





## RESEARCH ARTICLE

# Evaluation of antioxidant and anti-collagenase activity of *Rosa damascena* L. flower petal and receptacle extract [version 1; peer review: 1 approved, 1 approved with reservations]

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## Abstract

**Background:** *Rosa damascena* L. is the most notable species of the Rosaceae family in the world, and has been used in food, cosmetics, and the pharmaceutical industry. Bioactive compounds in this flower are known to have several activities, such as antioxidant, antimicrobial, and anti-inflammatory. In this study, the antioxidant and collagenase inhibitory activities of *R. damascena* L. petal and receptacle extracts were evaluated.

**Methods:** Ethanolic extraction of *R. damascena* L. petals (EERP) and *R. damascena* L. receptacles (EERR) were obtained, and bioactive compounds (flavonoids, phenolics, alkaloids, steroids, tannins, terpenoids, and triterpenoids) were classified by phytochemical screening. Antioxidant activities were analyzed by Ferric Reducing Antioxidant Power (FRAP) assay, while anti-collagenase analysis was examined through the inhibition of collagenase.

**Results:** Phytochemical test revealed the presence of flavonoids, phenolics, alkaloids, steroids, triterpenoids, triterpenes, and tannin. EERP showed higher FRAP activity ( $164.23 \pm 1.34 \mu\text{M Fe(II)}$ ) than EERR ( $12.85 \pm 6.19 \mu\text{M Fe(II)}$ ). EERP also had higher inhibitory activity of collagenase ( $\text{IC}_{50} = 115.48 \pm 1.78 \mu\text{M/mL}$ ) compared to EERR ( $\text{IC}_{50} = 141.96 \pm 6.13 \mu\text{M/mL}$ ).

**Conclusions:** *R. damascena* L. petal and receptacle ethanol extracts contain several components, such as phenolics, flavonoids, alkaloids, tannins, terpenes, triterpenoids, and steroids. These extracts exhibit antioxidant activity and collagenase inhibition. *R. damascena* L. petal extract showed higher antioxidant activity through FRAP assay and inhibitory activity of collagenase than *R. damascena* L. receptacle extract.

## Keywords


*Rosa damascena* L., Ferric Reducing Antioxidant Power, antioxidant, anti-collagenase

## Open Peer Review

Approval Status  

	1	2
version 1		
17 Jul 2020	<a href="#">view</a>	<a href="#">view</a>

1. **Ihsan Iswaldi**, Universitas Prasetya Mulya, Tangerang, Indonesia

2. **Oksana Sytar** , Kiev National University of Taras Shevchenko, Kyiv, Ukraine

Any reports and responses or comments on the article can be found at the end of the article.

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**Author roles:** **Elfitriani E:** Conceptualization, Investigation, Methodology, Writing – Original Draft Preparation; **Raif A:** Conceptualization, Investigation, Methodology, Resources, Writing – Original Draft Preparation; **Ginting CN:** Formal Analysis, Supervision, Visualization, Writing – Review & Editing; **Ikhtiari R:** Investigation, Methodology, Project Administration, Supervision, Validation, Writing – Review & Editing

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## Introduction

Antioxidants are compounds that can react with Reactive Oxygen Species (ROS) to prevent the oxidation of lipids, DNA, and proteins<sup>1</sup>. Several studies report that ROS correlates with several human diseases, such as cancer<sup>2</sup>, inflammation<sup>3</sup>, and the aging process<sup>4</sup>. In the aging process, ROS play a role in protein unfolding, lipid breakdown, DNA damage, fragmentation, and collagen disintegration<sup>5</sup>. In the body, ROS promote the activation of collagenase and block collagen synthesis, leading to a decrease in skin elasticity and acceleration of skin wrinkles. This mechanism by ROS is the major factor of degeneration collagen fiber<sup>6</sup>.

*Rosa damascena* L. is the most important species among the Rosaceae family of flowers. This species has many pharmacological properties and has been used in food, herbal medicine, and cosmetics since ancient times<sup>7</sup>. Previous research showed that *R. damascena* L. has anti-viral, anti-microbial, anti-cancer, anti-depressant, anti-inflammatory, anti-convulsant, and antioxidant activities<sup>8</sup>. Several studies report that phenolic compounds and flavonoids contributed to an increase in antioxidant activity<sup>9-11</sup>. In this study, we evaluate the antioxidant and anti-collagenase activity from ethanol extract of *R. damascena* L. petals and receptacles.

