



Preparation of silver nano particles using various chemical extracts of *Agaricus bisporus*

Mita R Patel^{1*}, Rasmikant A Patel², Necklin A Pithawala³, M Dhana Lakshmi⁴, Kespi A Pithawala⁵

¹ Principal, Government College, Santalpur, Patan, Gujarat, India

² Department of Chemistry, Municipal Arts and Urban Science College, Mehsana, Gujarat, India

³ Department of Biology, Gujarat Arts and Science College, Ahmedabad, Gujarat, India

⁴ School of Engineering and Applied Science Ahmedabad University, Gujarat, India

⁵ Department of Zoology, H and H B Kotak Institute of Science, Rajkot, Gujarat, India

Abstract

The present work examined the amalgamation of silver nanoparticles (AgNPs) by organic technique utilizing various chemical concentrates of *Agaricus bisporus*. Nanotechnology manages the Nanoparticles having a size of 1-100 nm in one measurement utilized essentially concerning medicinal science, nuclear material science, and some other well-known fields. Nanoparticles are utilized monstrosly because of little size, easy intrusion; remarkable physical properties and which are apparently appeared to change the performance of some other material which is in contact with these tiny particles. These particles can be arranged effortlessly by different chemical, physical, and biological approaches. Yet, the natural approach is the most developing methodology of planning, since, this strategy is simpler than alternate strategies, ecofriendly and less tedious so the Green synthesis was done by utilizing the aqueous solution of edible mushroom *Agaricus bisporus* and AgNO₃. Silver was of specific interest because of its reminiscent physical and chemical properties. A settled proportion of mushroom extract to metal particle was prepared and the shading change was watched which demonstrated the development of nanoparticles. The nanoparticles were portrayed by UV-Vis Spectrophotometer, FTIR, EDX, and SEM. The nanoparticles when formed were found to have the size extents from 60-80 nm which after some days grew up to larger sizes and flocked.

Keywords: silver nanoparticles, *Agaricus bisporus*, SEM, EDX, FTIR, antibacterial activity, antioxidant activity, green synthesis

Introduction

In late science Nanotechnology is a consuming field for the researchers. Nanotechnology manages the nanoparticles having a size of 1-100 nm in one measurement utilized essentially concerning medicinal science, nuclear material science, and all other known fields. Nanoparticles are utilized monstrosly because of its little size, introduction, physical properties, which are apparently appeared to change the performance of some other material which is in contact with these tiny particles. These particles can be arranged effortlessly by different chemical, physical, and biological approaches^[1]. Yet, the natural approach is the most developing methodology of planning, since, this strategy is simpler, ecofriendly and less tedious than alternate strategies. Nanotechnology is also being utilized in diagnosis, therapeutics and treatments for many disease and disorders^[2, 4]. It is an enormously powerful technology, which holds a huge promise for the design and development of many types of novel products with its potential medical applications on early disease detection, treatment and prevention. There is a need to develop a green chemistry approach for nano materials synthesis^[5, 9]. In these aspects synthetic methods based on naturally occurring biomaterials offers an alternative means for obtaining these nano particles^[10]. The bio- route for the synthesis of nanoparticles includes employing microorganisms such as *Pseudomonas stutzeri*, *Plectonemaboryanum UTEX485*, *Verticillium sp.*, *Fusariumoxysporium*, *Fusarium semitectum*, *MKY3* (yeast),

Thermomonospora sp.^[11, 12] and also an endless list of a variety of plants like *Avena sativa*, *Aloe vera*, *Ficus religiosa*, *Psidiumguajava*^[13, 16] etc. Only few mushrooms (spore bearing fruiting body of fungus) are used for this purpose i.e. *Volvariella volvacea*, *Pleurotus sajor*^[17]. In this work we have used various solvent extracts of edible mushroom *Agaricus bisporus* which is also known as button mushroom or Portobello mushroom for the synthesis of bio-functionalized silver nanoparticles (AgNPs). This mushroom proves its medicinal property as antioxidant and as antitumor agent^[18]. The present study focuses on the synthesis of Silver nanoparticles and its optimizations.

Materials and Method

Collection of mushrooms

Fresh mushrooms *Agaricus bisporus* (white button mushrooms) were procured from commercial sources

Preparation of fresh extracts of mushroom

After collection, the mushroom samples were cleaned by rubbing, scrapping and brushing. The removal of all foreign material was confirmed. Thereafter they were cut in small pieces of around 2 to 3cm across using machete. Then they were ground using motor and pestle. For preparing fresh extracts of mushroom Distilled water, Methanol, Isopropanol, Chloroform, Acetone (50:50v/v) were used as solvents. To every 1 gram of pulverized material,

50ml of solvent was used. The extracts were filtered through Whatman #1 filter paper.

Preparation of dry extracts of mushroom

After collection, the mushrooms samples were wrapped in newspaper and stored in moisture free open places. They were air-dried in shade that took 15 days or more. Mushroom samples were collected and cleaned by rubbing, scrapping and brushing. The removal of all foreign matters was confirmed. Thereafter they were cut in small pieces of around 2 to 3 cm across using a machete. Then they were ground using mortar and pestle. The smashed pieces were further dried at 45°C for an hour and immediately powdered in a grinder. The powder was collected and ground again at the end. For preparing extracts Distilled water, Methanol, Isopropanol, Chloroform, Acetone (50:50v/v) were used as solvents. For every 1 gram of powder, 50ml of solvent was used and was subjected to extraction using a reflux apparatus. After the completion of extraction, the supernatant was filtered through Whatman #1 filter paper.

Preliminary phytochemical screening

The freshly prepared extracts were subjected to standard phytochemical analysis to ensure the presence of following phytoconstituents viz. Alkaloids, saponins, steroids, phenols, glycosides, terpenoids and flavonoids.

Estimation of aggregate phenolic content in mushroom

Phenolic compound in mushroom concentrates are assessed by colorimetric test, in view of makers portrayed by Singleton and Rossi, 1965 with a few changes. Phenolic compound fills in as capable cancer prevention agents as a result of the hydrogen giving properties of their phenolic hydroxylic bunch; As well as by giving electrons to stop free radical chain response rising up out of oxidative anxiety. Take out ml of concentrate of mushroom and blend altogether with ml of Folin-Ciocalteu reagent. After mins, 1ml of 7.5% sodium carbonate (Na_2CO_3) is added and permitted to respond for 2 hours at room temperature. The absorbance is measured at 765 nm against a clear having all the reagent barring the specimen utilizing spectrophotometer. The aggregate phenols are evaluated by standard bend of Gallic corrosive. The aggregate phenol qualities are communicated as mg of Gallic corrosive counterparts (GAE)/g of dry concentrate.

