ISBN 978-93-86878-21-2
15th PHNOM PENH Int'l Conference on Food, Environmental, Biological & Medical Sciences (PFEBM-18)
Aug. 8-10, 2018 Phnom Penh (Cambodia)

Antioxidant, Antiglycation, and Antiaging Activities of Roselle Petals Extract *(Hibiscus sabdariffa L.)*

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Abstract**:** *Accumulation of radicals may lead to oxidative stresses, which is the main cause of aging. Antioxidant and antiglycation agents can protect the cells from oxidative stresses. An antioxidant source can be obtained from various sources, including plants. In this study we aimed to analyze the potential properties of roselle (Hibiscus sabdariffa. L) petals extract as antioxidant agent both via in vitro and in vivo assays. The roselle petals were extracted with 70% ethanol as solvent. The IC50 value of antiglycation activity of the extract was 527,9 ppm. The antiaging and antioxidant effects of roselle petals extract were analyzed in vivo, by using model organisms Saccaharomyces cerevisiae via spot assay. Following administration of roselle extract at concentration of 150 ppm, 300 ppm, 450 ppm, 600 ppm, 750 ppm, the viability of yeast cells at day-5 was higher than that the untreated group (0 ppm). In addition, yeast was found more tolerant toward H2O2 –induced oxidative stress conditions as treated with the roselle extract (300 ppm), particularly in the stationary phase. To our knowledge, this is the frist study to report the anti-aging and antioxidant properties of roselle petals by using yeast S. cerevisiae as model organism.*

Keywords: *Antioxidant, Antiaging, Antiglycation, Hibiscus sabdariffa. L, yeast*

1. Introduction

Human aging may serve as a main risk factor for diseases [1]. Aging-related diseases, such as Alzheimer, Parkinson, and diabetes, are considered as serious threats for aging population; thus, further efforts are required to deal with the challenges. Some factors associated with aging included genetic, lifestyle, environment, gene mutation, and disruption of immune system, and free radicals. Among these factors, free radicals are the foremost concerns. In addition, advanced glycation end product (AGEs) is also linked with th aging process [2].

Free radicals are molecule that has at least one unpaired electron and are therefore unstable and highly reactive. They are responsible for cell damages, disruption of cellular function, and cell death [3]. Furthermore, AGEs as a result of glycation are also reported as the inducer for aging. They are end product of reaction between amino acids and reducing sugars [4]. In excessive amount of AGEs, they may contribute to diabetes, Alzheimer, and aging. Presence of free radicals could accelerate aglycation process, in which the radicals are also product of aglycation [5]. Free radicals are hazardous components for human body; therefore, there is a need to deal with them. The use of antioxidants is common in reducing activity of free radicals since they enable to neutralize the radical molecules. In this regard, application of antioxidant is expected to inhibit aging process and alleviate cellular damages in human body.

Antioxidants can be available in two types, i.e. synthetic and natural. However, the use of synthetic antioxidants has been linked with some disadvantages as reported in butylated hydroxy toluene (BHT), one of synthetic compounds, that could be poisonous to experimental animals and carcinogenic. Hence, many food and medicine industries shift to use and develop natural antioxidants and search for new natural antioxidant sources [6]. Pratt and Hudson reported that most natural antioxidants are found in plants and often recognized as phenolic compounds present in almost plant parts such as wood, grains, fruits, leaves, roots, flowers, and pollen [7]. Roselle (*Hibiscus sabdariffa L*.) has attracted a great concern on its further use as antioxidant source.

Roselle (*H. sabdariffa L.)* has been reported to exert antioxidative properties. In Indonesia, the petals are harvested for raw material of roselle tea, known as red tea. Roselle contains methanol and polyphenol that can serve as anti-inflammatory agents, contributing to the reduced cell injury [8]. High content of antioxidant in roselle petal could be further use as inhibitor of free radicals. A previous study showed that roselle petal extract could attenuate level of malondialdehyde in rats after treated with waste cooking oil [9], suggesting that the extract demonstrated appreciable results as source of antioxidant.

However, studies on the use of roselle petal extract as antioxidant and antiaging has remained scarce. Previously, a research was performed focusing on antiaging and antioxidant properties of roselle petal extract with use of yeast *S. cerevisiae* as model. This current work aimed to evaluate the potential of rosell petal extract as antioxidant agent through *in vitro* and *in vivo* experiment.

