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# NOVEL *BALANITES AEGYPTIACA* MESOCARP SYNTHESIZED SILVER NANOPARTICLES: FORMATION, CHARACTERIZATION, ANTIMICROBIAL, CYTOTOXICITY AND ANTIVIRAL EFFECTS

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A novel, simple, cost effective, non-toxic and environmental friendly technique was developed for the synthesized silver nanoparticles using *Balauites aegyptiaca* (L.) Del mesocarp aqueous extract at 80 °C. UV-visible spectroscopic analysis was caried out to assess the fromation of the silver nanoparticles. The green synthesized nanoparticles were characterized using Zetasizer, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) equipped with Energy Dispersive Spectroscopy (EDS) in addition to Transmission Electron Microscopy (TEM). The antimicrobial, cytotoxic and antiviral actvities of the *Balauites aegyptiaca* nanoparticles have been investigated. This revealed that the nanoparticle suspension posessess cytotoxic activity against HepG-2 and MCF-7 cell lines, with $IC_{50}$ being 19.1 µg and 40.1 µg, respectively, in addidtion to moderate antiviral activity against HAV-10 virus.

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*Keywords*: Balauites aegyptiaca mesocarp aqueous extract, Silver nanoparticles, Characterization, Antiviral effect, Antimicrobial effect, Cytotoxicity effect.

## 1. Introduction

The use of medicinal plants as antibacterial, antiviral and anti-cancer therapeutic agents had significant progress in the past few decades. However, the emergence of resistant strains for the antimicrobial and antiviral agents and the non-specific effect of the anticancer drugs, in addition to their severe side effects, limited absorption and poor bioavailability reduce the clinical efficacy of currently used therapies. Thus, the need to discover and to develop new, alternative, or synergistic drugs from natural or synthetic origin remains urgent.

Recently, nanotechnology had an enormous impact on medical technology, significantly improving the activity, specificity, bioavailability and therapeutic index of various natural products [1]. Therefore, by using nanoscale carriers, the therapeutic value of natural products can be

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drastically improved [2]. Lately, plant extracts are being used as a process for the synthesis of silver nanoparticles that may find very important place in drug bioavailability [3].

*Balanites aegyptiaca* (L.) Delile (Balantiaceae, Zygophyllaceae), known as desert date, is a spiny shrub or tree that has many folk uses in various African countries [4]. It is widely used, in traditional medicine, to treat infectious diseases, psychoses, epilepsy, jaundice and rheumatism [5]. In addition, it is an important component of many popular preparations due to its antidiabetic, antiseptic, antimalarial, antisyphilitic and antiviral *(Herpes zoster)* activity [6]. The fruits are commonly used as a purgative, to remove intestinal parasites and sometimes to treat *Schistosomum japonicum* [7], while the aqueous extract of *B. aegyptiaca* demonstrated remarkable cytotoxic activity against stomach cancer cell SGC790 [8]. The spirostane steroidal saponins (SAP-1016 and SAP-884), isolated from *B. aegyptiaca*, had significant anti-proliferative activity that was comparable to the well-known anticancer agent, cisplatin, against both MCF-7 human breast cancer cells and HT-29 human colon cancer cells [9]. Another study proved that the mixture of balanitin-6 (28%) and balanitin-7 (72%), saponins from *B. aegyptiaca* kernels significantly increase the survival time of mice bearing murine L1210 leukemia grafts to the same extent reported for vincristine [10]. Lately, the fixed oil from the fruits had shown anticancer activity against lung, liver and brain human carcinoma cell lines [11]. The same study reported remarkable antimicrobial activity for the fixed oil against selected strains of Gram-positive and Gram-negative bacteria as well as antiviral activity against *Herpes simplex* virus [11].

The silver nanoparticles have various and important applications. Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that silver nanoparticles are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects [12]. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents [13]. In small concentrations, silver is safe for human cells, but lethal for microorganisms [14]. Antimicrobial capability of AgNPs allows them to be suitably employed in numerous household products such as textiles, as well as disinfection in water treatment, food storage containers, home appliances and in medical devices [15].

The previously mentioned ethnopharmacological uses and the reported pharmacological activities of *B. aegyptiaca* fruits combined with the technology of nanoparticles could lead to discover new promising entities of natural origin. This fact motivated us to investigate the anticancer, antibacterial and antiviral potential of the synthesized silver nanoparticles from *B. aegyptiaca* mesocarp aqueous extract.

A Novel method for biosynthesis of silver nanoparticls (AgNPs) was very cost effective, eco-friendly by using nontoxic sources as compared to the conventional chemical approaches, as well as simple applying and storage at room temperature and high stability. These facts motivated us to investigate the anticancer, antibacterial and antiviral potential of the synthesized AgNPs from *B. aegyptiaca* mesocarp aqueous extract. To the best of our knowledge, this is the first study describing the preparation of *B. aegyptiaca* silver nanoparticles and their biological effects.

## 2. Experimental

### 2.1. Plant material

*Balanites aegyptiaca* (L.) Delile fruits were collected from Halayeb Triangle, Egypt, in July-August 2012. The identity of the plant was verified by Mrs. Trease Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture and former director of the Orman Botanical Garden, Giza, Egypt. A voucher specimen (B.E.F.1) was deposited in the Herbarium of the Pharmacogonsy Department, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

### 2.2. Preparation of crude plant extract

The epicarps of the fruits were easily removed by fingers. Exactly 38 g of fruits were then exhaustively extracted with dist. $H_2O$ by maceration overnight (3 x 75 ml). The extract was filtered and the combined filtrates were immediately used for preparation of the nanoparticles and for the biological studies.

