Int J Pharm Bio Sci 2015 Oct; 6(4): (B) 782 - 785

Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

HPTLC FINGER PRINTING METHOD FOR THE DETERMINATION OF AMINO ACIDS FROM ETHANOLIC EXTRACT OF PALMYRA PALM FRUIT PULP (*Borossus flabellifer*)

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ABSTRACT

To identify the presence of various amino acids in ethanolic extract of palmyra fruit pulp, HPTLC finger printing analysis was carried out. The analysis was carried out by using CAMAG LINOMAT5 instrument which revealed the presence of five amino acids especially alanine, valine, methionine, leucine and phenylalanine with Rf values 0.16, 0.21, 0.27, 0.35, 0.37. The concentration of phenylalanine was maximum (36.56 mg/100mg) and methionine was present in minimum concentration (1.49 mg/100 gm).

KEY WORDS: **Amino acids, *Borossusflabellifer*, HPTLC, Ethanolic extract**

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INTRODUCTION

Amino acids, a class of biologically active compounds present in food and beverages, are important for human nutrition[1] and affect the quality of foods including taste, aroma, and colour[2]. Among different substances that constitute fruits, amino acids are becoming increasingly important and, for various reasons, their analytical determination is becoming more necessary[3]. The percentage of amino acids in fruits were affected by such as fruit maturity [4] mineral nutrition [5,6], water stress[7], climatic conditions, field treatments and light-dark transitions[8]. Palmyra palm or Asian toddy palm is botanically known as *Borasus flbellifer* L., belongs to the family *Arecaceae*[9]. The fresh pulp is reportedly rich in vitamins A and C[10]. The pulp of the mature fruit relieves dermatitis[10]. According to vijayakumari et al[11] the palmyra fruit pulp has good water and fat absorption properties. So PFP is used in bakery industry and in various food formulations. HPTLC fingerprinting is an ideal which allows comparisons between a standard and sample[12]. In combination with digital scanning profiling, HPTLC also provides accurate and precise RF values as well as a record of the separation in the form of a chromatogram with fractions represented as peaks with defined parameters including absorbance (intensity), RF, height and area [13]. No information is available regarding amino acid content of the palmyra fruit pulp. Therefore, the present attempt has been made to analyze free amino acids in ethanolic exract of palmyra fruit pulp through HPTLC analysis.

MATERIALS AND METHODS

The collected fruits were sorted out in order to remove damaged ones and were separated on the basis of the state of ripeness, similarity in shape and size. The fruits were then washed, weighed, peeled and pulped. The pulp was stored at 4^0C and some of the pulp was dried at 60^0C for 24 to 48 hrs. The dried pulp was finally milled using pulverizer to pass through 250 µm sieve. The samples were then packaged in polyethylene bags and kept in a refrigerator (4^0C) for further use[11].

(i) Preparation of extract

Palmyra fruit pulp powder was defatted with petroleum ether (60-80^0c) in a soxhlet apparatus. The defatted powder material (marc) thus obtained was further extracted with ethanol (95% v/v). The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary evaporator[11]. The extract was subjected to chromatographic analysis.

(ii) Apparatus

Spotting device: Linomat IV automatic sample

Spotter: CAMAG (Muttenz, Swizerland)

Syringe: 100µL Hamilton

TLC chamber: Glass twin trough chamber (20× 10× 4)

Densitometer: TLC scanner 3 with WinCATS software; CAMAG

HPTLC Plate: 20×10cm & 10x 10, TLC aluminum sheets silica gel 60F254; Merck KGaA

(iii) HPTLC conditions

According to sadasivam et al. [14] the filtrates (5µl of each) and the standards (2 µl each at a concentration of 1mg/ml) were coated on a pre-coated TLC aluminum silica gel – 60F 254 (Merck, Germany) (10cm x 10cm) (20cm x10cm). The TLC plates were developed with a solvent system consisting of n-butanol/ ethylacetate/ water/ acetic acid (1:1:1:1). The developed plates were stained using 0.3% ninhydrin in n-butanol as spraying reagent and the plates were heated at 100°C for 1min. These plates werescanned digitize and analyzed by using CAMAG software.

