

Observation of *Muntingia Calabura*'s Leaf Extract as Feed Additive for Livestock Diet

R. I. Pujaningsih^{1*}, B. Sulistiyanto¹ and S. Sumarsih¹

¹Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia.

*Corresponding E-mail: retno.marwoto@gmail.com

Abstract

Using of synthetic antioxidants in feedstuffs continuously can cause negative effect for the livestock. This study observed the constituent compounds of cherry leaf powder using format method of descriptive qualitative. Comparative study was done between young and old leaves to identify the content of antioxidant and antimicrobial. Based on the results of phytochemical tests that have been done, old cherry leaves contain compounds of flavonoids more than young cherry leaves. From the results of this study can be concluded that the results of old cherry leaf isolation using soxhlet extraction has antibacterial power against *E. coli* bacteria, and *S. aureus* at concentration of 75% have greater inhibitory ability.

1. Introduction

One cause of rancidity in animal feedstuffs is the activity of enzymes in the tissues of foods containing fat and microbial activity. Symptoms of rancidity with the process of fat oxidation begins with the emergence of taste, flatness, oily nature, then change the taste and aroma. The next turns into a musty smell, and the last stage becomes rancid, damaging vitamin content and poisoning. That's why antioxidants are needed to prevent and inhibit the oxidation process.

Based on the type, antioxidant is divided into two groups, natural and synthetic antioxidants. Natural antioxidant derived from plants because they contain phenolic compounds, nitrogen [1]. The use of synthetic antioxidants began to be limited because the results of research resumed that synthetic antioxidants such as BHT (Butylated Hydroxy Toluene) be able to poison experimental animals and are carcinogenic. Therefore the food and medicine industries are switching to develop natural antioxidants and seeking new natural antioxidant sources [3]. One of plant that can be used is cherry leaf (*Muntingia calabura*). The first attempt to study the antibacterial activity of *M. calabura* was carried out by using the leaves and fruits collected in the State of Puebla and State of Veracruz, Mexico. The result showed both MEMCL (methanol extract of *M. calabura* leaves) and MEMCFr (methanol extract of *M. calabura* fruits) exhibited antibacterial activity against *E. coli* and *S. aureus* with the recorded minimum inhibitory concentration of 512 and 356 1024 µg/mL, and 128 and 256 µg/mL, respectively[5]. Utilization of cherry leaves as feed additive avoids competition with humans whom prefer to consume the fruit than the leaves. Based on the results of phytochemical tests that have been done, cherry leaves qualitatively contain flavonoid compounds, triterpenoids, tannins, saponins and steroids in accordance with the results of phytochemical tests according to Zakaria [2].

Flavonoids are phenol compounds that can change when added by alkaline compounds or ammonia. In nature, flavonoids are water-soluble compounds. Flavonoids are active compounds that can be used as antioxidants, antibacterial, and anti-inflammatory because it can inhibit the activity of disease-causing bacteria [6].



Isolation of flavonoids can be done with several methods of extraction. Each method has a deficiency and an advantage in producing cherry leaf extract. Research related to tea leaf aging explained that older leaves had higher mean total flavonoid values compared with young leaves ie 54.08 mg QE / g dry matter weight and 33.54 mg QE / g dry matter weight, respectively. Further informed that old avocado leaves contain higher levels of flavonoids [8]. The higher the level of flavonoids, the antioxidant potential will be higher. Flavonoids are a natural antioxidant and have biological activity, as an antioxidant that can inhibit various oxidation reactions, and capable of acting as a reducer of hydroxyl radicals, superoxide and peroxy radicals. It can be assumed that the content of secondary metabolite compound of old cherry leaves has the ability as higher antioxidant than young leaves, so that antioxidant activity of old leaves is higher than the young leaves [2]. But the levels of these compounds in cherry leaves is not known quantitatively.

This study aims to observe the possibility of cherry leaf extract as feed additive that serves as antibacterial and antioxidant. Maceration, Soxhlet extraction and decoction were tested to get the best quality and quantity of cherry leaf extract. It is thought that older leaves contain more flavonoids that can function as organic feed additives.

2. Materials and Methods

Extraction is the separations of plants by using selective solvents through standard procedure. The purpose of all extractions is dissolved metabolites and leaving cellular cells insoluble (residue). The research was conducted in July - October 2017 at the laboratory of Feed Technology UNDIP and Biology laboratory of UNNES.

2.1. Materials

The cherry leaves used in this study are the leaves that are dark green and light green, still fresh, picked from cherry trees that grow in Tembalang District of Semarang. The leaves are then washed and dried with oven at 60° C for 24 hours. Next, it blend into powder. The solvents used were 96% ethanol, hexane, ether, petroleum ether, methyl chloride and alcohol.

