Preprint Title Synthesis, characterization and comparative anticancer potential of phytosynthesized mono and bimetallic nanoparticles using *Moringa oleifera* aqueous leaf extract.

Authors Shruti Gupta, Hemlata Hemlata and Kiran Kumar Tejavath

Publication Date 25 Aug 2020

Article Type Full Research Paper

ORCID® iDs Shruti Gupta - https://orcid.org/0000-0002-4934-4061; Hemlata Hemlata - https://orcid.org/0000-0003-1678-4957; Kiran Kumar Tejavath - https://orcid.org/0000-0001-7876-421X

Synthesis, characterization and comparative anticancer potential of phytosynthesized mono and bimetallic nanoparticles using *Moringa oleifera* aqueous leaf extract.

Authors:

Shruti Gupta[1], Hemlata[1], Kiran Kumar Tejavath[1]*

1. Department of Biochemistry, Central University of Rajasthan, NH-8, Bandarsindri, Ajmer 305817, Rajasthan, India.

***Correspondence address and Email: Department of Biochemistry, Central University of Rajasthan, NH-8, Bandarsindri, Ajmer 305817, Rajasthan, India. kirankumar@curaj.ac.in

Abstract

In this article we bring up facile one-step phytosynthesis of Silver (Ag), Gold (Au) and Ag/Au bimetallic nanoparticles by reduction of Silver nitrate and Tetrachloroauric acid solution, using aqueous leaf extract of *Moringa oleifera*. Physical characterization was done using different techniques including UV-Visible spectroscopy, FT-IR Spectroscopic Analysis, DLS, Zeta Potential, XRD, TEM and EDAX. These nanoparticles were evaluated for their cytotoxicity against human hepatocellular carcinoma cells (HepG2) and breast cancer cell line (MDA-MB-231 and MCF-7). Our data suggest these phytosynthesized nanoparticles showed a dose-dependent cytotoxic effect on all the cancer cell lines. However, AuNPs is seen to have higher cytotoxic potential with IC_{50} value in the range of 9.20-21.46µg/ml compared to that of Bimetallic NPs (Ag-Au NPs) with IC_{50} value of 37.22-49.94µg/ml. Whereas, Silver NPs (AgNPs) didn't show cytotoxic activity upto 60µg/ml in all the three cell lines for 24hrs. Hence, this study supports the effectiveness of phytosynthesized AuNPs for the development of anticancer agents.

Keywords:

Bimetallic Nanoparticles, Cytotoxicity, Gold, *Moringa oleifera*, Silver.

Introduction

Nanomaterials have proved to be the shining star of the present era that has touched every field of research. The nanomaterials formulated exhibit multiplicity in their physicochemical properties which comprise of electronic, catalytic, biological, magnetic, optical and so forth [1]. From the variety of nanomaterials, noble metal nanoparticles are the centre of attraction, owing to their outstanding properties that make them enormously useful in fields like food, pharmacy, textile, cosmetics, medication, catalysis, biosensors and theranostics [2]. Among the noble metal nanoparticles, Silver nanoparticles (AgNPs) and Gold nanoparticles (AuNPs) are the core of interest due to their stability and ease of preparation[3]. The versatile properties of silver nanoparticles that make it important in biological sciences are antimicrobial, antifungal, antioxidant and wound healing and that of gold nanoparticles include bio sensing, drug delivery, bio imaging and surface modification for attachment of ligands [4, 5]. Monometallic nanoparticles of silver and gold are intensively used from the past few decades but very few studies are available in literature on combination of these two noble metals as bimetallic nanoparticles which has proved to show better biocompatibility and eco-friendly [6], [7]. The bimetallic Ag/Au nanoparticles have also proved to combine the unique properties of Ag and Au nanoparticles and also have distinct advantages of their own [8, 9]. Bimetallic nanoparticles are designed in various forms in which the component monometals are differently arranged, such as core-shell, alloy and contact aggregated [10]. The process of synthesis of bimetallic nanoparticles plays a key role in its properties; which include physical synthesis, chemical synthesis and green synthesis. Instead of using the chemical and physical processes for nanoparticles synthesis, green synthesis of nanoparticles using plant sources is becoming more popular nowadays due to its nontoxicity, cost-effectiveness, and biocompatibility [11]. Earlier researchers have reported the synthesis of Ag/Au bimetallic nanoparticles using plant products and have also shown their applications, some of which include; catalytic activity associated with bimetallic nanoparticles prepared from pomegranate seed juice [12], antibacterial and antibiofilm activities associated with *Gloriosa superba* leaf extract [13], antiproliferative properties of gum kondagogu extract [14], cytotoxic potential of *Solidago canadensis* leaf extract [15], wound healing properties of *Madhuca longifolia* seed extract [4].