2. Materials & Methods

2.1. Extraction of Roselle Petal

The extraction was performed according to previous procedure [8]. Roselle petal was macerated using ethanol 70% with ratio of 1:5 (sample:solvent). Sample was soaked for 3×24 h while agitated twice a day to ensure solubility. The extract was taken and macerated twice with same solvent volume. The final extract was collected and evaporated using rotary evaporator at <60 °C to produce crude extract of roselle petal.

2.2. Antiglycation Test

The experiment was conducted according to former procedure [2]. In short, solution A containing BSA 20 mg/mL (80 µL) was reacted with glucose 235 mM (40 µL), fructose 235 mM (40 µL) and either 80 µL of sample or positive control (aminoguanidine) in 200 µL of phosphate buffer 0.2 M with pH 7.4. Corrected solution (solution Ao) and distilled water (80 µL) were used to substitute glucose and fructose. Control (solution B) was made consisting of BSA 20 mg/mL (80 µL), glucose 235 mM (40 µL), fructose 235 mM (40 µL), distilled water (80 µL) and added with 200 µl of phosphate buffer (0.2 M, pH 7.4) in a 1.5-ml tube. Solution Bo as corrected solution was made with use of distilled water (80 µL) to replace glucose and fructose. Each solution was incubated for 40 h at 60 °C, and 100 µl of the solution was transferred into microplate well 96. The fluorescence intensity was determined with excitation of 330 nm and emission of 440 nm. The measured absorbance was measured for inhibition and poltted in a linear regression to determine IC50. The % inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \left[1-\frac{(A-A0)}{(B-B0)}\right] \times 100\%$$

Where; A: fluorescence intensity of sample, Ao: fluorescence intensity of sample corrector; B: fluorescence intensity of control; Bo: fluorescence intensity of control corrector.

2.3. Determination of Yeast Aging

Yeast aging after treatment of roselle petal extracts was determined using spot assay according to modified method [10]. The modification was made on culture volume and determination of inoculation time and spotting time. S. cerevisiae BY4741 was cultured in liquid YPD medium for 24 h. Inoculum BY4741 was inoculated in YPD medium at initial OD of 0.1. Each culture (3 ml) consisted of YPD medium added with inoculum BY4741 (control) or added with various levels of roselle petal extracts (150, 300, 450, 600, and 750 ppm). The culture was made duplicate. In control, the culture was added with DMSO as much as maximum volume of the added extract. All cultures were then incubated for 1 and 5 days, followed by culture spotting in day 5. Spotting began with measurement of OD and the culture was adjusted to have OD = 1. Afterwards, serial dilution was made at 10-1, 10-2, 10-3 and 10-4 in microplate well 96, with maximum volume of 200 µl for each well. Each diluted suspension and culture (OD = 1) were spotted and incubated for 3 days in room temperature (25 °C).

2.4. Tolerance Test Against Oxidative Stress

In this experiment, oxidative stress was assessed using spot assay with modified concentrations according to previous method [10]. Diluted suspension (2 μl) of control and treatment groups was spotted in solid YPD medium containing H2O2 at 7, 8, and 9 mM.

3. Results and Discussion

3.1. Roselle Petal Extract

After maceration, the ethanolic extract of roselle petal was obtained, resulting in a brownish red and sticky paste with acid odor. The viscous extract was 198 g, which is equivalent to 39.6% of initial weight. The extractable yield was considered to contain bioactive compounds of the roselle petal based on solvent used. The result showed a higher yield in comparison with previous data, i.e. 33.83% [11]. This difference may result from dissimilar amount of simplisia and types of solvent. Additionally, previous studies also reported that drying temperature and length could also affect the extractable amount of roselle petal [12].

3.2. Antiglycation Activity

The antiglycation activity of roselle petal extract showed IC50 527.887 ppm, suggesting that the extract could exert a lower scavenging activity of AGEs compared to positive control, with IC50 35.992 ppm (Table 1). This can be simply explained that the treatment uses crude extract (not purified), which is associated with its low scavenging activity. Low purity means that other unwanted materials, such as components from solvent, may exist in the extract, thereby producing less powerful activity.

