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RESEARCH ARTICLE

Biosynthesis and characterization of silver nanoparticles and antimicrobial activity of *Trichoglottis tenera* (Lindl.) an endangered orchid of Western Ghats

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Abstract

This research paper deals with a rapid and eco-friendly method for green synthesis of nanoparticles. The research was also conducted to evaluate the therapeutic potentials of silver nanoparticles isolated from orchids plant extracts in five different concentrations (I-99 (ml) 1mM silver nitrate +1ml plant extract, II-98+2, III-97+3, IV-96+4, and V-95+5) and various time periods was used to study green synthesis of nanotechnology. The five isolated silver compounds were used to investigate the antimicrobial potential against ten clinical bacteria (*Escherichia coli*, *Vibrio cholerae*, *Serratia marcesens*, *Staphylococcus mutants*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Klepsiella pneumonia*, *Proteus vulgaris*, and *Pseudomonas aurogens*) and three fungal species *Aspergillus flavus*, *Candida albicans*, and *Trichophyton mentagrophytes.* Out of five isolated silver compounds, fourth and fifth were found to exhibit antimicrobial properties with potential to fight against *E.coli*, *Bacillus subtilis*, and *Salmonella typhi.* The result shows moderate activity in third and fourth silver compounds against *Shigiella sonehi*, *Vibrio cholerae and Pseudomonas aurogens.* The first and second isolated silver compounds were failed to show any antibacterial activity against *Klepsiella pneumonia*, *Staphylococcus mutans*, *Staphylococcus aureus*, and *Proteus vulgaris.* The synthesis of silver nanoparticles (AgNPs) was characterized by UV-visible spectroscopy, FTIR-Fourier Infrared spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD). The UV-Vis spectroscopy has given surface Plasmon resonance for the synthesis of silver nanoparticles in extract-I, extract-II, extract-III, extract-VI, and extract-V. It was generated in ten different time periods at 422 nm to 447 nm in all the five extracts. Scanning electron microscopy (SEM) images were obtained in different shapes like nano rods (500 nm).

INTRODUCTION

Medicinal plants are a promising source for novel antimicrobial compounds that are active against antibiotic resistant microorganisms. Many medicinal plants are considered to be potential antimicrobial crude drugs as well as a source for novel compounds with anti-microbial activity, with possibly new modes of action. The compounds may, kill antibiotic-resistant strains of bacteria such as *Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus* and *S. aureus* **(**David et al., 2006). The development of antimicrobial compounds from natural sources is considered to be a promising approach. Many focus on determining the antimicrobial activity of plant extracts found in folk medicine, essential oils or isolated compounds such as alkaloids, flavonoids, sesquiterpene lactones, diterpenes, triterpenes or

naphthoquinones. After detection of antimicrobial activity in the plant extract, some of these compounds were isolated or obtained by bioassay-guided isolation. Nanotechnology has become one of the fast developing fields of research in the past few decades. It has a multidisciplinary nature with tremendous potential to create new devices and materials with a wide range of applications. It is anticipated that nanotechnology will contribute to build a society with accessible clean energy and clean water, new industries and jobs, improved health care, and extended life span with high quality.

Nature has devised various processes for the synthesis of nano- and micro- length scaled inorganic materials which have contributed to the development of relatively new and largely unexplored area of research based on the biosynthesis of nonmaterial's (Dragieva et al., 1999). The number of higher plant species (angiosperms and gymnosperms) is estimated between 215,000 and 500,000 species. Of these, only about 6% has been screened for biological activity, and a reported 15% has been evaluated phytochemically (Fabricant and Farnsworth 2001; and Shankar et al., 2004a). In ancient Indian medical system (Ayurveda), gold is used as medicine in the preparation of nano level Swarna Bhasma to treat tuberculosis, anemia, and cough and also believed to prevent ageing (Neetu and Anand, 2012). Nanoparticles synthesis has many routed methods for the synthesis of silver nanoparticles; green synthesis using plant sources offers several advantages such as best in low-cost, non-toxic and eco-friendly agent (Aymonier et al., 2002; Sun and Xia, 2002). Silver have been as therapeutic agent for many diseases and an efficacious antibacterial and antifungal agent (Klaus et al., 1999). The Silver and Silver nanoparticles application is very important in medical industry such as typical ointments which prevent infection against burns and open wounds (Ip et al., 2006). Silver nanoparticles of size smaller than 100 nm contain about 10000–15000 silver atoms (Oberdorster et al., 2005; Verpoorte and Alfermann, 2000). They are prepared by engineering the metallic silver into ultrafine particles by numerous physical methods, which include spark discharging, electrochemical reduction, solution irradiation and cryochemical synthesis. The most widely used and known application of silver nanoparticles is in the medical sciences. These include typical ointments and creams containing silver to prevent infection of burns and open wounds (Chen and Schluesener, 2008). There are number of chemical and physical methods have been employed for the synthesis of silver nanoparticles (Ghorbani et al., 2011; Tien et al., 2008) but some particular methods have disadvantages because of the use of toxic chemicals and radiation. Biological synthesis of silver nanoparticles using microorganisms (Konishi et al., 23007; Kumar and Yadav, 2009) enzyme (Warheit et al., 2007) and plants or plant extracts (Raju et al., 2011; Raju et al., 2012; Raju et al., 2013a; Raju et al., 2013b; and Shankar et al., 2004a) has been suggested as possible alternative eco-friendly method.

Biosynthetic process of nanoparticles would be more useful, if nanoparticles are produced extracellularly using plants or their extracts in a controlled manner according to their size, shape and dispersity (Aymonier et al., 2002). Traditional method of biologically synthesized gold and silver nanoparticles is more convenient for pharmaceuticals and biomedical applications for their efficient antibacterial and antimicrobial properties and also in other applications like selective coatings for solar energy absorption, intercalation material for electrical batteries, optical receptors and catalysts in chemical reactions (Aymonier et al., 2002). The aim of this study, synthesized silver nano particles was used to evaluate the antibacterial activities (Gram positive and Gram Negative) of *Bacillus subtilis* (MTCC 441), *Proteus vulgaris* (MTCC 1771), *Serratia marcescens (*MTCC 8708), *Vibrio cholerae* (MTCC 3906), *Salmonella typhi* (MTCC 733), *Klebsiella pneumonia* (MTCC 7162), *Streptococcus mutans* (MTCC 890), *Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 424) *and Aspergillus flavus (*MTCC 2206)*Candida albicans* (MTCC 227), *Trichophyton mentagrophytes* (MTCC 7687), were respectively. The synthesized silver nanoparticles were characterized by various methods, using UV/Vis, FTIR, and SEM-EDX.

Material and Methods

Experimental Methods

Materials

Silver nitrate was purchased from Nice Laboratory Reagent Company in Cochin.

Collection of plant materials

Trichoglottis tenera plants were collected from Western Ghats during the month of July-2012. The collected plants were stored by refrigerator to continue my further studies.

Preparation of silver nitrate solutions

The molecular weight of the Silver nitrate-169.86gm was weighed per litre. This method was prepared by one molar silver nitrate solution. In other method of preparation 1mM solution of silver nitrate was weighed by 0.169.86 mg/liter. The Erlenmeyer flask (250 ml) containing 100 ml de-ionized water for dissolved $AgNO_3$ salts for preparation of 1mM silver nitrate solutions.

Preparation of plant extracts

The collected plant was identified by "The Rapinat Herbarium", St. Joseph's College (Autonomous), Tiruchirappalli-2. The plant was air dried at room temperature for four weeks until it turned into dried leaves. The dried leaves were subjected into pulverized with a mixer grinder to make it into smooth powder for preparation of plant extracts. The 5 gm of leaf was taken into an Erlenmeyer flask with 100 ml of sterile de-ionized water. The mixture was boiled for 5-10 min at 100ºC; finally the mixture was filtered and stored at 4°C for further studies.

Preparation of Silver nanoparticles with different concentrations of plant extracts

The preparation of 1mM aqueous silver nitrate solution was prepared by 0.017gm of $AgNO_3$ salt which was weighed and dissolved in 100 ml sterile de-ionized water. 1mM silver nitrate solution was taken into five number of Erlenmeyer flask containing 99 ml, 98 ml, 97 ml, 96 ml, 96 ml and plant extracts 1 ml, 2 ml, 3 ml, 4 ml, 5 ml was added by respective silver nitrate solution.

Biosynthesis of Silver nanoparticles

Biosynthesized silver nanoparticles used by plant extracts of *Trichoglottis tenera* were added by 1mM silver nitrate solution with different concentration of plant extracts. The reaction mixture of aqueous silver nitrate solution and plant extract a reduction of pure Ag^{2+} ions were monitored by UV-Visible spectrum in different time period. (Table-I).

Purification of Silver Nanoparticles

The obtained silver nanoparticles were then transferred to dialysis centrifuged tube (Tarsons -50 ml graduated centrifuge tube) centrifuged at 12,000 rpm for 20 min, after getting the pellet re-dispersed with double distilled water and the obtained single purified silver nanoparticles purified by repeated ultra cooling centrifugation for three to four times at 12,000 rpm for 20 min. Then, the centrifugation pellet was dried in hot air oven for further studies of SEM, and XRD.

