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Full Length Research Paper

Distribution of proteolytic and milk clotting enzymes in the plant of Sodom apple *Calotropis procera* (Ait.) R.Br. (Asclepiadaceae)

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The present study was undertaken to observe the distribution of milk clotting and proteolytic activities of the crude enzymes in the various parts (leaves, tender stem, matured stem, fruits, root and latex) of *Calotropis procera* (Sodom apple) or Bomubomu in Yoruba Language for use in the manufacturing of cheese and allied milk products and medicine. The results showed that milk clotting enzyme activities were evidenced in all the extract preparations except the root that did not show milk-clotting activity. The latex of the plant had the highest proteolytic and milk clotting activities at 35°C and pH 5.5. The root had higher proteolytic activity than stem, leaf or fruit. All parts of the plant possessed enzymes with proteolytic and milk clotting activities (except milk-clotting activity in root) which can be utilized in food industries and for medicinal purposes.

Key words: Cheese making, protein hydrolysis, enzyme activities, *Calotropis procera*.

INTRODUCTION

Calotropis procera (Ait.) R. Br., a wild growing plant of family *Asclepiadaceae*, is well known for its medicinal properties. Different parts of this plant have been reported to exhibit anti-inflammatory, analgesic, and antioxidant properties. It is found in most parts of the world in dry, sandy and alkaline soils and warm climate and is more common in south western and central India and western Himalayas. It is found in waste lands and grows as a weed in agricultural lands. In ancient Ayurvedic medicines, the plant *Calotropis procera* was known as “Rakta arka” (Verma et al., 2010).

The genus *Calotropis* R.Br. (Asclepiadaceous) is distributed in tropical and subtropical regions of Asia and Africa (The wealth of India, 1959). It is a common plant in Nigeria but it is more abundant in the northern part of the country (Sofowora, 1984).

For many years now, the normadic Fulani women of the Northern Nigeria use the part of the plant in the production of warankasi (a local soft cheese); the practice is still popular today even in almost all the parts of the country where fluid milk is abundant. The importance of this plant locally called “bomubomu” in South-West of Nigeria for use in the country as a cuddling agent in local cheese production came to limelight many decades ago in countries like India where the latex was used in Indian medicine as a blistering agent (Nadkarni, 1976). Jain et al. (1996) reported that *Calotropis procera* was used in traditional medicine as a purgative, anthelmintic, anticoagulant, anticancer as well as antipyretic, analgesic and antimicrobial agent. Fleurentin and Pelt (1982) also observed that the plant was used as an antiseptic for skin infection. Several studies have been carried out on the effects of various extracts of *Calotropis procera* on different organs of animals (Al-Robai et al., 1993a, b; Jain et al., 1996; Basu et al., 1997). Upon contact with eye, latex causes severe irritation, a burning sensation,