### 2.3. Synthesis of silver nanoparticles

For green synthesis of silver nanoparticles, the reagent silver nitrate (analytical grade, $AgNO_3$) in distilled water has been used as it is received from Techno Pharmchem, India, without further purification. *B. aegyptiaca* aqueous extract used to synthesize silver nanoparticles was centrifuged for 15 minutes at 5000 rpm at room temperature. This was followed by dropwise addition of 6 ml of the plant extract to the colourless aqueous solution of 0.001 mol/L silver nitrate at 80 °C, while stirring magnetically at 1000 rpm for 10 min. The change in colour was observed during this treatment, indicating the reduction of silver ions into silver particles and the formation of silver nanoparticles. The formation of AgNPs was indicated by the development of its characteristic yellowish-brown colour, due to excitation of surface plasmon resonance band in the UV-visible region.

### 2.4. Characterization of green silver nanoparticles

The synthesized silver nanoparticles (BAS) were characterized using UV-visible spectroscopy analyses with the help of Perkin Elmer UV-visible spectrometer Lambda 25, PerkinElmer, United Kingdom. The size of BAS nanoparticles was analyzed through Zetasizer, Nano series, HT Laser, ZEN3600 from Molvern Instrument, UK, while Thermo scientific, Nicolet 6700, FT-IR spectrophotometer was used for recording the infrared (IR) spectrum.

Transmission electron microscopy (TEM) JEM-1011, JEOL, Japan has been employed to characterize the size, shape and morphologies of formed biogenic synthesized silver nanoparticles. A drop of silver nanoparticles suspension was deposited on carbon coated copper grid and the film on grid was then dried. The TEM was operated and the measurements were performed at accelerating voltage of 100 KV.

Scanning electron microscopy (SEM) has been employed to characterize the shape and morphologies of formed biogenic synthesized silver nanoparticles using JEOL-FE SEM; and Energy Dispersive Spectrometer (EDS) analysis was performed for the confirmation of elemental silver. The samples were dried at room temperature and then analyzed for samples composition of the synthesized nanoparticles. Elemental analysis on single particles was carried out using Oxford Instrument, Incax-act, equipped with Scanning electron microscopy.

### 2.5. Antimicrobial screening

Antimicrobial activity of BAS was determined using the agar well diffusion assay method as described by Holder and Boyce, 1994 [16]. Seven bacterial (two gram positive and five gram negative) and two yeast strains, namely, *Bacillis subtilis* (RCMB 010067), *Staphylococcus aureus* (RCMB 010028), *Escherichia coli* (RCMB 010052), *Klebsiella pneumoniae* (RCMB 000111), *Pseudomonas aeruginosa* (RCMB 010043), *Salmonella typhimurium* (RCMB 010072), *Shegella dysentriae* (RCMB 010098), *Aspergillus fumigatus* (RCMB 02568) and *Geotricum candidum* (RCMB 05097).

The tested organisms were sub-cultured on nutrient agar medium (Oxoid laboratories, UK) for bacteria and Sabouraud dextrose agar (Oxoid laboratories, UK) for fungi. Ampicillin and Gentamicin were used as positive control for gram positive and gram negative bacteria, respectively, while Amphotericin B was used for fungi. The plates were done in triplicates. Bacterial cultures were incubated at 37°C for 24 h, while the fungal cultures were incubated at 25-30°C for 3-7 days. Antimicrobial activity was determined by measuring the zone of inhibition [17].

### 2.6. Evaluation of cytotoxic effect:

#### *2.6.1. Mammalian cell lines:*

HepG2 cells (human cell line of a well differentiated hepatocellular carcinoma isolated from a liver biopsy of a male Caucasian aged 15 years) were obtained from the American Type Culture Collection (ATCC). MCF-7 cells (human breast cancer cell line) were obtained from VACSERA Tissue Culture Unit.

### *2.6.2. Chemicals*

Dimethyl sulfoxide (DMSO) and crystal violet were purchased from Sigma (St. Louis, Mo., USA). DMEM, HEPES buffer solution, L-glutamine and gentamycin were purchased from (Bio Whittaker ® Lonza, Belgium).

### *2.6.3. Staining solution*

Crystal violet stain (1%) is composed of 0.5 % (w/v) crystal violet and 50 % methanol then made up to volume with distilled $H_2O$ and filtered through a Whatmann No. 1 filter paper.

### *2.6.4. Cytotoxicity assay*

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 µg/ml gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5% $CO_2$ and were sub-cultured two times a week. Cell toxicity was monitored by determining the effect of the test samples on cell morphology and cell viability. For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1 x $10^4$ cells per well in 100 µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% $CO_2$ for a period of 48 h. Three wells were used for each concentration of the test sample. Vinblastine was used as positive control for MCF-7 cells, while doxorubicin was used for HepG2 cells. Control cells were incubated without test sample and with or without Dimethyl Sulfoxide (DMSO). The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 24 h at 37°C, various concentrations of sample (50, 25, 12.5, 6.25, 3.125, 1.56 µg) were added, and the incubation was continued for 48 h and viable cells yield was determined by colourimetric method.

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain was removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested sample. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated [18-19].

## 2.7. Evaluation of antiviral activity using cytopathic effect inhibition assay

This assay was selected to show specific inhibition of a biologic function, i.e. cytopathic effect (CPE) in susceptible mammalian cells [20]. Monolayers of 10,000 vero cells, adhered at the bottom of the wells in a 96-well microtiter plate, were incubated for 24 hours at 37°C in a humidified incubator with 5% $CO_2$. The plates were washed with fresh Dulbecco's Modified Eagle's Medium (DMEM) and challenged with $10^4$ Herpes Simplex type II virus doses and simultaneously the cultures were treated with two-fold serial dilutions of tested compound in fresh maintenance medium and incubated at 37°C for two days. An infection control, as well as untreated vero cells control, was made in absence of the tested samples. Every 24 hours the observation under the inverted microscope was made until the virus in the control wells showed complete viral-induced cytopathic effect (CPE). Antiviral activity was determined by the inhibition of cytopathic effect compared to control i.e., the protection offered by the tested sample to the cells was scored [19].

