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Synthesis, characterization and antifungal activity of coated silver nanoparticles-nystatin and coated silver nanoparticles-clotrimazol

Anwar Adil Hameed , Mustafa Abdulghafoor Alheety

Department of chemistry, College of Science, University of Tikrit , Tikrit , Iraq

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Corresponding Author:

Name: Anwar Adil Hameed

E-mail:

Noor_2010912@yahoo.com

Tel:

Abstract

Two silver nanoparticles containing compounds were prepared by simple chemical reactions and characterized by X-ray diffraction (XRD), infra-red spectrum (FTIR) and atomic force microscope (AFM). These functionalized silver nanoparticles were used to study their biological activity against four types of fungi. These compounds proved to have high inhibition effect against all types of studied fungi. Due to the presence of silver nanoparticles which increases nystatin and clotrimazol interact with the fungi.

Introduction

Nano-sized particles known nanoparticles (NPs) are a very small materials that have at least one dimensions ranging from 1 to 100 nanometers [1]. In this range, nano materials have unique biological activity and other properties from their bulk form [2]. Its related AgNPs have distinctive physical and chemical properties, high thermal and electrical conductivity, chemical stability, non-linear optical properties, catalytic activity and surface enhanced Raman scattering [3,4]. These features made AgNPs able to use in production of drug delivery system, microelectronics, and medical devices [5,6], in addition to exhibit broad spectrum biological activity due to its toxicity to viruses, bacteria and fungi [7,8].

Silver nanoparticles have many shapes depending on the preparation method that used. Commonly shape is spherical nanoparticles, octagonal, prisms, and sheets are also popular [9]. Citrate reduction method has been used to prepare AgNPs due to its short reaction time and gives uniform size and shape [10,11]. Many diseases are caused by fungi. Pharmacological medicines for these sicknesses include the utilization of topical antifungal agents, for example, nystatin, clotrimazol, miconazole alternately cicloprox creams [12,13]. Silver was used as a safe inorganic antibacterial drugs, such as silvaden (silver sulfadiazine), because it has the ability to kill many types of bacteria or fungi [14]. Lately nano-sized particles of silver are being used as a smart new technology for preventing fungi and bacterial infection, and as a potential new health and environmental threat, therefore , it can be considered as a name of an antibacterial technology. That features because AgNPs can resist and fight microbial by inhibits cell growth due to the large surface which increases the silver that interact with the fungi [15].Synergistic effect of silver nanoparticles were studied by combining with antifungal drugs against *candida* species [16]. In this article, we have been establish a chemical reaction between (not synergistic effect) AgNPs with nystatin and clotrimazol. The AgNPs, as same as other nano materials, have large surface area and conformational entropy in polyvalent binding [17,18] that allow easily coordinate to flexible ligands such as anti-fungal drugs (nystatin and clotrimazol).Therefore, this coordinate complex would examine their anti-fungal and antibacterial activity.

Chemicals

Reagent grade silver nitrate and tri sodium citrate and sodium dodecyl sulfate (SDS) was bought from Sigma-Aldrich, clotrimazole and nystatine were purchased from Merck. These chemicals have been used without further purification.

Instrumentation

Infra-red spectra were recorded in WQF-510 spectrophotometer at a range between (400-4000 cm^{-1}) KBr discs, X-ray diffraction measurements ranging

(10-100) were recorded for nano materials by using (Shimadzu – XR – 6000) device with nickel - copper filter for the x-ray radiation (Cu Kα, λ = 1.5406 Å), Atomic Force Microscope (AFM images) were recorded by using PHYW type.

Synthesis of nano materials

Synthesis of silver nanoparticles (AgNPs):

Silver nitrate 0.1g was dissolved in 90 ml deionized water and heated with stirring to 95 ^{O}C. Then 5% (w/w) of SDS, was putted. 0.1 g tri sodium citrate in 10 ml of distilled water were slowly added and we noticed the colorless solution turned to blackish-grey. The solution was left overnight and decanted to separate AgNPs. AgNPs in 30 ml deionized water were then ultrasonicated at 100 watt for 10 minutes and centrifuged at 1500 rpm for 20 minutes to obtained AgNPs powder.

