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INVESTIGATION OF THE PROTEIN CONTENT OF *EICHHORNIA CRASSIPES* (MART.) SOLMS AND PISTIA STRATIOTES L. GROWING IN EGYPT

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لقد أسفرت نتيجة دراسة المحتوى البروتينى لأوراق ، وسيقان ، جذور نباتى ورد النيل وخس الماء عن الأتى:

١- أوراق وسيقان نبات ورد النيل تحتوى على أعلى نسبة من البروتين (٢٣,١٨٪)، يأتى بعدها أوراق وسيقان نبات خس الماء (١٧,٧٥٪) بينما تحتوى الجذور على نسبة أقل من المواد البروتينية وهى ١١,٦٨٪ ، ٩,٧٥٪ لنباتى ورد النيل وخس الماء على التوالى.

٢- أظهرت دراسة الاحماض الامينية للبروتين الموجود فى الاجزاء المختلفة للنباتين ، على احتواء هذه البروتينات على الاحماض الامينية الضرورية الأتية:

"الفينايل أمين ، اليوسين ، - أو الايزوليوسين ، الثربونين ، المثيونين ، التربتوفان ، الفالين إلى جانب الأحماض الامينية الآتية:

الهستيدين ، الارجينين ، الجلايسين ، حمض الاسبارتيك ، حامض الجليوتاميك ، البرولين ، الهيدروكسى برولين ، حامض ألفا أمينو بيوتريك. وقد لوحظ أن البروتين المفصول من نبات ورد النيل يحتوى على حامض الليسين بالإضافة إلى الاحماض الامينية سابقة الذكر.

٣- عينت الاحماض الامينية الضرورية للبروتين المستخلص من الأجزاء المختلفة للنباتين ، وقد أظهرت هذه الدراسة أن البروتين المفصول من أوراق وسيقان نبات ورد النيل تحتوى على أعلىنسبة من هذه الأحماض (١٣,٥٪). يأتى بعده البروتين المفصول من أوراق وسيقان نبات خس الماء (١٢,٢٪) ثم بعد ذلك البروتين المفصول من جذور نباتى ورد النيل (١٠,١٢٪) وخس الماء (٨,٦٪).

٤- عينت نسبة النيتروجين الكلى فى النباتين وكذلك نسبة النيتروجين القابل للذوبان فى الماء ووجد أن هذه النسبة كما يلى:

أ- ٤,٥٠٨٪ ، ٠,٨٢٥٪ لأوراق وسيقان نبات ورد النيل.

ب- ٣,١٥٥٪ ، ٠,٣١٦٪ لأوراق وسيقان خس النيل.

ج- ٢,٥٪ ، ٠,٣٣٦٪ لجذور نبات ورد النيل.

د- ٢,٩٣٪ ، ١,٤٥٪ لجذور نبات خس النيل.

٥- أجريت مقارنة بين المحتوى البروتينى للنباتين قيد البحث وبعض النباتات المستخدمة لتغذية الحيوانات ، وقد أظهرت المقارنة أن النباتين يمكن استخدامها كغذاء للحيوان.

A qualitative and quantitative study of the protein content of roots, stems and leaves of Eichhornia crassipes (Mart.) Solms (Family: Potederiaceae) and Pistia sratiotes L., (Family: Aracaceae), were carried out. This study revealed that these plants contain a large percent of nutritive proteins which include high percentage of essential amino acids covering the requirements of birds (Turkey) and animals.

INTRODUCTION

Eichhornia crassipes (Mart.) Solms (water hyacinth) and *Pistia stratiotes* L. (water lettuce) are among the aquatic plants which grow vigorously on the River Nile especially after the

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construction of the high dam in Aswan as a result of slow current of water. The removal of such plants from River Nile, branches and small canals represents one of the major problems of irrigation in Egypt and costs too much. At the same time, big amounts of these plants are removed every year without any benefit. So, it was found necessary to carry out a thorough investigation of these plants aiming for finding out the possibilities of their use in industrial fields.

The phytochemical screening of the different parts of such plants revealed the presence of protein materials (Biuret's test)[1].

Reviewing the current literature, little was reported[2-15] dealing with protein content of both plants.

So, a phytochemical study of protein content of both plants were carried out to find an industrial use of the removed plants from the River Nile.

EXPERIMENTAL

Materials

The leaves, stems and roots of both water hyacinth and water lettuce were separately collected, air-dried, powdered, sieved (sieve No. 10) and kept in brown bottles for this study.

