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BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING ENDOPHYTIC FUNGI *ASPERGILLUS CONCIUS, PENICILLIUM JANTHINELLUM* AND *PHOMOSIS SP.*

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ABSTRACT

The present study of seventy one endophytic fungi isolated from *Avicennia marina, Suaeda monica and Rhizophora mucronata* plant leaf. Among 34 genera and 71 species was identified by Lactophenol cotton blue mounting techniques. To induce the biosynthesis of silver nanoparticles ($AgNO_3$) using *Aspergillus conicus, Penicillium janthinellum* and *Phomosis* sp. And evaluate their antimicrobial potential. The characterization of silver nano particles on these fungi by FT-IR, UV, and Fre-SEM analysis to confirm the reduction and it is believed that protein might have played an important role in the stabilization of silver nanoparticles. The synthesized $AgNO_3$ were found to be extracellular polydispersed spherical or hexagonal particles ranging from 6-12mm, 8-14mm 10-16mm in size. Antibacterial activity was performed using a Agar well diffusion method against *Bacillus subtillus*, *E-coli, Enterobacter aurogens, Enterococcus, Klebseilla Pneumonia, Micrococus lutes, Salmonella typhi, Staphylococus aureus Streptococcus sp. Vibrio cholera.* $AgNO_3$ can be mycosynthesized extracelluraly using *A.Conicus, Penicillium janthinellum and Phomosis* sp. As the fungal system. Which is highly advantageous over chemical synthesis not only because in can be synthesized on a large scale, but because of the case of downstream processing and its biomedical activity.

INTRODUCTION: Nanotechnology is emerging field of science which involves synthesis and development of various nano materials. Silver compounds have also been used in the medical field to treat burns and a variety of infections. Commendable efforts have been made to explore this property using electron microscopy, which has revealed size, dependent interaction of silver nano particles with bacteria nano particles of silver have thus been studied as a medium for antibiotic delivery, and to synthesize composites for use as disinfecting filters and coating materials. However the bactericidal property of these nanoparticles depends on their stability in the growth medium, since this imparts greater retention time for bacterium nano particle interaction. There lies a strong challenge in preparing nanoparticles of silver stable enough to significantly restrict bacterial growth [1] (Li *et al*., 2007).

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Biologically synthesized silver nanoparticles have many applications, as in spectrally selected coatings for solar energy absorption, as intercelation material for electrical batteries, as optical receptors, as catalysts in chemical reactions and in bio-labelling.

Silver has been recognized to have inhibitory action on microbes present in medical and industrial process [2]. (Lok *et al.,* 2007). The most important application of silver and silver nanoparticles is in topical ointments to prevent infection against burn and open wounds. Chemical synthesis of nanopaticles leads to presence of traces of toxic chemical adsorbed on the surface which is undesirable in the medical applications of nanoparticles.

MATERIALS AND METHODS:

Collection of Soil Samples: The samples were collected from mangroves plant leaf at Karankadu Ramanathapuram District Plant leaf samples were collected employing sterile polythene bags [3] (Akinyanju and Fadayomi, 1989).

Isolation of Fungi: The plant leaf samples were surface sterilization and cut with 0.5 mm sized and were placed on potato dextrose Agar (PDA) and incubated at 30^0C for three days. Fungi was isolated from the mixed isolates from each plate and sub cultured on PDA. Sub culturing was continued until a pure isolate was obtained.

Characterization and Identification Isolates: Colonial morphology and microscopic examination of the various isolates of pure cultures were used to determine the reproductive and vegetative structures. Consequently, identification was done using onions [4] *et al.,* (1981). Spore identification was achieved by reference to spore atlases of Gregory [5] (1973) Anna [6] (1990) and Ainsworth *et al.,* (1973) [7].

Biosynthesis of Silver Nanoparticles: The fungal mat was washed thrice in deionized water to remove the unwanted material. Approximately 3.5 gm of fungal mat taken in a conical flask containing 100ml deionized water 10^{-1}mM $AgNO_3$ was added then it was incubated 37^oC for 3 days. After incubation period observed colour change.

