Suitability Of *Calotropis Gigantea* Extract As A Cleaning Agent For Dairy Storage Unit

H.Akshayan , Rita Narayanan, , G.SenthilKumar

Abstract— *Calotropis gigantea is a milkweed that is commonly available in parts of India, Srilanka, & Bangladesh. The latex of Calotropis gigantea contains the cardiac glycosides which belong to the alcohol soluble portion. The leaves of the milkweed Calotropis gigantea were used for the study. An alcoholic extract was prepared with 70% ethanol (v/v) and was centrifuged and freeze dried. The freeze dried product was diluted in phosphate buffer saline and partially purified extract was obtained through sephadex-G-100 chromatography. The protein content was analyzed by Biuret's method. The efficacy of the leaf extract was tested on samples from a dairy can and the results were recorded. It is concluded from the study that the leaf extract of Calotropis gigantea is suitable as a cleansing agent for dairy storage units.*

Index Terms— *Caloptropis gigantea, Sephadex-G-100, Biuret's method, Proteolysis,*

INTRODUCTION

1.1. MORPHOLOGY OF C. gigantea (The Wealth of India, 1992)

A tall shrub reaching 2.4-3 m. high; bark yellowish white, furrowed; branches stout, terete, more or less covered (especially the younger ones) with fine appressed cottony pubescence. Leaves 10-20 by 3.8-10 cm., sessile ,or nearly so, elliptic-oblong or obovate-oblong, acute, thick, glaucous-green. Clothed beneath and more or less above with fine cottony tomentum; base narrow, cordate, sometimes amplexicaul. Flowers inodourous, purplish or white, 3.5-5 cm. diam., in umbellate lateral cymes; peduncles from between the petioles, 5.9 cm. long, dilated at the base; pedicels much longer then the flowers, covered with cottony wool; buds ovoid. Calyx divided to the base; sepals 6 by 4 mm., ovate, acute, cottony. Corolla 2 cm. long or more; lobes 1.3-1.6 cm. long, deltoid-ovate, subacute, revolute and twisted in age; lobes of the corona 1.3cm. long by 5 mm. broad in the middle, shorter than the column, the back much curved towards the column above the obtuse spur, pubescent on the slightly thickened margin, the apex rounded (not bifid) with 2 obtuse auricles just below it. Follicles 9-10 cm. long, broad, thick, fleshy, ventricose, green. Seeds numerous, 6 by 5 mm., broadly ovate, flattened, narrowly margined, minutely tomentose, brown; coma 2.5-3.2 cm. long.

- *Akhsayan H is currently pursuing masters degree program in Food & Nutritional Biotechnology in SRM University,India, E-mail: akshayan2006@gmail.com*
- *Rita Narayanan is currently pursuing Doctorate degree program in TANUVAS,India, E-mail: ritanarayanan@yahoo.com*
- *Senthil Kuma G has completed Bachelors in Biotechnology in Anna University, India, Email: senthilbt8@gmail.com*

1.2. *Calotropis gigantea*

C. gigantea and *C. procera,* closely resemble each other, are found in the same regions, and are put to almost the same medicinal use. The latex of both the species contains the cardiac glycosides, calotropin, uscharin, calotoxin, calactin and uscharidin; gigantin has been reported only in *C. gigantea.* Calotropin, uscharin show digitalis like action on the heart. A bacteriolytic principle, capable of lysing *Micrococcus lysodeikticus* was also found in the latex. A non toxic proteolytic enzyme, calotropain (2-3%), has been isolated from the latex; it is more proteolytic than papain, ficin and bromelain, coagulates milk and digests milk, gelatin and casein. (The Wealth of India, 1992). Murthy *et. al* (1943) studied the chemical composition of *calotropis gigantea.* They reported that the cardiac poisons belonged to the alcohol soluble portion.

The latex of both the species contains some poisonous constituents due to which it has somewhat caustic effect on the mucous membrane and tender skin, and may cause secondary dermatitis. It acts as a drastic purgative and emetic, it is used as an arrow poison in Africa. The latex is highly toxic to rabbits, dogs and donkey if administered in high doses, but not to ruminants; it increases the heartbeat and respiration in animals leading to distress and death. It is reported to be used as an antidote for scorpion sting. (The Wealth of India, 1992)

Distribution: Throughout India, Ceylon.-Malay Islands, and S. China.

