Analysis of Vitamin C in Selected Medicinal Plants

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Summary: Vitamin C or ascorbic acid, a very useful constituent of redox mechanism is used in medicine and also added in manufactured food for anti-oxidation. A UV-spectrophotometric method was used for the determination of Vitamin C in 4 different medicinal plants. High amount of Vitamin C 160 mg/100 g was found in *Citrulus colcocynthis*, followed by *Hippophae rhamonides* oil 136.1 mg/100g. A relatively low concentration of Vitamin C was recorded in *Glycyrhiza glabra* 56.2 mg/100g and *Withinia somnifera* 51.50 mg/100 g. The presence of high concentration of Vitamin C in selected medicinal plants might be responsible for their therapeutic effects and uses in the traditional system of medicine

Introduction

Vitamin C or Ascorbic acid a water soluble vitamin is widely required in the metabolism of living being. Human body can not produced ascorbic acid nor synthesized during metabolic process [1, 2], and so must be obtained entirely through one's diet. Thus it is necessary to be constituent of the alimentary diet because it participates in the redox mechanism, allowing the hydrogen transport in the cellular respiratory chain level. According to US minimum daily requirement of 60 mg ascorbic acid per 250 mL container is required [3]. Vitamin C has been widely used in the pharmaceuticals, chemicals, cosmetics, and food industries by its bioactivity and antioxidant properties.

Its deficiency provokes fatigue and debility against blood vessels, teeth, bones, some ascorbate indication, difficult the hurt cicatrisation, the growth, the reproduction and the lactation [4-6].

Vitamin C is used as medicine and also added in manufactured food for anti-oxidation to conserve the product for a long time [7]. However, the excess of vitamin C can cause gastric irritation and diarrhoea, giving as metabolic product, the oxalic acid, which can cause renal problems [1, 8]. Thus, vitamin C determination is very important for various sectors of food and pharmaceutical industries.

For this purpose various methods have been developed for the analysis of ascorbic acid in pharmaceutical formulations, fruit juices, urine, plasma, *etc.* These methods include UV [9], fluorimetry, titration, *etc.* [10, 11]. Tulley has invented a UV method for the analysis of ascorbic acid [12] but its procedure is complicated and it only applies to the analysis of plasma samples. Recently, Kwakye [13], has developed a UV method for analysing ascorbic acid in commercial tablets by adding thiosulphate (0.04 % w/v) to the analysis of ascorbic acid in the multivitamin-mineral formulations containing interfering copper [9].

The oxidation of ascorbic acid during the sample preparation has caused much attention [9, 14] especially in the presence of Copper (II). It has been determined that 50 % vitamin C recovery obtained in those samples containing 0.2 ppm of Copper (II) concentration in the aqueous solution of vitamin C [9]. To date, however, no systematic research has been carried out regarding Copper (II) concentrations on the oxidation of ascorbic acid in aqueous solution. Also, there has not been any quantitative study on the oxidation of ascorbic acid during the sample preparation. Vitamin C degrades quickly and therefore there is special concern regarding the shelf life of these fortified foods. Consequently there has been considerable interest in alternative methods of determining the ascorbic acid content of food products.

In the present study, efforts have been made to analyse different types of medicinal plants and their parts for their vitamin C contents. The study will be very useful for providing a scientific data base besides knowing the amount of vitamin C. These medicinal plants are responsible for the therapeutic effects and their use in traditional system of medicine.

Results and Discussion

The solubility of vitamin C (ascorbic acid) in aqueous solution was determined by four factors *i.e.* pH, oxygen, time and temperature. All the solutions for the study were prepared in 0.05 M

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oxalic acid. These solutions were stored in volumetric brown flasks.

Table-1: Vitamin C Contents of the Selected Herbs.

Plant Species	Parts used	Vitamin-C, Content (mg/100g
Withina somnifera	Roots	51.50
Citrulus colcocynthis	Fruits	160.4
Hippophae rhamonides	Oil	136.1
Glycyrrhiza glabra	Roots	56.2

The acidity of ascorbic acid is based on "enol" group ionization and from C^3 and C^2 atoms, with their pKa values 4.17 and 11.57, respectively [15]. The undissociated ascorbic acid present in solution with pH lowers than 2 has maximum absorbance at 243 nm. At pH 4 above, 50 % of the molecules were dissociated and a maximum absorbance obtained at 250 nm. While from pH 5 to 10, almost all ascorbic acid was completely dissociated [16].

