Trichosporon Asahii Used in The Production of Silver Nanoparticles with Anticancer Effects

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Abstract
This study was conducted to evaluate the efficiency of silver nanoparticles anticancer activity which produced by T.asahii and characterization of the synthesized silver nanoparticles by UV-spectroscopy and scanning electron microscope. the resultes revealed the ability of T. asahii to produced silver nanoparticles. Within 2-4 hours, the reaction started, and coloration changed to a yellowish brown,also T. asahii was observed to produce well-dispersed, homogenous AgNPs with a variety of morphologies, the majority of which were spherical. A nanoparticle's average diameter was 43.17, and the size range of AgNPs was between (15.63-75.92 nm). The weight percentage of elemental constituents for AgNPs was 71.7, T. asahii average size of the nanoparticles generated from T. asahii was discovered to be 27nm . The resulting nanoparticles FTIR spectra reveal distanced twelve absorption bands ranging between 3453.99 to 461.79 cm-1, indicating the presence of twelve active functional groups. The results showed a significant inhibition of MCF-7 proliferation after 72 hrs, Dose and time depended cytotoxic cell was observed in AgNPs in the range of 6.25 -100 µg/ml concentration. AgNPs showed 100.0% of the cytotoxicity at the highest concentration (100 µg/ml) after 72 hrs. exposure while the lowest cytotoxicity showed at concentration 6.25µg/ml and 12.5µg/ml which were 72.2% and 73.4% respectively From the assay, the determined IC50 values for AgNPs were 72.2% .
Key words: Trichosporon asahii, AgNPs, Anticancer activity, MCF-7 tissue cultuer

1. Introduction

Trichosporon asahii is a pathogenic yeast that causes trichosporonosis, a deep-seated infection, in immunocompromised hosts. Pathogenic factors involved in this infection have not been investigated in detail, but morphological phenotype switching is thought to be important for T. asahii pathogenesis (Ichikawa Tomoe, et, al 2015).
The clinical manifestations of T. asahii infected patients were non-specific, the major types of infections were urinary tract infection, fungaemia and disseminated infection (Haitao, et al .2020).
The use of microorganisms in the synthesis of nanoparticles emerges as an ecofriendly and exciting approach, for production of NPs due to its low toxic, environmental compatibility, reduced costs of manufacture, scalability, and nanoparticle stabilization compared with the chemical synthesis (Correa-Llant et al ., 2013). Microbial source to produce the AgNPs shows the great interest towards the precipitation of nanoparticles due to its metabolic activity (Natarajan et al., 2010).
The biological method of synthesis of nanoparticles has proved to be better than some chemical or physical methods due to slower kinetics, which offers a better control over crystal growth and elimination of hazardous chemicals, making it an ecofriendly method (Husain et al ., 2015 ; Sanguiñedo Paula et al ., 2018). These methods of biosynthesis include the intracellular or extracellular production of nanoparticles of different elements by a variety of microorganisms and plant extracts (Husain et al ., 2015 ; Sanguiñedo Paula et al ., 2018) .
Silver salt and its colloidal formulations have been utilized to treat a wide range of diseases, such as ulcers, sepsis, burns, chronic wounds, acute epididymitis, and tonsillitis, and to prevent eye diseases in newborn children (Mohamed, et, al,2019 and Rabab, et, al, 2018) .Silver oxide nanoparticles exhibited antitumor properties in transplanted Pliss lymph sarcoma tumor models when administered by intravenous injection in the form of aqueous dispersions (Rutberg , 2008)
Cancer is a group of diseases, generating various pathological and metabolic changes in cellular environments. It is developed through diverse signaling mechanisms including cell proliferation, angiogenesis, and metastasis (Jason and Raymond , 2004) . A marginal increase in cancer cases within the last few years ends up mostly, with death (Raghunandan , et al. 2011).
Breast cancer is the most commonly occurring cancer in women, comprising almost one third of all malignancies in females. It is second only to lung cancer as a cause of cancer mortality, and it is the leading cause of death for American women between the ages of 40 and 55.1 The lifetime risk of a woman developing invasive breast cancer is 12.6 % 2 one out of 8 females in the United States will develop breast cancer at some point in her life (Richie et al , 2003).
MCF-7 cell variants are a novel tool for the study of breast cancer resistance to chemotherapy, because they appear to mirror the heterogeneity of tumor cells in vivo. As these cells are exposed to "selective" pressures in their environment (i.e., long-term exposure to TNF or other chemotherapeutic drugs)

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(Weldon et al , 2002).
The aim of this study is to evaluate the efficiency of silver nanoparticles anticancer activity which produced by T.asahii by screening for the different isolates of the yeast and characterization of the synthesized silver nanoparticles by UV- spectroscopy and scanning electron microscope.

