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In vitro Evaluation of Anti-inflammatory, Antimicrobial and Mosquito Larvicidal Efficacy of the Latex from *Calotropis procera*

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ABSTRACT

Biologically active compounds in the latex of *Calotropis procera* are found to possess vital pharmacological properties in Ayurvedic toxicology and therapeutics. Therefore, multiparous biological activities such as anti-microbial, anti-inflammatory and mosquito larvicidal properties of *C. procera* latex was evaluated in this study. Phytochemical analysis of the freshlatex of *C. procera* has indicated the presence of saponins, steroids, alkaloids and terpenoids. Anti-inflammatory efficiency of the latex from *C. procera* was analysed by proteinase inhibitory and membrane stabilization potential and compared with the standard drug Aceclofenac. Proteinase inhibitory potential was found to be 50.22% and membran estabilization potential was found to be 75.3% at a concentration of 250 µg/ml. Antagonistic activity of the latex against pathogenic bacteria and fungi illustrates that bacteria was found to be more susceptible when compared to the fungi tested. Inhibitory efficiency was found to be concentration dependant. Larvicidal efficiency of latex was investigated against denguevector *Aedes aegypti*. Percent mortality of *A.aegypti* was directly proportional to the concentration of latex. LC_{50} and LC_{90} values of *C.porcera* against *A. aegypti* was found tobe 85.113 and 162.35 µl/ml respectively. Morphological changes in the mosquito larva were observed on exposure to different concentration of the latex.

Key words: *Aedes aegypti, Calotropis procera, Mosquito larvicidal activity, Anti-microbial and Anti-inflammatory.*

Introduction

Plants are the primary source of biologically active natural products widely used in folk medicine. *Calotropis procera, a* small shrub of the family Apocynaceae, is rich in biologically active chemical compounds. *C. procera* is a member of the milk weed commonly known as Apple of Sodom or Madar shrub that grow up to a height of 4-5 meters with white or pink terminal flowers. Parts of the plant such as flowers, terminal leaf pairs, roots and latex have wide therapeutic applications (Dogara, 2023). Of the various parts of the *C. procera* plant, the most significant one which drew attention by the researchers is latex because of its medicinal properties and economic use for rubber production. The plant produces copious amounts of milky white endogenous latex. When the leaves or green parts are damaged, the latex secretion oozes out and has aprotective effect. The milky white latex is well known for its toxic as well as medicinal properties. Accidental exposure to the latex is reported to induce wide spectrum of ocular toxicity like congestion of eyes, iridocyclitis and sudden painless dim-

ness of vision with photophobia (Basak *et al*., 2009).

The latex plays an important defensive role by acting against nematodes, phytopathogenic fungi, herbivorous insects and cattle against grazing (Ramos *et al*., 2019). Apart from self-defence and toxic effects, the latex has been widely used in Indian traditional medicine for topological applications. It has been used for the treatment of skin diseases, rheumatism and aches (Arya and Kumar, 2005). The latex of *C. procera* is used for the relief of painful joints and swelling. The leaves and white flowers of the plant are used as a remedy for skin ailments, body pain etc., (Pandey *et al*., 2009). The latex is reported to be rich in biologically active compounds capable of promoting diverse benefits such as control of dermal fungal infections, antimicrobial activities and pain relief (Parabia *et al*., 2008).

Mosquitoes are responsible for the transmission of various lethal diseases to human. They not only cause diseases that afflict humans, but also transmit disease causing parasites to birds, dogs, horses, etc. In the recent years, the botanical derivatives are widely used in mosquito control programs. Phytochemicals that are tested against various life stages of mosquitoes, have the potential of growth and reproduction inhibitors, repellents and as oviposition deterrents. Therefore, plant-based products serve as good alternatives for synthetic chemical pesticides in mosquito control. The presence of mosquito larvicidal compounds in the latex of *C.procera* was reported by Ramos *et al.* (2019). Therefore, based on the above literature, in this study it was intended to analyse the anti-inflammatory, antimicrobial and mosquito larvicidal potential of *C.procera* latex.

