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Green Synthesis of Nanoparticles (Ag, Cu and Zn) from Plant Latex (*Musa paradisiaca* and *Croton variegatum*) and Evaluation of Antibacterial Activity

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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 accept 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.

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Keywords

Nanoparticles, *Musa paradisiaca*, *Croton variegatum*, Latex, Antibacterial assay.

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" (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*), Banana (*Musa paradisiaca*), Rubber tree (*Hevea brasiliensis*) and Croton (*Croton variegatum*) 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 re-agent, 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}m = 1nm), 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 10-100nm

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 eco-friendly 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).

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.

Musa paradisiaca (Banana)

Banana is an important economic and food crop. Banana plant belongs to genus *Musa* of the family "*Musaceae*", a monocotyledonous family. There are three commercially important species of banana: the *Musa acuminate* Colla or "desert banana" which is consumed ripe and raw; the second is the *Musa X paradisiacal* (syn. *Musa sapientum* L.) or "plantain", eaten green after cooking. The third is *Musa textiles* Nees, also called as *Abaca*, which is used as a fibre crop. Banana plants are vegetative propagated and are grown in all tropical agriculture systems.

Banana is a fruit eaten and cultivated by mankind from ancient times. It has a very good taste and nutritive value. It is enriched in carbohydrates, many important elements and vitamins. It belongs to genus Musa in the family "Musaceae" a monocotyledonous family with three genera, the other two genera are Enseteand Musella. There are two main species of bananas, the *Musa acuminate* Colla also called as "dessert banana" that is eaten ripe and raw and the Musa X paradisiacal to which "plantain" belongs is eaten green after cooking. The other species of this genus which is commercially cultivated is abaca or Manila hemp (*Musa textiles* Nees).

Taxonomical classification of *Musa paradisiaca* (Banana)

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

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Liliopsida

Order: Zingiberales

Family: Musaceae

Genus: Musa

Species: *Musa paradisiaca*

Croton variegatum (Garden croton)

Croton variegatum or garden croton is a species of plant in the genus *Codiaeum*, which is a member of family Euphorbiaceae. It is native to Indonesia, Malaysia, Australia, and the western Pacific ocean islands, growing in open forests and scrub. It is an evergreen shrub

growing to 3m tall has large, thick, leathery, shiny evergreen leaves, alternatively arranged. Consists of male and female flowers; the male flowers are white with five small petals and 20-30 stamens, the female flower is yellowish with no petals. The stamen contain milky sap that bleeds from cut stems, sometimes it is reddish white in colour (Huxley, 1992). The garden crotons should not be confused with *Croton*, a cosmopolitan genus also in the Euphorbiaceae, containing more than 700 species of herbs, shrubs and trees.

Taxonomical classification of *Croton variegatum* (Garden croton)

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

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Euphorbiales

Family: Euphorbiacea

Genus: Codiaeum

Species: *Croton variegatum*

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).

Sliver 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 anti-microbial 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 discuss 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 Banana (*Musa paradisiaca*) and Garden croton (*Croton variegatum*) 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 km^2 with a population density of 859 per km^2 and spread across 14 districts. The climate is characterized by tropical wet and dry with average annual rainfall amounts to 2,817 ± 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 two 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 Banana (*Musa paradisiaca*) and Garden croton (*Croton variegatum*) 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

Sliver nanoparticles

Stock solution was prepared by dissolving 1mMsliver nitrate ($AgNO_3$; 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 $AgNO_3$ 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 $AgNO_3$.

Copper nanoparticles

Stock solution was prepared by dissolving 2.49 g Copper sulphate ($CuSO_4$). 5 ml of latex solution of Banana (*Musa paradisiaca*) and Garden croton (*Croton variegatum*) is added to the 95ml of 100mM $CuSO_4$ solution and allowed to react in room temperature. The $CuSO_4$ nanoparticles will be formed after 1-2 hours.

Zinc nanoparticles

Stock solution was prepared by dissolving 2.87 g Zinc sulphate ($ZnSO_4$). 5ml of latex solution of Banana (*Musa paradisiaca*) and Garden croton (*Croton variegatum*) are added to the 95ml of 100mM $ZnSO_4$ solution and allowed to react in roomtemperature.

Test microorganisms

The organisms used comprise of two gram-negative organisms (*Klebsiella* and *E.coli*) and three gram-positive 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.

Staphylococcus species grow readily on ordinary media with a temperature range of 10 to 40°C, the optimum being 37°C and a pH of 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 pneumoniea, 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 µl of nanoparticle solution and 20 µl of control (stock solution) and sample (latex). The plates were incubated at 37^{0}C 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 Banana (*Musa paradisiaca*) and Garden croton (*Croton variegatum*).

Silver nanoparticles

Silver nanoparticles were synthesized from latex of Banana (*Musa paradisiaca*) and Garden croton (*Croton variegatum*). 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.

Copper nanoparticles

Copper nanoparticle were synthesized from latex of Banana (*Musa paradisiaca*) and Garden croton (*Croton variegatum*). 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 Banana (*Musa paradisiaca*) and Garden croton (*Croton variegatum*). Latex was added to 100 mMzinc 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.

