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Antioxidant Potential of Black, Green and Oolong Tea Methanol Extracts

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Abstract

Degenerative diseases and chronic diseases are often caused by oxidative stress. Oxidative stress caused by free radicals. Antioxidant as inhibitor are needed to prevent it which is one of antioxidant sources is tea. Tea processing generally produce various kinds of teas such as black, green and oolong tea. Tea processing affect the content of phenolic compounds. The aim of the research is to evaluate phytochemical content, total phenolic content of black tea, green tea and oolong tea extracts using catechin, quercetin, kaempferol, myricetin as standard, and to evaluate the antioxidative potency of black tea, green tea and oolong tea extracts compared to catechin, quercetin, kaempferol, myricetin. Phytochemical assay using modified Farnsworth method, the antioxidant activity were measured by by its 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging activity. Green tea extract contained highest phenolic and flavonoid. The highest antioxidant activity was green tea extract with $IC_{50}=0,487 \mu\text{g/mL}$. Green tea extract content phenol and flavonoid are higher compared to the other extracts, green tea extract has the highest antioxidant activity.

Keywords: antioxidant, black tea extract, green tea extract, oolong tea extract, total phenolic content

Introduction

Various degenerative and chronic diseases are often caused by oxidative stress. Oxidative stress is caused by free radicals. It highly reactive and unstable because of its unpaired electron in the outer atomic orbital. Free radical can react with the cells molecules by binding to it. It can oxidize the nucleic acid, proteins, fats and even the cells DNA and initiate the degenerative disease (Halliwell & Gutteridge, 2007). Free radicals are derived either from normal essential metabolic process in the human body or from external exposure (Bagchi & Puri, 1998). The antioxidants as oxidation inhibitor are needed to overcome the negative effect of the free radicals. Antioxidant inhibits the oxidation by reacting to reactive free radicals to form reactive substances that relatively stable. Endogenous antioxidant already present in the human body. However the exogenous antioxidant still needed if the free radicals present in copious amounts (Johnson, 2002). A balance between free radicals and antioxidant is necessary for proper physiological function. Antioxidant may exert their effect on biological systems by different mechanism, including electron donation, metal ion

chelation, co-antioxidants, or by gene expression regulation (Krinsky, 2000; Lobo et al., 2010).

There are two kinds of antioxidant based on its source, natural antioxidant and synthetic antioxidant. Synthetic antioxidants are carcinogenic when it consumed for long term. The needs of natural antioxidant that have fewer side effect and less toxic continues to rise. Natural antioxidants can protect the body against the damage caused by reactive oxygene species (ROS), inhibit the degenerative disease and inhibit the lipid peroxidase activity (Wiseman et al., 1997). Tea-one of the most popular beverage- components possess antioxidant activity. Most commercially prepared tea is obtained form the leaf of the plant *Camelia sinensis* (Satoh et al., 2005). Among tea, 69% of consumption is black tea, 28% of consumption is green tea and 3 % others is oolong tea (Cabrera et al., 2008). Teas of *C. sinensis* undergo different manufacturing processes. Green tea is produced by steaming (Japan) or panning (China) to prevent catechin oxidation by polyhenol oxidase. Oolong tea is semi-fermented while black tea is fully fermented (Eric et al., 2011). Many studies have shown that green, black and oolong tea has antioxidant properties (Xie et

al., 1993; Wiseman, 1997; McKay & Blumberg, 2002; Zhu et al., 2002; Higdon & Frei, 2003; Satoh et al., 2005). Tea processing will affect the phenol content and ultimately will affect the antioxidant activity of it (Higdon & Frei, 2003). Therefore, it become important to investigate the antioxidant activity of the extract of black tea, green tea, and oolong tea and test the phenol content based on the catechin, quercetin, kaempferol and myricetin standard.

