SYNTHESIS AND CHARACTERIZATION OF SILVER INCORPORATED AMINO-FUNCTIONALIZED MESOPOROUS SILICA NANOPARTICLES

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ABSTRACT

This study highlights a facile strategy for synthesizing MSN-based nanostructures using an ionic surfactant CTAB followed by surface modification of MSN through a sylilation process using APTMS (3-AminoPropyl TriMethoxy Silicate) . The amine functionalized MSNs were further incorporated with silver nanoparticles prepared by green route. The synthesized samples were further characterized by FTIR and HR-TEM analysis. From the FTIR, it was confirmed that the surfactant CTAB has been completely removed leading to the formation of mesoporous structure. It also confirms the presence of $–NH_2$ group and silver in MSN after functionalization. The mesoporous structure was also confirmed from HR-TEM data. The antibacterial and antifungal studies of the prepared samples were also studied.

CONTENTS

Page No.

LIST OF FIGURES

LIST OF ABBREVATIONS

APTMS	**3-aminopropyl trimethoxy silane**
FTIR	**Fourier Transform Infrared Spectroscopy**
HR -TEM	**High ResolutionTransmission Electron Microscopy**
MSN	**Mesoporous Silica Nanoparticles**
CTAB	**Cetyltrimethyl ammonium bromide**
TEOS	**Tetraethyl orthosilicate**
MSN-NH_2	**Amino functionalized MSN**
Nm	**Nanometer**
Ag –MSN-NH_2	**Silver incorporated Mesoporous silica Nanoparticles.**

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 NANOPARTICLES AND NANOSCIENCE

Nanoparticles are particles within the range of 1-100 nm and which is at least in one dimension. The physical, chemical and biological properties of these materials differ from the bulk materials. The term ‘nano science ‘is used to describe the control and manipulation of substances at nanometer level. They include the synthesis, characterization and application of nanoparticles. Nanoscience is present in almost all fields of science .The field of nanoscience is earning public awareness since 2000s. .They yield application in every fields such as physics ,material science ,chemistry, biology computer science and engineering and in whole field of science. Nanoscience have applications in human health and they yield better result especially in field of cancer treatment.

Several metals, dielectricals and semiconductors, nanoparticles have been synthesized in nanolevel. Nanoparticles are of great importance since they are the bridge between bulk materials and atomic or molecules. Nanochips are available easily in markets now. Nanoparticles also possess optical properties as they are small enough. In other semiconducting nanomaterials, that is, quantum dots, quantization of electronic energy level occurs.

1.2 SILVER NANOPARTICLES

Silver, due to its special optical , electrical, antimicrobial and antifungal properties have great impact since years. In the present work, silver nanoparticles are made by the reduction of silver nitrate using “Coleus aromaticus “. The plant is well known for its medicinal activities. It is the green synthesis of silver nanoparticles. Several factors which effect the formation of silver nanoparticles are concentration, pH, and temperature .Since green synthesis is used ,the wastage of materials is less .Toxicity of the metal is reduced by the use of plant extract .[1]

1.3 MESOPOROUS SILICA NANOPARTICLES

Mesoporous materials are ordered arrangement of nanochannels. They have pores with diameter in the range of 2-50 nm. Due to extremely small pore size they have enormous application in the field of biology. Mesoporous silica nanoparticles are synthesized in a number of ways .They are synthesized chemically or biologically. Biological synthesis of nanoparticles is done by microorganisms. It is a green and eco-friendly method [2] so that there are no side effects. In few years, mesoporous with different applications were synthesized. The mesoporous silica nanoparticles synthesis was achieved by adjusting the pH conditions, by differing the surfactants used. They are used as Nano carriers for several biomedical applications [3]. Mesoporous silica nanoparticles have a solid network with pore structure and large surface area .They has a honeycomb like structure and active surface. Mesoporous silica nanoparticles can be synthesized by sol – gel synthesis, colloidal precipitation and hydrothermal techniques.

1.31 SOL –GEL SYNTHESIS

It is a chemical method which uses a solution or colloidal particles to produce a gel. Subsequent metal alkoxides or chlorides undergo hydrolysis and simultaneous condensation to form a solid in liquid. It will form a gel on standing .Gel is dried by some processes which include shrinkage and subsequent densification. The gel is treated at very high temperature to perform polycondensation .Silica nanoparticles can also be synthesized by sol gel synthesis. TEOS in presence of mineral acid or base makes silica nanoparticles. Silanol are synthesized by the hydrolysis of TEOS. Later they undergo polymerization reaction to form silanol bridges and then the entire silica structure.

1.32 COLLOIDAL PRECIPITATION

When two solutions containing soluble salts are combined, an insoluble salt formation takes place .The process is called precipitation. When the range of the precipitate formed is within the nanometer range, a colloidal precipitation occurs .The disadvantage of this method is the aggregation of the formed nanoparticles. Surfactants or capping agents helps for stabilizing the nanoparticles formed by this method .In the production of mesoporous silica CTAB is the surfactant and it designs the size and shape of the nanoparticle formed and it also modifies nanoparticle surface.

1.33 HYDROTHERMAL TECHIQUE

At high pressure and high temperature, the reactions which take place in the presence of liquids or mineral solutions are called hydrothermal reactions. The high pressure is attained by heating substance with water at temperature higher than critical temperature of water in closed vessels. The closed vessel used here is called autoclave. A good autoclave must withstand under high temperature and pressure with corrosive solvent in it .Mainly water is used as the solvent in hydrothermal technique because of its capability to dissolve most of components in it .it is cheaper than any other solvent and also easy to use. It is non-toxic and stable. If we change the temperature and pressure water can act as a catalyst for the production of desired substance. Also water can be removed very easily after the completion

of the reaction .

This technique is very advantageous because it uses low temperatures. Also, the technique can be coupled with other processes like hot pressing, ultrasound,

electrochemistry, mechano – chemistry to increase its ability to synthesize new materials. Also the method doesn't need any catalyst, gives good yield and is not harmful. TiO_2 nanoparticles are made through this process.

1.34 GREEN SYNTHESIS OF MSN

Green synthesis of MSN refers to the most effective, less toxic and eco-friendly way of producing MSNs. It also reduces the formation of hazardous side products. The MSN produced in this way will be of high purity. The MSN which can be used under different pH conditions could be produced under this technique. MSN synthesized from hexafluorosilicicacid contains only small amount of waste products and is less toxic.

