



GREEN SYNTHESIS OF SILVER NANOPARTICLES USING TOMATO (*LYCOPERSICON ESCULENTUM*) EXTRACT AND EVALUATION OF THEIR ANTIFUNGAL ACTIVITIES

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Abstract

Nanoparticles exhibit unusual physical, chemical and biological activity due to their reduced small sizes and their potential to provide safe, eco-friendly, cost effective, high-stability, and high-loading-capacity nanoparticles. The current study was carried out to investigate the antifungal activity of silver nanoparticles synthesized using tomato fruit extract. AgNPs were characterized using UV-Vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and X-ray diffraction (XRD). The influence of process variables such as temperature, reaction, pH, AgNO₃ concentration and extract concentration was also investigated to optimize the biosynthesis of silver nanoparticles. The average size of synthesized AgNPs was 9.58 to 72.69 nm. The UV-vis absorption spectra confirmed the formation of the AgNPs with the characteristic peak at 370 nm and SEM micrograph acknowledged irregular particles in a nanosize range. FTIR measured the possible biomolecules that are responsible for stabilisation of AgNPs. XRD analysis exhibited the crystalline nature of AgNPs and showed face-centred cubic structure. The *in-vitro* antifungal activities of synthesized silver nanoparticles were studied by Agar well diffusion method and poisoned food method. In Agar well diffusion method showed the activity against *C. albicans* at the concentration of 400 and 500 mg.ml⁻¹, which exhibit the most potent concentration of silver nanoparticles against *C. albicans*. In poisoned food method, Absolute inhibition (100%) was observed when treated with AgNPs at concentration of 25%. The toxicity of synthesized silver nanoparticles were studied *in vivo*, there were no significant changes in mice weight during the study period. In conclusion. Manipulating the reaction parameters, particularly, the pH, tomato extract concentration, AgNO₃ concentration and temperature resulted in the formation of nanoparticles with various sizes and shapes. can potentially be used as an anti-candidal drug, also not exhibited toxicity for tested mice, but AgNPs toxicity *in vivo* still requires further research and investigation.

Key words : Silver nanoparticles, *Candida albicans*, *in vivo*, *in vitro*, toxicity.

Introduction

Oral candidiasis is one of the common fungal infections, affecting the oral mucosa caused by the yeast *Candida albicans* (Singh *et al.*, 2018). *Candida albicans* responsible for numerous types of oral infections (Vila *et al.*, 2020). Understanding the mechanisms used against antifungal drugs is imperative for patient treatment (Pristov and Ghannoum, 2019). The increased use of antifungal drugs, often for prolonged periods, has led to acquired antifungal resistance (Kulkarni *et al.*, 2018). Metal Nanoparticles (NPs) are of great scientific interest

as they bridge the gap between the bulk and atomic structures. NPs have unique physicochemical properties, *i.e.*, high surface area, high reactivity, tunable pore size and particle morphology (Ananda *et al.*, 2015; Siddqui *et al.*, 2015). Nanoparticles exhibit unusual physical, chemical and biological activity due to their reduced small sizes and they are applied in various disciplines including engineering agriculture, electronics, automotive, information and communication technologies, energy, textile, construction medical, and household products (Obiazikwor and Shittu, 2018). Nanoparticle structures consist of three layers: (i) core, (ii) shell, and (iii) surface (Rai *et al.*, 2010). To evaluate the synthesized

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nanomaterials, many analytical techniques have been used, including Ultraviolet Visible spectroscopy (UV-vis spectroscopy), X-Ray Diffractometry (XRD), Fourier Transform Infrared spectroscopy (FTIR), X-ray Photoelectron Spectroscopy (XPS), Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM), and so on (Ghosh *et al.*, 2012; Khan and Mushtaq, 2018). Due to their unique properties, AgNPs have been used extensively in various applications (Zhang *et al.*, 2016). Basically, the reduction of silver salts involves two stages (i) nucleation; and (ii) subsequent growth (Deepak *et al.*, 2011). Tomato was used as a reducing and capping agents for the biosynthesis of AgNPs. Sutradhar and Saha (2016) synthesized of ZnONPs in mass level, using tomato extract by both thermal method and under microwave irradiation using different power. Also Asmathunisha and Kathiresan (2013) investigated the rapid biosynthesis of silver and gold nanoparticles by *in vitro* callus and leaf extracts derived from tomato. Many *et al.* (2014) used tomato pomace for the synthesis of silver nanoparticles, and showed good antibacterial activity towards resistant pathogens (*B. subtilis*, *P. vulgaris*, *C. albicans*, *Pseudomonas*, *S. aureus*). The present investigation deals with the synthesis of silver nanoparticles and characterization using the aqueous extract of *Lycopersicon esculentum* (tomato fruits). The water extract of tomato fruits mostly contains proteins and antioxidant molecules such as phenolic compounds, glutathione and carotenoids (Keukens *et al.*, 1996) which are believed to act as stabilizing and reducing agents, respectively. With these nanoparticles, a preliminary test for antifungal activity was carried out by a diffusion method and poisoned food method.