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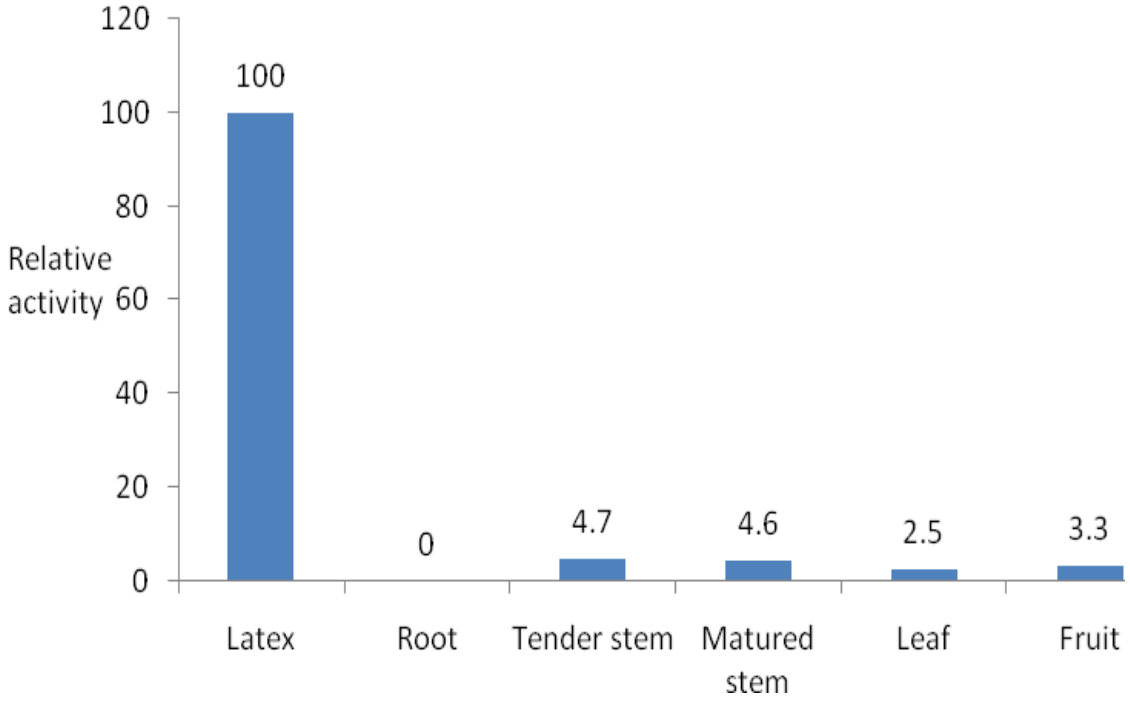

Figure 1. Milk clotting activity of the extracts from various parts of the plant.

oedema of eyelids and blurred vision (Matthayya, 1948; Wong, 1949; Crawford, 1958; Sugiki, 1966; Duke-Elder and Macfawl, 1972) with spontaneous recovery requiring no specific treatment (Grant, 1974). The latex has also been reported to be irritant, caustic, and depilatory when applied to the skin (Behl et al., 1966; Morton, 1962; Blohm, 1962; Nadkarni, 1976). The objective of this work is to screen various parts of the plant for isolation of milk clotting and proteolytic enzymes.

MATERIALS AND METHODS

The plant was obtained from a farm settlement around the Federal University of Technology, Akure, Ondo State, Nigeria. The latex was collected in a sterile container by breaking of the leaves while the other parts of the plant were obtained by up-rooting the plant. The roots were washed with water, while stems were cut into pieces and the leaves were removed.

Extraction of samples

Five gram each of root, tender stem, matured stem, leaf and fruit was crushed separately in pastel mortar and extracted with 25 ml of 0.05 M sodium acetate buffer, pH 5.5. One ml of the latex was mixed with 5 ml of 0.05 M sodium acetate buffer, pH 5.5. The extract was centrifuged at 3000 rpm for 15 min and the supernatant was stored in refrigerator for analysis.

Determination of milk clotting activity

Powdered milk (0.25 g) was weighed into a clean test tube and 0.75 ml of 0.05 M sodium acetate buffer pH 5.5 was added to it. The test tube was shaken vigorously until the milk dissolved and was placed in water bath for 10 min at 35°C. Thereafter, 1.0 ml of the crude extract was added and the time taken for the milk solution to clot was recorded.

Determination of the proteolytic activity

The extract was assayed for protease activity by a casein degradation method. Casein solution (1%) was prepared in 0.05 m citrate phosphate buffer pH 7.5 and heat-denatured at 100°C for 15 min in water bath, cooled and used as substrate. The reaction mixture consisted of 1 ml of the substrate and 1 ml of the extract was thoroughly mixed and incubated for 1 h at 35°C. The reaction was terminated by adding 3 ml of cold 10% trichloroacetic acid. The tubes were allowed to stand for 1 h at 2°C in a refrigerator to precipitate the undigested protein. The contents were centrifuged at room temperature for 30 min and absorbance of the supernatant measured at 280 nm (Ladd and Butler, 1972).

RESULTS AND DISCUSSION

Figure 1 shows the milk clotting activities of the various

Table 1. Ratio of activities.