2. Material and method

1- A Study of Isolates

One pathogenic yeast, T. asahii, was used in this study The first step To determine the effect of biosynthetic nanoparticles on each isolate, they were all activated on Brain heart infusion broth for 2 days at 25°C and examined for purity in the laboratory of Advanced Mycology at the Faculty of Science, University of Kufa-Iraq The second step involved morphological and microscopic investigation, which was used to verify the isolation's identity. The subsequent step involved the microorganism producing silver nanoparticles for use in subsequent research, characterization of biogenic AgNPs by a number of confirmatory assays, and testing its effectiveness on humans in vitro.

2- AgNPs Biosynthesised Utilizing Cell Free Supernatant

The silver nanoparticles were produced by T. asahii using silver nitrate (AgNO3) as a precursor. The cell-free supernatant of T. asahii was carefully mixed and equally distributed in sterilized tubes before being mixed with silver nitrate, which was added at a concentration of 10 mM. This process was carried out in complete darkness to prevent AgNO3 oxidation. The reaction mixture's pH was then raised to 8 using NaOH (2M). In a shaking incubator set to 37°C for 24 hours, the resultant solutions were shaken at 150 rpm.
After incubation period, the color change was observed. The mixture was then centrifuged at 6000 rpm for 25 minutes at 4 °C, After discarding the supernatant, the mixture was centrifuged three more times at the same speed and duration to eliminate any leftover supernatant. The pellet deposit at the bottom of the tube, which represents a collection of nanoparticles, was then dried in an oven at 40 °C for 24 hours

3- Anticancer activity of silver nanoparticles

3-1 Cell culture maintenance

Invasive breast ductal carcinoma is the primary tumor in MCF-7 cells, and they also show proliferative responses to estrogens, estrogen receptors, and progesterone receptors (Lacroix and Leclercq,2004; Charafe-Jauffret *et al.*,2006).
RPMI-1640 supplemented with 10% fetal bovine, 100 units/mL penicillin, and 100 g/mL streptomycin was used to maintain the MCF-7 Cell Line, which was donated by the Iraqi Center for Cancer and Medical Genetic Research at the University of Al-Mustansiriyah. Trypsin-EDTA was used to passage the cells, and they were reseeded at 80% confluence twice a week and cultured at 37 °C. (Sulaiman et al.,2018).

3-2Cytotoxicity assays of silver nanoparticles

Cytotoxicity Assays of silver nanoparticles were done to determine the cytotoxic effect of silver nanoparticles. Methyl Thiazolyl Tetrazolium (MTT) cell viability assay was done using 96-well plates. Cell lines were seeded at 1 × 104 cells/well. After 24 hrs. a confluent monolayer was achieved, cells were treated with tested nanoparticles at (6.25µg/ml, 12.5µg/ml, 25µg/ml, 50µg/ml,100 µg/ml) concentrations. Cell viability was measured after 72 hrs. of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of (MTT) stain and incubating the cells for 2.5 hrs. at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of 1%Dimethyl Sulphoxide (DMSO) followed by 37°C incubation for 15 min with shaking (Jabir et al.,2019). The absorbency was determined on a micro plate reader at 492 nm, the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:-
Cytotoxicity = A-B/A *100
where A is the optical density of control and B is the optical density of the samples. To visualize the shape of cells under the inverted microscope, 200 µL of cell suspensions were seed in 96-well micro-titration plates at density 1x104 cells mL-1 and incubated for 48 hrs at 37oC. Then the medium removed and then added tested components at (IC50). After the exposure time, the plates were stained with 50 µL of crystal violet and incubated at 37oC for 15 mins; the stain was washed gently with tap water until the dye was removed. The cell observed under an inverted microscope at 40X magnification microscope filed and photographed with a digital camera(Motulsky, 2003).

3-3 MTT Assay for Cell Viability Analysis

The MTT test is still one of the most useful and well-liked viability assays that rely on living cells converting substrate into chromogenic product. The MTT assay uses mitochondrial reductase to change the water-soluble yellow dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] into an insoluble purple formazan. The concentration of the formazan is then evaluated by optical density at 570 nm after it has been solubilized.
A sensitive test with outstanding linearity up to 106 cells per well is the end result. Similar to the alamar Blue assay, exposure to a toxin can induce cell stress without directly causing cell death, and minor changes in metabolic activity can result in significant changes in MTT. The assay has been standardized for cells grown in several wells, either adherent or non-adherent. A typical 96-well plate is used in the

protocol. To fit a new plate format, this can be scaled up. In a 96-well plate, distribute 500–10,000 cells each. Up to 106 cells, the assay is linearly good.

