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Antibacterial activity of silver nanoparticles synthesized by using whole plant extracts of *Clitoria ternatea*

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This study highlights the synthesis of silver nanoparticles using whole plant extracts of *Clitoria ternatea*. Antibacterial activity of silver nanoparticles was assessed by using disc diffusion method against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*, since *Bacillus* species and *S. aureus* strains may cause diarrhoea and an enteropathogenic form of *E. coli*, and *Klebsiella* species may cause food poising. The results of this study clearly indicate that silver nanoparticles synthesized from plant extracts of *Clitoria ternatea* has many pharmaceutical applications for the control of deadly pathogens.

Key words: Antimicrobial activity, nanotechnology, plant extracts, pharmacy, silver

Introduction

Nanotechnology is one of the exciting fields with many applications in the modern medicine (Xia *et al.* 2010). Nanoparticles are ranging in the size of 10-200 nm, and are in the solid state either amorphous or crystalline in nature (Gardea-Torresdey *et al*. 2002, 2003). Nanoparticles present a higher surface to volume ratio with decreasing size of nanoparticles (Song and Kim, 2009). Specific surface area is relevant for catalytic reactivity and other related properties such as antimicrobial activity of silver nanoparticles (Song and Kim, 2009). As specific area of nanoparticles is increased, their biological effectiveness can increase due to the increase in surface energy (Song and Kim, 2009). They are able to adsorb or encapsulate a drug or a chemical thus protecting it against chemical and enzymatic degradation (Shankar *et al*. 2003, 2004). In recent years, biodegradable polymeric nanoparticles have many applications in the modern medicine in terms of controlled drug delivery, targeting a particular organ or tissue or carriers of DNA in gene therapy, or in the delivery of proteins, peptides through a designated route (Sharma *et al.* 2007). In general, nanoparticles were synthesized from different synthetic polymers, polylactide (PLA), polyglycolide (PLG), and poly (D,L-lactide-co-glycolide) (PLGA) nanoparticles represent the most extensively investigated ones. Further some of the promising polymers approaches are poly (cyanoacrylate) (PCA), poly(alkylcyanoacrylate) (PACA), poly(ε-caprolactone) (PCL), and poly(ester-anhydride) (PEA) (Gardea-Torresdey *et al*. 2002, 2003). In addition to these polymers, natural biopolymers and macromolecules such as chitosan, sodium alginate, albumin, collagen and gelatin were also used for the synthesis for nanoparticles (Sharma *et al.* 2007). Among these, nanoparticles of proteinaceous origin, e.g albumin, collagen and gelatin have raised specific interest. They bear multiple modification opportunities for coupling e.g. targeting-ligands, crosslinkers, and shielding substances (Gardea-Torresdey *et al*. 2002, 2003). The synthesis of nanoparticles and their self-assembly is a cornerstone of nanotechnology (Zhang *et al.* 2008). Silver nanoparticles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnosis, antimicrobials and therapeutics, catalysis and microelectronics (Geethalakshmi and Sarada, 2010). However, there are many problems and toxicity of using metal oxide nanoparticles on the human health. The development of new chemical or physical methods has resulted in environmental contaminations since the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts (Zhang *et al.* 2008). Thus, there is a need for 'green nanotechnology' that includes a clean, nontoxic and environment-friendly method of nanoparticle synthesis. As an alternative to conventional methods, biological methods are considered safe and ecologically sound for the nanomaterial fabrication. Therefore, the use of green plants for similar nanoparticle biosynthesis methodologies is an exciting possibility which has compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis of nanoparticles. Use of plants for the synthesis of nanoparticles does not require high energy, temperatures, and it is easily scaled up for large scale synthesis and it is cost effective too (Shankar *et al.* 2003; Geethalakshmi and Sarada, 2010; Mukunthan *et al*. 2011; Vankar and Shukla, 2012; Ghosh *et al*. 2012).

