Green synthesis of silver, gold and silver-gold nanoparticles: Characterization, antimicrobial activity and cytotoxicity

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Abstract:
The present study reported a facile and rapid biosynthesis method for gold nanoparticles (GNPs) silver nanoparticles (AgNPs) and bimetallic heterogeneous sliver-aurum nanoparticles(AgAuNPs) using the leaves of Gmelinaarborea (ROXB) (Family Verbenaceae) extract. The aqueous leaves extract was used as biotic reducing and stabilizing agent of the growing nanoparticles. The synthesized gold nanoparticles (AuNPs),silver nanoparticles (AgNPs) and silver-gold core-shell nanoparticles(AgAuNPs) were characterized using UV-Vis spectroscopy (UV–Vis), Fourier transform infrared spectroscopy, (FT-IR), X-ray diffraction (XRD),transmission electron microscopy (TEM)and thermal gravimetric analyses (TGA). Several factors such as the extract amount, contact time and solution pH, as possible influences; were investigated to obtain the optimized synthesis conditions. The antimicrobial activity study revealed that while the aqueous extract at concentrations of0.8 and 4% (w/v) showed no effect on the antimicrobial activity, the produced nanoparticles, AuNPs, AgNPs and AgAuNPs inhibited thegrowth of Gram positive bacteria (Bacillus subtillus, Staphylococcus aureus), Gram negative bacteria (E. Coli and Pseudomonas aeruginosa) and Fungi (Candida albicans and Aspergillusniger). The cytotoxic activity against hepatocellular carcinoma (HePG2)was alsoevaluated.

Keywords:
Biological synthesis; Gmelinaarborea; gold nanoparticles; silver nanoparticles;silver-gold core-shell; nanoparticles, antibacterial activity; hepatocellular carcinoma

1. Introduction
Goldand silver nanoparticles are of great importance due to surface area and a high fraction of surface atoms. The scientific and technological implication of metal nanoparticles has made them the subject of intensive research. Both Ag and Au nanoparticles have beenstudied for biomedical applications of drug delivery,biomolecular recognition and molecular imaging (Sperlinget al., 2008 ; Wilson,2008).

Gold nanoparticles have been considered as an important area of research due to their unique and tunable surface plasmon resonance (SPR) and their applications in biomedical science including
tissue/tumor imaging, photothermal therapy and immuno-chromatographic identification of pathogens in clinical specimens (Huang, 2006). Gold nanoparticles offered advantages of stability, low toxicity, facile tunability of AuNP size, and the possibility of functionalization of its surface (Lukianova-Hlebe et al., 2012), therefore AuNPs can be successfully used in clinical diagnosis (Qin L. et al., 2017) cancer cell photothermolysis (Cho JH et al., 2017), bioimaging (Cho JH et al., 2017; Murphy C. Jet al., 2008) immunoassay (Cederquist K. B. et al., 2017) and antimicrobial activity (Pradeepa K. et al., 2017). Since the development of the concept of green nanoparticle (Siddiqi K. S and Husen A, 2017), there is a growing the need for environmentally benign metal–nanoparticle synthesis processes that do not use toxic chemicals in the synthesis protocols to avoid adverse effects in medical applications. Biological synthesis especially using plant extract are available for the synthesis of AuNPs and involve reduction of gold cations (Au⁺ or Au³⁺) to zerovalent (Au⁰) with a reducing agent (Pradeepa K et al., 2017; Khalil M. M. H et al., 2012).

Silver NPs (AgNPs) are known to be the most effective against bacteria and viruses (S. Galdiero et al., 2011; Lara H. H et al., 2010). In addition, microbes are unlikely to develop resistance against silver, because the metal attacks a broad range of target sites in the organisms (Pal S. et al., 2007). AgNPs target both the respiratory chain and the cell-division machinery, while concomitantly releasing silver ions (Ag⁺) that enhance bactericidal activity and finally leading to cell death (Prabhu S. and Poulose EK, 2012). The antimicrobial activity of AgNPs depends on their size (Lu Z. et al., 2013) and shape (Furno F. et al., 2004). Diverse applications of AgNPs include wound dressings, coating for medical devices and surgical masks, woven fabric microfiltration membranes, and nanogels (Li Y. et al., 2006; Atta AM et al., 2014).

