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Studying the Biological and Nutritional Role of the Saad Plant (*Cyperus rotundus* L.), and its Application in some Food Systems

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ABSTRACT

Cyperus rotundus L., a medicinal perennial from the Cyperaceae family, contains bioactive compounds like fatty oils, flavonoids, and terpenoids, offering anti-inflammatory and digestive benefits. This study analyzes its amino acid profile and pharmacological potential using advanced techniques to authenticate and differentiate its chemical composition. This study analyzed *C. rotundus* tubers from Iraq, assessing moisture, protein, fat, ash, fiber, and carbohydrates. Amino acids were quantified via HPLC to evaluate nutritional properties (AAS, EAAI, BV, PER), enhancing understanding of their therapeutic potential for traditional and modern medicine. The chemical analysis of *C. rotundus* L. tubers revealed a notable nutritional profile: 6.86% moisture, 12.40% protein, 5.13% fat, 68.13% carbohydrates, 1.33% ash, and 6.18% crude fiber. Using HPLC, the study identified 18 amino acids, including 8 essential ones, with glutamic acid (1202.03 µg/ml) being the most abundant, followed by leucine and isoleucine. Key nutritional indices were calculated, showing a biological value (BV) of 61.71, an essential amino acid index (EAAI) of 66.85, and a high amino acid score (AAS) of 131.21, indicating superior protein quality compared to FAO standards. The protein efficiency ratio (PER) further confirmed its nutritional potential. These findings highlight *C. rotundus* as a rich source of essential amino acids and high-quality protein, supporting its use in functional foods, dietary supplements, and medicinal applications. The study underscores the value in food technology and agriculture in improving nutrition and health.

Keywords: *Cyperus rotundus* L., Biological and nutritional, Application Saad plant

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INTRODUCTION

The perennial plant *C. rotundus* L., part of the Cyperaceae family, has round or rectangular tubers that are hairy and scaly. When fully mature, these tubers are white and have a pleasant flavor, although they can darken to brown or black and become damaged with age. The plant features long, cylindrical, smooth, and hollow stems that typically range from 50 to 160 cm long, with sharp triangular edges. At the base, the stems are surrounded by a dense layer of leaves. The stems culminate in spikelets, also known as inflorescences, which consist of three to nine irregularly arranged spikelets that branch out in an umbrella-like pattern [1, 2].

C. rotundus has a composition that includes 2.7% fatty oil. Additionally, it contains starch, sugars, resin, aromatic substances, and small amounts of alkaloids [3]. Glycerides, oleic acid, palmitic acid, and linoleic acid comprise the majority of its constituents [4]. The plant contains essential oils that makeup between 0.1% and 3% of its total weight, which can be used to alleviate stress and address digestive issues [5]. The root nodules are utilized in medicine [6], and they contain oils that are volatile, such as patchouli, eugenol, and terpenoids [7]. Other than a variety of active ingredients, including Phenols, Flavonoids, Essential Oils, ascorbic acid, and Linoleic Acid, the oily extracts of the sedge plant can inhibit the growth of harmful bacteria [8].

C. rotundus, commonly called walnut grass, is part of the Cyperaceae family and was initially found as a pest in India [9]. This traditional medicinal plant is native to China, Japan, and India, and has been employed to address inflammation and intestinal issues [10]. Studies have found that the plant contains oil, alkaloids, glycosides, saponins, flavonoids, tannins, starch, carbohydrates, proteins, and small amounts of manganese, chromium, and magnesium [11-15]. In vitro, the antimalarial efficacy of monoterpenes and diterpenes derived from the plant has been documented [9]. It possesses anesthetic properties [16] and impressive effects against fever [17]. Studies have demonstrated the pharmacological and therapeutic properties of the medicinal and botanical plants [9, 18]. The objective of this research was to identify and isolate the amino acids present in the plant Ampelopsis odorata through chemical analysis. This study aims to authenticate, differentiate, and chemically analyze the amino acids found in Ampelopsis odorata.

MATERIALS AND METHODS

Cyperus tubers He got it from the local markets of Baghdad, and the origin of the plant cultivation in Iraq is Anbar Governorate.

Estimation of the Chemical Composition of Cyperus Powder

The chemical composition of the ground's sedge plants was examined; this included:

The determination of moisture, protein, fat, ash and fiber via the methods described in [19].