Methodology

Plant material preparation, extraction and phytochemical content assay

Dried leaves green tea and black tea were obtained from Cisaruni Plantation, PTPN VIII, West Java, Indonesia and dried oolong tea was obtained from Tea Plantation, East Java. Extraction was performed based on maceration method using methanol 96% as the solvent (Widowati et al., 2011a; Widowati et al., 2013a; Widowati et al., 2014a; Widowati et al., 2014b). The extraction yielded 20.139 % of black tea, 22.11 % of green tea, and 17.39 % of oolong tea. The green tea, black tea, and oolong tea extract were tested by phytochemical assay using modified Fransworth method including terpenoid, phenol, steroid, triterpenoid, flavonoid, tannin, alkaloid, and saponin assay (Fransworth *et al.*, 1966; Widowati et al., 2010; Bera et al., 2014)

Total Phenol Assay

Briefly 25 μ L standard solution in 10 concentration level (500; 250; 125; 62,5; 31,25; 15,625; 7,81; 3,95; 1,98; and 0,98 μ g/mL) of catechin, quercetin, kaempferol, and myricetin and sample (extract from green tea, black tea, and oolong tea) in concentration of 500 μ g/mL were prepared for total phenol assay. Each standard and sampled was mixed with 125 μ L follin 10% and 100 μ L of Na_2CO_3 7,5 % in microplate. The reaction then incubated at 45°-50°C for 10 minutes. The absorbance was measured in 760nm of wavelength using microplate reader. The linear regression equation ($y = a + b$) was created based on the standard (catechin, quercetin, kaempferol, myricetin) absorbance value. The analysis of phenol content of sample was performed based on

the each of standard linear regression equation (Ivanova et al., 2005; Widowati et al., 2011a; Widowati et al., 2015).

DPPH scavenging activity assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity were analyzed based on the linear regression equation continued by median Inhibitory Concentration (IC_{50}) value determination. Ten concentration levels (500; 250; 125; 62,5; 31,25; 15,625; 7,8; 3,9; 1,9; and 0,9 μ g/mL) of black tea, green tea, and oolong tea extract as working solution was prepared for this assays. Briefly, 50 μ L samples (working solution) were added to a microplate followed by 200 μ L DPPH (Sigma-Aldrich) solution (0,077 mmol/L in methanol). The reaction was shaken vigorously and kept in the dark for 30 minutes at room temperatures. DPPH scavenging activity was determined by microplate reader at 517 nm. Methanol absolute was used as blanko. The IC_{50} value then determined. The radical scavenging activity of each sample was measured according to equation 1 (Han et al., 2004; Widowati et al., 2013b; Widowati et al., 2013c; Widowati et al., 2015).

$$S \quad \% = (A_c - A_s) / (A_c \times 100) \quad (1)$$

Description

A_s = sample absorbance

A_c = negative control absorbance (without sample)

Results

Phytochemical content of tea extracts

Table 1 shows the different phytochemical content, including terpenoid, phenol, steroid, triterpenoid, flavonoid, tannin, alkaloid, and saponin of green tea, black tea, and oolong tea methanol extract. All of phytochemical tested was found in the green tea extract. High level of phenol also found in green tea and oolong tea extract. Green tea also contains the highest level of alkaloid compared to the other tea extract. Otherwise, steroid and alkaloid did not observed in the black tea extract. Triterpenoid and tannin also not found in the oolong tea extract. Less content of saponin also found in all of the extract.

Table 1. Phytochemical content assay of black tea, green tea, and oolong tea methanol extract

Sample	Phytochemical assay							
	Terpenoid	Phenol	Steroid	Triterpenoid	Flavonoid	Tannin	Alkaloid	Saponin
Black tea	++	++	-	+	++	+	-	+
Green tea	+	++++	+	+	+++	+	+++	+
Oolong tea	+	++++	++	-	+++	-	+	+

Description:

++++ : very high content, +++: high content, ++: moderate content, +: low content, -: undetected

Phenolic compound of tea extracts

Statistical assay based on linear regression equation was performed to analyze the total phenol of black tea, green tea, and oolong tea based on the standard solution (catechin, quercetin, kaempferol

and myricetin). The highest level of total phenol was showed by green tea extract (Table 2). The degree of oxidation affected by the polyphenols profile of the tea. (Balentine et al., 1997).

