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Synthesis of silver nanoparticles using *Prosopis juliflora* extract: potential of antimicrobial and pollutants degradation performance

Yasir Anwar*, Mohammad Hussain Fakieh, Ihsan Ullah, Naser A. Alkenani, Mohammed A. Sharif

Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box: 80203, Jeddah 21589, Saudi Arabia, emails: yanwarulhaq@kau.edu.sa (Y. Anwar), mf1881@yahoo.com (M.H. Fakieh), ihsanknu@gmail.com (I. Ullah), nkenani@gmail.com (N.A. Alkenani), mhsharif1410@hotmail.com (M.A. sharif)

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ABSTRACT

This study was focused on the extract retrieval from *Prosopis juliflora* leaves and their use in the bio-synthesis of silver nanoparticles (AgNPs). The as-synthesized AgNPs were characterized by FTIR, FESEM, XRD and EDX analyses in order to determine the functional groups, morphology, crystal structure, and elemental composition of the samples. The extract produced metallic Ag and Ag_2O crystalline form of the AgNPs as revealed by XRD experiment. The synthesized AgNPs were used for their evaluation in the studies of antimicrobial as well as water decontamination from common pollutants. The synthesized AgNPs were found to catalyze the 2-nitrophenol (2-NPh) and methylene orange dye (MO) reduction by sodium borohydride with reaction rate constants of 0.352 and 0.134 min^{-1} in the water decontamination studies. The antimicrobial effect of the bio-synthesized AgNPs was studied using multi-drug resistant strain of *Staphylococcus aureus* as the model bacterial species and *Candida albicans* and *Candida tropicalis* as the model fungi strains. It was revealed that at a very dosage of the *Prosopis juliflora* extract-assisted synthesized AgNPs, the stated micro-organisms were unable to survive.

Keywords: Plant extract; Ag nanoparticles; Nitrophenol; Methyl orange; Antibacterial; Antifungal

1. Introduction

Plant extracts are increasingly gaining attention in the field of bio-nanotechnology because of their effective use in the nanoparticles synthesis [1–3]. In fact, a huge knowledge is available on the synthesis of metal nanoparticles using chemical, physical, and irradiative and bio-agents assisted methods [3–8]. The chemical methods for the nanoparticles synthesis involve the chemical solution deposition, co-precipitation method, and the usage of reducing agents which are directly introduced into the metal salt solution [9–14]. The physical methods for the nanoparticles synthesis are ball milling process of the pure metal, metal complex thermal decomposition, plasma arcing, thermal evaporation, pulsed laser desorption, in-situ reduction, spray pyrolysis, lithographic techniques, layer-by-layer growth, sputter deposition, molecular beam epistaxis and diffusion flame methods [15–27]. Both the above-mentioned procedures of chemical and physical routes to the nanoparticles synthesis are involved with the use of hazardous nature of chemicals such as expensive reducing chemicals and stabilizing agents and some types of radiations which are considered harmful to human health. Besides the successful nature of these methods in the synthesis of high quality nanoparticles, they are less practiced due to the high cost methods and environmental issues. In comparison with these methods, the bio-agents assisted nanoparticles synthesis methods are considered environment friendly [3]. Moreover, these methods

* Corresponding author.

are low cost, effective enough, eco-friendly and could be extended to the large scale production of the nanoparticles. In the bio-agents assisted methods, certain molecules act as both the reducing as well as stabilizing roles [28]. Mainly the use of fungi, bacteria, and plant extracts is considered in the bio-agents assisted methods.

Plant extracts consist of several molecules which act as reducing agents [29]. Phenolic acid, flavonoids, alkaloids and terpenoids are the secondary metabolites which play crucial role in the nanoparticles synthesis by reducing the ions to metal nano-clusters [2]. The concentration of both metal ions in salt solution and plant extract amount determines the rate of formation of the nanoparticles synthesis as well as effect the other properties [1].

