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ISOLATION AND DETERMINATION OF VITAMIN C (ASCORBIC ACID) IN DIFFERENT VARIETIES OF ROSES CULTIVATED IN QUETTA

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ABSTRACT

Isolation and determination of vitamin C (ascorbic acid) in different varieties of roses (rose hip fruit) cultivated in the District Quetta, Balochistan is described for the first time in this document. Research and different studies show that the rose hip contain vitamin C as an active ingredient 500-1000%. Six samples of roses that belongs to family "Rosaceae" was first selected and then used for the determination of ascorbic acid. Vitamin C (Ascorbic acid) content in different varieties of roses (Rose hip fruit) was prepared and then has been determined by using spectrophotometric and titrimetric method. The kinetic reaction between methylene blue (MB+) and vitamin C was studied in the spectrophotometric method with the carried measurement at maximum absorption $\lambda_{max.}$ 665nm. It was founded that the vitamin C content in six different varieties were 2556, 7323, 9011, 2167, 5045 and 5234 mg/100 g of dry weight sample, whereas the titrimetric method shows the following results as 2405, 7406, 9261, 2007, 5104 and 5020 mg/ 100 g of dry weight of the six samples respectively. Obtained results shown that the investigated roses are good source of vitamin C.

KEYWORDS: Ascorbic acid, Spectrophotometer, Roses, Titrimetric, rose hip fruit.

INTRODUCTION

Ascorbic acid (AA) also known as vitamin C is an organic nutrient that is very important for the normal functioning of a living body in order to produce a variety of biochemical functions.[1] It is a water-soluble vitamin usually not synthesized by the human body and must be provided in the form of diet (Marcus and Coulston 2001).[2] AA is an antioxidant coomonly found in inconstant amount in different fruits and vegetables.[3] The greatest amount of it is found in plants that mostly reached to maturity.[4] AA actively takes part in all processes of oxidoreduction present in the living cells.[5] The lack of AA causes the disease "Scurvy" that results in tooth loss, inflammated and bleeding gums.[6]

Since prehistoric times, rose hip are recognized as a medicine.[7] Rose is a flowering plant that contain hundreds of species and thousands of cultivars.[8] It belongs to genus '*Rosa'* and family '*Rosaceae'*.[9] The flowers of roses are found in different shapes, sizes and are also brassy and large, found in different colors.[10] Rose petals and rose hip both are fit for human consumption. Rose hips are an excessive source of vitamin C and have a little bit resemblance taste of sourness like a crab apple.[11-12] All roses produce hips but the *'Rugosa'* specie roses are the best and are said to be delicious tasting hip.[13] The rose hip is also known 'rose hep' and 'rose haw', is the accessory fruit of the rose plant. Its colors vary characteristically from green, orange to red and in some species ranges from dark purple to black.[14-15] The rose hip begins to form successfully after flowers pollination in early summer or spring seasons and in later summer ripen till autumn.[16] Figure 1 shows different rose cultivars (1–6) collected from the botanical garden of University of Balochistan for the assay of AA in their hips.

Figure 1: 'Rose hips' of different varieties of roses/ rose cultivars collected from the botanical garden, University of Balochistan, Quetta.

The ecological territories of Province Balochistan is very rich in plants growth due to its varied climate and soil conditions.[17] The rose flower is also used in different food and drinks (like jelly, jam and marmalade). They are also used in rose syrup, rose hip seed oil and in many make up products.[18-19] Rose hip oil is haul out from the fruits of the rose hip plants, whereas rose oil is extracted from the petals of roses.[20-21] Rose water are used in different Persian, middle eastern and south Asian cuisine to add distinguished smell and flavor to food.[22] Rose petals are also used to add flavors in different tea.[23] The therapeutic effect of plants is possible to explain through pharmacodynamic experimenting that the healing power of medicinal plants are not due to the chemicals they contain.[24]

This study presents the quantitative analysis of AA in the hips of different rose varieties cultivated in the Quetta District, Balochistan, Pakistan. Two analytical methods using UV-visible and titrimetric techniques were employed for the assay. Rose varieties were found rich in AA quantity and can be cultivated for commercial and pharmaceutical purposes in the climate of Quetta valley.

