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RESEARCH ARTICLE

The anthocyanin pigment extract from red rose as antibacterial agent

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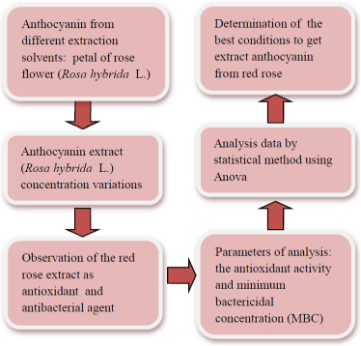
Graphical abstract



Abstract

Red rose consisting of anthocyanin pigment has been used as an antibacterial agent. However, there is no study on the anthocyanin pigment extract from red rose as the antibacterial agent. The effectiveness of the antibacterial agent can be affected by the solvent extraction and the flower shelf life. Here, we report the effects of solvent extraction and red rose (*Rosa hybrida* L.) shelf life on the antibacterial activity. Red rose concentrated extraction and randomized complete block design factorial was carefully used with factors of long display and solvent extraction. The extraction solvent (P) comprised of water, ethanol, and mixture of water-ethanol (1 : 1), while the red rose shelf life (M) consisted of 0, 2, 4 and 6 days. Moreover, pH, antioxidant activity and minimum bactericidal concentration (MBC) with four variations of concentrated concentration (100%, 50%, 25% and 12.5%) on *Escherichia coli*, *Salmonella thypi*, and *Pseudomonas sp.* were analyzed. After two days of shelf life using water as the solvent for extraction, the antioxidant activity achieved 79% at pH of 2.5. It was also demonstrated that it was able to kill all the investigated bacterias, which were *Escherichia coli*, *Salmonella thypi,* and *Pseudomonas sp*. with concentrated concentrations of 100%, 50% and 25%. When the concentrated concentration was 12.5%, the MBC value was constantly found to be 1.39×10^8 cfu/g for *Escherichia coli* and 9.53×10^7 cfu/g for *Salmonella thypi*.

Keywords: anthocyanin pigment, antibacterial, extract, red rose

INTRODUCTION

Red rose (*Rosa hybrida* L.) is one of the flowers that can easily found in Indonesia. In Indonesia rose is mostly used as a decoration, while it has a lot of health functions. Red rose has a higher antioxidant content than vitamins A and C. In higher concentration, it works better to reduce the value of serum glutamic oxaloacetic transaminase (SGOT) on rats administered with carbon tetrachloride (CCl_4) than the vitamins A and C (Saati, 2016).

Red rose was expected to have functioned as an antibacterial agent and therefore, has high potency to be used for natural preservation of food. Red rose contains flavonoid, organic acids, and antioxidants, which can prevent the growth of bacteria that make the food spoiled. Compared to other anthocyanin sources, such as grape, canna flower, spinach, dragon fruit, and purple cabbage, red rose contains the highest content of anthocyanin, which is 1.03 mg/mL with 1.14% yield (Saati *et al*., 2013).

The effectiveness of red rose as an antibacterial agent can be affected by extraction solvents and flower shelf life. It has been reported that polar extraction solvents such as methanol and ethanol are the best extraction solvent to extract anthocyanin (Budiarto in Wachid, 2004; Gokturk *et al*., 2004). However, the alcoholic solvent can leave residue through the process and may affect halal point on the food product. Other than alcoholic solvents, water is a non-alcoholic polar solvent that can be used to extract the red rose. Beside extraction solvent, the effectiveness of rose as the antibacterial substance can be affected by its shelf life. The content of anthocyanin in the red rose could vary with the shelf lifetime. Therefore, this study focuses on the effects of extraction solvent and the shelf life of the red rose on the antioxidant activity of several bacteria, such as *Escherichia coli*, *Salmonella thypi*, and *Pseudomonas sp.*

EXPERIMENTAL

Materials

The materials used in this study were a local red rose, *Escherichia coli, Salmonella typhi, Pseudomonas sp*., water, citric acid, ethanol 70%, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), methanol *p.a*, ethanol *p.a.*, sodium carbonate (Na_2CO_3) 2% in sodium hydroxide (NaOH) 0.1 N, copper(II) sulfate ($CuSO_4$) 0.5% in potassium sodium tartrate 1%, folin, broth media, agar media, and plate count agar (PCA).

