

# Natural Product Sciences



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3. Tea Flavonoids Induced Differentiation of Peripheral Blood-derived Mononuclear Cells into Peripheral Blood-derived Endothelial Progenitor Cells and Suppressed Intracellular Reactive Oxygen Species Level of Peripheral Blood-derived Endothelial Progenitor C Wahyu Widowati , Laura Wijaya , Dian Ratih Laksmitawati , Rahma Micho Widyanto , Pande Putu Erawijantari , Nurul Fauziah , Indra Bachtiar , Ferry Sandra	( PDF file / 6 pages) View 🔀   Down 🖏
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9. Cytotoxic, Anti-Inflammatory and Adipogenic Effects of Inophyllum D, Calanone, Isocordato-oblongic acid, and Morelloflavone on Cell Lines Muhammad Taher , Amnani Aminuddin , Deny Susanti , Nurul Iman Aminudin , Shamsul On , Farediah Ahmad , Hanisuhana	( PDF file / 7 pages) View 🎦   Down 🖏
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### Tea Flavonoids Induced Differentiation of Peripheral Blood-derived Mononuclear **Cells into Peripheral Blood-derived Endothelial Progenitor Cells and Suppressed** Intracellular Reactive Oxygen Species Level of Peripheral Blood-derived **Endothelial Progenitor Cells**

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Abstract - Endothelial dysfunction in atherosclerosis is associated with increasing oxidative stress that could be reversed by antioxidant. Therefore epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC) and catechin (C) of tea flavonoids were investigated for their roles in regenerating endothelial cell. Peripheral blood mononuclear cells (PB-MNCs) were isolated, plated and cultured in medium with/without treatment of EGCG, ECG, EGC and C. Results showed that among all EGCG, ECG, EGC and C concentrations tested, 12.5 µmol/L was not cytotoxic for peripheral blood-derived endothelial progenitor cells (PB-EPCs). Treatment of EGCG, ECG, EGC or C increased the percentages of CD34, CD133, VEGFR-2 expressions and suppressed hydrogen peroxide-induced percentages of reactive oxygen species (ROS) level in PB-EPCs. Taken together, our current results showed that EGCG, ECG, EGC or C of tea flavonoids could induce differentiation of PB-MNCs into PB-EPCs as well as protect PB-EPCs from oxidative damage by suppresing the intracellular ROS levels. Keywords - Tea flavonoids, Antioxidant, Endothelial progenitor cell, Differentiation, ROS, Apoptosis

#### Introduction

Endothelial dysfunctions play a role in the pathogenesis of atherosclerosis and its complications.<sup>1</sup> Increasing oxidative stress and disorder antioxidant mechanism, especially inactivation of nitric oxide (NO) by reactive oxygen species (ROS) such as superoxide anion radical  $(O_2-)$ , hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl radical (HO·), and lipid peroxides, may contribute to the endothelial dysfunction, intact vessel wall, up to the rupture of a lipid-rich atherosclerotic plaque.<sup>2</sup> Endothelial dysfunction

in atherosclerosis is associated with increasing oxidative stress that could be reversed by antioxidant therapy.<sup>3</sup> Antioxidants counter-balance the ROS production in cells and modify the regulatory pathways of cell growth.<sup>4</sup>

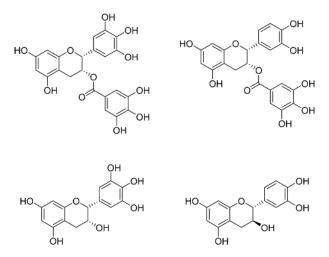
In several numbers of previous studies, tea consumption as a source of flavonoids was associated with low cardiovascular disease (CVD) and stroke-related mortality.<sup>5</sup> The mortality was significantly decreased along with the increase of tea flavonoids intake in a dose-dependent manner.<sup>6</sup> Some in vitro studies showed that flavonoids have strong ROS scavenging activity and prevent low density lipoprotein (LDL) to oxidize.<sup>1</sup> Atherosclerotic animal models study indicated that consuming dietary flavonoids can reduce atherosclerotic plaque development.<sup>8</sup> Flavonoids were reported to have anti-platelet, antiinflammatory, and antioxidant activities.9,10 In addition, an epidemiological study reported that consuming flavonoidcontaining foods and beverages could reduce CVD risk,

