



ISSN 2278-4136
JPP 2014; 3 (2): 221-224
Received: 13-06-2014
Accepted: 25-06-2014

Namam S. Al-Talabani
Chemistry Department, Faculty of
Science, University of Sulaimania, Iraq

Nazk M Aziz
Chemistry Department, Faculty of
Science, University of Sulaimania, Iraq

Vian M Saleh
Chemistry Department, Faculty of
Science, University of Sulaimania, Iraq

Investigation of biochemical constituents of Rhubarb (rewaz) extract in Iraq/Kurdistan region

Namam S. Al-Talabani, Nazk M Aziz, Vian M Saleh

Abstract

Rhubarb grows well along the coast and in cool sections of the central vally, it is an important medicinal herb have been shown to have a range of bioactivities relevant to human health. This study aims to find out the biochemical components of rhubarb which is anedible, wild and natural plant collected from the mountains. Surrounding Sulaimani City Kurdistan-Iraq. These components include protein, carbohydrate, sterol and organic acids. The ratio of nitrogen determined by using Kjeldahl method to estimate the amount of protein. The carbohydrate, organic acids and sterols determined by using HPLC. Levels of carbohydrate detected, show that the amount of monosaccharide's higher than the disaccharides ,and the percent of protein is 16.311%,organic acids were found to be decreased with decreasing molecular weight except for formic acid which is volatile and it was found to be higher. Our results clarify the biochemical composition of rhubarb, we found some types of sterol (stigma sterol 2.63 µg/ml, β-sitosterol 16.73 µg/ml, campe sterol 19.77 µg/ml and avenno sterol). we suggest that different biochemical constituents be used separately in clinical practice

Keywords: *Baphia pubescens*, marc, brewer's yeast, rotary evaporator.

1. Introduction

Rhubarb (rewash) as known in Kurdish is one of the most well-known and frequently used herbal medicines for the treatment of constipation, inflammation and cancer ^[1, 2]. Most of the published chemical investigations of the rhubarb plant have dealt chiefly with the organic acids present in high proportion in the freshly petiol or with the substances in the root responsible for the medicinal properties which have been held in high esteem from early antiquity ^[3].

Rhubarb has been clinically used for at least 2000 years as an antibacterial or laxative agent, the extensive phytochemical research on rhubarb has led to the isolation and identification of about 200 chemical compounds. It is known that genetic and environmental factors and their interactions affect the pharmaceutically important secondary metabolites in medicinal plants ^[4].



Correspondence:
Namam S. Al-Talabani
Chemistry Department, Faculty of
Science, University of Sulaimania,
Iraq

HPLC have been commonly used for the separation and determination of active components of rhubarb ^[5, 6]. Based on the content of biochemical compounds in rhubarb, pharmacologist carried out the research on the effect of different factors, for example temperature, sunlight and precipitation ^[7].

Rhubarb contains several nutrients including vitamins A and C, thiamin, riboflavin, niacin, potassium and phosphorus. 250 ml (1 cup) raw rhubarb contains 20 kilocalories (rhubarb is well known for its high quality fiber) [8].

Among its medical advantages, rhubarb can be used for the treatment of acidity in stomach and address constipation. In addition, it kills small worms inside intestine and help the liquidation in human's liver [9].

Rhubarb constituents showed extensive pharmacological activities including cathartic, diuretic, anticancer and hepatoprotective effects, as well as toxicological affects [10].

2. Materials and Methods

Part I: Finding out the Percentage of Nitrogen and Estimating the Ratio of Protein

To estimate the amount of protein in Rhubarb (rewas), the kjeldahl method [11] was used for determination of the ratio of Nitrogen which was the process of weighing 0.2 gm of the dried and ground Rhubarb in a glass bottle (50 ml), 4 ml of H₂SO₄ and 1.5 ml of concentrate Perchloric acid were added, then the bottle was set in a sand bath for 3 hours. After undergoing digestion, the volume was completed to 50 ml of distilled water, then the solution was distilled by using kjeldahl method and receptor reagent boric acid H₃BO₃. This was done by taking 10 ml from the digested substance and putting it in the distillation flask, 10 ml of NaOH was added (%30), then 5 ml of receptor reagent, which was boric acid, is added. The process of distillation continues until the color of the reagent changes from purple to green. Later on, the solution is titrated with H₂SO₄ (0.01 N). As a result the amount of Nitrogen is determined, the

ratio of protein is estimated by the application of the following two correlative equations [11, 12].

$\%N = n \times v \times 1.4 / \text{weight of the sample}$,

n represents normality of H₂SO₄

V represents volume of H₂SO₄

$\% \text{ Protein} = \%N \times 5.7$

Nitrogen: % 2.861

Protein: %16.311

Part II: Analysis of carbohydrate in Rhubarb

The aqueous extract carbohydrate was separated by FLC (Fast Liquid Chromatographic) under the following optimum conditions [13]:

Column: 5 μ m particle size (150 \times 4.6 mm I.D) NH₂ column,

Mobile phase were acetonitrile: water (75:25V/V)

Detection Refractive index RI detector

Flow rate 1.5 ml/min.

