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DETERMINATION OF AMINO ACIDS IN ACHYRANTHES ASPERA AND CISSUS QUADRANGULARIS BY HPLC

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ABSTRACT

Analysis of amino acids in *Achyranthes aspera* and *Cissus quadrangularis* by reversed phase high performance liquid chromatography (RP-HPLC) with UV detector was done. Derivatization of plant samples and 16 standard amino acids were achieved by adding o-phthalaldehyde (OPA). The obtained OPA derivatives were analyzed by RP-HPLC on a c-18 column with Sodium acetate buffer and Methanol (9:1) gradient elution. The common amino acids were separated in 30 min. Amino acid peak integration was achieved by using lab chromo HPLC software. The separation of standard amino acids and IS showed 17 fine peaks in chromatograms and Twelve amino acids in *Achyranthes* and fourteen amino acids in *Cissus* were detected by HPLC.

KEYWORDS: Reversed Phase High Performance Liquid Chromatography (RP-HPLC), Amino Acids, Derivatization, Achyranthes Aspera, Cissus Quadrangularis

INTRODUCTION

Plants are the major source of nutrition to all other living beings. A wide range of organic compounds, responsible for the biological activity of herb, are synthesized in plants, that are traditionally classified as primary and secondary metabolites. Primary metabolites are compounds that have essential roles associated with growth and development. These include acyl lipids, nucleotides, organic acids and amino acids. Plants synthesize amino acid from the primary elements by some collateral metabolic pathways.

There are many amino acids commonly referred to in human health .They are required by the body as it acts as a precursor. Eight amino acids are essential for humans as phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, and lysine. These amino acids are part of complex pathways and biological systems, must to be taken exogenously. Amino acids are precursors for the synthesis of secondary metabolites that confer beneficial physiological effects in consumers.

Cissus quadrangularis Linn. commonly known as hadjor, and ***Achyranthes aspera*** ("Prickly chaff flower) commonly known as apamarg are well-known plant drug in Ayurvedic, Unani, Siddha, Naturopathic & Home Remedies. These plants have been used in traditional Indian medicine for thousands of years to treat various disorders. Cissus is used in folklore medicine to heal bone fractures, throughout India while Achyranthes is used in the treatment of treatment of bleeding, asthma, oedema, in facilitating delivery, snake bite, renal complications, headache, leucoderma, skin diseases and pneumonia. In some studies it is also found that both plants are safe and effective in weight reduction.

The role of amino acids in the synthesis of secondary plant products is fairly known but there is a little information regarding quantitative production of various amino acids. Considering the fact, investigation of amino acids by HPLC from the *Achyranthes aspera* and *Cissus quadrangularis* has been carried out.

MATERIALS AND METHODS

3 gm powdered samples were hydrolyzed with 6M HCl at 150°C during 6 h, followed by rotary evaporation. Sample was re-suspended on 2 mL of Sodium citrate buffer pH 2.2. and derivatized by adding o-phthalaldehyde (OPA). The HPLC method precision and accuracy was evaluated using external and internal standards consisted on 16 standard amino acids given in kit (0.05 μmoles $mL^{-1}$for each amino acid) and was utilized to determine the retention times for each amino acid. An internal standard (IS) α-aminobutyric (0.05 μmoles mL^{-1}) was added to amino acid reference standard and each plant sample to normalize and quantify the amino acid content. The HPLC system (Shimadzu lab chromo2010 HT HPLC) having lab chromo software was used. The package used for analyzing results was and auto-sampling. Chromatographic analysis was carried out using a c-18 column at temperature: 30□C; injection volume 15 ul; mobile phase: Sodium acetate 0.1 M pH 7.2 and Methanol (9:1), flow rate 0.500 ml/min; separation time 30 min and UV detection at 254nm.

RESULT

The separation of standard amino acids and IS showed 17 fine peaks in chromatograms and twelve amino acids in *Achyranthes* and fourteen amino acids were detected in Cissus. The peak area of standards and samples were calculated to determinate amino acid concentrations.

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Acquired by	: Admin
Sample Name	: Mixture of standard amino acid
Sample ID	:Amino acid mixture
Tray #	: 2
Vail #	: 1
Injection Volume	: 20 µL
Conc.	: 0.05 µmole mL^{-1}
Data File Name	: Mixture of std. AA. data new.lcd
Method File Name	: Mixture of std. AA. Meth.lcm
Report File Name	: Default.lcr
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Data Processed	: 4/11/2015 1:16 PM

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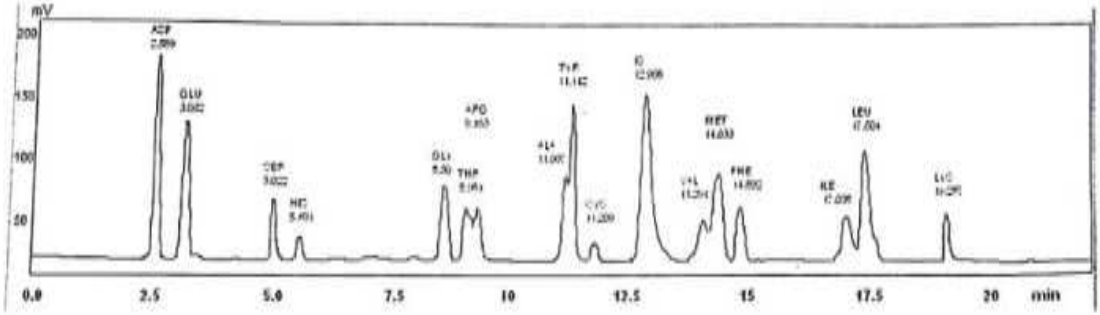