Synthesis of AgNPs-Nystatin (AgNPs-NY):

Silver nanoparticles (0.1g in 30 ml ethanol) were dispersed using ultrasonic device, then added to nystatin solution (1g in 50 ml of ethanolic 2% NaOH solution) slowly under vigorous stirring for 10 minutes. The solution was then refluxed for two hours until the solution completely turned to pale orange. Finally the solution was centrifuged and the precipitate was washed by ether and dried under vacuum oven at 60 ^{O}C for 24 hours.

Synthesis of AgNPs- Clotrimazol (AgNPs-CM):

Silver nanoparticles (0.1g in 30 ml ethanol) were dispersed using ultrasonic device, and added to clotrimazol solution (1g in 80 ml acetone) slowly under vigorous for 10 minutes. This solution was then refluxed for one hour. Grey precipitate was formed, filtrated, washed with acetone and finally dried under vacuum oven at 60 ^{O}C for 24 hours.

Results and discussions

FT-IR spectroscopy:

The FTIR spectrum of silver nanoparticles Fig.1, shows the many peaks at 3313 cm^{-1}, 1593 cm^{-1} and 1388 cm^{-1} which corresponding to stretching vibration of OH, C=O and C-O groups of the reducing agent, respectively [19].

While the FTIR spectrum of coated AgNPs-NY Fig.2 indicates shifting of stretching vibrations of OH, while (NH_2 overlap with OH) and C-O groups of nystatin Fig.3 frequencies in the coated AgNPs-nystatin, which proving the silver nanoparticles were successfully chemically coated with nystatin by NH_2 or/and OH groups as in the table 1.

In the FTIR spectrum of coated AgNPs-CM Fig.5 shows shifting of stretching vibrations of C-N group of clotrimazol Fig. 4 in the coated AgNPs-clotrimazol, table 2. The shifting refer that the silver nanoparticles were successfully chemically coated with clotrimazol as in the table 2.

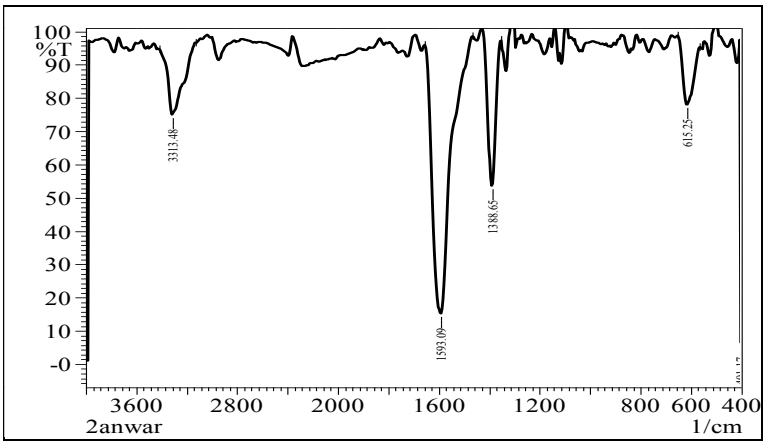

Figure 1: FTIR of AgNPs

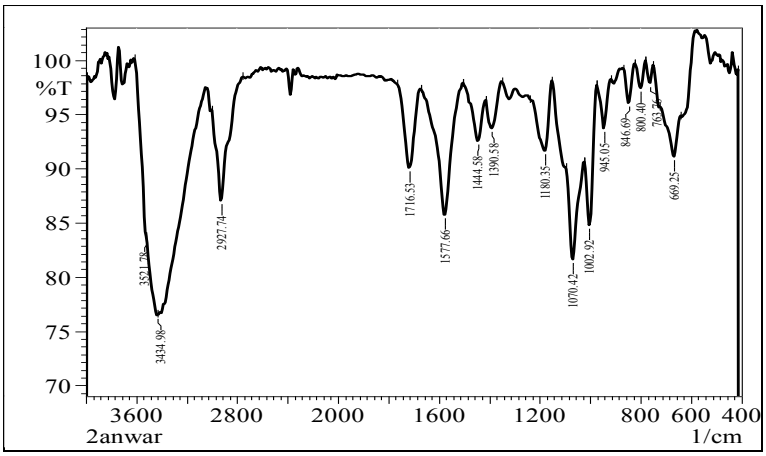

Figure 2: FTIR of Nystatin.