MATERIALS AND METHODS

All chemicals were analytical grade reagents used throughout without further purification obtained from Merck, Scharlau or stated otherwise. Deionized water (Elga, Purelab Option, High Wycombe, Bucks, UK) was used for cleaning and solution preparations. Glass and plastic ware used during experimental works were washed with commercially available surfactant, followed by rinsing with UHP water, then by soaking in 20% (v/v) aqueous hydrochloric acid for almost a week and again rinsing several times with deionized water. Spectrophotometric studies were performed throughout the experimental work employing a UV-visible double beam spectrophotometer ((Shimadzu, Model UV-1700, Japan).

The six rose hip flower materials were collected from the botanical garden present in the University of Balochistan, Quetta City during the end of the month of September 2018. The fresh rose hips were sorted out, washed, cleaned and dried at room temperature for about 15 days.

Iodine solution (0.005mol/L) was prepared by taking 2.0 g of potassium iodide and 1.3 g of iodine and dissolved them in 100 mL of distilled water in a 1 L volumetric flask followed by dilution with distilled water up to the mark. The concentration of this iodine solution was determined by titrating it with a standard solution of ascorbic acid.

Starch indicator solution (0.5% w/v) was prepared by taking 0.25 g of soluble starch in 50 mL of near boiling distilled water, stirred to dissolved and cooled before use.

Ascorbic acid stock solution (0.01mol/L) was prepared by taking 0.053 g of ascorbic acid in 30 mL of distilled water then stored in a dark in stopped glass bottle. From this stock standard solution, the working standard solutions of variable concentrations were prepared by diluting it in distilled water before use.

Methylene blue (MB) standard solution (0.001mol/L) was prepared by dissolving 0.016 g in 50 mL of distilled water.

Sample preparation

Six selected samples of rose hip were used for the determination of ascorbic acid. 2.5 g of each sample was washed, cleaned, dried and powdered by using mortar and pestle followed by the addition of 2 mL glacial acetic acid., vigorous stirring and filtration through a Buchner funnel. After this, the volume of the sample was made up to 100 mL by using distilled water. These samples were analyzed by using 2 methods *viz*. a Uv-visible spectrophotometric and a titrimetric method.

Spectrophotometric method

For the determination of AA in the samples, a previously reported spectrophotometric method [XX] was used with

slight modifications. Accordingly, fifty microliters of AA standard solutions / sample solutions were mixed with fifty microliter of methylene blue (0.001mol/L) solution and then diluted up to 10 mL with distilled water. Decrease in the absorbance of methylene blue was measured employing a double beam Uv-visible spectrophotometer with a $\lambda_{max.}$ of 665 nm. All measurements were made in triplicates and the average results are expressed in mg of AA per 100 g of dry weight sample.

For the evaluation of AA content in samples, a standard calibration curve was made between the %inhibition of absorbance and AA concentration over the range of $5 \times 10^{-6} - 1.25 \times 10^{-4}$ mol/L. To prevent the loss of AA in samples and standard solutions, the analyses were performed immediately due to the instability and possible oxidation of AA by atmospheric oxygen.

Titrimetric method

AA quantitative analyses in different samples were also performed by a previously reported [XX] titrimetric method. Accordingly, 10 mL of filtrate was taken in a titration flask and after the addition of 1 mL starch solution 0.5% (w/v), diluted up to 50 mL with distilled water and then titrated immediately to the end point with the 0.005 mol/L of standardized iodine solution. This procedure was repeated in triplicates for each analysis and the results are expressed in mg AA per 100 g of the dry sample.

RESULTS

Figure 2 shows a spectrophotometric standard calibration curve for AA. The curve was obtained between %inhibition calculated from the measured inhibition of absorbance spectrophotometrically at 665 nm and AA over the concentration range of $5.0 \times 10^{-6} - 2.0 \times 10^{-4}$ mol/L. The regression equation and coefficient of determination (R^2) were obtained as $y = 0.4494x + 0.195$ (where y is the %inhibition and x is the concentration of ascorbic acid in µM) and 0.9986 respectively. The regression equation was employed for the of AA in different cultivars of rose.

