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Regular Article

Silver nanoparticles synthesized by *in vitro* derived plants and callus cultures of *Clitoria ternatea*; Evaluation of antimicrobial activity

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This paper highlights for the first time the preparation and synthesis of silver nanoparticles from the callus and *in vitro* derived plant roots of white flowered variety of *Clitora ternatea.* The efficiency of antimicrobial activity of silver nanoparticles towards the clinical samples was assessed. Our study confirmed that these silver nanoparticles were found to be highly toxic and provides once again the pharmaceutical evidence of the medicinal plant *Clitoria ternatea*. Therefore, the use of silver nanoparticles should emerge as one of the novel approaches in many disease therapies, and applications of plant extract silver nanoparticles should be exploited for the medical treatment.

Key words: *Clitoria ternatea*, clinical samples, Dharwad, Karnataka, nanotechnology, silver nitrate.

Synthesis of ecofriendly silver nanoparticles is of great importance in the filed of nanotechnology (Ghosh *et al.* 2012; Xia *et al.* 2010; Zhang *et al.* 2008; Thirumurgan *et al.* 2009, 2010). Silver nanoparticles have attracted much attention due to their versatile applications in many areas such a medicine, textiles, sensors and detectors, catalysis, nanocomposites, agriculture and waste water treatment (Shanker *et al.* 2003a, 2003b; Hettiarachchi and Wickramarachchi, 2011). Being in nano scale, these nanoparticles have high surface: volume ratio. Therefore, they often show unique and considerably different physical, chemical, mechanical and biological properties compared to their macro scaled counterparts. Hence nanoparticles is an emerging area of nanoscience and critical technology with many applications in medical and industrial sectors. Production of silver nanoparticles using plants provides advancement over traditional chemical and physical methods as it is cost effective, easily scaled up for large scale, and no toxic chemicals were used during synthesis (Nabikhan *et al.* 2010; Malabadi *et al.* 2012a, 2012b; Gnanadesigan *et al.* 2011; Shankar *et al.* 2003a, 2003b; Geethalakshmi and Sarada, 2010; Mukunthan *et al.* 2011; Warisnoicharoen *et al.* 2011; Vankar and Shukla, 2012; Ghosh *et*

al. 2012). Green nanotechnology is very safe and utilizes non-toxic chemicals and simple solvent water for synthesizing nanoparticles (Malabadi *et al.* 2012a, 2012b; Sharma *et al*. 2007; Song and Kim, 2009; Gardea-Torresdey *et al*. 2002, 2003). This modern technique of green nanotechnology has facilitated the production of smaller silver nanoparticles with low toxicity to human and greater efficacy against bacteria (Malabadi *et al.* 2012a, 2012; Xia *et al.* 2010; Zhang *et al.* 2008; Song and Kim, 2009;Gardea-Torresdey *et al*. 2002, 2003; Shankar *et al*. 2003a, 2003b, 2004a, 2004b; Nabikhan *et al.* 2010; Geethalakshmi and Sarada, 2010; Farooqui *et al.* 2010; Elumalai *et al.* 2010; Khandelwal *et al*. 2010; Saxena *et al.* 2010; Sharma *et al*. 2007). Furthermore, nanoparticles are attractive alternative to antibiotics showing improved activity against multidrug resistant bacteria (Savithramma *et al.* 2011a, 2011b; Malabadi *et al.* 2012a, 2012b; Vankar and Shukla, 2012; Ghosh *et al*. 2012). Therefore, plant derived silver nanoparticles proved superior to other physical and chemical methods (Vankar and Shukla, 2012; Ghosh *et al*. 2012). Silver nanoparticles receive much more attention than silver nitrate because of their potential towards antimicrobial and antifungal activity. Silver shows oligodynamic effect towards various microorganisms at very low concentrations (Song and Kim, 2009; Gardea-Torresdey *et al*. 2002, 2003; Shankar *et al*. 2003a, 2003b, 2004a, 2004b; Nabikhan *et al.* 2010; Geethalakshmi and Sarada, 2010; Malabadi *et al.* 2012a, 2012b). In general silver ions can bind with a variety of negatively chaged molecules like RNA, DNA and proteins. The antibacterial property of silver nanoparticles increases because of their larger total surface area per unit volume (Song and Kim, 2009;Gardea-Torresdey *et al*. 2002, 2003; Shankar *et al*. 2003a, 2003b, 2004a, 2004b; Nabikhan *et al.* 2010; Geethalakshmi and Sarada, 2010). The mechanism of the silver nanoparticle bactericidal activity is effectively explained in terms of their interaction with cell membranes of bacteria by disturbing its permeability and respiratory function (Vankar and Shukla, 2012; Ghosh *et al*. 2012). Nanotechnology could be very helpful in regenerating the injurerved nerves. For biological and clinical applications, the ability to control and manipulation of nanoparticles for an extended period of time inside a cell can lead to improvements in diagnostic sensitivity and therapeutic efficiency.