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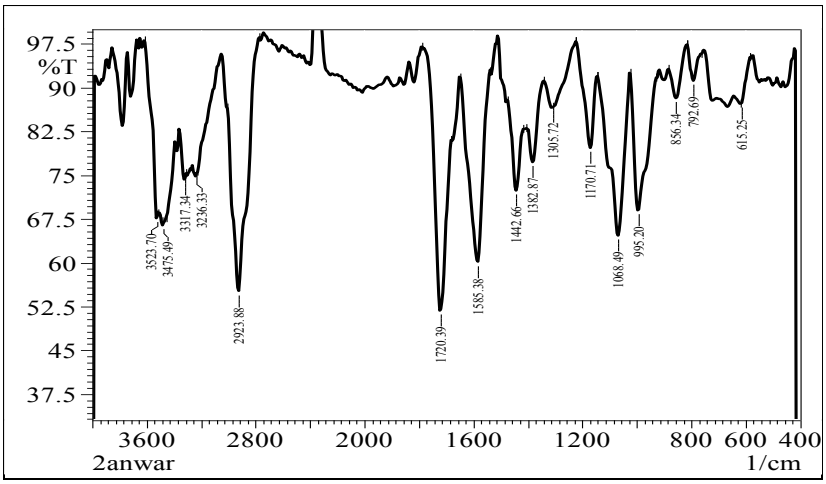

Figure 3: FTIR of AgNPs-NY.

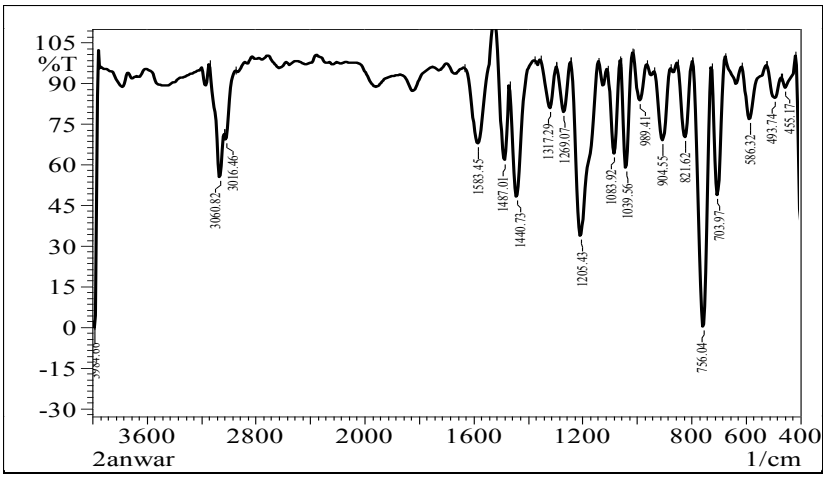

Figure 4: FTIR of Clotrimazole

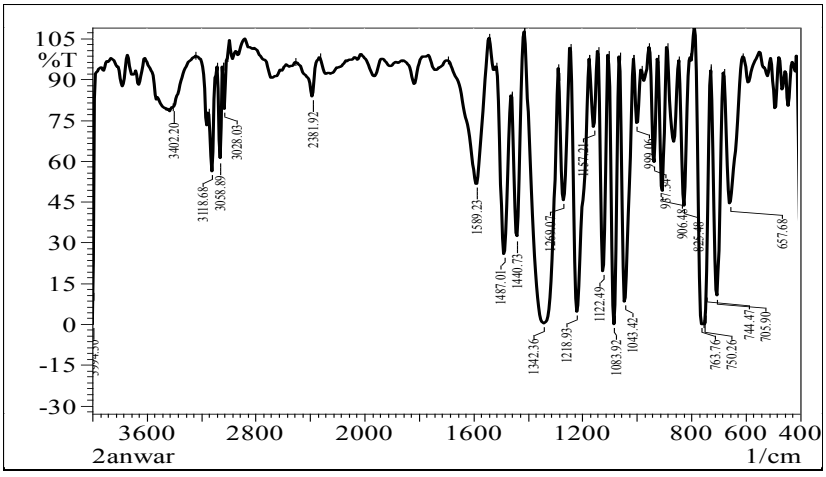

Figure 5: FTIR of AgNPs-CM

Table 1: FTIR frequencies in cm^{-1} of nystatin and coated AgNPs-NY.

