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Determination of *Ailanthus excelsa* Roxb., leaves collected from different agro climatic zones of Tamilnadu as fodder with reference to amino acid composition

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Abstract : *Ailanthus excelsa* Roxb., commonly known as tree of heaven, is a large deciduous tree native to India. Leaves are rated as highly palatable and protein rich nutritious fodder for sheep and goats and are said to augment milk production. Though it is rich in protein, some of the cattle may not prefer to feed on its leaves due to unpleasant odour. Therefore, we aimed to develop a value added product as protein supplement to animal feed using the leaves of *A. excelsa*. It is pertinent to analyse the amino acid variation to ascertain the protein rich nutritious fodder value of *A. excelsa* as an indicator. Thirty potential source of *A. excelsa* were screened for amino acid composition using chromatographic analysis. HPLC analysis of 30 potential sources of *Ailanthus excelsa* showed variation in amino acids and protein. Essential amino acids were found in 24 sources, non-essential amino acids in 28 sources and both in 22 sources. Among the essential amino acids reported in *A.excelsa*, threonine was found in 19 sources, followed by methionine in 6 sources, histidine in 7, phenylalanine in 6 and lysine, triptophan and leucine in only one source respectively. Among the nonessential amino acids proline, serine and glutamic acids were present in 19 sources each, aspartic acid 17 sources, cysteine HCl in 18 sources, norleucine in 9 and glycine in 3 sources respectively. The protein in leaves of *A. excelsa* collected during summer season was ranged from 0.55 mg/g to 3.45 mg/g with an average of 1.48mg/g and 0.4 mg/g to 3.9 mg/g with an average of 1.69 mg/g in winter season. Therefore it is found from the present study that among the thirty sources of *A. excelsa* leaves collected, twenty four were found to have essential amino acids with high total protein content and hence considered as highly protein rich nutritious fodder for cattle.

Key words: Amino acids, *Ailanthus excelsa*, nutrients, livestock, protein

1. Introduction

Globally, livestock production is increasing consistently and is expected to develop into the important agricultural sector in near future (Kaasschieter et al., 1992). Inadequate feed resources in terms of quantity and quality impede increased animal production. Feed is the most essential requirement for all livestock production and nutrient requirement could be a serious limitation (Fereja, 2016). High global population accompanied with demand for livestock products stimulates the need for higher yield per animal than increase in animal population and in such case livestock production has to remain competitive and continue to augment. It is essential to re-look the accessibility of nutrients particularly protein, amino acids and other biochemical factors as supplementary factor for nutritive establishment in fodder and other biological serves. The fodder potential of tree leaves lies in their nutritional efficiency and beneficial components which increase animal production.

Plants are a rich source of amino acids and their individual richness in plants is of great importance (Kumar et al, 2017). To synthesize proteins within the body, animals need raw amino acids as building blocks.

All animals require amino acids which are the building blocks of proteins required for optimal growth, reproduction, lactation, and maintenance (Wu, 2013; Wu et al., 2010; Kung Rode, 1996). Balancing rations for individual amino acids improves feed efficiency. The energy value of animal feeds is vital to meet the energy requirements in ruminants. From growth to production and reproduction, amino acids play a large part in the productivity of farm animals and can contribute significantly to the profitability of a farm. The composition of amino acids in feeds is very variable, so it is important to closely monitor feed quality to ensure that animals are consuming appropriate amounts of amino acids to maintain their health and productivity while maximizing profitability (Kaasschieter et al., 1992. The animal diet with insufficient quantities of essential amino acids limits the production of enough protein to support certain metabolic function. Amino acids are very much essential for animal health and chronic lack of essential amino acids in animal feed leads to infectious diseases. This can be prevented through dietary manipulations by providing alternate feeds or protein supplements.

