Protein and Carbohydrate Contents of *Salvadora oleoids*

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Summary: The chemical constituents of the leaves and the roots of *Salvadora oleoides* have been isolated and characterized. Their protein and carbohydrate content is studied in detail. The protein pattern from the leaves resembles that of other green leaves plant, however the protein fraction I is higher. The amino acid compositions of the leaf proteins and root peptides have been reported. Mono-, and disac-charides from both leaves and roots are also characterized. Their inorganic constituent is found to be very high, and has been analysed.

Introduction

Salvadora oleoides belongs to the family *Salvadoraceae.* The two species of this genus, i.e., *Salvadora percica* Linn. and *Salvadora oleoides* Decen are widely distributed in Pakistan.[1] Different parts of the plant have been used for various purposes,[2,3] particularly roots used in folk medicine for cure of blisters and rheumatism.[4,5]

The seeds have a high fat content and are used in the soap industry.[6,7] Gunde and Hilditch[8] have reported a detailed analysis of fatty acid composition of the seeds. Mitra and Misra[9] have reported that the seed-cake also contains 26.66% of crude protein and 16% ash. No detailed investigation have been conducted on roots and leaves of this plant.

In this commounication we are reporting the detail study on its carbohydrate and protein contents.

Experimental and results

Samples of the leaves and roots were collected from Thatta (Sind, Pakistan) region in the beginning of November. The samples were washed, and the leaves and roots were separately ground with water and shaken constantly for 8 hours. After leaving overnight the slurry of each was filtered. The dark orange coloured filtrates were concentrated in vacuo at 40°C to a thick syrup. These were left overnight at 4°C, and the white powder obtained by centrifugation was found to contain mostly inorganic salts. The protein and carbohydrate constituents of the residual syrups were studied.

Separation and Identification of Carbohydrates

The syrup from leaves and roots was subjected to an IR–120 mesh column 1.2x50 cm. The carbohydrates were eluted with deionised water. The fractions with positive carbohydrate tests were pooled together, and concentrated.

The carbohydrate fraction was subjected to a cellulose column (1.5 x 60 cm) chromatography. Elution was carried out with pure ethanol followed by a linear gradient of ethanol-water. Fractions of 1 ml. each were collected on an automatic fraction collector. An aliquot from each test tube was examined for the presence of sugar by Molisch test. The mono-and disaccharide separation was achieved. The analysis was carried out by paper chromatography and cellulose t.l.c. plates using ethyl acetate: acetic acid: formic acid:water (18:3:1:4) and phOH:H_2O (8:2) system. Authentic sugars were used as markers, and aniline phthalate as a developing agent.

The polysaccharides precipitated by alcohol were hydrolysed in 2N-HCl for 4 hours; HCl was removed in vacuo and the liberated sugars were identified on paper chromatography using the following solvent system[10,11]

Solvent system I	BuOH:EtOH:H_2O (4:2.2:1)v/v
Solvent system II	BuOH:Pyr.H_2O(1:1:1)v/v
Solvent system III	BuOH:Benz:Pyr:H_2O(5:1:3:3)v/v
Solvent system IV	BuOH:AcOH:H_2O (12:3:5) v/v

The carbohydrates analysed from leaves and roots are given in Table 1.

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Table I

Name of sugars	Leaves		Roots	
	Free sugar	Sugar on hydrolysis of extracts	Free sugar	Sugar on hydrolysis of extracts
Glucose	+	+++	+	+++
Fructose	+	++	+	++
Xylose	traces	+++	traces	+++
Galactose	traces	++		++
Galctouronic acid	+	++		
Mannose	+			
Sucrose	+		+	
Ribose		++		+++

Isolation of Proteins

Proteins and peptides were isloated from the residual syrup of the leaves and of the roots by cellulose column chromatography using solvent system II. A fast-moving yellow substance was eluted first, followed by the sugar, amino acids and protein/peptide fractions.

Electrophoresis of Proteins/Peptides

Electrophoresis was carried out, according to Maurer[12] using 7% acrylamide gel at 5 mA per tube, with bromophenol blue solution being used as marker. The gel tubes were developed by 1% amidoblack 10B in 7% acetic acid solution. The gel electrophoresis revealed that leaves have at least three major detectable proteins, SAL–1 SAL-II and SAL–III' (Fig.1) and that in roots two detectable peptide SAR–I and SAR–II.

The results are given below in Table II:

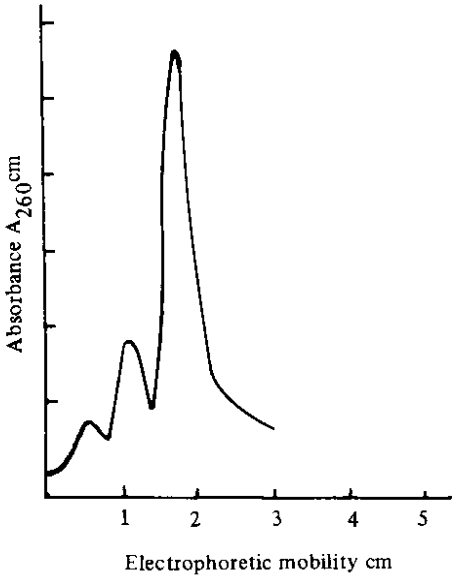

Fig. I Scan of proteins distribution in order of increasing mol. wt. on 7% polyacrylamide gel. The right most peak corresponds to leaf Fraction I-protein.

