Antioxidant and Antiaging Activities of Jasminum Sambac Extract, and its Compounds

by Wahyu Widowati

Volume 6 | Issue 1 | January-June 2016

Journal of Reports in Pharmaceutical Sciences

An Official Publication of Kermanshah University of Medical Sciences

Journal of Reports in Pharmaceutical Sciences 2018, 7(3), 270-285

Antioxidant and Antiaging Activities of *Jasminum Sambac* Extract, and its Compounds

Wahyu Widowati[a*], Widya Janeva B[a], Sri Nadya[a], Annisa Amalia[b], Seila Arumwardana[b], Hanna Sari W. Kusuma[b], Yukko Arinta[b]

[a]Medical Research Center, Faculty of Medicine, Maranatha Christian University, Jl. Prof. Drg. Surya Sumantri No. 65 Bandung 40164, West Java, Indonesia
[b]Aretha Medika Utama, Biomolecular and Biomedical Research Center, Jl. Babakan Jeruk 2, No. 9, Bandung 40163, West Java, Indonesia

Article Type:
Research Article

Article History:

Received: 2018-04-25
Revised: 2018-05-28
Accepted: 2018-06-27
ePublished: 2018-07-03

Keywords:
Jasmimum Sambac
Antioxidant
Antiaging
Anti-Collagenase
Anti-Elastase
Anti-Hyaluronidase

A B S T R A C T

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 ± 10.54 µg/mL) than eugenol (2.28 ± 0.12 µg/mL), but lower than hesperidin (226.34 ± 4.96 µg/mL). JSE showed lowest ABTS-activity (IC_{50}=39.20 ± 0.45 µg/mL) compared to hesperidin and eugenol (IC_{50}= 8.10 ± 0.60 and 1.56 ± 0.03 µg/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 µM Fe(II)/µg) respectively). JSE showed the lowest anti-collagenase activity (IC_{50}=339.30 ± 7.87 µg/mL), anti-elastase (IC_{50}=249.94 ± 16.51 µg/mL), and anti-hyaluronidase (IC_{50}=269.26 ± 90.52 µg/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.

[*]**Corresponding Author: Wahyu Widowati, E-mail:** wahyu_w60@yahoo.com

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-*β*-D-glucopyranoside, benzyl 6-*O*-*β*-Dxylopyranosyl- *β*-D-glucopyranoside (*β*-primeveroside), 2- phenylethyl *β*-primeveroside, 2-phenylethyl 6-*O*-*α*-L-rhamnopyranosyl- *β*-D-glucopyranoside (*β*-rutinoside) [17], dotriacontanoic acid, dotriacontanol, oleanolic acid, daucosterol, and hesperidin [18]. The volatile constituents consist of benzyl acetate, indole, E-E-*α*-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).

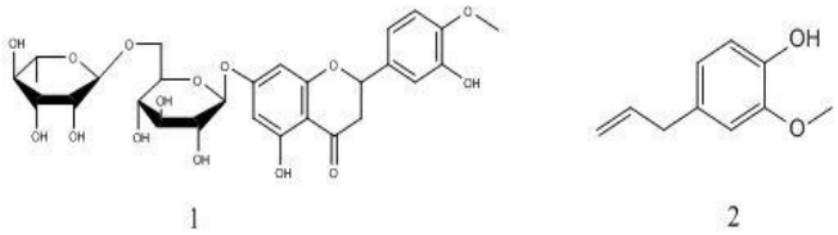

Fig. 1. Structure of two compounds contained in *J. sambac*; (1) Hesperidin (2) Eugenol

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 identifiy 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). Acetonytril 70% [Merck 100030] using to mobile phase (isocratical) 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_{50}).

Scavenging Activity (%) = (Ac-As)/Ac×100

As: sample absorbance

Ac: negative control absorbance (without sample)

ABTS-reducing activity

The antioxidant capacity JSE, compoundswere measured using the 2,2'-Azinobis-(3-ethylbenzo-thiazoline-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 $ABTS^{\bullet+}$ solution (Sigma Aldrich A1888) was diluted with 5.5 mM phospate 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 $ABTS^{\bullet+}$ 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 $ABTS^{\bullet+}$ absorbance in the presence of the test sample relative to the $ABTS^{\bullet+}$ absorbance in the absence of the test sample (negative control). ABTS-reducing activity (%) was then continued to be calculated as median inhibitory concentration (IC_{50}).