Red rose concentrate extraction

Randomized complete block design (RCBD) factorial with two factors were used for this study, which were extraction solvents (P) with 3 levels (water, ethanol, 1:1 water-ethanol) and red rose shelf life (M) with 4 levels (0, 2, 4, and 6 days of shelf life). Red rose concentrate extraction began with separating the rose petals and weight petals every 45 gram for a treatment. The petals were crushed by a blender, which was then added into a 300 mL solvent and 1% citric acid, then leave it for 24 hours at room temperature. After 24 hours, the red rose extract was filtered by a filter paper. The filtrate was poured into a glass container and was then evaporated by a rotary evaporator at 50-60 ^oC to get the red rose concentrate.

Minimum bactericidal concentration analysis

Minimum bactericidal concentration (MBC) analysis was done to all red rose concentrate treatment with four concentrations, which were 100%, 50%, 25%, and 12.5%. *Pseudomonas sp., Salmonella thypi,* and *Escherichia coli* were selected as the bacterias used in the analysis. The bacterias were cultivated on a Mueller Hinton agar (MHA) media at 37 ^oC for 24 hours. The bacterias were diluted with sterile water until equivalent to the McFarland 0.5 standard (108

CFU/mL). The bacterias were then diluted again on until achieved 106 CFU/mL. The red rose extract was applied on the bacterias in the test tube and they were incubated at 37 ºC. After 24 hours, the crude test tubes containing the bacterias were inoculated and incubated in a petri dish at 37ºC for 18-24 hours, and then the bacteria colony in each test tube was counted.

Antioxidant activity analysis

The 2,2-diphenyl-2-picrylhydrazyl (DPPH) was used as the radical to determine the red rose antioxidant activity. A certain amount of red rose concentrate (1 mL) was diluted in 9 mL 96% ethanol *p.a*. 4 mL. The solution was then added to 1 mL DPPH in methanol 0.20 M. After 10 minutes, the sample absorbance was analyzed by using a UV-Vis spectrophotometer, monitored at a wavelength of 517 nm. The antioxidant activity was then determined.

Statistical analysis

All data were analyzed by a statistical analysis (ANOVA). The data that showed influences in the statistical analysis were analyzed by using the Duncan's Multiple Range Test (DMRT) 5%. De garmo analysis was also used to determine the best treatment for the study.

RESULTS AND DISCUSSION

Pigment variety

The pigment that is usually found in the red rose is anthocyanin. The chemical structure of the anthocyanin is shown in Fig. 1. The color of anthocyanin under the acid condition is red, while at the base condition it changes to purple and blue (Samber, 2013).

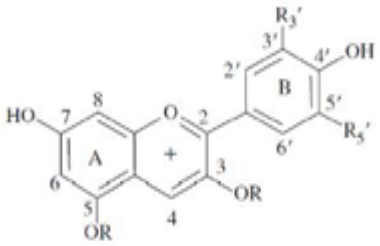


Fig. 1 Anthocyanin base structure (Samber, 2013).

Anthocyanin is the most common flavonoid in plants as a naturally occurring pigment responsible for most red colors in flowers, fruits, and vegetables (Blando *et al*., 2017). Anthocyanin compounds that are usually found are pelargonidin, cyanidin, peonidin, malvidin, petunidin, and delphinidin. The pigment characteristics of red rose cultivated in Batu, Indonesia are cyaniding glycoside (47%), malvidin glycoside (32%), and pelargonidin glycoside (18%), which could be analyzed by visible light spectroscopy at the range wavelength of 513-518 nm.

pH content of red rose concentrate

The extraction solvent and red rose shelf life affected the pH of the concentrate, as can be seen in Table 1. The highest concentrate pH, which was 2.85 was observed when the concentrate was obtained by using ethanol as the extraction solvent and 6 days flower shelf life. On the other hand, the lowest pH of 2.42 was observed on the concentrate where a mixture of water-ethanol (1:1) was used as the solvent and the red rose was fresh. The low pH was caused by the extraction process which produced some organic acids. Moreover, the citric acid was also added during the process to lower the pH.