Tree fodder as protein source is preferable to fulfil the crisis associated with the animal fodder during unfavourable seasons. *Ailanthus excelsa* Roxb., is a tree of heaven belonging to family Simaroubaceae, as an important source of dietary nutrients, alleviate the fodder scarcity. Ailanthus leaves are available in plenty, highly nutritional, palatable and considered as a suitable fodder for cattle by farmers. It even completely satisfies the maintenance requirement of cattle especially goats and sheep (Mandal, 1997).The leaves of *A.excelsa* enriched with nutrients, as a good source of protein and total free amino acids can be recommended as an efficient fodder for ruminants/cattle. It is the species which overcome the problem of feeding tree leaves to livestock through quantifying antinutritional factors which hamper the availability of protein in non- detectable amount (Sumathi et al., 2017). Brindha et al., (2019) reported that the leaves of *A.excelsa* with different bioactive compounds of antioxidant, anti-inflammatory, wound healing and other properties proving the assured use of this plant as suitable/palatable tree fodder. The present study aimed to supplement additional information on amino acid profile of leaves of *A.excelsa* suitable for animal fodder.

II. Materials and methods

Plant source

The leaf samples were collected from 30 sources of *A. excelsa* collected from different agro climatic zones of Tamil Nadu viz. Western, North western, Southern and Cauvery delta Zones and assembled at Field Research station of IFGTB, Kurumbapatti, Salem (Table no 1).

Table no 1. *A.excelsa* sources selected for the study

Plant source/ sources of *A.excelsa*	**Place of collection**	**Zones**
1,3,5,21, 33,89	Coimbatore, Erode, Pollachi	Western Zone
6,7,11,12,29	Virupatchi, Salem	North western Zone
23,25,27,31, 35, 39,43,47, 48, 54, 60,63,76,80	Dindigul, Theni, Palani	Southern Zone
86,87,91,92,93	Karur, Trichy	Cauvery Delta Zone

Processing of *A. excelsa* leaf sample

The leaves were washed thoroughly with tap water and shade dried for a week at room temperature (24 ± 2ºC). Completely dried leaf samples were ground to coarse powder using an electric blender, stored in an airtight container for amino acid analysis.

Estimation of Total Protein

The total protein of the leaves of ailanthus was quantified following the method of Lowry et al., (1951).10 mg of dried leaf powder was homogenized with 10- 15 ml of Trichloro Acetic acid (TCA) and

centrifuged at 5000 rpm for 10 min. Discarded the supernatant and again added 5 ml of TCA and centrifuged for another 10 min. Residue was dissolved in 5 ml of 0.1 N NaOH and 0.1 ml was pipetted out and made up with distilled water. Added 5 ml of reagent mixture [2ml of Na_2CO_3 in 0.1 N NaOH (Reagent A) + 1mL of 0.5 % of $CuSO_4$ in 1% Potassium Tartarate (Reagent B)] and incubated the sample for 20 minutes at room temperature. Then added 0.5 ml of Folin Ciocalteau reagent and incubated for 30 minutes. Blue colour was developed and OD was measured at 660nm. **Standard**: Bovine Serum Albumin

HPLC quantification of Amino acids

Acid hydrolysis of leaf samples were carried out according to the modified method of Lindroth and Mopper (1979). The dried ground samples (100 mg) were hydrolysed by 25 mL 6 N HCl, vortexed; the tubes were closed under vacuum and incubated at 110˚C in an oven for 24 and 72 h. After cooling at room temperature, the tubes were opened and the hydrolysate was filtered (0.45, urn, cellulose acetate), washed three times in distilled water, and then evaporated to dryness. The free residue was dissolved in citrate buffer pH 2.2 and filtered through anhydrous sodium sulphate followed by purification through filtration via Varian Bond Elute C_{18} solid phase extraction column. The purified samples were stored in sterile vials at 4°C for further chromatographic analysis. Amino acid analysis was performed by HPLC (Hitachi) using a reverse phase C18 column with a UV detector. A gradient mobile phase of sodium acetate 0.1 M pH 7.2 and acetonitrile (9:1) elute sample for amino acid separation through reverse phase C18 column. A 20µl of sample was injected each time and detected at 220nm. The samples and the mobile phase were filtered and sonicated for 20min before entering the column. Amino acids present in the samples were quantified by comparing the peak area of the samples with that of the standards.