The nanoparticles were characterized by UV-Vis spectroscopy, fourier transform infrared (FT-IR) spectroscopy and scanning electron microscope (SEM) analysis.

Characterization of Synthesized Silver Nanoparticles:

Ultra Violet Spectroscopy: The bioreduction of pour $AgNO_3$ are monitored using UV-Vis spectroscopy at regular intervals. During the reduction, 0.1ml of samples was taken and diluted several times with Millipore water. After dilution, it was centrifuged at 800 rpm for 5 minutes. The supernatant was scanned by UV-300 spectrophotometer (UNICAM). For UV-Vis 1601 schimodzu spectrophotometer, operated at a resolution of 420 nm.

FT-IR (Fourier Transform Infrared Spectroscopy) FTIR analysis of bioactive TLC Fractions: A known weight of sample (1 mg) was taken in a mortar and pestle and ground with 2.5 mg of dry potassium bromide (KBr). The powder so obtained was filled in a 2 mm internal diameter micro-cup and loaded onto FTIR set at 26^oC±1^oC. The samples were scanned using infrared in the range of 4000-400cm^{-1} using Fourier Transform Infrared Spectrometer (Thermo Nicolet Model-6700). The spectral data obtained were compared with the reference chart to identify the functional groups present in the sample.

Scanning Electron Microscopy (SEM): For SEM, the silver nanoparticle synthesized using Fungi was allowed to was allowed to dry completely and grounded well to a powder specimen is normally required to be completely dry. Since the specimen is at high vacuum. Living cells and tissues and whole, soft-bodied organisms usually require chemical fixation to preserve and stabilize.

Fixation is usually performed by incubation in a solution of a buffered chemical fixative, such as glutraldehyde. The fixed tissue is then dehydrated. The dry specimen was mounted on a specimen stub using and adhesive which as epoxy resin or electrically-conductive double-sided adhesive tape and sputter coated with gold palladium alloy before examination in the microscope.

Antibacterial activity of normal strain and silver nanoparticle synthesized strain (*Aspergillus conicus, Penicillium Janthinellum and Phomosis sp.*):

Preparation of Extract: 1 gm of silver nanoparticle synthesizing fungi were kept in the 10ml of organic solvent namely aqueous, n-Butanol and methanol, silver nanoparticle synthesizing fungi was grounded well with the help of mortar and pestle. The grained fungi was filtered through whatmann No.1 filter paper and the supernatant was collected, and stored for Antibacterial screening purpose.

Screening of Antibacterial Activity: The antibacterial activity of the aqueous and organic solvents (n-butanol and methanol) extracts from the normal strain and silver nanoparticle synthesized strain *(Aspergillus conicus, Penicillium Janthinellum, Phomosis sp)* were tested against the selected human pathogenic bacteria. The sterilized Nutrient agar medium was poured into each sterile petriplate and allowed to solidify using a sterile cotton swab. Fresh bacterial culture was spread over the plates by following spread plate technique. A well was cut on the solidified agar. The solvent extract was added into the each well. All the plates were incubated at 37^{o}C for 24-48hrs. After the inclubation, the zone of inhibition was observed.

RESULT AND DISCUSSION: The present study, number of fungi isolated from the plant leaf sample. Among the fungi isolated, seventy one identified. Among the identified isolates, only three fungi culture namely *A.conicus, Penicillium janthinellum* and *Phomosis* were predominantly found. *A. conicus, Penicillium janthinellum* and *Phomosis* were selected for the biosynthesis of silver nanoparticles abilities. This fungus dominantly presented in mangroves plant leaf sample so it was selected further study.

