

# Allergenic Protein Profile Of The Pollen Of *Datura* sp- A Comparative Study

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**Abstract:** The pollen of *Datura metel* has been found to have a role in causing allergy. The other two species of *Datura* i.e. *Datura innoxia* and *Datura stramonium* being similar to *Datura metel* may contribute a role to allergenic rhinitis. The present paper reports the comparative protein concentration study of mature and immature pollen of *Datura metel*, *Datura innoxia* and *Datura stramonium*. *Datura metel* and *Datura innoxia* showed a lower protein concentration in case of immature pollen than mature one. On the other hand *Datura stramonium* showed a higher protein concentration in immature pollen than mature one. From the SDS-PAGE electrophoresis study it was revealed that the three species shared five common proteins with molecular weights of 205.0 kDa, 97.4 kDa, 66.0 kDa, 59.4 kDa and 43.0 kDa. Apart from these five *Datura metel* and *Datura innoxia* shared another two other common pollen proteins and *Datura stramonium* and *Datura innoxia* showed a same result as previous. On the other hand apart from the fair common proteins, another protein was found to be common between *Datura metel* and *Datura stramonium*.

**Key words:** *Datura metel*, *Datura innoxia*, *Datura stramonium*, SDS-PAGE, pollens, proteins, allergenic proteins.

## Introduction

*Datura* is a herbaceous perennial plant of family Solanaceae, grown in tropical and temperate regions of the globe. *The Plant List* includes 103 scientific plant names of species rank for the genus *Datura*. Of these 13 are accepted species names. *The Plant List* includes a further 27 scientific plant names of infraspecific rank for the genus *Datura*. A number of species especially jimsonweed or thornapple (*Datura stramonium*) toloache (*Datura innoxia*) and *Datura metel* had been used by Mexican Indian people for various medicinal purposes from ancient era. Leaves are simple, alternate, dark green, broadly ovate, shallowly lobed and glabrous. Flowers are large, solitary, and trumpet-shaped. It has a sweet fragrance usually appreciated in the mornings and evenings, with a wide range of colours, ranging from white to yellow and light to dark purple. The flowers are hermaphrodite and are pollinated by insects. The fruit is covered with short spines in form of the capsule (Khaton et al., 2012). The medical value of *Datura* was proved from ancient era (Parveen et al., 2006). *Datura* can tolerate average range of soil with rich and moist even alkaline soil. It hardly survives under shade. It prefers a warm temperature and is distributed in warmer regions of the world (Drake et al., 1996). *Datura* probably is of American origin and widely cultivated in all tropical and subtropical regions for its beautiful flowers (Glottter et al., 1973). *D. metel* can also be found in East Asia or India, and is used in traditional Bangladeshi herbal medicine. In Traditional Chinese Medicine, the flowers of *D. metel* are known as baimantuoluo and used for skin inflammation and Psoriasis (Wang et al., 2008). In Ayurvedic medicine, seeds of *D. metel* are used to treat Skin rashes, Ulcers, Bronchitis, Jaundice and Diabetes (Agharkar et al., 1991). In Brazil, seeds are used for tea making which would serve as a sedative and flowers are dried and smoked as cigarettes (Agra et al., 2007). There are various species of *Datura* which are now cultivated for the production of secondary metabolites.

*Datura innoxia* is quite similar to *Datura metel*, to the point of being confused with it in early scientific literature. *D. metel* is a closely related Old World plant for which similar effects were described by Avicenna in eleventh century Persia. The closely related *Datura stramonium* differs in having smaller flowers and tooth-edged leaves *Datura innoxia* differs from *D. stramonium* and *D. metel* in having about 7 to

10 secondary veins on either side of the midrib of the leaf which anastomose by arches at about 1 to 3 mm. from the margin. No anastomosis of the secondary veins are seen in these 3 major species of *Datura*.

