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Screening of Latex Producing Plants for Their Milk Clotting Activity

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ABSTRACT

Latex of latex producing plants from *Euphorbiaceae, Asclepiadaccae, and Apocyanaceae* families were screened for their milk clotting efficiency. Latex enzymes were partially purified by salt or solvent precipitation. Partially purifiedproteases of *Calotropis gigantea* was found to possess high milk clotting activity (479±1 U/ml) and caseinolytic activity.However, partially purified *Allamandacathartica*latex proteases showed relatively low caseinolytic activity and therefore a high milk clotting index (53.585 ± 0.105). *Jatrophacurcus* showed no milk clotting activity even after 24 hours of incubation of enzyme with the substrate.High ratio of milk clotting tocaseinolytic activityexhibited by*A.cathartica*latex proteasesindicates the suitability of this plantas apotential vegetable milk coagulantsource.

Keywords: Latex, proteases, milk clotting and vegetable coagulant.

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INTRODUCTION

Cheese making begins with milk clotting which is widely achieved by rennin, extracted from the inner mucosa of the fourth stomach chamber (the abomasum) of slaughtered young, unweaned calves. Calf rennet has widely been used in cheese manufacture industry as it adds to flavour and texture of cheese during ripening. It is characterised by low proteolytic activity and high milk clotting activity [1].The increasing demand for cheese, the insufficient supply, high cost of rennet and associated ethical issues have led to the search for a suitable alternative to rennin [2]. In recent years, growing research interest has been directed towards finding several new proteases from plants and microbes with milk coagulation potential [3]. Plants are used as source of proteases due to its easy availability, efficient crude enzyme purification processes and isolation of natural coagulant. Plant coagulant usage increases the acceptability by the vegetarian population and has an advantage of improving their nutritional intake [4]. Proteolytic enzymes from plants receive attention due to its broad substrate specificity and activity in wide range of pH, temperature, presence of organic compounds and other additives.Plant latex proteases as bovine renin substitutes has been highly contemplated. Latex from plants belonging to the family of to *Euphorbiaceae, Asclepiadaccae, Moracceae, and Apocyanaceae* have been used worldwide as traditional medicine. Presence of abundant quantity and variety of proteases in latex makes it a potential plant coagulant source. Objective of present work was to screen and identify milk-clotting potential associated with five different latex producing plants belonging to *Euphorbiaceae, Asclepiadaccae, and Apocyanaceae* families.

MATERIALS AND METHODS

Chemicals and plant sources

All chemicals used were of analytical grade purchased from HI media laboratories Pvt.Ltd, Mumbai, India. *Calotropis gigantea, Allamanda cathartica, Plumeria rubra, Jatropa curcus, and Euphorbia antiquorum*from *Asclepiadaccae, Apocyanaceae, Euphorbiaceae* families respectively were collected from Gandhi Krishi Vighyana Kendra (GKVK), Hebbal, Bangalore and from Botanical society of Bangalore. The selected plants were identified and authenticated at National Centre of Ayurveda and Dietetics, Ashoka Pillar, Jayanagar, Bangalore and at University of Agricultural Sciences, Bangalore.

Preparation of crude enzyme

The latex from the selected plants were precipitated by salt [5]or solvent precipitation [6]. Latex was collected in the morning in clean beaker by breakingthe tender parts of the plant and left over night at 4^{o}C to allow the gummy substances to settle. The supernatant was decanted and centrifuged at 12000 rpm at 4^{o}C for 20 minutes. Supernatant was decanted and subjected to precipitation. The precipitate was further used for protein estimation and determination of milk-clotting activity.Latex ofall plants except *P. rubra*was subjected to salt precipitation alone, whereas *P. rubra*was subjected to solvent precipitation as salt fraction did not yield any results for milk clotting and protein estimation.

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Protein estimation

Protein content of crude enzymes was determined by Lowry's method [7]

Caseinolytic activity

Caseinolytic activity was assayed according to Murata *et al.,* [8].

Specific milk clotting activity

Milk clotting activity of the crude enzyme from all plant latex was performed by the method of Arima *et al.,*[9].The substrate(10% w/v of skim milk in 0.01M calcium chloride) at pH 6 was preincubated at 37^{o}C for 5 minutes. To 2 ml of substrateadded 0.2ml of the crude enzyme and curd formation was observed by manually rotating the test tubes time to time. The time taken for thevisible discrete particles to form was noted**.**

One clot unit was defined as the amount of enzyme that clots 10ml of the substrate within 40 minutes.

MCA (U/ml) =(2400/clotting time in sec) ×dilution factor.

Specific milk clotting activity was calculated as milk clotting activity per milligram of protein.

Milk clotting Index

Milk clotting index (MCI) was calculated by ratio of milk clotting activity to caseinoytic activity [10].

RESULTS AND DISCUSSION

Different levels of milk-clotting activity was observed with crude extracts of the plants studied. Firm curd separated from whey after addition of the crude enzyme preparation of latex of plants of *C. gigantea, P. rubra, A. cathartica, E. antiquorum*.Milk-clotting activity of *C. gigantea* was found to be maximum followed by *P. rubra*. Figure 1 is a representative picture of milk coagulation by *C.gigantea* latex crude enzyme. *J. curcus* did not show any milk clotting activity even after 24 hours of incubation of substrate with the crude enzyme. Fraction of precipitate obtained from solvent precipitation of *P. rubra* latex contained detectable level of protein and exhibited milk-clotting activity. Protein content of crude enzyme of *C. gigantea* was found to be maximum followed by *P. rubra.*Table1 shows protein content, MCA and specific milk-clotting activity of plants studied. Specific milk clotting activity was also found to be maximum in *C. gigantea*.

