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Comparative Study on Bioactivities of Four Rose Species for Anti-aging Cosmetic Applications

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When the skin is exposed to UVA/UVB rays, it stimulates the activity of the enzyme elastase. Wrinkles and sagging skin are the result of a breakdown in the skin's elastic fibre network. The aim of this research is to identify the most promising material for suppressing the enzyme elastase from common four rose (Damask, Bishop, Autumn and Lafont) species in Vietnam, as well as to compare the bioactivities of fresh and dried petals. The obtained results showed that Damask and Bishop petal extracts had the highest antioxidant activity in the DPPH assay with IC_{50} = 10.08 and 10.07 µg/mL, respectively. Damask rose displayed not only an outstanding elastase inhibition with IC_{50} = 261.42 ± 0.8 µg/mL but also a high total polyphenol content (TPC) value of 149.49 mg GAE/g extract. These results indicate that the *Rosa damascena* petals could be used as valuable material for anti-aging product development based on the high TPC and elastase inhibitory activities.

Keywords: Anti-aging, Antioxidant, Enzyme Elastase, *Rosa damascena* petals.

INTRODUCTION

In human body, with a coverage area of 1.7 m^2 and weighing about 15% of the total body weight, the skin plays as a barrier to protect from the external effects, especially UV radiation [1]. Skin aging is a combo of chronological aging, actinic damage and hormonal influences on the human body. Some common signals associated with aging include the change in appearance and structure of the skin [2]. Two main processes induce skin aging to consist of intrinsic, extrinsic aging and also stochastic processes. Due to changes in the metabolic processes, the random process generate free radicals sometimes harmful to the cell [3]. Sunlight plays a crucial part in human life and helps sustained life. When exposed to sunlight for a long time without any protection, UV radiation will cause several skin-related damages, from mild effects to serious damages [4]. The UV radiation not only causes skin aging, but also causes some damage to the skin, including skin cancer, sunburn cells, creation of free radicals [5] and DNA damage [6]. The generation of reactive oxygen species (ROS) after exposure to UV radiation may lead to photoaging stressors [7]. Those are indirect agents, which activate dermal enzymes, especially collagenase and elastase. These enzymes destroy collagen and degrade elastin, which directly affects the elasticity and durability of the skin. Moreover, the appearance of elastase and collagenase promotes wrinkles, dried skin and a leathery appearance [8].

In dermatology, few researches reported that the combination of some natural actives from plant extracts effectively prevents or alleviates the effects of UVB irradiation on the skin [9]. Plant extracts contain some active compounds such as polyphenols and triterpenoids that are able to inhibit collagenase, elastase and tyrosinase [10]. For that reason, they have been widely used as treatment ingredients in skin care products [11]. Specifically, these compounds have a strong antioxidative ability and prevent ROS formation [12]. In parti-cular, phenolic compounds in rose petals are characterized as phenolic acids and flavonoids, which account for large amounts in rose petals with 481.54 µg GAE × (mg samples)$^{-1}$. More specifically, gallic acid (GA) can inhibit matrix metallo-proteinase-1 (MMP^{-1}) and adjust to enhance the synthesis of elastin and procollagen. Quercetin is an important ingredient that inhibits aging enzymes such as collagenase enzymes [13]. In previous research, HPLC analysis showed that the main ingredient in the rose extract

sample was gallic acid (GA), in addition to other components, including quercetin and rutin [14]. Furthermore, the extract from rose petals is considered one of the promising future ingredients for the cosmetic industry. Hence, the antioxidant capacities, tyrosinase and elastase enzyme inhibition and other bioactivities of four widely distributed rose species in Vietnam were examined.

EXPERIMENTAL

All the chemicals and reagents used in this study were of analytical grade were procured from Merck, including gallic acid, kojic acid, ursolic acid, dimethyl sulfoxide, 2,2-diphenyl-1-picrylhydrazyl, Follin & Ciocalteu, enzyme elastase and enzyme tyrosinase. The dried plant materials were collected from Lam Dong and Vinh Phuc, Vietnam. After harvesting in these rose farms, fresh rose petals were washed and dried at 40 ºC for 12 h using a heat pump dryer until the humidity was below 12%. Finally, the raw materials were stored in dry places and crushed into small pieces before extraction (Table-1).

General procedure: Four rose species followed the extraction conditions as ethanol concentration of 96% (v/v) at 50 ºC, material-solvent ratio of 1:8 (g/mL), extraction period of 60 min and double extractions. After the maceration, the crude extract was concentrated in a vacuum rotary evaporator at 50 ºC to get the concentrated extract. Every batch of the extract was then homogenized to receive a uniform state. Then the concentrated extract was analyzed for the moisture content, total polyphenol and bioactivities.