Several types of transition metal nanoparticles such as silver (Ag), nickel, gold, platinum, iron, titanium, copper, and their oxides such as copper oxide, TiO_2, and Fe_3O_4 have been synthesized using extracts from natural sources [27,30–33]. It has been well documented that the shape, size and morphological features of the nanoparticles greatly affects the end properties of the nanoparticles. Among these, noble metal based nanoparticles are widely explored [33,34]. Ag nanoparticles are one of the noble metals family which is considered one of the useful product in the field of bio-nanotechnology [1,35]. Ag nanoparticles have certain unique properties such as antibacterial, anti-inflammatory and anti-fungal due to which it has been widely used in the biomedical field [1,2]. Owing to the good chemical stability, and environmentally benign nature of the Ag nanoparticles an increasing interest exists in their use in the composite fibers, fiber surface located catalysts, cosmetic products, and in electronic components [4,36,37]. Even though many established mechanisms are proposed for the Ag nanoparticles antibacterial mechanisms, a most common one is the release of the Ag^{1+} ions from the nanoparticles to the surrounding of the bacteria [38–40]. These Ag ions then interact with the nucleosides of the nucleic acid of bacteria by forming complexes [41,42]. Another mechanism simply states that the positively charged Ag ions make way to the cell wall or membrane of the bacteria and penetrates through it where it causes damage and leads to the cell death [43]. Another school of thought is the possibility of the Ag nanoparticles to bind the –SH (thiol) groups of the enzymes which results in the deficiency of the enzymes activity thus kills the bacterial cells [44]. In some recent studies, it was found that nanoparticles based bactericidal agents can show high activity against a broad spectrum of pathogenic bacteria and fungi [45,46]. This list also includes the antibiotic resistant bacteria and fungi [47].

Currently, the industrialization on one hand produces demanding stuff but on the other hand it is responsible for the production of the environmental pollution such as discharge of dyes and toxic metals and chemicals to nearby streams [14,45,48]. For their elimination purpose, either adsorbents are used to recover the toxic ions and dyes from the industrial or simulated wastewater or catalytic degradation is performed for the organic pollutants [49–52]. Mostly, phenolic compounds and other organic dyes are reduced to generate less toxic compounds. Even though, other transition metal [51,53] based catalysts could be used but Ag based nanoparticles are considered suitable due to the fact of their longer stability over time. Transition metal based nanoparticles such as copper, cobalt, and nickel gets oxidized which results in their lower catalytic activity [52].

In this article, we synthesized Ag nanoparticles by using *Prosopis juliflora* plant extract. This plant is a leguminous perennial, phreatophyte tree, which is widely found in the arid and semi-arid regions of world. Its leaves and bark extract has been previously identified to have allergens and biogenic amines [54]. Its potential has also been recognized in the phytoremediation of fluoride contaminated soil [55]. After preparation of the Ag nanoparticles by using its extract, additional experiments of bactericidal, fungicidal, and catalytic hydrogenation of 2-nitrophenol and methyl orange were carried out.

2. Experimental

2.1. Materials

Methanol solvent was obtained from the Sigma-Aldrich (USA). Silver nitrate ($AgNO_3$) salt was purchased from Merck, CAS # 7761-88-8, Ph Europe. 2-nitrophenol and methyl orange were purchased from BDH Chemicals, Poole, England. Pure water of resistivity less than 18 ohm was used for the solutions preparation. Sodium borohydride with 98% purity was purchased from Loba Chemie, India. Methicillin-resistant *Staphylococcus aureus* (MRSA), *Candida albicans*, and *Candida tropicalis* were provided by Microbiology Lab, King Abdulaziz University, Jeddah. Nutrient agar (HiMedia Laboratories, India) and broth medium are used for growth and maintenance of the microbial culture.

2.2. Prosopis juliflora plant extract

The plant leaves were obtained and thoroughly washed with water to remove the dirt impurities. Then the obtained leaves were dried in a shadow. After this process, the leaves were ground to make a powder form. The powdered form leaves were put into methanol and stirred for 24 h to extract the soluble compounds. The dark black color of extract was obtained by removing the leaves powder through filtration which was concentrated.

