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HPTLC screening of amino acids from alcoholic extracts of four molluscan species along the South East Coast of India

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ABSTRACT

The objective of the present study is to develop a rapid HPLTC method for simultaneous separation of amino acids in methanol and methanol: chloroform extract of molluscan species. The gastropods such as Lambis lambis, Tona dolium, Bursa rana and Natica marochiensis are collected from Tamilnadu coast, India for analysis. The HPTLC analysis of methanol and methanol: chloroform extracts of gastropod species shows the following 11 amino acids of total the 22 amino acid standards viz., Cysteine HCl, Histidine, Aspartic acid, Asparagine, Glutamine, Hy-proline, Alanine, Methionine, Threonine, Glutamic acid, Tryptophan. The analysis shows that valine is present only in L. lambis, proline in L. lambis and B. rana. Similarly, Tyrosine is present in T. dolium, B. rana and N. marochiensis. In the present study we infer that most of the amino acids present in methanol extracts are also present in methanol: chloroform extracts of different molluscan species. As amino acids are building blocks of protein this will aid to the identification of proteins and the followed HPTLC method is very efficient for quantification of amino acids.

Keywords: Mollusks, HPTLC, amino acids, gastropods.

INTRODUCTION

An appreciation of the potential applications of high end instruments for biomedical research has resulted in significant advances in biological sciences. For any newly isolated and synthesized compounds or drugs, elucidation of their biological potentials is exclusively important (**1**). Thus, optimized procedures should be used in modern day research to explore nature as a source of immeasurable value for obtaining molecules with extraordinary properties. Global interest in understanding the function of marine ecosystem has been accelerated in recent years as a number of therapeutically important molecules were isolated from the ocean with unique structures. This treasure of chemically diverse molecule is found in marine organisms which comprise approximately half of the total biodiversity on the earth which requires refined instruments for

analysis. Chromatography is a technique by which the complex mixtures can be resolved in to individual components (2). Wherein, the HPTLC analysis is an invaluable quality assessment tool for the evaluation of the compounds. It is generally realized that for monitoring quality, HPTLC fingerprinting is ideal which allows comparisons between a standard and sample [3]. Although the principles of TLC and HPTLC methods are identical, the kinetically optimized fine-particle layers feature in HPTLC separation is faster and more efficient and the results are more reliable and reproducible. In combination with digital scanning profiling, HPTLC also provides accurate and precise *R*F 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), *R*F, height and area [4]. Recent applications of high pressure thin layer chromatography for amino acid analysis in nanogram or ppm range using the simplest technique being worthy of mention, expand the usefulness of the method.

Amino acids are critical to life and have many functions in metabolism. They are the building blocks of proteins and serve as body builders and play a vital role in the metabolism of secondary metabolites (5). Since, the amino acids are used to a variety of applications in various industries such as, for the production of biodegradable plastics, chiral catalysts and in the synthesis of drugs and cosmetics. Therefore, the present attempt has been made to analyze free amino acids in methanol and methanol: chloroform extracts of four different molluscan species which including *L. lambis, T. dolium, B. rana* and *N. marochiensis* through HPTLC analysis.

EXPERIMENTAL SECTION

Chemicals and reagents

All the chemicals used in the experiment were of analytical grade. The amino acid standards were purchased from Sigma Aldrich, USA. All the solvents used in the experiment were procured from Merck Pvt. Ltd, Mumbai, India.

Apparatus

Rotary evaporator
Lyophilizer
Spotting device: Linomat IV automatic sample spotter; CAMAG (Muttenz, Swizerland)
Syringe: 100µL Hamilton (Bonadug, Swizerland)
TLC chamber: Glass twin trough chamber (20× 10× 4)
Densitometer: TLC scanner 3 with CATS software; CAMAG
HPTLC Plate: 20×10cm & 10x 10, TLC aluminum sheets silica gel 60F254; Merck KGaA

Preparation of Molluscan extracts

The molluscan animals such as *L. lambis, T. dolium, B. rana* and *N. marochiensis* were collected from Parangipettai coastal areas along the east coast of India. The collected samples were washed with distilled water and stored at 4°C until use. Further, the samples were homogenized and extracted with methanol and methanol: Choloroform (1:1). The extracted samples were centrifuged at 5000rpm x 15mins and supernatant was collected. The excess solvents were removed by using rotary evaporator and the samples were lyophilized. Finally, the lyophilized samples were used for amino acid analysis through HPTLC.