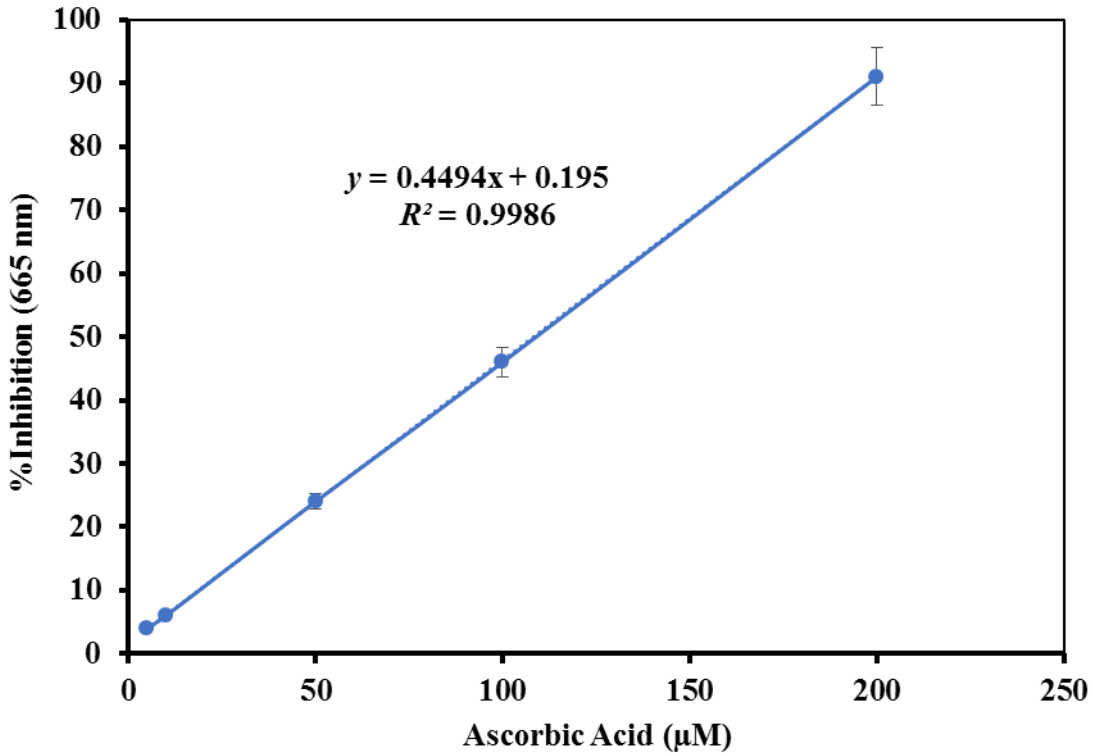

Figure 2: Standard calibration curve for ascorbic acid.

Similarly, antioxidant activity i.e. AA concentration was also analyzed by the spectrophotometric method. The results for the six samples of rose cultivars are given in Figure 3. The series of %inhibitions for 50, 100, 500, 1000 and 2000 µL for cultivar-1, 2, 3, 4, 5 and 6 are 1, 2, 8, 16 and 32; 4, 9, 15, 23 and 38; 6, 11.6, 14, 18 and 25; 1, 3, 5, 7.8 and 13; 3, 6, 10, 15 and 24; and 2, 5, 16, 28 and 48 respectively. The concentration of AA in each volume of each cultivar was determined using the aforementioned regression equation and the average quantity of AA is shown in Table 1. After calculation, the descending order of the average AA concentration for spectrophotometric method is as: 9011 mg/100 g dry weight (Cultivar 3) > 7323 mg/100 g dry weight (Cultivar 2) > 5234 mg/100 g dry weight (Cultivar 6) > 5045 mg/100 g dry weight (Cultivar 5) > 2556 mg/100 g dry weight (Cultivar 1) > 2167 mg/100 g dry weight (Cultivar 4). The highest averaged (n = 5) content of vitamin C was measured in sample/ cultivar 3 and the lowest content in cultivar 4.