Moringa oleifera belongs to family Moringaceae, it is a fast-growing plant native of Asian countries [16]. This tree has been used for ages due to its medicinal and nutritional properties [17]. Though the entire plant of *Moringa* is full of benefits, its leaves are the centre of attraction as they possess a high amount of bioactive compounds like flavonoids, saponins, tannins, phenolic acids, proteins, calcium, iron, vitamins, essential amino acids , omega fatty acids and antioxidant compounds [18]. These leaves are widely used in the traditional treatment of diverse diseases like; cancers, liver diseases, diabetes, rheumatoid arthritis, thyroid diseases, oxidative stress, inflamatory and depression [19]. Leaf extract of *Moringa oleifera* have been investigated to contain compounds like 4-(α-L-rhamnosyloxy) benzyl isothiocyanate, niazimicin and β-sitosterol-3-*O*-β-D-glucopyranoside which may be accountable for its anticancer activity [16, 20]. Previously different metal nanoparticles were synthesized using *Moringa oleifera* leaf extract which showed potent

anticancer activity; copper oxide nanoparticles against MCF-7, HeLa, Hep-2 and A549 [21]; nickel oxide nanoparticles against HT-29 [22]; gold nanoparticles against A549 [23] and silver nanoparticles against A431 [24]. However, unavailability of literature on the synthesis of Ag/Au bimetallic and other monometallic nanoparticles using *Moringa oleifera* leaf extract for comparative cytotoxic evaluation among these nanoparticles lead us to explore further.

Hence, in this study we report for the first time facile phytosynthesis of Ag/Au bimetallic nanoparticles using aqueous leaf extract of *Moringa oleifera* as a reducing and capping agent. The characterization of these particles was done using UV-Visible spectroscopy, Fourier infrared spectroscopy (FT-IR), Dynamic Light Scattering (DLS), Zeta Potential, X-Ray Diffraction (XRD), Transmission electron microscopy (TEM) and Energy Dispersive X-Ray Spectroscopy (EDAX). Further we show the comparative cytotoxic effect of Ag, Au and Ag/Au bimetallic nanoparticles against human cancer cell lines HepG2, MCF-7 and MDA-MB-231.

Methodology

Materials

Silver Nitrate ($AgNO_3$), Tetrachloroauric acid ($HAuCl_4$), 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's Modified Eagle Medium (DMEM), Trypsin- EDTA Solution, Penicillin/Streptomycin antibiotic, Dimethylsulfoxide (DMSO) were purchased from HiMedia, Fetal Bovine Serum (FBS) was purchased from Gibco and Milli Q water was used for preparing solutions and dilutions.

Preparation of *Moringa oleifera* aqueous leaf extract

The leaves of *Moringa oleifera* were collected from the Central University of Rajasthan campus, in January. The freshly collected *Moringa oleifera* leaves were thoroughly cleaned under tap water and then washed twice using Milli Q water. The leaves were shade dried at room temperature, powdered using a kitchen blender to get the fine powder. 5gm of *Moringa oleifera* leaf powder was added to 100ml of Milli Q water and boiled at 80°C for 15 mins. The extract so obtained was cooled and filtered using Whatman filter paper No 1. The obtained filtrate was treated as aqueous leaf extract and stored in 4° C for future use.

Synthesis of Ag, Au and Ag/Au bimetallic nanoparticles

The Ag, Au and Ag/Au bimetallic nanoparticles were synthesized using the aqueous leaf extract of *Moringa oleifera.* 2ml aqueous extract was added to 30ml of each of the solution of Silver Nitrate ($AgNO_3$) 1mM and Tetrachloroauric acid ($HAuCl_4$) 1mM. 7 ml of plant extract was added to 100ml of a mixture of Silver Nitrate (1mM) and Tetrachloroauric acid (1mM) in the ratio of 2:3 at room temperature. Change in colour of the reaction mixture was an initial indication of reduction of the salts to nanoparticles. Thus synthesized nanoparticles were subjected to centrifugation at 12000rpm for 25 minutes, followed by the dispersion of the

pellet in Milli Q water. The process of centrifugation and redispersion in the Milli Q water was repeated for thrice to ensure removal of traces free silver ions or gold ions in the respective metal nanoparticles.

Characterization of Nanoparticles

UV-Visible Spectroscopic measurements

UV-Visible spectra were carried out using BMG LABTECH SPECTROstar Nano for monitoring the synthesis of Ag, Au and Ag/Au bimetallic nanoparticles. A spectrum was recorded from a wavelength of 300nm to 1000nm against an absorbance ranging from 0 to 3 OD.

Dynamic light scattering (DLS) measurements and zeta potential

The hydrodynamic particle size, Polydispersity Index (PdI) and zeta potential of the phytosynthesized nanoparticles was measured by using Dynamic Light Scattering (DLS) (Malvern nanoZs, Malvern Instruments). The sample preparing steps included the dilution of the sample using Milli Q water and then sonicating the diluted sample for 20 minutes in an ultrasonic bath for the proper dispersion of the suspended nanoparticles. The temperature parameter of the machine was set at 25^{o} C with a light scattering a 90^{o}.

Fourier Transform Infrared Spectroscopy (FTIR) measurements

To identify the biomolecules bound on the surface of Ag, Au and Ag/Au bimetallic nanoparticles and thus involved in their capping and stabilization, FTIR spectroscopy was carried out. The nanoparticles were dried to powdered form and exposed to FTIR spectroscopy (PerkinElmer Spectrum Version 10.4.00, M/s Perkin Elmer Co., Waltham, Massachusetts, USA). The scanning range was 4000-400 cm^{-1}, with a resolution of 1 cm^{-1}.

X-Ray diffraction (XRD) analysis

The phase and crystal structure of the phytosynthesized Ag, Au and Ag/Au bimetallic nanoparticles was carried out by X-Ray Diffractometer (PANalytical-Empyrean) using Cu-Kα radiation with a wavelength of 1.540 Å at a scanning rate of 0.02° done in the region of 2θ values between 30° to 90° for Ag nanoparticles and between 10° to 90° for Au nanoparticles and Ag/Au bimetallic nanoparticles.

