Evaluation of anthocyanin extracted from *Hibiscus rosa sinensis* as a natural food colorant

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Introduction

Many species of *Hibiscus* are grown for their showy flowers or used as landscape shrubs. *Hibiscus* has medicinal properties [1]. "World Health Organization'' has recommended and traditional health and folk medicine systems has proved to be more effective in health problems worldwide. *Hibiscus rosa sinensis* is certain to emerge in the near future as a major player in the growing field of herbal health supplements and medicines both in daily self-care and in a professionally managed health care systems [2]. China rose or "Queen of tropics" is often a popular name for the gorgeous flowering plant *Hibiscus rosa sinensis*, as it is mainly found in south-east China and some islands in the Pacific and Indian Ocean. Traditionally, *Hibiscus* flowers have been reported to possess antitumor properties, as well as have been used as analgesic, antipyretic, anti-asthmatic, and anti-inflammatory agents. Research on extracts of stems, roots, leaves, and flowers from *Hibiscus* has revealed that its phytochemicals contributed to beneficial findings for human health such as antioxidant activity, which is the scavenging of free radicals that can lead to DNA damage [3].

Hibiscus rosa sinensis as shown in figure 01 is a brilliant scarlet tropical flower that has been traditionally used as food and medicine. It has a good supply of anthocyanin, B-vitamins like folic acid and niacin, minerals such as calcium, magnesium, iron, potassium, phosphorus, and zinc [4]. The red pigments contained in red flowers of the *Hibiscus* species are anthocyanin and have been widely used as colouring agents. Particularly, the red anthocyanin present in the calyces of Roselle *Hibiscus sabdariffa L*. has been used as food colorants and their major compounds are cyanidin-3-sambubioside and delphinidin-3-sambubioside [5].

Figure 1. *Hibiscus rosa sinensis*.

Methodology

Aqueous extraction. As shown in the figure 2. The first extraction method was performed by using 30 g of petals per 100 ml of distilled water. First, petals collected from hibiscus flowers were submerged in the water for 30 min. Then both water and petals were put into a blender and blended for 30 seconds. The resulting mixture was filtered through a cheesecloth and after that the sample was centrifuged at 4000 rpm for 10 min. Later Extract was filtered through the filter paper. After the filtration, the extract was rotary evaporated (45 °C, 60 rpm).

The Second extraction method was performed using 30 g of petals per 100 ml of hot water. Petals were collected from hibiscus flowers. After that, boiled water was poured in to them and rested for 10 min. The resulting mixture was filtered through cheesecloth after extraction sample was centrifuged at 4000 rpm for 10 min. The extract was filtered through the filter paper. After that filtration, the extract was rotary evaporated (45 °C, 60 rpm).

Preservation. The extract was freeze dried. One cup of extract and 1 cup of sugar were put into a clean saucepan. It was cooked over a medium heat for 10 to 15 min and it was stirred until it was slightly thickened and turned glossy.

After few minutes, citric acid was added to it and stirred well. Then the hot liquid was poured into sterilized bottles and seal immediately. One set of samples were stored in a cool, dark place at room temperature (26 ℃-31 ℃) and other at Refrigerated storage (4 ℃).

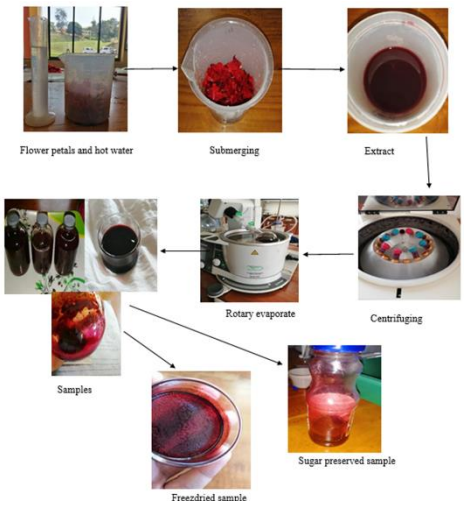

Figure 2. Processing steps of extraction and preservation.

Microbial and sensory evaluation, total flavonoid content, total phenolic content, total monomeric anthocyanin content and antioxidant activity of the final products were determined using AOAC standard methods.

Statistical Analysis. Statistical analysis was carried out with Minitab 19 statistical software. One-way ANOVA model used for data analysis in phytochemical and physiochemical properties. The level of significance ($p<0.05$) was used in this study. Results were expressed in terms of mean ± standard deviation (n=3).

Results and Discussion

Determination of the best extraction method. The weighed flower samples were subjected to blend method (treatment 1) and hot water extraction (treatment 2) separately to extract the anthocyanin. The flower extraction obtained from the hot water extraction produced the best extraction with a high shelf-life. It was observed that the extraction gained by the blend method showed less favourable phytochemical and physiochemical properties. Also, discoloration and ooze produced were observed visually. Therefore, the hot water extraction method was selected as the best extraction method.

Total flavonoid content. As ($P> 0.05$), there is no significant effect of treatment on the flavonoid content of the two samples under the 5% significance level. Flavonoid content of the blend and hot water samples were obtained as 11.5275 ± 3.255 mg/g and 14.90 ± 0.747 mg/g respectively, where the final product of Hot water method contains high flavonoid content.

Total phenolic content. As ($P> 0.05$), There is no significant effect of treatment on the phenolic compounds of the two samples under the 5% significance level. Phenolic content of the blend and hot water samples were obtained as 0.0349 ± 0.00015 mg/g and 0.0354 ± 0.0044 mg/g respectively, the final product of Hot water method contained the higher phenolic compounds.