All parts of the plant dried and taken with milk act as a good tonic, expectorant, and anthelmintic.-The leaves are applied to paralysed parts, painful joints, swellings; heal wounds.- The milk is caustic, acrid; expectorant, depilatory, anthelmintic; useful in

leprosy, scabies, ringworm of the scalp, piles, eruptions of the body, asthma, enlargement of the spleen and liver, dropsy;applied to painful joints, swellings.-The flowers are atomachic and good for the liver. (The Wealth of India, 1992)

Oil, in which the leaves have been boiled, is applied to paralysed parts, a powder of the dried leaves is dusted upon wounds to destroy excessive granulation and promote healthy action. (The Wealth of India, 1992)

The root, bark, and juice of this plant are used in medicine for their emetic, diaphoretic, alternative, and purgative properties. In the treatment of the dysentery, the dried bark of the root is stated to be an excellent substitute for Ipecacuanha. The bark, root, and dried milky sap may be used in small doses in certain cutaneous affections such as leprosy and secondary syphilis; the root-bark, in large doses, is an emetic. It is administered to promote secretions, and is stated to be useful in enlargements of the abdominal viscera, intestinal worms, cough, ascites, anasarca, etc. the flowers are considered digestive, stomachic, tonic, useful in asthma, catarrh, and loss of appetite. The powder of the root in 3 to 5 grains promotes gastric secretion and acts as a mild stimulant and may be given with carminatives is dyspepsia. It is also given as a febrifuge. (The Wealth of India, 1992)

The tincture from the leaves was tried as an antiperiodic in cases of intermittent fevers. It was found to be useful in many cases. The powdered root bark in doses of five grains was given to several cases of dysentery and was generally found to give relief. (The Wealth of India, 1992)

The plant is a popular remedy for snake-bite and scorpion-sting; but every part of it is quite useless in the antidotal and symptomatic treatment of either snake-bite or a scorpion sting. (The Wealth of India, 1992)

1.3. SCOPE OF THE STUDY:

The use of the synthetic detergents for the cleaning of the storage utensils is increasing day-by-day. However, these detergents eventually affect the aquatic ecosystem and lead to biomagnifications. Moreover, these detergents cause water pollution leading to various health hazards. Our study is focused to develop a biodegradable alternative to the chemical detergents, The *Calotropis gigantea* leaf extract.

Our study comprises of the following objectives:

- To obtain crude leaf extract of *calotropis gigantea.*
- To partially purify the leaf extracts.
- To test the efficacy of *calotropis gigantea* leaf extract as a cleansing agent for dairy equipment.

2. MATERIALS & METHODS

1000 grams of Fresh leaves of *calotropis gigantea* were weighed and washed thoroughly with tap water and cut in to small pieces and macerated separately by adding distilled water, till a homogenate liquid was obtained. The extracts were filtered in a double-net filter and treated with 100ml of 70% Ethanol and stirred well. The contents were stored overnight at 4°C, followed by decantation of the top-layer followed by centrifugation at 8000rpm for 5 minutes. The pellet was frozen at -30°C and then subjected to freeze drying at -48°C for 24 hours. The freeze dried pellet was diluted in phosphate buffer in 1:2 ratio and purified by gel filtration in a Sephadex G100 column (Andrews *et. al* 1965) followed by Protein estimation through Biuret (1948) method.

2.1. Rinsing Method:

2.1.1. Pre rinsing with buffer

500ml of sterile buffered distilled water was in 20L can. Then the can was rotated horizontally and shaken vigorously 25 times. 1ml of the buffer was withdrawn from the can and used for bacterial isolation by serial dilution method.

2.1.2. Rinsing with enzyme

10 ml of enzyme was taken in the pre-rinsed can. Then the can was rotated horizontally and vigorously shaken 25 times.8 complete rotations of the can were made during shaking operation to rinse sidewalls thoroughly and the enzyme was poured out.