Preparation of the standard Amino acids for HPTLC analysis

The 22 standard amino acids were prepared at the concentration of 1mg/ml in double distilled water and used for the further analysis.

Preparation of the sample for HPTLC analysis

The samples were dissolved in methanol at the concentration of 5mg/ml and centrifuged at 10,000rpm x 1min at 4°C. The supernatant was filtered through Whattman filter paper No.1 and the filtrate was used for HPTLC analysis.

HPTLC conditions

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

Fig: 1. HPTLC images under the excitation of respective wavelength of 22 standard amino acid and methanol and methanol: chloroform extracts of *Lambis lambis, Tona dolium, Bursa rana* and *Natica marochiensis*.

A. Tracks showing 22amino acid standards at wavelength 366nm (fluorescence)
B. Tracks showing 22amino acid standards at wavelength 254nm (UV)
C. Tracks showing four samples methanol and methanol: chloroform extracts at wavelength 366nm (fluorescence)
D. Tracks showing four samples methanol and methanol: chloroform extracts at wavelength 254nm (UV)

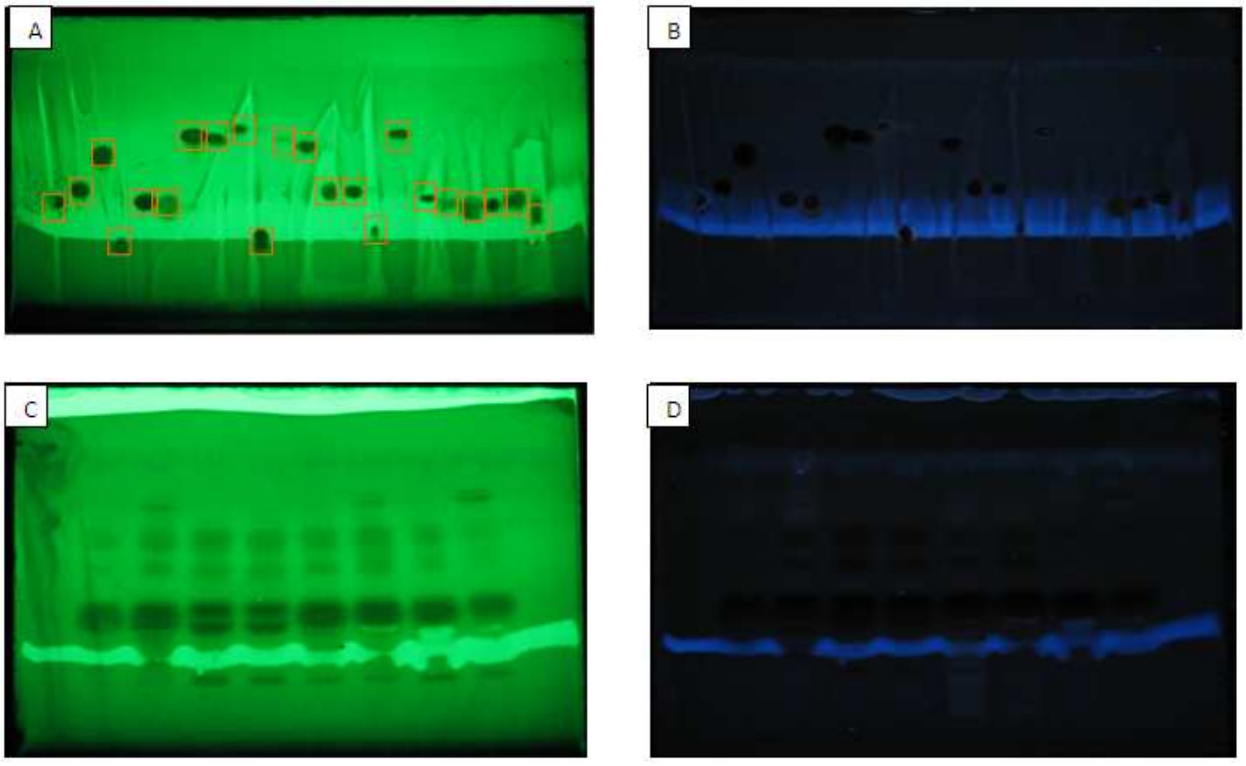


Fig.2. HPTLC scanning of all tracks at 490nm for 22 standard amino acids and methanol and methanol: chloroform extracts of *Lambis lambis, Tona dolium, Bursa rana* and *Natica marochiensis*.

