Identification and Quantification of Amino Acids in Medicinal Plant Samples from Western Ghats, Maharashtra using HPTLC Technique

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Abstract

This study focuses on the identification and quantification of 10 different amino acids in seven medicinal plants from the Western Ghats of Maharashtra, utilizing the High-Performance Thin Layer Chromatographic (HPTLC) technique. Notably, methionine was absent in all plant samples analyzed. Among the studied plants, *Glochidion ellipticum* demonstrated significantly high concentrations of lysine, threonine, valine, and leucine compared to other species. In contrast, *Bombax malabaricum* was found to have the lowest amino acid content.

Key words: Amino Acid Profiling, Medicinal Plants, HPTLC Analysis

1.Introduction

Since ancient times, medicinal plants have been instrumental in treating a wide range of ailments[1]. In recent years, herbal medicines have gained considerable attention as alternative therapies for preventing and managing various disorders, despite the largely unknown mechanisms underlying their efficacy. This growing interest has driven extensive research aimed at analyzing medicinal plants and uncovering their potential health benefits. This growing interest has driven extensive research aimed at analyzing medicinal plants and uncovering their potential health benefits[2].The therapeutic properties of these plants are primarily attributed to their diverse chemical constituents, which exert significant physiological effects on the human body. Among these, bioactive compounds such as alkaloids, tannins, saponins, steroids, flavonoids, amino acids, and terpenoids are recognized for their potent antioxidant activities[3].

Amino acids are crucial in protein synthesis and serve as precursors for secondary metabolites. They also engage in numerous physiological processes, including skeletal muscle function, atrophic conditions, sarcopenia, cancer, cell signalling, homeostasis, gene expression, hormone

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synthesis, and protein phosphorylation, and possess antioxidant properties[4-7]Therapeutically, amino acids are employed in treating brain metabolism and neurotransmission imbalances, enhancing immune function, and supporting cardiovascular and gastrointestinal health[8] They are also used in managing liver diseases, fatigue, skeletal muscle damage, cancer prevention, burns, trauma, sepsis, maple syrup urine disease, and diabetes[9]. A survey of the literature reveals that there is limited data on the identification and quantification of amino acids in medicinal plants[10-12], hence the present work is undertaken to identify and quantify ammino acids in a few medicinal plants from western ghats, Maharashtra.

Various analytical methods have been developed for amino acid determination. Early methods by Moore and Stein[13] involved derivatization and detection in the visible region using an amino acid analyzer, which, while reliable, are costly and time-consuming[14].Chromatographic techniques such as GC-MS[15] RP-HPLC[16] HPLC[17], and capillary electrophoresis[18] are also employed but are similarly time-intensive.In comparison, High-Performance Thin-Layer Chromatography (HPTLC) offers simplicity; it allows for the analysis of multiple samples at a relatively low cost and does not necessitate extensive clean-up procedures of crude samples, even for quantitative analysis.HPTLC enables the examination of the same sample at different wavelengths, providing a comprehensive profile of the plant sample[19,20].Consequently, HPTLC is globally utilized for characterizing crude plant drugs, pharmacologically active components, and other formulations[21] .

2. Experimental

2.1 Selection of plants

The selected plants were collected form Mahabaleswar and Bhimashankar region of Western Ghats from Maharashtra and were authenticated at Agharkar Research Institute (ARI, MACS), Pune.

The study deals with the analysis of *Glochidion ellipticum* **(bhoma)**, *Aspidium cicutarium* **(Kombad nakhi)**, *Mimosa pudica* **(lazalu bee)**, *Bergenia legulata* **(pashan bhed)**, *Mesua ferrea Linn* **(Nag keshar)**, *Curculigo orchiodies* **(Kali Musli)** *and Bombax malabaricum* **(shalmali kanta).** The parts selected from these plants were leaves, rhizome, seeds, stem, anther, stem and thorns respectively.

The names of medicinal plants along with their use in medicinal plants in different treatments are mentioned below:

Bhoma-cancer, Kombad nakhi-toncils and bleeding, Lazalu-astingent, Pashan bhed-renal sone and diuretic, Nagkeshar-bleeding and inflammation, kali musali- tonic, shalmali kanta-anticoagulant and inflammation.

