Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2017, 9 [12]: 45-52
[http://scholarsresearchlibrary.com/archive.html]

ISSN 0975-5071
USA CODEN: DPLEB4

Comparative Analysis of Ascorbic and Phenol Content Percentage in Leaves, Stems and Flowers of *Hibiscus rosa sinensis*

Pankaj K. Tyagi, Shruti Tyagi

Department of Biotechnology, Meerut Institute of Engineering and Technology, Meerut U.P. India

***Corresponding author:** Pankaj KT, Department of Biotechnology, Meerut Institute of Engineering and Technology, Meerut U.P. India. E-mail: pktgenetics@gmail.com*

ABSTRACT

Hibiscus Rosa sinensis is a well-recognized medicinal herb for its high phytochemical such as ascorbic acid and phenolic contents. Titration procedure was applied for determine the quantitative amount of ascorbic acid and phenolic content and tries to establish a relationship in both phytochemicals within plant parts such as leaves, stem and flowers. Hibiscus Rosa sinensis plant harvested into three different stages such as leaves, stem and flowers parts extracts. Ascorbic acid and phenolic content in plant parts were evaluated through titration methods and Folin-Ciocalteau reagent methods respectively. The leaves contained 65.48 ± 2.08 mg/100 g, ascorbic acid, stem had 46.98 ± 0.51 mg/100 g and flowers contained were 26.48 ± 1.44 mg/100 g. The phenolic content in leaves, stem and flower extracts were also calculated and found 43.31 ± 0.71 mg, 41.79 ± 1.20 mg and 35.25 ± 1.79 mg of Gallic acid equivalent (GAE) per gram respectively. Five replicates basic data of correlation indicated that the ascorbic acid and phenolic content in leaves, stem and flowers showing a non-significant, negative and positive relationship respectively. The high ascorbic acid contents in leaves showed that they are highly rich in vitamin C and good for the prevention and treatment of vitamin deficiency disease. Phenol content was also observed in higher amount in plant leaves as compare to stem and flowers. It is also observed that the different parts of plants contained different amount of ascorbic acids as well as phenolic content.

Keywords: Ascorbic acid; *Hibiscus rosa sinensis*; Phenolic content; Vitamin C; Phytochemicals

INTRODUCTION

Natural antioxidant agents have attracted much interest because of their ability to scavenge free radicals [1]. Free radical shave been implicated in the development of a number of disorders, including cancer, neurodegeneration and inflammation [2-4], giving rise to studies of antioxidants for the prevention and treatment of diseases. The presence of antioxidants such as phenolics, flavonoids, tannins and proanthocyanidins in plants may provide protection against a number of diseases; for example, ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders [5]. Medicinal plants are therefore being investigated for their antioxidant properties, and the demand for natural antioxidants and food preservatives is increasing [6]. *Hibiscus Rosa sinensis* are native to tropical Asia. A native of south eastern Asia (China), the plant is commonly found throughout the tropics and as a house plant throughout the world. Leaves and stems contain β-sitosterol, stigma sterol, taraxeryl acetate and three cyclo propane compounds and their derivatives. Flowers contain cyaniding diglucoside, flavonoids and vitamins, thiamine, riboflavin, niacin and ascorbic acid [7]. Quercetin-3-diglucoside, 3,7-diglucoside, cyanidin-3,5-diglucoside and cyanidin-3- sophoroside-5-glucoside have been isolated from deep yellow flowers; all above compounds and kaempferol-3-xylosylglucoside have been isolated from ovary white flowers. Vitamin is an organic substance which is needed in trace quantity for normal cell functions. The vitamins that cannot be synthesized internally by an organism are called essential vitamins, in their absence in the external medium, the cells cannot survive. A typical example of this is ascorbic acid which has trade name of vitamin C. Ascorbic acid functions in a number of biochemical reactions, mostly involving oxidation. Vitamin C has the chemical name ascorbic acid. It is a water soluble vitamin. Although it is important for good health, humans do not have the ability to make their own vitamin C and must obtain it through diet or take it in vitamin supplements. Citrus fruits, potatoes and some green plants are known to be good sources of vitamin C. Plants synthesize the compound for the growth, development, and protection of the plant. The exact pathway for its synthesis is not well understood. The objective of this study was to determine the amount of phytochemicals ascorbic acid and phenolic content of *Hibiscus Rosa sinensis* and comparison of this amount with respect to plant parts such as leaves, stem and flowers.

