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NOTE

Chemical Examination of the Seeds of *Tecoma Stans*

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In the present note we wish to report our results of chemical examination of the seeds of *Tecoma stans*.

Tecoma stans[1-3] belongs to *Bigmoniaceae*, which is commonly known as 'Pachagotla' in Telugu. It is found up to an altitude of 1500 meters in the hill areas. Its root is used as a powerful diuretic, vermifuge and used as antidote for snake bites and in beer making.

The seeds of plant were collected from Shidh Seeds Corporation, Dehradun (U.P.). 10 g of seed powder was refluxed with small quantity of calcium carbonate and 100 mL of distilled water for 1 h. The aqueous extract was separated by decantation and powder was further refluxed thrice with 50 mL of distilled water each time. The aqueous filtrates were combined and 10% solution of lead acetate was added till the precipitate was complete. It was filtered and H_2S gas was passed through the filterate. It was filtered and the filterate was neutralised with ammonia. This neutral solution was concentrated on water bath till the volume become 100 mL.

Identification of Reducing Sugars

For identification of sugars the spot of the concentrated test mixture and authentic sugars were applied on whatman No. 1 paper and chromatograms were developed in *n*-butanol : acetic acid : water (4:1:5, upper layer) solvent system. After developing the chromatogram was sprayed with anisaldehyde sulphuric acid reagent. The identify of test sugars were confirmed by comparision of their R_f values with those of authentic sugars. (Table-1)

TABLE-1
R_f VALUES OF SUGARS

S. No.	Name of Reducing Sugar	R_f Reported	R_f Observed
1.	Arabinose	0.21	0.20
2.	Galactose	0.16	0.16
3.	Glucose	0.18	0.18
4.	Rhamnose	0.37	0.38

The amount of reducing sugars were estimated (as glucose) by Fehling's method using methylene blue as indicator. Thus the percentage of reducing sugars were found to be present in the solution is 3.52% (as glucose).

Identification of Amino Acids

Isolation of Crude Protein: 100 g of defatted seed powder was macerated with brine solution at room temperature the mixture was centrifuged and supernatant liquid was decanted. The residue was again stirred with brine solution and centrifuged. This process is repeated till the liquid was negative to biuret test. To the combined supernatant, 6N HCl was added to precipitate the crude protein. The mixture was centrifuged and crude protein (6.0%) was obtained.

Acid Hydrolysis of Crude Protein: 100 mg of crude protein was hydrolysed by refluxing with 100 mL of 6N HCl for 20 h at 105–110°C. The solution was decolourised by animal charcoal and hydrolysate was dissolved in water (30 mL) filtered and concentrated to dryness. The excess of acid was removed by repeated dissolving in water and evaporations, finally dissolved in 10% isopropanol. The solution was subjected to descending paper chromatography[4, 5] developed in the solvent system *n*-butanol : glacial acetic acid : water (4:1:5, upper layer) and sprayed with ninhydrin in 95% butanol containing 5% 2N acetic acid. Amino acids were identified by co-chromatography with authentic samples. R_f values are reported in the Table-2

TABLE-2
PERCENTAGES OF AMINO-ACIDS

S. No.	Amino Acid	R_f Reported	R_f Obtained	Optical density	% age of amino acid
1.	Alanine	0.60	0.59	0.35	6.30
2.	Glycine	0.20	0.20	1.01	18.23
3.	Glutamic acid	0.51	0.51	1.32	23.56
4.	Isoleucine	0.80	0.79	0.38	6.82
5.	Methionine	0.71	0.72	0.33	5.80
6.	Phenyl alanine	0.55	0.55	0.28	5.06
7.	Proline	0.30	0.30	0.48	8.64
8.	Serine	0.18	0.17	0.40	7.20
9.	Threonine	0.24	0.26	0.25	4.32
10.	Tryptophan	0.63	0.62	0.19	3.42
11.	Tyrosine	0.42	0.42	0.18	3.25
12.	Valine	0,37	0.35	0.42	7.37

Quantitative Estimation of Amino Acids

The modified spectrophotometric method suggested by Moore and Stein[6] was used for the quantitative estimation of amino acids. Standard solutions of 0.05, 0.10, 0.15, 0.20 and 0.25% of glycine in 10% isopropanol were applied on whatman No. 1 paper and developed in *n*-butanol : acetic acid : water (4:1:5).

The paper was sprayed with ninhydrin solution. The spots were eluted with 5 mL of 10% isopropanol. The optical densities of known and unknown amino acid solutions were measured by UV at max. wave length (around 250 nm). A graph was plotted between optical density and concentration of glycine. The concentration of amino acids present in seed protein was obtained from the graph of glycine by interplotting their optical densities. The amino acids percentages were calculated from their concentration are presented in Table-2.

The the reducing sugars present in seeds of *Tecoma stans* is 3.52% (as glucose) which contain arabinose, galactose, glucose, and rhamnose. The percentage of various amino acids present in the crude protein (6.0%) were found to be alanine (6.30%), glycine (18.23%), glutamic acid (23.56%), isoleucine (6.82%), methionine (5.80%), phenyl alanine (5.06%), proline (8.64%), serine (7.20%), threonine (4.32%), trytophane (3.44%), tyrosine (3.25%) and valine (7.37%).

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