The pollen of *Datura* sp. has been found to have a role in causing allergy in sensitive patients. In spite of this the pollen of *Datura* sp. has till date not been considered to be a serious allergenic hazard (Parui and Mandal, 1998). One of the major reasons behind this is the entomophilous nature of the taxa. Surveys have reported the presence of entomophilous pollen from the air-spora (Agashe, 1989; Agashe *et al.*, 1983; Atluri *et al.*, 1992; Singh and Babu, 1982; Singh and Devi, 1992; etc.). The pollen of *Datura* has been reported in the air by several workers (Santra *et al.*, 1991; Jain *et al.*, 1992) and the allergenic potency has been proved (Santra *et al.*, 1991; Jain *et al.*, 1992; Parui and Mandal, 1998). Moreover, pollen grains tend to be distributed in dense concentrations around their sources and therefore tend to be of local occurrence (Gregory, 1961).

The pollen of *Datura metel* has already been proved to be allergenic (Mondal and Parui, 1998). With great resemblance with it to the other two species of *Datura* i.e. *Datura stramonium* L. and *Datura innoxia* M. may also have the significant role in causing allergy which is yet to be a matter of study. Being grown enormously and used hugely for the worship of Lord Shiva in India it is difficult to evade this pollen allergen especially by women who usually use it frequently. The pollen also may be a subject of causing risk in atopic persons who cultivate these plants in their garden for ornamental and medicinal purposes. In view of the significance of the role of pollen antigens in the diagnosis and therapy of allergenic patients, identification, isolation, purification and characterization of pollen allergens is a prerequisite for standardization (Bera *et al.*, 2016). Efforts are being made globally to standardize the pollen antigens as pollen collected from different source materials, stage of purity, time intervals and geographic places or with different storage periods have shown significant variation in their allergenic components (Singh *et al.*, 1993).

We have reported earlier the protein concentrations and protein profiles of the pollen of *Datura metel*, *Datura innoxia* and *Datura stramonium* individually (Bera *et al.*, 2015, 2016, 2017). The present paper reports a comparative study of protein concentrations during different seasons and a comparison between the similar and dissimilar allergenic proteins of the pollen of the three species of *Datura* i.e. *Datura metel* L., *Datura innoxia* M and *Datura stramonium* L.

## Materials And Methods

### Pollen collection

Pollen grains of *Datura stramonium* were collected in bulk from the plants growing in South Calcutta, West Bengal. Two types of pollen were collected – one from mature buds and the other from flowers which had finished blooming on the same day as well as different days of different seasons. Pollen grains from the anthers were sieved using different meshes (100, 200 and 300  $\mu$ m). All the samples were analyzed under the microscope, which revealed pollen purity varying from 90% to 95%.

### Protein extraction

Proteins from pollen were extracted according to the method of Singh *et al.* (1993) with slight modification (Mondal *et al.* 1997). The pollen was defatted with cold solvent ether and then dried in a vacuum desiccator. The defatted pollen was then used for protein extraction. Proteins were extracted in 0.2 M Tris HCl buffer (pH 7.4) by continuous stirring at 4<sup>0</sup>C for 20h. The extract was clarified by centrifugation at 12000 $\times$ g for 5 minutes at 4<sup>0</sup>C. The supernatant was collected. The samples were then stored at -20<sup>0</sup>C.

### Estimation of proteins

The protein concentration in the extract was estimated by the modified method of Lowry (Lowry *et al.*, 1951). A calibrated solution of bovine serum albumin was used as a standard.

## Gel electrophoresis

The protein sample was heated with an equal amount of sample buffer [0.06M Tris HCl (pH 6.8), 1% SDS, 10% sucrose, 0.5%  $\beta$ -mercaptoethanol, 0.01% Bromophenol blue] at 100°C for 3 min. The sample was loaded in the well of a 10% T mini-gel (8x7 cm gel) and the gel was run using Laemmli buffer system (1970) [0.05 M Tris, 0.192 M Glycine, 0.1% SDS, pH 8.4] at room temperature for 2hrs 30 min at 70V. The gel was calibrated using a marker mixture consisting of Myosin, Rabbit Muscle (205 kDa), Phosphorylase b (97.4 kDa), Bovine Serum Albumin (66 kDa), Ovalbumin (43 kDa), Carbonic Anhydrase (29 kDa) and Soyabean Trypsin Inhibitor (20.1 kDa). After electrophoresis, the gel was stained with 0.1% Coomassie Brilliant Blue R 250 and destained with methanol : acetic acid : water (4:1:5) mixture.

