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Identification of chemical compounds cherry leaves (*Muntingia calabura)* powder as a natural antioxidant

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Abstract

Antioxidant compound has a small molecular weight but is able to inhibit the oxidation reaction by preventing the formation of radicals. Using of synthetic antioxidants continuously can cause negative effect for the body. Based on the reason we needed another source of natural antioxidant by using plants, and one of plant that can we used is cherry leaf. Cherry leaf is an annual plant is easy to grow and contains a lot of phenolic compounds. This study showed that the cherry leaf extract contains flavanoid compounds, terpenoids, tannins and high antioxidant activity. Cherry leaf extract could be substitute synthetic antioxidants with further processing and identified constituent compounds. This study aims to determine the constituent compounds cherry leaf powder using format method of drying through cherry leaf extract at 50 °C and then homogenized with maltodextrin 8%, 0.3% tween 80 and dried at a drying temperature of 50 °C. The results obtained demonstrate the value of using the DPPH antioxidant activity of 80.50%. Identification of powdered Cherry leaf constituent compounds by GC-MS analysis shows the components of the volatile compounds such as geraniol (26,335%), eugenol (19,950%), citronellol (16,958%), α-amyrin (6,225%), myrcene (3,440%) and α-terpineol (7,356%). While the identification of constituent compounds by LC-MS analysis shows the phenol compounds: Gallic acid (18,607%), Catechin (14,077%), Quercetin (10,255%), Ellagic acid (9,626%) and Kaempferol (8,699%). Result of analysis functional compound with infrared wave length range of 520.74 to 3417.63 cm^{-3}, 15. The infra-red spectrum of functional compounds and the presence of broad bands at 3417.63 cm^{-3} can be attributed to (OH) stretching.

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Introduction

Antioxidants are chemical compounds that can donate one or more electrons to free radicals that inhibited free-radical reactions (Paiva and Gordon, 2002; Kikuzaki, *et al*., 2002; Carocho, 2013). Antioxidant compound has a small molecular weight but is able to inhibit the oxidation reaction by preventing the formation of radicals, thereby inhibiting cell damage associated with reactions within the body where the antioxidant status is an important parameter for monitoring the health of a person (Cai *et al*. 2004; Winarsi 2007).

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 (Cai *et al*, 2003; Wong *et al*, 2006; Chiang *et al*, 2013; Agati *et al*, 2012; 11-14). Synthetic antioxidants are often used in the food and pharmaceutical industry which consists of two types of butylated hydroxyanisole (BHA) and butylated hydroxyl- toluene (BHT) (EFSA, 2012). Using continuously of synthetic antioxidants can cause negative effect for the body, some results of laboratory studies on animals showed that BHA and BHT can cause cancer and tumors as well as both the preservative causes metabolic disorders in humans (Shebis *et al*, 2013). Therefore, we need alternative antioxidants that are safe for our body.

One of the plants that has potential as a source of natural antioxidants is the leaves of cherry (*Muntingia calabura*). This plant has fine hair gummy belonging to the group of glandular trichomes, produce secretions, and is the organ where bioactive compounds accumulate, which is associated with antioxidant activity (Kuntorini *et al,* 2013).

Several treatments have been performed to determine the compound in cherry leaves using extraction process by drying or wilting of cherry leaves at room temperature 27^{o}C for 1-2 weeks, then crushed and then used as raw materials extraction (Shih *et al,* 2006; Sani *et al*, 2012 ; Sridhar *et al,* 2011; Zakaria *et al,* 2014). The extraction of several studies show quantitative results that cherry leaves contains flavonoids, steroids, triterpenes, tannins and saponins (Zakaria, 2007).

Dried cherry leaves are extracted with methanol showed that tis leaves contains phenolic, saponins, tannins, flavonoids and anthroquinone (Siddiqua 2010 and Krishnaveni *et al* 2014). Whereas pharmaceutical research conducted Sindhe *et al,* 2013 report cherry leaf extract contains phenols, flavonoids and other compounds that can be used widely as a source of natural antioxidants for health.