Green synthesis of stable AgNPs with controlled size and shape is a very profitable approach. As a result, some novel methods have recently been developed using microbes such as bacteria and fungi (Das VL et al., 2014; A. Syed et al., 2013) or plant extracts from seeds, leaves, and tubers (Jagtap U. Band Bapat VA, 2013; Ghosh S. et al., 2012) for the synthesis of AgNPs. The use of plants for AgNPs synthesis is preferred over other biological processes because there is noneed to grow microbes, less expensive and it could be suitably scaled up for large-scale NP synthesis. Plant extracts possess many antioxidants, which act as reducing as well as capping agents (Mittal A. K et al., 2013).

Cancer is known to be the second leading cause of death in the world, so scientists attempt to discover harmless cancer therapy. In fact, most of the artificial agents being used currently in cancer therapy are toxic and can produce damage to the normal cells (Kampa M et al., 2000). Therefore, chemotherapy via nontoxic agents could be one solution for decreasing the risky effects of cancer.

Gmelina arborea (ROXB.) family verbenaceae (Kaswala R et al., 2012) having tremendous therapeutic potential that are not fully utilized. The leaves of Gmelina arboreahave some important chemical constituents which are responsible for the medicinal significance of the
herb. The hexane extract of *Gmelinaaborea* leaves exhibited vasorelaxant properties (Sylvie L.W et al., 2012). It contains a large concentration of alkaloids, luteolin as flavonoids as well as a variety of phytochemicals. The methanolic extract contains alkaloids, flavonoids, saponins, steroids, glycosides whereas chloroform extract contains alkaloids, saponins and steroids. So far, there is no report on the synthesis of nanoparticles using *Gmelinaaborea* leaf extracts. In this paper, we report on the synthesis of silver, gold and their bimetallic nanoparticles using hot water *Gmelina* leaf extracts as a simple, low-cost and reproducible method.

2. Experimental

2.1 Materials

HAuCl₄·H₂O 99.9% and silver nitrate AgNO₃ were purchased from Aldrich. Gmelina leaves were collected from Botanical Garden of Orman- Giza (Egypt). A stock solution of HAuCl₄ was prepared by dissolving 1.0 g HAuCl₄·H₂O in 50 ml deionized water in dark bottle. A stock solution of AgNO₃ (1 x 10⁻² M) was prepared by dissolving 0.084g in 50 ml de-ionized water. A 4.0 g of *Gmelina* leaf broth was boiled for 15 min, filtered and completed to 100 ml to get the extract. Deionized water was used throughout the reactions. Hydrochloric acid, sulphuric acid and sodium hydroxide used for pH monitoring were also purchased from Sigma-Aldrich.

2.2. Characterization of the nanoparticles

UV–visible spectra were recorded at room temperature using a Shimadzu 2600 spectrophotometer. X-ray diffraction (XRD) pattern was obtained using a Shimadzu XRD-6000 diffractometer with Cu Ka (λ = 1.54056 Å) to confirm the biosynthesis of nanoparticles. The size and morphology of the nanoparticles were examined and the TEM images were obtained on a JEOL-1200JEM for the TEM measurements. Fourier transform infrared (FTIR) spectra were recorded on a Nicolet 6700 FTIR spectrometer at room temperature. Thermogravimetric analyses were carried out with a heating rate of 10 °C/min using a Shimadzu DT-50 thermal analyzer.

2.3. Preparation of the aqueous extract

4.0g of fresh leaves of Gmelina was washed with tap water, followed by distilled water, boiled for 15 min and the resulting extract was filtered through filter paper and completed to 100 ml with deionized water to get the (4% w/v) extract.

2.4. Synthesis of gold nanoparticles.

For the synthesis of the gold nanoparticles, a certain volume of the *Gmelinaaborea* leaves extract (4% w/v) was added to the 0.05 ml HAuCl₄·H₂O solution at room temperature and the volume was adjusted to 10 ml with deionized water. The final concentration of Au³⁺ was 2.9 x 10⁻⁴ M and the reduction process of Au³⁺ to Au nanoparticles was followed by the change of the color of the solution from yellow to violet to dark pink and green depending on the extract concentration.
the nanoparticles prepared at different pH values, the pH of the solutions was adjusted using 0.1 N HCl and 0.1 N NaOH solutions.

