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NOTE

Detection of Amino Acids from The Stem Bark of *Juglans regia*

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Family *Juglandaceae* includes a valuable, medicinally useful species *Juglans regia*, growing in the forests of Himalayas in India. The root, stem, bark, leaves, seeds, cotyledons and seed oil are used to treat a variety of health complaints. Amino acids play an important role in plant biogenesis. Detection of amino acids was carried out on the stem bark of the species by paper chromatographic method using different mobile phases. Results show the presence of leucine, tyrosine, proline, methionine, ornithine, alanine, *etc*. in the different solvent systems.

Key Words: *Juglans regia*, Amino acids, Paper chromatography, Proline, Alanine.

Juglans regia is a woody, deciduous and frost-tender tree growing in the forests of Himalayas in India. It is a native of eastern Europe to North Asia *i.e.* Iraq, Mexico, Spain, Turkey, China, Nepal and India belonging to family *Juglandaceae*. The tree is in flower in June and seeds ripe in October[1].The stem bark is scented and resinous. All parts of the plant are medicinally important being used in folk medicines since long to treat various diseases[2]. The stem and root bark are anthelmentic, astringent and detergent[2]. The dried bark of the tree is used as a tooth cleaner[3]. The decoction of leaves, bark along with alum is used for staining wool brown[3]. The dried green husks contain 2.5-5.0 % ascorbic acid (vitamin C) which can be extracted and used as a vitamin supplement[3]. Juglone found mainly in the leaves and its derivatives show a wide spectrum of applications in the field of cosmetics, pharmacology and ecology[4]. Some extracts from the plant have shown anticancer activity[5]. Realizing the vast and valuable medicinal applications of the royal species attempts are made to analyze the plant material. The present work includes detection of amino acids from stem bark of the plant using paper chromatographic technique[6] and identifying them by comparing their R_f values with standard amino acids.

Whatmann filter paper no. 1 was used for paper chromatography. Amino acid kit (CHH laboratory reagent) was used for standard amino acids.

Extracts were prepared by using weighed quantity of powdered material in known volume of water and 9 % (w/v) aqueous sodium chloride solution. Repeated extraction of this material was carried out using chloroform to remove chlorophyll. The chloroform layer was separated and the remaining part was used for amino acids analysis.

Different solvent systems were tried to screen out the best mobile phase for separating the amino acids present in the plant material by paper chromatographic technique. (1) phenol:water (1:1), (2) water:butanol:acetic acid (5:4:1), (3) *n*-hexane: *n*-butanol:methanol:acetic acid:water (0.5:3:1:3:5), (4) *n*-butanol:acetone:water (1:3:2), (5) *n*-propanol:ammonia (7:3), (6) *n*-butanol:water:ethanol (2:1:1), (7) *n*-butanol:pyridine:ammonia (7:3), (8) pyridine:*iso*-propyl alcohol:acetic acid: water (8:8:1:30), (9) butanol:pyridine:acetic acid:water (6:10:1:3), out of these, [Phase-I]: pyridine:*iso*-propyl alcohol:acetic acid:water (8:8:1:3) and [Phase-II]: butanol:pyridine:acetic acid:water (6:10:1:3) solvent systems were found to be suitable for analysis.

Spraying reagent

Preparation of ninhydrin solution: It was prepared by mixing 0.0175 g of ninhydrin with 15 mL acetone.

The extracts were spotted on the chromatographic paper along with standard samples. The mobile phase was allowed to run to a certain height and the chromatogram was dried at room temperature. Ninhydrin solution was sprayed on the paper and again dried at room temperature. The R_f values of the amino acids of the experimental samples were determined and compared with the standards.

Tables 1 and 2 show the amino acids present in the plant material are glycine, L-isoleucine, L-ornithine monohydrochloride, L-histidine monohydrochloride, 2-amino-*n*-butyric acid and L-hydroxy proline are detected in the mobile phase-I: pyridine:isopropyl alcohol:acetic acid:water (8:8:1:3) whereas L-proline, L-tyrosine, DL-methionine, DL-norleucine, L-arginine monohydrochloride, DL-tryptophan and DL-phenyl alanine are detected in the mobile phase-II; butanol:pyridine-acetic acid:water (6:10:1:3). In plants proteins are stored in the form of amino acids and they have a important role in the metabolic pathways for synthesis of secondary metabolites.

TABLE-1
AMINO ACIDS DETECTED
PHASE-I: PYRIDINE:IPA:ACETIC ACID:WATER (8:8:1:3)

Name of amino acids	R_f value for standard amino acid	R_f value for plant extract	
		Water extract	Ethanol extract
Glycine	0.12	0.1	–
L-Histidine monohydrochloride	0.08	–	0.8
L-Isoleucine	0.93	0.95	–
DL-2-Amino *n*-butyric acid	0.89	–	0.89
L-Ornithine monohydrochloride	0.48	0.49	–
L-Hydroxy proline	0.79	–	0.8

TABLE-2
AMINO ACIDS DETECTED
PHASE-II: BUTANOL:PYRIDINE:ACETIC ACID:WATER (6:10:1:3)

Name of amino acids	R_f value for standard amino acid	R_f value for plant extract		
		Water extract	Acetone extract	Ethanol extract
DL-Phenyl alanine	0.78	–	–	0.8
L- Tyrosine	0.50	0.52	–	–
L-Arginine monohydrochloride	0.83	–	0.82	–
L-Proline	o.70	–	–	0.70
DL-Tryptophan	0.71	–	–	0.71
DL-Norleucine	0.82	–	0.80	–
DL-Methionine	0.83	–	0.82	–

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