## Results and Discussion

A variation in protein concentrations found in case of mature and immature pollen among all three species. *Datura metel* showed the least variation between the concentration of the pollen proteins of the mature and immature ones. Mature pollen exhibited the concentration of proteins of 260 ug/ml whereas the immature one showed 240 ug/ml of concentration. *Datura stramonium* followed the trend of *Datura metel* and showed the least concentration of protein i.e. 127ug/ml in case of immature pollen than the mature one which was found to be 162 ug/ml. On the other hand *Datura innoxia* pollen showed a higher concentration of protein i.e. 193.14 ug/ml in immature pollen where as mature pollen showed the concentration of 170.28ug/ml.

From the SDS-PAGE protein profile exhibited a total of 20 bands of proteins in case of *Datura metel*, 16 bands in case of *Datura stramonium* and 16 bands in case of *Datura innoxia*. Among these protein bands 5 proteins bands were found to be common in all the three species. These are d3, d9, d10, d11 and d14 in case of *Datura metel*, D'1, D'3, D'5, D'6 and D'8 in case of *Datura stramonium* and D1, D4, D9, D10 and D11 in case of *Datura innoxia*. The molecular weights of these five proteins were 205.0 kDa, 97.4 kDa, 66.0 kDa, 59.4 kDa and 43.0 kDa.

Apart from these common proteins among the three species, another two common protein bands d4 and d2 of *D. metel* had the same molecular weight with the protein bands D2 and D3 of *Datura innoxia* with the molecular weights 183.5 kDa and 108.2 kDa respectively whereas *D. metel* and *D. stramonium* shared another common protein band naming d20 (29.0 kDa) and D'9 (20.0 kDa) respectively along with the five common proteins. *D. stramonium* and *D. innoxia* shared two common proteins i.e D'4 (87.0 kda ) with D6 and D'10 (21.0 kda) with D15 respectively.

## Conclusion

The pollens of *Datura stramonium* and *Datura innoxia* which are still not reported to be allergenic show a great resemblance in protein profile to allergenic species *Datura metel* and may significantly be contributed to airspora. According to the previous report (Paruin and Mondal, 1998), two proteins (66.0 kDa and 59.4 kDa) have been found to be allergenic in *Datura metel*. These two proteins were also found to be common in *Datura stramonium* and *Datura innoxia*. According to Tilak (1989) , entomophilous pollen may be present in great amount near the source plant and may be responsible for causing a risk to sensitive patients. Further, the variation in the protein content as well as the profile with the different stages of maturity shows the need for proper standardization of the pollen extracts and designing standardized immunotherapeutic vaccines for effective allergen specific immunotherapy (AIT).

