IASJ المجلات الاكاديمية العراقية

Tikrit Journal of Pure Science

ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)

Journal Homepage: http://tjps.tu.edu.iq/index.php/j

Green synthesis of Silver Nanoparticles using the extract of *Rheum ribes* and evaluating their antifungal activity against some of *Candida sp.*

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https://doi.org/10.25130/tjps.v26i2.119

ARTICLE INFO.

Article history:

-Received: 4 / 12 / 2020

-Accepted: 15 / 12 / 2020

-Available online: / / 2021

Keywords: Silver nanoparticles, Green synthesis, *Rheum ribes*, Antifungal

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ABSTRACT

The synthesis of nanoparticles by green method using plant extracts and its application in the biomedical consider a prosperous field of research. Therefore, in this paper, Rheum plant was used as a reducing agent in synthesis silver nanoparticles and testing its effectiveness as anti-fungal. Silver Nanoparticles have been characterized using different techniques such as UV-visible spectroscopy, X-ray diffraction, Fourier transforms infrared spectroscopy and transmission electron microscope. Where the result of the examination UV-Vis showed absorption peaks of silver nanoparticles which formatted at wavelength (425) and XRD test confirmed the presence of silver nanoparticles clearly through compare peaks resulted with JCPDS card. It revealed the spectra of FTIR the existence of amine and aliphatic esters, that are involved in reducing and stabilizing AgNPs. Examination of TEM revealed that the silver nanoparticles were spherical shape and with an average size of 5-45 nm. Finally, the synthesized silver nanoparticles showed a good antifungal activity against some *Candida sp.* by observe inhibition zones that obtained.

Introduction

The nanotechnology field is one of the most active research areas in modern materials science. Nanoparticles display new or improved properties based on specific characteristics such as size, distribution, and morphology [1]. In the recent past years, there have been impressive developments in the nanotechnology fields, with numerous approaches developed to synthesize nanoparticles of particular shape and size depending on specific requirements, leads to rapidly increasing new applications of nanoparticles and nanomaterials [2]. Nanotechnology is an extremely efficacious technology and useful to design and development of several sorts of novel products with its potential medical applications on early disease detection, treatment, and prevention [3]. Nanotechnology involves the design, manipulation, production, and application of materials in the nanometer range 10^{-9}m [4]. Biological characteristics of NPs depend on its interaction with proteins, membranes, cells, DNA, and organelles and building a series of nanoparticle-biological interfaces attach with colloidal forces in addition to biophysicochemical interactions [5]. This leads to the formation of protein coronas, particle wrapping, intracellular uptake, and biocatalytic processes that could have biocompatible or bio-adverse outcomes Among all the nanoparticles developed and characterized, silver nanoparticle occupy significant position due to their several characterized that involve chemical stability, favorable electrical conductivity, and catalytic activity [6]. As a result it is commonly used in number of medical preparations acting as an antimicrobial against pathogens [7].

There are a variety of preparation techniques have been reported for the synthesis of nanoparticles include physical, chemical and biological. Among these techniques, biosynthesis of metal nanoparticles is of particular interest because of its benign advantages compared to the other processes, which including environmentally friendly, cheap cost, safe and rapid [8,9].

Candida species are ascomycetes belonging to the group of hemiascomycetes. Candida spp are part of the normal human microbiota, usually found in the genitourinary tracts, gastrointestinal, the oral cavity and to a lesser extent on the skin of most healthy humans [10,11]. The normally pose no threat but under certain circumstances, when patients are

immunocompromised, candida can become a pathogen [12]. While the incidence of Candida infections is increasing, the choice of suitable antifungal agents is limited due to the resistance of some species to several antifungals. Candida species can cause superficial to life-threatening candidemia and hospital-acquired infections in humans [13].

