A COMPARATIVE STUDY IN THE CONTENT OF AMINO ACIDS OF SHOOT AND ROOT OF MEDICAL HERBS OF *XANTHIUM STRUMARIUM* GROWING IN IRAQ

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ABSTRACT

Specimens of *X. strumarium* were collected from Mosul city for investigation and separation of Glycine, Asparagine, Aspartic, Glutamic and Arginine as amino acids by high-performance liquid chromatography (HPLC).
Separation process showed that the retention time of standard compounds was agreement with molecular weights of those standard, so the compounds that have high molecular weight took a longer retention time during the separation process compared to other.
In this study, the amino acid Glysine observed in both crude extract of *X. stramarium*, and also Asparagine and aspartic acid, while Glutamic acid was absent from those crude extracts.

Keywords: Natural products, Amino acids, HPLC, *X. strumarium*, Iraqi medicinal herbs.

1. INTRODUCTION

Plants synthesize a vast range of organic compounds that are traditionally classified as primary and secondary metabolites although the precise boundaries between the two groups can in some instances be somewhat blurred. Primary metabolites are compounds that have essential roles associated with photosynthesis, respiration, and growth and development. These include phytosterols, acyl lipids, nucleotides, organic acids and amino acids. (Crozier, 2006).
Although the amino acids are normally considered as primary metabolites, there are some unusual amino acids that are of restricted occurrence. Some antibiotics such as the penicillins are formed from small peptides.(Hanson, 2003)
More than 700 amino acids have been discovered in Nature and most of them are α-amino acids. The physiological importance of α-amino acids ensures a sustained interest in their chemistry particularly in pharmaceutical exploration for new drugs, and for their synthesis, reactions and physical properties. As is often the case when the chemistry of a biologically important class of compounds is being vigorously developed, an increasing range of uses has been identified for α-amino acids in the wider context of stereo selective laboratory synthesis (including studies of biomimetic synthetic routes). (Barrett and Elmore, 2004)
Some 20 amino acids commonly (actually, nineteen α-amino acids and one α-imino acid) are considered components of plant proteins and there are additional naturally occurring amino acids, such as ornithine and citrulline, that are not found in proteins (Mifl in, 1981). The amino acids are the basic building blocks of proteins that form the protoplasm, as well as enzymes that catalyze the biochemical processes of plants. (Pallardy, 2008).
amino acids are classified into 7 major classes based on side chain and ring structure present; Amino acids with aliphatic side chain. They are also called as aliphatic amino acids, Amino acids with side chain containing hydroxyl groups, sulfur atoms, acidic groups or their amide, basic groups, aromatic rings and Imino acids: they are proline and hydroxy proline.**(** Rao, 2006).
The properties of each amino acid are dependent on its side chain (—R); the side chains are the functional groups that are the major determinants of the structure and function of proteins, as well as the electrical charge of the molecule. Knowledge of the properties of these side chains is important for understanding methods of analysis, purification, and identification of amino acids. Amino acids with charged, polar or hydrophilic side chains are usually exposed on the surface of proteins. The nonpolar hydrophobic residues are usually buried in the hydrophobic interior or core of a protein and are out of contact with water.
About analysis of amino acids ,A range of chemical techniques is used to isolate and characterize amino acids, but three alternative techniques are the most frequently used for free amino acid analysis. The 1st, by applying ionic chromatography, ninhydrin derivatization and UV detection (Cunico and Schlabach 1983), has some problems with matrix interferences and detection limits. The 2nd is the separation of volatile amino acid derivatives by GC (Fantozzi and Montedoro 1974) and detection by FID and/or MS. This technique requires a careful sample extraction and pre concentration, as well as elaborate pre-column derivatization procedures. These steps pose some

problems with extraction and derivatization yields, facts that must be taken in account when assessing the reliability of final results. Also, some derivatives, (Vasconcelos and Chaves das Neves 1990), do not react efficiently with arginine. However, if correctly and carefully performed, these methods have high resolution power and sensitivity.
The 3rd is the separation of amino acid derivatives by HPLC and their detection by fluorescence (Godel *et al*., 1991). This HPLC technique offers the advantages of speed, ease of performance (absence of prior sample extraction steps), sensitivity similar to that found with GC, and simpler automation, which makes this method useful for processing a large numbers of samples. The main disadvantage is the multiplicity of peaks formed for some derivatives.
The aim of this study is to chemical comparative between roots and shoots of *Xanthium strumarium* a medical plants that growing in Iraqi Mosul city by use HPLC technique to determination of amino acids.