2.1.3. Post rinsing with buffer

500ml of sterile buffered distilled water was in 20L can. Then the can was rotated horizontally and shaken vigorously 25 times. 1ml of the buffer was withdrawn from the can and used for bacterial isolation by serial dilution method.

2.1.4. Plating rinse solutions

The samples were serially diluted up to 10^2 dilution. The dilutions were plated in Standard Plate Count agar and Violet Red Bile Agar. Then the dilutions were heated u to a temperature of 80°C and

then plated in order to identify the count of Thermophilic bacteria. A control trial was carried out with 0.5% Teepol. The samples were plated and the colony counts were tabulated.

3. RESULT AND DISCUSSION

Synthetic detergents are largely used in industries as a cleansing agent, in spite of the various disadvantages of synthetic detergents like insolublity in water, non biodegradable their persistence in the environment for a longer time leading to prolonged ill effects. These detergents directly or indirectly contribute to the various health hazards of the human and also affect the aquatic population. *Calotropis gigantea* is a milkweed which has a variety of medicinal and other applications. The present study aims at using the *Calotropis gigantea* leaf extract as an alternative to detergents used for cleaning dairy utensils.

4. CONCLUSION

The present investigation has elucidated the cleansing property of *Calotropis gigantea.* The leaves were used and a crude extract was obtained. To make this crude extract as cleansing agent the following treatment was done followed by 70% alcohol extraction where the cardiac glycosides present in the plant extract are separated out. The ethanolic extract was centrifuged and the pellets were freeze dried and the product was stored at -20°C. The freeze dried product was diluted in Phosphate Buffer saline and partially purified extract was obtained by Sephadex G 100 column chromatography. The above obtained extract was used for rinsing the cans and the efficiency was compared with that of the conventional dairy detergent.

Protein content of the sample was estimated to be 1.23g/dl. The proteolytic nature of the proteins in *Calotropis gigantean* can be exploited for easy removal of the casein biofilm sedimented on the wall of the container. The colonies were counted and the values were expressed statistically in Table I and Table II. The detergent has given an efficiency of 53.87% and 59.80% on the dilutions 10^{-1} and 10^{-2} respectively, whereas the leaf extract of *Calotropis gigantea* gave an efficiency of 83.117% and 88.174% on the dilutions 10^{-1} and 10^{-2} respectively.

5. References

• **Adetunji V O., and Salawu.O T.,** 2008. West African soft cheese 'wara' processed with *Calotropis procera* and *Carica papaya*: A comparative assessment of nutritional values. African Journal of Biotechnology. 7. pp. 3360-3362.

• **Allan G. Gornall, Charles J. Bardawill, Maxima M. David**. 1948. Determination of Serum Proteins by means of the Biuret reaction. The Journal of Biological Chemistry. 177. pp 751-766.

• **Andrews P.,** 1965. The gel filtration behavior of proteins related to thir molecular weights over a wide range. Biochemistry Journal. 96. 595-605.

• **Aworh O C., and Nakai S.** Technical note: Separation of Sodom apple proteinases by gel filtration. 1988. International Journal of Food Science and Technology. 23. pp 419-423.

• **Bharti S., Wahane V D., Kumar V L.,** 2010.Protective effect of Calotropis procera latex extracts on experimentally induced gastric ulcers in rat. Journal of Ethnopharmacology. 127. pp 440–444.

• **Bishnu Joshi, Sunil Lekhak, Anuja Sharma.,** 2009. Antibacterial Property of Different Medicinal Plants: *Ocimum sanctum,Cinnamomum zeylanicum, Xanthoxylum armatum* and *Origanum majorana.* Kathmandu university journal of science, engineering and technology. 5. pp 143- 150.

• **Brisca Renuga F, and Sahayaraj K.** 2009 Influence of botanicals in total head protein of Spodoptera litura. Journal of Biopesticides. 2. pp 52-55.

• **Carlos Edison Lopes, Maria Alice Gomes de Andrade Lima, N. Pereira Jr.** 2002. The influence of centrifugation on *Zymomonas mobilis* aggregation. Electronic Journal of Biotechnology. 5. pp 272-278.