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). 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). 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. 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 phenol glycoside 3- 5- 7- 4 - tetra - hydroxy flavone - 3 - rhamnoglycoside, an alkaloid called *clitorin* (MP 235^0 C) was extracted from the roots (Kulkarni *et al.* 1988; Rastogi and Mehrotra, 1991). 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).

Developed and developing countries show a great interest in indigenous medicine, and many developing countries use traditional medicines at the primary health care level (Malabadi *et al.* 2007). Many currently used drugs are expensive or not readily available and a major set back to their continued usage is the development of resistance (Malabadi *et al.* 2007). Use of herbal medicine is one of the common practices in India due to their wide pharmacological activities (Asolkar *et el*. 1992; Malabadi *et al.* 2007, 2010; Malabadi, 2005; Malabadi and Vijayakumar, 2005, 2007, 2008). Another reason is traditional herbal medicines are generally more acceptable from a cultural and spiritual perspectives (Malabadi *et al.* 2007, 2010; Malabadi, 2005; Malabadi and Vijayakumar, 2005, 2007, 2008). Herbal medicines provide people with a good alternative (Malabadi *et al.* 2007). This situation urgently forced scientists for searching new, inexpensive drugs that will be able to act for longer periods before resistance sets in. The present study was conducted to investigate antibacterial activity of silver nanoparticles synthesized from the whole plant extracts of *Clitoria ternatea* (L.) by preliminary bioassay screening. Antibacterial activity was evaluated by using the disc-diffusion assay, and minimal inhibitory concentration (MIC), values were determined by using the microdilution assay. The extracts were tested against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and the Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae*.

Materials and Methods

Plant material and preparation of extract

The different plant parts such as leaf, root, and stem of white flowered variety of *Clitoria ternatea* (L.) were collected from the field grown plants in Botanical Garden, Karnatak University, Dharwad, Karnataka state, India, and they were used for the following experiments. All the plant parts were washed three times with sterile distilled water. The plant material was sun dried and ground to make a fine powder. Further 4 grams of powder were taken into 250ml beaker and added 100ml of sterile distilled water and boiled for 20 min at 100^{o} C. The whole plant extract was collected in a separate beaker by standard filtration (Whatman filer paper) method.

Synthesis of silver nanoparticles

The procedure for the preparation of the silver nanoparticles has been adopted from Gardea-Torresdey *et al.* (2002, 2003), and Savithramma *et al.* (2011) with slight modifications**.** 1mM $AgNO_3$ (silver nitrate) solution was prepared and stored in amber colour bottle. 10ml of whole plant extract was taken in beaker separately and 50ml of 1mM $AgNO_3$ solution was added to the beaker drop wise with constant stirring at 50-60^{o} C and colour change was observed (Linga Rao and Savithramma, 2012). The colour change was checked periodically and the beaker was incubated at room temperature for 40 hours (Linga Rao and Savithramma, 2012). The color change of the whole plant extracts from yellow to brown indicated the presence and synthesis of silver nanoparticles from the whole plant extracts of *Clitoria ternatea*. The extract content was then centrifuged at 10,000 rpm for 20 min (Linga Rao and Savithramma, 2012). The supernatant was used for the spectrometric UV analysis and for the evaluation of antibacterial activity (Linga Rao and Savithramma, 2012). The spectrometric analyzed results highlighted the presence and reduction of silver ions in the tested samples.

Antibacterial activity

The test organisms in the investigations of antibacterial activity *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* were used in the following study (Malabadi *et al.* 2005). The number of cells in Mueller- Hinton (MH) broth cultures of each bacterial species was estimated using a serial dilution method (Lech and Brent, 1987). 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 *et al.* 2005). The plates were incubated overnight at 37^{o} C and colonies were counted. 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 *et al.* 2005). The optical density (OD) at 600 nm for each dilution was determined using spectrophotometer, and was used to indicate number of bacterial cells in cultures for the antibacterial screening and MIC determination (Malabadi *et al.* 2005).

