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Estimation of Chemical Compounds and Antioxidant Activity of Muntingia Calabura Extract

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Abstract : Cherry (*Muntingia calabura*) is particularly useful as a shade tree by the roadside. The leaves contains flavonoids, saponins, tannins and triterpen, steroid. The compounds in pharmaceuticals has a role as an antioxidant, anti diabetic, a bitter taste, antimicrobial, diuretic, etc. This research aims to determine the chemical compounds in cherry leaves that has properties as catcher free radicals. This research conducted through the extraction process using a randomized block design with various temperature (30, 40, 50, 60^{o}C), time (t) (30, 40, 50, 60 minutes) and aquadest as a solvent. The results of the best extraction treatment is at 50^{o}C for 60 minutes with the value of DPPH 96.86%. Based on analysis of GC-MS showed that volatile compounds consist of myrcene (5,927%), thymol (3,543%), α terpinol (11,831%), linalool (2,240%), geraniol (21,718%), nerol (4,375%), citronellol (12,837%), eugenol (17,498%), α lonone (1,413%), β sitosterol (7,806%), α Amyrin (3,167%), Lupelol (4,228%), α tocopherol (1,975%), dan β carotene (1,425%). Result analysis of LC-MS showed that consist of Fumaric acid (6,643%), Succinic acid (4,903%), Niacin (0,718%), Malic acid (2,863%), Cinnamic acid (4,945%), Pyridoxine (1,893%), Gallic acid (21,428%), Ascorbic acid (6,121%), Glucose (8,166%), Fructose (20,690%), Pantothenic acid (1,478%), Biotin (1,025%), Thiamine (1,158%), Kaempferol (6,825%), Catechin (14,407%), Quercetin (10,623%), Riboflavin (1,131%) and Folic acid (1,553%).
Keywords : Leaves of Muntingia calabura, extraction, antioxidant, DPPH, GC-MS, LC-MS.

Introduction

Cherry (*Muntingia calabura*), a member of family Elaeocarpaceae [1], native from Southern Mexico, the Caribbean, Central America and South America to the West [2]. This plant can spread quickly to the Asian mainland through the bird, so cherry also wellknown as hummingbirds [3]. In Indonesia, this plant is particularly useful as a shade tree by the roadside. Berry has long-stemmed, almost perfectly round, 1-1.5 cm diameter, green, yellow and finally red when ripe. Contains several thousand seeds are small, smooth, yellowish white; immersed in flesh and sweet juice once. People ussually consumed the fresh fruit, but leaves not widely used [4]. Though its leaves contain a beneficial compound, which was first used by the majority of the people of Peru with boil cherry leaves in water, it can treat fever, headaches and flu [5].

Based on the empirically experience, Zakaria in 2007 was reported that the Cherry leaves contains flavonoids, steroids, saponins, tannins and triterpen. Cherry leaves contains high in antioxidants [7], and in many pharmaceutical research have been reported that Cherry leaves have antioxidant activity [8,9,10]. Antioxidants are chemical compounds that can donate electrons to one or more compounds, oxidants, and turning the compound into a compound that oxidants are more stable. Because of its nature, this compounds can eliminate free radical in the body so it will not induce diseases [11].

Cherry leaves (*Muntingia calabura*) contains antioxidants that generally form by phenolic or pholifenols, the sinamat acid derivatives, flavonoids, tocopherols, coumarin and polifungsional acids. Flavonoids that have an antioxidant activity consist of flavonol, flavanon, flavones, isoflavones, catechins and kalkon [12]. Phenolic compounds that have antioxidant activity can be known through the way of extraction. Extractions are a way to separate a desired substance when it is mixed with others. The mixture is brought into contact with a solvent in which the substance of interest is soluble, but the other substances present are insoluble [13]. Components of active compounds from plants or animals can be extracted based on "Like Dissolved Like Theory" , compounds will be extracted depends on solubility [14].

There are several extraction methods i.e. maceration, soxhlet and using different solvents to identify the active compound in Cherry leaves [15, 16, 17,18]. Zakaria in 2007 reported that cherry leaves powder on soaked water for 72 hours at 40^{o}C, contains flavonoids, steroids, saponins, tannins and triterpen. Another studies conducted by Siddiqua in 2010 also reported that cherry leaves powder extracted in soxchlet using methanol, showed a high contents of phenolic, saponins, tannins and flavonoids. Similarly, Krishnaveni *et al.*, in 2014 reported that cherry leaves powder extracted using another solvent and maceration at 30-40^{o}C for 20 minutes produces an active compounds contain flavonoids, saponins and anthroquinone.

