Estimation Some of Metal Ions and Biological Constituents of Local Rheum Ribes (Rhubarb) of Kurdistan Region - IRAQ

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Abstract

It is an attempt to determine the levels of (1) minerals by using ICP spectrophotometer; (2) the fatty acids, by using (HBLC) chromatography technique; (3) vitamin C by using Redox titration method. Levels were found to be as bellow:

1. Metal ions levels were found to be gradually decreased from monovalent to divalent ions, whereas the levels of biological constituents were varied and resulted in :

Ash(fibers) = % 6.702 **,** Water %86, Linolenic acid 39.08 µg/ml , α- Linoleic acid 31.32 µg/ml, Arachidic acid 12.13 µg/ml, Erucic acid 4.1 µg/ml and Vitamin C 4.83 µg/ml.

2. As shown the levels of fatty acids were decreased with increasing unsaturated.

Keywords: metal ions, fatty acids, and ascorbic acid in Rheum Ribes (Rhubarb).

1. Introduction

Rhubarb (Rewas) as known in Kurdish, is a wild, edible and natural plant (Sabir, 2000). It is considered as a medicinal plant and used freshly for human consumption. Rhubarb can be eaten as fruit. While the leaves are toxic, the stalks are used in pies and other foods for their tart flavor. It mostly grows in spring season under the snow of the high mountains in the north and centre Asia. Its scientific name is Rheum which is a genus of about 60 perennial plants in the family Polygonaceae. The genus includes the vegetable rhubarb (Rheum rhabarbarum or Rheum x hybridum.) (Nieboer and Richardson,1980). The species have large somewhat triangular shaped leaves with long, fleshy petioles. The flowers are small, greenish-white to rose-red, and grouped in large compound leafy inflorescences, with long stem of about (15-40) centimeters. A number of varieties of rhubarb have been domesticated both as medicinal plants and for human consumption. Among its medical advantages, it can be used for the treatment of acidity in stomach and address constipation. In addition, it kills small worms inside intestine and helps the liquidation in human's liver (Chakravarty and Al-Rawi, 1964).

2. Aims

This paper aims to find out the chemical components of Rhubarb which is an edible, wild and natural plant collected from the mountains surrounding sulaimani city Kurdistan-Iraq.

3. Materials and Methods

3.1 Part I: Determination of Humidity & Ash ratio of the Sample

To estimate the amount of humidity (water) in Rhubarb, a specific weight of fresh Rhubarb was taken and dried by exposure to sunlight. After drying, it has been found that the ratio of humidity in Rhubarb is approximately 86 %.

To find out the ratio of ash, a sample of (5gm) of dried Rhubarb was taken and burned by Bunsen burner, then it was placed in muffle furnaces at 750 C° for 6 hrs. The sample was weighed after cooling in the room temperature, later on it was placed in the desiccators, finally, the ratio of humidity & ash and PH were calculated and found as follows (Martial et al., 2006).

3.2 Part II: The Estimation of Trace Elements By Using ICP-OES Method

2ml of HNO3 conc. is added to the dried and burned sample of the part I and added to D.W to dissolve the sample then the sample is filtrated. The filtrated sample was put in a volumetric flask and completed by D.W to 100ml. The sample was analyzed by using ICP Spectrophotometer instrument (Fallah et al.,2011; Frentiu etal.,2007; Ghanjaoui etal. 2011) and the concentration of element constituent of the Rhubarb was found (Gomez et al., 2007; Margui et al, 2005) as shown in below table (1).

3.3 Part III: RP-HPLC determination of Fatty Acids

Fatty Acids were separated on FLC (Fast Liquid Chromatographic) column, supelcosil LC -18, 3µm particle size(50 × 4.6mm ID)column,

Mobile phase: acetonitrile:aceton (59:41,v/v)

Detection: refractive index detector LC6-RID
Flow rate 1.0ml/min
Injection: 20µl saturated and unsaturated FAME fatty acid methyl ester
Extraction of the sample by using hydro distillation method:
Hydro Distillation Extraction method was used for extraction of essential oil. 1gm of crushed Rhubarb (rewas) were immersed in water then being boiled, the hot water helped to release the aromatic molecules from the plant, the molecules of these volatile oils evaporated. The steam was condensed in to liquid form, the oil was separated from water.
Fatty Acid Methyl Ester Preparation FAME:
Following the procedure adopted by Christie (1993), FAME was prepared by using 50 ml of oil sample dissolved in 950 ml hexane, then transmethylated using sodium methoxide. Utilizing the HPLC method, the oil is analyzed to find out its constituents, according to reference analysis procedure (Mcclements, 2007; Nikolova-Damyanova, 1997; Pettinella et al, 2006; Borch et al., 1975).
The concentration for each compound was quantitatively determined by comparison of the standard peak area with that of the sample.