Materials and Methods

Preparation of Tomato Fruit Extract

Tomato (*Lycopersicon esculentum* Mill) plants were grown in a greenhouse located at College of Sciences of Agriculture Engineering, University of Baghdad. The plants were kept in the 4°C temperature. Tomato fruits were harvested at the light red stage of ripeness. Calyces were removed and whole fruits were thoroughly washed and homogenized using a hand-held blender for 2 min. To prevent compositional changes, the homogenized extract was clarified by repeated (3×) centrifugations for 15 minutes at 15,000 and stored at 20°C for further experiments.

Preparation of mM AgNO₃ Solutions

One milimolar solution of AgNO₃ 0.017 g was

prepared by dissolving in 100ml deionized water (DIW). 2 and 3 milimolar solution was prepared by dissolving 0.034 and 0.051g each in 100 ml., and stored in coloured bottle in cool and dry place.

Green Synthesis of AgNPs

Three methods used in this study

Method A

AgNPs were synthesized by adding 25 ml of tomato fruit extract at 5, 10, 20 and 50% concentrations with pH of 5, 9 and 11 to a vigorously stirred 25 ml of 1, 2 and 3 mM silver Nitrite (AgNO₃) solution in a 250 ml flask. Reduction of AgNO₃ was observed from the progressive colour change at temperature 37, 75 and 100°C (Table 1). The setup was left for hours to complete the reaction, which was indicated by a stable yellowish brown colour of the mixture. To investigate the effect of various reaction conditions on the formation of nanoparticles, formation of nanoparticles was indicated by the development of yellowish brown colour in the reaction mixture. Nanoparticles obtained were purified by repeated centrifugation at various speeds (depending on the recovery) for 20 min, and stored at 4°C. (Lee, 2014).

Method B

Ten ml of aqueous fruit extract was added into 20ml aqueous solution of 1 mM AgNO₃ aqueous solution into 170 ml of deionized water for reduction into Ag⁺ ions at room temperature (Rajoriya, 2017). The reaction mixture was optimized at different controlled conditions for the green synthesis of AgNPs (Table 1).

Table 1: Reaction parameters applied in different treatment groups.

Treatment	Manipulated Parameter
1	pH of extract = 5, 9, 11
2	Extract concentration = 5%, 10%, 20%, 50%
3	Reaction temperature = 37°C, 75°C, 100°C
4	AgNO ₃ concentrations = 1, 2, 3 mM

Method C

Ten ml of aqueous solution plant extract was added to 90 ml of the 2 mM concentration of aqueous silver nitrate solution at a ratio of 1: 10 (v/v). The resulting mixture was continuously stirred and gradually saved at room temperature until reaction solution changed in color (Shankar *et al.*, 2004). AgNP were produced by reduction of silver nitrate solution by using fruit tomato extract. Ten milliliters of aqueous tomato extract was mixed with 10 ml of 3 × 10⁻³ M SDS solution and cooled in ice-cold water for few minutes. The solution was made alkaline (pH 11) with 0.15 N sodium hydroxide solution.

After that, 8 ml of 3×10^{-3} M aqueous silver nitrate was added into it dropwise with continuous stirring. The mixture was then heated for 20 min at 80°C. The color of the solution gradually changed from colorless to reddish yellow. The reddish yellow color indicated the formation of AgNP (Mittal *et al.*, 2014).

Characterization of Synthesized AgNPs

AgNPs obtained in each case were characterised by UV visible spectroscopy spectrophotometer (Systronics double beam spectrophotometer 2202, India), scanning electron microscopy (SEM) (ZEISS-EVO MA 15, Japan), X-ray diffraction (XRD) (Philips Analytical, Almelo, The Netherlands), and Fourier transform infrared spectroscopy (FTIR) (Perkin-Elmer 1725x, Japan).

