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Scavenging Capacity and Antibacterial Activity of Roselle Aqueous Extract and Wine Production

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Abstract

Roselle is an herbaceous medicinal plant where being native to Asia. The calyx of roselle action as well as its bioactive compounds and natural pigments. In this work, total phenolics, total flavonoids, scavenging capacity and antibacterial activity of roselle aqueous extract were investigated and applied as a substrate for wine production. Scavenging capacity of roselle aqueous extracts was increased dependently on dose of total phenolic compound in the extract. Roselle wine was produced and evaluated on pH, alcohol (%), total acids (%), total solids (°Brix), total viable counts of bacteria and yeast/mold for 10 days of fermentation. pH and total solids were significantly decreased from 0 day of fermentation, while alcohol, total acids and total viable counts of bacteria and yeast/mold were significantly increased until the end of process. Roselle aqueous extract at the concentration of 0.5 and 1 mg/ml inhibited the growth of *Escherichia coli*, *Salmonella* sp., *Bacillus* sp. and *Staphylococcus aureus* while wine products have no effect on antibacterial activity.

Keywords: Roselle, Roselle wine, Scavenging capacity, Antibacterial activity

1. Introduction

Roselle is the popular name of *Hibiscus sabdariffa* Linn. which belongs to *Malvaceae* family (Alarcon-Alonso et al., 2012). It was cultivated in many area, including Africa and Central America despite being native to Asia (Barhe & Tchouya, 2015). The outer ring of the fruit called calyx is commonly used in beverages and foods such as tea, syrup, jams and jellies (Mahadevan, Shivali & Kamboj, 2009; Borras-Linares et al., 2015). In many countries, the calyx is also applied as a traditional medicine to prevent hypertension, inflammation and liver disorders (Alarcon-Alonso et al., 2012). The aqueous extract of dried roselle flowers containing a high concentration of polyphenol which is prospective for treatment of leukemia and gastric carcinoma, hypolipiddaemia (Hopkins et al., 2013), antihypoglycaemia (Sachdewa, Nigam & Khemani, 2001) and antioxidant (Fernandez et al., 2012). Moreover, several reports are available on the consumption of the dried or fresh calyces, seeds and leaves of roselle in the preparation of beverages, fermented drinks (Da-Costa-Rocha et al., 2014). The non-alcoholic beverages have been made from a hot water extract of Roselle calyx and the product is usually sweetened with sugar and may be flavored with flavorings (Omemu et al., 2006). Other potentials of roselle calyx were the antibacterial action. The aqueous and ethanol extracts can inhibit food spoilage bacteria such as *Salmonella* Typhimurium DT104, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* (Chao & Yin., 2008) and *Klebsiella pneumoniae* (Liu et al., 2005).

Roselle calyx can also be used in wine production in Thailand according to its red variety and due to the very high duty on imported wine. The beneficial properties of wine, mainly polyphenols, are well established (Rosenzweig et al., 2017). Wine polyphenols are derived from the extraction of the skins or seeds of fruits during the maceration process. While the color components are readily extracted into the fermenting wine, and come to equilibrium before the end of fermentation, tannins and other polymeric polyphenols continue to be extracted as long as the skins are in contact with the wine (Sacchi, Bisson & Adams, 2005). Red wines have not been generally produced from tropical fruits because of the low content of extractable red pigments in red varietal grapes (Okoro & Emeka., 2007). Efforts towards producing red wine by adding synthetic red colorants or dyes are usually controlled, as their use and quantities are regulated by law to prevent toxicity in humans (USFDA, 1993). Thus, roselle is a potential red wine raw material according to the report of Ifie et al. (2012) During fermentation, physico-chemical analysis of the wines

indicated decrease in specific gravity, soluble solids, pH and color intensity. Sensory evaluation of the aged roselle wine in terms of color, flavor, taste and overall acceptability showed no significant difference compared to commercial wine samples.

