EXTRACTION AND PHYSICO-CHEMICAL STUDIES OF DIASTASE-LIKE ENZYME FROM PIPER BETLE PETIOLES: PART 1

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Received: 17 May, 1995 Accepted: 2 July, 1995

ABSTRACT: Petioles of the plant piper betle-bengal variety have been subjected for extraction employing standard procedure and the crude extract obtained has been evaluated for its diastase like activity and other physico-chemical properties to investigate further its possible biological and pharmacological activities.

INTRODUCTION

In recent times plant products are gaining lot of prominence for their potential biological and pharmacological properties. The nature and character of plant carbobydrolases have been under scientific investigations for more than half a century. More particularly leaves of Piper betle, a perennial dioecious creeper belonging to the family piperaceae and order piperales, used for chewing have been reported to be useful in catarrhal and pulmonary infections. The leaf extract has also been found to possess antiseptic and oral contraceptive properties. Interestingly, petiolous extract of Piper betle-Bengal variety in particular has been reported to be more effective in treating external wounds which may be due to the presence of chavicol’ an eugenol derivative or a diastase like enzyme that hydrolyses the oligosaccharides present in the cell wall of bacteria. This prompted us to study the physico-chemica properties of petiolous extract of piper betle-Bengal variety in a preliminary manner so as to evaluate further its possible biological and pharmacological activities in animal models.

MATERIALS AND METHOD

Fresh petioles of piper betle were air dried, powdered to a fine mass and extracted with phosphate buffer (pH 7.8) making use of a Balls-Tucker method and the crude extract concentrate was subjected for the various physicochemical studies.

DETECTION OF AMYLASE ACTIVITY

It was determined by a standard procedure using 0.05% crude extract solution in a phosphate buffer of pH 7.8 and starch solution (2%)

EFFECT ON VISCOSITY OF STARCH SOLUTION

It was studied by standard method using Ostwald viscometer and the pertinent data are given in table 1.

Effect of temperature and pH on the activity of extract

Freshly prepared starch solution (2% 25ml) buffer solution of standard pH (10ml) and
sodium chloride solution (0.2M; 1 ml) were taken into a stoppered tube, 22 mm in diameter and 200 mm long and the contents were mixed thoroughly and placed in a water bath maintained at required temperature. When the temperature of the mixture had reached the desired temperature measured amount of test solution of the crude extract was added and the time of addition was recorded and entire contents were mixed thoroughly. After 15 minutes hydrochloric acid (1.0M; 2 ml) was added to stop the reaction. The contents of the tube were transferred to a stoppered flask of 250 ml capacity, the tube was rinsed with 20 ml of distilled water and the washings were added to the flask. While stirring continuously iodine solution (0.05M; 10 ml) and sodium hydroxide solution (0.1M; 45ml) were added and allowed to stand in the dark at a temperature of 18C for 20 minutes and then 4 ml of 1:4 mixture of sulfuric acid and water was added and the contents were titrated against standard 0.1 M sodium thiosulfate solution. The procedure was repeated with the black and the potency of the substance being examined was calculated in units per milligram from the following expression.

\[
\text{Potency} = \frac{100}{\left(\frac{1}{5} (b-a) - 0.006\right) W}
\]

where ‘a’ and ‘b’ represent volume in milliliters of 0.1 M sodium thiosulfate used up in the titration against sample to be tested and blank respectively and ‘w’ represents amount of test sample in milligrams. The pertinent results are presented in tables 2, 3 and 4.

**RESULTS AND DISCUSSION**

It was observed that the amylase activity of the crude petiolous extract of piper betel was found to be 64 units per milligram.

It could be seen from Fig. 1 that the extract under investigation exhibited considerable reduction in viscosity of starch solution at all higher temperatures, the temperature at which maximum amount of starch digested (optimum temperature) was found to be 35C.

Studies on effect of temperature and pH on the amylase activity of the extract under investigation (Fig 2, 3 and 4) reveal that the temperature and pH have appreciable influence on the activity of the extract. The optimum temperature (t_{max}) for the maximum enzymatic activity was found to be 35C. The optimum pH (pH_{max}) using citrate buffer was found to be 5.6 to 5.8 and in case of phosphate buffer 7.9 which proved that pH_{max} for any enzyme is very much specific for each buffer system.

It could be inferred from the present preliminary study that the crude petiolous extract exhibit carbohydrolytic activity which is subject to confirmation through our future studies such as purification of enzymatic principle to the fullest possible extent, determination or biochemical properties and possible pharmacological properties which are in progress.