Antimicrobial activity

The antimicrobial activities of various concentration of plant extracts were synthesized silver nanoparticles were determined by standard disk diffusion assay using the microbial cultures. The synthesized silver nanoparticles were treated with various pathogenic bacteria like *Escherichia coli, Staphylococcus mutans, Shigielle sonegii, Salmonella typhi, Staphylococcus aureus, Proteus vulgaris, Vibrio cholera, Klepsiella pnemoniae, Pseudomonas aeruginosa*. The Mueller Hinton agar (HI media) was used for the studies of antimicrobial assay; Chloramphenicol (10µg) was used as control for the bacteria and the Sabouraud dextrose agar was used for antifungal studies. The fungi were used for the antifungal studies such as *Aspergillus fumigates, Candida albicans, Trichophyton mentagrophytes*. The standard antifungal agent *Nystatin* 10mg was used for antifungal studies. The plates were prepared using sterile 90mm Pertridishes (Malabadi, 2005). The pure bacterial species were periodically subcultured on every two weeks. Each bacterial species was cultured on Nutrient agar broth for antimicrobial studies and fungal species were cultured on Potato Dextrose Broth for antifungal studies. The impregnated silver nanoparticles discs were placed on the plates and incubated at 37ºC for bacterial culture for 24 hrs and 72 hrs fungi. The diameter of the zone of inhibition was measured in mm and the mean values were presented.

Statistical analysis
Statistical analysis was performed using statistical software packages SPSS 16.0 and experimental data were compared with student's *t* test. All experiments were repeated thrice and results were represented as means ±SD. *p*-value less than 0.05 were considered as level of significance.

Result and Discussion

The silver nanoparticles were synthesized by plant extracts exhibiting yellowish – brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Thirumurgan et al., 2010). Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. When exposed to herbal extracts the aqueous silver ions were reduced in solution, thereby leading to the formation of silver hydrosol. The silver ion (Ag+) is a bioactive and in sufficient concentration readily kills bacteria *in vitro*. Silver exhibits low toxicity in the human body, and minimal risk is expected due to clinical exposure by inhalation, ingestion, dermal application as silver and silver nanoparticles are used commonly for antimicrobial in a variety of industrial, healthcare and domestic applications (Maillard et al., 2012).

Characterization of Silver Nanoparticles

UV-VIS Spectral analysis
One of the most widely used techniques for structural elucidation of silver nanoparticles is characterized by UV-Vis spectroscopy (Sun et al., 2001). The pure silver ions were reduced and observed by UV-Vis spectrum was taking at 50 ml of the synthesized silver nanoparticles and it is compared with 50 ml of distilled water used as blank. A Perkin Elemer spectrophotometer at working range from 190 nm from 110 nm. The color reaction, monitored by UV – Vis spectrum at different time intervals was described in table.1. The first leaf extract was synthesis of silver nanoparticles which was generated at the peak variation of 425 nm and 426 nm. The second leaf extract was generated at peak variation of 414 and 422 nm. The third sample was showed in different peak variations at 422, 423, 428, 432, 435, and 437 nm, and fourth sample was also showed in different peak variation in different time periods at 436, 437, 438, 439, 440, 446, and 447 nm. The fifth leaf sample peak variation at 424, 430, 434, 438, 441, and 445 nm was showed in different time periods. All the peak variation found to locate at the interval between 414 and 447nm were detected by UV Vis spectrum light.

FTIR Spectral analysis
It is an easy way to identify the presence of certain functional groups in a molecule and also one can use the technique collection of absorption bands to confirm the identity of a pure compound. The FTIR spectral analysis, the synthesized silver nanoparticles solution was centrifuged at 10,000 rpm for 20 min. After the centrifugation pellet was subjected to wash with de-ionized water. This clear pellet was re-subjected to centrifuge at 10,000 rpm for 20 min, after the completion of centrifugation pellet was subjected again to wash with de-ionized water to get rid of clear silver nanoparticles. The centrifuged silver nanoparticles going to be assessed for FTIR analysis showed strong bands at 669 to 3464 cm which is first leaf extract that corresponds to stretch in OH band (3200-3600). The second leaf extract Ag NPs showed band between 667 and 3465 cm and likewise the third leaf extract showed strong band at 666 -3434 cm. The fourth leaf extract was showed the band at 668- 3904 cm. The fifth leaf extract showed the band at 685-3463 cm.

The alkenes' =C-H absorption between 675-100 cm^{-1}. Absorption 1000 cm^{-1} indicates alkenes' =C-H groups and intensity was strong. The carbonyl (C=O) absorption between 1690-1760cm^{-1}; this strong band indicates an aldehyde, ketone, carboxylic acid, ester, amide, anhydride or acyl halide. The peaks near 2100 and 2260 cm^{-1} were assigned to CºC stretching alkenes' group. The O-H or N-H group exhibit absorption wavelength between 3200 and 3600 cm^{-1}. The strong band indicates an alcohol, N-H containing amine or amide, or carboxylic acid and will also be observed a double in NH_2. An absorption wavelength above 3000 cm^{-1} indicates C=C, either alkenes' or aromatic rings. Confirm the aromatic ring by finding peaks at 1600 and 1500 cm^{-1} and C-H out-of-plane bending to give substitution patterns below 900 cm^{-1}. Confirm alkenes with absorption at 1640-1680 cm^{-1}. C-H absorption between 3000 and 2850 cm^{-1} is due to aliphatic hydrogen's. The aromatic compounds structure may also be confirmed from weak band and combination peak bands found from 2000 to 1600 cm^{-1}. The CN Nitrile groups are triple bond has been exhibit the absorptions wavelength at 2100-2260 cm^{-1} is small but exposed.

SEM (Scanning Electron Microscopy) analysis of silver nanoparticles

Scanning Electron Microscopy was characterized by size and morphology of the synthesized nanoparticles. Synthesis of silver nanoparticles suspension in water was used for the analysis of SEM. The freeze dried liquid silver nanoparticles suspension was fabricating a drop onto a clean electric stub and allowing water to completely evaporate. The high-energy electrons travel through the SiNx film of the open dish to interact with the specimen and backscattered electrons are detected in the vacuum chamber to construct a SEM image of the specimen. The silver nanoparticles were examined under a Philips XL-30 SEM at 12-16 kV with a tilt angle of 45°C. The particle size was found to be less than 500nm in all five experimental samples. The characterized nanoparticles showed 500 nm in nano rod shapes.

XRD (X- Ray diffraction) analysis of silver nanoparticles

The crystalline structure of the isolated silver nanoparticles was subjected to the analysis of X-ray diffraction studies in five isolated silver compounds. The X-ray diffraction studies were carried out by using XPERT-PRO 2.1 version. This analytical machine use wavelength intended at higher resolution the $K\alpha_1$ line is readily seen to be a double, which is labelled as $K\alpha_1$ and $K\alpha_2$. The three wavelengths are used in splitting anode cupper material (Cu) orbital energy $K\alpha_1$ (= 1.54056 Å) and $K\alpha_2$ (= 1.54439 Å) are very similar, and its third wavelength Kβ (= 1.39225 Å). The light focusing type is "line", of length unit- 12.0 mm and width unit -0.4 mm. The focusing of X-ray image is "taken off" of angle unit-6.0 deg. When x-rays radiation is encountered in any form of matter, they are partly transmitted and partly absorbed. The high intensity and narrow Kα -lines makes x-ray diffraction possible, since it's generally requires the use of monochromatic radiation. It was found experimentally that $I \propto x$ (I – intensity and x – distance) (web.stanford.edu). The particle size of the silver nanoparticles was carried out by using the equation Debye-Scherrer's from the 2θ values of the X-ray diffraction peaks is given below

Debye-Scherrer's equation:

$$D = k \lambda / \beta \cos \theta$$

K –constant
Λ –wave length of X-Ray,
B –FWHM (full width at half maximum)
θ – the Bragg's diffraction angle of the XRD peak.
D –particle diameter (size) (Das et al., 2009).

Antimicrobial studies

Disk diffusion is one of the oldest approaches to antimicrobial susceptibility testing and remains one of the most widely used antimicrobial susceptibility testing methods in routine clinical laboratories. It is suitable for testing the majority of bacterial pathogens, including the more common fastidious bacteria, which is versatile in the range of antimicrobial agents that can be tested and requires no special equipment (Jones, 1992 and (Malabadi, 2005). Silver is well known as one of the most universal antimicrobial substances (El-Shahaby et al., 2013). Silver nanoparticles were tested by antibacterial activities and were carried out by disc diffusion method (EUCAST, 2013). Hinton Agar (HI -Media Mumbai) is prepared according to the manufacturer's instructions and supplements are added after cooling to 42–45°C. Agar is dispensed in Petri dishes to achieve an even depth of 4.0 mm with a maximum variation of 0.5 mm.

