

ESTIMATION OF AMINO ACID CONTENTS**Dr. Arun Vats*****Komal Lata Nagpal****

ABSTRACT

Qualitative estimation of amino acids from various plant parts (roots, shoots and fruits) of all the four plant species have been carried out by paper chromatography and thin layer chromatography.

Amino acids have been estimated quantitatively by Spectrocolorimeter.

The maximum number of amino acids free as well as bound were found thirteen in fruits of *Solanum surattense*.

Out of 24 amino acids sixteen free and fourteen bound amino acids were detected in various plant samples tested.

Maximum total amount (46.2 mg/g.d.w.) of free amino acids was found in the fruits of *Solanum surattense*, while, minimum (19.8 mg/g.d.w.) in roots of *Barleria prionitis*. The amount of individual amino acid, however, showed considerable variation. The individual amount of methionin was found maximum (6.7 mg/g.d.w.) in fruits of *Solanum surattense* among all of the free amino acid detected in selected plant species while, minimum of glycine (1.6 mg/g.d.w.) in the roots of *Barleria prionitis*.

Maximum amount (39.3mg/g.d.w.) of the total bound amino acid was observed in the fruits of *Barleria prionitis*, while, minimum (14.6 mg/g.d.w.) in the roots of *Solanum surattense*. A large variation was shown in the amount of individual amino acids. The individual amount of aspartic acid was found maximum (5.8 mg/g.d.w.) in the shoots of *Solanum nigrum* among all the bound amino acids detected in selected plant species, whereas minimum (1.2 mg/g.d.w.) threonine in the roots of *Solanum surattense*.

In the present investigation, number of free amino acids were estimated more in all samples studied. Higher amounts of bound amino acids in the intact plant parts may be due to storage of proteins in these organs.

*Deptt of Botany, Seth G. L. Bihani S. D. College, Sri Ganganagar, India

**Deptt of Botany, M. L. Piramal P. G. College, Bagar, Jhunjhunu, India

INTRODUCTION:

Amino acids in plants are found in the free and bound state. All amino acids are made up of C, H, O and N. However, few possess sulfur also (cysteine and methionine). The general formula for amino acids is $R\cdot CH_2\cdot (NH_2)COOH$. The group R being either a simplest hydrogen atom (as in glycine) or a complex organic group. The group R may also show neutral basic or acidic character and include a hydroxyl, aromatic or sulfur containing substituents. An amino acid may be cyclic (aromatic) non cyclic (aliphatic) or heterocyclic. Due to the presence of at least one acid or carboxylic group (-COOH) and one or more basic or amino (-NH₂) group, these acids can behave as acids towards bases and a base towards acids, therefore they are amphoteric compounds. The compounds generally exist in two stereoisomeric forms, L-forms and D-forms. The asymmetry exists at α -carbon atom (except in glycine where R=H) only L-forms of amino acids are encountered in plant protein. However, the D-forms of many amino acids are found in cell wall, antibiotic peptide of many microorganisms and seaweeds.

ESTIMATION OF AMINO ACID CONTENTS:

The present investigation deals with qualitative and quantitative estimation of amino acid contents in different plant parts of *Barleria prionitis*, *Solanum nigrum* and *Solanum surattense*.

Materials and Methods

The roots, shoots and fruits of all the three selected plant species taken for present investigation were collected from study area.

Plant parts of *Barleria prionitis*, *Solanum nigrum* and *Solanum surattense* were collected in polythene bags. The samples were dried, powdered and then used for their estimation of amino acids.

Extraction Procedure

5 gm powdered material from each of the sample (roots, shoots and fruits) was macerated with 80% ethanol in a Waring blender. To each of these mixtures 80% ethanol was added to make up the volume up to 250 ml in each case and left overnight at room temperature. Each of the mixtures was filtered and the residue washed thrice with 80% ethanol. All the washings were pooled along with the filtered. To the filtrate chloroform (1:3 v/v) was added, shaken thoroughly and aqueous layer separated in a separating funnel (Awapara, 1948). The step was repeated thrice and the resulting aqueous layers from each of the samples were pooled and dried in a vacuum desiccator. Each of the residue thus obtained was dissolved in 10 ml 50% ethyl alcohol, dried, redissolved in 10% isopropanol and stored at 2°C.

