Research Article

Insecticidal Effects of Biosynthesized Silver Nanoparticles from Calotropis Species on *Tribolium casteneum*

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ABSTRACT

The aim of the study was to determine the insecticidal activity of silver nanoparticles synthesized from calotropis species (gigantean and procera) against *Tribolium castaneum*. Silver nano particles (AgNPs) were characterized by 200- 600 nm in the UV-VIS spectrum. SEM analysis of silver nanoparticles provided information about the surface, shape and size of the particles. The antibacterial property of silver nanoparticles was determined against the pathogenic bacteria such as *E.coli*, by agar well diffusion method. The formed silver nanoparticles are highly stable. The insecticidal activity of silver nanoparticles synthesized from the leaves of *C.gigantea* and *C.procera* against *Tribolium castaneum* was found to be highly effective.

Keywords: Silver nanoparticles, *Calotropis gigantea, Caltropis procera*, SEM analysis, insecticidal activity.

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INTRODUCTION

Calotropis is a genus of flowering plants in the Apocyanaceae, first described as a genus in 1810. It is native to southern Asia and North Africa. They are commonly known as milkweeds because of the latex they produce. It belongs to the asclepiadeaceae subfamily commonly, called as the dog bane family, comprises 347 genera and about 5100 species of flowering plants including herbaceous or shrubby climber[1-3]. The calotropis (erukkam in tamil) is a small genus belongs to the family consisting of two species, calotropis gigantean and *calotropis procera*. calotropis is a succulent and xerophytic shrub or small lacticiferous tree up to 2.5m, commonly known as "milkweed" or "crown flower" [4]. Calotropis species are most diverse in tropical and subtropical parts of Asia and south east Asia and extent into temperate areas.

The milky exudation from the plant is a corrosive poison. *Calotropis* species are poisonous plants; calotropin, a compound in the latex, is more toxic than strychnine. Calotropin is similar in structure to two cardiac glycosides which are responsible for the cytotoxicity of *Apocynum cannabinum*. Extracts from the flowers of *Calotropis procera* have shown strong cytotoxic activity. The extracts are also harmful to the eyes. Cattle often stay away from the plants because of their unpleasant taste and their content of cardiac glycosides.[5]

Aqueous, methanol, ethanol and petroleum ether extracts of the leaves of *C. gigantea* were reported to possess anti-Candida activity against clinical isolate of *Candida albicans, C. parapsilosis, C.tropicalis* and *C. krusei*. The aqueous extract of leaves of *C. gigantea* was reported to possess antibacterial activity against *Staphylococcus aureus*, *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Micrococcus luteus* and *Klebsella pneumonia*.[6] The aqueous extract of the latex of *C. gigantea* was reported to exhibit significantly inhibitory effect on *S. aureus, B. cereus, E. coli* and *C. krusei*. [7] Antifungal activity of *C. gigantea* was reported against plant pathogenic fungi like *Fusarium mangiferae,* that causes serious threat in mango cultivation.

The antibacterial activity of methanol extract from the root bark of C. gigantea and its petroleum ether, chloroform and ethyl acetate fractions. Both of methanol extract and its chloroform fraction showed activity against Sarcina lutea, B. megaterium and P. aeruginosa. Petroleum ether fraction showed activity against B. subtilis and Shigella sonnei, whereas ethyl acetate fraction showed activity against P. aeruginosa and E. coli.[8] The alcoholic extract of the flowers of C. gigantea was reported for analgesic activity in chemical and thermal models in mice. Root bark extract of C. gigantea was investigated for wound healing activity in Wistar albino rats. The cardenolide glycosides collected from the root C. gigantea were reported to carry cytotoxic activity against several human and mouse cell lines. Calotropin, frugoside and 4'– O-β-D glucopyransylfrugoside was found as the active principles. The hydroalcoholic (50:50) extract of aerial part of C. gigantea was studied for antidiarrhoeal activity against castor oil-induced-diarrhoea model in rats. Chitme et al. (2005) reported the anti-pyretic activity of the

water:ethanol (50:50) extract of C. gigantea roots. Anti-pyretic activity was studied by using yeast and TAB (Typhoid) vaccine induced pyrexia in Albino Swiss rats and rabbits. Methanol extract of C. gigantea root bark and its chloroform and petroleum ether fractions were evaluated for residual film toxicity, fumigant toxicity and repellent effect against several insects of larvae and adult of *Tribolium castaneum*.