Muller Hinton Agar (HI -Media Mumbai) has been complimented by (1.75% Casein hydrolysate, 0.2% Beef heart infusion, 0.15% Starch soluble, and 1.7% Agar, pH 7.3 + 0.2) (Praveen Kumar et al., 2013). The medium was prepared according to manufacturer's instructions, and sterilized with help of autoclave at 121°C 15psi pressure for 15min. The sterilized MH agar medium was dispensed by boiled petridishes at 30 ml in each to achieve an even depth of 4.0 mm with a maximum variation of 0.5mm. The bacterial inoculums was prepared by sterile nutrient broth and incubated at 37°C for the studies of antimicrobial activity. The solidified agar plate was kept in inoculation chamber for inoculation of bacterial suspension (10 CFU/ml) was spread (50 µl) evenly on the surface of agar plates. The paper disc was impregnated with different concentration of plant extract of synthesized silver nanoparticles were used for this study (Table: 3). *Chloramphenicol* is standard antibiotic (10 µg/disc) obtained from Hi-Media, Mumbai was used as positive controls. The negative control disc containing impregnated with Dimethyl sulfoxide (DMSO) used as negative controls. The diameter of inhibition zones was assessed in the antimicrobial

activity formed around the discs. The inoculated plates were incubated at 37°C for 24 h and the zone of inhibition was recorded.

The effect of synthesized silver nanoparticles (AgNPs) was assessed against the bacteria and fungi. The bacterial species of *Bacillus subtilis, Escherichia coli, Klepsiella pneumoniae, Shigiella sonehi* was assessed in the third, fourth and fifth extract they seem to have a relatively higher zone of inhibition towards the bacteria. The fungus of *Candida albicans* was revealed that inhibition zone found to be resistant in third, fourth and fifth extract. The moderate inhibition zone (3.69 + 0.15) was exhibited against the bacterial species *Bacillus subtilis* (MTCC 441) in two to fifth extracts. *Klebsiella pneumonia* (MTCC 7162) (3.70 + 0.15) and *Vibryo cholerae* (MTCC 3906) (3.37 + 0.15), were found to be significance activity was exhibited. The second, third, fourth and fifth extracts were showed remarkable inhibition against *Salmonella typhi* (MTCC 733), *Proteus vulgaris* (MTCC 1771), *Staphylococcus aureus* (MTCC 3160). *Serratia marcescens* (MTCC 8708) *Streptococcus mutans* (MTCC 890) and the fungal species of *Aspergillus flavus (*MTCC 2206) *Candida albicans* (MTCC 227) and *Trichophyton mentagrophytes* (MTCC 7687) were found to be moderate inhibition in the fourth and fifth extracts. In other words did not show any inhibition zone in first, second and third extracts of AgNPs.

Antibacterial properties inhibit the reproduction of bacteria, which is a microbe. The silver nanoparticles can "inactivate proteins, blocking respiration and electron transfer, and subsequently inactivating the bacteria. The antibacterial properties of the silver nanoparticles depend on the size of the particles; the smaller the particles the better the effect. The particle size is a major factor because the smaller the particle the greater the surface area, which allows for greater interaction with the bacteria (Araujo et al., 2011). The antibacterial properties of the silver nanoparticles depend on the size of the particles; the smaller the particles the better the effect. The particle size is a major factor because the smaller the particle the greater the surface area, which allows for greater interaction with the bacteria (Araujo et al., 2011). It has been reported that combined use of silver nanoparticles with antibiotics, such as *penicillin* G, *amoxicillin*, *erythromycin*, and *vancomycin*, resulted in enhanced and synergistic antimicrobial effects against Gram-positive and Gram-negative bacteria (e.g., *E. coli* and *S. aureus*) (Fayaz et al., 2010; Raj et al., 2009; and Shahverdi et al., 2007). Silver ions cause the release of K+ ions from bacteria; thus, the bacterial plasma or cytoplasmic membrane, which is associated with many important enzymes, is an important target site for silver ions. In addition to their effects on bacterial enzymes, silver ions caused marked inhibition of bacterial growth and were deposited in the vacuole and cell wall as granules. They inhibited cell division and damaged the cell envelope and contents of bacteria. Bacterial cells increased in size, and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers all exhibited structural abnormalities. Although beneficial as antimicrobial agents, silver nanoparticles have adverse effects on cells such as the production of reactive oxygen species which are toxic to both bacteria and eukaryotic cells (Carlson, 2008 and Park et al., 2009). Some studies have reported that the positive charge on the Ag ion is crucial for its antimicrobial activity through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles (Dibrov et al., 2002; Dragieva et al., 1999 and Hamouda et al., 2000). Ag nanoparticles accumulated in the bacterial membrane caused the permeability, resulting in cell death. However, because those studies included both positively charged Ag ions and negatively charged Ag nanoparticles, it is insufficient to explain the antimicrobial mechanism of positively charged Ag nanoparticles. Therefore, we expect that there is another possible mechanism (Araujo, 2011). Finally, silver ions interact with nucleic acids; they interact preferentially with the bases in DNA rather than with the phosphate groups, although the significance of this in terms of their lethal action is unclear (Joseph Wang, 2003).

Discussion

This is the right time for the green synthesis of nanoparticles are of great interest in the world due to eco-friendliness, economic prospects, feasibility and wide range of applications in nanomedicine, enzyme technology, nano-optoelectronics, nano robotics, nano wires etc. It is a new area of emerging technology in the scientific research world, where day-by-day developments is noted a bright future for this field. Now many medical facilities are available in the world, in this case drug delivery scientists are searching for the nanovehicle and nanodrug delivery system. The many mechanisms of drug delivery system would dramatically reduce drug dosage, the other way to improve in the drug absorption so that the patient can take a smaller dose, and yet have the same benefit, deliver the drug to the right place in the living system, increase the local concentration of the drug at the favorite site and limit or eliminate side effects (Mojtaba Salouti and Azam Ahangari, 2014). Even though there are many mechanisms attributed to the antimicrobial activity that has been exhibited by silver nanoparticles, the actual and

reliable mechanisms is not fully understood as the nanoparticles are found to act on different organisms in different ways. Nanoparticles of desired size and shape have been obtained successfully using living organisms- simple unicellular organisms to highly complex eukaryotes. The field of nano biotechnology is still in its infancy and more research needs to be focused on the mechanistic of nanoparticles formation which may lead to fine tuning of the process ultimately leading to the synthesis of nanoparticles with a strict control over the size and shape parameters (Prathna et al., 2010). This reliable protocol is very simple for the green synthesis of silver nanoparticles for social relevance to human beings.

S/No	Different time period (min)	$AgNO_3$ (ml) + Different conc. of plant extracts (ml)				
		Extract -I	Extract –II	Extract –III	Extract -VI	Extract -V
1	15	99 ml (1 mM $AgNO_3$) + 1 ml Plant leaf extract	98 ml (1 mM $AgNO_3$) + 2 ml Plant leaf extract	97 ml (1 mM $AgNO_3$) + 3 ml Plant leaf extract	96 ml (1 mM $AgNO_3$) + 4 ml Plant leaf extract	95 ml (1 mM $AgNO_3$) + 5 ml Plant leaf extract
2	30					
3	45					
4	60					
5	75					
6	90					
7	105					
8	120					
9	135					
10	150					

Table 1. The color reaction monitored by UV-Visible spectrum at different time intervals.

S.No	Functional Group	Type of Vibration	Compound type	Characteristic Absorptions (cm-1)	Intensity
1	=C-H	Bending	Alkene	675-1000	Strong
2	C=C	Stretch	Alkene	1620 - 1680	Variable
3	C□C	Stretch (M, W)	Alkyne	2100 - 2260	Variable, not present in symmetrical alkynes
4	C=O	Stretch	Carbonyl	1670-1820	Strong
5	N-H	Stretch	Amine	3300-3500	Medium (primary amines have two bands; secondary have one band, often very weak)
6	CN	Stretch	Nitrile	2210-2260	Medium
7	O-H	(Stretch, H-bonded)	Alcohol	3200-3600	Strong, Broad

Table 2. Characteristic IR absorption frequencies of organic functional groups.

Intensity: S- Strong, M - Medium, W- Weak, V- Variables.

S/No	Test microorganisms (Bacteria)	Diameter of inhibition zone (mm) using various solvents						
		Extract – I	Extract - II	Extract – III	Extract - IV	Extract - V	Positive Control	Negative Control
1	*Bacillus subtilis* (MTCC 441)	-	1.57 ± 0.11	2.66 ± 0.18	3.39 ± 0.19	3.69 ± 0.15	3.15 ± 0.12	2.29 ± 0.16
2	*Escherichia coli* (MTCC 443)	-	1.54 ± 0.11	3.18 ± 0.19	3.38 ± 0.19	3.35 ± 0.17	3.25 ± 0.18	2.30 ± 0.15
3	*Pseudomonas aeruginosa* (MTCC 424)	-	-	-	3.36 ± 0.18	-	3.27 ± 0.16	2.32 ± 0.15
4	*Proteus vulgaris* (MTCC 1771)	1.55 ± 0.10	1.56 ± 0.10	2.34 ± 0.19	-	2.36 ± 0.17	3.27 ± 0.15	2.33 ± 0.14
5	*Serratia marcescens* (*MTCC* 8708)	-	-	2.34 ± 0.15	-	-	3.26 ± 0.15	2.32 ± 0.14
6	*Vibrio cholerae* (*MTCC* 3906)	1.57 ± 0.11	1.58 ± 0.10	2.31 ± 0.15	3.37 ± 0.15	-	3.27 ± 0.14	2.34 ± 0.14
7	*Salmonella typhi* (*MTCC* 733)	-	1.57 ± 0.09	-	3.42 ± 0.10	2.32 ± 0.13	3.24 ± 0.16	2.36 ± 0.11
8	*Staphylococcus aureus* (MTCC 3160)	-	-	2.34 ± 0.11	3.67 ± 0.16	-	3.25 ± 0.17	2.38 ± 0.12
9	*Klebsiella pneumonia* (*MTCC* 7162)	1.58 ± 0.09	1.57 ± 0.09	2.35 ± 0.10	3.70 ± 0.15	3.34 ± 0.15	3.28 ± 0.18	2.40 ± 0.10
10	*Streptococcus mutans* (*MTCC* 890)	-	1.56 ± 0.09	2.38 ± 0.10	-	-	3.30 ± 0.17	2.42 ± 0.10

All the results are measured by mean of inhibition zone in mm ± S.D of three replicates.

