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Proximate Composition, Amino Acids and Sugar Contents of Leaves and Stems of *Ficus cordata* thunb. Subsp. *Salicifolia* (VAHL)

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ABSTRACT

Proximate composition, amino acids and sugar contents of leaves and stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) were investigated. The proximate composition evaluated were; the content of moisture, ash, crude fibers, lipids, carbohydrates, nitrogen and proteins. Also, free and protein amino acids beside free and combined sugars were determined. The results obtained showed that, the contents of moisture, ash and crude fiber in plant stem were higher than in plant leaves. While the contents of lipids, carbohydrates and proteins in plant leaves were higher than in plant stem. The nutritive value (200.64 kcal/100 g) of plant leaves was higher than of plant stem (164.05 kcal/100 g). Seventeen free & protein amino acids with different ranges of concentrations were detected in plant leaves and stem by using Amino Acid Analyzer. The separation of free and combined sugars contents in leaves and stem was achieved using High Performance Liquid Chromatography (HPLC), which revealed the presence of eleven free sugars and nine combined sugars.

Key words: Proximate analysis, Amino acids, Sugars, *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl).

1. INTRODUCTION

Ficus cordata Thunb. subsp. *salicifolia* (Vahl), belongs to family Moraceae. The Moraceae often called the mulberry family or fig family, are a family of flowering plants comprising about 50 genera and over 1500 species. Most are widespread in tropical and subtropical regions, less so in temperate climates **(Mahbubur Rahman and Khanom, 2013)**. A large number of these plants provide edible fruits. These include *Artocarpus heterophyllus*, *Ficus carica*, *Ficus glomerata*, *Morus alba*, and *Treculia africana*. Others are of medicinal importance; these include *Ficus sycamorus*, *Ficus polita* and *Ficus ingens*. The bark of *Antiaria toxicaria* is used for making garments and sacks. Many species yield good timber. *Morus australis* is grown for its leaves which are fed to silkworms. Many *Ficus* species are grown as shade trees. The bark of *Ficus nekbudu* serves as source of cloth. The wood of *Maclura aurantiaca* is suitable for making bows **(Akesa, *et al.*, 2016)**.

Ficus species have great economic importance. *Ficus carica* is used as a source of food due to its high nutritive value. *Ficus bengalensis* is rich in fibers that could be used in the manufacture of fabrics. *Ficus elastica* is considered as a rubber-yielding tree. *Ficus religiosa* has

a resinous substance secreted on the twigs, used in the manufacture of phonograph records, high-grade insulators, spirit varnish, sealing wax, drawing ink and watercolors **(Pakia, 2003; Kone and Atindehou, 2008)**.

Ficus cordata Thunb. subsp. *salicifolia* (Vahl) is a perennial evergreen small tree up to (8 – 15) m with milky latex; stem branching from the base, bark grey to grey brownish, mooth and in older parts peeling and with scales below. Leaves alternate, lanceolate, acute to acuminate tip, rounded base, entire margin, glabrous, shiny, lower surface paler than the upper surface, 5-20 cm long x 1 – 6 cm across. Monoecious flowers, male flowers sessile, stamen solitary; female flowers sessile, perianth 3 – 4 lobed and style equaling ovary. Fruit fig, compound, axillary 1 – 2, orange to red when ripe and ostiole prominent **(Boulos, 2002)**.

Proximate analysis of a food is the nutritional composition of that food and it is the estimation of the nutritive value of human food in its chemical form **(Smith, 2009).**

2. MATERIALS AND METHODS

2.1. Source and preparation of plant materials

The fresh aerial parts (leaves & stem) of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) were collected from Elba mountain habitat in the remotest south eastern corner of Egypt during the period of investigation 2014. The separated specimens were washed with distilled water and were shade dried at lab-temperature till constant weight; the dried parts were then grinded into fine powdery form; the plant was identified and stored in Herbarium, at Desert Research Center, Cairo, Egypt.

