Biochemical analysis and skin sensitivity test of the allergenic pollen of *Datura metel* L.

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Biochemical analysis in the form of quantitative estimation of carbohydrates, lipids, proteins and free amino acids and also identification of free amino acids by paper chromatography and thin layer chromatography of the pollen of *Datura metel* were performed. The protein profile was studied by SDS-PAGE and skin prick tests were performed with the pollen antigen of this plant. 29% of respiratory allergic patients showed positive response.

THE seriousness of allergic diseases in India began to be appreciated in the sixties as more than 10% of the population were estimated to suffer from major allergic disorders[1]. Aerial surveys of pollen and fungal spores have since been considered to be an essential aspect of the study of respiratory allergic diseases, as the role of pollen and fungal spores in causing respiratory disorders has been greatly recognized. However, in case of pollen, greater attention was given to anemophilous pollen by the aerobiologists, with the entomophilous pollen being neglected in their routine aeropalynological survey because of their rare occurrence. Contrary to this belief, recent surveys have reported the presence of entomophilous pollen from the air-spora[2-14]. According to Gregory[15], pollen grains tend to be distributed in dense concentrations around their sources and therefore tend to be local occurrence. This is seen more in case of entomophilous pollen, which remain in high concentrations in air near the source plants[16].

Datura metel L., an entomophilous plant, is one of the species of Solanaceae which is widely distributed in West Bengal, covering large areas of wastelands and road sides. Despite the production of abundant quantities of pollen, this plant has generally not been considered to be a serious allergenic hazard because of its entomophilous nature. However, the pollen of this plant has been reported in the air by several workers[11,17-19] and its allergenic potency has been proved[11,17]. In the present investigation, biochemical analysis in the form of quantitative estimation of carbohydrates, lipids, proteins and free amino acids, as well as identification of free amino acids by paper chromatography and thin layer chromatography (TLC), of the pollen *D. metel* was done to understand its chemical composition. Chemically the aeroallergens possess proteins, glycoproteins, lipoproteins or less often polysaccharides[11,20]. The protein profile was studied by SDS-PAGE and skin prick tests were performed with the pollen antigen of this plant.

Pollen grains were collected in bulk from the plants growing in and around Santiniketan, located about 160 km north-west of Calcutta and adjoining places of Birbhum district, in the morning hours before anthesis and then sun-dried. Pollen grains from dried anthers were sieved using different meshes (100, 200 and 300 µm).

100 mg of pollen sample were ground in a mortar at −20°C for 10 min. 5 ml of 2.5 N HCl was added to the resulting frozen powder and hydrolysed by keeping on a boiling water bath following Sadasivam and Manickam[21], cooled, neutralized with sodium carbonate and then centrifuged. The quantity of carbohydrate was determined by the method of Morris[22].

Lipids were extracted with chloroform : methanol (1 : 2 v/v) mixture and shaken with 0.9% NaCl to remove non-lipid contaminants[23]. The solvent layer was dried in vacuum and total amount of lipid weighed following the method of Itoh and Kaneko[24].

500 mg of pollen were crushed and homogenized with 5–10 ml of 0.5 M Tris buffer (pH 7.4) and centrifuged at 15,000 *g* for 25 min at 4°C. The supernatant was used for protein estimation by the method of Lowry *et al.*[25].

The free amino acids were extracted and quantified following the method of Sadasivam and Manickam[21]. Amino acids were extracted with 80% ethanol and quantified using ninhydrin solution as colouring reagent, with OD of the purple colour read at 570 nm against a reagent blank.

Paper chromatography was done on Whatman No. 1 filter paper with *n*-butanol : acetic acid : water (4 : 1 : 5 v/v) as eluant and 0.4% ninhydrin in acetone as spraying reagent[21].

Free amino acids were chromatographed on DC-Alufolien Kieselgel 60 aluminium sheets (Merck) using *n*-butanol : acetic acid : water (80 : 20 : 20 v/v) as the solvent system and 0.1% ninhydrin in acetone was used for detection of the amino acids.

The protein sample was boiled with equal amount of sample buffer [0.06 M Tris HCl (pH 6.8), 1% SDS, 10% sucrose, 0.5% β-mercaptoethanol, 0.01% bromophenol blue] at 100°C for 3 min. 10 µl of the sample containing 74 µg of protein was loaded in the well of a 10% mini-gel (8 × 7 cm gel) and the gel was run using Laemmli buffer system[26]. The gel was calibrated with a marker protein (molecular weight range – 29 kD to 205 kD) obtained from Sigma, USA. 0.1% Coomassie brilliant blue R 250 was used for staining the gel after electrophoresis and destained with methanol : acetic acid : water (4 : 1 : 5) mixture.

