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العدد 19

مجلة التربوي

مجلة علمية محكمة تصدر عن كلية التربية

جامعة المرقب

العدد التاسع عشر

يوليو 2021م

- **المجلة ترحب بما يرد عليها من أبحاث وعلى استعداد لنشرها بعد التحكيم .**
- **المجلة تحترم كل الاحترام آراء المحكمين وتعمل بمقتضاها .**
- **كافة الآراء والأفكار المنشورة تعبر عن آراء أصحابها ولا تتحمل المجلة تبعاتها .**
- **يتحمل الباحث مسؤولية الأمانة العلمية وهو المسؤول عما ينشر له .**
- **البحوث المقدمة للنشر لا ترد لأصحابها نشرت أو لم تنشر .**

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يشترط في البحوث العلمية المقدمة للنشر أن يراعى فيها ما يأتي :

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- يرفق بالبحث تزكية لغوية وفق أنموذج معد .
- تعدل البحوث المقبولة وتصحح وفق ما يراه المحكمون .
- التزام الباحث بالضوابط التي وضعتها المجلة من عدد الصفحات ، ونوع الخط ورقمه ، والفترات الزمنية الممنوحة للتعديل ، وما يستجد من ضوابط تضعها المجلة مستقبلا .

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- للمجلة الحق في تعديل البحث أو طلب تعديله أو رفضه .
- يخضع البحث في النشر لأولويات المجلة وسياستها .
- البحوث المنشورة تعبر عن وجهة نظر أصحابها ، ولا تعبر عن وجهة نظر المجلة .

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Effect Mesoporous silica silver nanoparticles on antibacterial agent Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus*

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الخلاصة

تم دراسة النشاط الميكروبى لجزيئات mesoporous Ag/NH_2-KIT-6(x) النانوية المصنعة بطريقة الأختزال الكيميائى ضد سلالتين من البكتريا السالبة والموجبة لصبغة جرام هما *P.aeruginosa* و *S.aureus* وأكدت النتائج المتحصل عليها القدرة العالية لجزيئات -Ag/NH_2 KIT-6(x) النانوية بمختلف تركيزات الفضة بها على تثبيط النمو الميكروبى لكلا من *P.aeruginosa* و *S.aureus* مقارنة بالمضادات الحيوية التى تم إستخدامها.

كما لوحظ وجود إختلاف فى درجة حساسية كلا الميكروبين للجزيئات النانوية وربما يعود هذا إلى إختلاف تركيب الجدر الخلوية لكلامنهما ولهذه الميكروبات أضرار كبيرة حيث أنها من الميكروبات الممرضة للإنسان لإرتباطها بالعديد من الأمراض الجهازية ويتضح من صور الميكروسكوب الماسح مدى التلف التى أحدثته جزيئات Ag/NH_2-KIT-6(x) النانوية للأغشية الميكروبية الخلوية.

نأمل فى المستقبل إستخدام أنواع مصنعة ونقية من الجزيئات النانوية فى صورة silica mesoporous لعلاج الالتهابات البكتيرية المقاومة للأدوية التي تسببها الأغشية الحيوية.

Abstract

Increase more attention to eco-friendly mesoporous silica silver nanoparticles featuring smaller particle sizes to enhance their remarkable antimicrobial properties. A simple chemical method was developed for synthesize high valence silver nanoparticles immobilized on the mesoporous silica nanomaterial, which showed strong antibacterial activity. Chemical reduction of silver ion has been regarded in the present work, and a

reducing agent , such as hydrazine was used to promote the reduction of the silver ion – precursor. The average particle size of the synthesized mesoporous silica-silver nanoparticles (Ag/NH_2-KIT-6(x)) with different concentrations of Ag (2.5 and 7%) calculated from Scherrer's equation for (1 1 1)-plane were 8 and 6.5 nm respectively. The synthesized materials were characterized using FTIR spectra, X-Ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and transmission electron microscopy (TEM), which revealed the mesoporous silica nanoparticles.

Antibacterial activities of mesoporous silica silver nanoparticles against Gram- negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus* were found to be increased with the increasing of Ag concentration in the Ag/NH_2-KIT-6(x). The maximum inhibition zone diameter when the concentration 7 % was used obtained against *P. aeruginosa* and *S. aureus* with diameters of 33 and 30 mm respectively**.** The antimicrobial activity of mesoporous Ag/NH_2-KIT-6(x) was evaluated also using the MIC&MBC tests. Biofilm forming from a variety of microbial pathogens can pose a serious health hazard that is difficult to combat. Nanotechnology, however, represents a new approach to fighting and eradicating biofilm-forming microorganisms. The biofilm formation by microplate method of both the untreated and the treated bacterial cells. Scanning electron microscopy observations clearly indicate that silver nanoparticles reduced the surface coverage by *P.aeruginosa* and *S. aureus*. thus prevent the biofilm formations. Mesoporous Ag/NH2-KIT-6 (7 %) exhibited excellent antbiofilm activity and prevented biofilm formation by *P.aeruginosa* and *S. aureus*. In conclusion the use of silver nanoparticles as antibacterial agent was according to be toxic against pathogenic bacteria and considered advantageous over other methods for control of pathogenic microorganisms, and it can be of great importance in developing novel drugs for curing many lethal diseases.

Introduction

Klevens *et al*. (2007) and Walker *et al*. (2009) increasing hospital and community-acquired infections due to bacterial multidrug-resistant (MDR) pathogens for which current antibiotic therapies are not effective and represent a growing problem. Antimicrobial resistance and biofilm development in affected patients is thus one of the major threats to human health, since it determines an increase of morbidity and mortality as a consequence of the most common bacterial diseases.

(Ruedas-Rama *et al*., 2012; Yildirim *et al*., 2013; Natarajan and Chen *et al*., 2014 and Selvaraj, 2014) mesoporous silica nanoparticles (MSNs) are well known for their biocompatibility, dispersibility, and chemical stability as carriers for drug/gene/antimicrobial agents. They have also been used as cell markers, catalytic substrates, absorbents, and matrix fillers**.** They can be synthesized with controlled size, shape and varying textural properties (e.g. pore size, surface area and porosity) for therapeutic uses **(Wu *et al*., 2013 and Qasim *et al*., 2014)**. Porous materials are of great interest because of their ability to interact with atoms, ions, molecules and nanoparticles not

only at their surfaces, but throughout the bulk of the materials. Therefore, the presence of pores in nanostructured materials greatly promotes their physical and chemical properties **(Han *et al*., 2011)**.