1.4 MODIFICATIONS OF MSN

MSN is an inorganic nanomaterial, which has a pore size of about a few nanometres. The pore size of MSN has the ability to capture large amount of molecules into it. The size, area and surface of the mesopores are selected in accordance with the molecules which are taken to bind with MSNs.

MSN surface can be modified by using amine functional groups. Many modifications from the aminated mesoporous silica has been made [4].Many researchers generated MSNs with the functional group acrylate.[5]. Aldehydic group introduced MSNs were also made. Amine functional group is more preferred because it is easier for transformation into other functional groups .The modification or the introduction of the functional groups occurs inside the pores of MSNs.

1.5 USES OF MESOPOROUS SILICA NANOPARTICLES.

1.51 MSN AS DRUG DELIVERY AGENTS.

MSNs have been used as the drug delivery agents. MSN particles modified with peptides have been known well for drug delivery system.[6]. The introduction of organic groups controls release and molecular recognition gives the mesoporous materials for the drug delivery. Any drug delivery system should have the ability to transport drug without any complication or loss of substance is a best drug delivery system. Also the release of suspected drug would be in a controlled manner .The modified silica particles have several features including stable mesoporous alignment large surface volume well defined pores and properties for specific delivery sites. They also act as a good host with different size, shapes and ease of use. MSNs are highly selective .All these properties account for the use of MSNs is a host for an efficient drug delivery system.[7].

1.52 MSN –USES IN CHEMOTHERAPY.

Cancer causes death of human. Chemotherapy is considered as the best treatment for cancer. But it doesn't kill the cancerous cells specifically. Also it causes severe adverse effects such as hair loss, migraine and loss of immunity. Most anticancer drugs have poor aqueous solubility, poor penetrating power and also they are restricted their entry through intra venous or oral routes. The proposal of MSNs had been a milestone in cancer therapy. MSNs are used to enhance their solubility and increase their permeability and thereby improve the efficiency of anti-cancerous drugs.

The network of MSN was functionalized to a stimulus responsive host

to increase the ability of drug release . Also, they are able to carry high loads and controlled drug delivery for chemotherapy .The size of MSNs allows for the EPR effect and the ligands on the surface of MSNs permits active targeting of specific molecular moieties on the surface of the cells. MSNs in their original form or modified form are used in treatment for liver cancer breast cancer, prostate cancer, lung cancer, colon cancer, brain cancer. Because of their incapability to permeate in living beings, there exists a non-usage of MSNs in clinical laboratories. MSNs can also be used in the diagnosis of cancer at early stages [8].

CHAPTER 2

REVIEW OF LITERATURE

Zhen-An Xiao et .al observed that in mild conditions (pH 6 -10) , by varying parameters and by adding suitable additives such as alcohols, amines etc. can prepare uniform, monodisperse and stable MSN. The particle size can also be controlled by 25 to 200 nm. The synthesis of MSN is a sol- gel process of silica in presence of surfactant. The high reaction temperature (60°C) is necessary for the synthesis of nano particles. [12].

Saher Rahmani et. al controlled the shape of MSN by adjusting the amount of EtOH as a cosolvent during the sol –gel process. The character of material differs, then function also changes .The formation of MSN of different structure, and morphology may increase the control of drug delivery and extend them [13].

Hong –Ping Lin et.al designed a simple synthesis on concept of kinetics to prepare the nanosize meso porous silica from a highly diluted liquid solution of sodium silicate .Because of properties such as high connectivity, large pores etc. MSNs can be used for the synthesis of high performance nano composites and for other applications. [14].

A Lodha et.al showed that drug loading does not require chemical functionalization of molecule. Also silica nano particles improved the solubility of water soluble drugs and increased the absorption and bio availability of compounds. Also the amorphous nature of water insoluble drug cyclosporine was not affected during the drug loading process as silica nanoparticle does not interact with cyclosporine. [15]

Hongyan He et.al highlighted that the platinum loaded MSN nano particles could be a promising drug delivery system. The drug carriers MSN-COOH bearing 1, 2 bi dentate carboxy groups were synthesized and were successful in binding oxaliplatin molecules with MSN. [16].

Chennan Liu et.al prepared MSN $–NH_2$ and modified alginate oligosaccharides on it. The efficiency of MSN – NH_2 - Cur -AOS nano particles was 91.25 %. As a result, the MSN NH_2 - Cur -AOS nano particles was easily absorbed by cancerous cells [17].

Tae- Hyunkin reported the ability of MSN– NH_2 with large pores and charged surface to deliver genes in rat stem cells. MSN– NH_2 were binded with Bore morphogenetic proteins 2.(BMP 2).to study their transfection efficiency .66% transfected produce BMP 2 pDNA developed may be a good system for bone tissue regeneration. [18].

Sheng Pan Peng et .al used MSN – NH_2 for removal of low concentration Malodorous aldehyde gases .They synthesized high specific area and adsorption capability MSN– NH_2without pores filing from a single step sol gel procedure .The usefulness of MSNs as malodorous aldehyde gases abatement materials exploits the high adsorption capability on amine surface. [19]

W .Naowanon et. al synthesized MSN– NH_2 under cyclohexane – water system using l arginine as base catalyst and C TAB as ionic surfactant. Later, they were utilized for the removal of Fe^{2+} and Cu^{2+} ions through adsorption. MSN– NH_2 have higher adsorption efficacy on Cu^{2+} (99%) Thermodynamic study shows that adsorption $Cu2^{+}$ and $Fe2^{+}$ were primarily by a physical adsorption and is a spontaneous process. [20].

Piege Qin et .al created amino functionalized MSN and demonstrated as useful sorbents of dispersive solid phase extraction for extraction and concentration of synthetic dyes from food stuff prior to HPLC . To achieve good extraction amount of MSN– NH_2, pH, adsorption time, desorption time, desorption level were investigated .The study was successfully applied to analysis of synthetic dyes in real samples [21].

HaiwenGuet.al worked in spherical mesoporous nano spheres modified by content tunable amino group was synthesized through a faule – cocondensation method. PA -6 MSN surface analysis shows strong interfacial affinity (covalent binding) may confine the mobility of PA 6 molecules along the tension axis. [22].

Hongyan He et.al prepared aldehyde group tuned catalyst and was incorporated onto MSN to behave as a hetero catalyst for oxidation of thio anisole to methyl phenyl sulfoxide under visible light .The catalyst exhibit catalytic efficiency compared to homogenous ones. [23].

Chia –Hung Lee et.al characterized NIR MSN suitable for in vivo optical imaging with high efficiency. MSN Trimethyl Ammonium with 50-100nm was prepared with modification of TA groups into MSN .Further ,Indo Cyanine Green adsorbed into it to render MSN –TA-ICG an efficient NIR Contrast agent for optical imaging [24].