## Legends to figures:

**Fig. 1 (A,B &C):** The plants of *Datura metel*, *Datura innoxia* and *Datura stramonium* respectively in full bloom.

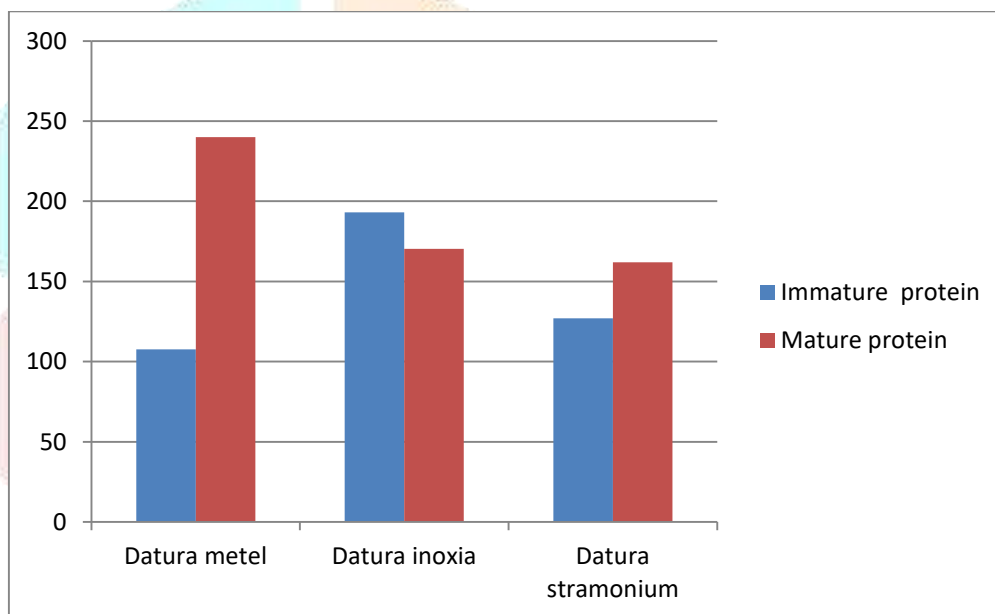
**Fig. 2 (A,B &C):** Light Microscopic images of pollen of *Datura metel*, *Datura inoxia* and *Datura stramonium* respectively to show 90% pollen purity.

**Fig. 3:** SDS-PAGE protein profile of the pollen of *Datura metel*, *Datura stramonium* and *Datura inoxia* marker (A) (66 kDa) BSA, (C), (E) & (G) 15µl, 10 µl and 5µl of protein sample respectively.

**Fig. 4:** Diagrammatic representation of the protein profile of the pollen of *Datura metel*, *Datura stramonium* and *Datura inoxia* marker (A) BSA, (B), (C) & (D) 15µl, 10 µl and 5µl of protein sample respectively.

**Table 1: Comparative Protein concentration of the pollen of *Datura metel*, *Datura inoxia* and *Datura stramonium* .**

Pollen Proteins	Mature (µg/ml)	Immature (µg/ml)
<i>Datura metel</i>	260	240
<i>Datura inoxia</i>	170.28	193.14
<i>Datura stramonium</i>	162	127



**Graph 1: Graphical representation of the protein production by mature and immature pollen of *Datura metel*, *Datura inoxia* and *Datura stramonium*.**

**Table2: SDS-PAGE protein profile of the pollen of *Datura metel***

M.W of Marker Proteins	Protein Bands	M.W in (kDa)
	d1	>205
	d2	>205
205	d3	205.0
	d4	183.5
	d5	172.7
	d6	152.0
	d7	129.7
	d8	108.2
97.4	d9	97.4
66	d10	66.0*

	d11	59.4*
	d12	56.2
	d13	49.8
43	d14	43.0
	d15	40.5
	d16	37.5
	d17	35.3
	d18	33.2
	d19	31.6
29	d20	29.0