Rai *et al*. (2012) nanoparticles are now considered a viable alternative to antibiotics and seem to have a high potential to solve the problem of the emergence of bacterial multidrug resistance. Metal nanoparticles of silver, copper, and gold have been according to be active against certain pathogenic bacteria and fungi. In particular, silver nanoparticles have attracted much attention in the scientific field.

Taraszkiewicz *et al*. (2012) and Franci *et al*. (2015) silver has always been used against various diseases, in the past it was used as an antiseptic and antimicrobial against Gram-positive and Gram-negative bacteria. Comparatively, silver nanoparticles have been intensely studied owing to their distinct properties such as conductivity, chemical stability, catalytic activity, nonlinear optical behavior, low cytotoxicity and strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities. These properties make them suitable for use as an antimicrobial agent in catheters, burns, severe chronic osteomyelitis, urinary tract infections and medical textiles.

Sweet and Singleton, 2011 and Rai *et al*. (2016) mesoporous silica silver nanobartecal were considered, particularly attractive for the production of a new class of antimicrobials opening up a completely new way to combat a wide range of bacterial pathogens. The production of mesoporous Silica - AgNPs is relatively inexpensive and the addition of these particles into goods such as plastics, clothing, creams, and soaps increase their market value **(Feng *et al*., 2000)**. The aim of the work presents an overview of mesoporous silica-silver nanoparticles preparation by chemical methods and the implications of their use in controlling pathogenic microbes as they were tested for their antibacterial efficacy for different multidrug-resistant bacterial strains.

Materials and Methods:

Chemicals

Pluronic P123 (triblock copolymer P123, $EO_{20}PO_{70}EO_{20}$, Mw of ≈5800), tetraethyl orthosilicate (TEOS, ≥99.0%),3-Aminopropyltrimethoxysilane (APTS, 97%), 2,4,6-trinitrobenzenesulfonic acid (TNBS, 1 M in H_2O), potassium tetraborate tetrahydrate ($K_2B_4O_7$, ≥99.5%), tetrabutylammonium bromide (≥98%), 1-butanol (≥99%), HCl (35wt. %), hydrazine hydrate, silver acetate(99.99%),1,2,4-trimethylbenzene solvents including methanol and ethanol were purchased from Sigma-Aldrich and used without further purification.

Synthesis of High Valence of Mesoporous Silica Silver Nanoparticles

Reported by **Qian *et al*. (2012) and Hassan *et al*. (2016)** the KIT-6 was synthesized with some modification. Ten g of Pluronic P123 were melted (75°C) and then dissolved in

300 ml distilled water and 35 ml HCl (35 wt. %). The mixture was sheared at 350 rpm for 1 h. at 35°C then, 10 ml of 1-butanol and 1 ml of 1, 2,4-trimethylbenzene were added at once to the stirred mixture for 30 min. at 35°C. After that, 20 g of TEOS was added to the stirred mixture and it was further stirred for 24 h. at 35°C. Then, the mixture was transferred to 500 ml Teflon autoclave and the hydrothermal process was carried out for 48 h. at 130°C under static conditions. The dried obtained white material after filtration and washing with mixture of water and ethanol was finally calcined in air atmosphere at 600 °C for 8 h.

Hassan *et al*. (2014) the obtained free surfactant KIT-6 was coupled with APTS to functionalize the KIT-6 pores with propylamine moieties as following, 2.0 g of APTS was added directly to 5.0 g of degassed KIT-6 in 150 ml dry toluene under mechanical stirring for 4 h, at ambient temperature and then refluxed for another 12 h. The obtained material NH_2-KIT-6 was recovered by centrifuge, washed several times with ethanol-water mixture (50/50 V/V) to remove unreacted APTS molecules and vacuum dried at 60°C overnight.

Weber *et al*. (**2000)** the amino groups functionalized KIT-6 was determined gravimetrically by using, 2, 4, 6-trinitrobenzenesulfonic acid. The degassed NH_2-KIT-6 (0.1 g) was suspended in double distilled water (1 ml) at 50°C for 2 h and left to cool. Then, the suspended NH_2-KIT-6 was further diluted with 5 ml of 100 mmol borate buffer ($K_2B_4O_7$; pH=9.3), and 1.5 ml of 1000 mmol TNBS solution was added and allowed to react for 4 h at 35 °C. After that, the NH_2-KT-6-was recovered by centrifuge at 14000 rpm for 5 min. The supernatant absorption was measured at 350 nm to determine the concentration of TNBS molecules. Reference samples were prepared in parallel composed of 6 ml of double distilled water of $K_2B_4O_7$ material without NH_2-KIT-6 material, and the concentration of amino was confirmed with TNBS solutions.

The doping of silver nanoparticle on NH_2-KIT-6 at different loading were prepared in dry toluene using tetrabutylammonium bromide as cationic surfactant and silver acetate as a silver precursor. Briefly, 1.0 g of NH_2-KIT-6 (1.628 mmol of $–NH_2$) was suspended in 10 ml of toluene under ultrasonic irradiation for 15 min, and 5 ml of aqueous containing 0.25% tetrabutylammonium bromide and 6 µl of hydrazine are added and the mixture was further sonicated for additional 5 min. A 0.138 g (0.81 mmol) of silver acetate in 5 ml of distilled water was injected into the sonicated mixture under vigorous stirring. The mixture color slowly turned from white to pink, ruby and dark red color, confirming formation of silver nanoparticles. The mixture temperature is raised to 45°C to ensure the compellation of reaction for 2 h, and the solvents were drained under vacuum at 80°C. After solidification, the obtained sample was washed several times with water-ethanol (90/5; V/V) until free tetrabutylammonium bromide was detected by $AgNO_3$. Finally, the obtained sample was dried under vacuum for 6 h. silver nanoparticles immobilized in NH_2-KIT-6 was stirred in 10 % HNO_3 and analyzed by Inductive Coupled Plasma (ICP). The samples were donated as Ag/NH_2-KIT-6(x), where x represents the weight ratio of Ag^o immobilized on NH_2-KIT-6 2.5 and 7 %.

Characterization of Mesoporous Silica Silver Nanoparticles

McCusker and Baerlocher, (2001) and Rai and Duran, (2011) the XRD patterns were performed in X'Pert PRO instrument using Cu Kα radiation at 40 kV and 30 mA. In powder XRD patterns, the positions of the peaks are determined only by the dimensions and shape of the unit cell and in general the intensities depend on the type of atoms and their position within the unit cell. Diffractograms were obtained from 0.5 to 10 (2Ɵ) at a scanning speed of 0.05 deg. min^{-1} and counting time of 5 s.