2.2. Proximate analysis and nutritive value

Proximate analysis of the leaves and stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) were determined including percentage of moisture content **(Rowell, 1994)**; total ash **(Brower and Zar, 1984)**; crude fibres **(British Pharmacopoeia, 1980)**; total carbohydrates **(Chaplin & Kennedy, 1994)**; total lipids, total nitrogen and protein contents **(James, 1995)**; Nutritive values of plant samples were determined according to the following equation **(Indrayan *et al*., 2005)**

Nutritive value = (protein × 4) + (carbohydrate × 4) + (fat × 9).

2.3. Investigation of free and protein amino acids

The investigation of free and protein-amino acids was accomplished according to **Pellet and Young, (1980)** and **Steven, *et al*., (1989)** by using Amino Acid Analyzer.

2.3.1. Preparation and identification of free amino acids

One gram of the defatted plant powder (leaves and stem) was extracted by boiling under reflux with 50ml of 50% ethanol for 3 times (each for 3 hours). The combined ethanolic solutions were filtered and treated with trichloroacetic acid solution (10%) for clarification. The supernatant fluid was concentrated under reduced pressure to 5ml. The residue was washed with distilled water. The volume of the filtrate was adjusted to 100 ml using distilled water. Five ml of diluted sample were dried at 70ºC, and then dissolved in 5ml loading buffer (0.2N sodium citrate

buffer pH 2). The sample was filtrated through 0.45 micropore filter and injected in Amino Acid Analyzer.

2.3.2. Preparation and identification of protein-amino acids

The defatted plant powder (leaves and stem) (0.1g) was dissolved in 10 ml of 6N HCl in a sealing tube. The mixture was hydrolyzed at 110ºC for 24 hours, filtered and the hydrolyzed protein-amino acids were obtained by evaporation of the hydrolysate to dryness. The residue was washed with distilled water. The volume of the filtrate was adjusted to 100ml, using distilled water. The investigation of protein-amino acid was completed as previously discussed for free amino acids.

2.4. Quantitative determination of free and combined sugars (Chaplin and Kennedy, 1994)

2.4.1. Extraction of free sugars

Twenty grams of the defatted plant powder (leaves and stem) were extracted with 80% ethyl alcohol, and filtered. The filtrate was clarified by Carrez reagent, filtered and then evaporated. The residue was dissolved in 3 ml of 10% aqueous isopropanol for chromatographic investigations.

2.4.2. Hydrolysis of combined sugars

Twenty grams of defatted air-dried plant powder (leaves and stem) were boiled under reflux for 3 hours with 25 ml of 6N HCl, then cooled and filtered. The HCl was evaporated under vacuum at 45ºC and the residue was dissolved in 10 ml of 10% isopropanol for chromatographic investigations.

2.4.3. HPLC of the free and combined sugars (Nagel, 1992)

The sugars (free and combined) of the plant were determined by using High Performance Liquid Chromatography (HPLC), Shimadzu Class-VPV 5.03 (Kyoto, Japan) equipped with refractive index RID-10A Shimadzu detector, LC-16ADVP binary pump, DCou-14 A degasser and Shodex PL Hi-Plex Ph column (Sc 1011 No. H70608), guard column Sc-Lc Shodex, and heater set at 80˚C. Separation and quantitation were carried out on an amino-bonded column with a mobile phase of CH_3CN and H_2O (80/20 v:v), whereas the extracted sugars were injected.

Standerd solution of individual sugars were prepared by diluting each analyzed sugar with deionized. Injected volume of each standerd was 20 µl.

3. RESULTS AND DISCUSSION

3.1. Proximate composition and nutritive value

Data presented in table (1) indicated that, the percentage of moisture and total ash content was the highest values in plant stem, while the lowest values in plant leaves. Higher ash content indicates that the total inorganic mineral is high (**Oloyede, 2005)**. Also, **Smith, (2009**) stated that, the high content of ash is useful in assessing the minerals present in the sample.

