DETECTION OF AMINO ACIDS FROM SEEDS OF CICER ARIETINUM (CHICK PEA) USING CHROMATOGRAPHIC TECHNIQUE.

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ABSTRACT: Cicer arietinum was a farmed produce developed in tropical, temperate & sub-tropical expanse. It was believed that the species originated in the southern Caucasus and northern Persia, south-eastern Turkey and Syria **(1)**. The phyto-chemical investigation of Chick pea plant (Cicer Arietinum) shows that the seeds contains amino acids, carbohydrates, fixed oils proteins, phytosterols, flavonoids glycosides alkaloids, tannins & phenolic compounds, saponins, iron, amino acids, sulphate, phosphate & chloride **(2)**. It also contains antioxidant. Protein contains amino acids as a basic and functional unit. Discovery of amino acids from water extracts of seeds of Cicer arietinum was performed in altered mobile phases by using technique called as paper chromatography. Amino acids take part in the metabolic trails for production of inferior metabolites & hence their existence was identified. Stem and branching part of Cicer arientinum were originate to be an affluent supply of a range of amino acids. Results which are achieved were match up with the model amino acids in comparative to respective mobile phases. The amino acid which are present are of 2 types essential & non essential.

Essential: Methionine, Histidine, Isoleucine, Phenylalanine, Threonine, Valine

Non Essential: Alanine, Arginine, Aspartate, Glutamic, Hydroxyl Proline, Serine, Tyrosine, Glycine

Keywords: Cicer Arietinum, Amino acids, Paper Chromatography, Phytochemical.

I. INTRODUCTION:

Previous few decades there has been a growing curiosity in the revision of curative plants & their customary applications in various areas of the world **(1)**. The seeds of Cicer arietinum is bring into play for a variety of purposes they taken as a food, also exploit by tradition as an Kaamoddiipak (Sanskrit word means increase sexual desire), for treatment of catarrh, lung disease, constipation, cholera, dyspepsia, diarrhoea, snakebite, flatulence, warts & sunstroke **(2)**. To lower down the cholesterol present in the blood acids are used for e.g. oxalic acid & malic acid. In India traditionally these acids were prepared by simple technique by sprinkling slight muslin above the harvest for the period of the night. In the daybreak the drenched cloth is wrung out, & the acids are pulling together & utilized as a drug to lower down the level of lipid. Seeds were also used to control the secretion of bile. The phyto-chemical examination of Cicer arietinum seeds clears about the presence of proteins, carbohydrates, fixed oils, amino acids, alkaloids, phytosterols, tannins & Phenolic compounds, glycosides, flavonoids, amino acids, saponins, phosphate, iron, chloride & sulphate **(6-7)**.

1.1 Plant profile:

Synonyms: Cicer album hort., Cicer arientinium, Cicer arietinum subsp. arietinum, Cicer edessanum Bornm., Cicer grossum Salisb., Cicer nigrum hort., Cicer physodes Rchb., Cicer rotundum Alef., Cicer sativum Schkuhr and Cicer sintenisii Bornm.

Taxonomic classification: Kingdom: Plantae; **Division:** Magnoliophyta; **Class:** Magnoliopsida; **Order:** Fabales; **Family:** Fabaceae; **Subfamily:** Faboideae; **Genus:** Cicer; **Species:** Cicer Arietinum

I. MATERIAL AND METHODS:

2.1 Collection of sample & its Identification:

The seed stuff was gathered from village Manjri, district of Pune, Maharashtra, India. It was validated from Botanical survey of India, Pune, & Maharashtra, India. Authentication No. Of plant material is AHMA S-068, Pune, India.

A paper chromatography technique was applied by using whatmann filter paper no 1. Ethanol, saline & water extract of specific concentrations were prepared by using from seed powder. If chlorophyll is exist it is do away with by uninterrupted dealing with chlorophyll. After treatment by discarding the layer of chlorophyll remaining extract is used for detection of amino acids. This chromatographic technique uses different mobile phases for detection of amino acids.