ABTS-reducing activity % = (Ac-As)/Ac×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^{3+}–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^{2+}–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 $CaCl_2$ 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,).

Inhibitory activity % = (Ac-As)/Ac×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).

Inhibitory activity % = (Ac-As)/Ac×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).

Inhibitory activity % = (Ac-As)/Ac×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	-

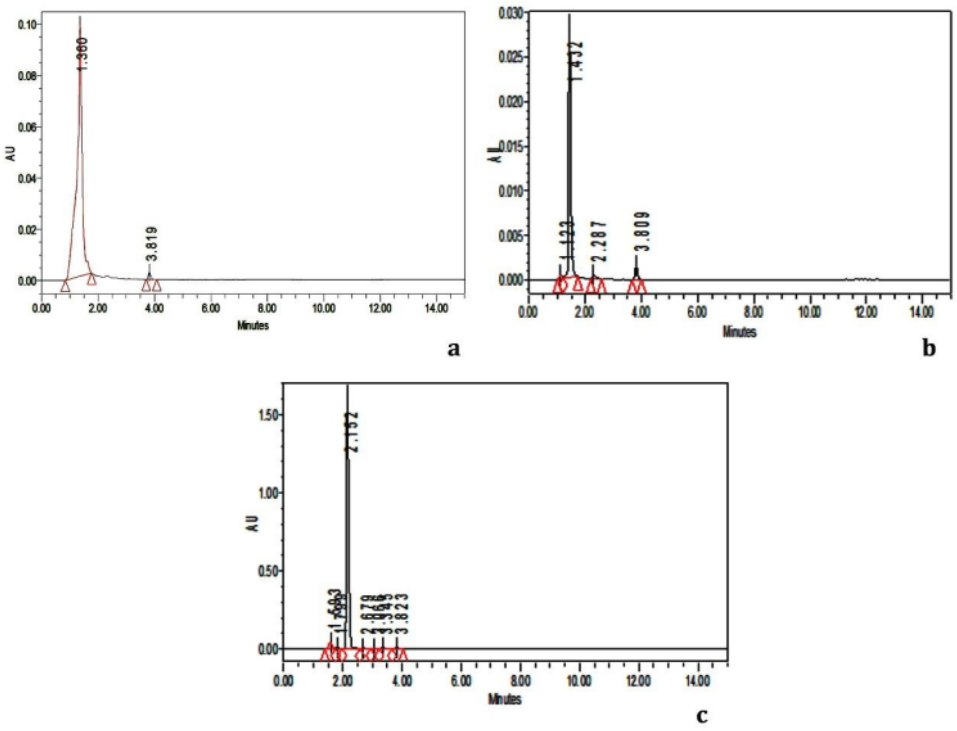

Fig. 2. Chromatogram of JSE, hesperidin, and eugenol from HPLC analysis; (a) JSE (b) Hesperidin (c) Eugenol

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 ± 10.54 µg/mL) than eugenol (2.28 ± 0.12 µg/mL), but lower than hesperidin (226.34 ± 4.96 µg/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^2	IC_{50} (µM)	IC_{50} (µg/mL)
JSE	y = 0.33x + 18.56	0.94	-	94.13 ± 10.54
Hesperidin	y = 0.07x + 24.05	0.96	370.71 ± 8.13	226.34 ± 4.96
Eugenol	y = 0.84x + 38.25	0.922	13.92 ± 0.74	2.28 ± 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_{50} 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_{50} value of ABTS-reducing activity of JSE, hesperidin, eugenol

Samples	Equation	R^2	IC_{50} (µM)	IC_{50} (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 ferroin analog, the Fe^{3+} complex of tripyridyltriazine $Fe(TPTZ)^{3+}$ to the intensely blue coloured Fe^{2+} complex $Fe(TPTZ)^{2+}$ 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_{50} 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_{50} value of Collagenase inhibitory activity of JSE, hesperidin, eugenol

Samples	Equation	R^2	IC_{50} (µM)	IC_{50} (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