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**Fig. 1.** Chemical Structures of catechins. Structures of (–)-epigallocatechin gallate (EGCG) (**a**), (–)-epicatechin gallate (ECG) (**b**), (–)-epigallocatechin (EGC) (**c**), and (+)-catechin (C) (**d**).

as well as increase endothelial function.<sup>7</sup>

The effects of green tea are mainly associated with polyphenols content, especially flavonoids with approximately 30% of dry weight of leaves.<sup>11</sup> Predominantly flavonoids consist of epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC) and catechin (C).<sup>12,13</sup> In our previous study, we found that EGCG enhanced proliferation of adipose tissue-derived mesenchymal stem cells (AD-MSCs) and differentiation of AD-MSCs into endothelial progenitor cells (EPCs). Tea flavonoids including EGCG, ECG, EGC and C could also have effects on peripheral blood-derived EPCs (PB-EPCs). Therefore we conducted a study in order to investigate proliferation and differentiation inducing properties of EGCG, ECG, EGC, and C (Fig. 1) on PB-EPCs.

#### Experimental

**General Experimental Procedure** – (–)-epigallocatechin gallate (EGCG), (–)-epicatechin gallate (ECG), (–)epigallocatechin (EGC), and (+)-catechin (C) with purity 95-99% (Fig. 1.) were purchased from Biopurify Phytochemical Ltd. (Chengdu, China). Human fibronectin was purchased from Roche (Basel, Switzerland). VascGrow<sup>TM</sup> medium was obtained from Stem Cell and Cancer Institute (Jakarta, Indonesia). Ficoll-Paque reagent was purchased from GE Healthcare (Uppsala, Sweden). Fluorescein isothiocyanate (FITC)-conjugated ulex europaeus agglutinin I (UEA-I), and 1,1-dioctadecyl-3,3,3,3-tetramethylindo carbocyanine-labeled acetylated LDL (DilacLDL) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The 4',6-diamidino-2-phenylindole (DAPI)

#### **Natural Product Sciences**

and 2'-7'-dichlorofluorescein diacetate (DCF-DA) were purchased from Invitrogen (Carlsbad, CA, USA). FcR Blocking and phycoerythrin (PE)-conjugated anti-CD133 antibodies were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). FITC-conjugated anti-CD45/PEconjugated anti-CD34 antibody was purchased from Becton Dickinson (Franklin Lakes, NJ, USA). PE-conjugated anti-vascular endothelial growth factor (VEGFR)-2/Kinase Domain Receptor (KDR) antibody was purchased from R&D System (Minneapolis, MN, USA). The 3-(4,5dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4sulfophenyl)-2H-tetrazolium (MTS) was purchased from Promega (Madison, WI, USA). Fluorescence-activated cell sorting (FACS) was performed using a FACS Calibur Flow Cytometer Becton Dickinson (Franklin Lakes, NJ, USA). Cells were observed using inverted fluorescent microscope (Axiovert 40 CFL, Zeiss).

**Isolation and Culture** – Briefly, total PB-MNCs were isolated from peripheral blood of healthy young human volunteers with Ficoll density gradient centrifugation. PB-MNCs were plated in human fibronectin-coated culture dishes and cultured using VascGrow<sup>TM</sup> at 37 °C in a humidified, 5% CO<sub>2</sub> incubator. New media was applied after 4 days of culture and the culture was maintained for 7 days. Prior to blood collection, all volunteers signed informed consent. All procedures were approved by the ethics committee of Stem Cell and Cancer Institute, Jakarta and the joint ethics committee of Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia and Immanuel Hospital, Bandung, Indonesia.

**Fluorescent Staining** – Fluorescent staining was performed to detect FITC-UEA-I and Dil-acLDL. To identify the uptake Dil-acLDL, adherent cells were incubated with 1 mg/mL Dil-acLDL at 37 °C for 4 hours. Cells were fixed with 3% paraformaldehyde for 10 minutes. Soon after cleansed, cells were incubated with 1 mg/mL FITC-UEA-I at 37 °C for 1 hour, followed by nucleus staining using DAPI. After staining, cells were observed using an Axiovert 40 CFL inverted fluorescent microscope from Carl Zeiss (Jena, Germany). Cells with double positive for both DilacLDL and FITC-UEA-I were defined as PB-EPCs.