The identity of both plants was established by Prof. Dr. El-Hadidy, Botany Department, Faculty of Science, Cairo University.

The authentic amino acids used in this study were obtained through the curtesy of BDH.

Preparation of protein[16]

Ten g of each dry powdered roots, stems and leaves of water hyacinth and water lettuce was separately stirred with 10% solution of sodium chloride for one hour. The mixture, in each case, was allowed to stand for 10 minutes. To the supernatant solution, an equal volume of 10% trichloroacetic acid was added and allowed to stand for 10 minutes. The precipitated protein $xs then filtered through whatmann filter paper No. 1 and washed successively with trichloroacetic acid, alcohol and ether. The protein isolated by gravimetric method was dried in a desiccator, weighed and the percentage of protein in each case was calculated and recorded in Table I.

Hydrolysis

100 mg of each isolated protein was separately hydrolyzed by heating with 6N HCl in a sealed tube at 100°C for 20 hours. After hydrolysis, HCl was removed by concentrating the hydrolysate at 40°C under vacuum to dryness. The residue was dissolved in 3 ml 10% isopropanol and saved for paper chromatographic investigation.

Paper chromatography

The different protein hydrolysates together with 1% solution of authentic amino acids in 10% isopropanol were chromatographed on whatmann filter paper No. 1, adopting the two dimensional technique. Development was done by n-butanol-acetic acid-water (12:3:5) (BAW) for the first run and phenol-ammonia (200:1) (PA) for the second and the chromatograms were sprayed with ninhydrin reagent. Results obtained were recorded in Table II.

Determination of total nitrogen content and protein content

The total and soluble nitrogen contents of each part under investigation were determined by Kjeldahl's method[18]. The percentage of each part was separately calculated and recorded in Table III.

Quantitative estimation of the essential amino acids of the protein under investigation

The ninhydrin method is applied for the quantitative estimation of the amino acids[19]. A standard curve for each of essential amino-acids viz., phenylalanine valine, threonine and leucine and/or isoleucine was made using serial concentration viz. 5, 10, 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160 and 200 ug. 2 ml of ninhydrin reagent[19] was added and the mixture in each case, was heated on a water bath for 20 minutes, 5 ml of 50% isopropanol was added to each tube and the bluish violet colour was measured after 15 minutes. The absorbance was

Table I: Percentage of protein in different parts of the examined plants.

Estimation Method	Water hyacinth		Water Lettuce	
	Leaves & Stems	Roots	Leaves & Stems	Roots
Gravimetric	23.10%	11.60%	17.10%	9.22%

Each value represents the mean of three.

Table II: Results of paper chromatographic investigation of protein hydrolysates.

Spot No.	Authentic amino acids	Colour ninhydrin	*Eichhornia crassipes* (Mart.)		*Pistia stratiotes* L.	
			Leaves & stolons	Roots	Leaves & stolons	Roots
1	L-Cysteine	violet	-	-	-	-
2	L-Lysine	violet	+	+	-	-
3	DL-Ornithine	violet	-	-	-	-
4	L-Histidine	grey violet	+	+	+	+
5	L-Arginine	violet	+	+	+	+
6	L-Glycine	red violet	+	+	+	+
7	DL-Aspartic acid	blue violet	+	+	+	+
8	DL-Serine	violet	+	+	+	+
9	L-Glutamic acid	purple	±	±	±	±
10	DL-Methionine	purple	+	+	+	+
11	DL-Threonine	violet	±	±	±	±
12	Hydroxy-proline	yellow	+	+	+	+
13	DL-Alanine	violet	±	±	±	±
14	L-Proline	yellow	+	+	+	+
15	2-aminobutyric acid	violet	+	+	+	+
16	L-Tyrosine	grey violet	+	+	+	+
17	B-Phenylalanine	grey violet	+	±	±	±
18	DL-Valine	violet	+	+	+	+
19	DL-Tryptophan	grey violet	+	+	+	+
20	Leucine and/or isoleucine	violet	+	+	+	+

+, present; -, absent; ±, present in traces.