Rhubarb is considered from medical plants because its contains a mixture of medicinal compounds such as tannins, glycosides, flavonoids, anthraquinone, volatile oils, and saponins that are biologically active compounds used in medicine due to their efficiency as antibacterial, antifungal, antioxidant, anticancer, wounds healing and immune-enhancing activity [14]. Numerous experimental and clinical studies have been performed related to the effects of medicinal plants in order to reduce the rates of diseases and treated all over the world [15,16].

Materials and Methods

Fungal strains

The Candida species isolates were collected and selected from many different clinical specimens at Al-Ramadi Teaching Hospital, where collected fourteen isolates revert to different species. The four Candida species isolates were identified based on their morphology also the Vitek 2 system was performed to identify the species and confirmation its diagnosis.

Preparation of plant extract

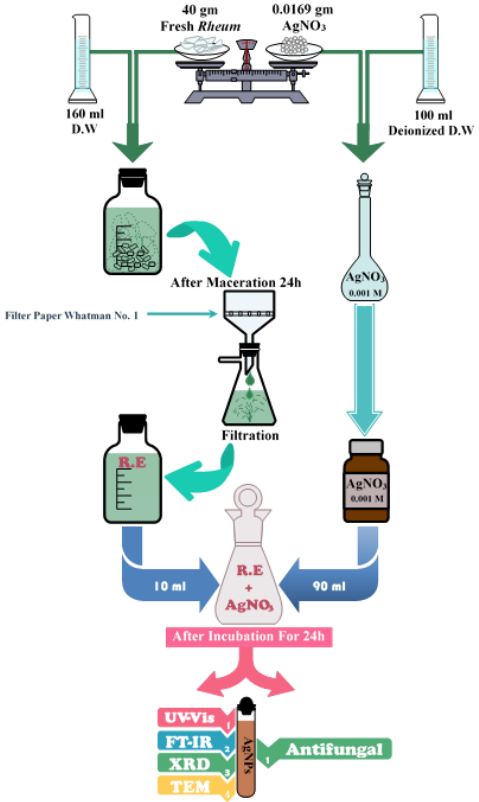
The fresh plants were collected from local markets then identified by a plant taxonomist in Salahuddin University Herbarium as *Rheum ribes* L. After that fresh plants washed many times with tap water and with distilled water to remove dust and soil particles from their surfaces. Weights 40 gm of fresh plant, then chopped to small pieces and maceration in 160 ml sterile distilled water for 24h as shown in scheme (1), then filtered with Whatman No.1 filter paper. Filtrate solution was collected with good storage and airtight at refrigerator temperature for further analysis [17].

Preparation of Nanoparticles

Nanoparticles (AgNPs) were prepared by the green synthesis method using plant extract of *Rheum ribes*, where 10 ml of plant extract was added to 90 ml of $AgNO_3$ solution at concentration 1mM as shown in scheme 1. After that, the combined solution were motivated dynamically at temperatures 25^{o}C; pH 9; and concentrations of 1 mM, with observing the gradual change in color of the solution [18].

Antifungal activity of nanoparticles test

Agar well diffusion method used to test the activity of nanoparticles as antifungal, where spread the suspension on sabouraud dextrose agar after compared with Macfarland tube (0.5) by cotton swab then made wells in each plate with diameter 6mm by cork borer, poured in each them 75 µl of nanoparticles solution and incubated overnight at 37^{o}C. After that, the inhibition zones surrounded wells were measured by Vernier caliper with millimeter-scale [19].



Scheme 1: Scheme explain Preparation procedure of Plant Extract, Metal Salts and Synthesis of Nanoparticles.

Results and Discussion

Identification of fungal strains

The Candida species isolates which collected show revert to different species and It has been identified four species which includes *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida glabrata* figure (1) based on their morphology also the Vitek 2 system was performed to identify the species and It has been confirmed diagnosis at the species level (appendix a).