2.2. Methods

This research is divided into 3 stages (sample preparation, extraction and data analyzing). The first stage is the making of cherry leaf *simplicia* powder. Cherry leaf *simplicia* powder used in this study is 300 grams for each of young and old leaves, that divided for 3 methods of extraction with the amount of each 100 grams. The second stage is extraction with maceration method, soxhlet extraction and decoction [7]. The data were analysed descriptively covering the physical quality of organoleptic from cherry leaf extract, the amount of rendement of each extraction method and the flavonoid content of cherry leaf extract [4] [5] [7].

2.2.1. Ekstraksi Maceration

One hundred grams of leaf *simplicia* powder cherry (each of young and old leaves) is put into vessel dark, then added 750 ml of solvent ethanol 96% and closed tightly as well protected from direct sunlight. Immersion process for 3 days while stirred every 8 hours. After 3 days, the mixture of *simplicia* and methanol was filtered so as to obtain the first macerate. The dregs were immersed with 250 ml of methanol for 1 day, filtered again and obtained a second macerate. The two macerates were applied overnight and then separated from the residue and concentrated using a rotary evaporator at a temperature of 40°C until a viscous ethanol extract was obtained. [9].

2.2.2. Soxhlet Extraction

Finely ground sample of each young and old leaves are placed in a porous bag made from filter paper which is placed in thimble chamber of the Soxhlet apparatus. Extraction solvents is heated (70°C) in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. As the liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again and the process is continued. Liquid extract that collected then concentrated by using rotary evaporator on temperature of 40°C until extract is obtained condensed ethanol [7].

2.2.3. Decoction Extraction

Simplicia cherry leaves of young and old leaves (each of 100 g) were extracted with water solvent at measured water temperature (96-98°C) for 30 minutes. The vessel is immersed in a boiling water bath. To obtain the condensed ethanol the rovac was used on temperature of 40°C upon collected liquid extract. [7]

2.2.4. Antimicroba test

The best extraction method then used to evaluate the antimicrobial activity of *E. coli* and *S. aureus*. The antimicrobial test was performed with an incubation time of 1 x 24 hour at a concentration of 75%.

2.3. Data collecting

The organoleptic parameters of the extract are descriptive, color, taste and smell [10]. Quantitative analysis of total flavonoids in cherry leaf extract was done by colorimetric method using aluminium chloride reagent [9]. Antimicrobial test was made for *E. coli* and *S. aureus*.

3. Results and Discussion

Many factors influence the quality of herbs and these include species variation, environmental conditions, and the time of harvesting, storage and processing. This study used the leaves of cherry (*Muntingia calabura*) due to the nature of the plant wherein leaves are the parts that are easy to collect in abundant throughout the year.

3.1. Organoleptic test

Old and young cherry leaf powder produced have a moisture content of 4.7% and 5%, respectively. Generally, water content of medicinal plant simplicia is maximal 10% [5]. Old and young cherry leaf simplicia obtained for 2.115 grams and 2.250 grams of 4.500 of cherry leaves, respectively. Organoleptic results of cherry leaf extract (*Muntingia calabura*) with maceration method, soxhlet extraction and decoction are the same. The texture is thick, dark green color and specific smell. [5][6][7].

3.2. Flavonoid

Studying from the total flavonoid content of all three extraction methods, the method that produced the highest total flavonoid percentage was the soxhlet extraction (Table 1). Based on the yield of cherry leaf extract, soxhlet extraction also yields more rendement than maceration method and decoction method, as well. This caused the total flavonoid content of soxhlet extraction is greater than the other two methods. In addition, the possibility of total flavonoids found in cherry leaves more easily sourced

with soxhlet extraction. In soxhlet extraction used less solvent than maceration method. So there is the possibility to reduce the compound that wasted within the solvent. Decoction is only suitable for extracting heat-stable compounds, hard plants materials (e.g. roots and barks) and usually resulted in more oil-soluble compounds compared to maceration and infusion [9].

Simplicia cherry leaf from old cherry leaf have a mean value of total flavonoids higher than young cherry leaf on all extraction methods. Factors affecting total levels of flavonoids in leaves are morphological and aged leaves, which will affect secondary metabolites and bioactive compounds produced [12]. The flavonoid content from decoction is the lowest because flavonoid compounds are not heat resistant and easily oxidized at high temperatures [2]. Flavonoids show different sensitivity in heat treatment depending on the structure. Regardless of its structure, flavonoids will be degraded at temperatures above 100° C. Flavonoids are heat sensitive due to hydroxyl groups and ketones, as well as unsaturated double bonds [11]. Table 1 showed the results of cherry (*Muntingia calabura*) leaf observation prior to become feed additive.

Table 1. Cherry (*Muntingia calabura*) leaf observation.

Observation	Old cherry leaf	Young cherry leaf
	----- % -----	
water content	4,7	5
rendement ^a on maceration method	26,58	25,43
rendement ^a on soxhlet extraction	28,92	27,78
rendement ^a on decoction method	26,25	25,11
flavonoid ^b by maceration method	0,19	0,16
flavonoid ^b by soxhlet extraction	0,22	0,18
flavonoid ^b by decoction method	0,16	0,14

^aSimplicia cherry leaf : 100 g

^bThe total flavonoid content is expressed as the number of grams of quercetin equivalent to each gram of the extract[7]

3.3. Antimicrobial test

The results of antimicrobial assays from cherry leaf extract with 1 x 24 hour incubation process can be explained in Table 2. Table 2 shows that extract from old cherry leaf produces higher inhibitory resistance to bacteria than the extract from young cherry leaf against *S. aureus* bacteria and bacteria *E. coli*. [5].

