Pak. J. Bot., 13(1): 99-101, 1981.

WATER SOLUBLE PROTEIN FROM *FAGONIA CRETICA* LINN.

G.A. SHAUKAT, M.A. MALIK, AND M.S. AHMAD.

Biochemistry & Natural Products Division, Nuclear Institute for Agriculture & Biology, P.O. Box No. 128, Jhang Road, Faisalabad.

Abstract

Water soluble protein was isolated and purified from the water extract fraction of dried *Fagonia cretica* plant. The acid hydrolysate of the material showed the presence of phosphate, sugar and amino acids in the molar ratios of lysine 1; threonine 2; aspartic acid 3; serine 3: glutamic acid 3.

Introduction

Fagonia cretica (Hooker, 1875; Stewart, 1972) locally known as 'Sachchi booti' grows wild and is abundantly distributed in different parts of Pakistan. The plant is collected in dry seasons as the water soluble contents of the plant are washed away with water in rainy season. The medicinal value of *F. cretica* is well documented, it is used as a constituent of herbal preparations for curing diseases of digestive system (Saeed, 1969). Its medicinal value lies in its action as emmenagogue and vasoconstrictor (Saeed, 1969). It is believed that it has some anticancer property and local hakims use the water extract for curing cancer of various organs (Khan, 1977). The water extract from the crushed plant brings down the number of leucocytes in leukaemic patients (Khan, 1977; Shaukat *et al*, unpublished data). Khan *et al* (1979) have reported the presenc of water soluble protein and free amino acids in the water extract of the fresh plant of *F. indica*. Here we report some of the preliminary studies on the isolation, purification and chemical nature of the water extract containing material from *F. cretica*.

Materials and Methods

Dried *F. cretica* plants were collected from Nawabshah, Sind. The plants were cut into small pieces, ground into a powder and 4g soaked in 100 ml of distilled water for 24 hrs in a refrigerator. The brown coloured supernatant collected by centifugation on a bench centrifuge was filtered through a Whatman No. 1 paper and passed through a charcoal celite column. Charcoal was washed before use with 20% acetic acid. The column was prepared by mixing 2g of washed dried charcoal and 2g of celite (British

Drug Houses, London) and pouring the slurry on a bed of 2 cm thick celite. The colourless effluent through column was collected and concentrated to 5-8 ml on a rotary evaporator at 37°C. Soluble protein was precipitated by adding 5 volumes of acetone and was collected by centrifugation washed twice with acetone and preserved, (yield 0.3%). The pigments were retained by the charcoal celite column.

Precoated silica gel plates (Macherey – Nagel + Co D-516 Duren-Germany) were used for thin layer chromatography. The following solvent systems (Lederer & Lederer, 1957) were employed; (A) chloroform; methanol; water (60: 35: 4) and (B) n-butanol; acetic acid; water (40: 20: 10). Compounds were detected on silica gel plates using ninhydrin for amino acid (Consden & Gordon, 1948), alkaline silver nitrate reagent for reducing compounds (Travelyan *et al.* 1950), aniline pthalate for reducing sugar (Partridge, 1949) and molybdate reagent for phosphoric esters (Hanes & Isherwood, 1949). TLC of the protein material in solvents A and B gave a single spot. The charcoal celite column was eluted with solvent, acetone; water; ammonia (70: 30: 0.2) mixture (100 ml). The eluent was dried up completely. Only a small residue of pigments was found.

The dried acetone precipitated material (100 mg) was hydrolysed in 6 N hydrochloric acid (5 ml) for 24 hrs at 110°C, for amino acid analysis. For sugar detection the acid hydrolysis was carried out at 100°C for 3 hrs in 2 N hydrochloric acid. The hydrolysates were dried repeatedly to dryness in vacuo and finally dried over sodium hydroxide. Inorganic phosphorous was detected from both the hydrolysates.

Table 1. Amino acid composition of water soluble protein of *Fagonia cretica*.

Amino acid	Residue percent	Molar ratio
Lysine	8.34	1.00
Threonine	16.71	2.00
Aspartic acid	25.12	3.01
Serine	25.15	3.02
Glutamic acid	24.68	2.96

Results

An aliquot from the hydrolysate was analysed for amino acid composition on E.E.L. Model 193 (Table 1). Tryptophane although destroyed during acid hydrolysis was found to be present in traces. The analysis of water soluble protein of *F. cretica* indicates the phosphatic amino glycosidic nature of the material. It is now known that some poly-

peptide compounds act as antitumor (Takeuchi *et al,* 1971). The work on the homogeneity of the protein precipitated by acetone from the water extract fraction of *F. cretica* is under investigation.

Acknowledgements

The authors wish to thanks Mr. S.M. Ifzal for the amino acid analysis and Messrs Anis Ahmad and M.Akram for assistance.

References

Consden, R. and A.H. Gordon. 1948. Effect of salt on partition chromatography. Nature, **162**: 180.

Hanes, C.S. and F.A. Isherwood. 1949. Separation of the phosphoric esters on the filter paper chromatograms. Nature, **164**: 1107.

Hooker, J.D. 1875. Flora of British India, **1**: 425 L. Reeve & Co., London.

Khan, A.M., A.H. Siddique and Z.H. Zaidi. 1979. Amino acid composition and protein contents of *Fagonia indica.* J. Chem. Soc. Pak., **1**: 139.

Khan, S.A. 1977. Cancer cure, a letter to the editor. The Pakistan Times, Lahore, August 12.

Lederer, E. and M. Lederer. 1957. Chromatography. Elsevier Publishing Company, London.

Partridge, S.M. 1949. Aniline hydrogen phthalate as a spraying reagent for chromatography of sugars. Nature, **164**: 443.

Saeed, H.M. 1969. Hamdard Pharmacopoeia of Eastern Medicine. Hamdard Academy, Karachi, 41 – 43 pp.

Stewart, R.R. 1972. An annotated catalogue of the vascular plants of West Pakistan and Kashmir. *In:* E. Nasir and S.I. Ali (Eds.) Flora of West Pakistan, University of Karachi, Pakistan, 433 p.

Takeuchi, T., S. Hori, M. Matsuzaki and H. Umezawa. 1971. Phenomycin, toxicity and distribution. J. Antibiotics, **24**: 197-199.

Travelyan, W.E., D.P. Procter and J.S. Harrison. 1950. Detection of sugars on paper chromatograms. Nature, **166**: 444..