# INVESTIGATION ON EMINIUM SPECULATUM

## Part 1. Compound and Free Amino Acid Composition

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(Received May 21, 1975; revised May 29, 1976)

**Abstract.** The free and bound amino acids of extracts of *E. speculatum* have been determined by paper chromatography and no evidence has been found for the occurrence of nonprotein amino acids.

*Eminium speculatum* locally kdown as "Laiyha" belongs to *Araceae* family which includes a number of plants having medicinal uses as well as fatal to human consumption.[1] The wild growth of this plant has been reported in Mosul, Kirkuk and Sulaimania during rainy season from December till May.[2] It also grows in Syria, Lebanon, Palestine and Egypt.

*E. speculatum* is known to kill fish and cause vomiting and diarrhoea.[3] Its use in snake-bite, piles and leprosy is mentioned in the literature.[4] Antaki[5] and Al-Malek[6] observed that the plant is harmful to intestine and causes ascites. Al-Rawi[2] and Mahran[7] have reported that *E. speculatum* is highly toxic, irritant and lowers the blood pressure.

Al-Bary[8] has reported the presence of alkaloids, steroids, saturated and unsaturated fatty acids and a physiologically active protein. None of these constituents was, however, isolated in pure form and characterised tentatively. Afzal *et al.*[9] have isolated and characterised an aliphatic ketone, laurone, from *E. speculatum*.

It was presumed that the toxicity of *E. speculatum* could be either due to some alkaloid, a special type of antienzyme protein or a nonprotein amino acid. This prompted us to investigate the amino acid composition of this plant.

## Experimental

The fresh plant was collected from Noran (20 km from Mosul) and dried in shade. The powdered plant material was exhaustively defatted for 10 hr with light petroleum (60-80°) in a Soxhlet extractor.

*Isolation of Proteins.* The defatted powder (10 g) was soaked in a 0.2% $Na_2CO_3$ solution (200 ml), overnight.[10,11] The mass was squeezed through double folds of muslin cloth. The clear fluid was centrifuged at 3000 rev/min for 20 min. The clear fluid was acidified to pH 4.0 with dilute $H_2SO_4$ and allowed to stand for 4 hr for complete precipitation of proteins. The proteins were centrifuged at 3000 rev/min for 20 min washed with distilled water (adjusted to pH 4), followed by two washings with 95% alcohol. The crude proteins were dried in an oven at 60° for 10 hr to give amorphous solid (0.57 g).

*Nitrogen Content.* Nitrogen was determined by the method of Markham.[12] This was found to be 0.65% on the basis of which the total protein was calculated as 4.06%.

*Acid Hydrolysis.* The crude proteins (100 mg) were hydrolysed under reflux with concd. HCl (3ml), for 20 hr.[13] The excess acid was removed by evaporation to dryness under reduced pressure. The resulting mass, containing the amino acid hydrochlorides was taken up in water (2 ml) and mixed with 95% ethanol (6 ml). This was allowed to stand for 10 min and then centrifuged. The clear supernatent was transferred quantitatively to a separating funnel and extracted with chloroform (25 ml). The organic extract was discarded and the aqueous layer, containing the amino acids, was evaporated to dryness. The dried mass was taken up in 10% isopropanol (1 ml). This solution was used for qualitative and quantitative analysis of bound amino acids.

*Isolation of Free Amino Acids.* The defatted powder (5 g) was triturated with distilled water (200 ml), for 3 hr. After filteration through muslin cloth, the extract was centrifuged to remove any suspended matter. Bulk of water was removed under reduced pressure and the residue was treated with alcohol – chloroform as described above. The aqueous layer was evaporated to dryness under reduced pressure and then taken up in 10% isopropanol (1 ml). This was used for analysis of free amino acids.

*Chromatographic Methods.* All comparisons were made by running unknown samples and reference amino acids side by side.

*Paper Chromatograms.* Ascending or descending chromatography was carried out on Whatman filter paper No. 1. Two-dimensional chromatograms were run by the ascending procedure on 25 × 25 cm. sheets The following solvent systems were used. (1) n-Butanol – acetic acid – water ; (12:3:5, v/v) ; for one dimensional chromatography.[14] (2) Phenol – water ; (80:20, w/v) ; for second dimension with system 1 as the first dimension solvent. (3) Pyridine – acetone – ammonia – water ; (45:30:5:20, v/v); for the first dimension.[15] (4) Isopropanol – formic acid-water ; (75:12.5:12.5, v/v) ; used as the second dimension solvent.

*Detection Reagent.* Ninhydrin 0.2% in acetone was freshly prepared and used as a routine reagent. All chromatograms were dried at 80° for 5 min before dipping in the reagent.

