Activity of Rose Flower Extract and Resepthakulum as Antioxidant and Anti-tyrosinase

Lobianna Nadeak, Ermi Girsang, Chrismis Novalinda Ginting, and Linda Chiuman
University of Prima Indonesia

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Abstract: Rose (rosa canina) is one of the flowers that are much in demand by the community because besides being an ornamental flower it can also be used as a cosmetic base material. Rose also contains a variety of substances that are treated as antioxidants, and free radical scavenging. The antioxidant activity test in this study used the parameters of diphenyl picrylhydrazin (DPPH) trapping activity and tyrosinase enzyme inhibition. Rose petals and base of rose contain flavonoids, phenols, tannins, triterpenoids, and alkaloids. At the same concentration of sample between the petals and the base of the rose produces different DPPH trapping activities, where the DPPH trapping activity on the rose petals was stronger than the base of the rose. IC_{50} value of rose petals <50 µg / ml while the base of rose > 50 µg / ml. It was seen that the effect of increasing tyrosinase inhibition was due to an increase in the concentration of the group extract and base of rose flowers, and the results were that at a concentration of 100 µg / ml there was a peak anti-tyrosinase activity.

1 INTRODUCTION

The characteristics of decreased beauty due to aging of the skin in humans are rough, dull skin surface, the appearance of brown spots, the appearance of skin wrinkles, weak skin flexibility which must occur in all human beings (Widowati, et.al., 2016). Excessive exposure to ultraviolet (UV) light increases the contribution of free radicals known as Reactive Oxygen Specices (ROS). This certainly affects the process of melanogenesis in the skin catalyzed by the enzyme tyrosinase. This enzyme regulates skin pigmentation through melamine synthesis. Increased UV radiation will increase melanin synthesis, which causes the risk of pigmentation or melanoma disorders (Lai, Wichers, Soler-Lopez & Dijkstra, 2018).

Bioactive compounds that exist in plants such as isoflavones, anthocyanins, and katesin have strong antioxidant activity against free radicals called neutralizing excessive Reactive Oxygen Species (ROS). The body has enzymatic and non-enzymatic antioxidant defense systems. The antioxidant enzymes are superoxide dismutase, catalase, and glutathione peroxidase. While non-enzymatic antioxidants are glutathione, tocopherol (Vitamin E), Vitamin C, b-carotein and selenium (Shalaby and Shanab, 2013; Ismail et al, 2020). Lately antioxidants have become something of interest in the medical world, known to have an effect on preventing premature aging (anti-aging) against free radicals (Garg, Khurana & Garg, 2017).

Rose plant (Rosa canina) contains vitamins (B, P, PP, E, K, and C), flavonoids, carotene, carbohydrates, and organic acids which most of these substances have properties as anti-oxidant, anti-inflammatory, free radical scavenging inhibits the oxidation process (Masek, Latos, Chrzescijanska & Zaborski, 2017). Flavonoid acid as an antioxidant and anti-tyrosinase is very beneficial against skin pigmentation (Zuo, et.al., 2018). Tyrosinase or polyphenol oxidase is an oxidireductase that plays a role in melanin biosynthesis and is the main pigment in hair, eyes and skin. The reaction of the tyrosinase enzyme with the L-DOPA substrate can produce an orange color. Inhibition of the activity of the tyrosinase enzyme is characterized by a reduction in the orange color that is formed or the result of a color reaction becoming more clear while simultaneously marking the occurrence of antioxidant activity (Fais, et al., 2009).

The tyrosinase enzyme is an enzyme responsible for skin darkening or melanogenesis. Tyrosinase in humans is a complex protein and is precisely folded, expressed and undergone post-translational modifications including Heavy glycolysation

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(Zolghadri, et al., 2019). Diphenyl picrylhydrazin (DPPH) compound is a free radical that is stable in aqueous or methanol solution and has a purple color (indicated by the absorption band in the methanol solvent at a wavelength of 515-520 nm). The DPPH has properties that are sensitive to light, oxygen, and pH, but are stable in the form of radicals so that it is possible to measure an accurate antioxidant activity. The antioxidant compounds will release hydrogen atoms to form radical antioxidant compounds. The DPPH which is a free radical that reacted with antioxidant compounds to form non-radical DPPH (Widowati, et.al., 2016). Mini rose showed the greater antioxidant activity in the ferric reducing antioxidant power (FRAP) and DPPH tests before digestion in vitro and together with cosmos as a source of phenolics with good antioxidant activity (de Morais, et.al., 2020). Dry rose tea can be used as a functional food to be a source of natural antioxidants (Kart & Çağındı, 2017).

