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Isolation and separation of proteins from the seeds of *Sysmbrium irio*

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ABSTRACT

In the present investigation we have attempted the isolation and separation of proteins from the seeds of *Sysmbrium irio*. Its seeds are used as an expectorant, restorative and externally as stimulating poultice. The protein content was found to be 18.25%. The amino acids were identified by Thin layer and Paper Chromatography. These studies revealed the presence of amino acids, namely- Serine, Glycine, Threonine, and Methionine, Leucine, Lysine, Proline, and Glutamic acid and two remained Unidentified.

Key words: *Sysmbrium irio*, Proteins, amino acids.

INTRODUCTION

Sysmbrium irio (Synonym- London Rocket) belongs1 to the Family Cruciferae and commonly known as "Khubkalan". It is used as an expectorant, restorative and used externally as a stimulating poultice. The plant is indigenous to northern India and its distribution extends through Afghanistan to Europe and the Canary Islands[2,3].

The seeds have a hot sharp taste. An extensive survey of literature has revealed that the fixed oil of S.irio has been reported partly[4]. The unsaponifiable part of the oil, however still remains to be studied for its components and its pharmacological activity. Protein fraction of the seed also needs its isolation and separation.

MATERIALS AND METHODS

The seeds of S. irio were dried in a hot air oven at 40-50°C for 48 hrs. The dried seeds were crushed to a coarse powder in an iron pestle & mortar. The powder was sieved & stored in air light container.

Chemical

Various chemicals used were potassium Iodide, Iodine, Metallic Mercury from CDH chemical. Sodium Hydroxide, Sodium Silicate, Conc. Sulfuric acid, Selenium, Copper Sulfate & Potassium Sulfate of analytical Grade were used.

Isolation of Proteins

The isolation of the proteins from seeds of S. irio was carried out by salt solution method. About 150 g defatted seeds of S. irio were crushed and soaked in 10%w/v Sodium Chloride solution for 10 hrs with occasional shaking. After 10 hrs maceration, the mixture was filtered & marc discarded. To the filtrate 0.5 N HCl was added slowly to get proteins precipitation (pH 3.0- 4.5).The proteins obtained were separated by filtration, washed, dried and weighed. The presence of organic nitrogen was found positive[5-7].

Determination of nitrogen content of proteins in seed[8]

Estimation method consisted of two parts:
(i) Conversion of nitrogen to ammonium Sulfate by Kjehldal method,

Table 1: Rf Values of Amino acids in Protein Hydrolysate

Spots Detected	Amino acids taken (Authentic)	RfValues of Authentic Amino acids	Spots from Sample
1.	Serine	0.13	0.13
2.	Glycine	0.23	0.25
3.	Glutamic acid	0.31	0.31
4.	Threonine	0.34	0.35
5.	Methionine	0.41	0.41
6.	Leucine	0.44	0.45
7.	Lycine	0.55	0.54
8.	Proline	0.64	0.64
9.	X	------	0.76
10.	Y	------	0.85

Solvent System: n-Butanol : Glacial Acetic acid : Water (8:2:2)
Adsorbent: Silica- Gel G
Detectron Reagent: Ninhydrin- Acetic acid Solution.

(ii) Estimation of ammonia by Nesslerization.
The seed protein of S. irio contains 10.80% nitrogen.

Hydrolysis of Proteins

About 2 g protein was mixed with 100ml HCl (6N) in a long flask, refluxed for 72 hrs. The hydrolysate was dissolved in 25ml of hot distilled water & filtered. The filterate was evaporated; residue obtained was dissolved in 25ml of 10% aqueous iso-propanol[9,10].

Thin Layer Chromatographic Studies of Hydrolyzed Protein

Approximately 0.10 ml of protein hydrolysate and authentic samples of amino acids were applied at the base line of TLC plates (Silica Gel- G), kept in TLC Chambers saturated with solvent system {n-butanol : Glacial Acetic acid : Water (8:2:2) }. The plates were air dried & sprayed with ninhydrin reagent and heated at 110 0C for about 10 minutes[11,12].

RESULTS AND DISCUSSION

10% w/v Sodium Chloride Solution extracted 15.96% of the proteins from defatted seeds of S. irio. The proteins gave positive test for nitrogen. Other characteristic tests for proteins were also positive. The nitrogen content of proteins was found to be 10.80%.

The Thin Layer Chromatographic studies (Table -1) revealed that protein hydrolysate of seed proteins of S.irio contained ten amino acids. Eight out of these were identified, of which four belongs to category of essential amino acids- threonine, methionine, lysine and leucine. Other four amino acids were serine, glycine, proline and glutamic acids.

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