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Estimation and Detection of Free Amino Acids from *Ficus benghalensis* Linn.

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Detection of amino acids from various extracts of *Ficus benghalensis* was carried out by using different mobile phases and different chromatographic techniques. The results obtained were compared with standard amino acids in the respective mobile phases. The distribution of free amino acids in aerial roots and leaves of *Ficus benghalensis* showed the presence of some common amino acids. Aerial roots showed the presence of nine amino acids and the leaves showed presence of eight amino acids by paper chromatography. Eight amino acids in the roots and ten amino acids in the leaves were detected by performing silica gel coated aluminium TLC plate. The estimation of proteins from different extracts of the plant was also carried out spectrophotometrically.

Key Words: *Ficus benghalensis*, Proteins, BCA method, Amino acids, BSA protein.

INTRODUCTION

Study of plant species for different medicinal resources is creating an increasing impact on today's era. The rapid development of different analytical techniques in recent years has enabled investigators to tackle some of the most challenging and fundamental problems in plant study and herbal medicine.

In India, *Ficus benghalensis* is commonly called as Banyan[1]. It is a very large tree up to 30 m in height with widely spreading branches bearing many aerial roots functioning as prop roots. Bark is greenish white; leaves are simple, alternate, often in clusters at the ends of branches. The fruits are small, crustaceous achenes, enclosed in the common fleshy receptacles[2]. Some phloem cells contain prismatic calcium oxalate crystals[3].

Literature survey reveals no reports on investigation of the amino acid pattern from aerial roots of *Ficus benghalensis*. Considering the lacuna, investigation of amino acids from aerial roots and leaves has been carried out.

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EXPERIMENTAL

Whattmann filter paper no. 1 for paper chromatography and precoated silica gel and aluminium plates for TLC technique were used. Chilled PBS (phosphate buffer saline), Lysis buffer (Dignam buffer C), BCA (bicinchoninic acid) kit, Albumin fraction V, BSA (bovine serum albumin) protein, DMEM solution (Dulbecco's modified eagles medium), 96-well plate, Elisa plate reader were used for protein estimation.

Growing aerial roots and leaves of *Ficus benghalensis* were collected, shade-dried and powdered. Three extracts were prepared by using weighed quantity of powder in known volume of ethanol, methanol and water. Repeated extractions of these extracts were carried out using chloroform to remove chlorophyll. The chloroform layer was separated and the remaining part was used for amino acid analysis. Chromatographic paper and precoated aluminium plates were used[4].

Two new mobile phases are used in paper chromatography technique for amino acid estimation.

Phase 1: phenol : water (1 : 1)

Phase 2: hexane : *n*-butanol : methanol : acetic acid : water (0.5 : 3.0 : 1.0 : 2 : 3.5)

Aerial roots and leaves comprise of different amino acids in different phases (Tables 1 and 2).

TABLE-1
AMINO ACIDS DETECTED IN THE PHASE PHENOL : WATER (1 : 1)

Name of amino acids	R_f for standard amino acids	R_f for amino acids of the roots	R_f for amino acids of the leaves
Alanine	0.167	0.161	—
Aspartic acid	0.374	0.387	—
Cysteine	0.070	0.064	—
Glutamic acid	0.103	0.096	—
Methionine	0.810	0.822	0.790
Ornithine	0.193	0.187	—
Proline	0.720	0.714	0.690
Threonine	0.451	0.477	0.433
Glycine	0.560	0.580	0.590

Precoated TLC chromatogram was carried out by developing in different mobile phases. The TLC plate was double run using first mobile phase as:

acetone : *n*-butanol : acetic acid : distilled water (10.5 : 10.5 : 3.0 : 6.0).

After the first run, the plate was dried at room temperature and kept for second run by adding 2.40 mL of ninhydrin solution in 21.0 mL of the above phase.