It was observed from the obtained data in table (1) that, the percentage of crude fibres in plant stem (18.23 ± 0.145) was higher than in plant leaves (16.33 ± 0.120). Fibers used as prepiotic, where it have the ability to promote bacteria fermentation in colon. Dietary fibres play an important role in human health, which consists mainly of cellulose, hemicelluloses and lignin, which exert different physiological effects on human health **(Zia-ur-Rehman, *et al*., 2003).** Food fiber promotes absorption of trace elements in the gut; reduce absorption of cholesterol and lower blood glucose in diabetic patients **(Aliyu, *et al*., 2009)**.

Data in table (1) showed that, the percentage of total carbohydrates and total lipids reached to (34.48 ± 0.153 and 1.80 ± 0.058), respectively in plant leaves, while reached to (30.60 ± 0.058 and 1.13 ± 0.033), respectively in plant stem. The highest percentage of total lipids content in leaves may be due to an increase in the metabolic rate, which leads to increase in carbohydrate concentrations which convert to lipid by oxidation reaction which agreed with **Stocker**, **(1960**).

It was also, observed that, the highest values for the percentage of total nitrogen and total protein were in plant leaves (1.87 ± 0.088) & (11.63 ± 0.555), respectively. Nitrogen is a universally occurring element in all living beings and major component of protein, therefore the concentration of protein is closely linked to the concentration of nitrogen in the plant.

The nutritive contents of the plant leaves and stem are listed in table (2); nutritive value of the plant was determined by multiplying the values obtained for protein, fat and carbohydrate by 4.00, 9.00 and 4.00, respectively and adding up the values. The percentage of nutritive value (200.64 kcal/100 g) of plant leaves was higher than of plant stem (164.05 kcal/100 g). The highest nutritive value in plant leaves due to high carbohydrates and fat accompanied by enough protein content therefore, the plant may be has some nutritional value **(Indrayan *et al*., 2005)**.

Table (1): Mean percentage values of proximate composition of leaves and stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) during the period of investigation (2014)

Item (%)	Mean ± SE	
	Leaves	Stem
Moisture	32.43 ± 0.384	36.63 ± 0.617
Total ash	3.33 ± 0.067	5.53 ± 0.120
Crude fiber	16.33 ± 0.120	18.23 ± 0.145
Total carbohydrates	34.48 ± 0.153	30.60 ± 0.058
Total lipids	1.80 ± 0.058	1.13 ± 0.033
Total nitrogen	1.87 ± 0.088	1.27 ± 0.088
Total protein	11.63 ± 0.555	7.87 ± 0.561

Each value in the table of proximate analysis was obtained by calculating the average of three experiments and data are presented as Mean±SEM.

Table (2): Mean percentage values of nutritive value of leaves and stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) during the period of investigation (2014)

Item	(Kcal /100 g)	
	Leaves	Stem
Nutritive value	200.64	164.05

3.2. Free and protein amino acids using Amino Acid Analyzer

3.2.1. Free amino acids

The separation of free amino acids in leaves and stem were achieved using Amino Acids Analyzer, the obtained results were tabulated in table (3), it was observed that, plant leaves and stem contain seventeen amino acids with different ranges of concentration. The exception was

absence of leucine in the plant leaves. The highest concentration of free amino acids in plant leaves was lysine (5.78 ppm) as essential amino acids and cysteine (6.30 ppm) as nonessential amino acids. The highest concentration of free amino acids in plant stem was theronine (3.09 ppm) as essential amino acids and serine (8.98 ppm) as nonessential amino acids.

3.2.2. Protein amino acids

Table (3) declared that, plant leaves and stem contain seventeen amino acids with different ranges of concentration. The exception was absence of cysteine in the plant stem. The highest concentration of amino acid in plant leaves and stem was leucine (31.83 & 25.31 ppm), respectively as essential amino acids and aspartic acid (48.95 & 44.51ppm), respectively as nonessential amino acids.

Amino acids are important biomolecules that both serve as building blocks of proteins and are intermediates in various metabolic pathways. They serve as precursors for synthesis of a wide range of biologically important substances including nucleotides, peptide hormones, and neurotransmitters. Moreover, amino acids play an important role in cell signaling and act as regulators of gene expression and protein phosphorylation cascade **(Wu, 2010)**, nutrient transport and metabolism in animal cells (**Wang, *et al*., 2013)**, innate and cell-mediated immune responses.