**MTS Assay** – This assay was done to quantify viable PB-EPCs under treatment of EGCG, ECG, EGC and C. Briefly,  $5 \times 10^3$  PB-EPCs were seeded in 96-well plates using serum-free medium for 24 hours. After EGCG, ECG, EGC or C treatment, PB-EPCs were added with 20 µL MTS and incubated at 37 °C in a humidified, 5% CO<sub>2</sub> incubator for 4 hours. Optical Density value was measured at 490 nm using a microplate reader from Biorad (Hercules, CA, USA).

**Apoptosis Assay** – PB-EPCs were cultured in 12-well plate and treated with EGCG, ECG, EGC or C for 24 hours. After that, PB-EPCs were collected, washed and stained with propidium iodide solution in phosphate buffer saline (PBS) for 15 minutes. The apoptotic cells were determined as SubG1 area using a fluorescence-activated cell sorting (FACS)Calibur flow cytometer from Becton Dickinson and presented as a percentage of total cells.

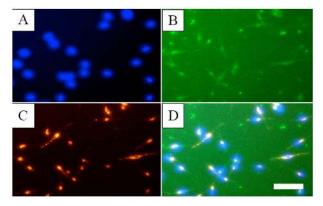
**Immunophenotyping** – PB-EPCs were detached using 2 mM ethylenediaminetetraacetate (EDTA) and pre-incubated for 15 minutes at room temperature with FcR Blocking. Then PB-EPCs were incubated at 4 °C with FITC-conjugated anti-CD45/PE-conjugated anti-CD34 and PE-conjugated anti-CD133 antibodies for 15 minutes, followed by PE-conjugated VEGFR-2/KDR antibody for another 40 minutes. Isotype-identical antibodies were also applied and used as controls to negative bindings. Each expression was analyzed using a FACSCalibur.

Intracellular ROS Assay – Treated PB-EPCs were incubated in 37  $^{\circ}$ C with 10  $\mu$ mol/L DCF-DA for 30 minutes and washed with PBS containing KCl solution. Level of intracellular ROS were measured using a FACSCalibur.

**Statistical Analysis** – Data are presented as mean and standard deviation. Statistical analysis was perfomed using IBM SPSS Statistics for Windows software version 20.0. (Armonk, NY, USA). One-way analysis of variance test was performed, followed by Duncan's post-hoc tests.

#### **Result and Discussion**

Lectin binding and Dil-acLDL uptake of the CD34<sup>+</sup>, CD133<sup>+</sup> and VEGFR-2<sup>+</sup> cells – Cultured cells were adherent with spindle-shaped morphology. Fig. 2B showed lectin binding property, while Fig. 2C showed Dil-acLDL uptake of the cells, marked by fluorescent cells in both Fig. 2B and 2C. These 2 characteristics



**Fig. 2.** PB-EPCs lectin binding and LDL uptake. PB-EPCs were seeded in 96-well plate and subjected to Fluorescent Staining as described in Experimental. A: DAPI, B: FITC-UEA-I, C: DilacLDL, D: Merge of A, B and C. White bar: 50 μm.

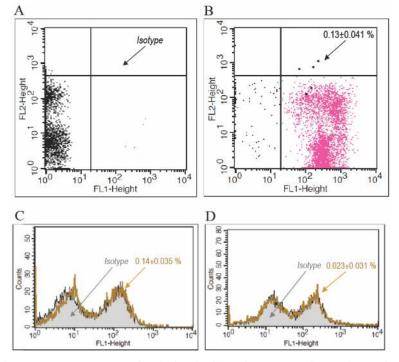


Fig. 3. PB-EPCs immunophenotypes. PB-EPCs were detached and subjected to Immunophenotyping as described in Experimental. A: isotype, B: CD34/45, C: CD133 and its isotype, D: VEGF-R2 and its isotype.