## Methods

### Sample extraction

*R. damascena* L. flowers were obtained from Bandung, Indonesia (-6.803662, 107.586633). In total, 1400 g and 700 g of petals and receptacles of *R. damascena* L. were dried and mashed, respectively. The dried samples (250 g petals and 90g receptacles) were soaked with 8000 mL and 3000 mL of 70% ethanol, respectively, and macerated. This solvent was replaced every 3 days and the extracted solution from the first to the last (15<sup>th</sup> day) was mixed and concentrated using a rotary evaporator at 50°C for 4 h. The extracts were labeled as ethanol extract of rose petals (EERP) and ethanol extract of rose receptacles (EERR).

### Phytochemical analysis

Phytochemical analysis was done to detect the presence of flavonoids, phenolics, tannins, steroids/triterpenoids, terpenoids and alkaloids. 10 mg of extracts were used for each analysis following the procedures of Widowati *et al.* without modification<sup>12</sup>.

### Ferric ion reducing antioxidant power (FRAP) activity

The antioxidant activity of EERP and EERR was investigated using FRAP assay following Sricharoen *et al.* with slight modifications<sup>13</sup>. Briefly, ferric chloride hexahydrate (Merck) and 2,4,6-tripyridyl-s-triazine (TPTZ) (Sigma) were mixed to form the FRAP reagent. 142.5 µL FRAP solution was added into 7.5 µL of sample extract and the mixtures were incubated at 37°C for 5 min. The absorbance of the reaction mixture was read at 539 nm. A set of concentrations 0–200 µg/mL of FeSO<sub>4</sub>·7H<sub>2</sub>O (Sigma) was used for calibration of the standard curve. The results were expressed as µM Fe(II)/µg extract.

### Anti-collagenase assay

Anti-collagenase activity of EERP and EERR was assessed by evaluating the inhibition of collagenase, following the procedure of Wittenauer *et al.*<sup>14</sup> 10 µL collagen from *Clostridium histolyticum* (0.01 U/mL, Sigma), 60 µL of 50 mM Tricine buffer pH 7.5 (containing 10 mM CaCl<sub>2</sub> (Merck) and 400 mM NaCl (TCL Co., Indonesia)), and 30 µL of extract (7.81 – 250 µg/mL) were mixed and incubated at 37°C for 20 min. After incubation, 20 µL of 1 mM N-[3-(2-Furyl)acryloyl]-leu-gly-Pro-Ala (FALGPA, Sigma) in Tricine buffer (Sigma) was added to the mixture. The collagenase inhibitory activities were measured at 335 nm by monitoring for 20 min after starting the reaction. The inhibition value was calculated according to Eq. (1). IC<sub>50</sub> values were determined from dose-effect curves.

$$\text{Collagenase inhibition activity (\%)} = \frac{A_{\text{negative control}} - A_{\text{extracts}}}{A_{\text{negative control}}} \times 100\% \quad (1)$$

### Data analysis

Statistical analysis using SPSS version 23 was carried out by normality test using numeric variable with Levene test and ANOVA and continued with post-hoc honestly significant difference, and Tukey test to the confidence level of 95% ( $\alpha = 0.05$ ). All data are expressed as mean  $\pm$  SEM.

## Results and Discussion

### Phytochemical analysis

Phytochemical investigations of *R. damascena* L. petal and receptacle extracts in ethanol expressed positive results of flavonoids, phenolic compounds, tannins, steroids, terpenoids, and alkaloids. The details are presented in Table 1.

**Table 1. Phytochemical test results for ethanol extracts of *Rosa damascena* L. petals and receptacles.** EERP, ethanolic extraction of *R. damascena* L. petals; EERR, ethanolic extraction of *R. damascena* L. receptacles.

