



DETERMINATION OF NUTRITIONAL COMPOSITIONS OF *RUMEX CHALEPENSIS* LEAVES: A NOVEL FINDING WITH IMPORTANT PRACTICAL IMPLICATIONS

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ABSTRACT

In this study, nutritional composition of *Rumex chalepensis* leaves was investigated. Results mineral compositions showed that concentration of Calcium (Ca), Phosphorus (P), Potassium (K), Sodium (Na), Magnesium (Mg), Zinc (Zn) and Selenium (Se) were 44.25, 9.98, 4713.91, 65.32, 81.13, 7.59 and 0.001 in mg/100g dry matter, respectively. The results of amino acids assay showed that content of Arginine and Carnitine were 1731.22 and 189.5 mg/g, respectively. The phytochemical screening of *R. chalepensis* leaf showed the presence of alkaloid, saponin, tannin, steroid, terpenoid, flavonoid, glycosides general, and glycosides with steroidal ring. The results of proximate composition (mg/g) in *R. chalepensis* leaf extract revealed the percentage of crude protein, crude fat, total ash, crude fibre, moisture content and carbohydrate in dry weight were 6.89%, 16.33%, 7.9%, 9.52%, 77.98% and 68.77%, respectively while the total gross energy was 654.53 kJ/100g. This study revealed that the *R. chalepensis* leaf can contribute useful amount of nutrients to human diet.

Keywords: *Rumex chalepensis*, Amino Acids, Phytochemical Screening, Mineral Compositions

INTRODUCTION

Medicinal plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to impede diseases comprising epidemics. The knowledge of their healing attribute has been transferred over the one hundred years within and among human communities [1, 2, 3]. The active ingredients produced during secondary vegetative metabolism are generally responsible for the biological characteristic of various plants utilized throughout the Earth for different objectives, such as cure of disease and pain alleviate. The use of medicinal plants has been greatly observed in majority developing countries as therapeutic agents for the maintenance of good health [4, 5].

Rumex chalepensis Mill, is a perennial herb greatly distributed in worldwide. *R. chalepensis* belonging to the Polygonaceae family are known to produce a large number of biologically important secondary metabolites, such as phenolic acids, flavonoid, anthraquinones [6], steroids [7], leucoanthocyanidins and glycosides [8].

The genus *Rumex* has attracted the attention of many researchers because of its medicinal properties. The genus *Rumex* leaves and roots have been used in traditional medicine for blood purification, constipation and inflammation [9]. The growing interest in numerous *Rumex* species has led to the

investigate of their biological activities. Traditionally, *Rumex* plants are used as antitumor, astringent antidermatitis [10], anti-inflammatory [11], diuretic, cholagogue, tonic, laxative agents [12] and bactericidal [13, 14].

The objective of this study was evaluated phytochemical compositions proximate, mineral, and amino acid of *R. chalepensis* leaves so that verify the usefulness of this plant as useful medicinal plant and also use it in drug formulation.

MATERIALS AND METHODS

Plant Preparation

The fresh samples of the mature plant leaves were collected from the area surrounding Hamun Lake, Zabol (31° 1' 43" N, 61° 30' 4" E), in Sistan and Baluchestan Provinces, Iran. A voucher specimen (accession no. 30148) was deposited in Ferdosi University Mashhad Herbarium (FUMH). It was appropriately sorted, leaf dried, powdered form and kept under refrigeration in an airtight plastic bottle before examination.

Preparation of Aqueous Extract

Ground sample (30 g) was mixed with 300 ml of distilled water using a shaking water bath for 24 h at room temperature. The extract was separated from the solid concentrate by filtering through Whatman No. 1 filter paper. The remaining residue

was re-extracted twice and the extracts were pooled. The solvent was removed under vacuum.