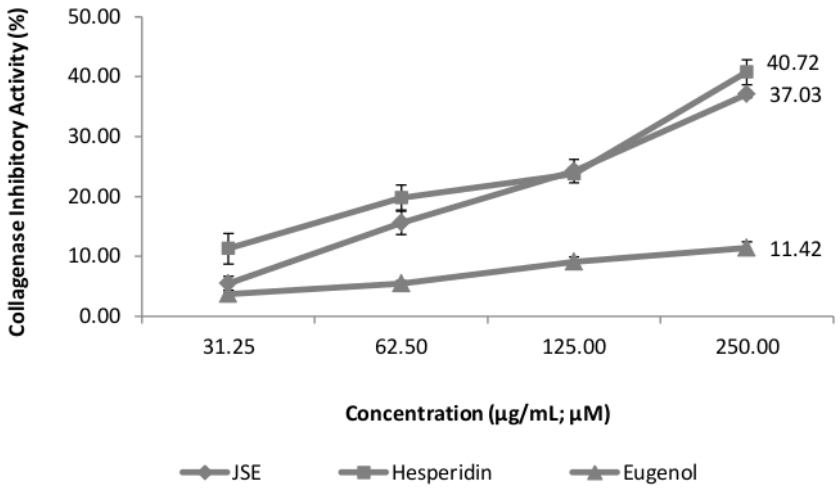

Fig. 3. Effect of JSE (µg/mL), hesperidin, eugenol (µM) on Collagenase inhibitory activity of JSE, hesperidin, eugenol (JSE, hesperidin, and eugenol were diluted in DMSO to reach final concentration of 31.25, 62.50, 125.00, 250.00 (μg/mL; μM))

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_{50} (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).

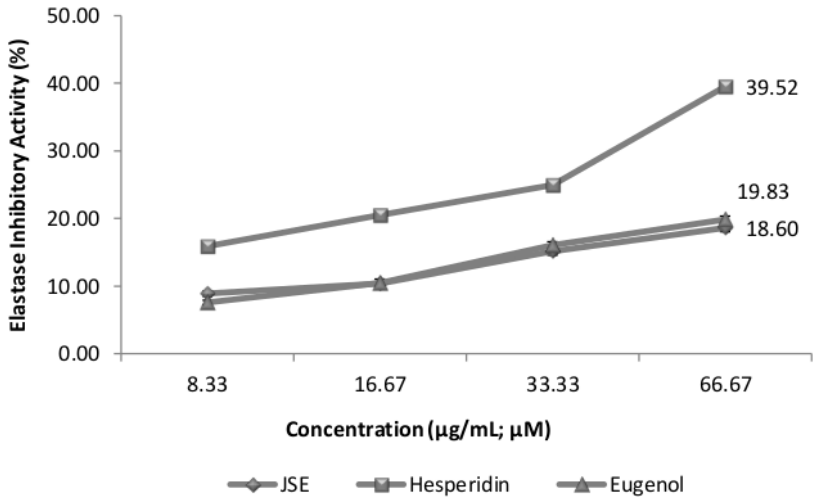

Fig. 4. Effect of JSE (µg/mL), hesperidin, eugenol (µM) on Elastase inhibitory activity of JSE, hesperidin, eugenol (JSE, hesperidin, and eugenol were diluted in DMSO to reach final concentration of 8.33, 16.67, 33.33, 66.67 (μg/mL; μM))

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_{50} 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_{50} value of samples (Table 6) show eugenol has highest inhibitory activity (IC_{50}= 34.52 ± 1.17 µg/mL), whereas hesperidin was moderate (IC_{50}= 57.46 ± 0.19 µg/mL), and JSE was the lowest (IC_{50}= 249.94 ± 16.51 µg/mL).