Detection method: Different methods were applied to test the biological activity of the extracts. Firstly, the total polyphenol content was analyzed by Folin & Ciocalteu assay. The antioxidant activity can be determined by three methods *viz*. β-carotene bleaching assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and TEAC free radical scavenging assay. In this study, the DPPH test helps screen for antioxidants, which can remove free radicals, such as polyphenols. Finally, the inhibitory activity of enzymes, including tyrosinase and elastase, was also determined.

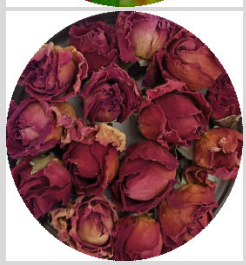
Total polyphenol content (TPC): Quantitative analysis of total polyphenol content was analyzed using the Folin-Ciocalteu reagent. The Folin-Ciocalteu reagent oxidizes the phenolic compounds to a blue coloured compound when undergoes electron transfer in a basic media. Using wavelength at 760 nm to measure the absorbance of the reference standard (gallic acid) and the extract samples [15]. The content of total polyphenols was estimated using eqn. 1:

$$TPE = GAE \times \frac{v}{m}$$

where TPC is total polyphenol contents in 1 g dried extract (mg GAE/g extract); v is the sample volume; and m is GAE sample content (mg/L).

Antioxidant activity: The DPPH free radical scavenging activity was carried out according to the method of Blois [16]. In brief, 120 µL of extraction solution was added to 180 µL of DPPH solution, mixed well, then incubated for 30 min in dark at 25 ºC and the density spectrophotometrically of sample (A_s) was measured at 517 nm. Positive validation was performed with 120 µL of vitamin C and 180 mL of DPPH solution. Next, the controlled solution was performed with 120 µL of extract and 180 µL of 80% methanol before measuring the absorbance

TABLE-1
FOUR ROSE SPECIES IN THE PREPARATION STEP

	Damask	Bishop	Autumn	Lafont
Fresh petals				
Dried petals				
Crushed petals				

(A_c). Finally, the blank solution was a mixture between 120 µL of 80% methanol and 180 µL of DPPH solution. The percentage of inhibition (I) was measured using eqn. 2:

$$I\ (\%) = \frac{A_c - (A_s - A_b)}{A_c}$$

where A_b, A_s, A_c are the absorbance of the blank, sample and control, respectively.

Enzyme elastase inhibitory activity: *N*-Succinyl-Ala-Ala-Ala-*p*-nitroanilide substrate hydrolyzes to coloured *p*-nitroaniline at the maximum absorption wavelength of 405 nm in the presence of enzyme elastase. The formation of *p*-nitroaniline is inhibited when an elastase inhibitor is present. Accordingly, as the sample concentration decreases, so does the colour. The anti-elastase activity of plant extracts was calculated using eqn. 3:

$$I\ (\%) = \frac{(A - B) - (C - D)}{A - B} \times 100$$

where A is the absorbance of blank containing enzyme, B is the absorbance of blank without enzyme; C and D is the absorbance of the sample containing enzyme and without enzyme, respectively.

Enzyme tyrosinase inhibitory activity: In the presence of tyrosinase enzymes, L-dopa will be oxidized to dopachrome with an absorption peak at a wavelength 475 nm. The formed dopachrome was reduced due to the presence of tyrosinase inhibitors. Therefore, the colour will also decrease depending on the test concentration of the sample. The anti-tyrosinase activity of the plant extracts was calculated by the same formula as shown in enzyme elastase inhibitory activity section.

RESULTS AND DISCUSSION

Extraction yield: Fig. 1 shows these rose extracts have different colours from moderate pink to dark purple due to a combination of carotenoid pigments, anthocyanidins and flavonoids. These extracts were stored in a dark bottle at low temperature and not exposed to air.