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

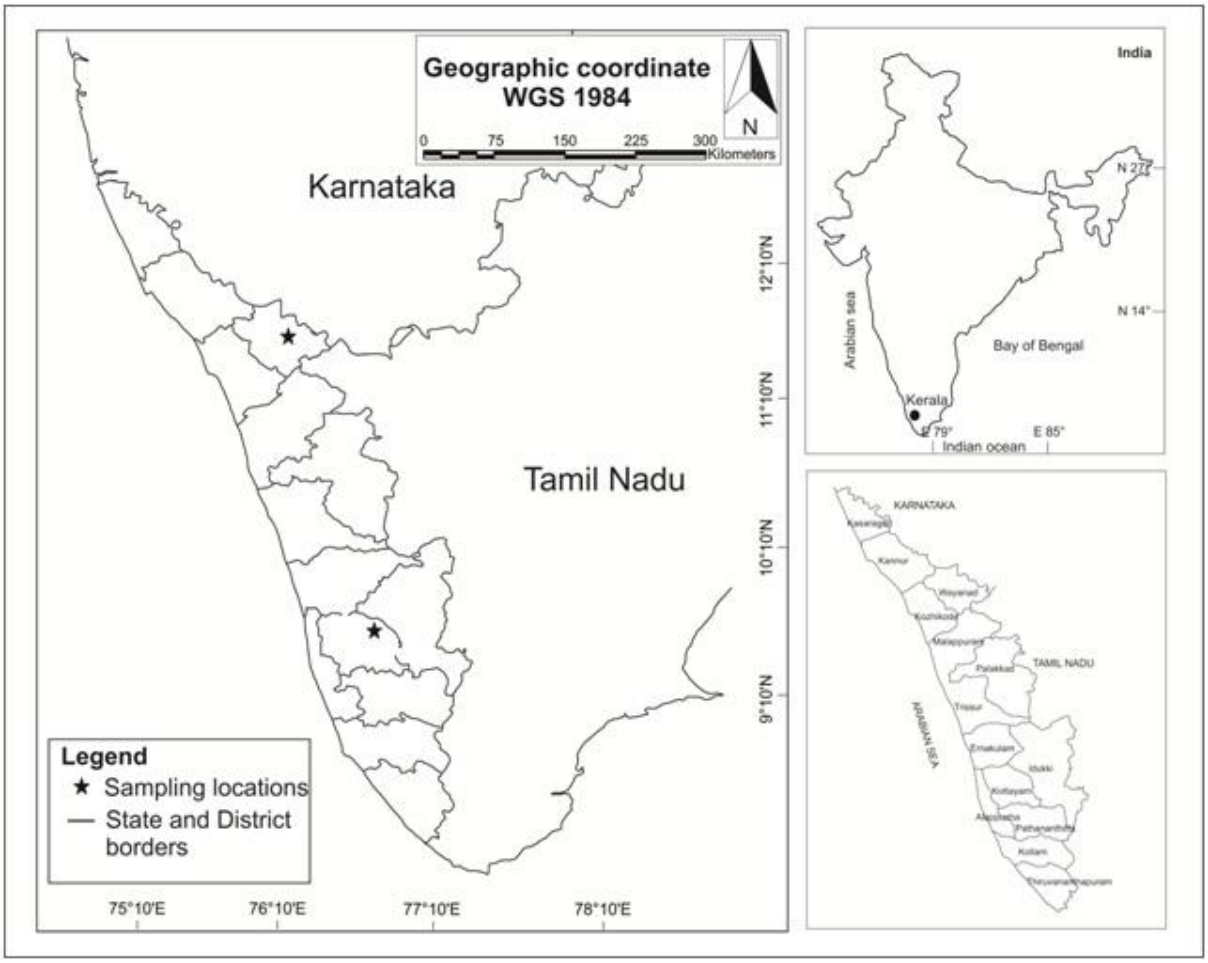


Table.1 Different vernacular names of *Musa paradisiaca* around the globe and India.

Language	Names
Scientific names	*Musa paradisiaca*
Name in various global languages	
French	Bananier
German	Banane
English	Banana
Name in various Indian languages	
Sanskrit	Kadali
Hindi	Kela
Urdu	Bonana
Marathi	Kela
Kannada	Baale
Gujarati	Kelphool
Malayalam	Vazha
Tamil	Vazhai

Table.2 Different vernacular names of *Croton variegatum* around the globe and India.

Language	Names
Scientific names	*Croton variegatum*
Name in various global languages	
French	
German	
English	Garden croton
Name in various Indian languages	
Sanskrit	
Hindi	
Urdu	
Marathi	
Kannada	
Gujarati	
Malayalam	Kroton
Tamil	

Table.3 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by *Musa paradisiaca latex*(1/50 dilution).

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

Table.4 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by *Musa paradisiaca latex*(1/100 dilution).

Microorganism	Nanoparticle	Control	Sample	Measure of zone of inhibition in mm		
				20	40	60
E.coli	Ag	10	8	11	12	13
	Zn	16	10	19	21	23
	Cu	12	10	13	16	20
Salmonella typhi	Ag	17	14	18	19	20
	Zn	15	10	18	20	23
	Cu	14	11	15	17	19
Staphylococcus aureus	Ag	16	10	15	18	20
	Zn	17	12	18	25	28
	Cu	16	10	21	23	25
Klebsiella species	Ag	10	10	11	14	16
	Zn	15	10	16	19	21
	Cu	13	10	11	13	18
Pseudomonas aeruginosa	Ag	10	8	11	13	16
	Zn	15	11	18	21	23
	Cu	11	10	12	13	15

Table.5 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by *Croton variegatum latex*(1/50 dilution).

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

Table.6 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by Croton variegatum *latex*(1/100 dilution).

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

Table.7 UV absorption spectrum of Silver nanoparticles formed from *Musa paradisiaca* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	1.176	1.373	0.603	0.327
1 hr	1.882	2.024	1.311	0.650
1 ½ hr	2.240	2.566	1.424	0.712
2 hr	2.481	2.902	1.581	0.764
2 ½ hr	2.677	3.201	1.913	0.914
Blank	0.431	0.365	0.243	0.128

Table.8 UV absorption spectrum of Copper nanoparticles formed from *Musa paradisiaca* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.513	0.402	0.193	0.631
1 hr	0.565	0.403	0.198	0.632
1 ½ hr	0.617	0.432	0.203	0.643
2 hr	0.821	0.518	0.267	0.679
2 ½ hr	0.899	0.593	0.289	0.698
Blank	0.037	0.035	0.047	0.470

Table.9 UV absorption spectrum of Zinc nanoparticles formed from *Musa paradisiaca* during different time of incubation**.**

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.541	0.241	0.110	0.043
1 hr	0.601	0.317	0.165	0.78
1 ½ hr	0.657	0.328	0.176	0.079
2 hr	0.663	0.330	0.160	0.065
2 ½ hr	0.569	0.284	0.120	0.062
Blank	0.000	0.000	0.000	0.000

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

Time	385 nm	435nm	560nm	680nm
½ hr	1.369	1.214	0.930	0.726
1 hr	1.380	1.233	0.938	0.756
1 ½ hr	1.398	1.265	0.964	0.783
2 hr	1.438	1.328	0.991	0.786
2 ½ hr	1.490	1.406	0.997	0.820
Blank	0.000	0.000	0.000	0.000

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

Time	385 nm	435nm	560nm	680nm
½ hr	0.150	0.145	0.178	0.604
1 hr	0.162	0.166	0.261	0.713
1 ½ hr	0.300	0.255	0.272	0.729
2 hr	0.333	0.267	0.279	0.739
2 ½ hr	0.427	0.366	0.280	0.751
Blank	0.000	0.000	0.000	0.000

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

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.788	0.678	0.577	0.446
1 hr	0.864	0.725	0.607	0.453
1 ½ hr	0.908	0.797	0.684	0.521
2 hr	0.927	0.801	0.697	0.527
2 ½ hr	0.993	0.885	0.731	0.534
Blank	0.000	0.000	0.000	0.000

Fig.2 Description of *Musa paradisiacal* a) plant bearing fruit, b) banana chips made from mature fruits, c) ripe banana plant, d) and f) plant with developing fruit, e) plant with flowers. Photo courtesy: Wikipedia.

Fig.3 Description of *Croton variegatum* a), c), d) and e) different varieties of garden croton b) developing flowers. Photo courtesy: Wikipedia.

Fig.4 Antibacterial activity study using well diffusion method of *Croton variegatum* 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.