Xi Feng Zhang et .al proposed that Ag nanoparticles can be synthesized by physical, chemical and biological processes. Ag nanoparticles has several bio applications such as antibacterial, antifungal, viral, anti-inflammatory, anti angio genic and anti-cancer agents .[25].

Liayawei et .al introduced methods of using Ag nano particles into virucidal agents and anticancer agents. Also, photo sensitizers, radio sensitizers and therapeutic agents in treatment in leukaemia, breast cancer, hepatocellular carcinoma etc. [26].

AqibJavaid et.al synthesized silver nanoparticles using green technological approaches due to their cost effective and eco-friendly nature. They synthesized nanoparticles from both gram negative and gram positive bacteria. It is considered as most attractive, simple, green and cost effective sources. [27].

Antarish saxena et.al reported the synthesized of silver nanoparticles, reducing Ag^{+} ions present in aqueous solution in extract of onion. Onion was good candidate for the synthesis of silver nanoparticles. Also, they studied antibacterial property of silver nanoparticles to E.Coli and Salmonella Typhimurium. [28].

N.Saifuddhin et.al composed a rapid, simple and green production of silver nano particles by using a combination of culture of supernatant of Bacillus Subtilis and microwave irradiation in water at absence of surfactant or soft temperature. The main outcome is that bio reduction method to produce nanoparticles is a good alternative to electrochemical methods. [29]

Elham Abbasi et.al prepared silver nanoparticles via a green synthesis .Such Ag nanoparticles can be used for spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries ,as optical receptors ,for bio labelling and as antimicrobials etc. AgNPs can be synthesized from Fusarium Oxyporum, Pseudomonas Stutzeri etc. [30].

Nat Tri Vo, et.al investigated about functionalized MSN by N^1-(3 Tri Methoxysilylpropyl)Di Ethylene Tri Amine (DAEAPTS). Tri functional groups on hd – MSN-NH_2 act as capping agent as well as reducing agent for silver production. Here Ag/ hd – MSN - NH_2 worked as nano reactors .The Ag / hd – MSN - NH_2 nanocatalyst show good reusability and high efficiency. [31].

Guilin dong et.al reported that an unprecedental catalytical performance for selective hydrogenation of di methyl oxalate to methyl glycolate. This is achieved by a catalyst based on AgNPs anchored inside the mesopores of amine derivatized silica nanospheres (MSN – NH_2) . This study points to be a promising route for development of high – performing Ag catalysed to be used in coal based MG production. [32].

Ali Saadet.al prepared Ternary nanocomposite of MSN (SBA -15), polypyrrole (pPy),and silver nanoparticles through a simple method consisting in oxidative Photo polymerisation of pyrrole in aqueous medium. Ag NO_3 is used as a photosensitizer. Strong bacterio static effect of nano composite was studied and was successful [33].

Chen Jinet.al designed Ag/AgBr loaded MSNs for rapid sterilization and acceleration of wound healing Ag/AgBr loaded nanostructure have photocatalysis effect which increases the antibacterial activity by producing reactive oxygen species (ROS).Ag^+ released will prevent bacterial infection and also stimulates immune functions. It is an effective strategy to prevent bacterial infection during wound healing.[34].

Kangli He developed a fast, convenient and highly sensitive method to quantify ACE. They include MSN– NH_2 absorbing a 6 –carboxy fluorescein modified aptamer of ACE. The

method is quite easy, good performance and sensitivity ,selectivity and provide an efficient reference. [35]

Juan Wang et. al introduced an injectable multi component polyamido amide dendrimer G3 cooperated biomimetric gel by using MSNs and dendrimer –templated AgNPs into a cross linked network. The elasticity and mechanical strengths can be modulated by introduction of MSN and silver nanoparticles [36].

CHAPTER 3

OBJECTIVES

- Preparation of mesoporous silica nanoparticles using ionic surfactant CTAB
- Functionalization of MSN using APTMS (3-aminopropyl trimethoxysilane)
- To incorporate silver nanoparticles to Amino functionalized MSN
- Characterization of the prepared samples using FTIR and TEM
- To study the antifungal and antibacterial properties of Ag incorporated amino functionalized MSN.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS.

Chemicals used:-

- Cetyltrimethylammonium bromide (CTAB)
- Ethanol (C_2H_5OH)
- Sodium Hydroxide (NaOH)
- Tetraethyl orthosilicate (TEOS)
- Hydrochloric acid (HCl)
- Distilled water
- 3-Aminopropyl trimethoxysilane
- Ammonium hydroxide (NH_4 OH)
- Silver nitrate.($AgNO_3$)
- Agar
- Muller-Hinton Nutrient broth
- Methanol
- Sterile distilled water
- Sterile cotton swabs
- Standard antibiotic (Amoxicillin - 1mg/ml)

- Laminar air flow
- Incubator
- Bacterial strains used – *Escherichia coli, Streptococcus* sp.

  Apparatus used:-

  - Round bottom flask
  - Glass beakers
  - Glass rods
  - Measuring jars
  - Watch glass
  - Filter paper
  - Magnetic stirrer
  - Syringes

4.2 EXPERIMENTAL METHODS

4.21 PREPARATION OF MESOPOROUS SILICA NANOPARTICLES USING CTAB

About 1g of CTAB(Cetyltrimethyl ammonium bromide) is taken and mixed with a solution of 3.5ml 2M NaOH , 3.5 ml ethanol and 50 ml distilled water. Using a magnetic stirrer, with constant stirring the mixture is heated to 60°C for 30 min at 500 rpm. To the solution, 2 ml

TEOS (tetraethyl ortho silicate) is added drop wise for about 10 min. Again, the solution was stirred for 2 Hours at 60°C. The obtained white precipitate is filtered and washed with hot water and then with ethanol HCl solution. The filtrate is heated in a Bunsen burner for about 4 hours to eliminate ionic surfactant. It is then kept for drying overnight at 50°C.

FIG 1;Stirring of MSN FIG 2: Synthesized MSN

4.22 FUNCTIONALIZATION OF PREPARED MSN USING APTMS

Aminated mesoporous silica nanoparticles are produced by a co condensation process which was already reported with some modifications [9]. TEOS and APTMS were taken 4ml and 1ml respectively and stirred for 2hrs .In another beaker, 0.2 g of CTAB was dissolved in 140 ml water using a magnetic stirrer for 20 min. 0.5 ml of ammonium hydroxide was added and stirred for 30 min. CTAB solution was stirred for another 4 hours and while stirring, the solution of TEOS and APTMS added drop wise. A milky white colour precipitate was obtained .It was filtered, washed. The filtrate is heated in Bunsen burner for about 4 hours to eliminate ionic surfactant. It is then kept for drying overnight at 50°C.