Groups / Compound	OH	NH_2		CH	C=O	COO-		C-O
		Sym.	Assym.	aliph.		Sym.	Assym.	
NY.	3434	–	–	2927	1716	1577	1444	1390
AgNPs-NY	3475	3317	3236	2923	1720	1585	1442	1382

Table 2: FTIR frequencies in cm^{-1} of clotrimazol and coated AgNPs-CM.

Groups / Compound	CH aromatic	C=C	C=N	C-N	C-Cl
CM	3060,3016	1583	1440	1205	821
AgNPs-CM	3118,3058,3028	1589	1440	1218	825

XRD pattern:

XRD pattern in the range of 10-100 (degree) of pure AgNPs shows many intense peaks due to high degree of crystallinity of this prepared nanoparticle. The main peaks at 2Θ=32.01°, 38.13 °, 46.09 ° and 57.67 ° were indexed with planes (101), (111), (200) and (220) respectively [20].

XRD patterns of nystatin and AgNPs-NY show less crystallinity than AgNPs-CM and clotrimazol Fig. 6. XRD pattern of nystatin gives main peaks at 2Θ=13.86 °, 20.48 ° and 20.00° while in the case of AgNPs-NY show these main peaks at 2Θ= 37.23°, 38.34°, 39.55°.

XRD pattern of clotrimazol gives these main peaks at 2Θ =12.58 °, 19.61 °, 20.86 °, 28.26 ° and 32.59 ° while AgNPs-CM gives 2Θ= 22.99 °, 16.32 °, 11.07 °, 15.47 ° and 28.88 °. All XRD patterns as illustrated in Fig. 6.

By Deby-scherrer equation ($Dc = K\lambda/\beta\cos\theta$), (β is the breadth of the investigational diffraction line at half of the maximum intensity, K is the shape factor constant= 0.9, and λ is the wavelength of X-ray source used in XRD instrument) [19]. The crystalline sizes (Dc) of AgNPs, AgNPs-CM and AgNPs-NY were found to be 44 nm, 34 nm and 7 nm, respectively. The increasing of AgNps sizes may due to the formation of aggregation of silver nanoparticles in a cluster form, which is then separated to be smaller grain sizes after reaction with clotrimazol and nystatin which may behave it seems to act as dispersing agent.