The multifunctional properties (colorant and bioactive properties) of roselle can be explored in the food and pharmaceutical industry, as natural ingredients to be incorporated into the food products (e.g. as a multifunctional ingredient) and pharmaceutical industries (e.g. as a natural colorant and medication for its bioactive properties) (Jabeur et al., 2017). From the application and medicinal values of anthocyanins, polyphenols of roselle can propose the antioxidant and antibacterial action through the wine products. Therefore, the aim of this work is to evaluate the antioxidant activity of aqueous extract of roselle, to estimate the chemical changes in wine production, to determine the antibacterial activity on roselle wine and aqueous extracts, and to prove the effect of roselle as a powerful natural plant.

2. Materials and methods

2.1 Preparation of roselle aqueous extract

Air-dried of roselle calyces were purchased from Muang, Nakhonsithammarat local market. A 50 g edible portion of roselle calyx was chopped and mixed with 100 ml of sterile distilled water at 25ºC for 12 hours, and then homogenized with blender. After filtration through a Whatman No. 1 filter paper, the filtrate was sterilized by passing through a 22-mm pore size and further freeze dried to fine powder (Chao & Yin., 2008). The powder was stored at 4 ºC until used. The substock solution of 10, 20, 30, 40 and 50 mg/ml were prepared by diluting absolute ethanol for total phenolic, total flavonoid and scavenging capacity test and 0.5 and 1 mg/ml of substock solution were prepared for antibacterial activity test.

2.1.1 Determination of total phenolics and total flavonoid contents of roselle aqueous extract

The amounts of total phenolics in roselle aqueous extract at a concentration of 10, 20, 30, 40 and 50 mg/ml were determined with the Folin-Ciocalteu reagent using the modified method of Lister and Wilson (2001). Twenty microliter of each concentration (three replicates), 100 µl of 2 N Folin-Ciocalteu's reagent were added and incubated at room temperature for 5 min. Thirty hundred microliter of sodium carbonate (Na_2CO_3) (25% w/v) was mixed and incubated at 45 ºC for 30 min. The absorbance of all samples were measured at 765 nm using UV–visible spectrophotometer. Aluminum chloride colorimetric method was used for flavonoid determination (Chang et al., 2002). One milliliter of sample (100 µg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. Then it was incubated at room temperature for 30 min and the absorbance of the reaction mixture was measured at 415 nm with UV-visible spectrophotometer.

2.1.2 Scavenging capacity of roselle aqueous extract

ABTS radical-scavenging activity of the extract was determined according to Re et al., (1999) The $ABTS.^+$cation radical was produced by the reaction between 5 ml of 14 mM ABTS solution and 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 h. Before use, this solution were diluted with ethanol to get an absorbance of 0.700±0.020 at 734 nm. The roselle aqueous extract at various concentrations with 1 ml of ABTS solution was homogenized and its absorbance was recorded at 734 nm. Ethanol blanks were run in each assay, and all measurements were done after at least 6 min. Similarly, the reaction mixture of standard group was obtained by mixing 950 µL of $ABTS.^+$ solution and 50 µl of BHT. The inhibition percentage of ABTS radical was calculated using the following formula: ABTS scavenging activity (%) = (A0 – A1)/A0 ×100 where A0 is the absorbance of the control, and A1 is the absorbance of the sample.

2.2 Roselle wine production

Roselle wine was prepared follow by modified method of Ifie et al., (2012). 5 g of roselle powder were dissolved in 1800 ml of distilled water into 2000 ml flask. Sucrose was added to the juice to adjust the soluble solid to 22 ºBrix. 250 ppm of sodium metabisulphite was added to inhibit the growth of bacteria and wild yeast. Dried wine yeast (*Saccharomyces cerevisiae*) was pitched in to the juice at 27±2 ºC (room temperature). Racking was done at room temperature immediately after the evolution of gases terminated; remaining yeast cells were removed from the fermenting to prevent further fermentation. Second racking was done with the introduction of bentonite slurry to aid racking and clarification. The second racking lasted for 10 days. The chemical and microbiological changes during wine production were evaluated on pH, titratable acidity, total solid, total alcohol, total bacteria and total yeast and fungal on 0, 2, 4, 6, 8 and 10 days of production.

pH and titratable acidity were determined by the method of AOAC (2000). Total titratable solid was expressed as percentage citric acid after titrating 10 ml of wine with 0.1 N sodiumhydroxide (NaOH) with phenolphthalein as an indicator. Total soluble

solids was determined using refractometer, while the alcohol percentage was estimated using ebulliometer. Total bacteria, yeast and fungal counts were determined by pour plate method (Harrigan and McCance, 1976). Total viable bacteria counts were carried out using plate count agar (PCA) and total yeast and fungal counts were grown on potato dextrose agar (PDA).