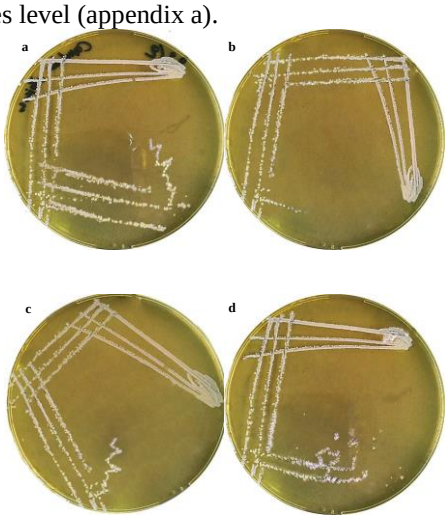


Fig.1: Candida spp. on SDA a) *Candida albicans*, b) *Candida glabrata*, c) *Candida tropicalis*, d) *Candida krusei*.

Green synthesis and characterization of AgNPs

Nanoparticles were prepared by using the green synthesis method by adding plant extract to the aqueous solution of $AgNO_3$, where the synthesis of the nanoparticles was confirmed using plant extract of *Rheum ribes* by change the colorless solution to brown-yellowish color figure (2), this indicates the formation of silver nanoparticles. Also formation and characterization of AgNPs were determined by many techniques, the ultraviolet-visible spectroscopy (UV-1800, Shimadzu) employed to identify nanoparticles absorption within the range (300-600) where figure (2) explain the UV-Vis spectrum of biosynthesized nanoparticles by using plant Rheum extract. Were the absorption peak was at the wavelength (425) nm, and it turns out this absorbance peak prove the formation of metallic silver nanoparticles located in the surface plasmon resonance of silver nanoparticles region [20].

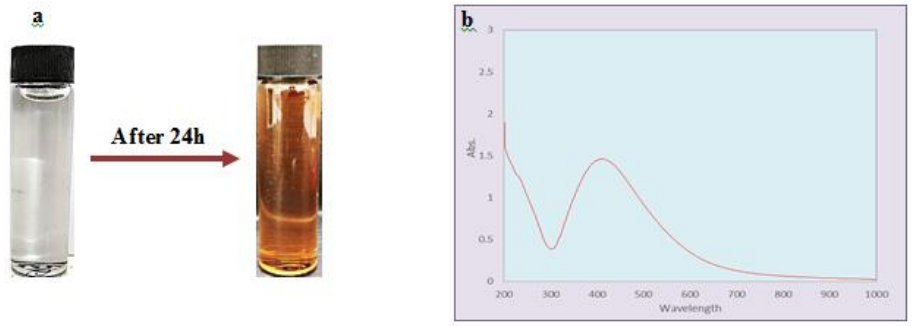


Fig.2: a)Explain color observation of AgNPs, b)UV-Vis spectrum of AgNPs synthesized.

The figure (3) show the results of the Fourier transform infrared spectroscopy (FTIR-600, Biotech) for treatment that used *Rheum* extract in preparation of silver nanoparticles. There are several peaks noticed in the figures shown below, some of them appear broad and strong peaks in the wavenumbers (3421-3213) cm^{-1} assigned to O - H stretching vibration in alcohol and carboxyl acid. While the active group C=C stretching vibration that found in the aromatic and amides compounds showed strong and sharp peaks at (1628-1593) cm^{-1}, on the other side, the bond S=O stretching vibration appeared as very sharp and strong peaks at the wavenumbers that ranged from 1390 cm^{-1} to 1361 cm^{-1}. As well as the medium and broad peaks at wavenumbers (1113-1034) cm^{-1} are mainly due to C-N stretching vibration in amines. The bond in the region between 829 cm^{-1} and 777cm^{-1} is attributed to the presence of active group of amines (N-H stretching vibration) in samples. The absorption bands at (526-501) cm^{-1} appeared as weak peaks that indicated to the bond S-S Disulfide stretching vibration that agree with Balakumaran [21].

