PAPER

NUTRITIONAL EVALUATION OF WILD PLANT *CISSUS ROTUNDIFOLIA*

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ABSTRACT

This study aimed to evaluate the nutritional and antinutritional components of *Cissus rotundifolia* leaves. They contain an appreciable amount of protein (12.5%$_{db}$), fat (7.45% $_{db}$), crude fiber (8.34 %$_{db}$) and minerals (16.32%$_{db}$). The protein fraction contains a relatively high level of essential amino acids, which accounted for 44.3% of the total amino acids. The fat contains a high concentration of unsaturated fatty acids that comprises 55.1% of total fatty acids. The mineral profile is composed of macro- and microelements. The antinutritional factors oxalate, phytate, tannins and cyanogenic glycosides are present at very low concentrations. *Cissus rotundifolia* leaves can be considered a potential source of nutritional components for healthy food purposes.

- Keywords: antinutrients, nutrients, *Cissus rotundifolia*, evaluation -

INTRODUCTION

Wild edible plants are species of plants that grow freely in the wild habitat without any agricultural treatments and can be consumed as a food (BELUHAN and RANOGAJEC, 2010). These types of plants are consumed worldwide, from developing and developed nations alike, and provide nutrition and food security for poor rural communities in several regions across the world (SUNDRIYAL *et al.*, 2003; AFOLAYAN and JIMOH, 2009) while serving as a diet supplement in Japan, Europe and North American (CHEN and QIU, 2012; BURLINGAME, 2000; REDZIC, 2006). Wild edible plants are rich in minerals, vitamins, dietary fiber, fatty acids and amino acids (BARROS *et al.*, 2010; LUCZAJ, 2010). The nutritional values of these plant species are comparable to, or even exceed, the corresponding domesticated types of plants (BURLINGAME, 2000; TARDIO *et al.*, 2006; AFOLAYAN and JIMOH, 2009). Moreover, wild edible plants are considered a good source of phytochemicals for human therapeutics (PENNY *et al.*, 2002; MASUDA *et al.*, 2003; VARDAVAS *et al.*, 2006). However, the presence of antinutritional principles in some species of wild plants, such as phytic acid, tannins, saponins, alkaloids and oxalates, can limit their exploitation (GUIL *et al.*, 1997; GUPTA *et al.*, 2005; LACHUMY *et al.*, 2010). Previous studies have shown that the corresponding domesticated types of these plants contain similar levels of antinutritional factors (SHAD *et al.*, 2013). Moreover, some of the antinutritional factors have therapeutic potential; for example, phytic acid has been shown to have anticancer and antioxidant activity (JARIWALLA, 2001; SHAMSUDDIN, 2002). Thus, the compositional analysis and nutritional evaluation of such wild plants are necessary for understanding their impacts on consumer's health (GUIL *et al.*, 1997). *Cissus rotundifolia* (Forsk) Vahl. is a perennial, evergreen, climber, wild plant and is a species of Cissus belonging to the family of Vitaceae (grape family). It is known as a common Arabian wax cissus, Peruvian Grape Ivy, Venezuelan Tree bine and locally (in south Saudi Arabia) as Algalaf.

This wild plant is commonly used as food thickeners in rural Nigeria. Moreover, it was found to have many therapeutic effects as hypoglycemic (ONYECHI *et al.* 1998), hypolipidemic (BELL *et al.*1993). In addition, its extract exhibits antibacterial activity (ALZOREKY and NAKAHARA, 2003). *Cissus rotundifolia* grows extensively in the southern region of Saudi Arabia, and their leaves only are widely consumed after cooking by local people as leafy vegetables. Although it is commonly used to prepare various dishes according to traditional dietary culture of locals, its nutritional potential has not been assessed. Therefore, this study aimed to evaluate the nutritional and antinutritional components of *Cissus rotundifolia* leaves (CRL). These data would increase the awareness about the exploitation of this renewable natural resource as a food.

MATERIALS AND METHODS

Sample collection and preparation

The leaves of *Cissus rotundifolia* (20 kg) were collected from the Abha region in southern Saudi Arabia. The leaves were washed with distilled water, dried in a hot air oven at 50°C to a constant weight, ground to a fine powder and stored in airtight plastic bags at 4°C until analysis.

Proximate composition analysis

The moisture, ash, crude lipid, crude fiber and crude protein (N×6.25) contents were determined according to the standard methods of (AOAC, 2000).

