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QUALITATIVE ANALYSIS OF AMINO ACIDS FROM THE SEEDS OF MORINGA OLEIFERA USING CHROMATOGRAPHIC TECHNIQUE.

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ABSTRACT: Moringa oleifera, often known as drumstick, is a well-known medicinal plant in Maharashtra that was chosen for chemical analysis in this study. Traditional medicine uses the bark, sap, roots, leaves, seeds, and flowers.

Its potential effects on insulin secretion and blood lipid profiles have been studied[12]. Different polyphenols found in leaf extracts are the subject of fundamental research to ascertain their possible effects on people.

No high-quality evidence has been found to suggest that Moringa has any effect on health or diseases, despite extensive exploratory research to establish whether its components have bioactive qualities[5]. Qualitative assays of amino acids were also investigated[6]. For qualitative analysis of seeds of Moringa Oleifera different chemical reagents are used.

Key words: Sap, Bark, Root, Leaves, Flowers, Seeds, Qualitative, Polyphenols, Chemical reagents

Introduction:

Native to Northern India's sub-Himalayan highlands, Moringa oleifera, often known as the miracle tree, horse radish tree, or drumstick tree, belongs to the Moringaceae family and is widely grown in tropical and subtropical climates[1]. Numerous nations, including India, the Philippines, Myanmar, and some regions of Africa, employ various plant components as wholesome food commodities[4].

Because of their enormous nutraceutical potential, research on Moringa has traditionally focused on its leaves and seeds. However, in recent years, there has also been an increased interest in flowers, mostly due to the encouraging results of a number of pharmacognostical investigations on flowers.

As, mature seeds have a high concentration of behenic acid, they produce 38–40% edible oil known as ben oil[14]. The refined oil is resistant to rancidity and is transparent and odourless. It is possible to boil the young

fruits and remove the oil by skimming it off the water's surface. Following oil extraction, the seed cake that is left over can be utilized as a flocculent to clean water or as fertilizer[13]. Another potential application for Moringa seed oil is as a biofuel[10].

Fig: 1 M. Oleifera plant

Fig: 2 M. Oleifera Seeds

I. Botanical information of the *Moringa oleifera*

Family name	:	Moringaceae
Botanical name	:	Moringa oleifera
Genus	:	Moringa
Species	:	Oleifera
Part used	:	Seeds, Flowers
Medicinal uses	:	Blood glucose regulation, Anaemia, Asthma, Diabetes, Liver, Cholesterol, Improves sleep, Inflammation, Astonic, Anticancer, Di-uretic, Antifungal, Anti-helminthic, Antibacterial, Anti Inflammatory, Antilarval, Antioxidant.

II. Extraction of Amino Acids in Seeds of *M. Oleifera*

a) Preparation of Sample Solution

After adding 50 g of M. oleifera seeds and 150 ml of 97% ethanol, the mixture was cooked on a water bath for 30 to 50 minutes. It was then centrifuged, cooled, and filtered. The residue was twice or three times washed with ethanol after the supernatant was decanted into a test tube. Heating on a water bath at 40–50 degrees Celsius reduces the volume of filtrate. Amino acid qualitative analyses were performed on the final sample.

III. Qualitative Tests of Amino Acids in Seeds of *M. Oleifera*

i) Xanthoproteic Reaction

In the ice bath, 1 ml of sample solution and 1 ml of HNO_3 were combined. The presence of amino acids (glycine, tyrosine, tryptophan, and arginine) was indicated by the sample solution turning yellow.

ii) Ninhydrin Reaction

1 ml of the ninhydrin reagent was added to 1 ml of the sample solution in a test tube. The presence of amino acids was suggested by the purple color that was seen.

iii) Lead Sulphide Test

2 ml of 40% NaOH solution and 1 ml of the sample solution were heated for two minutes. After cooling, 0.5 ml of sodium pulmbate solution was added to this solution. There was black precipitate. It shows that the sample solution contains the amino acid cystine.

iv) Glyoxylic Reaction

Glyoxylic acid and 2 ml of sample solution were combined, and then 2 ml of H_2SO_4 was gradually added along the test tube's side. The presence of the amino acid tryptophan was revealed by the appearance of a violet ring.

v) Sakaguchi Test

1 ml of 40% NaOH solution, two drops of 1% alcoholic naphthol, and a few drops of bromine water solution were combined with 3 ml of the sample solution. The development of a red hue suggested the presence of arginine.

