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Short Communication

SCREENING FOR PROTEOLYTIC AND CLOTTING ACTIVITIES OF SOME PLANTS IN NIGERIA

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ABSTRACT

Varieties of plants known to have medicinal values were analysed for both proteolytic and milk clotting activities, using IDF and Ratio method. The result of this screening test shows that all the samples under investigation had some degree of proteolytic (between 50 to 90units/ml) and milk clotting (120 to 1,580unit/ml) activities. *Calotropis procera* leaves and *Carica papaya* leaves shows higher values of proteolytic and clotting activity compared to the others. The relevance of higher ratio of milk clotting activity to proteolytic activity in dairy and food production are discussed.

Keywords: Medicinal plants, clotting activity.
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INTRODUCTION

Proteolytic enzymes are tools which have been used in medicine as well as in industries for many years. In recent years their utilities have become of vital importance due to the availability of active preparation with good solubility and stability (Hoffman, 1974). Trevan *et al* (1990) stress their importance due to the unique property of being active in wide range of temperature and pH. The wide diversity and specificity of these enzymes are used to a great advantage in developing effective therapeutic agents.

Besides their medicinal ability their use in detergent, textile, beer, food and diary industry can not be overemphasised (Rao *et. al.*, 1998). There are existing commercially available enzymes such as papain (Kimell and Smith, 1954), Bromelain (Takahesi *et. al.*, 1973) and Ficin (England *et. al.*, 1968) are available and of high degree of proteolytic activity, butt still there is need for new plant sources of these enzymes.

However, there are many plants especially of tropical origin that may serve as sources and this work therefore reports the screening of selected vegetative parts of various plants for their proteolytic and clotting activities.

MATERIALS AND METHODS

The leaves of *Hisbiscus sabdariffa*, *Musa sapientum*, *Aloe vera*, the leaves, stem and flowers of *Calotropis procera*, leaves of *Carica papaya*, *Mangifera indica*, *Moringa oleifera* and *Psidium guajava* (Mann *et. al.*, 2003) were collected in Zaria and its environs.

Preparation of Crude Enzyme

Twenty grams plant parts (e.g. leaf) of each sample were extracted with 100ml ice-cold acetone in the presence of glass powder ground using pestle and mortar. The slurry was filtered through Whartman filter paper No 1 and the residue spread on the filter paper and allowed to dry at 20^0C. This acetone dried powder was again grinded in

laboratory mortar and pestle, in 25ml ice cold 0.2M sodium phosphate buffer (pH 7.0) and the slurry was centrifuge at 400 x g for 10 minutes. The supernatant containing enzyme was transferred to 100ml volumetric flask and kept at –4°C till when required.

Protein Determination

Protein content of crude enzyme solution was carried out according to the procedure of Lowry *et. al.* (1951), using Bovine serum albumin as a standard.

Proteolytic Activity Assay Method

Proteolytic activity in enzyme solution was carried out according to the method described by (Onyike and Abdullahi, 2006) using Hammerstain casein as substrate. The reaction medium (2.0ml) was made up of 0.1M cystain-EDTA-phosphate buffer (pH 7.5), 1.0ml of enzyme sample and 0.5% casein. After incubation at 40°C for 30 minutes, the reaction was terminated by addition of 3ml of 5% TCA and the resultant precipitation was removed by centrifugation at 13,000 rpm for 20 minutes. Blanks were prepared for each sample in similar manner except for the addition of TCA to enzyme solution before mixing it with Hammestain casein.

One tyrosine unit is defined as the amount of enzyme that liberated one microgramme (1 µg) of tyrosine under the standard assay condition.

Clotting Activity Determination

The clotting activity was determined following procedure of IDF (1992) as described by Silva *et. al.* (2002). The reconstituted milk was obtained by dissolving low heat skim milk (DANO brand) in 10mM Aqueous Calcium Chloride (pH 6.5) so as to achieve concentration of 0.12 kg/l. The time taken for 0.12ml of each enzyme extract to concentrate 2ml of reconstituted milk were recorded, coagulation point was determined by manually rotating the test tube periodically at short time interval and checking for visible clot formation at 30°C.

One unit of milk clotting activity (Cu) is defined as the reciprocal of the time (minutes) necessary to start milk clotting.

Ratio of Milk Clotting-Protoelytic Activity

The ratio of milk clotting to proteolytic activity defined and determined as:

$$\text{Ratio} = \frac{\text{Milk clotting (unit/ml)}}{\text{Proteolytic activity (unit/ml)}}$$

RESULTS AND DISCUSSION

Ten plants traditionally used for medicinal purposes were investigated for their proteolytic and clotting activities. The ratio of milk clotting to proteolytic activity was also calculated. The comparison of their protein content, proteolytic activity, clotting activity and the ratio of milk clotting to proteolytic activity are presented in Table 1 and Figures 1, 2, 3 and 4 respectively.

