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Green synthesis of Nanoparticles (Ag, Cu and Zn) from Plant Latex (*Colocasia* esculenta; Ficus exasperata; Hevea brasilliensis) and Evaluation of Antibacterial Activity

Prem Jose Vazhacharickal* and Meera Krishnan

Department of Biotechnology, Mar Augusthinose College, Ramapuram, Kerala-686576, India

*Corresponding author

Abstract

Nanotechnology is the field of study of materials at nanoscale. It involves the production, manipulation, and use of materials ranging in size from less than one micron to that of individual atoms from not only chemical approaches but also biological materials. Silver, Copper and Zinc nanoparticles were successfully synthesised from silver nitrate, Copper sulphate and Zinc sulphate respectively through a simple green and natural route using latex of 5 different plant taxa. Nanoparticle formation was proved by UV-vis spectroscopy. The antimicrobial well diffusion method used was give information about the antibacterial activity of latex nanoparticles towards 5 different bacterial species by measuring the zone of inhibition. The use of two dilutions of latex solution was used for the comparative study of zone of inhibition. As nanoparticles have great application in medical world like gene therapy, cancer therapy, drug delivery, etc. So medical world also accepts the plant world for nanoparticle synthesis and mainly welcome the angiosperms for their potentiality of synthesis of non-polluted, environmentally acceptable, safety for human health nanoparticles.

Introduction

Many of the plant parts such as leaf, roots, stem, latex, flower etc. are used in traditional medicines. Here we are discussing about antimicrobial activity of nanoparticles formed from plant latex which is collected from different parts of 5 different plant species. Here we use dilution factor as an important parameter.

Since the last century nanotechnology is a known field of research. "Nanotechnology" was presented by Nobel laureate Richard P. Feyman during his well famous 1959 lecture "There's plenty of Room at the Bottom"

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Keywords

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(Feyman, 1960), there have been made various revolutionary developments in the field of nanotechnology. Nanotechnology produces various types of materials at nanoscale level. Nanoparticles (NP's) are wide class of materials that include particulate substances which have one dimension less than 100nm at least (Laurent et al., 2010). Metal nanoparticles are purely made of metal precursors. Due to the well-known localized surface plasmon resonance (LSPR) characteristics: these metal NP's have unique optoelectrical properties. NP's of alkali and noble metals; Cu, Ag, and Au have a broad absorption band in visible spectrum (Dreaden et al., 2012). There are various methods for synthesis of nanoparticles one of the important method is green synthesis. Green synthesis is the use of biological routes such as those involving microorganisms, plants, etc. for the synthesis of nanoparticles. As compared with other methods this method was easy, efficient, eco-friendly and eliminates the use of toxic chemicals, consume less energy and produce safer products and by products (Gardea *et al.*, 2002).

We uses dilution factor for finding the effect of dilution on nanoparticle formation. Dilution factor can be notated as (1: n+1), where the (n+1) represents the total volume of solute and solvent. Here we choose 1:50 and 1:100 dilutions for better results.

Latex is a stable dispersion (emulsion) of polymer micro particles in an aqueous medium. Latex as found in nature is either a milky or colourless fluid found in 10% of all flowering plants (angiosperms) [Anurag A. Agrawal; d Kotaro Konno, 2009]. It is a complex emulsion consisting of proteins, alkaloids, starches, sugars, oils, tannins, resins and gums that coagulate on exposure to air. Usually it is exuded after tissue injury. In most plants latex is white, but some have yellow, orange or scarlet latex (Mahlberg, 1993). Some specific plant parts or whole plant specifically angiospermic plants are used for the great synthesis of nanoparticles (Sharma *et al.*, 2007). Here we uses Taro (*Colocasia esculenta*), Sandpaper tree (*Ficus exasperata*) and Rubber tree (*Hevea brasiliensis*) and to collect latex.

Nanotechnology is an emerging field of science. It has increased applications in diverse area for the development of new materials at nanoscale levels (Paul *et al.*, 2015). Nanotechnology mainly consists of the processing of, separation, consolidation, and deformation of materials by one atom or one molecule (Prasad *et al.*, 2008). Nanoparticles has 1-100 nm in size and they possess novel physical and chemical properties (Sajeshkumar *et al.*, 2015a; Sajeshkumar *et al.*, 2015b; Vazhacharickal *et al.*, 2022). Nanoparticles bear antibacterial properties (Hajipour *et al.*, 2012).

Nanoparticle are particles of any shape with dimensions on the $1*10^{-9}$ and $1*10^{-10}$ (Carlos *et al.*, 2015). Metals like silver, copper and zinc has inhibitory effect on microbes. Nanoparticles synthesized by physical and chemical methods. They have draw back like expensive re-agent, hazards reaction condition, longer time, tedious process to isolate Nano particles. These lead to the development of new method for the synthesis of Nano particles which should be required, non-expensive reagent, less drastic reaction condition and Eco friendly (Kulkarni *et al.*, 2004).

Nanotechnology is the science deals with matter at the scale of 1 billionth of a meter $(10^{-9} \text{m} = 1 \text{nm})$, and is also the study of manipulating matter at the atomic and molecular scale. The word "nanotechnology" soon caught the attention of various media (TV networks, the internet, etc.) and the imagination and fascination of the community at large. Nanotechnology explores electrical, optical and magnetic activity as well as structural behaviour at the molecular and sub molecular level (Pickard *et al.*, 2008). Nanoparticles are defined as the particulate dispersion or solid particles with a size in the range of 10-100 nm

Green synthesis of nanoparticles

Synthesis of nanoparticles using biological methods is referred as greener synthesis of nanoparticles. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, and safe for human therapeutic use (Kumar *et al.*, 2009). Metals like silver, copper and zinc has inhibitory effect on microbes. Biological synthesis of metallic nanoparticles is inexpensive single step and ecofriendly methods. The plants and seeds are used successfully in the synthesis of various greener nanoparticles such as copper, silver, and zinc oxide (Kuppusamy *et al.*, 2016; Mishra *et al.*, 2014).