Biosynthesis of silver nanoparticle production: Fungal mat of *A. conicus, Penicillium Janthinellum* and *Phomosis* was mixed with silver nitrate solution and incubated in room temperature. The appearance of brown colour was due to the excitation of surface Plasmon vibrations. The control shows no change in colour of the mixture when incubated in the same conditions. The production of silver nanoparticles is high in *A.conicus, Penicillium Janthinellum* and *Phomosis* which sources dark brown colour *Phomosis*. Ahamed *et al*., (2003) reported that, cell free filtrate of *Penicillium sp.* was mixed with silver nitrate solution and incubated in dark in rotary shaker samples shows changed in colour from almost colourless to brown this is a clear indication of the formation of silver nanoparticles in the reaction mixture. The intensity of the colour was increased during the period of incubation. The appearance brown colour was due to the excitation of surface Plasmon vibrations.

Characterization of Silver Nanoparticle of UV-Vis Spectroscopy: Bio synthesis of silver nanoparticles using *A.conicus, Penicillium janthinellum* and *Phomosis* was monitored in the UV-Vis spectrophotometer. The UV-Vis spectra was recorded from the silver nitrate *A.Conicus, Penicillium Janthinellum* and *Phomosis.* UV-Vis spectra was recorded at 420nm after 24, 48 and 72 and 96 hours incubation (**Fig. 1**). In previous study synthesis of colloidal silver nanoparticles was initially performed by UV-Visible spectroscopic analysis. In UV-visible spectrum, a strong peak was observed between 400-200nm, indicate the presence of silver nanoparticles (Sastry *et.al*., 2003).

TABLE 1: ANTIMICROBIAL ACTIVITY OF BIOSYNTHESIS OF SILVER NANOPARTICILES USING A*SPERGILLUS CONCIUS.*

Sl. No.	Name Of The Microorganisms	Zone of Inhibition (Diameter in mm) Solvent		
		Ethanol	N-butanol	Aqueous
1.	*Micrococus*	12	10	10
2.	*Enterobacter*	10	14	11
3.	*Salmonella typhi*	10	13	-
4.	*Klebsiella pneumonia*	11	13	-
5.	*Staphylococus aureus*	13	12	-
6.	*Bacillus subtillus*	14	10	-
7.	*Vibrio cholera*	12	9	-
8.	*Streptococcus*	10	12	
9.	*E.coli*	9	10	10
10.	*Enterococus*	12	11	11

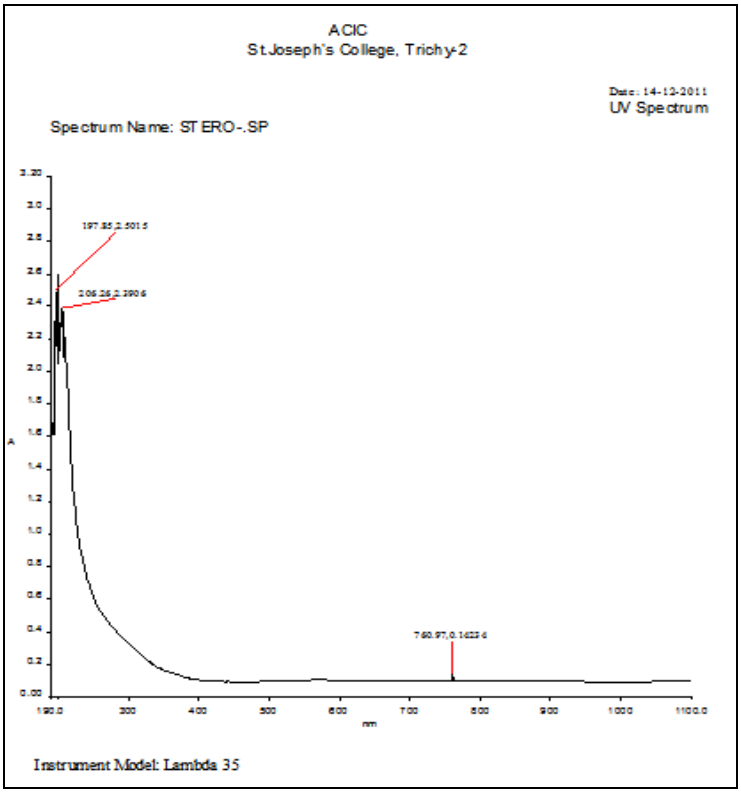
TABLE 1: ANTIMICROBIAL ACTIVITY OF BIOSYNTHESIS OF SILVER NANOPARTICILES USING *PHOMOSIS SP*

