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Research Article

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HPTLC screening of amino acids from *Acorus calamus* rhizome and *Ardisia solanacea* leaf from Kuttanad Wetlands, Kerala, India

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ABSTRACT

Acorus calamus L., a medicinal herb occasionally planted in the home gardens, and Ardisia solanacea Roxb., a wild edible fruit plant, are frequently found in the Kuttanad wetlands of Kerala state. High Performance Thin Layer Chromatography (HPTLC) technique was used to detect and quantify the amino acids present in the rhizome of Acorus calamus and in the leaves of Ardisia solanacea. Methanolic extracts of the test plants were subjected to HPTLC analysis where n-butanol: acetic acid:water (4:1:1) mixture was used in the mobile phase. Four amino acids, glutamic acid, L-cystine, lysine and serine were detected from A. calamus while alanine, glutamic acid and valine were detected from A. solanacea.

Key words: amino acid, *Acorus calamus*, *Ardisia solanacea*, HPTLC, methanolic extract

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INTRODUCTION

Amino acids are the “building blocks of the proteins”, which serve as body builders and play an important role in the metabolism [1]. Plant exudates and organ extracts often contain amino acids and ions as solutes [2] which have a major function in primary and secondary metabolic pathways. Amino acids have been analyzed using a number of procedures, including separation by high performance liquid chromatography (HPLC), gas chromatography, liquid chromatography and capillary electrophoresis [3]. HPTLC is emerging as a versatile, high throughput and cost-effective technology that is uniquely suited in assessing the identity and quality of botanical materials [4, 5]. Applications of HPTLC include phytochemical and biomedical analysis, herbal drug quantification [6], active ingredient quantification, fingerprinting of formulations [7, 8] and check for adulterants in the formulations.

Acorus calamus L. (Family: Araceae) is a semi-aquatic, perennial, tuberous herb, with creeping rhizomes, sword shaped leaves and spadix inflorescence. It has a long history in medicinal use. The plant (trade name ‘Sweet flag’), locally known as ‘Vayambu’, is grown traditionally in the home gardens of Kuttanad wetlands in Kerala, and the plant is also found in isolated thickets along marshy habitats and fringe areas of ponds and canals as garden escape. The rhizomes of sweet flag possess antioxidant property [9] and in Ayurvedic system of medicine, it is used in the treatment of diseases like fever, asthma and bronchitis. The rhizome is also used as a sedative and the natives use the rhizome in treating cough.

Ardisia solanacea Roxb., a native of India, is a glabrous shrub or small tree that reaches a height of 10-15 feet tall in nature. The genus *Ardisia* is the largest in the family Myrsinaceae, and approximately 500 species of evergreen shrubs and trees are found throughout the subtropical and tropical regions of the world. They are used as fruit, medicine or as ornamental plants and possess free radical scavenging potential [10]. *Ardisia* spp. produces several groups of biologically active phytochemicals, including saponins, coumarins and quinines and is a rich source of biologically potent compounds such as bergin and ardisin [11]. The objective of the present investigation was to detect the amino acids present in the rhizome of A. *calamus* and in the leaves of *A. solanacea* using HPTLC.

EXPERIMENTAL SECTION

Plant Materials

The taxonomic identity of *Acorus calamus* and *Ardisia solanacea* were confirmed by Dr. T. Shaju, Plant taxonomist, Division of Plant Systematics and Evolutionary Science, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, (JNTBGRI), Palode, Kerala, and the voucher specimens of the samples were deposited in the Herbarium of Environmental Resources Research Centre (ERRC), Thiruvananthapuram, Kerala. *A. calamus* rhizome and *A. solanacea* leaves were collected from Kuttanad wetlands of Kerala State, India.

Chemicals

TLC Silica gel 60 F_{254} plates were procured from Merck, Germany. Standard amino acids (aspartic acid, arginine, cystine, glutamic acid, glycine, histidine, hydroxyproline, lysine, serine, threonine, alanine, proline, alpha-amino butyric acid, leucine, isoleucine, methionine, valine, phenylalanine, tryptophan and tyrosine) and the solvents (analytical reagent grade) were procured from HiMedia Laboratories Pvt. Limited, Mumbai, India.

Preparation of plant extract

Stock solutions of samples were prepared by soaking 5 g of dried rhizome/ leaf powder in 100 ml volumetric flask with methanol for overnight and filtered through Whatman filter paper No.1. Working samples were prepared by taking 100 µl of stock solution and equally diluted (1:1 v/v) using methanol. Working samples of 2 µl from each sample preparations were used for HPTLC profiling.