Table 2. The average of total phenol level concentration of black tea, green tea, and oolong tea ($\mu\text{g}/\text{mg}$)

Sample	Catechin Equivalent (CE)	Quercetin Equivalent (QE)	Kaempferol Equivalent (KE)	Myricetin Equivalent (ME)
Black tea extract	14,33	4,50	4,33	4,17
Green tea extract	23,33	9,71	4,33	6,17
Oolong tea extract	16,08	5,09	3,33	4,67

DPPH Scavenging Activity

The most active extract in DPPH scavenging activity showed by green tea extract indicated by the lowest IC_{50} value compared to the other extract and standard (Table 3). DPPH is a stable free radical because of the unpaired electron. The unpaired electron of the radical becomes paired in the presence of hydrogen donor, decreasing the absorption in 517 nm of wavelength. The DPPH scavenging activity has been widely used to test

the compound ability as a free radical scavenger and antioxidant activity in food or plant extract (Sato et al., 2005). Previous study already obtained that the black tea has the antioxidant activity through DPPH free radical scavenging activity with $\text{IC}_{50} = 5,405 \mu\text{g}/\text{mL}$ (Widowati et al., 2011a), catechin (C) diluted in methanol with $\text{IC}_{50} = 8.11 \mu\text{M}$ (Evacuasiyany et al., 2014) and catechin diluted in DMSO with $\text{IC}_{50} = 7.02 \mu\text{g}/\text{ml}$ (Budiman et al., 2014).

Table 3. Inhibitory Concentration (IC_{50}) Value of Antioxidant DPPH Scavenging Activities of Tea extract and Standard

Samples	Replication	Linear equation	R^2	IC_{50} ($\mu\text{g}/\text{ml}$)	Average IC_{50}
Green tea extract	1	$Y = 4,299x + 48,70$	0,767	0,30	$0,487 \pm 0,258$
	2	$Y = 4,539x + 46,45$	0,735	0,78	
	3	$Y = 4,342x + 48,37$	0,751	0,38	
Oolong te extract	1	$Y = 7,363x + 12,93$	0,911	5,03	$5,005 \pm 0,060$
	2	$Y = 7,225x + 13,55$	0,918	5,04	
	3	$Y = 7,286x + 14,03$	0,911	4,94	
Quercetin	2	$Y = 6,592x + 3,575$	0,989	7,04	$4,279 \pm 0,065$
	3	$Y = 6,666x + 3,671$	0,987	6,95	
	1	$Y = 6,153x + 23,65$	0,901	4,28	
Kaempferol	1	$Y = 6,095x + 24,32$	0,893	4,21	7.154 ± 0.134
	3	$Y = 6,128x + 23,39$	0,878	4,34	
	2	$Y = 8,731x - 11,662$	0,823	7.06	
Myricetin	2	$Y = 8,063x - 8,924$	0,887	7.31	$4,496 \pm 0,058$
	3	$Y = 9,039x - 14,107$	0,827	7.09	
	1	$Y = 12,803x - 6,749$	0,829	4,43	
	2	$Y = 11,588x - 4,270$	0,809	4,69	
	3	$Y = 10,89x + 2,464$	0,719	4,37	

Discussion

Tea is one of the most widely consumed beverages in the world which is grouped into three main groups, including green, black, and oolong tea, according to the fermentation process. Fermentation of tea can affect the phytochemical content of different tea extract confirmed by the result that highest phenol and flavonoid content was observed in green tea (non fermented tea) and oolong tea (semi fermented tea) compared to black tea (fully fermented tea). In the fermentation process, the catechin is oxidized resulting thearubigin, theaflavin including theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3'-gallate (TF2B), and theaflavin-3,3'-digallate (TF3B) which is the key component of the black tea flavour (Leung et al., 2001; Yang & Landau, 2002; USDA, 2003). Phytochemical assay of green tea extract Catechins are a group of natural polyphenols found in green tea. Previous study of green tea extract using ethanol 70% resulted phenols (+++), triterpenoids (++) , steroid (-), terpenoids (+++), saponins (+), alkaloids (++) , flavonoids (++) , tannins (++) (Fanny et al., 2015). Different solvent concentration resulted different compounds, yield and bioactivities (Tiwari et al., 2011) The different solvent would result the different compound and bioactivity (Pujimulyani et al., 2004; Widowati et al., 2011b), validated by previous study that water extract of Forsythia koreana flowers exhibited a higher phenolic content than ethanol extract (Yang and Kang, 2011). Otherwise, theaflavins are another group of polyphenol pigment found in both black and oolong teas. Oolong tea that partially oxidized and retains a considerable amount of the original catechin (Nor & Mohd, 2003). Many studies have demonstrated that both catechin and theaflavin have strong free-radical-scavenging activity both *in vivo* and *in vitro* (Lai et al., 2001).