2.3 Antibacterial activity of roselle wine and aqueous extract

Four food spoilage bacteria, *Escherichia coli*, *Bacillus* sp., *Staphylococcus aureus*, and *Salmonella* sp. were supplied by Microbiology Laboratory of Science and Technology Faculty of NSTRU University, Nakhon Si Thammarat, Thailand. The roselle wine and aqueous extracts were subjected by disc diffusion assay (Jorgensen et al., 1999) with minor modifications. Briefly, four bacterial strains were grown in trypticase soy broth (TSB) at 37ºC for 16 h and cells were suspended in TSB to get 10^8 cfu/ml by using McFarland No. 0.5. Each bacterial test strains were swab onto mueller-hinton agar (MHA) medium. Then, 6 mm diameter filter paper discs with 50 µl of various concentrations of crude extracts were placed onto MHA. After incubation at 37ºC for 24 h, the antibacterial activity was measured in the diameter (mm.) of clear zone of growth inhibition.

2.4 Statistical analysis

Total phenolics, total flavonoids, scavenging capacity and antibacterial activity were expressed as mean±SD. Chemical and microbiological analysis during the roselle wine production was carried out using one way analysis of variance (ANOVA) using spss program at $p \leq 0.05$.

3. Results and discussions

3.1 Total phenolics, total flavonoid content and scavenging capacity of roselle aqueous extract

A large and diverse class of compounds, many of which occur naturally in a wide range of food and plants, is polyphenols and flavonoids, the largest and best studied group among polyphenols (Nishaa et al., 2012). A range of plant polyphenols is either being actively developed or currently sold as dietary supplements and/or herbal derived medicines. Although, many of them have properties including antioxidant, anti-mutagenic, anti-carcinogenic and anti-inflammatory effects that might potentially be beneficial in preventing disease and protecting the stability of genome (Furguson, 2001). Total phenolics and total flavonoid contents of roselle aqueous extracts were clearly increased with concentrations (Figure 1). Scavenging activity of roselle aqueous extract is presented in Figure 2. The extract exerted a concentration dependent scavenging and showed a maximum activity of 40.00±2.52 % inhibition at a concentration of 50 mg/ml. Roselle or *H. sabdariffa* revealed the presence of several interesting compounds, such as tocopherols, phenolic acids and flavonoids, including three different anthocyanins, 5-(Hydroxymethyl) furfural was the most abundant non-anthocyanin compound, while delphinidin-3-O-sambubioside was the major anthocyanin (Jabeur et al., 2017).

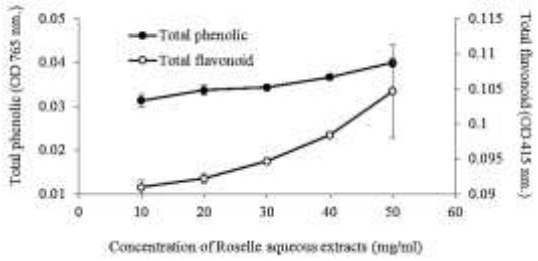

Figure 1. Total phenolics and total flavonoid contents of roselle aqueous extract**.**

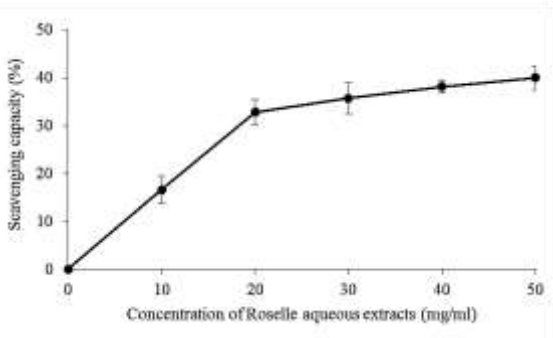

Figure 2. Scavenging capacity of roselle aqueous extract.