3. Results

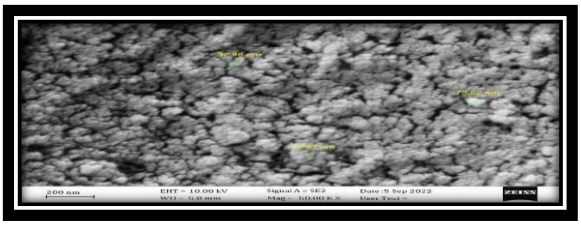
1-Biosynthesised nanoparticle characteristics

From silver nitrate, T. asahii produced silver nanoparticles. Within 2-4 hours, the reaction started, and the solution's coloration changed to a yellowish brown, indicating that T. asahii had Produced silver nanoparticles. These findings are in line with what Al-Inizi (2022) discovered. There are many methods for producing nanoparticles, including physical, chemical, and biological processes. The top-down physical technique and the bottom-up chemical and biological approach can both be used to create nanoparticles (Cao and Hu ,2009). The biological technique for the production of nanoparticles uses biological agents such as green algae, fungi, bacteria, actinomycetes, and plant extracts (Bhainsa and D'Souza, 2006; Saifuddin et al., 2009; Singhal et al., 2011 and Chaudhari et al., 2012).

A vast variety of resources are available for the production of nanoparticles using the biological technique. Enzymes that may hydrolyze metals and cause the enzymatic reduction of metal ions are secreted in great quantities by biological agents (Rai et al., 2009). The biological synthesis of nanoparticles was chosen as the focus because it is simple, quick, and ecofriendly. It also has controlled toxicity, control over size properties, and reasonable production processes (Vithiya and Sen, 2011). Utilizing microbes to create nanoparticles is one method to accomplish this goal (Bhattacharya and Mukherjee, 2008 and Gade et al., 2008).

2-Scanning Electron Microscope (SEM)

T. asahii was observed to produce well-dispersed, homogenous silver nanoparticles with a variety of morphologies, the majority of which were spherical. A nanoparticle's average diameter was 43.17, and the size range of AgNPs was between (15.63-75.92 nm) (Figure 1). SEM imaging and high-resolution topography data are produced by signal. Backscattered electrons have a larger energy than secondary electrons and originate from deeper locations, providing useful phase contrast data. Due to the fact that all points were covered, the SEM image could give us two main forms of information: the topographic structure of the surface and the distinction between various phases in the sample (Poole and Owens 2003).



WD= Working Distance, Mag= magnification, EHT= electron high tension (EHT) voltage

Figure (1): The shape of AgNPs was spherical, size between (15.63-75.92 nm) at Mag=50.00 KX.

Energy dispersive x-ray spectrophotometer (EDS)

The existence of elemental silver was found using Energy Dispersive-X-ray Spectroscopy (EDS), which kept track of the optical absorption peaks of silver elements. The discovery of elemental silver suggested that T. asahii converts the reaction mixture's reduced silver ions to silver. .Energy dispersive X-ray spectroscopy was used to determine the purity of silver, revealing the presence of a strong absorption peak at 3 keV, which is a feature of nano-crystalline silver. Also there were the spectrum contain signals from Carbon, molybdenum , Oxygen, and Chlorine were recorded in the spot profile mode .

The weight percentage of elemental constituents for AgNPs was 71.7,Thus, T. asahii can be considered capable of producing silver nanoparticles in a biological way. The weight percentage of Ag and other elements were listed in Table (1).

Table (1): The weight percentage of Silver and other elementals

Elements	Weight %
Silver	71.7
Carbon	15.6
Oxygen	1.4
Chlorine	15.2
Molybdenum	5.3
Total	100%

4.3.4. Analysis by X-Ray Diffraction (XRD)

XRD is a widely used analytical technique for analyzing molecular and crystal structures. The XRD examination of silver nanoparticles produced from *T. asahii* started position from °2Th= 8. 15 to end position °2Th=77.28, and revealed diffraction peaks at °2Th= 8.1°, 28.24°, 33.26°, 38.23°, 47.24°,56.37°, 58.21°, 66.80°, 68.50° and 77.53°. The XRD spectrum revealed that the produced silver nanoparticles were nanocrystals with a crystalline character. The peaks correspond to the planes (104), (198), (391), (74), (237), (107), (84), (53), (62) and (101) respectively, of silver crystal (Figure 2). Due to of the cubic and crystalline form of the nanoparticles, Scherrer's constant K value of 0.83 was chosen to compute the average size of silver nanoparticles generated from *T. asahii* average size of the nanoparticles generated from *T. asahii* was discovered to be 27nm .