Protein Bounded amino acids

Each of the residues (of original mixture in 80% ethanol) left after the extraction of free amino acids was hydrolysed with 6N HCl (30ml), Block *et al.* (1958), Khanna and Jain (1973), Khanna and Nag, (1973) at 100°C for 24 hour using a stoppered sealed tube. The mixture was filtered and the filtrate dried in a vacuum desiccator. The dried mass was dissolved in 10% isopropanol and stored at 2°C for further studies.

Qualitative Estimation**(a) One dimensional paper - Chromatography**

Each of the samples prepared and stored as above was studied separately by paper chromatography for qualitative determination. Each of the test samples (free and bound) along with the standard amino acids (alanine, arginine, asparagine, aspartic acid, citrulline, cystine, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lycine, methionine, ornithine, phenylalanine, proline, hydroxyproline, serine, threonine, tryptophan, tyrosine and valine) were applied (0.01ml) separately 2-3 cm above the edge of the Whatman No. 1 filter paper strips. The paper strips were developed by ascending chromatography technique in an organic solvent mixture n-butanol, acetic acid and water (60:20:20 Khanna and Jain 1973) for 18hr. in an air tight chromatography chamber which was earlier saturated with the solvent mixture. The developed strips were taken out, air dried, and sprayed with 0.25% of ninhydrin dissolved in acetone (Dent, 1948, Toennies and Kob, 1951). These paper strips were then heated in an oven at 80°C for 10 min. The resulting ninhydrin positive spots from each of the test samples were compared with those of the reference amino acids and their R_f values calculated ($R_f = \text{Total distance traveled by the substance} / \text{Total distance traveled by the solvent}$) five such replicates in each case were examined and mean value calculated.

(b) Two dimensional thin layer chromatography (TLC)

Thin glass plates (20 x 20 cm) coated with silica gel (30 gm/60 ml distilled water, wet thickness 0.2-0.3 mm), dried at room temperature were activated at 100°C for 30 min and cooled. About 0.01 ml of each of the extract samples (free and bound) was applied at one of the corners (leaving a margin of about 1" on both the vertical and horizontal edges) of the glass plates using a fine glass applicator. The plates were then developed in a solvent mixture of n-butanol, acetic acid, water (5:1:1) in first direction, in a chromatography chamber saturated with the solvent system. The developed plates were dried at room temperature. These plates were rotated at 90° and then redeveloped in the second direction using the

solvent mixture of phenol saturated with water.

The plates thus developed were allowed to dry at room temperature and sprayed with 25% of ninhydrin in acetone. The sprayed plates were left at $27\pm 1^\circ\text{C}$. The positions (R_f) and the colours of each of the ninhydrin positive spots were recorded.

Separate plates were developed for each of the test samples and also for the known reference amino acids. The respective amino acids were identified by comparing the relative positions and colours with those of the reference amino acids. Later, these results were compared with those of the recorded by one dimensional paper chromatography.

Quantitative Estimation

Different concentration (1 mg/ml to 9 mg/ml in 50% ethanol) of the standard reference amino acids, the presence of which was qualitatively confirmed in the test samples, were prepared and applied (0.01 ml) separately on Whatman No. 1 filter paper strips along with the test sample (free and bound) from each of the plant species. These various strips were developed (n-butanol, acetic acid, water; 6:2:2) and sprayed with ninhydrin reagent. Each of the ninhydrin spot from the known as well as from the test samples was cut, dissolved in 5 ml of 50% ethanol and optical density read (Spectrocolorimeter 103, Systronics India adjusted at 400 nm using 5 ml of 50% ethanol as blank). A regression curve of each of the known amino acid was worked out which followed the Beer's law. The concentrations of each of the qualitatively confirmed amino acid from the plant species in their free and bound fractions were separately determined (mg/gm dry weight) by comparing with those of their respective standard reference amino acids. Five such replicates of free and bound amino acids from each of the test samples were examined and mean values taken.