Trasmission Electron Microscopy (TEM) and EDAX (Energy-Dispersive X-ray Spectroscopy) analysis of synthesized nanoparticles

Trasmission electron microscopy (TEM) images were obtained in a FEI TECNAI G^2 S-Twin microscope at an accelerating voltage of 200kV. The electron diffraction pattern were recorded with a GATAN CCD camera. Energy dispersive X-Ray (EDX) spectra were recorded using Oxford Instruments X-Max^N SDD ($50mm^2$) system and INCA analysis software.

Cytological assays

Cell lines HepG2, MDA-MB-231, MCF-7 were cultured in DMEM media supplemented with 10% heat-inactivated FBS and 1% Penicillin/Streptomycin antibiotic. The cell lines were maintained in CO_2 incubator at 37°C and 5% CO_2. MTT assay was carried out to calculate the cell viability under the treatment of different concentrations of nanoparticles. For MTT assay, the cells were cultured in 96-well micro titre plates containing 10^3 to 10^4 cells per well for 24 hrs with 100µl of complete media. After 24 hrs, the cells were treated with different concentrations 05, 10, 20, 30, 40, 50 and 60 $\mu g.ml^{-1}$ of nanoparticles which were prepared in incomplete media. The cells were kept under treatment for 24 hrs after which they were subjected to MTT (20µl; 5mg/ml) for 4 hrs at 37°C, the formazan crystals thus formed were dissolved in 100µl of DMSO. Untreated cell lines are considered as the negative control. Then the plate was evaluated in the spectrophotometer at a wavelength of 570nm to check the absorbance in each sample, and cell viability was calculated using the following equation:-

% Cell viability = [Mean OD (Treated sample) – Blank OD / Mean OD (Control) – Blank OD] ×100

Statistical Analysis

The results of cytotoxicity of all the three phytosynthesized nanoparticles were compared to one another using Two-Way ANOVA test by Graph-Pad Prism Software. The thus calculated P values < 0.5 was considered significant and P values < 0.1 and 0.001 were considered highly significant.

Results and discussion

Moringa oleifera leaf extract is rich in various bioactive constituents which would play important role in exhibiting anticancer potential. Phytosynthesis of Ag, Au and Ag/Au bimetallic nanoparticles was achieved using *Moringa oleifera* aqueous leaf extract as the reducing and capping agent.

Synthesis of Ag, Au and Ag/Au bimetallic nanoparticles

Formation of Ag, Au and Ag/Au bimetallic nanoparticles was observed from the change of the color of the initial reaction mixture to final color change which indicates the reduction of individual salts to nanoparticles **(Figure 1a)**. This change in colour was observed in 10 mins for Ag nanoparticles from light yellow to dark brown colour and in 2 hours for Au nanoparticles from pale yellow to pink colour and Ag/Au bimetallic nanoparticle from yellow to reddish brown colour. The conversion of light yellow colour of the initial reaction mixture of $AgNO_3$ and plant extract to a dark brown colour indicated the reduction of Ag^+ to Ag^0, [25].On addition of plant extract to $HAuCl_4$ solution, change of pale yellow color to pinkish color similar changes in color was also observed in case of *Peganum harmala* L leaf extract [26]. The initial conformation for synthesis of Ag/Au bimetallic nanoparticles was visually indicated by transformation of pale yellow solution to reddish-brown color. Similar, colour change is also associated with the Ag/Au bimetallic nanoparticles synthesised from *Gloriosa superba* leaf extract and Trapa peel extract [13], [11]

Characterization of Ag, Au and Ag/Au bimetallic nanoparticles

UV-Visible spectroscopy analysis

After visual confirmation of colour change of the reaction mixture, the formation of the nanoparticles was further confirmed by measuring the UV-Visible spectra of the solution which gives surface plasmon resonance (SPR) absorption band. When measured using UV-Visible spectra from 300-1000nm the characteristic peak for aqueous leaf extract, Ag, Au, and Ag/Au bimetallic nanoparticles were seen at 350nm, 401nm, 537nm, and 532nm respectively (**Figure 1b)**. The incidence and intensity of SPR peaks depends on the shape, size, composition and structure of the nanoparticles formed. The appearance of a single blue shifted peak in range of 532nm for Ag/Au bimetallic nanoparticles indicates the formation of homogenous bimetallic construct of the nanoparticles rather than a physical mixture of both Ag and Au nanoparticles [27], [28].