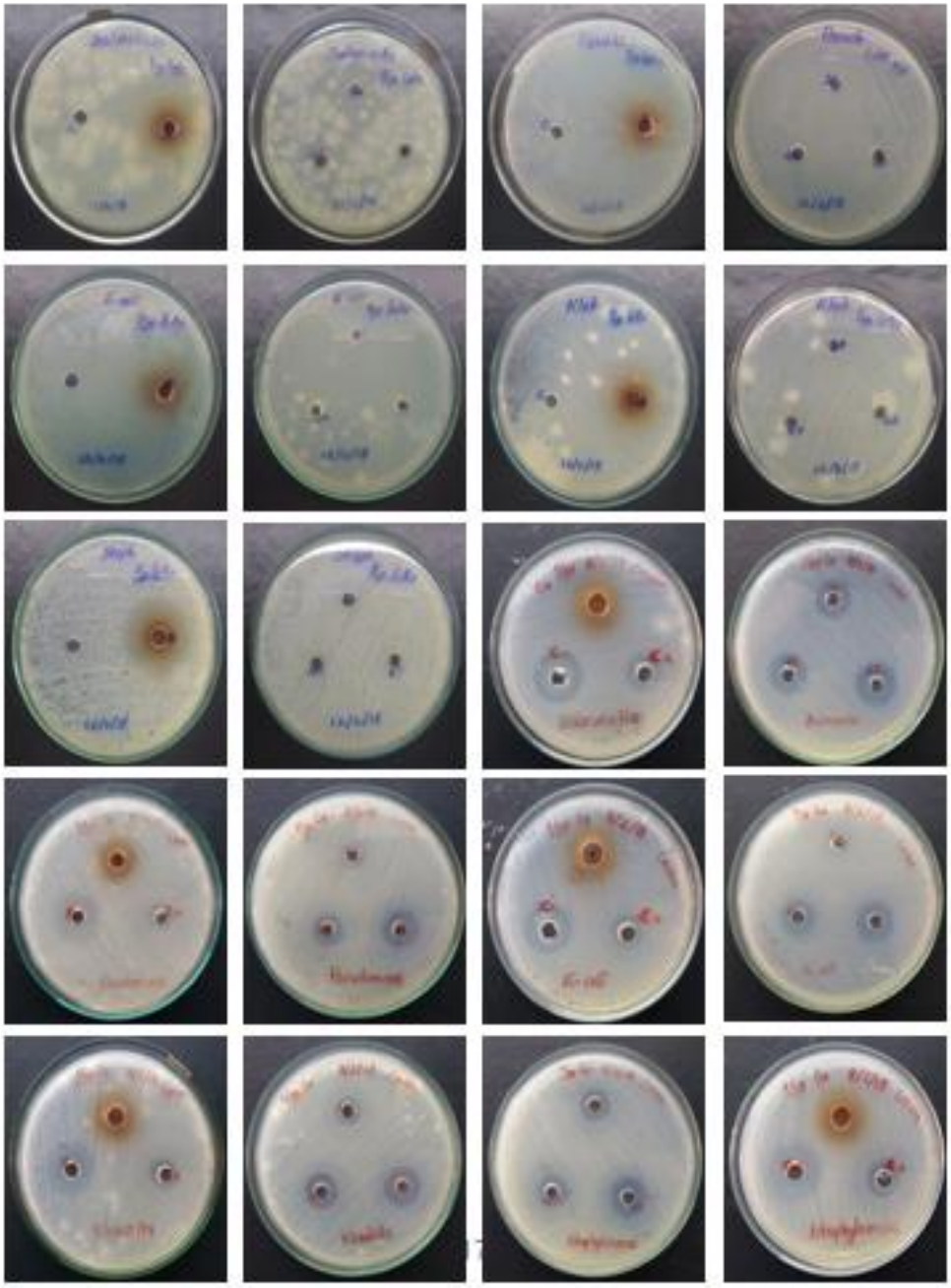


Fig.5 Antibacterial activity study using well diffusion method of *Croton variegatum* latex (1/50 dilution) nanoparticles (Zn) a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas 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.

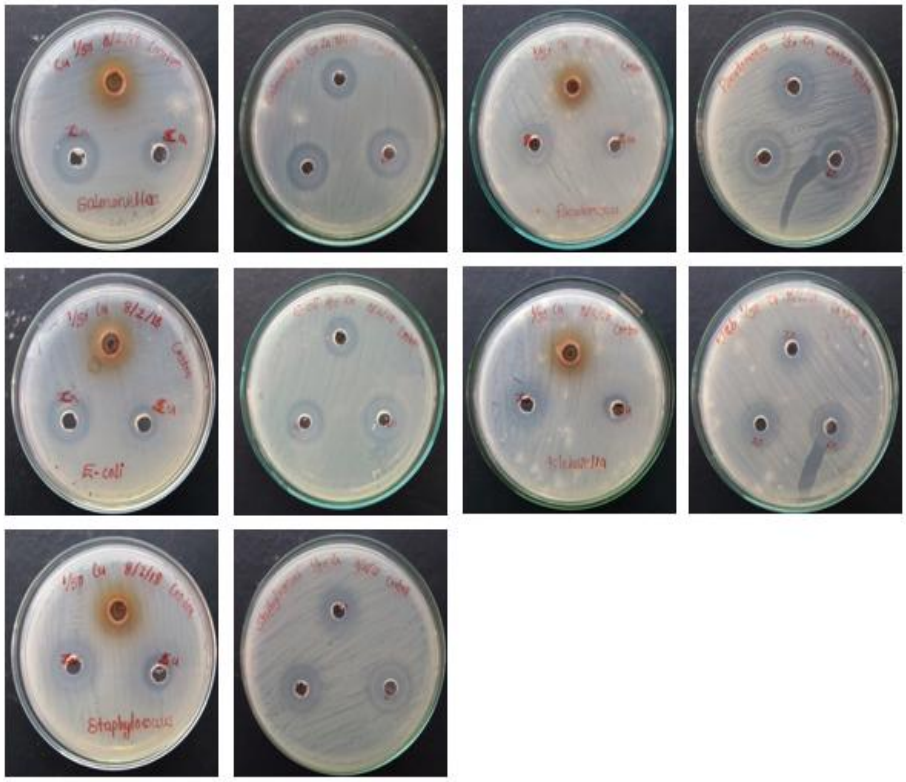


Fig.6 Antibacterial activity study using well diffusion method of *Croton variegatum* latex (1/100 dilution) nanoparticles (Ag) and (Cu) a) *Salmonella typhi* control plate (Ag), b) *Salmonella typhi* green synthesised nanoparticle test plate (Ag; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, 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.

