutes. The drying process is using by laboratory oven, and all treatments were repeated 3 times. After the leaves has dried, then 2 g of shredded dried leaves then brewed in 200 ml of hot water (95-98oC) and then was served to 15 panelists. Panelists were asked to give their honest opinion about the color, taste and aroma of kersen tea by giving a score of 1 to 5 (1 for least accepted until 5 for most accepted).

The Ability of Kersen Tea to Decrese High Blood Sugar Level in Hyperglicemic Mice.

A population of 10 male BALB/c mice was induced with 100mg/kg body mass of alloxan through their veins. 2 days after the injection, their blood sugar level was measured using Glucometer. Hyperglycemic mice have blood sugar level of 200 mg/dL or above. Hyperglycemic mice were then treated with kersen tea made by brewing 2 g of dried kersen leaves into 200 ml of hot water (95-98oC). The

tea was then left for 20 minutes until it has reach room temperature, then 1 ml was given to the mice orally by spuit. The mice's blood sugar level was measured using Glucometer at 120, 240, 360 and 480 minutes from it has given the tea to analyze whether kersen tea was effective to decrease high blood sugar level in hyperglycemic mice.

Data Analysis

All the data obtained were analyzed by using Co-Stat for the analysis of variance (ANOVA), one-way, where Honestly Significance Level (HSD) test was used to determine the significance difference ($P<0.05$) between different treatments. Results were expressed as means ± standard deviation (SD) of triplicate analyses, unless otherwise stated.

RESULTS AND DISCUSSION

Qualitative Analysis

In this experiment, the appearance of red color in the solutions was observed. The data collected is shown in Table 1. As can be seen, all kersen leaves samples positively contained flavonoids. This is shown by the appearance of red color in the solutions after being added by concentrated HCl and Mg. Concentrated HCl was used to hydrolyses flavonoids into its aglycone (Septyaningsih, 2010). Since all the samples contained flavonoids, then it can be used in the next experiments.

Tabel 1. Qualitative analysis of flavonoids in fresh kersen leaves

Sample	Repetition	Results
Fresh kersen leaves	1	+ flavonoid
	2	+ flavonoid
	3	+ flavonoid

The Effect of Leaves Position and Extraction Condition to Flavonoid Contents of Fresh Kersen Extract

The leaves position from one leaf segment gave significant effect to the flavonoid contents of fresh kersen leaves as shown on Table 2. There were significant differences between the 3 different kinds of leaves position. Middle leaves contained the highest flavonoid contents of 8.14 mg/extract compared to leaves on the buds or on the base. The leaves position from one leaf segment determines the maturity of the leaves. Young leaves were found on the buds, and it gets mature through the base. The more mature the leaves, the higher flavonoid it contained (Ainnurrohma, 2015). This findings also in line with other researches that mature cacao (Supriyanto et al., 2014), beluntas (Widyawati et al., 2011), breadfruit (Mu'nisa et al., 2012) and Acquilaria beccariana (Anwar et al., 2017) leaves contained the highest flavonoid contents.

Mature leaves have a greater ability to produce secondary metabolites, therefore a higher level of flavonoid was found in mature leaves. The middle leaves contained higher flavonoid contents then the leaves from the buds because secondary metabolites are synthesized along with the maturity of the leaves (Anwar et al., 2017). Leaves that get direct sunlight are able to synthesize more photosynthesis compounds such as primary and secondary metabolites, than those who do not get direct sunlight. Leaves are an important organ in plants because its chloroplast plays the role to perceive direct sunlight in order for the plant to make their own foods by photosynthesis. Exposure to direct sunlight will increase the production of secondary metabolites but if the exposure is too much it can cause the declining of secondary metabolites production (Biswal et al., 2003). This statement supported the outcome from these experiments where kersen leaves on the bud and on the middle has a higher flavonoid contents than leaves on the base of the segment. The highest flavonoids contents was found in the middle leaves because the leaves on the bud tend to get excessive sunlight, while the leaves on the base were more likely in shade and does not get enough sunlight. Abraham and Jaafar

(2012) also stated that very mature leaves has less amount of flavonoids contents. Several factors may affect the synthesize of flavonoids in leaves such as sunlight, temperature, drought and salinity (Harborne and Williams, 2000).