Preparation of standard amino acids

Standard amino acids alanine, arginine, aspartic acid, cystine, glutamic acid, glutamine, glycine, isoleucine, leucine, methionine, proline, serine, threonine and valine were prepared at a concentration of 1 mg/mL and phenylalanine, tryptophan and tyrosine were prepared at a concentration of 0.1 mg/mL in double distilled water and used for further analysis.

Calibration curve of amino acids

The working standard solution of amino acid (8 µL) was spotted to get different concentrations ranging from 120-660 ng/spot, using a micro syringe. The working standard solution was spotted as a sharp band on 6 mm width using spray technique with a Camag 100 µL sample syringe on prelocated silica gel aluminium plate 60F_{254}, (10 cm x10 cm) with 8 mm thickness using a CAmag Linomat V automatic sample applicator. A stock solution of standard amino acid of volume 8µL was spotted on to a TLC plate to obtain a concentration of 120, 280, 440 and 660 ng/spot of amino acids, respectively. The peak areas of different amino acid for each concentration were recorded and calibration curve was obtained by plotting peak areas of amino acid against applied concentration of amino acids. The result of linearity range and correlation range indicated that there was good correlation between peak area and the corresponding concentration of amino acids.

Instrumentation and chromatographic conditions

HPTLC was performed on 10 x 10 cm aluminium backed plates coated with silica gel 60F_{254} (Merck, Germany). Standard solution of alanine (10 µL) and sample solution (8,10,12 µL respectively) were applied to the plates as bands 6 mm wide and 10 mm from bottom edge of the same chromatographic plate by use of a Camag (Switzerland) Linomat V sample applicator equipped with a 100 µL Hamilton (USA) Syringe. Ascending development to a distance of 80 mm was performed at room temperature with n-butanol:acetic acid:water (4:1:1) as mobile phase, in a Camag glass twin-trough chamber previously saturated with mobile phase vapor. After development, the plates were air dried and then dipped in ninhydrin solution and the plates were dried in hot air oven. Quantitative analyses of the compounds were done by scanning the plates at 490 nm with a Camag TLC scanner with WINCAT software.

RESULTS AND DISCUSSION

For the detection of amino acids, various solvent compositions of mobile phase for HPTLC analyses were examined in order to achieve high resolution and reproducible peaks. The mobile phase with the composition of n-butanol:acetic acid:water (4:1:1) showed high resolution and repeated results which confirmed their efficiency and accuracy. The methanolic extracts of *A. calamus* and *A. solanacea* showed the presence of different amino acids with different Retention factor (R_f) values ranging from 0.04 to 0.43.

In the case of methanolic rhizome extract of *A. calamus*, four amino acids were detected. They were glutamic acid (Figure 1), lysine (Figure 2), serine (Figure 3) and L- cystine (Figure 4). Glutamic acid content was 10.82 ng/ µL and the lysine content were 9.43 ng/ µL. The serine and L- cystine contents in the samples were given in table 1. From the results it was concluded that L- cystine was the major amino acid present in the sample. It has been widely used in chemotherapeutic treatment for leukemia and is also a major constituent of biologically active molecules like glutathione [12].