2.3. Synthesis of Ag nanoparticles using Prosopis juliflora extract

Ag nanoparticles were synthesized using *Prosopis juliflora* extract and silver nitrate salt aqueous solutions. Briefly, a 500 mL stock $AgNO_3$ aqueous salt solution of 1 mM was prepared. Then 50 mL of this solution was taken in a volumetric flask. A plant extract solution of 10 mL was added to the flask containing $AgNO_3$ solution. The combined solution was kept at stirring. The visual change in color indicated the formation of the Ag nanoparticles. After completely reducing the Ag ions to the nanoparticles, the centrifugation process was performed to separate the Ag nanoparticles. Centrifuge was operated at 15,000 rpm for 10 min. The sediments (black color) were washed several times with ethanol and last dried in the vacuum oven and stored for further experiments.

2.4. Catalytic reduction studies

We carried out the degradation/reduction reactions of two model pollutants of MO and 2-NPh by a strong

reducing agent of sodium borohydride and the *Prosopis juliflora* extract assisted AgNPs were used as catalyst. The two aqueous solutions of 2-NPh and MO were prepared with the concentration of 0.15 mM and 0.05%, respectively. The reducing agent sodium borohydride was directly introduced into the reaction vessel with a weight of 0.672 g. For the catalytic reaction, 25 mL of 2-NPh and 50 mL of MO aqueous solutions were separately used. A quartz UV-cuvette was constantly filled for the absorbance measurements and again put the contents of it to the reaction vessel after measurement. To a UV-cuvette, 2 mL of the pollutant solution was charged for each measurement. Then UV-visible spectra in the range of 200–600 or 250–600 nm were recorded. A spectrophotometer of Thermoscientific (UK) was used for this purpose. After the first spectrum acquisition, the Ag nanoparticles were added to the reaction vessel. The addition of the Ag nanoparticles accelerated the reduction reaction as the solution became transparent after completion of the reaction. The reaction kinetics was continuously followed by recording the UV–Vis spectral data.

2.5. Biological studies

Antimicrobial properties of the *Prosopis juliflora* ethanolic extract assisted synthesized AgNPs were carried out against infectious bacteria and fungi. The antimicrobial properties of AgNPs were evaluated by carrying out modified colony forming unit method. Nutrient agar medium plates were prepared, sterilized, and solidified. About 1 μg/mL or lower amount of AgNP samples were added to each of the test tubes containing 9 mL of liquid medium (nutrient broth). Then 1 mL of infectious MRSA, *C. albicans* and *C. tropicalis* were added to 9 mL of tube containing liquid medium and incubated at 37°C for 24 h. The samples were serially diluted and then spread on nutrient agar. For the determination of colonies samples were incubated for 12 and 24 h. Number of colonies were counted using colony counter and experiment was repeated three times.

2.6. MTT assay (cytotoxicity assay)

Human MFC-7 cell line was exposed to green synthesized nanoparticles for the assessment of cell cytotoxicity assay. The cell line was exposed to different concentration of *Prosopis juliflora* extract (positive control), methanol (negative control), silver nanoparticles (AgNPs) and AgNPs + extract. The cell lines were treated to the final concentration of 50 μg/mL (extract), 25 μg/mL (AgNPs), and 30 μg/mL (AgNPs + extract). MFC-7 cells were transferred to Dulbecco's Modified Eagle Medium along with the treated materials and incubated for 24 h at 37°C with an adjusted 5% CO_2. The Dulbecco's Modified Eagle Medium was drained and replaced with that of MTT solution. The plates were incubated at 37°C for 24 h until the blue color was developed. Absorbance was measured using a Microplate reader at 570–690 nm.

2.7. Characterization and instrumental techniques

FESEM data were obtained using instrument JEOL, JSM-7600F (Japan). The Ag nanoparticles solution was dropped on the carbon tape, dried, and Pt coated prior to analysis. For the XRD analysis, the Ag nanoparticles solution was dried and the powder was subjected to analysis. ARL EXTRA from Thermoscientific (UK) was used for the acquisition of the XRD pattern. The instrument was operated at high voltage and copper K-alpha radiations were used. The catalytic tests were measured using the dye disappearance by double beam spectrophotometer of Thermoscientific (UK).