Materials and Methods

Sample collection

Latex of *Calotropis procera* was collected from Madurai suburb following the procedure described by Nenaah and Ahmed (2011) from the young twigs. The latex was collected in a sterile bottle. The bottle was shaken slightly during collection to avoid coagulation. The latex was dried and stored at 4 °C for further analysis.

Physicochemical analyses

The methods described by Krishnaiah *et al*. (2009) and Mikail (2010) were adopted for the determination of phytochemicals in the latex of *C.procera.*

Test for alkaloids

Latex (0.5 ml) was mixed with 2.5 ml of 2% hydrochloric acid. The solution was divided into 2 aliquots; to the first test tube which acted as a reference, 1 mL of distilled water was added. To the second test tube, 2 drops of Mayer's reagent (Potassium mercuric iodide solution) was added. Appearance of turbid white precipitate in the second test tube illustrates the presence of alkaloids.

Test for Tannins

Latex (0.5 ml) was boiled with 10 ml of distilled and filtered through a filter paper. Few drops of 0.1 % of $FeCl_3$ was added to the filtrate. Appearance of blackish blue colour indicated the presence of Gallic tannins and green blackish colour indicated catechol tannins.

Test for Flavonoids

Latex (0.5 ml) was added with one ml of 1 % ammonia solution and appearance of yellow colour illustrates the presence of flavonoids in the sample.

Test for Steroids

Latex (0.5 ml) was dissolved in 0.25 ml of acetic acid followed by the addition of 0.25 ml of chloroform. The solution was pipetted out into a dry test tube followed by the addition of 500 µl conc. H_2SO_4. A brown-red ring at the interface between the two liquids and a green supernatant indicated the presence of steroids.

Test for Terpenoids

Latex (2.5 ml) was mixed with 1 ml of chloroform followed by the addition of conc. H_2SO_4 on the sides of the tube, appearance of reddish-brown colour illustrates the presence of terpenoids in the sample.

Antimicrobial activity

Antimicrobial test was performed using agar well diffusion method on Muller Hinton agar and Sabouraud Dextrose agar for bacteria and fungi respectively. The plates were inoculated with selected bacterial (*Streptococcus pneumoniae*, *Bacillus cereus*, *Micrococcus luteus* and *Pseudomonas aeruginosa*) and fungal (*Fusarium oxysporum* and *Penicillium chrysogenum*) strains separately following spread plate technique. Wells of 7 mm in diameter were cut into these agar plates and 20 µl of latex aqueous was

added in each well. The plates were incubated for 24 hours at 37 ºC and were subsequently examined for the clear zones surrounding each well. Zone of inhibition was measured and recorded. Streptomycin discs were used as positive control for bacteria, while Clotrimazole discs were the selected antifungal references.

Anti-inflammatory analysis

Anti-inflammatory activity of *C. procera* latex was evaluated using membrane stabilization test and proteinase inhibitory activity described by Angajala *et al.* (2014).

Membrane Stabilization test

Human blood (10 ml) was collected from a healthy volunteer, transferred to heparinized centrifuge tubes and centrifuged at 3000 rpm for 10 min. After centrifugation, the pellet was washed and reconstituted to 10 % with normal saline. Two millilitres of latex at different concentrations (50, 100, 150, 200, 250 µl/ml) were mixed with equal volume of 10% v/v of RBC suspension and incubated in a water bath at 50 °C for 30 min. Reaction mixture was centrifuged at 2500 rpm for 5 min and absorbance of the supernatant was measured at 560 nm. A control was maintained without the addition of Latex.

