

DETERMINATION OF AMINO ACID CONTENTS IN *Achyranthes aspera*, *Cissus quadrangularis* AND *Moringa oleifera*

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ABSTRACT

Plants are excellent source for the discovery of new products of medicinal value for drug development. Reliable methods of standardization are needed for the analysis of crude plant products. Chromatography is widely used in search for biologically significant molecules such as amino acids. The amino acids are the basic units of proteins and play a vital role in the metabolism of secondary metabolites. Taking into consideration the above facts the qualitative estimation of amino acids from the seeds of *Achyranthes aspera* (Apamarg) and stem of *Cissus quadrangularis* (Hadjor) and leaves of *Moringa oleifera* (Sahjan) have been carried out by paper chromatography. Two mobile phases were selected after employing various mobile phases for the detection of amino acids by paper chromatography technique. The results were compared with the standard amino acids in both mobile phases. Twelve amino acids in *Achyranthes*, fifteen amino acids in *Cissus* and Sixteen amino acids in *Moringa* were detected by using two different mobile phases.

KEYWORDS: *Cissus quadrangularis*, *Moringa oleifera* and *Achyranthes aspera*, Paper chromatography, Mobile phase

The natural products obtained from plants provides a unique opportunity for the development of new drugs but due to their complex nature there is a need to isolate and purify the bioactive compounds from crude plant extract by advanced separation techniques and instrumentation. The compounds which are isolated from different natural plant sources by using various solvent systems and chromatographic techniques is very important. Practically most of them have to be purified by the combination of several chromatographic techniques. A number of sophisticated separation techniques have been used in the recent past, Paper Chromatography (PC) is one of them. *Achyranthes aspera* Linn. (Amaranthaceae), *Cissus quadrangularis* Linn. (Vitaceae) and *Moringa oleifera* Lamk. (Moringaceae) (Bhandari, 1990; Rastogi and Mehrota, 1993; Indian Medicinal Plants, 1995. Kirtikaran and Basu, 1993; Chopra et al., 1958.) are important plants possessing a wide biological activity. A wide range of organic compounds are responsible for the biological activity of herbs. They are traditionally classified as primary and secondary metabolites (Crozier A., 2006). Primary metabolites are compounds that have essential roles associated with growth and development. These include acyl lipids, nucleotides, organic acids and amino acids. Amino acids (Wu G., 2009; Rodríguez J. et al., 2014; Ma Xiaoli et al., 2015) selected for this study are included in primary metabolites. 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. keeping this in view, present study is designed to investigate (Chopra et al., 1958; Pral and Latha; 2002; Ayo, 2007) the amino acids in selected plants by the use of paper chromatography (Gasparic and Churacek, 1978).

MATERIALS AND METHODS

Cissus quadrangularis (stem) and *Moringa oleifera* (leaves) were collected from various parks of Bikaner where it is cultivated as ornamental plant whereas seed samples of *Achyranthes aspera* was purchased from the shop of herbal medicine. The fresh samples were dried separately and used for further analysis. Powdered plant material was separately homogenized in 90% ethanol and kept overnight. These mixtures were separately filtered and residual fractions washed thrice with 90% ethanol. The filtrate from each of the samples was extracted with chloroform in separating funnel. Upper aqueous layer was removed, concentrated dried and used for free amino acid analysis. For the analysis of bound amino acid each of the

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left over residual tissue fractions was hydrolyzed with 6N HCL for 24hours at room temperature. The mixture was heated at 100°C filtered, dried and dissolved in 50 % ethanol and used to test for their bound Amino acids. Standard solutions and samples of each selected plant was applied on the chromatographic sheet. Separation of amino acids was carried out in two different mobile phases {i.e. n-Butanol: Acetic acid: water (6:2:2) and n-Butanol : Acetone : Acetic acid : water(35:35:7:23)} using paper chromatography technique. The results obtained were compared with standards.