Mineral Assay

In this study, the mineral elements of the leaf plant were determined in the solution as follows-Na and K by flame photometry using NaCl and KCl to prepare standards. Minerals were assayed using the solutions obtained by dry ashing the leaf at 550 °C and dissolving it in 2 ml lanthanum chloride (5%) and 25 ml HCl (10%), boiling, filtering and making up to standard volume with deionized water. Phosphorus was assayed colorimetrically using a Spectronic instrument, with KH_2PO_4 as a standard. The Zn, Ca and Mg elements were evaluated by atomic absorption spectrophotometry. The detection limits for the metals in aqueous solution had been determined just before the mineral analyses using the methods of Techtron, giving the following values in $\mu\text{g/ml}$: K (0.002), Ca (0.01), Mg (0.004), Zn (0.001) and Na (0.03). The optimal analytical range was 0.1 to 0.3 absorbance units with coefficients of variation from 0.6-2.5%. The coefficients of variation percent were calculated [15]. The percentage contribution to energy due to protein (PEP%), because of total fat (PEF) and carbohydrate (PEC) as PEP%, PEF% and PEC%, respectively were calculated. Also the percentage utilizable energy due to

protein (UEDP%) was calculated. In addition Na/K, Ca/Mg, Ca/P and the millequivalent ratio of $[\text{K}/(\text{Ca} + \text{Mg})]$; the mineral safety index (MSI) of Zn, Ca, Na, P, and Mg were measured [16]. for measuring MSI, RAI is suggested adult intake; CV in the Table will represent measure value (CV) of calculating MSI from study results. In addition, The differences between the MSI of the samples and the standard MSI were measured.

Amino Acid Assay

In this study, Arginine and Carnitine profile were detected using the method described by Sparkman *et al.* [17]. Briefly, 3 g of the dried leaf was weighed into extraction thimble and the fat extracted with chloroform: methanol (2:1) mixture using soxhlet extraction apparatus [19]. Then, 1 g of the defatted sample was weighed into glass small tube. 8 ml of HCl (6N) was added and oxygen ejected by passing nitrogen to the small tube. The glass small tube was closed tightly and placed in an oven arrange beforehand at 105 °C for 24 hours. The small tube was allowed to cool before breaking open at the top and the content filtered. The filtrate was then evaporated to dryness and the residue dissolved with 3 ml acetate buffer and stored in plastic specimen bottles. 5 μl was distributed into the cartridge of the analyses which is designed to segregate and examine

the amino acid of the hydrolyzate. the value of Arginine and Carnitine present in the sample was calculated in g/100g protein from the chromatogram produced.

Phytochemical Screening

In this study measured the content of alkaloids, terpenes, saponins, tannins, glycosides, flavonoids and anthraquinone for phytochemical screening. For phytochemical screening were used the aqueous extract. According to standard methods of Trease and Evans [18], this include qualitative chemical analysis to identify the different classes of chemical component present. For alkaloids assay, one drops of Mayer's reagent were added to one ml of the extract. A creamy precipitate observed indicates the presence of alkaloids. two drops of Hager's reagent were added to one ml of the extract. A reddish brown precipitate observed indicates the presence of alkaloids. For terpenes assay, One ml of the extract was added to one ml of acetic anhydride followed by the addition of one ml concentrated sulphuric acid down the wall of the test tube to form a layer underneath. The test tube was monitored for red colouration appearance the presence of tri-terpenes. For saponins assay, we used 2 method include Frothing test and Emulsion test. In Frothing test, two ml of the extract in a test tube was forcefully shaken for 2 minutes. Frothing monitored in the three