Synthesis of silver nanoparticles

To 3 ml of 0.01mM AgNO_3 1ml of Mushroom extract was included and at interim of each 5 minutes colorimetric readings were taken at 410nm to discover the nearness of arrangement of nanoparticles the shading change meant that arrangement of nanoparticles. These were framed inside 15 minutes and these particles were steady for around one month.

Biosynthesis of silver nanoparticles

Synthesis of silver nanoparticles utilizing *Agaricus bisporus* concentrate was watched at the point when mushroom concentrate of various focuses was subjected to watery arrangement of 0.01M silver nitrate, a progressive change of shading was seen following 12 hours and changed its shading to rosy darker. This change of shading could be because of the development of silver nanoparticles of differing shape and size.

The development of decreased AgNPs response blend was additionally portrayed by UV-vis spectrophotometry.

Characterization strategies

The biosynthesized silver nanoparticles were portrayed by the accompanying strategies:

Visual observation

A change of shading from light yellow to ruddy dark colored was seen in the arrangement after noticeable illumination.

UV Spectrometric examination

The portrayal strategy includes ultra-violet and obvious spectroscopy. UV-Vis assimilation spectra were measured utilizing Systronic UV-117 spectrometer from 360nm to 700nm constantly and the mushroom powder extract was utilized as the reference for the standard adjustment.

Fourier transform infrared spectroscopy

FTIR examination was completed to decide the functional group show in mushroom extract and their conceivable inclusion in the synthesis of silver nanoparticles. FTIR examination was conveyed utilizing a FTIR SHIMADZU 8300 instrument with a wavelength scope of 4000 to 400 cm^{-1} . The outcomes were looked at for shift in functional peaks. A FTIR graph can be helpful for preliminary examination of surface science of biogenic nanoparticles (i.e. those chemicals that contain carbon). This method is broadly utilized for distinguishing proof of chemical residues, for example amine, carbonyl and hydroxyl functional group in a molecule. The FTIR examination was performed with reduced silver nanoparticles. All estimations were done in the range cm^{-1} at a resolution of 4 cm^{-1} . For this sample were sent for FTIR Analysis at Gujarat Laboratory, Ahmedabad. Sample with total of volume 1-2 ml were given in watery frame framed by creating AgNPs utilizing the reduction reaction of 3ml of 0.01 mM Silver Nitrate arrangement through 1 ml of Mushroom extract.

SEM (Scanning electron microscopy) and EDX (Energy dispersive X-ray analysis)

The surface morphology of silver nanoparticles was analyzed utilizing a filtering electron microscopy (6010 LA, Jeol). The morphological elements of incorporated silver nanoparticles from concentrate were considered by Scanning Electron Microscope (JSM-6480 LV). After 24Hrs. of the expansion of AgNO_3 the SEM slides were set up by making a spread of the arrangements on slides. At that point the specimens were subjected to SEM at a quickening voltage of 20 KV

Antibacterial action

Trial of microscopic organisms utilized

Human pathogens were obtained from the Department of Microbiology, Gujarat College, Ahmedabad. The Gram positive species namely *Staphylococcus aureus* MTCC-96, *Bacillus subtilis* MTCC-4411 and Gram negative species were *Escherichia coli* MTCC-425, *Salmonellatyphi* MTCC-733 were used to study the antibacterial activity. The Nutrient agar, Agar-agar type was used for growing the test bacterial strains and was maintained on corresponding agar slants at 4°C.

Preparation of microbial inoculums

The young bacterial cultures were prepared and used during the research period. The nutrient broth (NB) was prepared and then poured into several tubes. Then these tubes were sterilized. The pure bacterial strains were collected from the institute and then inoculated. The tubes were incubated at 37°C for 24-48 hours and the cultures were used for the experiments.

Antibacterial activity

The antibacterial exercises of the blend of silver nanoparticle, mushroom remove what's more, control were tried against the chosen bacterial strains. The 20ml of disinfected agar medium was filled each sterile petri plates and permitted to cement. The test bacterial societies were equitably spread over the proper media by utilizing a sterile cotton swab. At that point a well of 0.5cm was made in the medium by utilizing a sterile plug borer, 200µl of each as *Agaricus bisporus* extracts and also of silver nanoparticle were moved into partitioned wells. These plates were brooded at 37°C for 24 -48 hours. After brooding period, the outcomes were watched and measured the width of hindrance zone around the each well.

Anti-microbial affectability test on organisms (Positive control)

The anti-infection affectability test utilizing standard anti-infection agents (ampicillin, streptomycin and antibiotic medication) were investigated by the technique for Bauer *et al.*, (1996). The sanitized supplement agar medium was filled each sterile Petri plates and permitted to set. By utilizing a sterile cotton swabs, a new bacterial culture with known populace number was spread over the plates by taking after spread plate method. At that point the chosen standard anti-microbial circles to be specific ampicillin, streptomycin and antibiotic medication were put on the bacterial plates. At that point, the plates were hatched for 24 hours at 37°C. After the hatching time frame, the results were watched and the breadth of the restraint zone was measured around the segregates.

Assurance of antioxidant activity of mushrooms

DPPH radical-scavenging activity

DPPH radical-scavenging action was assessed by the strategy for Ravikumar *et al.* (2008) [19]. Quickly, 1.0 ml of combined AgNPs (50 µg/ml) was added to 1.0 ml of 0.16mM DPPH methanolic arrangement. Similarly straightforward 50 µg/ml mushroom and 0.1 M of AgNO₃ was likewise tried for antioxidant properties. The blend was vortexed for 1 min and left to remain at room temperature for 30 min in dull, and the absorbance was perused at 517 nm. Bring down absorbance demonstrated higher radical scavenging action. The capacity to scavenge the DPPH radical was figured utilizing the accompanying equation

$$\text{Scavenging activity (\%)} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100$$

Where, A control is the absorbance of the control (DPPH solution without AgNPs), A sample is the absorbance of the test sample (DPPH solution plus AgNPs) BHT was used as the standard. Each samples were performed for triplicate to minimize error.