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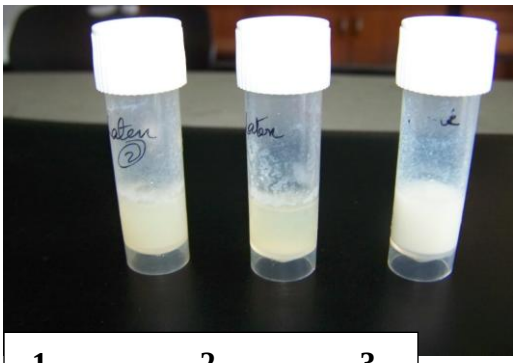
Table 1: **Protein content, MCA and Specific MCA of plants studied**

S.no	Plants	Protein (mg/ml)	MCA (u/ml)	Specific MCA(U/mg)
1	***C. gigantea***	44.65±0.649	479±1	10.73±0.18
2	***P. rubra***	30.75±0.25	101±1	3.285±0.005
3	***J. curcus***	8.475±0.125	Nil	Nil
4	***E.antiquorum***	22.07±0.35	6.16±0	0.28±0.003
5	***A.cathartica***	14.25±0.15	11.5±0.5	0.81 ± 0.03



1 & 2- *C.gigantea* crude latex protease with substrate, 3- Control

Figure 1: Milk clotting by *C.gigantea* latex protease

A closely related species to *C. gigantea, C. procera* has been extensively explored for its milk-clotting efficiency. Several studies on vegetable milk coagulants have reported high MCA associated with latex of this *Calotropis* species [11-13]. Comparison of the MCA and MCI of crude enzymes of plants studied in the present study confirmed the highest MCA possessed by *C. gigantea*latex.*E.antiquorum*exhibited low MCA in this study. However, its closely related species *E. niluvia* was reported to have significantly high milk clotting potential [14].

Table 2: MCA, Caseinolytic activity and MCI of the screened plants.

S.no	Plants	MCA (U/ml)	Caseinoytic activity (U/hour)	MCI
1	***C.gigantea***	479±1	63.1 ± 0.6	7.61 ± 0.07
2	***P.rubra***	101±1	31.25 ± 0.25	3.235 ± .005
3	***J.curcus***	-	-	-
4	***E.antiquorum***	6.16±0	1.465 ± 0.015	4.205 ± 0.045
5	***A.cathartica***	11.5±0.5	0.215 ± 0.015	53.585 ± 0.105

High proteolytic levels associated with vegetable coagulantsis a critical factor for not being able to identify ideal rennet substitute. In the current study, *C. gigantea*latex crude enzyme showed highest caseinolytic activity which was followed by that of*, P. rubra, E.antiquorum, A.cathartica andJ. curcus* (Table 2). Milk clotting index was highest for *A.cathartica* followed by *C. gigantea.* The ratio of milk clotting to proteolytic activity of proteases is an important standard for calf rennet's replacement. Though crude enzyme of *A.cathartica possess* low MCA when compared to *C. gigantea,* it slow proteolytic activity enhances its MCI and thereby its suitability as an ideal and effective vegetable milk coagulant. A

similarly low proteolytic activity associated with another species of Euphorbia was reported earlier [14].

CONCLUSION

Screening of latex producing plants for their milk clotting potential revealed crude enzyme from *A.cathartica* latex to be a promising vegetable milk coagulant. *C. gigantea* was found to have high milk clotting activity, however its caseinolytic activity was significantly high.Further purification and analysis of crude enzymes of *A.cathartica* and *C. gigantea*for their caseinolytic activity and milk clotting index may substantiate their suitability as vegetable milk coagulants.

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REFERENCES

[1] Sousa MJ, Ardo Y and McSweeney PLH. Int Dairy J 2001; 11: 327–45.
[2] Ahmed IAM, MorishimaI, Babiker EE and MoriN. Food Chem 2009a; 116: 395-400.
[3] Ahmed IAM, Morishima I, Babiker EE & Mori N. Phytochem 2009b; 70:483-91
[4] Sousa MJ and Malcatta FX. Le Lait 2002; 82:151-170
[5] Rajesh R, Gowda CDR, Nataraju A, Dhananjaya BL, KemaparajuK, Vishwanath BS. Toxicon 2005; 84-92.
[6] Indranil Chanda, Sanat Kumar Basu, Sadhan Kumar Dutta and Smriti Rekha Chanda Das. Trop J Pharm Res 2011; 10 (6): 705-11.
[7] Lowry OH, Rosenbrough NJ, Farr AL, Randall R J. J Biol Chem 1951;193: 265-275.
[8] Murata J, Satake M, Suzuki T. J Biochem 1963; 53:431–443.
[9] Arima Ya J, and Iwasaki SS. Methods in Enzymology. New York: Academic press. Milk clotting enzyme from *Mucor pusillus* var. Lindt. In E.G. Pearlman &Lorand (Eds). 1970; 446-459.
[10] Kumari M, Sharma A and Jagannadham MV. J Agr Food Chem 2010; 58: 8027–8034.
[11] Umar Dahot M, Yakoub Khan M, Memon AN. Journal of Islamic Academy ofSciences 1990; 3(4): 284-286.
[12] Aworh OC and Nakai S. J Food Sci 1996; 51(6): 1569-70
[13] Oseni OA and Ekperigin MM. International Journal of Biotechnology research 2013a; 1 (2):24-27.
[14] Shamkant B Badgujar and Raghunath T Mahajan. Indian J Nat Prod Res 2012; 3 (2):152-160.