Calculation

$$\text{Concentration of the sample} = \frac{\text{area of the sample}}{\text{area of the standerd}} \times \text{conc.of standard} \times \text{dilution Factor}$$

3.4 Part IV: Determination of Vitamin C:

To determine qualitatively and quantitatively the ratio of vitamin C in plants, there are various methods. The method adopted is redox titration (known as self-indicator) method which depends on the reduction of ascorbic acid by 2,6-Dichlorophenolindophenol (DCIP) (Hernández, et al., 2006).
The DCIP standard solution was prepared by dissolving 0.0245 gm of 2,6-dichloroindophenol in water and completed to 100 ml in volumetric flask and put it in burette. 50gm of juiced rhubarb was completed to 200 ml of D.W in a conical flask; 10 ml of the sample was taken, added to 5ml of dil. sulphuric acid and heated to 60C°. Later on the titration process was carried out.

Calculation

Determination of vitamin c by redox titration with 2,6-dichloroindophenol(DCIP):
Molecular weight=268.1gm/mole
Concentration=9.14*10-4mole/L
No. of mmole DCIP=No. of mmole ascorbic acid
(M×V)= (mg /M.wt) asc.
Mg (asc). = (M×V) ×M.wt asc.
Mg (asc). = $9.14\times10^{-4}\times1.5\times176.12\times200/10$
Results
% Ash (fibers) = 6.702 % **,** water = 86% **,** Vitamin C 4.83mg.

Table (1): Concentrations of metal ions in Rhubarb (ppm)

Elements	Concentration (ppm)
Co	0.006
Cr	0.0 26
Mo	0.031
Mn	0.346
Zn	0.410
Cu	1.833
Fe	5.072
Ca	65.63
Mg	73.040
Na	84.025
K	1186.5

Table (2): Types of fatty acids and their concentrations in Rhubarb

seq	subject	Retention time minute	Area	Concentration 25µg/ml
1	Linolenic acid	3.81	50850	39.08
2	α- Linoleic acid	4.96	63964	31.32
3	Arachidic acid	6.11	45272	12.13
4	Erucic acid	7.13	47624	4.1

4. Discussion

4.1 Aqueous layer

As %86 of the plant is water and % 6.72 fibers, the Rhubarb helps to decrease the constipation of intestines. In addition to that, its pH is 5.59, so it can be regarded as a weak acid and acceptable to be consumed by humans.

4.2 Metal Ions content

Metal ions are important for catalytic activity approved in plants. The significance of Rhubarb is rooted from the minerals of which it consists. Minerals which are inorganic nutrients, usually required in small amounts from less than 1 to 2500 mg per day, depending on the mineral, in addition to vitamins and other essential food nutrients. Metal ions levels were found to be gradually decreased from monovalent to divalent. The trace elements are essential components of enzyme systems and have affected the metabolism and tissue structure. Therefore, they have important roles in health and diseases. Understanding the metal ion content of plant mitochondria and metal ion interactions with the proteome are vital for insights into both normal respiratory function and the process of protein damage during oxidative stress (Tan YF et al., 2010). A selective change occurs in mitochondrial copper and iron content following in vivo and in vitro oxidative stresses, Mitochondrial respiratory chain pathways and matrix enzymes varied widely in their susceptibility to metal-induced loss of function, showing the selectivity of the process. Transition metal ions are essential in myriad biochemical functions by being incorporated into or associating with proteins to elicit functions in living cells. In plant mitochondria, key functions of metal cofactors include metabolism, electron transport, ATP synthesis, and the detoxification of reactive oxygen species (ROS). For example, copper (Cu) and iron (Fe) ions facilitate the transfer of electrons in the electron transport chain (Pascal and Douce ,1993), proteins of the tricarboxylic acid (TCA) cycle utilize metal ion cofactors to catalyze primary metabolic reactions (Jordanov et al. 1992), manganese (Mn) and Fe are required for antioxidant defense enzymes (Alscher, et al., 2002), and zinc (Zn) is required for the protein import apparatus in both carrier protein transport to the inner membrane(Lister et al., 2002) and presequence degradation(Moberg et al., 2003). Cobalt (Co) is known to substitute for other metal ions in the activation of NAD-malic enzyme and succinyl-CoA ligase from plant mitochondrial extracts (Macrae, 1971), but it is not known whether there is an in vivo requirement for trace amounts of Co for plant respiratory metabolism. It was proved that redox-inactive heavy metal cadmium exhibits strong affinity for oxygen, nitrogen, and sulfur atoms (Nieboer and Richardson, 1980) and can inhibit enzyme activity by direct blocking of protein function or displacement of natural metal centers. Murray et al., 1998) found that the hammerhead ribozyme does not require divalent metal ions for activity if incubated in high (> or =1 M) concentrations of monovalent ions. The exchange-inert metal complex Co $(NH3)3^{+}$ also supports substantial hammerhead activity. These results suggest that a metal ion does not act as a base in the reaction, and that the effects of different metal ions on hammerhead cleavage rates primarily reflect structural contributions to catalysis (DEMARTY et al., 2008).