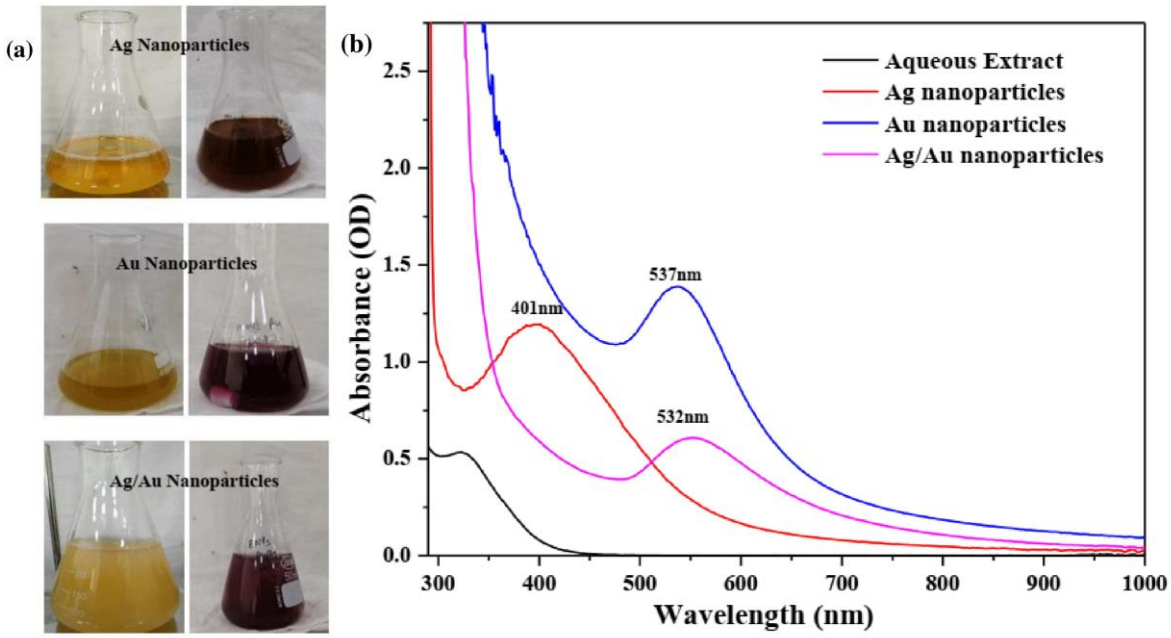

Figure 1: Formation of nanoparticles **a)** Color change during formation of different nanoparticles **b)** UV- Vis spectra of *Moringa oleifera* leaf extract and biosynthesized

Dynamic light scattering (DLS) measurements

Using DLS comparative hydrodynamic size distribution graph of phytosynthesized Ag, Au, and Ag/Au bimetallic nanoparticles were measured and are shown in **Figure 2 a , b and c** respectively. From the results, the calculated average size of Ag, Au, and Ag/Au bimetallic nanoparticles are 129 nm, 96 nm, and 109 nm, respectively. Larger size of monometallic nanoparticles are common in some cases which depends on the type of the phytoconstituents which are involved in the reduction of the metal nanoparticles [29]. The PdI index obtained for Ag, Au and Ag/Au nanoparticles were 0.305, 0.481 and 0.399 respectively **(Table 1)**. These values indicate monodisperse phase of the synthesised nanoparticles and can be prevented from aggregation [24]. The Zeta potential was observed to be -27mV, -36.9mV and -36.7mV for Ag, Au and Ag/Au

nanoparticles **(Figure 3)**.More negative potential values are associated with leaf extract mediated Ag and Au nanoparticles which are stable through a wide pH range.[30, 31].

Table 1: DLS, PdI and Zeta potential of biosynthesized Ag, Au and Ag/Au bimetallic nanoparticles.

Nanoparticles	**DLS**	**PdI**	**Zeta Potential**
Ag	129 nm	0.305	-27mV
Au	96 nm	0.481	-36.9mV
Ag-Au	109 nm	0.399	-36.7mV

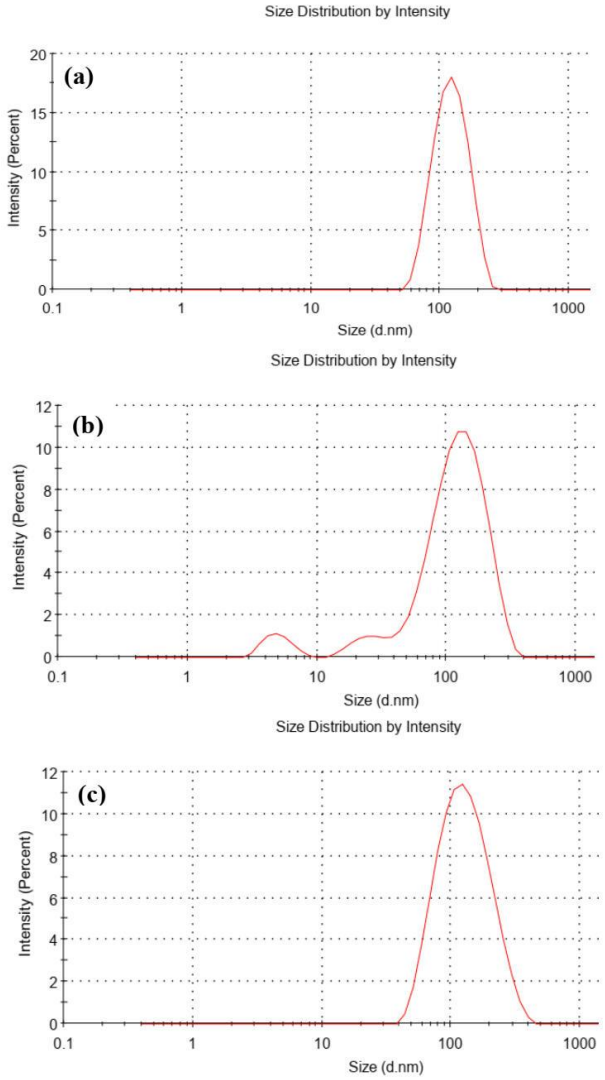

Figure 2: Dynamic Light Scattering (DLS) of biosynthesized **a)** Ag **b)** Au **c)** Ag/Au bimetallic nanoparticles at 25°C and light scattering at 90°.

