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Qualitative Analysis: Free Amino acid Composition of Leaf Extract (LE) and Leaf Protein Concentrates (LPC) of Some Wild and Cultivated Plant Species.

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Abstract

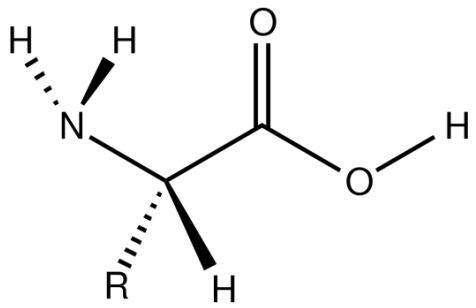
The objective of the present study is to search the alternative source of nutritional quality of dietary protein from the leaves of wild and cultivated plant species which are underutilized. Leaf protein concentrates (LPC) which is concentrated form of protein prepared from the foliage of plants is inexpensive and most abundant source of available protein and it is equivalent to most of the animal protein source. For the use of leaf protein concentrates (LPC) as a human and animal consumption it is necessary to have appropriate proportion of amino acid composition in it. The nutritive value of protein explained on availability and amount of various essential amino acids present in it. In the present communication describe the isolation of leaf protein concentrates (LPC) from the leaves of ten different plant species viz. *Brassica juncea* (L.) Czern. & Coss, *Brassica napus* L., *Brassica oleracea* var. *Botrytis* L. (cultivated) and *Chenopodium album* L, *Goniocaulon indicum*, (Klein ex Willd). C.B. CL, *Celosia argentea* L., *Vigna trilobata* (L.)Verde, *Digera muricata* (L.) Mart, *Tridax procumbens* L. and *Ocimum americanum* L. (wild), and its qualitative analysis by thin layer chromatography (TLC) technique for detection of different amino acid composition were done. In Leaf extract, the free amino acids like L-Tyrosine, DL-Valine and L-Argenine HCl Whereas in LPC, DL-Alanine was detected in most of the species. In the present study, maximum free amino acids were detected except proline in leaf extract as well as in leaf protein concentrates of different plant species. In general, the plant species under investigation showed all the essential amino acid except Histidine which was not detects in any of the species.

Key words: Leaf protein concentrates (LPC), Leaf Extracts (LE), Plant protein, Thin layer chromatography, Qualitative analysis, free amino acid.

Introduction: - Green plant leaves are an excellent source of protein. The leaves of few species mostly leafy vegetables are at present utilized by man. The protein which is in the concentrated form extracted from the plant leaves are known as leaf protein concentrates (LPC). LPC are being regarded as nutrition for animal and human consumption due to its availability and affordability. Leaf protein (LP) is one of the novel source widely accepted and recommended to increase availability of food/feed grade protein. Various reports and literature showed that LPC is a good source of amino acid, it also

contain polyphenols, but in some extent it also contains some other antinutrient factors like phytic acid, cynide and tannins which is somehow create major challenges to its use (Adeyemi & Osubor 2016). The extraction of protein directly from leaves has been suggested by Prof. N.W. Pirie for increasing the efficiency of using protein for human consumption. The green plant leaves contains variety of phytoconstituents which are medicinally important and the leaf protein extracted from green leaves can be successfully utilized as a human nutrition and for animal consumption.

Phytochemical investigations have revealed diverse kind of chemical constituents in various plants and have been listed as a source of valuable chemicals and some of them have been used as drugs. The protein is one of the most important nutrients in human and animal nutrition (Altschul 1958). They are particularly valuable as muscle and nerve builders, rather than as source of energy. Protein is the most important aspect of nutrition in human diet as it is connected with growth, maintenance and several other processes of life. The important characteristics of proteins are the presence of high nitrogen, sulphur and phosphorous content. The nutritive value of protein explained on its digestibility as well as on availability and amount of various essential amino acids. Amino acids (1) are the building blocks of protein, which in turn constitute the basic frame work of protoplasm.



