# SCREENING OF SOME PAKISTANI PLANTS FOR MILK CLOTTING ACTIVITY

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*SUMMARY: Ten plants were analysed for both milk clotting and protease activities. Nine plants were studied after classification into stems, leaves and flowers and one without this classification. During the course of screening Papaya leaves and Euphorbia caducifolia stem samples were found more potent for milk clotting activity.*

*Key Words: Milk coagulation, protease, papaya, euphorbia caducifolia.*

## INTRODUCTION

Rennet prepared from the abomasum (Fourth stomach from unweaned calves) was used in the production of cheese. But its supply had become less available, because instead of slaughtering calves to obtain rennet, they are preferably used to provide meat for human consumption. However the shortage of Calf's rennet was also highly increased due to religious restruction and ethnic regulations against the use of animal secretion in food paved the way for replacement of rennet from more convenient sources.

The possible alternative sources are higher plants and microorganisms. A plant derived substitute would be of particular value in view of its essentially unrestricted availability. Certain plants have been reported to yield promising rennet activity such as *Withania coagulans (1, 2); Ficus carica (3-6); Pumpkin (7, 8);* seeds of *Moringa oleifera (9); leaves of Calotropis procera (10);* seeds of *Ricinis communis; dried Papaya latex; Pineapple; cucumber; Benincasa cerifere; Galium verum; Pinquicule vulgaris; Drosera rotindifolia; and Ranunculus ligua (11).*

However, there are many plants especially of tropical origin, from which no work has been reported before. This work, therefore, deals with the screening of various plants for the isolation of milk clotting activity.

## MATERIALS AND METHODS

The plants were collected from Sindh University, New Campus and were processed on the same day. All reagents were used of analytical grade without further purification.

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**Extraction of milk clotting enzyme from plants**

20 G of plant sample was extracted with 100 ml ice-cold acetone in presence of some amount of glass powder using a pestle mortar. The slurry was filtered through Whatman No 1 filter paper and the residues were spread on filter paper and allow to dry at 20°C. This acetone dried powder was grinded in pestle mortar with 25 ml ice-cold 0.2 M citrate-phosphate buffer (pH 7.0) and the slurry was centrifuged at 4000 x g for 10 mins. The supernatant containing enzyme was transferred to 100 ml volumetric flask and this procedure was repeated twice. The total volume was made up with ice-cold citrate-phosphate buffer. This solution was used for estimating milk clotting activity.

**Preparation of substrate**

10 G of skimmed milk powder (Oxoid) was dissolved by stirring with glass rod in 100 ml of 0.05 M $CaCl_2$. The pH of milk substrate was adjusted to 5.8 with 0.1 N NaOH or HCl.

**Assay of milk clotting activity**

The milk clotting activity was assayed by the method of Berridge (12, 13). To 5.0 ml milk substrate pre-incubated for 15 mins at 50°C was added 0.5 ml enzyme sample and the time for milk clotting was determined. The end point was noted by appearance of milk clots.

The unit of milk clotting activity was defined as the amount of enzyme which clotted 1.0 ml of milk in one minute at 50°C.

**Skimmed milk coagulability by plant enzyme**

One half ml of an enzyme solution was added to 5.0 ml of pre-incubated skimmed milk (Oxoid) for complete coagulation at 50°C. The resulting coagulum was centrifuged at 4000 x g for 10 mins. In this experiment skimmed milk curd refers to the precipitate made by centrifugation of the coagulum.

Table 1: Milk clotting activity, protease activity and ratio of milk clotting to protease activity of tested plants.

| Name of plants | Protein (mg/ml) | Milk clotting activity (units/ml) | Protease activity (units/ml) | Ratio of milk clotting to protease activity |
|---|---|---|---|---|
| Opuntia phylloclades (Stem) | 0.52 | 120 | 05 | 24.00 |
| Cereus triangularis (Stem) | 0.10 | 160 | 06 | 26.60 |
| Aloe L. sp. (Stem) | 0.16 | 190 | 07 | 27.10 |
| Euphorbia caducifolia (Stem) | 0.52 | 600 | 05 | 120.00 |
| Calotropis procera (Leave) | 1.48 | 390 | 09 | 43.33 |
| Calotropis procera (Flower) | 0.92 | 170 | 06 | 26.33 |
| Papaya (Leave) | 1.60 | 1580 | 08 | 197.50 |
| Ficus bengalensis (Leave) | 1.68 | 380 | 07 | 54.30 |
| Ficus elastica (Leave) | 2.68 | 490 | 06 | 81.70 |
| Euphorbia hista (Whole plant) | 1.20 | 360 | 09 | 40.00 |