Cherry leaves (*Muntingia calabura*) can be used as a natural antioxidant in powder. Several studies have been done in the manufacture of natural antioxidants powdered such as from *Aloe vera* and guava leaves by microencapsulation process and foamat drying method. Microencapsulation is the food processor, from liquid to a solid material with the aim is to protecting sensitive components such as the active substance that is not resistant to external conditions such as temperature (Janusz *et al*, 2004; Atmane *et al,* 2006; Jafari *et al*, 2008). While Foam at drying is the drying method with foam, with the temperature under 100^{o}C and using fillers such as maltodextrin and Tween 80 (Narchi *et al.,* 2007; Ratti *et al*, 2008). The advantages of drying foam at are active compounds of the dried material is not damaged, the required drying time is shorter by producing a quality product (Rajkumar *et al,* 2007; Ratti *et al,* 2008; Kasma, 2007). The purpose of this study is to identify constituent compounds cherry leaf powder as a natural antioxidant by microencapsulation processed and foamat drying method.

Materials and methods

Sample preparation

Muntingia calabura leaves were obtained from National Agricultural Training Center (NATC), Malang, Indonesia. *Muntingia calabura* Leaves were used from cherry plants around 5 years old. After harvesting, the cherry leaves were washed by aquadest and was extracted at 50°C for 60 min. The filtrate obtained were then mixed with 8% maltodextrin, 0,3% tween 80 and homogenized using amixer at speed of 1800 rpm for 10 min. The rough obtained were scattered on the baking pan and dried at 50^{o}C for 4 h. The dried rough was blended and sifted using sieve 100 mesh.

Determination of antioxidant activity

Muntingia calabura powder were 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 (Khalaf *et al*., 2008)

Determination of phytocomponent

Gas chromatography and mass spectroscopy (GC-MS)

The volatile compound from the *Muntingia calabura* leaves powder analyzed for phytocomponent 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°C and final temperature was 280°C (5°C/minute), while the injector temperature was 300° 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°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 (Wartini, 2007)

Liquid chromatography and mass spectrometry (LC-MS)

Sample preparation was pipetted into a 15-mL glass tube and then 50 µL of IS working solution (3 µg/mL), 50 µL of 200 mM DTT in water and 50 µL of 1% formic acid were added. The solution was vortexed for 30 seconds then 1500 µL methanol was added. The solution mixture was vortexed for 60 seconds and centrifugated for 7 minutes at 3000 rpm**.** A part of the clear supernatant was transferred to glass vials and put on the rack of the auto-sampler kept at 25 °C. Ten microliters of *Muntingia calabura* leaves powder were injected and detected in the LC–MS/MS system. The compound of *Muntingia calabura* leaves powder were analyzed for phytocomponent using 8040LC/MS Shimadzu under the following conditions: column used were shim Pack FO-ODS (2mm Dx150mm, 8 µm) capilary voltage 3,0 kv and the initial column temperature was 35°C, while the sample Injection volum 1 µl. The flow gradient was 0/100 at 0 min, 15/85 at 5 min, 21/79 at 20 min, 90/100 at 24 min. The flow rate 0,5 ml/min, were sampling cone 28,0 V with solvent CH3ON(0,1% TFA)/ H2O (0,1%TFA) and MS focused ion mode are $[M]^+$, while collison energy 5,0 V and Desolvation gas flow 600 L/hr. The initial Desolvation temperature 350°C use fragmentation method low energy OID and Ionization ESI for Scanning 0,6 sec/scan (mz: 10-100) with Source temperature 100°C and Run time 80 minute (Elzanfaly, *et al*., 2017).

Determination of functional compounds

Muntingia calabura leaves powder was analyzed for its functional compound using FTI-R (Fourier Transforms Infrared). The Infrared spectra were recorded on FTIR-8400S (Shimadzu Deutchland GmbH) spectrophotometer in KBr and polyethylene pellets. Samples were weigh-in at 0.01 g and homogenized with 0.01 g KBr anhydrous by mortar agate. The mixture of sample and KBr were pressed by vacuum hydraulic (Graseby Specac) at 1.2 psi to obtained transparency pellet. Scanned sample passed through infrared, where its continuing wave by detector that connected to computer and given described of tested sample spectrum. Samples were usually scanned in the absorption area of 500-4000 cm-1. The results of analysis consisted of chemical structure, molecular binding form and certain functional group of tested sample as basic of spectrum type (Arum, 2012).

Result and discussion

Free radical scavening activity

The antioxidant activity of *Muntingia calabura* leaves powder determined using DPPH assay (%) was 80.50%, which higher than synthentic antioxidant such as BHT (butylated hydroxytoluene) at 70,5% as reported by Anilakumar, *et al* (2010). The high antioxidant activity in cherry leaf powder is correlated between encapsulation process and format drying method. The function of maltodextrin and tween 80 is to protect the active compounds, so that the phenolic compounds in the cherry leaves (Sindhe *et al*, 2013) did not loss in the drying process.