In vitro antifungal assay of AgNPs

The antifungal activity of the synthesized AgNPs was determined against *C. albicans*. The *in vitro* sensitivity test was carried out by using the agar well diffusion assay method as described by Bibi *et al.*, (2011), and by Poisoned food method (Grover and Moore (1962).

In vivo toxicity assay

Mice were used for the study and 20 mice were divided into four groups (n = 5). All the animals were acclimatized for two weeks under standard husbandry conditions, *i.e.*; room temperature of $25 \pm 1^\circ\text{C}$; relative humidity 45-55% and a 12:12 h light/ dark cycle. Mice were fed with standard laboratory food and had free access to drinking water (*ad libitum*). Each experimental group had separate set of animals in standard cages and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for two weeks prior to experimental protocol to minimize if any of non-specific stress before the delivery of AgNPs (AL-Jobori and Abdel-Kadoom, 2016). The different selected doses of 0, AgNO₃ (25%), AgNPs (25%) and AgNPs (50 %). The animals were weighed daily and the average weight was taken at the end of each of the three weeks, to record the change in weight by the effect of the treatments compared with the comparison treatment.

Statistical analysis

All the biological assays were performed in triplicate. The results are presented as mean with standard deviation. The mean values were further analysed for analysis of variance and least significant difference (LSD) at probability level $p \leq 0.05$ and ≤ 0.01 using SAS system.

Results and Discussion

Results

Synthesis of AgNO₃ Nanoparticles

Ag nanoparticles were synthesized by the addition of tomato fruit extract in different concentrations 5, 10, 20 and 50% at pH of 5, 9 and 11, to 1, 2, 3mM aqueous AgNO₃ at reaction temperature of 37°C, 75°C and 100°C nanoparticles was confirmed by changed the off White color of the solution to yellowish brown. The appearance of the deep yellowish brown color indicated formation of Ag nanoparticles (Fig. 1). They turned brown and the



Fig. 1: Test for Sunflower to show the synthesis of gold nanoparticles.

intensity of color was increased with the time of incubation.

Characterization of AgNPs

The formation of silver nanoparticles was further confirmed by UV- Visible spectrophotometer analysis. The UVvisible spectra (shown in Fig. 2) indicates a strong Plasmon resonance that is located at ~ 340 nm.

The formation of phase synthesized AgNPs was analyzed by X-ray diffraction, which confirmed the bio-reduction of metal nanoparticles is of elemental silver (Fig. 3). The XRD patterns obtained for the AgNPs showed a number of peaks at 25.3° , 42.5° , 50.7° , 54.0° and 76.8° in the 2θ range of 30° - 90° which were pertained to (111), (200), (220), and (311) of AgNPs, respectively.

FTIR results reveal absorption bands at 1936, 1882, 1803, 1764, 1656, 1602, 1510, 1392, 1222, 1188, 1085 cm^{-1} (Fig. 4). The vibrational bands correspond to the bonds such as C=O, C=C, C-N, C-O-C, C-O-H and C-Cl which were in the region range of 600–3822 cm^{-1} .

Scanning electron micrograph shows the variation in size of NPs by fruit extracts and concentrations of Ag ions. The particle size distribution revealed that the average size of the obtained sample at pH11, tomato extract 10%, AgNO₃ 2mM and temperature of 100°C was approximately 9.58-72.69 nm. (Fig. 5).

In vitro antifungal assay of AgNPs

The anti-microbial potential was assessed by determining the inhibition zone diameter. The sensitivity

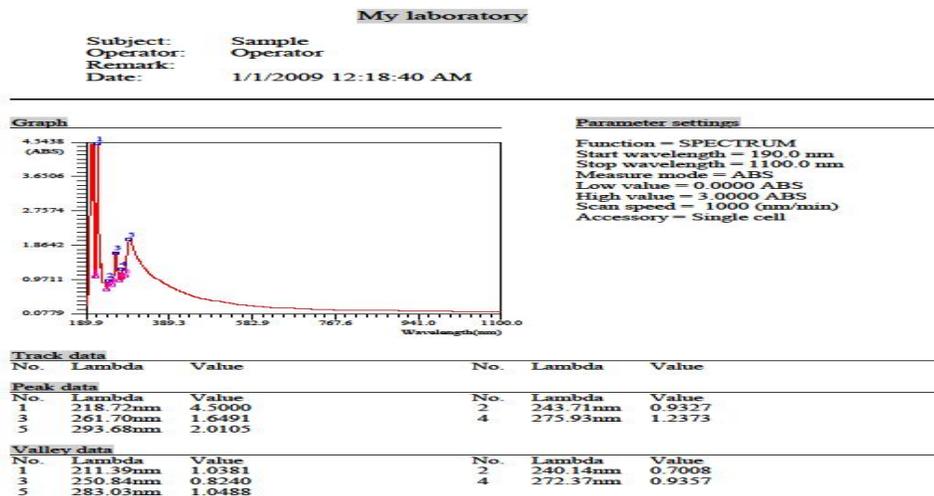


Fig. 2: UV-Visible spectrum of gold nanoparticles synthesized by Sunflower extract.