Previous studies have shown that phytochemical compounds contained in Rosa Damascena flower petals consist of alkaloids, flavonoids, tannins, carbohydrates, and amino acids (Tatke, Satyapal, Mahajan & Naharwar, 2015). It has also been found that rose petal extract contains high anthocyanins, flavonoids, polyphenols (Lee, et.al., 2018). Unlike the results of the phytochemical tests in this study, where the rose petals also contain triterpenoids and terpenoids.

Measuring the effectiveness of a compound in biological or biochemical functions capable of inhibiting the oxidation process by 50% (IC_{50}) was classified in several groups including <50 µg µg per milliliter (very strong); 50-100 µg µg per milliliter (strong); 101-150 µg µg per milliliter (moderate); > 150 µg µg per milliliter (weak) (Budaraga, Marlida & Bulanin, 2016). The IC_{50} value of extracted by DPPH method on black soybean and daidzein was 116.52 µg / mL and 109.34 µg / mL, repectively, which means it has moderate antioxidant strength (Kuswanto, 2018). Ethanol extract of cocoa beans and kojat acid can be used as inhibitors of enzymaticrosinase (Kurniasari, Djajadisastra & Anwar, 2018). Oil obtained from Nigela Sativa seeds (known as black cumin) is also often used as an anti-oxidant and anti-inflammatory (Bordoni, et.al., 2019). Manga waste (skin, seed coat, seeds) from the Colombian manga cultivar is a source of phenolic compounds that can be used as antioxidants and free radical cleaners (Castro-Vargas et al., 2019).

The antioxidant activity test of Rosa damascena rose petal extract was using standard ascorbic acid (iron reducing power test) and showed the highest antioxidant in the cream formula (Safia, et.al., 2019). The results show that rose extract has good potential for cosmetic development. Rose oil has the strongest antioxidant effect and a mixture of Rose oil, bergamot and patchouli (RBP) with a volume ratio of 7: 2: 1 produces the strongest antioxidant effect on the DPPH and ATBS Test [2,2-azinobis (3-ethylbenzothiazoline-6 sulphonic acid)] (Wongsukkasem, e.al., 2018). In that study also found that rose oil and bergamot have an antityrosinase activity around 28 ± 14.2% and 21 ± 10.7%. However, the study did not specifically explain whether there were differences in antioxidant concentrations between the base and rose petals.

2 METHOD

This research is an experimental laboratory study with data collection (random sampling) using samples of rose extract and receptacle. The study was conducted from July-September 2019 at the Biomolecular Laboratory and Biomedical Research Center (Aretha Medika Utama). The sample used was a rose obtained from the Source Seed Management Unit, Research Institute for Miscellaneous Plant Flowers in Malang and has gone through a process of determination. Rose was washed and dried in the sun to dry milled and extracted by maceration technique using 70% ethanol solvent for 3 days at room temperature. Next, the marinade was filtered to separate the filtrate and the residue. The obtained filtrate was evaporated with a motor unit that rotates the evaporation flask in 50 celcius degrees, so that a solid extract was received.

Rose petals has 1400 g net weight, simplicia powder of rose petals was 250 grams and the basic wet weight of rose flower was 700 g processed into simplicia powder, base of rose flowers about 90 grams each dissolved in 70% ethanol for 3 days by maceration method, so the extract of rose petal about 88.56 and 2.72 grams of rose base extract.

The tools and materials used in the phytochemical, DPPH trapping, and tyrosinase tests, respectively, are given in Table 1. Phytochemical tests are used to identify phenol compounds, steroids / triterpenoids, saponins, tannins, terpenoids, flavonoids, and alkaloids found in the lids, and bases roses.

Identification of phenol was carried out by dissolving extracts of petals and rose base 10 mg in ddH2O about 5 ml then adding 500% $FeCl_3$ solution about 500 µl. It is known that the sample solution contains phenol group compounds if one of the

primary colors is red or blue or the secondary colors are green, purple, or black.