Calotropis gigantea *Calotropis procera*

Figure 1: *Calotropis gigantea* and *Calotropis procera*.

***Calotropis procera* Ayurvedic uses:**

The parts of the plant used in Ayurvedic medicine are the leaves, fresh or dried, the roots and root bark, and the flowers. The powdered leaves are used for the fast healing of wounds, as a purgative and to treat indigestion. They are also used to treat skin disorders and liver problems. They are collected from September to February and are also used to treat piles when prepared in the form of a paste. Traditionally, the plant has been used as an antifungal, antipyretic and analgesic agent. The dried leaves used as an expectorant, and anti-inflammatory, for the treatment of paralysis and rheumatic pains. The dried latex and dried root are used as an antidote for snake poisoning. The tender leaves of the plant are also used to treat migraine. The capsulated root bark powder is effective against diarrhoea and asthma. [9] The pharmacological studies include reports of anticancer, antifungal and insecticidal activity of *C. procera*. The flowers of the plant exhibit hepatoprotective activity, anti-inflammatory, antipyretic, analgesic, and antimicrobial effects and larvicidal activity. The latex of the plant is reported to possess analgesic and wound healing activity, as well as anti-inflammatory and antimicrobial activity while the roots are reported to have anti-fertility and anti-ulcer effects. [10]

Nanoparticles

Nanoparticles are clusters of atoms in the size range of 1-10nm. “NANO” is a greek word synonymous to dwarf meaning extremely small. The use of nanoparticles is gaining impetus in the present century as they possess a defined chemical, optical, and mechanical properties. synthesis of nanoparticles is of much interest to the scientific community because of the wide range of application.[11] Nanoparticles of metals have been extensively studied for their potential application in catalysis, biological labelling, biosensor drug delivery, antibacterial and antiviral activity and detection of genetic disorders. Different types of nanomaterials like copper, zinc, titanium, magnesium, gold, alginate and silver have come up but silver nanoparticles have proved to be the most effective as it has good antibacterial efficacy against bacteria, viruses and other eukaryotic microorganisms. [12] The use of silver nanoparticle as a larvicidal agent instead of chemical insecticides is gaining importance because of their safety to users as well as non target species. several plants were screened successfully for the silver nanoparticle synthesis such as plumeria rubra, acacia Arabica, *Euphorbia tirucalli* and *Alstonia macrophylla*. In this study we are going to synthesize silver nanoparticles from the leaves of calotropis gigantean and procera species.[13]

Tribolium castaneum

Tribolium castaneum is a common and most destructive pest, found through out the world. This species has been found associated with a wide range of commodities including grain, flour, peas, beans, nuts, dried fruits and species. This pest attacks the germ part (embroyo portion) of the grain and their presence in stored food directly affects the quality and quality of the commodity. The insecticidal activity of calotropis flower extract against *Tribolium castaneum* has shown good insecticidal property.[14]

MATERIALS AND METHODS

Phytochemical Analysis

The leaves of *C.gigantea*n and c. procera were collected and allowed for complete shade drying. After complete shade drying, the dried leaves was pulverized into coarse powder and it was stored in an airtight glass container. At room temperature the powder of about 50 gms was mixed with known amount of methanol, ethanol and incubated overnight in a rotatory shaker. The aqueous extract was collected by using whatmann No 1 filter paper and again solvent was added to the residual mixture and incubated in

shaker for another 24 hrs. The extract was collected again using whatmann no 1 filter paper. Then it was mixed with the previous aqueous extract. Then it was used to carry out further phytochemical procedure.