TABLE I: IC50 Vitamin C Solution And Roselle Petal Extract

Sampel	Antiglikasi (IC50) ppm
Aminoguanidin	35,992
Ekstrak rosela	527,887

Based on *in vitro* experiment, roselle petal extract demonstrated a low antiglycation activity in comparison with previous reports. A study reported IC_{50} of antiglycation activity from several rhizomatous herbs such as pink and blue ginger *Curcuma aeruginosa* Roxb. (243.57 ppm), turmeric *Curcuma longa* (221.26 ppm), Java turmeric *Curcuma xanthorrhiza* Roxb. (221.60 ppm), white turmeric *Curcuma zedoaria* (236.38 ppm), cardamom (240.35 ppm), and ginger (207.95 ppm) [13]. Antiglycation activity of extracts isolated from plants was due to presence of high content of polyphenol. This compound enabled to inhibit formation of AGEs through disrupting reaction between monosaccharide and protein [14]. There was a strong correlation between amount of polyphenol and inhibition of protein glycation [15]. Some plausible mechanisms were proposed regarding the inhibitory activity of polyphenol against glycation process, i.e. (i) retarding initial glycation product (fructosamine), (ii) reducing formation of reactive carbonyl groups (dicarbonyl) in fructosamine or glucose, and (iii) alleviating formation of AGEs. Although there is a different result between *in vitro* and *in vivo* experiments, glycation model from *in vitro* test can be used to screen plant sources for antiglycation activity. Furthermore, in order to observe antioxidant and antiaging activity, *in vitro* assay is then performed to evaluate antioxidant activity of roselle petal extracts and their effects on lifespan expansion of yeast *S. cerevisiae* BY4741.

3.3. Yeast Aging

Aging test demonstrated that roselle petal extracts at all studied concentrations (150 ppm, 300 ppm, 450 ppm, 600 ppm, and 750 ppm) showed a remarkable effect on extending the yeast lifespan in comparison with absence of the extracts (0 ppm), as depicted in Figure 1. Lifespan of yeast treated with the extracts was much longer than that treated without extract, as observed in both day 1 and day 5. This suggests that roselle petal extract may

exhibit antiaging activity. Our experiment becomes the first to report antiaging activity of roselle petal extract using yeast as a model. Previous studies reported lifespan expansion of yeast after treatment with several plant sources such as *Cimicifuga racemosa, Valeriana officinalis L., Ginkgo biloba, Passiflora incarnate L., Apium graveolens L., Salix alba* [16].