3. Results and discussion

3.1. Synthesis of Ag nanoparticles pictorial representation

A simple plant extract method was used for the AgNPs synthesis as shown in Fig. 1. After collecting and gentle washing of the leaves of *Prosopis juliflora* (1 kg) with deionised water, they were dried and ground using mortar and pestle. Then 50 mL of ethanol was added and stirred for 24 h to ensure the solubility of the compounds into the solvent. The mixture was filtered and concentrated using heating treatment at 35°C.

The methanolic extract of the *Prosopis juliflora* was added to the tubes containing $AgNO_3$ salt solutions. Figs. 2a and

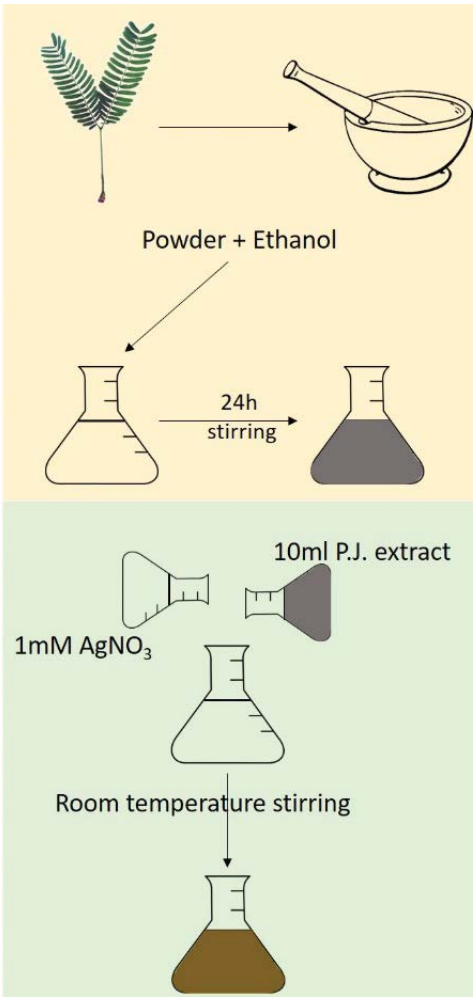

Fig. 1. Preparation steps of the Ag nanoparticles using *Prosopis juliflora*.

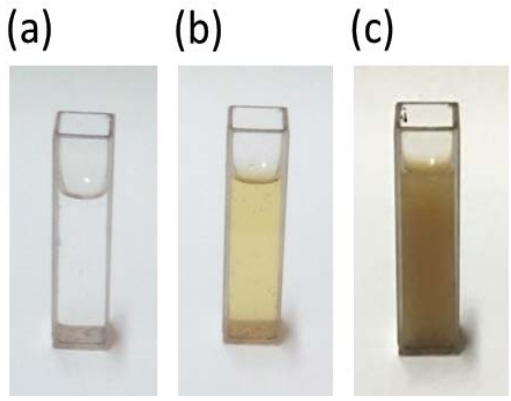

Fig. 2. Photographs of the (a) silver nitrate aqueous solution, (b) ethanolic extract of *Prosopis juliflora*, (c) mixed solution of the salt and extract after 5 min and precipitate formed after short time of stopping the stirring process.

b show the photographs of $AgNO_3$ aqueous solution and methanolic extract of *Prosopis juliflora*, respectively. Fig. 2c is the photograph of the mixed solution of the Ag salt and plant extract. It shows a brown turbid appearance after 1 h of the mixing. Fig. 2c shows the photograph of the cuvette *c* where the settled precipitate could be seen. This precipitate could be easily re-dispersed by shaking or filtered through cellulose filter paper or subjected to 10,000 rpm centrifuge process. After preparation of the Ag nanoparticles with extract in this solvent, the nanoparticles were centrifuged, re-dispersed in aqueous medium and used in further experiments.