Table 3: SDS-PAGE protein profile of the pollen of *Datura innoxia*

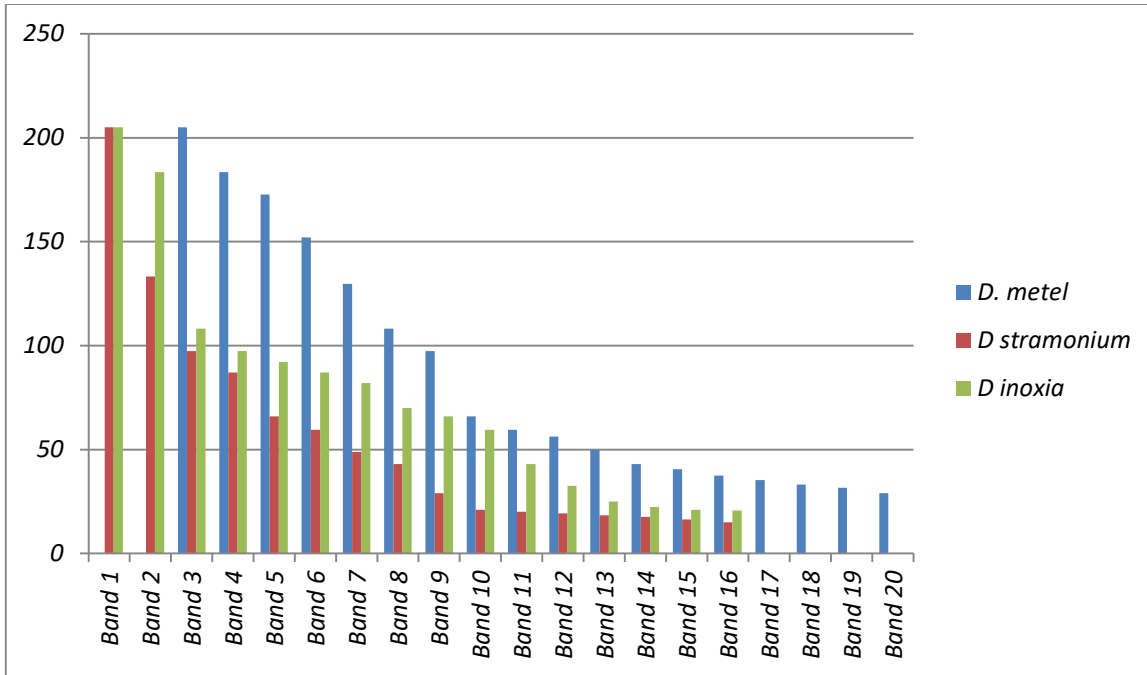
M.W of Marker Proteins	Protein Bands	M.W in (kDa)
205	D1	205.0
	D2	183.5
	D3	108.2
97.4	D4	97.4
	D5	92.1
	D6	87.0
	D7	82.0
	D8	70.0
66	D9	66.0*
	D10	59.4*
43	D11	43.0
29	D12	32.5
	D13	24.9
	D14	22.4
21.0	D15	21.0
	D16	20.6
14.3		

Table4: SDS-PAGE protein profile of the pollen of *Datura stramonium*

M.W of Marker Proteins	Protein Bands	M.W in (kDa)
205	D'1	205.0
	D'2	133.3
97.4	D'3	97.4
	D'4	87.0
66.0	D'5	66.0*
	D'6	59.4*
	D'7	48.8
43	D'8	43.0
29	D'9	29.0
	D'10	21.0
20.1	D'11	20.1
	D'12	19.2
	D'13	18.3
	D'14	17.6
	D'15	16.4
	D'16	15.0



14.3	
	Common proteins of all three species
	Common protins between <i>D. metel</i> and <i>D. inoxia</i>
	Common proteins between <i>D.metel</i> and <i>D. stramonium</i>
	Common proteins between <i>D.stramonium</i> and <i>D. inoxia</i>
	* Allergenic proteins among three species



Graph 2: Graphical representation of the SDS-PAGE protein profile of the pollen of *Datura metel*, *Datura stramonium* *Datura inoxia*