III. Results and discussion

3.1. HPLC profiling of Amino acids (AA) in *A.excelsa* leaves

Animal feed is the major expensive item and the inadequate availability of feed resources in both quality and quantity could be a serious limiting factor for livestock production (Kaasschieter et al., 1992). The fodder potential of tree leaves lies in their nutritional efficiency and the deficiency in any one of the nutrients may impair metabolism. Amino acids play a significant role in metabolic processes in the animal cells especially from growth to reproduction and significantly contribute to the farm profitability. Livestock require certain amounts of individual amino acids for production rather than protein. Animals have different requirement for each of the 10 essential amino acids, including: phenylalanine, valine, threonine, tryptophane, isoleucine, methionine, histidine, arginine, leucine, and lysine which cannot be synthesized by the animals and must be supplied from feed sources.

Amino acids are required for protein synthesis for maintenance, growth and productivity of animal (Sharma and Arora, 2013). Since, amino acid is very much essential for livestock the present study dealt with the HPLC analysis of the leaves of *A. excelsa* revealed the presence of essential amino acids in 24 sources, non essential amino acids in 28 sources (Fig 1). Out of 30 sources selected for the study, 22 sources contain both essential and non essential amino acids, 6 sources (1,11,23,31,66 and 89) contains only non essential amino acids and 2 sources (12 and 33) contain only essential amino acids (Table no 2). Though amino acids are reported in *A.excelsa*, their composition and quantity vary significantly. Among 30 sources, 3 sources contains one amino acid only, 5 accession with 2 amino acids, only one source with 3 amino acids, 5 sources with 5 amino acids, maximum of 9 sources found to have 6 amino acids, 5 sources with 7 amino acids and 2 sources found to have the maximum number of 8 amino acids (Table no 4 & Fig 2). Essential amino acids like histidine (1.03 mg/g), threonine (2.6 mg/g), methionine (0.35 mg/g), phenylalanine (1.03 mg/g), tryptophane (0.33 mg/g), leucine (65.97 mg/g) and lysine and nonessential amino acids like glycine (1.6 mg/g), norleucine (77 mg/g), aspartic acid (12.61 mg/g), cysteine HCl (0.61 mg/g), glutamic acid (2.6 mg/g), serine (2.6 mg/g), cystene (0.53 mg/g) and proline (2.6 mg/g) were quantified in the leaves of *A.excelsa*. Zheleznov et al., (1997) reported that the amino acid composition of amaranths with an extra ordinary high content of the single amino acid lysine was close to animal protein and lysine content was noted 2 and 3 times greater than that in wheat and maize.

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The amino acids contents varied among the leaves and stems of the herbal plants, Thyme (*Thymus vulgaris*), Rosemary (*Rosmarinus officinalis* L.) and Salvia (*Salvia officinalis* L.) (Hamad et al., 2014). Among the essential amino acids reported in *A.excelsa*, threonine was present is 19 sources, followed by methionine in 6 sources, histidine in 7, phenylalanine in 6 and lycine, triptophan and leucine in 1 sources respectively. Among the nonessential amino acids proline, serine and glutamic acids were present in 19 sources each, aspartic acid 17 sources, cysteine HCl in 18 sources, norleucine in 9 and glycine in 3 sources respectively (Table no 3). Glutamic acid and aspartic acid are the most abundant amino acids in plants (Kumar et al, 2017). Guoqiang and Jianqing (1997) showed that the healthy paulownia leaves found to contain threonine, Cysteine, valine, methionine, leucine, phenylalanine, lysine, histidine, and arginine. Lysine, methionine, and histidine have been identified most often as the most-limiting amino acids for ration supplementation in dairy cows (Schwab & Broderick, 2017).