The chemical composition of tea includes proteins, polysaccharides, polyphenols (catechins or flavan-3-ols, theaflavins, thearubigins, and proanthocyanidins), chlorophyll, minerals, and trace elements volatile compounds, amino and organic acids, lignins and alkaloid (Chen et al., 2009). Tea is well known for its health benefits because of its polyphenol bioactive content, especially catechin which have antioxidant activity that play a role in reducing the free radical effect (Vecchia et al., 1992; Bravo et al., 1998; Nagao et al., 2005). Catechin belong to flavonoid that possess the high antioxidant activity in biological system. (Gramza et al., 2005; Evacuasiyani et al., 2014)

According to the Table 2. the most active extract in DPPH scavenging activity was the green

tea extract showed by the lower of IC_{50} values compared to the catechin, quercetin, kaempferol, and myricetin. The green tea extracts have the higher antioxidant activity through DPPH scavenging activity and IC_{50} value compared to the black tea extracts (Widowati et al., 2011a). That result is in accordance to the phytochemical assay (Table 1) and total phenol assay (Table 2) showed that the green tea extract contains the higher total phenol content compared to black tea and oolong tea extracts and the higher flavonoid compared to the black tea extracts. High correlations were observed between antioxidants activities and polyphenol phytochemicals content. The antioxidants activities most probably might be contributed by polyphenols contents in the plant extracts (Bakar et al., 2009; Ling et al., 2010; Nor & Mohd, 2013).

The result of this study also confirmed that the green tea extract possesses the higher antioxidant activity compared to the catechin, quercetin, kaempferol and myricetin. Table 2 also confirmed that tea extract contains catechin and various compounds including quercetin, kaempferol and myricetin (Cabrera et al., 2006). Some research also investigated that black tea, oolong tea and green tea possess the antioxidant activity (Xie et al., 1993; Zhu et al., 2002). This study also in line with the research that polyphenol compounds have antioxidant activity (Higdon & Frei, 2003; Widowati et al., 2011a). Green tea is the good source of polyphenol especially for flavanol and flavonol compounds which is found in 30% of tea leaf dry mass (Wolfram et al., 2008). The antioxidant activity of green tea extracts was higher than oolong tea and black tea extracts (Gramza et al., 2005). Satoh *et al* (2005) also found that the green tea has the highest percent DPPH scavenging activity followed by roasted tea, oolong tea, and black tea respectively. That antioxidant activities have a strong correlation with the total phenolic content. The oxidation of flavanol in green tea may greatly contribute to the high antioxidant activities compared to the other kinds of tea (Von et al., 2000; Satoh et al., 2005).

Conclusion

The green tea methanol extract has the higher total phenol content compared to the other extracts (black tea and oolong tea). Green tea extract also shows the highest antioxidant DPPH scavenging activity. Hence, the need to exploit the potential of green tea in pharmaceutical industries arises.