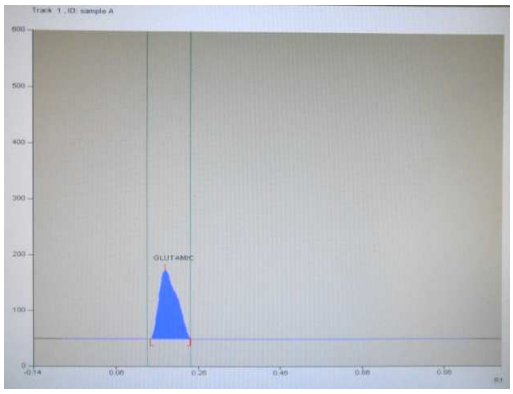

Figure1. Peak representing glutamic acid

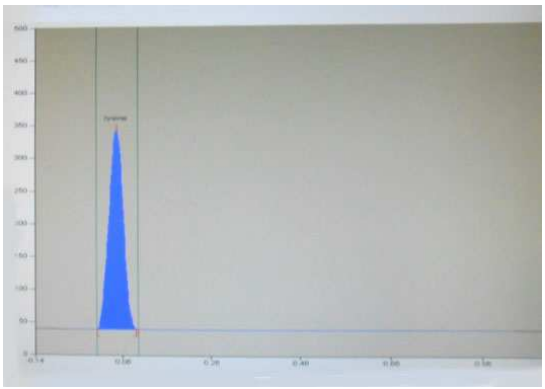

Figure 2. Peak representing lysine

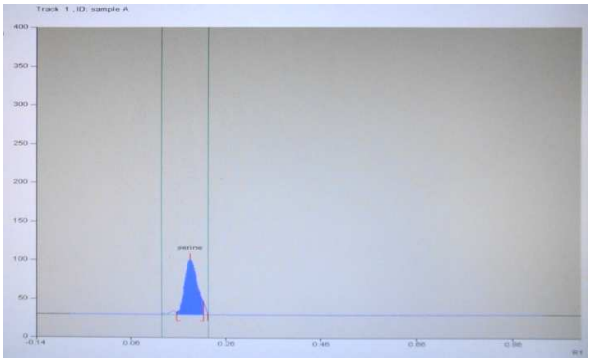

Figure 3. Peak representing serine

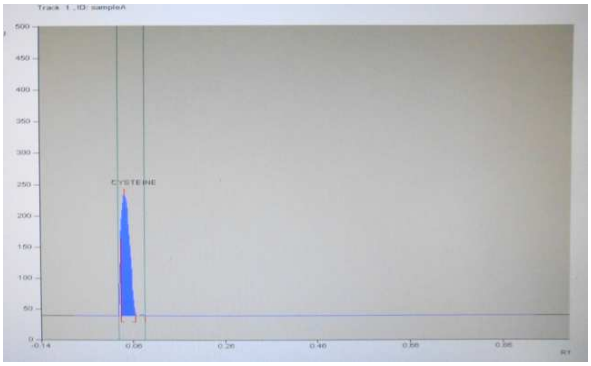

Figure 4. Peak representing cystine

Total seven amino acids were detected in *A. calamus* and *A. solanacea*. The alanine with the R_f value 0.22 is common in all vegetative parts of plants. The glutamic acid with R_f values 0.17 and 0.18 respectively were found in *A. solanacea* leaf and *A. calamus* rhizome. In the case of *A. solanacea* only three amino acids were detected. They were alanine (Figure 5), glutamic acid (Figure 6) and valine (Figure 7). Alanine content was 33.75 ng/ µL and glutamic acid and valine contents were given in table 1. Alanine was the predominant amino acid present in *A. solanacea*. Alanine is an important source of energy for muscle tissue, brain and central nervous system and strengthens the immune system by producing antibodies and helps in the metabolism of sugars [13]. Glutamic acid is responsible for the activation of brain [14] and this amino acid was found in both the plants.

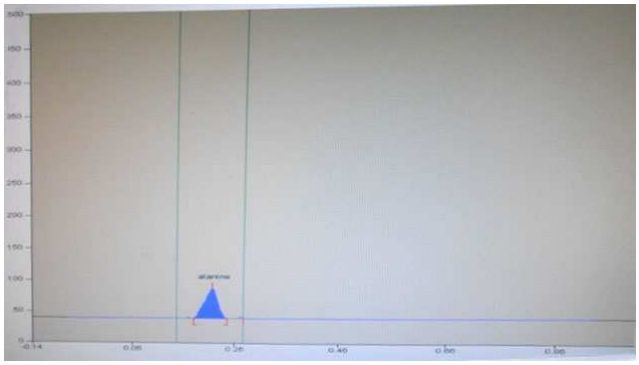

Figure 5. Peak representing alanine

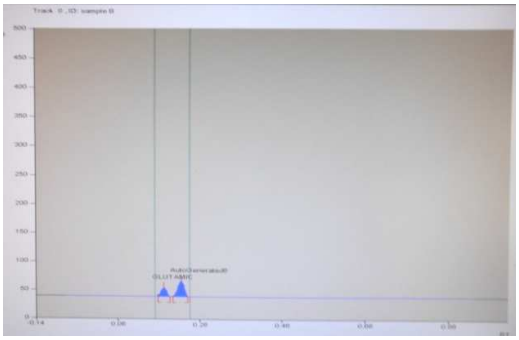

Figure 6. Peak representing glutamic acid

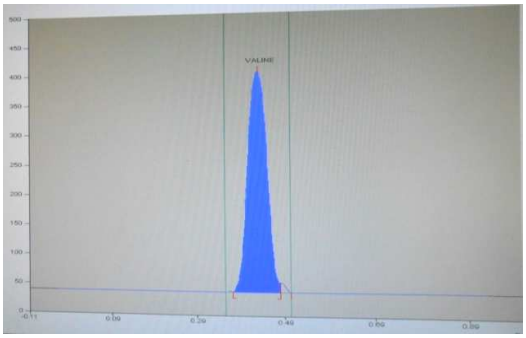

Figure 7. Peak representing valine

Table 1. Peak table for amino acid profile

Name of the plant	Amino acid	Retention factor (R_f)	Height	Area	Concentration (ng/µl)
A. calamus	Glutamic acid	0.18	113.44	3750.60	10.82
	L-cystine	0.04	231.26	3327.23	26.4
	Lysine	0.06	229.41	3953.79	9.43
	Serine	0.18	71.54	1518.36	5.4
A. solanacea	Alanine	0.22	46.89	966.78	33.75
	Glutamic acid	0.17	15.75	181.88	13.5
	Valine	0.43	214.28	6374.21	4.32