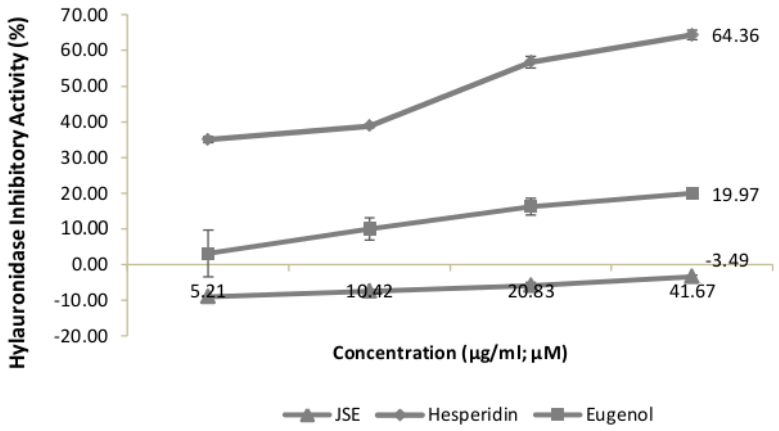

Fig. 5. Effect of JSE (µg/mL), hesperidin, eugenol (µM) on Hyaluronidase inhibitory activity of JSE, hesperidin, eugenol

(JSE, hesperidin, and eugenol were diluted in DMSO to reach final concentration of 5.21, 10.42, 20.83, 41.67 (µg/mL; µM))

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 IC_{50} values were shown in Table 7.

Table 6. The IC_{50} value of Elastase inhibitory activity of JSE, hesperidin, eugenol

Samples	Equation	R^2	IC_{50} (µM)	IC_{50} (µg/mL)
JSE	y = 0.17x + 8.01	0.94	-	249.94 ± 16.51
Hesperidin	y = 0.39x + 12.92	0.99	94.11 ± 1.63	57.46 ± 0.19
Eugenol	y = 0.20x + 7.11	0.92	210.25 ± 7.12	34.52 ± 1.17

Table 7. The IC_{50} value of Hyaluronidase inhibitory activity of JSE, hesperidin, eugenol

Samples	Equation	R^2	IC_{50} (µM)	IC_{50} (µg/mL)
JSE	y = 0.23x – 11.93	0.93	-	269.26 ± 90.52
Hesperidin	y = 0.83x + 32.59	0.90	21.08 ± 0.05	12.87 ± 0.03
Eugenol	y = 0.26x + 6.19	0.93	168.55± 19.32	27.67 ± 3.17

Figure 5 shows the anti-hyaluronidase activity of JSE, hesperidin, and eugenol. The highest activity of JSE at 41.67 µg/mL is hesperidin, and eugenol at concentration 41.67 µM are 64.36 ± 6.03%, -3.49 ± 2.38% respectively. These results are confirmed by IC_{50} value of JSE and its compounds, as shown in Table 7. Hesperidin showed lowest

IC_{50} value (12.87 ± 0.03 µg/mL) which indicate highest inhibitory activity, whereas eugenol is moderate (27.67 ± 3.17 µg/mL), and JSE is the lowest 269.26 ± 90.52 µg/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 ± 10.54 µg/mL), and lowest ABTS (39.20 ± 0.45 µg/mL) and FRAP activity in the highest concentration (65.46 ± 1.84 µM Fe (II)/µg) was lower compared to hesperidin and eugenol. Based on other study, if compound has IC_{50} value < 200 ppm or < 200 µg/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 µg/mL), nitric oxide (173.94 µg/mL) and hydrogen peroxide (125 µg/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 has done by Piwowarski et al. (2011) show those 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 inihibits 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 µM 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 µg/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.

References

[1] Raj K, Chanu SI, Sarkar S. Decoding complexity of aging. Cell Dev Biol. 2012;1:e117.
[2] World Health Organization. Interesting facts about ageing. http://www.who.int/ageing/about/facts/en/ (accessed march 2012). 2012
[3] Mukherjee PK, Maity N, Nema NK, Sarkarm BK. Bioactive compounds from natural resources against skin aging. Phytomed. 2011;19:64–63.
[4] Fulop T, Khalil A, Larbi A. The role of elastin peptides in modulating the immune response in aging and age-related diseases. Pathol Biol. 2012;60:28–33.

[5] Kurtz A, Oh S-J. Age related changes of the extracellular matrix and stem cell maintenance. Prev Med. 2012;54:S50–S56.

[6] Hsu M-F, Chiang B-H. Stimulating effects of *Bacillus subtilis* natto-fermented *Radix astragali* on hyaluronic acid production in human skin cells. J Ethnopharmacol. 2009;125:474–481.