Injection volume: 50 μ l

Preparation of the sample:

10gm of the sample was weighed, then dissolved in 50ml HPLC grade methanol: water (50:50 V/V), the sample was shaken and agitated in Ultrasonic bath for 10 minutes, filtrated, and then concentrated by evaporating the solvent with a stream of liquid N₂ until reached nearly 0.5 ml, then added some of mobile phase to reach 1 ml, filtration in whatman filter paper 0.25 μ m. Later on, 20 μ l was injected on HPLC column. The concentration for each compound was quantitatively determined by comparison the peak area of the standard with that of the sample, under the optimum separation conditions.

Calculation

$$\text{Concentration of the sample} = \frac{\text{Area of the sample}}{\text{Area of the sample}} \times \text{conc. of standard} \times \text{dilution Factor} \quad (\mu\text{g/ml}).$$

Part III: Estimation of Sterols

Active ingredient sterols

The extract was separated on FLC (Fast Liquid Chromatographic) column, LC -18, 3 μ m particle size (50 \times 4.6mm ID) column,

Mobile phase: 0.1% acetic acid in deionized water (solvent A) and acetonitrile (solvent B) using linear gradient from 0-100% B in 10 minutes, detection UV set 275nm, flow rate 1.2 ml/min, temperature 30 $^{\circ}$ C .

$$\text{Concentration of the sample} = \frac{\text{Area of the sample}}{\text{Area of the sample}} \times \text{conc. of standard} \times \text{dilution factor} \quad (\mu\text{g/ml})$$

Part IV: Estimation of active ingredient sterols

The extract was separated using FLC (Fast Liquid Chromatography) column, C-18, 3 μ m particle size (50 \times 4.6 mm ID), mobile phase 0.1% acetic acid in deionized water (solvent A) and acetonitrile (solvent B) using linear gradient from 0-100% B in 10 minutes, detection UV set at 275 nm, flow rate 1.2ml/min, temperature 30 $^{\circ}$ C.

Extraction

1 gm was dissolved in 5ml of hot water for two hours, then using Ultrasonic bath for 20 minutes to get all extract

Extraction:

1 gm was dissolved in 5ml hot water for 2 hours, then Ultrasonic bath for 20 minutes was used to get all extract dissolved in hot water 60 $^{\circ}$ C degree, then the extract was filtrated on filter paper no 1-0.5 mm to separate the fibers, then 20 μ l from the sample injected on HPLC system [14, 15].

The sequences of the eluted material of the standard were as follow, each standard was 25 μ g/ml.

dissolved in hot water 60 $^{\circ}$ C, then the extract were filtered on filter paper No. 1.05 mm to separate the fibers. 20 μ l from the sample was injected into HPLC system. Below are the separation conditions.

The sequence for each standard eluted material was 25 μ g/ml.

The separation occurred using liquid Chromatography Shimadzu 2010 LC equipped with binary delivery pump model 2010 Shimadzu. The eluted peaks were monitored by UV-Vis 2010 SPD spectrophotometer.

Calculation

$$\text{Concentration of the sample} = \frac{\text{Area of the sample}}{\text{Area of the sample}} \times \text{conc. of standard} \times \text{dilution factor} (\mu\text{g/ml})$$

Part V: The Separation and extracts of Organic Acids in Rhubarb (rewas)

Column: supelcogel c 610H (30 cm× 7.8 mm ID0 organic acid column)

Mobile phase: 0.1% H₃PO₄

Detection: UV at 210 nm

Flow rate: 1 ml/min

Injection volume: 20 μl

Extraction procedure:

Active ingredients were extracted according to the modified procedure reported by Lakshiminanayana *et al.* (2005), which was the fast liquid chromatographic (FLC, henceforth). The separation has been achieved as described below: 5 gm of the blended sample was extracted with ice-cold acetone until the sample became colorless (final volume 100 ml), then the crude extract (50 ml) was taken in separation funnel, 100 ml of the petroleum ether was added after had been mixed well. Later on, the lower polar layer were separated, the extract were repeated 3 times, total volume (200 ml), the extract was derided by using anhydrous sodium sulphate 20 gm and filtered by passing through Whatman No.1 filter paper [16]. The filtrated resultant was evaporated so as to dry using rotary evaporator (Buchi Switzerland) at 30 °C, and re-dissolved in specific volume of the mobile phase, and aliquot of 20 μl was used for HPLC analysis.

3. Results and Discussion

The mean value of the total protein of the whole leaf was 16.311 while table (1), (2) and (3) shows the amount of carbohydrates, sterols and organic acids.

Table 1: Different types of carbohydrate and their concentrations in Rhubarb.

Seq.	Subjects	Retention time minute	Area	Concentration 25 μg/ml
1	Fructose	1.26	59290	55.71
2	Glucose	2.41	52492	54.17
3	Sucrose	3.35	71975	18.76

Table 2: Sterols and their concentrations in Rhubarb.