2.5. Synthesis of silver nanoparticles.
For the synthesis of the silver nanoparticles, 0.5 ml of the Gmelina leaf extract was added to the 0.1 ml AgNO₃ solution and the volume was adjusted to 10 ml with de-ionized water. The final concentration of Ag⁺ was 1x10⁻⁴ M and the reduction process Ag⁺ to Ag⁰ nanoparticles was followed by the color change of the solution from yellow to brownish-yellow to deep brown within 24hrs depending on studied parameters such as the extract concentration, time, temperature and pH. The pH of the solutions was adjusted using 0.1N H₂SO₄ and 0.1N NaOH solutions.

2.6 Synthesis of AuAg bimetallic nanoparticles
For the synthesis of gold-silver nanoparticles, a 2.5 ml of plant extract (4g/ 100 ml deionized water) was added to 0.5 ml of AgNO₃ solution 1 x 10⁻² M and then after 24hrs 0.05 ml of HAuCl₄ solution 5.8 x 10⁻² M was added at room temperature and the volume was adjusted to 10 ml. The final concentration of Ag⁺ was (5 x 10⁻⁴ M) and the final concentration of Au³⁺ was 2.9 x 10⁻⁴ M and after 20min reduction process was followed by the color change of the solution from brownish-yellow to violet.

2.6. Cytotoxicity evaluation
In vitro anticancer activity evaluation of the nanoparticles was carried out against human cancer cell lines hepatocellular carcinoma (HePG2) using MTT method (Skehan P. et al., 1996). The relationship between drug concentrations and cell viability was plotted to calculate LC₅₀.
Potential cytotoxicity of the nanoparticles tested using cell line HePG2. Cells were plated in the 96-multiwell plate (10⁴ cells/well) for 24hrs before treatment with the extract or nanoparticles to allow attachment of a cell to the wall of the plate. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and atmosphere of 5% CO₂. After 48h, 0.5% of the water-soluble mitochondrial dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT⁺) was added. Incubation was continued for three more hours, the medium was removed and the water-insoluble blue formazan dye formed stoichiometrically from MTT⁺ was solubilized by acidic SDS. Optical densities were determined by a microplate reader (Tecan Infinite 200 Pro, Austria) at the wavelength of 570 nm. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specified compound.

2.7 Preparation of coated silver, gold and their bimetallic nanoparticles for antimicrobial assay
Two stock plant extracts(4% and20% w/v) were prepared by boiling 4.0g and 20.0g of Gmelina leaves for 15 min, filtered and completed to 100 ml with deionized water. A 2ml of the
extract solution (4% and 20% w/v) was used in the measurements and completed to 10 ml with deionized water. Different extract: Au\(^{3+}\) and/or Ag\(^{+}\) ratios were prepared by dilutions from stock solutions to obtain the samples denoted as group a, b, c, and d with final concentrations of the extract and the metal ions concentrations as follow: (a 1:0.8% extract only), (a 2; 4% extract only), (b 2, 4% extract and 1.45X10\(^{-3}\) M Au\(^{3+}\)),(b 3 ; 4% extract and 2.9X10\(^{-3}\)M Au\(^{3+}\) ) , (c 2; 4% extract and 5X10\(^{-4}\) M Ag\(^{+}\)),(c 3; 4% extract and 1X0\(^{-3}\)M Ag\(^{+}\)),(d 2; 4% extract and 1.45 X10\(^{-3}\)M Au\(^{3+}\) +2.5X10\(^{-3}\)M Ag\(^{+}\) ) and (d 3; 4% extract and 2.9 x10\(^{-3}\)M Au\(^{3+}\) +2.5x10\(^{-3}\)MAg\(^{+}\) )

### 2.8 Antimicrobial activity assay

The antimicrobial activity of the synthesized AuNPs, AgNps, and bimetallic nanoparticles was studied against the growth of Gram Positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923), Gram negative bacteria (*E. coli* ATCC 25922, *Salmonella typhimurium* and *Pseudomonas aeruginosa* ATCC 27858) and Fungi (*Candida albicans* ATCC 10231) in comparison with that of aqueous *Gmelina* leaf extract by using the standard agar well diffusion technique. The bacteria and fungi were maintained on nutrient agar medium and CzapeksDox agar medium, respectively. The agar media were inoculated with different microorganisms. After 24 h. of incubation at 30ºC for bacteria and 48 h. of incubation at 28 ºC for fungi, the diameter of inhibition zone (mm) was measured.

