Green Synthesis of Silver Nanoparticles Using the Extraction of some Plants Leaves

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DOI: https://doi.org/10.56807/buj.v2i3.110

Abstract

We have applied a green method for preparing silver nanoparticles (Ag NPs) using the extract of Ficus carica Leave . These extraction of plants leaves kept in an aqueous solution and used as a reducing agent and surfactant in the reaction. The structure and size of Ag nanoparticles are studied using XRD spectrums while an optical properties studied by UV-VISB spectrophotometer. The results showed that, the average grain size is in the range of 17-21nm using Debye- Scherer equation . In addition, we applied this study to investigate the antimicrobial activity of Ag NPs synthesized using Ficus carica L (FCL) extract. The obtained results are compared for both positive gram Escherichia coli (E. coli) and negative gram Staphylococcus aureus (S. aureus) bacteria. Finally, we have compared the antimicrobial activity of Silver nanoparticles with some antibiotic.

Keywords: *Silver Nanoparticles, Ficus carica, Antimicrobial , Escherichia, Staphylococcus.*

1. Introduction

The nanoparticles acquiring an especial interesting in modern research . They are atomic or molecular aggregates with at least one dimension between 1 and 100 Nm. They can highly modify their physical-chemical properties as compared to the bulk material. Actually, the most important properties of nanoparticles is the enhancement in the electrical, magnetic , optical and so on properties. Due to its unique characteristics, Silver nanoparticles (Ag NPs) is a novel material in antimicrobial, bio sensing, superconducting, and electronic materials. In biosynthetic approaches where plant extracts were used as a reducing and surfactant agents to produce silver nanoparticles. These green approaches has received great attention as a simple and reliable alternative to the conventional chemical and physical procedures for the synthesis of metal nanoparticles with controllable morphologies (Kim M, (2017) ,Mokhtar, (2019) Mehta1 B K, (2017) Huang J, (2007)) . In addition, the synthesis of nanoparticles using plant extracts has widely been explored for almost two decades (KiranmaiI (2007), Singh, (2012) , Arpita Roy (2017) Azat A, et al. (2016 Andreia . (2018), Mokhtar (2019).

Ficus carica L is an amazing and ancient source of medicines and food whose chemical and biological efficacy and medicinal uses have been tried(Barolo et al., (2014)). Ficus carica L used as treatment for diabetes(Perez et al., (2003)), hyperglycemia(El-Shobaki et al., 2010), hypertension(Alamgeer et al., (2017)), against throat diseases, antitussive,(Ziyyat et al., (1997)) cure hemorrhoids ,eliminate heart pain (Chunyan et al., 2008)and antimicrobial effect(Balestra et al., (2009), Debib et al., (2014), Jeong et al., (2009), Rashid et al., (2014)). Some of interesting

results that obtained by several authors to product a silver nanoparticles using *Ficus carica leaves are listed in table-1.*

Fig-1: Ficus carica leaves

Table-1 : silver nano particles using Ficus carica

References	AgNP Size
Sumon Das, Tamalika Chakraborty , (2018)	**21nm**
Prasoon Pal Singh, Chittaranjan Bhakat (2012)	**10 - 20 nm**
ACAY, H **, (2019)**	**17.30nm**
Andreia C &B ianca I. (2018)	**12.48nm &21nm**
Sagili J L ,…etc **(**2018)	**10-20nm**
Jaculin R A, …et al. ,(2018)	**5nm**
Arpita Roy (2017) Azat A, et al. (2016) KiranmaiI M. (2007), Andreia C &B ianca I. (2018)	**13nm**
S. Rajeshkumar (2016)	**10-20 nm**
Demet D G …..et al,(2016)	**50-120 nm**
KiranmaiI M. (2007),	**13nm**
Khwaja S S et al, *(2018)*	**20–80nm**
Azat A, et al. (2016)	**15 - 25 nm**
Harsh Kumar…et al, (2020)	**54–89 nm**

The most advantage here is the production of silver nanoparticle using an extraction of plant leaves . By other meaning the production here is using a naturally material without any additional chemical materials which means cheap, safety , nontoxic, green and naturally.

Escherichia coli is a gram-positive bacteria. It's the commonest cause of community and nosocomial urinary tract infection. E.coli get resistance of many antibiotic such as(Ampicillin, Cefalexin, Ciprofloxacin, Gentamicin, Nitrofurantoin, Trimethoprim and Cefpodoxime)(Bean et al., 2008).

