Green Synthesis of silver nanoparticles from Atriplex nummularia leaves extracted as antifungal against Candida albicans.

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ABSTRACT

Nanotechnology is one of the driving reasons for the upcoming knowledge revolution, particularly in the biomedical sectors and the development of antimicrobial alternatives This study included using the well diffusion method and the detection of the minimum inhibitory concentration to estimate the antifungal activity of green silver nanoparticles (AgNPs) synthesized by the green chemistry process from the leaf extract of *Atriplex nummularia* and evaluated their antimicrobial activities for three pathogenic isolates of *Candida albicans* through Minimum Inhibitory concentration (MIC). *Atriplex nummularia* leaves extract and silver nitrate were used to synthesize AgNPs; the aqueous plant extract acts as a reducing and stabilizing agent for silver nanoparticles.Fourier transforms infrared spectroscopy (FTIR),Field emission-scanning electron microscope (FESEM),X-ray diffraction (XRD), and Atomic force microscope(AFM) were used to evaluate the produced nanostructure. X-ray diffraction analysis was used to determine the AgNPs' crystalline nature. FTIR analysis appeared bundle the characteristic of the hydrogen-bonded OH group, which may be due to the formation of nanoparticles in the aqueous phase, FESEM appeared spherical in shape and around (17.50-127.87) nm in size,AFM ranged between (1-42.36) nm,XRD appear the average crystalline size of AgNPs was found between(6-26)nm silver nanoparticles appeared an antifungal activity at concentration of (512, 256, 128, 64) µg/mL respectively, while showing no inhibition and antifungal effects on study isolates at the concentration of(32,16,8,4,2)µg/ml. The antifungal activity of the produced silver nanoparticles from *Atriplex nummularia* leaves extract was proven that its simple, inexpensive, safe, and effective alternative to standard antifungal medicines

Keywords: silver nanoparticles; Atriplex nummularia; Candida albicans; Green Synthesis

1. Introduction

The processes of chemical, physical, and biological have previously been used to create several kinds of nanoparticles such as silver,gold,lead, and Platinum [1]. Because of the chemical characteristics of these particles and their limited capacity to generate drug-resistant strains, silver nanoparticles (AgNPs), as one of noble metallic nanomaterials, represent most significant candidates of choice in medical applications due to their wide spectrum of antimicrobial activity against a variety of medically important microorganisms. So, (AgNPs) have emerged as effective antimicrobial agents and an alternative remedy that may play a role in reducing multidrug resistance, and there has recently been a surge of interest from motivated scientists to adopt various metal nanostructures as a new antimicrobial model synthesized using green synthetic routes using biogenic matter and microbial sources. Nanomaterials produced by several physical and chemical methods are poisonous or damaging to the environment As a result, researchers prefer to use safer methods to make nanomaterials, such as the biological technique and green chemistry [2]. Due to its ease of manipulation, the green chemistry method has been widely used for the simple manufacturing of AgNPs [3]. Researchers are adopting green processes to manufacture diverse metallic nanoparticles in response to the growing need for ecologically acceptable nanoparticles. However, for the time being, leaves extract has been employed as a reducing agent in the creation of nanoparticles that may be useful [4]. Because of its antibacterial, antifungal, and antiparasitic properties, silver nanoparticles are widely employed in industry and medicine [1]. Silver nanoparticles are an important nanotechnology component, primarily because they do not change biosynthesis in living cells and so do not produce microbial resistance. Recent research has shown that silver nanoparticles can bind to cell walls and cause physical harm by affecting cellular respiration and preventing the usual process of budding, however the exact mechanisms of action of nano- silver are yet unknown [5-8]. There are two methodologies to synthesizing nanoparticles: bottom-up and top-down.The top-down method is the basis of the mechanical breakdown of most structures to the nanoscale structure. while on the contrary, the basis of the bottom-up method is the get-together of nanoscale structure is made up of atoms or molecules [9].