No.	Subjects	Retention time minute	Area	Concentration 25 μg/ml
1	Stigma sterol	1.24	111832	2.63
2	β-sit sterol	2.45	96069	16.73
3	Campe sterol	3.09	99143	19.77
4	Avenno sterol	4.27	81605	14.28

Table 3: Organic acids and their concentrations in Rhubarb

No.	Organic acids	Retention time minute	Area	Concentration 25μg/ml
1	Oxalic acid	2.26	784494	89.7
2	Citric acid	3.44	87233	37.29
3	Tartaric acid	4.27	91306	35.75
4	Formic acid	7.34	67869	59.1
5	Acetic acid	8.43	199841	11.33
6	Fumaric acid	9.25	148088	9.38

4. Discussion

Rhubarb is one of the oldest and most frequently used herbal medicines in China, Korea, Japan, and other Asian countries. In the view of far-ranging applications of rhubarb, although many studies on the chemical constituents, quality analysis, pharmacological activities, and clinical practices had been reported, there are still lots of questions waiting to be solved.

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.

Both protein and carbohydrate are found in Rhubarb which are sources of energy for human body. Rhubarb includes several types of Sterols which are ascribed to phytochemical in the class of Phytosterols, belonging to the group of lipids. Therefore, it has these benefits: anti-osteoarthritic, cytotoxic and apoptotic, antimicrobial ameliorating, antibacterial, and anti-inflammatory effects, along with anti-hyperlipidemic, anti-oxidant activities and having anti-genotoxic properties [17].

As its pH is 5.59, so it can be regarded as a weak acid and acceptable to be consumed by humans, Similar results for other products have been attained by George *et al.* and Mahmut Galiskan [19, 20].

To better evaluate the quality of rhubarb, suitable analytical methods need to be developed for the analysis of active ingredients in rhubarb.

5. References

- Xiao P, He L, Wang L. Ethano pharmacologic study of Chinese rhubarb. *J Ethano pharmacol* 1984; 10:275-293.
- Duck JA. Hand book of medicinal herbs. Edn 2 CRC press: Boca Raton, FL, 2002, 621.
- Clupepper CW, Cold well. *J S plant physiol* 1932; 7:447.

4. Hartmann T. from waste products to ecochemicals: fifty years research of plant secondary metabolism. *Phytochemistry* 2007; 68:2831-2846.
5. Ohshima Y, Ohno Y, Kajiyama K. High- performance liquid chromatographic separation of rhubarb constituents. *J Chromatogr* 1986; 360:303-306.
6. Kashiwada Y, Nonaka G, Nishioka I. Studies on rhubarb (*Rhei rhizoma*) XV. Simultaneous determination of phenolic constituents by high-performance liquid chromatography. *Chem pharm Bull* 1989; 37:999-1004.
7. Li L, Sun P, Feng CQ. Effects of climate factors on the contents of anthraquinones in *rheum tanguticum*. *Chin pharm* 2010; 43:4105-4107.
8. Chipman EW. Hints on rhubarb Production. *Agriculture Canada Kentville Bulletin*, 1974, 74-3, 6.
9. Charkravarty HI, Ali- Al rawi. Medical plant of Iraq. Edn 2, 1, Ministry of agriculture, Baghdad, 1964.
10. Zheng QX, WH-feng, Jian G, Hai-jiang N, Lin CS, Yang JS *et al.* Review of Rhubarb: Chemistry and pharmacology. *Chinese Herbal Medicines* 2013; 5(1):9-32.
11. Determination of ammonia and Kjeldahl nitrogen by indophenols method. 1976; 10:131-36.
12. Mohammad Amin, Flowers TH. Evaluation of Kjeldahl digestion method. *J of research science* 2004; 5(2):159.
13. Martin F, Chaplin. A rapid and sensitive method for the analysis of carbohydrate components in glycoproteins using gas-liquid chromatography. *Analytical Biochemistry* 1982; 123(1, 2):336-341.
14. Nyam KL. Physicochemical properties and bioactive compound of selected seed oil. *LWT. Food sci tech* 2009; 42:1396-1403.
15. Dalia A. Abdul, Majeed SN. Determination of steroid compounds in Hawthorn and Raspberry fruits in Sulaimani City Kurdistan region-north Iraq by HPLC technique. *Inter J of Scientific research* 2013; 2(5):563-5.
16. Shui G, leong LP. Separation and determination of organic acids and phenolic compounds in fruit juices and drinks by HPLC. *J of Chromatography* 2002; 977(1):89-96.
17. Jean L, Albert F, Marina H, Karin H. Plant sterols and blood cholesterol. *The EFSA journal*. 2008,781:1-12.
18. Tapola NS, Lyyra ML, Karvonen H. The effect of meat products enriched with plant sterols and minerals on serum lipid and blood pressure. *Inter J Food Nutr* 2004; 55(5):389-97.
19. George W, Alferd JP, Wakeman, Vickey HB. The organic acids of rhubarb (*Rheum hybridum*): III. The behavior of the organic acids during culture of excised leaves. *J Biol Chem* 1938; 126:43-54.
20. Galiskan M. The metabolism of oxalic acids. 2000; 24:103-106.