Proteinase inhibitory activity

The reaction mixture (2 ml) containing 0.06 mg trypsin, 1 ml of 20 mM tris-HCl buffer (pH 7.4) and 1 ml of latex at different concentrations (50, 100,150, 200, 250 µg/ml) was incubated at 37 °C for 5 min. One ml of 0.8 % (w/v) casein was added and incubated for 20 minutes. Two ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm using buffer as blank. Aceclofenac the commercially available drug recommended for anti- inflammatory activity was used as a reference.

Mosquito larvicidal activity

Mosquito larvicidal activity of the *C. procera*, against 4th instar larva of *Aedes aegypti* was determined following the method of Vijayakumar *et al*. (2015). *Aedes aegypti* eggs were collected from Indian Council of Medical Research (ICMR), Madurai, Tamil Nadu, India. Twenty larvae were introduced into a 500 ml glass beaker containing 250 ml tap water with different concentrations (25 µl, 50 µl, 75 µl and 100 µl/ml) of latex and covered with a mosquito net. A control group was maintained without the addition of latex. Mortality of the larvae was recorded after 24 h and no food was given during the treatment period. The control mortalities were corrected using Abbott's formula. The mortality percentage was calculated as the average of five replicates. The average larval mortality data were subjected to probit analysis (Veni *et al*., 2016) for calculating LC_{50}, LC_{90}, 95% confidence limits, regression equations, and chi-square values. Morphological changes in *Aedes aegypti* larvae on exposure to different concentrations of latex were observed after 24 h.

Results

Table 1 reveals the qualitative analysis of the phytochemical constituents in the latex and aqueous leaf extract of *Calotropis procera*. Steroids terpenoids, alkaloids, saponins were found to be present in the latex and leaf extract. Steroids and saponins were analysed by Salkowski test and Frothing test respectively. Tannins, cardiac glycosides and carbohydrates (reducing sugar) were found to be absent. The notable feature is the absence of flavonoids in the latex.

The antagonistic activity of *C.procera* latex was

Table 1. Phytochemical constituents in the leaf and latex of *C.procera.*

Phytochemicals	Name of the test	Response
Tannins	$FeCl_3$ test, Lead acetate test	-
Steroids	Salkowki test	+
Flavonoid	Ammonia	-
Saponins	Frothing test	+
Protein and amino acids	Ninhydrin	+
Alkaloids	Wagners test	+
Carbohydrates	Molischs test	-
Cardiac glycosides	Keller Killiani test	-
Terpenoids	Salkowski test (Modified)	+

tested for its antibacterial and antifungal activity against selected strains of bacterial and fungal pathogens. The results obtained are present in Table 2. Based on the zone of inhibition, the bacterial strains tested were found to be more susceptible to the latex than fungal pathogen. It is evident from the results that susceptibility of the bacterial strain *Streptococcus pneumoniae* was found to more pronounced followed by *Pseudomonas aeruginosa, Micrococcus luteus* and *Bacillus cereus* was found to be least susceptible to the latex of the four strains tested. No wide variation was observed on susceptibility of the two different fungal pathogens *Fusarium oxysporum* and *Penicillium chrysogenum* towards the latex.

Table 2. Antagonistis activity of *C. procera* latex against bacterial and fungal pathogen.

S. No.	Bacterial pathogens	Zone of inhibition (mm)
1.	*Streptococcus pneumoniae*	12.00 ±0.57
2.	*Micrococcus luteus*	6.33 ±0.33
3.	*Bacillus cereus*	8.00 ±0.25
4.	*Pseudomonas aeruginosa*	10.28 ±0.32
	Fungal pathogens	
5.	*Fusarium oxysporum*	7.00 ±0.17
6.	*Penicilliumchrysogenum*	5.00 ±0.18

Anti-inflammatory efficiency of the *C. procera* latex was determined by proteinase and membrane stabilization inhibitory activity. The latex was found to possess high anti-inflammatory efficacy. Proteinase inhibitory efficiency was found to be 50.22 % and inhibition for membrane stabilization was found to be 75.3 % at a concentration of 250 µg/ml (Table 3). However, Aceclofenac, a well-known anti-inflammatory agent exhibited 73.7 % proteinase inhibitory activity and 85.80 % of membrane stabilization inhibition at 250 µg/ml. Proteinase inhibitory and anti-inflammatory activity of the *C. procera* latex was comparatively lesser than the standard drug.