The old cherry leaf methanol fraction has higher concentration and stronger resistance to bacteria, whereas the young cherry leaf methanol fraction has lower concentration, indicating a small inhibitory power. This is due to the increasing levels of bioactive compounds that are increasingly bactericidal (microbial lethal agents), whereas lower levels are usually only bacteriostatic (agents that inhibit microbial growth, not microbial kill) [13].

Table 2. Inhibition's area of bacterial colony growth of *E.coli* and *S.aureus* after incubation for 1 X 24 hours with soxhlet extract

Bacteria	Diameter (cm)	
	Old cherry leaf	Young cherry leaf
<i>Escherichia coli</i>	1,2	1,1
<i>Salmonella aureus</i>	1,4	1,1

4. Conclusion

Based on the data, it can be concluded that old cherry leaf will give better effect as antimicrobial and antioxidant at a concentration of 75% than young cherry leaf.

Acknowledgments

This research was supported by Faculty of Animal Agriculture, Diponegoro University. We are thankful to our colleagues (Tito, Ditto, Endah, Ade, Erry, Reni) who provided expertise that greatly assisted the research.

References

- [1] Chiang C. J., Kadouh H., Zhou K. Q. 2013. Phenolic Compounds and Antioxidant Properties of Gooseberry as Affected by in Vitro Digestion," LWT-Food Science and Technology. **51** (2), 417-422. DOI: 10.1016/j.lwt.2012.11.014.
- [2] Zakaria, Z.A. 2007. Free Radical Scavenging Activity of Some Plants Available in Malaysia. Iranian Journal Of Pharmacology & Therapeutics. **6** :87-91.
- [3] Takashi, Miyake and Takayumi Shibamoto. (1997). Antioxidant Activities of Natural Compound Found in Plants. J. Agric. Food. Chem. **45**. 1819-1822.
- [4] Zakaria Z. A., Jais A. M. M., Mastura M., Jusoh M. S. H., Mohamed A. M., Jamil M. N. S., Rofiee M. S., Sulaiman M. R. (2007). In vitro antistaphylococcal activity of the extracts of several neglected plants in Malaysia. J Pharmacol **3**: 428-431.
- [5] Yasunaka, K., Abe, F., Nagayama, A., Okabe, H., Lozada-Perez, L., Lopez-Villafranco, E., Muniz, E.E., Aquilar, A., and Reyes-Chilpa R. (2005). Antibacterial activity of crude extracts from Mexican medicinal plants and purified coumarins and xanthones. J Ethnopharmacol **97**: 293-299.
- [6] Binawati, D. K., dan Amilah, S. 2013. Effect of Cherry Leaf (*Muntingia calabura L.*) Bioinsecticides Extract Towards Mortality of Worm Soil (*Agrotis ipsilon*) and Armyworm (*Spodoptera exiqua*) on Plant Leek (*Allium fistolum*). Wahana, **61**(2):51-57.
- [7] Harbone, J.B. 1998. Metode Fitokimia Penuntun Cara Modern Menganalisa Tumbuhan., ITB, Bandung.
- [8] Felicia, N., I. Wayan, R.Widarta, Ni Luh, A.Y. 2017. Pengaruh Ketuaan Daun dan Metode Pengolahan terhadap Aktivitas Antioksidan dan Karakteristik Sensoris Teh Herbal Bubuk Daun Alpukat (*Persea americana* Mill.). Jurnal Ilmu dan Teknologi Pangan (*Itepa*). **5**, no. 2, p. 85-94. ISSN 2527-8010.

- [9] Azwanida, N. N. 2015. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Med Aromat Plants* 4: 196. doi:10.4172/2167-0412.1000196.
- [10] Depkes R. I., 2000. Parameter Standar Umum Ekstrak Tumbuhan Obat, Departemen Republik Indonesia, Jakarta
- [11] Qiao, L, Y. Sun, R. Chen, Y. Fu, W. Zhang, X. Li, J. Chen, Y. Shen, and X. Ye. 2014. Sonochemical Effects on 14 Flavonoids Common in Citrus: Relation to Stability. *PloS ONE* **9** (2): e87766.
- [12] Farhoosh, R., G. A. Golmovahhed, and M. H.H. Khodaparast. 2007. Antioxidant activity of various extracts of old tea leaves and black tea wastes (*Camellia sinensis* L.). *Food Chemistry* 100: 231 – 236.
- [13] Volk, WA and Wheeler, MF. 1993. *Mikrobiologi Dasar*. Edisi V. Jilid 1. Diterjemahkan oleh Adisoemarto S. Jakarta: Erlangga.