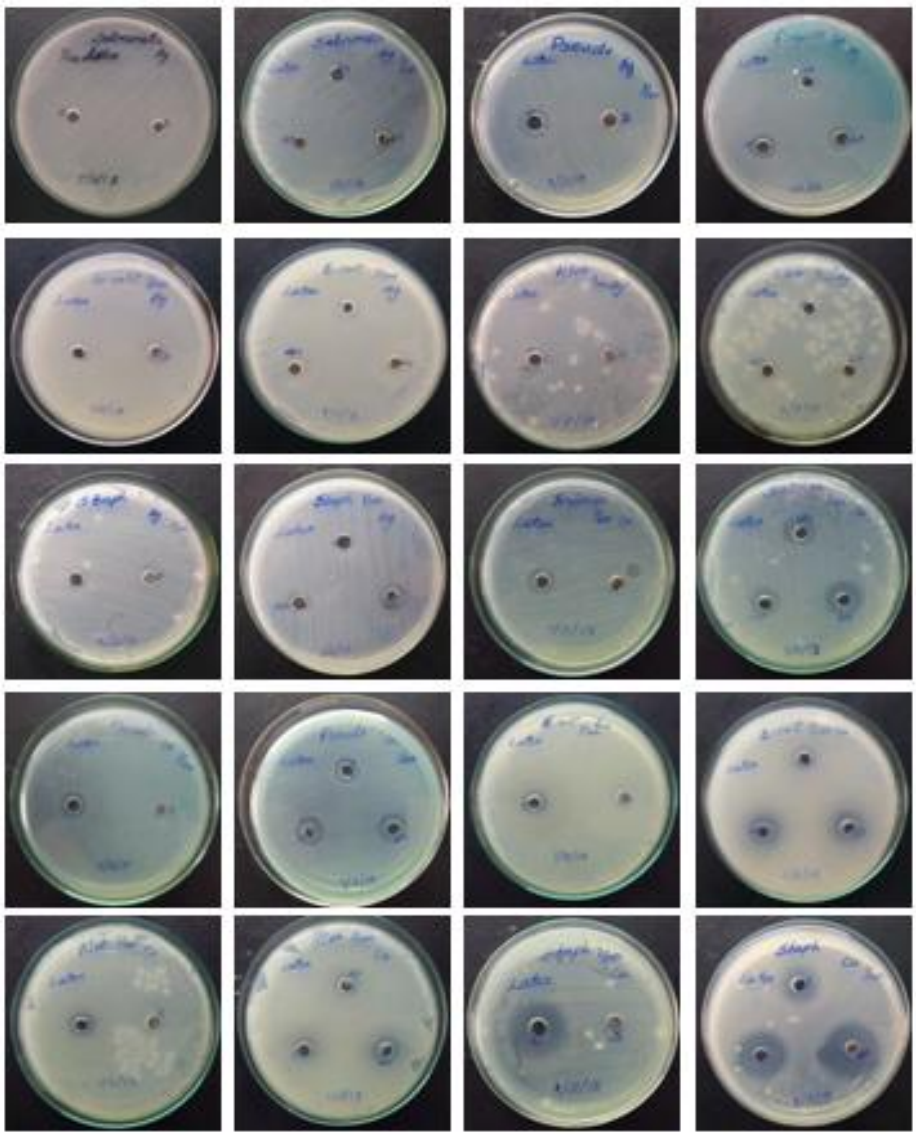


Fig.7 Antibacterial activity study using well diffusion method of *Croton variegatum* latex (1/100 dilution) nanoparticles (Zn) a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas 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.

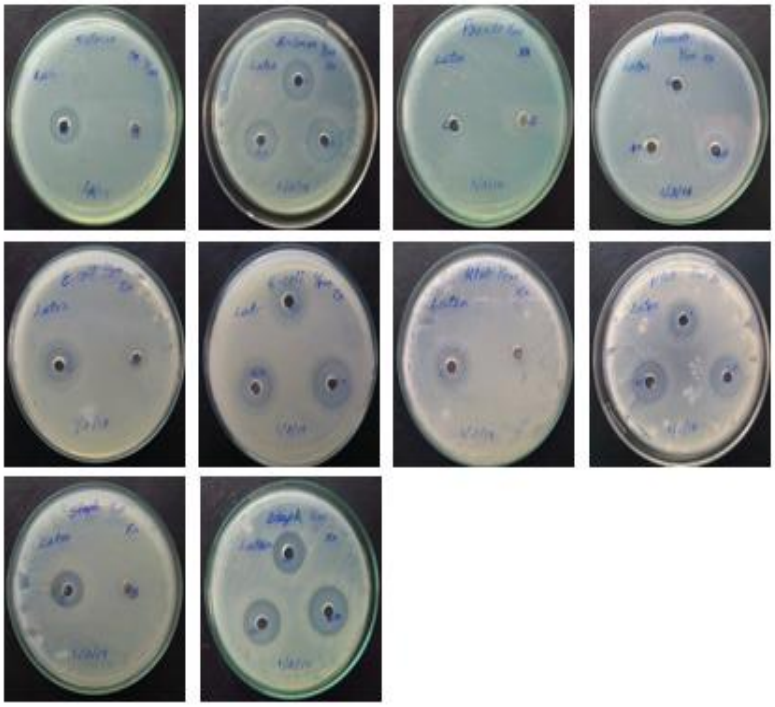


Fig.8 Antibacterial activity study using well diffusion method of *Musa paradisiaca* latex (1/50 dilution) nanoparticles (Ag) and (Cu) a) *Salmonella typhi* control plate (Ag), b) *Salmonella typhi* green synthesised nanoparticle test plate (Ag; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, 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.

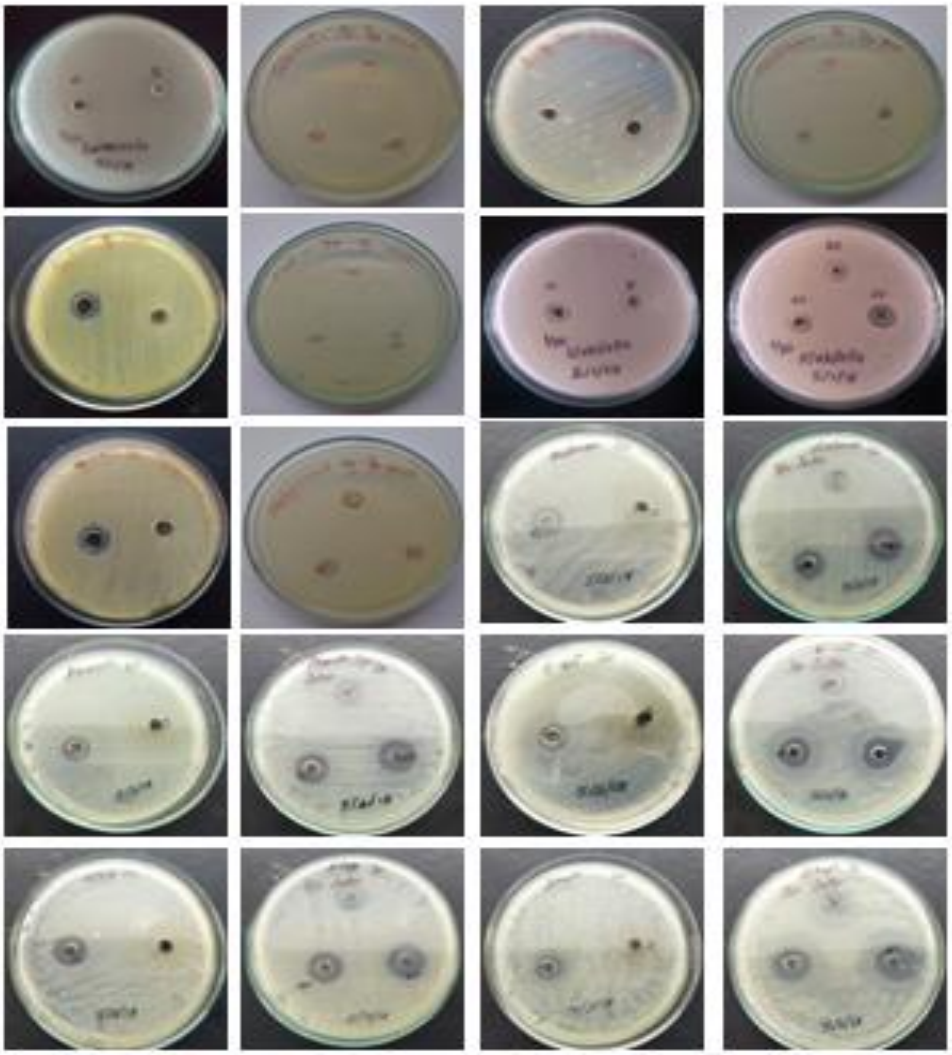


Fig.9 Antibacterial activity study using well diffusion method of *Musa paradisiaca* latex (1/100 dilution) nanoparticles (Ag) and (Cu) a) *Salmonella typhi* control plate (Ag), b) *Salmonella typhi* green synthesised nanoparticle test plate (Ag; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, 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.