**ACKNOWLEDGEMENTS**

The authors are highly thankful to the authorities of Jadavpur University, Calcutta for the facilities. One of the authors (GVSRS) is grateful to the University grants commission, New Delhi for the financial assistance.
Table – 1 time of fall of starch solution at various temperatures

| Time in minutes | 20°C | 25°C | 30°C | 35°C | 40°C | 45°C |
|-----------------|------|------|------|------|------|------|
| Blank 0 20      | 23.0| 19.5| 14.8| 12.8| 9.1 | 6.0 |
| 40 60 80        | 22.5| 19.0| 13.9| 11.4| 9.0 | 5.8 |
| 100 120         | 22.5| 18.6| 12.1| 10.1| 8.6 | 5.5 |
| 140 160 180     | 22.5| 18.6| 10.2| 8.8 | 8.6 |

| S.No | Temperature (oC) | a   | b   | (b-a) | Activity units/mg |
|------|------------------|-----|-----|-------|-------------------|
| 1.   | 10 20 25 30 35 40| 6.0 | 6.0 | 0.0  | 0.9  | 0.8  |
| 2.   | 6.1             | 7.0 | 0.4 | 0.3  | 0.9  |
| 3.   | 7.2             | 8.0 | 0.9 |
| 4.   | 6.8             | 7.2 |

Table – 2 Activity of extract* at various temperatures

| Time in minutes | 20°C | 25°C | 30°C | 35°C | 40°C | 45°C |
|-----------------|------|------|------|------|------|------|
| Blank 0 20      | 23.0| 19.5| 14.8| 12.8| 9.1 | 6.0 |
| 40 60 80        | 22.5| 19.0| 13.9| 11.4| 9.0 | 5.8 |
| 100 120         | 22.5| 18.6| 12.1| 10.1| 8.6 | 5.5 |
| 140 160 180     | 22.5| 18.6| 10.2| 8.8 | 8.6 |

*W=30 mg

| S.No | Temperature (oC) | a   | b   | (b-a) | Activity units/mg |
|------|------------------|-----|-----|-------|-------------------|
| 1.   | 10 20 25 30 35 40| 6.0 | 6.0 | 0.0  | 0.9  | 0.8  |
| 2.   | 6.1             | 7.0 | 0.4 | 0.3  | 0.9  |
| 3.   | 7.2             | 8.0 | 0.9 |
| 4.   | 6.8             | 7.2 |

Table – 3 Activity of extract* at various pH using citrate buffer

| Time in minutes | 20°C | 25°C | 30°C | 35°C | 40°C | 45°C |
|-----------------|------|------|------|------|------|------|
| Blank 0 20      | 23.0| 19.5| 14.8| 12.8| 9.1 | 6.0 |
| 40 60 80        | 22.5| 19.0| 13.9| 11.4| 9.0 | 5.8 |
| 100 120         | 22.5| 18.6| 12.1| 10.1| 8.6 | 5.5 |
| 140 160 180     | 22.5| 18.6| 10.2| 8.8 | 8.6 |

*W=25 mg

| S.No | Temperature (oC) | a   | b   | (b-a) | Activity units/mg |
|------|------------------|-----|-----|-------|-------------------|
| 1.   | 10 20 25 30 35 40| 6.0 | 6.0 | 0.0  | 0.9  | 0.8  |
| 2.   | 6.1             | 7.0 | 0.4 | 0.3  | 0.9  |
| 3.   | 7.2             | 8.0 | 0.9 |
| 4.   | 6.8             | 7.2 |
### Table – 3 Activity of extract* at various pH using phosphate buffer

| Time in minutes | TEMPERATURE |
|-----------------|-------------|
|                 | 20°C | 25°C | 30°C | 35°C | 40°C | 45°C |
| Blank           | 23.0 | 19.5 | 14.8 | 12.8 | 9.1  | 6.0  |
| 20              | 23.1 | 19.4 | 14.8 | 12.8 | 9.1  | 6.0  |
| 40              | 22.5 | 19.0 | 13.9 | 11.4 | 9.0  | 5.8  |
| 60              | 22.5 | 18.7 | 13.9 | 11.4 | 9.0  | 5.8  |
| 80              | 22.5 | 18.6 | 12.1 | 10.1 | 8.6  | 5.5  |
| 100             | 22.5 | 18.6 | 12.1 | 10.1 | 8.6  | 5.5  |
| 120             | 22.5 | 18.6 | 10.2 | 8.8  | 8.6  | 5.5  |
| 140             | 22.5 | 18.6 | 10.2 | 8.8  | 8.6  | 5.5  |
| 160             | 22.5 | 18.6 | 8.8   | 8.6  | 8.5  |

S.No | Temperature (°C) | a | b | (b-a) | Activity units/mg |
|-----|------------------|---|---|-------|-------------------|
| 1. 2. | 10 20 25 30 35 40 | 6.0 | 6.0 | 0.0 | 0.9 | 0.8 | 19.16 21.65 |
| 3. 4. | 45 | 6.1 | 7.0 | 0.4 | 0.3 | 0.9 | 45.05 61.73 19.16 |

*W=25 mg

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