RESULTS AND DISCUSSION

Among the intact plant parts i.e. roots, shoots and fruits of *Barleriaprionitis*, *Solanumnigrum* and *Solanumsurattense*, different types of amino acids were found. Out of twenty four amino acids sixteen free and fourteen bound amino acids were detected in various plant samples tested. The maximum number of amino acids free as well as bound were found thirteen in fruits of *Solanumsurattense* (Table- 6.1 and 6.2).

Maximum total amount (46.2 mg/g.d.w.) of free amino acids was found in the fruits of *Solanumsurattense*, while, minimum (19.8 mg/g.d.w.) in roots of *Barleriaprionitis*. The amount of individual amino acid, however, showed considerable variation. The individual amount of methionin was found maximum (6.7 mg/g.d.w.) in fruits of *Solanumsurattense* among all of the free amino acid detected in selected plant species while, minimum of glycine

(1.6 mg/g.d.w.) in the roots of *Barleriaprionitis*(Table 6.1).

Maximum amount (39.3mg/g.d.w.) of the total bound amino acid was observed in the fruits of *Barleriaprionitis*, while, minimum (14.6 mg/g.d.w.) in the roots of *Solanumsurattense*. A large variation was shown in the amount of individual amino acids. The individual amount of aspartic acid was found maximum (5.8 mg/g.d.w.) in the shoots of *Solanumnigrum* among all the bound amino acids detected in selected plant species, whereas minimum (1.2 mg/g.d.w.) threonine in the roots of *Solanumsurattense*(Table 6.2).

However, the amount of amino acids is different in various part of a plant due to some biochemical reactions going inside. In the present investigation number of free amino acids were more in all the samples. Higher amounts of bound amino acids in the intact plant parts may be due to storage of proteins in these organs.

TABLE - 6.1

Free Amino Acid contents (mg/g.d.w.) of various plant parts of selected plant species

S. No.	Amino Acid	R _f (x100)	<i>Barleriaprionitis</i>			<i>Solanumnigrum</i>			<i>Solanumsurattense</i>		
			Roots	Shoots	Fruits	Roots	Shoots	Fruits	Roots	Shoots	Fruits
1	Alanine	28	2.9	3.6	-	-	3.6	4.6	-	-	2.1
2	Arginine	14	-	-	3.7	2.9	-	-	-	-	3.9
3	Aspartic acid	25	3.7	4.5	-	4.6	5.8	6.2	2.5	3.7	2.5
4	Citrulline	23	2.4	3.8	3.4	-	-	3.7	2.1	2.8	-
5	Glutamic acid	35	-	4.6	-	3.8	2.9	4.1	4.9	3.4	3.8
6	Glycine	19	1.6	-	-	1.8	-	2.2	-	-	1.7
7	Histidine	13	2.7	3.1	3.9	4.9	4.5	3.1	-	4.7	2.9
8	Isoleucine	71	-	-	2.4	-	-	4.5	-	-	3.8
9	Leucine	72	-	-	4.5	2.7	1.9	-	3.4	2.4	-
10	Methionine	51	-	2.1	3.4	3.8	4.2	2.6	-	5.9	6.7
11	Phenyl alanine	65	-	3.8	5.7	-	2.5	4.9	4.7	5.1	4.5
12	Serine	33	-	-	4.3	-	3.7	-	-	3.2	2.7

13	Threonine	24	3.7	4.8	3.8	3.4	3.2	4.7	-	2.6	3.5
14	Tryptophan	62	-	3.5	2.4	-	1.8	3.6	4.4	-	-
15	Tyrosine	40	-	2.3	4.6	2.6	-	-	2.5	3.2	4.8
16	Valine	58	2.8	-	3.5	-	-	1.7	3.6	1.9	3.4
Total			19.8	36.1	45.6	30.5	34.1	45.9	28.1	38.9	46.2
Amino acid contents											
Total No. of amino acids			7	10	12	9	10	12	8	11	13

GRAPH – 6.1

Total Free Amino Acid contents (mg/g.d.w.) of various plant parts of selected plant species

