

Milk Clotting Potential of *Wrightia tinctoria* Latex on Buffalo (*Bubalus Bubalis*) and Goat (*Capra Hircus*) Milk

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Abstract: - Milk clotting potential of raw latex and crude enzyme extract (CEE) collected from *Wrightia tinctoria* fruits were examined in two different milk media. The raw latex and crude enzyme extract (CEE) exhibited excellent milk clotting activity against buffalo (*Bubalus bubalis*) and goat (*Capra hircus*) milk. The clotting potential was found to be higher for the crude enzyme extract and it showed good clotting activity against buffalo milk compared with goat milk. Effect of pH and temperature on the milk clotting activity (MCA) of raw latex and crude enzyme extract was studied. The results showed that the MCA of both raw latex and crude enzyme extract was increased with increasing the pH (up to 5) and the temperature. The highest MCA was found at 60 °C for crude enzyme extract. It was found that the addition of NaCl decreased the MCA and increased the clotting time of both raw latex and crude enzyme extract. Meanwhile the addition of CaCl₂ increased the MCA by decreasing the clotting time.

Keywords:- *Wrightia tinctoria*, Milk clotting activity, buffalo and goat milk.

I. INTRODUCTION

Cheese is an important milk product with rich nutrients and it is manufactured by the enzyme called rennet which produced from the calf stomach. It clots milk rapidly and used by the cheese production industries [1]. The production of the rennet is not enough to fulfill the need of emerging global cheese production. Hence, a lot of research works have been taken up to identify the alternative sources for the rennet, which are mainly focused on the plant and microbial coagulation enzymes [2]. Some of the animal rennet enzymes [3, 4] had been reported as the alternative for calf rennet. Many microbial enzymes prepared from fungus shows very good clotting activity [5]. Enormous of milk clotting enzymes had been purified from plants [6-9]. Because of the easy availability and simple purification methods, researches on plant rennet as clotting agent have been growing. [10]

Wrightia tinctoria belongs to *Apocynaceae* family is a small tree with milky latex, grows up to 15 meter and widely distributed in India and Burma. Leaves of this tree are opposite, variable, elliptic and are about 10-15 cm in length. White colored smelly flowers are seen in the branches with five lobes. The fruits are follicles in pairs and cylindrical in shape. The plant is rich in flavonoids, phenolic acids, alkaloids and phenol [11-12]. It has analgesic, anti-inflammatory,

anthelmintic, antiulcer, antidiabetic, anticancer activities [13] and is anciently used for healing of wounds [14].

II. MATERIALS AND METHODS

A. Chemicals, Plant material and milk source

All the chemicals (AG) were purchased from Merck, India, and used without further purification. The latex of *Wrightia tinctoria* were collected from the fruits of the plant in test tubes and stored at 4 °C in the laboratory. The milk samples from buffalo and goat were collected from the nearby farm houses in and around Irumbupalm village, Namakkal District.

B. Preparation of crude enzyme extracts (CEE)

10 ml of raw latex was mixed with 50 ml of 0.1 M Sodium acetate solution at pH 5.5. The mixture was kept at 4 °C for 24 hours and then filtered. The filtrate was centrifuged for 20 minutes at 2000 rpm. The brownish colored supernatant obtained was stored in a separate container and used for the study.

C. Milk clotting activity

The milk clotting activity of raw latex and crude enzyme extract (CEE) was determined as by the method of N. J. Berridge [15]. One milk clotting unit is determined as the amount of enzyme in 1 ml of extract clotting 10 ml of milk in 100 seconds at 30°C. The Milk clotting activity is calculated using the formula,

$$MCA = \frac{10 V}{Tc v}$$

Where, MCA- milk clotting activity, V- volume of milk, v- volume of enzyme and Tc- clotting time in seconds. The coagulated milk was then filtered using cheese cloth to get a semi solid cheese. Cheese prepared from the plant latex is shown in Figure.1.



Fig.1 Cheese prepared from plant latex.

D. Effect of pH and temperature on milk clotting activity

Effect of pH on milk clotting activity of raw latex and crude enzyme extract was determined at different pH values at the range of 1-7. The effect of temperature on milk clotting activity of raw latex and crude enzyme extract was measured in different temperatures between 30°C to 60 °C.