2. Materials and methods

Preparation of AgNO3 solution

Silver nitrate was purchased from Sigma (169.873 g/mol), 0.1725 gm of $AgNO_3$ was dissolved in 250 ml distilled water at room temperature to form 0.004 M (4 mM) then the solution stir in magnetic stirrer for 15 min to make the solution homogenous as shown in(Fig.1) then the solution kept in dark container to avoid oxidation

doi.org/10.31838/hiv22.02.192 Received: 01.05.22, Revised: 30.05.22, Accepted: 06.08.22

Fig.1: Preparation 4 mM of AgNO3 solution

Preparation leaves extract *of Atriplex nummularia*

Atriplex nummularia leaves were washed several times with water, then sterilized by 50% alcohol to eliminate foreign materials such as dust particles, fungal spores, and washed with distilled water more than 5 times, Grind the leaves after it dries and the weight is stable,5gm of finally grind of leaves were boiled in a cleaned and sterilized 250 ml Erlenmeyer flask for 45 min with 100 ml of sterile distilled water and then boiling the mixture for 5 min in water bath for the preparation of broth solutions. The leaves extract were and put it in the centrifuge at 5000 rpm for 10 minutes(Fig.2), it is ready to use [10]

Fig. 2: Steps Preparation leaves extract of Atriplex nummularia

Green Synthesis of silver nanoparticles (AgNPs)

AgNO3 put on a Magnetic Stirrer and inside magnetic bar for an 45 minutes at a temperature of 60 degrees Celsius then add 20 ml of aqueous leaves extract drop by drop to silver nitrate solution with a concentration of 0.004 M (4 mM), and watch changes in color from transparent to yellow to brown colour after 24 h (Fig.3) after 24 hours in aqueous

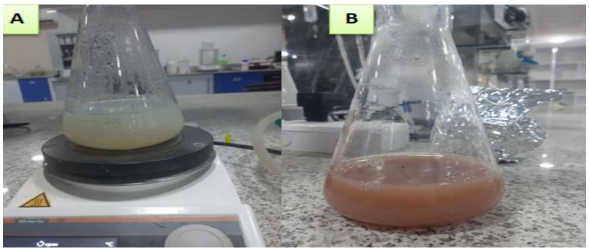


Fig.3. Formation of green silver nanoparticles(a)during add leaves extract(b)after 24 h.

Antifungal activity of AgNPs suspension

The AgNPs synthesized from *Atriplex nummularia* were tested for antifungal activity by well-diffusion method on the plate and by minimum inhibitory concentration method (MIC) using three *Candida albicans* isolates

Well diffusion method

silver nanoparticles synthesized from *Atriplex nummularia* were compared with artificial antimicrobial activities against *Candida albicans* isolates, about 20 ml The melted and chilled medium (Sabroud dextrose agar medium) was sterilized and poured in Petri dish, The plate was left overnight at room temperature for inspection pollution. Made 8 wells with a diameter of 5 mm using sterilized pasture Pipette into the agar plate,well 1-8 was loaded with a 50 µl replicated series. The concentration of fluconazole Suspension was (512µg / ml) that used as a positive control while (D.W) was used as a negative control plates were incubated at 25°C for 24 h inspecting the zones of inhibition surrounding the wells, each isolates tested triples and using a meter ruler, the diameter of inhibitory zones was measured, and the mean value for each organism was recorded and represented in millimeters. [11]

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of AgNPs was determined by the assays in sterile 96 well microtiter plates, 100 µl of serial two fold dilution from prepared AgNPs suspension was dispensed in the eight microdilution plate well, the last four pits were setup as controls. All wells were filled by 100µl of Yeast Extract–Peptone–Dextrose (YPD) broth then add 100µl of AgNPs suspension to the 1st well, mixed thoroughly and convey 100µl from the first well to the second well and so on respectively to the 8th well to do serial dilution to the prepared AgNPs suspension. One colony from overnight growing isolates were suspended in distilled water equal to McFarland tube No.0.5 (1-6X10^6cell\ml) to prepare isolates suspention, 10 µl of *Candida albicans* suspension was added to all wells and each isolate tested triplicate