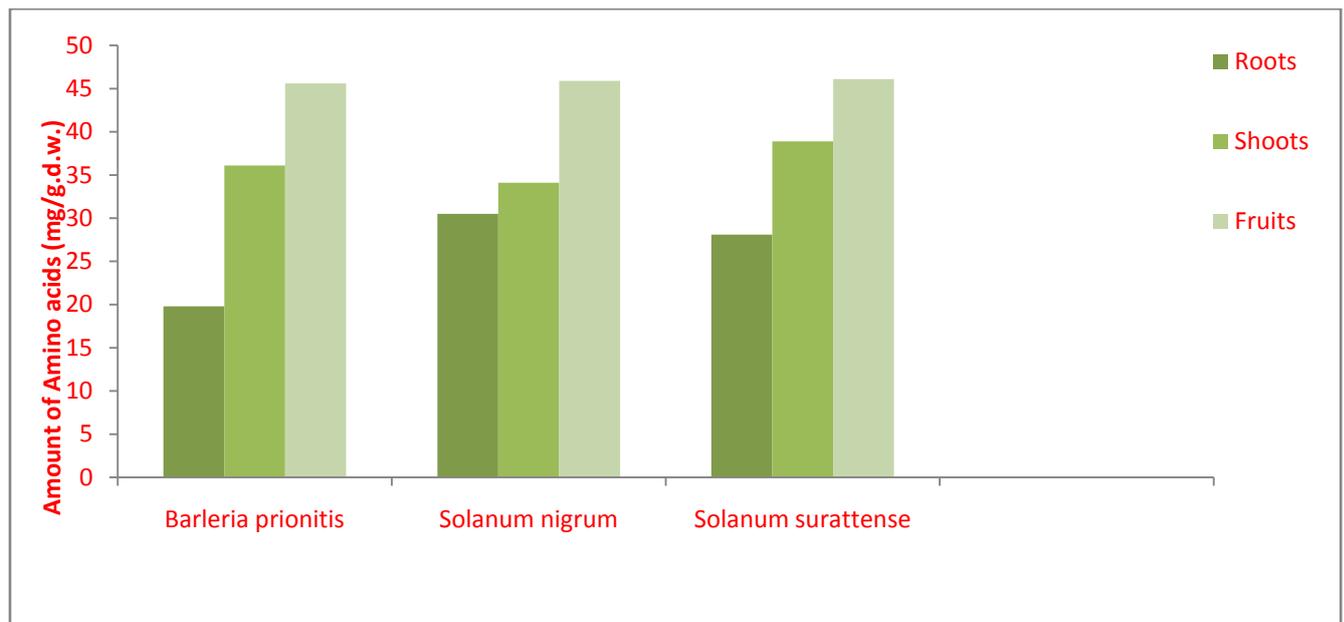


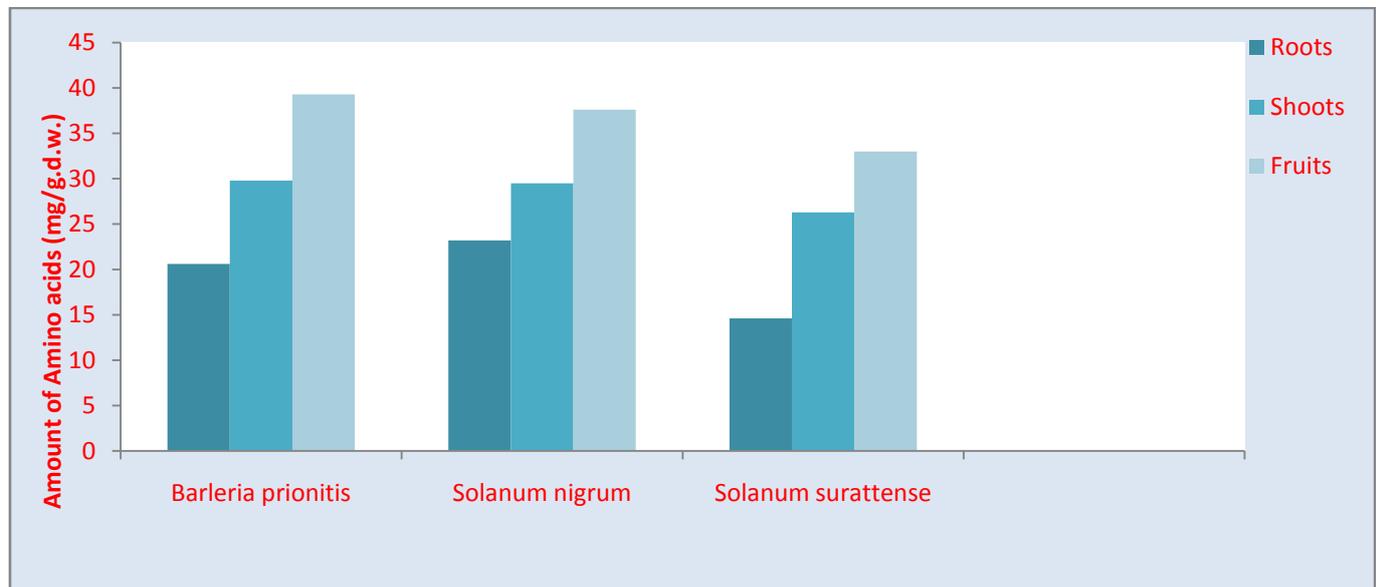
TABLE - 6.2

Bound Amino Acid contents (mg/g.d.w.) of various plant parts of selected plant species

S. No.	Amino Acid	R _f (x100)	<i>Barleriaprionitis</i>			<i>Solanumnigrum</i>			<i>Solanumsurattense</i>		
			Roots	Shoots	Fruits	Roots	Shoots	Fruits	Roots	Shoots	Fruits
1	Alanine	28	3.4	-	4.6	-	2.5	-	1.6	2.4	2.9
2	Aspartic acid	25	1.4	2.3	-	4.7	5.8	4.3	2.5	3.2	4.5
3	Citrulline	23	-	2.7	-	2.3	3.7	5.2	-	-	-
4	Glutamic acid	35	-	-	2.9	3.4	4.1	-	-	-	3.8
5	Glycine	19	2.6	3.5	4.8	-	-	3.9	2.7	3.5	-
6	Histidine	13	2.8	3.9	3.1	4.6	-	4.2	1.8	2.9	3.4
7	Lysine	11	1.7	2.4	3.5	3.2	3.6	4.2	-	-	-
8	Methionine	51	3.4	4.2	4.7	-	-	-	4.8	-	5.2
9	Phenyl alanine	65	-	-	2.1	-	2.6	3.3	-	3.6	-
10	Proline	27	-	-	-	-	3.5	4.1	-	2.5	-
11	Threonine	24	2.8	3.3	2.5	2.1	-	-	1.2	-	2.9
12	Tryptophan	62	-	2.5	3.7	-	-	2.1	-	2.4	3.2
13	Tyrosine	40	-	3.4	4.3	2.9	3.7	4.5	-	2.3	3.0
14	Valine	58	2.5	1.6	3.1	-	-	1.8	-	3.5	4.1
	Total Amino acid contents		20.6	29.8	39.3	23.2	29.5	37.6	14.6	26.3	33.0
	Total No. of amino acids		8	10	11	7	8	10	6	9	9

GRAPH – 6.2

Total Bound Amino Acid contents (mg/g.d.w.) of various plant parts of selected plant species



BIBLIOGRAPHY:

1. A number of workers have been investigated the quantitative and qualitative estimation of amino acids from a number of plant species. Dixit *et al.* (1974) reported six types of amino acids, lysine, phenyl alanine, serine, threonine and valine have been described from seeds of *Corchorusaestuans*.
2. Bhatnagaret *al.* (1976, 77) reported the presence of four types of amino acids as arginine, glutamic acid, histidine and valine in the seeds and pulp of *Citrulluscolocynthis*.
3. Harsh (1982) reported twelve free and eleven bound amino acids in various plant parts of *Citrulluscolocynthis*, *Corchorusdepressus*, *Fagoniacretica* and *Lyciumbarbarum* growing in arid zone of Rajasthan.
4. High amino acid contents have also been reported in germinated seeds of fodder plants (Kina, E.L., 1988), leguminous plants (Prakashet *al.*, 1987).
5. Otchereet *al.* (1988) estimated amino acids in seeds of *Griffoniasimplicifolia* (a leguminous shrub) by HPLC technique from Accra plains.
6. Gomes *et al.* (1988) have reported that the chemical composition of seeds of *Canavaliabrasiliensis* was similar to that other legume seeds.