Superoxide radical scavenging activity

The superoxide radical scavenging movement was measured by the strategy said by Nishimiki M. *et al.* (1972) [20]. Superoxide anions were created in a phenazine methosulfate nicotinamide adenine dinucleotide (PMS-NADH) framework through the response of PMS-NADH and oxygen. It was tested by the lessening of nitroblue tetrazolium (NBT). Every one of the arrangements utilized as a part of this test was set up in phosphate support (pH 7.4). 1ml of NBT (156µM), 1ml of NADH (468µM) and each 1ml of mushroom extract (10-50 µg/ml) and each AgNPs (10-50 µg/ml) were blended. The response was begun by including 1ml of PMS (60µM) and the blend was incubated at 25°C for 5min took after by estimation of absorbance at 560nm spectrophotometric ally. Diminished absorbance of the response blend demonstrated expanded superoxide anion rummaging movement. The rate hindrance was computed utilizing the equation,

$$\text{Inhibition (\%)} = [(A_0 - A_S) / A_0] \times 100 \dots \dots \dots (\text{Nishimiki M. et al. 1972}) [20]$$

Reducing power examine

The diminishing energy of the AgNPs was measured by the strategy for Oyaizu, (1986) [21]. Briefly, 500 µl of incorporated AgNPs (100, 200, 300, 400, 500 µg/ml) was blended with 2.5ml of phosphate cradle (0.2 M, pH 6.6) and 2.5ml of one percent potassium ferricyanide. Response blend was brooded at 50°C for 20 min. After brooding, 2.5ml of trichloroacetic acid (10%) was included, centrifuged at 650×g for 10 min. 2.5ml of upper layer was blended with 2.5ml of distilled water, 0.5ml ferric chloride (0.1%). Absorbance of the examples was perused at 700 nm. Expanded absorbance of the reaction mixture showed expanded reducing power. BHT was utilized as the standard.

ABTS+ scavenging capacity assay

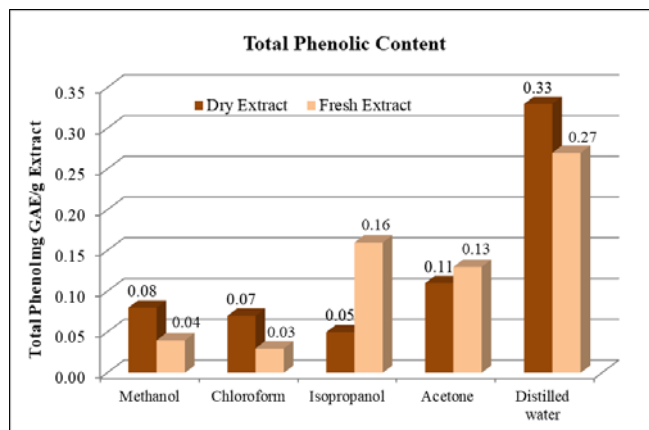
The ABTS decolonization tests were completed including the era of ABTS⁺ chromophore by oxidation of ABTS with potassium persulfate. The ABTS radical cation (ABTS⁺) was created by responding 7 mM stock arrangement of ABTS with 2.45 mM potassium persulfate and permitting the blend to dark condition at room temperature for no less than 6 hour prior utilize. Absorbance at 734 nm was measured following 10 minutes of incubation after mixing different concentrations of the *Agaricus bisporus* extracted (final concentration as 10, 15, 20 %; v/v) with 1 ml of ABTS⁺ solution. The ABTS⁺ searching limit of the filtrate was contrasted and that of vitamin C (50 µM), vitamin E (50 µM) and BHA (0.1 mg/ml).

Preparatory phytoconstituents examination

The phytochemical examination of *Agaricus bisporus* reveals that the extracts contain starch, glycosides, triterpenoids and phenolic mixes (Table-1) and (Graph-1). The phytochemical investigation of palatable mushrooms revealed the presence of major Phytoconstituents *viz.*, alkaloids, saponins, steroids, phenols, glycosides, terpenoids and flavonoids. Among the five solvents utilized for extraction, methanol extract demonstrated more number of phytoconstituents when contrasted with aqueous.

Table 1: Phytochemical analysis of fresh and dry extract of edible mushroom *Agaricus bisporus*.

Phytochemical	Test	Observation									
		Distilledwater		Methanol		Isopropanol		Chloroform		Acetone	
		Fresh extract	Dry extract	Fresh extract	Dry extract	Fresh extract	Dry extract	Fresh extract	Dry extract	Fresh extract	Dry extract
Alkaloids	Mayer`s	+	+	+	+	+	+	+	+	+	+
	Wagner`s test	+	+	+	+	+	+	+	+	+	+
	Dragendroff`s test	+	+	+	+	-	+	-	-	-	-
Carbohydrates	Molisch`s test	+	+	+	+	+	+	+	+	+	+
	Fehling`s test	+	+	-	-	-	-	-	-	-	-
	Benedict`s test	+	+	+	+	+	+	+	+	+	+
	Barfoed`s test	+	+	+	+	+	+	+	+	+	+
Glycosides	Keller-Killiani test	+	-	-	-	-	-	-	-	-	-
	Legal`s test	-	-	-	-	-	-	+	-	-	-
Protein/amino acids	Biuret test	+	+	+	-	+	-	+	-	-	-
	Millon`s test	-	+	-	+	-	-	+	+	-	-
Flavonoids	Alkaline reagent test	+	+	+	+	-	+	+	+	-	+
	Shinoda test	+	+	+	+	-	+	+	+	+	+
Phytosterols	Llibermaan-Burchard`test	+	+	+	+	+	+	+	+	+	+
Triterpenoids	Salkowski test	-	+	-	+	-	+	-	+	-	+
Phenolic compounds	FeCl ₃ test	+	+	+	+	+	+	+	+	+	+
Tannins	Lead acetate test	-	+	-	+	-	+	-	+	-	+
Saponins	Frothing test	+	+	+	+	+	+	+	+	+	+
	Emulsion test		+		+		+		+		+
Anthraquinones	NH ₄ OH test	-	-	-	-	-	-	-	-	-	-



Graph 1: Total phenolic content of dry extract and fresh extract of *Agaricus bisporus*.