FIG 3 Milky Emulsion

FIG 4 White precipitate

FIG 6 MSN -NH_2

FIG 5 Ignition

4.23 PREPARATION OF SILVER NANOPARTICLES AND ITS INCORPORATION ONTO MSN-NH_2

4.231 Preparation of silver nanoparticles using leaf extract and silver nitrate.

The procedure was based on already reported work with some modifications [10].Fresh and healthy leaves of coleus aromaticus is collected, washed and dried. About 10 g is weighed and transferred into a 250 ml beaker containing 100 ml distilled water. It is boiled for 20 min and filtered using Whatmann no.41 filter paper. 0.084 g of silver nitrate is dissolved in 500 ml distilled water. 2.5 ml, 5 ml, 7.5 ml extract was taken in different beakers and added 100ml silver nitrate to all of the solution. The mixture is kept undisturbed. The colour change from colourless to brown indicates the formation of silver nanoparticles.

4.232 Incorporation of silver nano particles to MSN - NH_2

2g MSN - NH_2 is soaked in 50 ml distilled water and stirred for 30 min. The solution of silver nitrate and leaf extract was added drop wise and continued the stirring for 1 hour. Brown colour precipitate is formed. It is filtered, washed and dried at 50°C. [11]

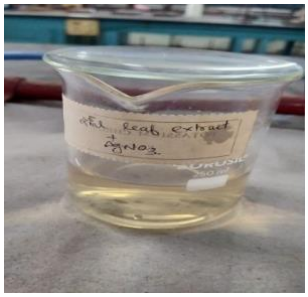
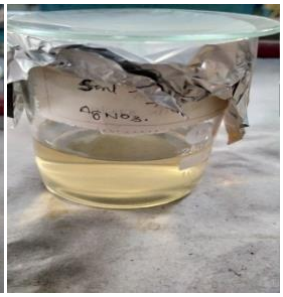
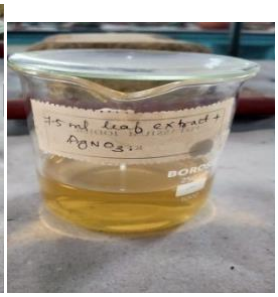
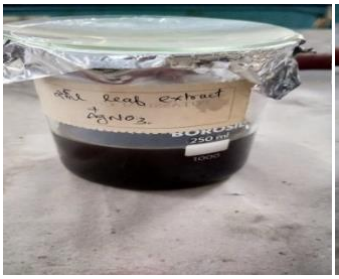





Fig 7 Fig 8 Fig 9 Fig 10



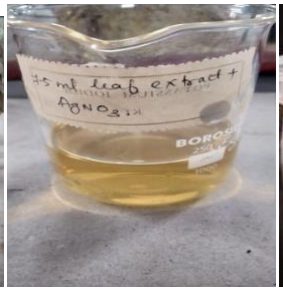



Fig 11 Fig 12 Fig 13 Fig 14

Fig 7: extract, Fig 8 :2.5 ml leaf extract + $AGNO_3$, Fig 9: 5 ml leaf extract +$AGNO_3$, Fig 10 ; 7.5ml leaf extract + $AGNO_3$..Fig 11,12,13 after 12 hrs. of mixing leaf extract and $AGNO_3$ .Fig 14 : synthesized MSN - NH_2 –Ag.

4.3 PHYTOCHEMICAL ANALYSIS OF PLANT EXTACT

4.31 PROCESSING OF PLANT MATERIAL

The plant material was thoroughly washed under running tap water followed by distilled water to remove dust and cut into small piece, dried under shade and pulverised in to fine powder using

motor and pestle. The powder was kept in plastic bags away from light, heat, moisture with proper labelling till further analysis.

4.32PREPARATION OF PLANT EXTRACTS

The phytochemical extraction was performed using polar solvent, methanol. Methanolic plant extract was prepared by using 100 % methanol as per the method explained by Mujeeb et al., 2014. For preparing methanolic plant extract 4 g of finely grounded leaves of the plant were taken in 100 ml conical flask. To this 50 ml of 100 % methanol was added and covered with aluminium foil. The conical flask was placed in shaker for overnight incubation at room condition. After incubation the extract was filtered using double layered Whatmann Filter paper No.1 in to 250 ml conical flask. The volume was made up to 200 ml by adding 100 % methanol and stored at $4^{o}C$ after proper labelling.

4.33 QUALITATIVE ANALYSIS OF PHYTO CONSTITUENTS

The methanol extract of plant sample (leaf) was analysed for the presence of the phytochemicals using standard phytochemical methods described by Kokate et al. 1995

- **Test for Alkaloids -** Dragendorff's Test: The sample extract (1ml) in chloroform was treated with 0.2 ml drops of Dragendorff's reagent and visualised brown precipitation confirmed the presence of alkaloid.

- **Test for Saponin:** Saponin was detected as per the method of Obadoni and Ochuko, (2001). 1ml plant extract was dissolved in water and shaken well. Froth formation, which lasts for a long time, shows the presence of saponin.

- **Test for Flavonoids -** Shinoda Test: 10 ml of extract was dissolved in methanol and a pinch of magnesium turnings were added to this followed by a few drops of concentrated hydrochloric acid. Formation of pink, red or magenta colour showed the presence of flavonoids.

- **Test for Steroid –** Liebermann - Butchard Test: To 1ml of extract added equal volume of chloroform and 1ml glacial acetic acid. To the solution add few drops of con. Sulphuric acid. Presence of green coloration confirms the presence of steroid.

- **Test for Terpenoid:** Extract (2ml) was treated with chloroform (1ml) and added a few drops conc. Sulphuric acid and observed reddish brown colouration for the presence of terpenoid.

- **Test for Phenolics:** Detection of phenolics was carried out as per the method discussed by Malick and Singh, (1980). To 1ml of plant extract, 2 ml of distilled water followed by addition of 1ml of 10% aqueous $FeCl_3$ solution. Deep blue or black colour detects the formation of phenol.

- **Test for Quinone:** To 2ml of extract few drops of con. Hydrochloric acid was added and the appearance of yellow precipitate indicates the presence of quinine.