2. MATERIALS AND METHODS

Five of standard Amino acids used to achieve the aims of the study by a series steps are:

A. Sample Preparation

After drying the samples, extraction process completed by 95% ethanol, and ethanolic solution concentrating by using a rotary evaporator (RVE).
(Grand et al., 1988)

B. Hydrolysis Amino Acids

Because Amino acids are found in the plant tissue and mammalian tissue, it hydrolyzed in order to cleave the peptide bonds in the proteins to release the free Amino for analysis using HPLC, the common method used for Amino acid hydrolysis is by adding 6N HCL and heating at 110ؠC for 24hrs or 145ؠC for 6hrs.
(Csapo et al., 2008).

C. Methods of Separation

After purification of crude by (0.1μM), filters membrane samples examined by LC-20 AD ShimadZu HPLC, which included C18(240 × 4.60mm) column chromatography. This technique used for separation processes, with 40 mM Na2SO4 , pH 2.65 as mobile phase, 1mL/min rat of flow and detection at λ = 210 nm wavelength., Separation process carry out in the chemical analysis lab. at Al-Kindy stat company in Mosul city based on the principle that mentioned from (Dionex, 2004).

D. Amino acids standard

Standard of (Glycine, Asparagine, Aspartic acid, Glutamic acid and Arginine) were suply from the Chemistry Department of Education college in Mosul University, one products of the Swiss Floka company as well as BDH Co. Standard solution was prepared by dissolving 0.1 g of the compound in 10 ml of ethanol.

