Quality Control of Herbs: Determination of Amino Acids in *Althaea officinalis, Matricaria chamomilla* and *Taraxacum officinale*

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Abstract: Analysis of raw materials and final products need reliable methods for the standardization of natural product drugs. Legal guideline also emphasizes on the qualitative and quantitative analyses of the plant constituents in an herbal product. In this study, thin layer chromatography (TLC) and amino acid analyzer was used for the determination of amino acids in plant extracts. Samples for this study were standards and aqueous extracts from *Althaea officinalis*, *Matricaria chamomilla* and *Taraxacum officinale*. Different amino acids in the extracts were detected through TLC. An automatic amino acid analyzer was used for the quantification of amino acids in the plant extracts under study.

Keywords: Plant extracts, amino acids, thin layer chromatography, amino acid analyzer.

INTRODUCTION

Determination of primary metabolites such as amino acids, carbohydrates along with the secondary metabolites is important for the quality control of herbs and herbal products (Qureshi *et al.*, 2011; Qureshi *et al.*, 2012). Analysis of amino acids has vitality in the field of biochemistry, food sciences (Sivoronov *et al.*, 1972; Skurikhin and Somin, 1983; Friedman *et al.*, 1988; Eizo, 1996), phyto-pharmaceuticals and pharmacy (Chu *et al.*, 1994; Bungau *et al.*, 2002; Misra and Pachauree, 2002; Culea *et al.*, 2006). Chromatographic methods can be used in search for bioactive compounds such as amino acids, carbohydrates, flavonoids, organic acids, peptides, tannins etc. Separation and identification of amino acids in complex samples like plant extracts have been often carried out using thin layer chromatography. This technique has various merits as: analysis of multiple samples can be performed at one time and also economical in terms of money and time (York *et al.*, 1990). As stationary phases silica gel (Ali and Qadry, 1987), modified silica gel (Bhushan and Ali, 1990) or polyamides (De Los Angeles Barcelon, 1982) have been employed. Mobile phase systems, which have been used most frequently, are: n-BuOH: Acetic acid: H_2O, Phenol: water, or n-BuOH: Acetone: Acetic acid: H_2O (Hodisan *et al.*, 1998). Determination of amino acids has been performed by automatic amino acid analyser utilizing ion exchange chromatography with post column derivatization using OPA (ortho phthaldialdehyde) (MÃ¸ller, 1993) or ninhydrin (Moore and Stein, 1951; Cynober and Coudray-Lucas, 1985). For quantification of amino acids after their separation by ion exchange chromatography, ninhydrin based detection method has been used most widely (Le Boucher *et al.*, 1997; Standara *et al.*, 1999).

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MATERIALS AND METHODS

Chemicals and reagents

Pre-coated silica gel TLC plates (20 cm *20 cm), acetone, acetic acid, n-butanol, ninhydrin, hydrochloric acid (37%), sodium hydroxide and ethanol were purchased from Merck AG (Darmstadt, Germany). Amino butyric acid (min. 99%), following amino acids: glycine (min. 98%), alanine (min. 98%), serine (min. 99%), proline (min. 99%), valine (min. 98%), threonine (min. 98%), cysteine (min. 98%), hydroxyproline (min. 98.5%), leucine (min. 98%), isoleucine (min. 98%), asparagine (min. 98%), aspartic acid (min. 98%), glutamine (min. 99%), glutamic acid (min. 99%), methionine (min. 98%), histidine (min. 99%), phenylalanine (min. 98%), arginine (min. 98%), tyrosine (min. 98%), lysine (min. 98%), tryptophan (min. 98%) and cystine, methanol, were obtained from Sigma-Aldrich GmbH, Germany, Plant material was delivered by Bionorica AG (Neumarkt i.d.Oberpfalz, Germany). All these chemical reagents were of analytical quality and used without further purification. Water was purified by a Nano Pure-unit (Barnstead, Boston, MA, USA).

Preparation of standards

All amino acid standards were prepared in water having a concentration of 1.0 mg/mL and stored at 2-8 °C.