- No activity

Table 3. Bioassay of different concentration of synthesised silver nanoparticles (Bacteria).

S/No	Test microorganisms (Fungi)	Diameter of inhibition zone (mm) using various solvents						
		Extract – I	Extract – II	Extract – III	Extract – IV	Extract – V	Positive Control	Negative Control
1	*Aspergillus flavus* (MTCC 2206)	-	-	-	1.31 ± 0.17	1.27 ± 0.11	2.30 ± 0.15	1.72+0.14
2	*Candida albicans* (MTCC 227)	-	-	-	1.32 ± 0.19	1.28 ± 0.12	2.32 ± 0.13	1.68+0.14
3	*Trichophyton mentagrophytes* (MTCC 7687)	-	-	-	1.30 ± 0.16	1.29 ± 0.11	2.28 ± 0.08	1.74+0.11

All the results are measured by mean of inhibition zone in mm ± S.D of three replicates.

- No activity

Table 4. Bioassay of different concentration of synthesised silver nanoparticles (Fungi).

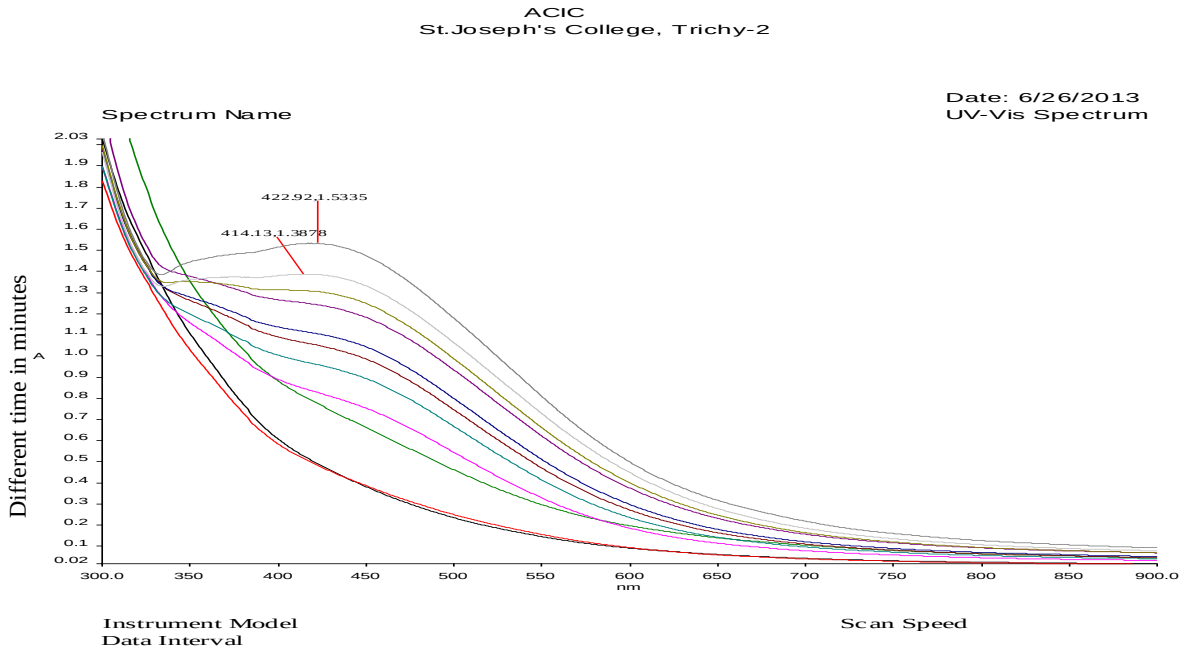

Figure 1. Showing the diagram of different time periods of peak variation of UV-Vis spectra of 1st plant extract.

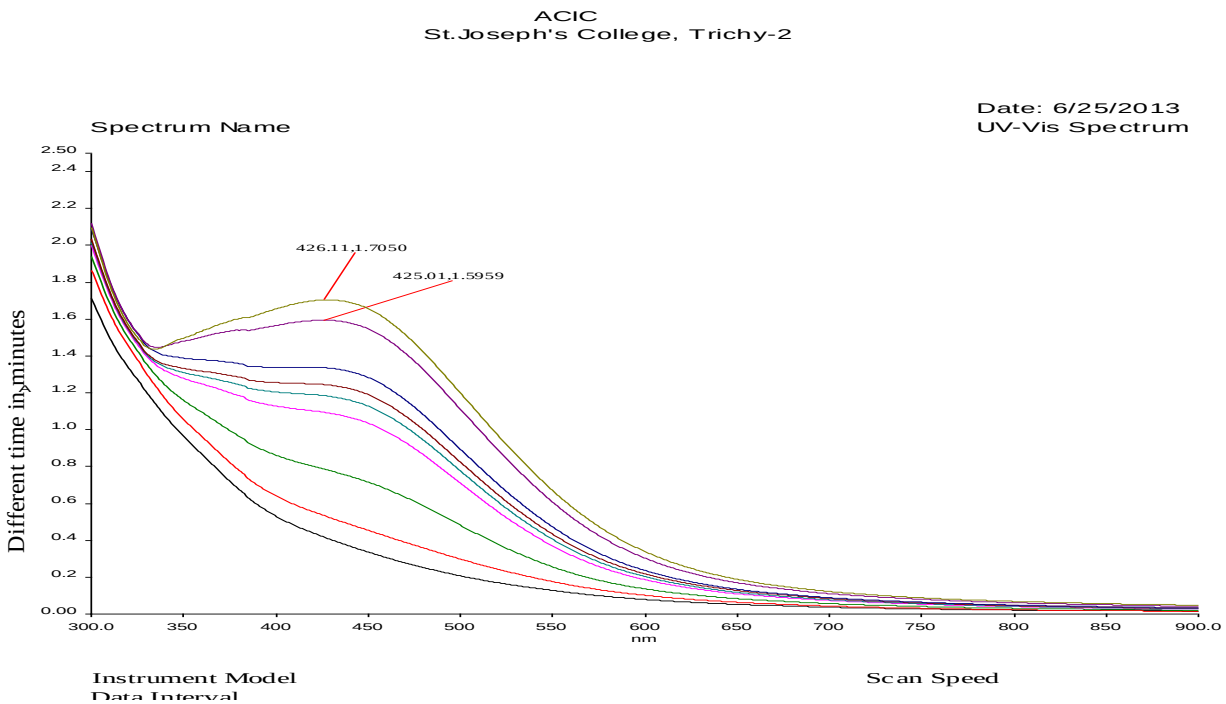

Figure 2. Showing the diagram of different time periods of peak variation of UV-Vis spectra of 2nd plant extract.

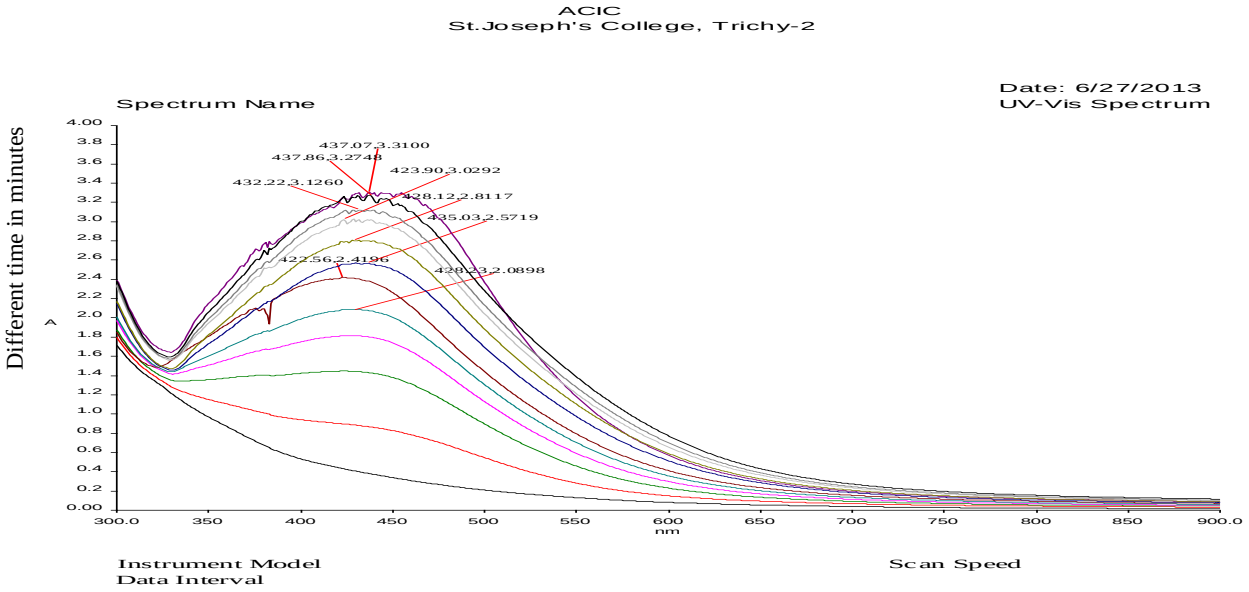

Figure 3. Showing the diagram of different time periods of peak variation of UV-Vis spectra of 3rd plant extract.