MATERIALS AND METHODS

Materials

The *Hibiscus Rosa sinensis* plants were collected from botanical garden of Chaudhary Charan Singh University, Meerut, India on January, 2017.

Reagents

The analytical grade reagents used for this research work included; 2.6 dichlorophenbolindophenol (blue dye), 20% glacial acetic acid, standard L-ascorbic acid and distilled water.

Sample preparation

Samples (Leaves, stems and flower) were washed, shade dried at room temperature and finely powdered with the help of a hand grinding mill. About 100 g of the powder was exhaustively extracted with ethanol. The extract was concentrated to a residue. The crude extract were stored in sterilized bottle and kept in a refrigerator for further use. 10 ml of each filtrate was mixed with 20% glacial acetic acid in a 100 ml standard flask which was made up to 100 ml with distilled water.

Dye preparation

The standard dye solution was prepared by dissolving 50 mg of blue dye in 50 ml of distilled water. The mixture was diluted to 200 ml, filtered and kept.

Preparation of standard ascorbic acid solution

This was prepared by dissolving 100 mg crystalline ascorbic acid in 50 ml of 20% glacial acetic acid and diluted to 100 ml with distilled water.

Titration procedures

10 ml of the ascorbic acid solution was titrated with the dye solution. Each drop of the dye in contact with the solution turns pink. The end point was reached when the pink colour lasts for 10 seconds. Similarly, 10 ml of each sample prepared was in turn titrated with the due and the titre values were noted.

Determination of total phenols

The total phenol content in the ethanolic extract of *Hibiscus Rosa sinensis* was determined with Folin-Ciocalteau reagent by the method [8]. To 2.5 mg of 10% Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (2% w/v) was added to 0.5 ml of each sample (3 replicates) of plant extract solution (1 mg/ml). The resulting mixture was incubated at 45°C with shaking for 15 min. The absorbance of the samples was measured at 765 nm using UV/visible light. Results were expressed as milligrams of Gallic acid (0-0.5 mg/ml) dissolved in water.

RESULTS AND DISCUSSION

Ascorbic acid content of *Hibiscus Rosa sinensis* was significantly influenced by plant parts and their interactions (Table 1). It is evident from Table 1 that ascorbic acid content of leaves was statistically higher 65.48 mg/100 g; as compared to stems 46.98 mg/100 g contained 19% lower ascorbic acid. Ascorbic acid content of flowers was statistically lower 26.98 mg/100 g. Ascorbic acid content was decreased gradually as leaves to stem and followed by flowers.

Ascorbic acid estimations

6.5 ml of the dye solution was needed to titrate 10 ml of the standard ascorbic acid solution which contained 1 mg of ascorbic acid per ml.

That is; 6.5 ml blue dye solution needed to titre 10 mg ascorbic acid.

Therefore, 1 ml ≡ (10/6.5) = 1.54 mg.

Table 1: Estimation of mean ± SE values of ascorbic acid (mg/100 g) and phenolic content (mgGAE/100 g) in different parts extract of *Hibiscus rosa sinensis* plant.

S. No.	Samples	Average titre volume (ml)	Ascorbic acid mg/10 ml	Ascorbic acid mean ± SE (mg/100 g)	Phenolic content mg/10 ml	Phenolic content mean ± SE GAE/100 g
1	*Hibiscus Rosa sinensis Leaves Extracts*	4.25	6.548	65.48 ± 2.08	4.124	43.31 ± 0.71
2	*Hibiscus Rosa sinensis Stems Extracts*	3.05	4.698	46.98 ± 0.51	4.343	35.12 ± 1.70
3	*Hibiscus Rosa sinensis Flowers Extracts*	1.72	2.648	26.98 ± 1.44	3.521	25.10 ± 1.70

In leaves extract

Leaves extract of *Hibiscus Rosa sinensis*, the average ml of the dye used was 4.25 ml.