The N_2-adsorption isotherms were performed at -196 °C in NOVA 3200 system instrument. The specific surface area was measured by applying the BET equation to the adsorption isotherm in regime ≥ 0.35 of P/P_0 value. The pore size distribution was calculated from Barrett–Joyner–Halenda (BJH) method. The total pore volume was considered to be the volume of liquid N_2 adsorbed at a relative pressure of 0.98. Prior to the analysis, the samples were outgassed under vacuum at 140°C for at least 16 h. High Resolution Transmission Electron Microscope images were obtained on a JEOL JEM 2100 microscope. Transmission Electron Microscope samples were prepared by sonication of the powder in ethanol and evaporating one drop onto a holey carbon film.

The FTIR spectra were recorded on a Nicolet iS10 Fourier transform infrared spectrophotometer. X-ray photoelectron spectroscopy (XPS) was carried out using a Thermo Scientific K-ALPHA USA instrument equipped with a dual X-ray source, using the AlKα radiation anode and a hemispherical energy analyzer. With an analyzer chamber pressure of 10^{-9} torr at a power of 250W. Metal' s concentration impregnated in the samples was quantitatively determined using standard methods of analyses including Inductive Coupled Plasma (ICP)**.** The ICP instrument is ICP-OES Perkin-Elmer Optima 2000 DV.

Microbiological Experiments:

Bacterial cultures

Two experimental bacterial strains, Gram positive *Staphylococcus aureus* and Gram negative *Pseudomonas aeruginosa* in addition were used in this study was isolated from hospitalized patients with indwelling catheter suffering from urinary tract infections (UTIs) by applying the standard methods of Harley . Bacterial cultures and Prescott (2002). Preserved at 4°C on nutrient agar slants. Colonies were identified by gram staining according to the conventional method, and the biochemical tests were done using API 20 test kits (bioMe´rieux, Inc., France).

Antibacterial susceptibility testing

Eight different antibiotics including Cefotaxime 30µg, Ceftriaxone 75µg, Amikacin 10µg, Nalidixic acid 30µg, Neomysin 30µg, Sulfamethoxazole-trimethoprim 25µg, Ampicillin 30µg and Colifuran 200µg as standard antibacterial agents were used and antibacterial

susceptibility test was performed by disk diffusion method on Mueller–Hinton agar plates according to **Kheiralla *et al*. (2014).**

Assessment of the antibacterial activity of mesoporous silica silver nanoparticles well diffusion method

The antibacterial susceptibility of strains was determined by agar well diffusion method as described by Abbaszadegan *et al*. **(2015)** Briefly, colonies from an overnight culture of the tested bacteria grown on nutrient agar were suspended in saline and turbidity was adjusted to 0.5 McFarland ($1x10^8$ CFU/ml). Plates of Mueller–Hinton agar were inoculated using a sterile swab to produce a confluent lawn of growth. Using sterile cork borer, the well (6 mm) was made into each Petri. A 50 ml of AgNPs suspended into dimethyl sulfaoxid DEMSO100 µg/ml to was added into the wells, then the plates were left at refrigeratorn for 3 hours to allow diffusion of the test sample and incubated at 37°C for 24 hours after which zones of inhibition were measured.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) Test:

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antimicrobial agents that inhibited 90 percent of the bacterial growth after an overnight incubation in comparison with the negative control **Abbaszadegan *et al*. (2015)**.The minimum inhibitory concentration and MIC of silver nanoparticles were assessed by the macro dilution broth susceptibility test according to the method based on the US National Committee for Clinical Laboratory Standards Guidelines **Kheiralla *et al.* (2014)** and **Karthikeyan *et al*. (2011).** Sterile Mueller–Hinton broth was used as diluents for the preparation of silver nanoparticles dilution. A serial dilution of the 0.01mg/ml silver nanoparticles solution was prepared within a desired range .One milliliter of each bacterial suspension that was prepared by suspending colonies from an overnight cultured tested bacteria on nutrient agar in sterile saline solution (0.85% NaCl) and adjusting turbidity to 0.5 McFarland ($1x10^8$ CFU/ml) was inoculated and then tubes were incubated for 24 h. at 37^oC, the control tubes without the silver nanoparticles were assayed simultaneously. To test the bactericidal effect a loopful from each tube was inoculated on Mueller–Hinton agar and incubated for 24 h. at 37^oC. **Ruparelia *et al*., (2008)** the nanoparticles concentration causing bactericidal effect was selected based on absence of colonies on the agar plate**.** The results were plotted as the mean value of 3 mutually independent experiments.

Effect of mesoporous silica silver nanoparticles on the biofilm formation of bacterial by microplate method:

The effects of mesoporous silica silver nanoparticles on the morphology of both *P. aeruginosa* and *S. aureus* were tested for biofilm formation. Bacterial strains were grown overnight in Trypticase Soy broth (TSB) supplemented with 0.25% glucose at 37^oC. After

that, the strains were diluted 1/200 in TSB supplemented with 0.25% glucose and 200 µl of this suspension was inoculated in triplicate to polystyrol microliter plates to them 100 µl of DEMSO containing 1µg of mesoporous silica silver nanoparticles (7%) was added treated also bacterial suspension were inoculated in triplicate without nanoparticles untreated and incubated overnight at 37^{o}C. Then, the cellswere decanted and wells were washed three times with phosphate buffer (PB), dried at room temperature and finally stained with 0.1% safranin. Absorbance of the adherent cells was measured at 490 nm using a microplate reader (Mindray, MR-96A). A strain was considered positive, if its absorbance value was > 0.12 **(Christensen *et al*. 1985)**.

Statistical analysis

(**SPSS, 2006**) the experiments were replicated three independent times and the data are presented as mean ±STD. Statistical analysis was carried out using Student's t test. Differences were considered statistically significant when P-value was less than 0.01.

After testing the data for normality, the differences in the treatments were tested using one-way analysis of variance (ANOVA 1) according to SPSS software. A post-hoc test was applied when differences were significant.

Results and Discussion

Yuan *et al*. (2017) overuse or misuse of antimicrobial agents has led to the development of multi-drug resistant bacteria. To overcome the limitations of conventional synthetic antimicrobial compounds, nanotechnology represents an alternative strategy in developing alternative antimicrobial agents that can efficiently kill bacterial cells and display immense potential for use in both medical and veterinary applications

Characterization of Mesoporous Silica Silver Nanoparticles

The sol-gel technique is one of the most important facile methods for porous preparation materials. The ordered cubic pore of silicate was synthesized using TEOS as silica precursor, HCl as a hydrolysis catalyst, P123 as structure directing agent (PO moieties as phase separation inducers; PEO moieties as a gelling agent) and butanol as co-surfactant. The pore construction morphology is determined by the balance between the silica coarsening and the structure freezing by P123 and butanol co-surfactant that create the cubic morphology of the obtained monolithic silica. The second phase includes grafting of APTS on the silica in toluene, forming covalent bond between the monolithic silica and amino-organosilane moieties **(Fig. 1)**. After that, the obtained amino-silicate (NH_2-KIT-6) was used as a platform to cap the reduced silver nanoparticle. In this stage, the silver acetate was embedded on NH_2-KIT-6, forming an Ag-NH_2-SiO_2 complex, and an appropriate amount of hydrazine was used to promote the decrease of the silver complex.