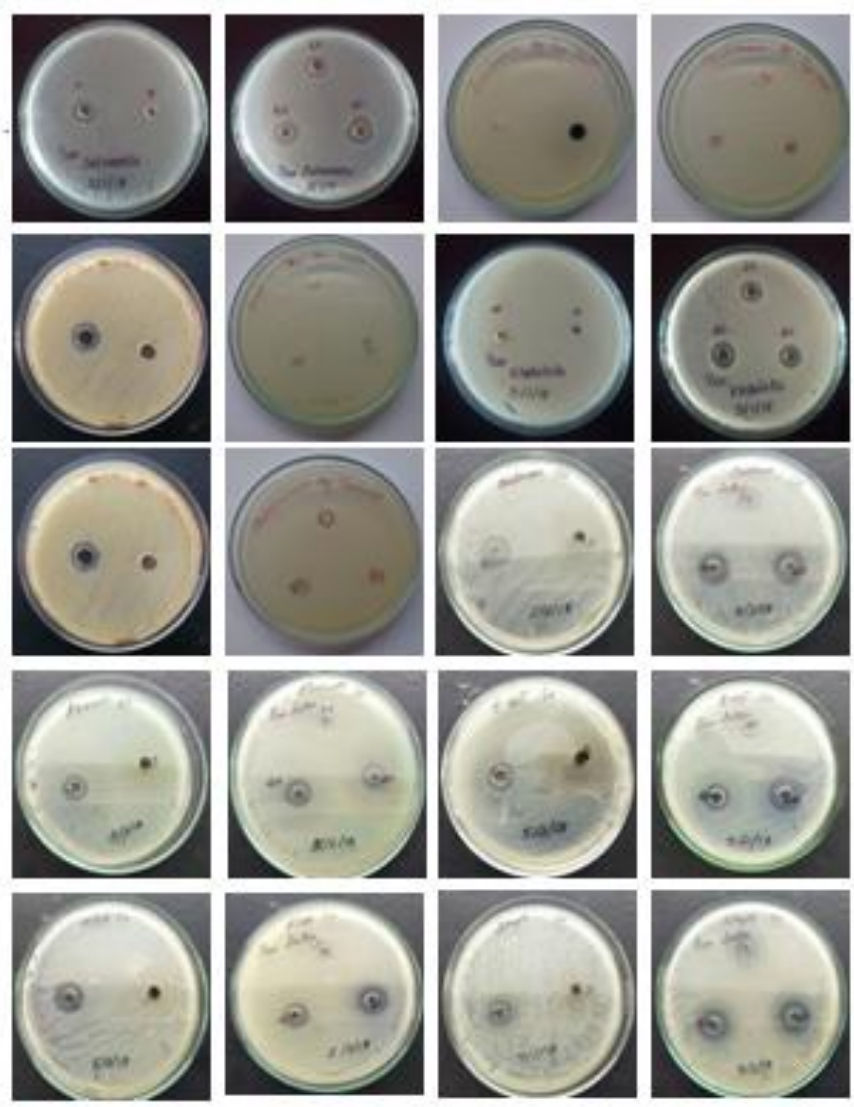


Fig.10 Antibacterial activity study using well diffusion method of *Musa paradisiaca* latex (1/50 and 1/100 dilution) nanoparticles (Zn) a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, 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).

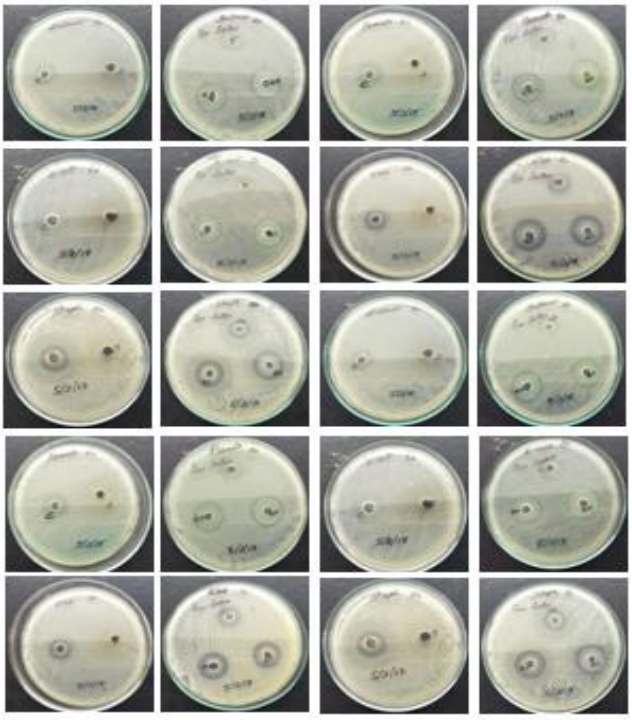


Fig.11 Silver nanoparticle formation of *Musa paradisiaca* latex under SEM imaging system with various resolutions.

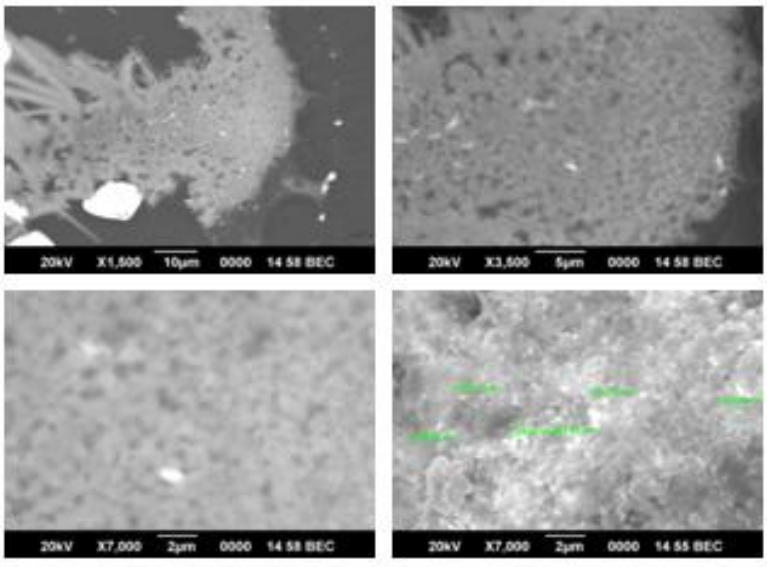


Fig.12 Copper nanoparticle formation of *Musa paradisiaca* latex under SEM imaging system with various resolutions.