Conclusion

The present work reports the green combination of AgNPs utilizing button mushroom. There are various strategies on blending nanoparticles, yet a large portion of them utilize costly chemicals and in this way are not practical. In addition, the buildups delivered are risky and lethal. This will bring about contamination which could prompt unanticipated impacts on regular biological system. In this work some colloidal AgNPs were acquired, while a few announced NPs were not much steady, they get total and settled down, and some are shaped oxide of Ag, or fragmented amalgamation. The retention crest in UV-VIS estimations fall in the Surface Plasmon Reverberation (SPR)

scope of 420–450 nm because of association of light and metal nanoparticles, when conduction electrons sway locally around nanoparticles at a positive recurrence (Figure-1) which firmly bolsters the development of AgNPs. The sizes of the AgNPs are similarly littler than other green combined AgNPs in the scope of 6-10 nm affirmed by SEM examination (Figure 2). Energy Dispersive X-Ray spectroscopy (EDX) is investigative method utilized for natural examination or, on the other hand concoction portrayal of test. Here EDX range obviously portrays that power of Ag line fixation as appeared in Figure-3. FTIR spectroscopy (Figure-4) is a helpful system to concentrate the core-shell morphology of AgNPs. The two wide groups at ~1642 and ~3400 are watched and are perceived as amide and hydroxyl and emerge because of - NH and - OH extend vibrations in the amide linkages of the protein correspondingly. An IR spectroscopic review has affirmed that the carbonyl gathering of amino corrosive buildup and peptides of proteins has solid capacity to tie metal, thus the proteins most perhaps may have shaped a topping on the Ag-NPs which moreover forestalls agglomeration of the particles, and along these lines the colloidal Ag-NPs are balanced out in the medium. Antioxidant activity of the AgNps formed is clear from the graphical Figures-5-8. We have tried antimicrobial action over *E. coli* and *S. aureus*, B. by plate dispersion technique. In all plates bacterial restraint zone appeared (Table-2-3). Different reports recommended that the AgNPs could deliver Ag particles which will harm the cell film, intrude on the metabolic movement, and along these lines prompt denaturation of protein lastly cell passing.