Table 2. The effect of leaves position to flavonoid contents of fresh kersen leaves extract

Leaves Position	Average flavonoid contents (mg/g extract)
Buds	5.56b[1)]
Middle	8.14a
Base	4.33c

Note :different letters at the back of the means value showing significant differences at 5% significance level (P<0,05).

Besides the leaves position, the other thing observed in the experiment was the condition during extraction, whether the extraction was conducted in light or dark condition. Data collected from this experiment is shown in Table 3. The condition during extraction gives non-significant differences on the total flavonoid contents of fresh kersen leaves extract. It is suspected that the heat from sunlight during extraction was not enough to damage the flavonoids on the extracts. Statistical analysis showed that there were no interactions between both factors (leaves position dan extraction conditions) to the total flavonoid contents of the extract, thus it can be said that the influence of these two factors were independent.

Table 3. The effect of extraction conditions to flavonoid contents of fresh kersen extract

Extraction conditions	Average flavonoid contents (mg/g extract)
Daylight	6.37a[1)]
Dark	6.65a

Note :different letters at the back of the means value showing significant differences at 5% significance level (P<0,05).

The result obtained from this experiment was middle leaves contained the highest flavonoid contents, and can be extracted in daylight or dark conditions. These samples were then used on the next experiments. Although the extraction conditions did not give significant differences to flavonoid contents, extraction in daylight is conducted on the next experiments because they are considered more practical.

The Effect of Sample:Solvent Ratio to Flavonoid Contents of Fresh Kersen Extract.

An experiment to find out the best sample:solvent ratio to yield the highest flavonoid contents was conducted with three different ratio; 1:5, 1;10 and 1;15. The sample:solvent ratio of 1:5 was made from immersing 1 g of shredded fresh middle leaves into 5 ml of hot water (95-98^{o}C) and so one for the other two treatments. Data collected from this experiment is shown on Table 4. Result shown that the sample:solvent ratio gave significant differences to flavonoid contents of kersen extract, with the ratio of 1:10 being the best out of all the other treatments. There were significant differences within the three treatments analyzed statistically using HSD at 5% significant levels. The sample:solvents ratio of 1:10 yielded the highest flavonoid contents of 5,27 mg/g extract, followed by the ratio of 1:15 and then 1:5.

Sample:solvent ratio of 1:10 were appointed to be the best ratio for the extraction of flavonoids. This findings also in line with other previous research by Liu *et al*. (2014) that stated a sample:solvents ratio between 1:8 until 1:12 gave the highest flavonoid contents extracted using MAE method. The addition of solvents after the optimal extraction point is reached will not yield more flavonoids. The best sample:solvents ratio can be determined by choosing based on whether the amount of the compound extracted is too small indicating there was not enough solvents used, this is shown on the experiment using a ratio of 1;5.

On the other hand, an experiment using a ratio of 1:15 also considered a non optimum ratio since the flavonoids extracted were lower and the purification cost of the solute is too expensive (Fengwei *et al*, 2014).

The result obtained from this experiment was middle leaves contained the highest flavonoid contents extracted in daylight conditions with sample:solvent ratio of 1:10 gives the highest flavonoid contents of fresh kersen leaves extract. These samples were then used on the next experiments.

Table 4. The effect of sample:solvents ratio to flavonoid contents of fresh kersen leaves extract..

Sample:solvent ratio	Average flavonoid contents (mg/g extract)
1:5	2,86c[1)]
1:10	5,27a
1:15	4,61b

Note :different letters at the back of the means value showing significant differences at 5% significance level (P<0,05).

The Effect of Temperature and Drying Time to Flavonoids Contents and Antioxidant Activity of Dried Kersen Leaves Extract.

This experiment was conducted to find out the best temperature and drying time to flavonoid contents and antioxidant activity of dried kersen leaves extract. The sample used in this experiment was those with the highest flavonoid content on the previous experiments. Data collected from this experiment is shown in Table 5. The result showed that drying temperature gave significant difference to flavonoid contents of dried kersen leaves extract, with drying temperature of 60ºC was the best treatment to achieve the highest flavonoid contents of 7,56 mg/g extract. The amount of flavonoid yielded from drying at 60ºC is higher than drying at 50ºC, because the sample was dryer and that makes more samples was used during the extraction procedure. The more sample used means the more flavonoids could be extracted. Whilst sample that was dried at 70ºC yielded a lower level of flavonoid contents, this is caused by the heat produced during drying breaks down flavonoids into other compounds. Degradation of flavonoids could happen at high temperature as the result of oxidation process that releases the double bonds in flavonoids, therefore the absorbance of some flavonoids could not be read and eventually gives a lower reading. These findings are also in line with Ainnurrohma (2015) that said flavonoids in Kumis kucing's leaves were reduced after drying at 70ºC because of the degradation of flavonoids into other compounds.