7. Sinha and Saran (1988) detected various amino acids and protein contents of leaves in *Panicum* spp. by using two dimensional chromatography technique.
8. Lefevreet *et al.* (1989) concluded that the values for glutamine and asparagin in stubble and roots of *Lolium perenne* (perennial rye grass) were twice than those leaves.
9. Joshi and Kumar (1993) found the quantitative estimation of glutamic acid, glycine, isoleucine, leucine, methionine, phenylalanine, serine, threonine and valine in different parts of *Salvadora persica*. They concluded that these amino acids were increase in amount in salinity conditions. While arginine, alanine, aspartic acid and asparagine were decrease in salinity.
10. Bains, N.S. and Harsh, M.L. (1996) have reported the thirteen free and eleven bound amino acids from various plant parts (Root, shoot and fruit) of *Citrullus lanatus* and *Withania somnifera* Linn.
11. Kubota *et al.*, (1998) estimated the two natural amino acids, (R₅)-3-[(methylthio) methylsulfinyl]-L-alanine and S-[(methylthio) methyl]-L- cysteine were isolated from the fruit of *Scorodocarpus borneensis* which was known to have a garlic like odour.
12. Acharya, J. (1999) reported free and bound amino acids from various plant parts (stem, leaf and fruits) of some arid zone trees.
13. Maheshwari, A. (2001) reported free and bound amino acids from various plant parts (roots, shoots and fruits) of *Crotalaria burhia*, *Capparis decidua* and *Ziziphus mauritiana*.
14. Ritu (2001) estimated the eighteen free and thirteen bound amino acids from various plant parts (roots, shoots and fruits) of *Heliotropium curassavicum*, *Parthenium hysterophorus* and *Lantana camara* collected from Indira Gandhi Canal irrigated area of Bikaner district.
15. Shahid, A. (2002) observed 13 free and 11 bound amino acids from various plant parts (roots, shoots and fruits) of *Acacia senegal*, *Acacia nilotica*, *Maytenus marginata*, *Parkinsonia aculeata* and *Prosopis cineraria*.
16. Harsh, G. (2002) has reported 18 free and 15 bounded amino acids from various plant parts (root, shoots and fruits) of *Abutilon indicum*, *Aerva persica*, *Euphorbia microphylla* and *Glinus lotoides* growing in Kodamdesar Pond area of Bikaner district.
17. Kapoor, B.B.S., Ranga, P.D. and Harsh, G. (2003) reported the production of free and bound amino acids from some arid zone Asteraceous medicinal plants.
18. Harsh, G. and Kapoor, B.B.S. (2004) observed free and protein bound amino acids in some arid zone plants like *Abutilon indicum*, *Aerva persica*, *Euphorbia microphyllus*

and *Glinuslotoides*.

19. Gir, R. (2006) has reported 18 free and 15 bound amino acids from various parts of some halophytic plants of waterlogged area of Hanumangarh district.
20. Khatri, S. (2007) has reported the amino acid contents from some plants species of Tal Chhappar Wildlife Sanctuary of Churu district.
21. Swami, S. (2008) has evaluated the amino acid contents from some medicinal plants of Sriganaganagar district.
22. Harisaranraj, R. and Suresh, R.K. (2009) has evaluated the amino acid contents from *Ruwolfiaserpentina* and *Ephedra vulgaris*.
23. Lohlum, S.A., Maikidi, G.H. and Soloman, M. (2010) has reported the amino acid profile from *Lophiralanceolata* seeds.
24. Gaffer, M.K. *et al.* (2010) has estimated amino acid contents from *Indigoferastragolina* leaves.
25. Gloria, A.O. *et al.* (2010) has estimated amino acid contents from *Aliumsativum*, *Zinziberofficinale* and *Capsicum frutescence*.
26. Gwfraz, M.A; Zeenat, A. and Tariq, H. (2011) has evaluated the amino acid contents from *Eruca sativa* seeds.