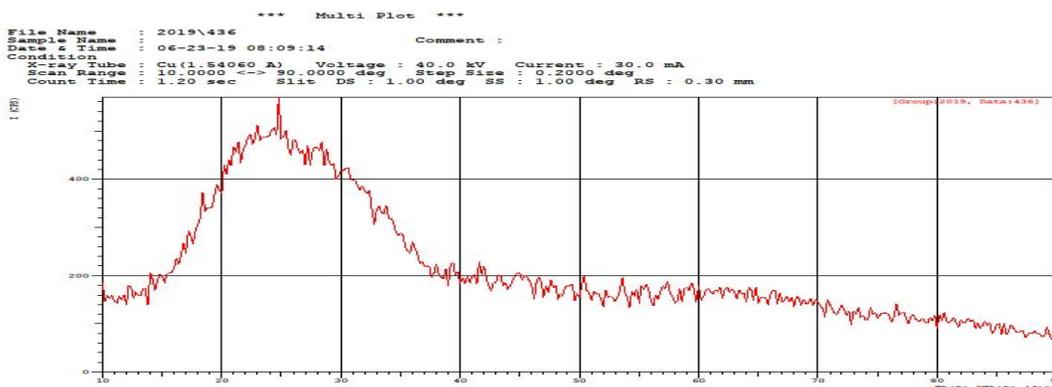


Fig. 3: XRD spectra of synthesis AgNPs.

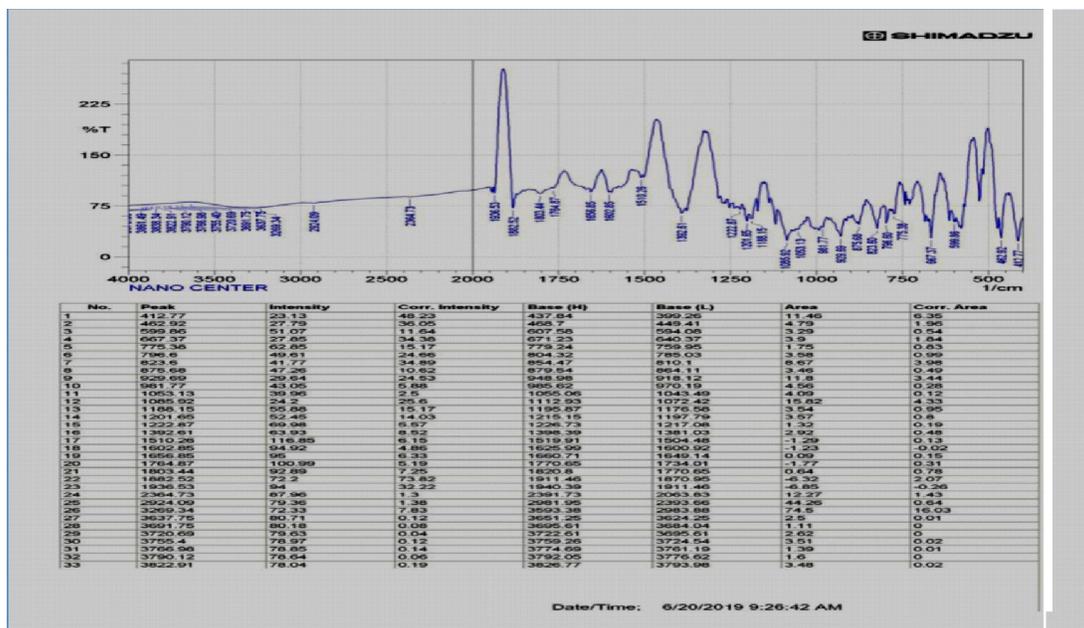


Fig. 4: FTIR absorption spectra of *Lycopersicon esculentum* -AgNPs.