The disc diffusion assay (Rasoanaivo and Ratsimamanga-Urverg, 1993) was used in the antibacterial screening procedure (Malabadi *et al.* 2005). MH agar base plates were prepared using sterile 90 mm Petridishes (Malabadi *et al.* 2005). MH agar was inoculated with a MH broth culture (10^{6}- 10^{8} bacterial ml^{-1}) of each bacterial species and poured over the base plates to form a homogenous layer (Malabadi *et al.* 2005). Filter paper discs (Whatman No 3 and 6 mm in diameter) were sterilized by autoclaving (Malabadi *et al.* 2005). These sterile paper discs were dipped in silver nanoparticle solution (10 µg/ml). These discs were air-dried under sterile conditions, and placed onto the seeded top layer of the MH agar plates. Each extract was tested in quadriplicate (four discs per plate), with a neomycin (5 µg ml^{-1}) disc as a reference or positive control. The plates were evaluated after incubation at 37^{o} C for 24h after which the zones of inhibition around each disc were measured (Malabadi *et al.* 2005). The ratio between the diameter of the inhibition zones (mm) produced by silver nanoparticle solution and the inhibition zone around the disc with neomycin (mm) was used to express antibacterial activity (Vlietinck *et al*. 1995; Malabadi *et al.* 2005). The activity of neomycin was included in this equation to adjust for plate-to-plate variations in the sensitivity of a particular bacterial strain (Rabe and Van Staden, 1997; Malabadi *et al.* 2005).

The microplate method of Eloff (1998a, 1998b) was used with slight modifications to determine the MIC values for silver nanoparticle solution with antibacterial activity (Malabadi *et al.* 2005). All extracts were initially tested at 12.5 mg ml^{-1} in 96-well microtitre plates and serially diluted twofold to 0.38 µg ml^{-1}, after which 100 µl bacterial culture (approximately 10^{6} bacteria ml^{-1}) were added to each well (Malabadi *et al.* 2005). The antibiotic neomycin was included as standard in each assay. Extract-free solution was used as blank control. The microplates were incubated overnight at 37^{o} C (Malabadi *et al.* 2005). 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^{o} C for 30 min (Malabadi *et al.* 2005). MIC values were recorded as the lowest concentration of extract that completely inhibited bacterial growth (Malabadi *et al.* 2005). 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 (Malabadi *et al.* 2005).

Results and discussion

The antibacterial activity of silver nanoparticles synthesized by using whole plant extracts of *Clitoria ternatea* showed positive results and it is presented in the table 1. Silver nanoparticles showed antibacterial activity against gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) as well as gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*). The water extracts presented very low antibacterial activity (Table 1). In the present study, the silver nanoparticles showed higher antibacterial activity against the tested pathogens (Table-1). The highest antibacterial activity was recorded with silver nitrate (Table-1). In our previous studies, methanolic extracts showed the greatest activity, and no activity was recorded with water extracts. Hexane and methanolic extracts of roots showed the highest and significant antibacterial activity against both Gram-positive and Gram-negative bacteria. No antibacterial activity was recorded with stem extracts (Malabadi *et al.* 2005).

Table-1: Antibacterial activity of whole plant extracts of *Clitoria ternatea*

Tested pathogens	Diameter of the inhibition zone (mm)			
	Control (plant - Ethanol extracts)	Silver nanoparticles	Silver nitrate $AgNO_3$	Methanol extracts
Bacillus subtilis	1	13	15	8
Staphylococcus aureus	1	15	16	7
Escherichia coli	3	10	13	6
Klebsiella pneumoniae	2	16	17	8