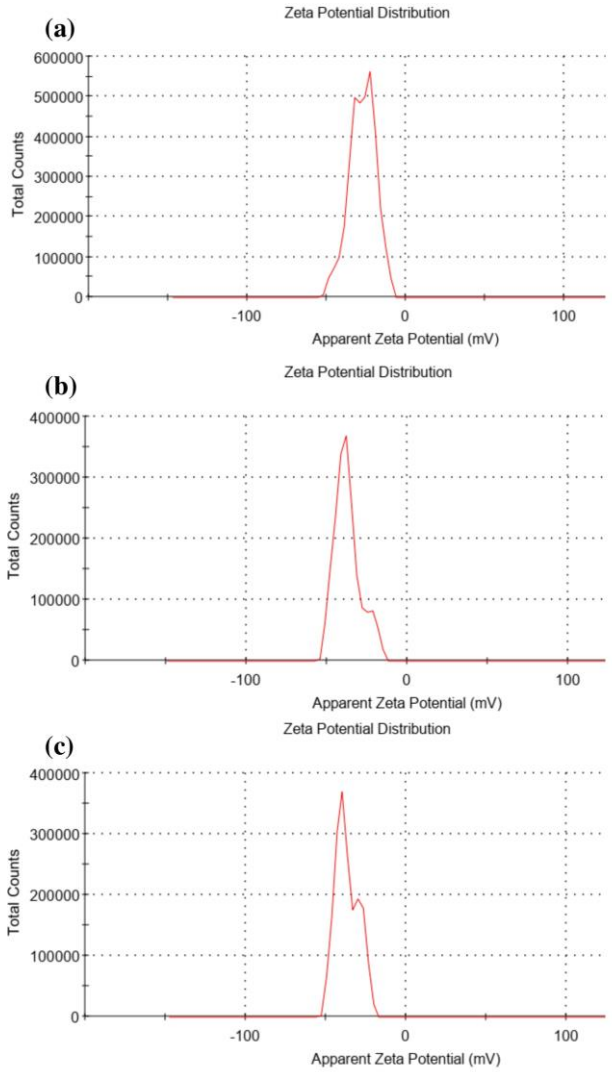

Figure 3: Zeta potential of biosynthesized **a)** Ag **b)** Au **c)** Ag/Au bimetallic nanoparticles at 25°C and light scattering at 90°.

FTIR Analysis

The Fourier transform infrared spectroscopy (FTIR) analysis was done to know the functional groups present in the aqueous leaf extract of *Moringa oleifera* involved in the synthesis of nanoparticles as a reducing and capping agent. Distinct clear peaks at 3326.37, 2931.3, 1579.58, 1380.07 and 1054.78 $cm^{-1}$was observed in leaf extract which were shifted in case of all the three nanoparticles synthesised (**Figure 4a-d)**. 3437.82, 3433.14 and 3278.52 cm^{-1} were observed in Ag, Au and Ag/Au nanoparticles which represents OH and N-H stretch of secondary amines, indicating them as the capping agents [24]. Peak at 2919.36, 2920.68and 2920.87cm^{-1} in these nanoparticles indicate involvement of H-C-H symmetric and asymmetric stretches and O-H of carboxylic groups [32]. The peaks at 1631.05, 1631.28 and 1580.71 cm^{-1} in the nanoparticles characterise the N-H bond of primary amines and also show a clear involvement of primary amines in their reduction and capping. 1379.83, 1383.8 and 1397.78 cm^{-1} peaks signify the N=O of nitro groups, but its lower intensity in case of Ag and Au nanoparticles than in aqueous extract shows the involvement of nitro groups in reduction process and higher intensity in case of Ag/Au bimetallic nanoparticles shows that nitro groups are capping agents. Peaks at 1025.68, 1025.82 and 1047.58 cm^{-1} are representative of C-O stretch of esters and ethers; their intensity represents their involvement in reduction for Ag and Au and capping of Ag/Au bimetallic nanoparticles respectively. Thus the overall FTIR spectrum shows the involvement of primary amines, esters, ethers and carboxylic acids in the overall reduction and capping of the nanoparticles [24]. These show that both proteins/peptides and carboxylic acid are present on the surface of the nanoparticles and are the capping biomolecules of the particles [33, 34].