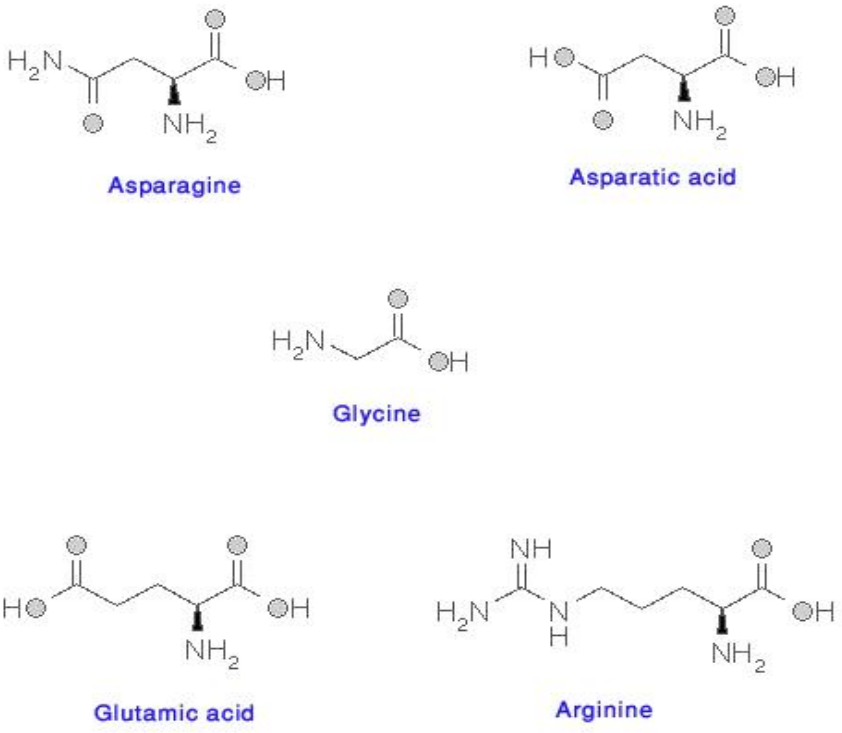
3. RESULTS AND DISCUSSION

After nitrate (NO_3) has been taken up by the plant, it may be subject to immediate reduction and assimilation into organic form in root tissue, or transported in the xylem to the leaves for reduction and further metabolism there. Nitrate may also be stored in the roots, in vacuoles for varying periods of time, before reduction or transport. Amino acids can be synthesized in roots and transported in the xylem to developing shoots, or formed in leaf tissue and transported in the phloem to sinks, such as developing seeds or apices (Pate and Layzell, 1990).
Based on the separation principle which mentioned by Dionex (2004), amino acids (Glycine, Asparagines, Aspartic acid, Glutamic acid and Arginine) were seprated from ethanolic extract of shoot and root of *X. strumarium.*
Separation process led to draw the curves of standard compounds of amino acids coupled with retention time of each compound, and the retention time which appearance on the Chromatogram of standard compounds was agreement with molecular weights of those standard, so the compounds that have high molecular weight took a longer time during the separation process compared to other compounds (Table, 1)

(Table,1) Molecular weight and retention time of amino acids under study.

Standards			Retention time (R_t) of compounds under study in crude of *X. strumarium*	
compounds	**Molcular weight**	**Retention time (min.)**	**Root**	**Shoot**
Glycine	**75.07**	**2.773**	**2.607**	**2.616**
Asparagine	**132.12**	**2.839**	**2.994**	**2.990**
Aspartic acid	**133.10**	**3.107**	**3.161**	**3.186**
Glutamic acid	**147.13**	**3.277**	*	*
Arginine	**174.20**	**4.074**	**4.449**	*

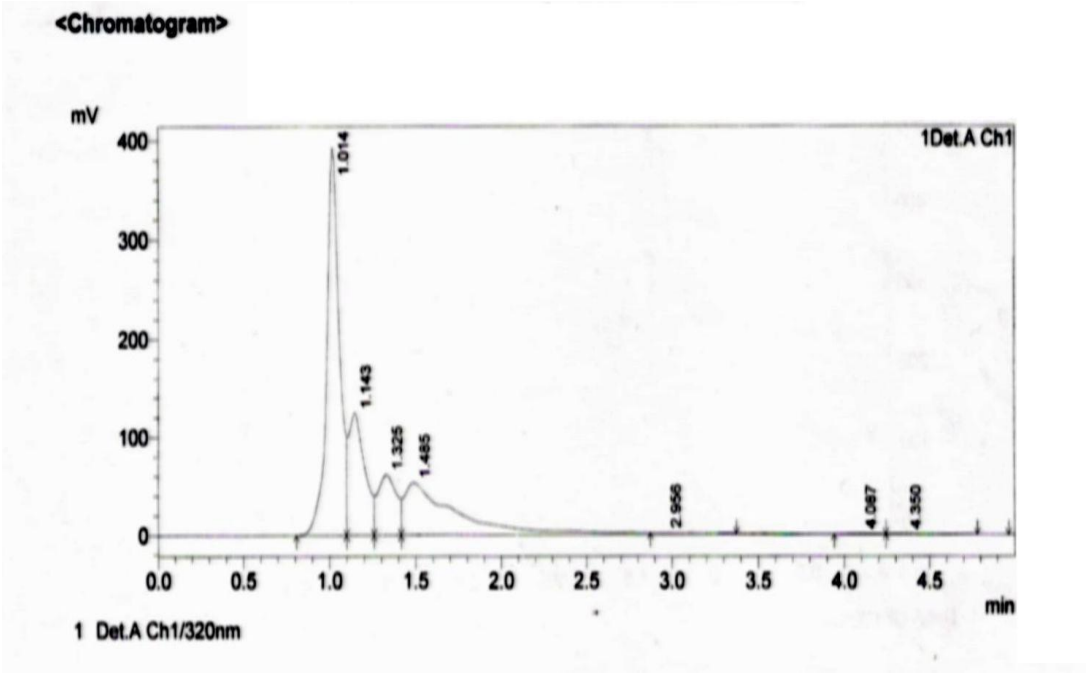
then retention time (R_t) of standard was adopted as conductor of their presence in the crude extracts. On this basis, the compounds that have been separated as amino acids (Fig. 1) are those that have retention time identical or different by (1 - 2) seconds from the retention time of standard.