The monolayers were fixed with formalin then stained with a 0.1% crystal violet solution and digital photos were taken using Olympus inverted microscope Model CKX41. Three independent experiments were assessed each containing four replicates per treatment. Acyclovir, which is clinically used for the treatment of herpetic viral disease, was used as appositive control under this assay system [21].

## 3. Results and discussion

### 3.1. Visual observation and UV-visible spectroscopy

The green synthesis of silver nanoparticles by *Balanites aegyptiaca* mesocarp aqueous extract has been achieved. Initial confirmation for synthesis of silver nanoparticles was confirmed by the formation of a yellowish-brown colour. Silver nanoparticles absorb radiation in the visible region of the electromagnetic spectrum (380–450 nm) due to the excitation of surface plasmon vibrations, and this is responsible for the yellowish-brown colour of silver nanoparticles in various media [22-24]. A photoraph of *Balanites aegyptiaca* aqueous extract, $AgNO_3$ solution and BAS, respectively is shown in Fig 1.

The measured UV-Vis spectrum confirmed the green synthesis of the silver nanoparticles by monitoring the reduction of pure $Ag^+$ ions into $Ag^0$ using UV-vis spectrophotometer. The combined vibration of electrons of metal nanoparticles in resonance with light wave caused free electrons for metal nanoparticles, which gives surface plasmon resonance (SPR) absorption band observed at 424.05 nm as shown in Fig. 2. Expansion of the peak indicated that the particles are poly-dispersed.

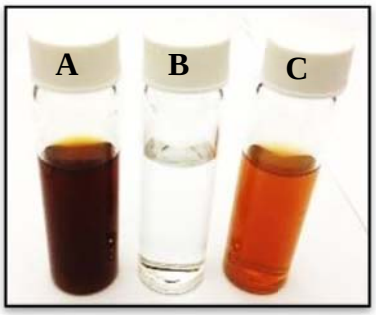


*Fig. 1: A: Balanites aegyptiaca aqueous extract; B: $AgNO_3$ solution; C: Balanites aegyptiaca nanoparticle suspension*

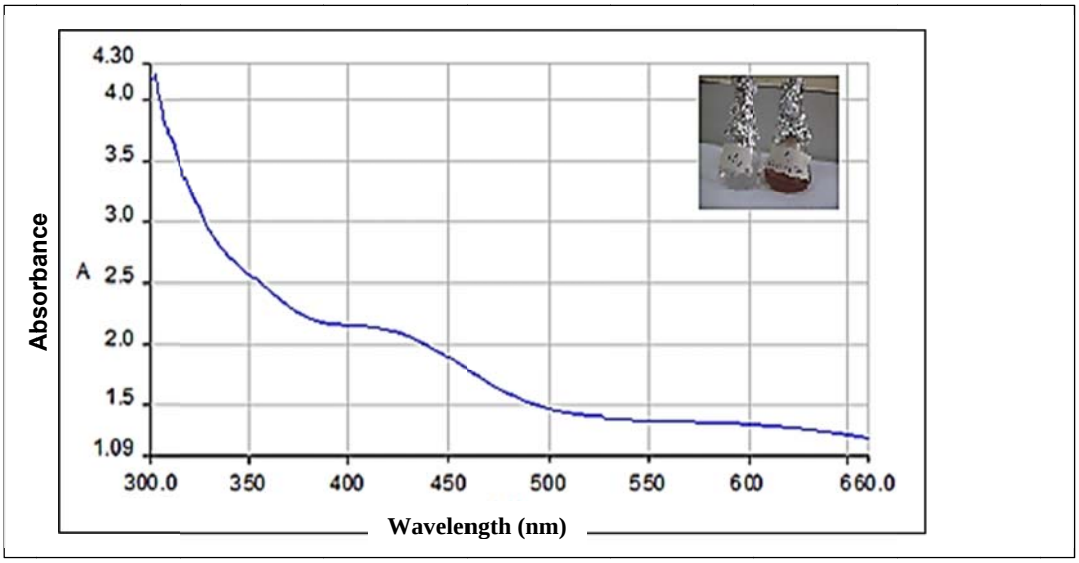


*Fig. 2: UV-vis absorption spectrum of silver nanoparticles synthesized by Balanites aegyptiaca mesocarp aqueous extract*

### 3.2. Particle size determination using Zetasizer

The average size of the formed silver nanoparticles was determined using Zetasizer. It was about 97 nm with mono-dispersity which can be observed clearly from the peak which indicates that the particles are fairly stable.