N–terminal Amino Acid

The purity of the proteins was confirmed by dansylation according to Gray and Hartley.[13] The terminal amino group was coupled with 1–dimethyl-aminonaphthalene-5-sulphonyl chloride (DNS–Cl). Identifica-

Table II

Leaf Protein	Approx %	Colour	M.W.
SAL–I	73	Dark brown	Very high*
SAL–II	22	Greenish brown	Very high*
SAL–III	5	Yellow	–
ROOT PEPTIDES			
SAR–I		Brown	7,000±1000
SAR–II		Dark brown	5,000±1000

*M.W. not measured.

tion was carried out on t.l.c. as DNS–amino acid,[14] using solvent system;benzene, pyridine, acetic acid (40:10:1 v/v) and observing the places under uv light at 254 or 343 nm). The results are given below:

N–terminal Amino Acid

Leaf protein		Root peptide	
SAL–I	SAL–II	SAR–I	SAR–II
Asp	Gly	Asp	Glu

Amino Acid Composition of Proteins

The amino acids present in protein were first identified by paper chromatography using Whatman Paper No.1 (6″ x 6″) according the solvent system modified by us.[15] A known quantity of the protein was hydrolysed with 6N–HCl in a sealed tube at 110°C for 18 hours and taken in a known quantity of 0.2M sodium citrate buffer of pH 3.25. An aliquot of it was analysed on Beckman 120C Automatic Amino Acid Analyser.

The amino acid compositions of the two leaf proteins (SAL–I and SAL–II) and the two root peptides (SAR–I and SAR–II) are shown in table III.

Discussion

Free sugars as glucose, fructose, mannose, sucrose and galactouronic acid were present in the water and

Table III

Amino Acid Composition of Leaf proteins and Root peptides

Names of the amino acids	Leaf proteins				Root peptides			
	SAL–I μ moles/kg		SAL–II μ miles/kg		SAR–I μ moles/kg		SAR–II μ moles/kg	
Gly	49.909	(22.6)*	141.197	(191.6)*	X		t	(164.2)*
Asp	137.923	(62.5)	78.729	(106.8)	204.01	(891.1)*	99.01	(164.2)
Thr	65.74	(29.8)	66.01	(89.6)	t		x	
Ser	115.383	(52.3)	t		24.92	(108.8)	x	
Glu	96.063	(43.6)	91.126	(123.)	x		504.02	(835.8)
Ala	182.213	(82.6)	t		x		t	
Cys	188.124	(85.30)	244.02	(331.1)	(–)		x	
Met	253.038	(114.7)	t		t		(-)	
Ile	574.233	(260.4)	46.68	(63.3)	(-)		(-)	
Tyr	122.762	(55.7)	(-)		(–)		(–)	
Phe	419.807	(190.4)	(–)		t		(–)	
Hyroxy pro	t	t	(–)		x		t	
His	t		t		(–)		(–)	
Arg	t	t	t		(–)		(–)	
Citrulline	(–)		(–)		p		(–)	
Amino butyric acid	(–)		(–)		(–)		p	
Pro	t		t		(–)		t	
Lys	(–)		69.02		(–)		(–)	

t = present in small amount
(–) = absent
p = present in considerable amount but quantitative analysis was not possible as the substance was not present in the standard.
Trp = was not assessed *Residues/1000

alcoholic extract of leaves; galactose and xylose were present in traces only. Each of the monosaccharides, particularly glucose and galactouronic acid increased in quantity on hydrolysis of the two extracts. This shows that these sugars are part of the di-saccharides, polysaccharides and/or are linked to other non-carbohydrate moiety. The above monosaccharides are found in the root extracts as well. Ribose is present in the hydrolysates of leaves and roots, indicating its presence as a moiety attached to some other component. Polysaccharide contents are higher in leaves which is expected; glucose, galactose and xylose form major constituent of these polysaccharides both in leaves and roots with slight variations.

A comparative study of the table III shows that the amino acids present in roots and leaves are not same The roots cells are able to synthesize only acidic amino acids (aspartic acid and glutamic acid) and serine. The other amino acids are present in traces include proline, hydroxy proline, glycine, alanine citrulline and methionine.

The result shows that phenylalanine is present in large amounts in the leaf proteins.

The leaf protein SAL–1 contains high percentage of isoleucine, cystine, methionine and phenyl-alanine, threonine and tyrosine, but small quantity of basic amino acids. SAL–II has a comparatively small quantity of sulphur containing amino acid, and contains lysine in addition. The variable electrophoretic behaviour can be explained due to these amino acid compositions.

SAL–I, SAR–I, and II on hydrolysis were found to contain glucose suggesting these to be glycoproteins, the molecular weight of these could well be lower than that shown by gel chromatography the carbohydrate moiety being responsible for the high figure[16]. SAL–I is present in higher quantity compared to SAL–II while SAL–III is present only in traces.

The soluble leaf protein pattern of this plant resembles with the others green-leaf plants. It has a major soluble protein fraction called Fraction I–protein and accounts for about 73% of the total protein. It is higher than reported for mesophytic plants.[17] This could be attributed to the epistatic genes for the other two protein fractions; probably required for the growth of the plant in harsh conditions (climate, salty soil, water scarcity etc); or the "chloroplast autonomy" is so directed to produce more of fraction I-protein in *Salvadora oleides* to enable to competete with the enhance rate of photosynthesis that amount to RBP–carboxylase due to the intense light condition.[18]

The bi-enzymic activity of the fraction[19] and detailed study on the polypeptide chains[20] are in progress and well be reported later.

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