Both water and ethanol can extract the active substances from red rose because of their similar characteristics. The good solvent for extraction process shall have high solubility towards the active substances, considering that the polar solvents dissolve polar solutes and non-polar solvents dissolve non-polar solutes (Vogel in Winarsih, 2005). The concentrate pH content increased as the red rose flower shelf life increased due to the increase in the glucose content (Saati, 2015).

Table 1 Values of red rose concentrate pH with extraction solvents and flower shelf life treatment.

Treatment		pH
Extraction Solvent	Red Rose Shelf Life (days)	
Water	0	2.49 ab
Water	2	2.50 ab
Water	4	2.51 ab
Water	6	2.83 d
Ethanol	0	2.52 b
Ethanol	2	2.60 bc
Ethanol	4	2.66 c
Ethanol	6	2.85 d
Water-Ethanol	0	2.42 a
Water-Ethanol	2	2.47 ab
Water- Ethanol	4	2.43 a
Water-Ethanol	6	2.84 d

Note: The same letter on the same column indicated the insignificant result according to DMRT 5%.

Antioxidant activity of red rose concentrate

The extraction solvent and red rose shelf life affected the antioxidant activity of the concentrate, as can be seen in Table 2. The highest antioxidant activity (81.57%) was obtained on the concentrate with water as the extraction solvent and fresh flower, while the lowest antioxidant activity (60.62%) was on the concentrate with ethanol as the solvent and 6 days flower shelf life. The concentrate extracted by water as the solvent has the highest antioxidant activity because water could extract antioxidant better than the ethanol; one of it was anthocyanin which is flavonoid that dissolves in water and a water-soluble vacuolar pigment (Raghvendra *et al*., 2011). It has been also reported that the anthocyanin in the red rose could be extracted with water (citric acid or lactic acid 1%) (Saati, 2016). Using the C_{18} Sephadex-G 25 column fractionation and HCl : H_2O (3:97) as the mobile phase, the isolate was confirmed to contain cyanidin, malvidin, and pelargonidin-glycoside.

Table 2 Values of red rose concentrate antioxidant activity with extraction solvents and flower shelf life treatment.

Treatment		Antioxidant Activity (%)
Extraction Solvent	Red Rose Shelf Life (days)	
Water	0	81.57 c
Water	2	79.17 bc
Water	4	63.82 a
Water	6	77.44 bc
Ethanol	0	75.14 bc
Ethanol	2	76.54 bc
Ethanol	4	73.14 b
Ethanol	6	60.62 a
Water + Ethanol	0	77.84 bc
Water + Ethanol	2	67.72 ab
Water + Ethanol	4	64.27 a
Water + Ethanol	6	63.67 a

Note: The same letter on the same column indicated insignificant result according to DMRT 5%.

The antioxidant activity decreased as the red rose shelf life increased because the moisture content in the fresh flower was higher than the one that has been saved for days. The high moisture content caused the fresh flower has higher antioxidant content as has been also reported by other groups (Ningtyas, 2010; Ozkan *et al.*, 2004).

Antibacterial analysis

Escherichia coli, Salmonella typhi, Pseudomonas sp. were used as the bacterias for the antibacterial analysis of the red rose. Four concentrations of red rose concentrate were applied, which were 100%, 50%, 25%, and 12.5%. The MBC analysis results showed that the red rose concentrate was able to kill all *Pseudomonas* sp. bacterial with all concentrations as can be seen in Fig. 2(A). The red rose concentrate was also able to kill all *Salmonella thypi* bacteria with 100% concentrate concentration as shown in Fig. 2(B). Furthermore, the 100% and 50% red rose concentrate were also able to kill all *Escherichia coli* bacteria as displayed in Fig. 2 (C). This result is in good agreement with others where *Rosa damascene* flower extracts, rosella (*Hibiscus sabdariffa* Linn.), and honje (*Etlingera elatior*) could inhibit the bacterial growth (Bohm, 2009; Handarini, 2014; Ningtyas, 2010; Ozkan *et al.*, 2010).