Larvicidal activity Larvicidal activity of *C. procera* latex against fourth instar larvae of *Aedes aegypti* is shown in Table 4. The percent mortality of *A. aegypti* was directly proportional to the concentration of *C. procera* latex. The maximum mortality (60%) of *Aedes aegypti* larvae was observed at 100 µl/ml of *C. procera* latex after 24 hrs of treatment. LC_{50} and LC_{90} values of *C. procera* latex against *Aedes aegypti* was found to be 85.113 µl/mL and 162.35 µL/ml respec-

Fig. 1. *Calotropis procera*

Table 3. Anti- inflammatory activity of *C. procera*latex

Concentration (µg/mL)	Percentage Inhibition			
	Membrane stabilization potential		Proteinase inhibitory activity	
	Standard	Standard	Latex	Latex
50	50.23 ± 0.60	42.38 ± 0.87	34.24 ± 0.34	36.2 ± 0.10
100	61.30 ± 0.50	45.24 ± 0.34	37.12 ± 0.46	45.6 ± 0.20
150	77.00 ± 0.25	50.17 ± 0.23	40.44 ± 0.13	54.8 ± 0.25
200	81.60 ± 0.20	61.69 ± 0.30	45.67 ± 0.64	71.2 ± 0.40
250	85.80 ± 0.40	73.70 ± 0.11	50.22 ± 0.21	75.3 ± 0.50

Table 4. Larvicidal activity of *C. procera* latex against the 4thinstar larvae of *Aedes aegypti.*

Concentration (µL/ml)	% Mortality	LC_{50} (95% LCL-UCL)	LC_{90} (95% LCL-UCL)	Regression Equation	X^2 (df=4)
25	13±0.00				
50	30±1.00	85.113	162.35	Y=4.577+3.85x	0.9973
75	45±1.30				
100	60±1.22				

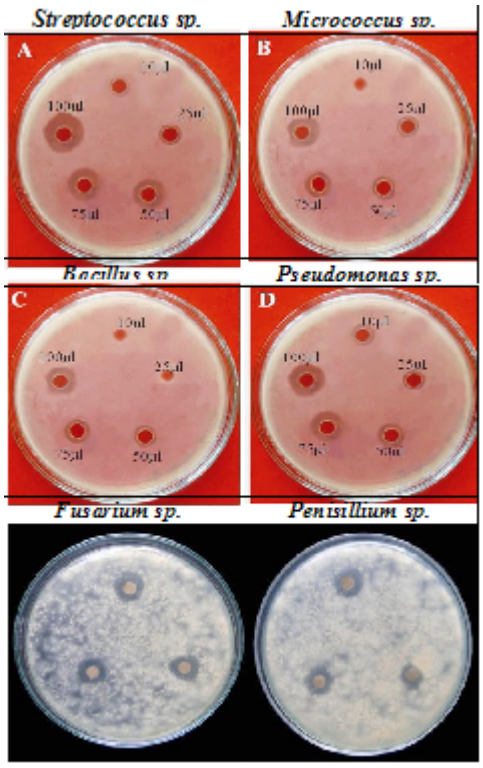

Fig. 2. Antimicrobial activity of *C. procera* latex- Well diffusion assay

tively. Regression analysis depicted that mortality rate (Y) of the larvae was positively correlated with the concentration of exposure (X) with a regression coefficient (R) value close to one (Table 4). Stereomicroscopic images of fourth instar *Aedes aegypti* larvae before and after 24 h exposure to 100 µl/ml of *C. procera*latex is presented in Figure 3. The untreated (control) larvae were found to be normal with no prominent morphological changes, whereas the larvae treated with 100 µl/ml of *C. procera*latex has shown progressive deformities with total disintegration of internal tissues of the larvae.