Sr.No.	Phase	Solvent system	Ratio / Proportion
1.	I	n – Butanol: Ethanol: Water	2 : 2 : 1
2.	II	Pyridine : Isopropyl alcohol: Acetic acid : Water	7:7:0.5:2
3.	III	n-Butanol: Acetone: Water	1: 1: 0.5
4.	IV	Butanol: Pyridine: Acetic acid: Water	3:5:0.5:1.5

Table 1. Solvent system suitable for the analysis.

2.2 Preparation of spraying reagent:

Detection of amino acids by paper chromatography technique uses ninhydrin solution as a spraying agent. Ninhydrin solution was arranged by dissolving 0.0174 g of ninhydrin in 15 ml acetone. Spots of standard samples & extract (Saline, Water, Acetone & Ethanol) were applied on the strips of chromatographic paper. Mobile part was allocated to run to a definite elevation & the chromatogram paper was desiccated at R.T. spraying agent was scattered over the paper & once again desiccated at R.T. from obtained data of distance covered by solute and solvent Rf values of experimental samples of amino acids were calculated & then it was compared with the standard one.

S. No.	Name of Amino Acids	Rf value for Water extract	Rf value for Saline	Rf value for standard amino acid
1	DL- Methionine	0.291	-	0.291
2	L- Histadine Monohydrochloride	0.092	-	0.100
3	DL- Iso- leucine	0.358	-	0.350
4	DL-Threonine	0.200	-	0.200
5	DL-Valine	-	0.325	0.316
6	L- Arginine Monohydrochloride	0.170	-	0.150
7	L- Glutamic acid	-	0.141	0.141
8	L- Hydroxy Proline	-	0.192	0.200

Table 2. Phase: I: n – Butanol: ethanol: water (2: 2: 1)

S. No.	Name of Amino Acids	Rf value for Ethanol extract	Rf value for Water extract	Rf value for standard amino acid
1	L-Histidine mono hydrochloride	0.8	–	0.8
2	L-Hydroxy proline	0.8	–	0.79
3	Glycine	–	0.1	0.12
4	L-Isoleucine	–	0.95	0.93

Table 3. Phase: II: Pyridine: Isopropyl alcohol: Acetic acid: Water (7:7:0.5:2)

S. No.	Name of Amino Acids	Rf value for Water extract	Rf value for Saline extract	Rf value for standard amino acid
1	DL-Alanine	0.208	-	0.200
2	L-Tyrosine	-	0.253	0.258
3	L- Hydroxy Proline	-	0.183	0.170
4	L- Glutamic acid	0.133	-	0.125
5	DL- Methionine	0.250	0.233	0.250

Table 4. Phase: III: n-Butanol: Acetone: Water (7:7:0.5:2)

S. No.	Name of Amino Acids	Rf value for Ethanol extract	Rf value for Acetone extract	Rf value for Water extract	Rf value for standard amino acid
1	L- Tyrosine	-	-	0.52	0.50
2	L-Arginine mono hydrochloride	–	0.82	–	0.83
3	DL-Methionine	–	0.82	–	0.83
4	DL-Phenyl alanine	0.8	-	-	0.78

Table 5. Phase: IV: Butanol: Pyridine: Acetic acid: Water (3:5:0.5:1.5)

II. RESULTS AND DISCUSSION

Proteins contain amino acids which are the basic unit in it. For synthesis of secondary metabolites during metabolic activity they play an important role. Stem & branching part of Cicer arietinum contain large amount of amino acids which is detected by using a technique is called as paper chromatography. The amino acids which were detected during analysis were in phase I- DL- Methionine, L- Histadine Monohydrochloride, DL- Iso- leucine, DL-Threonine, DL-Valine, L- Arginine Monohydrochloride, L-Glutamic acid, L- Hydroxy Proline during phase II- Glycine some were common in phase-I & III. In phase III- L-Tyrosine was the new one while again some were common with phase I & II. And in phase IV- DL-Phenyl alanine was new and some were again common with I, II & III

III. CONCLUSION

This study shows that water & saline extract of Cicer arietinum seam & branches contain about 11 amino acids.

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