3. Results and Discussion

Fourier transform infrared spectroscopy analysis (FTIR)

FTIR analysis of aqueous leaves extract of Atriplex nummularia and Silver nitrate (Green AgNPs) contains biomolecules responsible for converting silver ions into bio-formed silver nanoparticles [12, 13], that appeared ten different extensions of the bundles are (3848.84, 3435.58, 2353.61, 2081.03, 1633.27, 1505.01,1471.28, 1455.33,1045.72, 692.86)cm-1 as appearance of the peak at 3,436.51 cm-1 is due to vibration and stretch the OH bond of alcohols and phenols and the N-H expansion vibration of the primary amides of the protein is the bundle the characteristic of the hydrogen-bonded OH group, which may be due to the formation of nanoparticles from aqueous phase.

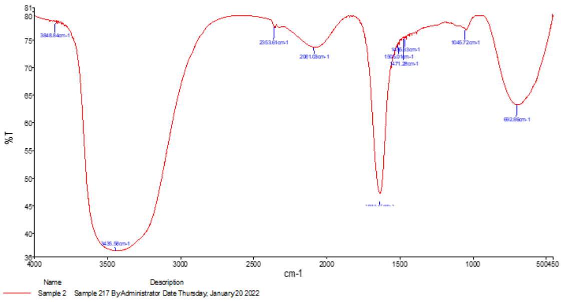


Fig.4.FTIR spectroscopy analysis of the formed silver nanoparticles by aqueous extract of Atriplex nummularia and silver nitrate (Green AgNPs).

(FESEM) Field-emission scanning electron microscopy analysis

The results showed various shapes and different sizes of these particles but the spherical shape prevailed [14] [15] and it was distributed Unilaterally (mono scattering) without agglomeration and with dimensions ranging between (17.50 -127.87) nm as in (Fig.5) the spherical shape of silver nanoparticles is one of the desirable properties of its relationship Its function and because it plays a crucial function in microorganism communication with the cell membrane [16].

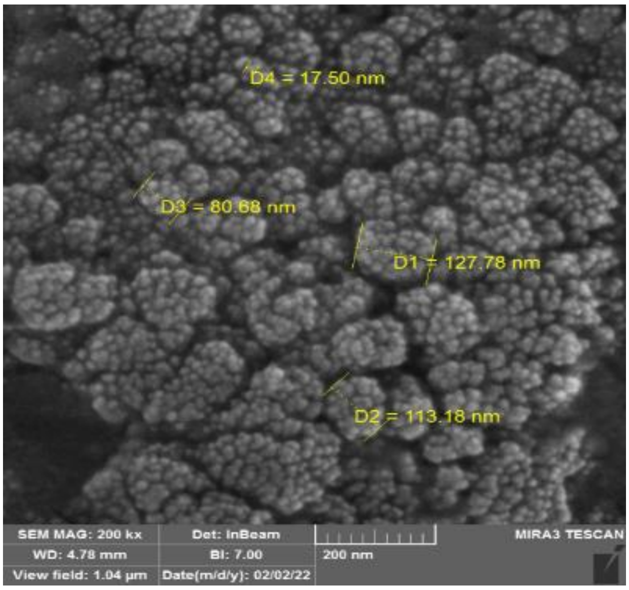


Fig.5.Examination (FESEM) with magnification forces of 200 kx Silver nanoparticles formed by leaves extract of Atriplex nummularia

Atomic force microscopy analysis

The results for green way showed two-dimensional and three-dimensional images of the topography of the surface of the silver particles,the nanoparticles have dimensions (0.39×0.39) µm and a height of 12 nm as shown in Figure (Fig.6.a), while it ranged between (1-42.36)nanometer as in the figure(Fig.6.b)