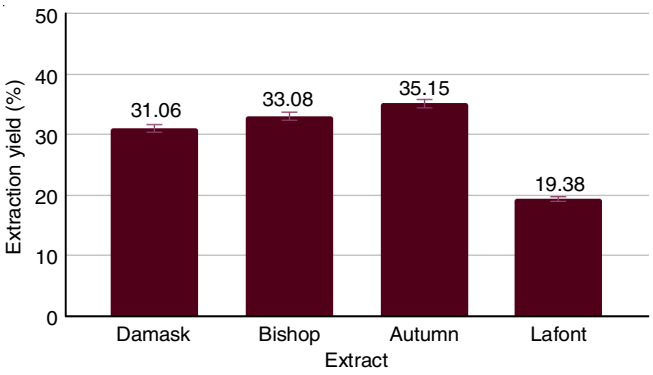


Fig. 1. Extraction yield of four different types of roses

Due to the difference in structure and content of active ingredients leads to different extraction efficiency of each type of rose. The extraction yield of Autumn rose and Bishop rose is highest with 35.15 ± 0.2 % and 33.08 ± 1.1 %, respectively, about 2 times higher than that of Lafont rose (19.38 ± 0.6 %). The difference in yield is due to the low fibre content of autumn roses, hence the extraction efficiency is high.

Total polyphenol content (TPC): Based on several studies, it has been shown that the presence of polyphenol compounds affect some plant activities including antioxidant activity, inhibition of tyrosinase enzyme and especially inhibition of elastase enzyme [17]. Among the studied four roses extracts, Lafont, Bishop and Damask rose extracts had a high total polyphenol content of 197.6 ± 0.8; 153.9 ± 2.5; 149.49 ± 1.2 mg GAE/g extract, respectively. On the other hand, Autumn rose extract had the lowest total polyphenol content (131.55 ± 4.3 mg GAE/g extract) despite the highest extraction yield (35.15%). This could be explained by the fact that the major ingredients in Autumn rose petals either not present or contains a very low level of phenolic compounds.

Antioxidant activity by DPPH assay: Damask and Bishop rose extracts with high total polyphenol content showed the highest antioxidant capacity by the DPPH method with IC_{50} = 10.08 ± 1.2; 10.07 ± 0.55 µg/mL, respectively (Fig. 2). Moreover, there is a similarity between antioxidant capacity and total polyphenol content. In contrast, the antioxidant ability (IC_{50} = 15.21 0.45 mg/mL) and total polyphenol content (TPC) of the Autumn rose extract are the lowest among all the tested extracts. This could be explained by the fact that the antioxidant capacity of rose petals may be related to the total phenolic content or the number of pigments present in the petals, which work as a free radical scavenger [18]. Therefore, based on the antioxidant results, measuring the total polyphenol content of flavonoids is a necessary and useful predictor of antioxidant capacity [19].

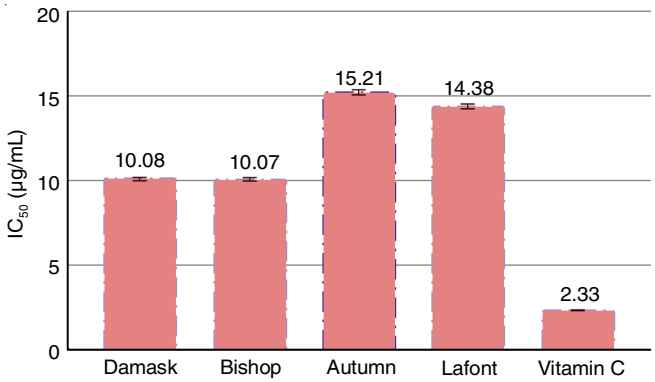


Fig. 2. Inhibition of DPPH oxidation of four different types of roses

On the other hand, the extract from Lafont rose can inhibit free radicals (IC_{50} = 14.38 ± 1.9 µg/mL) which is nearly 1.6 times lower than that of Damask rose (IC_{50} = 10.08 ± 1.2 µg/mL), despite a higher total polyphenol content than Damask rose. This result was similar to that of Belščak *et al.* [20], where the samples with the low phenolic content might show high antioxidant activity. This research showed that some methanolic soluble compounds (methylxanthine) or some pigment in fruits may react with DPPH radicals and affect the result of the DPPH assay. As a result, their antioxidant capabilities could not be predicted entirely based on their phenolic concentration [21].

Moreover, the antioxidant capacity of Damask and Bishop rose petals was significantly higher than that of other flowers, as investigated in previous studies [22]. In particular, the antioxidant activity of ethanol extract from red rose flowers (*Rosa damascena* Mill.) (IC_{50} = 15.49 µg/mL) [22] was 1.5 times lower than that of Damask and Bishop extracts. In addition, some colourful flowers including *B. kockiana* flowers (IC_{50} = 27.5 ± 5 µg/mL); *C. surattensis* petals (IC_{50} = 96.0 ± 16.0 µg/mL) also had a lower antioxidant capacity than all of the rose species that are investigated in this study [23].