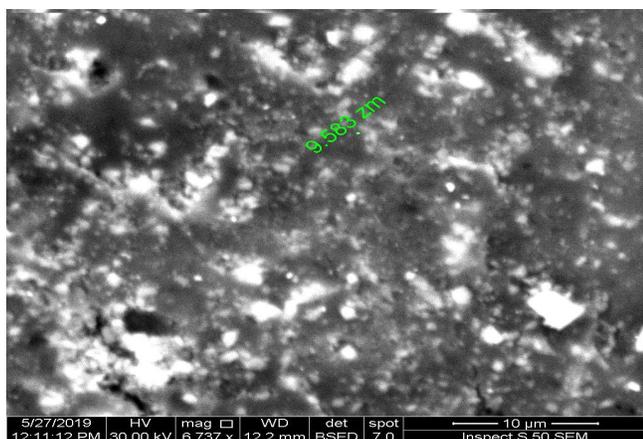


Fig. 5: SEM image of AgNPs synthesized from tomato fruits extract 10%, AgNO_3 2mM, pH11 and temperature reaction 100°C.

of fungal strains towards antibiotics with a clear zone around the well is tested using well diffusion method. Fig. 6 revealed that synthesized AgNP not effect on *C. albicans* at the concentrations of 50,100,150, 200 and 300 mg.ml^{-1} , but significant reduction in growth of *C. albicans* was observed at higher concentrations (400 and 500 mg.ml^{-1}) (Fig. 7) by increasing the inhibition zones from 10mm to 12mm (20%) and 14 mm (40%), respectively (Table 2).

Poisoned food method is mostly used to evaluate the antifungal effect against molds (kumar *et al.*, 2013). Table 3 and Fig. 8 and 9 revealed that AgNPs at concentration of 10 and 25% synthesized from tomato extract with concentrations of 10 and 40% caused inhibition in the growth of the *C. albicans* but not differ significantly from control treatments (tomato extract at concentration of 25% and molten agar Medium). While AgNP at concentration of 10% synthesized from tomato extract with concentration of 30% and AgNO_3 at concentration

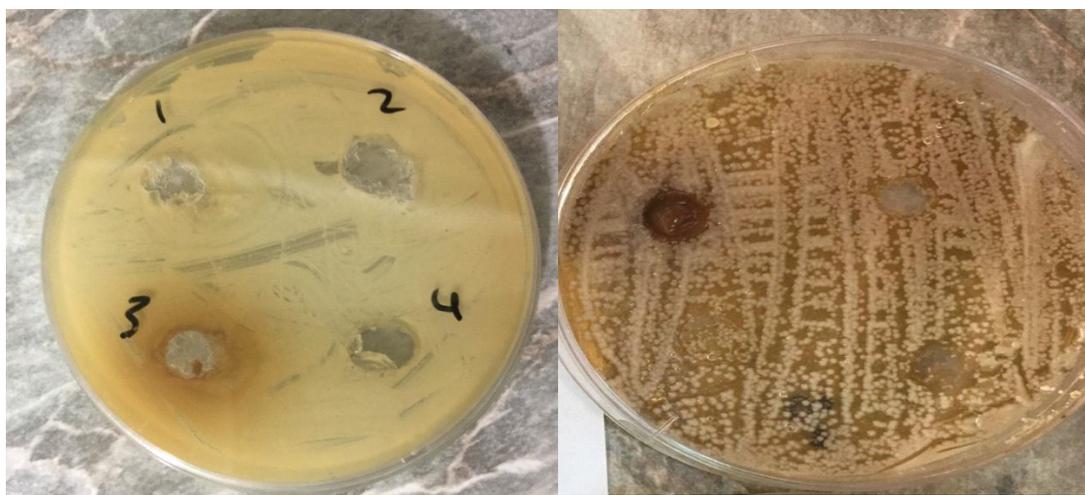


Fig. 6: Zone of inhibition by AgNO_3 nanoparticles at 50,100,150,200 and 300 mg.ml^{-1} on *C. albicans*.

Table 2: Testing of antifungal activity of synthesized AgNPs (zone of inhibition values in mm).

