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"Antioxidant and Antiaging Activities of Jasmimum sambac Extract, and Its Compounds"

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Title: **Antioxidant and Antiaging Activities of Jasmimum sambac Extract, and Its Compounds**

Authors: **Wahyu Widowati**

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## Antioxidant and Antiaging Activities of *Jasminum sambac* Extract, and Its Compounds

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### ARTICLE INFO

**Article Type:**  
Research Article

**Article History:**  
Received: 2018-00-00  
Revised: 2018-00-00  
Accepted: 2018-00-00  
ePublished: 2018-00-00

**Keywords:**  
*Jasminum Sambac*  
Antioxidant  
Antiaging  
Anti-Collagenase  
Anti-Elastase  
Anti-Hyaluronidase

### ABSTRACT

Aging is a complex process characterized by a progressive decline in physiological function, followed by dysfunction, and ultimately, death. Increase activity of hyaluronidase, elastase and collagenase, are documented in skin aging. Free radicals can stimulate skin aging through antioxidant system destruction, wrinkle formation, and melanogenesis. Antioxidant and anti-aging agents have been recently developed from herbal plants. In this study, we investigated the antioxidant and anti-aging activities of *Jasminum sambac* extract (JSE). The phytochemical assay was performed with modified Farnsworth method. Antioxidant assays were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenger, Ferric Reducing Antioxidant Power (FRAP) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)-reducing activities. Anti-aging properties were measured through inhibitory activities of collagenase, elastase, and hyaluronidase. Phytochemical analysis showed presence of phenols, triterpenoids, and flavonoids in low level, and terpenoids in high level. JSE showed higher DPPH-scavenging activity ( $IC_{50}=94.13 \pm 10.54 \mu\text{g/mL}$ ) than eugenol ( $2.28 \pm 0.12 \mu\text{g/mL}$ ), but lower than hesperidin ( $226.34 \pm 4.96 \mu\text{g/mL}$ ). JSE showed lowest ABTS-activity ( $IC_{50}=39.20 \pm 0.45 \mu\text{g/mL}$ ) compared to hesperidin and eugenol ( $IC_{50}= 8.10 \pm 0.60$  and  $1.56 \pm 0.03 \mu\text{g/mL}$ , respectively). The FRAP-reducing activity of JSE, hesperidin, and eugenol showed JSE was the lowest activity at highest concentration (65.46, 178.16 and  $402.42 \mu\text{M Fe(II)/}\mu\text{g}$ ) respectively). JSE showed the lowest anti-collagenase activity ( $IC_{50}=339.30 \pm 7.87 \mu\text{g/mL}$ ), anti-elastase ( $IC_{50}=249.94 \pm 16.51 \mu\text{g/mL}$ ), and anti-hyaluronidase ( $IC_{50}=269.26 \pm 90.52 \mu\text{g/mL}$ ) compared to hesperidin, and eugenol. Overall, JSE has low antioxidant activity compared to hesperidin and eugenol, as well as low anti-collagenase, anti-elastase, and anti-hyaluronidase activities.

## Introduction

Aging is a complex process characterized by a progressive decline in physiological function, followed by dysfunction, and ultimately death [1]. Aging can be affected by various factors, such as disease, injury, nutrition, exercise, stress, and environmental factors. World Health Organization (WHO) reported that the percentage of the world's population of people over 60 years old will be double from 11% to 22% between 2000 and 2050 [2]. Thus, many recent studies have focused on maintaining a healthy life by postponing aging.

Collagen is one of the major building blocks of the skin that maintains elasticity and strength of the skin. It is the main component of connective tissue, hair and nails [3]. Elastin is a protein found in connective tissue which is responsible for the elasticity of the skin and lungs [4, 5]. Degradation of elastin by intracellular elastase increases with age and/or repeated UV-radiation, leading to skin aging. Hyaluronic acid plays a role in retaining the moisture of the skin, as well as its structure and elasticity. It also facilitates the exchange of nutrients and waste products and is involved in rapid tissue proliferation, regeneration and repair [6]. Collagen, elastin and hyaluronic acid levels decrease during aging process, the increasing enzymes activity including collagenase, elastase and hyaluronidase, resulting loss of strength and flexibility of skin that generates wrinkles [7, 8].

It has been reported that skin aging occurs in presence of cumulative endogenous damage due to free radicals including reactive oxygen species (ROS) [9,10]. ROS are defined as oxygen-containing, highly reactive species. ROS are generated constantly during normal cellular metabolism

which is essential for biological functions. Excessive ROS causes oxidative stress and damage to biological molecules [11,12]. Previous studies have been investigated that continuous ROS exposure can stimulate skin aging through antioxidant system destruction, wrinkle formation, and melanogenesis [11]. ROS are usually removed from the body through antioxidant defense system [13]. Thus, maintaining antioxidant homeostasis is appropriate strategy to prevent skin aging [14].