extracts tested indicated the presence of saponins. In Emulsion test, 5 drops of extract were added to 3ml of the extract in a test tube and the mixture was vigorously shaken. A stable emulsion formed in the extract tested indicated the presence of saponins. For flavonoids assay, One ml of 10% NaOH was added to three ml of the aqueous extract. A yellow colouration showed the presence of flavonoids in the extract. For tannins assay, of the 1 ml of freshly prepared 10% KOH was added to 1 ml of the extract. A dirty white precipitate observed in each extract showed the presence of tannins. Of the 2 drops of FeCl_3 (5%) were added to 1 ml of the extract. A greenish precipitate showed the presence of tannins in the extract. For glycosides assay, of the 10 ml of 50% was added to 10 ml of the extract in a test tube. The mixture was heated in boiling water for 10 minutes. Of the 10 ml of Fehling's solution was added and the mixture was boiled. A brick-red precipitate was observed in the extract, showing the presence of glycosides. For anthraquinone assay, of the 1g of powdered leaf was boiled with 15 ml of FeCl_3 and 5 ml of dilute HCl for 10 minutes. The mixture was filtered while hot, cooled and the filtrate was shaken with equal volume of choloform. The layers were allowed to separate in a separating funnel, the choloform layer was transferred into another

test tube containing 5ml of 10% ammonia solution and the upper aqueous layer was observed for a bright-pink colour showing the presence of anthraquinones.

Proximate Analysis

In this study, total ash, fiber, moisture of extract were determined by the methods of the AOAC [19]. The nitrogen was measured by a Kjeldahl method and the crude protein content were calculated as $N \times 6.25$ [20]. Carbohydrate was determined by difference, rather than analysed directly. All the proximate results were illustrated in g/100 g dry weight. The energy values procured from crude protein ($\times 17$ KJ), crude fat ($\times 37$ KJ) and carbohydrates ($\times 17$ KJ) for each of the samples.

Statistical Analysis

Results were expressed as mean \pm SE of three independent experiments. Statistical significant differences were considered at $p < 0.05$ using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The nutritionally important minerals as well as the computed mineral ratios in *R. chalepensis* is shown in **Table 1**. Potassium (K) is a necessary mineral which must be consumed in the diet. It is essential for suitable nerve excitation and contraction in muscular tissues potassium.

In this study, the higher potassium content of 4713.91 mg/100g in the leaves of *R.*

chalepensis showed that the leaves of this plant are good diets for hypertensive patient because any diets rich in potassium can decrease the risks of hypertension and possibly strokes.

Magnesium (Mg) is essential for energy production, protein formation and cellular replication. The leaves of *R. chalepensis* contain 81.13 mg/100g of magnesium which show that it is a poor source of this mineral element. Idris *et al.* [21] evaluated nutrient content of the leaves of *R. acetosa*. They reported that Mg content in this plant was 73.56 mg/100g dry matter. The Mg content in *R. chalepensis* leaves was more than *R. acetosa*.

Zinc (Zn) is an necessary mineral that is naturally present in some foods and attainable as a dietary complement. Zn is as well as found in abundant cold lozenges and some over-the-counter drugs sold as cold remedies. Zn is mixed up in multiple aspects of cellular metabolism. It is needed for the catalytic activity of nearly 100 enzymes [22] and it plays a role in protein synthesis [23], wound healing [24], immune function [25], cell division and DNA synthesis [23].

Idris *et al.* [21] investigated nutrient content of the leaves of *R. acetosa*. They reported that *R. acetosa* have 2.66 mg/100g dry matter. The our results showed that *R. chalepensis* have more Zinc content as compared to *R. acetosa*. In our study, the

Zinc content was 7.59 mg/100g in the leaves of *R. chalepensis*. This result showed that the leaves of the plant have sufficient Zn content for using in dietary.

Sodium (Na) is essential for muscle contractions, maintaining pH balance, hydration, and nerve transmissions. Sodium regulates the fluid outside of the cells and is necessary to pump fluid into the cells as potassium carries by-products out. The leaf sodium content of 65.32 mg/100g as compare to the leaves of *R. acetosa* that evaluated by Idris *et al.* [21] was high sources of this mineral elements.