That is; 4.25 ml ≡1.54 × 4.25

10 ml of the *Hibiscus Rosa sinesis* leaves extract contained 6.548 mg ascorbic acid.

Therefore, 100 ml of leaves extract contain= 65.48 mg/100 g

In stems extract

Stems extract of *Hibiscus Rosa sinensis*, the average ml of the dye used was 3.05 ml.

That is; 3.05 ml ≡1.54 × 3.05

10 ml of the *Hibiscus Rosa sinensis* stems extract contained 4.698 mg ascorbic acid.

Therefore, 100 ml of stems extract contain= 46.98 mg/100 g.

In flowers extract

Flowers extract of *Hibiscus Rosa sinensis*, the average ml of the dye used was 1.72 ml.

That is; 1.72 ml ≡ 1.54 × 1.72

10 ml of the *Hibiscus rosa sinensis* flower extract contained 2.648 mg ascorbic acid.

Therefore, 100 ml of flower extract contain= 26.98 mg/100 g.

Phenolic content estimations

In the present study the phenolic content of leaves, stems and flowers extract of *Hibiscus Rosa sinensis* were found to be 43.31 ± 0.708 mg, 35.12 ± 1.702 mg, and 25.10 ± 1.701 mg of Gallic acid equivalent (GAE) per gram of extract respectively. Total

phenolic content of *Hibiscus Rosa sinensis* was significantly influenced by plant parts and their interactions (Table 1). It is evident from Table 1 that, phenolic content was higher in plant leaves than in stem and flower. The highest phenolic contents were recorded in leaves statistically followed by stems. Flowers, on the other hand contained the lowest phenolic compounds. Phenolic content of leaves was 8% higher than that of stem, and stems contained 10% more phenolic compounds compared to flowers. In recent years, attention has been focused on the antioxidant properties of plant-derived dietary constituents of food. Phenolic and flavonoids are secondary metabolites widely distributed in plants.

All in all, the comparative analysis of phenolic content and ascorbic acid are shown in Figures 1-4. The non-significant correlation (r= -0.122) was found in leaves extract between phenolic content and ascorbic acid while the stem and flower extract were showing negative (r= -0.858) and positive correlation (r=0.985) respectively.