2.2 Preparation of plant extract

5 g of air died plant sample was placed in the extractor of the Soxhlet apparatus and extracted with ethanol by continuous extraction for 8h, the obtained solution was concentrated on rota vapour. It was then mixed with methanol and used for HPTLC analysis

2.3 Determination of Amino acids by HPTLC technique

Each amino acid standard was prepared at a concentration of 0.5 mg mL^{-1} in methanol and water (50:50). Ninhydrin reagent was prepared by dissolving 0.6g of ninhydrin in 15 mL of glacial acetic acid and diluting it with 285 mL of iso-propanol. This reagent was used for post derivatisation of amino acids.

2.3.1 Instrument and conditions

A Camag HPTLC system with an automatic TLC sampler (Camag Linomat 5), TLC scanner 3, with UV cabinet and twin trough was used for the analysis. TLC plates of Merck, India were used for analysis with specification of 20 x 10 cm long, pre- coated with silica gel 60 F254, thickness of 0.2 mm. 100 µL syringe was used for sample spotting. The automated sampler Camag Linomat 5 was set to apply the sample spots (bands) of 8 mm length at an interval of 4 mm between the two bands and at a height of 7 mm from the bottom of the plate. Camag glass twin trough chamber was used for plate development. The developed plate was scanned using Camag TLC scanner 3 with Win CAST 1.4.4.6337 software.

2.3.2 Plate development and derivatization

The standard solutions of amino acids were prepared in methanol and water (50 :50). 15 µL of plant sample and 2 µL of standard amino acid were applied on the HPTLC plate using the Linomat-5 semi autosampler and sampling syringe. HPTLC twin trough chamber (20 X 10 cm) was pre-saturated with 15 mL of mobile phase for 10 min. The samples and standard mixtures

on the plate were run simultaneously over a migration distance of 7.5 cm. The mobile phase 1-butanol: glacial acetic acid: water (4: 1: 1) was chosen after several preliminary experiments. After development, the plate was dried in the oven at 85 ^{o}C for 5 min. the plate was sprayed with ninhydrin reagent and again dried in the oven for 5 min. the coloured spots on the HPTLC plates were then scanned with CAMAG TLC scanner linked to Win CAST software.

3. Results and Discussion

Ten different amino acids in 7 different plants were estimated in the present work. Their role human body is presented in Table 1

3.1 Analysis of plants for amino acid content

The mobile phase used for analysis of amino acids was selected on basis of prior literature reports of similar separation system and trials. To achieve effective separation of amino acids, various combination of mobile phase like 4:3:1, 4:3:3, 4:1:1 and 4:2:2 (1- butanol: glacial acetic acid: water) were tried. It was found, that 4:1:1 mixture of 1- butanol: glacial acetic acid: water gave good separation, with symmetrical and reproducible peaks of standard amino acids. The HPTLC plates were visualized at 366 and 512 nm wavelengths as shown in the Fig.1 and Fig. 2 respectively. The Rf values of the standards and colour of zone are given in Table 2

The HPTLC chromatogram of 10 amino acid standards and plant samples at 512 nm are shown in the Figs. 3 and 4 respectively.

The quantification of the amino acids present in the plants under study are compiled in the Table 3, the results are with an accuracy of +/- 2-4%.

An examination of Table 3 reveals that all the plant samples show absence of Methionine whereas, Aspartic acid (2.1 mg/g) and Proline (1.36 mg/g) were found in *Aspidium cicutarium* only. *Glochidion ellipticum* was found to be rich in Lysine (4.9 mg/g) among all the plant samples studied. *Mimosa pudica* was the only plant under study which showed presence of Histidine (0.05 mg/g). The highest amount of Threonine was found in *Aspidium cicutarium* (1.18 mg/g) whereas, it is absent in *Mesua ferrea* and *Curculigo orchiodies*. *Glochidion ellipticum* (0.85 mg/g) showed the highest Valine content whereas *Mesus ferrea* (0.12 mg/g) showed the least content.