Parts of plant extract	Milk clotting activity (units/ml)	Protease activity (units/ml)	Ratio of milk clotting to protease activity
Latex	6.67	0.052	128.27
Root	0.00	0.0344	0.00
Tender stem	0.314	0.0148	21.22
Matured stem	0.31	0.0147	21.09
Leaf	0.166	0.0141	11.77
Fruit	0.222	0.0118	18.81

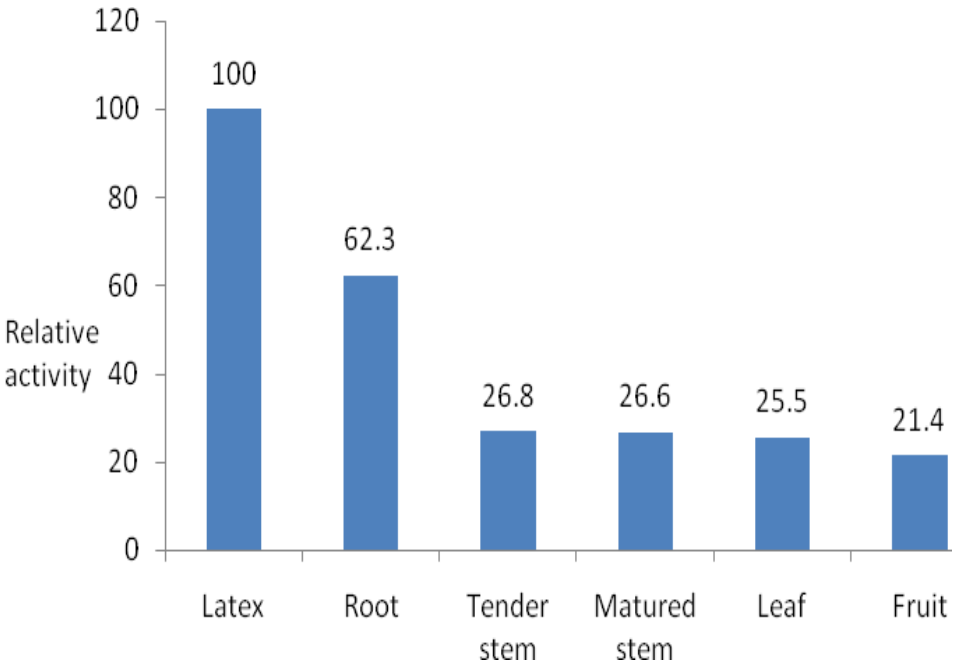

Figure 2. Proteolytic activity of the extracts from various parts of the plant.

parts of the plant with latex having maximum activities followed by stem, fruit and leaf in that order. The results obtained followed the trend observed by Dahot et al. (1990). Similarly, the ratio of milk clotting to protease activity (Table 1) was highest in latex in comparison to other parts followed by stem, fruit and leaf in that order in the plant. The ratio of activities is an important factor in cheese making (Dahot et al., 1990). The bark and latex of the plants have been used to brew and curdle milk (Dahot et al., 1990). All plant parts possessed proteolytic activities but the root did not show any observable milk clotting activity. In fact, the plant leaf has been used as clotting agent in the local production of “warankasi” (Ihekoroye and Ngoddy, 1985).

The observations from this study showed that all preparation contained protease activity and milk clotting activity apart from root which however was similar to those reported by Dahot et al. (1990) as seen from Figure 2. It is well known that almost all proteolytic enzymes clot milk, except the ratio between the milk clotting activity and protease activity, seemed to be very important in cheese making. The ratio of milk clotting to protease activity observed in this study was lower than those obtained by Dahot et al. (1990) for calotropis leaf collected from Sindh University which could be due to changes in environmental conditions and locations. Evidence from the work showed that the maturity or ageing of the stem has very little or no effect on the milk clotting and proteolytic activities of the plant.

Conclusion

In the crude extracts from different parts of the plant of Sodom apple, the milk clotting activity is generally distributed in all the parts of the plants except the root that could not be used to curdle milk in the local

production of cheese. It is also evidenced from the study that proteolytic enzymes are well distributed in all the parts of the plant and the extract from any part can be employed in proteolytic assays.

