Study on chemical Constituents, antioxidant properties and toxicity of *Hibiscus rosasinesis* flower extract

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Abstract

As per literature *Hibiscus rosasinesis* flower contain Vitamin C and since the flower is red in colour; it may contain anthocyanin. Both these ingredients are good antioxidants and very much essential for proper maintenance of human skin. Hence, the present paper deals with the ascorbic acid and anthocyanin content in *Hibiscus rosasinesis* flower; its antioxidant property and toxicity to ensure whether *Hibiscus rosasinesis* flower extract can be used for topical purpose. The acidified aqueous extract of fresh flowers, contain 0.5% to 1.0% anthocyanin while aqueous extract of fresh flowers contain 0.1% Vitamin C (Ascorbic acid). Vitamin C was estimated by iodometric titration while; Anthocyanin was estimated using UV-Visible Spectrometer. Since both these contents are essential for dermal cells, it can be concluded that *Hibiscus rosasinesis* flowers can find application in dermal activities like wound healing and anti-ageing. The ethanolic extract of the flowers were found to be non– toxic and exhibit antioxidant activity.

Keywords: *Hibiscus Rosasinesis*, Anthocyanin, Vitamin C, Antioxidant, Cytotoxicity

1. Introduction

Broad Knowledge about chemistry, biochemistry and molecular biology of the biosynthesis of flavonoids is already known, the most important group of flower color pigments is anthocyanin. The anthocyanins, a subclass of flavonoids, are important flower and fruit pigments which are responsible for the intense red and blue. Among flavonoids, the anthocyanins are the main colorant molecules; derivatives of pelargonidin provide the basis for orange-red hues, derivatives of cyaniding for red hues and derivatives of delphinidin for lilac to blue hues. Anthocyanins are believed to provide a broad variety of health benefits such as prevention of heart disease, inhibition of carcinogenesis, and anti-inflammatory activity in the brain. Anthocyanins have been shown to have some beneficial health effects on oxidative damage, detoxification enzymes, and the immune system, They have anti-platelet aggregation and anti-inflammatory properties as well. However, the stability of these anthocyanins is easily affected by structural modifications with hydroxyl, methoxyl, glycosyl, and especially acyl groups and by environment factors such as temperature and light. Anthocyanins are natural, water-soluble, non-toxic pigments. They have not been broadly used in foods and beverages even though they have been reported to be safe as dietary supplements\(^1\).

The anthocyanins have shown a higher antioxidant activity than vitamins C and E. Phenolic hydrogen atoms in these compounds are able to capture free radicals. It has also been reported, a linear correlation between the values of the antioxidant capacity and the anthocyanins content in blackberries, red raspberries, black raspberries and strawberries; additionally it has been described that the berry extracts posse a high scavenging activity towards reactive oxygen species chemically generated. The antioxidant activity of berries is directly propotional to the anthocyanins content. It is well known that the antioxidant activity of blues and red color is influenced by the total anthocyanin content of the plant material. It is well known that the presence of high content of anthocyanins contribute to antioxidant activity\(^1\).

Vitamin C (Vit. C) is one of the naturally occurring antioxidants in nature. Most plants and animals are able to synthesise Vit. C *in vivo* from glucose. Humans and certain other vertebrates lack the enzyme L-glucono-gamma lactone oxidase required for *in vivo* synthesis of Vit. C; hence, they must acquire it from natural sources such as citrus fruits, green leafy vegetables, strawberries, papaya and broccoli. The absorption of Vit. C in the gut is limited by an active transport mechanism and hence a finite amount of the drug is absorbed despite high oral dosage.\(^2\) Furthermore, bioavailability of Vit. C in the skin is inadequate when it is administered orally because it is a water-soluble vitamin that is lost in large amounts during...
food processing. The use of topical ascorbic acid is therefore favored in the practice of dermatology. Vitamin C is a potent antioxidant drug that can be used topically in dermatology to treat and prevent changes associated with photoaging. It can also be used for the treatment of hyperpigmentation. Because it is unstable and difficult to deliver into the dermis in the optimum dosage, research is being directed to find stable compounds of Vitamin C and newer methods of delivery of Vitamin C into the dermis. The visible changes associated with chronological aging and chronic sun exposure, especially to the face, head and neck areas are particularly concerning for a significant percentage of general population. This fact, along with the powerful influence of advertisement and the popular press, has led to an increasing demand for natural and efficient cosmetic ingredients that claim to reduce manifestatio ns of skin aging. Consequently new effective anti-aging agents are in high demand. Although many of the skin-protective claims attributed to botanical products still lack sufficient scientific evidence, the use of natural bioactives with potential antiaging and/or skin protective properties continues to receive attention from consumers.