Antioxidant properties derived from natural sources have been proposed for aging prevention [14]. Bioactive compounds contained in plants such as isoflavones, anthocyanins, and catechins may have promising antioxidant activity against ROS [15]. It has been reported that *Jasminum sambac* contain iridoidal glycosides [16], linalyl 6-*O*-malonyl- $\beta$ -D-glucopyranoside, benzyl 6-*O*- $\beta$ -Dxylopyranosyl- $\beta$ -D-glucopyranoside ( $\beta$ -primeveroside), 2-phenylethyl  $\beta$ -primeveroside, 2-phenylethyl 6-*O*- $\alpha$ -L-rhamnopyranosyl- $\beta$ -D-glucopyranoside ( $\beta$ -rutinoside) [17], dotriacontanoic acid, dotriacontanol, oleanolic acid, daucosterol, and hesperidin [18]. The volatile constituents consist of benzyl acetate, indole, E-E- $\alpha$ -farnesene, Z-3-hexenyl benzoate, benzyl alcohol, linalool, and methyl anthranilate [19, 20], eugenol, benzyl benzoate, cis-jasmone, geraniol, farnesol and trace amounts of isohytol and phytol [19, 20]. Thus, the flowers of *J. sambac* have been utilized as traditional medicines in Asia to treat various diseases including diarrhea, fever, conjunctivitis, abdominal pain, dermatitis, asthma, abscess, breast cancer, uterine bleeding, and toothache. This study we investigated the antioxidant and anti-aging activities of *J. sambac* compared to its compounds namely hesperidin and eugenol (Figure 1).

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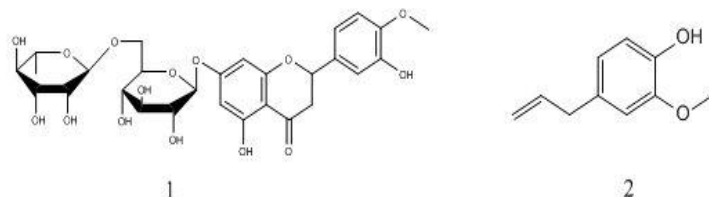


Fig. 1. Structure of two compounds contained in *J. sambac*; (1) Hesperidin (2) Eugenol

## Materials and methods

### Extract preparation

Flowers of *J. sambac* were collected from Brebes, Center Java, Indonesia. The plants were identified by herbarium staff, Department of Biology, School of Life Science and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. The dried simplicia of flower of *J. sambac* 70 g were extracted with distilled ethanol 70% (500 mL) using maceration technique. Ethanol filtrate was filtered, and wastes were re-macerated in triplicates. Macerates were concentrated using 50 °C rotavapor to obtain pasta form. The yield of *J. sambac* extract (JSE) was 18.28 g, it was stored at -20°C [8, 21, 22, 23, 24]. JSE was used as the experiment. Hesperidin [Chengdu Biopurify Phytochemicals Ltd 14021406] and eugenol [Sigma Aldrich E51791] were used as standard compounds.

### Qualitative phytochemical screening assay:

Content of *J. sambac* extract was evaluated by phytochemical screening assay using modified Farnsworth method. Phytochemical screening aim to identify qualitatively presence of phenols, steroids/triterpenoids, saponins, tannins, terpenoids, flavonoids, and alkaloids [8,24,25,26].

### High performance liquid chromatography (HPLC)

The analysis of chemical profiling of JSE by HPLC. Semi quantification JSE using the standard hesperidin [Biopurify Phytochemicals Ltd 14021406], and eugenol [Sigma Aldrich E51791]. HPLC analysis used the Hitachi Pump HPLC L-6200, Hitachi L-4000 UV detector and Reverse Phase Column C-18 (Phenosphere ODS-2, Phenomenex, 4.6 mm x 250 mm). Acetonitril 70% [Merck 100030] using to mobile phase (isocratic) with a flow rate of 1.0 mL/min. The samples were dissolved in methanol 70% (1 mg/mL) and filtered through a 0.22 µm syringe and injected 20 µL. UV absorbance was measured at 254 nm [27].