E. Effect of NaCl and CaCl₂ on milk clotting activity

Effect of NaCl and CaCl₂ on the milk clotting potential of raw latex and crude enzyme extract was determined by addition of various concentrations of NaCl and CaCl₂.

III. RESULTS AND DISCUSSION

The raw latex and crude enzyme extract (CEE) from the latex of *Wrightia tinctoria* exhibit excellent milk clotting activity

against buffalo and goat milk. The clotting potential is found to be higher for the crude enzyme extract and it shows good clotting activity against buffalo milk than goat milk.

A. Effect of pH and temperature

The effect temperature and pH on milk clotting activity of raw latex and crude enzyme extract are given in Table 1. The studies showed that the MCA of both clotting agents are increased with increase the pH up to 5, further increase in the pH causes decrease in MCA. The milk clotting potential of raw latex and crude enzyme extract is increased with increasing the temperature from 30 °C to 60 °C. The effect of pH and temperature are represented in the Figures 2 and 3 respectively. The highest MCA was found at pH 5 and 60 °C for crude enzyme extract in buffalo milk.

Temperature °C	MCA				pH	MCA			
	Buffalo milk		Goat milk			Buffalo milk		Goat milk	
	Raw latex	CEE	Raw latex	CEE		Raw latex	CEE	Raw latex	CEE
30	0.14	0.15	0.13	0.14	4	0.34	0.37	0.33	0.33
35	0.21	0.22	0.20	0.21	5	0.80	0.95	0.75	0.79
40	0.24	0.26	0.23	0.25	6	0.55	0.57	0.46	0.49
45	0.25	0.36	0.29	0.32	7	0.45	0.48	0.40	0.43
50	0.46	0.53	0.41	0.46	-	-	-	-	-
55	0.57	0.68	0.56	0.65	-	-	-	-	-
60	0.85	0.95	0.75	0.79	-	-	-	-	-