(1)

The proteins are the precursors in the formation of secondary metabolism molecules and these molecules were involved in cell signaling, gene expression, hormone synthesis,

phosphorylation of protein and antioxidant capacity (Moran-Palacio et al. 2014). The biological value of protein depends on the composition of amino acids (Longeneker 1963). The dietary protein provides a metabolic reserve of amino acids from which new body protein are synthesized. The consistent presence and proper composition of all the amino acids is the most important factor for the normal functioning and development of an organism. The deterioration in the health status takes place if the changes occur in the composition of amino acids (Agbadi et al. 2017). Bathurst (1953) studied the free amino acid and peptides (bound amino acid) of plant tissue by two-way chromatogram in different grasses. Mondal et al. (1998) analyzed the free amino acid content of four species of *Cassia* L. pollen by thin layer chromatography. Therefore in the light of all these published reports attempts were made in the present study to identify different types of amino acids qualitatively by using thin layer chromatography.

Materials and Methods: During the present investigation, ten different plants viz. *Brassica juncea* (L.) Czern. & Coss, *Brassica napus* L., *Brassica oleracea* var. *Botrytis* L. (cultivated) and *Chenopodium album* L, *Goniocaulon indicum*, (Klein ex Willd). C.B. CL, *Celosia argentea* L., *Vigna trilobata* (L.)Verde, *Digera muricata* (L.) Mart, *Tridax procumbens* L. and *Ocimum americanum* L.

(wild) were chosen as protein source. Analysis of oven dried leaf juices of experimental plants was also conducted to compare with that of LPC. The LPC were prepared by following the method suggested by N. W. Pirie (1966b). The LPC thus prepared was oven dried at 50 – 60^{0}C and the dried LE were used for qualitative analysis of amino acids.

Thin Layer Chromatography: - Secondary phytochemical screening was done according to the standard procedures adopted by the (Stahl 1969), (Harborne 1973, 1984, & 1998) and (Wagner & Bladt 1996).

Qualitative analysis of free amino acids was done by using thin layer chromatographic techniques.

a) Preparation of Sample: - It was done by the technique given by Singh and Tandan (1970). 1.0gm powdered plant material of dried leaf extract and leaf protein concentrate was taken in test tube and extracted in 10ml of 80% ethyl alcohol by boiling for about 5-10 min. over boiling water bath. Alcoholic extract was separated and residue was extracted with water. Both the extracts were combined and then evaporated to dryness over boiling water bath. The residue was dissolved in 2ml distilled water. This extract was directly used for detection of free amino acids.

b) Preparation of TLC plates: - The TLC plates were prepared by applying silica gel G slurry (1:2 w/v) in distilled water by aluminium applicator on 10 X 20 cm thin glass plate. The plates were activated by heating to 50^{0}C overnight. The spots were applied on these plates equidistantly with the help of micropipette or capillary tube, 2cm above the lower edge of plate. The distance between spots were 1.5cm, the line was drawn at the distance of 10cm from the spots. The spots were dried and then the plates were introduced in saturated sealed chamber containing solvent system.

c) Solvent System: - n-Butanol: Acetic acid: Water (4:1:1) (Harborne, 1998).