### DPPH scavenging activity

Fifty µL samples (0-2000 µg/mL for JSE, 0-2000 µM for compounds) was introduced in 96-well microplate and 200 µL of 0.077 mmol DPPH [Sigma Aldrich D9132] in dimethyl sulfoxide (DMSO) were added. The mixture was shaken vigorously and incubated in a dark room, at room temperature, for 30 min. After that, absorbance was measured at 517 nm using a microplate reader [Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific] was performed. For negative controls, 250 µL DPPH was used. For blank, 250 µL methanol was used [8,26,28,29]. The DPPH scavenging activity (%) was calculated as follows :

The scavenging activity (%) was then continued to be calculated as median inhibitory concentration (IC<sub>50</sub>).

$$\text{Scavenging Activity (\%)} = (\text{Ac-As})/\text{Ac} \times 100$$

As: sample absorbance

Ac: negative control absorbance (without sample)

#### **ABTS-reducing activity**

The antioxidant capacity JSE, compounds were measured using the 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt free radical assay [8,26,27,28,29]. ABTS was produced by reacting 14 mM ABTS and equal volume of 4.9 mM potassium persulfate (Merck EM105091) achieved final concentration of 7 mM ABTS in 2.45 mM potassium persulfate. The mixture was incubated in the dark room temperature for 16 h. The ABTS<sup>•+</sup> solution (Sigma Aldrich A1888) was diluted with 5.5 mM phosphate buffer saline (PBS) pH 7.4 and measured with microplate reader at 745 nm, resulting the absorbance of 0.70 ± 0.02. Briefly 2 µL sample (0-5000 µg/mL for JSE, 0-5000 µM for compounds) was added to 198 µL of ABTS<sup>•+</sup> solution, incubated for 6 min at 30 °C, and the absorbance was measured at 745 nm (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific). The percentage inhibition of ABTS radical (%) was expressed by the ratio of the reducing of ABTS<sup>•+</sup> absorbance in the presence of the test sample relative to the ABTS<sup>•+</sup> absorbance in the absence of the test sample (negative control). ABTS-reducing activity (%) was then continued to be calculated as median inhibitory concentration (IC<sub>50</sub>).

$$\text{ABTS-reducing activity \%} = (\text{Ac-As})/\text{Ac} \times 100$$

As: sample absorbance

Ac: negative control absorbance (without sample)

#### **FRAP reducing activity**

The ferric reducing antioxidant power assay (FRAP) of each standard solution and sample were measured according to a modified protocol [8,26,28,29]. The FRAP reagent was prepared by adding 2,4,6-tripyridyl-s-triazine (TPTZ) (Sigma 3682-35-7) and ferric chloride hexahydrate (Merck 1.03943.0250), forming the Fe<sup>3+</sup>-TPTZ complex. In 96-well microplate, 7.5 µL of samples (0-2000 µg/mL for JSE, 0-2000 µM for hesperidin, 0-250 µM for eugenol) were mixed with 142.5 µL FRAP reagent then incubated for 30 min at 37 °C. Antioxidant reduced to Fe<sup>2+</sup>-TPTZ at low pH was measured at 595 nm (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific). The results were expressed in µM Fe (II)/µg extract and compounds [8,26,27,28,29].

#### **Collagenase assay**

Collagenase inhibitory activity was measured according to modified method done by Sigma Aldrich Thring et al. (2009) and Widowati et al. (2016, 2017) [7,8,26,30]. Mixture was composed of 10 µL collagenase from *Clostridium histolyticum* [Sigma C8051] (0.01 U/mL in cold aquadest), 60 µL *Tricine* buffer (50 mM, pH 7.5, containing 10 mM CaCl<sub>2</sub> and 400 mM NaCl), 30 µL sample (0-1000 µg/mL for JSE, 0-1000 µM for compounds), then incubated at 37 °C for 20 min. After incubation, 20 µL of N-[3-(2-Furyl)acryloyl]-leu-gly-Pro-Ala substrate [Sigma F5135] (1 mM in *Tricine* buffer) was added. Absorbance was measured at 335 nm wavelengths (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific).

$$\text{Inhibitory activity \%} = (\text{Ac-As})/\text{Ac} \times 100$$

As: sample absorbance

Ac: negative control absorbance (without sample)

**Elastase assay**

Inhibitory activity of elastase was measured according to modified method by Sigma Aldrich and Thring et al. (2009) and Widowati et al. (2016, 2017) [7,8,26,30]. Approximately, 10 µL sample (0-1000 µg/mL for JSE, 0-1000 µM for compounds) was pre-incubated at 25 °C for 15 min with 5 µL *elastase from porcine pancreas* [Sigma 45124] (0.5 mU/mL in cold aquadest), 125 µL Tris buffer (100 mM, pH 8). After pre-incubation, mixture was then added with 10 µL N-Sucanyl-Ala-Ala-Ala-p-Nitroanilide substrate [Sigma 54760] (2 mg/mL in Tris buffer), incubated at 25 °C for 15 min. Absorbance was measured at 410 nm wavelength (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, USA).

$$\text{Inhibitory activity \%} = (\text{Ac-As})/\text{Ac} \times 100$$

As: sample absorbance

Ac: negative control absorbance (without sample)