Staphylococcus aureus is a gram-negative bacteria(Cohen et al., 2016, Heckel et al., 2017, Makovcova et al., 2017, Taylor and Unakal, 2019) .*Staphylococcus aureus* can be isolated from urinary tract infection (Abdallah et al., 2011, Baba-Moussa et al., 2008, Gad et al., 2009).

In this paper we have applied a green method for synthesizing silver nanoparticles (Ag NPs) using the extract of *Ficus carica Leaves* (*FCL*) *Leaves.* Also we have compared the antimicrobial activity of *Ficus carica L* Silver nanoparticles (FCLSNP) and some antibiotic on positive gram stain *Escherichia coli* and negative gram stain *Staphylococcus aureus* bacteria.

2. Materials and Experiments

2.1 Extract Preparation

Green leaves of *Ficus carica* were collected from Yemeni plants (Al-Baydha, Radaa). In the first step of preparing an extraction of plant, the *Ficus carica* leaves were washed with distilled water and left to dry naturally for a few days and kept in plastic boxes for further use. In the next step, two bickers filled with 100 ml of distilled water were heated at 60^{o}C in a magnetic stirrer. After that, 30 g and 40g of dried *Ficus carica* powder were poured in the heated water solution and kept for 25 minutes.

Fig-2: The preparation of (a) extract solution, (b) Ag NPs solution, and (c-d) Ag NPs powder

2.1.1 Synthesis Experiment: The synthesis procedure of Ag NPs can be described in the following steps. In the first step, 3g , 4 g and 1g of $AgNO_3$ (99% Al-Atheir Com.) was added to three barkers filled with 100 ml, and 100ml of distilled water each and kept to totally dissolve under vigorous stirrer for 20minuts. In the next step, 30ml and 40 ml of the *Ficus carica* extract solution was slowly added to the previous two solution and kept under vigorous stirrer only for 25 minutes at room temperature. We called them sample(B) and sample (A) respectively . The resultant solution was kept to naturally precipitate and the water were removed. In the final step, the obtained powder was washed twice with distilled water and methanol and then dried . The powder has kept in a small glass bottle for further use as shown in Fig. 2(a-d).

2.2 Antimicrobial Preparation

In first step, *Escherichia coli* and *Staphylococcus aureus* have isolated and identified from urine samples by (*New Med lab*)labs, Dhamar city. Then, resistance, isolated and identified *Escherichia coli* and *Staphylococcus aureus* have been used to measure the antimicrobial susceptibility of FCL extract and FCLSNP . In the next step, we used FCL extract (100 mg/L), FCLSNP concentrations (100, 75, 50, and 25)% and antibiotic (Ceftazidim, Cefepem, Clinomycin, Ceftazidim, Amoxyclave and Azetronem) to measure the inhibition zone size.

2.2.1 Preparation of (0.5) Mcfarland Standard

Preparation of 0.5 McFarland turbidity standard solution was carried out as cited in standard references. Add 0.5 ml of 1.0 %(wt/vol) anhydrous Barium Chloride (BaCl2) to 99.5 ml of 1.0% (vol/vol) cold pure sulfuric acid (H2SO4) solution. Stir to maintain a suspension and thoroughly mix immediately before the next step. Distribute about 5 ml McFarland Standard solution into screw-top tubes and store them in a dark at room temperature(Akinsiku et al., 2018).

2.2.2 Culture media and Antimicrobial assay

Mueller-Hinton agar (MH) (Hi-Media, Bombay, India) was respectively used for bacteria growth. Microbial cultures, freshly grown at 37°C the organisms were spread on MH agar plates by cotton swab. Antimicrobial activity was evaluated by measuring the inhibition zone size against the test organism(Capoor et al., 2007, Kali et al., 2016).

2.2.3 Antimicrobial Sensitivity Testing

Antimicrobial susceptibility was determined by the Kirby-Bauer disc-diffusion method performed on Muller-Hinton agar plates the disk strength (Baba-Moussa et al., 2008, Drew et al., 1972)and zone-size interpretation was in accordance with the Clinical and Laboratory Standards Institute(Tenover and Moellering Jr, 2007).