• **Clementina Barrera Bernal & Gabriela Vázquez & Icela Barceló Quintal & Anne Laure Bussy**. 2008. Microalgal Dynamics in Batch Reactors for Municipal Wastewater Treatment Containing Dairy Sewage Water. Water, Air, Soil, & Pollution. 190. pp 259–270

• **Daniel, M.Bollag, M.D.Rozycki, S.J.Edelstein.** 1996. Protein Methods Wiley-Liss Publication, New York. pp 21,94,271-275

• **Dilbagh Singh Pannu and M.P.Mathur.** 1990. Partial Purification and Characterization of Active Protease from Withania Coagulans. Indian Journal of Dairy Sciences. 43. pp 81-83

• Environmental, Health and Safety guidelines. 2007. DAIRY PROCESSING, World Bank Group.

• **Fayyaz Ahmad, Rafeeq A. Khan, Shahid Rasheed.** 1992. Study of Analgesic and anti inflammatory activity from plant extracts of *lactuca scariola* and *artemisia absinthium.* Journal of Islamic Academy of Sciences. 5. pp 111-114.

• **Ganesh Kumar. G, D.N Prasad, M.P.Tiwari.** 1998. Enzyme Detergents, The revolutionary Cleaning agents. Indian Dairyman. 50. pp 15-20.

• **Gaurav Lodhi, Hemant Kumar Singh, Kamlesh K. Pant, Zeashan Hussain.** 2009. Hepatoprotective effects of *Calotropis gigantea* extract against carbon tetrachloride induced liver injury in rat. Acta Pharmaceutica. 59. pp 89–96.

• **Jiafa Luo & Surinder Saggar & Rita Bhandral & Nanthi Bolan & Stewart Ledgard & Stuart Lindsey & Wentao Sun.** 2008. Effects of irrigating dairy-grazed grassland with farm dairy effluent on nitrous oxide emissions**.** Plant Soil. pp 119-130.

• **Jitendra B Jain*, Sheetal C Kumane & S Bhattacharya**. 2006. Medicinal flora of Madhya Pradesh and Chattisgarh – A Review. Indian Journal of Traditional Knowledge 5. pp. 237-242.

• **Kareem,S.O,; Akpan, I. and Ojo, O.P.** 2008. Antimicrobial activities of calotropis procera on selected Pathogenic Microorganisms. African Journal of Biomedical Research. 11. pp 105-110.

• **Kharkate S K, Karadbhajne V Y, and Gogte B B.,** 2005. Some resin based ecofriendly liquid detergents. Journal of Scientific & Industrial Research. 64. pp. 752-755.

• **Kuta F A.,** 2008 Antifungal effect pf Calotropis procera stem bark on Epidermophyton flocosum and Trichophton gypseum. African Journal of Biotechnology. 7. pp 2116-2118.

• **Lipscomb, I.P. H.E. Pinchin, R. Collin, K. Harris, C.W. Keevil**. 2006.The sensitivity of approved Ninhydrin and Biuret tests in the assessment of protein contamination on surgical steel as an aid to prevent iatrogenic prion transmission. Journal of Hospital Infection. pp 1-5

• **Mohammed Rahmatullah, Shamsuddin Sultan, Tanzila Taher Toma, Sayeda-a-Safa Lucky, Majeedul H. Chowdhury, Wahid Mozammel Haque, Mst. Eashmat Ara Annay, Rownak Jahan.** 2009. Effect of *Cuscuta reflexa* stem and *Calotropis procera* leaf extracts on glucose tolerance in glucose-induced hyperglycemic rats and mice. *Afr. J. Traditional,* Complementary and Alternative Medicines. 7. pp 109-112.

• **Mst Nazma Yesmin, Sarder Nasir Uddin, Sanzida Mubassara and Muhammad Ali Akond.** 2008 Antioxidant and Antibacterial Activities of .*Calotropis procera* Linn. American-Eurasian Journal of Agriculture & Environmental Science. 4.pp 550-553.

• **Muctar A.Mohamed, Charles B.O'Connor**.1996. Milk Coagulation by calotropis procera juice – Effetcs of juice storage time and temperature trial for cheesemaking. Indian journal of Dairy Science. 49. pp 277-285

• **Narendra Nalwaya, Gaurav Pokharna, Lokesh Deb, Naveen Kumar Jain.** 2009. Wound Healing Activity of Latex of Calotropis gigantea. International Journal of Pharmacy and Pharmaceutical Sciences. 1. pp 176-181

• **Naresh Kumar.C, Rita narayanan.** 2002. Milk clotting activity of *Calotropis gigantean.* Cherion. 5&6.pp 134-136.