This may be due the presence of terpenoids, which possess antifungal, antibacterial and anti-insect activities (Malabadi *et al.* 2005). This is a common feature particularly in the plants belongs to Lamiaceae. The significant and higher antibacterial activity of *Clitoria ternatea* are probably due to the presence of flavonoids in the plant (Malabadi *et al.* 2005). On the other hand antibacterial activity might be due to the presence of a phenol glycoside 3-5-7-4 tetra-hydroxy- flavone-3- rhamnoglycoside an alkaloid called clitorin (MP 235^{o} C) (Kulkarni *et al.* 1988). Reasons for high MIC values (data not presented) could be that the extracts tested are still in an impure form, or that active compound/s are present in very low concentrations (Malabadi *et al.* 2005). Nevertheless certain of the plant extracts warrant further investigation using bioassay-guided fractionation to characterize the active constituents (Malabadi *et al.* 2005). Therefore, the results of this study support to a certain degree, the traditional medicine uses of the plants evaluated and reinforce the concept that the ethanobotanical approach to screening plants as potential sources of bioactive substances is successful (Malabadi *et al.* 2005).

In the present study, synthesis of nanoparticles was successful by using silver nitrate as a reducing agent. Here silver has many unique properties of good conductivity, catalytic and chemical stability (Linga Rao and Savithramma, 2012). Silver has long been recognized as having inhibitory effect on bacterial strains and other microorganisms present in medical and industrial process (Song and Kim, 2009). The most important application of silver and silver nanoparticles is in medical industry such as topical ointments and creams containing silver to prevent infection against burns and open wounds (Song and Kim, 2009; Geethalakshmi and Sarada, 2010). Another widely used applications are medical devices and implants prepared with silver-impregnated polymers. In addition to this silver containing consumer products such as colloidal silver gel and silver-embedded fabrics are now used in sporting equipment (Song and Kim, 2009). It is well known fact that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Shankar *et al.* 2004). During formation of nanopartilces, the aqueous silver ions when exposed to herbal extracts were reduced in the solution, thereby leading to the formation of silver hydrosol (Linga Rao and Savithramma, 2012). As the extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to yellowish brown due to reduction of silver ion which indicated the formation of silver nanoparticles. The synthesis was confirmed by the spectrometric analysis. Ahmed *et al.* (2011) mentioned three different routes for the reduction of silver in plant extracts. The secondary metabolites present in plant systems may be responsible for the reduction of silver and synthesis of nanoparticles (Linga Rao and Savithramma, 2012). Another hypothesis is that electron released during glycolysis for the conversion of NAD to NADH led to transformation of silver nitrate to form nanoparticles and another mechanism is releasing of an electron when formation of ascorbate radicals from ascorbate reduces the silver ions (Linga Rao and Savithramma, 2012). These results are also confirmed with other previous reports and observed in *Cleodendrum inerme* (Farooqui *et al.* 2010), *Euphorbia hirta* (Elumalai *et al.* 2010), *Catharanthus roseus* (Mukunthan *et al.* 2011), *Dioscorea bulbifera* (Ghosh *et al*. 2012), Geranium (Shankar *et al.* 2003), *Citrus lemon* (Vankar and Shukla, 2012), and *Argimone maxicana* (Khandelwal *et al*. 2010).

The first report of plant synthesizing gold or silver nanoparticles appeared when *alfalfa* seedlings were shown to uptake gold or silver from metals-enriched nutrient media (Gardea-Torresdey *et al*. 2002, 2003). These studies demonstrated that Au(III) or Ag(I) ions were reduced in the solid media to Au (0) or Ag(0) by *alfalfa* plants, and then the metal atoms were absorbed into the plant, where growth of nanoparticles took place (Gardea-Torresdey *et al*. 2002, 2003). Another dimension was added to the 'green chemistry' approach for pure metal synthesis with the use of plant broths (Shankar *et al*. 2003, 2004). Shankar *et al* (2003, 2004) used lemongrass and geranium, and neem leaf plant extracts to induce the formation of gold nanoparticles or structures when reacted with aqueous chloroauric acid (Shankar *et al*. 2003, 2004). In another development, an uptake of high amounts of gold(III) ions by a leguminous shrub, *Sesbania drummondii*, has been demonstrated with subsequent reduction of Au(III) ions to Au(0) inside plant cells or tissues (Sharma *et al.* 2007). The nanoparticle-bearing biomatrix of *Sesbania* has the ability to reduce a hazardous and toxic pollutant, aqueous 4-nitrophenol (Sharma *et al.* 2007). In another study, there is a possibility of using live plants for the fabrication of nanoparticles. *Alfalfa* plants were grown in an $AuCl_4$ rich environment. The absorption of Au metal by the plants was confirmed by X-ray absorption studies (XAS), and transmission electron microscopy (TEM). Atomic resolution analysis confirmed the nucleation and growth of Au nanoparticles inside the plant and that the Au nanoparticles are in a crystalline state. Images also showed defects such as twins in the crystal structure, and in some cases icosahedral nanoparticles were found (Linga Rao and Savithramma, 2012). X-ray EDS studies corroborated that the nanoparticles are pure gold. This was the first report on the formation of gold nanoparticles by living plants and opens up new and exciting ways to fabricate nanoparticles