**Hyaluronidase assay**

Inhibitory activity of hyaluronidase was measured based on modified method of Sigma Aldrich and Tu and Tawata (2015) and Widowati et al. (2016; 2017) [8,26,31]. Briefly 25 µL samples (0-1000 µg/mL for JSE, 0-1000 µM for compounds) was pre-incubated at 37 °C for 10 min with 3 µL hyaluronidase from bovine testes type I-S [Sigma Aldrich H3506] (0.4 U/mL in 20 mM phosphate buffer, pH 7 containing 77 mM sodium chloride, and 0.01% bovine serum albumin), and 12 µL

phosphate buffer (300 mM, pH 5.35) at 37 °C for 10 min. After pre-incubation, 10 µL hyaluronic acid substrate (0.03% in 300 mM phosphate buffer, pH 5.35) [Sigma Aldrich H5542], then incubated at 37 °C for 45 min. Reaction was stopped using 100 µL acidic albumin acid (24 mM sodium acetate, 79 mM acetate acid, and 0.1% BSA). Mixture was left in room temperature for 10 min, and absorbance was measured at 600 nm wavelengths (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific).

$$\text{Inhibitory activity \%} = (\text{Ac-As})/\text{Ac} \times 100$$

As: sample absorbance

Ac: negative control absorbance (without sample)

**Results**

**Phytochemical Analysis**

Phytochemical analysis was done to determine content of compounds class in JSE. Compounds of class of JSE can be seen in Table 1. Phytochemical analysis shows the presence of phenols, triterpenoids, and flavonoids in low content, and terpenoids in high content.

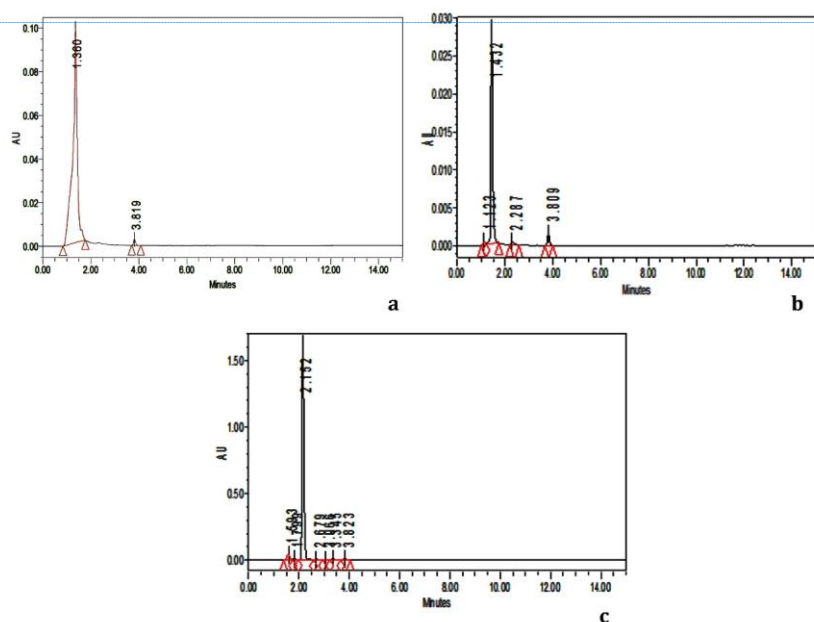
**HPLC Analysis**

HPLC analysis was evaluated to determine the compounds content of JSE. The HPLC standards using hesperidin and eugenol (Figure 2). Hesperidin has a retention time at 1.43 min, and eugenol at 2.15 min. The JSE peak at 1.36 min which was assumed that JSE contains hesperidin and doesn't contain eugenol.

**Table 1.** The result of qualitative phytochemical assay of JSE

Phytochemical content	Qualitative value of JSE
Phenols	+
Steroids/triterpenoids	-/+
Terpenoids	+++
Saponins	-
Flavonoids	+
Tannins	-
Alkaloids	-

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Fig. 2. Chromatogram of JSE, hesperidin, and eugenol from HPLC analysis; (a) JSE (b) Hesperidin (c) Eugenol

### DPPH scavenging activity

DPPH free radical scavenging activity can be used to determine antioxidant capacity of JSE and its compounds. This assay based on alcoholic DPPH solution reduction in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical from 2,2-diphenyl-1-picrylhydrazine (DPPH-H). The DPPH samples generally changes

purple colour into a colourless when antioxidant molecules quench DPPH free radicals [8, 28]. The  $IC_{50}$  of scavenging activities of JSE, hesperidin, and eugenol can be seen in Table 2. As shown in Table 2, the  $IC_{50}$  value of JSE was higher ( $94.13 \pm 10.54 \mu\text{g/mL}$ ) than eugenol ( $2.28 \pm 0.12 \mu\text{g/mL}$ ), but lower than hesperidin ( $226.34 \pm 4.96 \mu\text{g/mL}$ ). These results indicate moderate scavenging activity of JSE among samples.