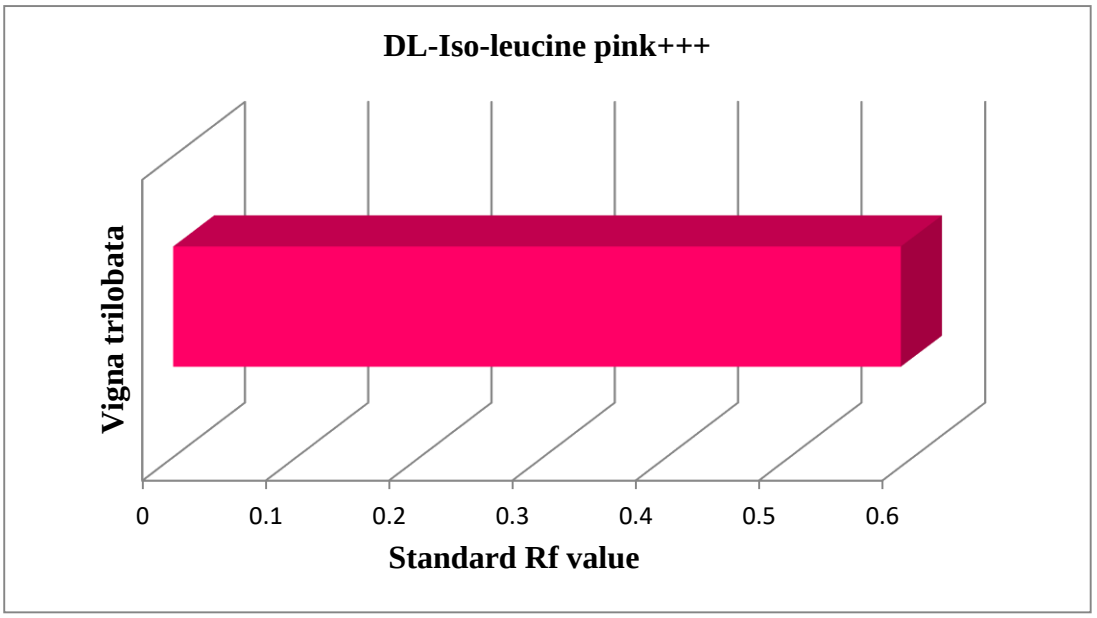
d) Detection: - The spots visualization was done by spraying ninhydrin reagent (Harborne 1998) and heating the plates at 105^{0}C for 10min. The observed spots were marked. These spots were compared with Rf values & colour of standard amino acids and these spots of amino acids were calculated and recorded. The Rf value of each spot can be calculate by using following formula,

$$R_f \text{ Value} = \frac{\text{Distance travelled by the solute (cm)}}{\text{Distance travelled by the solvent (cm)}}$$

Results and Discussion: - The results obtained during the present investigation were presented in following tables, figures and photo plates,

Free amino acids having 16 different Rf values were detected in all ten species in both leaf extracts and leaf protein concentrates. All the Rf values were compared with standards (Plate No. 1). In leaf extract, Amino acid L-Tyrosine, DL-Valine and L-Argenine HCl were detected in most of the species. L-Histidine HCl, L-Hydroxyproline, DL-Methionine, L-Ornithine HCl, L-Proline and DL-Tryptophan were not detected in any of the leaf extract species. *Brassica napus* and *Celosia argentea* showed maximum 8 and 7 amino acid respectively (Table No. 1 & Plate No. 2).