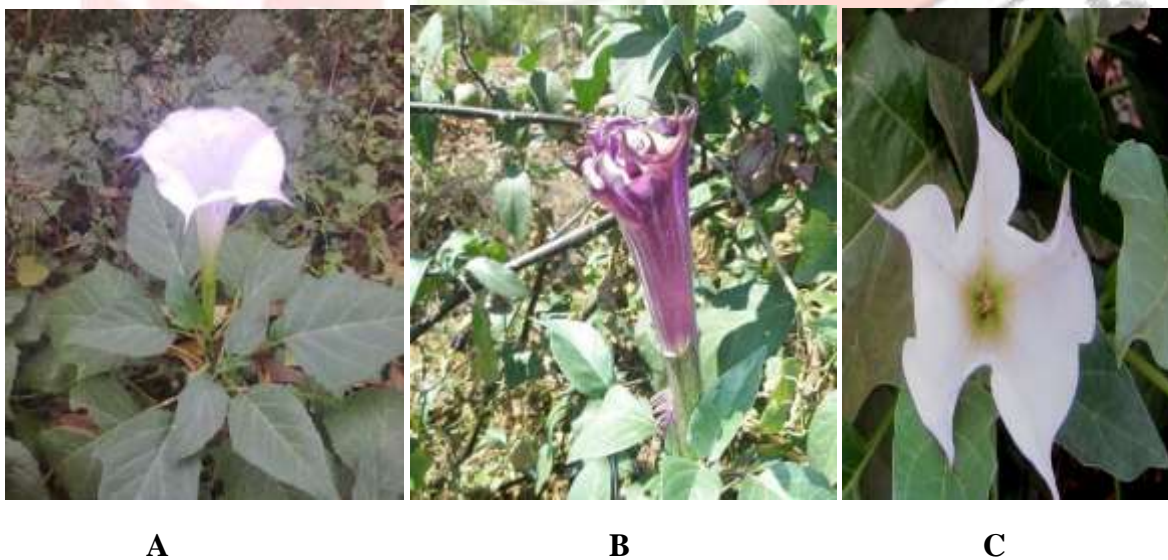
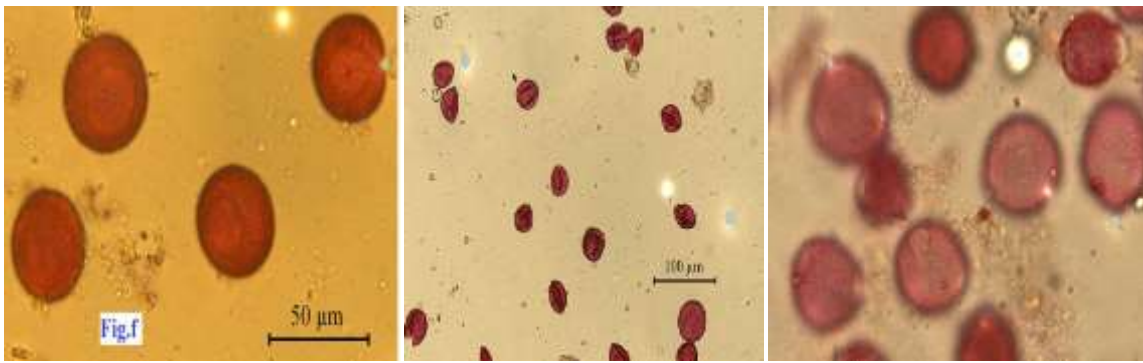