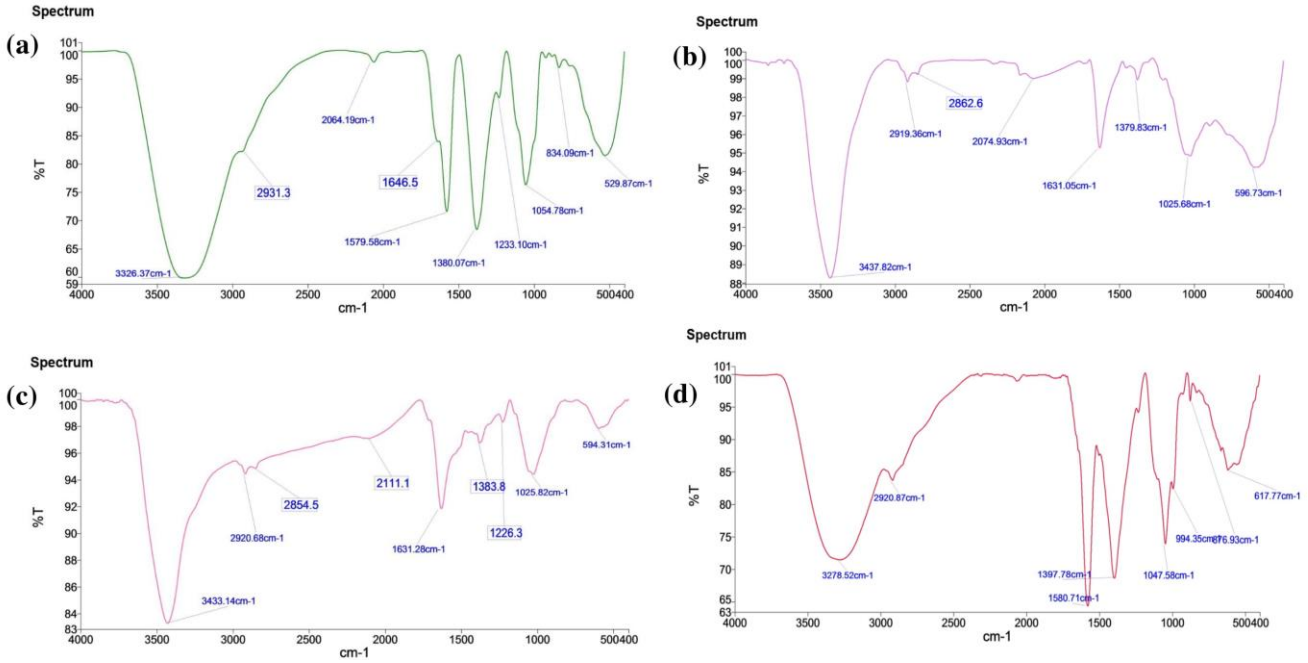

Figure 4: Fourier-Transform Infrared (FT-IR) spectra of **a)** Aqueous leaf extract of *Moringa oleifera* and **b)** Ag **c)** Au and **d)** Ag/Au bimetallic nanoparticles biosynthesized using *Moringa oleifera* leaf extract.

XRD analysis

The peaks obtained in the XRD analysis shown in **Figure 5a-c** had a characteristic pattern; this pattern of peaks confirmed the formation of different types of nanoparticles having crystalline structure. XRD pattern of Ag nanoparticles obtained diffraction peaks with 2θ values at 38.21°, 46.18°, 64.57° and 77.30° related to (111), (200), (220) and (311) planes respectively. This is in agreement with the silver crystals reported in International diffraction data centre (JCPDS file: 04-0783). The presence of these peaks indicated the face centered cubic (fcc) structure of Ag nanoparticles [35]. In Au nanoparticles also almost all the peaks diffraction with at 2θ values at 38.22°, 44.38°, 64.89°, 77.49°, and 81.81°, related to (111), (200), (220), (311) and (222) planes respectively, indicated the face-centered cubic structure of the phytosynthesized Au nanoparticles matched with diffraction data base (JCPDS 04-0784). XRD pattern of Ag/Au bimetallic nanoparticles with diffraction peaks at 2θ = 38.18°, 46.29°, 64.71° and 76.69°, and 85.68°, related to (111), (200), (220), (311) and (222) planes respectively. These results indicate the same lattice structure is present in the phytosynthesized Ag/Au bimetallic nanoparticles which are seen in individual monometals nanoparticles. Further, this results indicate the homogenous preparation of Ag and Au nanoparticles [27]. Apart from the Braggs peaks additional peaks are also found with 2θ values of 27.86°, 54.81°,57.50°, 67.38° and 32.28°, which are due to the phytoconstituents present in the plant extract [36].