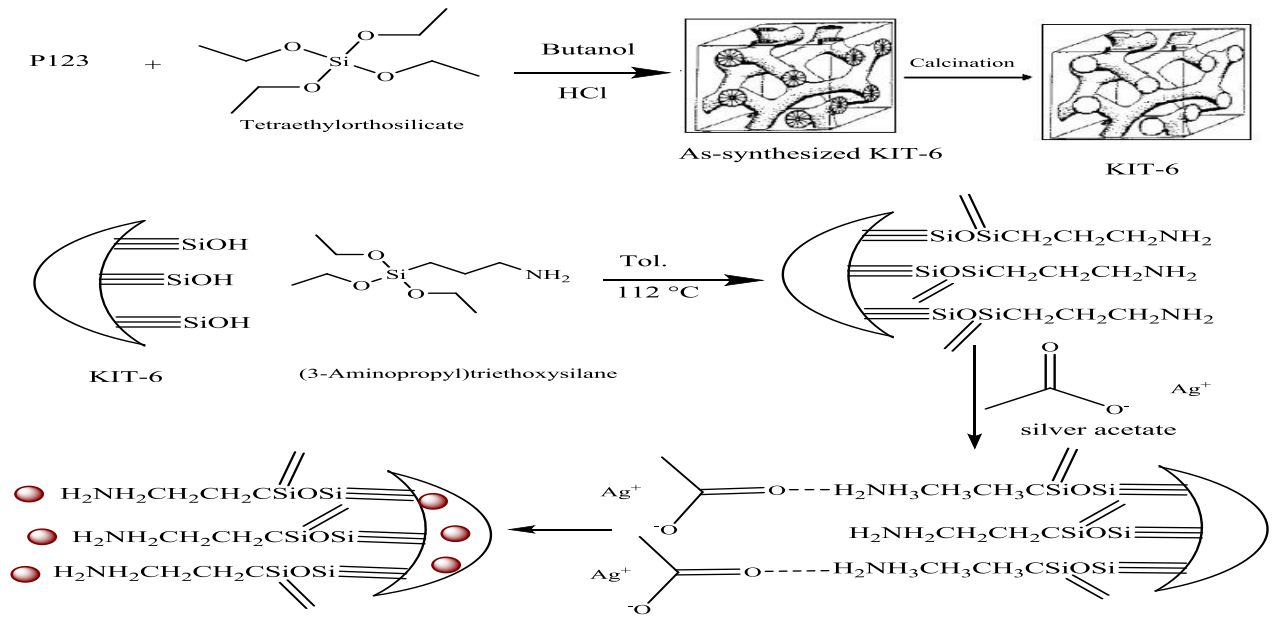


Fig.1: Schematic representation of the preparation of KIT-6, NH_2-KIT-6 and Ag/NH_2-KIT6(x).

The concentration of amino group per gram was of 1.45 mmol g^{-1} and the immobilized silver according to ICP analysis was of 7% and 2.5%, for Ag/NH_2-KIT-6(7 %) and Ag-NH_2-KIT-6(2.5%). The FTIR assembly details of the KIT-6 functionalized NH_2-KIT-6 and Ag/NH_2-KIT-6(x), were examined. Spectra of NH_2-KIT-6 and Ag immobilized on Ag-NH_2-KIT-6(7&2.5%) are shown in **Fig. 2** The KIT-6, NH_2-KIT-6 and Ag/NH_2-KIT-6 (7 and 2.5 %) exhibit the characteristic stretching broad bands associated with ≡Si-OH groups in the regime of 3450–3350 cm^{-1}, while the corresponding binding band for KIT-6 & NH_2-KIT-6, are observed at 1665 cm^{-1} and 1650 cm^{-1} for Ag/NH_2-KIT-6. The NH_2-KIT-6 exhibits the characteristic -CH_2 asymmetric and symmetric stretching bands near 2933 cm^{-1} and 2821 cm^{-1}, respectively. While asymmetric Si–O–Si stretching bands appaired near 1066 cm^{-1}, the band near 969 cm^{-1} is due to the Si–O bending in the Si–OH groups and asymmetric Si–O–Si stretching band appeared near 810 cm^{-1}. The vibration band at 3137 cm^{-1} in NH_2-KIT-6 is attributed to the stretching vibration of –NH_2 that is in hydrogen bonding with Si–OH. The presence of asymmetric –N–H bending vibration near 707 cm^{-1} confirms the incorporation of an amino group on the KIT-6 material. The band at 1380 cm^{-1} associated with –CH_2 vibration, can be seen for the samples containing aminopropyl groups containing silver metal **(Coates, 2000 and Wang *et al.*, 2005)**. However this band became more intense as Ag loading increase, implying free mobility of propylamine moieties after deposition Ag^0 on –Si-OH.

(Sajab *et al.*, 2011) Furthermorr, the significant reduction in band intensities at 3450–3350 cm^{-1}, conforming consumption of hydroxyl group after Ag anchoring in NH_2-

KIT-6 surface, suggesting that portion of silver acetate is incorporated on $-SiO_2$ moieties. Finally, the intensity of the characteristic absorption bands in the range 1350–960 cm^{-1} are also observed for all the samples, indicating slight changes in the pore structures after adsorption, which may be because of inherent disorder in the virgin and functionalized material structures**.**

The low angle XRD patterns of KIT-6, NH_2-KIT-6 and Ag/NH_2-KIT-6(2.5 and 7%) are shown in **Fig.3** The KIT-6 and NH_2-KIT-6 samples exhibit three well-resolved; one strong intense diffraction peak and two minor diffraction peaks that assigned to (2 1 1), (2 2 0) and (3 3 2) reflections, which can be indexed to $Ia\bar{3}d$, suggesting high ordered cubic mesoporous structure. It is clear that the intense peaks are shifted to lower angle after grafting APRTS molecule and more shift is noticed after Ag^{+1} loading. This behavior is resulted from increase the pore wall due to the grafting Ag^0 on propylamine moieties. In addition, the a_0 (unit cell parameter) is increased after Ag^0 immobilization on KIT-6, escorted by the shift of the intense peak (2 1 1) reflection to lower angle. The high angle XRD patterns of KIT-6, NH_2-KIT-6,Ag/NH_2-KIT-6(2.5, 7 %), are shown in **Fig. 4**. The XRD patterns of KIT-6 and NH_2-KIT-6 showed broad peak centered at 23.11°, while very weak peaks of the Ag^0 species in Ag/NH_2-KIT-6(2.5) materials is detected due to the high dispersion of silver on NH_2-KIT-6, while four peaks at 38.04°, 44.29°, 64.30^0 and 77.39° corresponding to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) plane-reflections of fcc Ag^0 nanostructure is noticed for Ag/NH_2-KIT-6(2.5%) material. The average particles size of AgNPs calculated from Scherrer's equation for (1 1 1)-plane were of 8 and 6.5 nm for Ag/NH_2-KIT-6(7 %) and Ag/NH_2-KIT-6(2.5 %), respectively.