Fig. 1: Detection of Free Amino Acid in LE

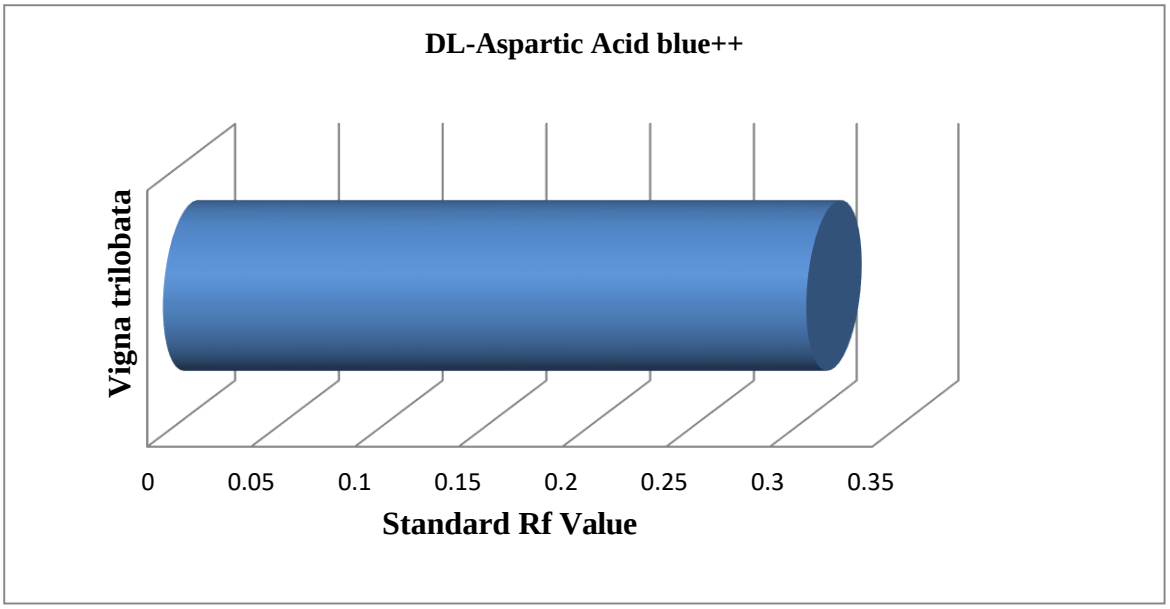


In LPC, DL-Alanine was detected in all the species. However, 2-Amino-n-Butyric acid, L- Cystein, DL-Dopa, Glycine, L-Histidine HCl, L-Hydroxyproline, L-Lycine HCl, DL- Methionine, L-Ornithine HCl, L-Proline and DL-Typtophan were not detected in any of the species. Six amino acids were present in most of the species whereas only one amino acid (DL- Alanine) was found in *Celosia argentea* (Table No. 2 & Plate No.3). In the present study, maximum free amino acids were detected except proline in leaf extract as well as in leaf protein concentrates of different plant species. The probable reason might be due to the low concentration in the prepared samples. It is also suggested that the increase in proline content is under physiological and pathological stress condition (Sadasivam & Manickam 1996).

Chibnall et al. (1963) reported the presence of methionine and tryptophan in Cabbage LPC (*Brassica oleracea* L.) as well as Wilson & Tilley (1965) in lucerne LPC. Byers (1971) also reported the presence of methionine from the LPC of Cabbage (*Brassica chinensis*). The amino acid which cannot be synthesised naturally in the body of an organism, such amino acid is called as essential amino acid and therefore these amino acids must be supplied in the diet from outer source. In human being there are total nine different essential amino acids were recorded and these are Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Histidine, Threonine, Tryptophan and Valine. Rajurkar & Gaikwad (2014) identified and quantified five amino acids
i.e. lysine, threonine, valine, isoleucine and tryptophan by using HPTLC technique in five different medicinal plant species. Adeyemi & Osubor (2016) also showed that the presence of seventeen different types of amino acids in water hyacinth LPC, and particularly they are rich in phynylalanine and leucine which is essential amino acid. Ngugi et al. (2017) also reported the presence of essential amino acids such as histidine, leucine, lysine and methionine in amaranth leaf protein concentrates but having lower amount of phynylalanine and tryptophan content. Agbadi et al. (2017) done comparative analysis of the presence of various amino acids in different parts of the plants e.g. roots, leaves and fruits sample of *Morinda citrifolia.* They showed that the leaves extracts contains highest amino acids as compared to the roots and fruits sample. Swarnakumari et al. (2019) has been successfully isolated the mixture of amino acids by using paper chromatogram from the leaves of *Alternanthera sessilis.* Mykhailenko et al. (2020) also reported that the aerial part of the plants i.e. *Crocus* and *Juno* leaves

extracts contains higher proportion of amino acids.