Application of nanoparticles

Nanoparticles has various applications. Nanoparticles have been used for constructing electrochemical and biosensors (Luo *et al.*, 2006). Metal nanoparticles embedded paints have good antibacterial activity (Kumar *et al.*, 2008). Current research is going on regarding the use of magnetic nanoparticles in the detoxification of military personnel in case of biochemical warfare (Salata, 2005).

One of the major opportunities for nanoparticles in the area of computers and electronics is their use in a special polishing process, chemical-mechanical polishing or chemical mechanical planarization, which is critical to semiconductor chip fabrication (Elechiguerra *et al.*, 2005).

Magnetic nanoparticles are also used in targeted therapy where a cytotoxic drug is attached to a biocompatible nanoparticle for tumor cell treatment (Pankhurst *et al.*, 2003). Porous nanoparticles have been used in cancer therapy.

Bioremediation of radioactive wastes from nuclear power plants and nuclear weapon production such as uranium has been achieved using nanoparticles (Duran *et al.*, 2007).

Silver nanoparticles

Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process (Morones *et al.*, 2005; Lok *et al.*, 2007). The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burns and open wound. Silver ions (Ag^+) and its compounds are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity towards animal cells (Prema, 2011).

In past ten years silver Nano particles have been one of the extensively studied Nano materials. It have physical, chemical, optical biological application and application in bio medicine, drug delivery, topical oilmen's and creams (Patcharaporin *et al.*, 2006).

Effective anti-bacterial activity is exhibited by copper Nano particles. Copper Nano particles has intensively clear cost effective and efficient bio synthesis technics (Min chung *et al.*, 2004).

Objectives

The main objectives of this study were

Synthesis of silver, copper, zinc nanoparticles using plant latex.

Characterization of nanoparticles by UV- Vis spectroscopy.

Analyse antimicrobial properties against gram –positive and gram – negative bacteria

Scope of the study

The study would enlighten the medical and pharmaceutical applications various green synthesised nanoparticles using plant latex against different microorganism which could be further explored.

Review of literature

Colocasia esculenta (Taro)

Taro or *Colocasia esculenta* is a tropical plant grown primarily for its edible corms, the root vegetables commonly known as taro. *Colocasia esculenta* is a member of family Araceae and are monocots, native to Southern India and Southern Asia, but is widely neutralised (Hill Albert, 1939; Kolchaar, 2006). It is a perennial, tropical plant initially grown as a root vegetable for its edible starchy corm, and as a leaf vegetable. It is used as food in African, Oceanic, and Indian cultures and is believed to have been one of the earliest cultivated plants. It is known by many local names, In Kerala it is called 'Chaemb'. Nigeria is the largest producer of taro in the world. The latex of *Colocasia esculenta* is an off-white coloured one and are spreaded along the body parts.

Taxonomical classification Colocasia esculenta (Taro)

Kingdom: Plantae-- planta, plantes, plants, vegetal

Subkingdom: Virdaeplantae

Division: Tracheophyta

Class: Magnoliopsida

Order: Alismatales

Family: Araceae

Genus: Colocasia

Species: Colocasia esculenta

Ficus exasperata (Sandpaper tree)

Sandpaper tree or *Ficus exasperate*, also called forest sandpaper fig, white fig, or sandpaper leaf tree, is a deciduous, and dioecious species of plant in the Mulberry family Moraceae, native to tropical Africa, from Senegal, Ethiopia to Angola, and southern Asia of India, Sri Lanka, and to Arabian countries such as Yemen. Sandpaper tree a small to medium sized tree in banyan group of figs, growing to 20-30 meters.

Flowers are unisexual and are pink, purplish or yellow, becomes orange or yellow, becomes orange or red at maturity (Berg, 1988). In Kerala it's called 'Therakam'.

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Besides sexual reproduction, the tree may grow with vegetative means propagated by seed and cuttings (Berg, 1988; Hijman *et al.*, 1984). The latex of *Ficus exasperata* is colourless and are found mainly in the stem portions.

Taxonomical classification of *Ficus exasperata* (Sandpaper tree)

Kingdom: Plantae-- planta, plantes, plants, vegetal

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Hamamelididae

Order: Urticales

Family: Moraceae

Genus: Ficus

Species: Ficus exasperata

Hevea brasiliensis (Rubber tree)

Hevea brasiliensis or the rubber tree is belonging to the family Euohorbiaceae. It is the most economically important member o genus *Hevea* because the milky latex extracted is the primary source of natural rubber. *H.brasiliensis* is a tall deciduous tree growing to a height of up to 43m in the wild, but cultivated trees are usually much smaller because drawing off the latex restricts the growth of the tree (Zhang *et al.*, 2008).

Taxonomical classification of *Hevea brasiliensis* (Rubber tree)

Kingdom: Plantae-- planta, plantes, plants, vegetal

Subkingdom: Viridiplantae

Division: Tracheophyta

Class: Magnoliopsida

Order: Malpighiales

Family: Euphorbiaceae

Genus: Hevea

Species: Hevea brasiliensis

Nanoparticles

Nano particles are particles of any shape with dimension on the $1*10^{-9}$ and $1*10^{-10}$ (Carlos *et al.*, 2015) the exhibit size related properties which is different from fine particles or bulk materials. Nano particles are a bridge between bulk materials and atomic or molecular structure (Mac Naught *et al.*, 1997). They possess unexpected optical properties they are small enough to confine their electrons and produce quantum effect. (Hewakuruppu *et al.*, 2013). Nanoparticles have higher specific surface area appropriate for catalysis. Nanoparticles synthesized by physical and chemical methods are highly expensive; require longer time need tedious process to isolate Nanoparticles. Thus new method was developed for the synthesis of Nanoparticles, green synthesis which is small in size, large surface area and eco-friendly (Kulakarni *et al.*, 2006).