Tracture and		Percentage of Viable P	B-EPCs (Mean±SD)	
Treatment	EGCG	ECG	EGC	С
Untreated	$100.00 \pm 8.27$ <sup>cA</sup>			
0.5% DMSO	$98.68 \pm 5.04$ <sup>cA</sup>			
12.5 µmol/L	$93.20 \pm 5.29$ <sup>cA</sup>	$94.00 \pm 2.27$ <sup>cA</sup>	$93.73 \pm 1.04$ <sup>cA</sup>	$93.39 \pm 0.33$ bcA
25 μmol/L	$78.28 \pm 4.02$ <sup>bA</sup>	$68.45 \pm 4.96$ <sup>bA</sup>	$69.23 \pm 4.32$ <sup>cA</sup>	$93.96 \pm 4.84$ bcB
50 µmol/L	$71.29 \pm 4.18$ <sup>bA</sup>	$62.18 \pm 2.27$ bA	$63.47 \pm 3.55$ <sup>bA</sup>	$86.21 \pm 5.81$ bB
100 µmol/L	$30.59\pm4.96~^{\mathrm{aA}}$	$32.43\pm4.96~^{\mathrm{aA}}$	$54.67\pm3.06~^{aB}$	$61.76\pm5.94~^{aB}$

Table 1. Effect of EGCG, ECG, EGC and C on PB-EPCs viability

PB-EPCs were treated with 12.5, 25, 50 or 100  $\mu$ mol/L of EGCG, ECG, EGC or C for 24 hours. MTS Assay was carried out as described in Experimental. Each treatment was done in triplicate. <sup>a,b,c</sup>Means in the same column containing the same superscript are not significant ( $p \ge 0.05$ ), while means in the same column containing different superscript in small letter indicate significant differences (p < 0.05). <sup>A,B</sup>Means in the same row containing the same superscript are not significant ( $p \ge 0.05$ ), while means in the same row containing different superscript in capital letter indicate significant differences (p < 0.05). Statistical analysis was performed based on Duncan's post-hoc test. SD: standard deviation.

 Table 2. Effect of EGCG, ECG, EGC and C on apoptosis of PB-EPCs

Treatment	Percentage of SubG1
Untreated	$11.68 \pm 1.20^{\ a}$
0.5% DMSO	$14.93 \pm 1.60$ <sup>a</sup>
EGCG	$14.48 \pm 6.83$ <sup>a</sup>
ECG	$14.57 \pm 7.67$ <sup>a</sup>
EGC	$12.31 \pm 3.00$ <sup>a</sup>
С	$15.97 \pm 3.62$ <sup>a</sup>

Ten thousands PB-EPCs were seeded in each 12-well plate and treated with 12.5  $\mu$ mol/L EGCG, ECG, EGC or C for 24 hours. After 24 hours, Apoptosis Assay was carried out as described in Experimental. Each treatment was done in triplicate. The apoptotic cells were determined on the basis of the SubG1 area. The data are presented as mean ± standard deviation. <sup>a</sup>Means in the same column containing the same superscript are not significant (p ≥ 0.05). Statistical analysis was performed based on Duncan's post-hoc test.

implied the cultured cells as PB-EPCs. The cells were further immunophenotyped for CD34, CD133 and VEGFR-2. Flow cytometric results showed that the cells expressed CD34, CD133 and VEGFR-2 (Fig. 3). Hence, our cultured cells were confirmed as EPCs.

EGCG, ECG, EGC and C reduced percentages of viable PB-EPCs – Based on the results of MTS assay (Table 1), PB-EPCs treated with higher concentration of EGCG, ECG, EGC, and C showed significant lower percentages of viable PB-EPCs. Among all concentrations of EGCG, ECG, EGC and C tested, 12.5  $\mu$ mol/L had the lowest potential in reducing viable PB-EPCs percentages. In accordance, under treatment of 12.5  $\mu$ mol/L EGCG, ECG, EGC or C, percentages of apoptosis resulted were almost similar to the untreated (Table 2). These results were similar to our previous research showing that >25