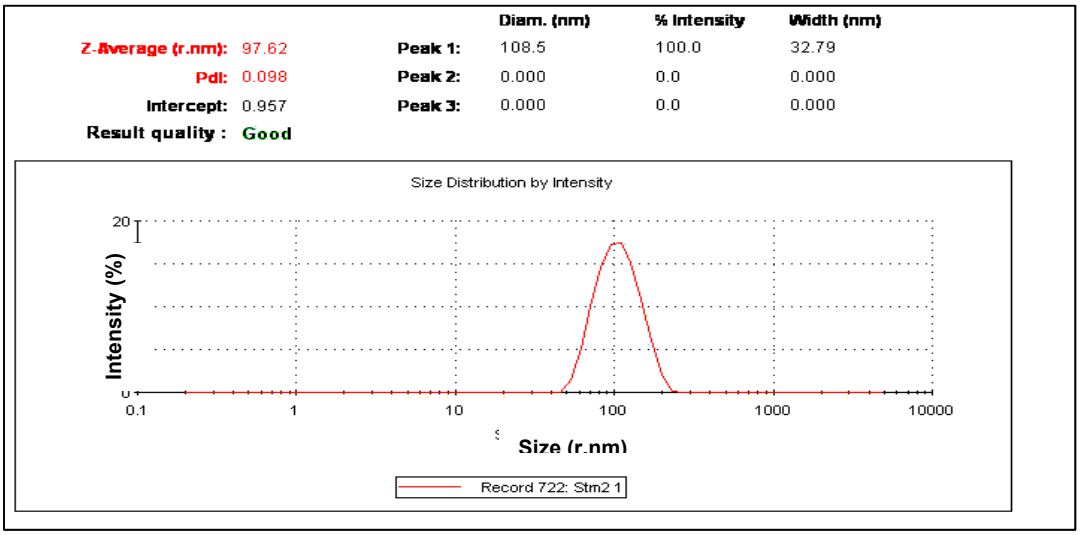


*Fig. 3: Zetasizer measurement of Balanites aegyptiaca silver nanoparticles size representing the average size.*

### 3.3. FTIR spectroscopy

The FTIR spectrum was used to identiy the biomolecules possibly responsible for the reduction of the $Ag^{+}$ ions and capping of the bio-reduced BAS. The Fig. 4 represents a comparison of the FTIR spectra of *Balanites aegyptiaca* mesocarp aqueous extract (A) and the prepared silver nanoparticles (B).

Fig. 4A represents the FT-IR spectrum of *Balanites aegyptiaca* mesocarp aqueous extract showing absorption peaks at 3254.58 $cm^{-1}$ corresponding to OH group and the peak at 1631.91 $cm^{-1}$ corresponding to C=O or/and C-C or/and N-H groups, whereas in Fig. 4B the FT-IR bands of green silver nanoparticles shows the peaks at 3266.90 $cm^{-1}$, 1633.19 $cm^{-1}$ and 1735.59 $cm^{-1}$ characteristic peaks for OH, C=O or/and C-C or/and N-H and C-H groups, respectively.

From the comparison between the FT-IR spectra in Fig. 4A and Fig. 4B, there is a shift in the peak position of Fig. 4B, which clearly indicates the presence of residual plant extract compounds as the reducing and stabilizing (capping) agent to the Ag NPs, i.e. the spectrum shows that the Ag NPs' banding with some groups in *Balanites aegyptiaca* compounds may cap the silver nanoparticles and form a layer on the surface of them which could result in reducing and stabilizing of the nanoparticles. FT-IR study indicates that the carboxyl (−C=O), hydroxyl (−OH), and amine (−NH) groups in *Balanites aegyptiaca* extract are mainly involved in reduction of silver ions to silver nanoparticles, which also confirmed that the protein present in *Balanites aegyptiaca* acts as a reducing agent and stabilizer for the silver nanoparticles, which prevents the agglomeration.

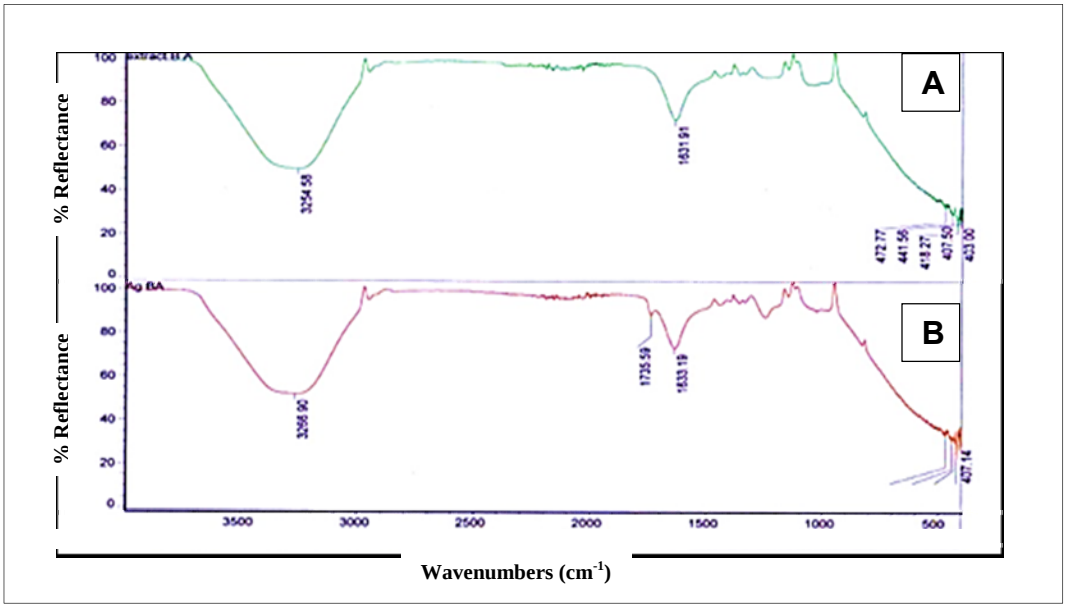


*Fig. 4: FTIR spectra of (A): Balanites aegyptiaca aqueous mesocarp extract; (B): BAS nanoparticle suspension prepared from the extract.*