Sl. No.	Name Of The Micro Organisms	Zone of Inhibition (Diameter in mm) Solvent		
		Ethanol	N-butenol	Aqueous
1.	*Micrococus*	10	13	11
2.	*Enterobacter*	11	14	12
3.	*Salmonella typhi*	12	13	13
4.	*Klebsiella pneumonia*	13	11	10
5.	*Staphylococus aureus*	14	12	10
6.	*Bacillus subtillus*	10	10	11
7.	*Vibrio cholera*	9	15	12
8.	*Streptococcus*	11	12	13
9.	*E.coli*	12	10	14
10.	*Enterococus*	13	12	10

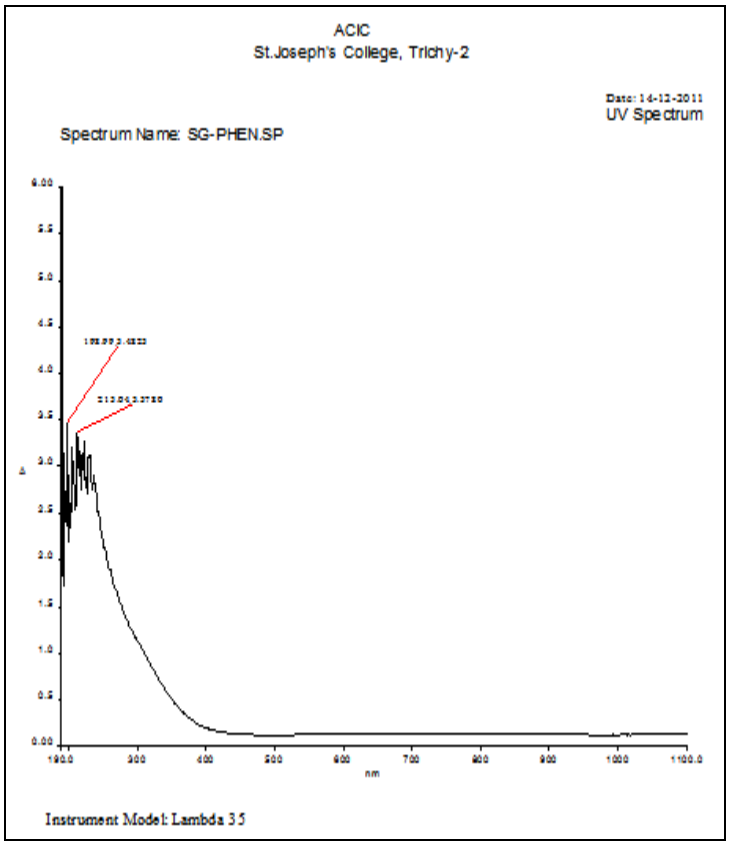
TABLE 1: ANTIMICROBIAL ACTIVITY OF BIOSYNTHESIS OF SILVER NANOPARTICILES USING *PENICILLIUM JANTHINELLUM*