• **Ogugua Aworh C & Muller H G.,**1987. Cheese-making Properties of Vegetable Rennet from Sdodm Apple (Calotropis rocera). Food Chemistry. 26. pp 71-79.

• **Pharmacia fine chemicals.** 1978. Sephadex, Gel Filtration in theory and practice. User manual, Uppasala, Sweden.

• **Pathak.**A.K, A.Argal. 2007. Analgesic activity of calotropis gigantea flower. Fitoterapia. 78. Pp 40-42

• **Pradeep Deshmukh, T. Jennifer Fernandes, Akarte Atul, Emmanuel Toppo.** 2009.Wound healing activity of *Calotropis gigantea* root bark in rats. Journal of Ethnopharmacology. pp 178–181

• **Purcifull D E, Christei S R, Batchelor D L.** 1975. Preservation of Plant Virus Antigens by Freeze-Drying. Florida Agricultural Experiment Stations Journal .5849. pp 1202-1205.

• **Rupal Vasant A. and Narasimhacharya. A.V.R.L.** An investigation on the termiticidal effects of certain weed

plants. *'Prajna' - Journal of Pure & Applied Sciences.* 16. pp 1-8.

• **Schwarzenbeck. N. Borges J. M. Wilderer**.**P. A.** 2005. Treatment of dairy effluents in an aerobic granular sludge sequencing batch reactor. Applied Microbiology and Biotechnology. 66. pp 711–718.

• **Sorimuthu Pillai Subramanian and Venkatesan Saratha**. 2010 Evaluation of Antibacterial Activity of *Calotropis gigantea* Latex Extract on Selected Pathogenic Bacteria. Journal of Pharmacy Research. 3 . pp 517-521

• **The Wealth of India**. A Dictionary of Indian Raw Material and Industrial Products. Volume-III.Council of Scientific and Industrial Research, New Delhi; 2004. pp. 78-81

• **Toliwal, S D Darshan Patel, Patel J V & Kalpesh Jadav**. 2009. Utilization of by-product of oil processing industry in liquid detergent. Indian Journal of Chemical Technology. 16. pp. 373-376.

• **Tripathi B. D. and Alka R. Upadhyay.** 2003.Dairy Effluent Polishing By Aquatic Macrophytes. Water, Air, and Soil Pollution. 143. pp 377–385.

• **Usha,K. Singh B., Praseetha P., Deepa N., Agarwal D. K., Agarwal R., & Nagaraja A.** 2009. Antifungal activity of Datura stramonium, Calotropis gigantea and Azadirachta indica against Fusarium mangiferae and floral malformation in mango. European Journal of Plant Pathology. 124. pp 637–657

Table I: Colony count in pre rinse and post rinse using Detergent

Dilution	**10^{-1}**		**10^{-2}**	
Sample Type	Pre Rinse	Post Rinse	Pre Rinse	Post Rinse
Total Viable Count	29.4 ± 1.14	4.4 ± 0.89	19.6 ± 1.14	2.4 ± 1.14
Aerobic Count	18.6 ± 1.14	2.8 ± 1.14	14.8 ± 0.83	1 ± 0.70
Coliform Count	23.6 ± 1.14	6.4 ± 2.07	23.6 ± 1.04	1.6 ± 0.548

Table II: Colony count in pre rinse and post rinse using Calotropis gigantea

Dilution	**10^{-1}**		**10^{-2}**	
Sample Type	Pre Rinse	Post Rinse	Pre Rinse	Post Rinse
Total Viable Count	27.55 ± 5.95	12.833 ± 3.11	20.833 ± 2.48	5.83 ± 1.485
Aerobic Count	9.6 ± 1.14	3.2 ± 1.3	5.75 ± 0.95	1.5 ± 0.577
Coliform Count	60.75 ± 1.7	35 ± 2.16	27.5 ± 1.29	16.75 ± 1.5