The presence of high concentration of vitamin C in selected medicinal plants might be responsible for their therapeutic effects and uses in the traditional system of medicine [17].

As in our samples, the concentration of vitamin C varies which may be due to the following facts.

1) The stability of vitamin C in aqueous solution.
2) Sensitivity towards heat and light.
3) In solution vitamin is oxidized due to dissolved oxygen.

As it is already discussed that the medicinal plants samples have high vitamin C contents which support the conclusion of the works of Suner *et al*., [19] in which he concluded that the ascorbic acid is stable in solid form but oxidized in solution by dissolved oxygen according to the equation.

The active parts of the selected medicinal plants used for the analysis of vitamin C contents showed variable amounts of the ascorbic acid, therefore the study carries important information's beside other therapeutic effects of the analysed medicinal plants. This study also provides a scientific data base along with the reported constituents isolated from the medicinal plants.

Experimental

Reagents

All the reagents used were of analytical grade

i. HPLC grade methanol was purchased from E. Merck Germany.

ii. USP grade ascorbic acid reference standard (RS) was purchased from May and Baker Ltd England.

Double distilled water was used throughout the experiment.

Instrument

A Hitachi UV-Vis Spectrophotometer model U-2000 (Japan), with a 1.0 cm optical path quartz cell was used for spectrophotometer measurements.

Sampling

Four different medicinal plants samples were after collection were dried under shade and then processed. All the medicinal plants were obtained from Medicinal Botany Centre (MBC) PCSIR Labs Complex, Jamrud Road, Peshawar, KPK, Pakistan for estimation of their vitamin C contents.

Preparation of Stock Solutions

Ammonium Molybdate (5 %m/v) Solution

Weigh 5 g of ammonium molybdate and dissolve it in 100 mL of distilled water.

Oxalic Acid (0.05 M) Solution

Weigh required quantity of oxalic acid, freshly prepared solution containing 0.02M, EDTA and then make up the volume 100 mL with distilled water.

Sulphuric Acid (5 % v/v) Solution

Weigh 5 mL of concentrated sulphuric acid and make up the volume 100 mL with distilled water.

Meta Phosphoric Acid with Acetic Acid Solution

Dissolve with shaking required quantity of *meta* phosphoric acid pellets in required quantity of acetic acid and then make the volume 100 mL with distilled water.

Standard L-Ascorbic Acid (0.1 % w/v) Solution

Weigh 0.1 g of L-ascorbic acid and dissolved in oxalic acid (0.05 M) solution freshly prepared and make up the volume 100 mL.

Preparation of Different Standard Solution

Take 0.5, 1, 2, 3, 4, and 4.5 mL of standard L-ascorbic acid (0.1 % w/v) solution in separate 25 mL volumetric brown flasks added 4.5, 4, 3, 2, and 0.5 mL of oxalic acid (0.05 M) solution in each volumetric brown flask. Then add separately *meta* phosphoric acid with acetic acid 0.5 mL, sulphuric acid (5 % v/v) solution 1 mL and ammonium molybdate solution 2 mL in each volumetric brown flask and make up the volume 25 mL with distilled water.

Preparation of Sample Solutions

Accurately weighted 1 g of each sample in a 25 mL conical flask, add 10 mL of oxalic acid (0.05 M) solution and placed the samples for 24 h.

After 24 h, the samples were filtered through a 0.45 µm filter paper. Then 2.5 mL of each sample was transferred to a separate 25 mL volumetric brown flask, 2.5 mL of oxalic acid (0.05 M) solution. Then added separately *meta* phosphoric acid with acetic acid 0.5 mL, sulphuric acid (5 % v/v) solution 1 mL and ammonium molybdate solution 2 mL in each volumetric brown flask and make up the volume 25 mL with distilled water.

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