RESULTS AND DISCUSSION

Qualitative determination of amino acids from chromatogram specified total 12 amino acids in *Achyranthes* and 16 amino acids in *Moringa* were detected in two different mobile phases {i.e. n-Butanol: Acetic acid: water (6:2:2) and n-Butanol : Acetone : Acetic acid : water(35:35:7:23)} whereas 15 amino acids in *Cissus* were detected in single mobile phase i.e. in n-Butanol : Acetone : Acetic acid : water(35:35:7:23). In *Achyranthes* Methionine, Lysine, Arginine, Serine, Alanine, Valine, Leucine, Threonine, Histidine, Aspartic acid, Glutamic

Table 1 : Amino Acids Identified in *A. aspera* in Mobile Phase n-Butanol : Acetic Acid :Water (6:2:2)

Name of the Amino Acids	R _f values		
	Std .Amino Acids	Amino Acids in <i>Achyranthes</i>	Amino Acids in <i>Moringa</i>
Methionine	0.066	0.063	0.068
Lysine	0.088	0.090	0.086
Arginine	0.210	0.210	0.211
Serine	0.223	0.220	0.224
Alanine	0.222	0.221	0.618
Valine	0.620	0.622	0.621
Leucine	0.581	0.583	0.583
Threonine	0.617	0.619	0.618
Tryptophan	0.668	ND	0.666
Isoleucine	0.710	ND	0.711
IS (Alpha Amino Butyric Acid)	0.893	0.891	0.899

Table 2 : Amino Acids Identified in the Selected Plants in Mobile Phase n-Butanol: Acetone: Acetic Acid :Water (35:35:7:23)

Name of the Amino Acids	R _f values		
	Std .Amino acids	Amino acids in <i>Achyranthes</i>	Amino acids in <i>Moringa</i>
Histidine	0.055	0.057	0.051
Aspartic acid	0.077	0.073	0.075
Lysine	0.083	0.081	0.089
Serine	0.141	0.139	0.144
Arginine	0.215	0.212	0.217
Glutamic Acid	0.302	0.300	0.306
Cysteine	0.333	0.336	0.332
Tyrosine	0.455	ND	0.457
Leucine	0.577	0.581	0.572
Valine	0.619	0.623	0.616
Phenylalanine	0.750	ND	0.755
IS (Alpha Amino Butyric Acid)	0.866	0.890	0.860

**Table 3 : Amino Acids Identified in *Cissus Quadrangularis* in Mobile Phase n-Butanol:
Acetone: Acetic Acid :Water (35:35:7:23)**

Name of the Amino Acids	Rf values	
	Std. Amino ACIDS	Amino Acids in Plant Part
Histidine	0.055	0.057
Methionine	0.066	0.065
Lysine	0.083	0.081
Serine	0.141	0.139
Arginine	0.215	0.212
Cystine	0.333	0.336
Glutamic Acid	0.302	0.300
Glycine	0.397	0.401
Alanine	0.419	0.421
Leucine	0.577	0.581
Valine	0.619	0.623
Threonine	0.622	0.617
Isoleucine	0.710	0.713
Tyrosine	0.722	0.727
Phenylalanine	0.750	0.755
IS (Alpha amino butyric acid)	0.866	0.883

Acid, Cysteine were found among them five amino acids (i.e. valine, threonine, methionine, leucine, and lysine) are essential amino acids.

Histidine, Methionine, Lysine, Serine, Arginine, Cystine, Glutamic Acid, Glycine, Alanine, Leucine, Valine, Threonine, Isoleucine, Tyrosine, Phenylalanine were reported in *Cissus* among them seven amino acids (i.e. phenylalanine, valine, threonine, isoleucine, methionine, leucine, and lysine) are essential amino acids.

In *Moringa* Methionine, Lysine, Arginine, Serine, Alanine, Valine, Leucine, Threonine, Tryptophan, Isoleucine, Histidine, Aspartic acid, Glutamic Acid, Cysteine ,Tyrosine, Phenylalanine were reported among them all the eight essential amino acids (i.e. phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, and lysine) are present.

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.

CONCLUSION

All the selected plants were found rich in various amino acids. This study also established the fact that the *M. oleifera* contains all the eight essential amino acids and *C.*

quadrangularis contains seven essential amino acids. hence it may be suggested that intake of *Moringa* leaves powder and *Cissus* stem powder is beneficial for human health due to presence of a good number of essential amino acids.

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