Among 30 sources of *A. excelsa*, the amino acid lysine was reported in one source, methionine and histidine in 7 sources only with lesser amount compared to other amino acids. Among the afore mentioned three limiting amino acids, lysine was rich (4.8 mg/g) compared to other two (1.03 and 0.33 mg/g). Milk protein production was reduced due to the amino acid lysine which was the first-limiting amino acid (Rulquin and Verite, 1993). Sniffen et al., (2001), reported that less of methionine and lysine reduced the milk protein. The animal fodder *Cnidoscolus aconitifalius* (Chaya) was rich in the amino acids lysine, leucine, phenylalanine and valine which were comparable to soybean meal and alfafa hay (Anil et al., 2010). Arginine, glutamine, glutamate, glycine, and proline, leucine and tryptophan participate in cell signalling, gene expression, and metabolic regulation (Wu et al., 2014). The two amino acids Lysine and tryptophan allow the animal body to produce complete protein and eliminates malnutrition (Bjarnason and Vasal,1992). Lack of lysine and tryptophan in animal feed may also leads to occurrence of disease like kwashiorkor a severe disorder caused due to deficiency of good quality protein. This health issues and low growth performance due to lack of essential amino acids can be meet out through dietary manipulations by supplementing various alternate common feeds to animals. The presence of essential and non essential amino acids required for protein synthesis; for the growth performance and for heath benefit of animals. Leaves of *A.excelsa* can be a suitable alternate to fulfil the requirements of nutrition in terms of essential amino acids.

Table no 2. Essential and non essential amino acids in leaves of *A.excelsa*

S.No	Amino acids (Essential/ Non essential)	Accession of *A.excelsa*	No of sources
1	Sources with essential amino acids	3,5,6,7,12,21,25,27,29,33,35,39,43,47,48, 54,60,63,80,86,87,91,92,93	24
2	Sources with non essential amino acids	1,3,5,6,7,11,21,23,25,27,29,31,35,39,43,47,48,54,60,63, 66,80,86,87,89,91,92,93	28
3	Sources with both essential and non essential amino acids	3,5,6,7,21,25,27,29,35,39,43,47,48,54,60,63,80,86,87,91,92,93	22
4	Sources with essential amino acids only	12, 33	2
4	Sources with non essential amino acids only	1,11,23,31,66,89	6

Table no 3. HPLC quantifications of amino acid of *A.excelsa* leaves

S.No	**Amino acid**	**No of sources**	**Sources of *A.excelsa***	**mg/g**
	Essential amino acids			
1	Methionine	6	5,12,25,35,48,63	0.35
2	Lysine	1	47	4.8
3	Threonine	19	3,5,6,7,21,25,29,39,43,48,54,60,63,80,86,91,92,93	2.60
4	Phenylalanine	6	7,27,29,33,47,87	1.03
5	Histidine	7	3,12,29,33,35,43,93	1.03
6	Tryptophan	1	7	0.33
7	Leucine	1	47	65.97
	Non-essential amino acids			
8	Glycine	3	1,29,31	1.6
9	Aspartic acid	16	6,7,11,21,27,25,29,31,39,47,48,54,60,63,80,86, 93	12.16
10	Cysteine HCl	18	5,6,11,21,25,27,29,31,39,47,48,54,60,63,80,86,91,92	0.61

11	Cystene	12	5,6,11,21,31,39,47,54,60,80,86,92	0.53
12	Proline	19	3,5,6,7,21,25,27,29,39,43,48,54,60,63,80,86,91,92, 93	2.60
13	Serine	19	3,5,6,7,21,25,27,29,39,43,48,54,60,63,80,86,91,92,93	2.60
14	Glutamic acid	19	3,5,6,7,21,25, 27,29,39,43,48,54,60,63,80,86,91,92,93	2.60
15	Norleucine	9	1,3,23,27,29,35,76,87, 89	77