*Standard Solutions*

*Solution A.* It contained leucine, phenylalanine, tryptophan, valine, proline, hydroxyproline, threonine, glycine, aspartic acid and lysine (5 m moles/l of each), in hydrochloric acid (0.1N).

*Solution B.* It contained isoleucine, methionine, tyrosine, alanine, glutamic acid, serine, arginine, histidine and cystine, (5 mmole/l of each), in HCl (0.1N).

The two solutions were neutralised with an equal volume of NaOH (0.1N), before use. Each solution (20 μ l was spotted on a separate sheet and chromatographed in the same manner as the unknown. The unknown chromatograms were compared against the standard spots.

*Standard Amino Acid Curves*

The standard curves of various individual amino acids were prepared by using pure and dry amino acids (B.D.H.) as described by Giri *et al.*[16] The protein hydrolysate and the free amino acid extracts were chromatographed in solvent system (3) and (4) using the two dimentional chromatographic procedure. The chromatograms were developed and the coloured spots were extracted and the optical densities were measured at 540 nm using Spectronic 20 absorption photometer. The concentration of each amino acid in the unknown mixture was read from its respective standard curve, prepared above.

## Results and Discussion

The components of the free amino acid extract and the protein hydrolysate are shown in Tables 1 and 2 respectively.

TABLE 1. FREE AMINO ACID OF *Eminium speculatum.*

| Spot No. | Amino acid | μg/0.01 ml | Corrected amount μg | mg/100 g |
|---|---|---|---|---|
| 1 | Cystine | 30.0 | 36.0 | 72.0 |
| 2 | Lysine | 40.0 | 48.0 | 96.0 |
| 3 | Aspartic | 52.0 | 62.4 | 124.8 |
| 4 | Glycine | 70.0 | 84.0 | 168.0 |
| 5 | Alanine | 64.0 | 76.8 | 153.0 |
| 6 | Serine | 50.0 | 60.0 | 120.0 |
| 7 | Valine | 102.0 | 122.0 | 244.0 |
| 8 | Leucine | 120.0 | 144.0 | 288.0 |

In solvent system 1, the amino acids possessing low *Rf* values were not resolved as compact and discrete spots. However, good resolutions were obtained by using phenol – water as the second-dimentional solvent. Solvent system 3 and 4 gave excellent separations and constituted a handy means of identification of unknown mixtures when used with standards A and B. Since sulphur-containing amino acids, cystine and methionine tend to undergo oxidation to cysteic acid and methionine sulphone respectively, this was ceased by prior treatment of spots on the paper with 30% $H_2O_2$ (20 ml).

The extraction procedure described above results in the loss of individual amino acids as shown by automated ion exchange chromatography.[17] The values obtained by this method were multiplied by a factor of 1.2 in order to account for the losses occurred in the extraction procedure. Figs. 1–4.

A number of extraction procedures were used employing varying proportions of alcohol – water mixtures to extract nonprotein nitrogeneous substances and the extracts were examined by chromatography. No nonprotein amino acid could be detected in these extract, on comparison with the 20 known protein amino acids.