Conc. mg.ml^{-1}	NPs Inhibition zone(mm)	Antifungal activity (%)	Tomato extract 25% conc. Inhibition zone(mm)	AgNO_3 2mM Inhibition zone(mm)	Antifungal activity (%)	Control without add (Agar medium) Inhibition zone(mm)
50	10	0	10	10	0	10
100	10	0	10	10	0	10
150	10	0	10	17	70	10
200	10	0	10	14	40.3	10
300	10	0	10	15	50	10
400	12	20	10	22	120	10
500	14	40	10	23	130	10
P-value	0.0419 *	0.0001 **	NS	0.0001 **	0.0001 **	NS



Fig. 7: Zone of inhibition by AgNO₃ nanoparticles at 400 and 500 mg.ml⁻¹ on *C. albicans*.



Fig. 8: Anti-fungal activity of tomato extract at concentration of 25% by food poisoned method.

Table 3: Anti-fungal activity of synthesized AgNPs by food diffusion method.

Tomato extract conc. 25% (Control)	Molten agar medium (Control free)	AgNO ₃ Conc. 25%	NPs conc. 10% synthesis from tomato extract conc. 10%	NPs conc. 10% synthesis from tomato extract conc. 30%	NPs conc. 25% synthesis from tomato extract con. 40%	NPs conc. 25 % synthesis by (10 ml AgNP + 90ml distilled water)
80	80	33	75	72	75	0
LSD				7.092 **		

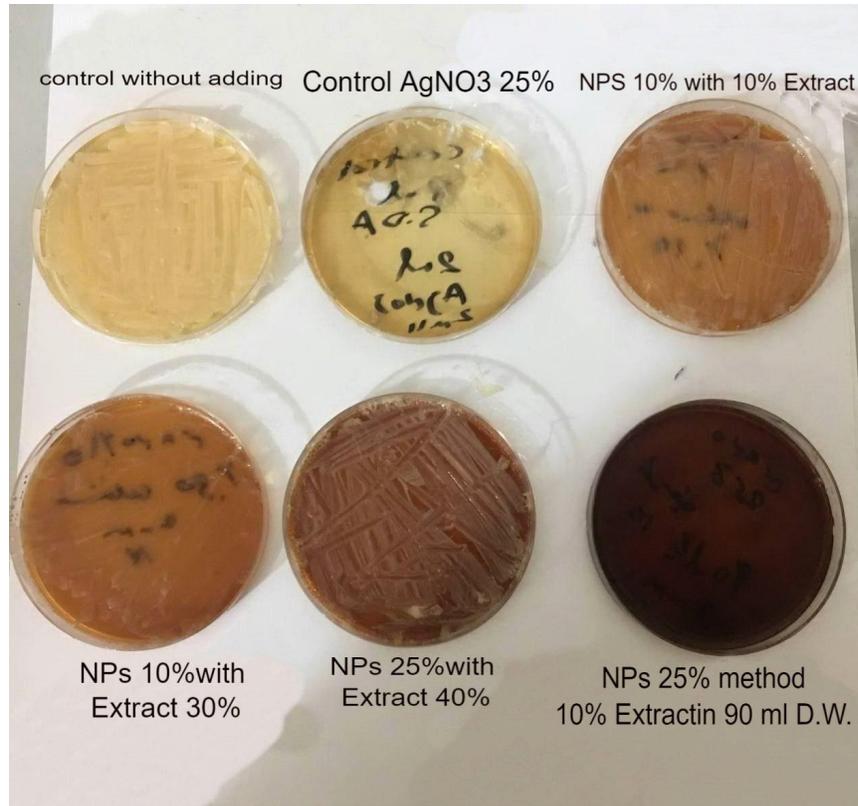


Fig. 9: Anti-fungal activity of molten agar Medium ,AgNO₃ at concentration of 25% and NPs at concentration of 10 and 25% by food poisoned method.

Table 4: Body weight changes during 3 weeks oral administration of silver nanoparticles.

Week	Mean \pm SD				LSD value
	Control Weight(g)	AgNO ₃ 25% Weight(g)	AgNPs 25% Weight(g)	AgNPs 50% Weight(g)	
Week1	35.33 \pm 2.31	34.33 \pm 1.52	35.67 \pm 2.88	34.33 \pm 1.53	N.S
Week2	34.33 \pm 3.21	34.00 \pm 1.00	35.00 \pm 3.60	33.00 \pm 1.00	N.S
Week3	35.67 \pm 3.05	33.67 \pm 1.52	34.00 \pm 1.73	32.00 \pm 1.00	N.S
LSD value	N.S	N.S	N.S	N.S	

* ($P \leq 0.05$)

of 25% significantly inhibited its growth. Absolute inhibition (100%) was observed when treated with AgNPs at concentration of 25%.