 Table 3. Effect of EGCG, ECG, EGC and C on PB-EPCs immunophenotypes

51		
CD34 (%)	CD133 (%)	VEGFR-2 (%)
$52.86\pm8.44~^{\mathrm{b}}$	$0.63\pm0.02~^{ab}$	$0.22\pm0.02$ $^{a}$
$28.96\pm9.68$ $^{\rm a}$	$0.36\pm0.14$ $^{a}$	$1.78\pm0.15$ $^{\rm c}$
$51.09\pm2.77$ $^{\rm b}$	$0.83\pm0.41~^{ab}$	$1.22\pm0.19$ $^{b}$
$60.48\pm2.60$ $^{\rm b}$	$0.61\pm0.08~^{ab}$	$2.42\pm0.13$ $^{\rm c}$
$54.70\pm3.74$ $^{\rm b}$	$1.13\pm0.71$ $^{\rm b}$	$1.21\pm0.22$ $^{\rm b}$
$62.24\pm9.22$ $^{\rm b}$	$1.01\pm0.13$ $^{ab}$	$1.47\pm0.33$ $^{bc}$
	$52.86 \pm 8.44^{\text{b}}$ $28.96 \pm 9.68^{\text{a}}$ $51.09 \pm 2.77^{\text{b}}$ $60.48 \pm 2.60^{\text{b}}$ $54.70 \pm 3.74^{\text{b}}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$

Isolated-PB-MNCs were cultured with addition of 12.5 µmol/L EGCG, ECG, EGC or C. PB-EPCs Culture was then carried out as described in Experimental. PB-EPCs were then detached for Immunophenotyping as described in Experimental. Each treatment was done in triplicate. The data are presented as mean  $\pm$  standard deviation. <sup>a,b,c</sup>Means in the same column containing the same superscript are not significant (p  $\ge$  0.05), while means in the same column containing different superscript in small letter indicate significant differences (p < 0.05). Statistical analysis was performed based on Duncan's post-hoc test.

 $\mu$ g/mL green tea extract induced cytotoxic in PB-EPCs<sup>14</sup>, while 12.5  $\mu$ g/mL was not cytotoxic for EPCs.

EGCG, ECG, EGC and C increased expressions of CD34, CD133 and VEGFR-2 – EPCs are defined by their cell surface expressions, including CD34, CD133 and VEGFR-2<sup>15,16</sup>. CD34 and CD133 indicate the plasticity of the cells bearing the stem cell characteristic, whereas VEGFR-2 indicates endothelial characteristic. Based on our current flow cytometric results, EGCG, ECG, EGC or C increased the percentages of CD34, CD133 and VEGFR-2 expressions (Table 3). The highest percentage of CD34 expression was induced by C, the highest percentage of CD133 expression was induced by EGC, meanwhile the highest percentage of VEGFR-2 expression was induced by EGC, These results suggested that EGCG, ECG, EGC

Table 4. Effect of EGCG, ECG, EGC and C on ROS of H <sub>2</sub> O <sub>2</sub> -induced PB-EPCs				
Treatment	ROS Level (%)	Ratio of All to Negative Control (%)	Ratio of All to Positive Control (%)	
Untreated (negative control)	$7.20\pm1.65$ $^{\rm a}$	$99.95 \pm 22.97$ <sup>a</sup>	$22.81 \pm 5.24$ <sup>a</sup>	
H <sub>2</sub> O <sub>2</sub> (positive control)	$31.55 \pm 1.10^{\ e}$	438.15 ± 15.22 °	$99.99 \pm 3.47$ °	
$EGCG + H_2O_2$	$12.92\pm0.70$ $^{\rm c}$	$179.49 \pm 9.67$ °	$40.96 \pm 2.21$ °	
$ECG + H_2O_2$	$10.83 \pm 2.35$ bc	$150.46 \pm 32.62$ bc	$34.34 \pm 7.44$ bc	
$EGC + H_2O_2$	$18.66 \pm 2.81$ <sup>d</sup>	$259.21 \pm 39.09$ <sup>d</sup>	$59.15 \pm 8.92$ <sup>d</sup>	
$C + H_2O_2$	$8.85\pm1.13~^{ab}$	$122.96 \pm 15.73$ <sup>ab</sup>	$28.06 \pm 3.59$ <sup>ab</sup>	

Table 4. Effect of EGCG, ECG, EGC and C on ROS of H<sub>2</sub>O<sub>2</sub>-induced PB-EPCs