3.2 Chemical and microbiological changes during roselle wine production

Table 1 shows the results of chemical and microbiological changes during wine processes. The results displayed the changes in pH during fermentation which led towards the acidic range. The results equally show that the pH significantly decreased from an initial value of 2.74±0.06 on 0 day to pH of 2.54±0.06. Theoretically, sugars are converted to alcohols, then alcohols to aldehydes, aldehydes to ketones, and ketones are finally converted to acids during fermentation (Opara & Rexford, 2012) and the percentage of total acid of roselle wine was increased from 0.46±0.02 to 1.07±0.08 during the fermentation. Total solids were significantly decreased during the fermentation progress. The initial value of 22.5±0.70 ºBrix was obtained on 0 day and 16.35±0.49 ºBrix at the end of the fermentation. The decrease in total solid values

may be attributed to utilization of the sugars for growth and other metabolic activities by the organism. There was a steady increase in percentage of alcohol from an initial value which was not detectable at day 0 to a value of 6.40±0.07% at the 10th day. The increasing alcohol in the wine is due to the conversion of sugar, which has been shown to reduce as fermentation proceeded.

Table 1. Chemical and microbiological changes during the roselle wine production

Days	Chemical changes			
	pH	Alcohol (%)	Total acids (%)	Total solids (°Brix)
0	2.74±0.06	0.00	0.46±0.02	22.5±0.70
2	2.65±0.07*	2.60±0.14*	0.75±0.01*	18.90±0.71*
4	2.64±0.03*	3.55±0.07*	0.81±0.03*	18.40±0.28*
6	2.61±0.01*	4.75±0.07*	0.86±0.06*	17.65±0.77*
8	2.56±0.04*	5.60±0.14*	1.04±0.10*	16.85±0.63*
10	2.54±0.06*	6.40±0.07*	1.07±0.08*	16.35±0.49*
Days	**Microbiological changes**			
	Total bacteria (10^4 cfu/ml)		**Total yeast and fungi (10^4 cfu/ml)**	
0	1.54±4.42		1.27±3.53	
2	62.00±8.73*		3.50±4.59	
4	48.50±9.19*		2.82±1.41	
6	31.82±8.48*		4.12±1.41	
8	48.15±9.49*		41.50±6.00*	
10	34.57±9.44*		39.40±4.77 *	

Values are mean±SD for three replicates; *$P \leq 0.05$ as compared to the 0 day.

Microbiological changes in roselle wine production were observed from 0 to 10 days. There was a steady increase in viable bacteria and yeast counts from initial values of 1.54±4.42x10^4 cfu/ml and 1.27±3.53x10^4 cfu/ml to maximum values of 34.57±9.44x10^4 cfu/ml and 39.40±4.77x10^4 cfu/ml respectively, on the last day of wine production. However, from the report of Opara and Rexford, (2012), the population density started to decrease on the 6th day and continued until the 11th day. The decrease in population density could be a result of depletion of some nutrients especially nitrogen and phosphate which have been reported to enhance growth of yeast during wine fermentation. These elements were not added to the culture medium. Another reason may be due to the accumulation of toxic metabolites. It is known that consumption of glucose by yeast results in acidic metabolites.

3.3 Antibacterial activity of roselle wine and aqueous extract

Roselle wine and aqueous extracts (0.5 and 1 mg/ml) were evaluated on the antibacterial activity by disc diffusion method. Both concentrations of aqueous extract of roselle were inhibited all tested microorganisms (*E. coli*, *Bacillus* sp., *S. aureus* and *Salmonella* sp.). The highest clear zones of 0.5 and 1 mg/ml of aqueous extract of roselle were found to inhibit *E. coli* at 3.58 and 4.08 mm., respectively, which was greater than ethanol control. However, roselle wine product has no effect on antibacterial activity (Table 2). Similar to the report of Chao and Yin (2008), roselle calyx aqueous and ethanol extracts and protocatechuic acid effectively and dose-dependently inhibited the growth of *S.* Typhimurium DT104, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, and *B. cereus* in ground beef and apple juice. The mechanism of this action is not completely understood but it has been proposed that the extract contains phenolic compounds including flavonoids and cyaniding which have been reported to exhibit antimicrobial activities (Diarra et al., 2013).