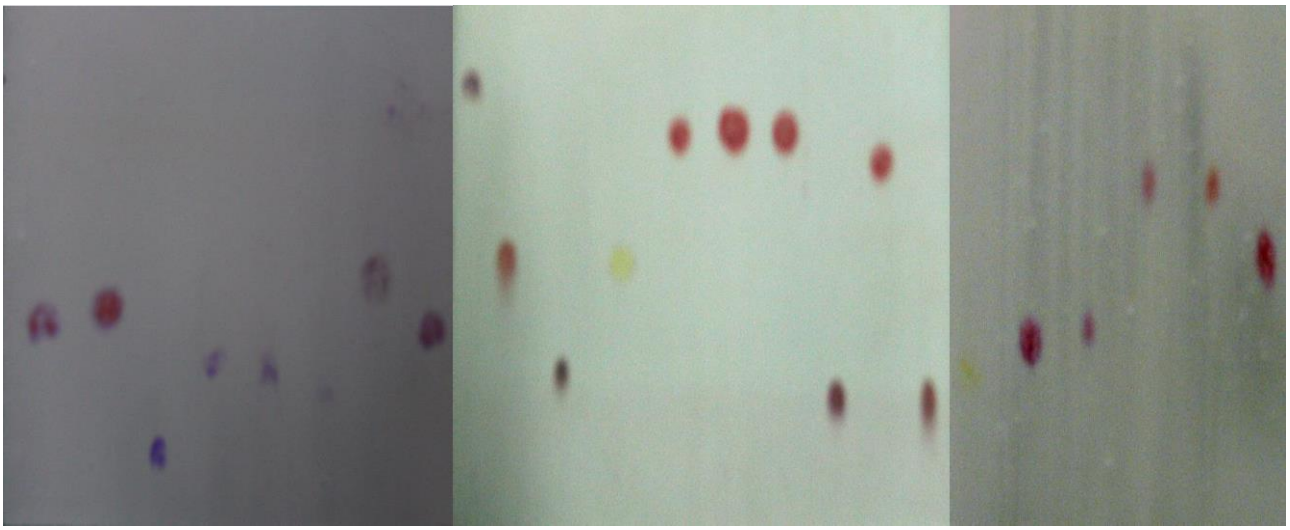
Fig. 2: Detection of Free Amino Acid in LPC



Conclusion: - on the basis of results obtained during the present study it was conclude that, as for as the free amino acid is concerned, the leaf extracts (LE) as well as leaf protein concentrates (LPC) of vegetable crop species under investigation showed maximum number of amino acids as compared to non crop plant species. In general, the plant species under investigation showed all the essential amino acid except histidine which was not detects in any of the species and thus the LPCs prepared from the leaves of these plant species which are underutilised have potential and can be exploited as nutrient supplement for animals, ruminants as well as for human beings.

PLATE – 1

TLC analysis for detection of amino acid standards



1. DL-Alanine, 2. DL-2-Amino-n-Butyric acid, 3. L-Argenine HCl, 4. DL-Aspartic Acid,

5. L-Cystein HCl, 6. L-Cystein, 7. DL-Dopa, 8. L-Glutamic acid, 9. Glycine,

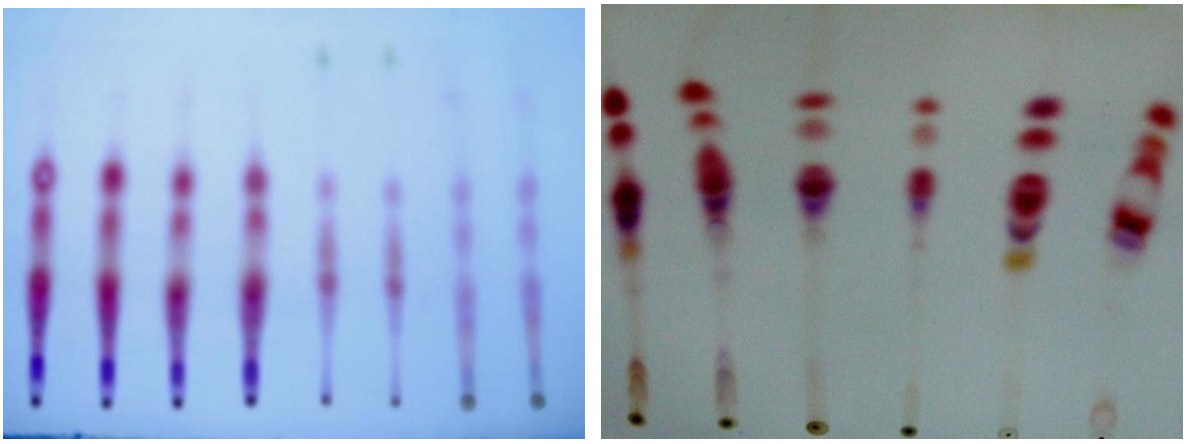
10. L-Histidine HCl, 11. L-Hydroxyproline, 12. DL-Iso-leucine, 13. DL-Nor-leucine,