Free radicals are highly reactive molecules with unpaired electrons that can directly damage various cellular structural membranes, lipids, proteins, and DNA. The damaging effects of these reactive oxygen species are induced internally during normal metabolism and externally through various oxidative stresses. The production of free radicals increases with age, while the endogenous defense mechanisms that counter them decrease. This imbalance leads to the progressive damage of cellular structures, and thus, results in accelerated aging. Antioxidants are substances that can provide protection from endogenous and exogenous oxidative stresses by scavenging free radicals. Topical antioxidants are available in multivariate combinations through over-the-counter skin care products that are aimed at preventing the clinical signs of photoaging.

World Health Organization has been promoting research in identifying newer bioactive molecules from natural sources, Hibiscus rosasinesis, known colloquially as rose mallow, Chinese hibiscus, China rose and shoe flower, is a species of flowering plant in the family Malvaceae, native to East Asia. As per literature Hibiscus rosasinesis flower contain Vitamin C and since the flower is red in colour; it may contain anthocyanin. Both these ingredients are good antioxidants and very much essential for proper maintenance of human skin. Hence, the present paper deals with the ascorbic acid and anthocyanin content in Hibiscus rosasinesis Flower; its antioxidant property and toxicity to ensure whether Hibiscus rosasinesis flower extract can be used for topical purpose.

2. Materials and Methods
2.1 Estimation of Vitamin C by Iodometric titration

2.1.1 Equipment Needed
1. Burette and stand
2. 100 mL or 200 mL volumetric flask
3. 20 mL pipette
4. 10 mL and 100 mL measuring cylinders
5. 250 mL conical flasks

2.1.2 Reagent Preparation
Iodine solution: (0.005 mol L⁻¹): 2 g of potassium iodide added to a 100 mL beaker. 1.3 g of iodine added into the same beaker mixed with distilled water and swirled for a few minutes until iodine dissolved. Transfer the iodine solution to a 1 L volumetric flask, making sure to rinse all traces of solution into the volumetric flask using distilled water. The solution is made up to 1 L with distilled water.

Starch indicator solution: (0.5%): Weighed 0.25 g of soluble starch and added it to 50 mL of near boiling water in a 100 mL conical flask. Stirred it to dissolve and cooled before using.

2.1.3 Sample Preparation
Fresh flowers of Hibiscus rosasinesis were collected, weighed, crushed in mortar-pestle and added to 100 ml Distilled water. This solution was stirred for 30 min on magnetic stirrer. Then the solution was filtered and the filtrate was preserved for further analysis at 20°C to 25°C. The flowers were authenticated to be those of Hibiscus Rosasinesis at Blatter Herbarium, St. Xavier’s College, Mumbai.

2.1.4 Procedure for analysis
Sample Preparation: A 20 mL aliquot of the sample solution was pipetted into a 250 mL conical flask. 150 mL of distilled water and 1 mL of starch indicator solution was added. The sample was titrated with 0.005 mol L⁻¹ iodine solution. The endpoint of the titration was identified as the first permanent trace of a dark blue-black colour due to the starch-iodine complex. Repeated the titration with further aliquots of sample solution until concordant results (titres agreeing within 0.1 mL) were obtained.

Standard Preparation: 10mg of standard ascorbic acid was dissolved and diluted to 100ml in volumetric flask with distilled water. A 20 mL aliquot of the standard solution was pipetted into a 250 mL conical flask. 150 mL of distilled water and 1 mL of starch indicator solution was added. The standard was titrated with 0.005 mol L⁻¹ iodine solution. The endpoint of the titration was identified as the first permanent trace of a dark blue-black colour due to the starch-iodine complex.

Calculation for vitamin C
Assay = Sample Reading × Standard Weight × 100
Standard Reading Sample Weight

2.2 Estimation of Anthocyanin

2.2.1 Equipment needed
1. UV-Visible Spectrophotometer
2. Magnetic Stirrer
3. 250ml beaker
4. 50ml volumetric flasks
5. 5ml Bulb Pipette

2.2.2 Reagent Preparation
Potassium Chloride Buffer 0.025M (pH 1.0): Weighed 1.86g potassium chloride. 900ml distilled water was added. pH adjusted to 1.0 with concentrated hydrochloric acid. This solution was transferred to 1000ml volumetric flask. Volume made up to the mark with distilled water.

Sodium Acetate Buffer 0.4M (pH 4.5): Weigh 54.43g sodium acetate. 900ml distilled water was added. Adjusted pH to 4.5 with concentrated Hydrochloric Acid. This solution was transferred to 1000ml volumetric flask. Diluted upto the volume with distilled water.