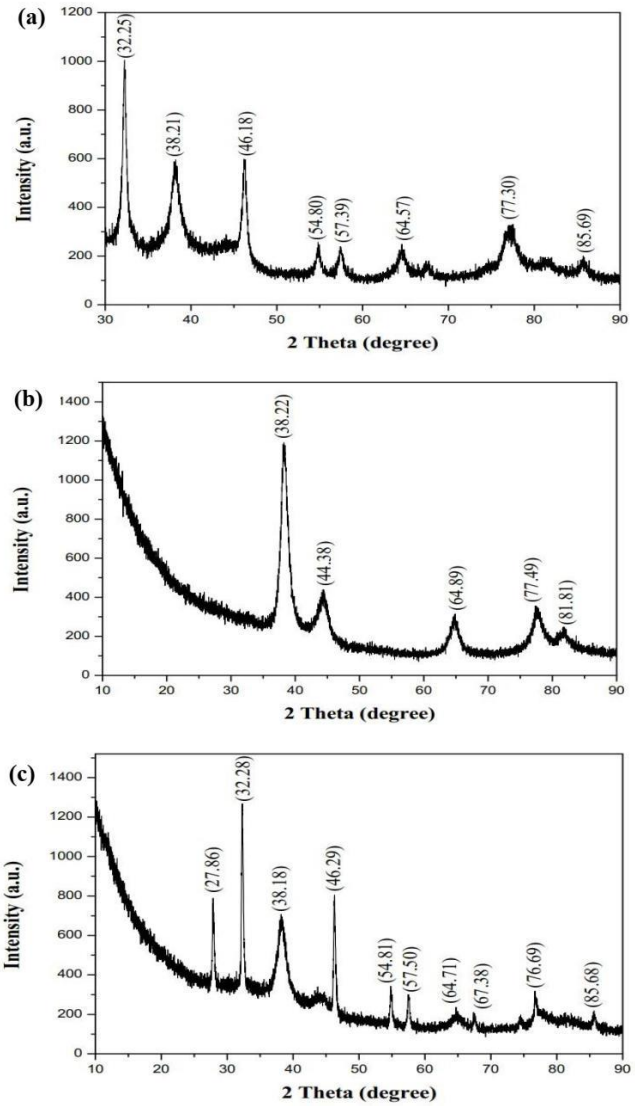

Figure 5: X-Ray Diffraction (XRD) pattern of **a)** Ag **b)** Au and **c)** Ag/Au bimetallic nanoparticles biosynthesized using *Moringa oleifera* leaf extract.

EDAX analysis and Morphology of Ag, Au and bimetallic Ag/Au bimetallic nanoparticles

The elemental analysis done using EDAX of the Ag and Au and nanoparticles showed peaks at 3 KeV, 2.3 KeV and this suggested peak of silver and gold of silver nanoparticles **(Figure 6a& b.)**. Ag/Au showed peaks at both 3KeV and 2.3 KeV **(Figure 6c)**. Additionally peaks of carbon and oxygen were seen which are present in the plant extract and the aqueous solution. Similar results were also reported in case of other biosynthesised mono and bimetallic nanoparticles [37].

Morphology of Ag/Au bimetallic nanoparticles analysed by TEM showed a size of the nanoparticles between 11-25 nm and associated with a hexagonal, triangular, spherical shape. Selected Area Electron Diffraction (SAED) **(Figure 7c)** confirms the crystalline nature of the Ag/Au nanoparticles and correlates with TEM results **(Figure 7a and b)**. Combination of hexagonal, triangular and spherically shaped bimetallic nanoparticles are also seen in biosynthesised nanoparticles from *Volvariella volvacea* and Trapa peel extract [38], [11].

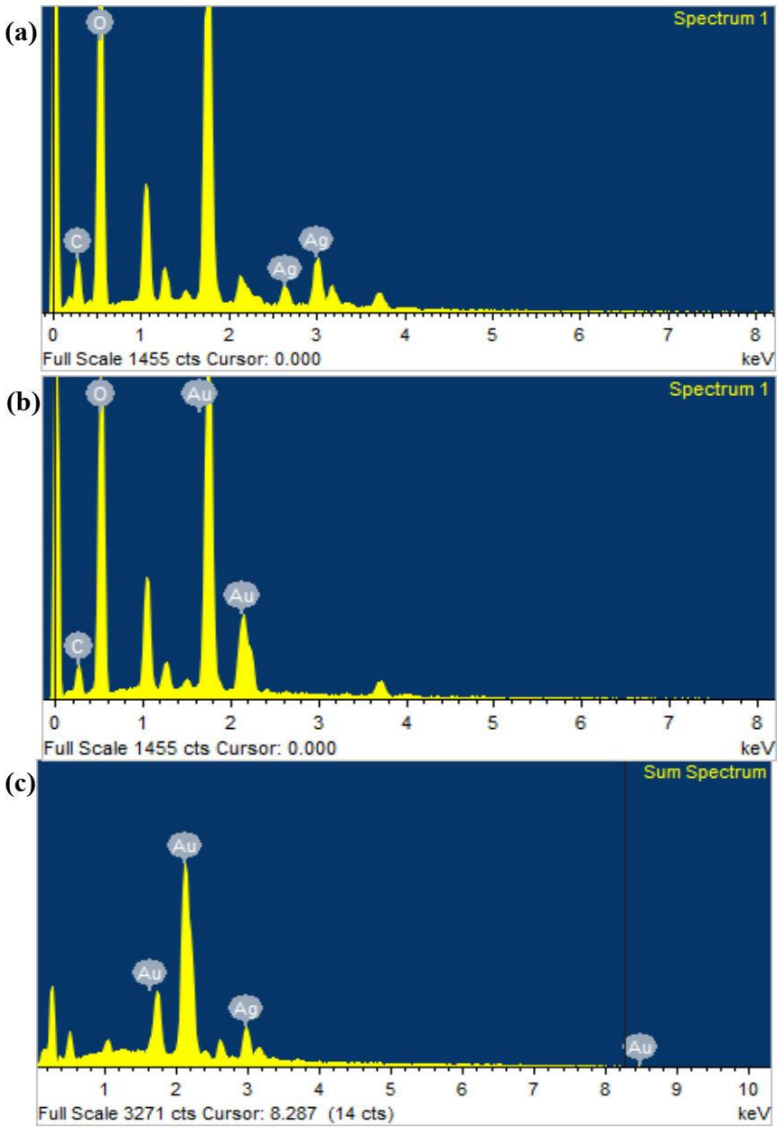

Figure 6: Energy Dispersive X-Ray Spectroscopy (EDAX) spectrum of **a)** Ag **b)** Au **c)** Ag/Au bimetallic nanoparticles biosynthesized using *Moringa oleifera* leaf extract.