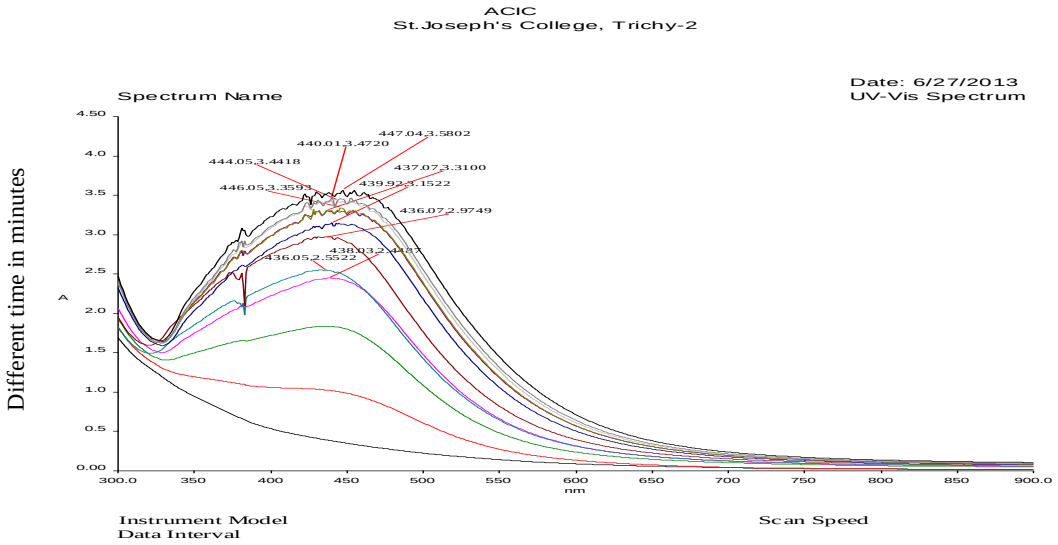

Figure 4. Showing the diagram of different time periods of peak variation of UV-Vis spectra of 4th plant extract.

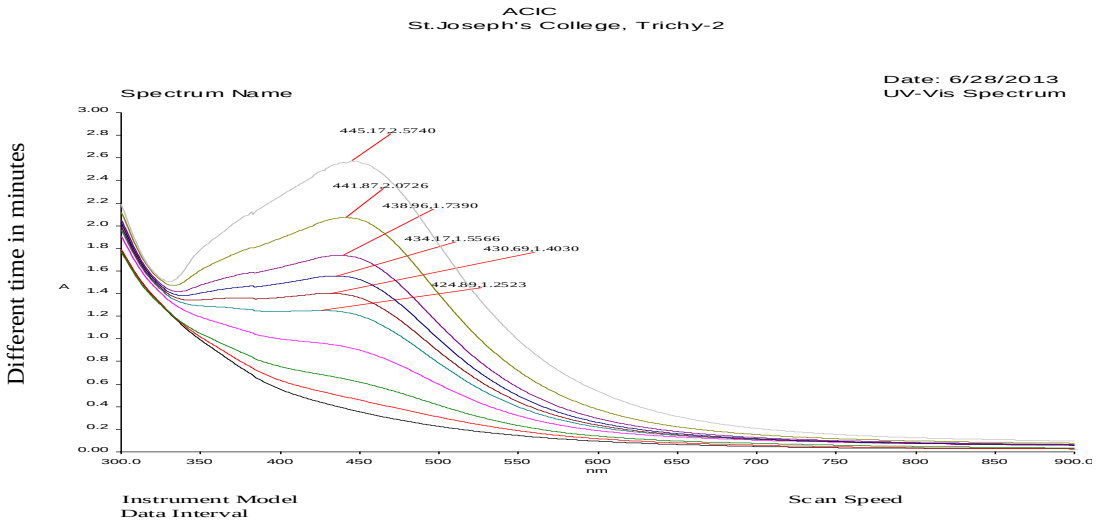

Figure 5. Showing the diagram of different time periods of peak variation of UV-Vis spectra of 5th plant extract.

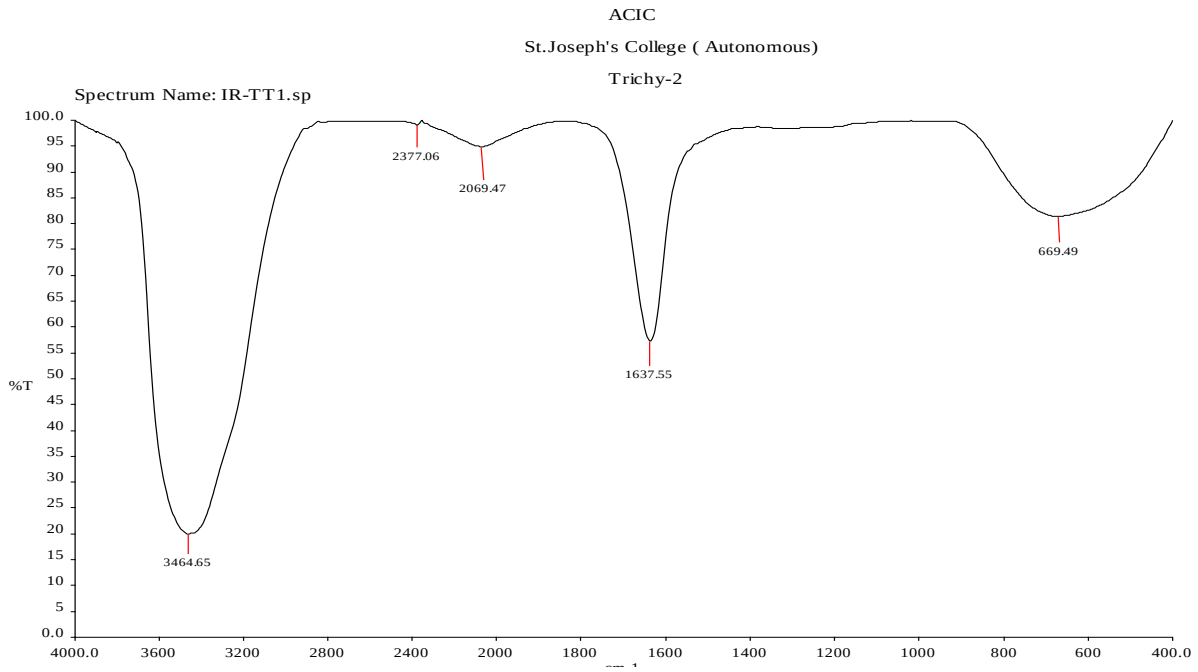

Figure 6. Showing the diagram of different time periods of peak variation of FTIR spectrum in 1st plant extract.

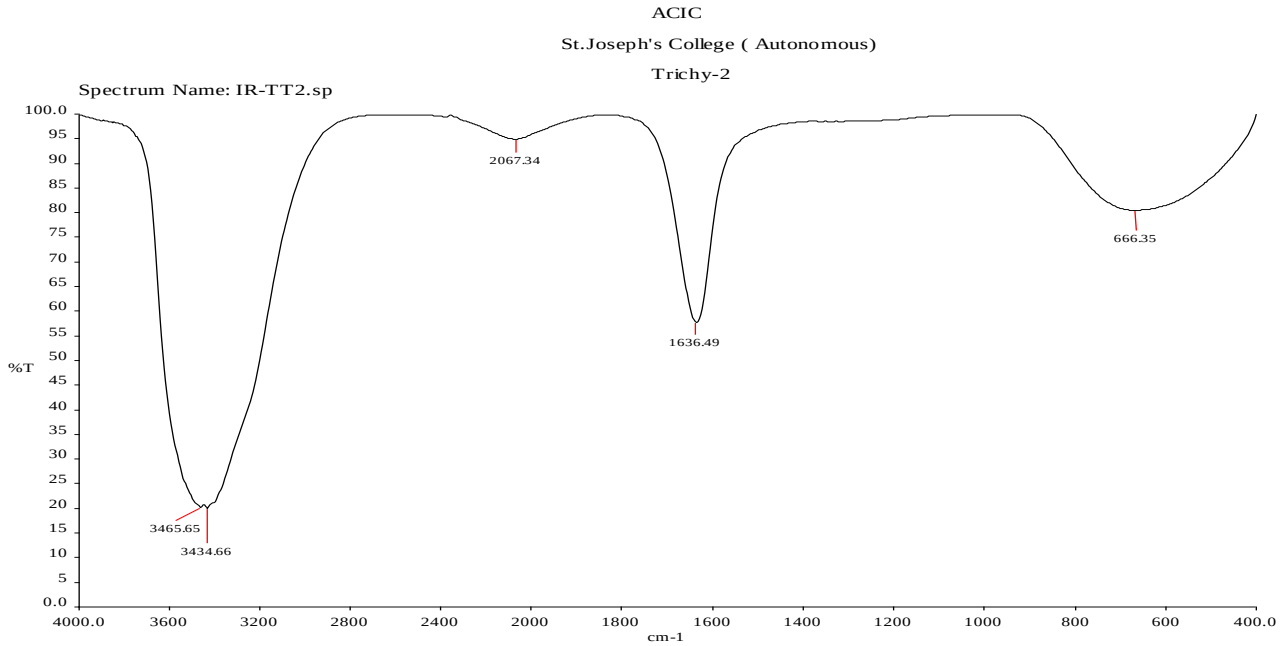

Figure 7. Showing the diagram of different time periods of peak variation of FTIR spectrum in 2nd plant extract.