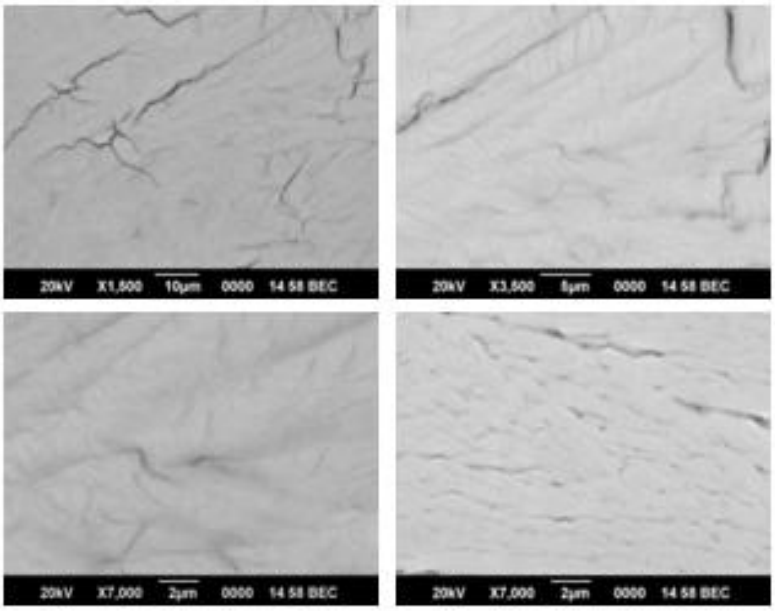


Fig.13 Zinc nanoparticle formation of *Musa paradisiaca* latex under SEM imaging system with various resolutions.

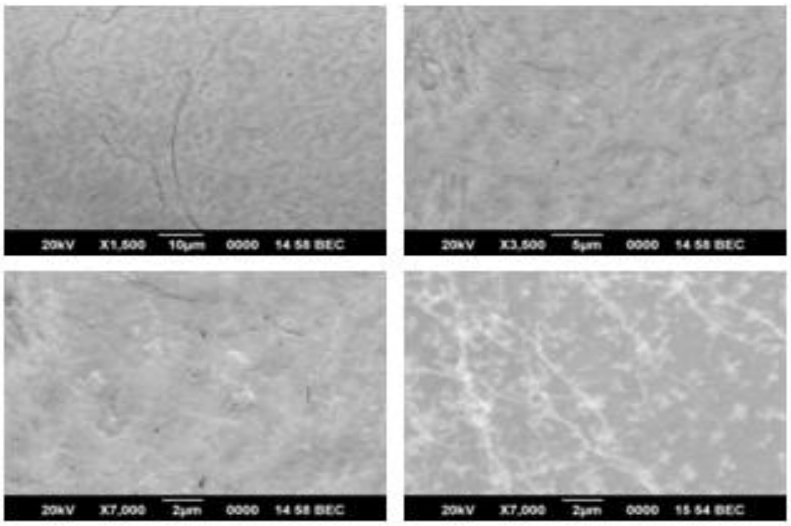
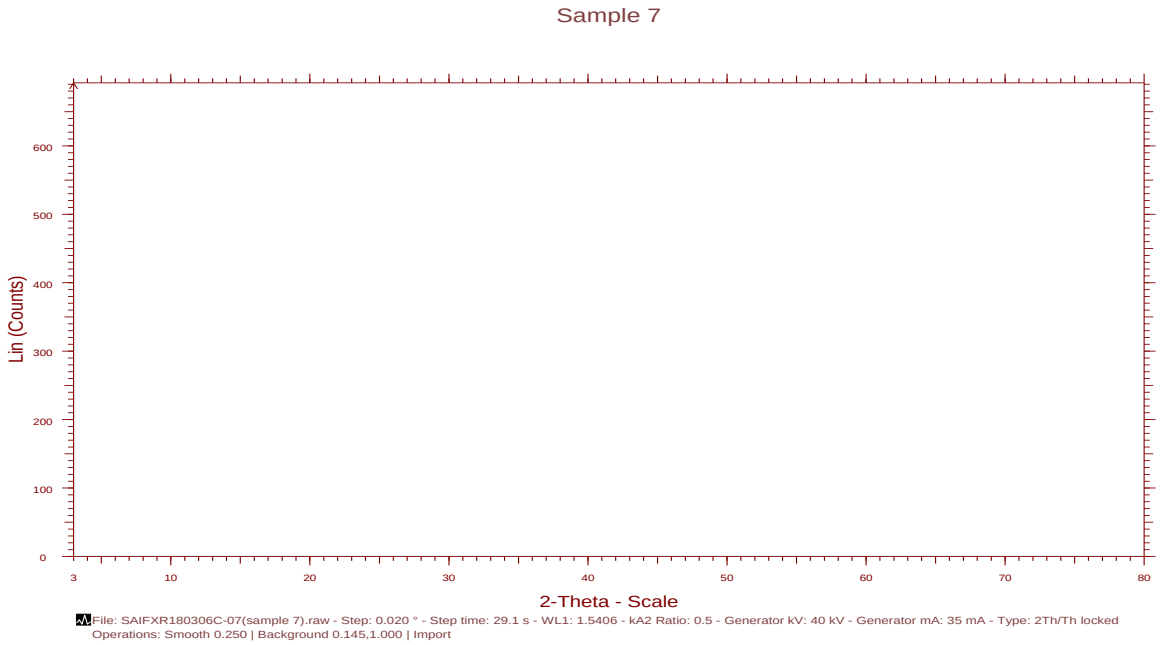


Fig.14 Silver nanoparticle formation of *Musa paradisiaca* latex under XRD imaging system.