The high content of flavonoid from cherry leaves may be referred as a natural antioxidant and has biological activity due to the rough surface of its leaves. Cherry leaves has glandular trichome, is a secretory accumulation of bioactive compounds associated with antioxidant activity [7].

Based on the data of the studies have been conducted on cherry leaves mostly identified as flavonoids, which are phenolic compounds group is dispersed in nature, and and derived from higher plants [12], this study will be performed to determine compounds contained in cherry leaves as a result of using extraction process. This extraction will be performed using variations in temperature and long time of extraction. Data are expected in extraction studies will provide an overview of the power catchers of free radicals and compounds that constitute it.

2. Experiment

2.1. Plant materials

Cherry leaves *(Muntingia calabura)* were obtained from National Agricultural Training Center (NATC), Malang. The old Cherry leaves *(Muntingia calabura)* were used from cherry plants around 5 years old.

2.2. Extraction

The cherry leaves were washed after collection and 50 gr of leaves were sliced. Added 200 ml of water distilated with various temperature (30°C, 40°C, 50°C and 60^{o}C) and then mixed on a hot plate, with the various length of time the extraction process (30, 40, 50 and 60 minutes). Separate filtrate and residue from extract using rotary vacuum evaporator at 40^{o}C for 1 hour. Sentrifuge filtrate (5500 rpm for 10 minutes) to precipitate impurities entrained, in order to obtain a supernatant and precipitate were analyzed.

3. Analysis chemical compound

Determination of antioxidant using 2,2-diphenyl-2-picrylhydrazyl (DPPH) [20]

The cherry leaves extract was analyzed for its antioxidant activity using DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging assay. Sample (200 g) was dissolved in 100 mM Tris–HCl buffer (800 µl,

pH 7.4) and then added 1 ml 500 µM DPPH. The solution was homogenized for 20 minutes in dark room. Spectrophotometry was used to determine the absorbance at 517 nm.

Gas chromatography and mass spectroscopy (GC-MS) [20]

Volatile compound from the cherry leaves was analyzed using GC-MS QP2010S-Shimadzu under the following condition: column used were Rtx-5MS, 30 m length and inner diameter of 0.25 mm and the initial column temperature was 70^{o}C and final temperature was 280^{o}C (5^{o}C/minute), while the injector temperature was 300^{o} C with split mode injector and split ratio of 72.6 and pressure of 14.0 kPa. The flow rate was 40 ml/minute and the flow within the column was 0.50 ml/minute. The detector temperature was 300^{o}C and using Helium as the gas carrier with EI (Electron Impact); and the samples volume injected was 1µl. Compounds were identified by comparing retention indices/comparing mass spectra of each compound with those of authentic samples and library.

Liquid chromatography and mass spectrometry (LC-MS) [21]

LCMS analysis use model Shimadzu LCMS-8040LC/MS dengan tipe column shim Pack FO-ODS (2mm D x 150mm, 8 µm) capilary voltage 3,0 kv and column temperature 35^{o}C for sample Injection volum 1 µl with flow gradient 0/100 at 0 min, 15/85 at 5 min, 21/79 at 20 min, 90/100 at 24 min and flow rate 0,5 ml/min. Sampling cone 28,0 V with solvent CH3ON(0,1% TFA)/ H2O (0,1%TFA) and MS focused ion mode are [M]+, Collison energy 5,0 V and Desolvation gas flow 600 L/hr with Desolvation temperature 350^{o}C use Fragmentation method low energy OID and Ionization ESI for Scanning 0,6 sec/scan (mz: 10-100) with Source temperature 100^{o}C and Run time 80 minute

4. Statistical analyses

The data were analyzed by using SPSS ver. 18 using level of significance (α) 0.05. Significant differences between treatment were determined by Duncan Multiple Range Test (DMRT).

Result and Discussion

Temperature and time of cherry leaves extraction affect the contents of bioactive compounds that have an antioxidant activity (Table 1).