The white-flowered variety of *Clitoria ternatea* (L.) (Leguminosae) commonly known as the *butterfly pea* is a perennial ornamental twinning herb conspicuous for its large papilionaceous corolla (Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004; Malabadi *et al.* 2005, 2007). The foliage and pods are eaten by livestock. The natives in parts of Sri Lanka and India consume the green pods as vegetables. The plant is considered to be a good brain tonic and is useful for throat, eye infections, skin diseases, urinary troubles, an ulcer, antidotal, in improving memory and intelligence. This genus has 70 species of which three are from India (Polhill *et al*. 1981). It is an important medicinal and a forage plant. The plant is also a good soil binder because of its twinning stem and rhizomatous roots (Kirtikar and Basu, 1935; Chopra *et al.* 1956; Asolkar *et al*. 1992; Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004; Malabadi *et al.* 2005, 2007; Malabadi and Nataraja, 2003). In our previous study, antibacterial activity of silver nanoparticles were synthesized by using whole plant extracts of field grown plants of *Clitoria ternatea* has been tested (Malabadi *et al.* 2012a). Cytotoxicity of silver nanoparticles showed good results (Malabadi *et al*. 2012a, 2012b). The present study was conducted to investigate the antibacterial activity of silver nanoparticles synthesized from the callus and root extracts of *in vitro* derived plants of *Clitoria ternatea* (L.) by preliminary disc

diffusion assay screening. This study is extended for synthesizing nanoparticles from *in vitro* derived plant roots and callus cultures of *Clitoria ternatea* to meet the requirements of various medical applications. The extracts were tested against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and the Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, and fungal strain such as *Candida albicans.*

Materials and methods

The *in vitro* regeneration of white flowered variety of *Clitoria ternatea* has been done according to our previously published protocols (Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004). In brief**,** fresh seeds of white flowered variety of *Clitoria ternatea* (Linn.) were collected from the ripe-pods of plants growing in Botanical Garden of Karnatak University, Dharwad, India. Seeds were surface sterilized with 70% ethanol for 5 min and 0.1% $HgCl_2$ for 5 minutes, rinsed three times with sterile double distilled water and germinated aseptically on MS basal medium (Murashige and Skoog, 1962) with 3.0% sucrose (Analar - grade) and 0.7% agar (Difco-bacto) in 145mm X 25mm glass culture tubes containing 15 ml of the medium under cool white fluorescent light (100 µ mol m^{-2} s^{-1}) at 25 ± 2^0 C with a relative humidity of 55-60%.

Leaf segments of 5.0 mm^2 from 10-day-old aseptically grown seedlings were placed on MS basal medium supplemented with 5.71 µM IAA and 9.3 µM Kn for the induction of embryogenic tissue (Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004). The embryogenic callus was separated and again subcultured on MS supplemented with 5.71 µM IAA and 9.3 µM Kn for further proliferation (Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004). This embryogenic tissue was used for the maturation experiments. The maturation medium was a modified MS basal medium in which all macroelements, except ammonium nitrate and potassium nitrate were reduced to half strength with 1.0 g/l^{-1} meso-inositol, 1.5 g/l^{-1} casein hydrosylate (CH) and 0.5 g/l^{-1} L-glutamine. Both casein hydrosylate (CH) and L-glutamine were filter sterilized (Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004). The maturation medium contained 3.0% sucrose, 0.7% agar and supplemented with 9.3 µM Kn and 5 % (V/V) coconut water (CW). The embryogenic callus after 3 weeks was transferred to modified MS basal medium supplemented with 9.3 µM Kn and 5% (V/V) Coconut water for the induction of somatic embryos (Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004). After 2-3 weeks, the embryogenic callus produced globular and heart shaped somatic embryos. The average number of somatic embryos per each cultured leaf explant was found to be 26 (Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004). The conversion of somatic embryos into plantlets was high (80%) when matured somatic embryos were separated and cultured on half strength MS basal medium without growth regulators (Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004). If left undisturbed, the somatic embryos matured while still attached to the callus and germinated to produce shoots, but after 2 weeks instead of further growth recallused. In a third experiment, the matured somatic embryos were separated and germinated on half strength MS basal medium without growth regulators (Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004). Somatic embryos germinated into plantlets within 2-3 weeks. Plants with well developed roots were transferred to pots containing vermiculite and hardened for one week at 80% relative humidity and 25±2^0C. The hardened plants were transferred to a green house (Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004). The

callus and root extracts of *in vitro* derived plants were used for the following experiments.