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References

- Bakar MFA, Mohamed M, Rahmat A, Fry J (2009) Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*). *Food Chem* 113:479-483.
- Bagchi K, Puri S (1998) Free radicals and antioxidants in health and disease. *EMHC* 4: 350-360.
- Balentine D, Wiseman S, Bouwers L (1997) The chemistry of tea flavonoids. *Crit Rev Food Sci Nutr* 37: 693-704.
- Bera TK, Chatterjee K, Ghosh D (2014) In-vitro antioxidant properties of the hydro-methanol extract of the seeds of *Swietenia mahagoni* (L.) Jacq. *Biomarkers Genomic Med* 20: 1-7
- Bravo L (1998) Polyphenols:chemistry, dietary sources, metabolism, and nutritional significance. *Nutr Rev* 56: 317-333.
- Budiman I, Tjokropranoto R, Widowati W, Rahardja F, Maesaroh M, Fauziah N (2014) Antioxidant and anti-malarial properties of catechins. *British J Med Med Research* 5(7): XX-XX
- Cabrera C, Artacho R, Gimenez R (2006) Beneficial effects of green tea-a review. *J Am Coll Nutr* 25(2): 79-99.
- Chen H, Zhishuang Q, Lingling F, Peng D, Xin Z (2009) Physicochemical properties and antioxidant capacity of 3 polysaccharides from green tea, oolong tea, and black tea. *J Food Sci* 74(6): 469-474.
- Evacuasiyany E, Ratnawati H, Liana LK, Widowati W, Maesaroh M, Mozef T, Risdian C (2014) Cytotoxic and antioxidant activities of catechins in inhibiting the malignancy of breast cancer. *Oxid Antioxid Med Sci* 3(2):141-146
- Eric W, Eu Y, Pei P, Yon P (2011) Antioxidant and antibacterial properties of green, black, and herbal teas of *Camellia sinensis*. *Pharmacognosy Res* 3(4): 266-272.
- Fransworth N (1966) Biological and phytochemical screening of plants. *J Pharm Sci* 55: 225-276.
- Gramza A, Pawlak-Lemanska K, Korczak J, Sowicz E, Rudzinska M (2005) Tea extracts as free radical scavengers. *Polish J Environ Studies* 14(6):861-867.
- Halliwell B, Gutteridge J (2007) Free radicals in biology and medicine. 4th ed. Clarendon Press, Oxford, UK
- Han S, Lo S, Choi Y, Kim J, Baek S (2004) Antioxidant activity of crude extract and pure compounds of *Acer ginnala* Max. *Bull Korean Chem Soc* 25(3): 389-391.
- Higdon J, Frei B (2003) Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Cri Rev Food Sci Nutr* 43:89-143.
- Ivanova D, Gerova D, Chervenkov T, Yankova T (2005) Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *J Ethnopharmacol* 96:145-150.
- Johnson P (2002) Antioxidant enzyme expression in health and disease: effects of exercise and hypertension. *Comp Biochem Physiol* 133: 493-505.
- Krinsky N (1992) Mechanism of action of biological antioxidants. *Proc Soc Exp Biol Med* 200: 248-254.
- Lai K, Yalun S, Ruoyun C, Zesheng Z, Yu H, Zhen-Yu C (2001) Theaflavins in black tea and catechins in green tea are equally effective antioxidants. *JN* 2001: 2248-2251.
- Leung L, Su Y, Chen R, Zhang Z, Huang Y, Chen ZY (2001) Teaflavins in black tea and catechins in green tea are equally effective antioxidants. *J Nutr* 131:2248-2251.
- Ling J, Mohamed M, Rahmat A, Abu Bakar M (2010) Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (*Boesenbergia rotunda*, *B. puchella* var *attenuata* and *B.armeniaca*). *J Med Plant Res* 4(1): 27-32.
- Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* 4(8):118-126.
- McKay D, Blumberg J (2002) The role of tea in human health: an update. *J Am Coll Nutr* 21: 1-13.
- Nagao T, Komine Y, Meguro S, Hase T, Tanaka Y, Tokimitsu I (2005) Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. *Am J Clin Nutr* 81: 122-129.
- Nor Q, Mohd F (2013) Phytochemicals and antioxidant properties of different parts of *Camellia sinensis* leaves from Sabah Tea Plantation in Sabah, Malaysia. *Int Food Res J* 20(1): 307-312.
- Pujimulyani D, Wazyka A, Anggrahini S, Santoso U (2004). Antioxidative properties of white