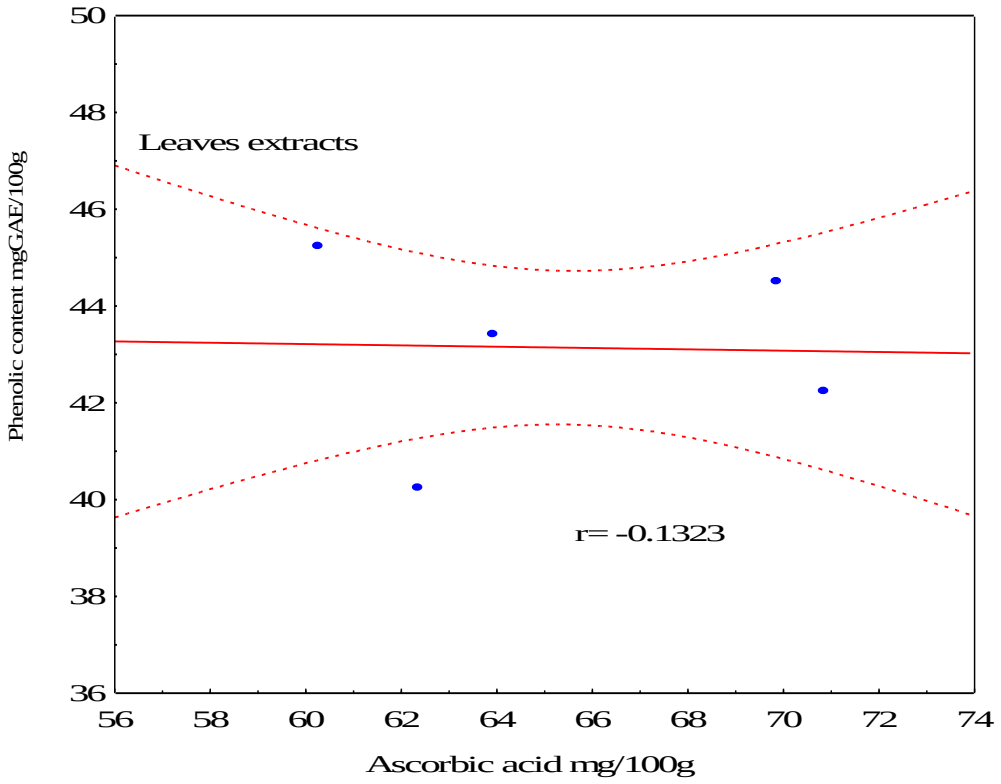

Figure 1: Comparative correlation analysis between ascorbic acid and phenolic content in leaf extracts of *Hibiscus rosa sinensis.*

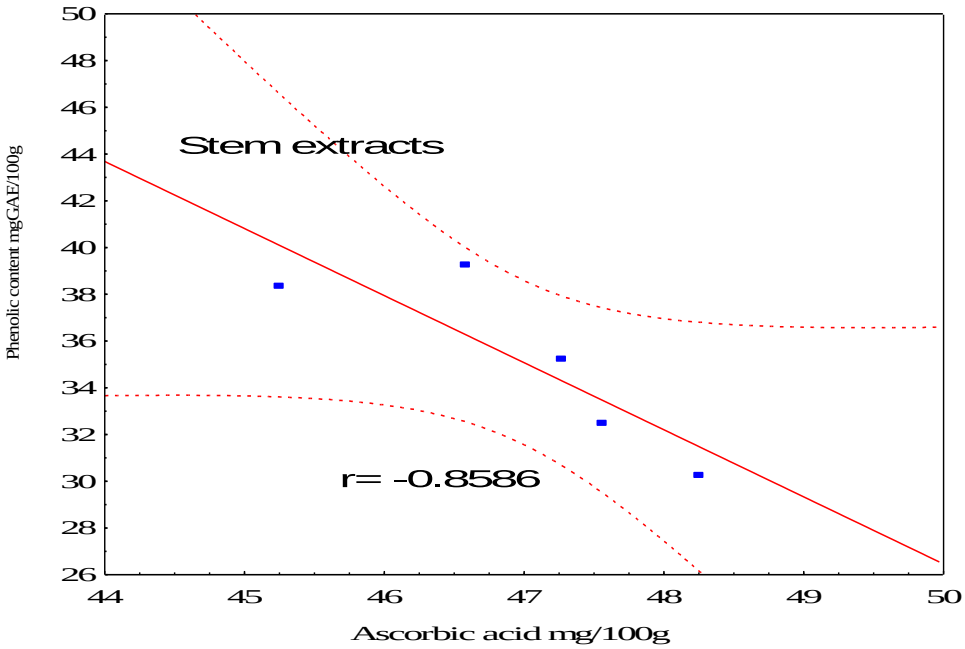

Figure 2: Comparative correlation analysis between ascorbic acid and phenolic content in Stem extracts of *Hibiscus rosa sinensis.*