Pollen antigens prepared according to the method of Sheldon *et al.*[27] were tested on 98 patients suffering from naso-bronchial allergy as well as healthy volunteers at Calcutta Medical Research Institute, Calcutta. Negative and positive controls were also performed. The negative

control was the buffer saline in which the allergen was resuspended and positive control was histamine acid phosphate injection diluted with buffered saline to 1 : 10,000, i.e. 1 µg of histamine acid phosphate. Results were recorded after 15 to 20 min and depending on the wheel diameter, the tests were graded 1+ to 4 +.

The pollen of *D. metel* was found to contain a high level of protein (22.0%) and a low level of free amino acids (0.81%) and lipids (6.3%) (Figure 1). The carbohydrate level was found to be 13.2%. Stanley and Linskens[20] reported the protein level in pollen ranging between 5.9% and 28.3% of pollen residue. Thus protein level of 22% in the pollen of *D. metel* is quite high when compared to this.

Seven types of free amino acids were detected in the pollen of *D. metel* apart from two other unknown amino acids which could not be identified. The seven amino acids present in the free form included amino-*n*-butyric acid, arginine, aspartic acid, glutamic acid, isoleucine, methionine and proline (Table 1, Figure 2) among which proline was present in highest amount as has been reported earlier in most pollen[20]. Proline is used by pollen during growth, possibly in tube or wall protein formation, or in a more fundamental metabolic reaction associated with the sexual process[28]. The other major amino acids are amino-*n*-butyric acid and arginine.

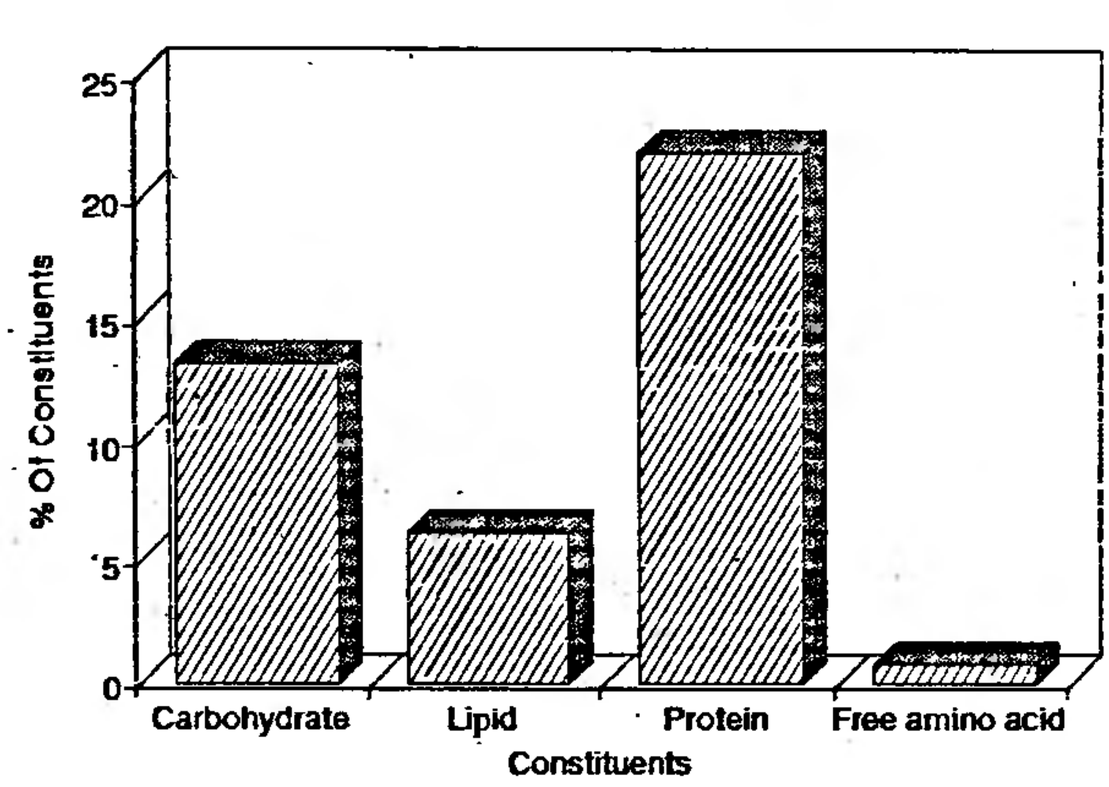

Figure 1. Biochemical composition of the pollen of *D. metel*.