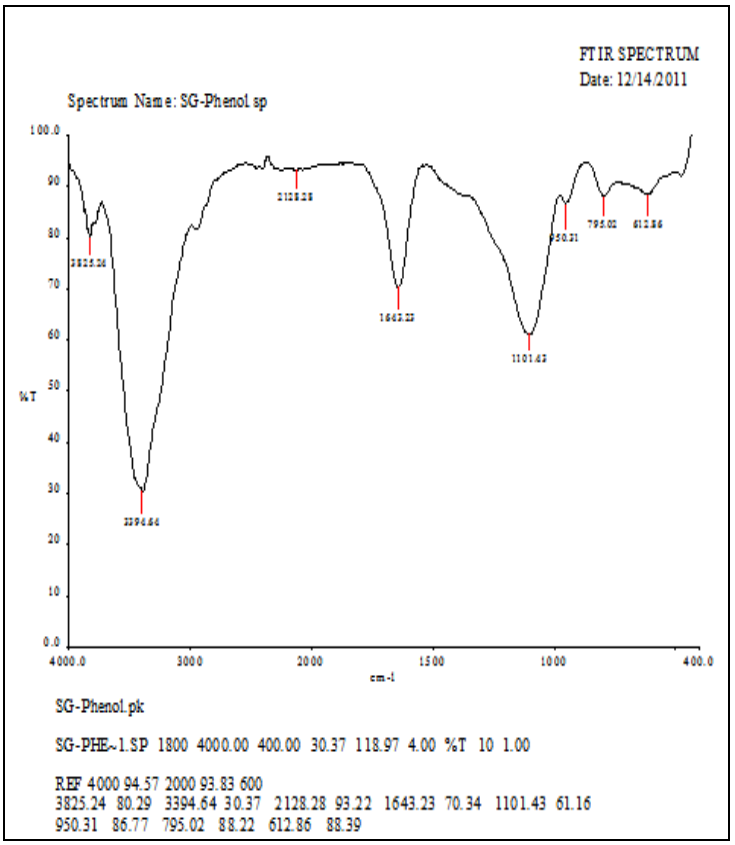
Sl. No.	Name Of The Micro Organisms	Zone of Inhibition (Diameter in mm) Solvent		
		Ethanol	N-butenol	Aqueous
1.	*Micrococus*	7	10	-
2.	*Enterobacter*	10	9	-
3.	*Salmonella typhi*	8	8	-
4.	*Klebsiella pneumonia*	11	10	-
5.	*Staphylococus aureus*	9	9	-
6.	*Bacillus subtillus*	6	7	-
7.	*Vibrio cholera*	7	6	-
8.	*Streptococcus*	10	11	-
9.	*E.coli*	10	9	-
10.	*Enterococus*	9	11	-

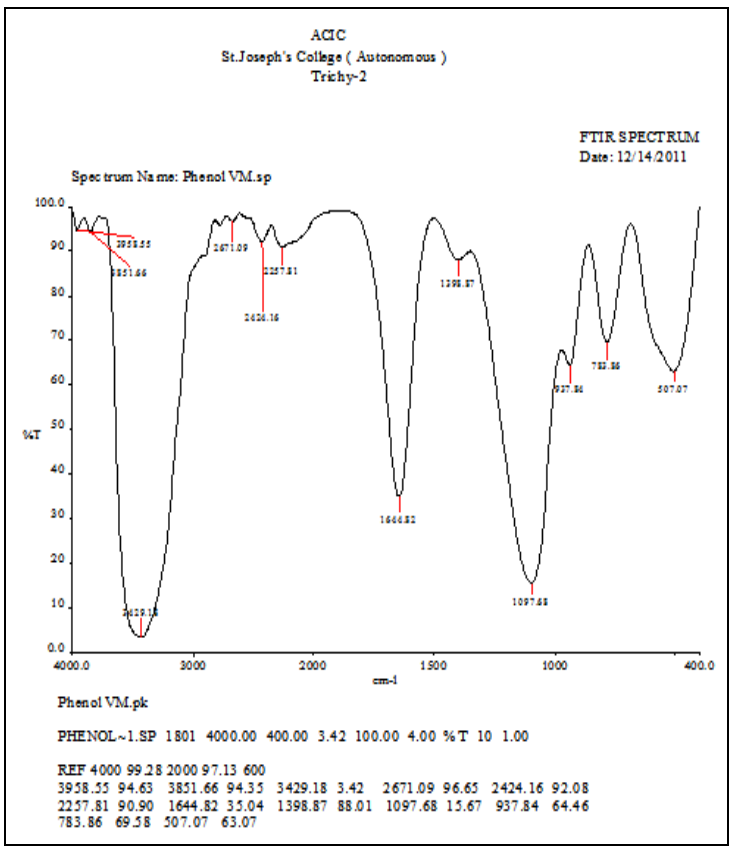
Identifications of functional group using FT-IR: FT-IR measurements of the freeze dried samples were carried out to identify the possible interactions between silver and bioactive molecules, which may be responsible for synthesis and stabilization (capping material) of silver nanoparticles. The amide linkages between amino acid residues in proteins give rise to well known signatures in the infrared region of electromagnetic spectrum (**Fig. 1**).