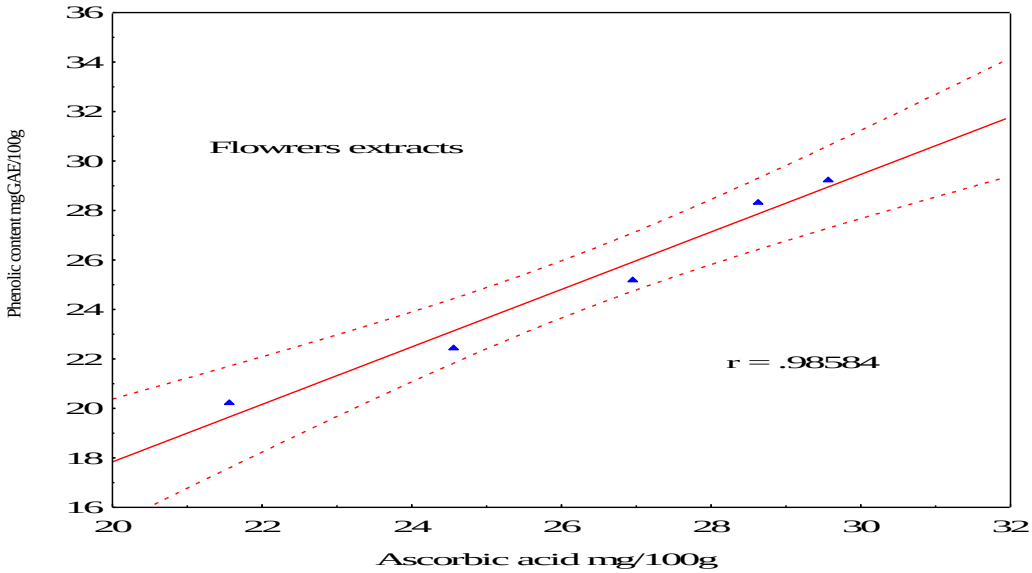

Figure 3: Comparative correlation analysis between ascorbic acid and phenolic content in flowers extracts of *Hibiscus rosa sinensis.*

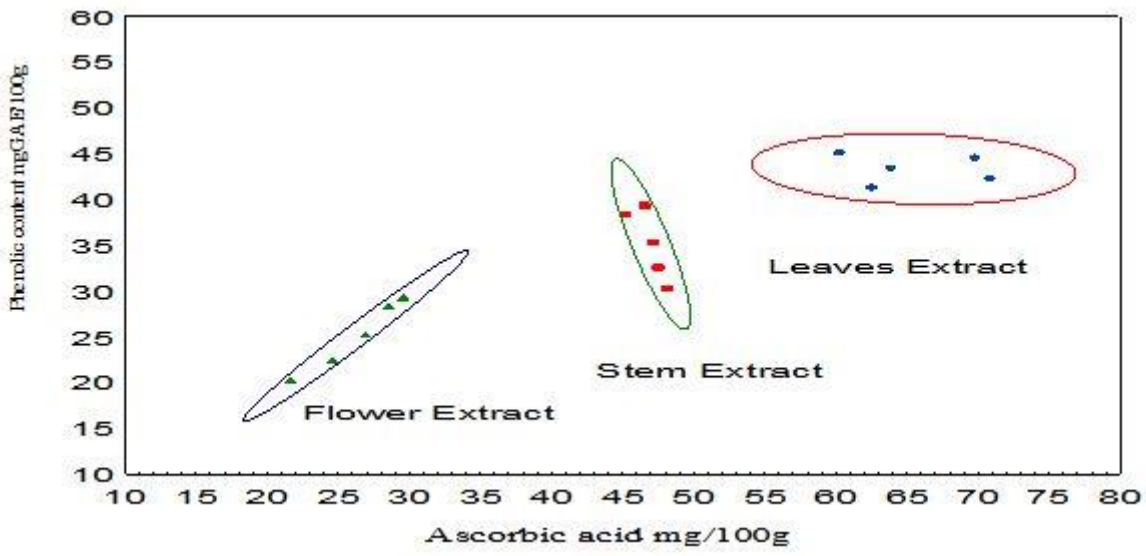

Figure 4: Comparative analysis of ascorbic acid and phenolic content in plant leaves, stem and flower extracts.

The highest initial phenolic content was found in leaves of *Hibiscus Rosa sinensis*. This finding is in agreement with that the I. candida contains higher phenolic compounds in leaves 1.423% compared to stem 0.516% [9] and the higher phenolic content in leaves than in stems of *A. paniculata* [10]. Furthermore, the highest initial flavonoid content is attributed to young leaves, followed by young stems and also confirmed that phenolic content was higher in early growth stage compared with matured stages [11]. Ascorbic acid content in *Hibiscus Rosa sinensis* varied within plant parts. The ascorbic acid of plant leaves were in higher amount and decreases very slowly in respect to plant parts as stem; the lowest amount of ascorbic acids were observed in flowers. This finding is in concord with that of the ascorbic acid content of three herbs such as parsley (*Petroselinum crispum*), dill (*Anethumgra veolens*) and lovage (*Levisticum officinale*) were affected by storage duration [12].