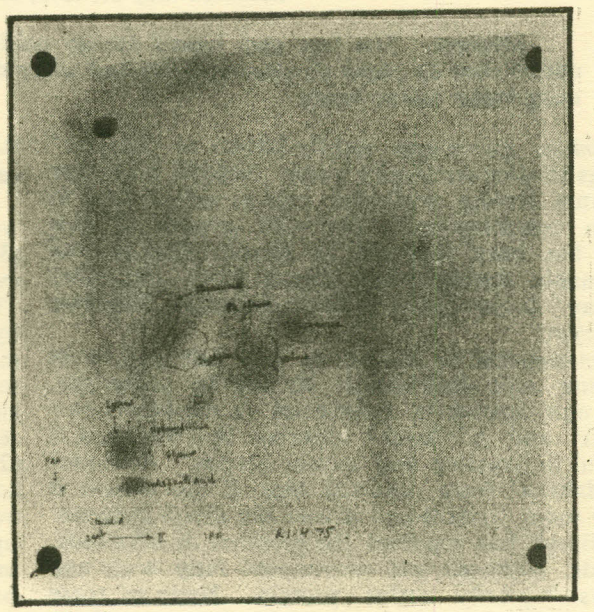

Fig. 1

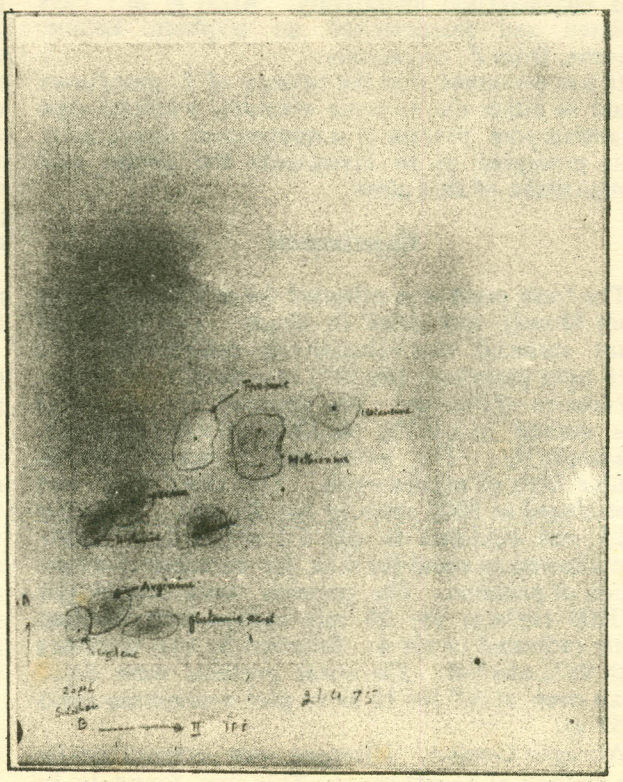

Fig. 2.

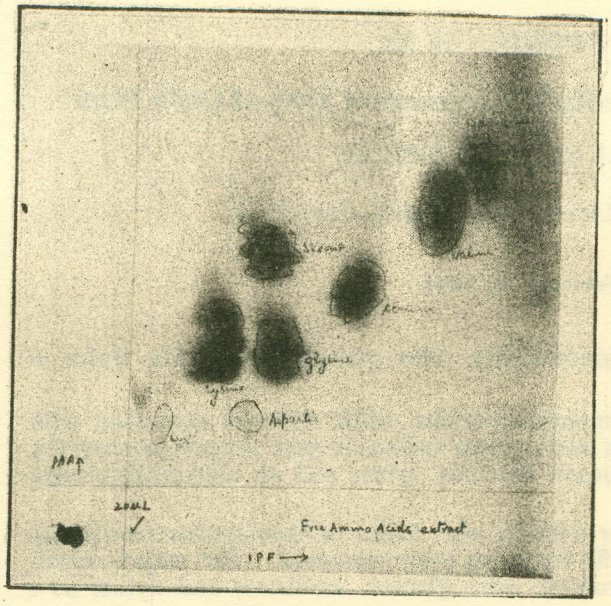


Fig. 3.

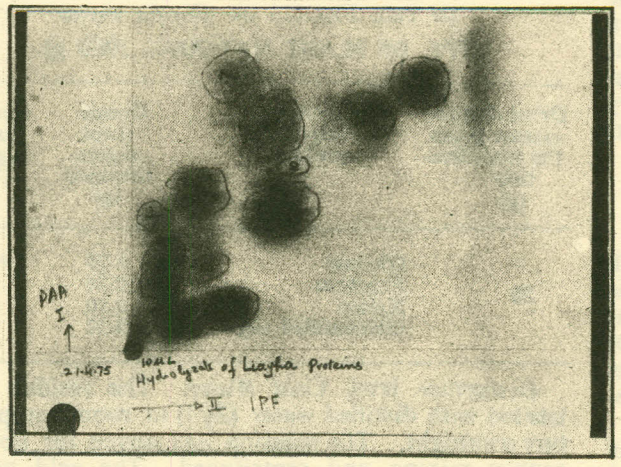


Fig. 4.

**Acknowledgements.** We are deeply indebted to Dr. A.R. Al-Hassoo, Medical Research Laboratories, University of Mosul, for his cooperation and the use of Spectronic 20 spectrophotometer.

TABLE 2. AMINO ACID COMPOSITION OF *Eminium speculatum* PROTEINS.

| Spot No. | Amino acid | µg/0.01 ml | Corrected amount µg | mg/100 g |
|---|---|---|---|---|
| 1 | Histidine | 16.0 | 19.2 | 1.92 |
| 2 | Lysine | 31.0 | 37.2 | 3.72 |
| 3 | Aspartic | 50.0 | 60.0 | 6.0 |
| 4 | Serine | 80.0 | 96.0 | 9.6 |
| 5 | Glycine | 30.0 | 36.0 | 3.6 |
| 6 | Glutamic | 120 0 | 144.0 | 14.6 |
| 7 | Threonine | 30.0 | 36.0 | 3.0 |
| 8 | Tyrosine | 60.0 | 72.0 | 7.2 |
| 9 | Alanine | 131.0 | 157.0 | 15.72 |
| 10 | Proline | Not determined | | |
| 11 | Methionine | 42.0 | 50.4 | 5.04 |
| 12 | Isoleucine | 112.0 | 134.4 | 13.44 |

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