A. 22 tracks showing the standard amino acid profile at 490nm.
B. Four samples of methanol and methanol: chloroform extracts profile at 490nm.

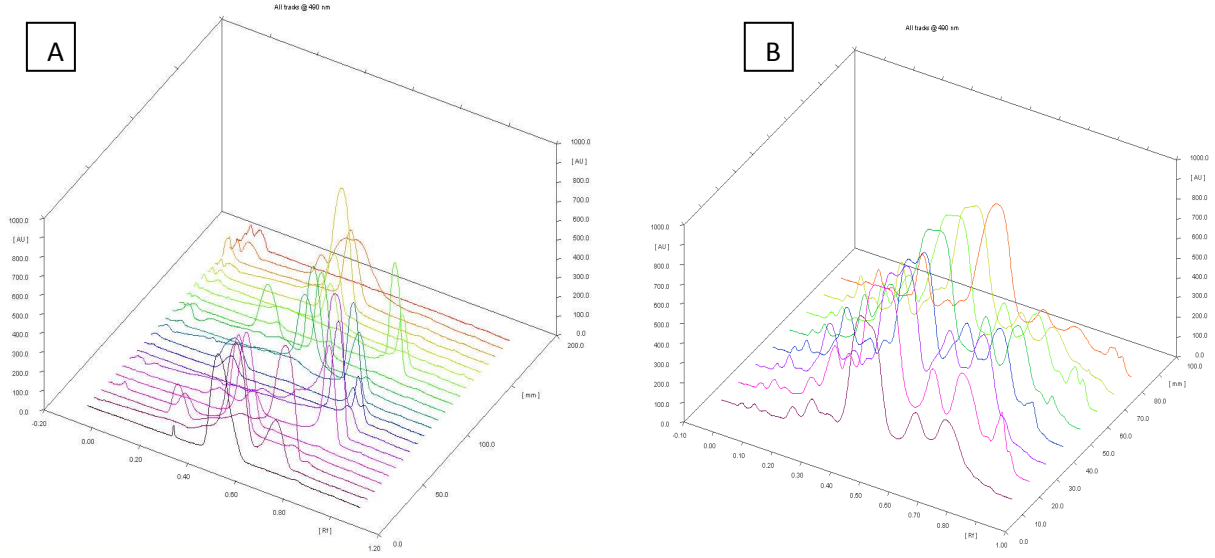
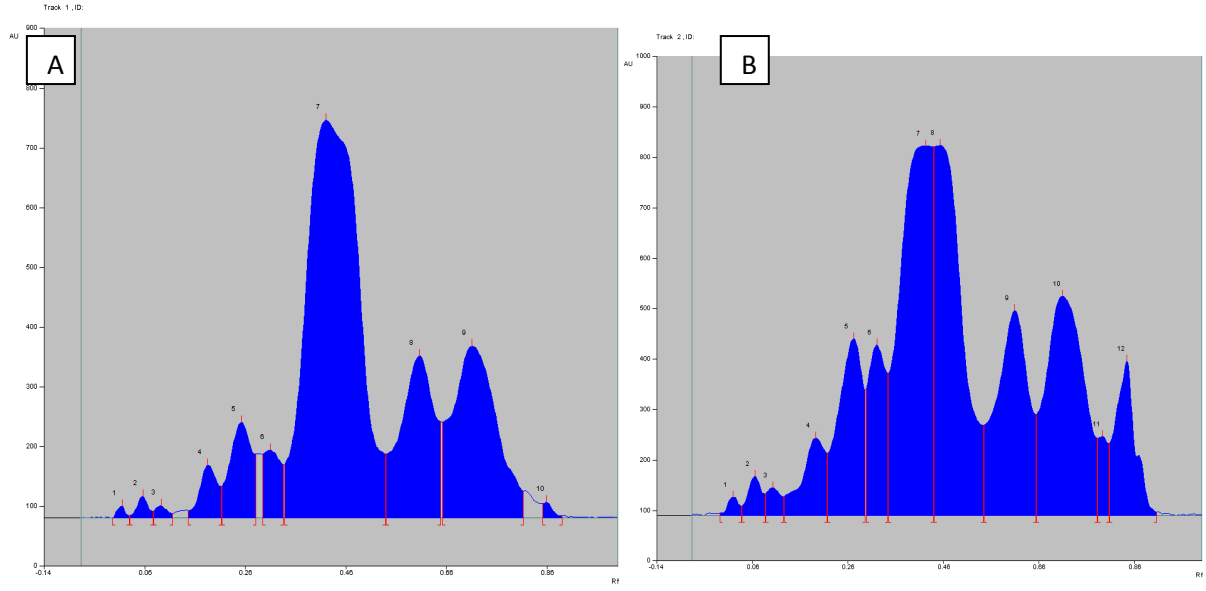