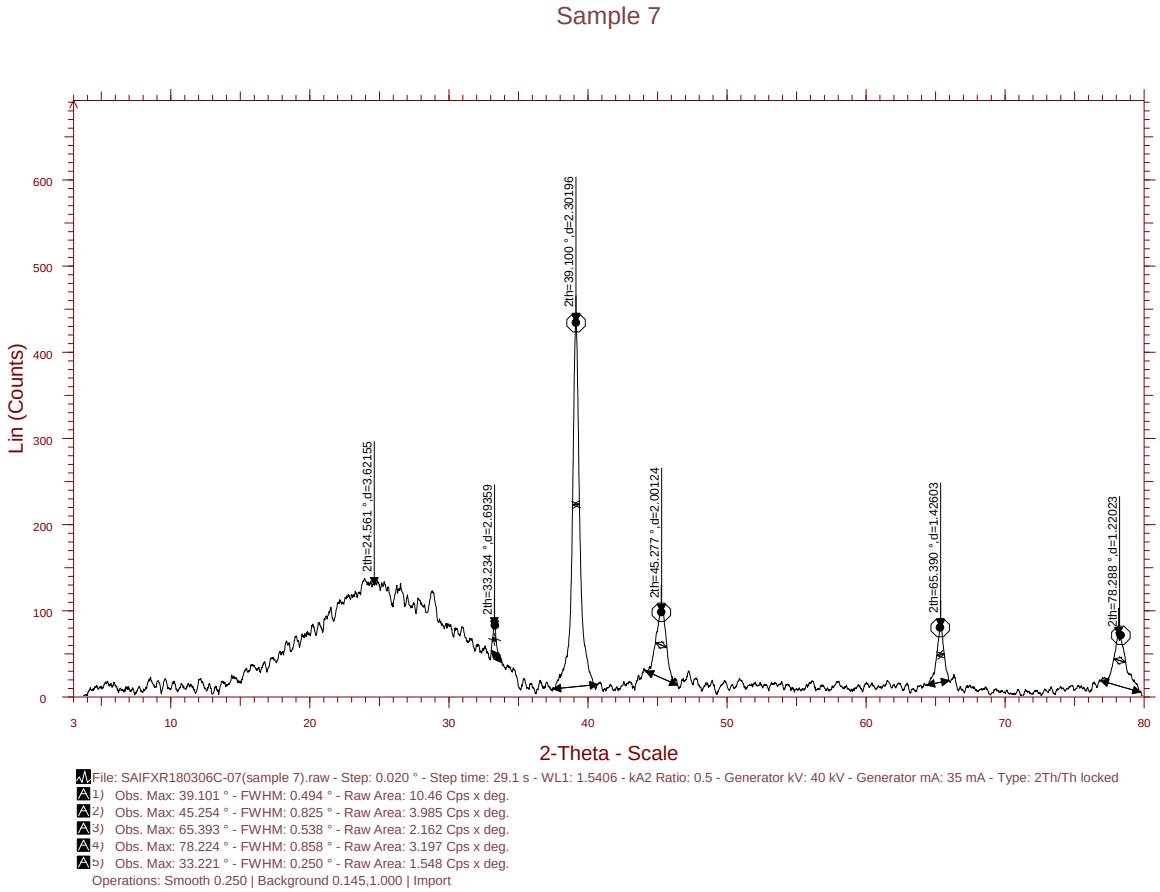
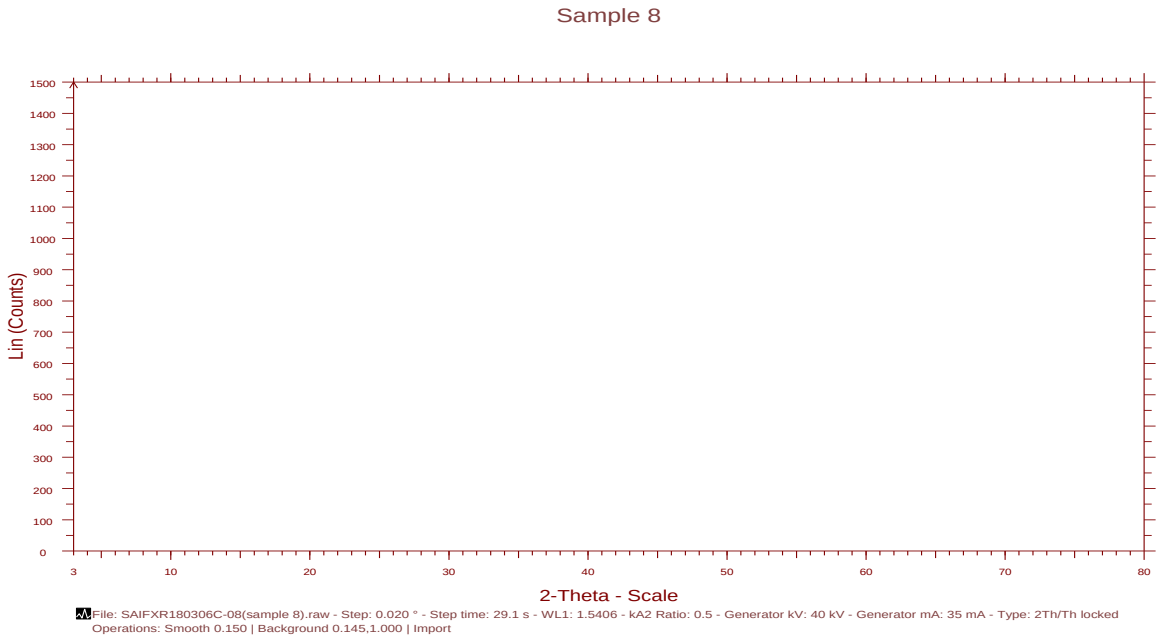


Fig.15 Copper nanoparticle formation of *Musa paradisiaca* latex under XRD imaging system.



