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Spectrophotometric and Chromatographic Analysis of Amino Acids present in Leaves of *Ailanthus excelsa*

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Abstract: *Ailanthus excelsa* leaves was analyzed for Amino acid contents. The dried leaves were grounded in the pastel and mortar; ethanol was added, filtered and centrifuged. The qualitative Amino acids were determined by paper chromatography. The chromatogram was developed with mobile phase n-butanol: acetic acid: water (80:20:10). The each spot of amino acids was visualized by spraying agent 0.1% Ninhydrin solution in Acetone. Three amino acids were identified by paper chromatography. The quantitative analysis of Amino acids was carried out by Spectroscopic technique. Detection was carried out at 570 nm.
Keywords: *Ailanthus excelsa*, Amino acid, Spectrophotometer**,** Paper chromatography.

1. Introduction

Recently, in India several scientists have reported the therapeutic importance of the chemical constituents of plants used in ancient Indian medical system. Mutalic [1] paper on Research Needs and Traditional Medicine in South East Asia Region has emphasized for research in traditional medicine. *Ailanthus excelsa* of the Acanthaceae family has been used in cough, asthma , bronchitis, tuberculosis, inflammation and allergy [2-5]. Several active constituents have also been isolated from different parts of *Ailanthus excelsa* [6]. Though the plant is used in the treatment of jaundice in Bengal, more evidence is needed to substantiate its pharmacological effects. From preliminary phytochemical analysis it was found that the extract showed positive response for the presence of flavanoids, tannins, alkaloids, reducing sugars and saponins [7].

Amino acids are compounds containing one or more amino groups and one or more carboxylic acid groups.[8] They are of great biological importance since they play an important role as growth promoting factors and in nitrogen metabolism.[9] Some of the amino acids are supported to act as precursors of some secondary metabolites like alkaloids etc. They occur in plants both in free state and basic units of proteins and other metabolites. It is a common convention to group plant amino acid in to protein and non protein constituent. These so called protein amino acid also occur as free amino acids.[10,11] Many free amino acids have been isolated from plant sources, but only a few of them occur on a wider scale. Examples are amino-butyric acid, aminoadipic acid, pipecolic acid and acetylornithine.[12]

Generally, 70 to 80 % alcohol extract of the test plant was prepred and used for the estimation of free amino acids. Ninhydrin is the standard reagent was most widely used for the detection of amino acid or nitrogen containing compounds. If the normal method is employed for the development; it imparts yellow colour to proline and hydroxyproline and violet colour

to all other amino acids. Report are availabe on the use of the other spray reagents such as alkaline bromothymol blue solution containing formaldehyde, aromatic aldehyde, isation and Folin's reagent for detection of amino acid.[13]

1.1-Separation of Amino acids

Among the different seperating methods, paper chromatography is comparatively convenient and gives good seperation. It is considered [14,15] useful for qualitative and rough quantitative work. Workers have employed different types of Paper Chromatography technique. Consden and co workers[16] have used descending paper chromatography. Williams and Kirby[17] have employed ascending circular of horizontal chromatography. Paper chromatography has also been advocated as a useful technique, especially seperation of amino acids running very close to each other in one solvent.

In this study, circular paper chromatography was used to seperate and identifies amino acid content in *Ailanthus excelsa*.

2. Experimental

2.1-Extraction of Amino acids

Alcoholic extract containing crushed *Ailanthus excelsa* powder was filtered and concentrated at room temperature for 2 hours.

2.2-Paper chromatography of Amino acids

The extract was spotted on whatman No.1 chromatographic grade filter paper along with standardized mixture of known amino acids on the same chromatogram. The chromatogram were developed using n-butanol : acetic acid : water (80:20:10). The spot of amino acids were visulised by spraying with Ninhydrin and were identified by their characteristic colour and comparison of R_f value with those of aunthetic samples.

2.3-Determination of free amino acids content by spectroscopic technique

2.3.1-Instrumentation

The UV-Visible double beam spectrophotometer with matched quartz cells (1cm), Model: PharmaSpec1700, Make: Shimadzu Corporation, Kyoto, Japan.

2.3.2-Procedure

Two gm of the crushed *Ailanthus excelsa* powder was weighed and ground in a pastel and mortar with a small quantity of washed sand. To these homogenate 5 to10 ml of ethanol (80%) was added, filtered and centrifuged. The extract was used for the quantitative estimation of total free amino acids.