- saffron extract (*Curcuma mangga* Val) in the β -carotene bleaching and DPPH-radical scavenging methods. *Indonesian Food Nutr. Progress.* 2004, II(2): 35-40.
- Satoh E, Tohyama N, Nishimura M (2005) Comparison of the antioxidant activity of roasted tea with green, oolong, and black teas. *Int J Food Sci Nutr* 56(8): 551-559
- Tiwari P, Bimlesh K, Kaur M, Kaur G, Kaur H. (2011) Phytochemical screening and Extraction: A Rev Inter Pharm Sci 1(1): 98-106
- USDA. (2003) USDA database for the flavonoid contents of selected foods Beltsville. US Department of Agriculture.
- Vecchia C, Negri E, Francheschi S, D'Avanzo B, Boyle P (1992) Tea consumption and cancer risk. *Nutr Cancer* 17(1): 27-31.
- Von G, Joubert E, Hansmann C (2000) Comparison of antioxidant activities of rooibos tea (*Aspalathus linearis*) on red blood cells of Japanese quails. *Gen Physiol Biophys* 19: 365-371.
- Widowati W, Ratnawati H, Rusdi U, Winarno W, Immanuel V (2010) Phytochemical assay and antiplatelet activity of fractions of velvet bean seeds (*Mucuna pruriens* L.). *Hayati J Biosci* 17(2): 85-90.
- Widowati W, Herlina T, Ratnawati H, Mosef T, Risdian C (2011a) Antioxidant activities and platelet aggregation inhibitor of black tea (*Camellia sinensis* L.). *Med Plants* 3(1): 21-26.
- Widowati W, Sardjono CT, Wijaya L, Laksmiawati DR, Darsono L (2011b) Free Radicals Scavenging Activities of Spices and Curcumin. *Proceeding of The Second International Symposium on Temulawak*, ISBN No. 978-979-25-1209: 179-181
- Widowati W, Ratnawati H, Mozefis T, Pujimulyani D, Yellianty Y (2013a) Hypolipidemic and Antioxidant Effects of Black Tea Extract and Quercetin in Atherosclerotic Rats. *Int J Med, Pharm Scie Engineer* 7(10):64-71
- Widowati W, Wijaya L, Teresa L, Bachtiar I, Yellianty Y, Laksmiawati R (2013b) Antioxidant, anticancer, and apoptosis-inducing effects of Piper extracts in HeLa cells. *J Exp Integr Med* 3(3): 225-230.
- Widowati W, Mosef T, Risdian C, Yellianty Y (2013c) Anticancer and free radical scavenging potency of *Catharanthus roseus*, *Dendrophthoe petandra*, *Piper betle* and *Curcuma mangga* extracts in breast cancer cell lines. *Oxid Antioxid Med Sci* 2(2): 137-142.
- Widowati W, Ratnawati H, Wahyudianingsih R, Yellianty Y, Maesaroh M, Pujimulyani D, Mozef T (2014a) Effect of green tea extract and epigallocatechin-3-gallate potency on lipid profile and coronary artery morphology of dyslipidemic rats. *Oxid Antioxid Med Sci* 3(2):147-152
- Widowati W, Widyanto RM, Husin W, Ratnawati H, Laksmiawati DR, Setiawan B, (2014b) Nugrahenny D, Bachtiar B. Green tea extract protects endothelial progenitor cells from oxidative insult through reduction of intracellular reactive oxygen species activity. *Iran J Basic Med Sci* 17 (9):703-709
- Widowati W, Ratnawati H, Husin W, Maesaroh M (2015) Antioxidant properties of spice extracts. *Biomed Engineer* 1(1): 24-29
- Wiseman S, Balentine D, Frei B (1997) Antioxidants in tea. *Crit Rev Food Sci Nutr* 37: 705-718.
- Wolfram S (2007) Effects of green tea and EGCG on cardiovascular and metabolic health. *J Am Coll Nutr* 6(4): 373S-388S.
- Xie B, Shi H, Chen Q (1993) Antioxidant properties of fractions and polyphenol constituents from green, oolong, and black tea. *Proc Nat Sci Repub China* 17:77-84.
- Yang C, Landau J (2002) Effect of tea consumption on nutrition and health. *J Nutr* 130: 2409-2412.
- Yang XN, Kang SC. 2011. *In vitro* antioxidant activity of the water and ethanol extracts of *Forsythia koreana* flowers. *Nat Prod Res.* 2011 Aug 24. Online available
- Zhu Q, Hackman R, Ensunsa J, Holt R, Keen C (2002) Antioxidative activities of oolong tea. *J Agric Food Chem* 50: 6929-6934.