[7] Thring TSA, Hili P, dan Naughton DP. Anti-collagenase, anti-elastase, and anti-oxidant activities of extract from 21 plants. BMC Compl Altern Med. 2009;9:1-11.

[8] Widowati W, Fauziah N, Herdiman H, Afni M, Afifah E, Kusuma, HSW, et al. Antioxidant and anti-aging assays of *Oryza sativa* extracts, vanillin and coumaric acid. J Nat Remed. 2016;16:88-89.

[9] Yasui K, Sakurai H. Chemiluminescent detection and imaging of reactive oxygen species in live mouse skin exposed to UVA. Biochem Biophys Res Commun. 2000;269:131-136.

[10] Park JS. Walnut husk ethanol extract possess antioxidant activity and inhibitory effect of matrix metalloproteinase-1expression induced by tumor necrosis factor alpha in human keratinocyte. Kor J Aesthet Cosmetol. 2013;11:715-719.

[11] Kim DS, Jeon BK, Mun YJ, Kim YM, Lee YE, Woo WH. Effect of *Dioscorea aimadoimo* on anti-aging and skin moisture capacity. J Orient Physiol Pathol. 2011;25:425-430.

[12] Mruka DD, Silvestrini B, Mo MY, Cheng CY. Antioxidant superoxide dismutase–a review: its function, regulation in the testis, and role in male fertility. Contraception. 2002;65:305-311.

[13] Moini H, Packer L, Saris NEL. Antioxidant and prooxidant activities of α-lipoic acid and dihydrolipoic acid. Toxicol Appl Pharmacol. 2002;182:84-90.

[14] Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004;74:2157-2164.

[15] Kaur C, Kapoor HC. Anti-oxidant activity and total phenolic content of some Asian vegetables. Int J Food Sci Technol. 2002;37:153-161.

[16] Zhang Y-J, Liu Y-Q, Pu X-Y, Yang C-R. Iridoidal glycosides from *Jasminum sambac*. Phytochem. 1995;38:899-903.

[17] Inagaki J, Watanabe N, Moon JH, Yagi A, Sakata K, Ina K, et al. Glycosidic aroma precursors of 2-phenylethyl and benzyl alcohols from *Jasminum sambac* flowers. Biosci Biotechnol Biochem. 1995;59:738-739.

[18] Zhang ZF, Bian BL, Yang J, Tian XF. [Studies on chemical constitutents in roots of *Jasminum sambac*]. Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica. 2004;29:237-239.

[19] Younis A, Mehdi A, Riaz A. Supercritical carbon dioxide extraction and gas chromatography analysis of *Jasminum sambac* essential oil. Pak J Bot.2011; 43:163-168.

[20] Edris A, Franz C, Chizzola R. Isolation and characterization of the volatile aroma compounds from the concrete headspace and the absolute of *Jasminum sambac* (L.) Ait. (Oleaceae) flowers grown in Egypt. Eur Food Res Technol. 2008;226:621–626.

[21] Widowati W, Mozef T, Risdian C, Yellianty Y. Anticancer and free radical scavenging potency of *Catharanthus roseus, Dendrophthoe petandra, Piper betle*, and *Curcuma mangga* extracts in breast cancer cell lines. Oxidants Antioxid Med Sci. 2013;2:137-142.

[22] Widowati W, Wijaya L, Wargasetia T, Bachtiar I, Yellianty Y, Laksmitawati D. Antioxidant, anticancer and apoptosis-inducing effects of Piper extracts in HeLa cells. J Exp Integr Med. 2013;3:225-230.

[23] Laksmitawati DR, Widyastuti A, Karami N, Afifah E, Rihibiha DD, Nufus H, Widowati W. Anti-inflammatory effects of *Anredera cordifolia* and *Piper crocatum* extracts on lipopolysaccharide-stimulated macrophage cell line. Bangladesh J Pharmacol. 2017;12:35-40.

[24] Adnyana I, Abuzaid A, Iskandar E, Kurniati N. Pancreatic lipase and a-amylase inhibitory potential of mangosteen (*Garcinia mangostana* Linn.) pericarp extract. Int J Med Res Health Sci. 2016;5:23-28.