The process of identifying steroids / triterpenoids was done by dissolving 10 mg into a drip plate, adding glacial acetic acid until submerged for 10-15 minutes, then adding one drop of concentrated H_2SO_4. If the solution produces blue green, this portion of the population contents the more chemical elements of the steroid class. Whereas if the solusion produces purple / red / orange color, this portion of the population contents the more chemical elements of the triterpenoid group.

Table 1: Materials and tools.

Materials	Tools
1. Phytochemical Test	
Rose Petal Extract; Rose base extract; FeCl3 (1% in ddH2O) (Merck,103943); Aquades (ddH2O); HCl 2 N (Merck 1090631000); HCl 1 N (Merck 109057); H2SO4 pekat (Merck 1007310510); Amil alcohol (Merck 1009751000); Vanilin (Sigma-Aldrich, W310727); Mg/Zn powder (Merck 1058151000, 1087560250); Dragendorff reagents (potassium iodide Merck 207969; and bismuth nitrate Merck 248592).	Test tube; Spatula; Mikropipet (1-10 µl, 50- 200 µl, 100-1000 µl) (Eppendorf); Tips 10, 200, 1000µl (NEPTUNE); Waterbath (Hanyang); and Drip plate..
2. DPPH Trapping Test	
Rose Petal Extract; Rose base extract; 2,2 Diphenyl-1-picrylhydrazyl (DPPH) (Sigma D9132); Methanol absolute (Merck 1060092500); DMSO (Merck 1029522500); and Akuades (ddH2O).	pH meter (OHAUS Starter300 portable); Erlenmeyer Tube; Spatula; Magnetic stirrer and hot plate (Thermo Fisher Scientific); Multiskan Go Reader (Thermo Fisher Scientific, 1510); Incubator (ESCO, IFA-32-8); Micropipette (1-10 µl, 50- 200 µl, 100-1000 µl) (Eppendorf); 96 well-plate (Costar, 3596); Falcon tube 15 ml (SPL, 50015); Falcon tube 50 ml (SPL, 50050); Analytical Balance (AXIS); Tube Effendorf 1,5 ml (SPL, 60015-1); Vortex (WiseMix, VM-10); and Tips (1-10 µl, 50- 200 µl, 100-1000 µl) (Borusil).
3. Tyrosinase Test	
Rose Petal Extract; Rose base extract; Potasium dihydrogen phosphate (Merck 104873); Dipotasium hydrogen phosphate(Merck 105104); Tyrosinase from Mushroom (Sigma T3824); L-DOPA (3,4-Dihydroxy-L-phenylalanine) (Sigma D9628); Potasium Hydroxyl (Sigma P5958); and Aquades (ddH2O).	pH meter (OHAUS Starter300 portable); Erlenmeyer Tube; Spatula; Magnetic stirrer and hot plate (Thermo Fisher Scientific); Multiskan Go Reader (Thermo Fisher Scientific, 1510); Incubator (ESCO, IFA-32-8); Micropipette (1-10 µl, 50- 200 µl, 100-1000 µl) (Eppendorf); 96 well-plate (Costar, 3596); Falcon tube 15 ml (SPL, 50015); Falcon tube 50 ml (SPL, 50050); Analytical Balance (AXIS); Tube Effendorf 1,5 ml (SPL, 60015-1); Vortex (WiseMix, VM-10); and Tips (1-10 µl, 50- 200 µl, 100-1000 µl) (Borusil).

Identification of saponins in the sample is the presence of foam which is always stable, after going through the process of dissolving 10 mg of petals and rose flower base extract using ddH2O in a heat-resistant glass container, heated to boiling for 5 minutes, filtered, stirred vigorously and the addition of 1 N hydrogen chloride solution. The identification of tannins can be done by entering a 10 mg sample into a heat-resistant glass container containing 2 N hydrochloric acid with a volume of 2ml. Heating is carried out in a water medium which lasts for half an hour. After that, add the type of alcohol pentanol ($C_5H_{11}OH$) with a volume of 500 µl. The pentanol layer will produce a tannin group compound if one of the layers is orange or red.