PB-EPCs were treated with 12.5  $\mu$ mol/L of EGCG, ECG, EGC or C for 30 minutes. PB-EPCs were then treated with H<sub>2</sub>O<sub>2</sub> with final concentration of 200  $\mu$ mol/L for 1 hour. Treated-PB-EPCs were subjected to Intracellular ROS Assay as described in Experimental. Each treatment was done in triplicate. The data are presented as mean±standard deviation. <sup>a,b,c,d</sup>Means in the same column containing the same superscript are not significant (p ≥ 0.05), while means in the same column containing different superscript in small letter indicate significant differences (p < 0.05). Statistical analysis was performed based on Duncan's post-hoc test.

and C induced differentiation of peripheral blood mononuclear cells (PB-MNCs) into PB-EPCs. Our previous results also showed that ECGC induced differentiation of AD-MSCs into EPCs<sup>17</sup>. In addition, resulted PB-EPCs showed that VEGFR-2 was the most elevated marker, indicating high endothelial characteristic.

EGCG, ECG, EGC and C suppressed H<sub>2</sub>O<sub>2</sub>-induced ROS level in PB-EPCs - DCF-DA has been used for several studies dealing with the effect of ROS in cell culture<sup>18,19</sup>. DCF trapped within the cells was measured to represent the intracellular ROS level<sup>20,21</sup>. Compared with the untreated, induction of H2O2 increased percentages of ROS level in PB-EPCs significantly (Table 4). By pretreatment of EGCG, ECG, EGC or C, H2O2-induced percentages of ROS level was suppressed. These results were consistent with our previous results. Green tea extract that was supposed to contain flavonoids including EGCG, ECG, EGC and C, suppressed H<sub>2</sub>O<sub>2</sub>-induced ROS level in PB-EPCs<sup>14</sup>. Among all investigated flavonoids, C had the highest ROS suppressing capacity in PB-EPCs, since C was reported to have high 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and high superoxide dismutase activities<sup>22</sup>.

Taken together, our current results showed that EGCG, ECG, EGC or C of tea flavonoids, could induce differentiation of PB-MNCs into PB-EPCs as well as protect PB-EPCs from oxidative damage by suppresing the intracellular ROS levels. In addition, concentration of 12.5  $\mu$ mol/L was not cytotoxic for EPCs. This study suggests that tea flavonoids might be related to PB-EPCs production and protection.

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#### **Natural Product Sciences**

Received September 18, 2015 Revised November 18, 2015 Accepted November 25, 2015

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

#### KARYA ILMIAH : JURNAL ILMIAH

	Tea Flavonoids Induced Differentiation of Peripheral Blood-derived Mononuclear Cells into Peripheral Blood-derived Endothelial Progenitor Cells and Suppressed Intracellular Reactive Oxygen Species Level of Peripheral Blood-derived Endothelial Progenitor Cells 8 Orang					
Nama-nama Penulis		Vijaya, Dian Ratih Laksmitawati, Rahma Micho wijantari, Nurul Fauziah, Indra Bachtiar, Ferry				
Status Penulis	: Penulis Pertama / <del>Penulis k</del>	<del>e /</del> Penulis Korespondensi **)				
Identitas Jumal Ilmiah	: a. Nama jurnal	: Natural Product Sciences				
	b. Nomor ISSN	: 1226-3097				
	c. Vol., No., Bulan, Tahun	: Vol. 22 No. 2, 2016				
	d. Penerbit	: The Korean Society of Pharm				
	e. DOI Artikel (jika ada)	: 10.20307/nps.2016.22.2.87				
	f. Alamat Web Jurnal	: <u>http://www.e-nps.or.kr/</u>				
	g. Terindeks di	: Scopus Q3, SJR 0.322				
Kategori Publikasi Jurnal Ilmiah (beri tanda √yang dipilih)	Jurnal Ilmiah Internasional / Internasional Bereputasi **)					
(berrianda V yang alphin)	Jurnal Ilmiah Nasiona	Jurnal Ilmiah Nasional Terakreditasi				
	Jurnal Ilmiah Nasiona	1 / Nasional terindeks ***)				

#### HASIL PENILAIAN (Peer Review):

			Nilai Mak			
No	Komponen Yang Dinilai		Internasional / Bercputasi	Nasional Terakreditasi	Nasional ***)	Nilai Akhir Yang Diperolch *)
a.	Kelengkapan unsur isi karya	(10%)	4			315
b.	Ruang lingkup dan kedalaman pembahasan	(30%)	12			117
c.	Kecukupan dan kemutakhiran data/ informasi dan metodologi	(30%)	12			1115
d.	Kelengkapan unsur dan kualitas penerbitan	(30%)	12			117
	Total	100%	40		2	38,4