Amino acids analysis

The defatted samples (0.2 g) were hydrolyzed with 6 N HCl (10 mL) in a sealed tube at 100°C for 24 hours. The hydrolyzates were completed to 25 mL with deionized water. Five ml of each hydrolyzate were evaporated until free from HCl vapor and dissolved in citrate buffer (CSOMOS and SIMON-SARKADI, 2002). The identification and determination of amino acids were conducted using the amino acid analyzer AAA-400 (INGOS, Czech Republic) equipped with an (OSTION LG ANB, INGOS) ion-exchange column (200 x 3.7 mm) and a flow photometer detector. The elution was carried out using a different pH gradient of sodium-citrate buffers. Chromatographic data processing including calculation of retention times and peak areas of separated amino acids were performed using AMIK software 3.0 (Czech Republic). A mixture of standard amino acids (INGOS, Czech Republic) was utilized as external standards.

Fatty acid analysis

The lipids were extracted according to the method outlined by EGAN *et al.* (1981) and GRESSLER *et al.* (2010). Briefly, 10 g of the sample was digested with 10 ml of hot concentrated HCl using a boiling water bath and vigorous stirring before the color of the content turned brown. The lipid was extracted by shaking with 30 ml of diethyl ether and was repeated three times. The solvent was evaporated and the total amount of lipid was gravimetrically estimated. The fatty acid was transmethylated into their corresponding methyl esters (RADWAN, 1978). The lipids (50 mg) were redissolved in 2 mL benzene, aliquots of 2 mL of methanolic sulfuric acid (1%, v/v) were added and the tubes were stoppered with nitrogen and kept in a water bath at 90°C for 90 min. Water (8 mL) was added, the methylated fatty acids were extracted with 5 ml petroleum ether and the mixture was evaporated to dryness. Two microliters of the

fatty acid methyl esters solution were injected into a HP (Hewlett Packard) 6890 GC, coupled with a splitless injector mode, a flame- ionization detector (FID) and a HP-5 column (5% diphenyl, 95% dimethyl polysiloxane, 30 m, 0.32 mm ID, 0.25 μm film thickness). The following operating conditions were used: injector temperature 220°C, oven temperature program: initial temperature 150°C for 2 min, raised to 200°C at a rate of 10 °C /min, then increased to 250°C at a rate of 5°C /min and held at 250°C for 9 min, detector temperature: 250 °C, carrier gas was nitrogen at a flow rate of 1 ml/min. The mixture of fatty acid standards was subjected to the same treatments of the samples and used to identify and quantify the fatty acids in the samples.

Mineral analysis

The samples were digested as described by AMIN *et al.* (2013). Briefly, leaf powder (0.5 g) was digested with 4 ml of concentrated nitric acid and 1 ml of perchloric acid, cooled and filtered with Whatman No.42 filter paper. The supernatant was completed to 50 ml with distilled water. The blanks were carried out using the same procedure. The mineral concentrations of the digested diluents were determined against a multielement standard solution (Campro Scientific, Berlin, Germany) using Inductively Coupled Plasma-Optical Emission Spectrophotometry ICP-OES (Varian 720-ES, Varian Inc, Palo Alto, CA, USA).

Determination of antinutrients

The content of oxalate was measured using the titrimetric method of SANCHEZ-ALONSO and LACHICA (1987). Phytic acid in leaves was quantified according to the method of LUCAS and MARKAKAS (1975). The spectrophotometric method described by SARKIYAKI and AGAR (2010) was used to estimate the amount of cyanogenic glycoside in leaves. The tannin content was estimated using spectrophotometric analysis according to the method of POLSHETTIWAR *et al.* (2007).

Statistical analysis

All measurements were achieved in triplicate and the results were expressed as the mean value ± standard deviation of three measurements, using SPSS 13.0 (SPSS Inc., IL, USA).

RESULTS AND DISCUSSION

Proximate compositions

The nutritional composition of the leaves (Table 1) was compared with those of the most widely consumed foods (wheat, rice and potato) throughout the world. This comparison is justified by the fact that in the countries of origin leaves are used in two forms: fresh and sundried powder. The latter one is consumed as a partial replacer of wheat flour, corn flour and rice, to overcome a deficient of these foods.

The determined nutrients of the leaves were superior to those of wheat, rice and potato. This emphasizes their value as a good source of nutrients. A relatively high ash content in the leaves was associated with the amount of mineral elements.

Amino acid composition and protein quality

By the amino acid analysis (Table 2) fifteen amino acids were identified in CRL protein fraction. Among the detected amino acids, eight of essential amino acids (EAAs), which amounted to 358.5 mg/g crude protein, was identified. This exceeded the value of EAAs that is recommended by FAO for adults (2013). The amount of EAAs comprised 44.3% of the total individual amino acids, which is a ratio similar to that reported for the domesticated vegetable kale leaves (LISIEWSKA *et al.*, 2011). The present analyses also indicated that the protein in CRL contained a considerable level (69.9 mg/g protein) of aromatic amino acids (AAA) (histidine, phenylalanine and tyrosine), which is much higher than the AAA scoring pattern recommended by FAO for adults (38 mg/g) (2013). Similar to previous studies performed on many domesticated vegetable species (LISIEWSKA *et al.*, 2011; KMIECIK *et al.*, 2009), glutamic acid was the major amino acid identified in CRL protein. Cysteine, methionine and tryptophan were excluded in this study because they were destroyed during acid hydrolysis. All individual EAAs in leaf proteins (Table 2) compared favorably with the corresponding amino acid reference that is recommended for adults by FAO (2013) except for histidine, which had a score slightly below what is recommended. Therefore, CRL can be considered a good source of balanced protein.