14. L-leucine, 15.L-Lycine HCl, 16. DL-Methionine, 17. L-Ornithine HCl,

18. DL-B-Phenyl alanine, 19. L-Proline, 20. DL-Serine, 21. DL-Threonine,

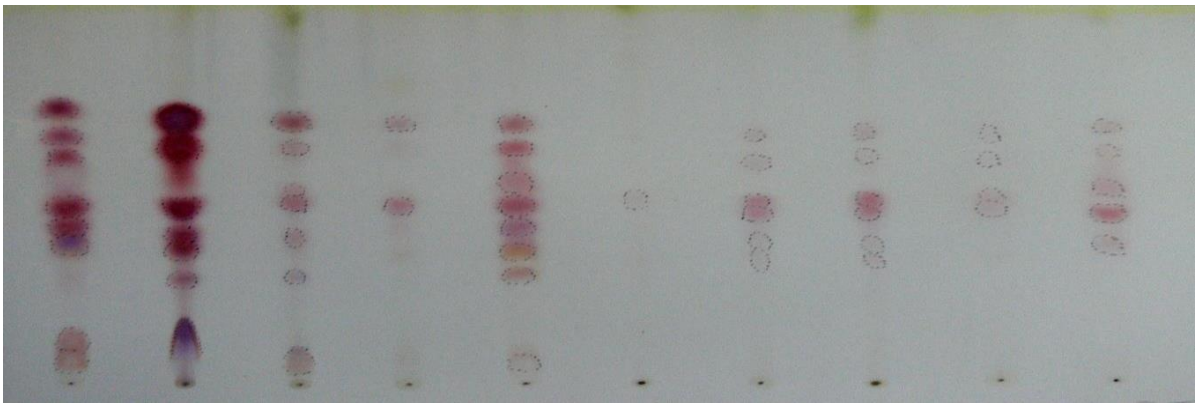
22. DL-Tryptophan, 23. L-Tyrosine, 24. DL-Valine.

PLATE:-2. TLC analysis for detection of free amino acids of leaf extract



1 2 3 4 5 6 7 8 9 10

PLATE:-3. TLC analysis for detection of free amino acids of leaf protein concentrates



1 2 3 4 5 6 7 8 9 10

1. *Brassica juncea*, 2. *Brassica napus*, 3. *Chenopodium album*, 4. *Goniocaulon indicum*,

5. *Brassica oleracea*, 6. *Celosia argentea*, 7. *Vigna trilobata*, 8. *Digera muricata*, 9. *Tridax procumbens*, 10. *Ocimum americanum*.

Table No:-1. TLC analysis for detection of free amino acids of leaf extract (LE) of various plants.

Standard Amino acids	Color	Standard (Rf)	*Brassica juncea*	*Brassica napus*	*Chenopodium album*	*Goniocaulon indicum*	*Brassica oleracea*	*Celosia argentea*	*Vigna trilobata*	*Digera muricata*	*Tridax procumbens*	*Ocimum americanum*
DL-Alanine	pink++	0.41	-	++	++	-	-	-	-	-	-	-
DL-2-Amino-n-Butyric Acid	pink+++	0.45	-	-	-	-	+++	+++	+++	-	+++	+++
L-Argenine HCl	blue+++	0.10	+++	+++	-	-	+++	+++	+++	-	-	+++
DL-Aspartic Acid	blue++	0.31	-	-	-	++	-	-	-	-	-	-
L-Cystein HCl	blue++	0.27	-	++	++	-	-	-	-	-	-	-
L-Cystein	blue+	0.24	+	+	-	+	-	-	-	-	-	-
DL-Dopa	pink++	0.54	-	-	-	-	-	++	++	-	-	++
L-Glutamic Acid	pink+++	0.40	-	-	-	-	-	-	-	-	-	+++
Glycine	pink++	0.32	-	++	++	-	-	++	-	-	-	-
L-Histidine HCl	purple+++	0.10	-	-	-	-	-	-	-	-	-	-
L-Hydroxyproli ne	yellow++	0.30	-	-	-	-	-	-	-	-	-	-
DL-Iso-leucine	pink+++	0.59	-	+++	-	-	-	-	+++	+++	-	+++
DL-nor-leucine	pink+++	0.61	+++	-	-	-	-	-	-	-	-	-
L- leucine	pink++	0.62	-	-	-	-	-	++	-	-	++	-
L-Lycine HCl	brownish+++	0.08	-	+++	-	-	-	+++	-	+++	+++	-
DL-Methionine	pink+++	0.52	-	-	-	-	-	-	-	-	-	-
L-Ornithine HCl	brownish blue++	0.07	-	-	-	-	-	-	-	-	-	-
DL-B-Phenyl alanine	purple+++	0.64	-	-	-	-	+++	-	-	-	-	-
L-Proline	yellow++	0.27	-	-	-	-	-	-	-	-	-	-