Discussion

Exploitation of plant and plant products for therapeutic applications stretches back the earliest stage of civilization. Phytochemicals still remain as the main source of drugs and extensively exploited in drug discovery. The latex of *C. procera* is well known for its toxic and medicinal properties. Though the latex is said to be toxic, proper and precautious use of the latex is said to be perfect remedy for various ailments. *C. procera* has important medicinal properties with proven pharmacological potential. The re-

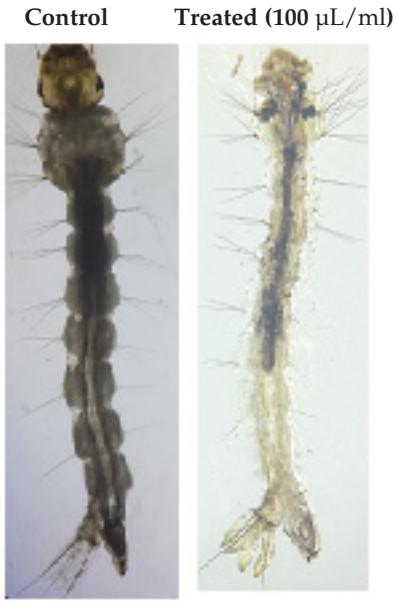

Fig. 3. Morphological changes in the4thinstar larvae of *A. aegypti* before (Control) and after (Treated) exposure to *C.procera* latex.

sult of the qualitative phytochemical study of latex and the leaves of *C. procera* revealed the presence of alkaloids, saponins, terpenoids and steroids. Tannins were found to be absent in leaves and latex (Table 1). Importance of the phytochemicals in the different parts of *C. procera* have been reported by Mungole *et al*. (2010). Besides the afore mentioned chemicals, presence of anthocyanins, proteolytic enzymes, cardenolides, and triterpenoids were reported by Kumar and Basu (1994). The medicinal value attributed to plants is a function of the bioactive phytochemical constituents (Rajasingam, 2023).

Plants containing phytochemicals have been found to have anticancer properties (Nanasombat and Teckchuen, 2009). The broad pharmacological profile of *C. procera* could be interesting for the pharmaceutical industry to develop new drugs.

The therapeutic potential of the latex from the shrub *Calotropis procera* has been scientifically proven (Belemlilga *et al*., 2016; Macedo *et al*., 2012). The use of these plants has advantages, such as a low cost, biodegradability and a lack of ecological harm (Zhu *et al*., 2013). In addition, medicinal plants are also sources of bioactive compounds derived from primary and secondary metabolism, enabling the development of new drugs (Ribeiro *et al*., 2013).

Antimicrobial potential of the *C. procera* latex re-

vealed that bacteria were more susceptible than the fungi tested. Of the four bacteria analysed, *Streptococcus pneumoniae* was found to be more susceptible to the latex and of the two fungi tested *Fusarium oxysporum* was found to be more susceptible than *Penicillium chrysogenum*. Banso (2009) illustrated that phytochemicals exhibit antimicrobial activity against a number of microorganisms. Latex and plant leaf extracts have been reported to be active against both gram positive and gram-negative bacteria (Ashafa and Afolayan, 2009). Sodipo *et al*. (1991) reported that tannins prevent the development of microorganisms by making proteins unavailable for the growth of the organism. Furthermore, facilitate the precipitation of microbial protein. The latex was found to possess anti-fungal and anti-bacterial efficiency, reinforcing the hypothesis of its defensive role.

Anti-inflammatory efficiency of *C. procera* latex was determined by proteinase inhibitory and membrane stabilization assay and compared to that of the standard drug Aceclofenac. Anti-inflammatory effect of the protein fraction recovered from the latex of *C. procera* was reported by Alencar *et al.* (2006). Larvicidal activity of *C. procera* latex was monitored against 4th instar larvae of the mosquito species *Aedes aegypti* 24 h post-treatment. Probit analysis was used to analyse the data from bioassay experiments. The obtained results agree with present study.