Silver nanoparticles

Silver has inhibitory effect on microbes. They prevent infection against burn and open wounds. They are highly toxic to micro-organism exhibiting strong biocidal effect. (Tippayawat *et al.*, 2016). Application of plant extract for the synthesis of silver Nano particles is more advantageous because of its resource availability, security, reaction rate and convenience. Factors including pH, dosage of plant extracts, dosage of silver ions, reaction temperature and time affect synthesis of Silver nanoparticles. Plant extracts act as a reducing agent has an important role in capping and stabilizing of Nanoparticles (Rao *et al.*, 2017; Mody *et al.*, (2010).

Copper nanoparticles

Copper Nano particles have high optical, catalytic, mechanic and electrical properties. They are cheap high yielding and have short reaction time under normal reaction condition. Copper nanoparticles have antimicrobial activities against various bacterial and fungal strain from any researchers (Kulkarni *et al.*, 2006). It is used in various fields including agricultural, industrial, engineering and technical fields. Effective anti-bacterial activities are exhibited by copper nanoparticles. They are cost effective and have efficient bio synthesize techniques. Copper nanoparticles have less cost than silver and gold nanoparticles.

Zinc nanoparticles

Zinc nanoparticles have wide application; various synthetic methods have been employed to produce ZnNps (Song *et al.*, 2007). Zinc nanoparticles can produced from zinc oxide and zinc sulphate.

Zinc nanoparticles has several medicinal uses, it harm skin, stomach, intestine and lymphatics system and they probably induces tumours. Zinc nanoparticles has antibacterial effect on microbes and it mainly depends up on the size and the presence of visible light. Zinc nanoparticles are used in the optical devices, sensors, catalysis, biotechnology, DNA labelling, drug delivery, medical, chemical and biological sensors (Devasenan *et al.*, 2016).

Anti-microbial activity

Anti-microbial agent is a substance that kills microorganisms or stops their growth. Anti-microbial medicines are grouped according to the micro-organisms they act. Antibiotic are used against bacteria, antifungal are used against fungi.

They are also classified on the basis of their function. The agents that kill microbes are called microbicidal; those that merely inhibit their growth are called biostatic (Kingston *et al.*, 2008). The use of anti-microbial agents for the treatment of infection is known as anti-microbial therapy. The use of antimicrobial medicines for the prevention of infection is known as antimicrobial prophylaxis. (Saxon *et al.*, 2014).

Anti-bacterial activity

Medicinal and aromatic plants are used on a large scale in medicine against drug resistant bacteria (Poole *et al.*, (2002). Anything that destroys bacteria or suppresses their growth or their ability to reproduce. Heat, chemicals such as chlorine, and antibiotic drugs all have antibacterial properties. Many antibacterial products for cleaning and handwashing are sold today (Knetsch *et al.*, 2011).

Nanoparticles (NPs) are increasingly used to target bacteria as an alternative to antibiotics. Nanotechnology may be particularly advantageous in treating bacterial infection. In this review, be discus and antibacterial mechanisms of nanoparticles against bacteria and the factors that affect nanoparticle formation (Huh *et al.*, 2011).

Agar well diffusion

The agar diffusion test (Kirby-Bauer antibiotic testing, KB testing or disc diffusion antibiotic sensitivity testing) is a test of antibiotic sensitivity of bacteria. It uses antibiotic discs to test the extent to which bacteria are affected by those antibiotics (Bonev *et al.*, 2008). In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is incubated. If antibiotic stops the bacterial growth or kill the bacteria, there will be an area around the wafer where the bacteria do not show growth (Brown and Kothari, 1975). This is called a zone of inhibition.

The size of this zone depends on many factors, one being how effective the antibiotic is at stopping the growth of the bacterium. Another factor is diffusion of the antibiotic within the agar medium and varies based on the molecular configuration of the antibiotic (Mohanty, 2010). Here we uses well diffusion method, where the wells were made on the agar plate and directly added the sample.

Dilution factor

There are many ways to express the concentration and dilution of a sample. One of the best method is dilution factor, To make a dilute solution without calculating concentration, we can rely on a derivation of the formula;

Dilution factor (DF) = (Final volume/Solute volume)

Expressing the dilution as a ratio of parts of the solute to the total number of parts is common. The Dilution factor (DF) can be used alone or as the denominator of the fraction, for example, a DF of 10 means a 1: 10 dilution or 1 part of solute + 9 parts diluents, for a total of 10 parts. Here we use a DF of 50 and 100.

Hypothesis

The current research work is based on the following hypothesis

Plant latex of Taro (*Colocasia esculenta*), Sandpaper tree (*Ficus exasperata*), and Rubber tree (*Hevea brasiliensis*) shows antibacterial activity.

These plant latex could be used in formulating different kind of nanoparticles (silver, copper and zinc) and their antibacterial activity of the nanoparticles vary widely.

Materials and Methods

Study area

Kerala state covers an area of $38,863 \text{ km}^2$ with a population density of 859 per km² and spread across 14 districts.

The climate is characterized by tropical wet and dry with average annual rainfall amounts to $2,817 \pm 406$ mm and mean annual temperature is 26.8° C (averages from 1871-2005; Krishnakumar *et al.*, 2009). Maximum rainfall occurs from June to September mainly due to South West Monsoon and temperatures are highest in May and November.