DL-Serine	pink+++	0.34	+++	-	-	-	-	-	-	-	-	-
DL-Threonine	pink++	0.37	-	-	-	++	++	-	-	-	-	-
DL-Tryptophan	pink+++	0.65	-	-	-	-	-	-	-	-	-	-
L-Tyrosine	pink+++	0.65	+++	+++	+++	+++	+++	-	+++	+++	+++	+++
DL-Valine	pink+++	0.51	+++	-	+++	+++	+++	+++	+++	+++	+++	-
Total Bands			6	8	5	5	6	7	6	4	5	6

High color intensity +++, Intermediate color intensity ++, Low color intensity +, Absent -.

Table No:-2. TLC analysis for detection of free amino acids of leaf protein concentrates (LPC) of various plants

Standard Amino acids	**Color**	**Standard (Rf)**	***Brassica juncea***	***Brassica napus***	***Chenopodium album***	***Goniocaulon indicum***	***Brassica oleracea***	***Celosia argentea***	***Vigna trilobata***	***Digera muricata***	***Tridax procumbens***	***Ocimum americanum***
DL-Alanine	pink++	0.41	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
DL-2-Amino-n-Butyric Acid	pink+++	0.45	-	-	-	-	-	-	-	-	-	-
L-Argenine HCl	blue+++	0.10	+++	+++	-	-	-	-	-	-	-	-
DL-Aspartic Acid	blue++	0.31	-	-	-	-	-	-	++	++	-	-
L-Cystein HCl	blue++	0.27	-	+++	-	-	++	-	-	-	-	-
L-Cystein	blue+	0.24	-	-	-	-	-	-	-	-	-	-
DL-Dopa	pink++	0.54	-	-	-	-	-	-	-	-	-	-
L-Glutamic Acid	pink+++	0.40	+++	-	-	-	+++	-	-	-	-	-
Glycine	pink++	0.32	-	-	-	-	-	-	-	-	-	-
L-Histidine HCl	purple+++	0.10	-	-	-	-	-	-	-	-	-	-
L-Hydroxypro line	yellow++	0.30	-	-	-	-	-	-	-	-	-	-
DL-Iso-leucine	pink+++	0.59	+++	-	-	-	-	-	+++	-	-	+++
DL-nor-	pink+++	0.61	-	-	-	-	-	-	-	+++	-	-

leucine												
L- leucine	pink++	0.62	-	++	+++	-	++	-	-	-	++	-
L-Lycine HCl	brownish+++	0.08	-	-	-	-	-	-	-	-	-	-
DL-Methionine	pink+++	0.52	-	-	-	-	-	-	-	-	-	-
L-Ornithine HCl	brownish blue++	0.07	-	-	-	-	-	-	-	-	-	-
DL-B-Phenyl alanine	purple+++	0.64	+++	-	-	-	-	-	-	-	-	-
L-Proline	yellow++	0.27	-	-	-	-	-	-	-	-	-	-
DL-Serine	pink+++	0.34	+++	+++	-	-	+++	-	-	-	-	-
DL-Threonine	pink++	0.37	-	++	+++	-	-	-	++	++	++	-
DL-Tryptophan	pink+++	0.65	-	-	-	-	-	-	-	-	-	-
L-Tyrosine	pink+++	0.65	-	-	-	-	-	-	+++	+++	-	+++
DL-Valine	pink+++	0.51	-	-	+++	-	+++	-	+++	+++	+++	+++
Total Bands			6	6	5	1	6	1	6	6	4	4

High color intensity +++, Intermediate color intensity ++, Low color intensity + Absent -.

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