IV. Qualitative Estimation of Amino Acids in Seeds of M. Oleifera. by Two Dimensional Paper Chromatography Method .

The two-dimensional ascending Paper Chromatography method was used to assess the amino acid content of the chosen sample. The stationary phase for paper chromatography was Whatmann No. 1 chromatographic paper.

A 4:1:5 (v/v) ratios of n-butanol, acetic acid, and water was the initial solvent system. The second solvent system had a 4:1 (v/v) ratio of phenol to water. The reagent ninhydrin was used as the color developer. Using a basis line, origins were made in the lower left corner of a square sheet of Whatmann No. 1 chromatographic paper (18 x 18 cm) at the intersection of two lines that were 2 cm from the two corners of the sheet.

A capillary tube was used to spot the sample solution (an ethanolic extract of the sample) on the baseline, and a drier was used to desiccate it. The chromatographic tank was used to develop the paper in the first dimension using the ascending technique with a 4:1:5 (v/v) ratio of n-butanol, acetic acid, and water. The chromatographic paper was removed and allowed to dry after seven hours.

Phenol: water, 4:1 (v/v), was used to produce the second dimension of the dry paper while it was twisted at a right angle. Following the solvent system's operation, the paper was removed and allowed to air dry overnight. The ninhydrin spray reagent was then used to find the amino acid sites.

V. RESULT & DISCUSSION : QUALITATIVE TESTS OF AMINO ACIDS IN SEEDS OF M. OLEIFERA.

The qualitative tests of amino acids in seeds of M. Oleifera. were examined and the results are shown in table below.

Table 1 Qualitative Tests of Amino Acids in Seeds of M. Oleifera.

Sr. No.	Test	Observations	Conclusion
1.	Xanthoproteic Reaction	Yellow color	Glycine, Tryptophan and Tyrosine is Present
2.	Ninhydrin Reaction	Purple color	Amino acids are Present
3.	Glyoxylic Reaction	Violet ring	Tryptophan is present
4.	Sakaguchi Test	Red color	Arginine is present
5.	Lead sulphide Test	Black colour precipitate	Cystine is present

VI. ONE DIMENSIONAL METHOD OF PAPER CHROMATOGRAPHY FOR SEPARATION AND IDENTIFICATION OF AMINO ACIDS.

Using the solvent system n-butanol: acetic acid: water, 4: 1: 5 (v/v), the amino acids in M. Oleifera seeds were separated using the one-dimensional paper chromatography method and compared to standard amino acids

VII. SEPARATION AND IDENTIFICATION OF AMINO ACIDS COMPOSITION BY THIN LAYER CHROMATOGRAPHY METHOD

Using an aluminium pre-coated silica gel plate (Merk Co. Inc., Kieselgel F256) and the solvent system n-butanol: acetic acid: water, 4: 1: 5 (v/v), the amino acids in M. Oleifera seeds were separated using the Thin Layer Chromatography method and compared to standard amino acids.

VIII. QUANTITATIVE DETERMINATION OF AMINO ACIDS CONTENT IN SEEDS OF M. OLEIFERA.

The ninhydrin assay method and two-dimensional paper chromatography were used to quantitatively assess the amino acid content of M. Oleifera seeds.

Two-dimensional paper chromatography was used to extract the sample (100 µl) using a first eluting solvent system with n-butanol: acetic acid: water, 4:1 (v/v) and a second solvent system with phenol: water, 4:1 (v/v). After air drying, the paper sheet was sprayed with a 0.2% ninhydrin reagent. After that, it was placed in an oven set to 100°C for two minutes. The amino acid dots turned purple on the sheet. The Rf values of typical amino acids were compared to the measured Rf values of purple spots. Using a UV visible spectrophotometer, clear six-color spots were cut and eluted with methanol to measure absorbance. Using the ninhydrin and ethylenediamine tetra acetic acid (EDTA) reagents, the absorbance values of six amino acids in the sample solution were determined at 570 nm.