### 3.4. TEM analysis of silver nanoparticles

TEM images of the green silver nanoparticles synthesized by *Balanites aegyptiaca* extract are shown in Fig. 5 (A-D). These observations indicate that the morphology of nanoparticles is highly variable with a variety of shapes being spherical (Fig. 5A), rodlike (Fig. 5B), triangular (Fig. 5C), cone-shaped (Fig. 5D) or irregular. These findings are in agreement with the UV-vis spectrophotometric measurements, which in Fig. 2 show a broad surface plasmon resonance band that is due to aggregation and adsorption of compounds in *Balanites aegyptiaca* extract onto the surface of Ag nanoparticles. Our results and findings are in agreement with those of Mallikarjuna et al., 2011 [25], who reported on the green synthesis of silver nanoparticles using *Ocimum* leaf extract and their characterization. They reported that the silver nanoparticles are surrounded by a faint thin layer of other materials, which they suppose are capping organic material from *Ocimum* leaf broth in addition to few agglomerated particles.

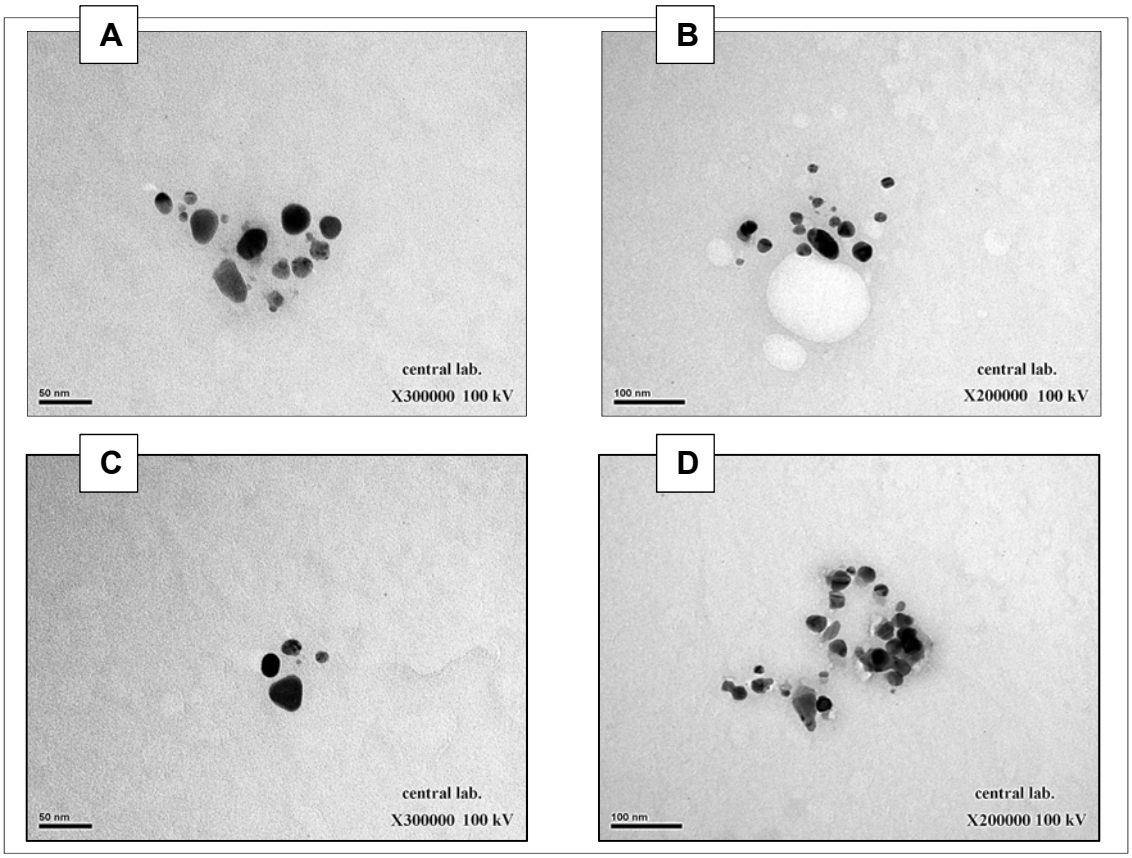


*Fig. 5 (A-D): TEM images of the BAS nanoparticles.*

### 3.5. SEM analysis of silver nanoparticles

Scanning electron microscopy technique has provided further insight into the morphology details of the silver nanoparticles synthesized by *Balanites aegyptiaca* extract. **Fig. 6** shows the SEM micrographs of the biogenic synthesized silver nanoparticles. It detected that silver nanoparticles possess spherical and irregular forms dispersed with fair aggregations.