Table 1. Antioxidant Activiy of Effect Combination Temperature and Time Extraction *Muntingia Calabura* Leaves

\Treatment	Antioxidant activity %)
Combination 30^{o}C for 30 minutes	59,07
Combination 30^{o}C for 40 minutes	65,83
Combination 30^{o}C for 50 minutes	67,76
Combination 30^{o}C for 60 minutes	70,99
Combination 40^{o}C for 30 minutes	74,32
Combination 40^{o}C for 40 minutes	77,40
Combination 40^{o}C for 50 minutes	80,81
Combination 40^{o}C for 60 minutes	84,88
Combination 50^{o}C for 30 minutes	87,87
Combination 50^{o}C for 40 minutes	90,21
Combination 50^{o}C for 50 minutes	94,40
Combination 50^{o}C for 60 minutes	96,86
Combination 60^{o}C for 30 minutes	95,55
Combination 60^{o}C for 40 minutes	94,76
Combination 60^{o}C for 50 minutes	88,78
Combination 60^{o}C for 60 minutes	85,98

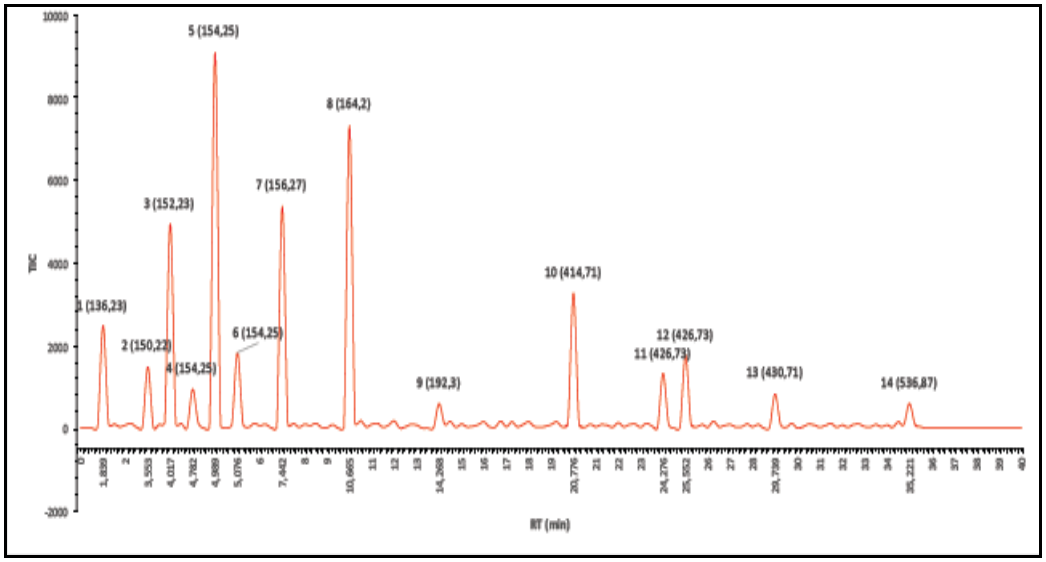

Figure 1. GC-MS Chromatogram Of Muntingia Calabura Leaves Exctract

Based on table 1, the value range of antioxidant activity from Cherry leaves extract are between 59,07 – 96,86%. The combination of 50^{o}C and 60 minutes shows the highest percentage of antioxidant activity. According to [22] the high antioxidant activity of a plant can be influenced by the content of phenolic compounds and the treatment. In studies that have been done by [6; 10] cherry leaf extract contains a high phenol compound.

The effect of temperature and time of cherry leaves extraction greatly influences the fluctuation antioxidant activity. The highest antioxidant activity (96.96%) achieved by combination 50^{o}C in 60 minutes but but after that it decreased. The increase of antioxidant activity due to the solubility of the active component of the material is possible because the cell wall damaged by heating [23]. Another factor influencing the antioxidant activity due to heat can also be caused by the use of aqua distilated as a solvent in which the hydrophilic antioxidant cherry leaves so many bioactive antioxidants extracted.

Studies have shown that this class of flavonoid compounds identified in cherry leaves contain high phenol [8.19]. The composition of volatile compounds from the best combination 50 ° C in 60 minutes shown in figure 1. The chromatogram results shows chemical compounds that consist of myrcene 5.927%, thymol 3.543%, α-Terpineol 11.831%, Linalool 2.250%, geraniol 21.718%, nerol 4.375% , citronellol 12.837%, 17.498% eugenol, α-lonone 1.413%, 7.806% β-sitosterol, α-Amyrin 3.167%, 4.228% Lupeol, α-Tocopherol 1.975% and 1.45% β-Carotene. The sequence detection of compounds by GC-MS can be seen in Table 2.