Synthesis of silver nanoparticles

The callus and roots of *in vitro* derived plants of *Clitoria ternatea* was oven dried at 50° C and ground to make a fine powder. Further, 25 grams of powdered callus and roots were taken separately in two 250ml beaker containing 100ml of sterile distilled water. Mixture was boiled for 10 min at 100°C (Malabadi *et al.* 2012a, 2012b). The callus and root extracts were collected separately in two beakers by a standard filtration (Whatman filer paper) method. 1mM $AgNO_3$ (silver nitrate) solution was prepared and stored in amber colour bottle. 10ml of callus and root extracts were taken in beaker separately and 50ml of 1mM $AgNO_3$ solution was added to the beaker drop wise with constant stirring at 50-60° C and colour change was observed (Malabadi *et al.* 2012a, 2012b; Linga Rao and Savithramma, 2012). The colour change was checked periodically and the beakers were incubated at room temperature for 40 hours (Malabadi *et al.* 2012a, 2012b; Linga Rao and Savithramma, 2012). The color change of the callus and root extracts from yellow to brown indicated the presence and synthesis of silver nanoparticles. The extract contents were then centrifuged at 10,000 rpm for 20 min (Malabadi *et al.* 2012a, 2012b; Linga Rao and Savithramma, 2012). The silver nanoparticles were isolated and concentrated by repeated (4-5 times) centrifugation of the reaction mixture at 10, 000 g for 10 min. The supernatant was replaced by distilled each time and suspension stored for the further use. The supernatants were used for the spectrometric UV analysis and for the evaluation of antibacterial activity (Malabadi *et al.* 2012a, 2012b; Linga Rao and Savithramma, 2012). The spectrometric analyzed results highlighted the presence and reduction of silver ions in the tested samples. The reduction of silver ions was monitored by measuring the absorbance of the reaction mixture in a range of wavelength from 300 to 600 nm using spectrocolorimeter to find the absorbance peak (Malabadi *et al*. 2012a, 2012b).

Stabilization of silver nanoparticles

The protocol for the stabilization of silver nanoparticles has been adopted from previous published papers (Gardea-Torresdey *et al*. 2002, 2003; Shankar *et al*. 2003a, 2003b, 2004a, 2004b; Sharma *et al*. 2007) with slight modifications in our experiments. In this method, 0.9 g of polyvinyl alcohol (PVA) was dissolved in 100ml sterile distilled water at 100° C by vigorously stirring to form homogenous solution (Nabikhan *et al.* 2010; Malabadi *et al.* 2012b). An aqueous solution of 20 ml of silver nanoparticles synthesized by callus and root extracts was added with PVA solution (Nabikhan *et al.* 2010). This solution was then allowed to stir in a flask for about 10 min, and then the solution was purged with nitrogen. A fresh solution of $5x10^{-3}$ M was prepared and introduced drop by drop into PVA-$AgNO_3$ solution (Nabikhan *et al.* 2010; Malabadi *et al.* 2012b). The solution was then stirred for 15min under inert atmosphere at room temperature of 28°C. Silver nanoparticles were also similarly prepared in the absence of PVA. The color intensity at 420 nm was measured separately for callus and root extracts stabilized with and without PVA (Nabikhan *et al.* 2010; Malabadi *et al*. 2012b).