The lyophilized nanoparticles samples were analyzed in FT-IR to identify the possible biomolecules responsible for the reduction of the Ag^+ ions by the cell filtrate. The representative spectra of nanoparticles obtained manifest absorption peak located at about 3843.68cm^{-1} (-NH group of amines). 3597.73 cm^{-1} (-OH group of phenols), 2080 65cm^{-1} (aromatic-CH stretching) 1631.66cm^{-1} (-NHCO of amide) and 767.16cm^{-1} (C-Cl) Naveen *et al*., (2010).







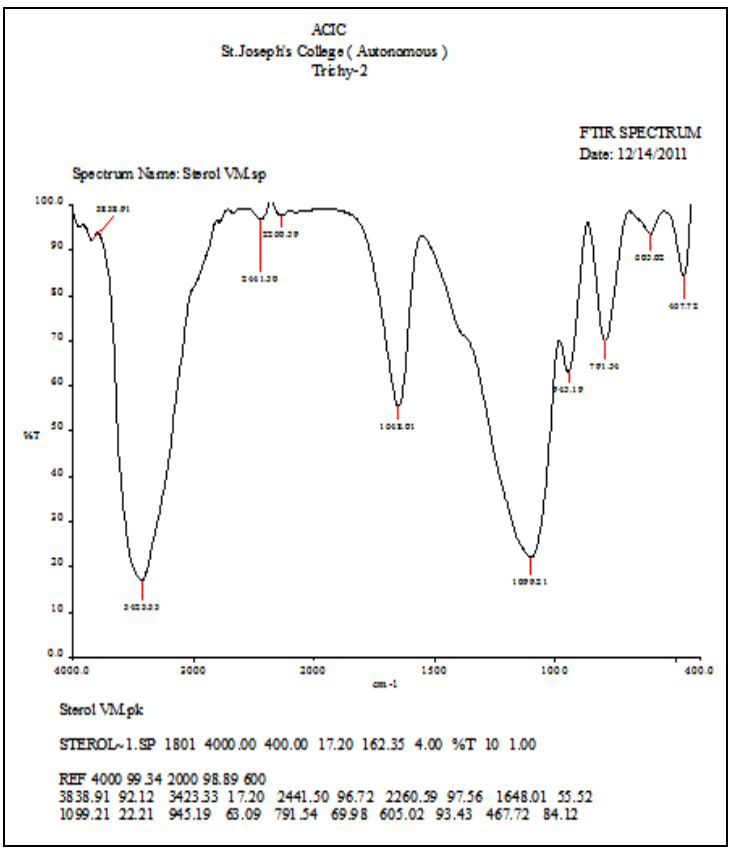
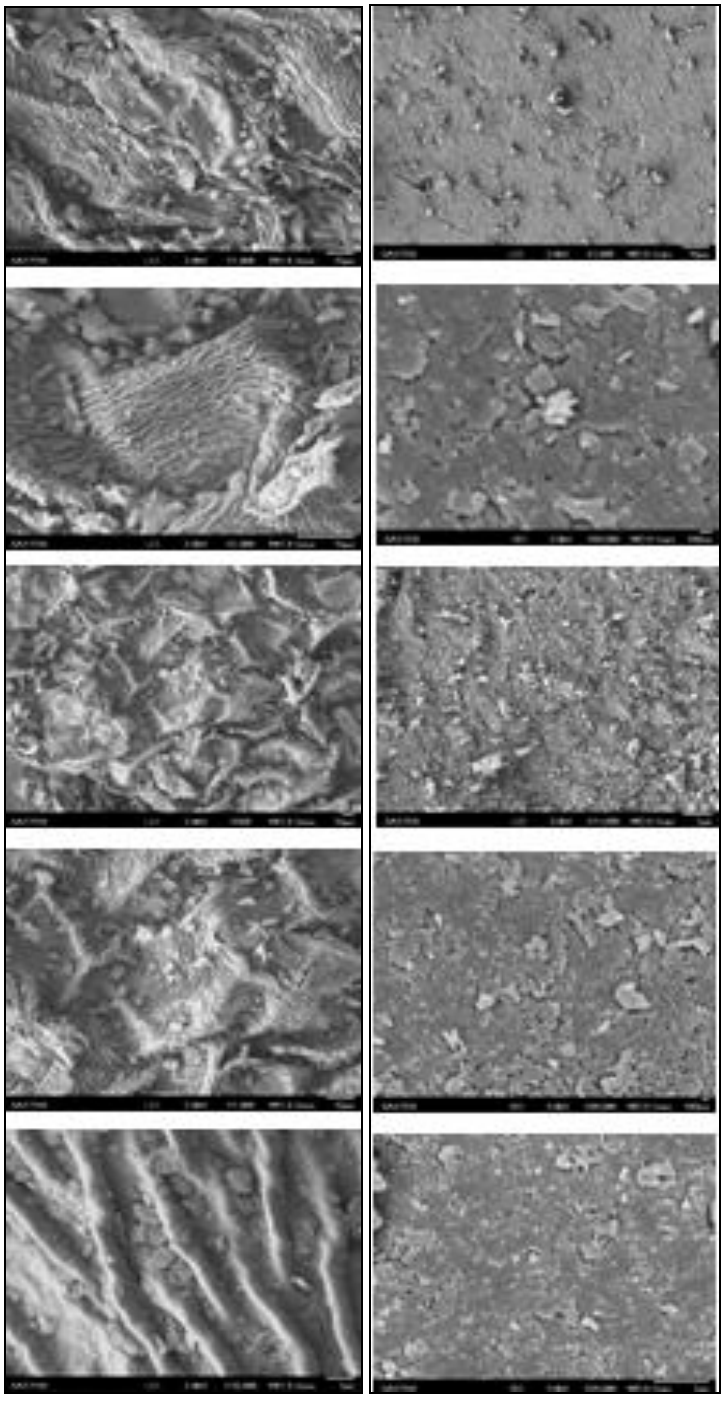