Table 2. The  $IC_{50}$  value of DPPH scavenging activity of JSE, hesperidin, eugenol

Samples	Equation	R <sup>2</sup>	IC <sub>50</sub> (μM)	IC <sub>50</sub> (μg/mL)
JSE	$y = 0.33x + 18.56$	0.94	-	$94.13 \pm 10.54$
Hesperidin	$y = 0.07x + 24.05$	0.96	$370.71 \pm 8.13$	$226.34 \pm 4.96$
Eugenol	$y = 0.84x + 38.25$	0.922	$13.92 \pm 0.74$	$2.28 \pm 0.12$

**ABTS - reducing activity**

ABTS-reducing activity can be used to determine antioxidant capacity of JSE and its compounds. The assay is based on the discoloration of ABTS by antioxidant compounds, the ABTS is generated by reacting a strong oxidizing agent (potassium permanganate/potassium persulfate) with the ABTS salt. The reduction of blue-green ABTS

radical by hydrogen-donating antioxidant compounds [8,26]. The ABTS-reducing activity of JSE, hesperidin, and eugenol can be seen in Table 3. Table 3 shows that the IC<sub>50</sub> value of JSE was the highest (39.20 ± 0.45 µg/mL) compared to hesperidin and eugenol (8.10 ± 0.60 and 1.56 ± 0.03 µg/mL, respectively). Based on the results, JSE exhibited lowest ABTS-reducing activity among samples.

**Table 3.** The IC<sub>50</sub> value of ABTS-reducing activity of JSE, hesperidin, eugenol

Samples	Equation	R <sup>2</sup>	IC <sub>50</sub> (µM)	IC <sub>50</sub> (µg/mL)
JSE	y = 1.21x + 2.73	0,99	-	39.20 ± 0.45
Hesperidin	y = 3.65x + 1.86	0.99	13.27 ± 0.99	8.10 ± 0.60
Eugenol	y = 4.89x + 3.36	0.97	9.54 ± 0.20	1.56 ± 0.03

**Ferric reducing antioxidant power (FRAP) activity**

FRAP activity can be used to determine antioxidant capacity of JSE and its compounds. The FRAP activity of JSE, hesperidin, and eugenol can be seen in Table 4. The FRAP activity method is based on the reduction of a ferrioxalate complex of tripyridyltriazine Fe(TPTZ)<sup>3+</sup> to the intensely blue coloured Fe<sup>2+</sup> complex Fe(TPTZ)<sup>2+</sup> by antioxidant in acidic medium [8,28]. In this study, FRAP activity of JSE, hesperidin and eugenol at the highest concentration are 65.46 ± 1.84, 178.16 ±

2.00 and 402.42 ± 3.46 µM Fe(II)/µg respectively. These results indicate JSE has lowest FRAP activity among samples.

**Collagenase assay**

Collagen, the major component of the skin, is degraded by the enzyme collagenase. Inhibition of collagenase activity delays the process of forming pre-collagen fibre and subsequently the wrinkling process [3,8,26]. The inhibitory activity of JSE, hesperidin, and eugenol can be seen at Figure 3, while the IC<sub>50</sub> values are shown in Table 5.

**Table 4.** FRAP activity of JSE, hesperidin, eugenol

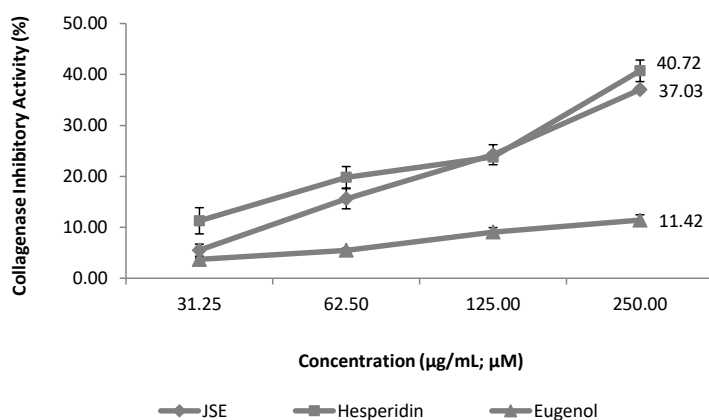
Sample	Concentration (µM)	Concentration (µg/mL)	FRAP Activity (µM Fe(II)/µg)
JSE	-	2000	65.46 ± 1.84
		1000	32.11 ± 0.69
		500	15.22 ± 0.39
		250	5.94 ± 0.72
Hesperidin	2000	1221.13	178.16 ± 2.00
	1000	610.57	103.87 ± 1.04
	500	305.28	57.81 ± 0.43