Enzyme inhibitory capacity

Tyrosinase inhibition: Tyrosinase plays a crucial role in the biosynthesis of melanin, which is responsible for pigments of the skin, eyes, hair and coloured parts of the eyes in mammals [23]. Melanin is important for the prevention of UV damage to the skin. However, when the accumulation of melanin is excessive, skin hyperpigmentation disorders, including melasma and malignant melanomas, are formed. Therefore, tyrosinase inhibitors such as hydroquinone and kojic acid are used against skin disorders [24]. This study evaluated the ethanol extract of roses during the oxidation of dihydroxyphenylalanine (L-dopa).

Table-2 showed that the inhibitory activity (IC_{50}) against the tyrosinase enzyme was in the range of 1006.21-1756.71 µg/mL. Specifically, three of four tested rose species, Damask rose, Autumn rose and Lafont rose, showed an insignificant inhibition of tyrosinase activity at a concentration of 1000 µg/mL with I% = 41.8 ± 1.2; 27.4 ± 0.81; 3 ± 0.56 %, respectively. The highest inhibition activity (IC_{50}) was found to be 1006.21 ± 4.8 µg/mL and belonged to Bishop extract. It contains a diverse range of secondary metabolic products, including phenolics, whose antioxidant capacity and phenolic compound composition differs widely. Therefore, tyrosinase inhibition may be related to antioxidant activity [25]. However, this activity of rose was much lower than that of other positive controls and other medicinal plant extracts *e.g.* the tyrosinase inhibition activity of kojic acid (IC_{50} = 6.66 µg/mL) and arbutin (IC_{50} = 42.0 ± 0.8 µg/mL) [26]. Some previous studies [26] on flavonoids showed quercetin is a good tyrosinase inhibitor, while kaempferol was a weaker inhibitor *e.g.* in *H. laricifolia* Juss. extract has the only active compound (quercetin) for tyrosinase inhibition and then this extract showed strong activity against tyrosinase (IC_{50} = 122.1 ± 4.1 µg/mL). In contrast, total kaempferol content in rose extract was much higher than total quercetin content (accounting for 75.03-99.64 % of total flavonoids) [27].

TABLE-2
ENZYME INHIBITORY CAPACITY OF FOUR ROSE SPECIES OF VIETNAM

Rose species	Tyrosinase inhibition, IC_{50} (µg/mL)	Elastase inhibition, IC_{50} (µg/mL)
Damask rose	1351.20	261.42
Bishop rose	1006.21	499.16
Autumn rose	1579.22	326.80
Lafont rose	1756.71	617.69
Kojic acid	6.66	–
Ursolic acid	–	115.98

Therefore, the rose extract does not seem to have tyrosinase enzyme inhibitory activity.

Elastase inhibition: Elastin is an elastic protein found in connective tissues in the skin. This protein helps maintain tissue's shape. Exposure to sunlight increases the expression of elastase that hydrolyzes elastin fibers [28]. This can lead to decreased skin elasticity, causing wrinkles and sagging [29]. On this basis, inhibiting the enzyme elastase can prevent wrinkles and maintain skin elasticity. Based on the results of Table-3, Damask rose extract showed the highest inhibitory activity with IC_{50} = 261.42 ± 0.8 µg/mL, followed by autumn rose (IC_{50} = 326.80 ± 1.2 µg/mL). Damask rose extract has the good antioxidant capacity and also an outstanding ability to inhibit the enzyme elastase, about 2 times lower than that of standard ursolic acid (IC_{50} = 115.98 ± 1.2 µg/mL) and 0.8 times higher than that of autumn rose extract.

TABLE-3
BIOACTIVITY OF FRESH AND DRIED ROSE OF VIETNAM

Rose species	TPC (mg GAE/g extract)	DPPH assay, IC_{50} (µg/mL)	Tyrosinase inhibition, IC_{50} (µg/mL)	Elastase inhibition, IC_{50} (µg/mL)
Fresh rose	182.5	8.85	1092.74	590.36
Dried rose	149.5	10.08	1351.20	261.42

The results also showed that extracts of Lafont rose had insignificant elastase inhibitory activity IC_{50} = 617.69 ± 3.4 µg/mL. Meanwhile, the total polyphenol content and antioxidant capacity of Lafont rose petals were much higher than that of other extracts but the elastase inhibition was still insignificant. A study conducted by Pientaweeratch *et al.* [30] showed that there was not any correlation between the TPC and antiaging properties. Similarly, the lavender and witch hazel leaves extracts had high antioxidant capacity but undetectable or low elastase inhibitory assay [31].

