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Extraction and physico-Chemical studies of Diastase-Like Enzyme from piper betel petioles: Part II

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ABSTRACT: *Crude enzyme extract obtained from the petioles of the plant piper betel- Bengal variety as been evaluated for various physico-chemical studs such as estimation of protein content, thin layer chromatography, optical activity and tests for the presence of thiol groups, disulphide and peptide linkages and the results are discussed.*

INTRODUCTION

In view of the biological and pharmacological importance of *piper betel* in general a need as been felt to undertake an investigation on extraction of diastase like enzyme and its phyisico chemical properties which ma exhibit significant biological and pharmacological properties. As a step in this direction and in continuatioin of our work[1] we now report some of the physico-chemical properties of the crude petiolous extract of the plant piper betel- Bengal variety. Its evaluation for the possible antimicrobial properties it, however, in progress.

MATERIALS AND METHODS

Extraction of the crude enzymatic principle from the petioles of *piper betel* leaves for the purpose has been effected by the standard method [2-4] and the crude extract concentrate has been subjected for the various physico-chemical studies as follows.

Estimation of Protein content

It was determined by Folin-Lowry method 5,6 using bovine serum albumin solution (0.1mg/ml) as standard for comparison to prepare the 'Standard curve' of optical density versus amount of peotein. The protein content of the test sample was computed from the standard curve (Gig.1) thus prepared for the purpose, using the data presented in the table.1.

Thin Layer chromatography

It has been aimed to estimate qualitatively the products formed when the crude extract under investigation as reacted with starch substrate using maltose solution (0.5% w/v) as know standard for comparison.

The phosphate buffer solution of the crude enzyme extract (5ml; 0.1 % w/v) was added to a test tube containing starch substrate (5ml; 1% w/v) which was maintained at 35oC in water bath. Te contents were mixed thoroughly for few minutes. After 6 ours, the test tube containing the reaction mixture was taken out for the water bat and cooled rapidly. Now a chrotogram[7] of the reaction mixture was developed on activated silica gel plate employing maltose solution (0.5% w/v) as standard for comparison.

Estimation of Optical Activity

An accurately measured quantity of the crude extract (0.05% w/v) was tested for optical rotation using spectropolarimeter having cell length 0.1 decimeters and the degree of optical activity[7] was calculated fro t following expression.

$$[\alpha]^t{}_D = \frac{100.\ \alpha}{1.C}$$

Where $[\alpha]^t{}_D$ α, 1 and C represent specific rotation, observed rotation, length of the cell in decimeters and concentration in gm/100 ml of the test sample respectively.

Test for thiol groups, disulfide and peptide linkages

A chemical investigation for the presence of thiol (-SH) groups, disulphide (-S-S-) and peptide (CONH) linkages in the crude enzyme extract was effected by the standard procedures [8].

RESULTS AND DISCUSSION

It was observed that the amount of protein present in the crude enzyme extract was estimated to be 31.3%) (w/w).

Qualitative stud of the resulting products of the search crude extract mixture employing thin layer chromatography with maltose solution as standard for comparison revealed that the reaction product formed from the starch solution in the presence of crude extract was 'maltose like product', since it exhibited R_f value as that of 'maltose standard' further confirming the starch splitting nature of the crude enzyme extract under investigation.

The crude enzyme extract was found to be dextrorotatory with the specific rotation of 60^o.

The various specific chemical tests performed for the purpose revealed the absence of thiol groups and disulphide linkages in t crude extract under investigation, however, the presence of peptide linkages has been confirmed.

From these preliminary findings it could be concluded that the carbohydrolytic activity, optical activity and the presence of proteinaceous matter having peptide linkages confirm the enzymatic nature of the extract under investigation. Though the efforts in the purification of the crude enzyme extract to the fullest possible extent are on, however, an evaluation for the possible antimicrobial activity of the crude extract, on the other and, is in progress.

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Table -1: Estimation of protein content.

Sl. no	Type of sample	Bovine serum Albumin (0.1mg/ml) Solution in ml	Crude extract (0.1mg/ml) Solution in ml	Distilled water in ml	$CusO_4$ Reagent in ml	Folin Reagent in ml	Optical density	Protein content in mg

1	Blank	-	-	1.0	5.0	0.5	0.00	0.0000
2	S.S-1	0.1	-	0.9	5.0	0.5	0.03	0.0121
3	S.S-2	0.2	-	0.8	5.0	0.5	0.02	0.0732
4	S.S-3	0.4	-	0.6	5.0	0.5	0.14	0.0411
5	S.S-4	0.6	-	0.4	5.0	0.5	0.21	0.0622
6	U.S-1	-	0.4	0.6	5.0	0.5	0.04	0.0125
7	U.S-2	-	0.8	0.2	5.0	0.5	0.09	0.0250

S.S – Standard sample, U.S – Unknown Sample.

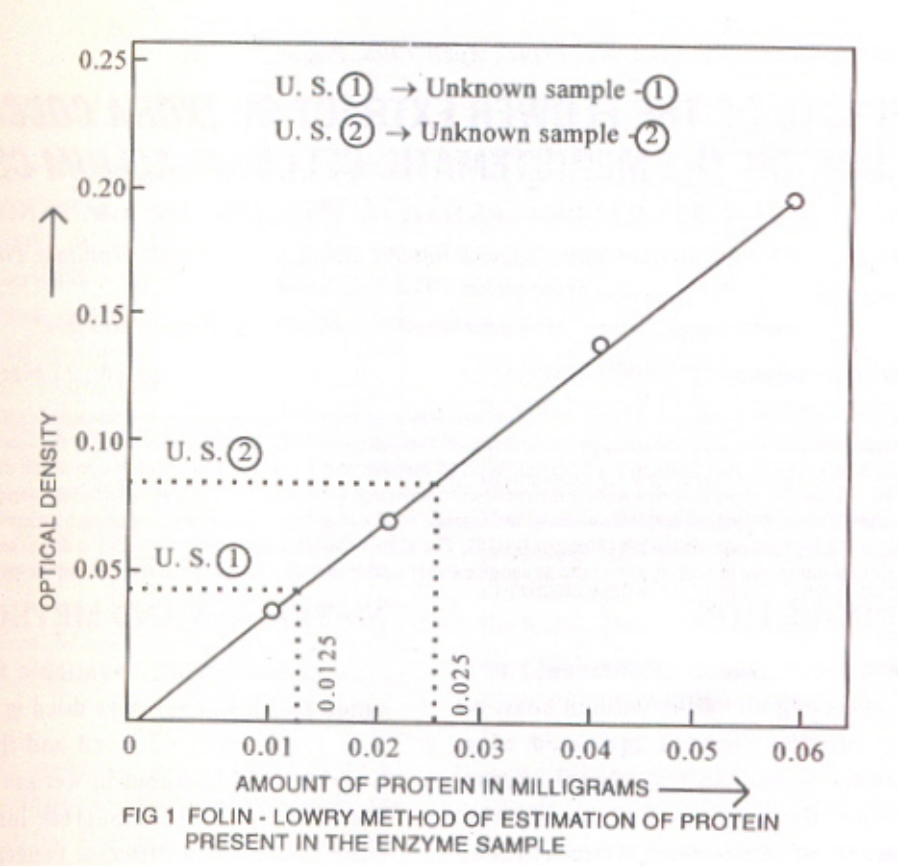

FIG 1 FOLIN - LOWRY METHOD OF ESTIMATION OF PROTEIN PRESENT IN THE ENZYME SAMPLE

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