Test for Alkaloids (Mayer's Test):

2.0ml of extract was measured in a test tube to which picric acid solution was added. The formation of orange coloration indicated the presence of alkaloids.

Test for Cardiac Glycosides (Keller-Killani Test):

5ml of plant extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer which shows the presence of Cardiac glycosides.

Test for Tannins:

The substance (extracts) mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of Tannins.

Test for Saponins:

Froth test for saponins was used. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Test for Steroids:

One gram of the test substance (plant extracts) was dissolved in a few drops of acetic acid. It was gently warmed and cooled under the tap water and a drop of concentrated sulphuric acid was added along the sides of the test tube. Appearance of green colour indicates the presence of Steroids.

Test for Terpenoids (Salkowski Test):

5ml of each plant part extract was mixed in 2 ml of chloroform, and concentrated H2SO4 (3ml) was carefully added to form a layer. Formation of reddish brown coloration at the interface shows the positive results for presence of terpenoids.

Test for Reducing Sugars:

Benedicts and Fehlings test: 2ml of the extract was mixed with beneditcs reagent and fehlings A and B solution and then it was kept in boiling water bath for 5 mins. appearance of reddish brown color indicates the presence of reducing sugar.

Test for Proteins:

Leaf extract (500µl) was taken and 0.1 ml of Millon's reagent was added. Brown coloration shows positive result.

Quantitative Tests

Leaf extracts in four different solvents (water, methanol, ethanol, ethyl acetate) were used for experimental purpose.

Phenol Test: Leaf extract (1ml), 2ml of distilled water and 0.5 ml of Folinciocalteu's reagent were taken in a test tube. After 3 minutes 2 ml of 20% sodium carbonate solution was added. The tubes were kept in boiling water bath for 1 minute and cooled.The solution was diluted one fold and O.D was taken at 680 nm.

Flavonoids Test: Leaf extracts (1ml) was mixed with 4 ml of distilled water and 0.3 ml of 5% NaNo2.After 5 minutes 0.3 ml of 10% Alcl3 was added. Then 2 ml of 1M NaOH was added. The solution was diluted and O.D measured at 520 nm.

Antibacterial Activity of Silver Nanoparticle Against *E.coli*

The crude extracts were screened for antibacterial activity using agar well diffusion method described by Russel and Furr (1977). Agar cup method was followed in which culture of E coli was spreader out onto Muller Hinton agar plates. Wells were made on the plates with a cork borer (diameter 1.2 cm) to which different extraction were added in specific volume (1ml) along with control (ethyl acetate).the plates were incubated for 24 hours at 370C.

Biosynthesis of Silver Nanoparticle

Freshly collected leaves of c. gigantea and *C.procera* were washed with double distilled water, and finely cut the leaf. a known amount (50gms) of leaf was added with 200 ml of deionised water and boiled for 15 mins in a water bath. The mixer was then cooled and filtered using whatmann no 1 filter paper. For the synthesis of silver nanoparticles, a known concentration of leaf extract was interacted with 1mM AgNo3 solution at a define mixing ratio to made up 100 ml volume in 250ml lerlenmeyer flasks. The flasks were incubated in a rotatory shaker at 120 rpm speed for a desired time at 28°C.

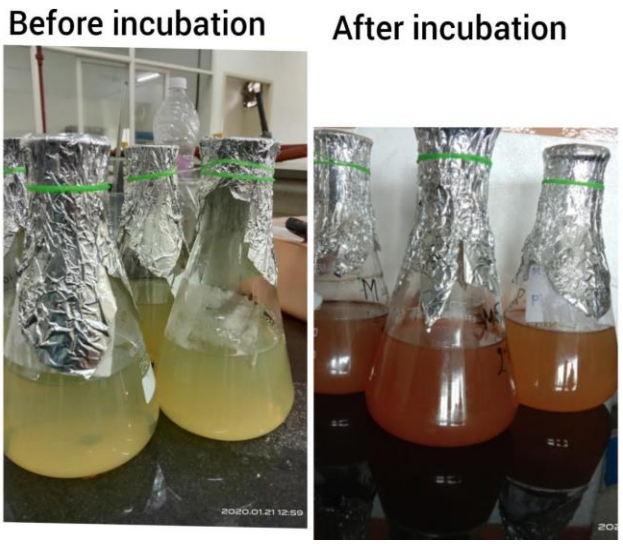

Figure 2: Colour changes indicating the presence silver nanoparticles after incubation.