[25] Bera TK, Chatterjee K, Ghosh D. In vitro antioxidant properties of the hydro-methanol

extract of the seeds of *Switenia mahagoni* (L.) Jacq. BGM. 2015;7:18-24.

[26] Widowati W, Rani AP, Hamzah RA, Arumwardana S, Afifah E, Kusuma HSW, et al. Antioxidant and antiaging assays of *Hibiscus sabdariffa* extract and its compounds. Nat Prod Sci. 2017;23:192-200.

[27] Widowati W, Darsono L, Suherman J, Yelliantty Y, Maesaroh M. High Performance Liquid Chromatography (HPLC) analysis, antioxidant, antiaggregation of mangosteen peel extract (*Garcinia mangostana* L.). Int J Biosci Biochem Bioinform. 2014;4:458.

[28] Rusmana D, Wahyudianingsih R, Elisabeth M, Balqis B, Maesaroh M, Widowati W. Antioxidant activity of *Phyllanthus niruri* extract, rutin and quercetin. Indones Biomed J. 2017;9:84-90.

[29] Widowati W, Widyanto RM, Husin W, Ratnawati H, Laksmitawati DR, Setiawan B, et al. Green tea extract protects endothelial progenitor cells from oxidative insult through reduction of intracellular reactive oxygen species activity. Iran J Basic Med Sci. 2014;17:702–709.

[30] Utami S, Sachrowardi QR, Damayanti NA, Wardhana A, Syarif I, Nafik S, et al. Antioxidants, anticollagenase and antielastase potentials of ethanolic extract of ripe sesoot (*Garcinia picrorrhiza* Miq.) fruit as antiaging. J Herbmed Pharmacol. 2018;7:88-93.

[31] Tu PTB, Tawata S. Antioxidant, antiaging, and anti-melanogenic properties of the essential oil from two varieties of *Alpinia zerumbet*. Molecules. 2015;20:16723-16740.

[32] Irshad M, Chaudhuri PS. Oxidant-antioxidant system: role and significance in human body. Indian J Exp Biol. 2002;40:1233–1239.

[33] Blois MS. Antioxidant determination by the use of stable free radical. Nature. 1958;181:1199.

[34] Thaakur SR. Free radical scavenging activity of *Jasminum sambac*. JGTPS. 2014;5:1658–1661.

[35] Shekhar S, Prasad MP. Studies on antioxidant properties of *Jasminum* species by FRAP assay. Int J Pure App Biosci. 2015;3:52-57.

[36] Chiu TM, CC Huang, TJ Lin, JY Fang, NL Wu, CF Hung. 2009. In vitro and in vivo anti-photoaging effects of an isoflavone extract from soybean cake. J Ethnopharmacol. 2126:108-113.

[37] Kim JH, Cho YH, Lee BC, Pyo HB. Cosmetic composition comprising extracts of *Pterocarpus santalinus* capable of inhibiting activity. Korea Patent. 2007. http://patent.ipexl.com/KR/1020050076940.htmL.

[38] Kim S, JH Chung. Berberine prevents UV-induced MMP-1 and reduction of type I procollagen expression in human dermal fibroblasts. Phytomed. 2008;15:749-753.

[39] Maity N, NK Nema, MK Abedy, BK Sarkar, PK Mukherjee. 2011. Exploring *Tagetes erecta* Linn flower for the elastase, hyaluronidase and MMP-1 inhibitory activity. J Ethnopharmacol. 2011;137:1300-1305.

[40] Moon HI, J Lee, PZ Ok, JH Chung. The effect of flavonol glycoside on the expressions of matrix metalloproteinase-1 in ultraviolet-irradiated cultured human skin fibroblasts. J Ethnopharmacol. 2005;101:176-179.

[41] Satardekar KV, Deodhar MA. Anti-ageing ability of Terminalia species with special reference to hyaluronidase, elastase inhibition and collagen synthesis in vitro. Int J Pharmacogn Phytochem Res. 2010;2:30-34.

[42] Aslam MN, Lansky EP, Varani J. Pomegranate as a cosmeceutical source: Pomegranate fractions promote proliferation and procollagen synthesis and inhibit matrix metallo proteinases-1 production in human skin cells. J Ethnopharmacol. 2006;103:311-318.