Shown in **Fig. 5** the HRTEM images of the prepared samples **.** The KIT-6 and NH_2-KIT samples exhibited well 3D cubic ordered mesoporous materials. The Ag^0 immobilized NH_2-KIT showed ordered/disordered mesopores, however the irregular domains become predominant as Ag^0 loading increased due to the significant adsorption of Ag^0 particle on amino or silanol groups. Moreover, the AgNPs appeared highly dispersed on NH_2-KIT and took the direction of KIT-6 pores, and the particle size distribution of silver nanobarticales was of 4-9 nm size.

Shown in **Fig. 6** the N_2 adsorption-desorption isotherms of KIT-6, NH_2-KIT-6 and Ag/NH_2-KIT-6(2.5, 7 %) materials and the textural properties are collected in **Table 1.** All samples displayed Type IV isotherms with pronounced capillary condensation at high relative P/P_0 and H1 hysteresis loop, indicating large uniform channel-like mesoporous with narrow pore size distribution. The BET of KIT-6 was of 705 m^2 g^{-1}, however, after grafting APRTS, the surface area decreased to 503 $m^2g^{-1,}$ and the sharp decrease is noticed after immobilization of silver nanobarticales. The BET of Ag/NH_2-KIT-6(2.5, 7%) was of 315 m^2 g^{-1} and 280 m^2 g^{-1}, implying good dispersion of AgNP in the inter-connected pore of KIT-6.

Shown in **Fig. 7** the UV-Vis absorption of NH_2-KIT and Ag/NH_2-KIT-6(2.5,7%) materials.the bare NH_2-KIT- didn't show any UV-Vis absorption, while Ag/NH_2-KIT-6(2.5

%) and Ag/NH_2-KIT-6(7 %) showed UV-Vis absorption at 413 nm and 417 nm, respectively due to the Mie Plasmon resonance excitation of silver nanobarticales **(Kim *et al*., 2006)**. As the concentration of silver nanobarticales increased, the Plasmon resonance peak showed slight red shift, indicating the silver nanobarticales particle becomes larger somewhat. However, the low difference in silver nanobarticales particle size is attributed to the chemical coordination and steric effect of amino groups grafted on KIT-6 materials that obstruct the further growth of silver size.

The XPS spectra were used to demonstrate chemical environment for carbon, nitrogen and silver elements **Fig. 8.** The high resolution XPS of C 1s is showed three peaks of binding energy at 284.28, 284.6 and 286.24 eV, appoint to C-C, C-H, and C-N sp^3 carbon, respectively. The XPS spectra in the N 1s binding energy range displayed the spin orbital single for NH_2-KIT-6 and doublet for Ag/NH_2-KIT-6(7%) at 399.7 and 401.2 eV, indicating that electron transfer from nitrogen atom to metallic Ag^0. These values are in agreement the presence of $–NH_2$ and coordinated $–NH_2$, as reported for other immobilized Ag^0. The XPS profile of Ag 3d is curve-fitted into doublet spin-orbital with ≈6 eV separation, **Ferraria *et al*. (2010)** centered at 367.6 and 373.5 eV, which assigned to Ag $3d_{5/2}$ and Ag $3d_{3/2}$ present on NH_2-KIT-6. In this study absence of the peaks at ~368 and ~374 eV indicates that all silver atoms are reduced **(Gebeyehu *et al*., 2016)**.

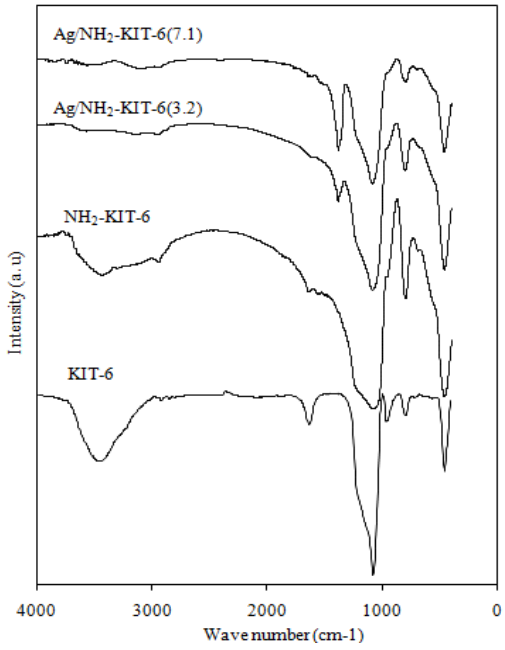


Fig. 2: FTIR spectra of calcined KIT-6, NH_2-KIT-6, Ag/NH_2-KIT-6 (2.5 & 7%) materials.

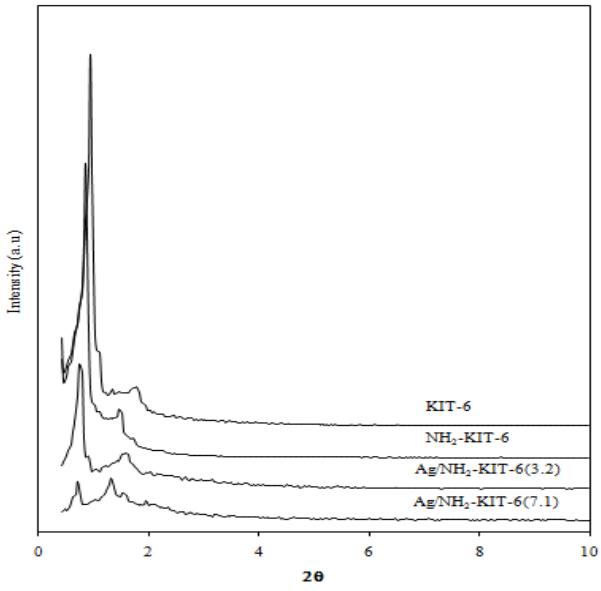


Fig. 3: Low XRD patterns for KIT-6, NH_2-KIT-6 and Ag/NH_2-KIT-6 (2.5 & 7%) materials.