It is well known *Hibiscus Rosa sinensis* contain a range of key nutrients with medicinal value including vitamin C. It has been reported that the ascorbic acid in the body aids in iron absorption from the intestines. It is important for connective metabolism especially the scar tissue, bones and teeth [13]. In addition to its physiological functions, it is necessary as an anti-stress and protector against cold, chills and damp [14]. It prevents muscle fatigue and scurvy that is characterized by skin hemorrhages, bleeding gums, fragile bones, anemia and pains in joints and defects in skeletal calcification [14]. The function of ascorbic acid also accounts for its requirement for normal wound healing [15]. It acts also as antioxidants in the skin by scavenging and quenching free radical generated by ultra violet radiation stabilization. The production of collagens is also dependent on vitamin C. It helps in the promotion and restoration of skin and improvement of fine wrinkles [16]. Thus, our research findings confirm that *Hibiscus Rosa sinensis* is a potential medicinal herb, and a good source of phytochemicals ascorbic acid and phenolic content and both phytochemicals may be harvested freshly from leaves stem and flowers for getting vitamin C or other purpose.

REFERENCES

1. Saeed, N., Khan, MR., and Shabbir, M., an Antioxidant activity, totalphenolic and total flavonoid content of whole plant extracts *Torilis leptophylla* L., BMC Complement. *Altern. Med.* **2012.** 12:221-221
2. Halliwell, B., Oxidative stress and cancer: Have we moved for-ward? *Biochem. J.* **2007.** 401:1-11.
3. Halliwell, B., Oxidative stress and neurodegeneration: Where are we now? *J. Neurochem*, **2006.** 97:1634-1658.
4. Ferguson, L.R., Chronic inflammation and mutagenesis. *Mutat. Res. Fund. Mol*. **2010.** 690:3-11.
5. Gulcin, I., Antioxidant activity of food constituents: An overview. *Arch. Toxicol*. **2012.** 86:345-391.
6. Peschel, W., et al. An industrial approach in the search of natural antioxidants from vegetable and fruits wastes. *Food Chem.* **2006.** 97:137-150.
7. Ghani, A., Medicinal plants of Bangladesh with chemical constituents and uses. 2nd edn, Asiatic Society of Bangladesh, Dhaka **2003.**
8. Tyagi, S., Role of phytochemicals on biosynthesis of silver nanoparticles from plant extracts and their concentration dependent toxicity impacts on *Drosophila melanogaster*. *Biological Insights*. **2006.** 1:21-28
9. Males, Z., et al. Quantitative analysis of phenolic compounds of *Inula candida* (L.) *Cass. Periodicum Biologorum.* **2010.** 112: 307-310.
10. Rafat, A., Philip, K., and Muniandy, S., Antioxidant potential and content of phenlic compound in ethanolic extract of selected parts of Andrographis paniculata. *Journal of Medicinal Plant Research*. **2009.** 4:197-202.
11. Ghasemzadeh, A., et al. Changes in phytochemical synthesis, chalcone synthase activity and pharmaceutical qualities of sabah snake grass in relation to plant age. *Molecules*. **2014.** 19:17632-17648.
12. Catunescu, GM., et al. The effect of cold storage on some quality characteristics of minimally processed parsley (*Petroselinum crispum*), dill (*Anethum graveolens*) and lovage (*Levisticum officinale*). *Bulletin UASVM Agriculture* **2012.** 69:1843-5246.
13. Okwi, D.E., Emenike, IN., Evaluation of the phytonutrients and vitamins contents of Citrus fruits. *International J. Molecular Medicine and Advance Sciences.* **2006.** 2(1):1-6.
14. Okwu, D.E., Phytochemicals, vitamins and minerals contents of two Nigerian medicinal plants. *International J. Molecular Medicine and Advance Sciences.* **2005.** 1:375-381.
15. Okwu, D.E., Phytochemicals and vitamins content of indigenous spices of south eastern Nigeria. *J. Sustainable Agriculture and Environment*. **2004.** 6(1):30-37.
16. Roger, G.D.P., Encyclopedia of medicinal plants (Vol. 1). Education and Health Library Editorial Safeliz S.L. Spsin. (eds) **2002.** 265: 153-154.