(Linga Rao and Savithramma, 2012). It has also showed that how it is possible to link materials science and biotechnology in the new emerging field of nanobiotechnology. Silver has more microbial efficacy and more effective in the presence of proteinaceous material and inorganic binding proteins that associated with inorganic structures *in vivo* using routine molecular biology techniques (Linga Rao and Savithramma, 2012). Silver nanoparticles synthesized from plant species are highly toxic and inhibited the growth of the tested bacterial species (Savithramma *et al.* 2011; Linga Rao and Savithramma, 2012). This has got direct applications in the medical science. Similar observations were also found in *Allium cepa* (Saxena *et al.* 2010), *Argimone mexicana* (Khandelwal *et al.* 2010), and *Artocarpus heterophyllus* (Thirumurgan *et al.* 2009, 2010; Linga Rao and Savithramma, 2012). Therefore, synthesis of silver nanoparticles using medicinal plants were found to be highly toxic against tested bacterial species and the rate of the toxicity is illustrated in the table-1. Ahmed *et al* (2011) mentioned that the pathogenic effect of nanoparticles can be attributed to their stability in the medium as a colloid, which modulates the phosphtyrosine profile of the pathogen proteins and arrest its growth (Linga Rao and Savithramma, 2012). The growth of the tested microorganisms was inhibited due to the presence of peptidoglycan, a complex structure and contains teichoic acids or lipoteichoic acids which have a strong negative charge (Linga Rao and Savithramma, 2012). This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less silver to reach cytoplasmic membrane than gram negative bacteria (Ahmed *et al.* 2011). Silver nanoparticles have an ability to interfere with metabolic pathways (Warsnoicharoen *et al*. 2011). The findings of Sereemaspun *et al.* (2008) suggested that the inhibition of oxidation based biological process by penetration of metallic nano sized particles across the microsomal membrane (Linga Rao and Savithramma, 2012). The molecular basis for the biosynthesis of these silver crystals is speculated that the organic matrix contain silver binding properties that provide amino acid moieties that serve as the nucleation sites (Prabhu *et al*. 2010; Savithramma *et al.* 2011). The fundamental mechanism of biological nanoparticle synthesis is not fully understood (Song and Kim, 2009). For gold nanoparticles synthesized extracellularly by the fungus *Fusarium oxysporum*, it was reported that the reduction occurs due to NADH-dependent reductase released into the solution (Song and Kim, 2009). In case of neem leaf broth, it was reported that terpenoids are believed to be the surface active molecules stabilizing the nanoparticles and reaction of the metal ions is possibly facilitated by reducing sugars and/ or terpenoids present in the neem leaf broth (Song and Kim, 2009). The results with *Capsicum annuum* L. extract indicated that the proteins which have amine groups played a reducing and controlling role during the formation of silver nanoparticles in the solutions, and that the secondary structure of the proteins changed after reaction with silver ions (Song and Kim, 2009). On the other hand the average size of silver nanoparticle size has decreased with increasing the silver nitrate concentration. The reason of decrease in particle size with silver nitrate concentration is not clear (Song and Kim, 2009). It is considered that the particle size and shape are dependent on various conditions such as plant type, nanoparticle type, reaction temperature and composition (Song and Kim, 2009). Therefore, the synthesis of silver nanoparticles using whole plant extracts of *Clitoria ternatea* showed high antibacterial activity which has many practical applications in the medical science. This method is potentially exciting for the large scale synthesis of silver nanoparticles, and it is ecofriendly, simple and economical route to synthesized silver nanoparticles.