Peak	Name	Retention Time
1.	ASP	2.589
2.	GLU	3.052
3.	SER	5.022
4.	HIS	5.401
5.	GLY	8.50
6.	THR	8.981
7.	ARG	9.163
8.	ALA	11.007
9.	TYR	11.142
10.	CYS	11.209
11.	IS	12.908
12.	VAL	13.291
13.	MET	14.033
14.	PHE	14.892
15.	ILE	17.058
16.	LEU	17.504
17.	LYS	19.287

Verified
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Acquired by	: Admin
Sample Name	: Cissus amino acid
Sample ID	: Amino acid Cissus.
Tray #	: 2
Vail #	: 1
Injection Volume	: 20 µL
Conc.	:
Data File Name	: Cissus amino acid data new.lcd
Method File Name	: Cissus amino acid Meth.lcm
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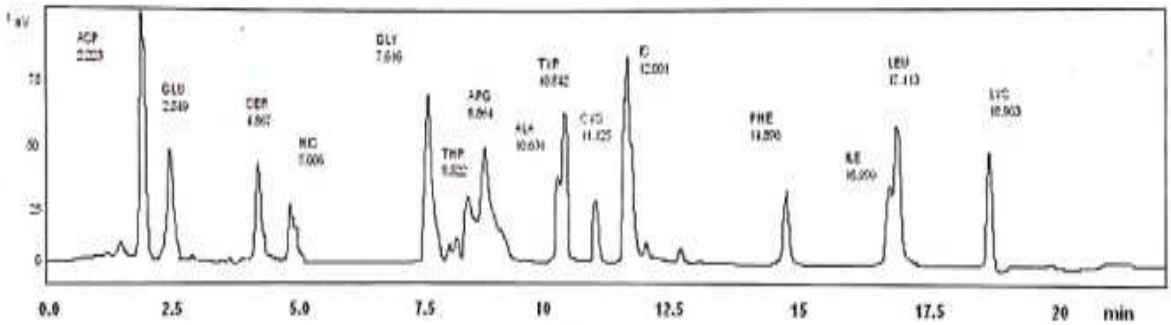

Peak	Name	Retention Time	Area(mm^2)	Conc.($\mu g\ g^{-1}$)
1.	ASP	2.223	18.799	1858.66±18
2.	GLU	2.549	7.741	769.52±09
3.	SER	4.867	6.222	666.27±04
4.	HIS	5.006	2.001	148.71±12
5.	GLY	7.616	14.733	1589.44±22
6.	THR	8.522	2.911	233.59±08
7.	ARG	8.864	9.159	833.76±25
8.	ALA	10.631	ND	ND
9.	TYR	10.842	8.236	801.44±19
10	CYS	11.125	1.699	131.95±04
11	IS	12.001	15.183	1626 16±33
12	PHE	14.896	2.971	151.07±35
13	ILE	16.999	ND	ND
14	LEU	17.113	7.015	721.35±19
15	LYS	18.963	5.357	556.84±29

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Acquired by	: Admin
Sample Name	: Achyranthes amino acid
Sample ID	: Amino acid Achy.
Tray #	: 2
Vail #	: 1
Injection Volume	: 20 µL
Conc.	:
Data File Name	: Achyranthes amino acid data new.lcd
Method File Name	: Achyranthes amino acid Meth.lcm
Report File Name	: Default.lcr
Data Acquired	: 11/2/2016 2:15 PM
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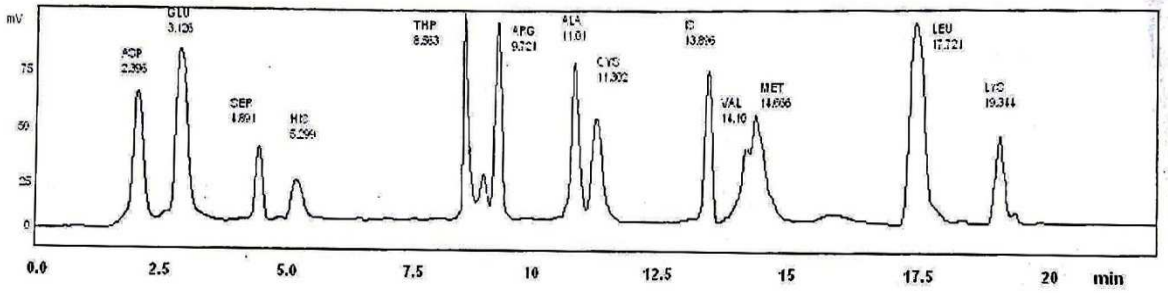

Peak	Name	Retention Time	Area(mm^2)	Conc. ($\mu g\ g^{-1}$)
1.	ASP	2.396	10.257	985.36±44
2.	GLU	3.125	14.338	1996.61±09
3.	SER	4.891	6.149	458.09±14
4.	HIS	5.299	1.201	151.21±13
5.	THR	8.563	13.632	1126.34±03
6.	ARG	9.721	12.996	967.09±27
7.	ALA	11.010	8.421	788.62±22
8.	CYS	11.302	5.229	521.06±08
9.	IS	13.896	6.529	796.36±14
10.	VAL	14.100	ND	ND
11.	MET	14.666	5.173	196.56±06
12.	LEU	17.721	20.671	2444.12±15
13.	LYS	19.344	4.225	176.89±16

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CONCLUSIONS

High concentrations of aspartic acid, glutamic acid, serine, glycine, arginine, tyrosine, lysine and leucine found predominate in the cissus and leucine, glutamic acid, threonin, arginine ,aspartic acid in achyranthes. Amino acids from essential amino acids i.e. phenylalanine, threonin, leucine, and lysine in cissus and theronine & leucine in achyranthes are found in considerable amount. Specific metabolic processes in which these amino acids participate may be related to the therapeutic properties of plants as per their use in traditional medicine.

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