**LEMBAR HASIL PENILAIAN
SEJAWAT SEBIDANG atau PEER REVIEW**

KARYA ILMIAH : JURNAL ILMIAH

Judul Karya Ilmiah (Artikel) : Antioxidant Potential of Black, Green and Oolong Tea Methanol Extracts
 Jumlah Penulis : 6 Orang
 Nama-nama Penulis : **Wahyu Widowati**, Tati Herlina, Hana Ratnawati, Gabriella Constantia, I Dewa Gde Sathya Deva, Maesaroh Maesaroh
 Status Penulis : Penulis Pertama / ~~Penulis ke-....~~ / Penulis Korespondensi **)
 Identitas Jurnal Ilmiah : a. Nama jurnal : Biology, Medicine, & Natural Product Chemistry
 b. Nomor ISSN : 2089-6514
 c. Vol., No., Bulan, Tahun : Vol 4; No.2; 2015
 d. Penerbit : Sunan Kalijaga State Islamic University & Society for Indonesian Biodiversity
 e. DOI Artikel (jika ada) : 10.14421/biomedich.2015.42.35-39
 f. Alamat Web Jurnal : sciencebiology.org/index.php/BIOMEDICH
 g. Terindeks di : DOAJ

Kategori Publikasi Jurnal Ilmiah: Jurnal Ilmiah Internasional / Internasional Bereputasi **)
 (beri tanda \checkmark yang dipilih)
 Jurnal Ilmiah Nasional Terakreditasi
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HASIL PENILAIAN (Peer Review) :

No	Komponen Yang Dinilai	Nilai Maksimal JURNAL ILMIAH			Nilai Akhir Yang Diperoleh *)
		Internasional / Bereputasi <input checked="" type="checkbox"/>	Nasional Terakreditasi <input type="checkbox"/>	Nasional ***) <input type="checkbox"/>	
a.	Kelengkapan unsur isi karya (10%)	2			1,6
b.	Ruang lingkup dan kedalaman pembahasan (30%)	6			5,6
c.	Kecukupan dan kemutakhiran data/ informasi dan metodologi (30%)	6			5,5
d.	Kelengkapan unsur dan kualitas penerbitan (30%)	6			5,5
	Total	100%	20		18,2

Catatan Penilaian ARTIKEL oleh Reviewer :

a. Kelengkapan dan kesesuaian unsur... *Paper ditulis dengan baik, hasil sesuai judul penelitian, mengikuti landasan penelitian.*

b. Ruang lingkup & kedalaman pembahasan... *Penelitian tentang potensi berbagai jenis teh (oolong, hitam, hijau) sebagai antioksidan secara in vitro dan uji fitokimia.*

c. Kecukupan & kemutakhiran data serta metodologi... *Ida dasar penelitian baik membahas, mengukur kadar fenol, sinyawa fitokimia berbagai ekstrak teh.*

d. Kelengkapan unsur dan kualitas penerbit... *Jurnal internasional terindeks DOAJ, Penerbit Sunan Kalijaga State Islamic University & Society for Indonesian Biodiversity.*

e. Indikasi plagiasi
Tidak terdapat indikasi plagiarisme atau self plagiarisme.....
.....
.....

f. Kesesuaian bidang ilmu ..Paper bidang bio kimia , biomedis sesuai dengan bidang ilmu peneliti
.....
.....
.....

REVIEWER 1



(Prof. Dr. Chrismis Novalinda Ginting, M.Kes)
NIK : 0115127801
UNIVERSITAS PRIMA INDONESIA

LEMBAR HASIL PENILAIAN
SEJAWAT SEBIDANG atau PEER REVIEW

KARYA ILMIAH : JURNAL ILMIAH

Judul Karya Ilmiah (Artikel) : Antioxidant Potential of Black, Green and Oolong Tea Methanol Extracts
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Kategori Publikasi Jurnal Ilmiah: Jurnal Ilmiah Internasional / ~~Internasional~~ Bereputasi **)
 (beri tanda ✓ yang dipilih)
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HASIL PENILAIAN (Peer Review) :

No	Komponen Yang Dinilai	Nilai Maksimal JURNAL ILMIAH			Nilai Akhir Yang Diperoleh *)
		Internasional <input checked="" type="checkbox"/>	Nasional Terakreditasi <input type="checkbox"/>	Nasional ***) <input type="checkbox"/>	
a.	Kelengkapan unsur isi karya (10%)	2			1,7
b.	Ruang lingkup dan kedalaman pembahasan (30%)	6			6,8
c.	Kecukupan dan kemutakhiran data/informasi dan metodologi (30%)	6			9,4
d.	Kelengkapan unsur dan kualitas penerbitan (30%)	6			9,5
Total 100%		20			18,4

