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STUDIES ON CARBOHYDRATE AND AMINO ACID COMPOSITION OF *THESPESIA POPULNEA* AND *T. POPULNEOIDES*

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Abstract

Amino acids and carbohydrates present in the seeds of *Thespesia populnea* and *T. populneoides* were studied. The two species were found to have marked differences in amino acid composition. Alanine, methionine, tryptophan and arginine were found to be present in *T. populneoides* only. In *T. populnea* the carbohydrate composition showed the presence of common sugars except mannose detected in polysaccharide form. Differences in the composition of amino acids and carbohydrates provides a basis for distinction of the 2 species.

Introduction

Thespesia populnea (L.) Sol. ex Corr. and *T. populneoides* (Roxb.) Kostel of the family Malvaceae are important taxa both from pharmacognostic and taxonomic point of view. Whereas the chemical constituent and medicinal importance of *T. populnea* have been reported (Nadkarni, 1954; Chadha, 1976; Hopkins & Chrisholm, 1960; Osman *et al.*, 1968; Rajiah & Subbaram, 1974; Gibbs, 1974), there does not appear to be any report on pharmacognostical, phytochemical or biochemical studies on *T. populneoides*. Although *T. populneoides* was described by Roxburgh as early as 1832 (under *Hibiscus populneoides*) the later studies have only been reported under the name of *T. populnea*. Taxonomically there has been a controversy whether *T. populneoides* is synonymous with (Hutchinson, 1947; & Borssum 1966) or distinct from *T. populnea* (Fosberg & Sachet 1972; Abedin 1977). The present paper describes the phytochemical studies on the two taxa which confirm the views of Roxburgh (1832) Fosberg & Sachet (1972) & Abedin (1977).

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Table 1. Separation and identification of carbohydrates of *Thespesia populnea* (A) and *T. populneoides* (B) with respect to standard samples in different solvent systems.

Solvent system	BAW_1			BAW			PPWA			EAW			EPW		
Standard samples	Rfs.* Std.**	Rfs. A	Rfs. B	Rfs. Std.	Rfs. A	Rfs. B	Rfs. Std.	Rfs. A	Rfs. B	Rfs. Std.	Rfs. A	Rfs. B	Rfs. Std.	Rfs. A	Rfs. B
Raffinose	0.091	+(0.089)		0.085	+(0.083)		0.121	+(0.118)		0.084	+(0.087)		0.220	+(0.222)	
Trehalose	0.125	+(0.124)		0.137	+(0.131)		0.149	+(0.146)		0.129	+(0.127)		0.249	+(0.250)	
Lactose	0.129		+(0.128)	0.150		+(0.148)	0.167		+(0.163)	0.236		+(0.249)	0.304		+(0.307)
Maltose	0.141			0.171			0.208			0.185			0.294		
Galactose	0.206		+(0.213)	0.241		+(0.244)	0.232		+(0.230)	0.310			0.340		
Sucrose	0.222	+(0.224)		0.239	+(0.233)		0.230			0.322	+(0.326)		0.329	+(0.329)	
Glucose	0.228			0.254			0.331			0.334			0.331		
Fructose	0.247			0.279			0.234			0.360			0.330		
Arabinose	0.252			0.278			0.241			0.363	+(0.367)		0.343		
Mannose	0.225	+(0.254)		0.270	+(0.264)		0.237	+(0.236)		0.335			0.352		
Ribose	0.330			0.307			0.318			0.402			0.380		
Rhamnose	0.412			0.316			0.396			0.429			0.386		

* Rfs. = Rf values., ** Std. = Standard.