Fig.3. HPTLC digital scanning profiles of *Lambis lambis, Tona dolium, Bursa rana* and *Natica marochiensis* methanol and methanol: chloroform extracts.

A. Lambis lambis methanol extract. B.Lambis lambis methanol: chloroform extract. C. Tona dolium methanol extract. D. Tona dolium methanol: chloroform extract. E. Bursa rana methanol extract. F. Bursa rana methanol: chloroform extract.G. Natica marochiensis methanol extract. H. Natica marochiensis methanol: chloroform extract.

B. Peaks representing the corresponding the amino acids in the sample. Peak.1: Cysteine HCl (Rf 0.02), Peak 2,3,4: non-standard amino acids , Peak 5: Histidine (Rf 0.28), Peak 6: non-standard amino acids, Peak 7,8: amino acids having same migration points Aspartic acid, asparagines, Glutamine, hy-proline (Rf 0.46), Peak 9: Alanine, threonine, methionine, glutamic acid (Rf 0.52), Peak 10: Valine (Rf 0.70, Lambis lambis methanol and methanol: chloroform extract), tyrosine (Rf 0.74, Tona dolium, Bursa rana and Natica marochiensis methanol and methanol: chloroform extracts), Peak 11: Proline (Rf 0.79,methanol: chloroform extract of Lambis lambis and methanol chloroform extracts of Bursa rana), Peak 12: Tryptophan (Rf 0.82, all the four molluscan species except in the methanol extract of Tona dolium).