All these results show that Damask rose extract showed significant inhibitory effects on elastase activity, at least *in vitro*. Besides, Damask rose was also proved to be a potential extract with a relatively high total polyphenol content (TPC = 149.49 ± 1.7 mg GAE/g extract) and good extraction efficiency (31.06%), especially with outstanding antioxidant activity with IC_{50} = 10.08 ± 1.2 µg/mL. As a result, it can be used in cosmetics as a plant-based material that benefits from the UV irradiation.

Comparision of bioactivities between fresh and dried Damask petals: The drying process is one of the most important steps before extraction. The dried materials are easy to store and transfer. However, the drying process operates under high temperatures for a long time, which causes the odour of the material to evaporate easily and some active compounds can be denatured. Therefore, the fresh material was extracted in the following part to compare with the dried Damask rose.

Table-3 shows that the total polyphenol content (TPC) in dried Damask rose petals was lower than that in fresh rose petals (182.5 ± 2.12 mg GAE/g extract). Youssef & Mokhtar [32] studied the effect of drying methods on the total phenolic content. It was observed that the rose extracts of dried petals always showed a lower concentration of total phenolics than

those from fresh petals. A study conducted by Zhang *et al*. [33] on bitter melon leaf (*Momordica charantia*) showed that the loss of total phenolic content during drying of extract from freeze-dried leaves was 4.3-9.7%, compared to the fresh materials. Based on the research of Mrad *et al*. [34], a decrease in total polyphenol content can be attributed due to the binding of polyphenols with other compounds (proteins), which cannot be extracted or determined by Folin & Ciocalteu's phenol assay.

Moreover, fresh Damask rose extract had the highest antioxidant activity IC_{50} with 8.85 ± 1.1 µg/mL, just only 3.8 times lower than the vitamin C positive control (IC_{50} = 2,328 µg/mL) but marginally higher than that of dried Damask rose extract (IC_{50} = 10,08 ± 1.2 µg/mL). Dorozko & Kunkulberga [35] showed that the scavenging activity of DPPH radicals for an extract from a fresh petals sample was higher than in dried samples.

The tyrosinase inhibitory activity of the fresh rose (IC_{50} = 1092.74 ± 5.8 µg/mL) had greater than that of the dried one (1358.2 ± 6.4 µg/mL). A study about lavender species conducted by Hsu & Chang [36] also showed that drying (including oven-drying and freeze drying) destroyed the lavender's inhibitory activities. The water extract of freshly prepared lavender showed the strongest tyrosinase inhibition. In addition, it was indicated that the inhibitory compounds were unstable under drying conditions [36]. Moreover, some studies showed a moderate correlation between tyrosinase inhibitory activity and their phenolic contents [37]. Tyrosinase inhibitory activity might depend on the hydroxyl groups. Because that could form a hydrogen bond to the enzyme's active site, the antioxidant activity may be one of the important mechanisms responsible for tyrosinase inhibition [38].

Following Table-3, the elastase inhibitory capacity of extract from fresh material (with IC_{50} = 590.36 ± 5.48 µg/mL) was about 2 times lower than that of extract from dried petals similar to the result of Lafont rose. Therefore, it showed that the total polyphenol content and the antioxidant capacity are for reference only and the ability to inhibit elastase might depend on the inhibitors' concentration. Mota *et al*. [38] reported the bioactivities of elderberries (*Sambucus nigra* L.) which showed that fresh and dried berries had different percentages of elastase under the same extraction conditions inhibition. Specifically, the fresh berries gave lower elastase inhibition (I% = 17.7 ± 0.3%) than the dried one (I% = 31.6 ± 1.4%). Apparently, the drying process before extraction may increase of inhibitory compounds for enzyme elastase [38].

As shown above, the ability to inhibit the enzyme tyrosinase might be related to antioxidant capacity [38] and the inhibition of elastase depended on the inhibitor concentration. In this case, the inhibitory effects of roses on tyrosinase activity depended not only on species but also on the preparation methods. In particular, drying processes affect the phenolic content, antioxidant and enzyme inhibitory activity in Damask rose petals differently. The dried petals were chosen to be a good material for extracting and developing outstanding bioactivities, especially the elastase inhibitory activity. In addition, to utilize natural herb materials, the drying procedure is a convenient way to store and deliver the materials before further processing [36].

Conclusion

Among the four common roses (Damask, Bishop, Autumn and Lafont) in Vietnam, Damask petals have the best enzyme inhibitory activity and good antioxidant activity. However, the total polyphenol content is not as high as Lafont roses. Therefore, Damask rose has the potential to be used in cosmetics with antiaging effects. It will be a potential product in the skin care industry, particularly antiaging.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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