However, recent studies indicated the concern about the use of metal oxide nanoparticles for the human therapy. Engineered nanomaterials like the nanoparticles are so small that they can pass through the skin, lungs and intestinal tract with unknown effects to human health. Metal-based nanoparticles have been linked to both environmental and animal toxicity in a variety of studies (Xia *et al.* 2010). One of the best example is the use of 500 nm TiO_2 particles have some ability to cause DNA strand breakage (Xia *et al.* 2010). Furthermore, 20 nm TiO_2 nanoparticles are capable of causing complete destruction of super-coiled DNA (Xia *et al.* 2010). In addition to the increased potential for DNA damage from engineered metal oxide nanoparticles, another concern for their application in cosmetics is the potential for inhalation, ingestion, and penetration through the skin (Xia *et al.* 2010). Once in the blood stream, nanomaterials can be circulated inside the body and are taken up by organs and tissues such as the brain, liver, spleen, kidney, heart, bone marrow, and nervous system (Xia *et al.* 2010). With their stability, the damage of these nanoparticles to human tissues and organs can occur through a traditional ROS pathway, or through accumulation that can impair their normal functions (Xia *et al.* 2010). *In vitro* studies on BRL 3A rat liver cells exposed to 100-250 µg/ml of $Fe3O_4$, Al, MoO_3 and TiO_2 nanoparticles revealed significant damage from ROS in these cells (Xia *et al.* 2010). Carbon nanotubes have also been shown to be toxic to kidney cells and inhibit cell growth (Xia *et al.* 2010). The stability of nanomaterials in the environment has also been linked to brain damage and mortality in several aquatic species (Xia *et al.* 2010). Therefore, as an alternative approach to hazardous metal-based nanoparticles, organic nanoparticles have been isolated from English ivy (*Hedera helix*) (Xia *et al.* 2010). The results indicated that the ivy nanoparticles were more efficient in blocking UV light, less toxic to mammalian cells, easily biodegradable, and had a limited potential to penetrate through human skin (Xia *et al.* 2010). When compared to TiO_2 nanoparticles, the English ivy (*Hedera helix*) nanoparticles showed decreased cell toxicity, and were easily degradable, indicating that they provided a safer alternative to these nanoparticles (Xia *et al.* 2010). Ivy, a root-climber, is an evergreen plant belongs to the genus *Hedera* (Zhang *et al.* 2008). Ivy can affix itself to, and extend its growth upward on, rocks, fences, trees, and many other surfaces (Zhang *et al.* 2008). In this “climbing” process, ivy uses adhering disks of the aerial rootlets developed from the stem to affix to the surface (Zhang *et al.* 2008). Removal of climbing ivy from a surface can be difficult, even after plant death at the root (Zhang *et al.* 2008). Charles Darwin reported in 1876 that ivy rootlet secretes yellowish matter while climbing a surface (Zhang *et al.* 2008). Ivy plants secrete nanoparticles for surface climbing. HPLC/MS analysis suggests empirical formulas for the 19 prevalent compounds of organic composition from the secreted nanoparticles (Zhang *et al.* 2008). The study suggests that the weak adhesion and hydrogen bonds are the likely forces for ivy surface climbing (Zhang *et al*. 2008). The finding may inspire new method to synthesize nanoparticles from ivy plants biologically, or new climbing mechanisms for engineering applications (Zhang *et al*. 2008).

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