The highest concentration of Leucine was observed in *Glochidion ellipticum* (1.55 mg/g) and was found to be absent in *Mimosa pudica* and *Bombax malabaricum.* Serine was

found only in *Curculigo orchiodies* (0.41 mg/g) and *Mimosa pudica* (0.13 mg/g). *Bergenia legualata, Mesua ferrea* and *Curculigo orchiodies* showed the significantly high concentration of Alanine. *Meusa ferrea* (9.46 mg/g) showed the highest Alanine content. From the Table 2 it can be summarized that *Glochidion ellipticum* is found to be rich in Lysine, Threonine, Valine and Leucine with significantly high concentrations as compared to other plants studied whereas, *Bombax malabaricum* contains lowest concentration of amino acids Leucine and Threonine.

Conclusion:

HPTLC studies of different medicinal plants from Western Ghats of Maharashtra for various ammino acids estimation revealed that *Glochidion ellipticum* was found to be rich in Lysine, Threonine, Valine and Leucine with significantly high concentration as compared to other plants. Methionine was found to be absent in all the plants while *Bombax malabaricum* was found to contain the lowest amount of amino acids

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Figures:

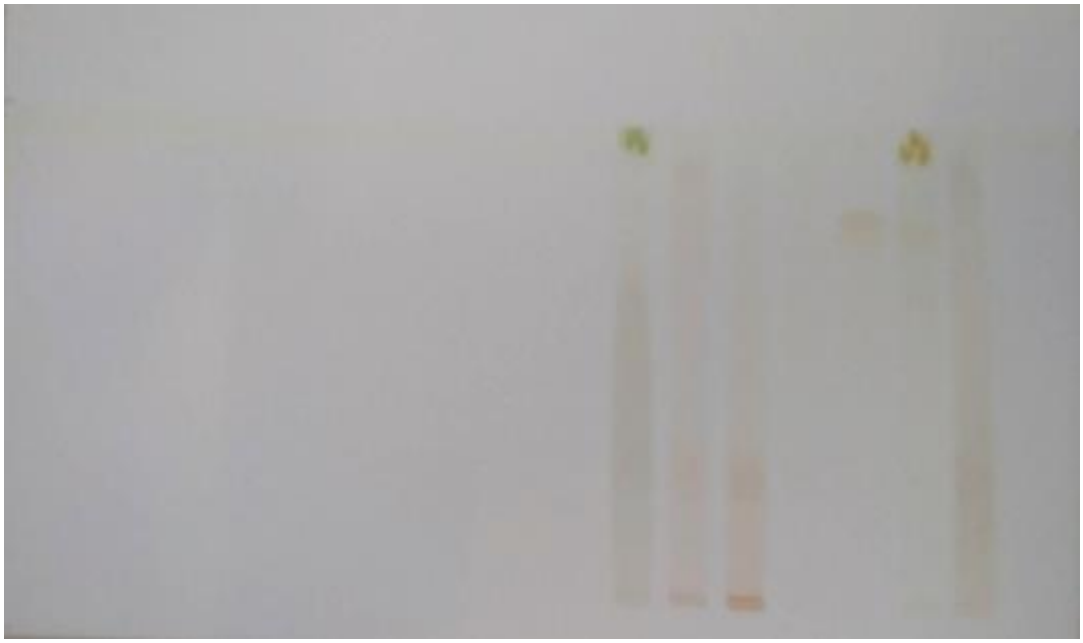

Fig.1 HPTLC plate with standards and plant samples before derivatization and visualized at 366 nm