Table 1 - Proximate composition (g/ 100g) of CRL compared with wheat, rice and potato.

Constituent (%)[a]	CRL	Wheat[b]	Rice[b]	Potato[c]
Moisture	93.1±0.2	12.6	13.0	75.7
Crude protein (dry basis)	12.5±0.1	11.3	7.70	8.27
Crude fat (dry basis)	7.45±0.1	1.80	2.20	1.11
Crude fiber (dry basis)	8.34±0.2	13.2	2.20	9.94
Ash (dry basis)	16.3±0.2	1.70	1.20	3.98

[a]Values are expressed as the means ± SD of three separate determinations).
Source: [b]KOEHLER and WIESER (2013); [c]GUMUL *et al.* (2011)

Table 2 - Amino acid profile of CRL protein.

Amino acids	mg/g protein[a]	FAO Pattern 2013	% of total
Essential amino acids			
Histidine	16.4±0.2	15	2.03
Isoleucine	47.5±0.2	30	5.88
Leucine	96.6±0.4	59	11.9
Lysine	38.7±0.1	45	4.79
Phenylalanine	37.9±0.1		4.69
Threonine	23.7±0.2	23	2.94
Valine	69.9±0.6	39	8.65
Arginine	27.4±0.2		3.39
Non-essential amino acids			
Alanine	98.5±0.7		12.1
Aspartic acid	64.9 ± 0.1		8.03
Glutamic acid	127.3±0.6		15.7
Glycine	97.7±0.6		12.0
Proline	7.77±0.1		0.96
Serine	38.2±0.2		4.72
Tyrosine	15.5±0.1		1.92
Total EAAs[b]	358.5		
Total non- EAAs	450.0		
Total individual amino acids (mg/g protein)	808.5		
Total AAA[c]	69.9		
% of EAAs	44.3		
% of Non- EAAs	55.7		

[a]Values are expressed as the means ± SD of three separate determinations on dry weight basis;
[b]Essential amino acids;
[c]Aromatic amino acids (phenylalanine+ histidine +tyrosine).

Fatty acid profile of CRL

The data in Table 3 show that 12 fatty acids were determined in the leaf lipidic extract, four out of which are unsaturated fatty acids and comprised more than half (55.1%) of the total fatty acid content. This high level of unsaturated fatty acids makes the CRL of main health interest. Palmitic acid, oleic acid and linoleic acid were the three major components present in the leaves, representing 31.7%, 25.7% and 22.9% of the total individual fatty acids, respectively. Palmitic acid is commonly found in both animal and plant foods. WHO (2003), reported that, dietary intake of palmitic acid increases the risk of cardiovascular diseases. However, in moderation, palmitic acid may not be entirely bad, as it does display mild antioxidant and anti-atherosclerotic properties (CHO *et al.*, 2010). The high proportion of both oleic acid (omega-9 fatty acids) and linoleic acid (omega-6 fatty acids) in leaves raises the biological value; therefore, consuming the leaves could be healthy and meet a part of the essential fatty acids requirements. The data also show that the leaf lipids contain odd-numbered fatty acids (tridecylic, pentadecanoic and pentadecenoic acid) in its composition. Such fatty acids have been found in many daily consumed foods such as human milk (NISHIMURA *et al.*, 2013; KOLETZKO *et al.*, I988), ruminants milk (BREVIK *et al.*, 2005), fish (ATEŞ *et al.*, 2013), and commonly consumed vegetables (BATISTA *et al.*, 2011). Concerning the impact of odd-numbered fatty acids on health, MARTYSIAK-ZUROWSKA (2008) reported that there is no risk of presence of odd-numbered fatty acids in food as it is found in mother's milk and ruminant's milk.

Table 3 - Fatty acid composition of CRL.