Extraction

Powdered plant material (1.0 g) was refluxing in water (20.0 mL) for two hours (Dukhanina *et al.*, 2006). Extracts were centrifuged at 14.6 *1000 g.

Screening test (Ninhydrin test) for amino acids in plants

Aliquots of each extract (250 µL) were placed separately in a test tube, neutralized with 0.1 N NaOH and combined with an equal amount of ninhydrin solution (1 mg/mL in water).

Thin layer chromatography

1 μL of standard solutions and samples of each was applied on the pre-activated silica plate (Ali and Qadry, 1987). Separation was achieved by double development (Hodisan *et al.*, 1998) in a glass chamber pre-saturated with n-BuOH: acetone: acetic acid: H_2O (35:35:10:20) as a mobile phase (Gaspar Randic *et al.*, 2004). Total distance of migration was 18 cm. After the first elution the plate was air dried and eluted for the second time in the same direction, along the same distance and with the same eluant. Visualization of the spots was achieved by spraying 2% ninhydrin dissolved in acetone: n-BuOH (1:1 v/v) on the plate which was then dried at 105-110°C for 15 minutes. Amino acids in the extracts were identified by comparing the R_f values and the colour of gained spots from samples with those of reference standards.

Quantification of amino acids through amino acid analyzer

Extracts were adjusted to pH 2 with 0.1 M HCl solution. Quantification of amino acids was performed by the Institute of Amino Acid Analytics Kuhlmann GmbH (Ludwigshafen, Germany) using an automatic amino acid analyzer according to the method described in the European Pharmacopoeia (Pharmacopoeia).

RESULTS

Qualitative investigation of plant extracts

Screening test using ninhydrin as a detecting reagent identified different amino acids in *Althaea*, *Taraxacum* and *Matricaria*. Results from the TLC experiments are tabulated in table 1. R_f-values of the detected amino acids were calculated and compared with those obtained from the TLC of amino acids standards. Threonine, glutamic acid, alanine, hydroxyproline, proline, glycine, aspartic acid, asparagine, serine and glutamine are identified in all three plant extracts. Valine, cysteine, methionine, tyrosine, leucine and isoleucine are visualized in *Taraxacum* and *Matricaria* while lysine and arginine are found in *Althaea* and *Matricaria*. Finally, phenylalanine and tryptophan can be detected only in *Althaea* while histidine cannot be identified in any plant.

Quantification of amino acids

The results of quantification are given in table 2. Asparagine showed the highest concentrations, i.e. 800 mg/mL and arginine was detected in the amount of 540 mg/mL in *Althaea.* Proline occurred in the amount of 340 mg/mL in *Taraxacum* and 185 mg/mL in *Matricaria*, respectively.

Table 1: Results of TLC analysis of amino acids

#	Name of Standards	Colour	R_f value	Tarax	Alth	Matri
1	L-Threonine anhydrous	Purple	0.503	++	++	++
2	Aminobutyric acid	Purple	0.507	++	++	++
3	L-Phenylalanine anhydrous	Purple	0.755	ND	+	ND
4	L-Glutamic acid anhydrous	Purple	0.463	++	++	++
5	L-Valine anhydrous	yellowish purple	0.633	++	ND	++
6	L-Alanine anhydrous	Purple	0.469	++	++	++
7	L-Cysteine anhydrous	brownish yellow	0.721	+	ND	+
8	L-Histidine-HCl monohydrate	purple	0.100	ND	ND	ND
9	Hydroxy-L-proline anhydrous	brownish yellow	0.422	+	+	+
10	L-Proline anhydrous	lemon yellow	0.381	++	+++	++
11	Glycine anhydrous	reddish purple	0.401	+	+	+
12	L-Aspartic acid anhydrous	bluish purple	0.367	++	+++	++
13	L-Methionine anhydrous	yellowish purple	0.693	+	ND	+
14	L-Asparagine anhydrous	orange brown	0.340	++	+++	++
15	L-Tyrosine anhydrous	purple	0.727	+	ND	+
16	L-Serine anhydrous	purple	0.400	+	+	+
17	L-Leucine anhydrous	yellowish purple	0.733	+	ND	+
18	L-Lysine-HCl anhydrous	purple	0.133	ND	+	+
19	L-Arginine-HCl anhydrous	purple	0.187	ND	+++	+
20	L-Tryptophan anhydrous	yellowish purple	0.780	ND	+	ND
21	L-Isoleucine anhydrous	yellowish purple	0.713	+	ND	+
22	L-Glutamine anydrous	purple	0.393	+	+	+