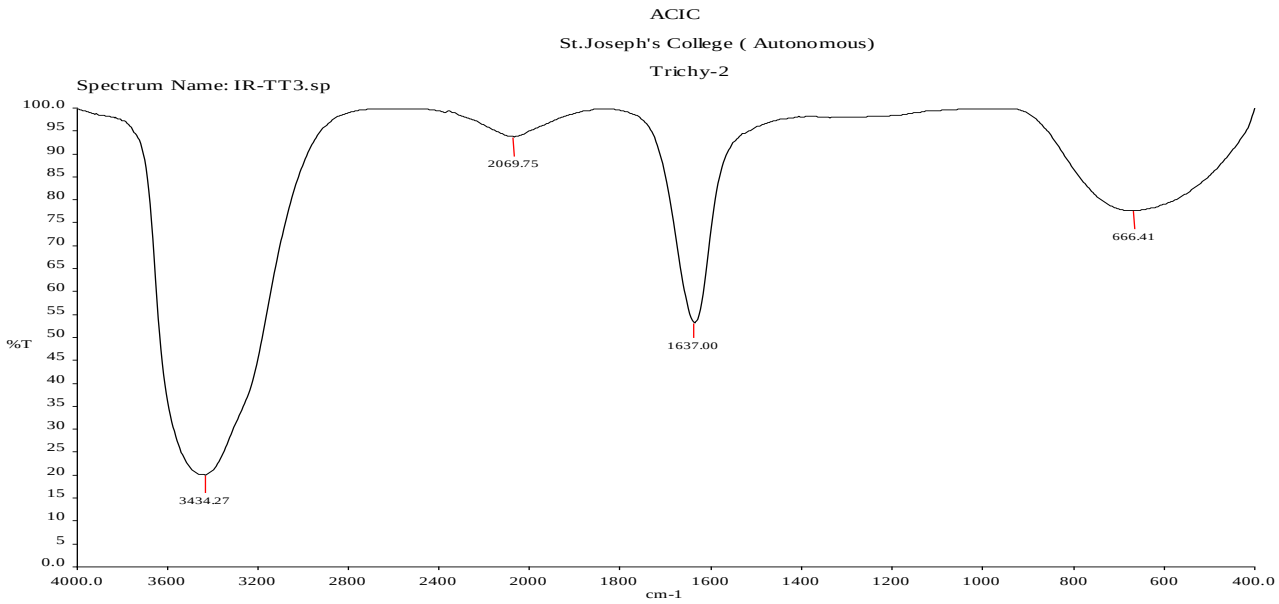

Figure 8. Showing the diagram of different time periods of peak variation of FTIR spectrum in 3rd plant extract.

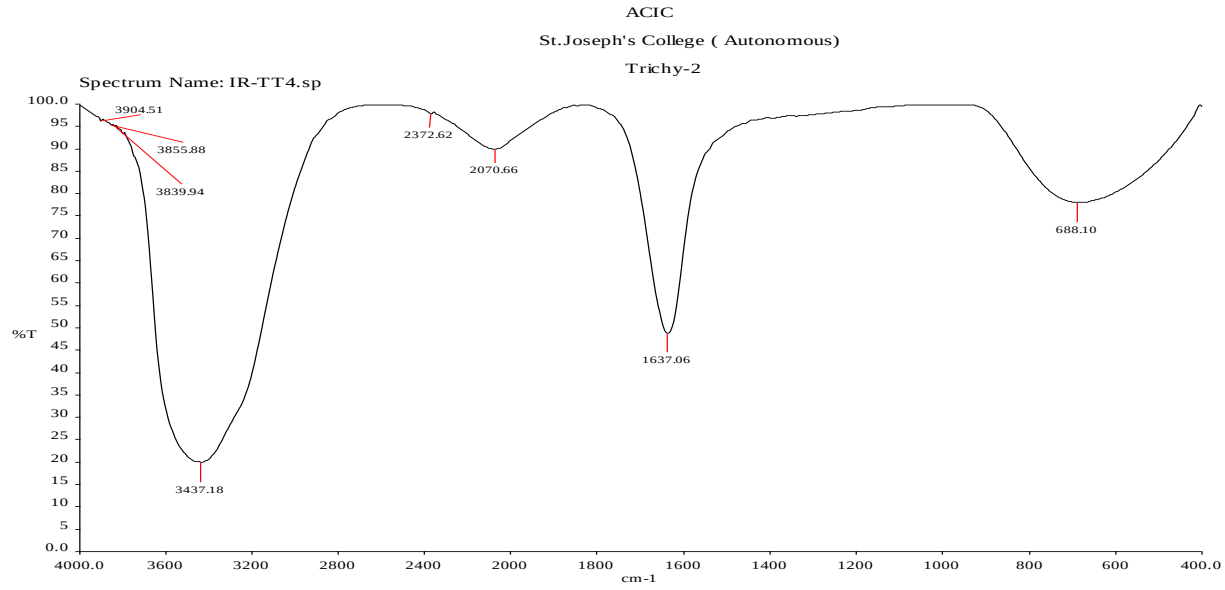

Figure 9. Showing the diagram of different time periods of peak variation of FTIR spectrum in 4th plant extract.

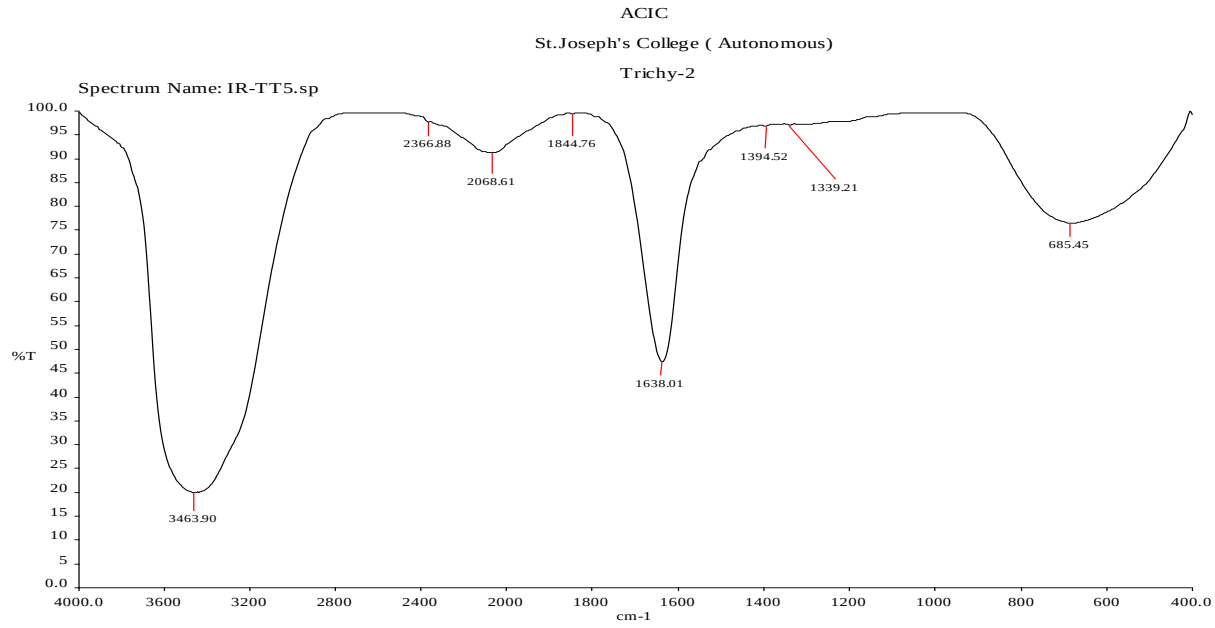

Figure 10. Showing the diagram of different time periods of peak variation of FTIR spectrum in 5th plant extract.