1. To 0.2, 0.4, 0.6, 0.8, 1.0 ml of extract 1.0 ml ninhydrin solution was added.
2. 2 ml of distilled water Contents test tube were heated in boiling water bath for 20 minute to which 5 ml of the 80% ethanol was added and contents were mixed.
3. After 15 min the intensity of the purple colour against a reagent blank on a spectrophotometer at 570 nm was recorded. The colour is stable for 1 hour.
4. The reagent blank as above by taking 0.1 ml of 80% ethanol instead of the extract was prepared.

3. Construction of a calibration curve

50 mg Leucine in 50 ml distilled water was dissolved in volumateric flask. 10 ml of this stock standard was taken and diluted to 100 ml in another volumetic flask for working standard solution. Procedure for the sample was performed and O.D. was measured. Standard curve using absorbance versus concentration was drawn to find out the total free amino acids in the sample and to express as percentage equivalent of leucine in given sample.

4. Results and Discussion

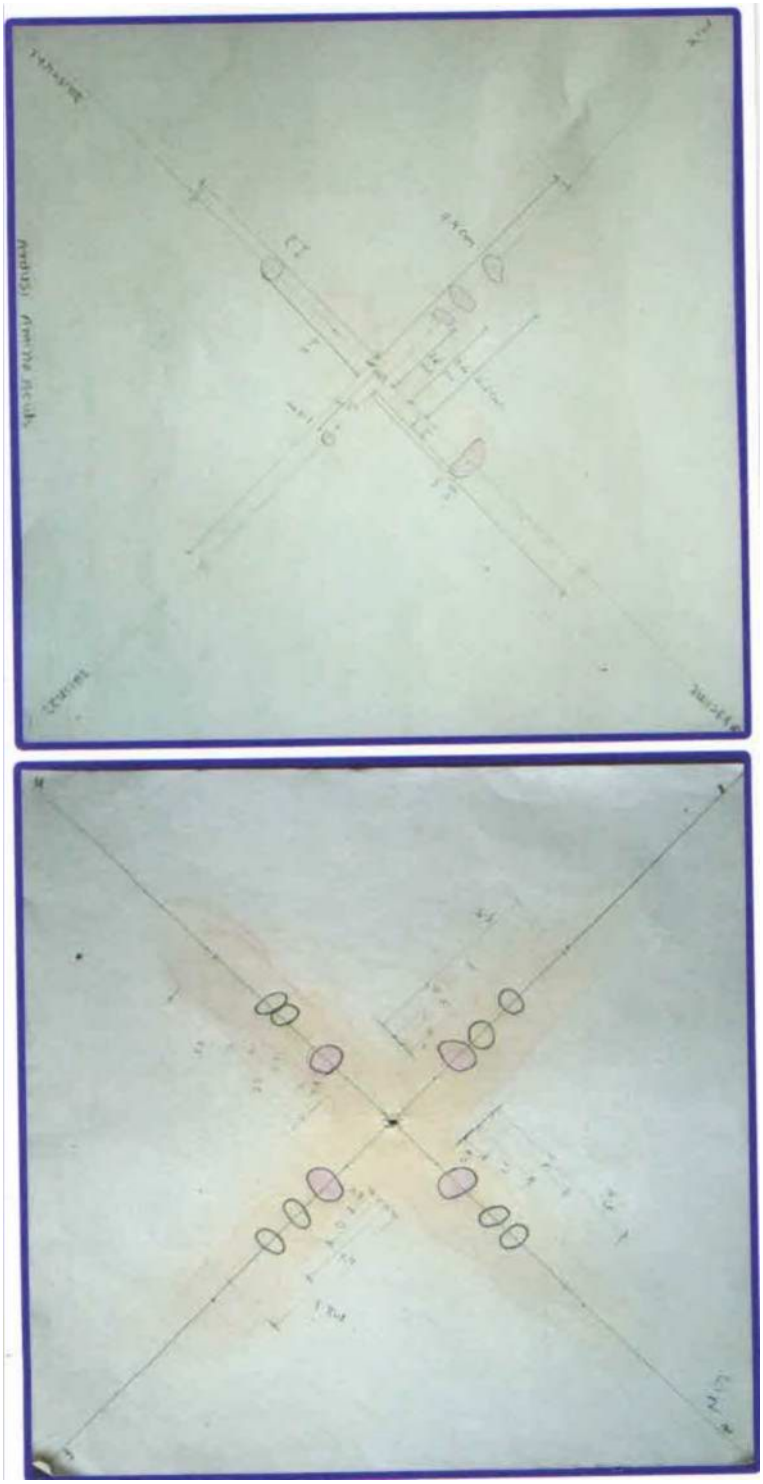
4.1 Paper chromatography of Amino acid

Three amino acids have been isolated from of *Ailanthus excelsa* by the paper chromatographic method. Amino acids have been identified by their characteristic colors, reported R_f values and by paper chromatography with authentic sample. The result are recorded in Table no.1 and shown in chromatogram. The amino acids found to be present in alcoholic extract of *Ailanthus excelsa* are Leucine, Glycine, and Tyrosine.

Table-1 R_f values of Amino acids of *Ailanthus excelsa*

NO.	R_f value	Color	Amino acid