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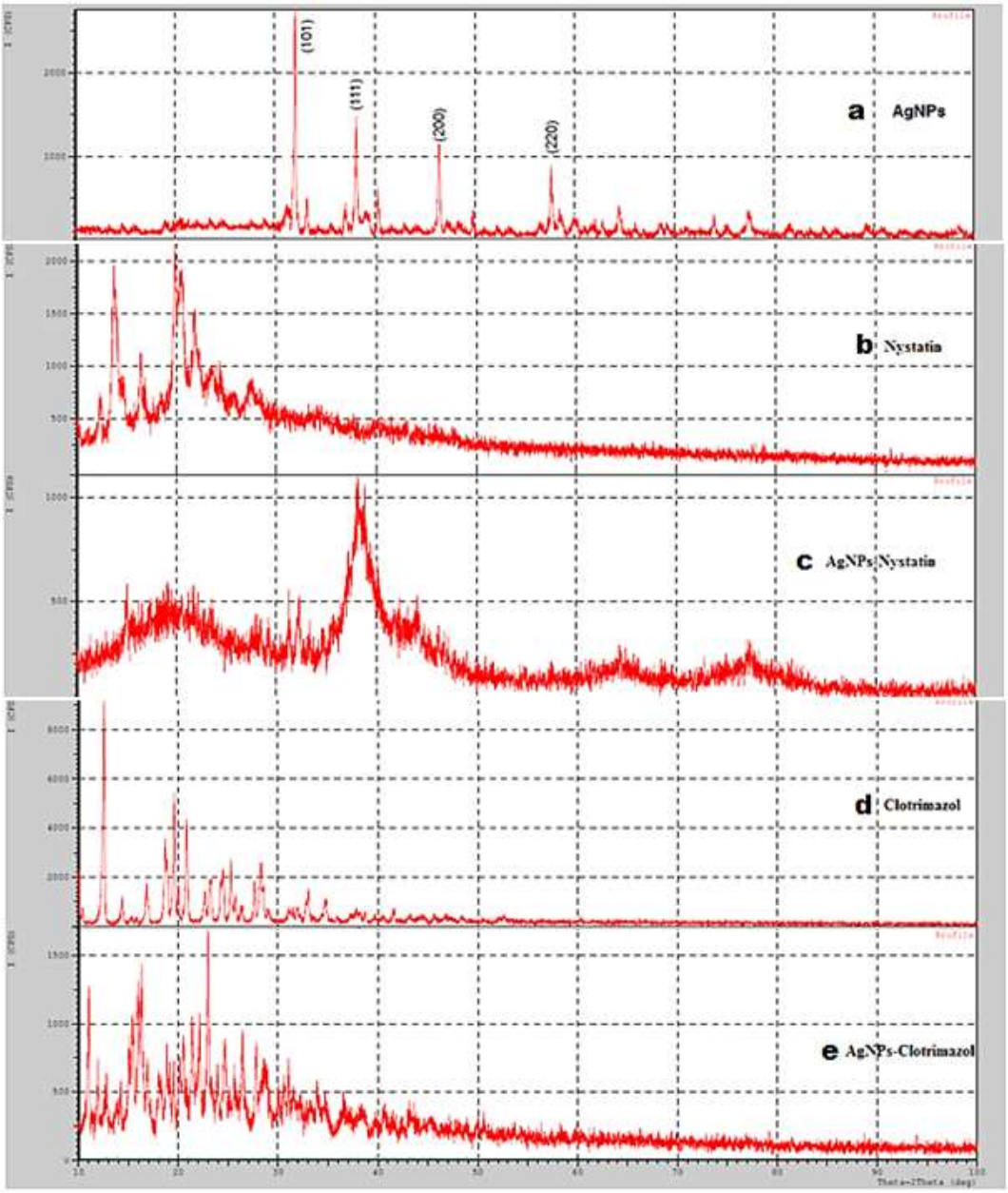

Figure 6: XRD pattern of (a) Pure AgNPs, (b)Nystatin, (c) AgNPs- Nystatin, (d) Clotrimazol and (e) AgNPs-Clotrimazol

Atomic force microscope (AFM):

AFM measurements of the prepared nano materials (AgNPs, AgNPs-Nystatin and AgNPs- Clotrimazol) Fig. 7 (a, b and c) give maximum height (11.61 nm, 3.46 nm and 3.73 nm), sequentially. We noticed the diameter size and maximum height of silver nanoparticles are bigger than other nanomaterial due to the formation of silver nanoparticles aggregation with the time. This proves that the classical antifungal drugs behave as dis-joining agent of accumulated AgNPs by coating of silver nanoparticles.

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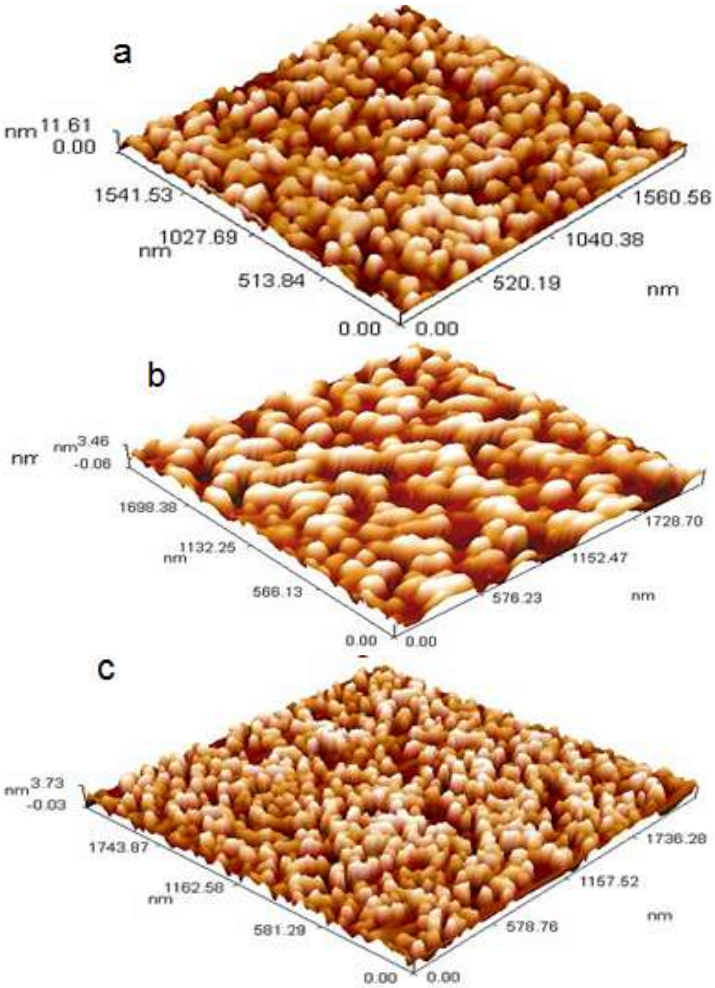