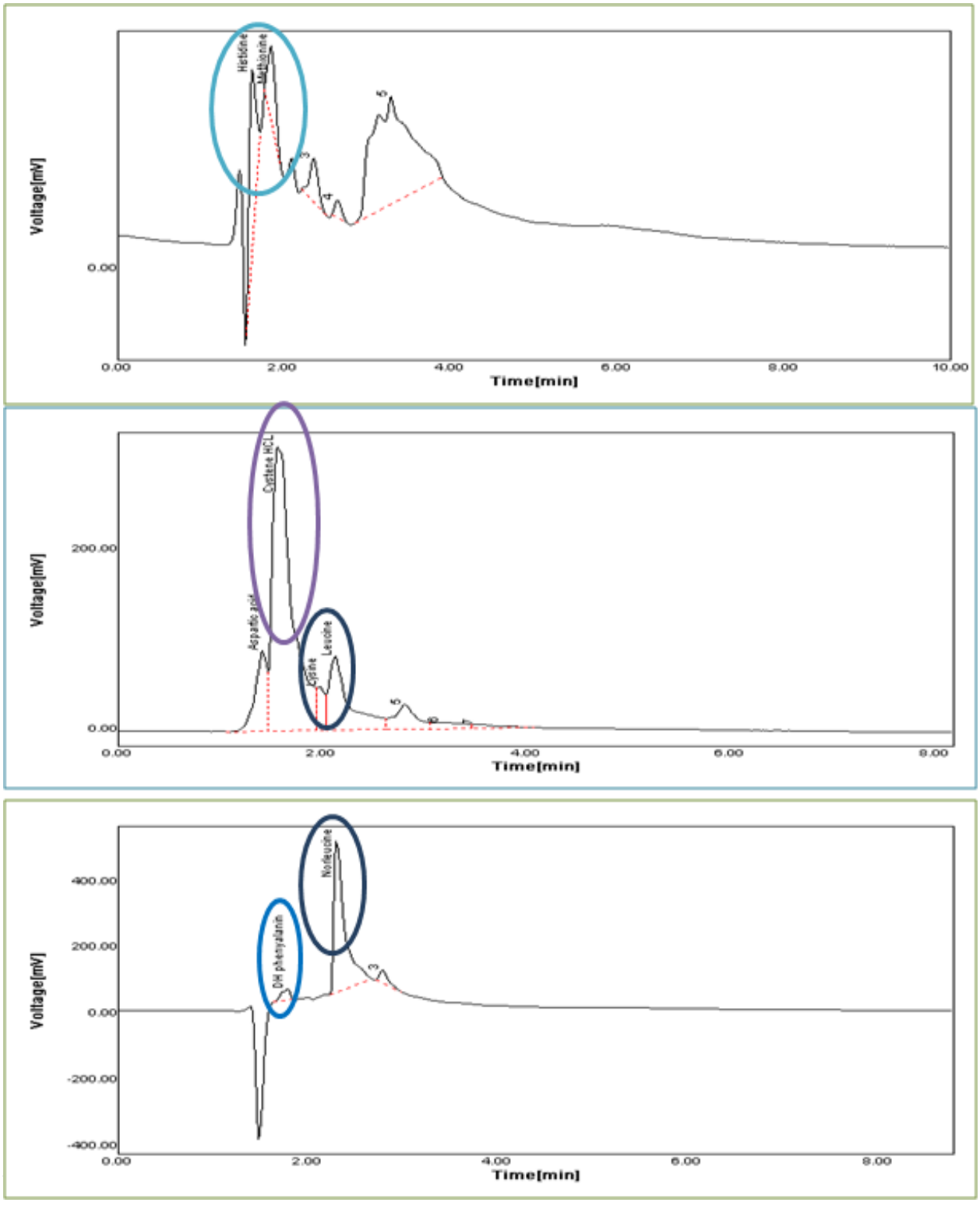

Fig 1 . HPLC characterization of amino acids of *A.excelsa*

Table no 4. HPLC profiling of amino acid in the leaves of *A.excelsa* sources

S. No	Accession number	Location	Amino acids	No. of essential amino acids	No. of non essential amino acids	Total No. of amino acids
1.	1	Coimbatore	Glycine, Norleucine	-	2	2
2.	3	Coimbatore	Aspartic acid, Histidine, Threonine Proline, Norleucine	2	3	5
3.	5	Erode	Aspartic acid, CysteineHcl, Cystene, Methionine, Glutamic acid, Serine, proline, threonine	2	6	8
4.	6	Salem	Aspartic acid, CysteineHcl, Cystene, Glutamic acid, Serine, proline, threonine	1	6	7
5.	7	Salem	Aspartic acid, Glutamic acid, Serine, proline, threonine, Phenylalanine, Tryptophan	2	5	7
6.	11	Salem	Aspartic acid, Cysteine HCl, Cystene, Glutamic acid, Serine	-	5	5
7.	12	Salem	Histidine, Methionine	2	-	2
8.	21	Pollachi	Aspartic acid, Cystene, CysteineHcl, Glutamic acid, Serine, proline, threonine	1	6	7
9.	23	Palani	Norleucine	-	1	1
10.	25	Palani	Aspartic acid, Cysteine HCl, Glutamic acid, Serine, proline, threonine	1	5	6
11.	27	Palani	Aspartic acid , Cysteine HCl, Glutamic acid, Serine, Proline, threonine, Norleucine, Phenylalanine	2	6	8
12.	29	Virupachi	Histidine, Glycine	1	1	2