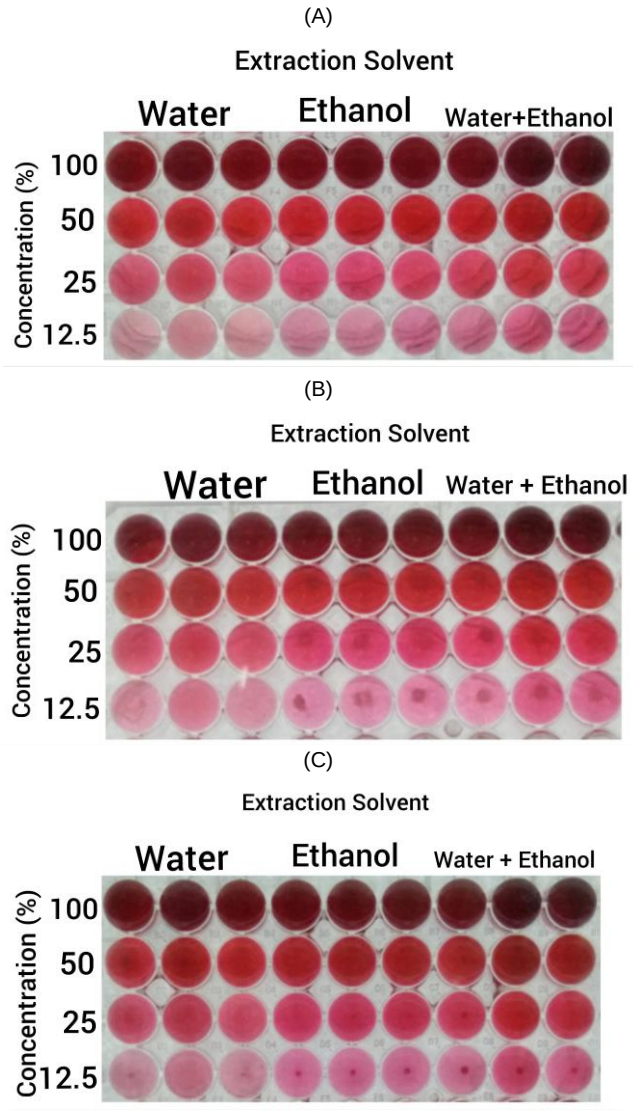


Fig. 2 Result of 100%, 50%, 25%, 12.5% concentration's red rose concentrate MBC analysis on *Pseudomonas* sp. (A), *Salmonella thypi* (B), and *Escherichia coli* (C).

The influence of the extraction solvent and flower shelf life in the *Salmonella thypi* at 50% concentration of red rose concentrate on the MBC values can be seen in Table 3. The mixture of water-ethanol (1:1) and ethanol gave similar high MBC values, which were 1.47×10^8 and 1.46×10^8 cfu/g, respectively. The lowest MBC value was observed on the concentrate extracted by water, which value was 0 cfu/g. On the other hand, the highest MBC value, which was 9.20×10^7 cfu/g, was obtained when the flower was in fresh condition with 0 day shelf life, and the lowest one was obtained on the concentrate after 6 days shelf life, which was 5.5×10^7 cfu/g.

Table 3 MBC values of *Salmonella thypi* in the presence of 50% concentration of red rose extracts with various extraction solvents and durations of red rose shelf life.

Treatment	MBC Value (cfu/g)
Extraction solvent	
Water	0 a
Ethanol	1.46×10^8 b
Water-Ethanol	1.47×10^8 b
Rose shelf life	
0 day	9.20×10^7 b
2 days	8.30×10^7 ab
4 days	6.30×10^7 a
6 days	5.50×10^7 a

Note: The same letter on the same column indicated insignificant result according to DMRT 5%

The MBC values of *Escherichia coli* and *Salmonella thypi* when the 25% concentration of red rose extract was used are shown in Table 4 and Fig. 3. Table 4 shows that concentrate with the highest MBC values of *Escherichia coli* and *Salmonella thypi* were those extracted by using ethanol, where the values were 1.47×10^8 and 1.86×10^8 cfu/g, respectively. On the other hand, using water as the solvent did not give any MBC values for both bacterias.