***In vivo* AgNPs Toxicity**

There were no significant ($P < 0.05$) dose-related decreases in the body weight of mice in the same week (1 or 2 or 3 final week) when exposure to AgNO₃ at concentration of 25% and AgNPs at concentrations of 25 and 50% compared with control, which ranged between 34.33 - 35.67 at first week, 33.00 - 35.00 at second week and 32.00- 35.67 at final week (Table 4). No significant ($P < 0.05$) changes were observed in mice weight after three weeks with a slight decrease in the weight of mice from 32g (first week) to 31g (second week) then 30g (final week) when treated with high dose (AgNPs 50%), and with slight decreases in the weight of mice from 34.00 and 35.00g to 33.67g and 34.00g from second week to final week treated with AgNO₃ and AgNPs, respectively. However there were no dose-related changes in the body weight of untreated mice (Table 4).

Discussion

The biosynthesis of nanoparticles by plants could be a better candidate for the low-cost and large-scale production of nanoparticles due to it being environmentally safe (Makarov *et al.*, 2014). The tomato extract was used as reducing as well as stabilizing agent for the synthesis of AgNPs. Numerous compounds have been extracted from tomato phenolic compounds, phytoalexins, protease inhibitors, and glycoalkaloids (Friedman, 2002), antioxidants like ascorbic acid, vitamin E, carotenoids and flavonoids, glutathione and carotenoids, vitamins, *i.e.* A, B and C; phytosterols (Keukens *et al.*, 1996). In this study, the appearance of a yellowish brown color in the reaction solution was a clear indication of the formation of AgNPs in the reaction mixture (Fig. 1), as described previously (Many *et al.*, 2014; Sutradhar and Saha, 2016; Zia *et al.*, 2016). The plant extracts were reported to act as reducing and capping agents, thereby reducing the

silver ions (Ag⁺) to metallic silver (Ag⁰) (Obiazikwor and Shittu, 2018).

The characterization of the green synthesized AgNPs using the aqueous fruits extract of tomato was achieved using techniques such as UV-vis spectroscopy, FTIR analysis, SEM, and XRD analysis (Sutradhar and Saha, 2016; Abdul Jalil *et al.*, 2017; Obiazikwor and Shittu, 2018 Rahman *et al.*, 2019). The surface

plasmon resonance phenomena (SPR) absorbance is sensitive to the shape, size, and nature of particles present in the solution, and also depends upon the inner particle distance and the surrounding media. Spectral analysis revealed the SPR absorption of green synthesized AgNPs at 340 nm, the spectrum of the sample was obtained for wavelength range and the band was noticed between 320-475 nm. (Fig. 2), which was within the typical wavelengths reported for AgNPs. This results were in agreement with the results of Gebru *et al.*, (2013) who stated that metal nanoparticles exhibit weak absorbance around 300–400 nm, the absorption peak at 370 nm corresponds to the transverse plasmon vibration in the Ag nanoparticles. Sutradhar and Saha (2016) reported that the UV vis spectra of ZnONPs showed the absorption peak at 322 and 334 and 360 nm. The variation in spectra may be due to the number of particles and the size distribution in the solution (Zhou and Wang, 2012).

SEM image showed the high density nanoparticle synthesized by fresh tomato extract were relatively irregular in shape. This confirmed the development of silver nanostructures. Scanning electron micrograph shows the variation in size of NPs, the size might also vary due to the reduction in aggregation of the growing NPs and even the peak intensity enhances in response to pH, temperature, concentration of tomato extract and concentration of AgNO₃. The SEM image showed the nanoparticle in the range of 9.58 -72.69 nm (Figure 3) when used tomato extract at 10% concentration, AgNO₃ at 2mM, pH 11 and temperature reaction at 100°C. This size of particle confirms the presence of nanoparticle. This result agreed with other research (Many *et al.*, 2014; Das *et al.*, 2016; Halbandge *et al.*, 2017). The results of the FTIR spectroscopy of tomato extract and its AgNPs synthesized after the bioreduction (Fig. 4) revealed that the bonds such as C=O, C=C, C-N, C-O-C, C-O-H and C-Cl which were in the region range of peaks at 600–3822 cm⁻¹. Many reports describe that the NPs synthesised by reacting AgNO₃ with biological solutions are face centered cubic with minor variations in peak

values depending upon nature of extract; metabolites present and binding properties (Mitra *et al.*, 2012). FTIR spectra indicate the presence of phenolic hydroxyl groups in the structure of flavonoids, that are supposed to be responsible for the reduction of silver ions in to silver during the synthesis of AgNPs (Zargar *et al.*, 2014). The major content in flavonoids could reduce silver nitrate more successfully (Rodríguez-Luis *et al.*, 2016).