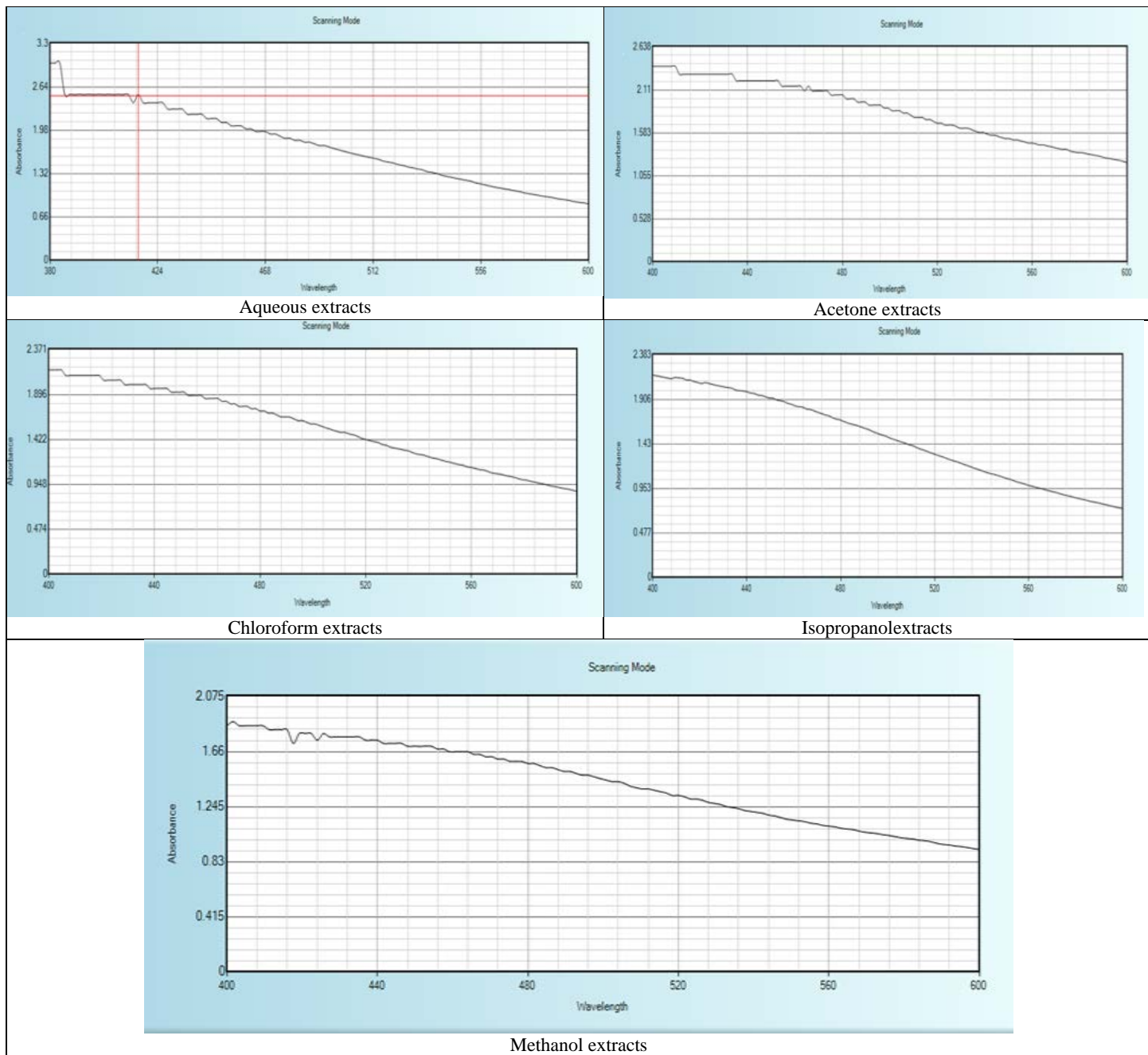


Fig 1: UV-Vis spectra of AgNP synthesized from various mushroom extracts

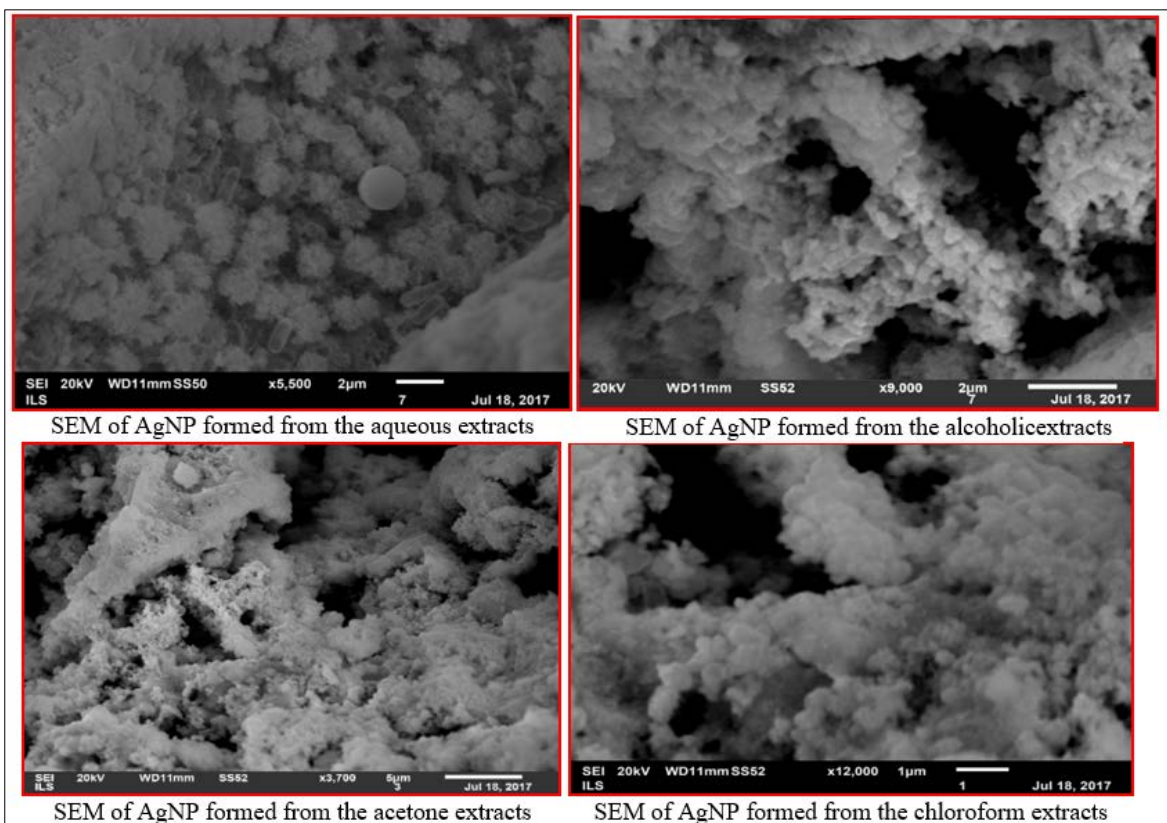


Fig 2: SEM of AgNP formed from the various extracts of Agaricus

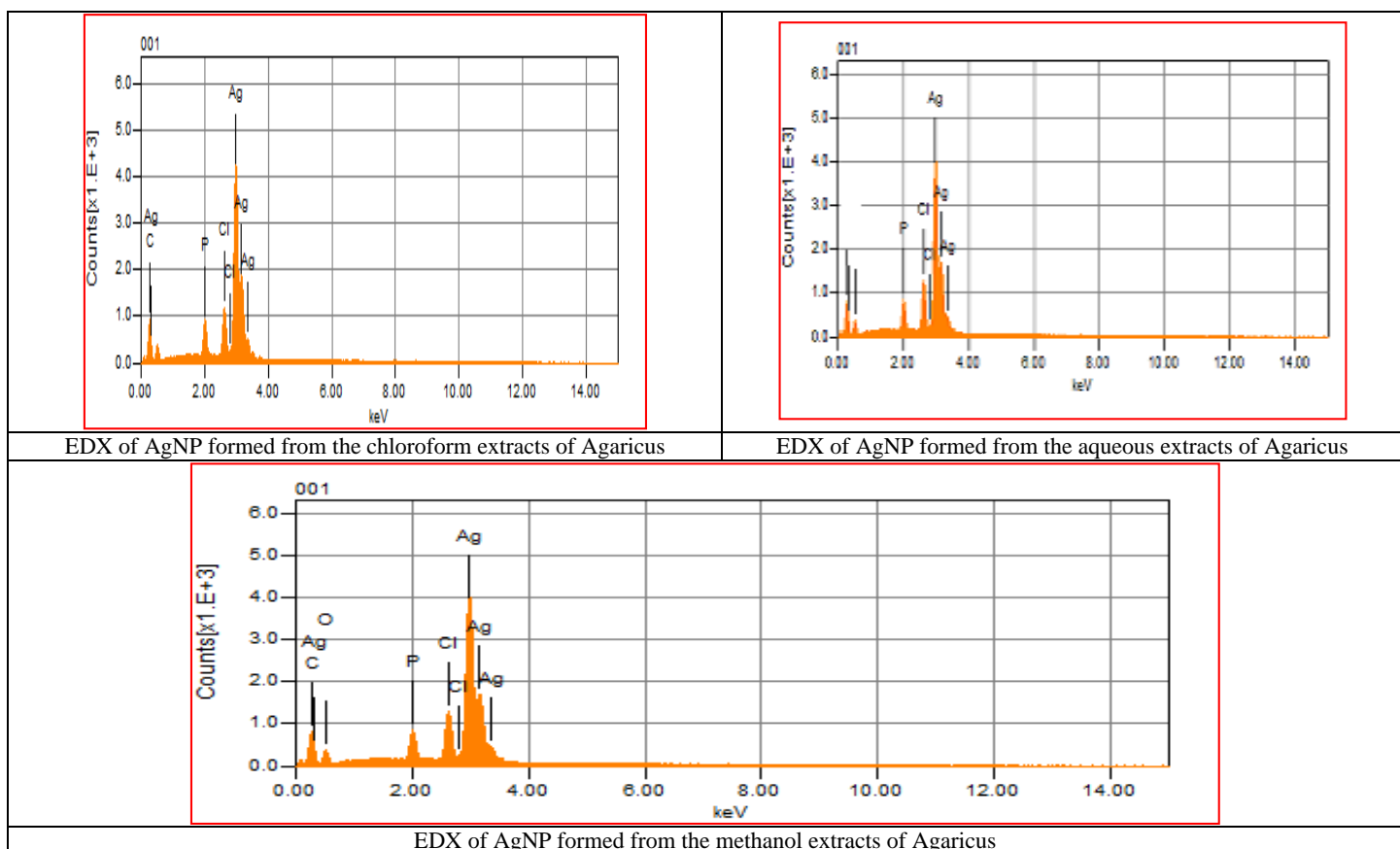
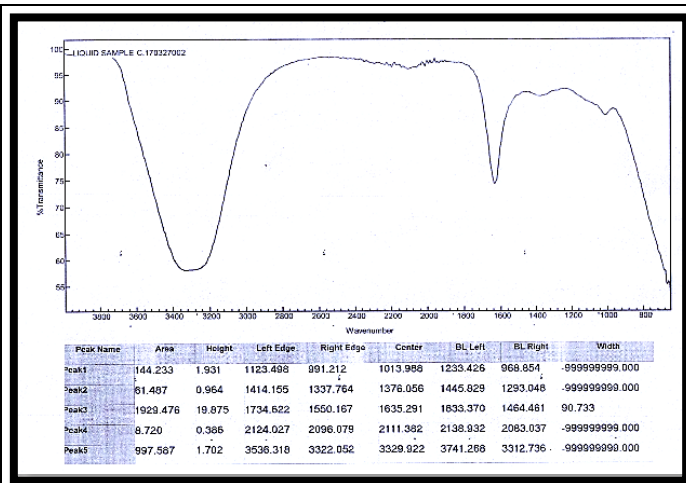
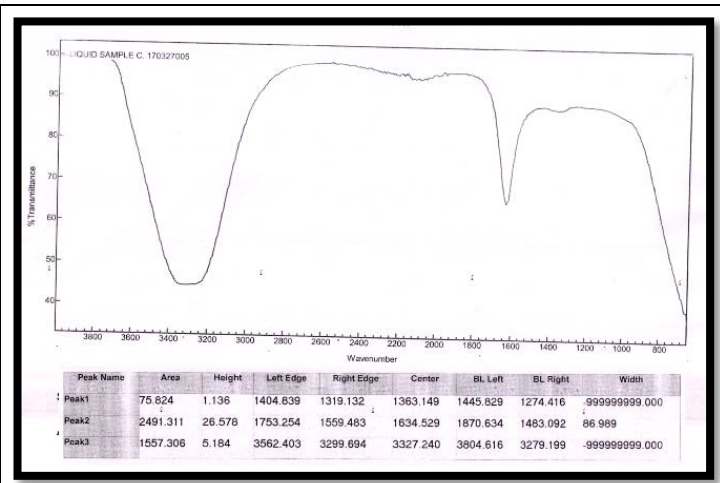