	250	152.64	29.72 ± 0.77
Eugenol	250	41.05	402.42 ± 3.46
	125	20.52	249.69 ± 4.80
	62.50	10.26	155.13 ± 1.95
	31.25	5.13	82.17 ± 1.71

**Table 5.** The IC<sub>50</sub> value of Collagenase inhibitory activity of JSE, hesperidin, eugenol

Samples	Equation	R <sup>2</sup>	IC <sub>50</sub> (μM)	IC <sub>50</sub> (μg/mL)
JSE	y = 0.13x + 4.82	0.95	-	339.30 ± 7.87
Hesperidin	y = 0.13x + 9.12	0.97	326.72 ± 22.95	198.09 ± 14.01
Eugenol	y = 0.03x + 3.39	0.92	1370.88 ± 190.53	225.10 ± 31.20

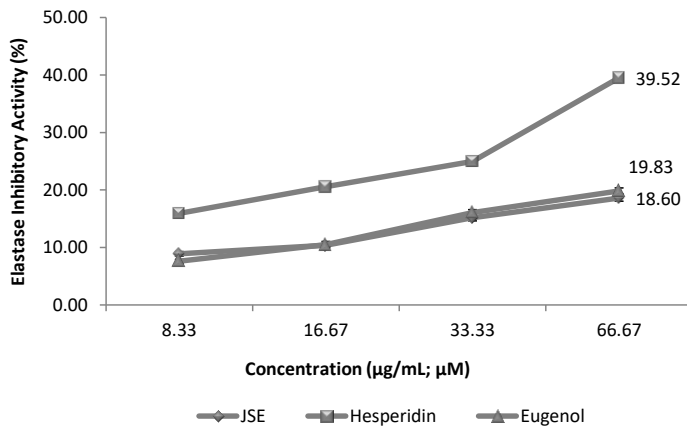


**Figure 3.** Effect of JSE (μg/mL), hesperidin, eugenol (μM) on Collagenase inhibitory activity of JSE, hesperidin, eugenol

(JSE, hesperidin, and eugenol were diluted in DMSO to reach final concentration of 31.25, 62.50, 125.00, 250.00 (μg/mL; μM))

The inhibitory activity of JSE was comparable with hesperidin and eugenol. Figure 3 shows the anti-collagenase activity of JSE, hesperidin, and eugenol. The highest inhibitory activity was hesperidin (40.72 ± 2.12%). JSE possess moderate anti-collagenase activity among samples (37.03 ±

0.54%). However, as shown in Table 5, hesperidin show the highest anti-collagenase activity with IC<sub>50</sub> (198.09 ± 14.01 μg/mL), whereas eugenol was moderate (225.10 ± 31.20 μg/mL), and JSE was the lowest (339.30 ± 7.87 μg/mL).



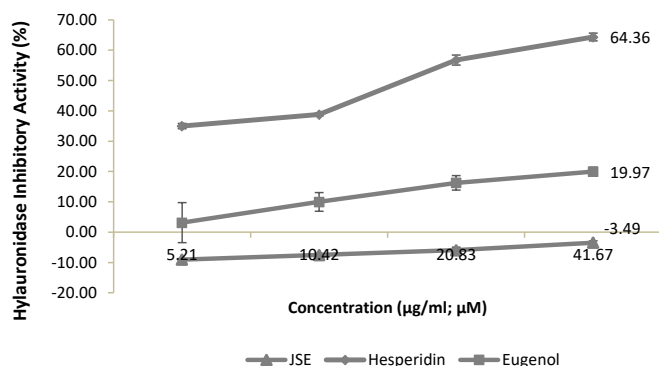
**Figure 4.** Effect of JSE (µg/mL), hesperidin, eugenol (µM) on Elastase inhibitory activity of JSE, hesperidin, eugenol

(JSE, hesperidin, and eugenol were diluted in DMSO to reach final concentration of 8.33, 16.67, 33.33, 66.67 (µg/mL; µM))

**Elastase assay**

Elastin is a protein found in connective tissue which is responsible for the elasticity of the skin and lungs [4,5]. This protein is catalyzed by the enzyme elastase. Degradation of elastin by intracellular elastase increases with age and/or repeated UV-radiation, leading to skin aging [6]. The elastase inhibitory activity of JSE, hesperidin, and eugenol can be seen at Figure 4, while the IC<sub>50</sub> values are shown in Table 6.

Figure 4 shows the anti-elastase activity of JSE, hesperidin, and eugenol. The highest activity of JSE at 66.67 µg/mL is 18.60 ± 0.55%, the highest activity of hesperidin, eugenol at 66.67 µM are 39.52 ± 0.71%, 19.83 ± 0.52%, respectively. The IC<sub>50</sub> value of samples (Table 6) show eugenol has highest inhibitory activity (IC<sub>50</sub>= 34.52 ± 1.17 µg/mL), whereas hesperidin was moderate (IC<sub>50</sub>= 57.46 ± 0.19 µg/mL), and JSE was the lowest (IC<sub>50</sub>= 249.94 ± 16.51 µg/mL).