In conclusion, the latex of *C. procera* is considered as environmentally safe, less expensive, economical as well as practical in application with minimum care by individuals and communities.

Conflict of Interest: None

References

Afolayan, A.J. and Ashafa, T.O. 2009. Chemical composition and antimicrobial activity of the essential oil from *Chrysocoma ciliate* L. leaves. *J Med Plants Res.* 3: 390-181.

Alencar, N.M.N., Oliveira, J.S., Mesquita, R.O., Lima, M.W., Vale, M.R., Etchells, J.P. and Ramos, M.V. 2006. Pro and anti-inflammatory activities of the latex from *Calotropis procera* (Ait.) R. Br. are triggered by compounds fractionated by dialysis. *Inflamm Res*. 55: 559-564.

Angajala, G., Ramya, R. and Subashini, R. 2014. In-vitro anti-inflammatory and mosquito larvicidal efficacy of nickel nanoparticles phytofabricated from aqueous leaf extracts of *Aegle marmelos* Correa. *Acta Trop.* 135: 19-26.

Arya, S. and Kumar, V. L. 2005. Antiinflammatory Efficacy of Extracts of Latex of *Calotropis procera* Against Different Mediators of Inflammation. *Mediators Inflamm.* 4: 228-232.

Ashafa, T.O. and Afolayan, A. J. 2009. Assessment of the antimicrobial activity of the root extracts from *Chrysocoma ciliata* L. *African J Microbiol Res*. 3(11): 700-703

Banso, A. 2009. Phytochemical and antibacterial investigation of bark extract of *Acacia nilotica. J Med Plant Res*. 3(5): 082-085.

Basak, S., Bhaumik, A., Ayan Mohanta and Singhal, P. 2009. Ocular toxicity by latex of *Calotropis procera* (Sodom apple). *Indian J Ophthalmol.* 57(3): 232-232.

Belemlilga, M. B., Traoré, A., Ouédraogo, S., Kaboré, A., Tamboura, H.H. and Guissou, I.P. 2016. Anthelmintic activity of *Saba senegalensis* (A.DC.) Pichon (Apocynaceae) extract against adult worms and eggs of *Haemonchus contortus. Asian Pac J Trop Biomed.* 6(11): 945-949.

Dogara, A.M. 2023. A systematic review on the biological evaluation of *Calotropis procera* (Aiton) Dryand. *Futur J Pharm Sci.* 9(1).

Krishnaiah, D., Devi, T., Bonno, A. and Sarbartly, R. 2009. Studies on phytochemical constituents of six Malaysian Medical Plants. *J Med Plants Res.* 3(2): 607-072.

Kumar, V.L. and Basu, N. 1994. Anti-inflammatory activity of the latex of *Calotropis procera*. *J Ethnopharmacol.* 44(2): 123-125.

Macedo, I.T.F., Bevilaqua, C.M.L., de Oliveira, L.M.B., Camurça-Vasconcelos, A.L.F., Morais, S.M., Machado, L.K.A. and Ribeiro, W.L.C. 2012. *In vitro* activity of *Lantana camara*, *Alpinia zerumbet*, *Mentha villosa* and *Tagetes minuta* decoctions on *Haemonchus contortus* eggs and larvae. *Vet Parasitol.* 190(3-4): 504-509.

Mikail, H.G. 2010. Phytochemical screening, elemental analysis and acute toxicity of aqueous extract of *Allium sativum* L. bulbs in experimental rabbits. *J Med Plants Res*. 4(4): 322-326.

Mungole, D.S., Kamble, R., Kanfade, H., Chaturvedi, A. and Zanwar, P. 2010. Active phytochemical potentiality of in-vitro regenerated plantlets of *Canscorade currens* (Dalzell). *Indian J Sci Technol*. 3(6) : 679-683.