### 3 Results and discussion

#### 3.1 UV–visible spectroscopy and TEM Studies

##### 3.1.1 Effect of concentration of Gmelina leaves extract

The primary variable in the reaction condition was the concentration of the *Gmelina* leaf extract. The formation and stability of gold nanoparticles were followed by Uv-visible spectrophotometry. Figure 1 shows the Uv-visible spectra of gold nanoparticles formation using constant HAuCl\(_4\) concentration 2.9x 10\(^{-4}\)M with different concentrations of extract from 0.2 to 3 ml. The inset shows photos of the color change of gold nanoparticles with changing the Gmelina leaves extract concentration. As is clear from the inset in Figure 1a the color changed from pale yellow to violet to dark pink and green depending on the extract concentration. As shown in Figure 1b, UV–vis spectra showed that in the range of low amounts of the leaf extract (0.2–2 ml in 10 ml solution), the absorption spectra exhibit a gradual increase of the absorbance accompanied with a shift in the \(\lambda_{\text{max}}\) of SPR band absorption peak from 566 to 534 nm. With an increase in the quantity of extract, the full width at half maximum (FWHM) decreases supporting the reduction in particle size. Further addition of higher amounts of the extract, the \(\lambda_{\text{max}}\) was shifted to longer wavelengths and the green color of the AuNPs solution was developed (inset of Fig. 1) with a slight decrease in absorbance (*Khali M.M.H. et al., 2014*). This is most likely due to changes in the dielectric properties of the layer immediately surrounding the gold nanoparticles (*Mulvaney P, 1996*) and to some small amount of particle aggregation.
Figure 1: UV-Vis absorption spectra of (4% w/v) of the Gmelina leaf extract (a) The color change of gold solution formed using different concentrations of plant extract, (b) UV-vis spectra of gold nanoparticles using constant HAuCl$_4$ concentration $2.9 \times 10^{-4}$M with different concentrations of extract from 0.2 to 3 ml.

For silver nanoparticles prepared using Gmelina leaf extract, the SPR band of silver nanoparticles formed with different extract concentrations from 0.2 to 1.5 ml at room temperature after 24 hrs as there was no color developed within the first 2 or 3 hrs at room temperature. The color of the solutions changed from pale yellow to deepbrown depending on the extract concentration. As the concentration of the Gmelina leaf extract increases, the absorption peak gets more sharpness and the blue shift was observed from 458 to 441 nm. The blue shifted and sharp narrow shape SPR band indicating the formation of aspherical and homogeneous distribution of silver nanoparticles was observed (Khalil M.M.H et al., 2014). It should be mentioned that the extract absorption has a maximum at about 400 nm and could contribute to the absorption of AgNPs at high extract concentration.

Figure 2: (a) The color change of silver solution formed using different concentrations of Gmelina extract, (b) UV–vis spectra of silver nanoparticles using constant AgNO$_3$ concentration (1x10$^{-4}$M) with different concentrations of extract from 0.2 to 1.5 ml.
Since the formation of AgNPs needs 24h at least for the reaction to finish, formation of AuAgNPs was carried out by addition of AuCl\textsubscript{4}\textsuperscript{-} solution to the AgNPs using successive reduction process (Mallik K et al., 2001). AgNPs firstly prepared by adding 2.5 ml of plant extract (4%) to 0.5ml of silver nitrate (10\textsuperscript{-2} M), and left for 24hrs at room temperature to ensure formation of AgNPs), Fig 3, followed by addition of a 0.05ml of AuCl\textsubscript{4} (5.8 x 10\textsuperscript{-2} M) to the solution in Ag: Au 1:1 molar ratio. The UV–visible spectrum of the AgAuNPs exhibited a clear peak for the AuNPs while a band corresponding to silver nanoparticles is not observed using this concentration ratio. Similar spectra for the Au/Ag bimetallic solution using Neem leaf broth (Shankar S.S et al., 2004) and Persimmon (Diopyros kaki) leaves (Song J. Yand Kim B.S, 2008) were observed and this can be attributed to the formation of gold nanoparticles layer formed a thin uniform film around the gold nanoparticles, leading to considerable damping of a distinct silver plasmon vibration band at ca. 430 nm and the surface plasmon absorption band showed only one peak which result from the metal of the shell. The damping of silver plasmon upon the formation of Au(Ag) nanoparticles could be also explained with the consideration of the standard reduction potential of AuCl\textsubscript{4}/Au pair (0.99 V, vs SHE) which is higher than that of Ag+/Ag pair (0.80 V, vs SHE), then silver nanoparticles can be oxidized by the addition HAuCl\textsubscript{4} (Sun Y et al., 2002). Successive reduction is usually carried out to prepare “core-shell”-structured bimetallic nanoparticles (Mallik Ket al., 2001; Chen H.M et al., 2006). Previous studies have shown that co-mixed particle solutions contain two absorption maximums and not the presence of a single peak as was observed in our case.