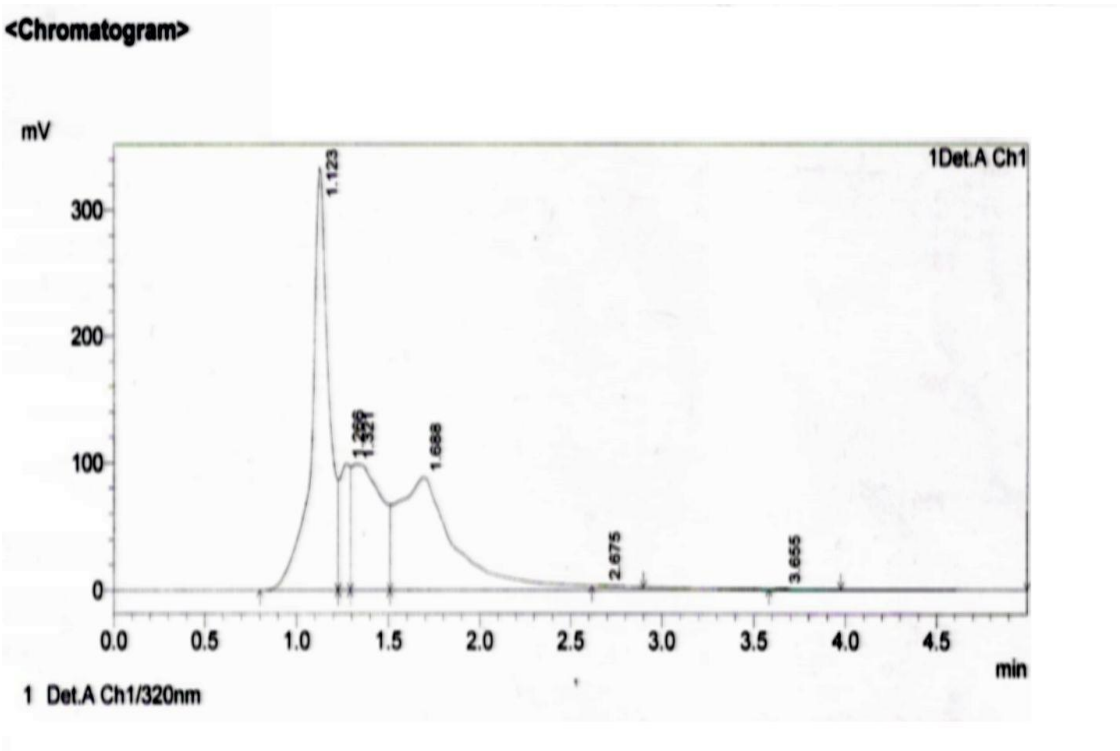
(Fig. 1) Formula of the amino acids that have been separated in the current study.

Glycine is known to be involved in several important reactions of intermediary metabolism. Glycine can enter into organic acid metabolism by transamination reactions which are of widespread occurrence in microorganisms and plant tissues.(Sinha and Cossins1964).