Screening of antibacterial activity

The test pathogenic organisms used in the investigations of antibacterial activity were *Bacillus subtilis* and *Staphylococcus aureus*, and the gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* and fungal strain *Candida albicans* (Malabadi, 2005; Malabadi *et al*. 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008;

Malabadi *et al.* 2012). Tenfold serial dilution of overnight MH broth cultures were prepared and 100 µl of each dilution were spread onto MH agar plates using a glass spreader (Malabadi, 2005; Malabadi *et al.* 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008; Malabadi *et al*. 2012a, 2012b). The plates were incubated overnight at 37° C. Following the assumption that each living bacterial cell will grow into a separate colony on the plate, the number of cells present per milliliter of the original overnight cultures was calculated (Malabadi, 2005; Malabadi *et al*. 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008; Malabadi *et al.* 2012a, 2012b). The optical density (OD) at 600 nm for each dilution was determined using spectrophotometer, and used to indicate numbers of bacterial cells in cultures for the antibacterial screening and MIC determination (Malabadi, 2005; Malabadi *et al.* 2012a, 2012b).

The antibacterial activity was done by disc-diffusion assay method (Malabadi, 2005; Malabadi *et al*. 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008; Malabadi *et al.* 2012a, 2012b). Filter paper discs (Whatman No 3 and 6 mm in diameter) were sterilized by autoclaving. In this method, 1000 µl of silver nanoparticles prepared from callus and rhizome extracts was mixed in 1.0 ml of sterile distilled water and then applied to sterile paper discs of 5mm diameter (Whatman Filter papers) (Malabadi *et al.* 2012a, 2012b). These discs were air-dried under sterile conditions. Similarily 1000 µl nanocomposite of silver nanoparticle and PVA prepared from callus and root extracts was mixed in 1.0 ml of distilled water and applied to sterile paper disc. These discs were then placed on Muller Hinton Agar swabbed with clinical strains of bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*, and the gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* and fungal strain *Candida albicans* at a concentration of 10^6 bacteria/ml for bacteria (Malabadi, 2005; Malabadi *et al.* 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008; Malabadi *et al.* 2012a, 2012b). The plates were incubated at 37° C for overnight. The zone of inhibition was measured in millimeter after 24 h of incubation and recorded. Each extracts of callus and root were tested in quadruplicate (four discs per plate), with a silver sulphadiazine (1mg ml^{-1}) disc as a reference or positive control (Malabadi, 2005; Malabadi *et al*. 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008; Malabadi *et al.* 2012a, 2012b). The ratio between the diameter of the inhibition zones (mm) produced by callus and root extracts and the inhibition zone around the disc with silver sulphadiazine (mm) was used to express antibacterial activity. The activity of Silver Sulphadiazine was included in this equation to adjust for plate-to-plate variations in the sensitivity of a particular bacterial strain (Malabadi, 2005).

The antibiotic silver sulphadiazine was included as standard in each assay. Extract-free solution was used as blank control (Malabadi, 2005; Malabadi *et al*. 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008). The microplates were incubated overnight at 37° C (Malabadi et al. 2012a, 2012b). As an indicator of bacterial growth, 40µl p-iodonitrotetrazolium violet (INT) (Sigma) dissolved in water were added to the microplate wells and incubated at 37° C for 30 min (Eloff, 1998a, 1998b). MIC values were recorded as the lowest concentration of extract that completely inhibited bacterial growth (Malabadi, 2005; Malabadi *et al.* 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008; Malabadi *et al.* 2012a, 2012b). Since the colorless tetrazolium salt is reduced to a red colored product by biologically active organisms, the inhibition of growth can be detected when the solution in the well remains clear after incubation with INT (Eloff, 1998a, 1998b).

Results and discussion

In our present study, the antimicrobial activity of *in vitro* derived plant roots and callus cultures of *Clitoria ternatea* extract was evaluated and results were presented in table-1. As the root and callus extract was mixed in the aqueous solution of the silver nitrate, the color changed into brown due to the reduction of silver ion to silver which indicated the formation of stable silver nanoparticles. Furthermore, stabilization of silver nanoparticles has played an important role in the antimicrobial activity. In the present study, the highest antimicrobial activity was recorded with stabilized nanoparticles against non-stabilized silver nanoparticles. Therefore, stabilization with polyvinyl alcohol (PVA) has increased the antimicrobial activity against tested clinical samples (Table-1) which also confirms with our previous findings with *C. speciosus* (Malabadi *et al.* 2012b).