REFERENCES

Al-Robai AA, Abo-Khatwa AN, Danish EY (1993a). Toxicological studies on the latex of the usher plants Calotropis procera (Alt). R. Br. In Saudi Arabia.III.

Al-Robai AA, Abo-Khatwa AN, Danish EY (1993b). Toxicological studies on the latex of the usher plants (procera(Ait). R. Br. In Saudi Arabia. 11 Ultra structural changes in the cardiac muscle and Variation in the blood serum composition of Treated Albino rats. Arab Gulf J. Sci. Res., 1(13): 425-440.

Basu A, Sen T, Pal S, Mascolo N, Capasso F, Chaudhuri AKN, Nagchaudhuri AK (l997). Studies on the Anti-ulcer activity of the chloroform fractions of *Calotropis procera* root extract, Phytotherapy Res., 11(2): 1 63165.

Behl PN, Captain RM, Bedi BMS, Gupta S, (1966). Skin-Irritant and Sensitizing Plants Found in India. New Delhi: PN Behl.

Bharti S, Wahane VD, Kumar VL (2010). Protective effect of Calotropis procera latex extracts on experimentally induced gastric ulcers in rat. J. Ethnopharmacol., 127(2): 440-444

Blohm H (1962). Poisonous Plants of verecudia. Cambridge, mass: Harvard University Press.

Crawford HE (1958). Crown flower keratoconjunctivitis. Hawaii Med. J., 17(3): 244-245.

Dahot MU, Khan MY, Meimon AN (1990). Screening of some Pakistani Plants for milk clotting activity. J. Islamic Acad. Sci., 3(4): 284-286.

Duke-Elder S, Macfaw PA (1972). System of Ophthalmogy. Vol. XIV. London: Herry Kinpton.

Effects of usher latex on the fine structures, oxygen consumption and $Na^{+}K^{+}$ Transporting AtPase Activity of Albino Rat kidneys.Arab-Gulf Journal-of -Scientific-Research, 11(3): 441-455.

Fleurentin J, Pelt JM (l982). Repertory of drugs and Medicinal plants of Yemen J. Ethnopharmacol., 5: 85-108

Grant WM (1974). Toxicology of the Eye, 2nd edn. Springfield, Ill.: CC Thomas. pp. 15-27.

Ihekoronye AI, Ngoddy PO (1985). Integrated food Science and Technology for the tropics. Macmillan Education Ltd. London. p. 357.

Jain SC, Sharma R, Kain R, Shanna RA (1996). Antimicrobial Activity of *Calotropis procera.* Fitoterapia. 67(3): 275-277.

Ladd JN, Butler JHA (1972). Short-term assay of soil proteolytic enzyme activities using proteins and dipeptide substrates. Soil Biol. Biochem., 4: 19-39.

Matthayya RES, (1948). Madar keratites. Proc. All-ludi Oplithal. Soc., 10: 44

Morton JF (1962). Ornamented plants with poisonous properties. II. Proc. Fla. St. Hort. Soc., 75: 484.

Nadkarni KM (1976). Indian materia medica, 3rd Education (Revised and enlarged by Nalkarni A.K.) Vol. 1 and 2 Bombary: Popular Poakashar.

Sofowora A (1984). Medical plants and Traditional Medicine in AfricaJohn Wiley and Sons Limited, lbadan. pp. 142-146.

Sugiki S (1966). Crowflower or cornflower. Achiever Ophthal., 75: 736.

The Wealth of India (1959). Raw Materials, Vol. II, CSIR, New Delhi, pp. 20-23.

Verma R, Satsangi GB, Shrivastava JN (2010). Ethno-Medicinal Profile of Different Plant Parts of Calotropis procera (Ait.) R. Br. Ethnobotanical Leaflets, 14: 721-42.

Wong WH (1949). Crownflower kera to conjunctivitis. Hawaii Med. J., 8: 339.