Catatan Penilaian ARTIKEL oleh Reviewer :

- a. Kelengkapan dan kesesuaian unsur.
Jurnal ini sudah cukup lengkap dan mengikuti panduan jurnal yang baik dan ada kesesuaian antara unsur dan isinya.
- b. Ruang lingkup & kedalaman pembahasan
Ruang lingkup dan pembahasan yang digunakan sudah mendalam
- c. Kecukupan & kemutakhiran data serta metodologi.
Secara umum metodologi sudah memadai dan lengkap, serta kemutakhiran data sudah terpenuhi
- d. Kelengkapan unsur dan kualitas penerbit

Jurnal sudah memiliki PBB (Inggris), terindeks ISI 2009-6514 dan juga sudah memituti regulasi yang baik.

e. Indikasi plagiasi

Tidak ditemukan adanya unsur plagiasi.

f. Kesesuaian bidang ilmu

Sudah sesuai dengan bidang ilmu yang diteliti penulis.

REVIEWER 2



(Prof. Dr. Ermi Girsang, M. Kes)

NIK : 0117057501

UNIVERSITAS PRIMA INDONESIA

LEMBAR HASIL PENILAIAN
SEJAWAT SEBIDANG atau *PEER REVIEW*

KARYA ILMIAH : JURNAL ILMIAH

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Kategori Publikasi Jurnal Ilmiah: Jurnal Ilmiah Internasional / ~~Internasional Bereputasi~~ (**)
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HASIL PENILAIAN (*Peer Review*) :

No	Komponen Yang Dinilai	Nilai Maksimal JURNAL ILMIAH			Nilai Akhir Yang Diperoleh *)
		Internasional / Bereputasi <input checked="" type="checkbox"/>	Nasional Terakreditasi <input type="checkbox"/>	Nasional (***) <input type="checkbox"/>	
a.	Kelengkapan unsur isi karya (10%)	2			1,65
b.	Ruang lingkup dan kedalaman pembahasan (30%)	6			5,7
c.	Kecukupan dan kemutakhiran data/informasi dan metodologi (30%)	6			5,45
d.	Kelengkapan unsur dan kualitas penerbitan (30%)	6			5,5
	Total	100%	20		18,3

Catatan Penilaian ARTIKEL oleh Reviewer :

a. Kelengkapan dan kesesuaian unsur... *Paper ditulis dengan baik, hasil sesuai judul penelitian, mengikuti kaidah penelihan*

Jurnal ini sudah cukup lengkap dan mengikuti panduan jurnal yang baik dan ada kesesuaian antara unsur dan telrnya.

 b. Ruang lingkup & kedalaman pembahasan

Penelitian tentang potensi berbagai jenis teh (oolong, hitam, hijau) sebagai antioksidan secara in vitro dan uji fitokimia

Ruang lingkup dan pembahasan yang digunakan sudah mendalam

c. Kecukupan & kemutakhiran data serta metodologi. Ide dasar penelitian baik membahas, mengukur kadar fenol, senyawa fitokimia berbagai ekstrak teh.

Secara umum metodologi sudah memadai dan lengkap, kemutakhiran data sudah terpenuhi

d. Kelengkapan unsur dan kualitas penerbit. Jurnal internasional terindeks DOAJ, Penerbit Sunan Kalijaga State Islamic University & Society for Indonesian Biodiversity

Jurnal sudah memakai PBB (Inggris), terindeksi ISSN 2089-6514 dan juga sudah memiliki regulasi yang baik

e. Indikasi plagiasi



Tidak terdapat indikasi plagiarisme atau self-plagiarisme

tidak ditemukan adanya unsur plagiasi

f. Kesesuaian bidang ilmu

Paper bidang biokimia, biomedis sesuai dengan bidang ilmu penulis

Sudah sesuai dengan bidang ilmu yang diteliti penulis

Medan, Reviewer 2 	Medan, Reviewer 1 
(Prof. Dr. Ermi Girsang, M.Kes) NIK : 0117057501	(Prof. Dr. Chrismis Novalinda Ginting, M.Kes) NIK : 0115127801
UNIVERSITAS PRIMA INDONESIA	UNIVERSITAS PRIMA INDONESIA