Source of latex

Crude latex was obtained by cutting the green stems and fruits of five plants of different genus. Milky white and watery latex both are collected in sample containers and are directly used for better results without storing.

Sample collection

Fresh latex of Taro (*Colocasia esculenta*), Sandpaper tree (*Ficus exasperata*) and Rubber tree (*Hevea brasiliensis*) are collected from Pala, Kottayam district of Kerala state, India. The latex were collected 2ml Eppendorf tubes, transported with ice packs and analysed for nanoparticle formation capabilities.

Preparation of latex solution

1/50 Dilution: For solution with 50 Dilution factor (DF), mix 1 ml of crude latex with 49ml of distilled water.

1/100 Dilution: For solution with 100 Dilution factor (DF), mix 1ml of crude latex with99ml of distilled water.

Synthesis of nanoparticles

Silver nanoparticles

Stock solution was prepared by dissolving 1mMsliver nitrate (AgNO₃; Merck, Mumbai, India) and volume made up to 250 ml with distilled water.5 ml of latex solution was added to 95 ml of 1mM AgNO₃ solution and allowed to react at room temperature. The formation of nanoparticle increases in the presence of sun light. Dark brown colour indicates the formation of AgNO₃.

Copper nanoparticles

Stock solution was prepared by dissolving 2.49 g Copper sulphate (CuSO₄). 5 ml of latex solution of Taro (*Colocasia esculenta*), Sandpaper tree (*Ficus exasperata*), and Rubber tree (*Hevea brasiliensis*) is added to the 95ml of 100mM CuSO₄ solution and allowed to react in room temperature. The CuSO₄ nanoparticles will be formed after 1-2 hours.

Zinc nanoparticles

Stock solution was prepared by dissolving 2.87 g Zinc sulphate (ZnSO₄). 5ml of latex solution of Taro (*Colocasia esculenta*), Sandpaper tree (*Ficus exasperata*) and Rubber tree (*Hevea brasiliensis*) are added to the 95ml of 100mM ZnSO₄ solution and allowed to react in room temperature.

Test microorganisms

The organisms used comprise of two gram-negative organisms (*Klebsiella* and *E.coli*) and three grampositive organisms (*Staphylococcus*, *Bacillus* and *Micrococcus*). The test organisms were obtained from the Department of Biotechnology, Mar Augusthinose College, Ramapuram.

Escherichia coli

These are gram negative, facultative or anaerobic rods (commonly abbreviated *E.coli*) commonly found in the lower intestine of warm blooded organisms. The organisms are relatively heat sensitive and are readily destroyed at high temperature. The optimal temperature for growth is 37°C. *E. coli* is responsible for intestinal tract infection and diarrhoea.

Staphylococcus species

These are spherical in shape, non-motile, gram positive and facultative anaerobes which are positive in the catalase test. The coagulase test is used to broadly demarcate Staphylococcus species into coagulase positive and coagulase negative species. Staphylococus species grow readily on ordinary media with a temperature range of 10 to 40°C, the optimum being 37°C and a pHof 7.4-7.6. *S. aureus* strains have emerged resistant to the penicillinase-stable penicillins (cloxacillin, dicloxacillin, methicillin, nafcillin, and oxacillin).

Klebsiella species

The genus Klebsiella consists of non-motile, capsulated rods that grow well on ordinary media forming large, dome shaped, mucoid colonies of varying degrees of stickiness. *Klebsiella species* are widely distributed in nature, occurring both as commensals in the intestines and as saprophytes in soil and water. *Klebsiella species* can cause diseases like pneumoniae, ozena and rhinoscleroma.

Micrococcus species

These are positive cocci which occur mostly in pairs, tetrads or irregular clusters. They are catalase and oxidase positive. They are aerobic with a strictly respiratory metabolism. They are parasitic on mammalian skin and are ordinarily non-pathogenic.

Bacillus species

The genus Bacillus consists of anaerobic bacilli forming heat resistant spores. They are gram positive but tend to be decolourised easily so as to appear gram variable, or even frankly gram negative. They are generally motile with peritrichous flagella. *Bacillus anthracis*, the causative agent of anthrax, is the major pathogenic species. *B. cereus* can cause food borne gastroenteritis. Some species may be responsible for opportunistic infections.

Characterization of nanoparticles

UV-Vis spectroscopy

The periodic scans of the optical absorbance between 345 and 700nm with a UV- Vis spectrophotometer (Model 118, Systronics, Mumbai, India) at a resolution of 1 nm were performed to investigate the reduction rate of silver ions by the extract. The reaction mixture was diluted 20 times and used for **UV-Vis** spectrophotometry. Deionised water was used to adjust the baseline. The reduction of Ag^+ , Cu^{2+} and Zn^{2+} was monitored periodically by using a UV- Vis Spectrophotometer and the UV- Vis spectra of the reaction solutions were measured in the range of 375-760 nm.

SEM-XRD analysis

SEM-EDX Analysis was carried out in instrument JSM 6390 with acceleration voltage 20 kV. SEM reveals

information about the sample including external morphology, chemical composition and crystalline structure and orientation of materials making up the sample.

SEM provides detailed high-resolution images of the sample by rastering a focused electron beam across the surface and detecting secondary or back scattered electron signal. The XRD imaging of the silver, copper and zinc nanoparticles was performed to confirm the presence of elemental metal signal and provides quantitative compositional information.

Antibacterial assay

Antimicrobial assay was performed by agar well diffusion method. The broth cultures of each organism were aseptically swabbed on Muller Hinton agar plates using sterile cotton swabs.

Wells of 7 mm diameter were made in the inoculated plates using sterile cut tips and wells are filled with 20, 40 and 60 μ l of nanoparticle solution and 20 μ l of control (stock solution) and sample (latex). The plates were incubated at 37^oC for 24 hours after which the diameter of zones of inhibition were measured.