Test	Result			
	Observation	EERP	Observation	EERR
Flavonoids	Yellow/ Orange	(+)	Yellow/ Orange	(+)
Phenolics	Green/Blue/Black	(+)	Green/Blue/Black	(+)
Tannins	Red/ Orange Layer	(+)	Yellow gold Layer	(+)
Steroids/Triterpenoids	Orange	(+) Triterpenoids	Green	(+) Steroids
Terpenoids	Purple	(+)	Green/ Gray	(-)
Alkaloids	Orange	(+)	Orange	(+)

The results of phytochemical screening showed steroids are detected in EERP by the bluish-green color, while in the same test, EERR gave a red color indicated it contained triterpenes. Although there was a difference between chemical compounds, both samples contained phenol and flavonoids that act as antioxidants and anti-collagenases. In previous research, Patil *et al.*<sup>15</sup> found that the ethanol extract of *R. damascene* Mill L. contains total phenolic and flavonoid compounds at a higher level than acetone, aqueous, and chloroform extracts.

The antioxidant and anti-collagen activity of flowers depends on the compounds it contains and the solvent used. Phenolics are well known to have antioxidant capability<sup>9-10,16</sup>, as does flavonoids and alkaloids<sup>17</sup>. Steroids, terpenoids, and triterpenoids also have been reported to have antioxidant activity<sup>18</sup>. Muccilli *et al.*<sup>19</sup> reported that tannins also play a role in blocking oxidative chain reactions. The solvent extract also has a contribution to increasing antioxidant and anti-collagenase activity. Ethanol extract of *R. damascena* L. petals has been shown to have a lower IC<sub>50</sub> value than acetone when evaluated by DPPH<sup>15</sup>. Ethanol extracts also show the highest phenolic content and antioxidant activity than water extracts of *R. spinosissima*<sup>9</sup>.

#### Antioxidant activity

Total antioxidant activity of EERP and EERR were further evaluated by FRAP assay. The FRAP value was obtained by plotting the standard curve of FeSO<sub>4</sub> at a concentration between 0.78 and 50 µg/mL (Figure 1).

The antioxidant activity of both extracts is presented in Figure 2. In this test, the antioxidant activities were obtained based

on the capacity to reduce ferric (III) to ferrous (II). The result demonstrates that EERP has higher antioxidant capacity compared to EERR.

Table 2 showed FRAP values for different concentrations of EERP and EERR. The data showed normal distribution and was significantly different ( $p > 0.005$ ). Both extracts showed higher antioxidant activity with higher concentrations (Figure 2). The highest antioxidant activities were observed at 50 µg/mL (EERP:  $164.23 \pm 1.34$  µM/mL; EERR:  $12.85 \pm 6.19$  µM/mL).

#### Anti-collagenase activity

Collagen is the most abundant protein responsible for conferring skin thickness, strength, and elasticity<sup>20</sup>. This metalloproteinase enzyme has a vital role in collagen degradation, which increases aging signs<sup>21</sup>. The percentage of collagenase inhibition of EERP and EERR found in the present study is presented in Table 3. Anti-collagenase activity showed a concentration-dependent manner, where higher concentrations of the extracts increased the anti-collagenase (and thus antiaging) activity of extracts. At the highest concentration (250 µM/mL) EERP showed the highest inhibition activity compared to EERR ( $91.52 \pm 3.44$  µg/mL and  $77 \pm 2.03$  µg/mL, respectively).

The IC<sub>50</sub> percentage can be seen in Table 4. The IC<sub>50</sub> value of EERR is higher than EERP ( $141.96 \pm 6.13$  µM/mL and  $115.48 \pm 1.78$  µM/mL, respectively). This indicates that EERR has less activity to impede collagenase activity than EERP; however, these results show that there is potential for EERR to inhibit collagenase. Park *et al.*<sup>22</sup> found that *R. damascena* extract impacted

