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UV Spectrophotometric Analysis Profile of Ascorbic Acid in Medicinal Plants of Pakistan

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Abstract: There is a growing interest in the food industry and in preventive health care for the development and evaluation of natural antioxidants from medicinal plant materials. Ascorbic acid (Vitamin C) an important precursor of redox mechanism is used in medicines and food products. On account of its anti oxidant a property, UV-spectrophotometric procedure was applied for the determination of this phytochemical in 29 different medicinal plants of Pakistan. The procedure showed variable occurrence of the ingredient in the selected herbs. High yield was detected in the herb *Jasmine nudiflorum* (202.74 mg/100 g), *Psidium guajuava* (145.75 mg/100 g), *Cupressis sempervirens* (141.43 mg/100 g) whereas low yield was found in *Zyzipus jajuba*, (19.21 mg/100 g) *Lactuca sativa* (22.07 mg/100 g) and *Conyza japoniea* (24.49 mg/100 g). The others have concentrations between 26.99 and 134 29 mg/100 g. The main purpose of the study was to quantify vitamin C contents in medicinal herbs utilizes as a potential source of ascorbic acid.

Key words: Medicinal plants · Ascorbic acid · UV Spectrophotometer

INTRODUCTION

Ascorbic acid, a water soluble vitamin has been implicated in the biological processes by regulating metabolic reactions. Human body cannot produce nor synthesize the precursor [1] and acquired from exogenous sources. According to US standards minimum daily requirement of 60 mg per 250 mL of ascorbic acid is required [2]. Ascorbic acid has been widely used in the pharmaceutical applications, chemicals, cosmetics and food industries due to bioactivity and antioxidant properties.

Natural ascorbic acid is vital for the body performance. Lack of ascorbic acid impairs the normal formation of intercellular substances throughout the body including collagen, bone matrix and tooth dentine [3]. Apart from catalytic role, ascorbic acid also increases the organism resistance against microorganism and participates in the antibody formation. Problems associated with its deficiency include fatigue and debility against blood vessels, teeth, bones, some ascorbate indication, hurt cicatrisation, growth, reproduction and lactation [4-6].

The oxidation of ascorbic acid during the sample preparation has received much attention [7, 8] especially in the presence of Copper (II). It has been determined that 50% ascorbic acid recovery obtained in those samples containing 0.2 ppm of copper (II) concentration in the aqueous solution of ascorbic acid [7]. For finding solution to problems linked with quantification of ascorbic acid, 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.

For estimation of ascorbic acid in pharmaceutical formulations, fruit juices, urine, plasma, various methods have been reported which include UV [7], fluorimetry, titration, etc. [9, 10]. The HPLC and fluorimetry methods have been demonstrated with good sensitivity and

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specificity, but their implementation requires specialized equipment, reagents and standards. In recent years Kwakye [11] UV method for analysing ascorbic acid in commercial tablets in the presence of multivitamin-mineral formulations containing interfering copper [12] has been reported.

In the present study, investigations have been carried out to analyse different types of medicinal plants for their ascorbic acid contents and generate a scientific database for public awareness about the maximum or minimum concentration in medicinal plants. These finding will be useful for the users to know the daily uptake of Vitamin C while utilizing medicinal plants.

MATERIALS AND METHODS

Reagents: All the reagents used were of analytical grade. HPLC grade methanol was purchased from E. Merck Germany. 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: Certified and verified specimens of medicinal plants were obtained from Medicinal Botanic Centre (MBC) (PCSIR) Labs, Peshawar Khyber Pakistan.

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 up to 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 1mL and ammonium molybdate solution 2 mL in each volumetric brown flask and make up the volume up to 25 mL with distilled water.

Preparation of Sample Solutions: Accurately weighted 1g of each sample in a 25 mL conical flask, add 10 mL of oxalic acid (0.05 M) solution and the samples was placed under shade for 24 h for extraction of Vit C contents.

After 24 h, the samples were filtered through a 0.45μm 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 1mL and ammonium molybdate solution 2 mL in each volumetric brown flask and make up the volume 25 mL with distilled water.

Each sample was then analysed for Vitamin C at 760 nm compared with the standard one [13].