The absorbance values of the six amino acids—Arginine, Aspartic acid, Glycine, Lysine, Threonine, and Valine - measured by the afore mentioned method were used to create the standard curves for these amino acids. Standard amino acid curves were used to determine the percentages of amino acids in M. Oleifera seeds.

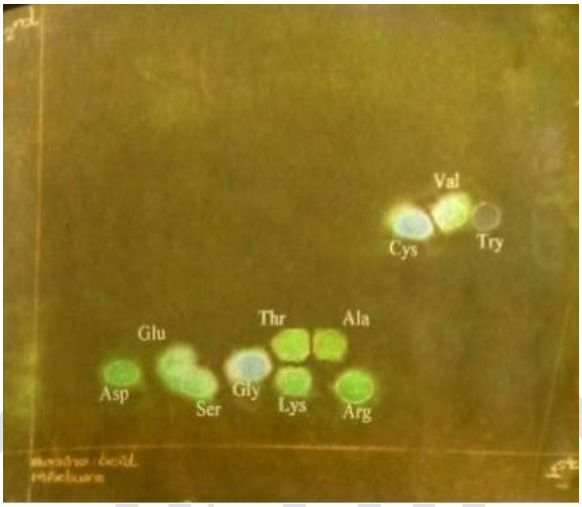

Fig: 3 Amino acids mixture by two dimensional paper chromatography method

IX. RESULTS AND DISCUSSION

Solvent 1= n-butanol: acetic acid: water, 3.5:0.5:4. 5 (v/v)
solvent 2= phenol: water, 3E4:1(v/v)

The amino acids content in seeds of M. Oleifera was evaluated by two dimensional ascending Paper Chromatography techniques. It follows that eleven amino acids—glutamic acid, aspartic acid, glycine, serine, lycine, threonine, arginine, alanine, valine, cystine, and tryptophan—are present in the ethanol extract of M. Oleifera seeds. The two-dimensional Paper Chromatogram of an eleven-amino acid mixture was used to identify these amino acids.

The table below provides a description of the two-dimensional Paper Chromatogram results and Rf values.

Table 2: Rf Values of Standard Amino Acids and Sample Solution of the Seeds of M.Oleifera

Amino Acid	Sample Solution		Standard Amino Acids	
	Solvent -1	Solvent -2	Solvent -1	Solvent -2
Aspartic acid	0.14	0.16	0.13	0.16
Glycine	0.15	0.38	0.14	0.36
Alanine	0.20	0.53	0.22	0.54
Arginine	0.10	0.60	0.11	0.61
Cystine	0.44	0.71	0.45	0.68
Valine	0.45	0.73	0.44	0.75
Glutamic acid	0.14	0.23	0.14	0.24
Threonine	0.20	0.48	0.21	0.49
Tryptophan	0.45	0.80	0.47	0.82
Lycine	0.09	0.46	0.09	0.45
Serine	0.08	0.34	0.10	0.32

X. CONCLUSION

The results indicate that plants are a rich source of different phyto-constituents with a range of pharmacological characteristics. Calcium, iron, potassium, magnesium, manganese, and zinc are among the essential minerals found in moringa. The M. Oleifera seeds had the greatest potassium level (255 ppm)[19]. This fact confirmed that M. Oleifera seeds have hypertension or blood pressure-lowering effects and function as circulatory enhancers. Numerous essential and non-essential amino acids can be found in M. Oleifera seeds. To determine whether the seeds are suitable as a comprehensive nutritional supplement for humans, it is necessary to examine how processing affects the seeds' nutritional contents.

Foods containing amino acids must be ingested in amounts and ratios that closely resemble the pattern the body needs. A wide range of phytochemistry is present in M. Oleifera plant seeds. Moringa Oleifera seeds also contain beneficial secondary metabolite chemicals, including reducing sugar, phenolic compounds, polyphenol, alkaloids, flavonoids, steroids, and terpenes. The widespread use of this plant has a lot of potential for many therapeutic uses and could serve as an alternative.

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