The content of phenolic compounds in natural antioxidants associated with raw material as in the photosynthesis process by the plant, so it will affect the antioxidant activity (Foyer *et al.*, 2003; Patras *et al.*, 2009, Naiyana *et al.*, 2010).

Identification chemical compound of Muntingia calabura leaves powder by GC-MS

A typical gas chromatogram of *Muntingia calabura* powder is shown in Fig. 1 and list of the compounds identified appears in Table1. Fourteen compounds of *Muntingia calabura* powder were identified using GC-MS and the major compound identified was geraniol (26.335%), citronellol (16.958%) and eugenol (19.950%). All three compounds are relatively volatile. Associated with the process of making liquid extract, cherry leaves were heated, reported by lutfiadi *et al* (2007) this condition make them more volatile. Geraniol and citronellol including monoterpenoid compound and eugenol is flavanoid that largest compound in leaf cherry (Sindhe *et al.,* 2013).

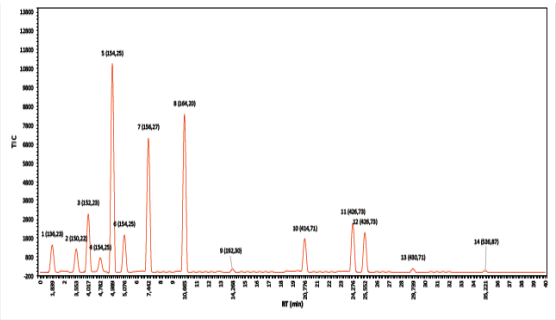

Fig. 1. GC-MS chromatogram of *Muntingia calabura* leaves powder.

Source: Results of laboratory analysis (Triswaningsih, 2016)

Identification chemical compound of Muntingia calabura leaves powder by LC-MS

Cherry leaves powder compound identified by LC-MS is shown in Fig. 2 and the details of the identification of compounds in Table 2. Twenty compounds identified using LC-MS with the main compounds are gallic acid (18,607%), catechins (14,077%), quercetin (10.255 %), ellagic acid (9,626%) and kaempferol (8,699%). As reported by Espín *et al*, (2005) that five main compounds in the leaves of the cherry powder including the phenolic (gallic acid), flavanoid (quercetin, kaempferol) and polyphenols (ellagic acid).

Cherry leaf powder constituent compounds contain lots of the phenolic and flavanoid so it is great as a source of natural antioxidants (Sindhe *et al*., 2013).

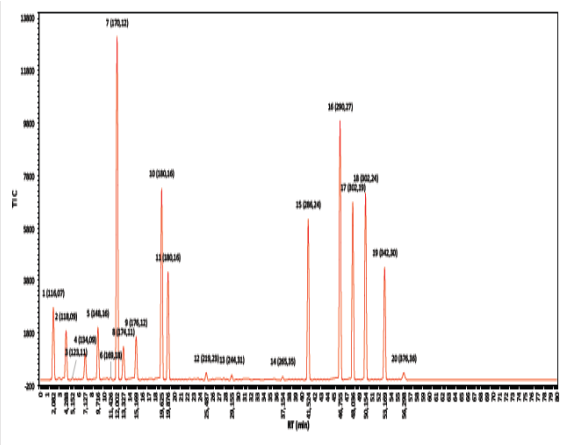

Fig. 2. LC-MS chromatogram of *Muntingia calabura* leaves powder.

Source: Results of laboratory analysis (Triswaningsih, 2017)

Identification antioxidant compounds by FTI-R

The infrared spectrum of *Muntingia calabura* powder as shown in Fig. 3 was in the wave length range of 520.74 to 3417.63 cm-3, with fifteen functional compounds detected (Table 3). According to table 3 and Fig. 3, the presence of broad bands at 3417.63 cm-3 can be attributed to (OH) stretching. The wave length at 2931.60 to 1726.17 cm-3; 1643.24 to 1514.02 cm-3 can be attribute stretching vibration, nitrogen group and eter (R-O-R), respectively.

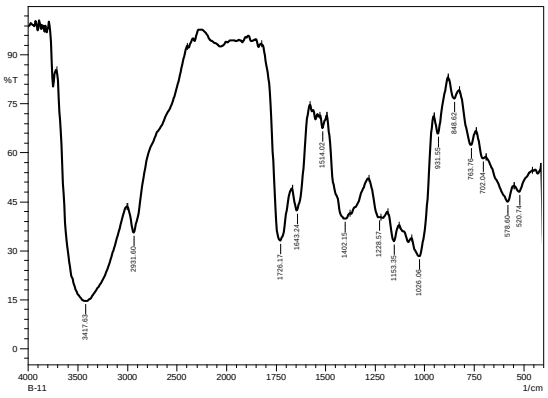

Fig. 3. The infrared spectrum of *Muntingia calabura* leaves powder.