FIGURE 1: FTIR SPECTRA

Scanning Electron Microscope (SEM): Fungal species were inoculated in the silver nitrate was selected and it was characterized by scanning electron microscope. Scanning electron microscope analysis was used measure the size of silver nanoparticles. In this analysis the size nanoparticles 80μm and 120μm, size silver nanoparticle obtained as *A. Conicus, Penicillium Janthinellum* and *Phomosis* (**Fig. 3**). In previous study the size of synthesized nanoparticles was found to be 58.33 ± 17.88nm by SEM analysis [8] (Shaligram *et.al*., 2009).



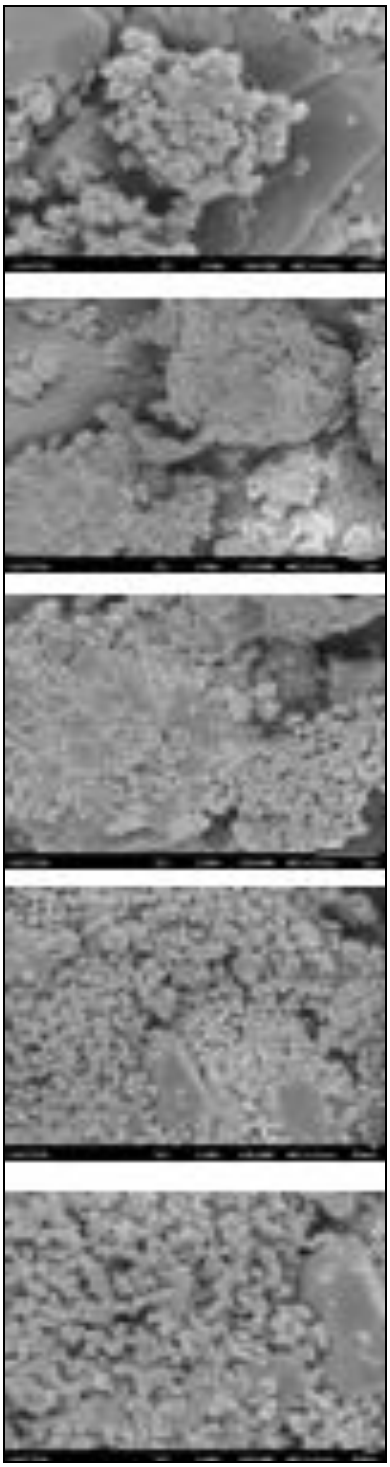


FIGURE 2: SCANNING ELECTRON MICROSCOPE

Screening of antibacterial activity by Agar Well Diffusion Method: The organic solvents extracts of *A.Conicus, Penicillium Janthinellum* and *Phomosis* shows better zone of inhibition 12mm, 8mm, 6 mm reagent in n-Butanol with *Vibrio cholerae, Salmonella typhi* and *Staphylococcus aureus* than methanol (**Fig. 3**). There are no significant results in aqueous extracts of *A. Conicus, Penicillium Janthinellum* and *Phomosis.* The results were compared with silver synthesized

strain *A. Conicus, Penicillium Janthinellum* and *Phomosis.* Silver synthesized *A. Conicus, Penicillium Janthinellum* and *Phomosis* shows better zone of inhibition (Plate-6). It was observed in n-Butanol as 10 nm in diameter for *Vibrio cholerae* and *Staphylococcus aureus* followed by *Salmonella typhi* (15 mm in diameter) and the methanol extracts shows moderate activity against tested pathogens 10 mm, 5 mm in diameter. The aqueous extracts of silver synthesized *A. Conicus, Penicillium Janthinellum* and *Phomosis* shows significant results as 7.5mm, 7.5mm, and 6mm rather than n-Butanol and methanol (**Fig. 4**). In previous study, the biological synthesis of silver nanoparticles was found to be most active against the clinically isolated human pathogenic bacterias. The results proved that silver nanoparticles showed maximum activity at least concentration, which revealed silver nanoparticles as novel antibacterial agent. (Thirumurugan *et al,* 2009).

TABLE 2: DETECTION OF VARIOUS FUNCTIONAL GROUPS BY FT-IR FROM *A. CONICUS, PENICILLIUM JANTHINELLUM* AND *PHOMOSIS*

S. No.	Group frequency cm^{-1} of the sample	Functional Group Assignment
1.	3406.98	N-H Stretch, Primary Two Bands, Amine N-H Stretching
2.	2925.89	Chelating Compound Co-H Stretching vibration free OH
3.	2861.34	C-H alkalines, C-H stretching vibrations two band (aldehyde)
4.	2356.28	Hydrocarbon chromophone, C-H Stretching (Alkane)
5.	2145.24	-N = C = N- stretching vibrations, diamides
6.	1731.49	Cyclic, β lactams, dilute solution
7.	1647.67	C-C alkene / ketone stretching β dilution, -N=N- stretching
8.	1559.32	N-H, amine salt, β diketone, primary amide -N-H, Coo^{-} aromatic
9.	1449.90	Aromatic
10.	1379.47	Coo- anion, OH-Phenol (Sulfonyl Chlorides)
11.	1073.88	(C-F) Halogen Compound C-X stretching Vibrations

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