Figure 7: 3D AFM image of (a) AgNPs; (b) AgNPs-NY and (c) AgNPs-CM.

Studying of Antifungal activity:

The anti-fungal activity was studied using the prepared nanomaterials Fig.8 against four types of fungal (*Candida albicans, Candida tropicalism, Candida Pampislosis and Candida Glabrata).* The test was proved using hole diffusion method by prepared nanomaterials solution 8 $mg.ml^{-1}$ DMF and 50 $mg.ml^{-1}$ for other compounds.

The results Table 3 shows good anti-fungal growth in this diluted concentration while other materials give the same inhibition zone but with a five times to nano materials concentration. These results are due to the presence of AgNPs which ease the enters of clotrimazol and nystatin in the fungi cell or behave as drug delivery particles that allow to receive this ligands to the cell which will coordinate to minerals in fungi cells as (K^{+}, Na^{+}, Cu^{+2} and Zn^{+2}), that need it for growing. Inhibition zone was illustrated in table 3 and Fig. 9, 10.

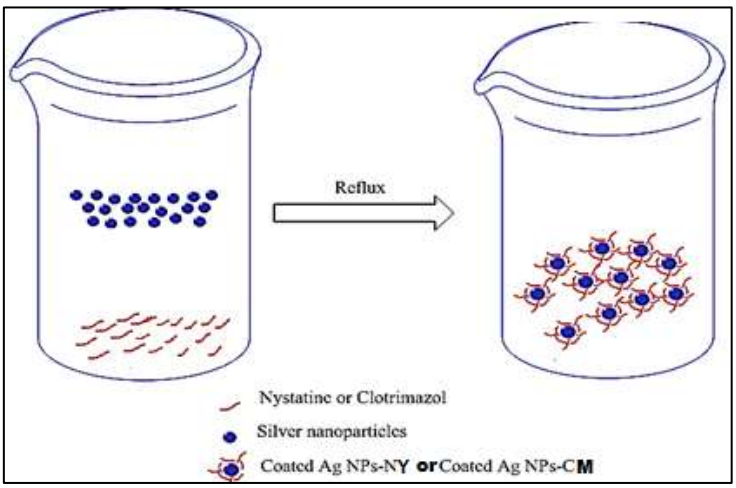

Figure 8: Graphical image of chemical method

Table 3: Inhibition zone of each material with their concentrations

Inhibition zone (mm)				Conc.(mg)	Material	Seq.
F4	F3	F2	F1			
14	15	18	12	50 mg	AgNPs	1
31	30	29	30	50 mg	CM	2
34	40	35	39	8 mg	AgNPs-CM	3
30	34	30	33	50 mg	NY	4
35	32	31	33	8 mg	AgNPs-NY	5