TABLE1: Results of Parameter Analyses of Various plants

Plant Species	Protein Content (mg/ml)	Proteolytic Activity (unit/ml)	Milk Clotting Activity (unit/ml)	Ratio of Milk Clotting Activity To Proteolytic Activity
Hisbiscus Sabdariffa	0.52	50	120	2.4
Musa Sapientum	0.1	60	160	2.6
Aloe Vera (leaves)	0.16	70	190	2.7
Calotropis Procera (Stem)	0.52	80	400	8
Calotropis Procera (leaves)	1.48	90	1000	12
Calotorpis Procera (Flower)	0.92	60	170	2.6
carica papaya (leaves)	1.6	80	1580	19.7
Mangifera indica (leaves)	1.68	70	380	5.4
Moringa Oleifera (leaves)	2.68	60	490	8.1
Psidium Guajava (leaves)	1.2	90	360	4

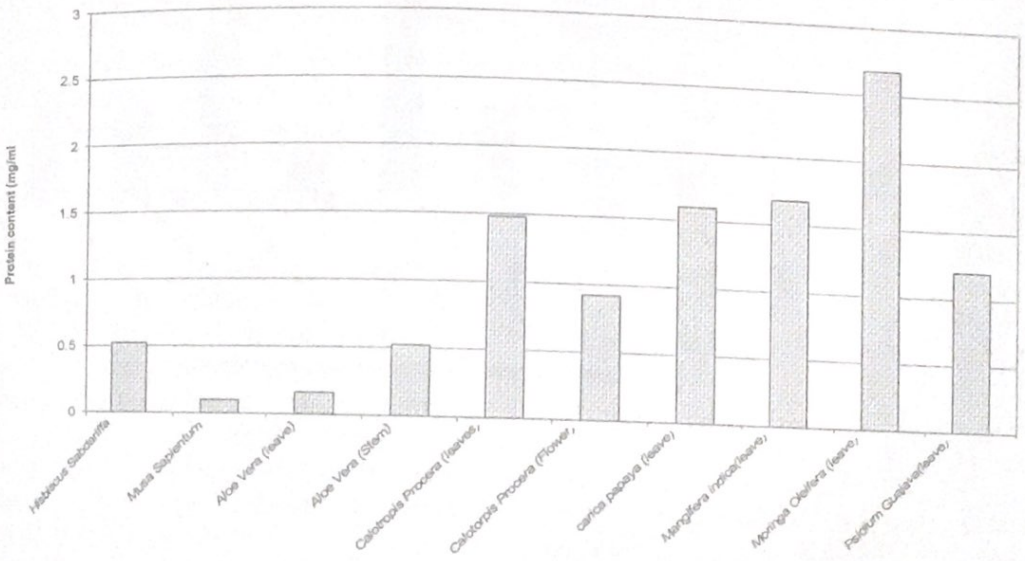

Fig 1: Protein content of various plants (mg/ml)

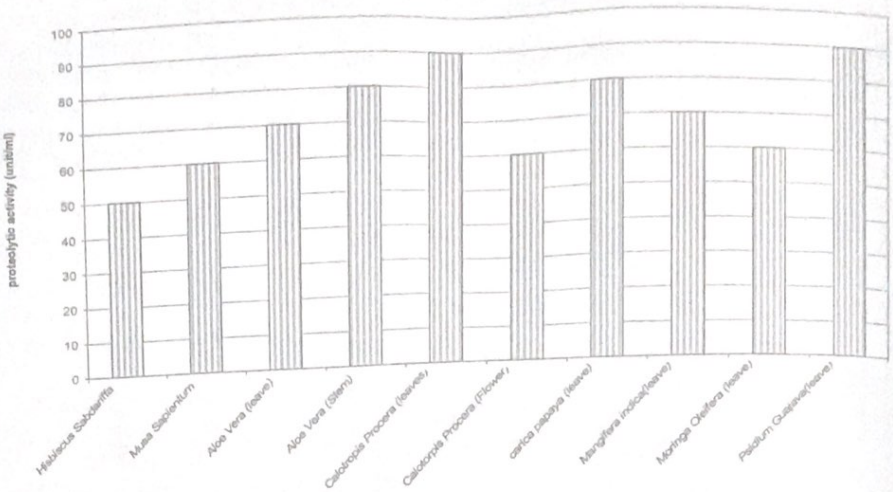

Fig 2: Proteolytic activity of the plants (unit/ml)