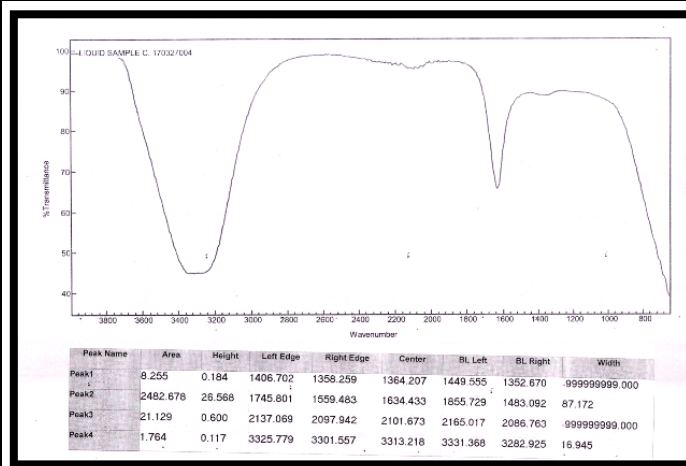
Fig 3: EDX of AgNP formed from various extracts of Agaricus



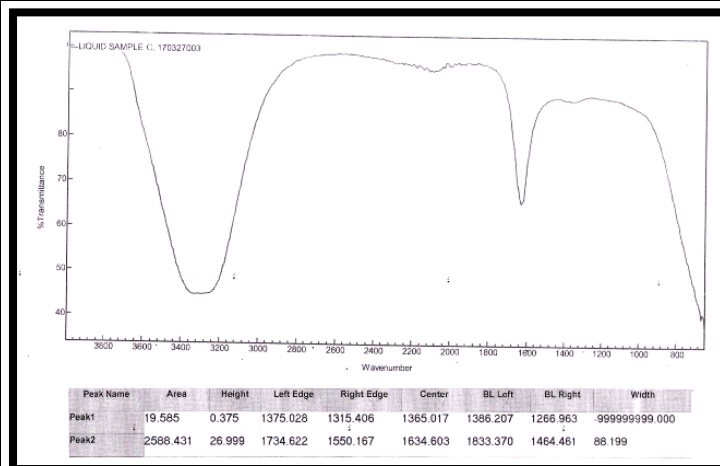
FTIR-of AgNP, [Aqueous extracts of mushroom]



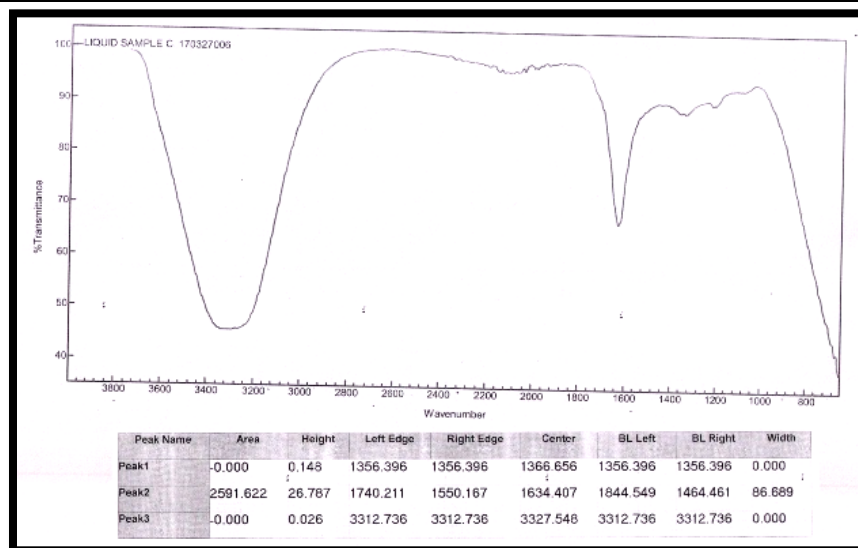
FTIR-of AgNP, [Chloroform extracts of mushroom]



FTIR-of AgNP, [Methanol extracts of mushroom]



FTIR-of AgNP, [Isopropanol extracts of mushroom]



FTIR-of AgNP, [Acetone extracts of mushroom]