Catatan Penilaian ARTIKEL oleh Reviewer: a. Kelengkapan dan kesesuaian unsur Penulitian Mumenuhi standard karya ilmink atadumis, muto da yang digunakan secuai dan tepat digunakan pada penulitian Ini b. Ruang lingkup & kedalaman pembahasan Penelitian tentang pemangaatan senyawa flavonoid teh (EECE 55C, ECE, C) melindungi stress orgidatig EPCs yang diindutri radiial bebas c. Kecukupan & kemutakhiran data serta metodologi. Ide dasar penelitiran baik membahas meranisme Benyawa flavonoid mengindutsi diperensiasi BMC menjadi EPC, menekan ROS dari EPCs d. Kelengkapan unsur dan kualitas penerbit. Jumal M& terindeks. Suppur A 3 SJA 0,322. Penerlait

Korean Society	of Pharma Lognosy	
c. Indikasi plagias Simlan ty ind	dex sebecur 15% . Tiday	terdapat indibac 1 plagiarism cubau self - plagiarism
f. Kesesuaian bida	ang ilmu Paper bidang bi	enedis sesuai dungan birdang ilmu penulic.

**REVIEWER** 1

Zam B.

(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)	: Tea Flavonoids Induced Differentiation of Peripheral Blood-derived Mononuclear Cells into Peripheral Blood-derived Endothelial Progenitor Cells and Suppressed Intracellular Reactive Oxygen Species Level of Peripheral Blood-derived Endothelial Progenitor Cells
Jumlah Penulis	: 8 Orang
Nama-nama Penulis	: Wahyu Widowati, Laura Wijaya, Dian Ratih Laksmitawati, Rahma Micho Widyanto, Pande Putu Erawijantari, Nurul Fauziah, Indra Bachtiar, Ferry Sandra
Status Penulis	: Penulis Pertama / <del>Penulis ke</del> / Penulis Korespondensi **)
Identitas Jurnal Ilmiah	: a. Nama jurnal : Natural Product Sciences
	b. Nomor ISSN : 1226-3097
	c. Vol., No., Bulan, Tahun : Vol. 22 No. 2, 2016
	d. Penerbit : The Korean Society of Pharm
	e. DOI Artikel (jika ada) : 10.20307/nps.2016.22.2.87
	f. Alamat Web Jurnal : <u>http://www.e-nps.or.kr/</u>
	g. Terindeks di : Scopus Q3, SJR 0.322
Kategori Publikasi Jurnal Ilmiał (beri tanda √ yang dipilih)	: <b>Jurnal Ilmiah Internasional</b> / Internasional Bereputasi <b>**</b> )
(beri ianaa x yang aipitin)	Jurnal Ilmiah Nasional Terakreditasi
	Jurnal Ilmiah Nasional / Nasional terindeks ***)

#### HASIL PENILAIAN (Peer Review ) :

			Nilai Mak	simal JURNAI	LILMIAH	
No	Komponen Yang Dinilai		Internasional / Bereputasi	Nasional Terakreditasi	Nasional ***)	Nilai Akhir Yang Diperoleh *)
a.	Kelengkapan unsur isi karya	(10%)	4			3,6
b.	Ruang lingkup dan kedalaman pembahasan	(30%)	12			11,5
c.	Kecukupan dan kemutakhiran data/ informasi dan metodologi	(30%)	12			1,6
d.	Kelengkapan unsur dan kualitas penerbitan	(30%)	12			11.12
	Total	100%	40			38,2

Catatan Penilaian ARTIKEL oleh Reviewer :

a,	Kelengkapan dan kesesuaian unsur. Kelengkapan unsur 167 Artocel Juluh entry lengten dan kerdupat Keretwatan antura unsur dan 100 Mya
	antura Ungur dan lanya
b.	Ruang lingkup & kedalaman pembahasan Ruang berhasan Induh memerdai dun terdapat Kedalaman analija data
	den pembahalan
c.	Kecukupan & kemutakhiran data serta metodologi. Setura umum metodologi sudah memadai dan lengkap, kemutukhiran olata Juga fuluh terpenuhi.
	Juga Suduh perperuhi.
d.	Kelengkapan unsur dan kualitas penerbit