2.3 Structure of silver nanoparticles

we have used XRD Diffraction to study the structural characterizations Fig. 3(a) shows

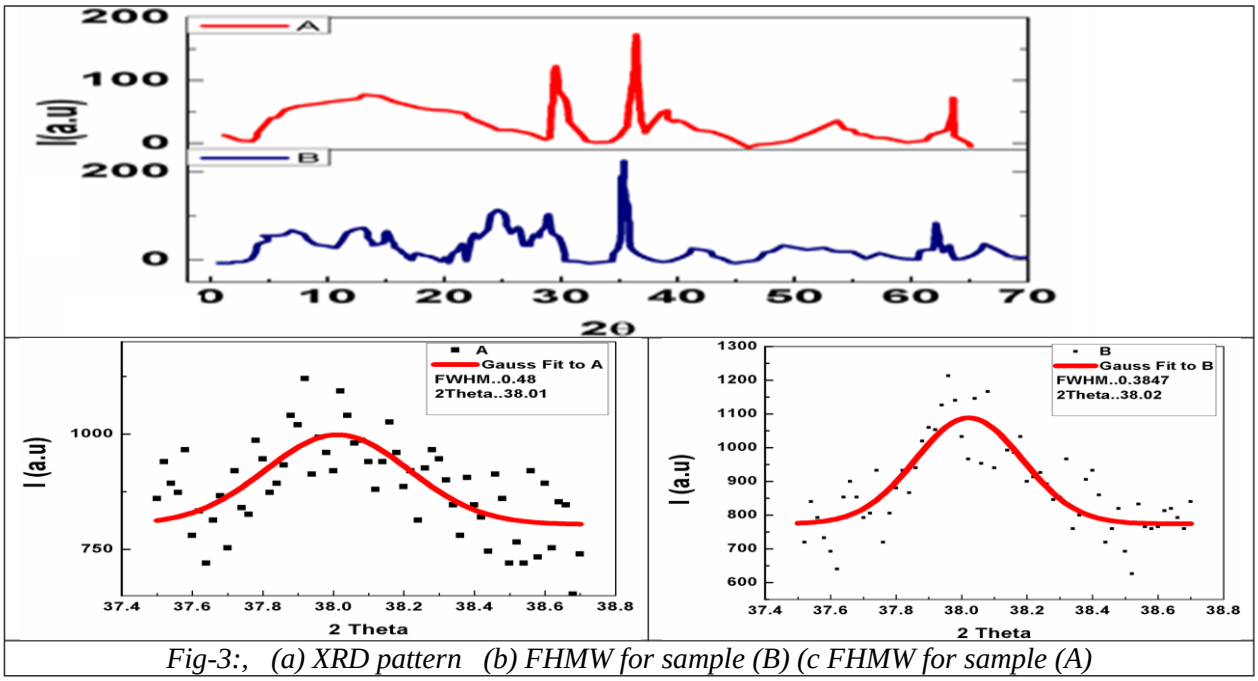

Fig-3:, (a) XRD pattern (b) FHMW for sample (B) (c FHMW for sample (A)

Table(2) Structural properties of standard and synthesized silver nanoparticles.

Samples	2θ (degree)	FWHM (β)	d(Å) (111)	Lattice Parameters (Å)	Dislocation density (ð) × 10^{17}(m^{-2})	Crystallite size (D) (nm)	Micro-strain(ε) × 10^{-3}
A	**38.1**	**0.48**	**2.365**	**4.0862**	**0.037**	**17.492**	**0.722**
B	**38.02**	**0.3847**	**2.364**	**4.0862**	**0.021**	**21.8**	**0.586**

Table(3): Antimicrobial sensitivity tests

Antimicrobial	***Escherichia coli***	***Staphylococcus aureus***
***FCL**	Resistance	Resistance
***FCLSNP**	Sensitive	Sensitive
Cefepem	Resistance	Resistance
Clinomycin	Resistance	Resistance
Ceftazidim	Resistance	Resistance
Amoxyclave	Resistance	Sensitive
Azetronem	Sensitive	Resistance

**FCL= Ficus carica L *FCLSNP = Ficus carica L Silver nanoparticles*

the X-Ray Diffraction pattern of our prepared Ag powder. The XRD diffraction pattern showed three characteristic diffraction peaks which is similar to those of pure silver. This result confirms the reduction process from silver nitrate to silver nanoparticles using our prepared plant extraction. The high-intensity peaks reflects simple fact that, the silver nanoparticles have high crystallinity.