Cystine is required for skin formation, for elasticity and texture of skin, detoxification of body, important in collagen production, slow down the aging process, deactivates free radicals and has the capacity to neutralize toxins. It also aids in protein synthesis and presents cellular change [13]. Valine is involved in many metabolic pathways and is considered indispensable for protein synthesis and optimal growth [15]. Amino acids from medicinal plant, *Cassytha filiformis* were screened using HPTLC method [16] and HPTLC fingerprints can also be used for documentation and quantification of chemical markers to identify morphological and geographical variation in herbal raw materials [17, 18] and this also offers better resolution and estimation of active constituents with reasonable accuracy in a shorter time [19].

CONCLUSION

Different amino acids, glutamic acid, lysine, serine and L-cystine were detected from *A. calamus* rhizome and glutamic acid, alanine, and valine were detected from *A. solanacea* leaf in appreciable quantities. The results indicate the possibility of utilizing these otherwise underutilized plants as food supplement including cattle feed.

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REFERENCES

[1] MD Swati; SK Gayatri; CT Rasika; AK Asha; R D Nirmala; PS Jyoti, *J. Chem. Pharm. Res.*, **2011**, 3(3), 192-195.
[2] CR Warren; MA Adams, *J. Expt. Botany*, **2000,** 51 (347), 1147-1157.
[3] WI Menzel; WP Chen; AD Hegeman; JD Cohen, *Methods Mol. Biol*., **2012,** 918,165-78.
[4] RD Chaudhary. Herbal Drug Industries, Fourth Reprint, Eastern Publishers, New Delhi, **2006**, 71, 473-474.
[5] M Irmgard, W Steffen, U Abraham, R Eike, *J. Pharm. Biomed. Anal.*, **2008**, 48 (3), 587-591.
[6] GS Sushma; BA Devi; CH Madhulatha; KU Kumar; P Harathi; NS Subramanian; M Ramadevi, *J. Chem. Pharm. Res.*, **2013**, 5(3), 98-104.
[7] S Thennarasan; S Murugesan; T S Subha, *J. Chem. Pharm. Res.*, **2014**, 6(1), 674-677.
[8] M Ritu; KT Ashok, *J. Chem. Pharm. Res.*, **2013**, 5(7), 240-245.
[9] S Manju; R P Chandran; PK Shaji; GA Nair, *Int. J. Pharm. Pharm. Sci*., **2013**, 5 (4), 376-380.
[10] RP Chandran; S Manju; MV Vysakhi; PK Shaji; GA Nair, *J. Pharm. Res*., **2013**, 6 (5), 555- 558.
[11] H Kobayashi; E de Mejia, *J. Ethnopharmacol.,* **2005**, 96 (3), 347-354.
[12] PS Chandrachood; TV Gadkari; AA Bhave; RC Torane; S Deore; NR Deshpande; RV Kashalkar, *Der Pharma Chemica*, **2011**, 3 (4), 449-453.
[13] SA Gaikwad; A Kale; K Mundhe; NR Deshpande; JP Salvekar, *Int. J. ChemTech. Res*. **2010**, 2 (2),1092-1094.
[14] G Bist; H Sandhu, *J. Indian Chem. Soc.,* **1990**, 67, 1002-1003.
[15] V Madhavan; H Basnett; AC Kumar; SN Yoganarasimhan, *Indian J. Pharm. Sci*., **2008**, 70 (6), 789-800.
[16] J Harborne, A Harborne. Phytochemical methods: a guide to modern techniques of plant analysis. Springer, **1998**.
[17] SK Srinivas; AS Aparna, *E. J. Chem.*, **2012**, 9(1), 313-317.
[18] SK Sripathi; G Poongothai; P Lalitha, *J. Chem. Pharm. Res.*, **2011**, 3(5), 544-549.
[19] PD Sethi. High performance thin layer chromatography: quantitative analysis of pharmaceutical formulations; CBS Publishers & Distributers, New Delhi, **1996,** 10-60.