The antioxidant was be measured by IC_{50} number (the concentration of the smaple solutions required to inhibit free radical DPPH property by 50%. Sample with an IC_{50} below 200 ppm indicates that the sample has a high antioxidant activity. This is shown on all the kersen extract used in this experiment. The best drying temperature that achieve the highest antioxidant activity was 60ºC with IC_{50} of 53,98 ppm. This research is in line with the flavonoid contents of the extract, whereas extract with the highest flavonoid contents has the lowest IC_{50} number.

Table 5.The effect of drying temperature to flavonoids contents and antioxidant activity of dried kersen leaves extract.

Drying temperature (ºC)	Average flavonoid contents (mg/g extract)	IC_{50} (ppm)
50	6.12c	70,21a[1)]
60	7,56a	53.98c
70	6.39b	61,75b
Vit. C (100 ppm) for comparison		21,04d

Note :different letters at the back of the means value showing significant differences at 5% significance level (P<0,05).

Drying time also gave significant differences to flavonoid contents and antioxidant activity of dried kersen extract. Data collected from this experiment is shown in Table 6. Kersen leaves that were dried for 60 minutes gave the highest flavonoid contents of 7.75 mg/g extract and in line with the lowest number of IC_{50} of 54,82 ppm. The longer the drying time will evaporate more water from the sample. Syafrida *et al.* (2018) said flavonoid are sensitive to heat, the higher the drying temperature and the longer the drying time will decrease the flavonoid content in sample. After statistically analyzed, drying temperature and time gives positive correlations to total flavonoid contents of kersen leaves extract. The optimum temperature for drying kersen leaves was 60ºC for 60 minutes.

1n this experiment, vitamin C was used to compare the antioxidant activity of kersen leaves. Vitamin C is known to have a high antioxidant activity and it is commonly used everyday to boost immune systems. The second and third C atom in Vitamin C could act as electron donor that can act as radical scavenging. The IC_{50} of vitamin C observed was 21,04 ppm which means vitamin C has a very high antioxidant activity. The IC_{50} of vitamin C is lower than all the kersen leaves samples, however all of them has an IC_{50} that were lower than 200 ppm which means that all samples has high antioxidant activity.

Table 6. The effect of drying time to flavonoid contents and antioxidant activity of kersen leaf extract.

Drying time (minutes)	Average flavonoid contents (mg/g extract)	IC_{50} (ppm)
60	7,75a[1)]	54,82b[1)]
120	6,36b	64,52a
180	5,96c	66,61a
Vit C (100 ppm) for comparison.		21,04c

Keterangan :huruf berbeda di belakang nilai rata-rata menunjukkan perbedaan yang nyata pada taraf kesalahan 5% (P<0,05).

The Effect of Temperature and Drying Time to Color, Flavor and Aroma of Dried Kersen Leaves Tea.

Temperature and drying time gave significant difference to color of kersen tea, and non significant difference to flavor and aroma of kersen tea. Data for this experiment is shown in Table 7. According to Kartika (1987) color is an important factor to the assessment of foods. Consumers tend to pick foods from their color. If the food has a vibrant color, they tend to judge that the food will have a good taste. In this experiment panelist tend to prefer kersen tea that was dried at 60ºC for 60 minutes. They tend to like tea with a bright green color rather than darker green or chocolate. Samples of kersen leaves that was dried in a higher temperature tend to have darker color (from dark green to chocolate), this happens because of the heat that cause the degradation of chlorophyll. Chlorophyll are unstable during heating and it is hard to keep the compound undamaged (Deman, 1997).

The chlorophyll in leaves are linked with protein. Heat can cause the denaturation of protein and chlorophyll will be released. During heating, the Mg ion in the central of chlorophyll structure will be replaced with H ion, this cause the degradation of chlorophyll into pheophytin. As a result of this degradation process, the color of leaves will change from bright green into darker green or even chocolate (Putri *et al.,* 2014).