Table 1: Antibacterial activity of in vitro derived plants, and callus cultures of *Clitoria ternatea* on the clinical samples by disc diffusion method

Clinical samples	**Diameter of inhibition zone** (mm)					
	Plant extracts	Silver nanoparticles	Silver Nitrate ($AgNo_3$)	Methanol	Ethanol	Water
Bacillus subtilis	**Root** +PVA -PVA **Callus** +PVA -PVA	**Root** 23 16 **Callus** 18 12	10	8	3	0
Staphylococcus aureus	**Root** +PVA -PVA **Callus** +PVA -PVA	**Root** 21 14 **Callus** 20 10	11	6	2	0
Escherichia coli	**Root** +PVA -PVA **Callus** +PVA -PVA	**Root** 20 14 **Callus** 21 11	13	8	4.5	0
Klebsiella pneumoniae	**Root** +PVA -PVA **Callus** +PVA -PVA	**Root** 17 15 **Callus** 19 7	8	5.3	4.3	0
Candida albicans	**Root** +PVA -PVA **Callus** +PVA -PVA	**Root** 23 16 **Callus** 21 13	7	6	3.5	0

Root extract derived silver nanoparticles showed the highest toxicity as compared against callus extracts. This might be due to the presence of phenol glycoside 3-5- 7- 4 - tetra - hydroxy flavone - 3 - rhamnoglycoside, an alkaloid called *clitorin* (MP 235^0 C) which has been extracted from the roots (Kulkarni *et al.* 1988; Rastogi and Mehrotra, 1991). The root has a sharp bitter taste with antihelmintic, analgesic, antipyretic, and anti-inflammatory properties (Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004; Malabadi *et al.* 2005, 2007). The plant is used for curing severe bronchitis, asthma, hectic fever (Mandal *et al*. 2003), and also as a tonic against ulcers of the cornea and tuberculoses (Cooke, 1908; Nadkarni, 1982). Roots are emetic and are used by the local tribes to cause abortion. Root paste is applied on the stomach of cattle for urinary and abdominal swellings, sore throat, mucous disorders and fever (Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004; Malabadi *et al*. 2005, 2007). A root juice is given in cold milk to remove phlegm in chronic bronchitis. The seeds contain oil and a bitter resinous principle which were used as powerful purgative (Malabadi *et al.* 2005). The plant has thus been evaluated extensively for various pharmacological activities (Malabadi, 2002, 2003; Malabadi and Nataraja, 2001, 2002a, 2002b, 2004; Malabadi *et al.* 2005, 2007). The results of this study also clearly indicated that silver nanoparticles synthesized from root and callus extracts of *Clitoria ternatea* has many pharmaceutical applications for the control of deadly pathogens (Malabadi *et al.* 2012). The significant and higher antibacterial activity of *Clitoria ternatea* are probably due to the presence of flavonoids in the plant (Malabadi *et al.* 2005; Malabadi *et al.* 2012).

Nanoparticles exhibit completely new properties based on specific characteristics such as size, distribution and morphology. As specific surface area of nanoparticles is increased, their biological effectiveness can increase in surface energy (Satyavani *et al.* 2011; Malabadi *et al.* 2012a, 2012b). The most widely used and known applications of silver and silver nanoparticles are include topical ointments and creams containing silver to prevent infection of burns and open wounds (Malabadi *et al*. 2012a, 2012b). Production of nanoparticles can be achieved through different methods. However, chemical approaches can not avoid the use of toxic chemicals in the synthesis protocol. Biological methods of nanoparticles synthesis using microorganisms, enzymes, and plant or plant extract have been suggested as possible green nanotechnology alternatives to chemical and physical methods. The antimicrobial activity in terms of inhibition zone significantly varied with test microbes and the type of the extracts. This differential antimicrobial activity of silver nanoparticles can be attributed to their differential sizes and shape: the antimicrobial activity increases with decreasing size of the silver nanoparticles (Nabikhan *et al*. 2010). The mechanism for the antimicrobial action of silver ions is not properly understood; however, the effect of silver ions on microbe can be observed by the structural and morphological changes (Vankar and Shukla, 2012). Recently, silver nanoparticles have been used in agriculture to extend the maintenance of asparagus leaves (from 2 to 21 days) and to increase the ascorbate, chlorophyll, and fiber contents of leaves, and decrease seed abscission in borage (*Borago officinalis* L.) (Seifsahandi *et al*. 2011). Silver nanoparticles are novel silver compounds composed of clusters of silver atoms developed using nanotechnology. Silver nanoparticles have many different applications in electronic, optical, catalytic, and medicinal industries. The silver nanoparticles show efficient antimicrobial