**Fig. 5.** Effect of JSE ( $\mu\text{g/mL}$ ), hesperidin, eugenol ( $\mu\text{M}$ ) on Hyaluronidase inhibitory activity of JSE, hesperidin, eugenol (JSE, hesperidin, and eugenol were diluted in DMSO to reach final concentration of 5.21, 10.42, 20.83, 41.67 ( $\mu\text{g/mL}$ ;  $\mu\text{M}$ ))

### Hyaluronidase assay

Hyaluronic acid plays a role in retaining the moisture of the skin, as well as its structure and elasticity. Hyaluronic acid levels decrease during aging process [6]. Hyaluronidase inhibitory activity

can be used to determine antiaging capacity of JSE, its compounds. The hyaluronidase inhibitory activity of JSE, hesperidin, and eugenol can be seen in Figure 5, while the  $\text{IC}_{50}$  values were shown in Table 7.

**Table 6.** The  $\text{IC}_{50}$  value of Elastase inhibitory activity of JSE, hesperidin, eugenol

Samples	Equation	R <sup>2</sup>	$\text{IC}_{50}$ ( $\mu\text{M}$ )	$\text{IC}_{50}$ ( $\mu\text{g/mL}$ )
JSE	$y = 0.17x + 8.01$	0.94	-	$249.94 \pm 16.51$
Hesperidin	$y = 0.39x + 12.92$	0.99	$94.11 \pm 1.63$	$57.46 \pm 0.19$
Eugenol	$y = 0.20x + 7.11$	0.92	$210.25 \pm 7.12$	$34.52 \pm 1.17$

**Table 7.** The  $\text{IC}_{50}$  value of Hyaluronidase inhibitory activity of JSE, hesperidin, eugenol

Samples	Equation	R <sup>2</sup>	$\text{IC}_{50}$ ( $\mu\text{M}$ )	$\text{IC}_{50}$ ( $\mu\text{g/mL}$ )
JSE	$y = 0.23x - 11.93$	0.93	-	$269.26 \pm 90.52$
Hesperidin	$y = 0.83x + 32.59$	0.90	$21.08 \pm 0.05$	$12.87 \pm 0.03$
Eugenol	$y = 0.26x + 6.19$	0.93	$168.55 \pm 19.32$	$27.67 \pm 3.17$

Figure 5 shows the anti-hyaluronidase activity of JSE, hesperidin, and eugenol. The highest activity of JSE at 41.67  $\mu\text{g/mL}$  is hesperidin, and eugenol at concentration 41.67  $\mu\text{M}$  are  $64.36 \pm 6.03\%$ ,  $-3.49 \pm$

$2.38\%$  respectively. These results are confirmed by  $\text{IC}_{50}$  value of JSE and its compounds, as shown in Table 7. Hesperidin showed lowest  $\text{IC}_{50}$  value ( $12.87 \pm 0.03 \mu\text{g/mL}$ ) which indicate highest

inhibitory activity, whereas eugenol is moderate ( $27.67 \pm 3.17 \mu\text{g/mL}$ ), and JSE is the lowest  $269.26 \pm 90.52 \mu\text{g/mL}$ .

## Discussion

Plants have been widely used to treat various diseases due to its medicinal properties. Therefore, phytochemical analysis on plants is required to observe compounds class that might possess medicinal activities. The present study showed JSE contained phenols, triterpenoids, and flavonoids in low content, and terpenoids in high content. In this study, HPLC analysis showed that JSE contain hesperidin. Previous phytochemical analysis showed bioactive compounds in *J. sambac*, namely iridoidal glycosides [16], and hesperidin [18], based on the HPLC result indicate that JSE doesn't contain eugenol, this result was not in line with previous research that *J. sambac* contained eugenol [20].

Antioxidant is used as antiaging prevention due to its involvement ROS in skin aging process. UV radiation absorbed by the skin promotes overproduction of ROS, as well as oxidative stress. Oxidative damage results in lipid peroxide formation, mitochondrial and DNA damage, and protein and gene modification which alter protein structure and function [32]. Excessive ROS leads to the activation of hyaluronidase, collagenase and elastase, which can further contribute to skin aging [3]. In the present study, JSE showed moderate DPPH-scavenging activity ( $94.13 \pm 10.54 \mu\text{g/mL}$ ), and lowest ABTS ( $39.20 \pm 0.45 \mu\text{g/mL}$ ) and FRAP activity in the highest concentration ( $65.46 \pm 1.84 \mu\text{M Fe(II)/}\mu\text{g}$ ) was lower compared to hesperidin and eugenol. Based on other study, if compound has  $\text{IC}_{50}$  value  $< 200 \text{ ppm}$  or  $< 200 \mu\text{g/mL}$  it has strong antioxidant activity [33]. Based on standards, that indicates JSE has high antioxidant activity toward DPPH-scavenging activity and ABTS-reducing activity. Referring to previous study, *J. sambac* possess moderate scavenging effect in the