Fig: 1



D

E

F

Fig: 2

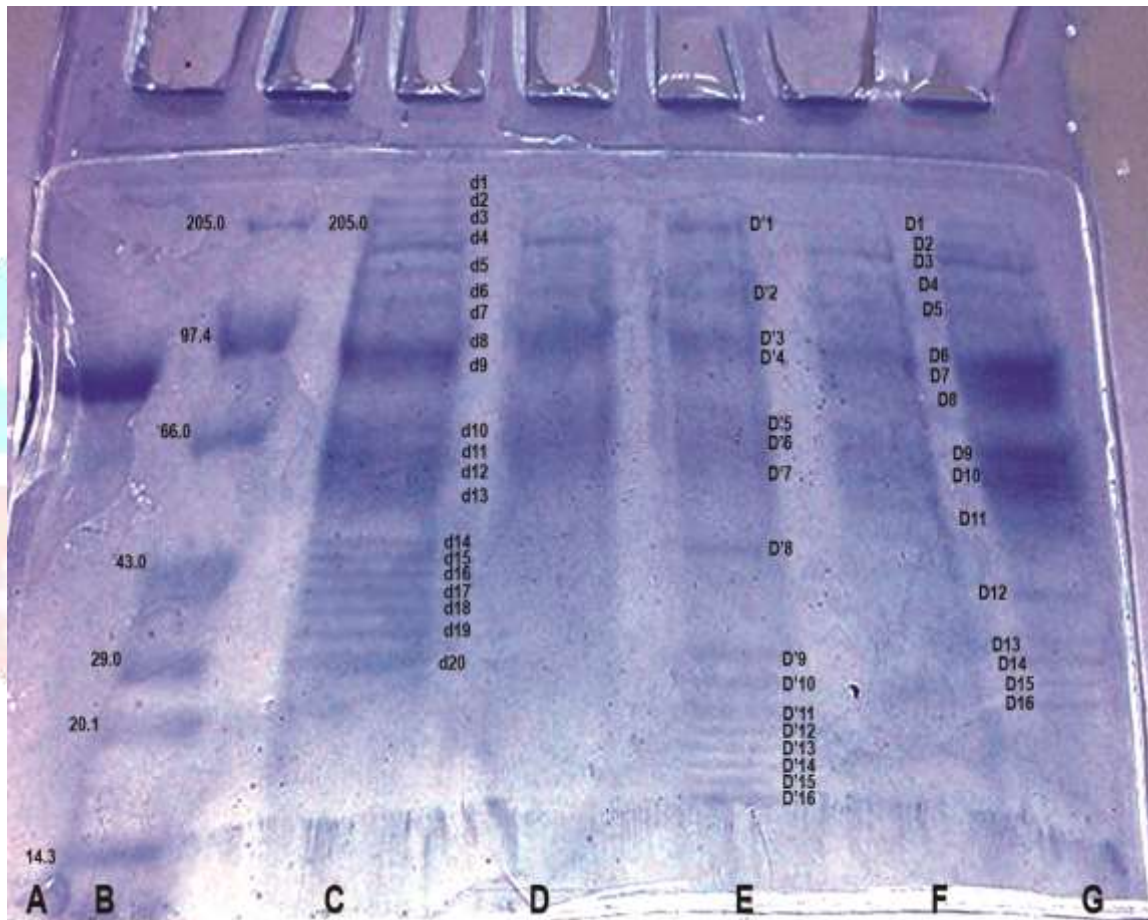


Fig: 3

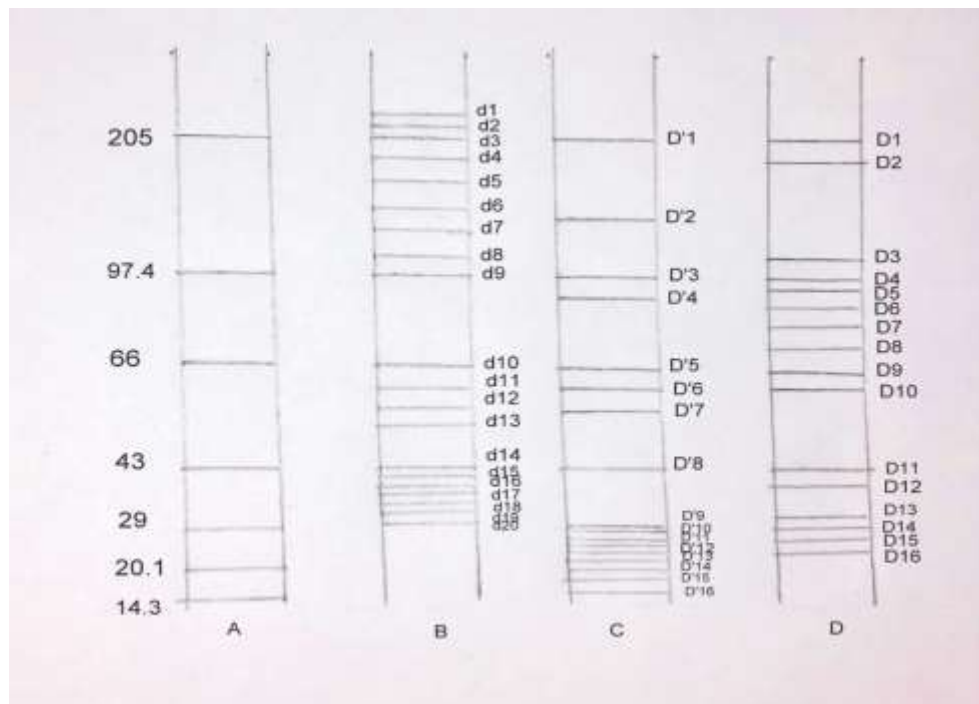


Fig: 4

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