Characterization of silver nanoparticle

Change in colour was observed in the silver nitrate solution incubated with the leaf extract. The UV-visible spectrum of this solution was recorded in perkin-elmer spectrophotometer, from 200-600nm, at an interval of 24hr,48hr,72hr. After desired reaction periods. The broth containing silver nanoparticles was centrifuged at 10000 rpm for 15 min, following which the pellet was re-dispersed in sterile distilled water to get rid of any uncoordinated biological molecules. The process of centrifugation and redispersion in sterile distilled water was repeated thrice to ensure better separation of free entities from the metal nanoparticles. The *C.gigantea* and *C.procera* leaf extract embedded with silver nanoparticles was freeze dried, powdered and used for the insecticidal activity

Insecticidal Activity of Silver Nanoparticle Against *Tribolium castenum*

To study the insecticidal activity, residual film method was performed here. In this method the formulated insecticide (silver nanoparticles) is diluted in a volatile solvent acetone and the insecticide solution is deposited on a glass surface of the petri dish. Petri dish are most commonly used for insecticide efficacy. The solvent is allowed for uniform spreading in the petri dish by swirling it gently, so that the insecticide is spread evenly over the entire surface leaving a residual film. The target test insects (*Tribolium castenum*) are then released onto the film of the toxicant in the container. Thereafter the known numbers of insects are exposed on for a 18- 48 hrs depending upon the mortality rate. Here the dose were calculated by dividing the actual amount of AgNps (in mg) present in the 1ml with the area of the petridish (7cm diameter) and it was expressed as amount per square centimetre (mg/cm2). By this way we got 5 doses, that were 0.714, 1, 1.28,. 57,1.85mg/cm2. When the petridishes were dried, 10 beetles were released in each petridish with three replication. A negative control with using the same no. of insects and solvent only. Insect mortality was recorded for 24 hours and 48 hours after treatment. (Fig 3)

Figure 3: Petridishes with Silver Nanoparticles.

RESULTS AND DISCUSSION

Phytochemical analysis

This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important phytochemicals such as terpenoids, phenol, Flavonoids, reducing sugar, protein, alkaloids, and tannins were present in the methanolic and aqueous extracts of gigantean and *C.procera*. The phytochemical screening and quantitative estimation showed that the leaves of gigantean and *C.procera* were rich in phenol, tannins, terpenoids, flavonoids, alkaloids, etc. (Table 1, and 2)

Table 1: Presence of Phytochemicals in Methanolic and aqueous extraction

Phytochemicals	*C.gigantea*		*C procera*	
	Methanolic extraction	Aqueous extraction	Methanolic extraction	Aqueous extraction
Flavonoids	-ve	-ve	-ve	-ve
Alkaloids	+ve	+ve	+ve	+ve
Phenol	+ve	+ve	+ve	+ve
Terpenoids	+ve	+ve	+ve	+ve
Steroids	+ve	+ve	+ve	+ve
Saponins	-ve	-ve	-ve	-ve
Reducing sugar	++ve	++ve	+ve	++ve
Protein	+ve	+ve	++ve	+ve
Glycosides	+ve	+ve	+ve	+ve
Tannins	+ve	+ve	+ve	+ve

Table 2: Quantitative estimation of phenol and flavonoids (O.D at 680nm)

Extraction	*C.gigantea*		*C.procera*	
	Phenol	Flavonoids	Phenol	Flavonoids
Aqueous	0.15	Nil	0.16	Nil
Methanol	0.18	0.02	0.20	0.03

Determination of antibacterial activity of silver nanoparticles synthesized from *C.gigantea* and *C.procera*

From the above study, it is proved that the experimental plants *(C.gigantea* and *C.procera)* showed strong antibacterial activity against Gram positive bacteria *E.coli*. The antibacterial activity of *C.gigantea* and *C.procera* are given in Table 3. From the results, it was found that Ag Np exhibited significant antibacterial activity compared to the other extraction of *C.gigantea* and *C.procera*. From these results, we can conclude that some of the components from the Ag Np exhibit synergistic action against bacteria.