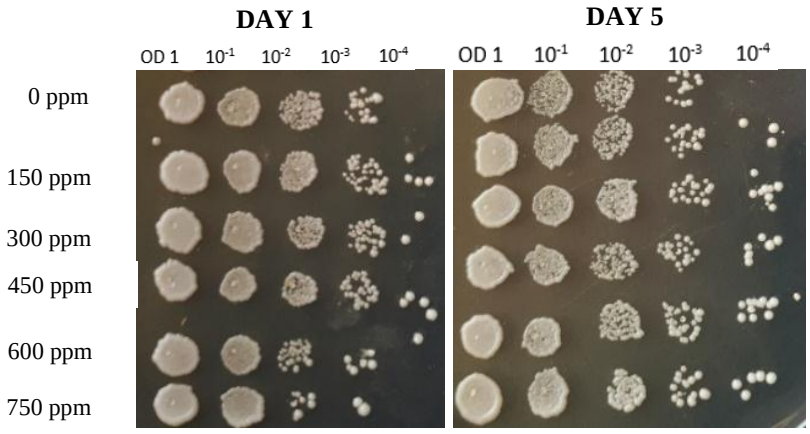
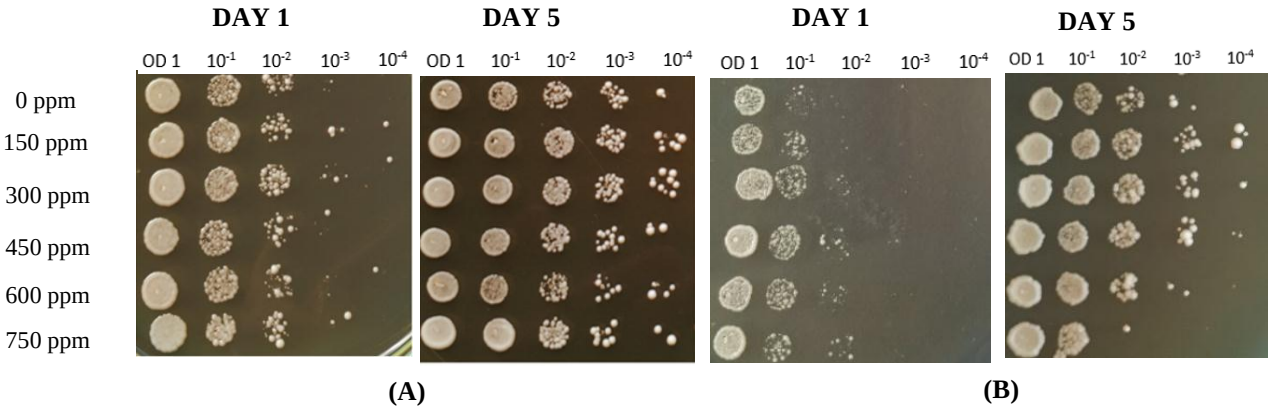
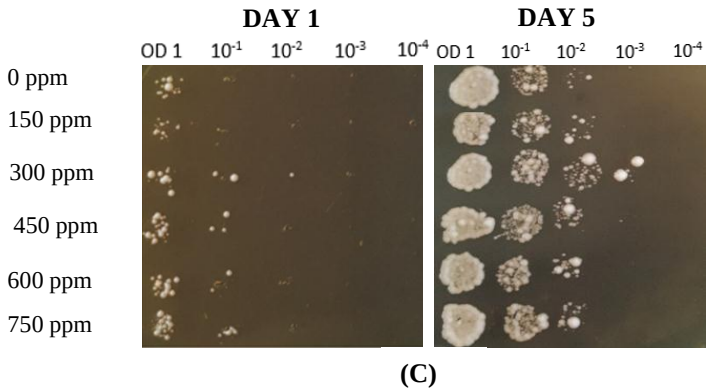


Fig. 1: Influence of roselle petal extracts on lifespan of yeast *S. cerevisiae* BY4741 observed using spot assay.

3.4. Tolerance Test

The results showed that roselle petal extracts could prolong yeast lifespan under stressed condition due to presence of H_2O_2 (Figure 2). Oxidative stress constitutes a key factor of aging process, while oxidative free radicals may damage cellular components such as DNA, protein, carbohydrate and lipid [17]. Therefore, this current work was designed to evaluate parameters associated with possible effects of antioxidant on yeast cells. The results also demonstrated that viability of yeast after treatment exposure was found to be higher than that treated with control treatment (Figure 2). We found that roselle petal extract at 300 ppm was regarded as the most appreciable result in term of its response to various levels of oxidative stress H_2O_2 (7 mM, 8 mM, 9 mM) in day 5. This indicates that antioxidant exerts a pivotal contribution to antiaging properties of roselle petal extract. Previous study reported that *Passiflora incarnate L*., and *Salix alba* could also enhance yeast viability under exposure to oxidative stress H_2O_2 4 mM in 4-days culture [16].





(C)

Fig. 2: Influence of roselle petal extracts on viability of *S. cerevisiae* BY4741 after exposed to H_2O_2 at 7 mM (A), 8 mM (B), and 9 mM (C)

In general, possible mechanisms of antiaging activities in yeast were proposed, including activation of antioxidant-related genes, regulation of genes associated with aging pathways, mitochondrial activities, transduction signals toward nutritional components in medium [10][18]. Based on our data, we stated that activation of antioxidant- and antiaging-related genes in yeasts possibly accounted for antiaging properties of roselle petal extracts. Hence, further studies are needed to uncover regulation of aging pathways on S. cerevisiae after treated with roselle petal extracts.

4. Conclusion

Crude extract of roselle petal could exert antiglycation activity at IC50 527.887 ppm. Antiaging feature of the extract was responsible for the raising viability of the yeasts and the enhancement of their tolerance against oxidative stress H2O2 at various levels (7 mM, 8 mM, 9 mM), in which the concentration of extract at 300 ppm was found to promote the best result.

5. Acknowledgements

This work is funded by Indonesia Endowment Fund for Education (LPDP) scholarship.

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