Source: Results of laboratory analysis (Triswaningsih, 2016)

Table 1. Major pytho-components from *Muntingia calabura* leaves powder using analyzed GC-MS.

Peak	Compound	% RA
1	Myrcene	3,440
2	Thymol	2,928
3	α-Terpineol	7,356
4	Linalool	1,826
5	Geraniol	26,335
6	Nerol	4,690
7	Citronellol	16,958
8	Eugenol	19,950
9	α-lonone	0,396
10	β-Sitosterol	4,234
11	α-Amyrin	6,225
12	Lupeol	4,999
13	α-Tocopherol	0,449
14	β-Carotene	0,208

Source: Results of laboratory analysis (Triswaningsih, 2016).

Table 2. Major pytho-components from *Muntingia calabura* leaves powder using analyzed LC-MS.

Peak	Compound	% RA
1	Fumaric acid	3,924
2	Succinic acid	2,685
3	Niacin	0,124
4	Malic acid	1,432
5	Cinnamic acid	2,856
6	Pyridoxine	0,148
7	Gallic acid	18,607
8	Dehydroascorbic acid	1,817
9	Ascorbic acid	2,359
10	Glucose	10,365
11	Fructose	5,856
12	Pantothenic acid	0,393
13	Biotin	0,267
14	Thiamine	0,190
15	Kaempferol	8,699
16	Catechin	14,077
17	Ellagic acid	9,626
18	Quercetin	10,255
19	Maltose	6,092
20	Riboflavin	0,393

Source: Results of laboratory analysis (Triswaningsih, 2017)

Table 3. Function compound of *Muntigia calabura* leaves powder analyzed by using FTIR.

No	Wave length (cm^{-3})	Functional compound	Type of vibration
1	520.74	Nitro-NO_2	NO_2 rock
2	578.6	Nitro-NO_2	NO_2 bend aromatic
3	702.04	Methyl benzenes	Alkane C-C skeletal vibration
4	763.76	Cyclobutanes	Alkane C-C skeletal vibration
5	848.62	Straight-chains alkanes	Alkane C-C skeletal vibration
6	931.55	Branched alkanes	Alkane C-C skeletal vibration
7	1026.06	CH_3Z, where Z=-CR_3	CH_3 rocking vib
8	1153.35	CH_3-CO-	Rocking vib
9	1228.57	-O-CH_3	Rocking CH_3
10	1402.15	-SO-CH_3	asym CH_3 def vib
11	1514.02	Aliphatic azoxy compounds – $N=N^+-O^-$	Electron-withdrawing group on N-O nitrogen
12	1643.24	Keton R-CO-R	C=C strecth konj.
13	1726.17	Saturated aliphatic ketones	sat. Methyl ketones 1730-1700cm^{-1}
14	2931.60	-CH_2- (acyclic)	Alkane C-H stretching vibration
15	3417.63	Quinone oximes	O-H str

Source: Results of laboratory analysis (Triswaningsih, 2016).

Conclusion

Cherry leaf powder constituent compounds identified by GC-MS; LC-MS; FTI-R, consist of phenolic compounds, polyphenols, and flavonoids. The functional group that is identified as OH str, aromatic, ether and DPPH assay value is higher than the synthetic antioxidant (BHT). So the cherry leaves powder can be used as a natural antioxidant.

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References

Agati G, Azzarello E, Pollastri S, Tattini M. 2012. Flavo- noids as Antioxidants in Plants: Location and Functional Significance. Plant Science **196**, 67-76.

DOI: 10.1016/j.plantsci.2012.07.014

Anilakumar KR, Sudarshanakrishna KR, ChandramohanG, Ilaiyaraja N, Khanun F, Bawa AS. 2010. Effect of *Aloe vera* (L.) gel extract on antioxidant enzymes and azoxymethane-induced oxidative stress in rat. Indian Journal of Experimental Biology **48**, 837-842.

Arum YP. 2012. Isolasi dan Uji Daya Antimikrobia Ekstrak Daun Kersen *(Muntingia calabura)*. Under Graduates thesis, Universitas Negeri Semarang. Jurnal MIPA **35(2)**, 165-174 (In Bahasa Indonesia)

Atmane M, Muriel J, Jo lS, Stephane D. 2006. Flavour Encapsulation and Controlled Release – A Review. Review International Journal of Food Science and Technology **41**, 1–21.