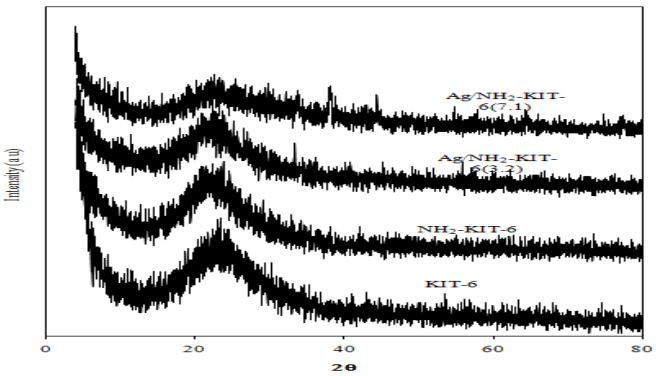


Fig. 4: High XRD patterns of KIT-6, NH_2-KIT-6 and Ag/NH_2-KIT 6 (2.5 & 7%) materials.

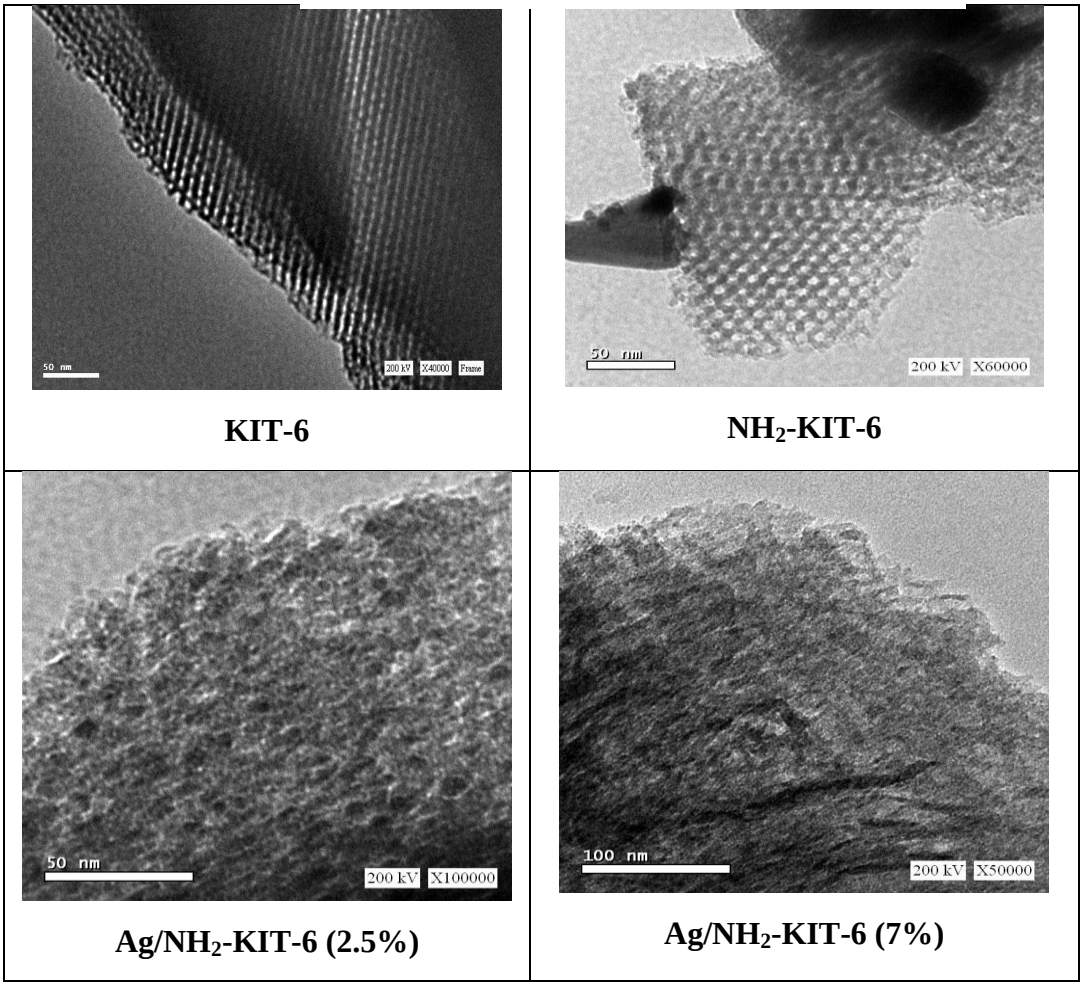


Fig. 5: High Resolution Transmission Electron Microscope images of KIT-6, NH_2-KIT-6 and Ag/NH_2-KIT-6 (2.5&7%) materials.

Table 1: The physicochemical characterization and textural of KIT-6, NH_2-KIT-6 and NH_2-KIT-6 (2.5 & 7 %).

Sample	d_{211}(Å)	a_0 (nm)	W_T	S_{BET} [a] ($m2g^{-1}$)	D_p [b] (nm)	V_p (cm^3g^{-1})
KIT-6	93.87	22.99	2.735	705	8.76	0.889
NH_2-KIT-6	102.61	25.13	5.945	503	6.62	0.62
Ag/NH_2-KIT-6(3.2)	119.23	29.21	8.865	315	5.74	0.46
Ag/NH_2-KIT-6(7.1)	124.27	30.44	10.04	280	5.18	0.39

S_{BET}, specific surface area; D_p, pore diameter; W_T, pore wall thickness [$a_o / \sqrt{2}$, (where $a_o = \sqrt{6}$ a]

[a] Values obtained from XRD studies.

[b] Values obtained from N_2-adsorption results.