Statistical analysis

The results were analyzed and descriptive statistics were done using SPSS 12.0 (SPSS Inc., an IBM Company, Chicago, USA) and graphs were generated using Sigma Plot 7 (Systat Software Inc., Chicago, USA).

Results and Discussion

Synthesis of nanoparticles

Nanoparticles were synthesized from the latex of Taro (*Colocasia esculenta*), Sandpaper tree (*Ficus exasperata*) and Rubber tree (*Hevea brasiliensis*).

Silver nanoparticles

Silver nanoparticles were synthesized from latex of Taro (*Colocasia esculenta*), Sandpaper tree (*Ficus exasperata*) and Rubber tree (*Hevea brasiliensis*). Latex (1/50, 1/100 dilutions) was added to 1mM silver nitrate solution and kept to reaction to take place. A colour change was observed from colourless to dark brown. This occurred as a result of the reduction of silver ions present in the solution.

Language	Names
Scientific names	Colocasia esculenta
Name in various global languages	
French	Songe
German	Taro
English	Taro
Name in various Indian languages	
Sanskrit	Aaluki
Hindi	Arvi
Urdu	Dasheen
Marathi	Aalu
Kannada	Kesavedantu
Gujarati	Arvi
Malayalam	Chempu
Tamil	Sempu

Table.1 Different vernacular names of *Colocasia esculenta* around the globe and India.

Table.2 Different vernacular names of Ficus exasperate around the globe and India.

Language	Names
Scientific names	Ficus exasperata
Name in various global languages	
French	
German	
English	Sandpaper tree
Name in various Indian languages	
Sanskrit	Karapatra
Hindi	Gobla
Urdu	Anjeerdashthi
Marathi	Karvat
Kannada	Garagatti
Gujarati	Dhedumbar
Malayalam	Therakam
Tamil	Maramthinniatti

Language	Names
Scientific names	Hevea brasiliensis
Name in various global languages	
French	Arbre a caoutchouc
German	
English	Rubber tree
Name in various Indian languages	
Sanskrit	
Hindi	
Urdu	
Marathi	
Kannada	
Gujarati	
Malayalam	Rubber
Tamil	

Table.3 Different vernacular names of *Hevea brasiliensis* around the globe and India.

Table.4 Zone of inhibition against various bacteria (*E. coli, Klebsiella species, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by Colocasia esculenta *latex*(1/50 dilution).

Microorganism	Nanoparticle	Control	oparticle Control Sample Measure of zone of inhibition mm			nibition in
				20	40	60
E.coli	Ag	10	8	12	13	14
	Zn	9	9	10	12	14
	Cu	9	8	9	9	9
Salmonella typhi	Ag	9	9	13	15	16
	Zn	10	9	16	20	22
	Cu	9	8	11	12	10
Staphylococcus aureus	Ag	21	13	14	15	17
	Zn	9	8	13	15	19
	Cu	12	9	13	14	19
Klebsiella species	Ag	8	11	12	13	15
	Zn	11	9	16	18	22
	Cu	9	10	9	9	11
Pseudomonas	Ag	12	9	12	14	19
aeruginosa	Zn	30	8	19	22	24
	Cu	33	9	30	34	35

Microorganism Nanoparticle (Control	Sample	Measure of zone of inhibition in mm		
				20	40	60
E.coli	Ag	10	8	11	12	13
	Zn	10	9	10	11	12
	Cu	11	10	15	16	17
Salmonella typhi	Ag	17	14	12	14	17
	Zn	10	9	12	13	14
	Cu	17	11	19	21	23
Staphylococcus aureus	Ag	16	10	15	18	20
	Zn	11	8	9	10	11
	Cu	23	10	24	26	30
Klebsiella species	Ag	10	10	11	14	16
	Zn	10	9	10	12	13
	Cu	15	11	20	21	22
Pseudomonas	Ag	10	7	11	13	16
aeruginosa	Zn	12	8	10	11	13
	Cu	11	9	13	14	15

Table.5 Zone of inhibition against various bacteria (*E. coli, Klebsiella species, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by *Colocasia esculenta latex* (1/100 dilution).

Table.6 Zone of inhibition against various bacteria (*E. coli, Klebsiella species, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by *Ficus exasperate latex* (1/50 dilution).

Microorganism	Nanoparticle	Control	Sample	Measure of zone of inhi mm		hibition in
				20	40	60
E.coli	Ag	13	8	12	13	13
	Zn	21	10	19	21	23
	Cu	10	9	11	12	13
Salmonella typhi	Ag	12	9	12	13	14
	Zn	23	10	21	23	25
	Cu	10	9	11	12	13
Staphylococcus aureus	Ag	17	11	13	16	18
	Zn	18	10	24	25	28
	Cu	11	9	12	13	14
Klebsiella species	Ag	11	9	9	11	12
	Zn	21	8	18	22	24
	Cu	10	9	9	10	11
Pseudomonas	Ag	22	9	18	21	23
aeruginosa	Zn	11	10	11	14	16
	Cu	10	9	10	11	12

Microorganism	Nanoparticle	Control	Sample	Measure of zone of inhibition in		
				mm		
				20	40	60
E.coli	Ag	12	9	12	13	16
	Zn	20	10	19	21	23
	Cu	11	8	10	11	12
Salmonella typhi	Ag	13	10	11	13	13
	Zn	19	9	22	23	24
	Cu	10	8	10	11	12
Staphylococcus aureus	Ag	12	9	14	16	18
	Zn	25	10	25	28	30
	Cu	12	10	11	12	13
Klebsiella species	Ag	12	9	11	12	17
	Zn	23	10	22	24	22
	Cu	10	8	10	9	10
Pseudomonas	Ag	22	10	19	22	23
aeruginosa	Zn	11	10	11	12	14
	Cu	10	9	11	12	13

Table.7 Zone of inhibition against various bacteria (*E. coli, Klebsiella species, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by *Ficus exasperate latex* (1/100 dilution).