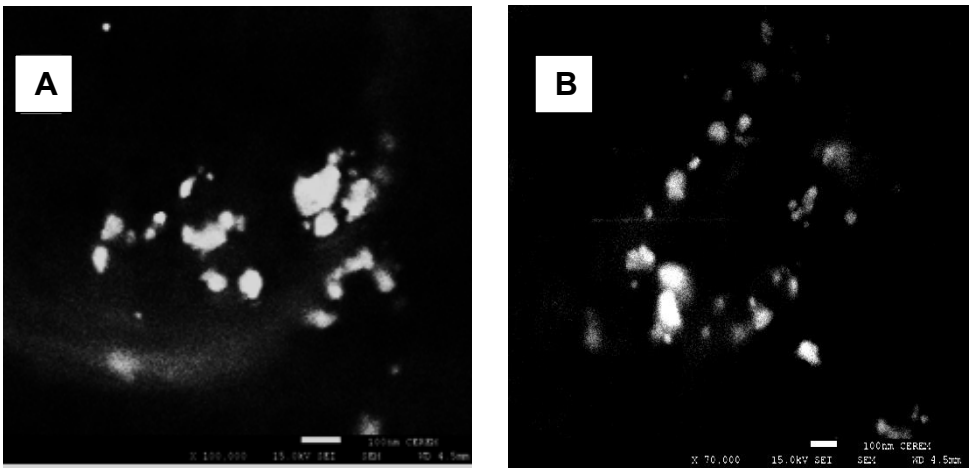


*Fig. 6 (A-B): SEM images of spherical and irregular BAS nanoparticles.*

### 3.6. EDS analysis of silver nanoparticles

Energy-dispersive spectroscopic (EDS) analysis was used to verify the presence of silver in the BAS nanoparticles suspension. Figure 7 shows peaks confirming that the silver does exist in the suspension. Signal of the Ag atoms in the nanoparticles are observed at 3 keV confirming the reduction of silver ions to silver nanoparticles, whereas signals of carbon and oxgen atoms are seen in the range of 0.0-0.5 keV. This indicates the presence of the plant extract (as a capping agent) on the surfaces of the AgNPs. These results are in accordance with the results of FTIR analysis presented in Fig. 4. Resuts of EDS analysis are shown in Fig. 7, wheras Table 1 presents the percentage of the elements present in the suspension.

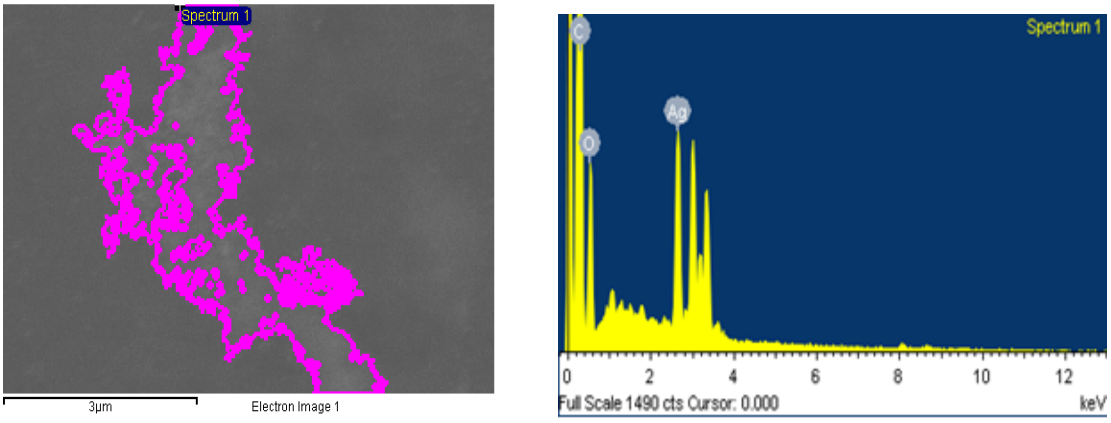


*Fig.7: EDS pattern of Balanites aegyptiaca nanoparticles with four dominant peaks for carbon, oxygen and Ag atoms, respectively.*

*Table 1: EDS results showing percentage of elements present in Balanites aegyptiaca nanoparticle suspension*

| Element | Weight% | Atomic% |
|---|---|---|
| C K | 73.18 | 84.93 |
| O K | 15.64 | 13.62 |
| Ag L | 11.18 | 1.44 |
| Totals | 100.00 | |

### 3.7. Antimicrobial activity study

BAS nanoparticle suspension was tested against different gram positive and gram negative bacteria, in addition to fungi. It showed moderate activities against most of the tested microorganisms, with the highest being on the gram positive bacteria *Bacillis subtilis*. This is followed by the gram negative bacteria, *Escherichia coli*, *Klebsiella pneumoniae*, *Shegella dysentriae* and *Salmonella typhimurium*, respectively. In addition, it exhibited moderate antifungal activity against *Aspergillus fumigates*. BAS nanoparticle suspension was inactive against the gram positive bacteria *Staphylococcus aureus* and the gram negative bacteria *Pseudomonas aeruginosa* as well as the fungus *Geotricum candidum*. The antimicrobial activity of this extact is less than that of standard as reflected by lower size of the zone of inhibition relative to the standard,

therefore no minimum inhibitory concentration was determined. Results of antimicrobial studies are compiled in Table 2 and presented in Fig. 8 (A-C).

*Table 2: Antimicrobial activity of BAS nanoparticle suspension using diffusion agar well diffusion assay method.*

| Tested microorganisms | BAS | Positive control | | |
|---|---|---|---|---|
| | M ± S.D.[a] | Amphotericin B | *Ampicillin* | *Gentamicin* |
| *Bacillis subtilis* (RCMB 010067) | 15.1 ± 0.25 | NT | 32.4± 0.10 | NT |
| *Staphylococcus aureus* (RCMB 010028) | NA | NT | 27.4± 0.18 | NT |
| *Escherichia coli* (RCMB 010052) | 14.2± 0.63 | NT | NT | 22.3± 0.18 |
| *Klebsiella pneumoniae* (RCMB 000111) | 14.0± 0.37 | NT | NT | 20.2± 0.25 |
| *Pseudomonas aeruginosa* (RCMB 010043) | NA | NT | NT | 17.3± 0.15 |
| *Salmonella typhimurium* (RCMB 010072) | 11.2± 0.44 | NT | NT | 25.4± 0.18 |
| *Shegella dysentriae* (RCMB 010098) | 12.3± 0.58 | NT | NT | 17.3± 0.15 |
| *Aspergillus fumigatus* (RCMB 02568) | 11.3± 0.37 | 23.7± 0.10 | NT | NT |
| *Geotricum candidum* (RCMB 05097) | NA | 28.7± 0.22 | NT | NT |

Samples were tested at a concentration of 100 µl; [a]Data are expressed in the form of (M ± S.D.): mean ± standard deviation; Diameter of the inhibition zone (mm) beyond the well diameter of 6 mm; NT: not tested; NA: no activity.

