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Characteristics Bioactive Compound of *Muntingia calabura* Leaves in Grow Up Height Different (Distric Area)

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Muntingia calabura has the vertiue from the fruits and the leaves. As long time the leaves just become the trash. The place where *Muntingia calabura* trees grow up may affect the bioactive components of kersen's leaves produced. This study aims to determine the characteristics of the bioactive components of kersen leaves at different heights as well as their potential as kersen leaf tea. The total phenol and flavonoid content was also analyzed to support the potential of kersen leaf tea as an herbal tea. The research method used was a completely randomized design with one factor, namely the difference in the height of the growing places of the *Muntingia calabura* (kersen) trees taken from Jember, Bondowoso and Situbondo Regency. The bioactive compounds found in *Muntingia calabura* leaves analyzed using FTIR were confirmed as functional groups at wavelengths 3362.48, 2920.96, 2850.30, 1623.51, 1447.40, 1214.17, 1101.28 dan 680.77 cm^{-1} that was alcohol OH bond, C-H bond, aldehide group, alkene group, aromatic group, eter, C-O bond, C-H bond and C-N bond. The results showed that the *Muntingia calabura* leaves from grow up of the height different consist of total fenolic content between 4.066,10 – 5.914,92 mg/100 GAE and total flavonoid content between 22,34 – 31,44 mg/g.

Keywords: *Muntingia calabura*; different area; FTIR; total fenolic; total flavonoid

1. Introduction

Based on a literature survey, it was found that plants containing high levels of flavonoids, tannins and polyphenols were reported to have multibiological effects including antioxidant activity and antidiabetic properties, namely cherry leaves (*Muntingia calabura*). *Muntingia calabura* is a medicinal plant that has anti-diabetic properties. The bioactive components found in this plant are flavonoids, phenolics and tannins. Kersen leaves have functional properties as antihyperglycemia [1], anti-inflammatory [2], anti-proliferative [3] and antibacterial [4].

Research on different growing heights will affect the contain of the tea yield and on the evaluation of brewing tea [5]. Research on the contain of vitamin E and its potential as an antioxidant at different altitudes to grow affected the vitamin E content and antioxidant activity of Moringa leaves from the coast to the mountains [6]. This is in line with a study reported by [7] that the chemical content of Moringa leaves increases with the increase in the growing site of Moringa leaves. Studies on the characteristics of the bioactive components of kersen leaves at different heights of growing places have never been carried out, therefore the research team proposes research on the characterize the bioactive components of kersen leaves at different heights and their potential as herbal teas. The difference in the height of the place to grow will used as a reason studied so that this study uses a one factor pattern (independent variable) completly random design. It is possible that the difference in the height of the kersen leaves in several districts, namely Jember, Bondowoso and Situbondo, will have an effect on the bioactive components of the observed kersen leaves. Kersen leaf bioactive components will analyzed using FTIR method, total phenol and total flavonoids were also analyzed.

2. Material and Methods

2.1 Materials and Tools

The necessary materials were kersen leaves obtained from Jember, Situbondo and Bondowoso Distric. The raw materials for chemical analysis needed where alcohol 70%, ethyl acetate, H_2SO_4, distilled water, folin ciocalteu, Na2CO3 and Gallic acid. The equipment used likes FTIR equipments, erlenmeyer, hot plate, volume pipette, micropipette, and spectrophotometer, blender and beker glasses.

2.2 Kersen Leaves Ground Preparation

Kersen leaves obtained from Jember, Situbondo and Bondowoso Distric then dried in food dehidrator. Dried kersen leaves mill in blender then shifted with 80 mesh. Kersen leaves ground will used for analysis parameters.

2.3 Research Design

This study was conducted by completely randomized design with one factroe (district area) were Jember, Situbondo and Bondowoso.

2.4 Analysis Parameters

Bioactive compound analyzed by using FTIR. Chemical analysis : total phenolic content and total flavonoid.

2.4.1. FTIR Analysis

5 gram sampel (ground kersen leaves) was pour in to the equipment then results analysis detected by sensor with output graph FTIR (Fourier Transform Infrared Spectroscopy).

2.4.2. Determination of total phenol content

The analysis of the total polyphenol content was performed spectrophotometrically using the modified folin ciocalteu method [8]. The sample extract with a certain volume was put into a test tube, then added with distilled water to a volume of 5 mL. Afterward, a 0.5 mL of follin ciocalteau was added into the test tube, then vortexed, and allowed to stand for 5 minutes. Then added Na2CO3 (7% as much as 1 mL., Distorted, and allowed to stand for 60 minutes in a dark space. The absorbance value was measured using the spectrophotometer at a wavelength of 765 nm. The total polyphenol content in the extracted sample was calculated using a standard curve made from Gallic acid (GA) in some concentrations. The total polyphenol content in the material was expressed as mg GAE/g of sample.

2.4.3. Determination of total flavovoid content

The analysis of flavonoid content was performed spectrophotometrically using the Morby method [9]. All spectra were measured on a Beckman DB-G spectrophotometer equipped with a Sargent model SRL recorder. The wavelength calibration of the spectrophotometer was carried out with a Holmium Oxide Filter (supplied by Beckman Instruments), which has $^{\lambda}$ max$^{'s}$ at 279.3, 287.6, 333.8, 360.8, 418.5, 536.4 and 637.5 nm. For convenience, spectroscopic grade methanol without added reagent was used as reference. It is useful (but not necessary) to have available four matched standard silica cuvettes of 1 cm path length in addition to the reference cuvette.

2.5 Data Analysis

The experiments were carried out in triplicates and data obtained from experiments were analysed using the SPSS (version 16.0). One way ANOVA test was used to obtain the significant difference between each factor. The means were compared using Tukey at the 5% significance level.