XRD as a powerful nondestructive technique for characterizing crystalline materials provides information on structures, phases, preferred crystal orientations, and other structural parameters, such as average grain size, crystallinity, strain, and crystal defects (Salari *et al.*, 2019). The XRD clearly indicates the silver nanoparticles formed by tomato extract, reduction of silver ions by XRD pattern clearly exhibited the presence of silver nanoparticles (Fig. 5). Existence of peaks (111), (200), (220) and (311) matched with the standard Joint Committee for Powder Diffraction Set JCPDS) data-04784 (Vijayaraghavan *et al.*, 2012). Interestingly, most of researchers (Das *et al.*, 2014; Lee, 2014; Zia *et al.*, 2016; Halbandge *et al.*, 2017; Salari *et al.*, 2019) that synthesized the nanoparticles using plant extracts seem to obtained a similar crystal structure.

The agar well diffusion method was employed for screening of antifungal activities of silver nanoparticles compared with silver nitrate at the concentrations of 50,100,150, 200,300,400 and 500mg. ml⁻¹ without add (Agar medium) control with no inhibition zone. But significant reduction in growth of *C. albicans* was observed at higher concentrations (400 and 500 mg.ml⁻¹) (Fig. 7) by increasing the inhibition zones from 10mm to 12mm (20%) and 14 mm (40%), respectively (Table 2). Similar results obtained by Ali and Abdallah (2020) who found that standard disk diffusion method revealed that AgNPs displayed anti-candidal activity (11.33-mm inhibition zone). The antifungal potency of the plant extract and AgNPs increased with increasing their corresponding concentrations (Hafez *et al.*, 2017). Several mechanisms were reported to explain the mode of anti-candidal activity of AgNPs. These include the capacity of AgNPs to damage the membrane permeability barrier and to destruct the membrane lipid bilayers, resulting in the leakage of ions, along with forming pores and dissipating the electrical potential of the membrane. In addition, AgNPs were shown to block the cell cycle at G2/M phase in *C. albicans* (Kim *et al.*, 2009), to increase the production of reactive oxygen species (ROS), and to decrease the activity of metal-based antioxidant enzymes (Dantas *et al.*, 2015). Metal ions are released by some metal oxide NP's, are absorbed through cell membrane

followed by interaction with proteins and nucleic acids damaging enzyme activity (Zakharova *et al.*, 2015). Therefore, they can be used as potent biocide against harmful pathogens.

Silver nanoparticles synthesized utilizing fresh tomato extract showed higher antifungal activity against *C. albicans*. (Table 3, Fig. 8, 9) AgNP at concentration of 10% synthesized from tomato extract with concentration of 30% and AgNO₃ at concentration of 25% significantly inhibited its growth by 10 and 59%, respectively. Absolute inhibition (100%) was observed when treated with AgNPs at concentration of 25%. Sohal *et al.*, (2019) reported that the synthesized silver nanoparticles showed highest% of mycelia growth inhibition of pathogenic fungi *Fusarium moniliforme* by 77.08±0.72 (p<0.0001). Hafez *et al.*, (2017) tested the inhibitory effect of AgNO₃ on fungi using poisoned food technique, they found that the MIC of plant extract and AgNPs was 0.1%, and concluded that the poisoned food technique was more sensitive and accurate than the agar well diffusion technique.

There is an increasing interest in the analysis of potential nanoparticle toxicity, it is becoming necessary for a better understanding of the mechanisms of silver NPs biological interactions and their potential toxicity (More *et al.*, 2018). Results of this study revealed that there were no significant (P < 0.05) dose-related decreases in the body weight of mice in the same week (1 or 2 or 3 final week) or after three weeks when exposure to AgNO₃ at concentration of 25% and AgNPs at concentrations of 25 and 50% compared with control (Table 4). It should be noted that in a range of oral investigations, no effects on body weight were reported (Sardari *et al.*, 2012; van der Zande *et al.*, 2012; Espinosa-Cristobal *et al.*, 2013), these results were in line of the results of current study. On other hand, was disagreement with others (Kim *et al.*, 2010; Hadrup *et al.*, 2012; Shahare and Yashpal, 2013). Therefore, AgNPs toxicity *in vivo* still requires further research and investigation.

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