Phosphorus (P) is a major mineral and most of it is stored in human bones. Lesser amounts are found in human teeth, DNA, and cell membranes throughout human body. Phosphorus is essential for many biochemical reactions to take place, such as converting the foods huamn eat into energy. Of the 9.98 mg/100g dry matter was recorded as phosphorus concentration in the leaves of *R. chalepensis*. Phosphorus content in *R. chalepensis* leaves was 9.98 mg/100g dry matter. Based on the Guil-Guerrero *et al.* [26], for good calcium and phosphorus intestinal utilization, Ca/P ratio necessity be close to one. The leaves of *R. chalepensis* had a high ratio (4.43). This shows that the leaves of *R. chalepensis* are good sources of calcium over that of phosphorus; but, the diet based on this

leaves required to be complemented with other food material rich in phosphorus.

Calcium (Ca) is a mineral that helps human build and maintain strong bones and teeth. Good calcium intake throughout human life be able help to prevent osteoporosis. Calcium content in the leaves of *R. chalepensis* was found to be 44.25 mg/100g dry matter. Idris *et al.* [21] reported that *R. acetosa* was involved 53.25 mg/100g calcium in leaves. It seems that the this plant is good source for human diets.

Selenium (Se) is an necessary mineral and micronutrient. It is essential to human health and found in many foods. Selenium content in the leaves of *R. chalepensis* was found to be 0.001 mg/100g dry matter.

The value of Na/K ratio (0.01 mg/100g dry matter) was lower than 0.6, the value that favours non-enhancement of high blood pressure disease in human. the high amount of K/Na ratio (72.16 mg/100g dry matter) obtained in our study suggests that bringing the ratio down would need the consuming of food sources rich in sodium. The Ca/Mg value in our study were 0.54 mg/100g dry matter. The milliequivalent ratio of $[K/(Ca+Mg)]$ (37.59) was comparably lower than 2.2 recommended, which means the sample would promote hypomagesaemia in human [27].

The amino acids content in *R. chalepensis* leaf extract is shown in **Table 2**. The values

of Arginine and Carnitine were 1731.22 and 189.5 mg/g, respectively. L-Arginine (Arg) is the substrate for the synthesis of nitric oxide, the endothelium-derived relaxing factor necessary for regulating vascular tone and hemodynamics [28]. Carnitine is a conditionally necessary nutrient that plays a critical role in fatty acid metabolism and energy production [29].

In our study, the concentration of zinc in this plant was very high when compared with those of zinc-rich foods of plant origin, such as mushrooms, green peas (boiled) and sesame seeds [30].

The mineral safety index (MSI) values of the leaf extract are shown in **Table 3**. The standard MSI for the elements are Na (4.8), Mg (15), P (10), Ca (10), K (4.8) and Zn (33). For Ca, P, Mg, Na, K and Zn the MSI values ranged from 0.019-6.356, with all the differences between the standard and calculated MSI values being positive. The implication of the positive values is that no abnormally high level of the minerals were present in the leaf extract. For all mineral that calculated MSI were lower than standard MSI and therefore within the USRDA [16].

The results of phytochemical screening of *R. chalepensis* leaf extract are shown in **Table 4**. The aqueous leaf extract of *R. chalepensis* contains alkaloid, saponins, tannin, terpenoids, steroid, flavonoid and

cardiac glycosides as their major secondary metabolites.

The proximate composition (mg/g) in *R. chalepensis* leaf extract shown in **Table 5**. The Kjeldahl test determines the total nitrogen in a sample. The only major component of most food which contains nitrogen is protein (fat, dietary fibre and carbohydrate do not contain nitrogen). If the amount of nitrogen is multiplied by a factor depending on the kinds of protein anticipated in the food the total protein can be measured. This value is known as the "crude protein" content [31]. The crude protein content (6.89%) was comparably higher than the values reported from leaves of *R. acetosa* (3.70%) [21].

The ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. The total ash content of *R. chalepensis* leaf was 7.9 %.

Crude fat is the term used to refer to the crude mixture of fat-soluble material present in a sample. Crude fat also known as the ether extract or the free lipid content, is the traditional measure of fat in food products. In our study the value of the crude fat was 16.33%.