Table 2. Major Pytho-Components of Muntingia Calabura Leaves Extract for 50 ^{o}C at 60 Minutes

Peak	Compound	Composition (%)
1	Myrcene	5.927
2	Thymol	3.543
3	α-Terpineol	11.831
4	Linalool	2.250
5	Geraniol	21.718
6	Nerol	4.375
7	Citronellol	12.837
8	Eugenol	17.498
9	α-lonone	1.413
10	β-Sitosterol	7.806
11	α-Amyrin	3.167
12	Lupeol	4.228
13	α-Tocopherol	1.975
14	β-Carotene	1.425

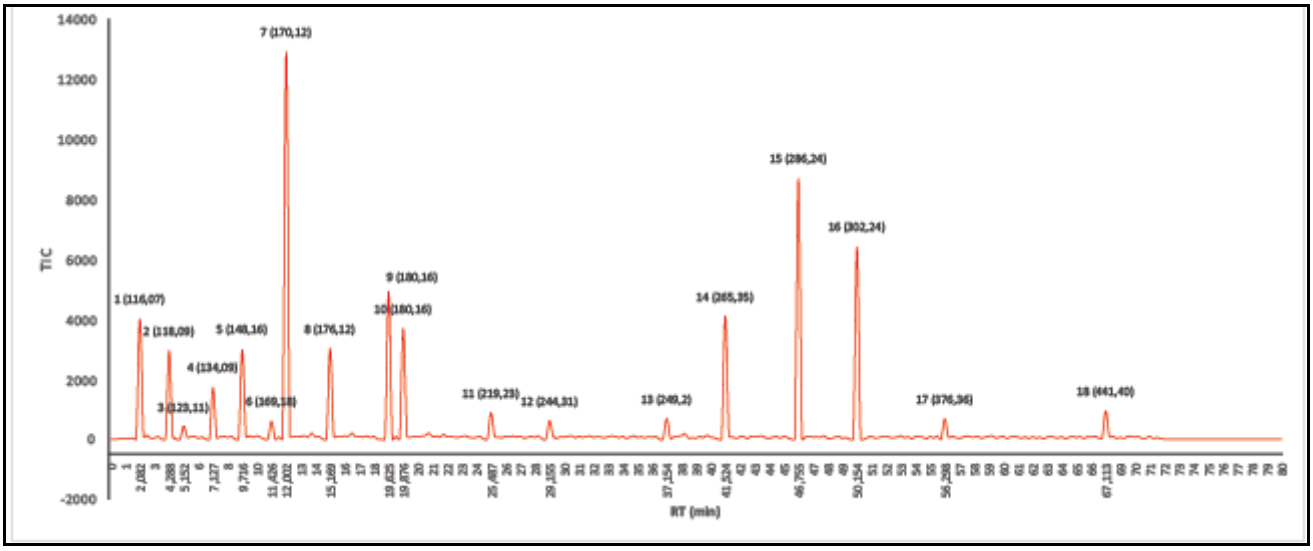

Figure 2. LC-MS Chromatogram of Muntingia Calabura Leaves Exctract

Table 3. Major Pytho-Components of Muntingia Calabura Leaves Extract for 50 °C at 60 Minutes

Peak	Compound	Composition (%)
1	Fumaric acid	6,643
2	Succinic acid	4,903
3	Niacin	0,718
4	Malic acid	2,863
5	Cinnamic acid	4,945
6	Pyridoxine	1,893
7	Gallic acid	21,428
8	Ascorbic acid	6,121
9	Glucose	8,166
10	Fructose	20,690
11	Pantothenic acid	1,478
12	Biotin	1,025
13	Thiamine	1,158
14	Kaempferol	6,825
15	Catechin	14,407
16	Quercetin	10,623
17	Riboflavin	1,131
18	Folic acid	1,553

Chemical compound from the best combination 50 ° C in 60 minutes with the analysis LC-MS as can see in figur. The chemical compound showed that consist of Fumaric acid (6,643%), Succinic acid (4,903%), Niacin (0,718%), Malic acid (2,863%), Cinnamic acid (4,945%), Pyridoxine (1,893%), Gallic acid (21,428%), Ascorbic acid (6,121%), Glucose (8,166%), Fructose (20,690%), Pantothenic acid (1,478%), Biotin (1,025%), Thiamine (1,158%), Kaempferol (6,825%), Catechin (14,407%), Quercetin (10,623%), Riboflavin (1,131%) and Folic acid (1,553%). The sequence detection of compounds by LC-MS can be seen in Table 3.

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