order towards the DPPH radicals ( $122 \mu\text{g/mL}$ ), nitric oxide ( $173.94 \mu\text{g/mL}$ ) and hydrogen peroxide ( $125 \mu\text{g/mL}$ ) when compared to ascorbic acid [34]. Following the results, Shekhar and Prasad (2015) found that *Jasminum* species, including *J. sambac*, showed higher FRAP-activity compared to ascorbic acid [35]. Low activity of JSE in the present study might be due to low of flavonoids detected. It has been reported that flavonoids are the largest group among plants with bioactive properties [36]. Flavonoids decrease the early activation of signaling pathway in response to UV induced injury. The quercetin, polyphenol, flavonoid and flavonol, ellagic acids from natural resources, produces the free radicals scavenging activity, inhibition of elastase activity and matrix metalloproteinases (MMPs) expression, and increase expression of procollagen type I [37,38,39,40].

The low antioxidant activity was indeed associated with low anti-aging activity. Based on previous study, antioxidant obtained from plant extract, inhibit elastase, hyaluronidase, collagen synthesis, lipid peroxidation activity, protein expression of procollagen and prevent MMPs gene induction and due to this fibrillin fibre length elongated to maintain elasticity of skin [41,42]. MMPs are part of a group of transmembrane zinc containing endopeptidases which include collagenases and gelatinases. Collagenases are metalloproteinases capable of cleaving other molecules found within the cell for example collagenase-2 (MMP-8) can cleave aggrecan, elastin, fibronectin, gelatine and laminin as well as collagen [43]. Another proteolytic system involved in the degradation of the extracellular matrix (ECM) is that of serine proteases one of which is elastase. Elastase, a member of the chymotrypsin family of proteases, is responsible primarily for the breakdown of elastin which is vital for giving elasticity to arteries, lungs, ligaments and skin [44, 45, 46, 47]. In terms of anti-aging, finding inhibitors of elastase enzymes can be useful to prevent loss of

skin elasticity. In the present study, JSE showed lowest activity of anti-collagenase, anti-elastase and anti-hyaluronidase compared to hesperidin and eugenol. From our best knowledge, there is no studies regarding anti-aging activity of *J. sambac*. However, the activity of JSE in the present study was very low, which might be due to absence of tannins. Tannins is likely found in the most active extract. Recent study done by Piwowarski et al. (2011) show that twelve tannin-rich plants possess anti-collagenase and anti-hyaluronidase activity [48].

In this study, hesperidin showed highest activity. Hesperidin is a flavanone glycoside (a subclass of flavonoids) that is found abundantly in citrus fruits. Hesperidin possesses vitamin-like activity that decrease capillary permeability (vitamin P), leakiness, and fragility. It also showed antioxidant, anti-inflammatory, anticarcinogenic, and antiallergic properties [49, 50, 51]. The study done by Wilmsen et al. (2005) showed that hesperidin significantly reduced the level of the DPPH which was comparable to antioxidant trolox [52]. It has been reported that hesperidin significantly inhibits the ROS of the yeast [53]. Hesperidin also inhibits the formation of advanced glycation endproducts (AGEs) that possess potentially harmful effects on biological function, and are associated with aging and many degenerative illnesses [54]. Hesperidin derived from Citrus genus extended the lifespan of yeast at doses of 5 and 10  $\mu$ M as compared to non-treated yeast.

Eugenol in the present study showed moderate antioxidant and anti-aging activity. However, previous studies showed good antioxidant activity of eugenol. Referring to Widowati et al. (2013), eugenol showed good antioxidant activity ( $IC_{50}$  of DPPH= 3.8  $\mu$ g/mL) [22]. Eugenol had the most powerful antioxidant activity and radical-scavenging activity compared to butylated hydroxyanisole, butylated hydroxytoluene, a-

tocopherol, and trolox [55]. Eugenol inhibit lipid peroxidation by trapping active oxygen species such as  $O_2^-$  or hydroxyl radicals, rather than by breaking the free radical chain reaction [56].

### Conclusion

Overall, JSE showed low antioxidant activity compared to hesperidin and eugenol, as well as low anti-collagenase, anti-elastase, and anti-hyaluronidase.

### Acknowledgments

This research was supported by Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, Indonesia for a research grant, laboratory facilities, and research methodology. We are thankful to Rismawati Laila Qodariah, Rizal Rizal, Fajar Sukma Perdana, Ni Luh Wisma Ekayanti for their valuable assistance.

### Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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