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

Suvarna Pachpore and Kapil Punjabi

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* Fower; 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 posses 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 [2, 3]. 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 photoageing. 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.[2] 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 manifestations of skin aging. Consequently new effective antiaging 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 [3, 4].

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 [5].

World Health Organization has been promoting research in identifying newer bioactive molecules from natural sources [6]. *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* Fower; 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 [7]

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 100ml Distilled water. This solution was stirred for 30min on magnetic stirrer. Then the solution was filtered and the filtrate was preserved for further analysis at $20^{o}C$ to $25^{o}C$. 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

$$\text{Assay} = \frac{\text{Sample Reading}}{\text{Standard Reading}} \times \frac{\text{Standard Weight}}{\text{Sample Weight}} \times 100$$

2.2 Estimation of Anthocyanin [8]

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): Weighed 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 $6^{O}C$ 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

$$\text{Monomeric anthocyanin Pigment (mg/L)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1}$$

Where,

A = (A516-A700) pH 1.0 – (A516-A700) pH 4.5

MW= molecular weight (449.2)

$$\text{DF (dilution factor)} = \frac{\text{Weight of sample} \times 5}{100 \times 50}$$

E = molar absorptivity (26,900)

2.2.4 Cytotoxicity of extract [9]

In vitro cytotoxicity of the extract was evaluated on non-cancerous cell line like Vero (Monkey kidney cell line). To evaluate effect cell toxicity colorimetric MTT assay was performed. Known number (1×10^4 cells) of cells were seeded into tissue culture grade 96 well plates in a volume of 100 µl of culture medium and incubated for 24hrs. Cells were then treated with various concentrations (10.0 – 0.6 mg/ml) of ethanolic extract of *Hibiscus rosasinesis* for 24 hrs. After exposure cells were washed with phosphate buffered saline and 100 µl of 0. 5% of MTT (HiMedia Laboratories Pvt. Ltd., India) dissolved in Sterile DMEM was added to all the wells and plates were incubated for 4hrs. After 4hrs, all the contents of wells were removed and 100 µl of Dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals. Absorbance of colour solution was measured on Multimode reader (Synergy HTX Multimode reader, BIOTEK India) using a test wavelength of 490 nm. Toxicity against Vero was determined, percentage toxicity was estimated for each concentration, and IC50 value was calculated by plotting the percentage toxicity versus concentration of extract in mg/ml

2.3 Antioxidant activity [10]

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical is stable free radical which gets reduced when reacts with antioxidants. DPPH accepts hydrogen from antioxidant and colour change observed is from purple to yellow absorbance is read at 517 nm. The capacity to scavenge the DPPH radical is calculated using the following equation:

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

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 517nm.

3. Results and Discussion

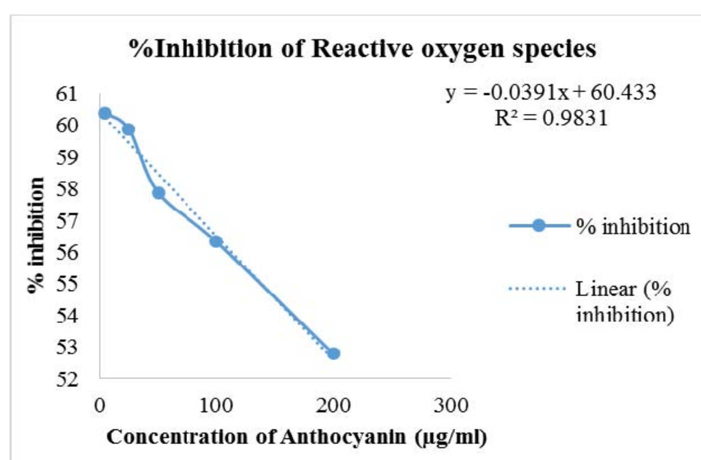
3.1 Vitamin C content in aqueous extract of fresh flowers:

Sr. No.	Name	Weight	Burette Reading	Result
1	Standard	10.6mg	3.9ml	0.11%
2	Sample	4.9951g	2ml	
3	Standard	10.2mg	8.9ml	0.15%
4	Sample	3.9645g	5.3ml	

3.2 Anthocyanin content in acidified water of fresh flowers

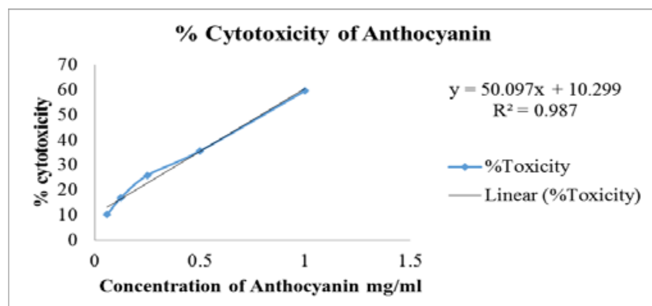
It is observed that 3.99gm (rounded to 4gm) flowers contain 0.037mg/L anthocyanin which means that 4gm flowers contain 0.92% (rounded to 1.0%) anthocyanin while 2.6 gm flowers contain 0.011mg/L anthocyanin which means 2.6gm flowers contain 0.42% (rounded to 0.5%) anthocyanin.

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.

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6. References

1. Padma SV, Jyoti S. Evaluation of Anthocyanin Content in Red and Blue Flowers; International Journal of Food Engineering. 2010; 6(4):7.
2. Pumori ST. Vitamin C in dermatology; Indian Dermatol Online J. 2013; 4(2):143-146.
3. Walingo KM. Role of Vitamin C (Ascorbic Acid) on Human Health - A Review. African Journal of Food Agriculture Nutrition and Development.
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.
5. Bogdan A, Baumann L. Antioxidants used in skin care formulations; Skin Therapy Lett. 2008; 13(7):5-9.
6. WHO. The promotion and development of traditional medicine. Geneva, 2011, 622.
7. Determination of Vitamin C Concentration by Titration. University of Canterbury, New Zealand.
8. Naji E, Kershi RM, Luca R. Free Radical Scavenging Activity and Anthocyanin in flower of Adenium Obesum collected from Yemen. Journal of Pharmacy and Phytotherapeutics. 2013, 1:5. (ISSN 2321: 5895)
9. Freshney RH. Culture of animal cells. Cell Proliferation. 2010; 15(2.3):1.
10. Tailor CS, Goyal A. Antioxidant Activity by DPPH Radical Scavenging Method of *Ageratum conyzoides* Linn. Leaves; American Journal of Ethnomedicine. 2014; 1(4):244-249.