3.2. Morphology

Fig. 3a shows the FESEM image of the *Prosopis juliflora* methanolic extract assisted synthesized AgNPs. It can be seen that polydispersed AgNPs were deposited on a plastic substrate. The size of the nanoparticles ranged between 70 and 105 nm. The EDX spectrum is shown in Fig. 3b. It shows clear signals of the Ag from the AgNP in the sample. The signals of carbon and oxygen might arise from the extract and substrate on which the AgNPs were deposited.

To explore the crystal structure of the synthesized AgNPs, the sample was centrifuged for particles accumulation and XRD experiment was carried out.

3.3. XRD and FTIR studies

Fig. 4 shows the XRD pattern of the AgNPs prepared by using *Prosopis juliflora* extract. There existed two types of crystals of silver compounds. The reflections tagged with # represent the (111), (200), (220), and (311) of cubic phase crystal structure of Ag_2O. In addition to these, the reflections tagged with * represent the (111), (200), (220), and (311) of the cubic phase of metallic Ag.

FTIR spectroscopy was performed for both the samples of pure extract and the AgNPs. For better understanding, we tabulated the important peaks of the spectra in Table 1. It is important to note that phenolic and alcoholic peak of compounds were present in the leaf extract. These peaks

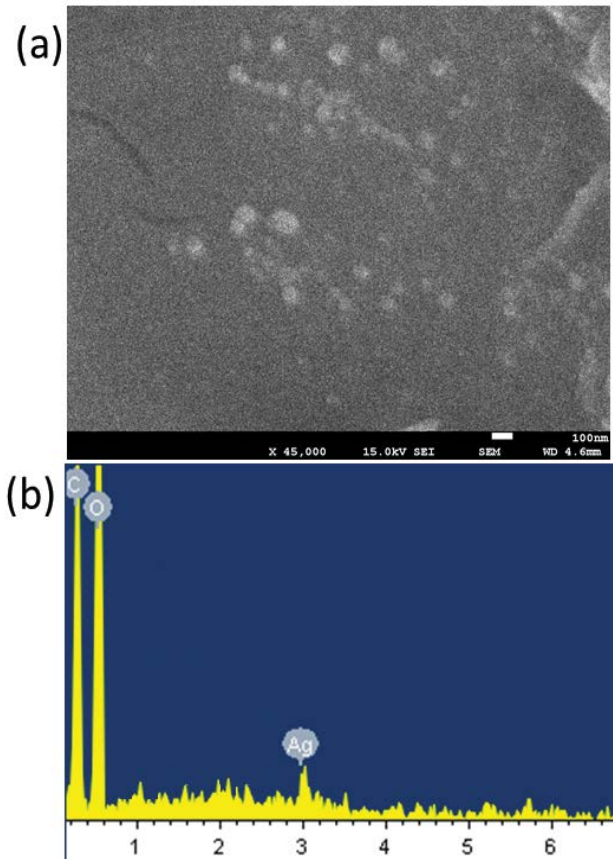

Fig. 3. FESEM image (a) and EDX spectrum (b) of the synthesized AgNPs using *Prosopis juliflora* extract.

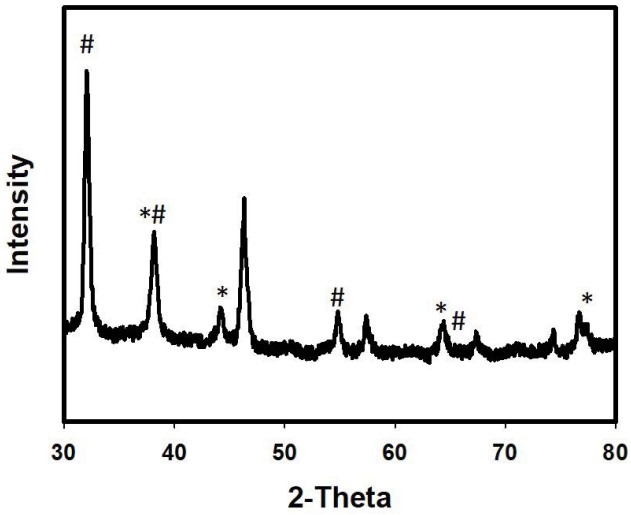

Fig. 4. XRD pattern of the AgNPs obtained through $AgNO_3$ treatment with *Prosopis juliflora* extract. The # and * represent reflections of Ag_2O and Ag, respectively.

shifted to new positions in the FTIR spectrum of extract-assisted synthesized AgNPs, suggesting that the alcoholic and phenolic functional groups bearing compounds were responsible for the synthesis of the nanoparticles.