- **Test for Cardiac glycosides -** Killer Killiani test: To 1ml of each extract a few drops of glacial acetic acid and ferric chloride and 3 - 4 drops of concentration sulphuric acid were added. The appearance of blue-green colour indicates the presence of glycosides.

- **Test for Carbohydrates -** Molisch's test: About 10 ml of the plant extract was taken and added 1 ml water and added two drops of 1% alcoholic solution of α-naphthol. After that it was added with 1 ml concentrated sulphuric acid along the sides of the test tube so that it forms a heavy layer at the bottom. Formation of deep violet colour at the junction of the two liquids indicates the presence of carbohydrates.

4.4 CHARACTERISATION TECHNIQUES

4.41 FTIR ANALYSIS

FTIR spectrometer is used to study the chemical composition of the synthesized mesoporous silica nanoparticles. Spectra was recorded from 500-4000 cm^{-1} using Perkin-Elmer LS-55-Luminiscence spectrometer.

4.42 HR TEM ANALYSIS.

HR TEM can be used for the study of surface morphology of synthesized MSN particles.

This technique images crystallographic structure with atomic scale precision. HR TEM provides highest resolution of about 0.08 nm. The face contrast imaging is the basis of image formation in HR TEM. The instrument can operate at Bright – field, Dark - Field. JOEL 3010 with a UHR pole piece operates at a voltage of 300 kV is used .The instrument works under a vacuum in the range of 10^{-5} to 10^{-6} pa.

4.5 ANTIFUNGAL ASSAY BY AGAR DIFFUSION METHOD

To the petriplate, 20 ml of Muller Hinton Agar was poured after solidification. 100µl of test pathogen *(Escherichia Coli* and *Strephylococcus sp)*. were swabbed on 2 separate plates .Four wells of 6 mm diameter introduced into Agar medium 100µml of extracts were filled. (100 µL / mL and 50 µL / mL concentration, antibiotic solution (amoxicillin) (positive control) and solvent blank-methanol (negative control). The plates were incubated for 24 hours at 37°C. After incubation the diameter of inhibitory zones formed around each disc is measured.

4.6 ANTIFUNGAL ASSAY BY AGAR DIFFUSION METHOD

Agar well diffusion method is also used for performing antifungal assay of sample. Potato dextrose agar was made use for this. 20ml of sterile medium was poured into assay plate and solidified. Once the medium solidifies, four wells, each of 9mm in diameter were cut out from agar, a sterile swab was used to evenly distribute the fungi (*C.albicans*) over the agar surface. The test volumes 50 µL and 100 µL from 1 µL / mL of the sample were added to the respective wells. Clotrimazole was used as the positive control and the solvent (methanol) used for sample dilution was used as the negative control. The plates were incubated at room temperature for 24 hours. The zone of inhibition was measured.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACT.

QUALITATIVE ANALYSIS OF PHYTOCONSTITUENTS

The preliminary phytochemical analysis of plant extracts of leaf of *Coleus aromaticus* in methanol polar solvent was done by colour tests. The results were presented in the Table 1

- **Test for Saponin:** Saponin is not detected in *coleus aromaticus* leaf. Saponin is bioorganic compounds having at least one glycosidic linkage (C- O -sugar bond) at C-3 between a glycone and a sugar chain. Hydrolysis of saponin molecule produces two portions, a glycone and a sugar moiety. Saponin exhibit medicinal properties such as anti-inflammatory, antibacterial, antifungal, antiviral, insecticidal, anticancer etc.

FIG .15**.** Preliminary phytochemical analysis of saponin in *coleus* leaf

- **Test for Flavonoids -** Shinoda Test: Methanolic extract of leaf of *coleus aromaticus* showed the absence of flavonoids. Chemically flavonoids are based upon a fifteen-carbon skeleton consisting of two benzene rings linked via a heterocyclic pyrene ring. They can be divided into a variety of classes such as flavones, flavonols, flavanones and others.

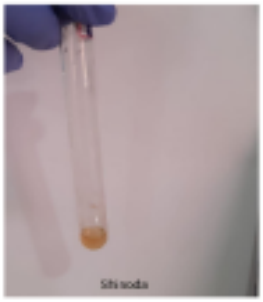


FIG.16**.** Preliminary phytochemical analysis of flavonoids in *coleus* leaf

- **Test for Steroid:** Liebermann - Butchard Test: In *Coleus*, the presence of steroid was not detected in the methanolic extract. Steroids have the fundamental structure of four carbon rings called the steroid nucleus with a vast array of biological activities.

FIG .17**.** Preliminary phytochemical analysis of Steroid in *Coleus*

- **Test for Phenolics**-Methanolic extract of *coleus* leaf showed negative test to phenol. Phenolic compound from plants have several biological effects such as anti-inflammatory, antioxidant properties and can play an important role in prevention of diseases.

FIG.18. Preliminary phytochemical analysis of Phenolics in *coleus* leaf

- **Test for Cardiac glycosides** (Killer Killiani test) - leaf of *coleus* showed absence of cardiac glycosides in methanolic extracts. Cardiac glycosides are steroids having the ability to exert specific powerful action on the cardiac muscle. A very small amount can exert a beneficial

simulation on diseased heart. These compounds are primarily valuable in the treatment of congestive heart failure.

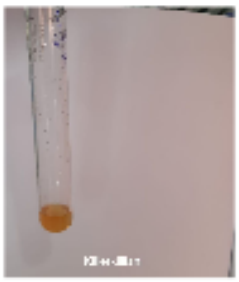


FIG 19. Preliminary phytochemical analysis of Cardiac glycosides in *coleus* leaf

- **Test for Carbohydrates** - Molisch's test: In the methanolic leaf sample showed the presence of carbohydrate. In Molisch's test the carbohydrate undergoes dehydration upon the introduction of concentrated hydrochloric or sulphuric acid, resulting in the formation of an aldehyde. This aldehyde undergoes condensation along with two phenol-type molecules resulting in the formation of a purple or reddish-purple coloured complex at the junction. It is usually present inside the plant cells as compact insoluble granules which may be spherical, ovoid or compact crystals and which have a distinctly layered structure.

FIG.20. Preliminary phytochemical analysis of Carbohydrates in *coleus.*

- **Test for Quinone**

The methanolic extract of leaf sample showed the absence of Quinone.

FIG. 21**.** Preliminary phytochemical analysis of Quinone in *Coleus***.**

- **Test for Terpenoid**

The methanolic extract of leaf sample showed the absence of Terpenoid.

FIG 22**.** Preliminary phytochemical analysis of Terpenoid

- **Test for Tannin**

Leaf showed absence of tannin in methanolic extracts.