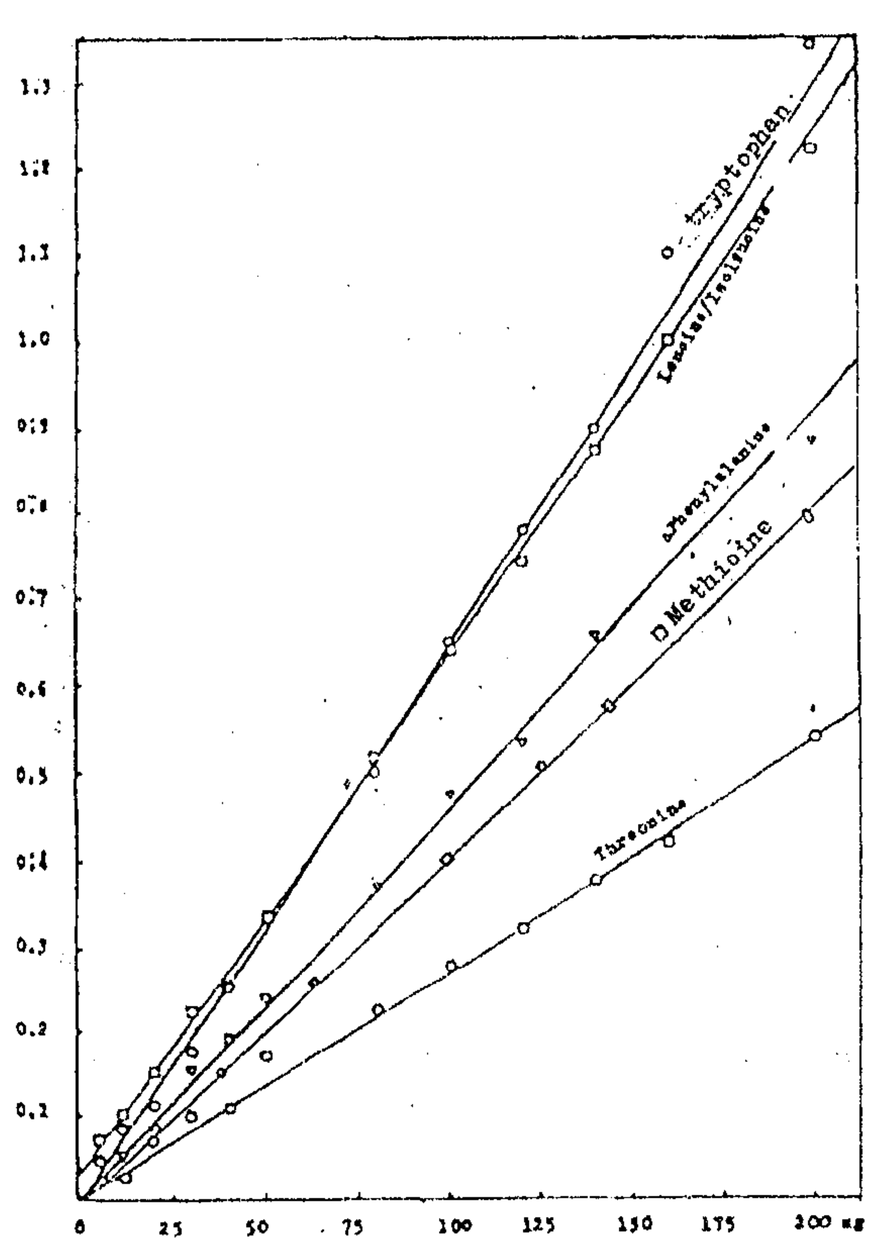
Table III: Percentage of the total and soluble nitrogen as well as protein content of the different parts under investigation.

	T.N	Percentage of S.N.	Protein
Water hyacinth			
L & S	4.51	0.852	23.18
R	2.50	0.636	11.68
water Lettuce	3.16	0.316	17.75
R	2.93	0.450	9.75

T.N.: Total nitrogen
S.N.: Soluble nitrogen
L & S: Leaves and stolons
R.: Roots.
Each value represents the mean of three experiments.

plotted against concentration. The results obtained are shown in Fig. 1.

Fig. 1



Recovery curve of each amino acid was done under the same condition and the percentage of the recovery was determined.

The concentration of each essential amino acid, was determined from the corresponding calibration curve and after correction from the recovery curves. The results are recorded in Table IV.

RESULTS AND DISCUSSION

From Table IV, one may conclude that the protein content of the different parts of water hyacinth are higher than those of water lettuce. The different parts can be arranged according to their protein content in a descending manner as follow: leaves and stolons of water hyacinth (23.0%), leaves and stolons of water lettuce (17.16%), roots of water hyacinth (11.68%), and finally the roots of water lettuce (9.75%).