The obtained XRD peak positions appeared at 38.14°, 44.32°, 64.52. They can be indexed as (111), (200), (220), crystal planes of Ag respectively. All these diffraction peaks can be indexed to the face-centred cubic (FCC) crystalline structure of pure Ag NPs.

Our calculated results showed that the average grain size is in the range of 15-21nm using Debye- Scherer equation .

The average nano particles is calculated from (FWHM) of broadening of the diffraction peaks using Debye–Scherrer's formula which was first invented by Scherrer in 1918 and then subjected to different modifications. Debye-Scherer equation is written as follows:

$$\boldsymbol{\delta = \frac{0.9\,\lambda}{B\cos\theta}} \ldots\ldots\ldots(1)$$

Where δ is the grain size diameter, B(2θ) is FWHM (Full Width at Half Maximum) in radians and can be determined using Gaussian curve, λ = 1.54 nm (a tool characteristic) and θ is the Bragg angle. However, the value of (0.9) is the constant of proportionality (Scherer constant) and generally chosen between (0.89 – 0.94) The detailed variation of the peak position (2θ), full width at half-maximum (FWHM, β) value, d value, a value, average crystallite size (D), The dislocation density (δ) value and micro-strain (ε) along the (011) plane of CDS are presented in Table 2. These results are calculated according to the following relations:

$$\varepsilon = \beta\cos\frac{\theta}{4} \quad \ldots(2)$$

$$\delta = \frac{1}{D2} \ldots(3)$$

$$\text{dhkl} = \left[\frac{h2 + k2 + l2}{a2}\right] - 0.5 \quad \ldots(4)$$

$$2\text{dsin}\,\theta = n\,\lambda \quad \ldots(5)$$

The chemistry of leaves constituents confirm the availability of several active chemical compounds in its leaves and maybe they are the most responsible compounds for reducing silver precursor to silver ions surfactant. Moreover, silver nanoparticles in this size range are an excited area in many applications such as antibacterial applications in water purification or in medical and pharmaceutical applications as well as in environment fields (Mokhtar, (2019)). .

In addition, the average sized of nanoparticles can be calculated Hall-Williamson equation:

$$\beta\cos\theta = (k\lambda/D) + 4\varepsilon\sin(\theta) \ldots(6)$$

2.4 Optical Properties:

Because the depletion of the sample in the previous measurement, other samples were prepared under the same conditions. We have monitored the produced silver nanoparticles using the UV-Vis Spectrophotometer at a wavelength range of 340 -650 nm. From Fig-4 , the obtained curves show the extract of *Ficus carica* reduced and produced AGNP. The Gaussian-shape of the Samples (A&B) peaks at 455 nm and 434nm Respectively, suggest the presence of uniform spheres of the individual silver nanoparticles. In fact this was confirmed by a color change in the reaction mixture to reddish brown which refer to the surface plasmon resonance (SPR). The SPR is affected by three factors namely; the particle size, dielectric medium and chemical neighborhood surroundings (KiranmaiI (2007), Singh, (2012) , Arpita Roy (2017) Azat A, et al. (2016 Andreia . (2018), Mokhtar (2019).

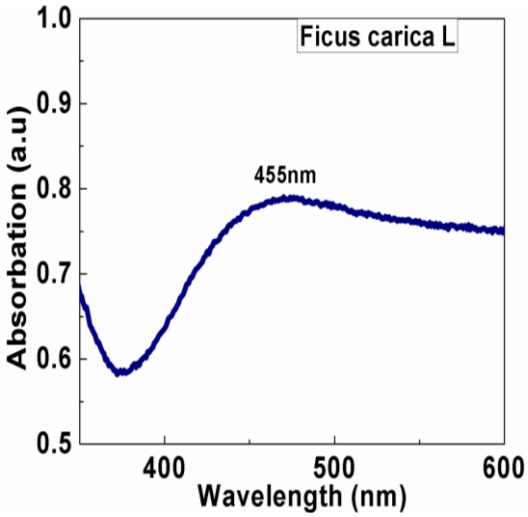

Fig-4: UV-VISB for AgNP using Ficus carica

2.5 Antimicrobial Effect

2.5.1 Sensitivity susceptibility of (FCL) and (FCLSNP)

The antimicrobial sensitivity tests show that FCLSNP is a susceptibility against microbial organisms but, FCL extract is not susceptibility as shown in fig -5(a). It's very clear that, FCL shows resistance effect against *E.coli* which is agreed with some previous study (Rashid et al., 2014) .