**Protease assay method**

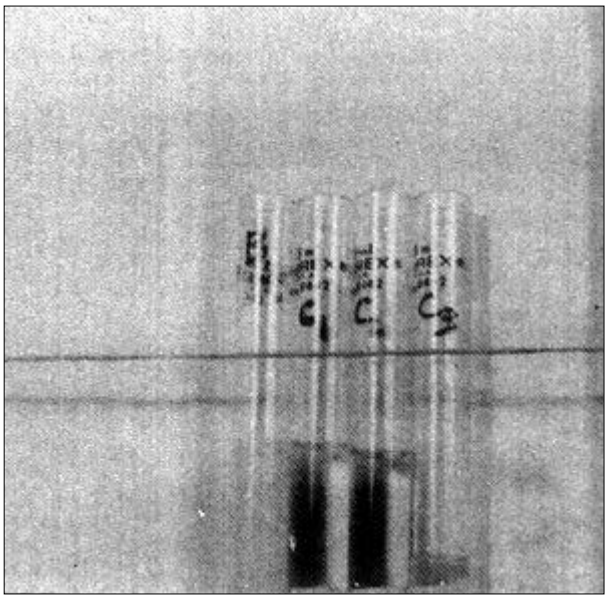
Protease activity in enzyme solution was determined according to the method described earlier (14).

One unit of the protease activity was defined as the amount of enzyme that liberated 1 μg of tyrosine under the standard assay conditions.

**Determination of protein**

Protein content of enzyme solution was determined by the method of Lowry *et al.* (15), using bovine serum albumin as a standard.

Figure 1: Skimmed milk coagulability by commercial enzyme. B. inactivated enzyme; $C_1$: Rennin; $C_2$: Pepsin; $C_3$: Trypsin.



**Ratio of milk clotting to protease activity**

The ratio of milk clotting to protease activity was defined as follows.

$$\text{Ratio} = \frac{\text{No of milk clotting activity units/ml}}{\text{No of protease activity units/ml}}$$

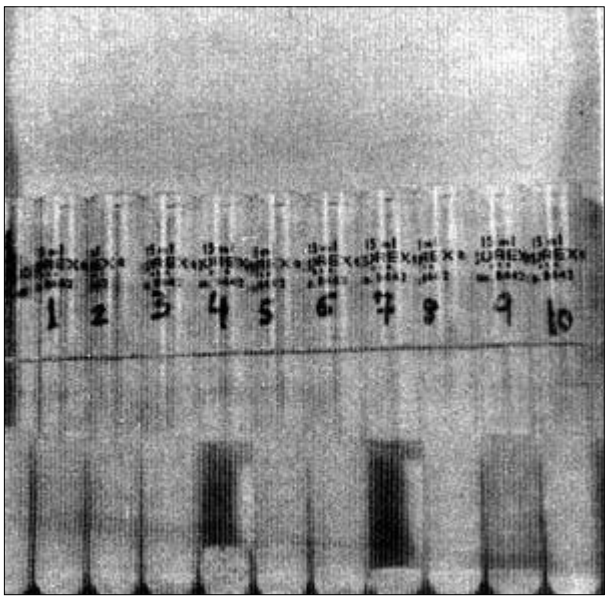
## RESULTS AND DISCUSSION

Ten plant traditionally known to have some milk clotting action were investigated for milk clotting and protease activities. The ratio of milk clotting to protease activities were calculated. The comparison of activities and the ratio is presented in Table 1.

Out of ten plants nine plants were studied after classification into stem, leave and flower, whereas one was used without this classification. The results shown in Table 1 indicate that all preparation contained both milk clotting and protease activities. It is well known that almost all proteolytic enzymes clot milk, but the ratio between the milk clotting activity and protease activity is seemd to be very important in cheese making. In this regard it seems that *Papaya* leaves and *Euphorbia caducifolia* stem samples posses higher ratio of milk clotting to protease activity in comparison to other plants.

Figures 1 and 2 show the skimmed milk coagulability of commercial enzymes (rennin, pepsin and trypsin supplied by Sigma) and various plant samples, while no coagulation was observed either on the addition of water (B in Figure 2) or the enzyme (rennin) treated at 100°C for 5 minutes (B in Figure 1). The resulting coagula were separated clearly, after centrifugation at 4000 x g for 10 mins, into skimmed milk curd and whey. It was observed for Figures 1 and 2 that the coagulation of protein in skimmed

Figure 2: Coagulation of skimmed milk by the crude enzyme from various plants, *B. Water; 1. Opuntuo phylloclades; 2. Cereus triangularis; 3. Aloe L. ps.; 4. Euphorbia caducifolia; 5. Calotropis procera; 6. Calotropis procera; 7. Papaya (Carica papayalinn); 8. Ficus bengalensis; 9. Ficus elastica; 10. Euphorbia hista.*



milk was due to the reaction caused by the enzyme, because curd was clearly formed with commercial and two plant enzyme but not on heating the enzyme at 100°C, water and other plant enzyme which posses low milk clotting activity. However, it was not clear whether the coagulation of skimmed milk with *Papaya* leaves and *Euphorbia caducifolia* stems was due to a single enzyme action or the combined action of several enzymes. Further studies on characterization and properties of the purified enzyme and the mechanism of milk coagulation from these plants would be interesting from enzymological view point and this will be reported in the near future.

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