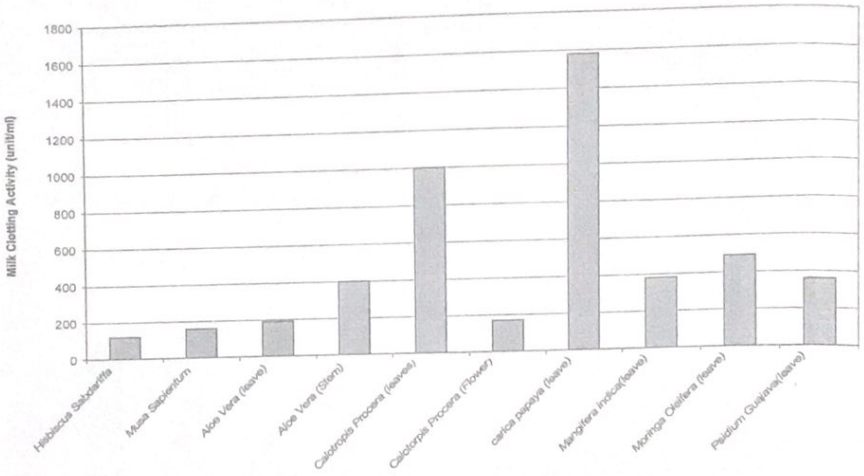

Fig 3: Milk Clotting Activity of various plants (unit/ml)

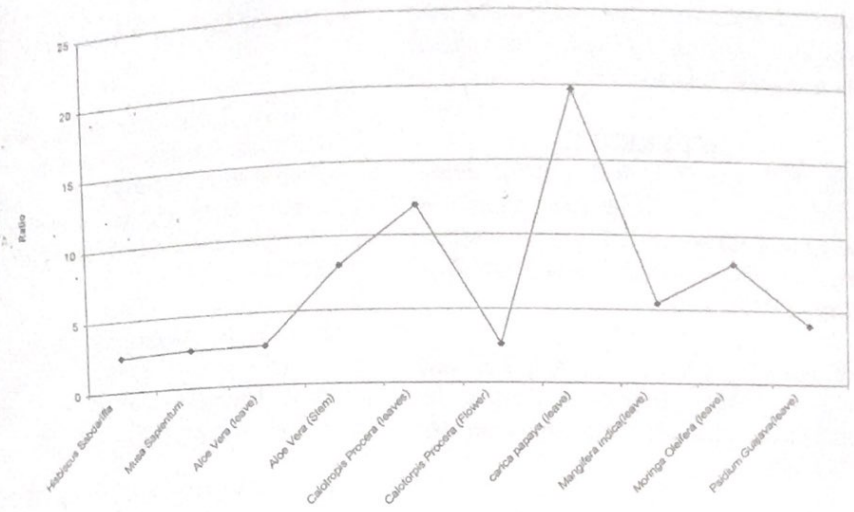

Fig 4: Milk Clotting activity ratio of Proteolytic activity

The results show that all plant preparations under investigation contain both proteolytic and clotting activities. It is well known that almost all proteolytic enzymes clot milk (Hoffman, 1974), but the ratio of milk clotting activity to proteolytic activity is seen to be very important parameter in dairy industry especially in cheese making. Several plants proteolytic enzymes have been reported (Silva *et. al.*, 2002) to clot milk. However, many are unsuitable for cheese production owing to their excessive proteolytic character (low ratio), which decrease cheese yield and produce bitter flavours.

Mornga oleifero contained 2.7mg/ml protein which was significantly higher ($P<0.05$) than the other plants and parts. *Carica papaya*, *Mangifera indica* and *Calotropis procera* had contents in the range of 1.58 to 1.65 mg/ml which were insignificantly different ($P>0.05$). The least protein content was recorded in *Musa sapientum* (0.18mg/ml).

The highest proteolytic activity was recorded in leaves of *Psidium guajava* and *Calotropis procera*. These were followed closely by stem of *Calotropis procera* and leaves of *Carica papaya*. The least proteolytic activity was 50unit/ml in *Hibiscus sabdaniffo*.

Mink clotting activity was significantly highest ($P<0.05$) in leaves of *Carica papaya* followed by the leaves of *Calotropis procera*. The least were recorded in *Hibiscus sabdariffo*, *Musa sapientum*, *Aleovera* and flower of *Calotropis procera*. Similar trends were recorded in ratio of milk clotting to proteolytic activity of the plants.

In this regard it seems that *Carica papaya* and *Calotropis procera* leaves posses higher ratio of milk clotting to proteolytic activity compared to other plants under investigations. Cheese production starts with milk coagulation, which involves the proteolytic cleavage of an insoluble milk casein, limited proteolysis (high ratio) is useful indicator of enzymes appropriateness for use in cheese making compared to excessive proteolysis (low ratio) (Chen and Zall, 1986). However, it was not clear whether proteolytic activities of the enzymes in these plants were due to single enzyme and or multiple action.

CONCLUSION

The higher ratio of clotting activity to proteolytic activity of *Carica papaya* and

Calotropis procera leaves would make them excellent enzyme sources in cheese manufacturing industry.

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