Estimation of carbohydrates

Estimate the percentage of carbohydrates using the following formula.

carbohydrates percentage = 100- (moisture percentage+ ash percentage + protein percentage + fiber percentage + fat percentage).

Estimation of Amino Acids Present in Cyperus Protein

High-performance chromatography equipment (HPLC) was used to determine the amino acids, and the main column was made. This approach has the following benefits:

• Generally speaking, using a small reaction system may lower reagent consumption rates.

• The use of costlier reagents that yield lower background levels (from post-column derivatisation) permits increased sensitivity.

As long as the unreacted derivatisation reagent is separated in the column, it doesn't matter whether it is found.

Preparation of Protein Standards

It has been prepared a dilution series of BSA (or another standard protein) in the expected range (e.g., 0–2000 µg/mL) (Table 1):

Table 1 Preparation of BSA Standard Solutions for Protein Assay

Tube	BSA Concentration (µg/mL)	Volume of Stock (µL)	Volume of Buffer (µL)
1	0 (Blank)	0	1000
2	250	250	750
3	500	500	500
4	750	750	250
5	1000	1000	0

Adjust volumes based on stock concentration.

Sample Preparation

- Dilute Cyperus protein extracts fall within the standard curve range.
- Perform at least duplicate/triplicate measurements for accuracy.

Protein Assay (Example: Bradford Method)

- It was Added 100 µL of each standard/sample to a cuvette/microplate well.
- It was Added 1 mL of Bradford reagent, mixed, and incubated 5–10 min at RT.
- It was measured absorbance at 595 nm (for Bradford).

Data Collection and Plotting the Standard Curve

A standard curve was established using bovine serum albumin (BSA) at concentrations of 0, 250, 500, 750, and 1000 µg/mL to quantify protein concentration based on absorbance at 595 nm. The blank (0 µg/mL) showed an absorbance of 0.000, while increasing BSA concentrations resulted in progressively higher absorbance values: 0.150 (250 µg/mL), 0.320 (500 µg/mL), 0.480 (750 µg/mL), and 0.650 (1000 µg/mL). The linear increase in absorbance with protein concentration indicates a consistent and measurable relationship, confirming the validity of the assay for protein quantification. This standard curve can be used to interpolate unknown sample concentrations by comparing their absorbance values to the established trend (Table 2).

- Graph Absorbance (Y-axis) vs. Protein Concentration (X-axis).
- Perform linear regression to obtain the equation:

y=mx+cy=mx+c

(Where yy = absorbance, xx = concentration, mm = slope, cc = intercept)

Example trendline equation: y=0.0006x+0.02, R2=0.998

Primary Column Conditioning

Primary column conditioning enhances sensitivity for small sample analyses before amino acid derivatization. Reagents like PITC, phthalaldehyde, and dansyl chloride are used, with PITC reacting rapidly at room temperature. Reverse-phase chromatography separates derivatized amino acids but requires hydrophobic modification for permeability. A Shim-pack XR-ODS column and Prominence FLC system analyze PITC derivatives, detected via PITC detector. Key reagents (methanol, PITC, amino acid standards) were sourced from Aldrich Chemical Co. This method enables high-throughput, precise separation of amino acids [20, 21].

Table 2 Absorbance Measurements of BSA Standards at 595 nm

Standard (µg/mL)	Absorbance (595 nm)
0	0.000
250	0.150
500	0.320
750	0.480
1000	0.650

Chromatography System

The HPLC system comprised a Shimadzu LC-6A pump, Spd-6AV UV detector (254 nm), and SIL-6A gradient controller. A Rheodyne 7125 injector (20 µL loop) delivered samples to a Shimpack XR-ODS column (50 × 4.6 mm, 3µm). Separation used a linear gradient (0–20 min) of Solvent A (5% methanol/0.1N sodium acetate buffer, pH 7.0) and Solvent B (pure methanol) at 1 mL/min. Data was processed via RC-6A [22].

Extraction

A 1g dried sample was mixed with 50 ml alcohol-water solution, then hydrolyzed in 30 ml 6N HCl at 110°C for 6 hours. The concentrate was reduced to 5ml via nitrogen evaporation, mixed with 5ml citric acid buffer (pH 2.2), and 20µL was injected into the HPLC column [23].