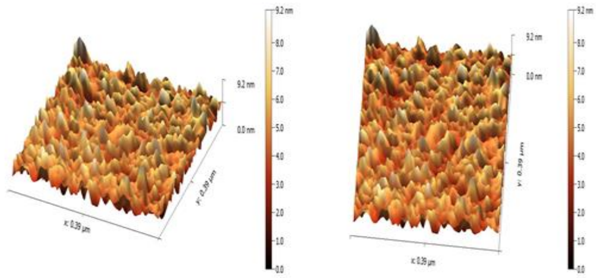
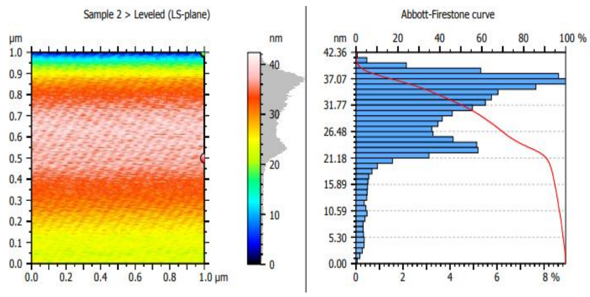


Fig.6.a Show two and three-dimensional surface topography of green silver nanoparticles under atomic force microscopy.



(Fig.6.b)Atomic force microscopy diagram showing the average size of green silver nanoparticles.

(XRD) X-ray diffraction analysis

The results of the X-ray diffraction analysis of bio-manufactured(green synthized) by aqueous extract and having (2-Theta) angles(26.408, 28.615, 30.999, 32.384, 35.773, 38.238, 39.983, 43.621) as in(fig.7) it confirms that the extract of the leaves has reduced the entire silver ions in the form of silver metal only and without the presence of oxides or other impurities, which indicates the success of the preparation on the one hand and also proves that the *Atriplex nummlaria* leaves extract has a high reducing ability and prevents the oxidation of silver and preserves it without turning it into oxides, as noted in anther hand. The absence of an angle (64,77) because the silver nanoparticles are less crystallized due to their presence in Nano-size and spherical shape [17], the average crystalline size of AgNPs was found between(6-26)nm by the calculation that done by using the equation: $D = {K\lambda}/_{\beta} \cos\theta$ (Debye-Scherrer)

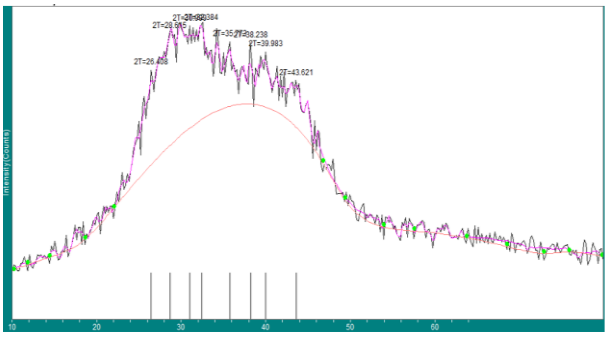


Fig.7.Crystalline levels produced by green produced silver nanoparticles measured by XRD spectrometry.

Well diffusion method

The antifungal activity of serial dilutions of the generated AgNPs from A triplex nummular by green method was evaluated using three different isolates of Candida albicans as a model, and the effects were measured as an inhibitory zone on the plates using the well diffusion method, at varied concentrations of AgNPs (512, 256, 128, 64, 32, 16) µg/mL, the AgNPs activity against C. albicans was tested, the result showing that the (AgNPs) solution have an antifungal activities at well No. 1, 2,3,4 in concentration of(512,256,128,64µg/ml) respectively *(Fig. 8)*

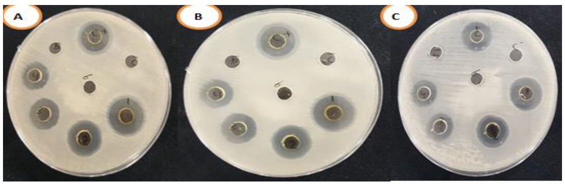


Fig. 8: The antifungal activity of prepared Silver nanoparticles against different isolates (a,b,c)of Candida albicans.

