

Chemical Examination of the Seeds of *Cassia tora*

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The carbohydrates and proteins have been analyzed from the seeds of *Cassia tora*. The seeds having high nutritional properties are useful in cattle feed industries.

Key words: Chemical examination, *Cassia tora*.

INTRODUCTION

Cassia tora belongs to Leguminosae, which is commonly known as *Punwar* in Hindi. Seeds contain a glycoside and a pleasant smelling fixed oil. The oil is reported to contain oleic, linoleic, palmitic and lignoceric acids¹⁻³. The seeds also contain β -sitosterol⁴, gums⁵, oxytocic principle⁶, chrysophanol⁷ and some crystalline substances⁸.

EXPERIMENTAL

The seeds of the plant were collected from nearby area of Khandwa city. 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 H₂S gas was passed through the filtrate to remove the excess of lead acetate. It was again filtered and the filtrate was neutralized with ammonia. The neutral solution was concentrated on a water bath till the volume became 100 mL.

Identification of Reducing Sugars

For identification of reducing sugars the spot of the concentrated test mixture and authentic sugars were applied on Whatmann No. 1 paper and chromatograms were developed in *n*-butanol : pyridine : water (6 : 4 : 3). After developing the chromatogram it was dried and sprayed with *p*-amino phenol phosphoric acid reagent (0.15 g *p*-amino phenol in 20 mL ethanol and 10 mL 50% H₃PO₄). Finally it was dried in an air oven at 105°C for 15 min. R_f values of the sugars were calculated and tabulated in Table-1. The identity of test sugars was confirmed by comparison on their F_{T0} values with those of authentic sugars.

TABLE-1
R_f VALUES OF REDUCING SUGARS

Name of reducing sugar	R _f reported	R _f observed
Xylose	0.82	0.81
D-glucose	0.70	0.70
D-galactose	0.62	0.61
Raffinose	0.29	0.29

The amount of reducing sugars was estimated (as glucose) by Folin and Wu method¹⁰, by colorimeter using red filter (620 nm). Thus the percentage of reducing sugars found to be present in the solution was 3.14% (as glucose).

Isolation of Crude Protein: 100 g of defatted seed powder was mercerated 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 protein (15.8%) was obtained.

Acid Hydrolysis of Crude Protein: 100 mg of crude protein was hydrolyzed by refluxing with 100 mL of 6 N HCl for 20 h at 105° and 100°C. The solution was decolorized 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. The solution was subjected to purification by ion exchange chromatographic method described by Thompson *et al.*¹¹

Chromatographic Identification of Amino Acids: The mixture of amino acid was dissolved in 10% isopropanol. The amino acid mixture was analyzed by two-dimensional paper chromatography on Whatmann filter paper No. 1. The following solvent systems were employed as described by Block *et al.*¹² and Stein *et al.*³: (1) Phenol : Water : Ammonia (50 : 20 : 1) in first direction; (2) Butanol : Acetic acid : Water (4 : 1 : 5) in second direction (at 90° from the first direction).

The chromatograms after development were dried in air followed by blowing hot air to remove traces of phenol. Authentic samples of pure amino acids (10–20 µg) dissolved in isopropanol were also run under identical conditions (for reference along with the test samples.).

The spots were located by spraying with ninhydrin solution (0.2% in acetone w/v). The various coloured spots thus obtained were identified by comparing their R_f values with those of authentic samples (Table-2).

Quantitative Estimation of Amino Acid: The modified spectrophotometric method suggested by Moore and Stein¹³ was used for the quantitative estimation of amino acids. Standard solution of 0.25%, 0.20%, 0.15% and 0.05% of glycine and test mixture in 10% isopropanol were applied on Whatman No. 1 paper and developed in *n*-butanol : acetic acid : water (4 : 1 : 5) in one direction^{12, 13}. The chromatograms were dried, sprayed with ninhydrin (0.2% in acetone), dried in an air oven followed by blowing hot air to remove traces of phenol. The spots

were eluted with 5 mL of 10% isopropanol. The optical densities of known amino acid solutions were measured by spectrophotometer at max. wavelength (250 mm). 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. Percentage of amino acids was calculated from their concentration (Table-2).

TABLE-2

S.No.	Amino Acids	percentage of Amino Acids
1.	α -alanine	8.2
2.	Aspartic acid	10.23
3.	Argenine	9.54
4.	Citrulline	0.89
5.	Cystine	3.12
6.	Glycine	7.23
7.	Gultamic acid	9.28
8.	Histidine (e)	6.29
9.	Hydroxyproline	0.31
10.	Isoleucine	4.20
11.	Leucine	7.37
12.	Lycine	8.42
13.	Phenyl alanine	1.60
14.	Proline	3.26
15.	Serine	2.82
16.	Threonine	5.29
17.	Tyrosine	2.61
18.	Tryptophane	2.63
19.	Valine	5.75
20.	Unidentified spot	0.98

RESULTS AND DISCUSSION

Thus the reducing sugars in the seeds of *Cassia tora* (3.14%) contain xylose, D-glucose, D-galactose and raffinose. The percentages of various amino acids present in the crude protein (15.8%) were found to be alanine (8.2%), aspartic acid (10.23%), argenine (9.54%) cituline (0.89%), cystine (3.12%), glycine (7.23%), glutamic acid (9.28%), histidine (6.29%), hydroxy proline (0.31%), isoleucine (4.20%), leucin (7.37%), lysine (8.42%), phenyl alanine (1.6%), proline (3.26%), serine (2.82%), threonine (5.29%), tyrosine (2.61%), tryptophane (2.12%), valine (5.73%). One unidentified amino acid having per cent composition (0.98%) was also present.

The total per cent composition of essential amino acids in the protein

hydrolysate of the seeds was 51.09%. It was observed that essential amino acid contents of the protein hydrolysates are comparable with those of groundnut seeds. The above seeds may be used as supplementary protein sources. The presence of very rarely available citrulline in the seeds of *Cassia tora* was remarkable.

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