(2) NH_2-KIT-6

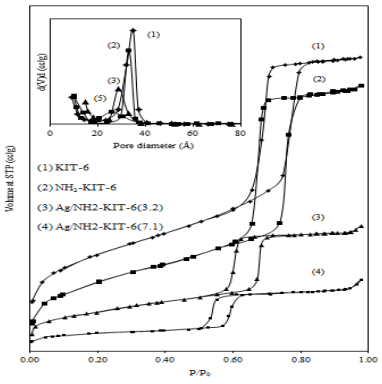


Fig. 6: Nitrogen adsorption/desorption and pore size distribution of KIT-6, NH_2-KIT-6 and Ag/NH_2-KIT-6 (2.5 & 7 %)

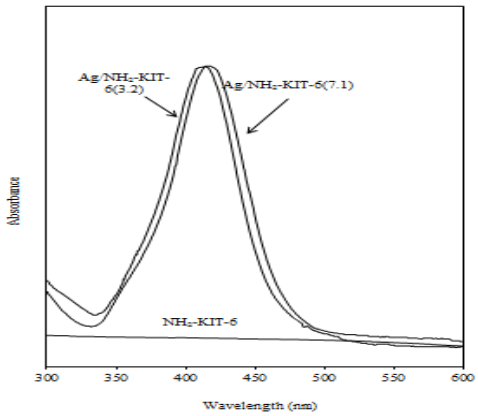


Fig. 7: UV-visible absorption spectra of NH2-KIT-6, Ag/NH2-

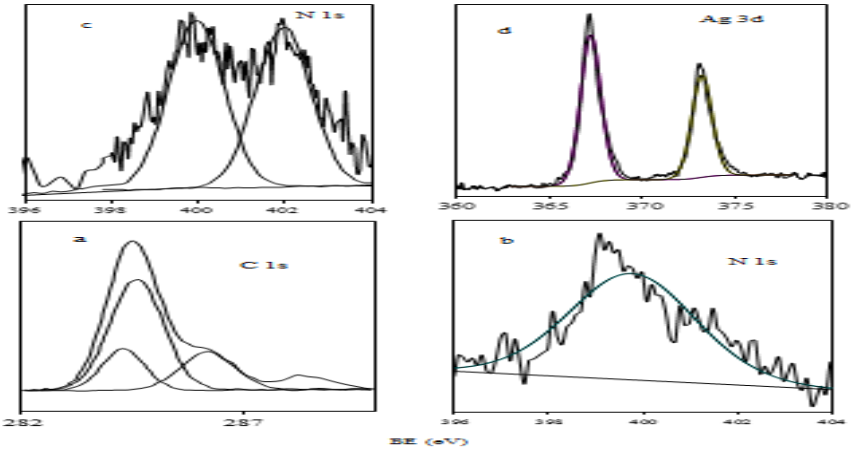


Fig. 8: XPS of **a:** C 1s, **b& c:** N 1s and **d:** Ag/NH2-KIT-6 (7 %) material.

Antibacterial Activity

Antibiotic susceptibility test was performed against two bacterial strains, *S. aureus* as Gram positive & *Pseudomonas aeruginosa* as Gram negative strains **Table 2**. *Pseudomonas aeruginosa* was found to be resistant to Cefotaxime, Nalidixi acid and F while *S. aureus* was found to be resistant to Nalidixi acid and Sulfamethoxazole-trimethoprim. The antibacterial activity was affected with the concentrations 2.5 &7% of Ag in Ag/NH2-KIT-6(x). The maximum inhibition zone diameter obtained is 33 and 30 mm when using 7 % against *P. aeruginosa* and *S. aureus* respectively.

Shen *et al*. (2014) the present results reported that the mesoporous Ag/NH2-KIT-6(x) exhibited good antibacterial activity and inhibited the growth of *P. aeruginosa and S. aureus* effectively. Whereas the mesoporous silica-KIT-6 and NH_2-KIT-6 exhibited no bacterial inhibitory effects due to the absence of metal silver nanoparticles in the composite material. APTS modification procedure plays a very important role in the immobilization of silver nanoparticles onto $mSiO_2$. Because the amino group of APTS could absorb a larger amount of Ag+ ions tightly through the complex and electrostatic interactions between Ag+ ions and amino functional groups. However, there only a small amount of Ag+ ions could be absorbed onto $mSiO_2$ APTS modification and the created Ag nanoparticles could easily break away from the channels of $mSiO_2$ during the filtration and washing procedure**.** The synthesized AgNPs appeared to be highly dispersed on NH_2-KIT6 and took the direction of KIT-6 pores, and the particle size distribution of mesoporous AgNPs was of 4-9 nm. In general, for nanoparticles to be effective, their typical size should not be larger than 50 nm. More precisely, mesoporous silver nanoparticles with size between 10 and 15 nm have increased stability, biocompatibility and enhanced antimicrobial activity **(Yacaman *et al*., 2001)**. Some studies have revealed that the antibacterial action of AgNPs is more effective against *S. aureus* and *K. pneumoniae* when nanoparticles of smaller diameter (<30nm) are used **(Collins *et al*., 2010)**.

Morones *et al*. (2005)The antibacterial effect of AgNPs as proposed is due to their smaller particles size that apparently has superior penetration ability into bacteria, especially in Gram-negative. Antimicrobial efficacy of AgNPs was evaluated by many researchers against a broad range of microbes, including MDR and non-MDR strains of bacteria, fungi, and viruses **(Malarkodi *et al*., 2013)**.

Rai *et al*. (2012) Nano-sized metal particles are now well-established as a promising alternate to antibiotic therapy because they possess unbelievable potential for solving the problem associated with the development of multidrug resistance in pathogenic microorganisms, hence also regarded as next-generation antibiotics.

Table 2: Antibacterial activity of Ag/NH2-KIT-6(x) versus antibacterial activity of standard antibiotics against *S. aureus* and *P. aeruginosa*.

Antibacterial agent	**Inhibtion zone (mm)**	
	S. aureus	***P. aeruginosa***
Ag/NH2-KIT-6 **(7%)**	30.0±0.0a	33.0±2.0a
Ag/NH2-KIT-6 **(2.5%)**	24.0±0.0bc	25.0±0.0b
Cefotaxime	13.0±0.0d	0.0±0.0f
Ceftriaxone	29.0±1.0a	17.0±0.0d
Amikacin	23.0±0.0bc	20.0±0.0c
Nalidixi acid	0.0±0.0e	0.0±0.0f
Neomysin	21.0±1.0bc	15.3±0.6e
Sulfamethoxazole-trimethoprim	0.0±0.0e	17.0±0.0d
Ampicillin	24.0±1.0b	17.0±0.0d
Colifuran	18.7±8.1c	0.0±0.0f
F value	**50.671*** **	**844.590*** **

The antimicrobial activity of Ag/NH2-KIT-6 (x) was also evaluated using the macrodilution broth susceptibility method. MIC and MBC of silver nanoparticles shown best results with the concentration 7 % of Ag/NH2-KIT-6 than the concentration 2.5 % of Ag/NH2-KIT-6 **(Table 3)**. The MIC and MBC for the Ag /NH2-KIT-6 (2.5%) against tested bacteria *P. aeruginosa* and *S. aureus* differ in their values, but MIC equals MBC at the concentration (7%).