RESULTS

Ascorbic acid concentration from 29 different medicinal plants was determined with UV-Visible spectrophotometer. It can be seen from the Table 1 that the high contents of Vitamin C were found in *J. nodiflurum, R. communis, C. sempervirens, R. alucolatus, C. nocturnum, F. esculantum* as 202.74, 145.74, 141.43, 45.48, 91.144 and 134.80 mg/100g DW(dry weight). While moderate value of Vitamin C was found in *R. aluculatus, C. noctornum, C. fistula, R. indica, O. latifolia, E. heliscopia, T. stanz, F. parviflora, N. tazetta* and *O. europea* as 95.55, 91.14, 85.55, 82.12, 74.71, 72.47, 71.99, 69.29, 65.19 and 64.84 mg/100g respectively However very low concentration of ascorbic acid was recorded for *S. media, G. tricorne, F. bengumera, V. thapsus, P. forum, C. chrysthanum, T. officinale, P. crispa, B. ciba, L. sativa and Z. jajuba* as 48.65, 47.15, 45.16, 30.33, 39.43, 33.91, 35.05, 26.99, 69.29, 22.06 and 19.2 mg/100g respectively.

Table 1: Concentration of Vitamin C in Medicinal Plants mg/100g

Name	Absorption	Concentration
Jasmine nudiflorum	1.864	202.74
Riecinus commnis	1.322	145.74
Ficus bengumera	0.362	45.169
Ficus pomela	0.338	42.634
Policaria crispa	0.188	26.988
Narcissus tazetta	0.553	65.199
Fagophyrum esculantum	1.216	134.80
Cassia fistula	0.747	85.548
Cherry chrysthanum	o.255	33.913
Ruscus hypophylum	0.524	62.202
Taraxicum Officinale	0.266	35.052
Verbascum Thapsus	0.221	30.339
Rumax aluculatus	0.365	45.489
Fomaria parviflora	0.592	69.297
Bombax ciba	0.143	69.297
Stellaria media	0.395	48.653
Pectus forums	0.307	39.432
Olea europea	0.549	64.839
Rosa indica	0.714	82.116
Euphorbia heliscopia	0.622	72.473
Oxalis latifolia	0.644	74.714
Castrum nocturnum	0.800	91.144
Pridium guajava	1.321	145.75
Cupressis sempervirens	1.280	141.43
Withinaia somnifera	0.361	45.029
Galium tricorne	0.381	47.154
Tecoma stonz	0.618	71.999
Lactuca sativa	0.142	22.067
Zizyphus jajuba	0.115	19.211

Table 2: Different Standard Solutions and Their Concentrations At (mg/100g)

S.No	Absorption	Concentration
1	0.3	40
2	0.62	80
3	1.03	120
4	1.52	160

DISCUSSION

As in our samples, the concentration of ascorbic acid varies which may be due to the following facts such as stability of ascorbic acid in aqueous solution and sensitivity towards heat and light. In solution, vitamin is oxidized by dissolved oxygen.

As it is already discussed that the medicinal plants have high ascorbic acid contents which support the conclusion of the work of Suner *et al.* [1, 13] who concluded that ascorbic acid is stable in solid form but oxidized in solution by dissolved oxygen.

The solubility of ascorbic acid in aqueous solution was determined on the basis of four factors i.e pH, oxygen, time and temperature. 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 [14]. The undissociated ascorbic acid present in solution with pH lowers than 2 has maximum absorbance at 243 nm. At pH above 4, 50% of the molecules were dissociated and a maximum absorbance obtained at 250 nm. While from pH 5-10 range, almost all ascorbic acid was completely dissociated [15].

During analysis of ascorbic acid some factors such as temperature, solvent, pH, light and metal ions (Cu^{+2} and Fe^{+3}) degrade the ascorbic acid [13, 16]. With increasing Cu (II) concentrations, the rate of oxidation of ascorbic acid obviously increases which demonstrates that copper (II) can greatly accelerate the oxidation of ascorbic acid in aqueous solution [7].

The mean concentration of copper (II) in drinking water is 60 µg / L (0.06 ppm) [16]. As it is already known that water is comparatively abundant in fruits and vegetables than medicinal plants. This might explain the reason why the ascorbic acid oxidizes quickly in fruits.

CONCLUSION

Our results showed that the samples species were rich in ascorbic acid constituents, which demonstrated that the screened species might have good antioxidant activity and could be a good source of natural antioxidants. The UV spectrophotometric method enabled the identification of a wide range of ascorbic acid present in medicinal plants without time-consuming sample preparation. Therefore, the quantitative analysis of ascorbic acid in the species could be helpful for explaining the relationships between total antioxidant activity and total ascorbic acid content of the plants. Obviously, to confirm the beneficial effects of these plants, it is necessary to carry out further studies about their in vivo activity and bioavailability.

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