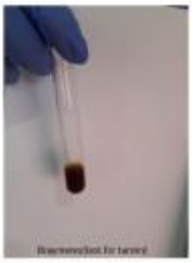


FIG 23**.** Preliminary phytochemical analysis of Tannin in *coleus*.

- **Test for Alkaloid**

In leaf methanolic extract showed high quantity of Alkaloid**.**

FIG. 24**.** Preliminary phytochemical analysis of Alkaloid in *coleus* leaf**.**

Table 1. Phytochemical analysis carried out in leaf samples of. *Coleus.* in methanol polar solvent.

Phytochemical tests	**Methanolic leaf extract**
Saponin	Negative
Flavanoid	Negative
Steroid	Negative
Phenolics	Negative
Cardiac glycosides	Negative
Carbohydrates	Positive
Quinone	Negative
Terpenoid	Negative
Tannin	Negative
Alkaloid	Positive

5.2 FTIR ANALYSIS OF MSN, MSN-NH_2, Ag-MSN-NH_2

The FTIR spectra of pure CTAB, MSN synthesised using CTAB as surfactant and MSN-NH_2 is shown in Fig.25 & 26.Two bands appeared at around 1080 cm^{-1} and 799 cm^{-1} in all samples corresponding to the asymmetric and symmetric stretching vibration of Si-O-Si respectively. The band observed around 960 cm^{-1} was due to surface Si-OH groups. The peaks at 3012cm^{-1}, 2914cm^{-1}& 2848cm^{-1} which are characteristic peaks of CTAB, disappeared in the spectra of MSN.

The modified silica were confirmed by FTIR analysis(fig 27) .The band at 1638 cm^{-1}corresponds to N-H bending vibration of amine group which confirms that MSN was modified to MSN-NH_2.The band at 3467 cm^{-1} corresponds to O-H Stretching band of the surface silanol group. The peaks at 1113 cm^{-1} corresponds to the siloxane vibration of $(SiO_2)_n$ groups ,799 cm^{-1} shows asymmetric vibration of Si-O –Si and 407 cm^{-1} show Si-O- Si bending vibration.

From the FTIR spectra of Ag-MSN -NH_2 (fig 28), the peak at 3470 cm^{-1} corresponds to OH stretching H bonded alcohols and phenols of the plant extract. The peak at 470 cm^{-1} corresponds to the silver nano particles stretching.

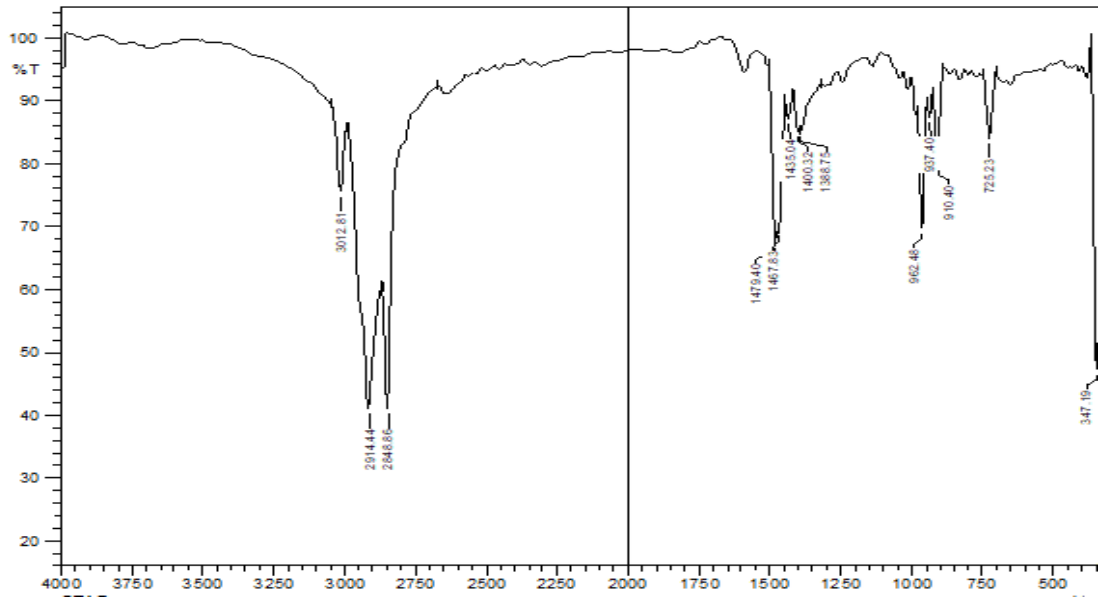


FIG 25: FTIR Spectrum of CTAB.

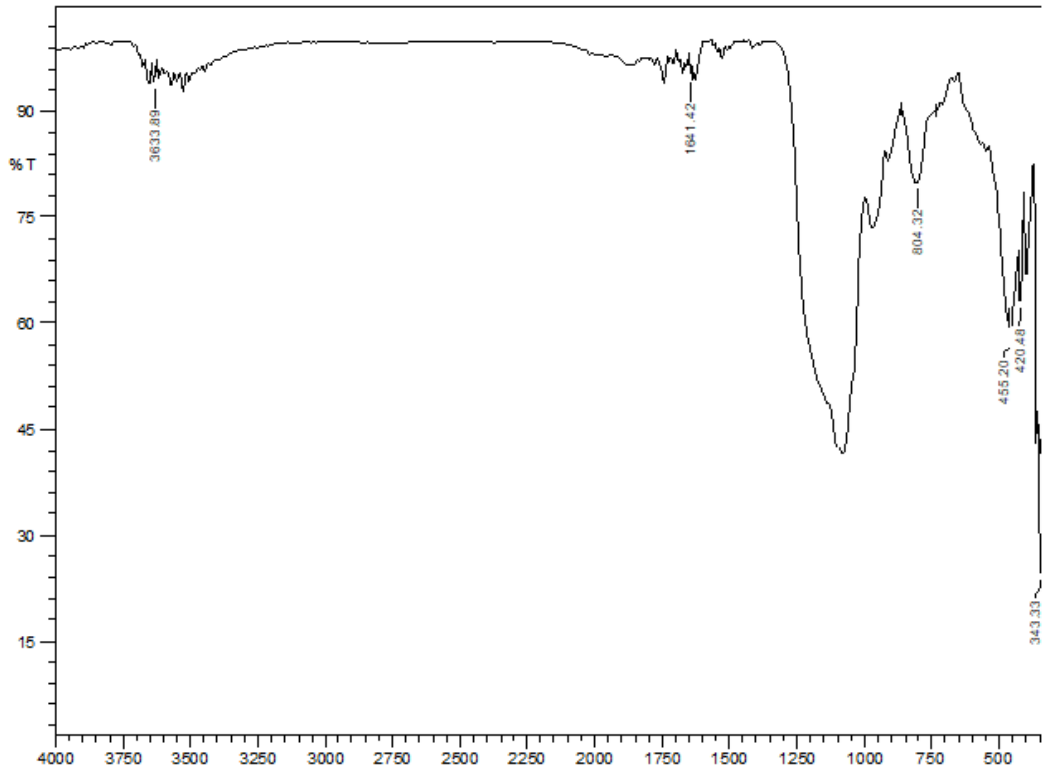


Fig 26: FTIR Spectrum of MSN synthesized using CTAB.