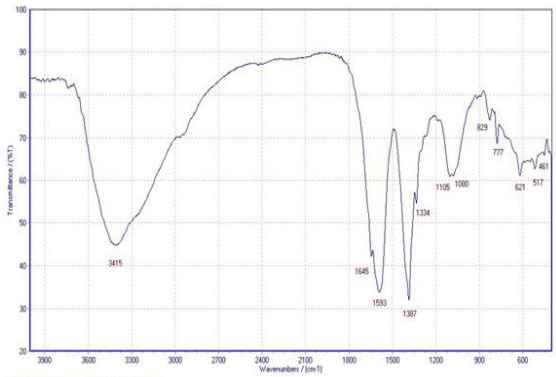


Fig.3: FT-IR Spectrum of AgNPs synthesized.

The figure 4 exhibit X-Ray diffraction (XRD-6000, Shimadzu) with 2 theta angles that ranged from 20-80°; this patterns was similar to the Bragg's reflection of silver nanocrystal, which may be index to the (111), (200), (220), (311) when compared with cards of the Joint Committee on Powder Diffraction Standards (JCPDS). The crystal structure nature of the synthesized silver nanoparticles is face center cube (FCC) that agreement with results of previous studies [22,23].

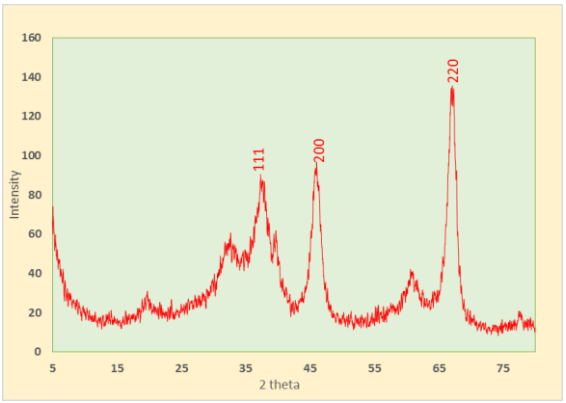


Fig.4: XRD pattern of AgNPs synthesized.

The TEM (Phillips CM10) analysis of silver nanoparticles synthesized by using the Rheum extract plant as a reducing agent proved that shape was spherical with different sizes. Figures (5) show the particle sizes and shapes of the treatment groups. Where noticed the particle size in range (5-30) nm. these data correspond with Tawfeeq *et al.* and Muthuraman *et al.* [24,25].

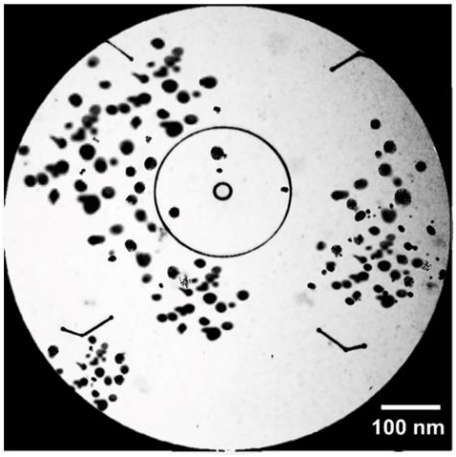
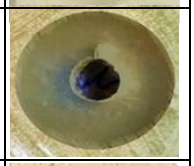
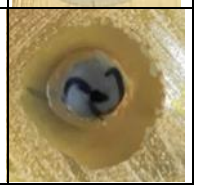


Fig. 5: TEM image of AgNPs synthesized using *Rheum ribes* extract.