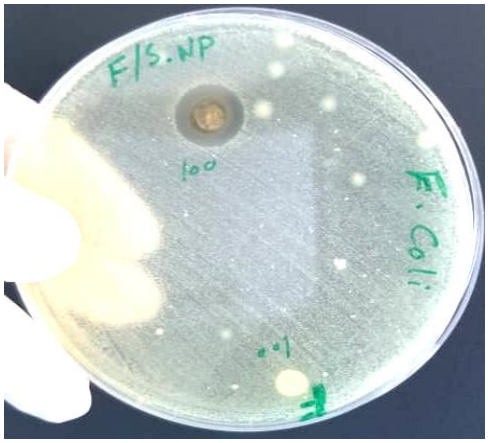

Fig-5(a): Effects of FCL Nano –particle extract is more susceptibility than FCL extract

2.5.2 The effect of (FCLSNP) on *Escherichia coli* and *Staphylococcus aureus.*

Fig- 5(b-c) shows a high antimicrobial inhibitor effect of each FCLSNP concentration (100,75,50 AND 25)% on *Escherichia coli and Staphylococcus aureus*. According of zone-siz, FCLSNP shows higher antimicrobial inhibition effect on *Escherichia coli (*gram +ve) more than *Staphylococcus aureus* (gram -ve *)* which is agree with reference,(Al Askari et al., 2013) . The comparative study is shown in Fig-6.

. Fig- 5 (b) Effect of (FCLSNP) on *Escherichia coli*

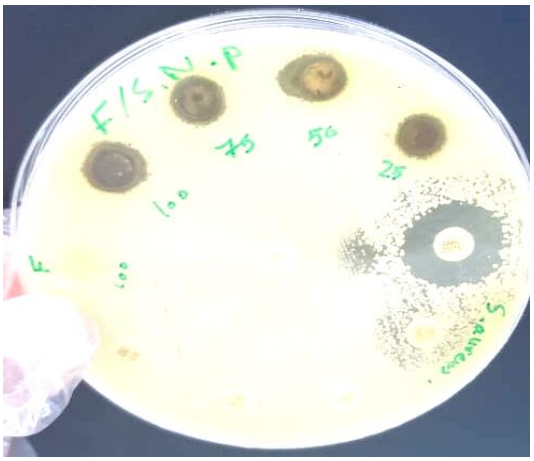

Fig- 5 (c): Effect of (FCLSNP) on *Staphylococcus aureus.*

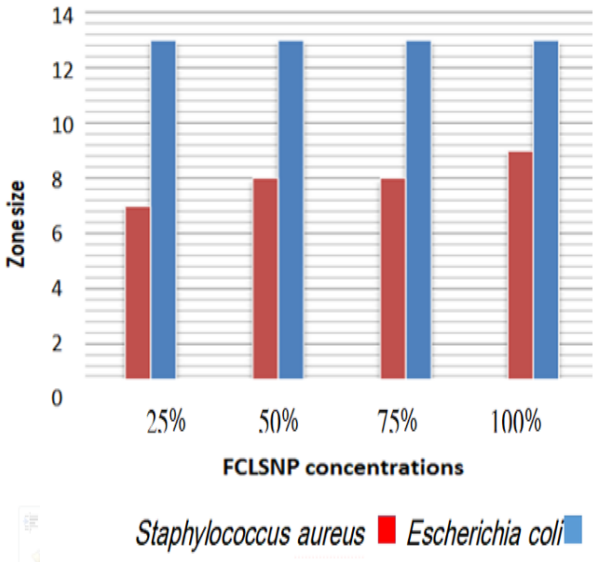

Fig-6: Effect of FCLSNP concentrations on *Escherichia coli* and *Staphylococcus aureus(* acoording to zone size **)**

3. Conclusions

This study has presented the synthesis of highly crystalline Ag NPs with average grain size is in the range of 15-21nm using Debye-Scherer equation and 25-27nm Hall-Williamson using the extraction *Ficus carica L.* These leaves are being proved as reducing and capping agents for reducing silver ions to silver nanoparticles without any additional hazardous chemicals In addition, we applied this study to investigate the antimicrobial activity of Ag NPs synthesized using *Ficus carica L* (FCL) extract. The obtained results are compared for both positive gram *Escherichia coli* (E. coli) and negative gram *Staphylococcus aureus* (S. aureus) bacteria.

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