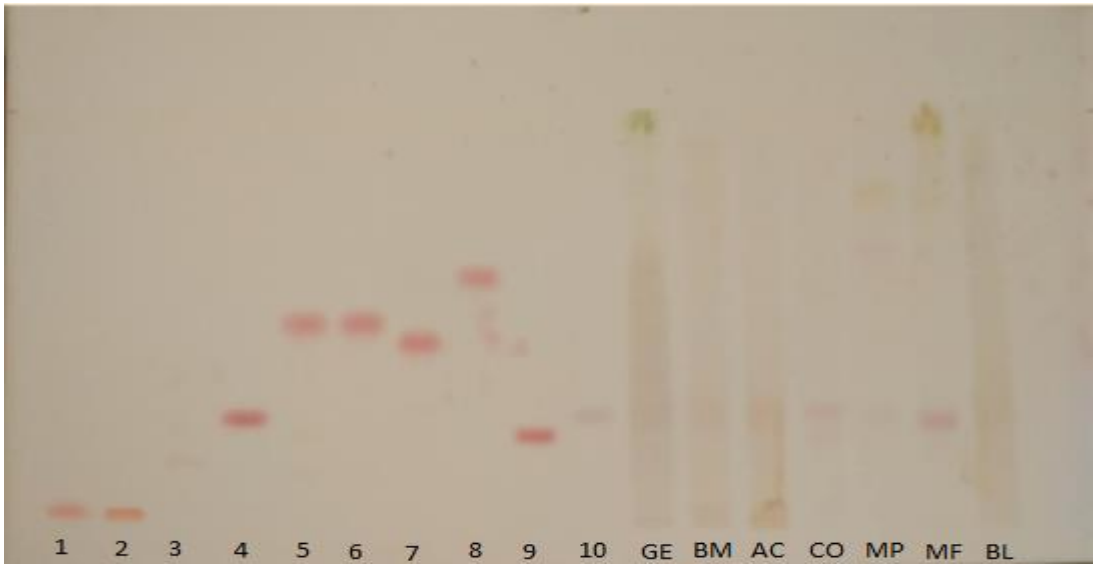

Fig. 2 HPTLC Plate with standards and plant sample after derivatization with ninhydrin and visualized at 366 nm