Fig 4: FTIR of AgNP synthesized from various mushroom extracts

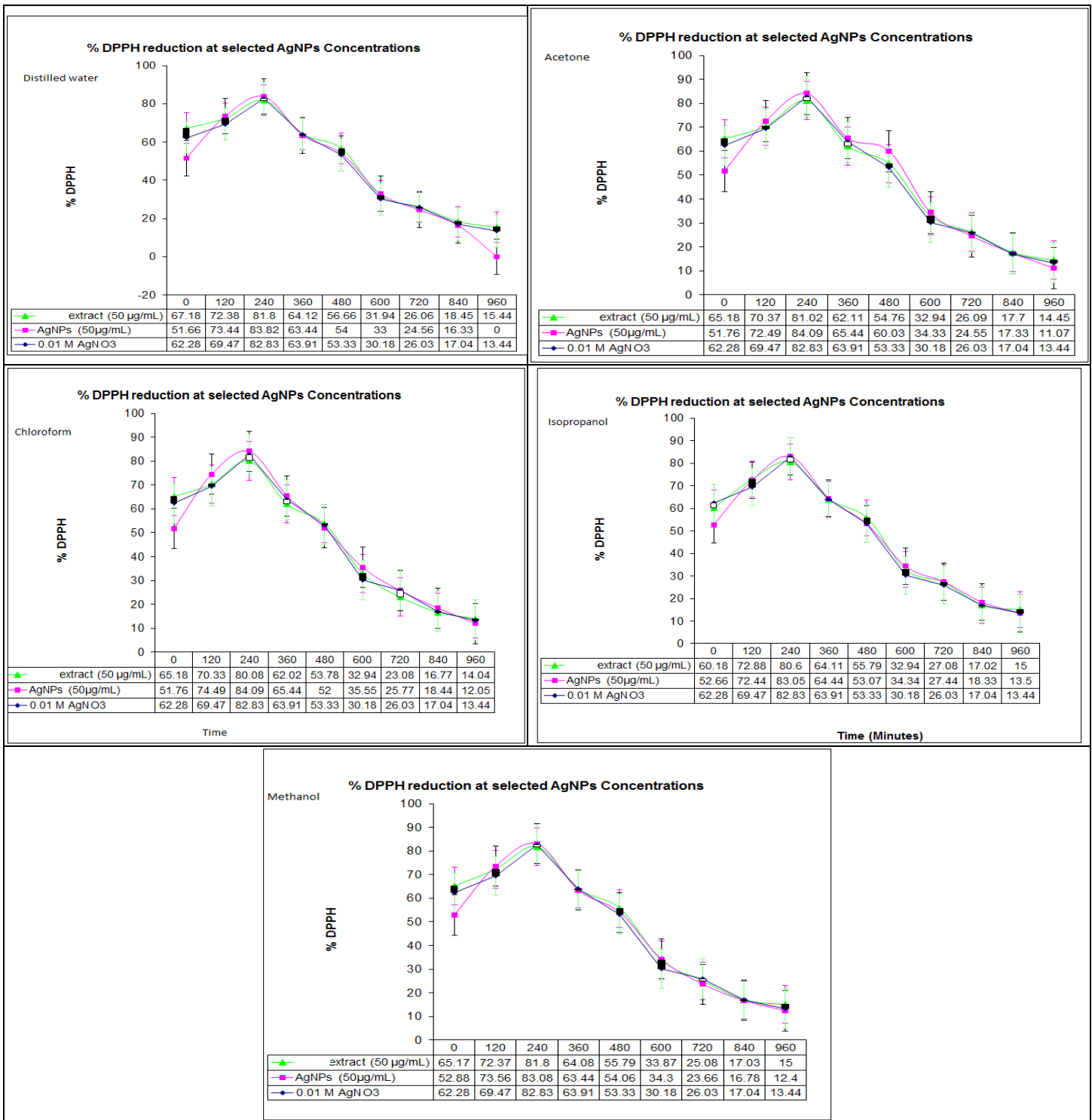
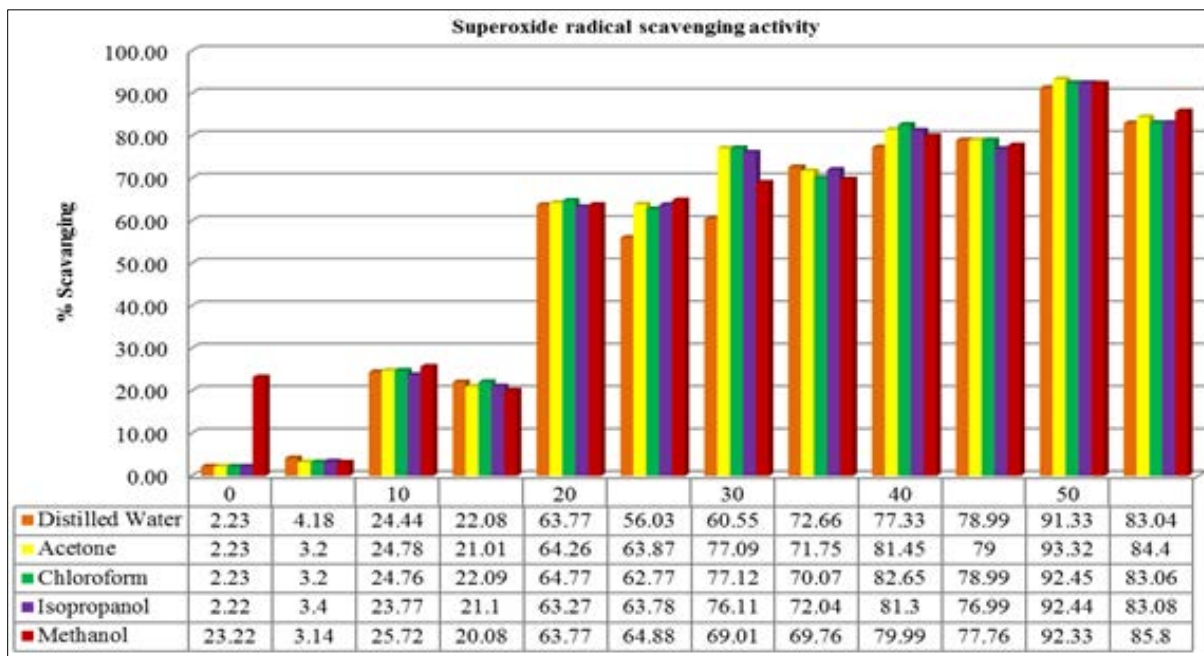
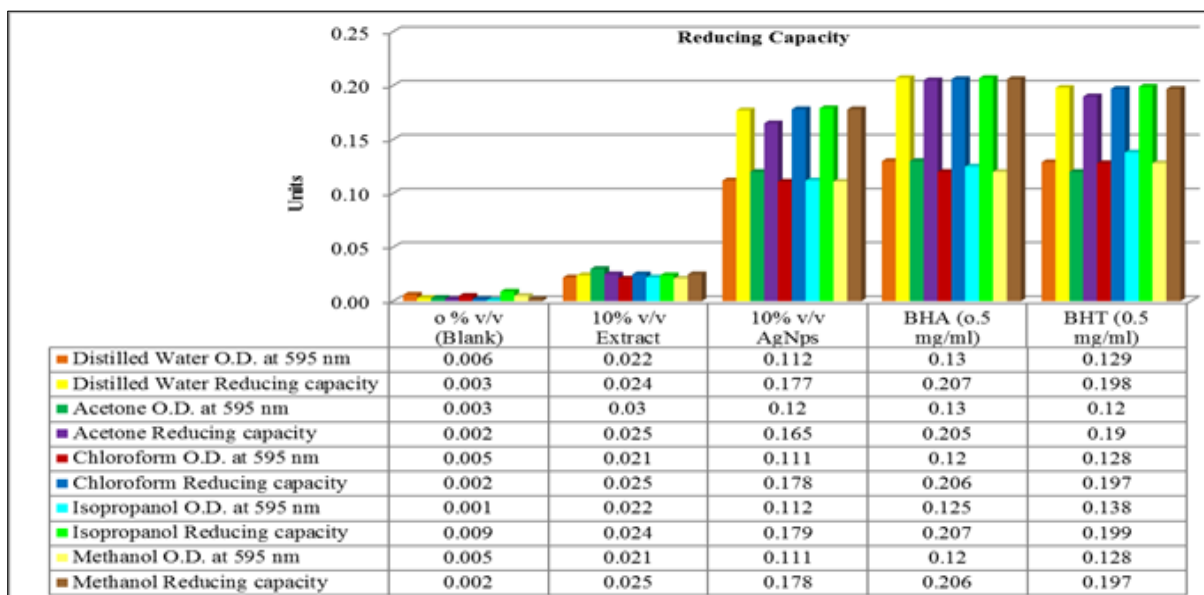