Table.8 Zone of inhibition against various bacteria (*E. coli, Klebsiella species, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by *Hevea brasiliensis latex* (1/50 dilution).

Microorganism	Nanoparticle	Control	Sample	Measure of zone of inhibition in mm		
				20	40	60
E.coli	Ag	9	8	10	11	12
	Zn	20	10	22	25	28
	Cu	11	10	12	18	22
Salmonella typhi	Ag	9	8	11	12	13
	Zn	23	11	27	30	32
	Cu	17	10	20	22	24
Staphylococcus aureus	Ag	8	7	9	10	11
	Zn	15	10	19	21	25
	Cu	19	11	20	22	25
Klebsiella species	Ag	9	8	10	11	12
	Zn	21	11	23	25	27
	Cu	12	10	15	17	20
Pseudomonas	Ag	10	9	11	12	13
aeruginosa	Zn	25	9	27	30	32
	Cu	14	10	18	20	25

Microorganism	Nanoparticle	Control	Sample	Measure of	of zone of inl mm	nibition in
				20	40	60
E.coli	Ag	10	9	11	12	13
	Zn	21	9	22	25	28
	Cu	14	10	16	18	20
Salmonella typhi	Ag	10	8	11	12	13
	Zn	25	10	27	30	32
	Cu	14	10	17	22	25
Staphylococcus aureus	Ag	8	9	1	11	12
	Zn	19	10	21	24	25
	Cu	14	10	17	22	25
Klebsiella species	Ag	9	8	10	11	12
	Zn	22	10	24	26	28
	Cu	11	9	12	15	18
Pseudomonas	Ag	10	9	11	12	14
aeruginosa	Zn	25	10	26	29	31
	Cu	18	9	21	23	24

Table.9 Zone of inhibition against various bacteria (*E. coli, Klebsiella species, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by *Hevea brasiliensis latex* (1/100 dilution).

Table.10 UV absorption spectrum of Silver nanoparticles formed from *Ficus exasperata* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
1⁄2 hr	0.394	0.380	0.301	0.299
1 hr	0.572	0.515	0.392	0.314
1 ½ hr	0.633	0.531	0.470	0.387
2 hr	0.698	0.601	0.503	0.417
2 ½ hr	0.817	0.758	0.600	0.486
Blank	0.000	0.000	0.000	0.000

Table.11 UV absorption spectrum of Copper nanoparticles formed from *Ficus exasperata* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.122	0.120	0.140	0.528
1 hr	0.134	0.167	0.203	0.540
1 ½ hr	0.157	0.183	0.214	0.670
2 hr	0.196	0.197	0.220	0.762
2 ½ hr	0.291	0.232	0.259	0.772
Blank	0.000	0.000	0.000	0.000

Time	350 nm	385 nm	435 nm	560 nm
¹ ∕2 hr	0.303	0.114	0.106	0.077
1 hr	0.326	0.177	0.108	0.082
1 ½ hr	0.339	0.202	0.112	0.087
2 hr	0.350	0.209	0.129	0.089
2 ½ hr	0.359	0.210	0.143	0.093
Blank	0.000	0.000	0.000	0.000

Table.12 UV absorption spectrum of Zinc nanoparticles formed from *Ficus exasperata* during different time of incubation.

Table.13 UV absorption spectrum of Silver nanoparticles formed from Colocasia esculenta during different time of incubation.

Time	385 nm	435nm	560nm	680nm
1⁄2 hr	0.537	0.575	0.415	0.301
1 hr	0.540	0.576	0.440	0.307
1 ½ hr	0.580	0.588	0.441	0.308
2 hr	0.582	0.602	0.449	0.333
2 ½ hr	0.587	0.641	0.466	0.347
Blank	0.000	0.000	0.000	0.000

Table.14 UV absorption spectrum of Copper nanoparticles formed from Colocasia esculenta during different time of incubation.

Time	385 nm	435nm	560nm	680nm
1⁄2 hr	0.129	0.117	0.066	0.457
1 hr	0167	0.125	0.106	0.498
1 ½ hr	0.198	0.143	0.113	0.500
2 hr	0.223	0.153	0.115	0.508
2 ½ hr	0.228	0.158	0.117	0.512
Blank	0.000	0.000	0.000	0.000

Table.15 UV absorption spectrum of Zinc nanoparticles formed from Colocasia esculenta during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
1⁄2 hr	0.122	0.090	0.065	0.032
1 hr	0.130	0.095	0.066	0.039
1 ½ hr	0.138	0.099	0.068	0.041
2 hr	0.145	0.106	0.085	0.066
2 ½ hr	0.215	0.159	0.121	0.077
Blank	0.000	0.000	0.000	0.000

Time	385 nm	435nm	560nm	680nm
1⁄2 hr	1.122	0.870	0.659	0.480
1 hr	1.140	0.993	0.700	0.515
1 ½ hr	1.164	1.013	0.743	0.545
2 hr	1.172	1.039	0.790	0.586
2 ½ hr	1.184	1.059	0.816	0.596
Blank	0.000	0.000	0.000	0.000

 Table.16 UV absorption spectrum of Silver nanoparticles formed from Hevea brasiliensis during different time of incubation.