(ND: Not detected, +: visible, ++: clearly visible, +++: intense spot, Tarax: *Taraxacum officinale*, Alth: *Althaea officinalis*, Matri: *Matricaria chamomilla*)

Table 2: Amino acids quantification results through amino acid analyzer

Plant name	L-Aspartic acid	L-Threonine	L-Serine	L-Asparagine	L-Glutamic acid	L-Glutamine	L-Proline	Glycine	L-Alanine	L-Valine
Althaea officinalis	37	2	*	800	8.8	*	14.7	0.5	10.1	1.5
Matricaria chamomilla	45	31	38	97	42	27	185	10.7	43	38
Taraxacum officinale	24	15.3	16.6	62	17.8	17	340	4.3	31	31

	L-Cystine	*L-Methionine*	*L-Isoleucine*	*L-Leucine*	*L-Tyrosine*	*L-Phenylalanine*	*L-Histidine*	*L-Tryptophan*	*L-Lysine*	*L-Arginine*	*Aminobutyric acid*
Althaea officinalis	0.6	*	0.5	*	0.6	0.7	1.2	7.2	1.9	540	31
Matricaria chamomilla	*	*	27	25	21	24	15.9	8.6	24	23	52
Taraxacum officinale	*	*	17.1	16.4	10.1	14.2	4.6	3.4	4.2	16.9	43

(Limit of quantification: 0.05mg/L, unit: mg/L), *=<0.05mg/L

DISCUSSION

Qualitative investigation of plant extracts

All considered plant extracts were screened for amino acids employing ninhydrin. They prove the presence of amino acids in *Althaea*, *Taraxacum* and *Matricaria*. The obtained results were verified by thin layer chromatography (TLC). For all three herbal drug preparations, several signals were detected. Especially for *Althaea* two very dominant spots were determined with an R_f-value of 0.19 and 0.35, respectively. However, the limits of the employed method are highlighted if the signals are compared with the standards. In fact, TLC-separation of amino acid standards showed clearly separated basic analytes (histidine, arginine and lysine) with R_f values <0.190. In this case, signal assignment of arginine could be clearly done for Althaea. No clear separation, on the other hand, could be detected for amino acids with R_f values >0.190, which makes spot identification in the sample difficult. Unfortunately, this concerns the most interesting part of the TLC chromatogram. Nevertheless, a trial concerning spot assignment on the basis of R_f-values and colour of spots is presented in table 1. Apart from a few studies that report different values, these results widely agree with previously published data (Diez, 1961; Samofal *et al.*, 1985; Blazekovic and Stanic, 2004; Gaspar Randic *et al.*, 2004).

Quantification of amino acids

Semi quantitative information from TLC delivered higher concentrated spots for asparagine and arginine only in *Althaea officinalis*. No dominant signals were gained for the other two plant extracts.

For quantification, analytes were separated and analysed through ion exchange chromatography using a pH dependent gradient and post column derivatization with ninhydrin. Detection of derivatized amino acids was performed at 570 nm and at 440 nm. The results of quantification are given in table 2. These data confirmed results from qualitative and semi quantitative investigations, i.e. out of twenty-one investigated amino acids, asparagine and proline are the leading amino acids in *Matricaria* and *Taraxacum* while the highest amount of asparagine and arginine were found in *Althaea*.

Limit of detection

The limit of detection of the TLC technique was 0.1 µg/µL and the limit of quantification of the amino acid analyzer was established at 0.05 µg/mL.

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