Table 1
Peak positions in the FTIR spectra

Sample	Peak position	Functional group
Prosopis juliflora extract	3,410	–OH (alcoholic compounds)
	2,936	Ph–OH (carboxylic group stretching of phenolic compounds)
AgNP	3,468	–OH (alcoholic compounds)
	2,730	Ph–OH (carboxylic group stretching of phenolic compounds)

3.4. 2-nitrophenol and methyl orange degradation

Fig. 5a shows the UV-visible spectra of the 2-NPh reduction reaction. For the reduction of the 2-NPh, sodium borohydride as a reductant was used. It is a strong reducing agent, however, incapable of completing the reduction of the 2-NPh in short time as mentioned in earlier reports [10,52,56,57]. In the presence of suitable catalyst, the 2-NPh could be effectively reduced by sodium borohydride [58]. It is evidenced from Fig. 4 that the main peak of the 2-NPh at 413 nm started to decrease with the addition of *Prosopis juliflora* extract-assisted synthesized AgNPs. The decrease in the intensity of the 413 nm peak was due to the conversion of the 2-NPh to the 2-APh [37]. The spectra indicate that the reaction was completed in 17 min as the peak intensity at 413 nm vanished completely. We also tested the *Prosopis juliflora* extract-assisted synthesized AgNPs in the degradation of the MO dye. Fig. 5b shows the UV-visible spectra of the MO reduction reaction in an aqueous medium. Similar to the 2-NPh, the sodium borohydride alone was unable to reduce the MO dye molecules [59]. Upon incorporation of the *Prosopis juliflora* extract-assisted synthesized AgNPs to the MO dye solution containing sodium borohydride, the color of the solution started to disappear with time. As evidenced by the UV-visible spectra, the peak located at the 464 nm disappeared after 26 min of time. Such changes in the UV-visible spectra of the MO indicated that AgNPs successfully catalyzed the reduction reaction of the MO in its aqueous solution. The data from Figs. 5a and b were treated using the pseudo-first order reaction kinetics. A plot between the $\ln(C/C_0)$ and time (t) for these two reactions is shown in Fig. 5c. The linear fitting of the data suggested that the reaction rate constants for the reduction of the 2-NPh and MO were 0.352 and 0.134 min^{-1}.

3.5. Biological studies

Fig. 6 shows the results of the antibacterial and fungicidal effects of the *Prosopis juliflora* extract-assisted synthesized AgNPs. The petri dishes in the first row of Fig. 6 shows the micro-organisms (from left to right: C1: *Candida albicans*, C2: *Candida tropicalis* and C3: Methicillin-resistant *Staphylococcus aureus* [MRSA]) grown in the form of colonies. *Candida albicans*, *Candida tropicalis* are the two infectious disease causing fungi while Methicillin-resistant *Staphylococcus aureus* is an antibiotic-resistant strain of the bacteria. The control samples in the first row of Fig. 6 shows the colonies of the micro-organisms on nutrient plate agar. The second row images in Fig. 6 show the AgNPs treated micro-organisms (from left to right: T1: *Candida albicans*, T2: *Candida tropicalis*, and T3: MRSA). It is clear from the second row images