Antifungal activity

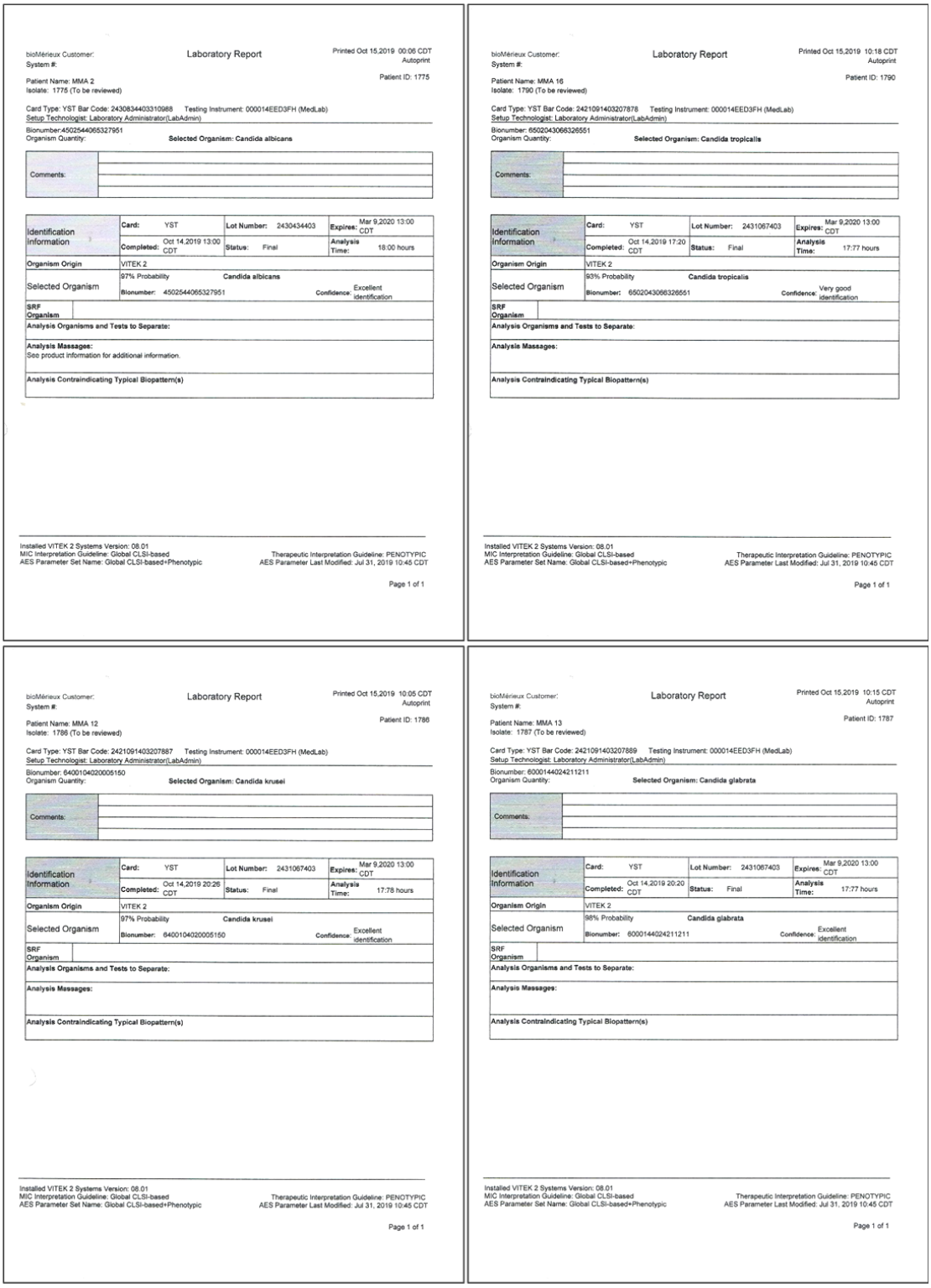
In this study, silver nanoparticles were successfully synthesized by the biological method using plant extract of Rheum as reduce agent as shown in the procedure mentioned in the scheme (1). AgNPs exhibited antimicrobial activity against pathogenic microorganisms with varying degrees, which may be due to their higher surface activity, as submitted by the diameters of the inhibition zone, that agree with what obtained by Hamidi *et al*., and Maeah [26,27]. Where nanoparticles showed a good antifungal activity against *Candida tropicalis* greater than other species then it followed by *Candida krusei*, after that *Candida albicans*, and *Candida glabrata* came last as shown in table (1). One of the possible modes of action of the plant mediated silver nanoparticles might be to attach to the cell surface and disrupt the cell membrane and interact. After penetration, Ag NPs release silver ions which interacted with DNA, proteins and sulfur containing cell constituents, therefore, the organisms were prevented.