From Table II, it can be concluded that all proteins of the different parts of both plants under investigation contain 14 amino acids namely, L-arginine, DL-aspartic acid, L-glycine, L-histidine, 2-aminobutric acid, DL-methionine, DL-serine, B-phenyl alanine, L-proline, DL-tryptophan, DL-threonine, DL-valine and leucine and/or isoleucine. L-lysine is present in the proteins separated from different parts of water hyacinth in addition to the previously mentioned

Table IV: Percentage of essential amino acids in proteins of different parts of plants under investigation.

Essential amino acids (%)	Water hyacinth		Water lettuce	
	Leaves & stolons	Roots	Leaves & stolons	Roots
Phenyl-alanine	4.1	2.0	3.7	2.5
Tryptophan	1.3	0.66	1.1	0.9
Threonine	2.0	0.50	1.6	0.8
Leucine and/or Isoleucine	4.1	4.66	3.0	2.1
Methionine	2.0	2.30	2.8	2.3

Each reading represents the mean of two experiments.

amino acids, while DL-alanine is present in those isolated from the different parts of water lettuce. Hydroxyproline and L-glutamic acid are present in small amount in all proteins isolated from different parts of plants under investigation. L-tyrosine is preset in protein of leaves and stolons of water hyacinth, while other parts of investigated plants contain traces of this amino acid.

The results obtained by K-Jeldahl's method (Table III) agree to a great extent with those obtained by precipitation method used for the isolation of proteins.

From Table IV, it can be concluded that the proteins of the different parts under investigation can be considered as a valuable dietetic proteins as they contain 6 essential amino acids, namely phenylalanine, threonine, tryptophan, methionine, leucine and/or isoleucine. According to the essential amino acid content in proteins, the different parts can be arranged in a decreasing order as follow: leaves and stolons of water hyacinth (13.5%) leaves and stolons of water lettuce (12.2%), roots of water hyacinth (10.12%) and roots of water lettuce (8.6).

The percentage of the protein content of the different parts of the plants under investigation when compared with other fodder plants[20-24], of common use for animals, were found to be of equal or slightly low values as shown in Table V. But due to the high percentage of mineral content of water hyacinth[24-29], it is more preferable to use the isolated protein materials.

Table V: Protein content of some common fodder plants.

Food staff	Protein percent
Clover	50.00
Barely food	12.13
Fine wheat feed	12.50
Linseed meal	24.00
Peas	22.00
Lentils	25.00
Chick pea	22.47
Lupine seed	32.81
Vicica species	15.33
Water hyacinth leaves & stolans	23.10
Water lettuce leaves & stolans	17.70
Water lettuce roots	11.60

Concerning the proteins isolated from the different part of water hyacinth, our results are in agreement with those reported by Boyd (1970), in containing L-glutamic acid, L-glycine, L-histidine, DL-threonine, B-phenylalanine, L-lysine, L-leucine and/or isoleucine, L-tyrosine, L-proline, DL-serine and DL-valine. In addition to the previously mentioned amino acids, L-arginine, DL-aspartic acid, DL-methionine, hydroxy proline, 2-aminobutyric acid and DL-tryptophan were found in this plant. Our findings also differ from those reported by El-Serafy[6] (1981) in the percentage of protein content being 13.3% in leaves and stolons and 17.1% in roots. Concerning the percentage of

protein content of water hyacinth (*Eichhornia crassipes*), our findings are also in agreement with that of Reddy[24] (1977) (14-16% protein) and higher than those reported by Boyd and Meginty (1981)[5] (13.2% proteins). Our work on the protein content of water lettuce, can be considered as a first report for such investigation.

Concerning the amino acids content of the protein of water lettuce (Table III) our findings are in good agreement with those published by Boyd[3] (1970) in containing: L-arginine, DL-aspartic acid, DL-alanine, L-glycine, L-glutamic acid, histidine, L-threonine, L-tyrosine, L-leucine and/or isoleucine, B-phenylalanine, L-proline, DL-serine and DL-valine and differ in the absence of lysine and the presence of DL-tryptophan, hydroxy-proline, methionine and 2-aminobutyric acid.

So, one can recommend that the proteins isolated from the different parts of both plants under investigation could be used as additive for fodder of animals and birds while the dried water lettuce could be used as fodder.

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