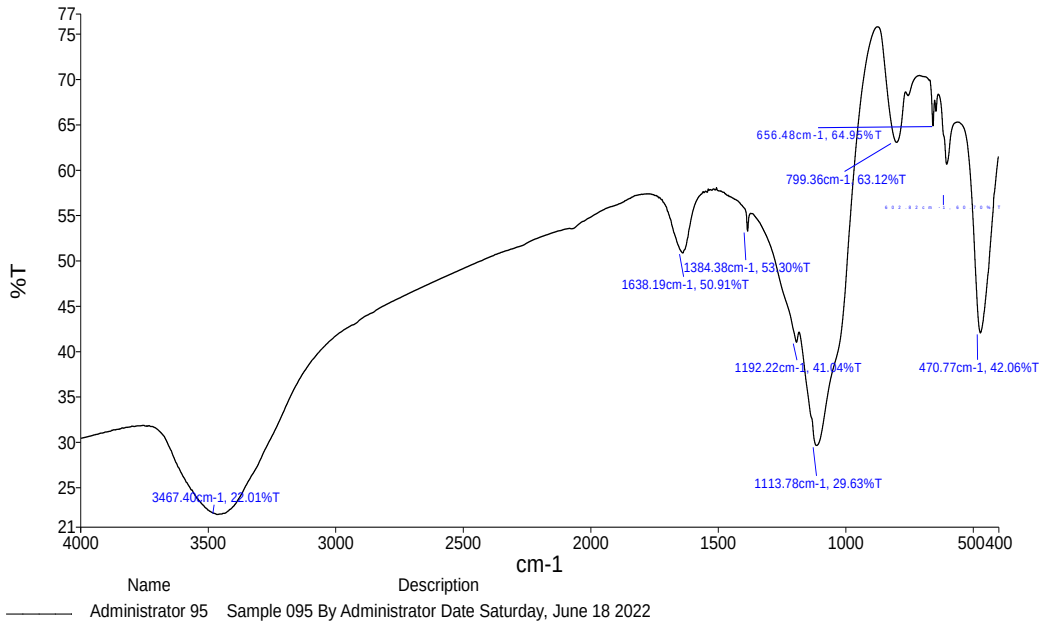


FIG 27: FTIR spectrum of MSN-NH_2.