Table1: Explain Antifungal activity of silver nanoparticles.

Microorganisms	Inhibition Zone (mm)	
Candida albicans	17	
Candida krusei	18	
Candida tropicalis	19	
Candida glabrata	17	

Conclusion

The method of green synthesis of nanoparticles by plant extract proved its competence in the synthesis of silver nanoparticles. Also, silver nanoparticles that were obtained showed antimicrobial effects against yeast and thus it can be investigated for further medicinal applications where the efficiency of nanoparticles against *Candida tropicalis* greater than other species, then it followed by *Candida krusei*, after that *Candida albicans*, and *Candida glabrata* came last.



bioMérieux Customer: Laboratory Report Printed Oct 15,2019 00:06 CDT Autoprint
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Patient Name: MMA 2
Isolate: 1775 (To be reviewed)
Card Type: YST Bar Code: 2430834403310988 Testing Instrument: 000014EED3FH (MedLab)
Setup Technologist: Laboratory Administrator(LabAdmin)
Bionumber:4502544065327951
Organism Quantity: Selected Organism: Candida albicans
Comments:
Identification Information: Card: YST Lot Number: 2430434403 Expires: Mar 9,2020 13:00 CDT
Completed: Oct 14,2019 13:00 CDT Status: Final Analysis Time: 18:00 hours
Organism Origin: VITEK 2
Selected Organism: 97% Probability Candida albicans
Bionumber: 4502544065327951 Confidence: Excellent identification
SRF Organism
Analysis Organisms and Tests to Separate:
Analysis Massages: See product information for additional information.
Analysis Contraindicating Typical Biopattern(s)
Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based
AES Parameter Set Name: Global CLSI-based+Phenotypic
Therapeutic Interpretation Guideline: PENOTYPIC
AES Parameter Last Modified: Jul 31, 2019 10:45 CDT
Page 1 of 1

bioMérieux Customer: Laboratory Report Printed Oct 15,2019 10:18 CDT Autoprint
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Patient Name: MMA 16
Isolate: 1790 (To be reviewed)
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Setup Technologist: Laboratory Administrator(LabAdmin)
Bionumber: 6502043066326551
Organism Quantity: Selected Organism: Candida tropicalis
Comments:
Identification Information: Card: YST Lot Number: 2431067403 Expires: Mar 9,2020 13:00 CDT
Completed: Oct 14,2019 17:20 CDT Status: Final Analysis Time: 17:77 hours
Organism Origin: VITEK 2
Selected Organism: 93% Probability Candida tropicalis
Bionumber: 6502043066326551 Confidence: Very good identification
SRF Organism
Analysis Organisms and Tests to Separate:
Analysis Massages:
Analysis Contraindicating Typical Biopattern(s)
Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based
AES Parameter Set Name: Global CLSI-based+Phenotypic
Therapeutic Interpretation Guideline: PENOTYPIC
AES Parameter Last Modified: Jul 31, 2019 10:45 CDT
Page 1 of 1