3. Results and Discussion

3.1 FTIR Results

The first microscopic characterization performed was FTIR analysis. FTIR absorption analysis was carried out to determine the functional groups contained in the three samples. The IR transmittance spectrum of the samples is shown in Figures 1-3.

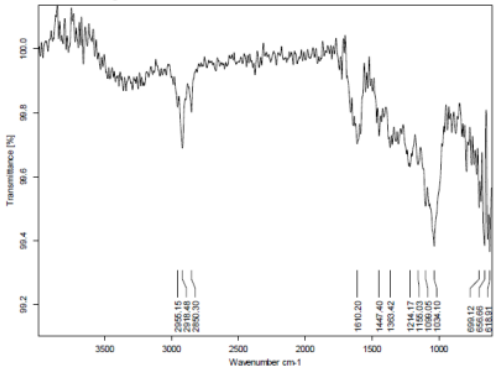

Figure 1. IR Transmittance spectrum of kersen leaves ground from Jember

The sampel of kersen leaves were come from Jember Distric have fungtional groups contain strong C-H bond which is represented with the wavenumber 2955.15, 2918.48 and 2850.30 cm^{-1}. Also contain C=C bond which is represented with the wavenumber 1610.20 cm^{-1}, C-N bond which is represented with the wavenumber 1214.17 cm^{-1}, C-OH cyclic bond which is represented with the wavenumber 1099.05 cm^{1}. Our report has similar results of the analysis of the kersen leaf extracts with FTIR reported by [10] show wavenumber 2927 $cm^{-1}$of a C-H function group. The absorption value is 1704 cm^{-1} which is the C=O function group, the absorption value of 1620 cm^{-1} is a functional group of C=C, the absorption value of 1451 cm^{-1} is a stretched C-C function group and an absorption number of 1042 cm^{-1} is a functional group of C-OH cyclic.

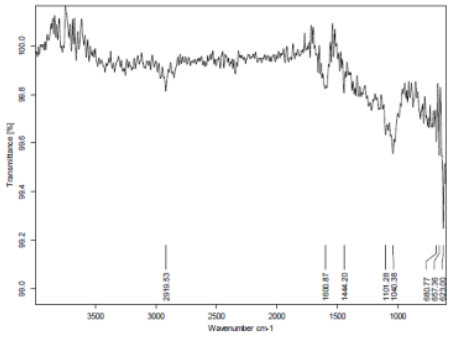

Figure 2. IR Transmittance spectrum of kersen leaves ground from Situbondo

The sampel of kersen leaves were come from Situbondo Distric have fungtional groups contain strong C-H bond which is represented with the wavenumber 2919.53 cm^{-1}. Also contain C=C bond which is represented with the wavenumber 1600.87 cm^{-1} and C-O bond or alcohol ester which is represented with the wavenumber 1101.28 cm^{1}.The sample of kersen leaves ground were collected from Situbondo has similar wavenumber of IR bands with Jember area. The IR bands of sample of kersen leaves ground were collected from Bondowoso Distric can be seen in Figure 3.

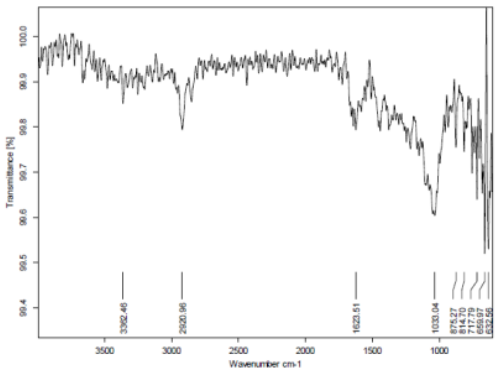

Figure 3. IR Transmittance spectrum of kersen leaves ground from Bondowoso

The sampel of kersen leaves were come from Bondowoso Distric have fungtional groups contain strong C-H bond which is represented with the wavenumber 2920.96 cm^{-1} and OH group which is represented with the wavenumber 3362.48 cm^{-1}. Also contain C=C bond which is represented with the wavenumber 1623.51 cm^{-1} and C-O bond or alcohol ester which is represented with the wavenumber 1033.04 cm^{1}. This results has similar report with research was conducted by [11] where IR bands at 3412 and 1705 cm^{1} are characteristic of the O-H and C=O stretching modes derived from the group of compounds contained in flavonoids, tannins, terpenoids, saponins, and polyphenols.

3.2 Chemical Properties

Parameter chemical of kersen leaves ground determinated by determination of total phenol and total flavonoid content. The results showed that the area of origin of kersen leaves would affect the total phenol and total flavonoids levels. Similar results with obtained by [12] showed that the composition of metabolites were influenced by variations in the height of growing place, carbon dioxide levels, the presence of insects and pathogens bacteria. The highest percentage of total phenol obtained from Bondowoso District, however the highest percentage of total flavonoid obtained from Situbondo District. The average test results of percentage of total phenol and total flavonoid can be seen in Table 1.

Table 1. Percentage of Total Phenol and Total Flavonoid Kersen Leaves

District Area (Grow Up Height Different)	Total Phenol	Total Flavonoid
Jember	51.93^{b}	22.33^{a}
Bondowoso	59.15^{b}	27.24^{b}
Situbondo	40.66^{a}	31.44^{c}

Note: Figures followed by the different superscript letters indicate significantly different with Duncan's test 5%.

4. Conclusion

Kersen leaves has potential as an antioxidant because it has high total phenol and total flavonoids content which was confirmed by the results of FTIR analysis to have a functional group with IR spectra at 3362.48 wavenumber absorption representing the OH group, 2850.30-2955.15 which represents the CH group, 1600.87-1623.51 which represents group C = C and 1033.4 representing the CO group.

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