Biosynthesis of silver nanoparticles from *C.gigantea* and *C.procera* :

Now a days, synthesis of silver nanoparticles using plant extracts were getting more popular. Aqueous extracts of leaves to AgN03 solution, the colour of the reaction medium changed rapidly from colourless to brown 1:4 ratio. That brown colour indicated that surface Plasmon vibrations, typical of silver nanoparticles. The control of Ag N03 solution without plant extract showed no colour change. The UV- vis spectrum of silver nanoparticles solution with the *C.gigantea* and *C.procera* leaf extract, Table 4. While no absorbance peak was observed in control, a characteristic surface Plasmon absorption peak at 400 nm was observed 24hrs that attained maximum intensity after 72hrs. After 72 hrs of incubation, no change in intensity at 450nm was observed indicating complete reduction of silver ions.

Table 3: Antibacterial activity of *C.gigantea* and *C.procera* against *E.coli (*Zone of inhibition*)*

Extraction	Bacterial culture	*C.gigantea*	*C.procera*
Aqueous	*E.coli*	1.5cm	1.4 cm
Methanol	*E.coli*	1.4 cm	1.25cm
Ag nanoparticle	*E.coli*	2.8cm	2.45cm

Table 4: UV- Visible absorption spectra of silver nanoparticles synthesized from *C.gigantea* and *C.procera*

Absorption spectrum	C. gigantea Hours			*C.procera* Hours		
nm	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
300	1.35	0.20	0.8	1.40	0.15	0.10
350	1.22	1.21	1.18	1.17	1.15	1.05
400	1.25	1.24	1.20	1.31	1.22	1.20
450	0.7	0.5	0.4	0.10	0.9	0.5

The SEM analysis shows that *C.gigantea* and *C.procera* leaf extract treated with 1mm silver nitrate solution for 72hrs. The surface deposited silver nanoparticles are seen clearly at higher magnification in silver nitrate treated *C.gigantea* and *C.procera* leaf extract. The silver nanoparticles solution is extremely stable for nearly 50 days with only a little aggregation of particles in solution *(*Fig 4, 5).

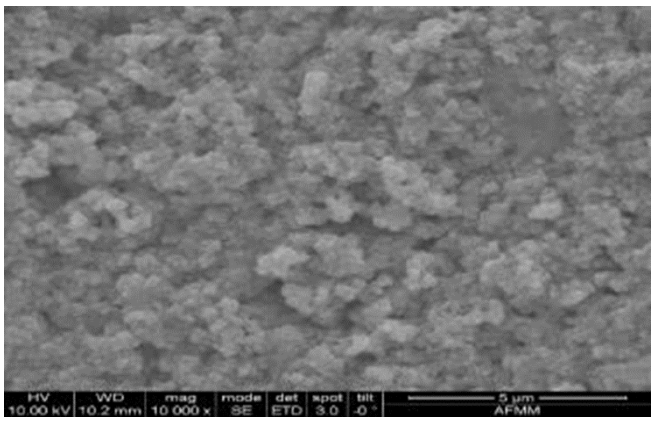

Figure 4: Scanning electron micrograph of silver nanoparticles synthesized from *C.gigantea*