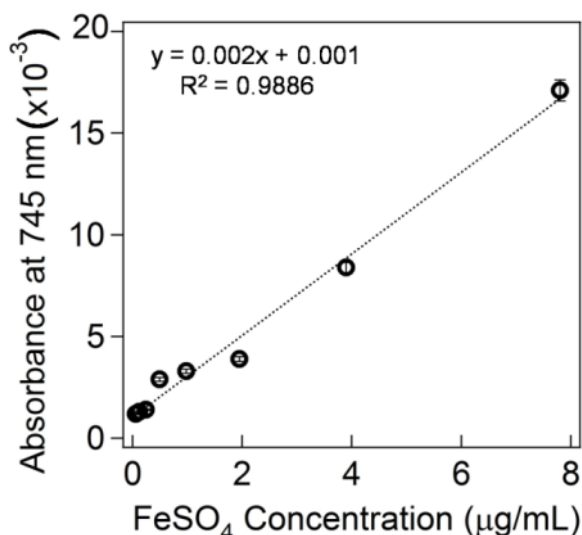
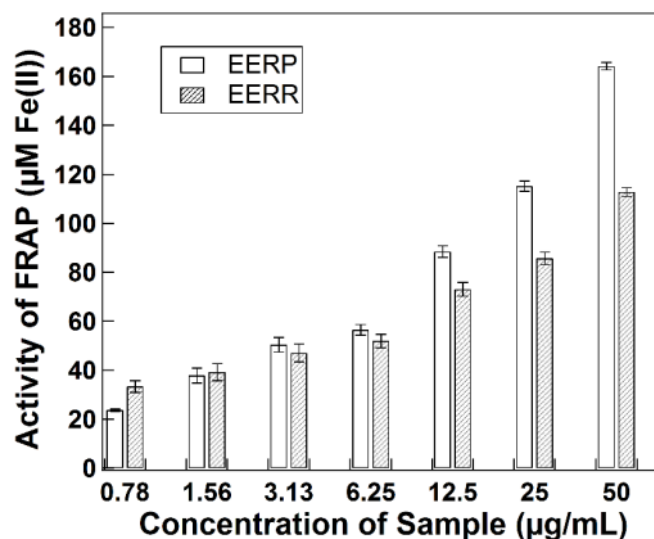


Figure 1. Standard curve of FeSO<sub>4</sub>.



**Figure 2. Effect of various concentrations of EERP and EERR in FRAP activity.** EERP, ethanolic extraction of *Rosa damascena* L. petals; EERR, ethanolic extraction of *R. damascena* L. receptacles.

**Table 2. FRAP activities of EERP and EER.** EERP, ethanolic extraction of *Rosa damascena* L. petals; EERR, ethanolic extraction of *R. damascena* L. receptacles.

Concentration (µg/mL)	FRAP activity (µM Fe(II))	
	EERP	EERR
0.78	23.70±3.88 <sup>a</sup>	33.35±4.83 <sup>a</sup>
1.56	37.83±5.53 <sup>ab</sup>	39.25±6.73 <sup>a</sup>
3.13	50.43±6.32 <sup>bc</sup>	47.07±7.25 <sup>a</sup>
6.25	56.63±5.66 <sup>c</sup>	51.97±7.33 <sup>a</sup>
12.50	88.50±7.75 <sup>d</sup>	73.08±9.71 <sup>b</sup>
25.00	115.20±8.24 <sup>e</sup>	85.68±9.28 <sup>b</sup>
50.00	164.23±1.34 <sup>f</sup>	112.85±6.19 <sup>c</sup>

Data presented in the form of mean ± SEM. Different lowercase letters indicate a significant difference in  $p < 0.05$  (Post Hoc Tukey HSD Test).

**Table 3. Anti-collagenase activity of EERP and EERR.** EERP and EERR were diluted in tricine buffer to reach the final concentration of 7.81; 15.63; 31.25; 62.50; 125.00; 250.00 (µg/mL). EERP, ethanolic extraction of *Rosa damascena* L. petals; EERR, ethanolic extraction of *R. damascena* L. receptacles.

Concentration (µg/mL)	Inhibition activity (%)	
	EERP	EERR
7.81	11.81±1.76 <sup>a</sup>	13.32±2.36 <sup>a</sup>
15.63	21.09±1.49 <sup>a</sup>	17.38±2.04 <sup>ab</sup>
31.25	21.68±2.50 <sup>a,b</sup>	19.08±1.04 <sup>b</sup>
62.50	33.01±2.42 <sup>b</sup>	33.74±2.27 <sup>c</sup>
125.00	56.46±7.67 <sup>c</sup>	46.19±1.92 <sup>d</sup>
250.00	91.52±3.44 <sup>d</sup>	77.04±2.03 <sup>e</sup>

Data presented in the form of mean ± SEM. Different lowercase letters indicate a significant difference in  $p < 0.05$  (Post Hoc Tukey HSD Test).