1.	0.42	violet	Glycine
2.	0.31	violet	Leucine
3.	0.56	violet	Tyrosine

Figure-1: Photograph showing paper chromatography of amino acid from methanol extract constituents of Ailanthus excelsa



Solvent system: - n-Butenol: Acetic Acid: Water [4:1:5]
Spraying Agent: - 0.1% Ninhydrin

4.2 Spectroscopic technique of Amino acid

Three amino acids were identifying by paper chromatography, which are Leucine, Glycine, and Tyrosine in the sample of leaves of *Ailanthus excelsa*. The total free amino acid in sample was quantitatively identified by spectroscopic technique using the lucine as the standard sample. In spectroscopic technique, result shows that the 223 mg free amino acids found in 100 gm of sample of leaves of *Ailanthus excelsa*.

Table-2 Standard Lucine Curve

Sr. No.	Concentration(ml)	Absorbance
1	0.2	0.05
2	0.4	0.127
3	0.6	0.232
4	0.8	0.315
5	1	0.507

Figure-2 Standard Lucine Curve

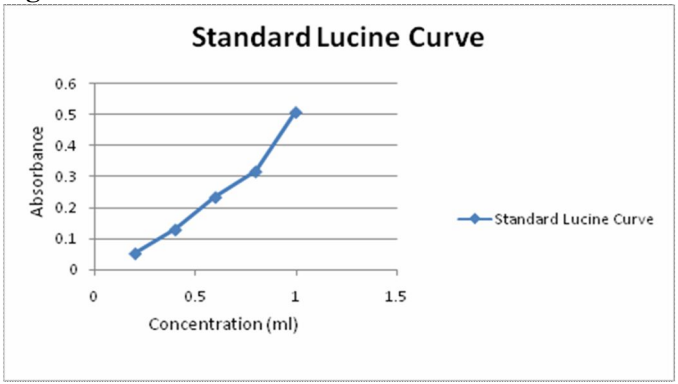
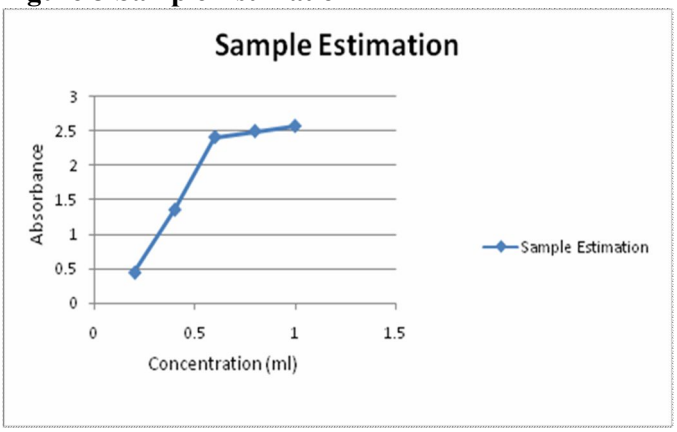


Table-3 Sample Estimation

Sr. No.	Concentration(ml)	Absorbance
1	0.2	0.447
2	0.4	1.355
3	0.6	2.403
4	0.8	2.489
5	1	2.563

Figure-3 Sample Estimation



5. Conclusion

By using routine paper chromatography technique and the spectrophotometric technique to identified the amino acids by qualitatively and quantitatively. Simple analytical paper chromatography technique gives the good separation of amino acid using proper mobile phase. The total amount of amino acids present in the sample extract from the leaves was identified by the simple spectrophotometric technique.

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