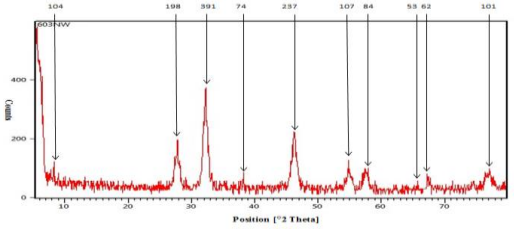


Figure (2): Silver nanoparticles produced from T. asahii , analysis by XRD diffraction pattern.

4.3.5. Fourier Transform-Infrared Spectroscopy (FTIR)

The possible functional groups of biomolecules in *T. asahii* were identified using FTIR analysis. The present study focuses on an investigation by FTIR in the mid-IR spectrum reported by (Nandiyanto *et al.*, 2019), and they divided it into four distinct zones:

1. The single bond region 2500-4000 cm^{-1}, (O-H, N-H, C-H).
2. The triple bond region 2000-2500 cm^{-1}, (C≡ C, C≡ N).
3. The double bond region 1500-2000 cm^{-1}, (C=C, C=O, C=N).
4. The fingerprint region 600-1500 cm^{-1}.

The resulting nanoparticles FTIR spectra reveal distanced twelve absorption bands ranging between 3453.99 to 461.79 cm-1, indicating the presence of twelve active functional groups as showed in Figure (3)

The frequency of each functional group is quantified. Data (wavenumber) was taken from a source (Coates, 2000). The specific functional group which appeared in AgNPs were illustrated in (Table 2). These results do not match what the researcher reached (Al-Inizi, 2022) who found that The nanoparticles' FTIR spectra reveal distanced nine absorption bands ranging between 3745.69 to 561.66 cm-1, indicating the presence of active functional groups

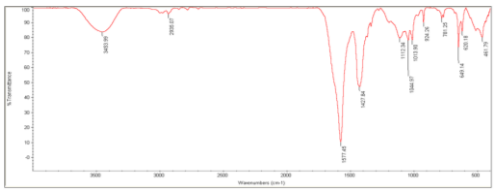


Figure (3): The Twelve Absorption Bands That Appeared through FTIR Analysis Revealed Functional Groups in AgNPs and Regions for Each Band.

Table (2): Functional Groups in AgNPs, Their Waveform Number According to Current Screening Results and Evaluation of Those Groups by Source (Coates, 2000).

Peak	X (cm^{-1})	Functional Group / Assignment
1	3453.99	Hydroxy group, H-bonded OH stretch
2	2935.07	Methylene C-H asym./sym. Stretch
3	1577.45	Aromatic nitro compounds
4	1427.84	Carbonate ion
5	1112.34	Sulfate ion
6	1044.97	Phosphate ion
7	1013.90	Phosphate ion
8	924.26	Silicate ion
9	781.25	Aliphatic chloro compounds, C-Cl stretch
10	649.14	Alkyne C-H bend
11	620.18	Alkyne C-H bend
12	461.79	Aryl disulfides (SS stretch)

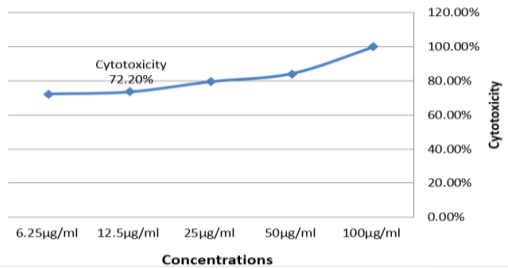
2-Anticancer activity (MTT assay) of AgNPs.

MTT assay is a colorimetric assay based on a color change through cell metabolism activation, the cytotoxic effects of AgNPs produced by T. asahii on human cancer cell lines (MCF-7) were studied for 72 hrs.

The results showed a significant inhibition of MCF-7 proliferation after 72 hrs, positive control of 100% lysed cells was added. The proliferation of cells were significantly lower compared to the untreated control cells. Dose and time depended cytotoxic cell was observed in AgNPs in the range of 6.25 -100 µg/ml concentration. AgNPs showed 100.0% of the cytotoxicity at the highest concentration (100 µg/ml) after 72 hrs. exposure while the lowest cytotoxicity showed at concentration 6.25µg/ml and 12.5µg/ml which were 72.2% and 73.4% respectively From the assay, the determined IC50 values for AgNPs were 72.2% . (Table 3) (Figure 4) .

Table(3): Cytotoxicity of Breast cancer cell lines using MTT assay according to different doses of AgNP

Concentrations	Cytotoxicity
100µg/ml	100.0%
50µg/ml	84.3%
25µg/ml	79.5%
12.5µg/ml	73.4%
6.25µg/ml	72.2%



Figure(4): Cytotoxicity effect of AgNPs nanoparticles on MCF-7 cell line. Usen differents concentrations .

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