Terpenoids are identified by entering a 10 mg sample into a drip plate, adding vanillin to taste, adding concentrated H_2SO_4 to one drop and then homogenizing, if it produces a purple color then the sample contains compounds of terpenoid class. Identification of flavonoids was carried out by dissolving a sample of 10 mg of petal extract and rose flower base in a test tube containing 2 N hydrogen chloride. Add sufficient magnesium or zinc, then heat for 5-10 minutes, cool and filter. After that, add pentanol with a volume of 1 ml. Extract samples and rose petals will produce flavonoid class compounds if the sample gives either red or orange color.

Identification of alkaloids by dissolving samples of rose petals and base extracts in 5 ml ddH2O evaporated in a water bath. After producing a precipitate, immediately add 5 milliliters of 2N hydrochloric acid. The resulting solution is divided into 2 test tubes. The first tube is called a comparator by inserting 3 drops of 2N HCl. While the second tube solution is transferred as much as one drop to the drop plate, then give 3 drops of Dragendorff reagent. Identify alkaloids (+) if they form an orange precipitate.

A total of 200 µL DPPH 0.077 mmol in methanol was added with a rose sample extract and 50 µL receptacle each on the microplate. The mixture was incubated at room temperature for 30 minutes to obtain the absorbance value at a wavelength of 517 nm using a microplate reader. For negative control, 250 mL DPPH was used, while for blanks, 250 mL absolute DMSO was used (Widowati, et.al., 2016). The antioxidant activity by DPPH (%) method is:

$$\text{DPPH trapping activities (\%)} = 1 - \frac{\text{absorbance of the sample}}{\text{absorbance control}} \times 100 \quad (1)$$

Zulghadar described that the method of inhibiting tyrosinase enzyme activity was carried out with minor changes (Zolghadri, et.al., 2019). A mixture of aqueous solution consisting of 20 µL samples of rose extract and receptacles (concentration 100 µg / mL; 50 µg / mL; 25 µg / mL; 12.50 µg / mL; 6.25 µg / mL; 3.13 µg / mL), 20 µL of the Tyrosinase from Mushroom enzyme (125 U / mL), and 140 µL of

potassium phosphate buffer (20 mM, pH 6.8) were incubated at room temperature during a quarter of an hour. Besides that, it was also prepared for controls containing only 20 µL enzymes and 160 µL phosphate buffer and blanks which only contained 160 µL phosphate buffer and 20 µL samples. Further, the solution mixture was added as much as 20 µL of L-DOPA substrate (1.5 mM) and re-incubated at room temperature for 10 minutes. Absorbance was measured using a wavelength of 470 nm. The percentage of inhibitory activity was calculated using the following formula:

$$\% \text{ inhibition} = \frac{C-S}{C} \times 100 \quad (2)$$

where C is the enzyme absorbance activity without a sample, and S is the enzyme absorbance activity by the addition of the tested sample.

The obtained data the experiment was processed using the SPSS program with the One-Way ANOVA test and continued by the Post Hoc Test using the Tukey HSD test with a confidence level of 95% (α = 0.05). The DPPH and anti-tyrosinase activity test results were followed by an analysis of the linear regression equation to determine the value of Inhibition Concentration 50 (IC_{50}). Overall, the stages of the rose extraction process can be seen in Figure 1.

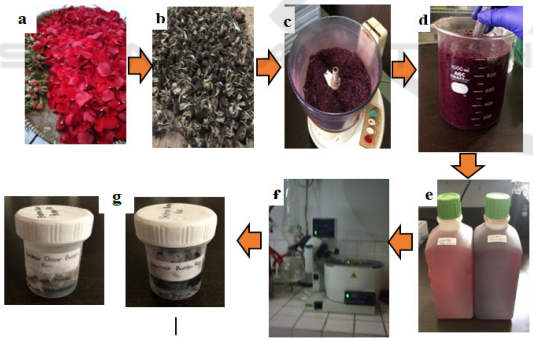

Figure 1: Extraction of the Rose petals and base of roses Procedure: (a) separation, (b) dry separation, (c) grinded, (d) immersed in 70% ethanol, (e) accommodated ethanol, (f) ethanol filtrate evaporation, (g) 70% ethanol extraction yield.