Table.17 UV absorption spectrum of Copper nanoparticles formed from *Hevea brasiliensis* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
1⁄2 hr	0.190	0.168	0.257	0.607
1 hr	0.307	0.244	0.269	0.655
1 ½ hr	0.349	0.339	0.273	0.717
2 hr	0.370	0.375	0.330	0.742
2 ½ hr	0.378	0.410	0.343	0.754
Blank	0.000	0.000	0.000	0.000

Table.18 UV absorption spectrum of Zinc nanoparticles formed from *Hevea brasiliensis* during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
1⁄2 hr	0.755	0.575	0.407	0.259
1 hr	0.763	0.577	0.411	0.279
1 ½ hr	0.815	0.590	0.452	0.281
2 hr	0.865	0.639	0.465	0.304
2 ½ hr	0.893	0.683	0.489	0.315
Blank	0.000	0.000	0.000	0.000

Table.19 Biochemical characterization of the organisms used in the study.

Organisms	Ι	MR	VP	С	GS	U	0	CL	COG	NR
Salmonella typhi	-VE	+VE	-VE	-VE	-VE	-VE	-VE	+VE	_	+VE
Pseudomonas aeruginosa	-VE	-VE	-VE	+VE	-VE	-VE	+VE	+VE	-VE	+VE
Staphylococcus aureus	-VE	+VE	+VE	+VE	+VE	+VE	-VE	+VE	+VE	+VE
E. coli	+VE	+VE	-VE	-VE	-VE	-VE	-VE	+VE	_	+VE
Klebsiella pneumoniae	-VE	-VE	+VE	+VE	-VE	+VE	-VE	+VE	_	+VE

(I- Indole, MR- Methyl Red, VP- Voges Proskauer, C- Citrate, GS- Gram Staining, U- Urease, O- Oxidase, CL- Catalase, COG-Coagulase, NR- Nitrogen Reductase).

Organisms	Zone of Inhibition (mm)								
	AMP	AMP CHL ENO ERY GEN KAN							
Salmonella typhi	1.7	3.2	_	_	_	-	_	1.17	
Pseudomonas aeruginosa	-	_	22-28	_	16-21	-	_	-	
Staphylococcus aureus	27-35	19-26	22-28	22-30	19-27	19-26	26-37	24-30	
E. Coli	16-22	21-27	28-36	_	19-26	17-25	_	18-25	
Klebsiella pneumoniae	32	_	_	16	_	_	16	14	

Table.20 Antibiotic susceptibility test of the organisms used in the study.

AMP: Ampicillin; CHL: Chloramphenicol; ENO: Enonacin; ERY: Erythromycin; GEN: Gentamycin; KAN: Kanamycin; PEN: Penicillin; TET: Tetracycline.

Fig.1 Map of Kerala showing the various sample collection points.



Fig.2 Description of *Colocasia esculenta (Taro) a) plant in natural habitat, b) plant with mature and young leaves, c) corms, d) corms cut opened, e) stem packed for sale*. Photo courtesy: Wikipedia.



Fig.3 Description *Ficus exasperata* a) tree bearing mature fruits, b) and c) plant with young and mature leaves, d) and e) half matured fruit. Photo courtesy: Wikipedia; indiabiodiversity.org.



Fig.4 Description of *Hevea brasiliensis (Rubber tree)* a) different plant parts, b) seeds, c) flowers with developing young leaves, d) latex harvested from mature tree. Photo courtesy: Wikipedia.



Fig.5 Antibacterial activity study using well diffusion method of *Colocasia esculenta* latex (1/50 dilution) nanoparticles (Ag) and (Cu)a) *Salmonella typhi* control plate (Ag), b) *Salmonella typhi* green synthesised nanoparticle test plate (Ag; 20, 40 and 60 μl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e)*E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test, k) to t) above mentioned organisms plate in same order for Cu nanoparticles.



Fig.6 Antibacterial activity study using well diffusion method of *Colocasia esculenta* latex (1/50 dilution) nanoparticles (Zn) a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e)*E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test.



Fig.7 Antibacterial activity study using well diffusion method of *Colocasia esculenta* latex (1/100 dilution) nanoparticles (Ag) and (Cu)a) *Salmonella typhi* control plate (Ag), b) *Salmonella typhi* green synthesised nanoparticle test plate (Ag; 20, 40 and 60 μl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test, k) to t) above mentioned organisms plate in same order for Cu nanoparticles.



Fig.8 Antibacterial activity study using well diffusion method of *Colocasia esculenta* latex (1/100 dilution) nanoparticles (Zn)a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 20, 40 and 60 μl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e)*E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test.



Fig.9 Antibacterial activity study using well diffusion method of *Ficus exasperate* latex (1/50 dilution) nanoparticles (Ag) and (Cu)a) *Salmonella typhi* control plate (Ag), b) *Salmonella typhi* green synthesised nanoparticle test plate (Ag; 20, 40 and 60 μl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test, k) to t) above mentioned organisms plate in same order for Cu nanoparticles



Fig.10 Antibacterial activity study using well diffusion method of *Ficus exasperate* latex (1/50 dilution) nanoparticles (Zn)a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test.



Fig.11 Antibacterial activity study using well diffusion method of *Ficus exasperate* latex (1/100 dilution) nanoparticles (Ag) and (Cu) a) *Salmonella typhi* control plate (Ag), b) *Salmonella typhi* green synthesised nanoparticle test plate (Ag; 20, 40 and 60 μl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test, k) to t) above mentioned organisms plate in same order for Cu nanoparticles.



Fig.12 Antibacterial activity study using well diffusion method of *Ficus exasperate* latex (1/100 dilution) nanoparticles (Zn)a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test.



Fig.13 Description Antibacterial activity study using well diffusion method of *Hevea brasiliensis* latex (1/50 dilution) nanoparticles (Ag) and (Cu)a) *Salmonella typhi* control plate (Ag), b) *Salmonella typhi* green synthesised nanoparticle test plate (Ag; 20, 40 and 60 μl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test, k) to t) above mentioned organisms plate in same order for Cu nanoparticles.