RESULTS AND DISCUSSION

1. Finger printing profile of amino acids

HPTLC amino acid profile of ethanolic extract of palmyra fruit pulp was recorded in Figure 1 and Table 1. The result showed the presence of

five amino acids. The concentrations of these amino acids were alanine (16.49mg), valine (15.28 mg), methionine (1.49), leucine (69.64) and phenylalanine (35.56). 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[15].Valine is, involved in many metabolic pathways and is considered indispensable for protein synthesis and optimal growth [16]. Leucine is the only dietary amino acid that has the capability to stimulate muscle protein synthesis and is the most important amino acid for muscle building[17]. Aromatic amino acids apart from their structural function in proteins, are precursors of many important biological compounds such as neurotransmitters and hormones in the human body[18]. Methionine, as sulphur containing amino acids, is known to possess antioxidant property[19]. The results indicated that the pulp contains an appreciable amount of amino acids.

Figure 1
Densitogram display for ethanolic extract of palmyra fruit pulp

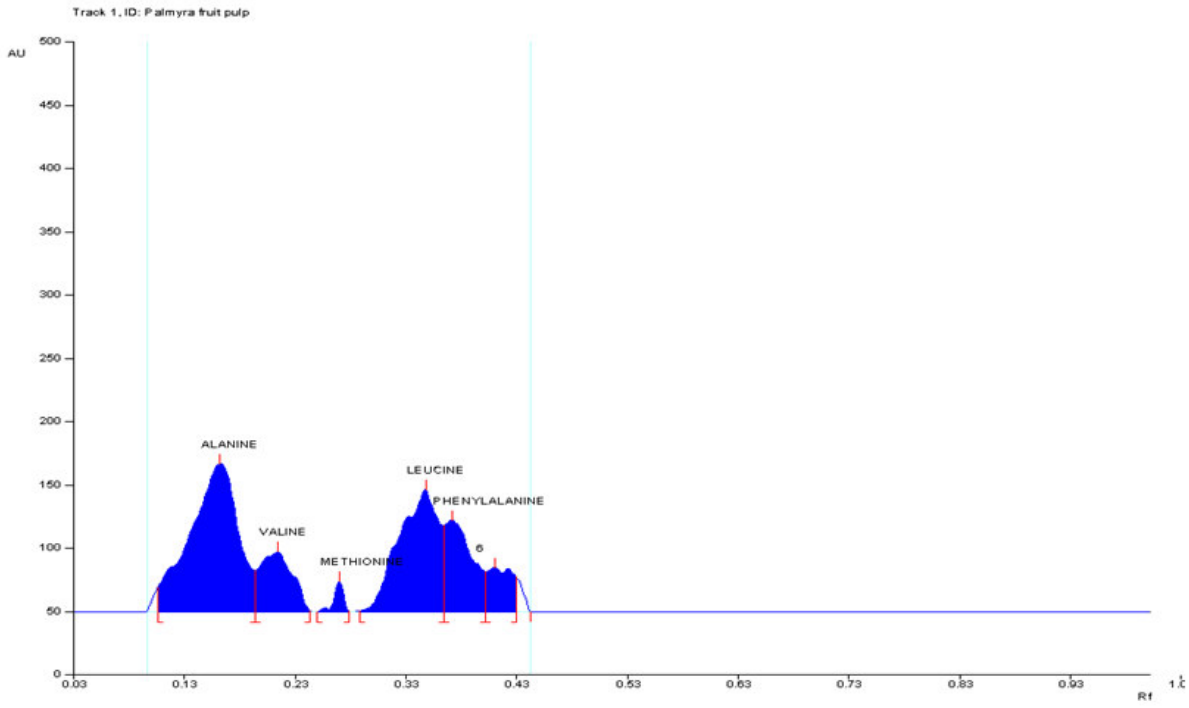

Table 1
Peak table with Rf values, height, area and concentration

Amino acids	Rf	Peak Height	Peak Area	Concentration (mg/100gm)
Alanine	0.16	116.815	4246.90	16.49
Valine	0.21	47.710	1184.69	15.28
Methionine	0.27	24.851	171.65	1.49
Leucine	0.35	96.485	2915.43	69.64
Phenylalanine	0.37	72.419	1530.72	36.56

CONCLUSION

Compared to TLC, the HPTLC method is more feasible for development of chromatographic finger prints to determine major active constituents of medicinal plants. The separation and resolution are much better than TLC. Different amino acids, alanine, valine, methionine, leucine and phenyl alanine were detected from the ethanolic extract of the palmyra fruit pulp.

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