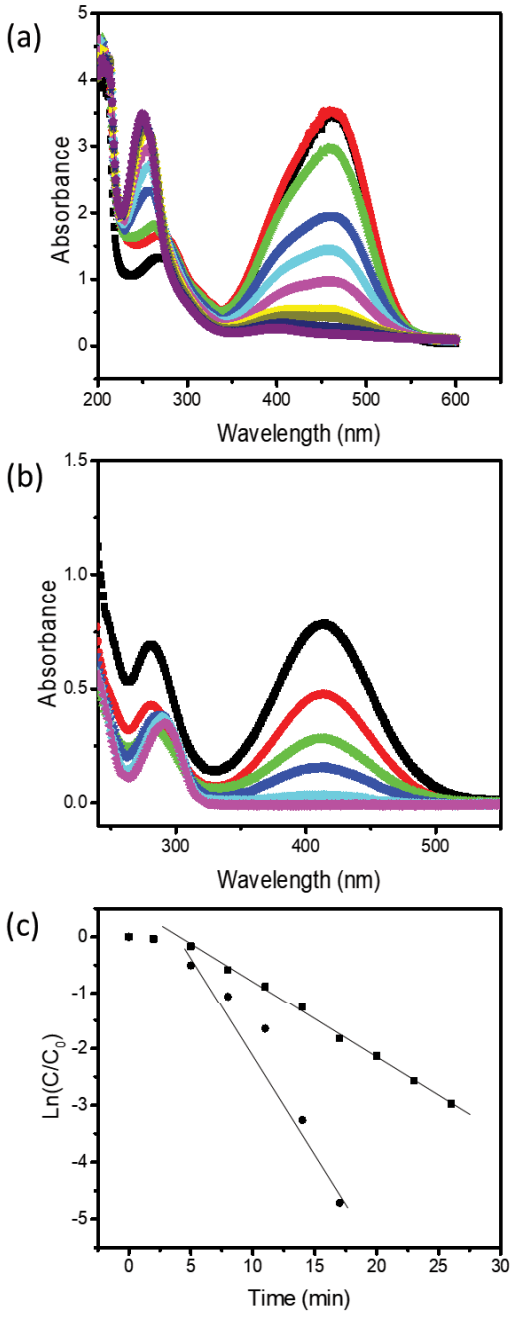

Fig. 5. UV-visible spectral evidence of 2-NPh (a) and MO (b) reduction reaction by $NaBH_4$ catalyzed by *Prosopis juliflora* extract-assisted synthesized AgNPs. Kinetic plot of $Ln(C/C_0)$ vs. time for the two reactions.

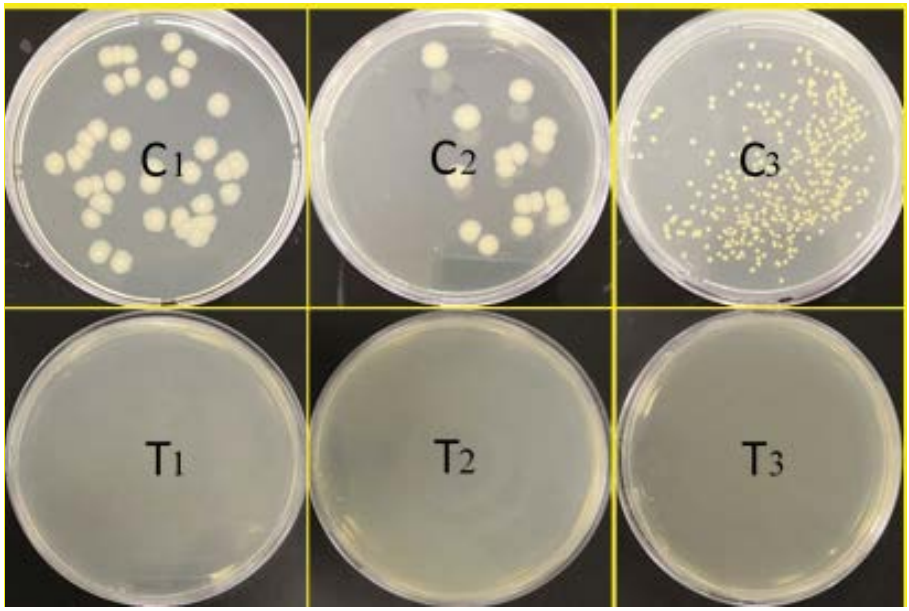

Fig. 6. Growth of *Candida albicans*, *Candida tropicalis* and Methicillin-resistant *Staphylococcus aureus* on nutrient plate agar showing inhibition in the presence of biosynthesis silver nanoparticles. T stands for treated and C stands for control.