**Figure 3:** UV–Visible spectra of AgAu bimetallic nanoparticles 2.5 ml of plant extract (4%) was added to(0.5 ml)of silver nanoparticles using constant concentration (1 x 10\textsuperscript{-2} M) and then after 24hrs (0.05 ) ml of gold nanoparticles using constant concentration (2.9x 10\textsuperscript{-4} M) was added at room temperature.

The nanoparticles products were further characterized using Transmission Electron Microscopy(TEM). The TEM images confirm the formation of thenanoparticles, the gold
nanoparticles were spherical with an average size of 10 nm, Fig. 4(a), while the AgNps were in the range of 70 nm and exhibited a range of different morphologies with irregular contours, Fig. 4(b). The core-shell silver-gold nanoparticles Au(Ag)NPs, Fig. 4(c) exhibit surface morphologies closed to AgNPswith obvious light portion for most nanoparticles supporting the formation of gold layer on AgNps seeds. It can be seen that the size of AgAuNps are smaller than AgNPsand that can be explained by the oxidation of AgNps upon addition of AuCl₄⁻ before its reduction Au⁰ and form a layer on the AgNps.

Figure 4. TEM images of (a) Au NPs. (b) AgNPs (c) AgAuNPs nanoparticles.

3.1.2 Effect of contact time

The effect of contact time between the Au³⁺ ions and Ag⁺ with the extract was studied at room temperature to follow the rate of reaction and the time necessary to complete the reduction process. Fig 5a shows the formation of AuNPs started within 5 min and increased with time. Absorption band showed no change after 35 minutes indicating the complete reduction of Au³⁺.

The reaction between Ag⁺ and the reducing material in the extract was followed for one week, Fig 5b. It can be seen that the absorption spectra and the color intensity of the solution increased with contact time and decreased slightly after 5 days. This result implies that the silver nanoparticles prepared by this green synthesis methodis very stable without aggregation. It is pertinent to note that in previousstudies the time span required for reduction of silver ions ranged from 24 to 48 h (Chandran S.P et al., 2006)
The rate of the Gmelina leaf extract mediated biosynthesis of AuAgNPs is studied by monitoring the absorption intensity of the SPR for 6 days. As shown in Fig.5c the bioreduction started within 60 min then increased dramatically and after 4 days there was nearly no change in the absorption intensity indicating that the reaction was completed within 4 days.

### 3.1.3. Effect of pH

The pH of the extract used in the biosynthesis of nanoparticles is a critical factor affecting the size, shape and composition of the nanoparticles (Singh M et al., 2009). With the help of UV-visible spectroscopy and TEM analysis, the impact of Gmelina leaf extract solution pH on the biosynthesis of Au, Ag and AgAu nanoparticles was investigated within the range of pH (3-10), Figure 6. It can be seen that the absorbance increases with increasing pH from 3 to 10 with a blue shift in the spectra. Furthermore, the particles formed in the acidic medium were unstable and precipitated within 12 h while the particles prepared at pH 9 were stable for one week.
Figure 6: UV-Vis spectra of (a) AuNPs (b) AgNPs (c) AuAgNPs formed using different pH of Gmelina extract.