13.	31	Dindigul	Aspartic acid, Cysteine HCl, Cystene, Alanine, Glycine, Norleucine	-	6	6
14.	33	Coimbatore	Histidine, Phenylalanine	2	-	2
15.	35	Theni	Histidine, Methionine, Norleucine	2	1	3
16.	39	Theni	Aspartic acid, Cysteine HCl, Cystene, Glutamic acid, Proline, Serine, Threonine	1	6	7
17.	44	Theni	Histidine, Glutamic acid, Proline, Serine, Threonine	2	3	5
18.	47	Theni	Aspartic acid, Cysteine HCl, Cystene, Lysine, Leucine, Phenylalanine	3	3	6
19.	48	Theni	Aspartic acid, Cysteine HCl , Methionine, Proline, Threonine, Serine	2	4	6
20.	54	Theni	Aspartic acid, Cysteine HCl, Cystene, Proline,Threonine, Serine	1	5	6
21.	60	Theni	Aspartic acid, Cysteine HCl, Cystene, Proline, Threonine, Serine	1	5	6
22.	63	Theni	Aspartic acid, Cystene HCl, Methionine, Threonine, Proline, serine	2	4	6
23.	76	Theni	Nor leucine,	-	1	1

24.	80	Theni	Aspartic acid, Cysteine HCl, Cystene, Proline, Threonine, Serine	1	5	6
25.	86	Trichy	Aspartic acid, Cysteine HCl, Cystene, Proline, Threonine, Serine	1	5	6
26.	87	Karur	Phenylalanine, Norleucine	1	1	2
27.	89	Coimbatore	Nor leucine,	-	1	1
28.	91	Karur	Aspartic acid, Cystene HCL, , Proline, Threonine, Serine	1	4	5
29.	92	Karur	Aspartic acid, Cysteine HCl, Cystene, Methionine, Proline, Threonine, Serine	2	5	7
30.	93	Karur	Aspartic acid, Histidine, Proline, Threonine, Serine	2	3	5