[43] Raffetto JD, Khalil RA. Matrix metalloproteinases and their inhibitors in vascular remodeling and vascular disease. Biochem Pharmacol. 2008;75:346-349.

[44] Kim Y, Uyama H, Kobayashi S. Inhibition effects of (+)-catechin-aldehyde polycondensates on proteinases causing proteolytic degradation of extracellular matrix. Biochem Biophys Res Commun. 2004;320:256-261.

[45] Baylac S, Racine P. Inhibition of human leukocyte elastase by natural fragrant extracts

of aromatic plants. Int J Aromather. 2004;14:179-182.

[46] Melzig MF, Löser B, Ciesielski S. Inhibition of neutrophil elastase activity by phenolic compounds from plants. Pharmazie. 2001;56:967-970.

[47] Siedle B, Cisielski S, Murillo R, Löser B, Castro V, Klaas CA, et al. Sesquiterpene lactones as inhibitors of human neutrophil elastase. Bioorg Med Chem. 2002;10:2855-2861.

[48] Piwowarski JP, Kiss AK, Kozłowska-Wojciechowska M. Anti-hyaluronidase and anti-elastase activity screening of tannin-rich plant materials used in traditional Polish medicine for external treatment of diseases with inflammatory background. J Ethnopharm. 2011;137:937-941.

[49] Garg A, Garg S, Zaneveld LJD, Singla AK. Chemistry and pharmacology of the Citrus bioflavonoid hesperidin. Phytother Res. 2001;15:655–659.

[50] Chen MC, Ye YY, Ji G, Liu JW. Hesperidin upregulates heme oxygenase-1 to attenuate hydrogen peroxide-induced cell damage in hepatic L02 cells. J Agric Food Chem. 2010;58:3330–3335.

[51] Elavarasan J, Velusamy P, Ganesan T, Ramakrishnan SK, Rajasekaran D, Periandavan K. Hesperidin-mediated expression of Nrf2 and upregulation of antioxidant status in senescent rat heart. J Pharm Pharmacol. 2012;64:1472–1482.

[52] Wilmsen PK, Spada DS, Salvador M. Antioxidant activity of the flavonoid hesperidin in chemical and biological systems. J Agric Food Chem. 2005;53:4757-4761.

[53] Sun K, Xiang L, Ishihara S, Matsuura A, Sakagami Y, Qi J. Anti-aging effects of hesperidin on *Saccharomyces cerevisiae* via inhibition of reactive oxygen species and UTH1 gene expression. Biosci Biotech Biochem. 2012;76:640-645.

[54] Li R, Cai L, Xie XF, Yang F, Li J. Hesperidin suppresses adjuvant arthritis in rats by inhibiting synoviocyte activity. Phytother Res. 2010;24:S71-S6.

[55] Gülçin İ. Antioxidant activity of eugenol: a structure–activity relationship study. J Med Food. 2011;14:975-985.

[56] Ogata M, Hoshi M, Urano S, Endo T. Antioxidant activity of eugenol and related monomeric and dimeric compounds. Chem Pharm Bull. 2000;48:1467-1469.

Antioxidant and Antiaging Activities of Jasminum Sambac Extract, and its Compounds

ORIGINALITY REPORT

20%	17%	14%	8%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	e-sciencecentral.org Internet Source	4%
2	Submitted to Universitas Diponegoro Student Paper	3%
3	www.myhealthfulworld.com Internet Source	2%
4	scialert.net Internet Source	2%
5	id.123dok.com Internet Source	2%
6	ejurnal.litbang.pertanian.go.id Internet Source	2%
7	www.hindawi.com Internet Source	2%
8	eprints.skums.ac.ir Internet Source	2%
9	D Pujimulyani, L Suryani, A Setyawati, A Amalia, R L Qodaniah, H S W Kusuma, W	2%

Widowati. " Elastase, Hyaluronidase and Tyrosinase inhibitor activities antiaging of extract and its fractions ", IOP Conference Series: Earth and Environmental Science, 2019

Publication

Exclude quotes	On	Exclude matches	< 2%
Exclude bibliography	On		