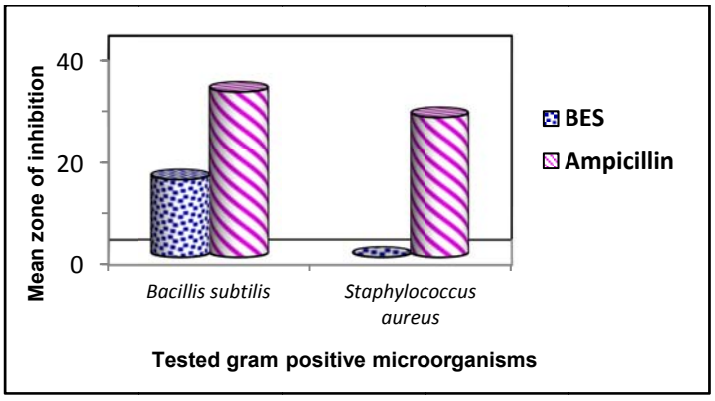


*Fig. 8A: Antimicrobial activity of BAS nanoparticle suspension against gram positive bacteria.*

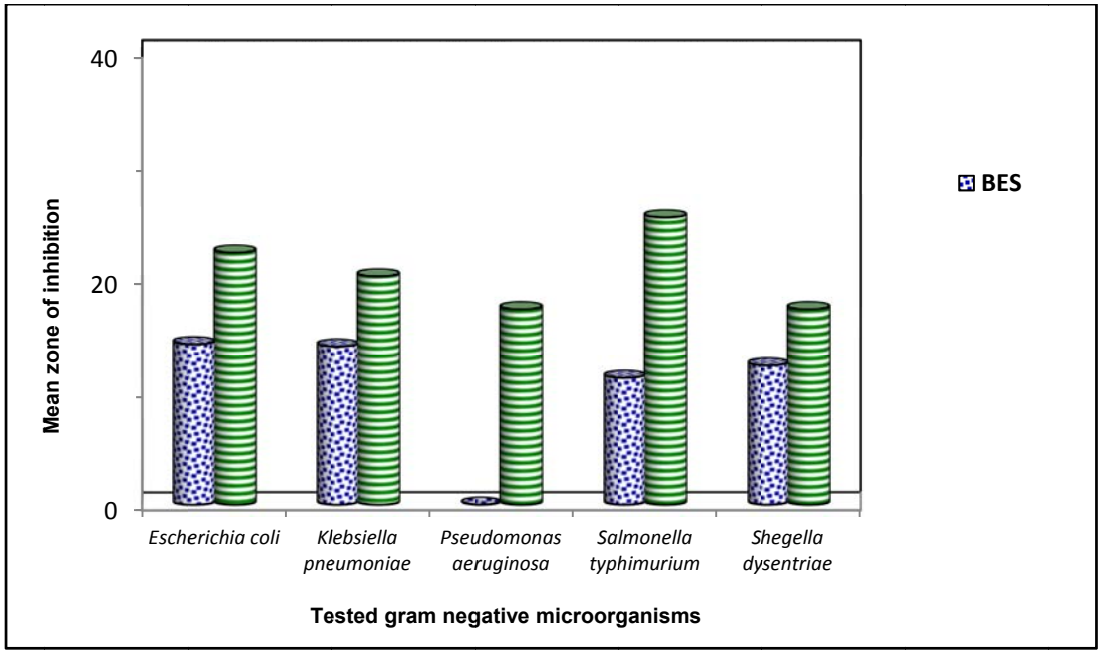


*Fig. 8B: Antimicrobial activity of BAS nanoparticle suspension against gram negative bacteria.*

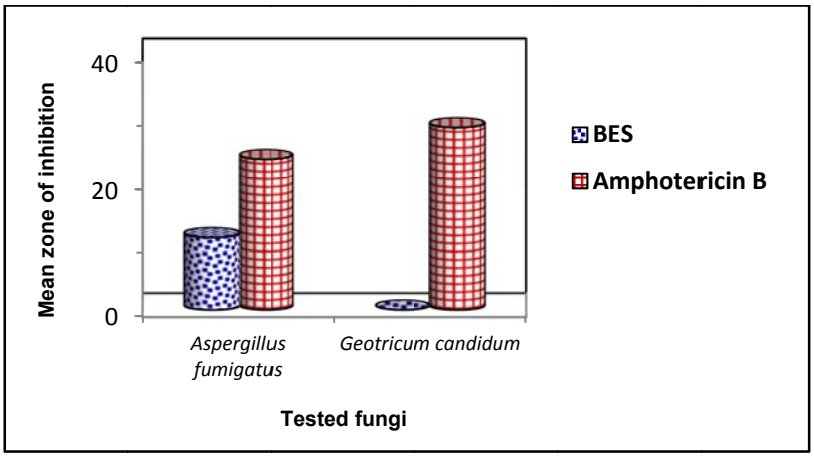


*Fig. 8C: Antimicrobial activity of BAS nanoparticle suspension against different fungi.*

### 3.8. Cytotoxicity study

According to Al Ashaal et al. 2010, the fixed oil of *Balanites aegyptiaca* fruits exhibits anticancer activity against lung, liver and brain human carcinoma cell lines [11]. These reported results stimulated our interest to find out the effect of BAS nanoparticles on other cancer cell lines as breast carcinoma cells (MCF-7) and the hepatocllular carcinoma cells HepG-2. The BAS nanoparticle suspension showed cytotoxic activity against both tested cell lines, with $IC_{50}$ being 19.1 µg and 40.1 µg in case of HepG-2 and MCF-7 cell lines, respectively. The results obtained in this study are in agreement and confirmed the previous investigations reporting the cytotoxic activity of *Balanites aegyptiaca* [11, 26]. Results of cytotoxicity study are presented in Fig. 9 and 10.