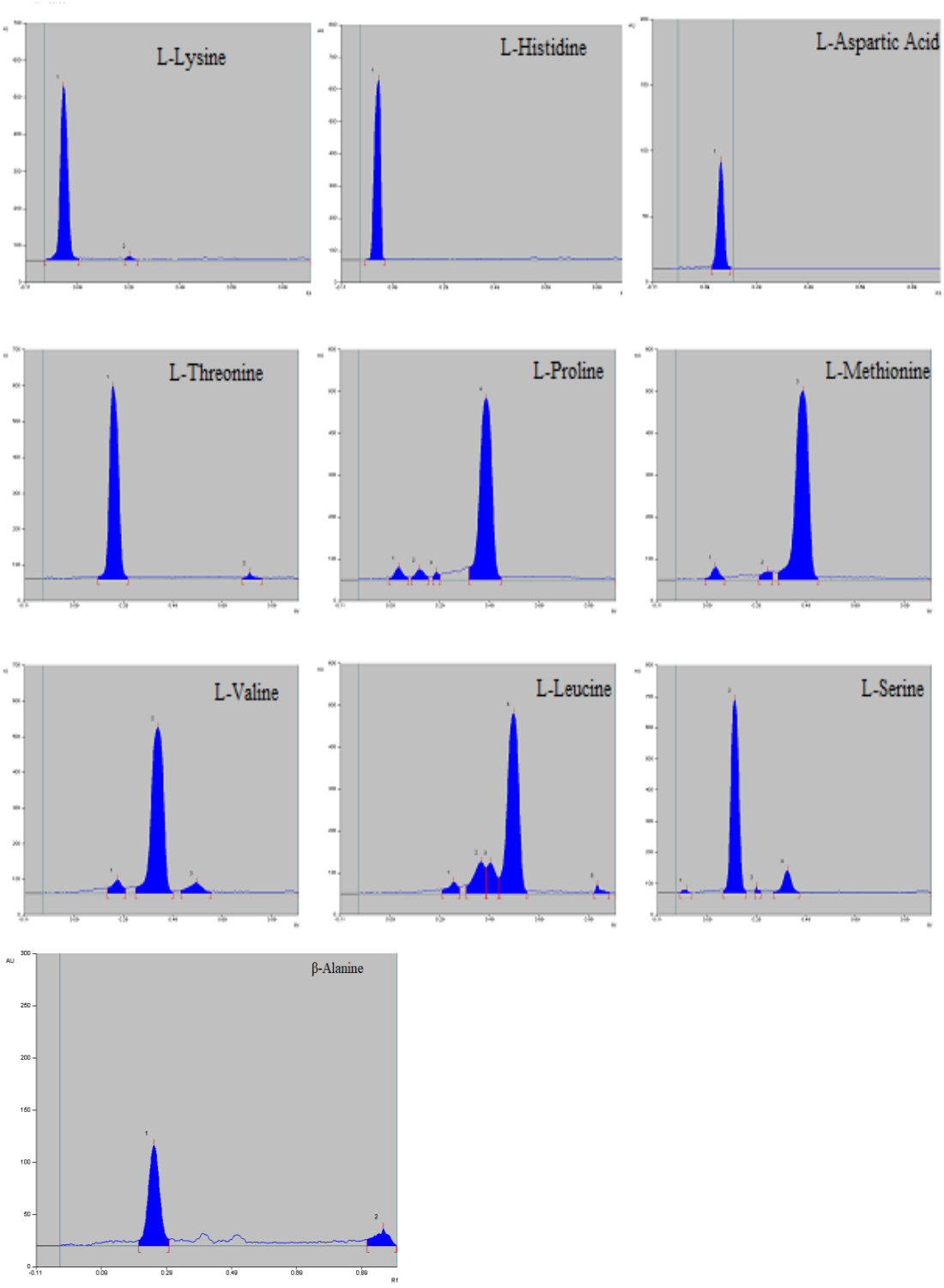

Fig.3 HPTLC chromatogram of standard Amino Acids

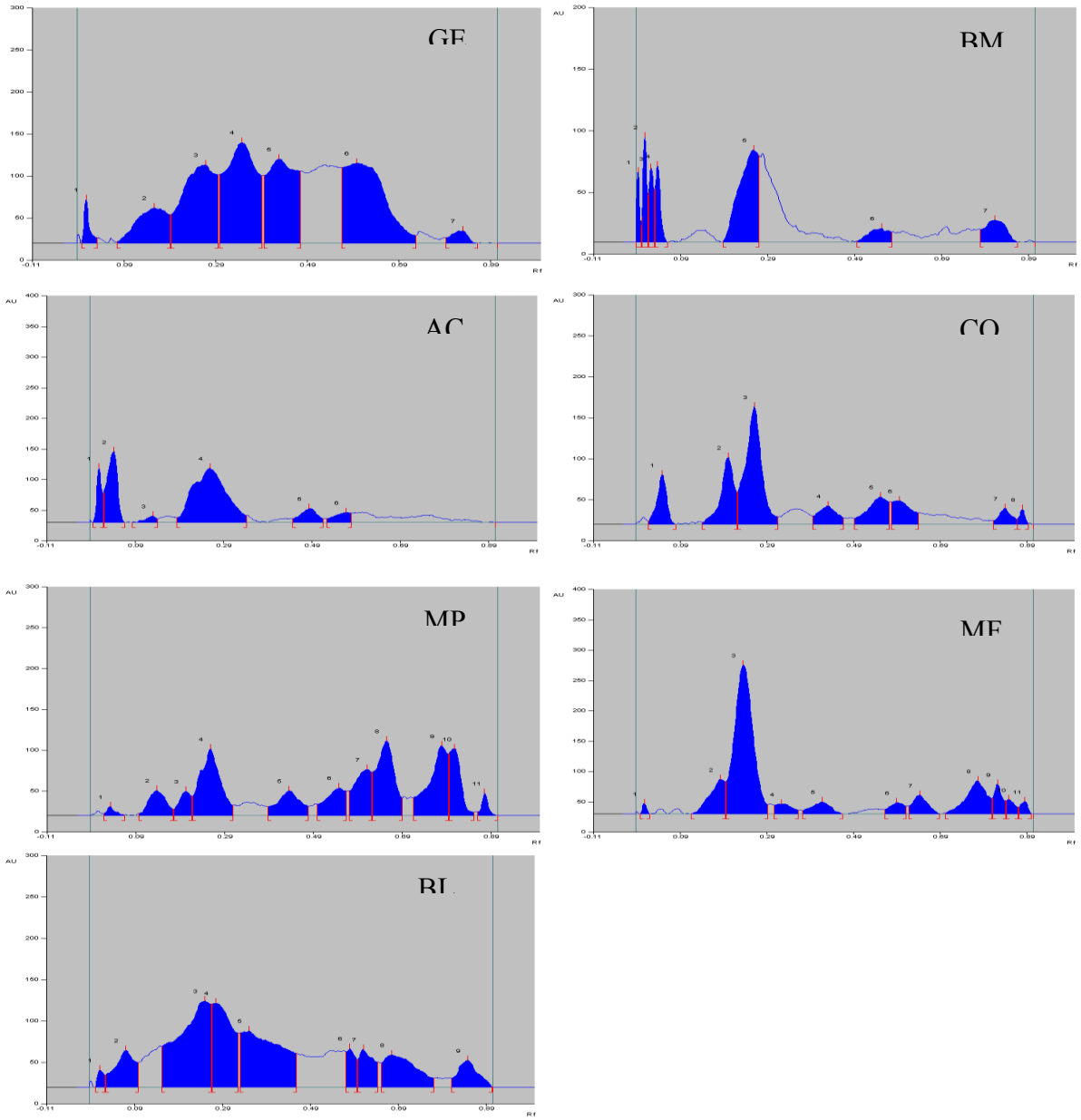

Fig. 4 HPTLC chromatogram of *Glochidion ellipticum (GE), Bombax malabaricum (BM),Aspidium cicutarium (AC),Curculigo orchiodies (CO),Mimosa pudica (MP),Mesua ferrea (MF), Bergenia legulata (BL)*

Tables:

Table 1: Role of Amino Acids in human body

Sr. No	Amino acid	Role in human body
1	Alanine	Helps in metabolism of sugar and organic acids, enhances immunity and gives energy to the brain and central nervous system.
2	Aspartic acid	Helps in proper functioning of RNA and DNA, associated synthesis of immunoglobulin and antibody.
3	Histidine	It regulates the uptake of metals like Fe, Cu, Mo, Zn and Mn. It also helps in formation of metal containing enzymes.
4	Leucine	It helps in regulating blood sugar levels, stimulates growth of bone tissues and muscles and formation of growth hormones.
5	Lysine	Responsible for Ca uptake, help in building muscle protein and formation of collagen.
6	Methionine	Helps in single carbon transfer and detoxification of liver
7	Proline	Helps in maintaining strength of heart muscle, proper functioning of joints and tendons
8	Serine	Important functioning of brain, CNS and maintaining overall physical and mental health of body