From Table 1 and Fig.9 the highest inhibition area.is observed in AgNPs solution of 512 µg/ml and decrease with Decreased nanoparticle concentration up to the fourth dilution (64 µg/mL) with a Statistically significant differences (P < 0.05) between different Inhibition and (Control fluconazole well C+), Distal water(C-) as negative control, while well No.5(532 µg/ml) and No.6 (16 µg/ml)show no inhibition.

Table 1: Inhibition distance zones for a suspension of AgNPs compared to a solution of fluconazole as a control in Candida albicans by Well-diffusion method.

Well No. suspension	Concentration of the suspension µg/ml	Isolate 1 Mean inhibition zone(mm)	Isolate 2 Mean inhibition zone(mm)	Isolate 3 Mean inhibition zone(mm)
1- AgNPs	512	23.33333	22.66667	22. 33333
2- AgNPs	256	19.33333	19	20.33333
3- AgNPs	128	18.33333	17.66667	17.66667
4- AgNPs	64	15.33333	14.66667	15.33333
5- AgNPs	32	0	0	0
6- AgNPs	16	0	0	0
Fluconazole suspension C+	512	20.66667	20	20.33333
Distilled water C -	0	0	0	0
L.S.D.Interaction P < 0.05=1.404				

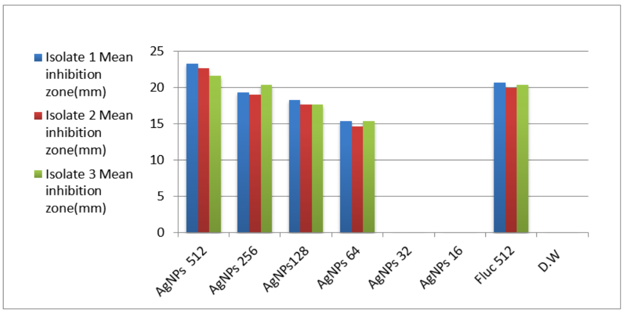


Fig.9: means of inhibition zones of AgNPs and Fluconazole at P < 0.05=1.404.

Minimum inhibitory concentration (MIC):

The MIC values showing a clear inhibitory effect of AgNPs on *Candida albicans* growth depending on the size/shape of AgNPs, with no inhibition effects at the lowest concentrations (32 µg /mL, 16 µg /mL, 8 µg /mL, 4 µg /mL, 2 µg /mL).concentrations, 64 µg/ml of AgNPs is the MIC concentration that inhibits the growth *Candida albicans* on YPD broth (complete inhibition) as shown in(Fig.10) to confirming the effect of complete inactivation of the prepared AgNPs suspension, samples from all micro-wells (in the MIC test) were re-inoculated onto YPD agar plates And incubated for 24 hours at 25 °C, no growth was observed from the first four wells) 1,2,3,4 (indicates the complete inhibition effects of the as-prepared AgNPs Commentary on the study isolates.

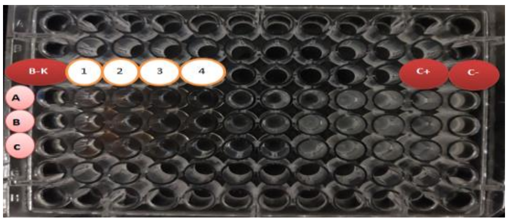


Fig.10: Microdilution method for AgNPs suspension, column (B-K) are control blank only medium, column (C+)are control positive, column (C-)are control negative,rows (A,B,C) represented tested study isolates, dilution 4 (64 ug\ml) represent the MIC for this suspension.

4. Conclusion

In this study, a green chemical procedure was used to synthesize silver nanoparticles from *Atriplex nummularia* leaves extract in a rapid, ecologically friendly, and acceptable manner. The resulting particles are spherical in shape, and this procedure does not require the use of any chemical reagents. The antifungal efficacy of silver nanoparticles generated from *Atriplex nummularia* leaf extract by green chemistry was demonstrated in this study, making this approach an easy, affordable, safe, and effective alternative to standard antifungal medicines.

5. References

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