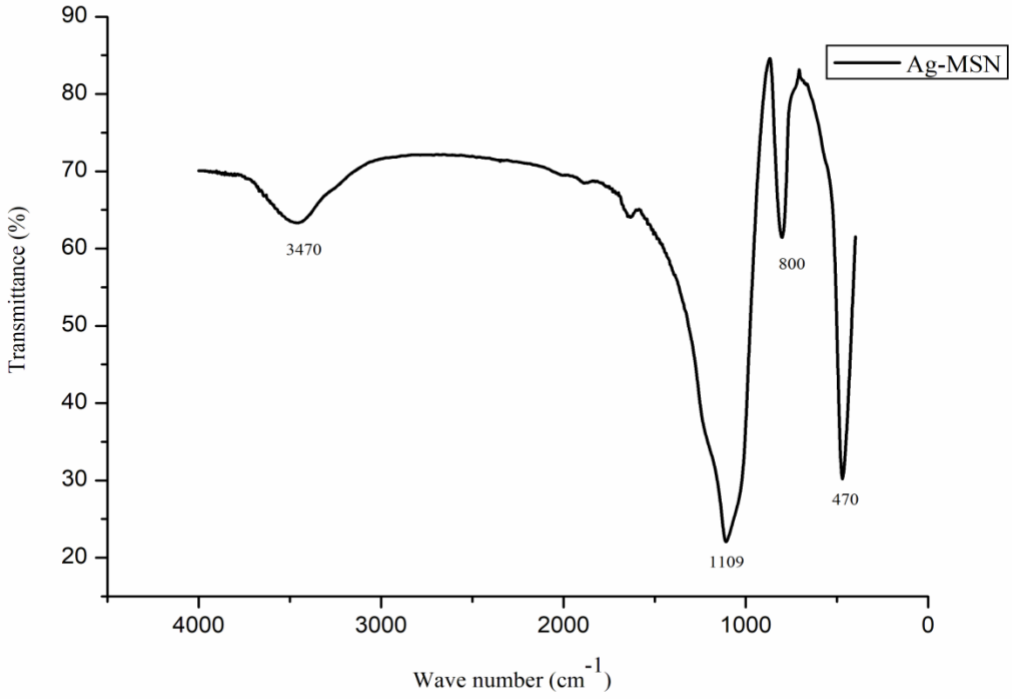


FIG 28 : FTIR analysis of Ag-MSN-NH_2

5.3HR TEM ANALYSIS OF MESOPOROUS SILICA NANOPARTCLES

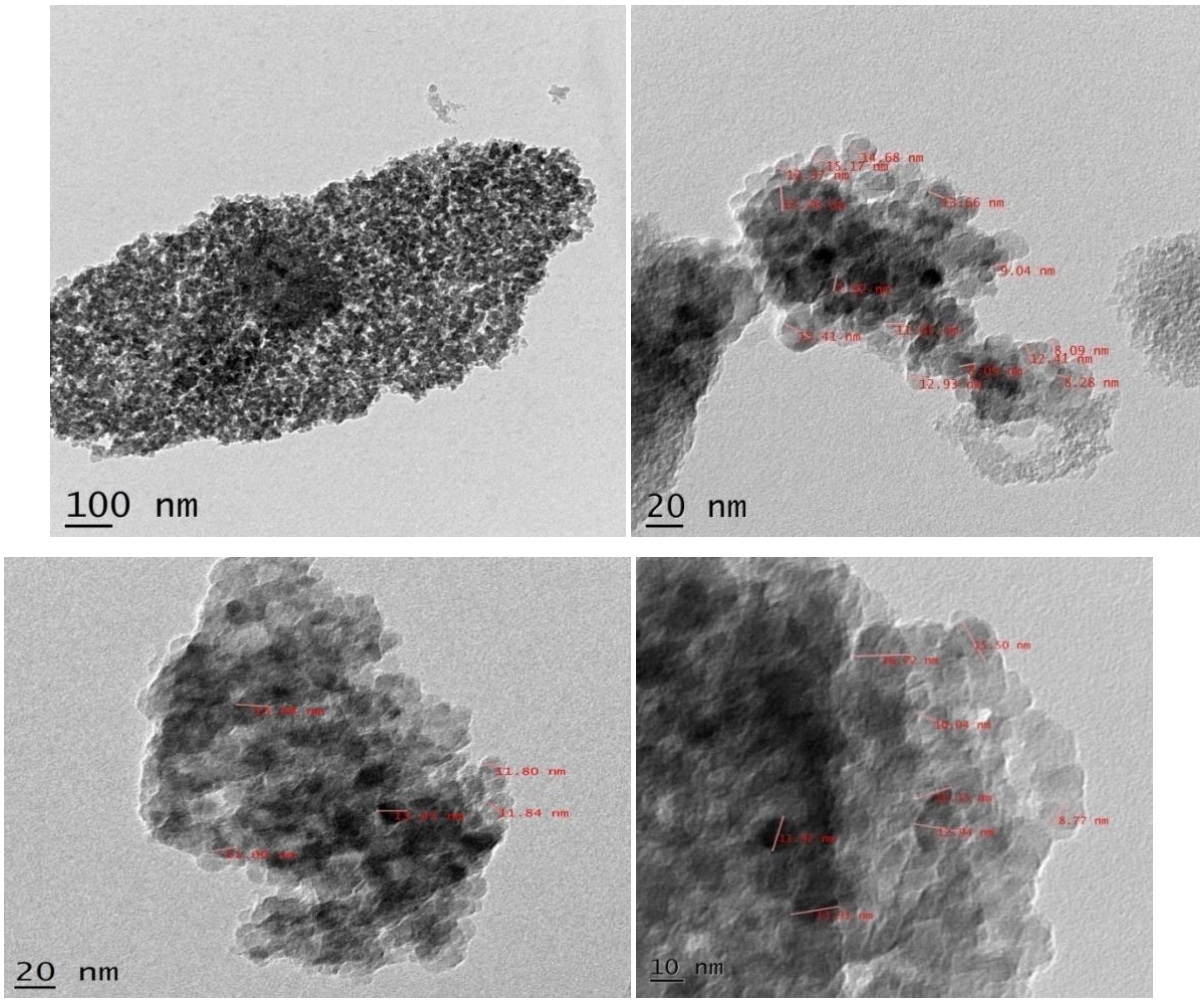


FIG 29: HR-TEM images of prepared MSN.

The surface topology of MSN was studied by using HR TEM. It gives high resolution images of MSN. These images show a round grain like structure for silica particles formed. It appears as stacking of several round grains like structures together. Small pores formed in SiO_2 can be seen and mesoporous nature was confirmed by the pore size which is in between

2-50 nm

5.4 ANTIFUNGAL AND ANTIBACTERIAL ANALYSIS

The antifungal property of Ag –MSN NH_2 powder was studied using agar diffusion method against selected human pathogen such as *C. albicans* and antibacterial study using E*scherichia coli* and *Strephylococcus* sp. These two different pathogens have also tested with commercially available antibiotic (Amoxicillin) and results were indicated. Antibiotic solution is positive control and solvent blank (methanol) is the negative control. The extracts used against the pathogenic organisms have showed varied degree of antibacterial activity and antifungal activity against the pathogens.

5.41 ANTIBACTERIAL ANALYSIS

Table 2

Sl no	Organism	Positive Control	Negative Control	Test 1(T_1) (50µl from 1 mg /ml)	Test 2 (T_2) (100 µl from 1 mg/ml)
1.	*Strephylococcus*	1.6 cm	0 cm	0.5cm	1.5 cm
2.	*E.Coli*	1 cm	0 cm	0.8cm	1.2 cm

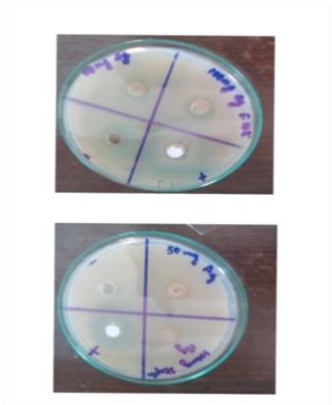


FIG 30: Antibacterial study of Ag –MSN-NH_2

5.42 ANTIFUNGAL ANALYSIS

The antifungal analysis of the sample is done by using the pathogen *C. Albicans*. The pathogen was also tested with commercially available antibiotic. Results are indicated in table.

Table 3

Sl no	Organism	Positive control	Negative control	Test 1(T_1) (50µl from 1 mg /ml)	Test2(T_2) (100 µl from 1 mg/ml)
1	*C.Albicans*	1.6 cm	0 cm	0 cm	0 cm

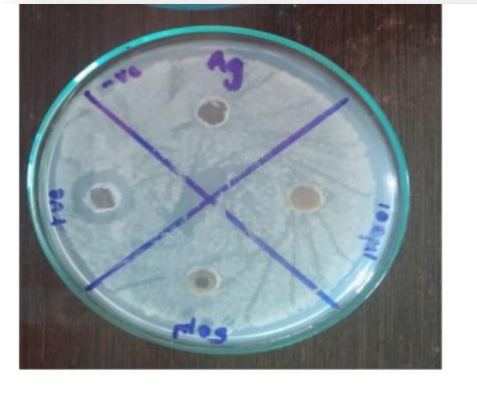


FIG 31 : Antifungal analysis of Ag –MSN-NH_2

CHAPTER 6

CONCLUSION

This study highlights the synthesis and characterization of silver incorporated amino functionalized mesoporous silica nanoparticles. The functionalization is achieved using APTMS. The prepared MSN, MSN–NH_2 , Ag–MSN-NH_2 is confirmed by FTIR analysis and surface morphology using HR –TEM which confirms mesoporous structure. This study also focuses on the anti-bacterial and antifungal properties of Ag–MSN–NH_2. The finding suggests that the as prepared Ag-MSN- NH_2 is having antibacterial properties but lacks antifungal properties.

CHAPTER 7

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