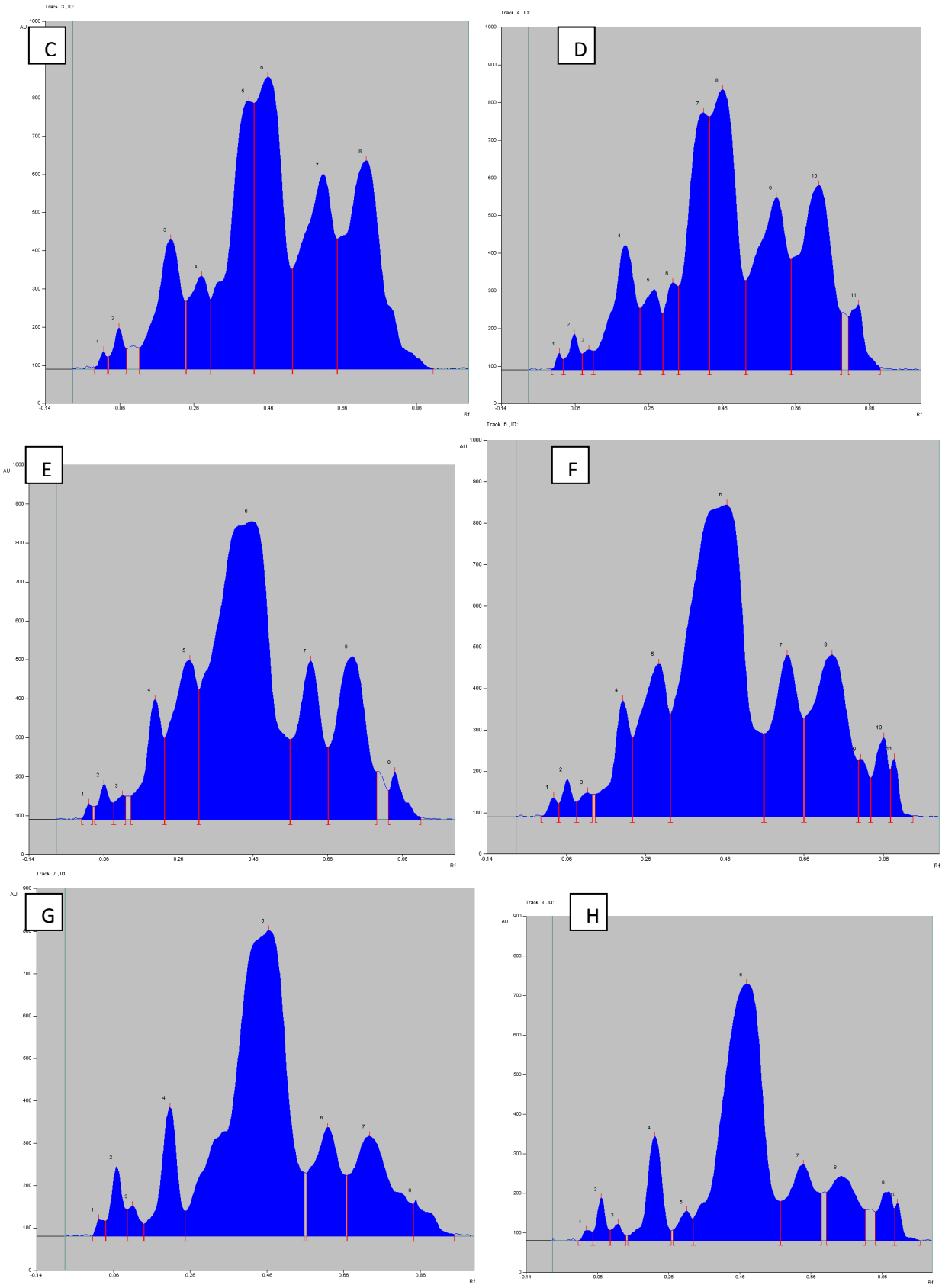
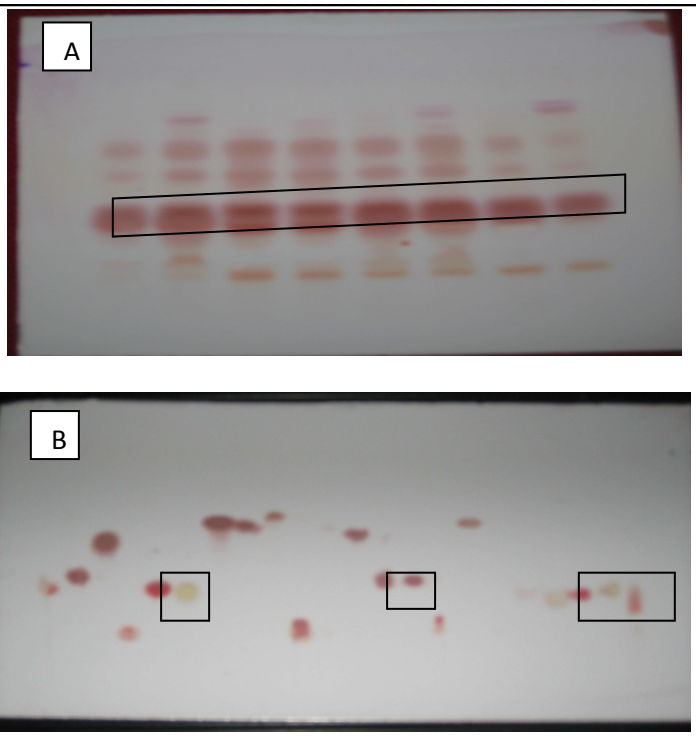


Fig. 4: The markings show the amino acids having very similar Rf values merged to form a blurred bands (same migration point) A. Samples methanol and methanol: chloroform extracts of *Lambis lambis, Tona dolium, Bursa rana* and *Natica marochiensis*, B. 22 standard amino acids.