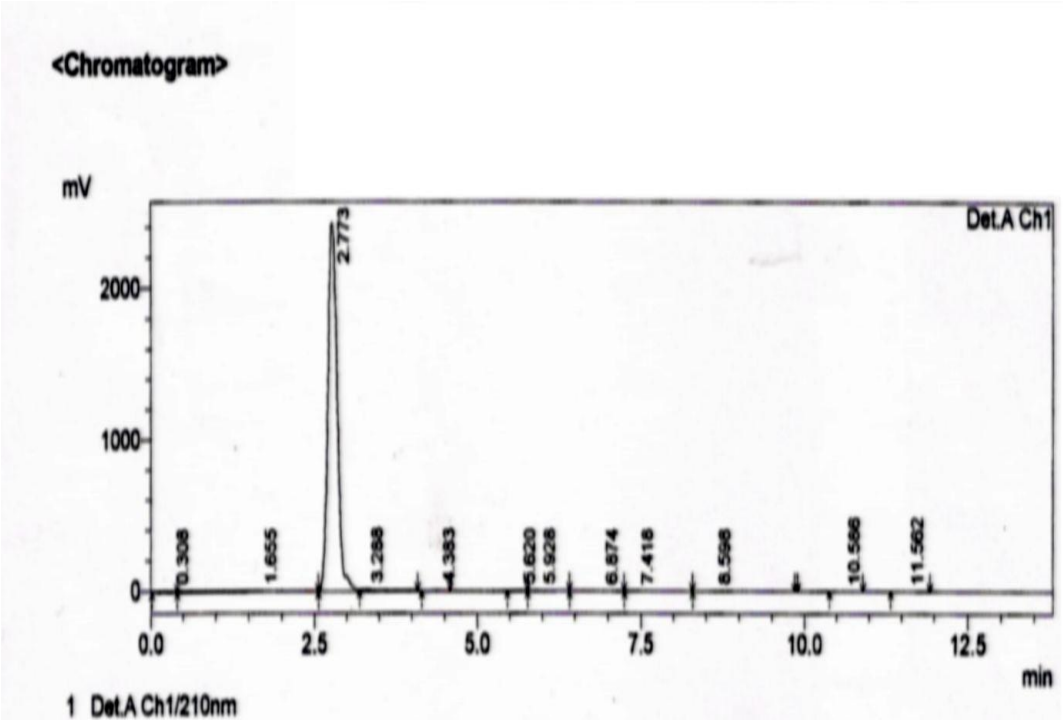
In this study the amino acid Glysine observed in both crude extract of X. stramarium, and the separatiobn showed that the R_t 2.607 and 2.616 min were very close to the R_t 2.773 min of Glycine standard (Fig. 2, 3 and 4) . This result apply to to the Asparagine which identified by R_t 2.990 and 2.994 min depending on the R_t 2.839 min. of Asparagine standard. (Fig. 5)



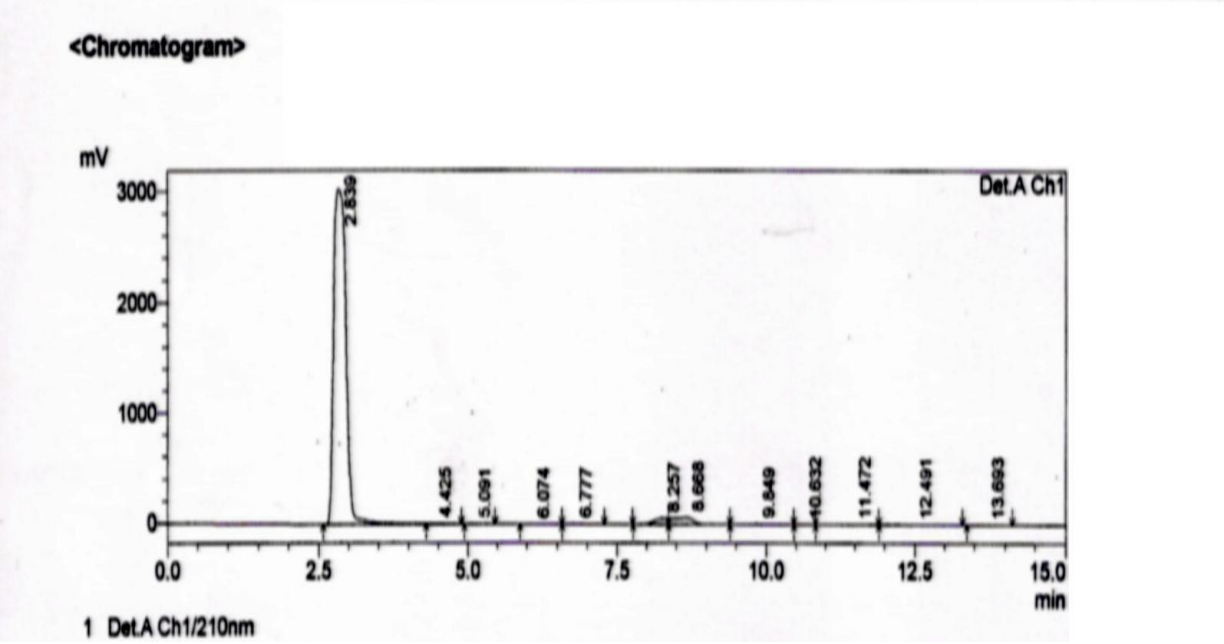
(Fig. 2) Chromatogram of Leaves extract by use 40 mM Na_2SO_4, pH 2.65 mobile phase in HPLC technique.