The effect of temperature and drying time gave a non significant difference to flavor and aroma of kersen tea. This was probably caused by there were no additional flavor enhancer such as sugar or other flavoring added to the tea,

so the tea will taste bland. No other components that can improve the tea's aroma were also added. Nowadays the tea company added many other components such as dried aromatic flowers or other aroma enhancer that could make the aroma of tea more acceptable to consumers.

The result obtained from this experiment was kersen tea made from middle leaves that was dried at 60°C for 60 minutes and were extracted at daylight condition on 1:10 of sample:solvent ratio gives the highest flavonoids, antioxidant contents, and color that is preferred by panelist. These samples were then used on the next experiments.

Table 7. The effect of drying temperature and time to color, flavor and aroma of kersen tea

Drying temperature (°C)	Drying time (minutes)	Color	Flavor	Aroma
		Average Score	Average Score	Average Score
50	60	2,08ab[1)]	1,56a[2)]	2,02a[2)]
50	120	2,00ab	1,67a	2,00a
50	180	1,91b	1,75a	2,02a
60	60	2,40a	1,71a	2,00a
60	120	2,22ab	1,6a	2,04a
60	180	2,13ab	1,73a	2,02a
70	60	2,18ab	1,75a	2,00a
70	120	2,18ab	1,82a	1,93a
70	180	2,35ab	1,51a	2,08a

Keterangan :huruf berbeda di belakang nilai rata-rata menunjukkan perbedaan yang nyata pada taraf kesalahan 5% (P<0,05).

The Ability of Kersen Tea to Decrese High Blood Sugar Level in Hyperglicemic Mice.

This experiment was conducted in order to find out whether kersen tea made with the best ingredients and processing procedure could reduce high blood sugar level in hyperglycemic BALB/c mice. A population of 10 healthy male mice was used for this experiments. Data collected is shown in Figure 1. It is shown that kersen tea was able to decrease blood sugar level of hyperglycemic mice from 272.1 mg/dL to 250.2 mg/dL after being treated for 480 minutes. Kersen leaves tea were able to decrease high blood sugar level by 8,04%. Even though the final blood sugar level of mice is still higher than 200 mg/dL (still considered being hyperglycemic) but it has proven that kersen tea was able to decrease blood sugar level. The ability of kersen tea is lower than the ability of synthetic anti diabetic drugs that can directly decrease blood sugar level, but by consuming kersen tea periodically could prevent or decrease diabetes mellitus. Future research is needed to find out the best doses for consuming kersen tea in order for it to be more effective and efficient on decreasing high blood sugar level.

The controlled mice were given aquadest and treated the same as mice that was given kersen leaves tea. The increasing high blood sugar level in controlled mice until 240 minutes probably caused by the alloxan effect. After 240 minutes the blood sugar level of controlled mice decreases, this is due to the reparation of cell in the mice's body so it was able to produce its own insulin again.

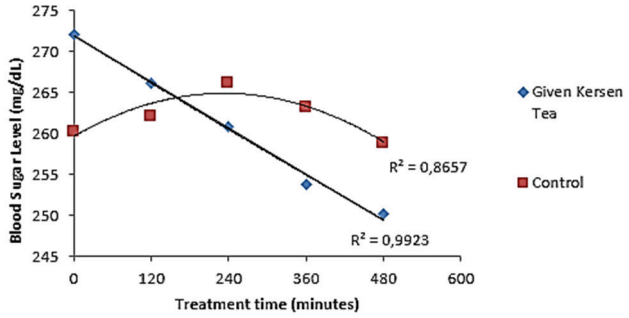

Figure 1. The changes of mice's blood sugar level during 480 minutes of treatment

CONCLUSION

Kersen leaves positively contained flavonoids after being tested by both quantitative and qualitative analysis. Sample from middle leaves that was dried at 60oC for 60 minutes and was extracted in daylight condition with the sample:solvents ratio of 1:10 gives the highest falvonoid contents (7,75 mg/g extract), highest antioxidant activity (IC50 of 54,82 ppm), and tea color that was preferred by panelist. Kersen tea was able to decrease high blood sugar level of hyperglycemic BALB/c mice from 272.1 mg/dL to 250.2 mg/dL, or by 8.04%.

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