*** Where F1= *Candida albicans*, F2= *Candida tropicalis*, F3= *Candida pampislosis*, F4= *Candida glabrata***

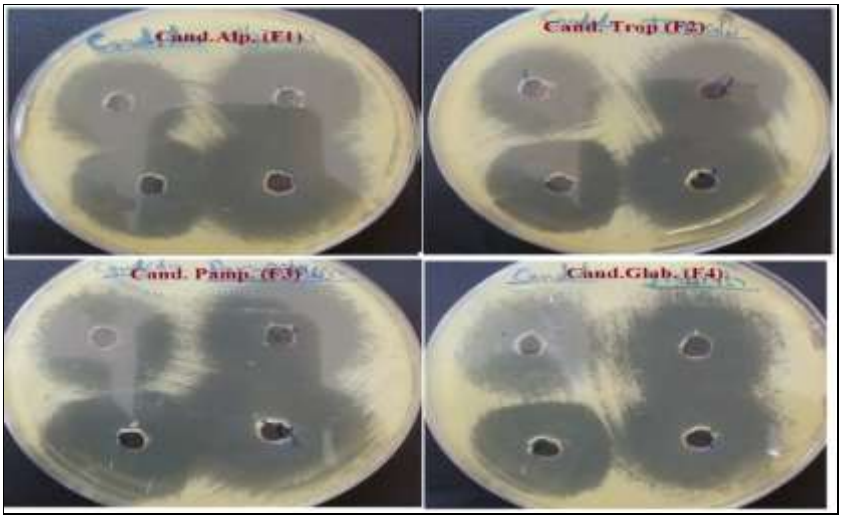

Figure 9: The images of Petri dishes with inhibition zone for NY and ANPs-NY, CM, AgNPs-CM

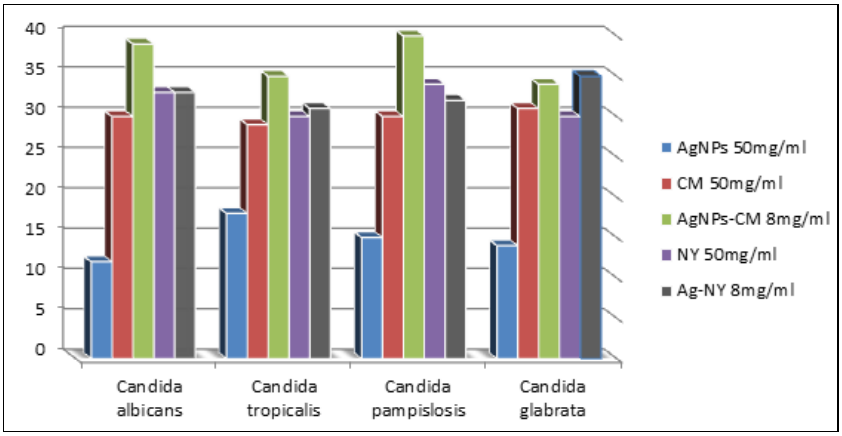

Figure 10: Diagram of inhibition zone for four types of fungi

Conclusions

Two nanomaterials derived from common antifungal drugs with silver nanoparticles have been successfully prepared by a chemical reaction method. In conclusion, the results of this study show that the silver nanoparticles have a main role in the enhancement of common antifugal drugs (Clotrimazole, Nystatine) although it is not has a good antifungal activity on the studied fugals. Also the results show that AgNPs-CM has a better antifungal activity than AgNPs-NY at the same conditions. This work, still very important to the development of novel antimicrobial agents containing nanomaterials.

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تحضير وتشخيص التاثير المضاد للفطريات لدقائق الفضة النانوية – نيستاتين ودقائق الفضة النانوية – كلوتريمازول

انوار عادل حميد ، مصطفى عبد الغفور الهيتي
قسم الكيمياء ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

الملخص

تم تحضير مركبات نانوية جديدة محتوية على دقائق الفضة النانوية عبر تفاعلات كيميائية بسيطة وتم تشخيصها بواسطة حيود الأشعة السينية، طيف الأشعة تحت الحمراء، ومجهر القوة الذرية. استعملت هذه المركبات المحتوية على دقائق الفضة النانوية لدراسة فعاليتها الحيوية ضد اربع انواع من الفطريات.

هذه المركبات المحتوية على دقائق الفضة النانوية اظهرت قابلية تثبيط عالية ضد جميع انواع الفطريات المدروسة وهذا يعزى الى وجود دقائق الفضة النانوية والتي تمتلك مساحة سطحية عالية والتي تزيد الارتباط بين النستاتين والكلوتريمازول مع الفطريات.

الكلمات المفتاحية: دقائق الفضة النانوية، النيستاتين، الكلوتريمازول ، مضاد للفطريات