Fibre is not a single chemical entity; it is an extremely complex mixture of poorly

characterized constituents. Despite the fact that our knowledge current understanding about fibre is in a state of development, the most useful concept at demonstrate is that of "dietary fibre". Dietary fibre is considered to be plant-cell skeletal remains that are resistant to digestion. The crude fibre value in our study, was 9.52%. Moisture content express the amount of water present in a moist sample. Moisture content can be expressed on wet or dry basis. The moisture content measured in *R.chalepensis* leaf was 77.98%.

In our study, the carbohydrate content in *R.chalepensis* leaf was 68.77%. Carbohydrates in vegetables will vary between individual pieces and depending on cooking method. Most vegetables have a high water content which will be absorbed when they get boiled. And so the carbohydrate content of each vegetable will lower compared to the same weight when raw.

Energy is not a nutrient but is required in the body for physiological functions, metabolic processes, heat production, muscular activity, synthesis of new tissues and growth. It is released from food components by oxidation. The main sources of energy are carbohydrates, fats, proteins. The daily energy requirement for an adult is between 2500-3000 kCal (10455-12548 kJ) depending on his physiological state while

that of infants is 740 kCal (3094.68 kJ)(18). Higher carbohydrate content of 68.77% in the *R.chalepensis* leaf give rise to higher energy value of 654.53 kJ/100g in the sample. This findings shown that the leaf of this plant are good energy sources.

This study has recommended some biochemical knowledge on the mineral elements, proximate composition, amino acids and phytochemistry of *Rumex chalepensis* leaf. There are shows that the plant leaf are good sources of mineral elements, nutrients, phytochemicals and very rich in amino acids. And so, these results confirm the scientific basis for the use of this plant in folklore and it can give practical amount of nutrients to the human diet.

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Table 1: the Mineral Compositions and Some Masured Mineral Ratios in *Rumex chalepensis* leaf extract

Mineral elements	Concentration (mg/100g dry matter)
Calcium (Ca)	44.25 ± 0.01
Phosphorus (P)	9.98 ± 0.03
Potassium (K)	4713.91 ± 2.34
Sodium (Na)	65.32 ± 0.54
Magnesium (Mg)	81.13 ± 0.00
Zinc (Zn)	7.59 ± 0.06
Selenium (Se)	0.001 ± 0.01
Na/K	0.01
K/Na	72.16
Ca/P	4.43
Ca/Mg	0.54
[K/(Ca+Mg)]*	37.59

*milliequivalent. The data are mean ± SE

Table 2: The Amino Acids Content in *Rumex chalepensis* Leaf Extract (g/100g).

Amino acids	Values (mg/100g ± SE)
Arginine	1731.22± 0.3
Carnitine	189.5± 0.2

Table 3: The Mineral Safety Index of Na, K, P, Ca, Mg and Zn for the *Rumex chalepensis* Leaves Extract

Elements	TV	CV	D
Sodium (Na)	4.8	0.035	+4.76
Potassium (K)	4.8	0.084	+4.71
Phosphorus (P)	10	0.063	+9.93
Calcium (Ca)	10	0.032	+9.96
Magnesium (Mg)	15	0.019	+14.98
Zinc (Zn)	33	6.356	+26.64

TV = Table value, CV = Calculated value, D = Difference (TV – CV).

Table 4: Phytochemical Screening of *Rumex chalepensis* Leaf Extract

chemical compounds	aqueous extracts
Alkaloid	+
Saponnin	+
Tannin	+
Steroid	+
Terpenoid	+
Flavonoid	+
Glycosides general	+
Glycosides with steroidal ring	+

The values: present in moderate quantity(+); absent (-).

Table 5: The Proximate Composition (mg/g) in *Rumex chalepensis* Leaf Extract

Parameters	Concentration (% Dry weight)
Crude protein	6.89 ± 0.00
Crude fat	16.33± 0.04
Total ash	7.9± 0.05
Crude fibre	9.52 ± 0.00
Moisture content*	77.98± 0.09
Carbohydrate	68.77 ± 0.22
Energy kJ/ 100g	654.53 ± 0.11

The data are mean values ± SE of triplicates. *Value expressed as % wet weight