Derivatization Procedure

Ten microliters of either standard or unknown samples were combined with 10 microliters of PTIC reagent. After one minute, 50 microliters of 0.1M sodium acetate at pH 7.0 were added. The mixture was thoroughly mixed, stirred, and then placed in an ultrasonic bath for ten minutes. The resulting extract was filtered through a 0.2-micron filter (catalog number 16534), and 20 microliters were injected into the HPLC column. The quantitative concentration of the chemical was determined by comparing the peak area of the standard to that of the sample [24].

Nutrition Properties

Amino Acid Score (ASS)

The amino acid composition was calculated using the following formula based on the article by Mossé et al. [25]. The percentage of essential amino acids in the sample was divided by the percentage of essential amino acids recommended by the FAO to calculate the amino acid value (Table 3).

Table 3 Standard Amino Acids Table

No.	Amino acids	Area µv	Concern.25 µg/ml each
1	Aspartic acid Asp	187032	577.91
2	Glutamic acid Glu	239261	1202.03
3	Serine Ser	152331	348.57
4	Arginine Arg	198756	582.17
5	Aspargine, Asn	142682	283.88
6	Cysteine cys	145792	207.13
7	Alanine Ala	203771	295.13
8	Proline Pro	182321	417.48
9	Glycine Glc	171002	443.29
10	Threonine ,Thr	169894	296.140
11	Tyrosine Tyr	168098	648.13
12	Valine Val	151763	382.38
13	Methionine Met	199770	268.87
14	Histidine , His	170222	160.10
15	Isoleucine Ile	186112	769.22
16	Leucine Leu	167901	727.63
17	Phenylalanine Phe	174751	715.88
18	Lysine Iys	117472	690.66

The Essential Amino Acid Index (EAAI)

Use the following equation to determine the essential amino acid based on the findings of Ijarotimi *et al.* (2013):

$$EAAI) = \sqrt[9]{\frac{[Lys\ x\ Threo\ x\ Val\ x\ Meth\ x\ Isoleu\ x\ leu\ x\ Phynylal\ x\ Histi\ x\ Trypt]a}{[Lys\ x\ Threo\ x\ Val\ x\ Meth\ x\ Isoleu\ x\ leu\ x\ Phynylal\ x\ Histi\ x\ Trypt]b}}$$

If a group of amino acids is represented in the sample / (group of amino acids in the standard protein (eggs or casein).

Nutritional Index (NI)

The value of the nutritional index was estimated according to what was reported by (ljarotimi, 2013) as in the following equation:
Nutritional index (%) = (EAAI * % protein) /100

The Biological Value (BV)

According to Muneb *et al*. [26], the biological value was calculated using the following formula:
Bv = 1.9 * EAAI-11.7

Protein Efficiency Ratio (PER)

Three equations were used to evaluate protein efficiency according to what was reported by Vioque *et al.* [27] They are:
PER1=-0.684+0.456×Leu-0.047
PER2=-0.468+0.454×Leu-0.105×Tyr
PER3=-1.816+0.435×Met+0.78×Leu+0.211×His-0.944×Tyr

RESULTS AND DISCUSSION

The composition of *C. rotundus* tubers is detailed in Table 4. The moisture, protein, fat, carbohydrates, ash, and crude fiber contents of *C. rotundus* were measured at 68.13%, 5.13%, 6.86%, 1.33%, and 6.18%, respectively.

Table 4 Chemical composition of Cyperus tubers used in the experiment

%	Ingredients
68.13	Moisture
5.13	Fat
12.40	Crude Protein
1.33	Ash
6,18	Crude Fiber
6.86	Carbohydrate
100	Total

According to previous research [31], the analysis was carried out at the postgraduate laboratory of the University of Baghdad's College of Agriculture.

These results differ from previous findings, which reported a moisture content of *12.91%* and protein, carbohydrate, ash, and fiber contents of 23.67%, 41.72%, and 11.60%, respectively. Researchers have highlighted the nutritional value of various food combinations, and due to their high dietary value, *C. rotundus* tubers can be effectively incorporated into children's diets [28]. Because of the high protein and fat content, as well as the high quality of the active ingredients, Parsley is ideal for preserving foods' content [29] and increasing the shelf life of specific foods, such as jams [30].