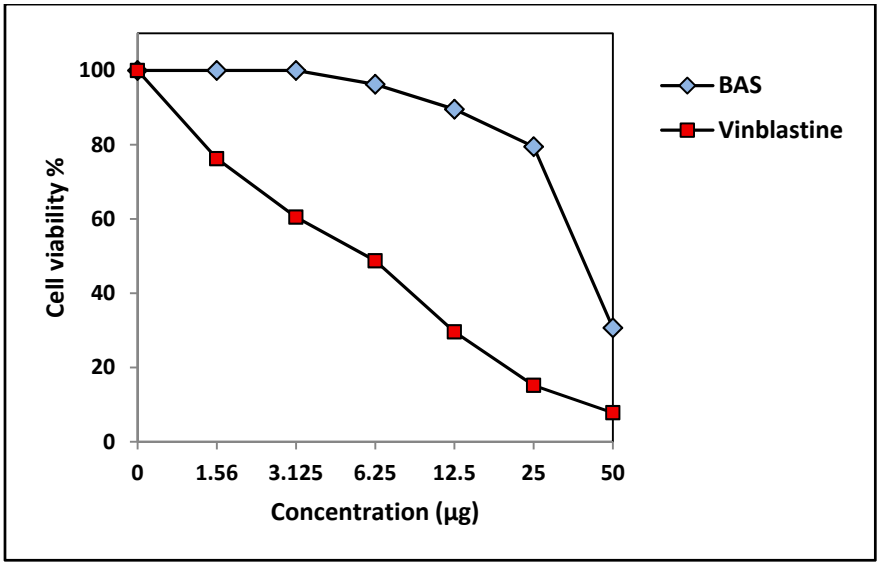


*Fig. 9: Cytotoxic activity of BAS nanoparticle suspension and vinblastine against MCF-7 cell line.*

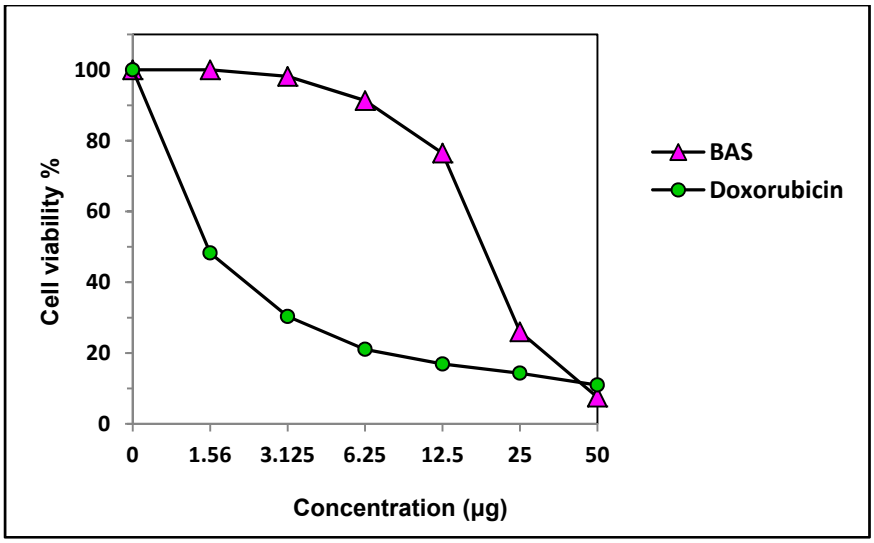


*Fig. 10: Cytotoxic activity of BAS nanoparticle suspension and doxorubicin against HepG-2 cell line.*

### 3.9. Evaluation of antiviral activity

Preliminary screening showed that *Balanites aegyptiaca* oil had antiviral activity [11]. Therefore, BAS nanoparticle suspension was tested against two viruses, namely the Hepatitis A virus (HAV-10) and the *Herpes simplex* virus (HSV-1). The results showed moderate antiviral activity against HAV-10 virus, wheras it was inactive against HSV-1 virus.

## 4. Conclusion

In conclusion, our study describes the first synthesis of silver nanoparticles using *Balauites aegyptiaca* (L.) Del mesocarp extract. The method is rapid, non-toxic, environmental friendly and of low cost. The herin reported results suggest that *Balauites aegyptiaca* mesocarp extract plays an important role in the reduction and stabilization of silver without the need of external stabilizing/capping agents. The formation of AgNPs was determined by UV-vis spectroscopy, where surface plasmon absorption maxima can be observed at about 424 nm. In addition, by the use of Zetasizer an average size of silver nanoparticles was defined as 97 nm. The green silver nanoparticles were characterized using FTIR spectroscopic, TEM, SEM and EDS

techniques. The synthesized AgNPs showed moderate activities against most of the tested gram positive and gram negative bacteria as well as fungi. Furthermore, the nanoparticle suspension showed cytotoxic activity against both tested cell lines, with $IC_{50}$ being 19.1 µg and 40.1 µg in case of HepG-2 and MCF-7 cell lines, respectively in addidtion to moderate antiviral activity against HAV-10 virus. All these findings suggest that the nanoparticles synthesized from *Balanites aegyptiaca* may be a promising candidate for a wide variety of applications in pharmaceutical and biomedical fields as well as in nanomedicine.

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