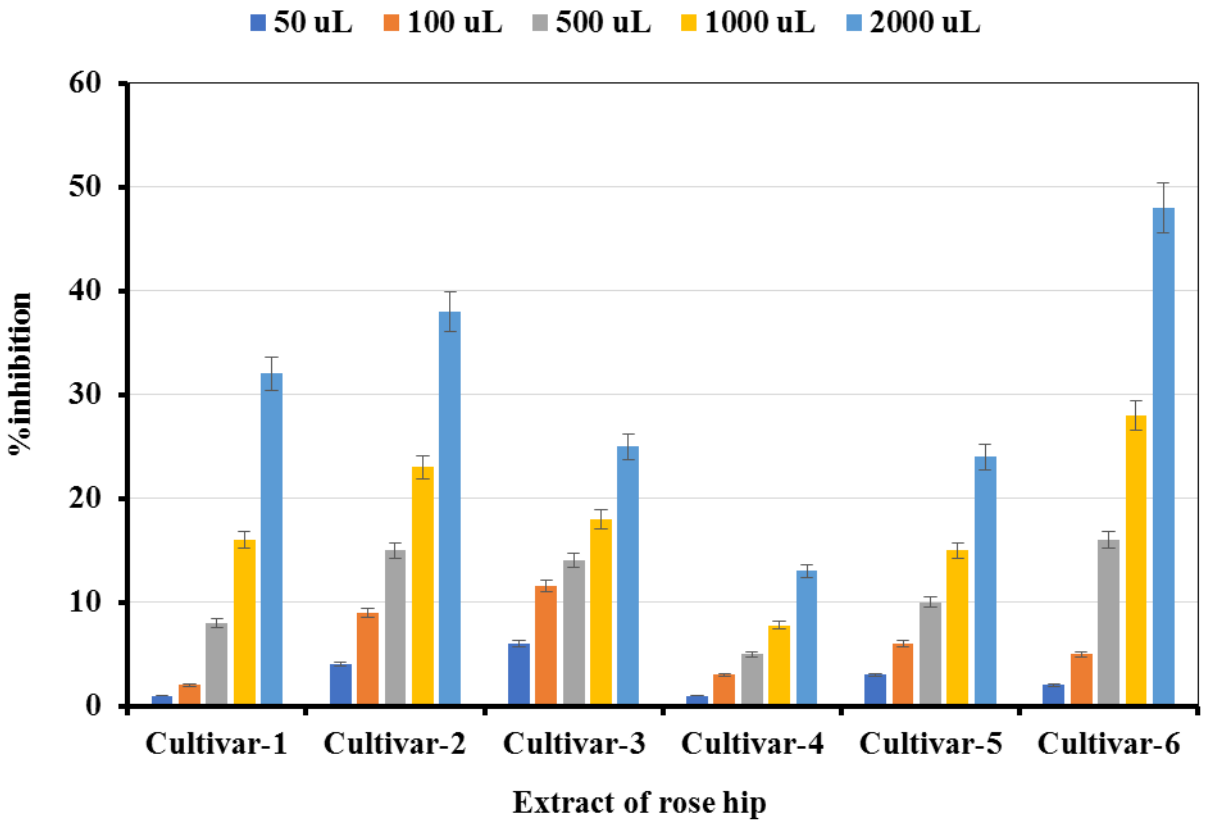

Figure 2: The content of vitamin C in six different varieties of rose hip determined by spectrophotometric and titrimetric methods. Results obtained are expressed in (mg/100g of dry weight of sample).

Roses	Spectrophotometry (mg/100g)	Titrimetric (mg/100g)
Cultivar 1	2556	2405
Cultivar 2	7323	7406
Cultivar 3	9011	9261
Cultivar 4	2167	2007
Cultivar 5	5045	5104
Cultivar 6	5234	5020

Similarly, using titrimetric procedure, almost the same results were achieved for the six cultivars. According to titrimetric results shown in Table XX, the descending order of averaged AA content (n = 5)/100 g of dry weight in the cultivars are as: 9261(Cultivar 3) > 7406 (Cultivar 2) > 5104 (Cultivar 5) > 5020 (Cultivar 6) > 2405 (Cultivar 1) > 2007 (Cultivar 4).

UV-visible spectrophotometric results that are attained for the determination of AA are well agreed with the results obtained by using titrimetric method. To check whether the spectrophotometric method is significantly different from titrimetric method or not, paired student *t*-test was applied on the results of the both methods at 95% confidence limit. The calculated t-test value i.e. 0.2997 is very low than the t-distributed value i.e. 2.5706 at 95% confidence limit. Therefore, it can be concluded that the two methods are not significantly different. This indicates that the spectrophotometric method was based on methylene blue can be used as an alternate to the titrimetric method for the determination of vitamin C content in rose hip fruit.