9	Theronine	Supports in smooth functioning of digestive system and liver metabolism. It is part of elastin, collagen, and enamel protein.
10	Valine	Responsible for smooth functioning of nervous system, muscles, immune system and cognitive

Table 2: Rf values of standard amino acids and colour of zone detected with Ninhydrin reagent

Amino Acid	Rf value	Colour
L- Lysine	0.04	Yellow
L- Histidine	0.03	Brown
L-Aspartic Acid	0.15	Pale Violet
L-Threonine	0.26	Brown
L- proline	0.48	Brown
L-Methionine	0.45	Brown
L-Valine	0.43	Brown

L-Leucine	0.59	Brown
L-Serine	0.20	Brown
β-Alanine	0.25	Violet

Table 3: Concentration of amino acids in plant samples under study

Amino Acids Concentration (mg/g)										
Plant Name	Lysine	Histidine	Aspartic acid	Threonine	Proline	Methionine	Valine	Leucine	Serine	Alanine
Glochidion ellipticum	4.90	ND	ND	1.17	ND	ND	0.85	1.55	ND	ND
Aspidium cicutarium	1.23	ND	2.1	1.18	1.36	ND	ND	0.11	ND	ND
Mimosa pudica	ND	0.05	ND	0.65	ND	ND	0.21	ND	0.13	ND
Bergenia legulata	ND	ND	ND	0.81	ND	ND	ND	0.17	ND	ND
Mesua ferrea	ND	ND	ND	ND	ND	ND	0.12	0.11	ND	9.46
Curculigo orchiodies	0.62	ND	ND	ND	ND	ND	0.13	0.23	0.41	4.77
Bombax malabaricum	0.32	ND	ND	0.58	ND	ND	ND	ND	ND	ND

ND = Not Detected

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