3 RESULTS AND DISCUSSION

It was found that phytochemical screening for rose petals and base of rose contained flavonoids, phenols, tannins, triterpenoids and alkaloids, without saponins. Rose petals contain terpenoids but not for base of the rose. Antioxidant activity by trapping DPPH in rose petal extract was always higher than rose base extract at various concentrations. This result can be seen in Table 2, where the data is presented in the form of an average ± standard deviation. The difference in the percentage of DPPH and anti-tyrosinase trapping activity at each concentration (expressed in µg / ml) indicates that F count> F table with p <0.001 <α = 0.05. The total antioxidant activity of rose petals and base of rose extracts will increase with the increase of theconcentration. At the maximum concentration, rose extract showed the highest antioxidant activity.

Table 2: Statistics of Antioxidant Activity of Rose Petals Extract and Base of Rose (Average, Post Hoc Test Results of Tukey HSD Test).

Final Concentration (µg/ml)	DPPH arrest rate (%)	
	rose petals	base of rose
6.25	44.36±0.92^{a}	33.91±0.37^{a}
12.50	48.17±0.80^{b}	38.00±2.44^{b}
25.00	55.03±0.50^{c}	44.14±0.49^{c}
50.00	62.59±1.50^{d}	50.32±0.42^{d}
100.00	76.76±0.32^{e}	64.80±0.58^{e}
200.00	103.98±0.03^{f}	90.66±0.74^{f}

Anti-tyrosinase activity in base of rose (br) was higher than rose petal (rp) extracts, except at a concentration of 6.25 µg / ml. This can be seen in Table 3. The highest anti-tyrosinase activity of roses at a concentration of 100 µg / mL.

Table 3: Statistics of Inhibitory Activity of Tyrosinase Extract of Rose Petals (rp) and Base of Rose(br) (Mean, Post Hoc Test Results of Tukey).

Final Consentration (µg/ml)	Average tyrosinase inhibition (%)	
	rp	br
3.125	20.56±1.59^{a}	20.95±1.69^{a}
6.25	24.59±0.72^{b}	23.95±1.16^{a}
12.5	28.43±2.04^{b}	29.00±2.35^{b}
25	35.03±1.61^{c}	36.40±1.84^{c}
50	44.94±1.40^{d}	45.65±0.75^{d}
100	66.33±0.72^{e}	69.25±1.51^{e}

Antioxidant activity by trapping DPPH in rose petal extract is higher than base of rose extract. This can be seen in Table 4 where the average IC_{50} value of rose petal extract was 14.89 µg / mL; while in the basic extracts of roses, the average IC_{50} value was 52.81 µg / mL. In the anti-tyrosinase (A-TS) activity test on base of rose, it is more effective than rose petals, it can be seen that IC_{50} values are 62.27 µg / mL and 58.66 µg / mL for rose petals and base of rose, reapectively.

Table 4: IC_{50} Value of DPPH Trapping and Anti-tyrosinase Activity from rose petal and base of rose extracts.

Sample	Regression equation		R^2		IC_{50} (µg/ml)		IC_{50} Average (µg/ml)	
	DPPH	A-TS	DPPH	A-TS	DPPH	A-TS	DPPH	A-TS
rp 1	y = 0.2968x + 45.71	y = 0.4427x + 22.529	0,9933	0.9941	14,45	62,05	14.89	62,27
rp 2	y = 0.2987x + 45.605	y = 0.4576x + 21.795	0,9859	0.9759	14,71	61,64		
rp 3	y= 0.3002x + 45.351	y = 0.4596x + 20.998	0,9904	0.9904	15,49	63,10		
br 1	y = 0.283x + 35.845	y = 0.4765x + 22.628	0,9889	0.9848	50,02	57,69		
br 2	y = 0.2809x + 34.876	y = 0.4899x + 21.74	0,9921	0.9804	53,84	57,69		
br 3	y = 0.2875x + 34.318	y = 0.4772x + 20.863	0,9920	0.9946	45,70	61,06	52.81	58,66

4 CONCLUSION

Comparison of antioxidant activity through DPPH trapping based on IC_{50} value of rose petal extract has a value of 14.89 µg / ml and rose base extract has 52.81 µg / ml. This shows that the rose petals are stronger than the base of the rose. The IC_{50} value of the antitirosinase test results on rose petal extract and rose base have antitirosinase activity of 62.27 µg / ml and 58.66 µg / ml, respectively. These results indicate that the base of the rose is more effective than rose petals

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