Table.1 Effect of pH and temperature on MCA

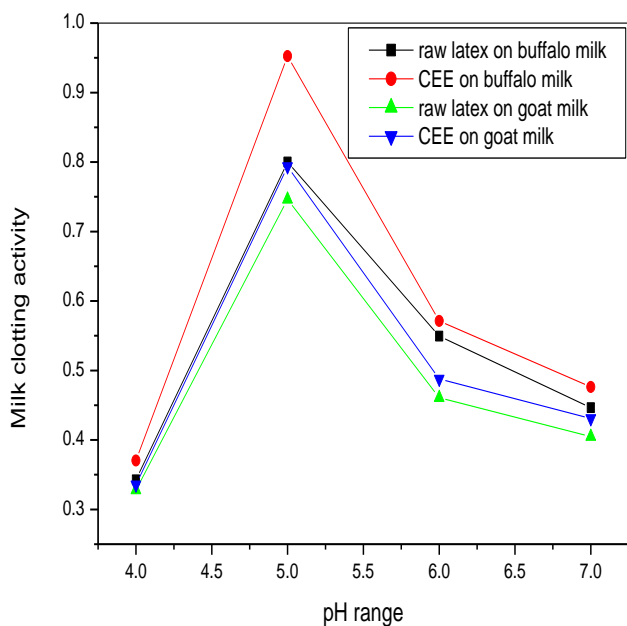


Fig.2 Effect of pH on milk clotting activity

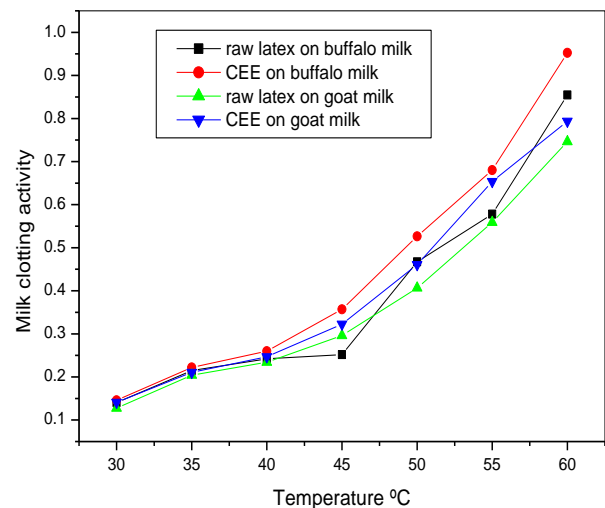


Fig.3 Effect of temperature on milk clotting activity

B. Effect of NaCl and CaCl₂ on milk clotting activity

The change in milk clotting activity for different NaCl and CaCl₂ concentrations are given in Table. 2 and illustrated in Figure 4 and Figure 5 respectively. Results indicate that the addition of NaCl decreased the MCA by increasing the clotting time. This is because, the added NaCl increased the coagulation time and lowers the curdling process, and hence it can be used to make soft cheese. Meanwhile the addition of CaCl₂ increased the MCA by decreasing the clotting time.

NaCl	MCA				CaCl	MCA			
	Buffalo milk		Goat milk			Buffalo milk		Goat milk	
	Raw latex	CEE	Raw latex	CEE		Raw latex	CEE	Raw latex	CEE
0	0.78	0.95	0.64	0.80	0	0.78	0.95	0.64	0.80
5	0.46	0.51	0.41	0.48	5	0.95	1.05	0.88	0.93
10	0.27	0.32	0.27	0.28	10	1.09	1.25	1.03	1.08
15	0.16	0.19	0.16	0.17	15	1.35	1.47	1.12	1.20

Table.2 Effect of NaCl and CaCl₂ on MCA

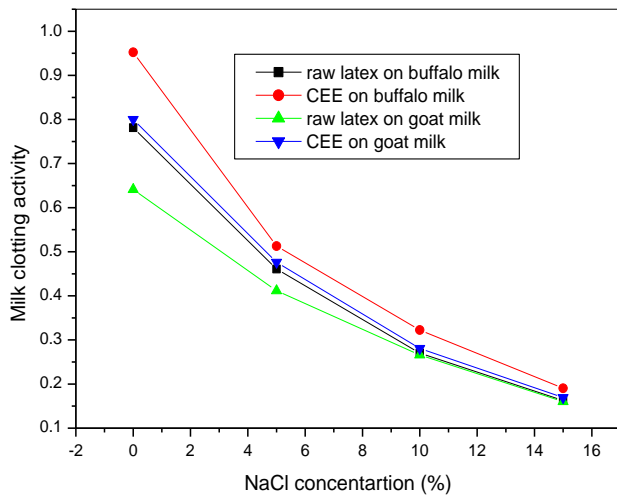


Fig.4 Effect of NaCl on Milk clotting activity

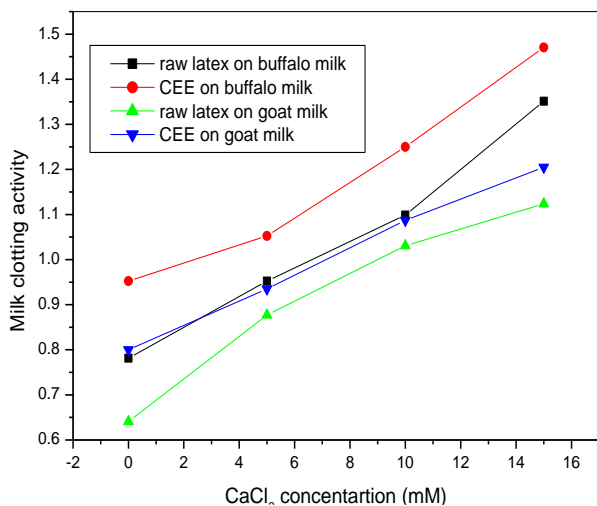


Fig.5 Effect of CaCl₂ on milk clotting activity

IV. CONCLUSION

The raw latex and crude enzyme extract from *Wrightia tinctoria* fruits exhibited excellent milk clotting activity against buffalo and goat milk. The clotting potential of this plant coagulant was found to be higher for the crude enzyme extract and it showed good clotting activity against buffalo milk compared with goat milk. The MCA of both raw latex and crude enzyme extract was increased with increasing the pH and the temperature. It was found that the addition of NaCl decreased the MCA and CaCl₂ increased the MCA by influencing the clotting time. To conclude, the plant latex and

the crude enzyme extract shows an appropriately high milk clotting activity and can be used as an alternant for animal rennet.

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