of the nutrient plates agar that the *Prosopis juliflora* extract-assisted synthesized AgNPs at a concentration of 1 µg/mL produced very high percentage inhibition against infectious fungi and bacteria as the micro-organisms were unable to form colonies. Even though, when tested the lower concentrations of the *Prosopis juliflora* extract-assisted synthesized AgNPs, an inhibition in the fungal and bacterial growth was observed, but not as effectively as its 1 µg/mL concentration. Our results suggested that these green synthesized AgNPs could be used as antimicrobial agents for cleaning the environment as well as for medical purposes. Our results are in-line with the previous report which showed that similar or lower concentrations of the AgNPs could play the antifungal and antibacterial role. They proved that the AgNPs exhibit no cytotoxic effects on human fibroblasts at such concentrations [60].

There are several explanations for the AgNPs mode of action. Metal depletion is one of the AgNPs mode of actions that forms irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by progressive release of lipopolysaccharide molecules and membrane proteins [60,61]. Although their inference involved some sort of binding mechanism that involves interaction between AgNPs and component(s) of the outer membrane is still unclear. Ag-generated free radicals were reported by using the electron spin resonance of Ag nanoparticles [62]. Antimicrobial mechanism of AgNP is related to the formation of free radicals followed by free radical-induced membrane damage.

3.6. MTT assay results

Cell toxicity assay was done in order to check silver nanoparticles effect against MCF-7 cell line. Results showed that silver nanoparticles have more cytotoxic effect compared with positive control as shown in Fig. 7. Several studies

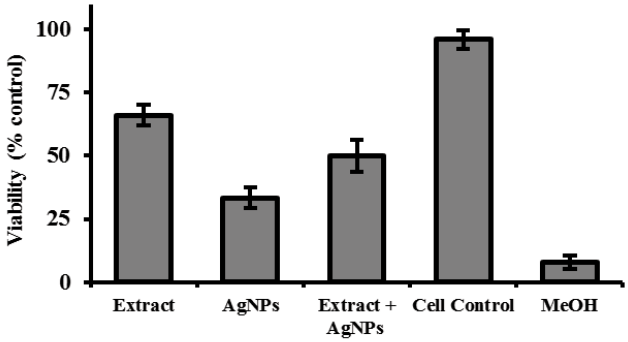

Fig. 7. Cell viability assay of MCF-7 cell lines against silver nanoparticles along with *Prosopis juliflora* extract (positive control), methanol (negative control).

showed that silver nanoparticles have a cytotoxic effect on different human cancer cell lines [2,63–66]. Our study confirms that silver nanoparticles have the ability to kill cancer cells. Some studies show that toxicity of silver nanoparticles have directly relation to their concentration [65]. The more the concentration of silver nanoparticles, the more it has cytotoxicity to the human cell lines. In this study, we used the Ag nanoparticles with *Prosopis juliflora* extract (positive control), methanol (negative control).

4. Conclusions

We showed that the *Prosopis juliflora* extract could be effectively used for the synthesis of AgNPs. The FESEM analysis showed irregular sized AgNPs. EDX analysis confirmed that the sample was composed of organic carbon and inorganic Ag metal. The crystalline nature of metallic Ag and Ag_2O were observed from the synthesized AgNPs. The prepared AgNPs successfully catalyzed the reduction

reactions of the 2-NPh and MO by using a reducing agent of sodium borohydride with reaction rate constants of 0.352 and 0.134 min^{-1}. Moreover, the AgNPs were also tested in the antifungal and antibacterial studies. Results revealed that the prepared AgNPs through the plant extract (green route) could be potentially used against the infection causing fungi strains of *Candida albicans*, *Candida tropicalis*, and bacterial strain of methicillin-resistant *Staphylococcus aureus*.

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