Fig 5: Antioxidant activity of AgNP synthesized from various mushroom extracts



Extract and AgNPs Concentration (µg/mL)

Fig 6: Superoxide radical scavenging activity of AgNP synthesized from various mushroom extracts



Extract and AgNPs Concentration (µg/mL)

Fig 7: Reducing capacity of AgNP synthesized from various mushroom extracts

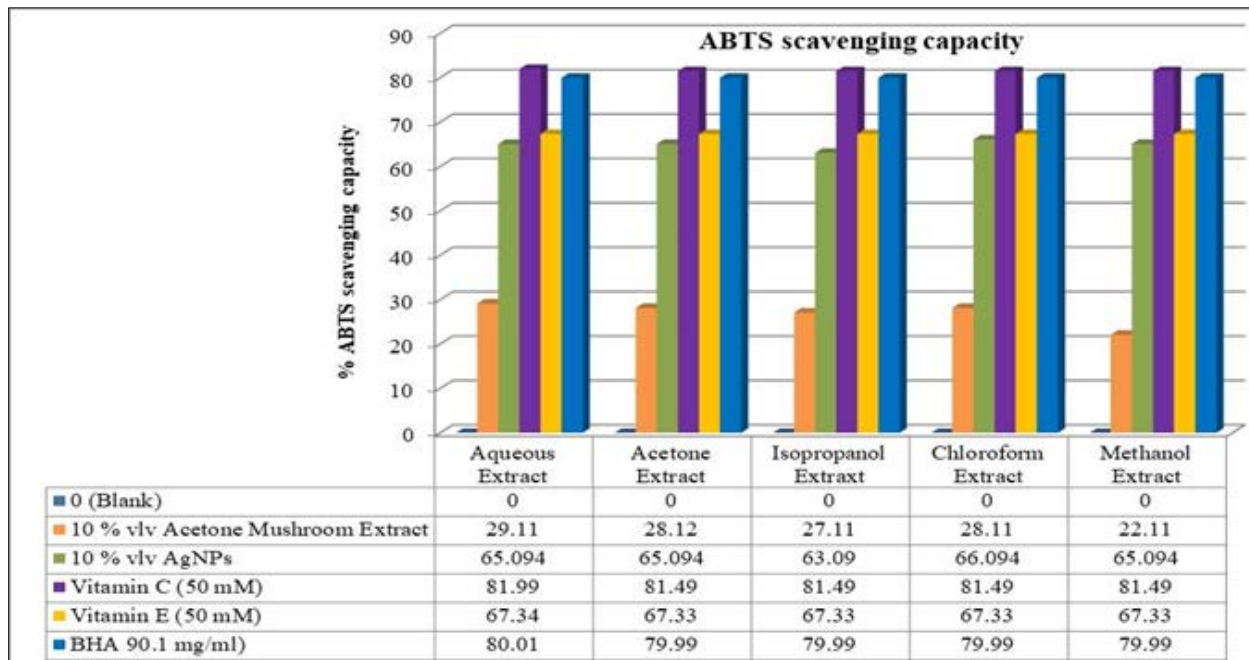


Fig 8: ABTS scavenging capacity of AgNP synthesized from various mushroom extracts

Table 2: Diameter of Inhibition zone (mm) for various microorganisms

Microorganism	Solvent extract	Diameter of Inhibition zone (mm)				
		Nanoparticle@ Dry Extract	Nanoparticle@ FreshExtract	Strepto-mycin	AgNp	AgNO ₃
<i>Staphylococcus aureus</i> MTCC-96	Methanol	08mm	07mm	-	09mm	05mm
	Acetone	10mm	10mm	-	-	05mm
	Isopropanol	09mm	08mm	-	-	05mm
	Chloroform	09mm	07mm	-	07mm	05mm
	Distilled water	10mm	09mm	20mm	-	05mm
<i>Salmonella typhi</i> MTCC-733	Methanol	05mm	05mm	-	-	05mm
	Acetone	06mm	15mm	-	06mm	05mm
	Isopropanol	08mm	07mm	-	-	05mm
	Chloroform	07mm	05mm	-	07mm	05mm
	Distilled water	08mm	07mm	20mm	-	05mm
<i>Bacillus subtilus</i> MTCC-4411	Methanol	08mm	07mm	-	07mm	04mm
	Acetone	10mm	10mm	-	-	04mm
	Isopropanol	09mm	08mm	-	-	04mm
	Chloroform	09mm	08mm	-	-	04mm
	Distilled water	10mm	09mm	20mm	-	04mm
<i>Escherchia coli</i> MTCC-425	Methanol	05mm	04mm	-	-	04mm
	Acetone	08mm	05mm	-	05mm	04mm
	Isopropanol	06mm	04mm	-	-	04mm
	Chloroform	06mm	04mm	-	05mm	04mm
	Distilled water	08mm	05mm	26mm	-	04mm

Table 3: Antibiotic zone inhibition (mm)

Strain	Amoxicillin	Chloramphenicol	Ag Np	AgNO ₃
<i>Staphylococcus aureus.</i>	15mm	00mm	06mm	03mm
<i>Salmonella typhi.</i>	14mm	00 mm	07mm	05mm
<i>Bacillus subtilus.</i>	16mm	00 mm	10mm	06mm
<i>Escherchia coli.</i>	15mm	00 mm	09mm	04mm

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