2.2.3 Extraction of anthocyanin:
The anthocyanins were extracted according to the methodology of Lees and Francis (1972). Fresh flowers of
Hibiscus rosasinesis as it is (i.e. without crushing) were treated with 100ml distilled water acidified with conc. HCl to pH 1.0. This solution was placed in refrigerator at temperature below 6°C and wrapped with aluminium foil for overnight. The next day the solution was filtered and this resulting extract was used for anthocyanin test.

Sample preparation and anthocyanin test
Two 5 ml aliquots were removed from the filtrate, each placed in a 50 ml volumetric flask, the volume completed with two buffer solutions: potassium chloride buffer 0.025 M (pH 1.0) and sodium acetate buffer 0.4 M (pH 4.5) respectively. Absorbance was then measured simultaneously at 516 nm and 700 nm after 15 minutes of incubation at room temperature. Absorbance readings were made at room temperature against distilled water as blank was used for measurements.

Calculation for anthocyanin content

\[
\text{Monomeric anthocyanin} = \frac{A \times MW \times DF \times 1000}{\epsilon \times 1}
\]

Where,
\[
A = (A_{516} - A_{700}) \text{ pH 1.0} - (A_{516} - A_{700}) \text{ pH 4.5}
\]
\[MW = \text{molecular weight (449.2)}\]
\[DF = \text{Weight of sample x 5/100 x 50}
\]
\[\epsilon = \text{molar absorptivity (26,900)}\]

DPPH radical scavenging activity (%) = \[\frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}}\] *100

In this study DPPH radical scavenging activity was assessed by a DPPH method, 100µl of sample (i.e ethanolic extract of flower) was mixed with 1 ml of 0.1 mM DPPH (Sigma Aldrich, USA) in ethanol solution. This was left for incubation in dark condition at room for 30 mins. Ascorbic acid (Vitamin C) was used as the positive control for the assay. The DPPH free-radical was measured by reading absorbance at 517 nm.

3. Results and Discussion

3.1 Vitamin C content in aqueous extract of fresh flowers:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name</th>
<th>Weight</th>
<th>Burette Reading</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard</td>
<td>10.6mg</td>
<td>3.9ml</td>
<td>0.11%</td>
</tr>
<tr>
<td>2</td>
<td>Sample</td>
<td>4.9951g</td>
<td>2ml</td>
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</tr>
<tr>
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<td>Standard</td>
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<td>8.9ml</td>
<td>0.15%</td>
</tr>
<tr>
<td>4</td>
<td>Sample</td>
<td>3.9645g</td>
<td>5.3ml</td>
<td></td>
</tr>
</tbody>
</table>

3.2 Anthocyanin content in acidified water of fresh flowers
It is observed that 3.99gm (rounded to 4gm) flowers contain 0.037mg/L anthocyanin which means that 4gm flowers contain 0.92% (rounded to 1.0%) anthocyanin while 2.6 gm flowers contain 0.011mg/L anthocyanin which means 2.6gm flowers contain 0.42% (rounded to 0.5%) anthocyanin.

2.3 Antioxidant activity
The EC 50 value for the sample was found to be 266.8 µg/ml. This indicates that the fresh flower extract shows good free radical scavenging activity.

3.4 Toxicity

The toxicity of ethanolic extract of *Hibiscus Rosasinesis* flowers was determined on normal cells (non-cancerous) like vero. Determination of toxicity is important from the therapeutic application point of view. In this case the IC50 of anthocyanin was found to be 0.79 mg/ml. The IC50 is the concentration at which 50% of the cell population is lost to the toxic effects of the drug. Thus, this study confirmed that fresh flowers of *Hibiscus rosasinesis* contain Vitamin C (0.1%) and Anthocyanin (0.5% to 1.0%). Both these ingredients exhibit good antioxidant activity as they show positive results for DPPH radical scavenging assay. Since IC50 was found to be at a high concentration as compared to the concentration at which antioxidant activity was observed, the anthocyanin extracted from *Hibiscus rosasinesis* can be used as an antioxidant agent.

4. Conclusion

Present study suggest that since *Hibiscus rosasinesis* flower contain Vitamin C and Anthocyanin, the fresh flowers can be evaluated to study their efficacy against UV radiation on skin cells. *Hibiscus rosasinesis* flower possesses significant antioxidant activity and plays a major role in controlling oxidation. Thus, from the results of DPPH antioxidant assay we can conclude that *Hibiscus rosasinesis* can serve as an easily accessible source of natural antioxidants; hence can be used as an effective intervention for free radical mediated disease. By summarizing all the results in this experiment we can conclude that *Hibiscus rosasinesis* has antioxidant activity with less or no toxicity.

5. Acknowledgement

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6. References

4. Leonel ER, Diana ER, Brittany G, Diana MC, David R, Bertold F et al. Role of Anthocyanins in Skin Aging and UV induced Skin Damage; Anthocyanins in Health and Disease; CRC net Base.