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NOTE

Chemical Investigation of the Seeds of *Schleichera Trijuga*

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In the present work, the authors report the chemical investigation of the seeds of *Schleichera trijuga.*

Schleichera trijuga[1–3] belongs to the natural family of Sapindaceae and is known as "Kusum" in Hindi and "Posuku" in Telugu. It is distributed throughout the Indo-Malaysian region. A medium sized to large, deciduous or nearly evergreen tree, up to 32.3 m in height and 2.4–3.7 m in girth, usually with clear bole of 0.6 m, found in the sub-Himalayan tract from Kashmir to West Bengal and in the Central and Peninsular India, up to an altitude of 900 m, especially in the moisture localities, also recorded from the Garo hills.

Seeds of *Schleichera trijuga* yield a fatty oil called macassar oil, which is used for rheumatism, skin troubles and also for culinary and lighting purposes; flowers yield a dye, and bark is used in applications for itch, pain in the back and loins, inflammations and ulcers. The tree is an important host for Kusmilac. The seeds of *Schleichera trijuga* were procured from Shidh Seeds Corportion, Dehradun (U.P.), India.

Air dried powdered seeds (20 g) were extracted with dilute aqueous calcium carbonate solution (200 mL), by refluxing for 3–4 h on steam bath. The reaction mass was allowed to cool and settled. The supernatant liquid was decanted and the process of refluxing, settling and decanting was repeated three more times using 100 mL of distilled water each time. All the aqueous extracts were combined and to it was added 10% solution of lead tetraacetate to ensure complete precipitation. The mass was filtered and hydrogen sulphide gas was bubbled through the filtrate to precipitate excess lead tetraacetate as lead sulphide. Again the mass was filtered and the filtrate was neutralized with ammonia solution. The neutralized aqueous solution was concentrated on water bath under reduced pressure to result in a residual syrupy mass which is used for the identification of reducing sugars.

Identification of Reducing Sugars

Using Whatman No. 1 paper and mobile phase n-butanol : acetic acid : water (4 : 1 : 5 v/v)[4], chromatograms were developed for both test mixture and authentic sugars; dried and sprayed with aniline hydrogen phthalate[5] reagent when colour sopts were observed in a few minutes. The identity of test sugars was confirmed by comparison of their R_f values with those of authentic sugars (Table-1).

TABLE-1
MOBILE PHASE (1) n-BUTANOL : ACETIC ACID : WATER (4 : 1 : 5 v/v)
Schleichera trijuga

S. No.	Name of Sugar	R_f value observed	R_f value reported
(1)	D-Glucose	0.18	0.18
(2)	D-Fructose	0.23	0.23
(3)	L-Rhamnose	0.36	0.37
(4)	Lactose	0.09	0.09
(5)	D-Galactose	0.16	0.16
(6)	Maltose	0.11	0.11
(7)	D-Arabinose	0.20	0.21

The amount of reducing sugars was estimated (as glucose) by Fehling's method using methylene blue as indicator. Thus the percentage of reducing sugars found to be present in the solution is 13.5% (as glucose).

Identification of Amino Acids

Isolation of Crude Protein: 100 g of defatted seed powder was macerated with sodium chloride solution at room temperature and the mixture was centrifuged and the supernatant liquid was decanted. The residue was repeatedly stirred with sodium chloride solution and decanted till the last washing liquid was negative to biuret test. To the combined supernatant liquid, 6 N hydrochloric acid was added to precipitate the crude protein. The mass was centrifuged and crude protein 21% 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 hydroysate 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 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, 0.10, 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. 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 interplotting their optical densities. The amino acids percentages calculated from their concentration are presented in Table-2.

TABLE-2
PERCENTAGE OF AMINO-ACIDS

S. No.	Amino acid	R_f Reported	R_f Obtained	Optical density	% of amino acid
1.	Alanine	0.60	0.59	0.35	7.40
2.	Glycine	0.20	0.20	1.01	16.00
3.	Glutamic acid	0.51	0.51	1.32	18.50
4.	Isoleucine	0.80	0.79	0.38	7.20
5.	Methionine	0.71	0.72	0.33	8.20
6.	Phenyl alanine	0.55	0.55	0.28	4.10
7.	Proline	0.30	0.30	0.48	7.20
8.	Serine	0.18	0.17	0.40	6.20
9.	Threonine	0.24	0.26	0.25	5.42
10.	Tryptophan	0.63	0.62	0.19	5.60
11.	Tyrosine	0.42	0.42	0.18	4.30
12.	Valine	0.37	0.35	0.42	6.45
13.	Histidine	0.08	0.08	0.80	3.72

Thus the reducing sugars present in seeds of *Schleichera trijuga* is 13.5% (as glucose) which contain D-glucose, D-fructose, L-rhamnose, lactose, D-galactose, maltose and D-arabinose. The percentages of various amino acids present in the crude protein (13.5%) were found to be as follows:

Amino Acid	% of amino acid
Alanine	7.40
Glycine	16.00
Glutamic acid	18.50
Isoleucine	7.20
Methionine	8.20
Phenyl alanine	4.10
Proline	7.20
Serine	6.20
Threonine	5.42
Tryptophan	5.60
Tyrosine	4.30
Valine	6.45
Histidine	3.72

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