Preparation of ninhydrin solution: It was prepared by mixing 1.75 g of ninhydrin to colliding, acetone and *n*-butanol as 0.75 : 12.5 : 12.5 mL respectively.

Aerial roots and leaves elucidate typical pattern for amino acids (Table-3).

TABLE- 2
AMINO ACIDS DETECTED IN THE PHASE HEXANE : N-BUTANOL : METHANOL : ACETIC ACID : WATER (0.5 : 3 : 1 : 2 : 3.5)

Name of amino acids	R_f for standard amino acids	R_f for amino acids of the roots	R_f for amino acids of the leaves
Alanine	0.300	0.311	—
Aspartic acid	0.122	0.120	0.123
Arginine	0.360	0.380	—
Glutamic acid	0.600	0.620	0.610
Threonine	0.650	0.650	0.710
Histidine	0.500	—	0.412

TABLE-3
SEMI-QUANTITATIVE ESTIMATION OF AMINO ACIDS BY TLC

Name of amino acid	Quantity in roots (μg/mL)	Quantity in leaves (μg/mL)
Glutamine	050.0	200.0
Glycine	020.0	050.0
Glutamic acid	200.0	100.0
Alanine	100.0	030.0
Proline	150.0	010.0
Leucine	010.0	010.0
Tyrosine	050.0	200.0

Bicinchoninic acid (BCA) Assay for Total Protein Content: Measurement of protein synthesis *in vitro* has been used as a basic description of toxicity. BCA assay is the faster and easier method to determine the total protein content. This assay is based on the reduction of Cu^{2+} to Cu^{1+} by protein at an alkaline pH 9. Two BCA molecules chelate the Cu^{1+} ion to form a purple coloured complex, which can be quantified spectrophotometrically[5].

2 mg/mL standard BSA protein solution was prepared using mili Q water. This standard was added in 96-well plate in an order of 0, 2, 4, 6, 8 and 10 μL each and blank form also done. The volume of each well was made up to 10 μL using mili Q water. Two wells were prepared for carrying out blank reading, one for water and another for coloured DMEM solution. 10 μL of each extract was added in each well. In each well 200 μL of the reagent was added with the help of multiple micropipettes. The 96-well plate was incubated at 37°C for 30 min. The absorption of the developed colour was measured at 562 nm on Elisa plate reader and protein concentration of the extracts was determined (Table-4).

TABLE 4
PROTEIN ESTIMATION OF DIFFERENT EXTRACTS FROM *FICUS BENGHALENSIS*

Extract	Conc. of std. BSA (μg/μL)	Average absorbance	Average absorbance of extracts	Conc. of protein in extract (μg/μL)
Methanol-roots	800	0.526	1.284	2415.7
Water-roots	1200	0.703	0.618	1169.2
Ethanol-roots	1600	0.854	0.913	1717.0
Water- leaves	2000	0.929	1.025	1926.7

RESULTS AND DISCUSSION

In paper chromatography phase one, leaves show presence of Met, Pro, Thr, Gly while in roots, apart from these Ala, Cys, Asp, Glu and Orn are noticed. Phase two in addition to phase one elucidates Asp, Glu and His in leaves and Arg in roots. The present investigation indicates that aspartic and glutamic acids are present in both extracts of roots and leaves.

The results obtained from TLC study of the aerial roots show the presence of cysteine, lysine, glutamine, glutamic acid, glycine, alanine, tyrosine and proline. In leaves along with these methionine and leucine are present in very minute amounts. In case of roots, glutamic acid, proline and alanine are found in major amount as compared to the other amino acids. In case of leaves glutamine and tyrosine are present in the same concentration (200 μg/mL) while glutamic acid (100 μg/mL) explains higher concentration as compared to other amino acids.

Protein estimation data reveal the presence of 1717, 2415 and 1169 μg/mL of proteins in ethanol, methanol and water extracts of roots respectively. Water extract of leaves makes clear the appearance of 1962 μg/mL of proteins.

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