activity compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. Therefore, silver is ideally suited for effective control of germs, molds and fungus. Its benefit over the use of antibiotics can be used as a powerful strategy to combat the increasing spread of multidrug resistance resulting from broad use of antibiotics. Therefore, clinical efficiency of antibiotics has been compromised (Ghosh *et al*. 2012). In the synthesis of silver nanoparticles, generally silver nitrate is used as the salt precursor (Hettiarachchi and Wickramarachchi, 2011). Reduction of Ag (I) to Ag (o) can be achieved by chemical, electrochemical, and phytochemical reduction as well as thermal, ultrasound, microwave, gamma and electron irradiation (Hettiarachchi and Wickramarachchi, 2011). However, chemical methods of synthesizing silver nanoparticles are simpler than physical methods; they have several disadvantages over physical methods (Hettiarachchi and Wickramarachchi, 2011). Chemical reduction leaves the residual reducing agents and hence the final product needs further purified. Furthermore, chemical methods may associate with environmental toxicity and biological hazards (Hettiarachchi and Wickramarachchi, 2011). Therefore, plant derived silver nanoparticles play an important role in medical applications. Recently silver nanoparticles were synthesized from calli cells of *Citrullus colocynthis* (Satyavani *et al.* 2011). The callus extract reacted with silver nitrate solution confirmed silver nanoparticles synthesis through the steady change of greenish colour to reddish brown (Satyavani *et al.* 2011). They also confirmed that plant based silver nanoparticles possess considerable anticancer effect compared with commercial nanosilver (Satyavani *et al.* 2011). The reduction of the metal ions through the callus extracts leading to the formation of silver nanoparticles of fairly well defined dimensions. Use of silver nanoparticle should emerge as one of the novel approaches in cancer therapy and when the molecular mechanism of targeting is better understood, the applications of silver nanoparticles are likely to expland further (Satyavani *et al.* 2011). Therefore, synthesis of silver nanoparticles from plant is evolved into an important branch of nanotechnology. The approach appears to be cost effective alternative to conventional methods of assembling silver nanoparticles. The silver nanoparticles prepared are found to be stable over a period of one month at room temperature and showed no signs of aggregation. This might due to stabilization of silver nanoparticles. Generally metal nanoparticles aggregate among themselves and progressively grow into larger clusers and eventually precipitates, deviating from nanoscale (Hettiarachchi and Wickramarachchi, 2011). This avoids the effectiveness of synthesized nanoparticles and discourages its applications (Hettiarachchi and Wickramarachchi, 2011). Coalescence may be prevented by adding a cluster stabilizer. Synthetic polymers such as polyvinyl chloride, polyvinyl alcohol, and polyvinyl pyrrolidone are already in usage as stabilizing polymers of silver nanoparticles (Hettiarachchi and Wickramarachchi, 2011). Due to non-biodegradability and toxicity of synthetic polymers, at presently a considerable attention has been drawn on the use of natural polymers to stabilize silver nanoparticles (Hettiarachchi and Wickramarachchi, 2011). Furthermore, especially in medical applications, additional steps are necessary to remove the synthetic polymer after synthesis, which makes the synthesis process much complex and less economic. Once the stabilizer is eliminated there is a tendency to diminish the stability of synthesized silver nanoparticles, altering the effective particle size (Hettiarachchi and Wickramarachchi, 2011). Therefore, natural

polysaccharides being a environment benign, biodegradable, highl abundant and low cost, they are more favorable to be utilized as a stabilizer for synthesized silver nanoparticles (Hettiarachchi and Wickramarachchi, 2011). Therefore, development of a green method to synthesize silver nanoparticles is desired. Furthermore, these green methods are very low cost, fast, efficient and generally lead to the formation of crystalline nanoparticles with a variety of shapes (spheres, rods, prisms, plates, needles, leafs or dendrites), with sizes between 1 and 100nm (Kouvaris *et al*. 2012). These features mainly depend on the process parameters, such as the nature of the plant extract and the relative concentrations of the extract and metal salts reaction, pH, temperature and time of reaction, as well as the rate of mixing of plant extract and metal salts (Kouvaris *et al*. 2012). It is concluded from the present findings that, the biosynthesized silver nanoparticles of callus and root aqueous extract of *in vitro* derived plants of *Clitoria ternatea* provided potential toxicity against tested clinical samples which could be used for prevention of several deadly diseases. Therefore, these silver nanoparticles have potential applications in the biomedical field this simple procedure has several advantages such as cost effetiveness, compatibility for medical and pharmaceutical applications, as well as large scale commercial production.

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