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f. Kesesuaian bidang ilmu
Jumal ini sudah fisuai denzen bizleng. Tunu yang stifekuni oleh penuliI

**REVIEWER 2** 

p. •

(Prof. Dr. Ermi Girsang, M. Kes) NIK : 0117057501 UNIVERSITAS PRIMA INDONESIA

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

#### KARYA ILMIAH : JURNAL ILMIAH

	Tea Flavonoids Induced Differentiation of Peripheral Blood-derived Mononuclear Cells into Peripheral Blood-derived Endothelial Progenitor Cells and Suppressed Intracellular Reactive Oxygen Species Level of Peripheral Blood-derived Endothelial Progenitor Cells 8 Orang					
	Wahyu Widowati, Laura Wijaya, Dian Ratih Laksmitawati, Rahma Micho					
	Widyanto, Pande Putu Erawijantari, Nurul Fauziah, Indra Bachtiar, Ferry Sandra					
Status Penulis	Penulis Pertama / Penulis ke/ Penulis Korespondensi **)					
Identitas Jurnal Ilmiah	: a. Nama jurnal : Natural Product Sciences					
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	g. Terindeks di : Scopus Q3, SJR 0.322					
Kategori Publikasi Jurnal Ilmiah (beri tanda √ yang dipilih)	: Jurnal Ilmiah Internasional / Internasional Bereputasi **)					
(beri landa 🔍 yang dipilih)	Jurnal Ilmiah Nasional Terakreditasi					
	Jurnal Ilmiah Nasional / Nasional terindeks ***)					

HASIL PENILAIAN (Peer Review ):

			Nilai Maksimal JURNAL ILMIAH			
No	Komponen Yang Dinilai		Internasional / Bercputasi	Nasional Terakreditasi	Nasional ***)	Nilai Akhir Yang Diperoleh *)
a.	Kelengkapan unsur isi karya	(10%)	4			3,55
b.	Ruang lingkup dan kedalaman pembahasan	(30%)	12			11,6
c.	Kecukupan dan kemutakhiran data/ informasi dan metodologi	(30%)	12			11,55
d.	Kelengkapan unsur dan kualitas penerbitan	(30%)	12			11,6
	Total	100%	40			38,3

Catatan Penilaian ARTIKEL oleh Reviewer :

a. Kelengkapan dan kesesuaian unsur. Penulitian momenului standard karya Umiah akademir, metodo yang digunakan jesuai dan tepat digunakan pada penulihian ini. Kelengkapan unsur Gi artikel sudah cukup lengeap alan terdapat keseguaran antara unsur dan leinya. b. Ruang lingkup & kedalaman pembahasan.

Penulihan tentang pemanfaatan cenyawa flavonvid teh ( EECG , EEC, ECG, C) me-... Undungi stress orsidalis EPCs young dürdytes radikal bebas Ruang baharan sudah memadai dan terdapat kedalaman analisy dato dan pembahasan c. Kecukupan & kemutakhiran data serta metodologi. Ide da sar perulikan batk nembahos milconisme sanyawa flavonoid menginduka diferensiasi BMC menjadi EPC, menekan Rix dari tPCs Secara unum metodologi sudab memadai dan lengkap, kemunthiran data juga sudah terpenuhi d. Kelengkapan unsur dan kualitas penerbit Jurnal NPs ternolets sapus Q3 SJR 0322 Penerbit the Korean Society of Pharm kvalitas penerloit sudah memenuhi hardah dan kvalitas jarnal sudah terindikasi dengan cank sapes as six 0,322 dan masuk kategori bereputas; yang back ..... e. Indikasi plagiasi ..... Limilarity index Seberar 15%, Trdak terdapat Indikari plagiarism atau self. plagicitim. Jejanh ini belum terlihat adanya unfur playiasi v f. Kesesuaian bidang ilmu Paper bidang biomedis Servai dungan bidang (Imu penulis -Jurnal ini sudah sesuai dengan bidang Timu yang delakuns oleh penulis 

Medan. Reviewer 2

(Prof. Dr. Ermi Girsang, M.Kes) NIK : 0117057501 UNIVERSITAS PRIMA INDONESIA

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