Table 2. Antibacterial activities of roselle aqueous extract and wine product

Sample	Inhibition zone (mm.)			
	EC	***BC***	***SA***	***SM***
Roselle wine product (6.4% alcohol)	-	-	-	-
Aqueous extract of roselle (0.5 mg/ml)	3.58	3.42	3.3	3.25
Aqueous extract of roselle (1 mg/ml)	4.08	3.92	3.5	3.5
Ethanol (99.99%)	3.42	2.58	2.66	3.25
Absolute solution alcohol (6.4%)	-	-	-	-
Distilled water	-	-	-	-

- no inhibition zone; EC=*E. coli*; BC=*Bacillus* sp.; SA=*S. aureus* and SM=*Salmonella* sp.

4. Conclusions

Part of health benefits, plants are a source of pigments and pharmaceutical compounds that can be used as natural food and drug colorants. Today, the nutritional and bioactive components of plants gain much interest not only among scientists, but also in people's life styles. *H. sabdariffa* or roselle calyx is one of the plants which is commercially applied into food products. From our report, roselle was claimed to have a strong effect on scavenging and antibacterial activity due to polyphenol compounds. When roselle calyx was included in the raw material of wine production, the wine produced from roselle has been found to be a very good raw material as it can give great color and flavor. Also, high acidity of roselle wine gives it an edge in terms of storability and its resistance to microbial spoilage. In addition, roselle is readily available and cheap. Thus, it can be a good raw material for wine industry.

5. Acknowledgements

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6. References

Alarcon-Alonso, J., Zamilpa, A., Aguilar, F. A., Herrera-Ruiz, M., Tortoriello, J. & Jimenez-Ferrer, E. (2012). Pharmacological characterization of the diuretic effect of *Hibiscus sabdariffa* Linn (Malvaceae) extract. *Journal of Ethnopharmacology*, *139*(3), 751-6.

AOAC Method (1990). Official methods of analysis (15th ed.). Washington, DC, USA: Association of Official Analytical Chemists.

Barhé, T. A., & Tchouya, G. R. F. (2015). Comparative study of the anti-oxidant activity of the total polyphenols extracted from Hibiscus sabdariffa L., Glycine max L. Merr., yellow tea and red wine through reaction with DPPH free radicals. *Arabian Journal of Chemistry*, *9*, 1-8.

Borrás-Linares, I., Fernández-Arroyo, S., Arráez-Roman, D., Palmeros-Suárez, P. A., Del Val-Díaz, R., Andrade-Gonzáles, I., & Segura-Carretero, A. (2015). Characterization of phenolic compounds, anthocyanidin, antioxidant and antimicrobial activity of 25 varieties of Mexican Roselle (*Hibiscus sabdariffa*). *Industrial Crops and Products*, 69, 385-394.

Chang, C., Yang, M., Wen, H. & Chem, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, *10*, 178-82.

Chao, C. Y. & Yin, M. C. (2008). Antibacterial effects of roselle calyx extracts and protocatechuic acid in ground beef and apple juice. *Foodborne Pathog Dis*, doi: 10.1089/fpd.2008.0187.

Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., & Heinrich, M. (2014). *Hibiscus sabdariffa* L. – A phytochemical and pharmacological review. *Food Chemistry*, *165*, 424-443.

Diarra, M.S., Block, G. & Rempel, H. (2013). In vitro and in vivo antibacterial activities of cranberry press cake extracts alone or in combination with β-lactams against *Staphylococcus aureus*. *BMC Complementary and Alternative Medicine*, doi: 10.1186/1472-6882-13-90.

Ferguson, L.R. (2001). Role of plant polyphenols in genomic stability. *Mutation Research*, *475*, 89-111.

Fern´andez Arroyo, S., Herranz-L´opez, M., Beltr´an-Deb´on, R., Borr´as-Linares, I., Barraj´on-Catal´an, E. & Joven, J. (2012). Bioavailability study of a polyphenol enriched extract from *Hibiscus sabdariffa* in rats and associated antioxidant status. *Molecular Nutrition & Food Research*, *56*(10), 1590-5.

Harrigan, W. F. & McCance, M. (1976). Laboratory Methods in Food and Dairy Microbiology. Academic Press Inc., London, pp. 25-28.