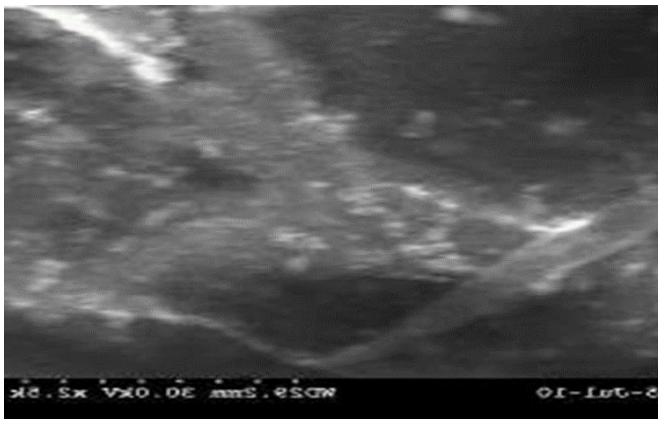

Figure 5: Scanning electron micrograph of silver nanoparticles synthesized from *C.procera*

Insecticidal activity of *C.gigantea* and *C.procera* against T. casteneum

This study describes the toxic effect of silver nanoparticles synthesized from *C.gigantea* and *C.procera* against adults of *T.casteneum* (Fig 6).

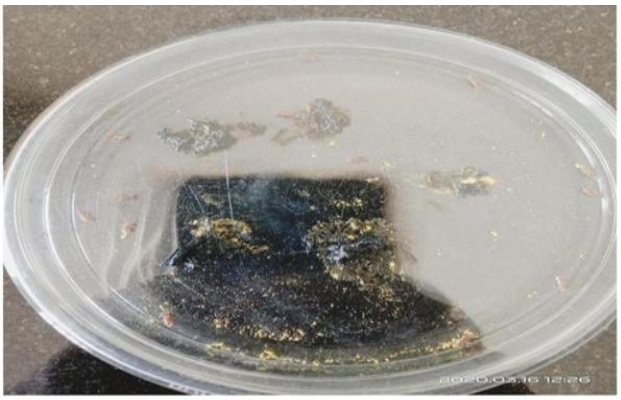

Figure 6: Insecticidal activity of silver nanoparticles of *C.gigantea* and *C.procera*

The mortality rate of *T.casteneum* and the effectiveness of silver nanoparticles was determined and it was found to be increased with increased exposure time, the maximum residual toxicity was observed with 1.85 mg/cm2 for the

adults of *T.casteneum* respectively, after 48hrs of exposure. No mortality was observed in control. Results of this study demonstrated the toxicity of silver nanoparticles was susceptible to kill the *T.casteneum* with high concentration (1.85 mg/ cm2). This proved that when the concentration of silver nanoparticles was increased, the mortality of *T.casteneum* decreased *(*Table 5).

Table 5: Insecticidal activity of silver nanoparticles of *C.gigantea* and *C.procera*

Silver nanoparticles of *C.gigantea* against adult stage of *T.casteneum*			**Silver nanoparticles of *C.procera* against adult stage of *T.casteneum***		
	Mortality Rate			**Mortality Rate**	
Dosage	24hrs	48hrs	Dosage	24hrs	48hrs
0.714	Nil	2	0.714	Nil	1
1.0	1	4	1.0	1	3
1.28	4	5	1.28	4	4
1.57	4	6	1.57	5	5
1.85	10	Nil	1.85	9	1

SUMMARY AND CONCLUSION

The renewable source of *C.gigantea* and *C.procera* can be used as an effective reducing agent for the synthesis of AgNps. The biological reduction of metal would be boon for the development of clean, non toxic, and environmentally acceptable green approach to produce metal nanoparticles. The formed AgNps are highly stable. In this study we have demonstrated the insecticidal Activity of silver nanoparticles synthesized from the leaves of *C.gigantea* and *C.procera* against *Tribolium castaneum*, which proves that this particular nanoparticle may be used as a potent insecticide. Hence, the overall results suggested that the AgNps from calotropis species have potential insecticidal effect which might be used in pest control. Studies to be done in future to identify the effects on human beings who uses the stored grains treated with silver nanoparticles synthesized from calotropis species.

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