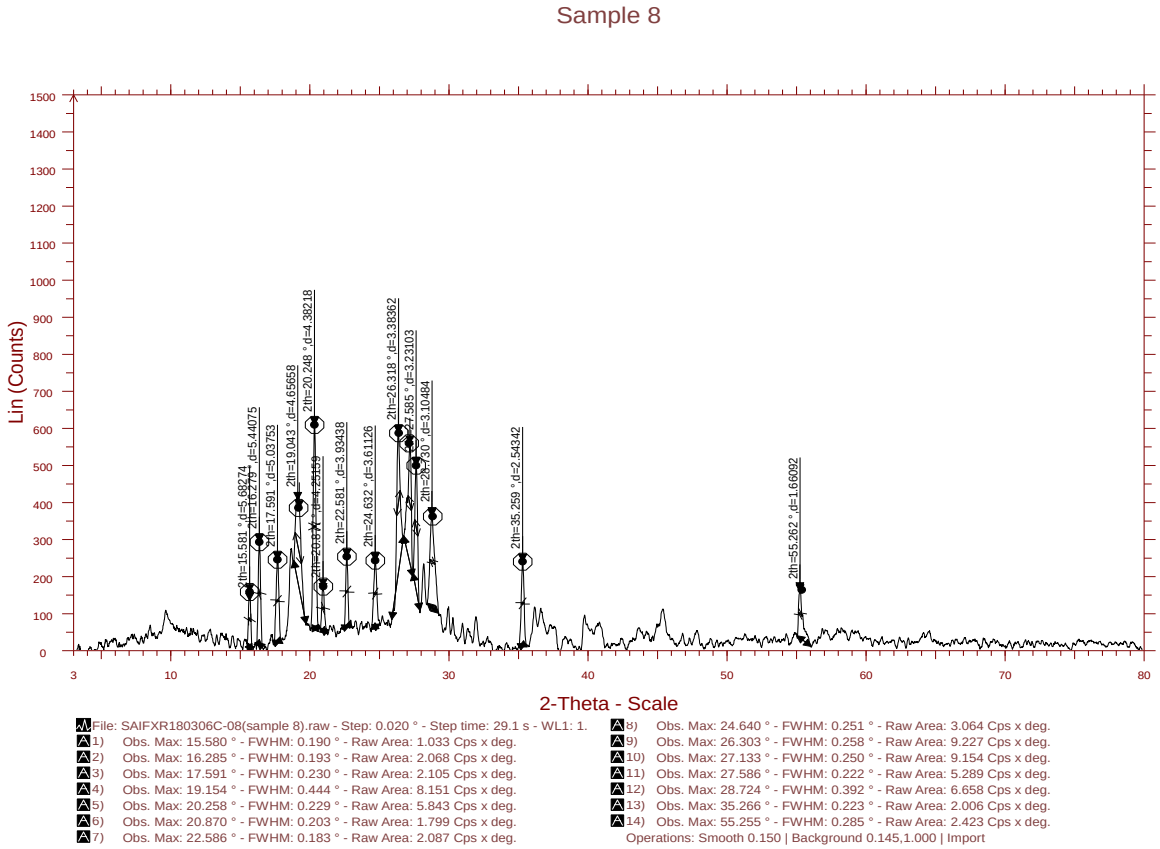
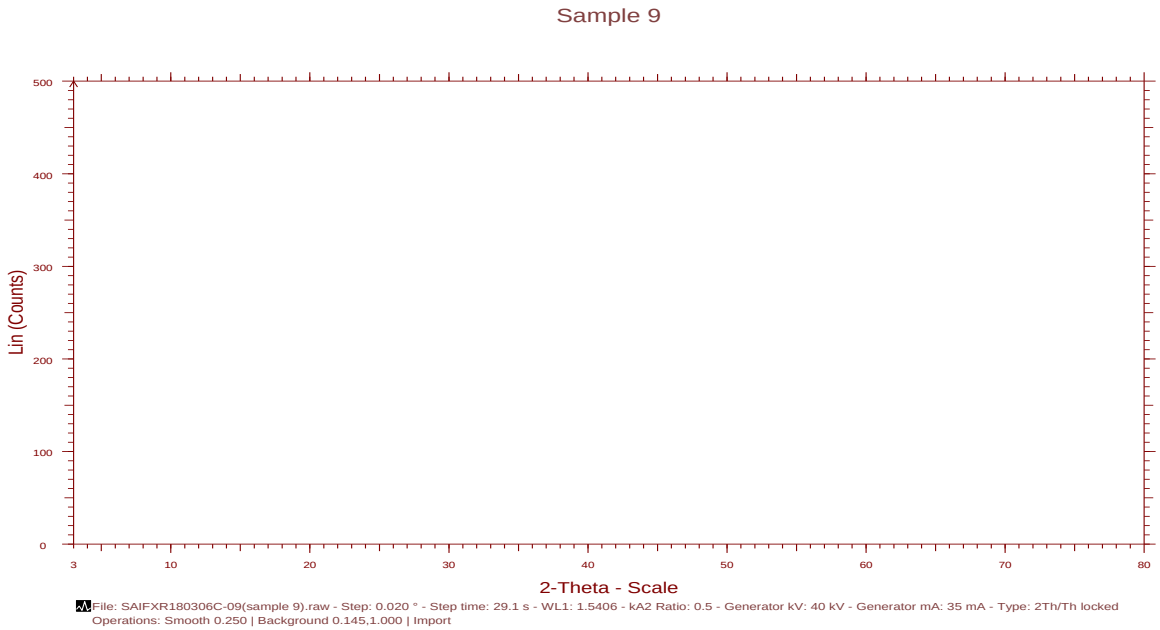
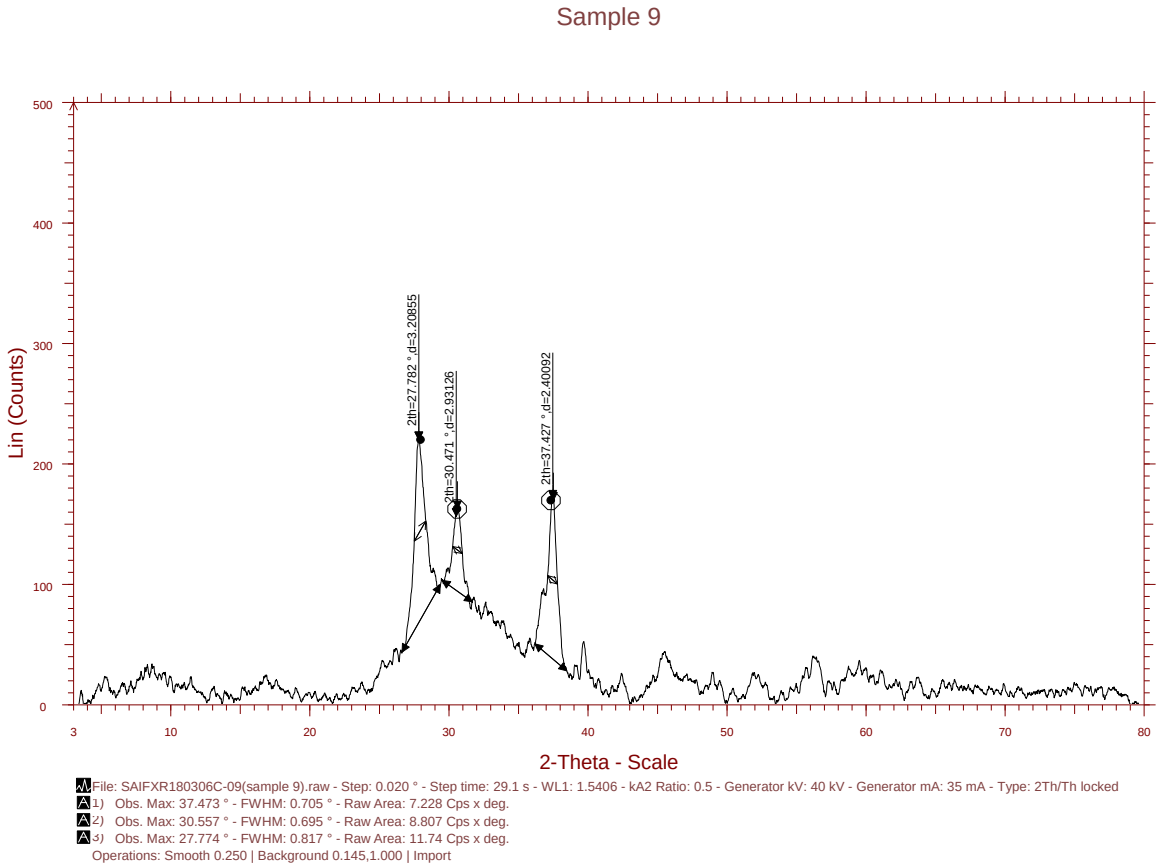


Fig.16 Zinc nanoparticle formation of *Musa paradisiaca* latex under XRD imaging system.





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 *Musa paradisiaca*. The intensity of the peak at 435nm was increased with time until the reduction completes. The maximum peak was found to be 385nm for *Croton variegatum.* 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 *Musa paradisiaca* and *Croton variegatum*. 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 *Musa paradisiaca* and *Croton variegatum*. The intensity of peak at 350nm was increased with time until the reduction completes.

Antibacterial assay

The latex of Banana (*Musa paradisiaca*) and Garden croton (*Croton variegatum*) 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 Gram-negative 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 *Musa paradisiaca* and *Croton variegatum* 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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