Aspartic acid (L-aspartate) thought to help promote a robust metabolism, it used to generating cellular energy, treat fatigue and depression and used to generate adenosine triphosphate (ATP) and the fuel that powers all cellular activity. Aspartic acid plays an important role in the citric acid cycle, or Krebs cycle, during which other amino acids and biochemicals (such as sparagine, arginine, lysine, threonine and isoleucine) are synthesized (**Eid, *et al*., 2011)**. Quantitative and qualitative changes in the synthesis of protein of the plants may be due to response to water deficient **(Pessarakli, 1995).**

Table (3): Free and protein amino acids in leaves and stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) using amino acid analyzer

Name of Amino Acids	Free & Protein Amino Acids (ppm)			
	Leaves		Stem	
	Free	Protein	Free	Protein
	Essential Amino Acids			
Theronine	0.90	18.09	3.09	17.34
Valine	1.04	23.30	0.95	18.33
Methionine	4.06	0.18	0.03	4.80
Isoleucine	0.87	18.87	0.75	14.76

Leucine	-	31.83	1.52	25.31
Phenyl alanine	1.16	21.84	0.85	17.85
Histidine	0.05	11.53	1.56	12.31
Lysine	5.78	17.44	1.84	17.77
Arginine	0.61	16.19	0.63	15.99
Non-Essential Amino Acids				
Aspartic acid	2.45	48.95	1.41	44.51
Serine	1.02	20.46	8.98	19.44
Glutamic acid	2.55	44.35	1.95	38.40
Proline	1.55	29.20	2.01	27.85
Glycine	0.01	23.25	0.09	17.04
Cysteine	6.30	2.14	1.26	-
Alanine	1.02	21.78	0.85	16.08
Tyrosine	0.10	9.33	0.89	0.56

3.3. Free and combined sugars using HPLC

3.3.1. Free sugars

The separation of the free sugars contents in leaves and stem was achieved using High Performance Liquid Chromatography (HPLC), which revealed the presence of eleven free sugars (Table 4). The maximum percentage of free sugars was glucose (351.1 mg/100g) and sucrose (328.4 mg/100g) in leaves and stem, respectively. Meanwhile, the minimum percentage was xylose (28.64 mg/100g) and galactose (10.6 mg/100g) in leaves and stem, respectively. These results are in agreement with the observations of ***Ackerson, (1981)*** and ***Munns & Weir, (1981)***; they observed a considerable increase in glucose (reducing sugars) as well as sucrose (non-reducing sugar) with water stress. Also, some conversion of glucose to sucrose may have occurred during overnight rehydration in prestressed leaves.

Weib and Alt, (2017) reported that, glucose, fructose, and sucrose belong to the group of the most frequently-occurring lower-molecular carbohydrates in plants.

3.3.2. Combined sugars

Investigation of the hydrolyzed combined sugars contents in leaves and stem was achieved using (HPLC), which revealed the presence of nine sugars (Table 4). The maximum percentage of hydrolyzed sugar was raffinose (620.5 mg/100g) and arabinose (568.3 mg/100g) in leaves and stem, respectively. Meanwhile, the minimum percentage was lactose (48.9 mg/100g) and sucrose (45.3 mg/100g) in leaves and stem, respectively.

Table (4): Free and combined sugars in leaves & stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl)

Name of Sugars	Free and Combined Sugars (mg/100g)			
	Leaves		Stem	
	Free	Combined	Free	Combined
Raffinose	214.2	620.5	128.3	265.1
Sucrose	100.5	246.3	328.4	45.3
Maltose	148.3	308.1	78.3	254.1
Lactose	65.4	48.9	67.4	52.4
Glucose	351.1	567.2	157.4	247.6
Xylose	28.64	201.3	68.3	-
Galactose	38.3	410.3	10.6	178.2
Mannose	35.7	226.3	24.3	410.2
Fructose	214.3	-	117.8	165.4
Arabinose	241.9	574.1	210.6	568.3
Manitol	150.6	-	126.1	-

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