The size of the AuNPs was followed by measuring TEM at pH 5 and pH 9, Figure 7. These results were consistent with several studies which reported that at low pH, the gold nanoparticles prefer to aggregate to form larger nanoparticles rather than to nucleate and form new nanoparticles (aggregation of nanoparticles is favored over the nucleation). However, higher pH facilitates the nucleation and subsequent formation of a large number of nanoparticles with a smaller diameter. Increasing the pH increase would change the electrical charges of biomolecules which might affect their capping and stabilizing abilities and subsequently the growth of the nanoparticles (Veerasamy et al., 2011).
It can be seen that absorbance increases with increasing pH to 8. In previous studies, it was shown that the size and shape of biosynthesized nanoparticles could be manipulated by varying the pH of the reaction mixtures. A major influence of the reaction pH is its ability to change the electrical charges of biomolecules which might affect their capping and stabilizing abilities and subsequently the growth of the nanoparticles. This result was confirmed by the TEM measurement carried out at pH 5.5 and pH 9, the size and shape of Au AgNPs were affected by changing the pH of the reaction medium. The size of the AuNps were with the average size 15 nm at pH 5.5 while at pH 9, smaller size in the range of 8 nm were found with different morphologies. The decrease in the nanoparticles size was also observed for AgNPs and AgAuNps, Figure 7.

3.2. X-Ray diffraction study:

X-ray diffraction data provides information about crystallinity, crystallite size, orientation of the crystallites and phase composition and aid in molecular modeling to determine the structure of the material (Joshi R et al., 2008). Advantages of XRD are the simplicity of sample preparation, rapidity of measurement, analyze mixed phases and determine sample purity. Its limitations are a requirement of homogenous and powdered material, peak overlays lead to unclear data. The green-synthesized nanoparticle was clearly analyzed using XRD measurements. Fig. 8 shows the XRD patterns of the formed nanoparticles Au, Ag and AgAuNps, respectively. The XRD patterns of Au, Ag and Ag NPs (Fig. 8) show four peaks of (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) facets of face-centered cubic crystal structure. The broadening of Bragg’s peaks indicates the formation of nanoparticles. The unknown peaks marked with x, Fig 8 b and c, could be due to crystalline bioorganic compounds from the extract.
Figure 8: X-Ray diffraction patterns of (a) AuNPs and (b) AgNPs (c) AuAg prepared with aqueous *Gmelina* leaf extract.

### 3.6 Fourier transform infra-red spectroscopy (FTIR):

FTIR spectrum was used to identify the possible function groups of biomolecules in the Gmelina leaf extract that might be responsible for bioreduction and coating of AuNPs, AgNPs, and AgAuNPs, Fig 9. The spectrum of the FTIR spectra of Gmelina leaf extract showed a broad band at 3408 cm$^{-1}$. This band attributed to the OH groups in the biomolecules. The IR bands at 2920 and 2852 cm$^{-1}$ due to C–H stretching vibration modes in hydrocarbon chains. The IR bands at 1381 and 1732 cm$^{-1}$ were characterized as C–O and C=O stretching modes of the carbonyl functional group. The stronger band at 1642 cm$^{-1}$ was characterized as C=O of the amide groups of protein in plant, 1516 cm$^{-1}$ due to stretching vibrations of –C–C– in aromatic rings. Medium bands at 1069 cm$^{-1}$ due to the C–O–C and C–OH vibrations are observed. These assignments were consistent with the isolated flavonoids as luteolin, kaempferol, isoquercitrin, quercetin-3-O-robinobioside from Gmelina extract using 90% methanol by Ghareeb et al. (Ghareeb M.A et al., 2014). The FTIR of AuNPs showed a shift in the absorbance peaks from 3408.4 to 3412.00 cm$^{-1}$ and from 1732 to 1713 and from 1652.5 to 1623.0 cm$^{-1}$ and 1382.2 to 1419.5 cm$^{-1}$. In the case of Ag NPs, a large shift in the absorbance peak with decreased band intensity was observed from 3408.4 to 3404.7 cm$^{-1}$ and 1382.2 to 1320 cm$^{-1}$. The spectra also illustrate a prominent shift in the wave numbers corresponding to amide (1652.5–1600 cm$^{-1}$), validates that free amino (NH$_2$) groups in compounds of the Gmelina leaf extract have interacted with AgNPs surface.
making AgNPs highly stable. Similar shifts were observed in the IR spectrum of AuAgNPs, the IR spectrum, Fig 9(d) indicating the capping of the nanoparticles with the extract constituents.