The concentration of Ag/NH2-KIT-6 (7%) shows 0.0045 and 0.041µl/ml for both MIC and MBC against *P. aeruginosa* and *S. aureus* respectively, i.e. completely inhibited, which indicated that the MIC and MBC of Ag/NH2-KIT-6(7%) are equal **(Table 3).** The obtained result agree with **Ansari *et al*. (2011)** whom showed that the AgNPs of 5–10 nm dimension display both bacteriostatic as well as bactericidal effects against *S. aureus,* MSSA and MRSA.

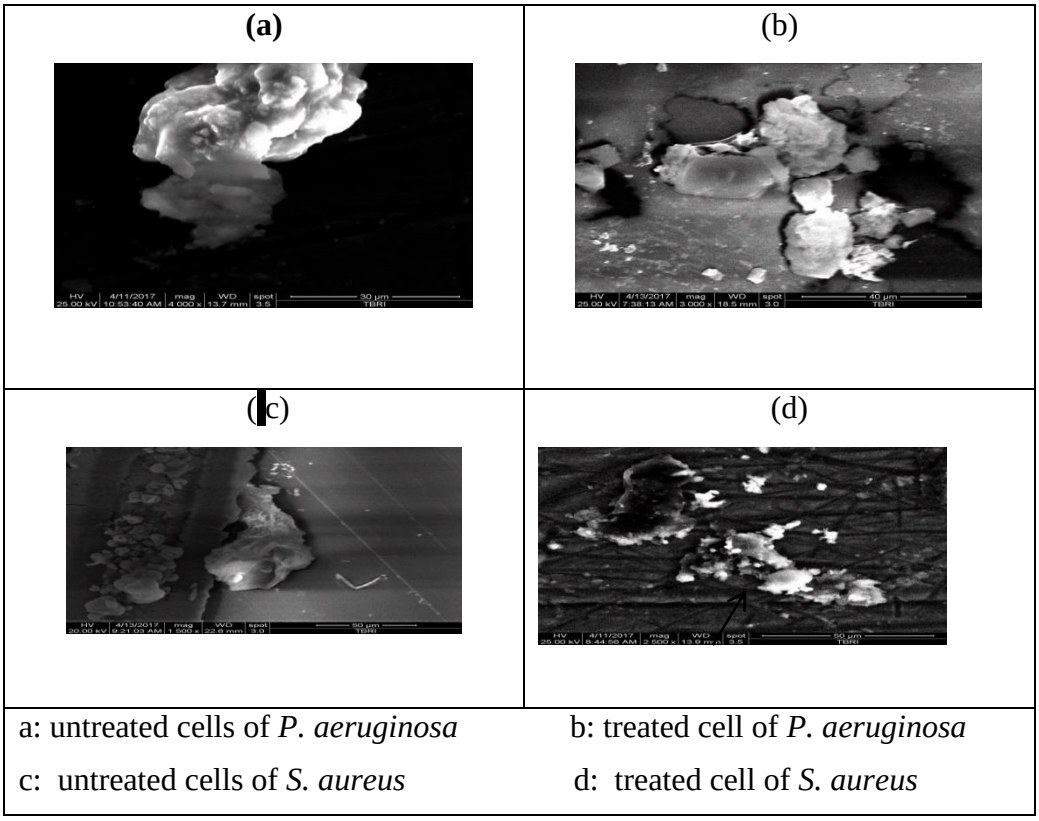
Gram-negative bacteria are more sensitive against nano Ag/NH_2KIT-6 than gram-positive bacteria, also **Klapiszewski *et al* .(2015)** and **Yuan *et al*. (2017)** demonstrated that, AgNPs were more effective against Gram-negative *P. aeruginosa* than Gram-positive *S. aureus*, which could be explained by differences in membrane structure and the cell wall composition, which influence bacterial susceptibility to AgNPs.

Table 3: The MIC and MBC of mesoporous silver nanoparticles (2.5 and 7%) against *P. aeruginosa* and *S.aureus***.**

Ag/NH_2-KIT-6 (x) (µg/ml)		**Tested Bacterial**	
		P. aeruginosa	***S.aureus***
2.5%	**MIC**	0.0045	0.041
	MBC	0.013	0.37
7%	**MIC**	0.0045	0.041
	MBC	0.0045	0.041

According to the obtained results, Mesoporous Ag/NH2-KIT-6 (7 %) exhibited excellent antibiofilm activity and prevented biofilm formation by *P.aeruginosa and S. aureus* qualitatively by micropate method **Fig 10**. Similar results were also reported by **Kalishwaralal *et al*. (2010)** against *P. aeruginosa* and *S. epidermidis* biofilms and found that 100 nM of AgNPs resulted in a95–98 % reduction in biofilm. **Thirumurugan *et al*. (2016)** reported that silver nanoparticles mostly had an inhibitory on biofilm formation as an average of 60.67 ± 2.52 against *E.coli* fllowed by 40.33 ± 5.033 for *P.aeruginosa* and 34.33 ± 9.71 against *S. aureus* were obtained.

Fig. 10: SEM micrographs of mesoporous Ag/NH_2-KIT-6 (7%) on the biofilm formation of *P. aeruginosa* and *S. aureus* cells.



a: untreated cells of *P. aeruginosa* b: treated cell of *P. aeruginosa*

c: untreated cells of *S. aureus* d: treated cell of *S. aureus*

Conclusion

In this study¸ Antibacterial activity of mesoporous Ag/NH2-KIT-6(x) nanoparticles has been demonstrated in several investigations, In the present study, mesoporous nanoparticles showed good antibacterial activity against all the tested pathogens comparison with standard antibiotics . The results of MIC and MBC tests revealed a higher MIC value for *P. aeruginosa* comparing to the other tested pathogens. This may be due to the differences in bacterial cell walls, since Gram-negative bacteria have thinner cell wall comparing to Gram-positive bacteria. In agreement, **Kim *et al.*** However, in our study, the MBC values were identical for all the pathogens. It has been previously stated that bactericidal property of mesoporous nanoparticles is dependent on the concentration and size of nanoparticles and also the initial bacterial concentration. **Guilhelmelli, *et al*. (2013)** The susceptibility to a biocide varies markedly between planktonic cells and biofilms. Biofilms are microbial communities consisting of cells attached to biotic or abiotic surfaces, embedded in an exopolymeric matrix. These structures are well known for their remarkable resistance to diverse chemical, physical, and biological antimicrobial agents and are one of the main causes of persistent infections. Nanotechnology may provide the answer to penetrate such biofilms and reduce biofilm formation by the use of ‘‘nanofunctionalization’’ surface techniques to prevent the biofilm formation.

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