Table 1. Free amino acid composition of the pollen of *D. metel*

Amino acid	µmoles/mg dry wt
Amino-*n*-butyric acid	0.142
Arginine	0.168
Aspartic acid	0.030
Glutamic acid	0.018
Isoleucine	0.100
Methionine	0.064
Proline	0.216
Unknown	0.068

The protein profile of *D. metel* by SDS-PAGE revealed 12 bands (Figure 3) between the molecular weight range of 18 kD and 127.2 kD. These bands were designated as DM1 to DM12. Among these, the most prominent bands were DM3 (87 kD), DM6 (59.4 kD), DM9 (40.5 kD) and DM11 (31.6 kD). DM1 (127.2 kD), DM2 (97.4 kD), DM8 (49.8 kD) and DM12 (18 kD) were present as faint bands.

The skin prick tests with the antigen of *D. metel* revealed 29% of the patients to be positive. Out of 98 patients tested, 58 were male and 40 were female. Sixteen patients had rhinitis only and the remaining had bronchial asthma alone or with rhinitis. The patients

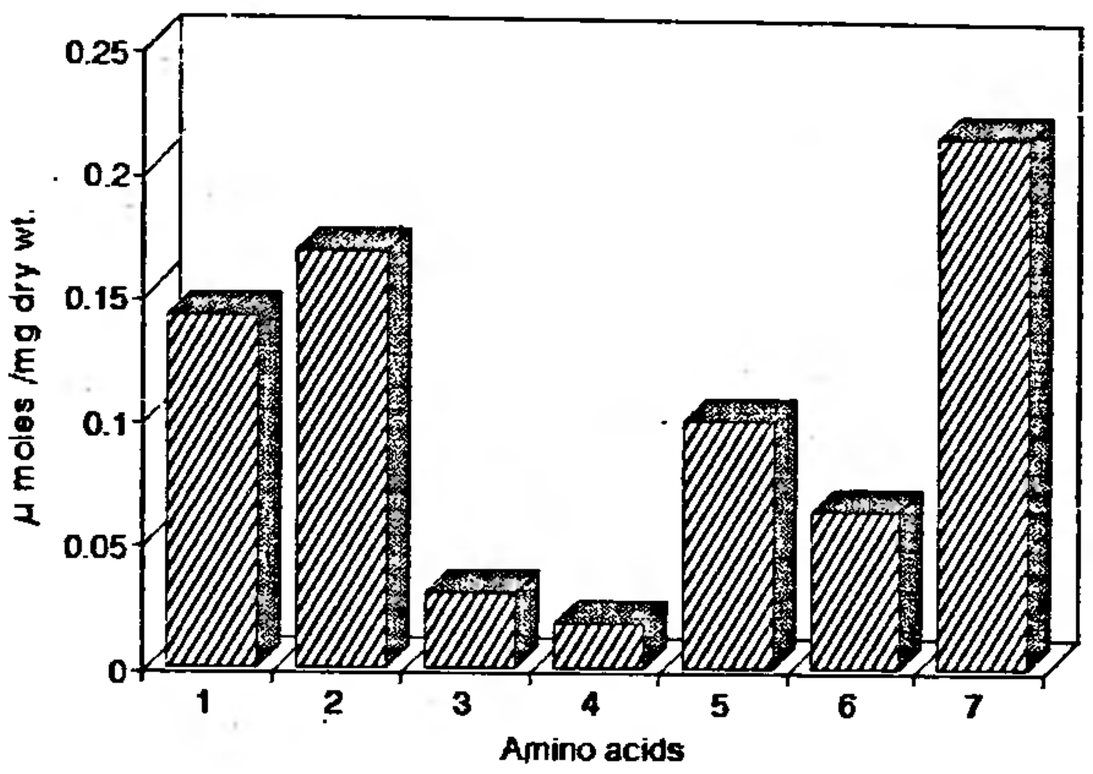

Figure 2. Free amino acid composition of the pollen of *D. metel*. (1) Amino-*n*-butyric acid, (2) arginine, (3) aspartic acid, (4) glutamic acid, (5) isoleucine, (6) methionine and (7) proline.