BAW_1 = Butanol: acetic acid: water upper layer, (4:1:5): BAW = Butanol: acetic acid: water (12:3:5): PPWA = Propanol: Pyridine: water : acetic acid (8:8:4:1): EAW = Ethyl acetate: acetic acid: water (3:3:1): EPW = Ethyl acetate: pyridine: water (10: 4: 3): All solvents were from E. Merck-Darmstadt Analytical grade; pyridine was distilled over ninhydrin and water used was double distilled de-ionized. The separations were achieved in above solvent systems with better separations in BAW_1

Materials and Methods

Seeds from mature fruits of *T. populnea* and *T. populneoides* growing at the Karachi University Campus were collected and ½ Kg of each were separately ground and extracted in ethyl alcohol. The alcoholic extracts were concentrated in vacuo at 20°C and the viscous dark brown materials of *T. populnea* and *T. populneoides* obtained were partitioned in ethyl acetate and distilled water fractions. The water extracts of *T. populneoides* and *T. populnea* showed the presence of carbohydrates and amino acids.

The water soluble fractions of *T. populnea* and *T. populneoides* were separated on cation exchange resin IR 120 X 8 column 30 X 1.59 cm. at 55°C. The separation was achieved by first eluting the column with 0.02N acetic acid followed by water and then with 0.5N ammonia. The acid and water fractions were pooled together, evaporated and carbohydrate contents determined. The ammonia fraction was evaporated and amino acids were analyzed. The free amino acid fraction eluted from the IR 120 column was subjected to paper chromatography. Analysis was also carried out on Beckman 120-C Automatic Amino Acid Analyser.

Acetone was added to the water extracts of the two species in order to identify the presence of polysaccharides. In *T. populnea* there was no insoluble fraction, and that monosaccharide sugars were only present, while in *T. populneoides* the precipitates indicated the presence of polysaccharides. Acetone insoluble fraction (1 mg) was hydrolysed in 2N H_2SO_4 in a sealed tube for 4 hours at 100°C. The hydrolysed sample was paper chromatographed. The carbohydrate contents were identified by paper chromatography (Whatman No. 1) using the solvent systems as given in Table 1.

Results and Discussion

Studies on carbohydrate fraction showed the presence of only monosaccharides in *T. populnea* and that *T. populneoides* contains polysaccharide as well (Table 1). The sugars reported in these species commonly occur in other plants. Mannose is known to be present in polysaccharide form in plant (Alston & Turner, 1963; Gibbs, 1974). Our studies show that mannose is present in free form in *T. populnea* and that in this species no soluble polysaccharide could be identified. This probably could be the reason for its free state. Lactose has been reported in a few plants (Gibbs, 1974) and its presence in *T. populneoides* is reported herein.

Our results show that alanine, methionine, tryptophan and arginine are absent in *T. populnea* (Table 2). These are however, present in *T. populneoides*. On the other hand homocitrulline and lysine are present in *T. populnea* and absent in *T. populneoides*. Further, quantitative differences were found in both the taxa. The amino acids reported

Table 2. Free Amino acid composition of *Thespesia populnea* and *T. populneoides*.

Amino acid	Test plant	
	T. populnea (μ g/100 mg)	*T. populneoides* (μ g/100 mg)
Cys 0_3H	12.23	10.91
Asp	8.38	2.04
Thr.	0	1.21
Ser	10.20	1.33
Glu	2.32	1.49
Pro.	0	traces
Gly	9.12	7.52
Ala	0	2.58
Homocit	present	–
Val	18.38	2.04
Ile	18.37	11.42
Leu	2.04	8.63
Met	0	traces
Tyr	4.08	–
Trp.	0	present
Phe	6.12	9.37
Lys.	3.24	1.15
His	1.08	2.15
Arg	0	4.48

herein are common in plants except homocitrulline. Cystine/cysteine is estimated as cysteic acid. Cystine is the reduced form and it is probable that cysteine is present in *T. populneoides* instead of cystine in other plants as suggested by Gibbs (1974). Similarly tyrosine has been reported as proteogenic amino acid in various families (Gibbs, 1974). Studies presented here confirms our earlier differentiation of *Thespesia* spp. on morphological basis (Abedin, 1977).

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