Fatty Acid	FA (µg/g)[a]	% of total
Caprylic acid (C8:0)	7.56±0.3	0.23
Capric acid (C10:0)	12.4±0.2	0.38
Lauric acid (C12:0)	35.6±0.2	1.09
Tridecylic acid (C13:0)	63.1±0.1	1.93
Myristoleic acid (C14:1)	101.2±0.2	3.09
Myristic acid (C14:0)	39.8±0.2	1.21
Pentadecenoic acid (C15:1)	110.5±0.1	3.38
Pentadecanoic acid (C15:0)	92.8±0.2	2.83
Palmitic acid (C16:0)	1036.5±0.4	31.7
Linoleic acid (C18:2c)	750.2±0.1	22.9
Oleic acid (C18:1c)	841.5±0.5	25.7
Stearic acid (C18:0)	181±0.7	5.53
Total unsaturated fatty acids	1803.4	
Total saturated fatty acids	1468.8	
Total individual fatty acids	3272.1	
% of total unsaturated fatty acids	55.1	
% of total saturated fatty acids	44.9	

[a]Values are expressed as the means ± SD of three separate determinations on dry weight basis.

Mineral content of CRL

The contents of both macro- and microelements in leaves are presented in Table 4. Calcium, which is required for the formation of bone and neurological function (BRINI *et al.*, 2013), was the predominant element in leaves (15.1 mg/g). A modest consumption of 66.5 g of leaves per day would satisfy the adult daily requirement of calcium (1,000 mg/day), according to the Institute of Medicine (2011). Therefore, CRL could be a good source of calcium. Sodium was the second abundant element found in CRL, followed by potassium. Potassium and sodium play an important role in regulating blood pressure and body acid-base balance (CLAUSEN *et al.*, 2013; SIDDHURAJU *et al.*, 2001). An appreciable concentration of magnesium was determined in the leaves. Magnesium is needed to prevent heart disease and growth retardation (CHATURVEDI *et al.*, 2004). CRL could be considered a rich source of iron and an intake of 47.4 g of leaves could satisfy the recommended adult dietary intake (6 mg/day) of iron according to the Institute of Medicine (USA, 2001). Zinc, which is a component of many enzymes and a wide array of cellular and biochemical processes (KARCIOGLU, 1982; COLEMAN, 1992), is present in a moderate amount in leaves. Significant amounts of both copper and chromium, which are a component of many respiration enzymes and glucose tolerance factor, respectively (SANDS and SMITH, 2002; MERTZ, 1993), were observed in the leaves (FAILLA *et al.*, 2001; KELVAY, 2000).

Antinutritional factors

The edibility of any wild plant depends on the content of anti-nutritional factors. Analyses were carried out in CRL and results are shown in Table 5. The oxalate content was equal to 3.05mg/100 g, value lower than that reported (14.9 g/100 g) in common green leafy vegetable spinach (*Spinacia oleracia*) (YADAV and SEHGAL, 2003). The phytate level (0.76 mg/100 g) in leaves was found to be less compared with that reported in domesticated crops of *Solanum indicum* (695.8 mg/100 g, ABEROUMAND, 2012), lima beans (234 mg/100 g, EGBE and AKINYELE, 1990) and underutilized green leafy vegetables (0.92–13.06 mg/100 g, GUPTA *et al.*, 2005), indicating that the lower phytic acid content in CRL will provide a better bioavailability of minerals. The estimated tannin value in leaves is considerably lower compared with those (0.59 mg/100 g) reported in lima beans (*Phaseolus lunatus*) by EGBE and AKINYELE (1990). The detected level of cyanogenic glycosides (0.023 mg/100 g) can be consider inappreciable compared with those of lima beans (colored) (3120 mg HCN/kg) (SPEIJERS, 1993) and is much lower than the reported lethal dose (3.70 HCN mg/kg bw) for mouse (CONN, 1979). These results reveal that antinutritional factors exist in CRL, but at lower levels compared with many daily-consumed foods.

Table 4 - Mineral composition of CRL.

Mineral	Concentration[a]
Macroelements	*mg/g*
Calcium (Ca)	15.1±0.2
Magnesium (Mg)	3.55±0.1
Sodium (Na)	11.2±0.2
Potassium (K)	8.09±0.3
Microelements	*µg/g*
Iron (Fe)	126.6±3
Zinc (Zn)	51.6±0.3
Manganese (Mn)	31.3±0.6
Copper (Cu)	3.21±0.3
Chromium (Cr)	2.38±0.2

[a]Values are expressed as the means ± SD of three separate determinations on dry weight basis.

Table 5 - Antinutrients contents in CRL.

Compound	Content (mg/100g) [a]
Oxalate	3.05±0.1
Phytate	0.76±0.1
Tannins	0.26±0.1
Cyanogenic glycosides	0.023±0.0

[a]Values are expressed as the means ± SD of three separate determinations on dry weight basis.

CONCLUSIONS

The present study serves as a basis to encourage the local communities to exploit the nutritive potentials of the wild plant *Cissus rotundifolia.* Results of analyses demonstrated good nutritional qualities and CRL could, thus, contribute to overcome the nutritional deficiency especially in arid climates. Therefore, it is now imperative that a nutritional database of this wild plant is set up to retain the information for a better management and conservation of this natural resource and habitats related to it.

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