Table 4 MBC values of *Escherichia coli* and *Salmonella thypi* in the presence of 25% concentration of red rose extracts with various extraction solvents.

Bacteria	Treatment	MBC Value (cfu/g)
Escherichia coli	Water	0 a
	Ethanol	1.35×10^8 b
	Water-Ethanol	1.29×10^8 b
Salmonella thypi	Water	0 a
	Ethanol	1.86×10^8 b
	Water-Ethanol	1.67×10^8 b

Note: The same letter on the same column indicated insignificant result according to DMRT 5%.

Figure 3 shows that the highest MBC value of *Escherichia coli* was obtained on the red rose concentrate with 2 days shelf life, which was 8.04×10^7 cfu/g, and the lowest one was the concentrate with 6 days shelf life, which was 5.23×10^7 cfu/g. In the case of *Salmonella thypi*, the highest MBC value was 1.06×10^8 cfu/g when the concentrate has 0 day shelf life, while the lowest MBC value was 7.23×10^7 cfu/g when the concentrate has 6 days shelf life.

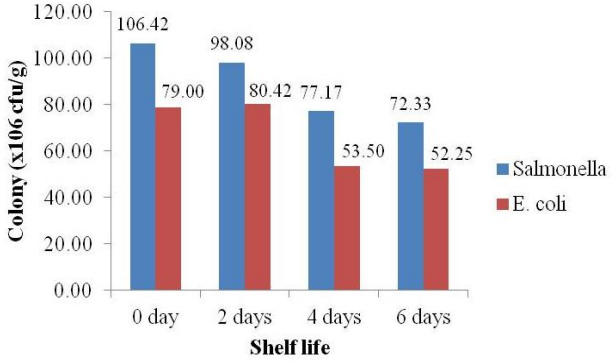


Fig. 3 MBC values of *Escherichia coli* and *Salmonella thypi* in the presence of 25% concentration of red rose extracts with various durations of red rose shelf life.

The influence of the extraction solvent and flower shelf life on the MBC values of *Escherichia coli* and *Salmonella thypi* when using 12.5% concentration of red rose extract can be seen in Table 5. All the results showed different values because each bacteria has different characteristics and its own sensitivity to the antibacterial agent (Kuswanto in Warziki, 2015). Red rose concentrate with the highest concentration gave the smallest MBC value. The smaller MBC value means that there are more bacterias that were killed by the antibacterial agent (Warziki, 2015).

Table 5. MBC values of *Escherichia coli* and *Salmonella thypi* in the presence of 12.5% concentration of red rose extracts with various extraction solvents and durations of flower shelf life.

Treatment		MBC Value (cfu/g)	
Extraction Solvent	Shelf Life (days)	*Escherichia coli*	*Salmonella thypi*
Water	0	1.44×10^8 a	1.14×10^8 b
Water	2	1.39×10^8 a	9.53×10^7 ab
Water	4	1.38×10^8 a	9.47×10^7 ab
Water	6	1.69×10^8 a	7.53×10^7 a
Ethanol	0	2.44×10^8 b	2.71×10^8 f
Ethanol	2	2.19×10^8 ab	1.79×10^8 cd
Ethanol	4	1.28×10^8 a	1.51×10^8 c
Ethanol	6	1.72×10^8 a	1.25×10^8 bc
Water-Ethanol	0	1.99×10^8 ab	2.27×10^8 e
Water-Ethanol	2	1.59×10^8 a	1.88×10^8 d
Water-Ethanol	4	1.34×10^8 a	1.39×10^8 bc
Water-Ethanol	6	1.40×10^8 a	1.28×10^8 bc

Note: The same letter on the same column indicated insignificant result according to DMRT 5%.

CONCLUSION

The effectiveness of red rose concentrate extracted by water as an antibacterial agent was higher than the rose concentrate extracted by ethanol or the mixture of water-ethanol (1:1). It was able to kill all bacterias (*Escherichia coli*, *Salmonella thypi*, *Pseudomonas sp*.) with 100%, 50%, and 25% concentrate concentration. When the concentration of the extract was 12.5%, the MBC values for *Escherichia coli* and *Salmonella thypi* were 1.39 10^8 cfu/g and 9.53 10^7 cfu/g, respectively.

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