Hopkins, A. L., Lamm, M. G., Funk, J. L. & Ritenbaugh, C. (2013). *Hibiscus sabdariffa* L. in the treatment of hypertension and hyperlipidemia: a comprehensive review of animal and human studies. *Fitoterapia*, *85*, 84-94.

Ifie, I., Lisa, J.M., Peter, H. & Gary, W. (2016). *Hibiscus sabdariffa* (Roselle) extracts and wine: phytochemical profile, physicaochemical properties, and cabohydrase inhibition. *Journal of Agricultural and Food Chemistry*, *64*, 4921-4931.

Ifie, I., Taiwo, O. O. & Johnson, O. A. (2012). Production and quality attributes of vegetable wine from Hibiscus sabdariffa Linn. *African journal of food science*, *6*(7), 212-215.

Jabeur, I., Eliana, P., Lillian, B., Ricardo, C. C., Marina, S., Beatriz, M., Oliveira, P. P., & Isabel G.F.R. Ferreira. (2017). *Hibiscus sabdariffa* L. as a source of nutrients, bioactive compounds and colouring agents. *Food Research International*, *100*, 717-723.

Jorgensen, J. H., Turnidge J. D., & Washington J. A. (1999). Antimicrobial susceptibility tests. Dilution and disk diffusion methods. ASM Press (Washington DC). p. 1526-1543.

Lister, E. & Wilson, P. (2001). Measurement of total phenolics and ABTS assay for antioxidant activity (personal communication). Lincoln, New Zealand: Crop Research Institute.

Liu, K. S., Tsao, S. M. & Yin, M. C. (2005). In vitro antibacterial activity of roselle calyx and protocatechuic acid. *Phytotherapy Research*, *19*, 942–945.

Mahadevan, N., Shivali, K. P., & Kamboj, P. (2009). *Hibiscus sabdariffa* Linn. – An overview. *Natural Product Radiance*, *8*(1), 77–83.

Nishaa, S., Vishnupriya, M., Sasikumar, J. M., Hephzibah, P. C. & Gopalakrishnan, V. K. (2012). Antioxidant activity of ethanolic extract of *Maranta arundinacea* L. tuberous rhizomes. *Asian Journal of Pharmaceutical and Clinical Research*, *5*(4), 85-88.

Okoro, C. E. (2007). Production of red wine from roselle (*Hibiscus sabdariffa*) and pawpaw (*Carica papaya*) using palm-wine yeast (*Saccharomyces cerevisiae*). *Nigeria Food Journal*, *25*(2), 158-164.

Omemu, A. ., Edema, M. O., Atayese, A. O., & Obadina, A.O. (2006). A survey of the microflora of *Hibiscus sabdariffa* (Roselle) and the resulting "Zobo" juice. *African Journal Of Biotechnology*, *5*(3), 254-259.

Opara, C. & Rexford, N. C. (2012). Production of wine from zobo (*Hibiscus sabdariffa*) flower juice. *Journal of Biochemical Technology*, *3*(4), 436-437.

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Re. R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-evans, C. (1999). Antioxidant activity applying an improved ABTS radical decolorization assay. *Free Radical Biology and Medicine*, *26*, 1231–1237.

Rosenzweig, T., Nir, S., Konstantin, R., Uriel, E., Anna, P., Benzi, G., Maria, S., & Elyashiv, D. (2017). Red wine and wine pomace reduced the development of insulin resistance and liver steatosis in HFD-fed mice. *Journal of Functional Foods*, *34*, 379-389.

Sacchi, K. L., Bisson, L. F. & Adams, D. O. (2005). A review of the effect of wine making techniques on phenolic extraction in red wines. *American Journal of Enology and Viticulture*, *56*(3), 197–206.

Sachdewa, A., Nigam, R. & Khemani, L. (2001). Hypoglycemic effect of *Hibiscus rosa sinensis* L. leaf extract in glucose and streptozotocin induced hyperglycemic rats. *Indian Journal of Experimental Biology*, *39*(3), 284-6.

USA, Food and Drug Administration (1993): FDA/

CFSAN Brochure on Food colour facts. Retrieved from www.cfsan.fda.gov/~ Ird/colour fac.html)

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