From superimposition study a band (Rf 0.02) corresponding to Cys-HCl is visible in all the eight samples, which indicate the presence of Cysteine HCl in the molluscan extracts. Band (Rf 0.28) corresponding to Histidine is visible in all the samples indicate the presence of Histidine. Similarly band with Rf value 0.46 corresponding to the amino acids Aspartic acid, Asparagine, Glutamine, Hy-proline indicates their presence in all the eight samples. Band (Rf 0.52) corresponding to Alanine, Methionine, Threonine, Glutamic acid is visible in the all samples. Band (Rf 0.70) corresponding to the Valine indicates the presence of Valine in methanol and methanol: chloroform extracts of *Lambis lambis*. Band (Rf 0.74) corresponding to the tyrosine is present in the methanol and methanol: chloroform extracts of *Tona dolium, Bursa rana and Natica marochiensis.* Band (Rf 0.79) indicates the presence of Proline in the methanol: chloroform extract of *Lambis lambis* and methanol: chloroform extracts of *Bursa rana.* Band (Rf 0.82) corresponding to Tryptophan is visible in methanol and methanol: chloroform extracts of all the four molluscan species except in the methanol extract of *Tona dolium*.

DISCUSSION

Proteins are linear, large complex molecules, heterogeneous polymers genetically mandated with 20 different building blocks of all living organisms, which residues linked by covalent peptide bonds (-CO-NH-) into the polypeptide chain(6). Protein is essential for normal function, growth and maintenance of body tissues. Its content is considered to be an important tool for the evaluation of physiological standards [7]. They are utilized to form various cell structures, of which they are key components and they serve as source of energy [8]. All amino acids have different role that helps body normal function and growth. The amino acid, tryptophan plays an important role in the brain as a precursor of the neurotransmitter, serotonin, which has a major effect on the feeding behavior of animals [9]. Valine is involved in many metabolic pathways and is considered indispensable for protein synthesis and optimal growth [10]. Histidine is also an indispensable amino acid involved in many metabolic functions including the production of histamines, which take part in allergic and inflammatory reactions. It plays a very important role in maintaining the osmoregulatory process and is related to energy production or is used in other metabolic pathways during certain emergencies/ harsh conditions [11]. Thus through this study we have indentified the amino acids present in the methanol and methanol: chloroform extracts

Lambis lambis, Tona dolium, Bursa rana and *Natica marochiensis* such as Cysteine HCl, Histidine, Aspartic acid, Asparagine, Glutamine, Hy-proline, Alanine, Methionine, Threonine, Glutamic acid, Tryptophan each having particular role. Further many compounds have been detected in flora forms than in fauna. Recently, HPTLC analysis of amino acids revealed higher levels of essential amino acids, such as phenylalanine, leucine, tyrosine, isoleucine, tryptophan, methionine, valine, threonine, arginine, histidine, lysine in female prawns when compared to the male prawns, *Macrobrachium rosenbergii* [12]. Plumbagin a useful antifertility agent was also detected from *Drosera burmannii* an insectivorous hairy herb by comparison with the reference standard using HPTLC [13]. Also, the different parts of the tree *Boswellia serrata* (*Linn.*) have been extracted with different solvents and thus HPTLC has been used for separation of different components [14]. Earlier, Erin Muller *et al*., used HPTLC analysis to analyze neutral lipids in the digestive gland-gonad (DGG) complex of the marine snails *Ilyassa obsoletus* and*Littorina littoria* infected with larval trematodes [15].

By the use of multiple solvent systems, one can verify the identification of different spots of the TLC plates [16]. Further Carol and Perscott [16] stated that several of the amino acid derivatives have identical migration rates in different solvent system. As stated above some amino acids like Aspartic acid, Asparaginine, Glutamine, Hy-proline (RF 0.42) and Alanine, Methionine, Threonine, Glutamic acid(Rf 0.52) have same migration point.(Fig.4. A,B) The presence of unidentified spots is not unaccepted because besides 22 standard amino acids, there are a vast number of non-standard amino acids present.

CONCLUSION

Although TLC is a conventional method, the HPTLC method is more practical, the separation and resolution are much better and the results are much more reliable and reproducible than TLC [17]. It is generally realized that for monitoring quality, HPTLC finger printing is ideal which involves comparison between standard and samples [3]. Carol & Prescott have made an attempt in analyzing free amino acids in marine and fresh water samples using conventional TLC method [16]. Furthermore, the colorful pictorial HPTLC image provides extra, intuitive visible color or fluorescence parameters for parallel assessment on the same plate. In the present study, the proposed HPTLC fingerprint method combined with digital scanning profiling was used for standardization. In this way HPTLC is feasible for the development of chromatographic finger print and to determine the amino acid composition of any samples in an effective manner.

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