Monomeric anthocyanin content. As ($P< 0.05$), there is a significant effect of treatment on the antioxidant activity of the two samples under the 5% significance level. The final product of Hot water method contains high anthocyanin content as shown in Table 2.

pH. As ($P> 0.05$), There is no significant effect of treatment on the pH of the two samples under the 5% significance level. The final product of blend method has a high pH.

Table 1. Phytochemical properties of the extract.

Property	*Blend method*	*Hot water method*	*P – value*
Total Flavonoid Content / TFC (mg/g)	11.5275 ± 3.255	14.9038 ± 0.747	0.155
Phenolic compounds (mg/g)	0.0349 ± 0.00015	0.0354 ± 0.0044	0.861
Anthocyanin (mg/g)	24.83 ± 0.170	67.93 ± 0.173	0.009
Anti-oxidant activity % / IC 50	91.62 ± 8.963	54.34 ± 10.955	0.010

pH	5.14 ± 0.004	5.02 ± 0.0001	0.429

Table 2. Results of Anthocyanin content.

Parameter	*Blend method*	*Hot water method*	*P-value*
Anthocyanin content (mg/g)	24.8295 ± 0.170	67.9255 ± 0.173	0.009
After 7 days, anthocyanin content (mg/g)	11.8992 ± 0.002	53.2872 ± 0.133	0.005

Antioxidant activity. As shown in the figure 3 final product from blend method had higher IC_{50} value which corresponds to a lower antioxidant activity of the samples than samples from hot water extraction method.

Therefore, based on the phytochemical analysis, it can be concluded that, hot water extraction method is the best method over blend method to extract anthocyanin from *Hibiscus* flowers.

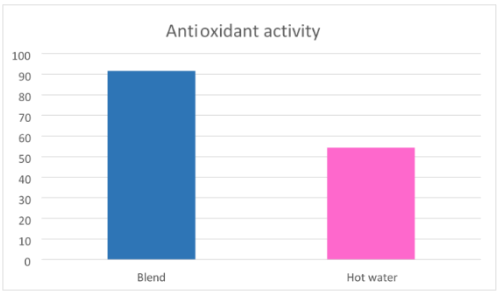

Figure 3. Antioxidant activity (IC_{50} %).

pH values of the samples stored at room temperature and refrigerated condition were measured by using an electronic pH meter on the 1st day and after the 7th day from the production. As ($P<0.05$), it can be concluded that there was a significant difference in pH in refrigerated samples and samples stored at room temperature.

Sensory evaluations for the *Hibiscus* Aloe Vera drink. Sensory evaluation was conducted for three selected Aloe Vera beverage samples incorporated with colorant extracted from, *Hibiscus* using hot water extraction method. 30 un-trained panelists were involved for the sensory evaluation using 9-point hedonic scale. However, colouring among all other treatments that were subjected to sensory evaluation.

The estimated median rank score of sensory attributes for aroma, texture, taste, mouth feel (consistency), colour and overall acceptability are shown in table 3. As ($P< 0.05$), there is a significant effect of the treatment on the aroma, mouth feel, and overall acceptability of the product. According to the Friedman test results, estimated median value for the above sensory attributes were similar to the control.

Overall Acceptability. From the results of sensory evaluation which was done for the assessment of overall acceptability, sample A has gained the highest mean rank which is formulated by commercially available colorant. Meanwhile, sample C has obtained the lowest mean ranks.

Table 3. Results of sensory evaluation.

Parameter	Sum of Ranks			P value
	Sample A	Sample B	Sample C	
Aroma	57.0	34.0	29.0	0.000
Texture	43.5	39.0	37.5	0.607
Taste	45.0	36.0	39.0	0.331
Mouth feel	56.0	38.5	25.5	0.000
Color	43.0	39.5	37.5	0.675
Overall acceptability	48.5	44.0	27.5	0.000

Therefore, it can be concluded that sample A is the best with respect to the overall acceptability. According to the sensory profile, sample A is the most preferred colorant among all other samples that were subjected to sensory evaluation. However, sample B and C also showed better sensory properties. But when considering the overall acceptance, sample A

had shown the highest mean rank value than sample B and C. Comparing Hibiscus samples, B is the best colorant compare to sample C. Based on the variation of the pH values, the refrigerated storage was selected as the best storage condition as there was a significantly favourable difference in most of the quality parameters in the product stored in room temperature. It can be concluded that refrigeration is the best storage condition as they noticed, ooze/foam produced due to the fermentation of the product with the time.

Conclusion

The aim of extracting anthocyanin from *Hibiscus rosa sinensis* was to produce a natural food colorant which has the potential to substitute artificial food colorants. Ultimately by evaluating its phytochemical properties, hope to provide a better alternative to synthetic food colorants with harmful carcinogenesis effects. The *Hibiscus* sugar preserved coloring can be stored 7 days under the refrigerated condition which means longer shelf life compared to the colorant stored at room temperature. Artificial preservatives were not used for the colorant to enhance shelf life. In the sugar preserve technique only lemon juice and sugar were used as natural preservatives.

According to overall appearances and results obtained, the best extraction method is the hot water extraction method and among the three samples A, B and C the hot water extracted sugar preserved colorant (B) was selected as the best sample. The major reason for that is, samples produced from blend method got rejected by panellists during sensory evaluation

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