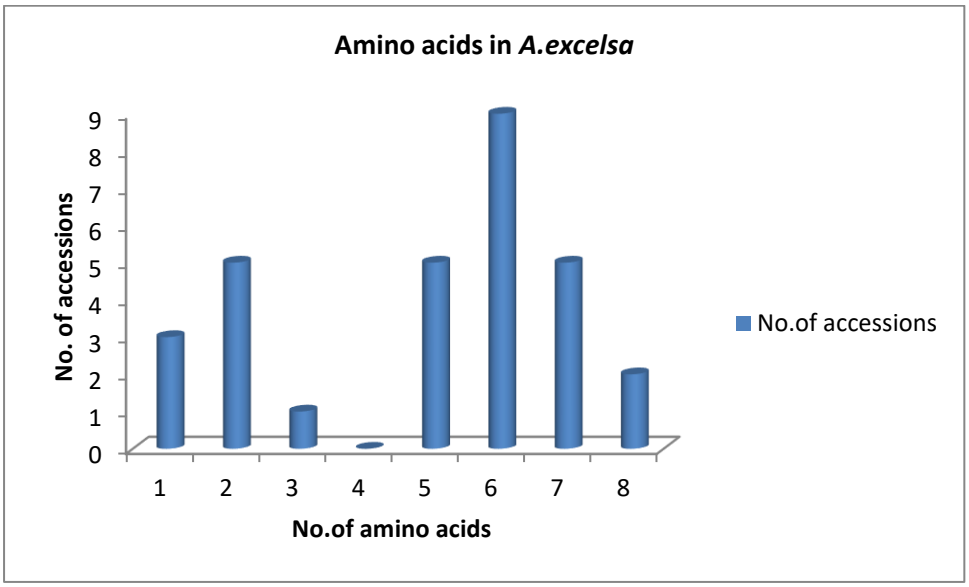

Fig 2. **Amino acid in the *A.excelsa* sources**

It is necessary to re-look the availability of nutrients especially protein and other biochemical factors as an additional tool for nutritive establishment/fodder and other biological serves. Fodder trees are an important source of supplementary protein, vitamins and minerals in developing countries (Baumer, 1992). Salem et al., (2006) reported that fodder trees are the locally produced protein supplement for ruminants during the inadequate supply of foliage. Chadokar and Kantharaju (1980) also reported Glyricidia as protein rich source that enhanced feed Intake and weight of ewes and lambs. As per the report of Bhandari and Gupta (1972) the leaves of *Ailanthus excelsa* are protein rich nutritious fodder and palatable tree yields an average of about 500-700 kg of green leaves twice a year and also, found to be suitable fodder for cattle, sheep and goats and are said to augment milk production (Chandra, et al., 2013). Graham Andrews (1998) reviewed and reported the scenario of the overseas experience in using the trees and shrubs as a sole source of fodder for livestock production. *Prosopis juliflora* cultivated for livestock in Peru reported to produce seven tonnes of high protein pods per hectare in a year (Felker, 1981). In the present study total protein was quantified for both summer and winter season collected leaves and high percentage of total protein content was recorded in almost all sources of *A.excelsa*. The protein ranged from 0.55 mg/g to 3.45 mg/g with an average of 1.48mg/g during summer season. The lowest protein was recorded in Palani and highest in Trichy. Protein quantified during winter season showed that the minimum of 0.4 mg/g was recorded in Virupatchi and a maximum of 3.9 mg/g in Uttarakhand accession with an average of 1.69 mg/g. Leucaena and Gliricidia foliage yields are higher in the wet season (Aregheore, 1995; Balogun and Otchere, 1995). Their leaves provide protein-rich supplements to traditional

village diets to increase small ruminant productivity. Leaves of *Tephrosia candida* a perennial shrub, is a good source of protein for ruminants (Babayemi et al., 2003a). High protein in the diet and especially in the forage should be desired as it largely determines the intake and digestibility (Babayemi et al., 2003b). Leaves of *Tephrosia candida* a perennial shrub, is a good source of protein for ruminants (Babayemi et al., 2003a). Over all protein content was measured high during winter season than leaves collected during summer season. Hence, irrespective of the seasonal variation *A.excelsa* may be considered as a protein source for animal feed.

IV. Conclusion

Protein rich supplements increase animal productivity and amino acids are essential for animal health as well. Ailanthus other than its nutritional wealth, it is also rich in amino acids which are essential for animal production and their health. Though variation in amino acids and protein were observed in the thirty sources of *A.excelsa*, twenty four sources were found to have essential amino acids with high total protein. Due to the presence of highly variable composition of amino acids and protein, it is very essential to assure the fodder/feed must contain appropriate amino acid for their health, growth and production. *Ailanthus excelsa* with all such desired quality of being a fodder may be consider as an alternate tree fodder for livestock.

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