Cai YZ, Sun M, Corke H. 2003. Antioxidant Activity of Betalains from Plants of the Amaranthaceae, Journal of Agricultural and Food Chemistry **51(8)**, 2288-2294.
DOI: 10.1021/jfo30045u

Cai Y, Luo Q, Sun M, Corke H. 2004 Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Science **74**, 2157-2184.
DOI: 10.1016/ j.lfs. 2003. 09.047

Carocho M, Ferreira ICFR. 2013. A Review on Anti- oxidants, Prooxidants and Related Controversy: Natural and synthetic compounds. Screening and Analysis Methodologies and Future Perspectives. Food and Chemical Toxicology **51**, 15-25.
DOI: 10. 1016/j.fct.2012.09.021

Chiang CJ, Kadouh H, Zhou KQ. 2013. Phenolic Com- pounds and Antioxidant Properties of Gooseberry as Af- fected by *in Vitro* Digestion," LWT-Food Science and Te- chnology **51(2)**, 417-422.
DOI: 10.1016/j.lwt.2012.11.014

EFSA. 2012. Scientific Opinion on the Reevaluation of Buty- lated Hydroxytoluene BHT (E 321) as a Food Additive. EFSA Panel on Food Additives and Nutrient Sources Ad- ded to Food (ANS). European Food Safety Authority Journal **10(3)**, 2588.
www.efsa.europa.eu/en/efsajournal/doc/2588.pdf

Elzanfaly ES, Hanan AM. 2017. A Liquid Chromatography/Tandem Mass Spectrometric Method for Determination of Captopril in Human Plasma: Application to a Bioequivalence Study. Journal of Applied Pharmaceutical Science **7(02)**, 008-015.

Espín J, Tomás-Barberán F. 2005. Constituyentes bioactivos no-nutricionales de alimentos de origen vegetal y su aplicación en alimentos funcionales, in n.d. (Ed.), *Alimentos funcionales*. Fundación Española para la Ciencia y la Tecnología [FECYT], Madrid, 101-153.

Foyer CH, Noctor G. 2003. Redox Sensing and Signal- ling Associated with Reactive Oxygen in Chloroplasts, Pe-roxisomes and Mitochondria. Physiologia Plantarum **119(3)**, 355-364.
DOI: 10. 1034/j.1399-3054.2003.00223.x

Jafari SM, Assadpoor E, He Y, Bhandari B. 2008. Encapsulation Efficiency of Food Flavours and Oils during Spray Drying. Journal Drying Technology **26**, 816-835.

Janusz A, Ewelina M. 2004. Mikroencapsulation of Oil Matrix/Water System During Spray Drying Process-Proceedings of the 14th International Drying Symposium (IDS 2004). C: 2043-2050

Kasma I. 2007. Kajian Pengolahan Bubuk Instant Bubuk Wortel Dengan Metoda Foammat Drying. Balai Pengkajian Teknologi Pertanian Sumatera Barat. Buletin Teknologi Pascapanen Pertanian **3**, 20-25.

Khalaf NA, Shakya AK, Al-Othman A, El-Agbar Z, Farah H. 2008. Antioxidant Activity of Some Common Plants. Turky Journal Biology **51**, 55.

Kikuzaki H, Hisamoto M, Hirose K, Akiyama K, Taniguchi H. 2002. Antioxidants Properties of Ferulic Acid and Its Related Compound. J. Agric. Food Chem **50**, 2161-2168.

Krishnaveni, Marimuthu, Ravi Dhanalakshmi. 2014. Qualitative and Quantitative Study of Phytochemicals in *Muntingia calabura* L. Leaf and Fruit. World Journal of Pharmaceutical Research **3(6)**, 1687-1696.

Kuntorini, Evi Mintowati, Setya Fitriana dan Maria Dewi Astuti. 2013. Struktur Anatomi Dan Uji Aktivitas Antioksidan Ekstrak Metanol Daun Kersen (*Muntingia Calabura*). Prosiding Semirata FMIPA Universitas Lampung, 2013. Semirata 2013 FMIPA Unila (In Bahasa Indonesia)

Lutfiadi R, dan Supriyadi. 2007. Model Matematik Perubahan Senyawa Geraniol Teh Hijau Selama Penyimpanan. www.ejournal-unisma.net **8(1)** (In Bahasa Indonesia)

Naiyana P, Sunisa S, Worapong U, Saowakon W. 2010. Effect of Thermal Processing and Protein Nutriens on Antioxidant Activity of Tom-Kha Paste Extract. Asian Journal Food AfrgroIndustry **3 (04)**, 389-399.