Fig.14 Antibacterial activity study using well diffusion method of *Hevea brasiliensis* latex (1/100 dilution) nanoparticles (Ag) and (Cu)a) *Salmonella typhi* control plate (Ag), b) *Salmonella typhi* green synthesised nanoparticle test plate (Ag; 20, 40 and 60 μl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test, k) to t) above mentioned organisms plate in same order for Cu nanoparticles.



Fig.15 Antibacterial activity study using well diffusion method of *Hevea brasiliensis* latex (1/50 and 1/100 dilution) nanoparticles (Zn)a) Salmonella typhi control plate (Zn), b) Salmonella typhi green synthesised nanoparticle test plate (Zn; 20, 40 and 60 μl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species*control, j) *Staphylococcus species* test, k) to t) above mentioned organisms plate in same order for Zn nanoparticles (1/100 dilution).



Copper nanoparticles

Copper nanoparticle were synthesized from latex of Taro (*Colocasia esculenta*), Sandpaper tree (*Ficus exasperata*) and Rubber tree (*Hevea brasiliensis*). Latex was added to 100 mM copper sulphate solutions and kept to reaction to take place. A colour change was observed from blue to pale yellow. This occurred due to the reduction of copper ions present in the solution.

Zinc nanoparticles

Zinc nanoparticles were synthesized from latex of different plants Taro (*Colocasia esculenta*), Sandpaper tree (*Ficus exasperata*) and Rubber tree (*Hevea brasiliensis*). Latex was added to 100 mM zinc sulphate solution and kept to reaction to take place. A colour change was observed from colourless to pale brown. This

occurred due to the reduction of zinc ions present in the solution.

Characterization of nanoparticles

Silver nanoparticles

UV spectrometry

Synthesised Silver nanoparticles were characterized by UV-VIS spectrophotometry. The maximum peak was found to be 435nm for *Colocasia esculenta*. The intensity of the peak at 435nm was increased with time until the reduction completes. The maximum peak was found to be 385nm for *Hevea brasiliensis*, and *Ficus exasperata*. The intensity of the peak at 385nm was increased with the time until the reduction completes.

Copper nanoparticles

UV spectrometry

Synthesized copper nanoparticles were characterized by UV-VIS spectrophotometry. The maximum peak was found to be at 680nm for *Colocasia esculenta*, *Ficus exasperate* and *Hevea brasilensis*. The intensity of the peak at 680nm was increased with the time until the reduction completes.

Zinc nanoparticles

UV spectrometry

Synthesized zinc nanoparticles were characterized by UV-VIS Spectrophotometry. The peak was found to be 350nm for *Colocasia esculenta*, *Ficus exasperate* and *Hevea brasilensis*. The intensity of peak at 350nm was increased with time until the reduction completes.

Antibacterial assay

The latex of Taro (*Colocasia esculenta*), Sandpaper tree (*Ficus exasperata*) and Rubber tree (*Hevea brasiliensis*) showed growth inhibitory effects against *salmonella*, *pseudomonas*, *staphylococcus*, *E.coli* and *Klebsiella*.

The nanoparticles are widely used in Pharmaceutical and biological applications, so the green synthesis of nanoparticle plays an important role. The use of plant latex makes an economical sense. The latex part of a plant contains various organic compounds such as alkaloids, cardiac glycosides, tannins, sterols and triterpenes. These compounds play role in the reduction of Silver nitrate, Copper sulphate and Zinc sulphate to Silver, Copper and Zinc respectively. Recent days there are certain studies going on for production of nanoparticle from plant latex. These reports only explain about any of the following nanoparticle silver, copper, or zinc not about all these. Here we discuss about these three nanoparticles formed from five different plant latex and also the antimicrobial activity of each. From the experiment, Copper nanoparticle formed from Taro latex of 1/50 dilution shows a zone of inhibition ranging between 33 to 35mm in size against Pseudomonas. The garden croton latex directly shows antimicrobial activity against all the test microorganisms used and shows the zone of inhibition in between 9 to 23mm in size. The Zinc nanoparticle of Rubber latex has an inhibitory effect on Salmonella compared to other microbes.

UV- Vis spectroscopic study of the Coloured sample solution confirmed the synthesis of nanoparticles. Silver nanoparticles become an important application the field of microbiology such as antibacterial activities, The copper nanoparticles are applied to biosensors and electrochemical sensors, The Zinc oxides are used in the manufacture of rubber and cigarettes(as filter) and the calamine lotion is made out of Zinc oxide. Some of the plants we selected are used in traditional medicines.

Comparing with crude latex, The latex nanoparticles exhibits higher antibacterial activities against Gramnegative as well as Gram-positive bacteria than the use of crude untreated latex alone. The addition of three different volumes of nanoparticle solution in the well of the agar plate is helped to identify the variation in the size of the zone of inhibition. The well containing 60µl of latex nanoparticle shows more inhibitory effect towards bacteria. The exact antimicrobial activity of the nanoparticles is still in debate. The use of two dilutions helps us to compare the effect of dilution factor on antimicrobial activity of latex nanoparticle and also the effect on nanoparticle formation. The 50DF of latex form nanoparticles faster, and shows zone of inhibition in larger size compared to 100DF of latex.

Antibacterial assay

The SEM-XRD analysis proved the effective formation of silver, copper and zinc nanoparticles in all the samples.

The results showed that latex of *Colocasia esculenta*, *Ficus exasperate* and *Hevea brasiliensis* with two dilutions are used to synthesis Silver, Copper and Zinc nanoparticles. And the formed nanoparticles show antibacterial activity against both Gram negative and Gram positive bacteria. The biosynthesis of nanoparticles is cost efficient, pollutant free and simpler to synthesis.

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