Estimation of Amino Acids in the Protein of the Cyperus Plant

The percentages of amino acids in the Cyperus plant and those suggested by the FAO/WHO [32] are shown in table 5. A total of 18 amino acids, including 8 essential ones, were found in the plant. The highest percentage of the plant extract was recorded by the amino acid glutamic acid (1202.03 micrograms/ml), which was followed by isoleucine, leucine, phenylalanine, lysine, tyrosine, arginine, aspartic acid, glycine, proline, valine, serine, threonine, alanine, asparagine, methionine, cysteine, and histidine. The results aligned with his conclusions [33].

Table 5 Demonstrates the amount of amino acids in Cyperus plant extracts and contrasts them with the amino acids found in soybean protein isolate, milk, and egg proteins

Amino acids	Soybean protein isolate **%	Milk and egg %	FAO/WHO* %	Plant extracts µg/ml
Arginine	6.6	-	-	582.17
Aspartic	9.9	-	-	577.91
Asparagine	1.2	1.7	1.1	283.88
Alanine	3.4	-	-	295.13
Isoleucine	6.8	8.6	6.6	769.22
Proline	-	-	-	417.48
Tyrosine	3.2	-	-	648.13
Threonine	3	4.7	3.4	296.140
Cysteine	4.5	-	-	207.13
Serine	4.2	-	-	348.57
Valine	1.1	6.6	3.5	382.38
Phenylalanine	5.2	-	-	715.88
Glycine	3.4	-	-	443.29
Glutamic	17	-	-	1202.03
Lysine	5.2	7	5.8	690.66
Leucine	4.1	5.4	2.8	727.63
Methionine	1.1	-	-	268.87
Histidine	2.3	2.2	1.9	160.10
EAA	-	51.2	33.9	4010.88
NEAA	-	-	-	5005.72
TAA	-	-	-	9016, 6

(EAA) Essential amino acids, (NEAA) Non-essential amino acids, (TAA) Total amino acids.
* FAO/ WHO [24], **Wang *et al* [34]

The most prevalent amino acids found in Cyperus tubers include the essential amino acids glutamic acid, isoleucine, leucine, phenylalanine, lysine, and arginine. These results align with previous studies conducted on peanuts [35]. The table shows that Cyperus extract is rich in essential amino acids needed by the human body, including isoleucine, leucine, phenylalanine, and lysine. Among these, glutamic acid and isoleucine make up the majority of the essential amino acids, comprising 44% of the extract. This is comparable to celery seeds, which contain 45% essential amino acids. Additionally, it has been noted that aspartic acid and glutamic acid are the most prevalent components in seed dust, with high concentrations of histidine, valine, and tryptophan also documented [36-38].

Nutritional Properties

Table 6 presents the nutritional properties of various date palm species, focusing on the theoretical biological value (BV) that was examined. This number (61.1719) reflects the protein composition of the body. The investigation also explored three different theoretical ratios of protein efficiency, resulting in the amino acid scores (AAS) shown in Table 4. The Protein Efficiency Ratio (PER) serves as a measure of protein quality, with values below 1.5 indicating low quality and values above 2 indicating high quality [39, 40]. The study reported that the protein efficiency ratio (PER) of palm kernels from three different varieties of date palm—Zahdi, Khadrawi, and Barban—was 0.75, 0.865, and 0.82, respectively. In contrast, the value of PER1 was 331.06 [41, 42]. The investigation demonstrated that the value of the PER2 was 261.82 compared to the three other values of the kernels of the three date palm varieties Zahdi, Khadrawi and Barban. The values of the core groups of the three different date palm varieties were 0.12%, 0.59% and 0.53% respectively [43, 44]. The value of PER3 was found to be 108.71, the lowest among the three values for PER1 and PER2. In comparison, the results for the Zahdi, Khadrawi, and Barban date palm varieties were 3.54% and 1.68%, respectively. The research also recorded a nutritional index (NI) value of 829%, significantly higher than the NI values for the three date palm varieties, which were 43.71%, 69.20%, and 37.99%. The essential amino acid composition (EAC) serves as one of the indicators of nutritional value. When compared to the essential amino acid index (EAAI) values for the Zahdi, Khadrawi, and Barban varieties, which were approximately 111.68, 110.09, and 108.55, respectively, the study revealed different nutritional values. The EAAI value for the samples was estimated to be around 66.85 [45-47].