It was reviewed that the interactions between calcium ions and cell walls play a key role in plant physiology. Calcium ions are involved in many mechanisms: for example, stabilization of cell wall structures, acidic growth, ion exchange properties, control of the activities of wall enzymes. All these properties originate from the tight binding of calcium ions to the pectins present in the cell walls. The factor most important for controlling wall behavior is the density of non-diffusible charges and, due to its high affinity; calcium can significantly affect this factor. We also discuss the theoretical ion exchange models in relation to the sRNA (DEMARTY et al., 2008).

4.3 Vitamin -C

Ascorbic acid (vitamin C) has well-documented roles in many aspects of redox control and anti-oxidant activity in plant cells. This Botanical Briefing highlights recent developments in another aspect of l-ascorbate metabolism: its function as a precursor for specific processes in the biosynthesis of organic acids. Our understanding of l-ascorbate metabolism, is to cover biosynthesis, translocation and functional aspects. The role of l-ascorbate as a biosynthetic precursor in the formation of oxalic acid, l-threonic acid and l-tartaric acid is described, and progress in elaborating the mechanisms of the formation of these acids is reviewed. The potential conflict between the two roles of l-ascorbate in plant cells, functional and biosynthetic, is highlighted.The presence of an enough amount of ascorbic acid in ruherb which is an active antioxidant, acting as a reducing agent to reverse oxidation in liquids (Debolt et al., 2007). Recent advances in the understanding of l-ascorbate catabolism and the formation of oxalic and l-tartaric acids provide compelling evidence for a major role of l-ascorbate in plant metabolism. Combined experimental approaches, using classic biochemical and emerging 'omics' technologies have provided recent insight to previously under-investigated areas.

4.4 Fatty acids

Levels of fatty acids were decreased with increasing unsaturated. The existence of some fatty acids as mentioned above has its specific significance. For instance, Gamma Linolenic Acid (GLA) is needed for important bodily functions such as the maintenance of hormonal balance and healthy skin structure, Arachidonic Acid (AA) which is found in the membrane s of nerves helps to transmission of messages in the central nerve system. Thus, the storage of oils is a generalized characteristic in higher plants, which has the main function of serving as an energy source to the embryo during the heterotrophic stage (Debolt et al., 2007). Previous to the activation of the photosynthetic machinery (Ovando-Medin, 2010), such a stage is fundamental in the success or the failure of the embryo to germinate, emerge and establish as a new plant (Bewley and Black, 1994).

The patterns in the total content and in the fatty acids composition of the oil among species are differing in habit, habitat and relatedness. Hypotheses have been proposed to explain the patterns found. Oils are formed by an extension of the membrane-lipid biosynthetic pathway common to all plant tissues (Voelker and Kinney, 2001), but in contrast to membrane lipids, there is great fatty acid diversity. The acyl chains of fatty acid range from 8 to 24 carbons, varying in degree of saturation (number of double bonds), spatial arrangement (*cis*, *trans*) and in functional groups. Many plant species accumulate seed oils with unusual fatty acids. A huge amount of information is available on fatty acids diversity in plants, but the main interests are centered in the search for fingerprints useful in plant taxonomy (Sharma, 1993)and in the improvement for high accumulation of seed oils and for the production of novel(unusual) fatty acids or those industrially important (Hosamani and Katagi, 2008).Molecular biology tools are enabling to scientists to elucidate biochemical mechanisms implicated in the fatty acid diversity.

5. Conclusion

The purpose behind shedding light or selecting Rhubarb is that it is a natural plant growing abundantly in mountainous areas in spring time. Because of its use as fruit or its significance in medical treatments, the paper tries to find out the chemical components and nutrient values of Rhubarb.

6. Acknowledgements

Firstly, I would like to express my deep gratitude and appreciation to Dr. Hamid Ghaffuri at Baghdad University for his revision and efforts in writing this paper. My thanks are also due to both the head of Chemistry department, School of Science and the presidency of the University of Sulaimani for supporting and funding this project.

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