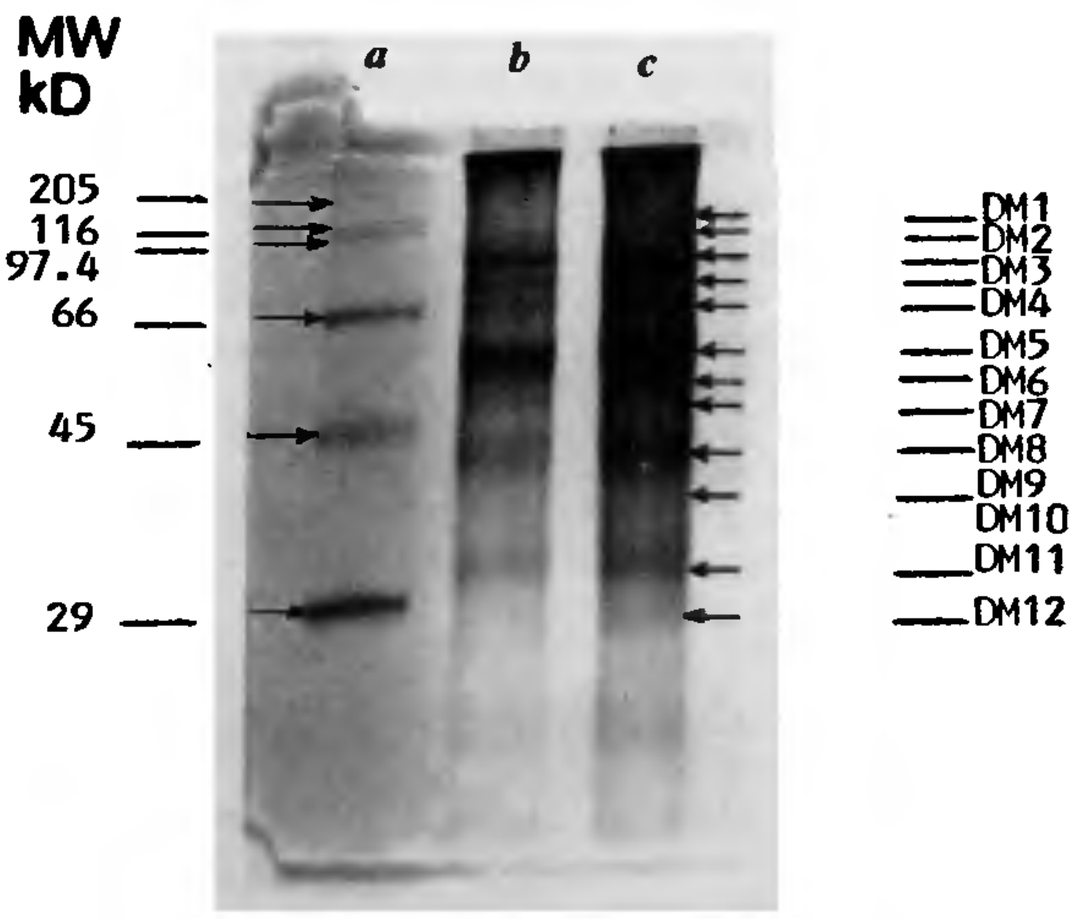

Figure 3. SDS-PAGE of pollen extract of *D. metel* (*a*) represents marker proteins (*b* and *c*) contain 60 µg and 74 µg of 0–80% ammonium sulphate pellet fraction of crude homogenate. It represents DM1 to DM12.

Table 2. Results of skin tests against the antigen of *D. metel*

Antigen tested	Total number of tests	Patients (%)					Normal persons (%)		
		Negative	1+	2+	3+	4+	Negative	1+	2+
Datura metel	98	71.0	16.3	10.6	2.1	0.0	90	10	0

Table 3. Age-wise distribution of patients who showed positive skin response to *D. metel* pollen antigen

Age group	Male	Female	Total
1–10	1	–	1
11–20	2	2	4
21–30	4	1	5
31–40	5	4	9
41–50	2	2	4
51–60	3	2	5
Total	17 (60.7%)	11 (39.3%)	28 (100%)

tested were between the age group of 9 and 56 years. Tables 2 and 3 give the results of the skin tests. Of the 29% patients showing positive response, 16.3% gave 1+ reaction, 10.6% gave 2+ reaction and 2.1% gave 3+ reaction. 4+ reaction was observed in none of the patients.

The pollen of *D. metel* thus reveals a high level of proteins (22%) and high level of proline, amino-*n*-butyric acid and arginine. SDS-PAGE of protein of the pollen showed 12 bands between molecular weight range of 18 kD and 127.2 kD. Results of skin tests showed positive response in 29% patients with 16.3% patients giving 1+ reaction, 10.6% giving 2+ reaction and 2.1% showing 3+ reaction. Although there are earlier reports of allergy of *D. metel* pollen, the present observation concerning perceptions of cause of respiratory allergy due to *D. metel* pollen supports the notion that *D. metel* should not be dismissed as a serious allergen.

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Methyl farnesoate stimulates ovarian maturation in the freshwater crab *Oziotelphusa senex senex* Fabricius

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Methyl farnesoate stimulation of ovarian maturation in the crab, *Oziotelphusa senex senex* is demonstrated. Greater mean oocyte diameter and mean ovarian indices of the crabs that received methyl farnesoate provide evidence that methyl farnesoate acts as a reproductive hormone in crustacea.

SINCE the discovery of the mandibular organs (MO) in