Table 6 Nutritional properties of Cyperus plant

Cyperus plant	Nutritional properties
131.219133	AAS
66.855	EAAI
829.002	NI
61.1719	BV
331.06828	PER1
261.82237	PER2
108.714	PER3

(AAS) amino acid score, (EAAI) amino acid index, (NI) nutritional index, (BV) theoretical biological value, (PER 1,2,3) protein conversion efficiency.

Chemical Composition of Cyperus Tubers

The chemical analysis of *C. rotundus* tubers revealed the following composition: moisture (68.13%), protein (12.40%), fat (5.13%), carbohydrates (6.86%), ash (1.33%), and crude fiber (6.18%). These results highlight the tuber's nutritional profile, with a high moisture content and significant protein levels, making it a potential source of dietary nutrients. The carbohydrate content was calculated using the formula: carbohydrates percentage = 100 - (moisture + ash + protein + fiber + fat) (Table 7).

Table 7 Chemical Composition of Cyperus Tubers

Component	Percentage (%)
Moisture	68.13
Protein	12.40
Fat	5.13
Carbohydrates	6.86
Ash	1.33
Crude Fiber	6.18

Amino Acid Profile

Using high-performance liquid chromatography (HPLC), 18 amino acids were identified, including 8 essential amino acids. Glutamic acid was the most abundant (1202.03 µg/ml), followed by isoleucine (769.22 µg/ml) and leucine (727.63 µg/ml). The amino acid composition was compared to FAO/WHO standards, showing a high proportion of essential amino acids, particularly glutamic acid, isoleucine, and leucine (Table 8).

Table 8 Amino Acid Composition of Cyperus Tubers

Amino Acid	Concentration (µg/ml)
Glutamic Acid	1202.03
Isoleucine	769.22
Leucine	727.63
Phenylalanine	715.88
Lysine	690.66

Nutritional Properties

The nutritional indices were calculated to assess the quality of *Cyperus rotundus* protein. The essential amino acid index (EAAI) was 66.85, the biological value (BV) was 61.17, and the amino acid score (AAS) was 131.21. The protein efficiency ratio (PER) values were 331.07 (PER1), 261.82 (PER2), and 108.71 (PER3), indicating high protein quality (Table 9).

Table 9 Nutritional Properties of Cyperus Tubers

Parameter	Value
EAAI	66.85
BV	61.17
AAS	131.21
PER1	331.07
PER2	261.82
PER3	108.71

These results demonstrate the potential of *Cyperus rotundus* as a nutrient-rich food source, with high-quality protein and essential amino acids, supporting its use in dietary and medicinal applications.

Recommendations

This research on *C. rotundus* L. marks a significant advancement in our understanding of its chemical composition and nutritional benefits. The comprehensive chemical assessments, which examine moisture, protein, fat, carbohydrates, ash, and fiber, provide a detailed overview of the plant's macronutrient profile. Furthermore, the identification of 18 amino acids, including 8 essential amino acids, highlights its potential as a valuable source of protein and vital nutrients.

TUtilizing advanced methods such as high-performance liquid chromatography (HPLC) ensures accurate measurement of amino acid levels, with glutamic acid identified as the most abundant amino acid. The study also evaluates key nutritional metrics, including biological value (BV), essential amino acid index (EAII), amino acid score (AAS), and protein efficiency ratio (PER), emphasizing the plant's promise as a source of high-quality protein. Given these findings, this research serves as an important reference for future inquiries in nutritional science, food technology, and agricultural practices. It is strongly recommended for publication and dissemination among experts in agriculture, food science, and nutrition.

CONCLUSION

In conclusion, the study of *Cyperus rotundus* tubers offers valuable insights into their chemical composition and nutritional properties. The analysis showed a high moisture content of 68.13%, along with significant levels of protein (12.40%), fat

(5.13%), and essential amino acids, particularly glutamic acid, isoleucine, and leucine. The presence of 18 amino acids, including eight essential ones, highlights the plant's potential as a nutrient-rich food source. Advanced techniques such as HPLC confirmed the accuracy of these findings, while nutritional indices like BV, EAAI, and PER emphasized its high-quality protein content. These results suggest that *C. rotundus* could be effectively incorporated into food products to enhance nutritional value, especially in regions that need affordable, nutrient-dense food sources. Further research and application in food technology and agriculture are strongly recommended.

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