(Fig. 3) Chromatogram of roots extract by use 40 mM Na_2SO_4, pH 2.65 mobile phase in HPLC technique.



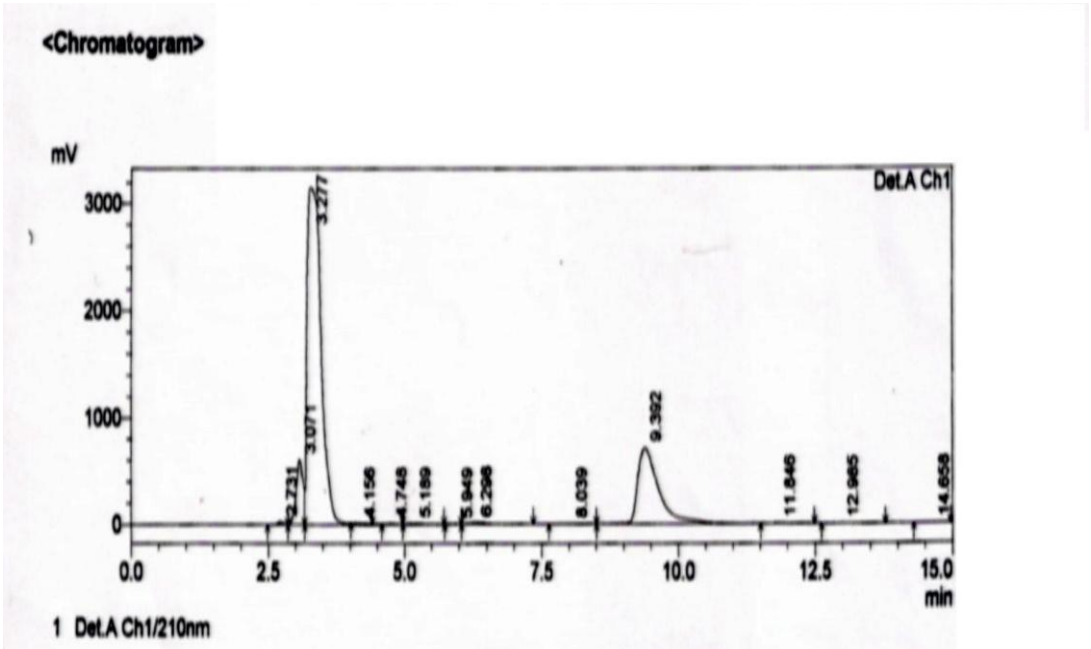
(Fig. 4) Chromatogram of Glycine Standard, that appeared at 2.773 minutes by use 40 mM Na_2SO_4, pH 2.65 mobile phase in HPLC technique.



(Fig. 5) Chromatogram of Asparagine Standard, that appeared at 2.839 minutes by use 40 mM Na_2SO_4, pH 2.65 mobile phase in HPLC technique.

In the same context , the curves that appeared at 3.161 and 3.186 min. (Table, 1) refer to the Aspartic acid because those values were identical to retention time 3.107 min. of Aspartic acid standrad (Fig. 6)