DISCUSSIONS

The results obtained shows that the rose hip is very rich in content of vitamin C. Similar observations were noticed by Rasanu *et al.*, (2005),[25] who investigated that the content of ascorbic acid in different growing stages of flowers and fruits of cherry, apricot and apple. The medicinal and nutritional influence of these investigated species were better understood by the help of the results that were attained in this research. Vitamin C is ubiquitous antioxidant that are found in plants and animal's cells. It plays a significant role in the detoxification of triggered oxygen that was acting as an antioxidant by reducing superoxide, hydroxyl radicals and hydrogen peroxide or by reducing singlet oxygen.[26,27]

In this study, rose hips of selected species were investigated while concerning the content of vitamin C

they contain. The determination of the content of vitamin C was proceed by using two methods spectrophotometric and titrimetric. The titrimetric method was based on iodometric titration that was mostly used for the plant material analysis. This method was generally preferable when high concentration of vitamin C is considered.[28-29] An accurate and specific determination of the nutrient content in plants is tremendously vital in order to understand the relation between nutrient intake and human health, for this reason important restraints should be taken in the employment of the methods that have been established for the analysis of specific plants.[30-31] For the determination of ascorbic acid in plant materials several spectrophotometric methods have been reported (Bajaj and Kaur, 1981; Klein and Perry, 1982).[32] The spectrophotometric method used in this research is based on the measurement of decreasing absorption at λmax = 665nm intensity of methylene blue (MB+) that reduces to the colorless leucomethylene blue (CMB+). This method is formerly used in the determination of vitamin C in hawthorn fruits under some optimized conditions (Tahirovic et al., 2012).[33] The indirect spectrophotometric determination of ascorbic acid reaction occurs when added in increasing amount consume (MB+) and decreasing the concentration of (MB+). The results found the decrease in absorption linearly with the increase in concentration of ascorbic acid. The calibration graph was drawn by plotting the absorbance in contradiction of ascorbic acid concentration and then the amount of ascorbic acid obtained from referring this calibration curve. The obtained curve was linear in the concentration range between 5×10^{-6} – 1.25×10^{-4} mol/L for ascorbic acid standards. Calibration curves were also obtained for the six cultivars samples with the following correlation factor of r^2 = 0.999, 0.9998, 0.999, 0.9959, 0.9981 and 0.9916 respectively. For the comparison of the results attained the iodometric method was used as a reference method and these results were articulated in mg AA per 100 g of the dry sample. As the results of the six different varieties of rose hip were 2405, 7406, 9261, 2007, 5104 and 5020 mg/ 100 g of dry weight of sample respectively. The averaged results of the two methods were found not significantly different by applying paired student t-test at 95% confidence level.

CONCLUSION

The aim of this research engrossed on the isolation and determination of AA content in different roses cultivars founded and collected from the University of Balochistan, Quetta were examined. The scientific approval also supports the intake of vitamin C during unadorned and chronic ailment for falling the risk of diseases. The National Research Council also recommends intake of 75 mg per day of vitamin C as a minimum compulsion. This research study revealed that the high content of vitamin C is present in different varieties of rose hip fruits. These are safe to use as a consumption of vitamin C and therefore are not at risk. The accurate and precise evaluation of vitamin C is necessary for human body. The results found in this study will be supportive for the intake of vitamin C from the rose hip fruit for cure of several ailments. For the determination of ascorbic acid content in rosehip was built on the spectrophotometric method reaction takes place between methylene blue and ascorbic acid. The results obtained were associated with the iodometric determination and results shows good pact. In this research first data were given for the content of AA in six different varieties of roses. The higher content of vitamin C was found in sample 3. The content of AA in rose hip is much higher than founded in plants by other investigations. According to our knowledge, this is the first time the content of vitamin C was investigated in different varieties of roses (rose hip fruit) in Balochistan. The results obtained from this study will help to comprehend better about the influence of the vitaminCc that can be useful for the pharmaceutical, cosmetic, food and many more industries in the development of new inventions.

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