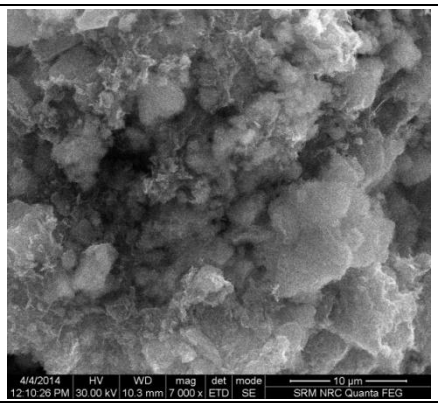

Fig 11. SEM images of the nanoparticles are synthesized from an aqueous solution of 1 mM $AgNO_3$ - Nano fibers.

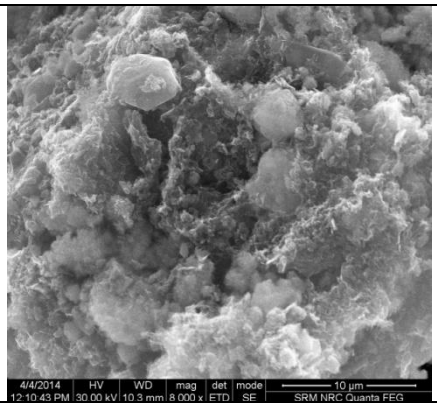

Fig 12. SEM image is shows the morphologies of surface functionalized silver nanoparticles powders in 1 mM $AgNO_3$ in aqueous solution

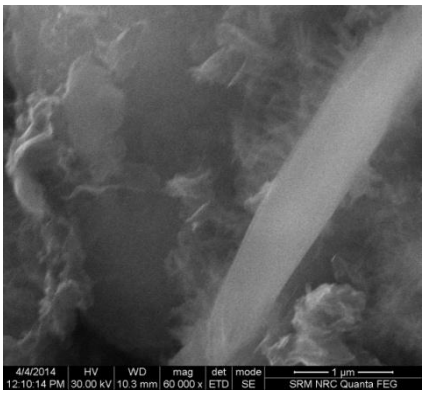

Fig 13. SEM images of the nanoparticles are synthesized from an aqueous solution of 1 mM $AgNO_3$ – Nano rods shape.

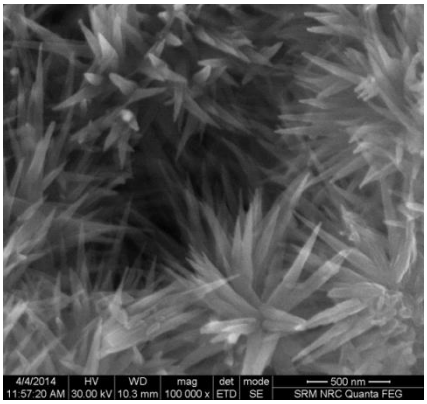

Fig 14. SEM images of the nanoparticles are synthesized from an aqueous solution of 1 mM $AgNO_3$ - Nano fibers.

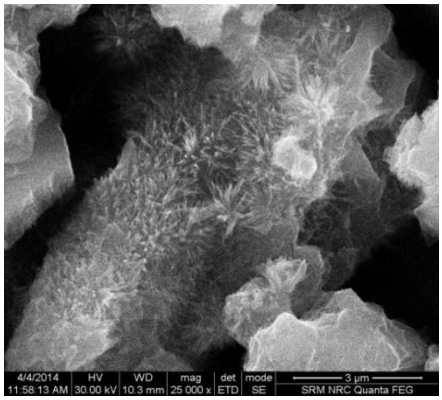

Fig 15. SEM images of the nanoparticles are synthesized from an aqueous solution of 1 mM $AgNO_3$ – morphology view.

Figures: SEM images are taken from different concentration of synthesized silver nanoparticles.