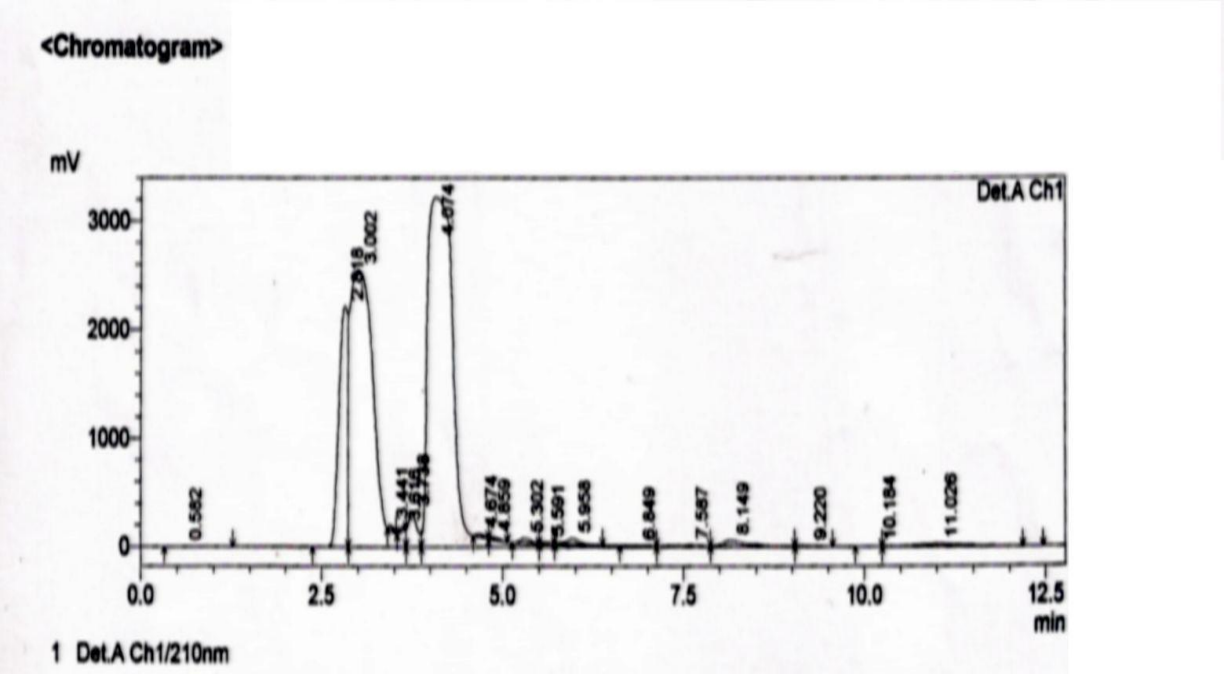
Asparagine, isolated from asparagus as early as 1806, was the first amide to be identified (Lam *et al.* 1996). It serves not only as a protein precursor, but as a key compound for nitrogen transport and storage because of its stability and high nitrogen-to-carbon ratio (2 N to 4 C for asparagine, versus 2 N to 5 C for glutamine or 1 N to 5 C for glutamate). The major pathway for asparagine synthesis involves the transfer of the amide nitrogen from glutamine to asparagine.



(Fig.6) Chromatogram of Aspartic acid Standard, that appeared at 3.107 minutes by use 40 mM Na_2SO_4, pH 2.65 mobile phase in HPLC technique.

About Glutamic acid, the results did not show curve has a nearby retention time of Glutamic standard (Fig. 2, 3 and 7) this result can not be an indicator of the absence of metabolic path of this compound, because of Glutamate plays a key role in protein synthesis and degradation. Glutamate is the central transit site for the interchange of amino nitrogen amongst amino acids. Several different aminotransferses mediate the transfer of amino nitrogen to and from glutamate through fully reversible reactions. (Kelly and Stanley, 2001)

That mean the α-amino group can be transferred to a wide variety of 2-oxo acid acceptors to form amino acids, and similarly the α-amino group can be transferred back to form glutamate when 2-oxoglutarate and other amino acids are available. For example The a-amino group of glutamate may be transferred to oxaloacetate to form aspartate by aspartate aminotransferase (Lea *et al*., 2007)

