NOTE

Chemical Investigation of the Seeds of *Glircidia maculata*

E. SREEDEVI and J.T. RAO*
Department of Chemistry
Dr. Hari Singh Gour University, Sagar-470 003, India

The reducing sugars and proteins have been analysed from the seeds of *Glircidia maculata*. The seeds having high nutritional properties are used in cattle feed industries.

Glircidia maculata[1-3] belongs to leguminosae, which is commonly known as 'Ration' in English and is widely cultivated in India as a shady and cooling tree. Leaves are used in the treatment of urticaria, rash and burns. Aerial parts are used as spasmodic, CNS depressant and diuretic etc.

Analysis of reducing sugars: The seeds of the plant were collected from Shidh Seeds Sales 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 the 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 precipitation was complete. It was filtered and H_2S gas was passed through the filtrate to remove the excess of lead acetate. It was again filtered and the filtrate was neutralised with ammonia. This neutral solution was concentrated on water bath till the volume became 100 mL.

Identification of reducing sugars: For idenification of sugars the spots of the concentrated test mixture and authentic sugars were applied on Whatman No. 1 paper and chromatograms were developed in an *n*-butanol : acetic acid : water (4 : 1 : 5, upper layer) solvent system. After developing, the chromatogram was sprayed with anisaldehyde sulphuric acid reagent. The identity of test sugars were confirmed by comparison of their R_f values with those of authentic sugars (Table-1). The amounts of reducing sugars were estimated (as glucose) by Fehling's method using methylene blue as indicator. Thus the percentage of reducing sugars as found to be present in the solution is 6.78% (as glucose)

TABLE-1

S. No.	Name of reducing sugar	R_f reported	R_f observed
1.	D-glucose	0.18	0.18
2.	D-fructose	0.23	0.23
3.	Lactose	0.09	0.09
4.	D-galactose	0.16	0.16

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 the supernatant liquid was decanted. The residue was again stirred with brine solution and centrifuged. This process was repeated till the liquid was negative to biuret test. To the combined supernatant, 6 N HCl was added to precipitate the crude protein. The mixture was centrifuged and crude protin (9.6%) was obtained.

Acid Hydrolysis of Crude Protein: 100 mg of crude protein was hydrolysed by refluxing with 100 mL of 6 N HCl for 20 h at 105–110°C. The solution was decolorised by animal charcoal and the hydrolysate was dissolved in water (13 mL), filtered and concentrated to dryness. The excess of acid was removed by repeated dissolving in water and evaporation, and finally dissolving 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% to 2 N acetic acid. Amino acids were identified by co-chromatography with authentic samples. R_f values are reported in Table-2.

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%, 0.15%, 0.20%, 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 amino acid solutions were measured by UV at max. wavelength (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 interpolating their optical densities. The amino acid percentages calculated from their concentration are presented in Table-2.

<div align="center">TABLE-2</div>

S.No.	Amino acid	R_f reported	R_f observed	Optical density	Percentage of amino acids
1.	Arginine	0.39	0.38	0.86	9.06
2.	Glutamic acid	0.51	0.51	0.52	5.48
3.	Glycine	0.20	0.20	0.91	9.58
4.	Histidine	0.08	0.08	0.80	8.42
5.	Isoleucine	0.80	0.81	0.83	8.74
6.	Leucine	0.61	0.60	0.62	6.53
7.	Lysine	0.12	0.13	0.95	10.01
8.	Phenylalanine	0.55	0.54	0.77	8.11
9.	Threonine	0.24	0.24	0.82	8.64
10.	Tryptophan	0.63	0.63	0.79	8.32
11.	Tyrosine	0.42	0.41	0.74	7.79
12.	Serine	0.18	0.18	0.88	9.27

Thus the reducing sugars present in the seeds of *Glircidia maculata* are 6.78% (as glucose) containing D-glucose, D-fructose, lactose and D-galactose. The percentages of various amino acids present in the crude protein (9.6%) were found to be arginine (9.06%), glutamic acid (5.48%), glycine (9.58%), histidine (8.42%), isoleucine (8.74%), leucine (6.53%), lysine (10.01%), phenylalanine (8.11%), threonine (8.64%), tryptophan (8.32%), tyrosine (7.79%) and serine (9.27%).

ACKNOWLEDGEMENT

REFERENCES

1. The Wealth of India: A Dictionary of Raw Materials and Industrial Products, CSIR Publications, New Delhi, Vol. IV, p. 138 (1950).
2. V.S. Agarwal, Economic Plants of India, Kailash Prakashan, Calcutta, p. 155 (1986).
3. K.K. Asolkar, K.K. Kakkar and O.J. Chakre, Second Supplement to Glossary of Indian Medicinal Plants with Active Principles, Publications and Information Directorate (CSIR), p. 329 (1992).
4. E. Leadrer and M. Leadrer, Chromatography, Elsevier, p. 247 (1957).
5. K.V. Giri, S. Radhakrishnan and C.S. Vidyanathan, *Anal. Chem.*, **24**, 1677 (1952).
6. W.H. Stein and S. Moore, *J. Biol. Chem.*, **176**, 367 (1948).