![FTIR spectra of (A) Gmelina leaf extract (B) gold nanoparticles (C) silver nanoparticles (D) gold-silver nanoparticles.](image)

Figure 9: FTIR spectra of (A) Gmelina leaf extract (B) gold nanoparticles (C) silver nanoparticles (D) gold-silver nanoparticles.

3.7 Thermal gravimetric analysis (TGA):

The TGA plot of the capped AuNPs, capped AgNPs and AuAgNPs prepared using Gmelina leaf extract Fig. 11(a,b,c) showed a steady weight loss in the temperature range of 40-650 nm depending on the nanoparticles type. The amount of extract molecules present along with AuNPs, AgNPs, and AuAgNPs was calculated by TGA (Fig. 10). The figure indicates that the weight loss takes place in three regions in the case of AuNPs. The first region appears around 200 °C (~10% weight loss) and the second region being around 340 °C (~35% weight loss) and the third region around 500 °C (~30% weight loss) thus corresponding to a total weight loss of 75%. In case of AgNPs, the weight loss also occurs in four regions, the first at 200 °C (~15% weight loss), the second at around 335 °C (~30% weight loss), the third region at around 520 °C (~25% weight loss), and the fourth region around 600 °C giving a total weight loss of 76%. In case of AuAgNPs, the weight loss occurs also in four regions, the first around 200 °C (~14% weight loss), the second at around 335 °C (~26% weight loss), the third region at around 590 °C (~20% weight loss), and the fourth region around 620 °C (~7% weight loss) giving a total weight loss of 67%. These data suggest that the AuAgNPs are more thermally than AuNPs and AgNPs.
Figure 10: TGA of (a) capped AuNPs using Gmelina leaf extract (b) capped AgNPs, (c) AuAgNPs.

3.5 Cytotoxicity Activity

Liver cancer is the third most common cause of death in cancer. The death rate is increasing and 85% people are affected in developing countries, more commonly men (Siegel et al., 2013). Actually, in vitro cytotoxicity assays are widely used to chemicals including cancer chemotherapeutics, pharmaceuticals, biomaterials, natural toxins, antimicrobial agents and industrial chemicals because they are rapid and economical. These cytotoxicity tests measure the concentration of the substance that damages components, structures or cellular biochemical pathways, and they also allow direct extrapolation of quantitative data to similar in vitro situations (Cree (Ed.) I.A, 2011). The in vitro anticancer activity evaluation of the newly synthesized nanoparticles was carried out against human cancer cell lines hepatocellular carcinoma (HePG2) using MTT method (Dwivedi A.D and Gopal K, 2010). Doxorubicin hydrochloride is one of the most effective anticancer agents was used as a reference drug in this study. The relationship between drug concentrations and cell viability was plotted to calculate IC$_{50}$, the value which corresponds to the concentration required for 50% inhibition of cell viability and the data are shown in Table 1. According to the data in Table 1, the aqueous extract and the AuNps prepared using the extract are cytocompatible, while the AgNps and AuAgNps have cytotoxic activity with IC$_{50}$ of 11.4 and 2.98 µg/ml that is considered to be promising cytotoxic compared to the reference Doxorubicin (Singh et al., 2010) evaluated cytotoxic and genotoxic of glycolipid-conjugated silver and gold nanoparticles on HePG2 cells. They found that both nanoparticles are found to be cytocompatible up to 100 mM metal concentrations and the gold nanoparticles are more cytocompatible than the same concentrations of silver nanoparticles. Particle size (Pan Y. et al., 2007), surface modification, type of ligand for modification, charge of the nanoparticles (Goodman C.M et al., 2004) are important parameters that control binding to cytotoxicity. It should be mentioned that the cytotoxic activity using HepG-2 assay for the pure flavonoids
(luteolin, kaempferol, isoquercitrin, quercetin-3-O-robinobioside) isolated from Gmelina arborea Roxb. showed that the tested compounds have cytotoxic activity with IC$_{50}$ ranging from 3.38 to 15.70 μg/ml (Ghareeb M. A et al., 2014).