Narchi A, Ch, Vial AG, Djelveh. 2007. Effect of the Formulation on The Continuous Manufacturing of Foamed Products. Proceedings of European Congress of Chemical Engineering (ECCE-6) Copenhagen. 1-17.

Paiva MF, Gordon MH. 2002. Effect of pH and Ferric Ions on The Antioxidant Activity of Olive Polyphenols in Oil-in-Water Emulsions. JAOCS **79(6)**, 571-576.

Patras A, Burton NP, Pieve SD, Butler F. 2009. Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blacberry purees. Innovative Food Science and Emerging Technologies **10**, 308-313

Rajkumar P, Kailappan R, Viswanathan R, Rghavan GSV. 2007. Drying characteristics of foamed alphonso mango pulp in continous type foammat dryer. Journal of Foo Engineering **79 (4)**, 1452-1459.

Ratti C, Kudra T. 2008. Process and Energy Optimazition In Drying Foamed Material. Canmet Energy Technology Centre- Varennes, PQ, Canada J3X 1S6 Department of Soils and Agri-Food Engineering, Laval University, Quebec, QC, Canada G1K 7P4. Journal Drying Technology **66**, 47.

Sani MH, Mohd ZA, Zakaria T, Balan LK, The, Salleh MZ. 2012. Antinociceptive Activity of Methanol Extract of *Muntingia calabura* Leaves and the Mechanisms of Action Involved. Research Article Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine Article ID 890361, 10.

Shebis Yevgenia, David Iluz, Yael Kinel-Tahan, Zvy Dubinsky, Yaron Yehoshua. 2013. Natural Antioxidants: Function and Sources. Food and Nutrition Sciences **4**, 643-649.

Shih Cheng-Dean, Jih-Jung Chen, Hsinn-Hsing Lee†. 2006. Activation of Nitric Oxide Signaling Pathway Mediates Hypotensive Effect of *Muntingia calabura* L. (Tiliaceae) Leaf Extract. The American Journal of Chinese Medicine **34(5)**, 857–872.

Siddiqua Ayesha KB, Premakumari, Rokeya sultana, Vithya and Savitha. 2010. Antioxidant Activity and Estimation of Total Phenolic Content of *Muntingia calabura* by Colorimetry. International Journal of Chem Tech Research CODEN(USA): IJCRGG ISSN : 0974-4290 **2(1)**, 205-208.

Sridhar M, Thirupathi K, Chaitanya G, Ravi Kumar B, Krishna Mohan G. 2011. Antidiabetic Effect of Leaves of *Muntingia calabura* L, Normal and Alloxan Induced Diabetic Rats. *Pharmacologyonline* **2**, 626-632.

Wartini NM. 2007. Komparasi Metoda Separasi dan Pengaruh Curing Terhadap Senyawa Penentu Flavor Pada Ekstrak Falor Daun Salam. Disertasi.Program Pasca Sarjana Universitas Brawijaya.Malang (In Bahasa Indonesia).

Winarsi H. 2007. Antioksidan Alami dan Radikal Bebas. Yogyakarta: Kanisius. (In Bahasa Indonesia).

Wong CC, Li HB, Cheng KW, Chen F. 2006. A Sys- tematic Survey of Antioxidant Activity of 30 Chinese Medicinal Plants Using the Ferric Reducing Antioxidant Power Assay. Food Chemistry **97(4)**, 705-711.
DOI: 10.1016/j.foodchem.2005.05.049

Yuslinda, Elka. 2012. Penetuan Aktivitas Antioksidan Dari Beberapa Ekstrak Sayur-sayuran Segar dan Dikukus Dengan Metode DPPH. *Scientia* **2(1)**, 1. (In Bahasa Indonesia).

Zakaria ZA. 2007. Free Radical Scavenging Activity of Some Plants Available in Malaysia. Iranian Journal of Pharmacology & Therapeutics **6(1),** 87-91.

Zakaria ZA, Mohd Hijaz Mohd Sani, Manraj Singh Cheema, Arifah Abdul Kader, Teh Lay Kek, Mohd Zaki Salleh. 2014. Antinociceptive activity of methanolic extract of *Muntingia calabura* leaves: further elucidation of the possible mechanisms. Research article BMC Complementary and Alternative Medicine **14**, 63.