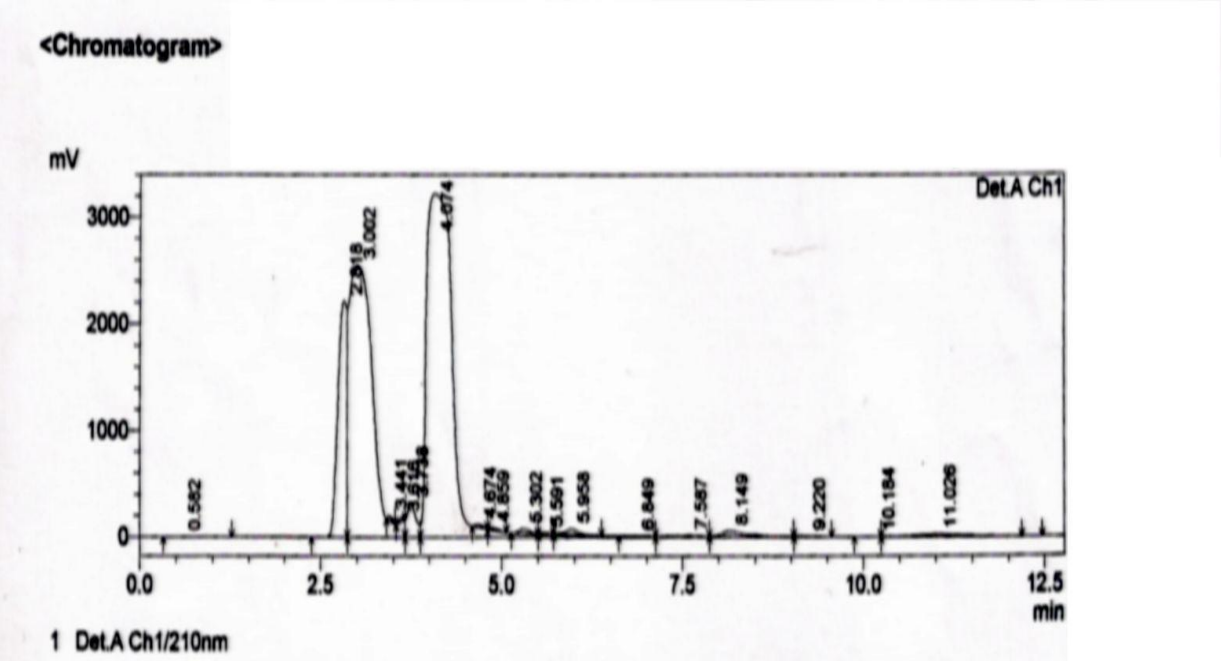


(Fig. 7) Chromatogram of Glutamic acid Standard, that appeared at 3.277 minutes by use 40 mM Na_2SO_4, pH 2.65 mobile phase in HPLC technique.

Glutamate is the precursor of arginine and is metabolized via acetylated derivatives to ornithine, citrulline, and arginosuccinate in a nine-step process (Slocum, 2005). Arginine has a high N:C ratio (4:6), and along with asparagine (2:4) acts as a major nitrogen storage compound in higher plants, where it occurs in both the protein and soluble form. (Forde and Lea, 2007)

L-Arginine is an amino acid that has numerous functions in animal and plant kingdom. In mammals, arginine is used to make very active compounds such creatine, L-glutamate, and L-proline, and can be converted to glucose and glycogen if needed. In plants arginine residues form a significant store of nitrogen in seeds. The degradation of arginine releases this nitrogen supporting the growth of the seedlings during germination. (Pintus *et al*., 2009) Arginine may also act as precursors of polyamines, which can play an important role in the response of plants to stress. (Alca´zar et al., 2006).

The results also showed that the Arginine was absent from the extract of shoot while it was separated from root extract of *X. strumarium,* Arginine identified through R_t 4.449 min based on 4.074 the retention time of their standard , It is important to say that the deferent in the retention time (about 3 sec.) between Arginine and their standard, Possibly due to the Optical isomerism: All the amino acids except glycine have at least one asymmetric carbon atom because of this they exhibit optical isomerism. Presence of single asymmetric carbon atom gives rise to two optical isomers. One isomer is the mirror image of the other isomer. If a carbon atom is linked to four different groups through covalent bonds then it is called as asymmetric carbon. The two mirror images of amino acid serine are L-serine and D-serine. Further, the optical isomers of amino acids are optically active. They are capable of rotating plane polarized light. Some amino acids rotate plane polarized to left and some rotate the plane polarized light to right.**(** Rao, 2006)



(Fig. 8) Chromatogram of Arginine Standard, that appeared at 4.074 minutes by use 40 mM Na_2SO_4, pH 2.65 mobile phase in HPLC technique.

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