Table 1. Cytotoxicity (IC50) of aqueous extract of Gmelina and the nanoparticles

<table>
<thead>
<tr>
<th>Item</th>
<th>IC$_{50}$/μg</th>
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<tbody>
<tr>
<td>Gmelina</td>
<td>&gt;50</td>
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<tr>
<td>GmelinaAuNps</td>
<td>&gt;50</td>
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<tr>
<td>GmelinaAgNps</td>
<td>11.4</td>
</tr>
<tr>
<td>GmelinaAuAgNps</td>
<td>2.98</td>
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<tr>
<td>Doxorubicin HCl</td>
<td>1.2</td>
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3.6. Antimicrobial assay:

Two concentrations of Gmelina leaf extract were prepared 4% and 20% as used for all experiments. These stock solutions were used to prepare different solutions for the study of the antimicrobial activity of Gmelina leaf extract and the nanoparticles. First, two concentrations of Gmelina leaf extract (a1; 0.8% w/v a2; 4% w/v) were prepared by dilution and examined in comparison with that of newly formed nanoparticles solutions synthesized using the same Gmelina leaf extract concentrations (b2 & b3). The results of the antimicrobial assay were shown in Fig. 11. Samples of Gmelina leaves extract at concentrations of 4% w/v, respectively have no antimicrobial activity against all tested strains. Also, sample b2 with extract concentrations 4% w/v and Au$^{3+}$ (1.45X10$^{-3}$ M ) showed no antimicrobial activity. However, increasing Au$^{3+}$ concentration in sample b3 (2.9X10$^{-3}$ M HAuCl$_4$), the prepared AuNps showed antimicrobial activity against Bacillus subtilis, E. coli, Pseudomonas aeruginosa and A. niger. This activity may be due to the increase in the yield of the capped AuNPs. It is believed that due to their large surface area, nanoparticles have more penetration power into microorganisms and if the active constituents of the plant extract can be delivered to the interior of the microbes higher antimicrobial activity could be recorded (Kvitek L. et al., 2014).

Silver ions, as well as AgNps, were known to have strong antimicrobial activities (Furno F et al., 2004). The antibacterial activity of different solutions synthesized using 4% w/v plant extract added to different Ag$^+$ concentrations (c1, 5X10$^{-4}$ M Ag$^+$) and (c2, 1X10$^{-3}$ M Ag$^+$) demonstrated that AgNPs has antibacterial activity against both Gram positive and Gram negative bacteria, and the results are depicted in Figs.11. These results agreed with previous work (Kim J.S et al., 2007; Bindhu M.R and Umadevi M, 2013), the activity of these solutions was mainly due to the different amounts of AgNPs formed upon addition of different concentrations of Gmelina extract. The Gram-negative bacteria E. coli was less sensitive to AgNPs compared with S. aureus. This may
be due to the characteristics of certain bacterial species (Bindhu M.R and Umadevi M, 2013). The difference in sensitivity of Gram-positive and Gram-negative bacteria to AgNps was due to the difference in thickness (Gram positive is 50% higher than Gram-negative) and constituents of their cell membrane structure (Kim J.S et al., 2007) thus, large doses is required for Gram-positive bacteria. As well, antibacterial activity of different solutions synthesized using 4% w/v plant extract added to different Ag⁺ and Au³⁺ concentrations (d = 1.45 X10⁻³ M Au³⁺ +2.5X10⁻³ M Ag⁺) and (d = 2.9 x10⁻³ M Au³⁺ +2.5x10⁻³ MAg⁺) demonstrated that AgAuNPs has antibacterial activity against both Gram positive and Gram negative bacteria.

Figure 11 Antimicrobial activities of different concentrations of Gmelina leaf extract, AuNPs, AgNPs and AgAuNPs against A. niger, Basillussubtillus, Candida, E. coli, pseudomonas, Staph aureus.
Conclusion

This work presented synthesis consisting of a reduction of the silver and gold ions using a Gmelina leaf extract for the first time. The silver, gold and AgAu-core shell nanoparticles were characterized using different techniques. The toxicity effects of AgNps, AgNps as well as AgAu core-shell bimetallic NPs on the liver carcinoma cell line (HePG2) were examined and found that the IC$_{50}$ of AgAuNps (2.98mg/ml) is higher than AgNps (11.4mg/ml) and AuNPs (>50mg/ml) or Gmelina (>50mg/ml). The antimicrobial activity of the prepared nanoparticles were studied and the activity was found to depend on the concentration of nanoparticles and their sizes.

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