**Table 4. IC<sub>50</sub> value of the anti-collagenase activity of EERP and EERR.** EERP, ethanolic extraction of *Rosa damascena* L. petals; EERR, ethanolic extraction of *R. damascena* L. receptacles.

Samples	Equation	R <sup>2</sup>	IC <sub>50</sub> (µg/mL)
EERP	y = 0.3208x + 12.944	0.991	115.48±1.78
EERR	y = 0.2598x + 13.146	0.990	141.96±6.13



metalloproteinase transcription by suppressing AP-1 activation. AP-1 is a protein activated by UVB penetration to the skin, and initiates changes of the extracellular matrix, including collagen, elastin, and proteoglycans degradation. Our results show that ethanol extracts of petals and receptacles of *R. damascena* L. possess anti-collagenase activity.

## Conclusion

*R. damascena* L. petal and receptacle ethanol extracts contain several components, such as phenolics, flavonoids, alkaloids, tannins, terpenes, triterpenoids, and steroids. The pharmacological effects of these extracts exhibit antioxidant activity and collagenase inhibition. *R. damascena* L. petal extract showed higher antioxidant activity through FRAP assay and inhibitory

activity of collagenase than *R. damascena* L. receptacle extract.

## Data availability

### Underlying data

Figshare: Raw data Analysis of anticollagenase an IC50 value.xlsx, <https://doi.org/10.6084/m9.figshare.12585626.v1><sup>23</sup>

Figshare: Raw data FRAP Activity of Extracts of Rosa Damascene Petals and Receptacles, <https://doi.org/10.6084/m9.figshare.12585671.v1><sup>24</sup>

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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Version 1

Reviewer Report 11 January 2022

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 **Oksana Sytar** 

Department of Plant Biology, Institute of Biology, Kiev National University of Taras Shevchenko, Kyiv, Ukraine

Dear Authors,

The article "Evaluation of antioxidant and anti-collagenase activity of *Rosa damascena* L. flower petal and receptacle extract" is original research work and can be indexed after major revision.

Please, see some comments below:

- In Introduction part it would be good to add more information about previous studies with representatives family Rosaceae.
- It would be good to admit that research studies were done with identification of flavonoids and phenolics in the leaves of some representatives family Rosaceae but less current studies about petal biochemical composition (e.g. Sytar *et al.*, 2015<sup>1</sup>; Sytar *et al.*, 2018<sup>2</sup>). Also for *Rosa damascena* was studied well biochemical composition of essential oils but not petal which can be source of essential oil depending from method of extraction (Mahboubi *et al.*, 2011<sup>3</sup>; Piotrowicz *et al.*, 2021<sup>4</sup>).
- In the part material and methods for line "Phytochemical analysis was done to detect the presence of flavonoids, phenolics, tannins, steroids/triterpenoids, terpenoids and alkaloids." - please add information if it was used spectrophotometrically or other kind of methods.
- Please, admit that evaluation of anti-collagenase activity of *Rosa damascena* petals was done for the first time. Add information why it is important to study anti-collagenase activity.
- In the part where is written about Phytochemical test results for ethanol extracts of *Rosa damascena* L. petals and receptacles, please add a sentence that the method of visual assessment was recommended to use for pre-screening of anthocyanins content and rutin for some plants (Sytar *et al.*, 2014<sup>5</sup>).

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**Is the work clearly and accurately presented and does it cite the current literature?**

Partly

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Plant secondary metabolites, especially plant natural compounds with healthy effects

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Reviewer Report 12 August 2021

<https://doi.org/10.5256/f1000research.27327.r86477>



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**Ihsan Iswaldi**

Food Business Technology Study Programme, Universitas Prasetiya Mulya, Tangerang, Indonesia

This article can be accepted in this form after the affirmation of several issues:

1. Tannins are highly polymerized compounds of polyphenols and flavonoids are also chemically defined families of polyphenols. So, the authors do not need to mention polyphenols in line with flavonoids and tannins because the last two are classified as polyphenols. Unless the authors want to mention the classes of polyphenols, then write them in sequence.
2. The authors should explain why they only used FRAP assay for antioxidant activity. Why did not they carry out DPPH, ABTS, TEAC, ORAC, and TBARS methods as well and compare the results?

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Food Chemistry and Analysis; Bioactive Ingredients; Analytical Separation

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