bioMérieux Customer: Laboratory Report Printed Oct 15,2019 10:05 CDT Autoprint
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Patient Name: MMA 12
Isolate: 1786 (To be reviewed)
Card Type: YST Bar Code: 2421091403207887 Testing Instrument: 000014EED3FH (MedLab)
Setup Technologist: Laboratory Administrator(LabAdmin)
Bionumber: 6400104020005150
Organism Quantity: Selected Organism: Candida krusei
Comments:
Identification Information: Card: YST Lot Number: 2431067403 Expires: Mar 9,2020 13:00 CDT
Completed: Oct 14,2019 20:26 CDT Status: Final Analysis Time: 17:78 hours
Organism Origin: VITEK 2
Selected Organism: 97% Probability Candida krusei
Bionumber: 6400104020005150 Confidence: Excellent identification
SRF Organism
Analysis Organisms and Tests to Separate:
Analysis Massages:
Analysis Contraindicating Typical Biopattern(s)
Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based
AES Parameter Set Name: Global CLSI-based+Phenotypic
Therapeutic Interpretation Guideline: PENOTYPIC
AES Parameter Last Modified: Jul 31, 2019 10:45 CDT
Page 1 of 1

bioMérieux Customer: Laboratory Report Printed Oct 15,2019 10:15 CDT Autoprint
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Bionumber: 6000144024211211
Organism Quantity: Selected Organism: Candida glabrata
Comments:
Identification Information: Card: YST Lot Number: 2431067403 Expires: Mar 9,2020 13:00 CDT
Completed: Oct 14,2019 20:20 CDT Status: Final Analysis Time: 17:77 hours
Organism Origin: VITEK 2
Selected Organism: 98% Probability Candida glabrata
Bionumber: 6000144024211211 Confidence: Excellent identification
SRF Organism
Analysis Organisms and Tests to Separate:
Analysis Massages:
Analysis Contraindicating Typical Biopattern(s)
Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based
AES Parameter Set Name: Global CLSI-based+Phenotypic
Therapeutic Interpretation Guideline: PENOTYPIC
AES Parameter Last Modified: Jul 31, 2019 10:45 CDT
Page 1 of 1

Appendix a: Reports of VITEK 2 System test

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التوليف الأخضر لجسيمات الفضة النانوية باستخدام مستخلص الريواز وتقييم فعاليتها كمضاد فطري ضد بعض انواع المبيضات

مصطفى مهدي عودة ، هاجر علي شريف ، بري لطيف محمد
قسم علوم الحياة ، كلية العلوم ، جامعة كركوك ، كركوك ، العراق

الملخص

يعتبر تركيب الجسيمات النانوية بالطريقة الخضراء باستخدام المستخلصات النباتية وتطبيقها في الطب الحيوي مجالًا بحثيًا مزدهرًا. لذلك، في هذه الدراسة، تم استخدام نبات الريواز كعامل مختزل في تخليق جسيمات الفضة النانوية واختبار فعاليتها كمضاد فطري. حيث تم تشخيص جسيمات الفضة النانوية باستخدام عدة تقنيات مثل التحليل الطيفي للأشعة فوق البنفسجية UV-Vis، وحيود الأشعة السينية XRD، والتحليل الطيفي بالأشعة تحت الحمراء FTIR، والمجهر الإلكتروني النافذ TEM. حيث أظهرت نتيجة الفحص بالأشعة فوق البنفسجية UV-Vis قمة امتصاص لجسيمات الفضة النانوية عند الطول الموجي (425) نانومتر، وأكد اختبار XRD وجود جسيمات الفضة النانوية بوضوح من خلال مقارنة القمم الناتجة مع بطاقات JCPDS. بينما كشف التحليل الطيفي FTIR عن وجود مجاميع الفعالة للاسترات الأمينية والأليفاتية، التي تشارك في اختزال واستقرار الجسيمات النانوية AgNPs. واظهرت الصورة المجهرية TEM لجسيمات الفضة النانوية انها كروية الشكل وبحجم يتراوح بين 5–45 نانومتر. من خلال ملاحظة مناطق التثبيط أظهرت جسيمات الفضة النانوية المُصنَّعة نشاطًا مضادًا جيدًا للفطريات ضد بعض انواع المبيضات.