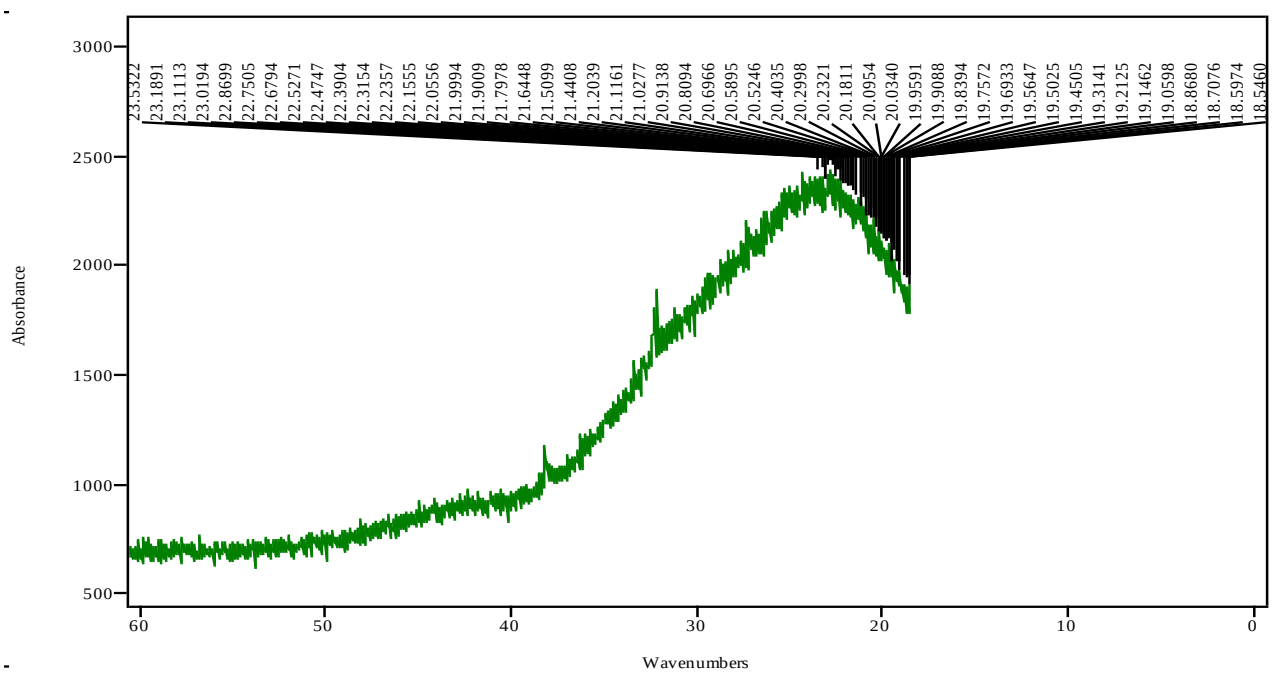

Fig 16. Sample-I

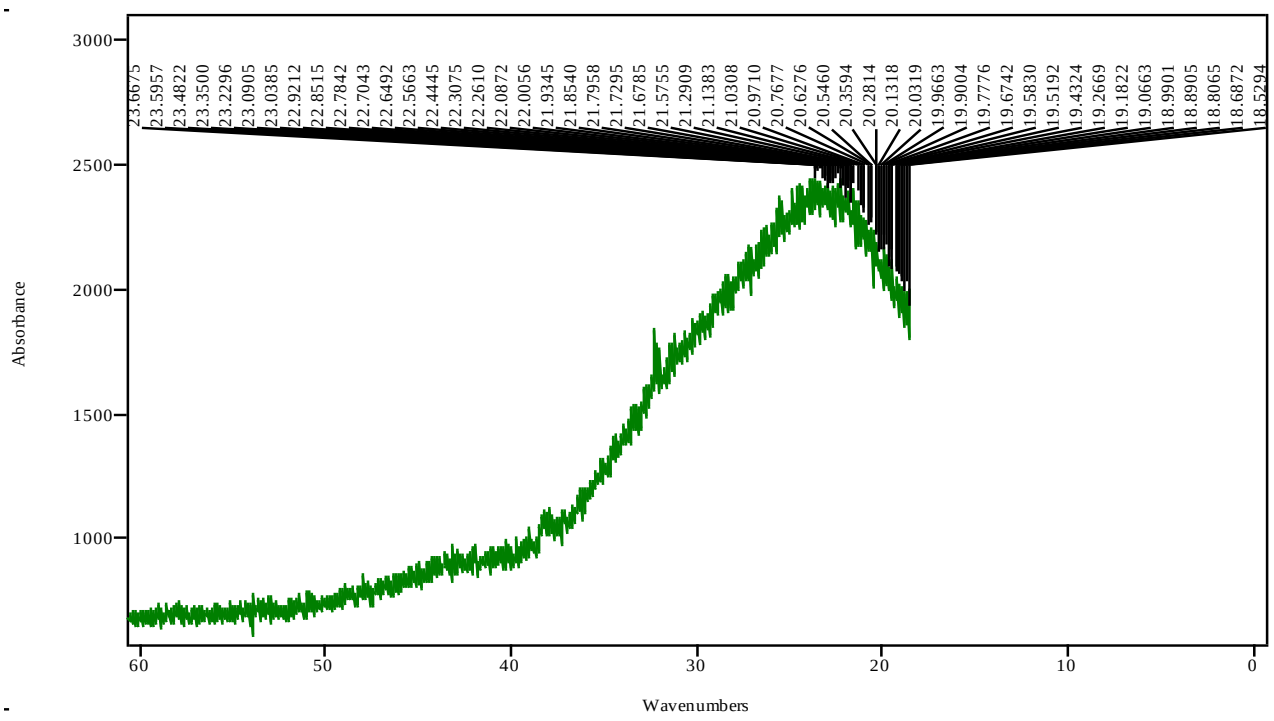

Fig 17. Sample-II

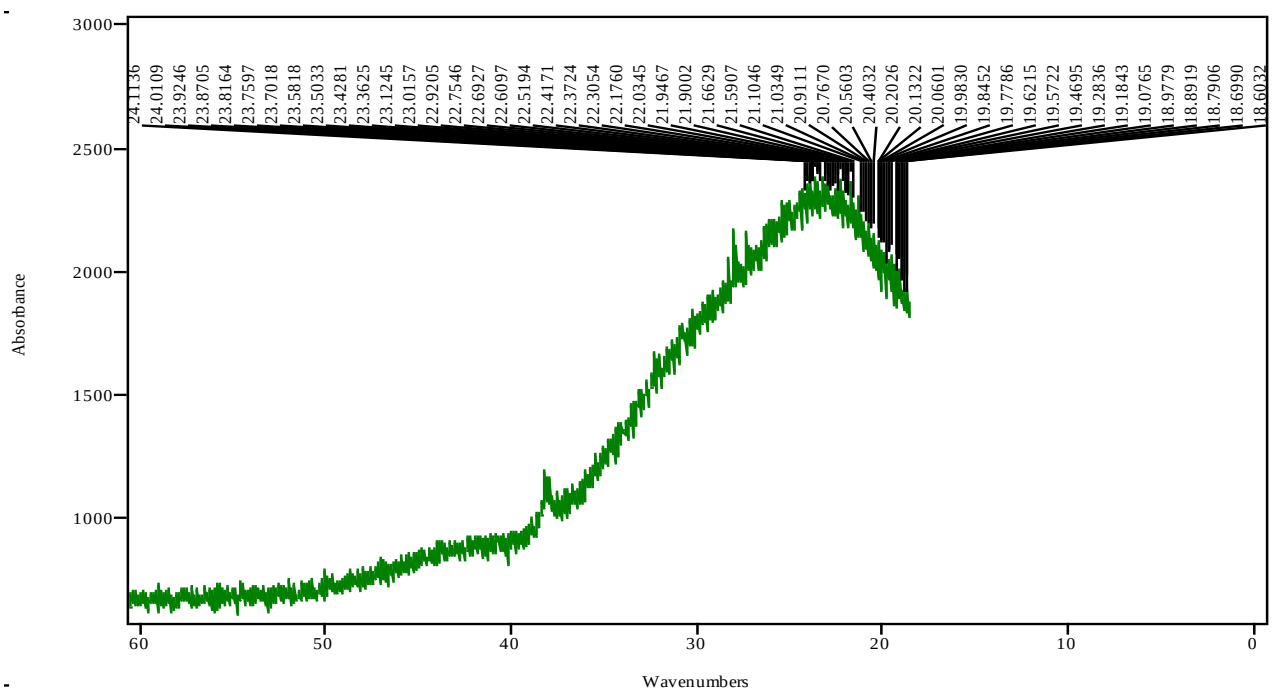

Fig 18. Sample-III

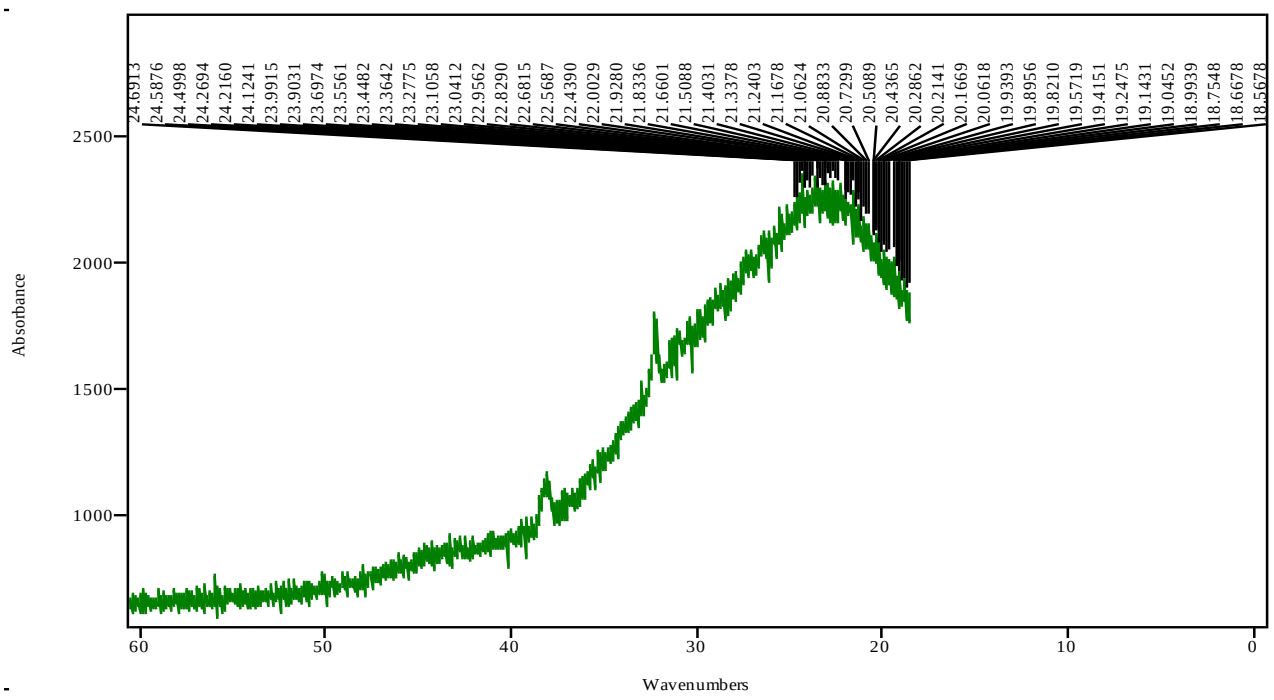

Fig 19. Sample-IV

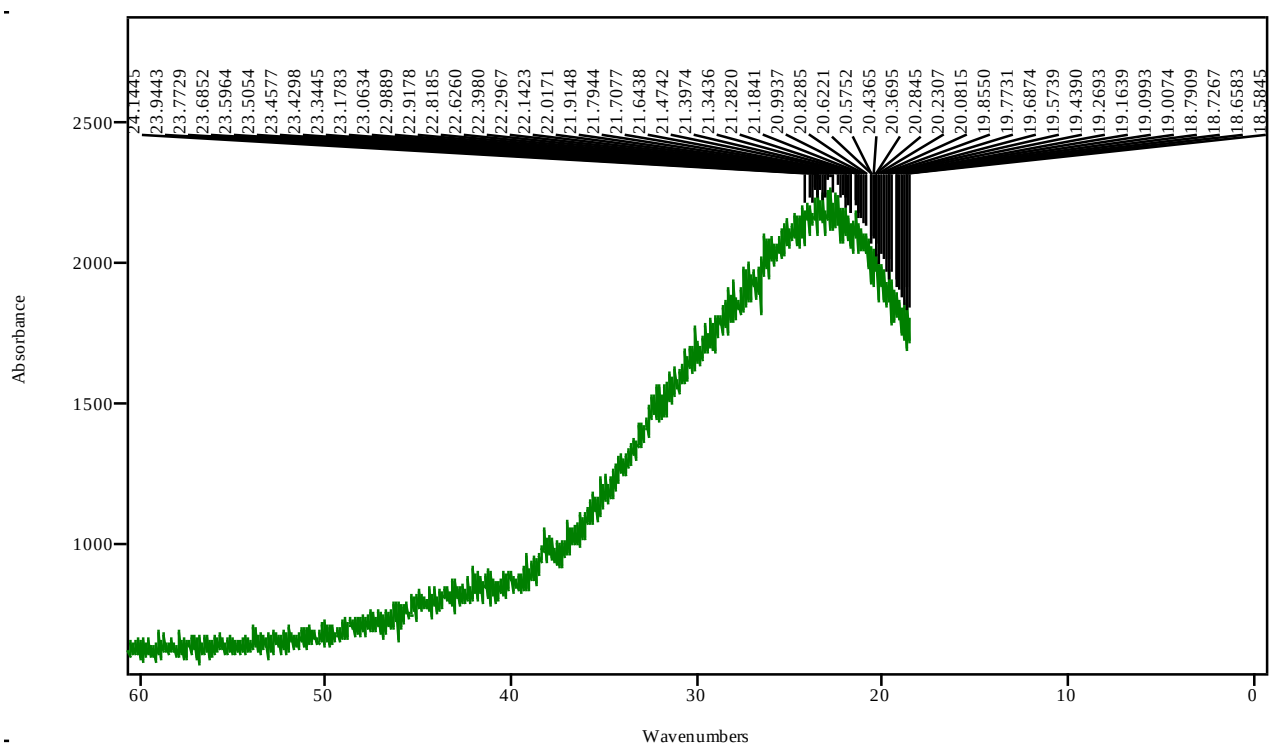

Fig 20. Sample-V

X-ray diffraction pattern of the different concentration of synthesized silver nanoparticles from plant extracts.

Acknowledgement

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