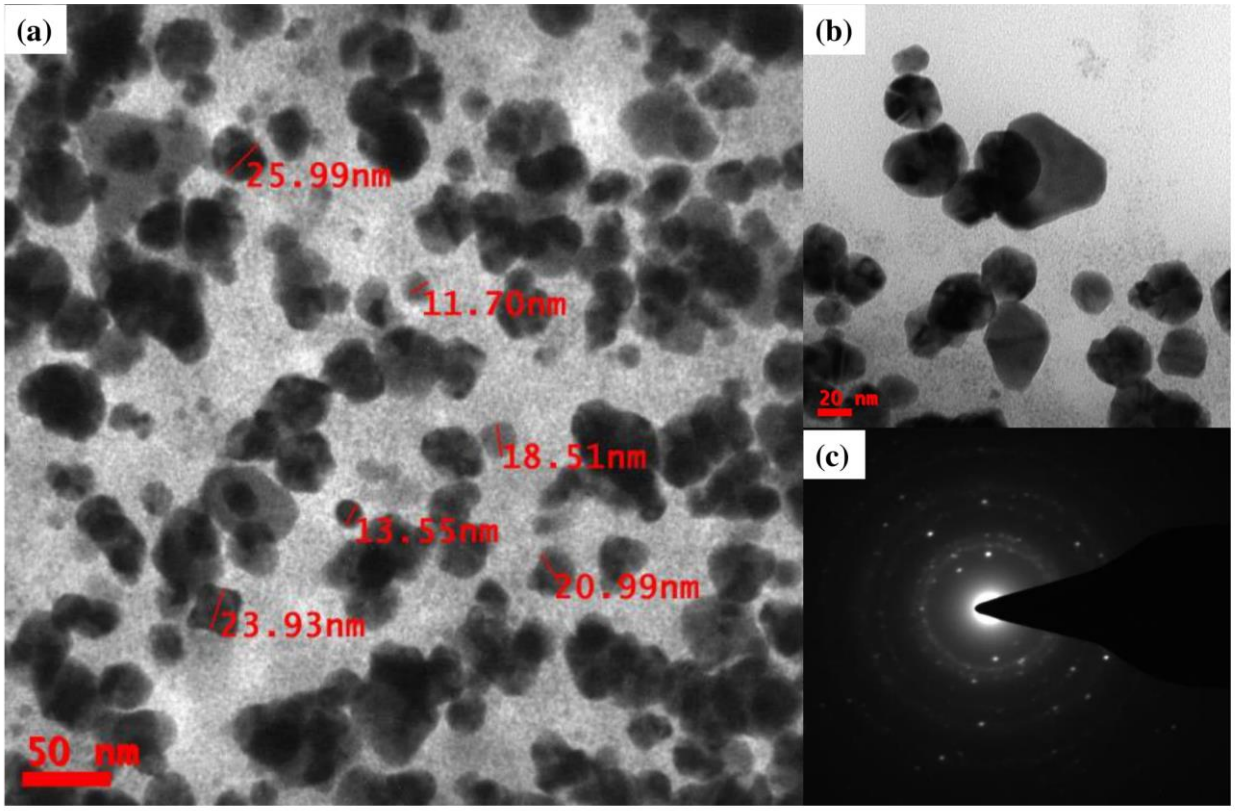

Figure 7: Morphological analysis of biosynthesized Ag/Au bimetallic nanoparticles **a) and b)** Transmission Electron Microscopy (TEM) image **c)** SAED image.

Cytotoxic effects of the synthesized nanoparticles

To evaluate the cytotoxic effect of Ag, Au and Ag/Au bimetallic nanoparticles on the survival percentage of the cancer cells, they were tested on different cell lines i.e.; HepG2, MDA-MB-231, and MCF-7. Concentrations of the nanoparticles initially used for all three cell lines were 150, 125, 100, 75, 50, 25, 10µg/ml, but after seeing their response to such concentrations some modifications were made in the concentration of nanoparticles used for different types of cell lines (results not shown). Final concentration of nanoparticles used for all the cell lines tested was in the range of 05-60µg/ml. The IC_{50} values were calculated from the logarithmic dose dependent curve **(Table 2)**. The results were represented in graphs as the percentage survival of cells post treatment compared to the negative control (untreated cells). At a concentration of 20 µg/ml the Au showed a cell viability of 50.24% while Ag/Au and Ag nanoparticles showed 79.2% and 85.58% viability respectively and at 60 µg/ml the cell viabilities turns to be 27.4%, 10.18% and 63.88% for Au, Ag/Au and Ag nanoparticles respectively on HepG2 cells **(Figure 8a)**. MDA-MB-231 also responded in a similar manner as that of HepG2 when treated with 20 µg/ml Au nanoparticles 50.4% cell viability was obtained and 69.39% and 92.97% cell viability in case of Ag/Au and Ag nanoparticles respectively. At 60 µg/ml of the three nanoparticles, the cell viability was reduced to 28.96%, 45.44% and 78.17 % for Au, Ag/Au and Ag nanoparticles respectively **(Figure 8b)**. Further, upon treatment with 10 µg/ml of Au nanoparticles, MCF-7 cell lines the cell viability was found to be 49.32%. At the same concentration Ag/Au and Ag nanoparticles showed 71.84% and 90.67% cell viability respectively. On

increasing the concentrations of Au, Ag/Au and Ag nanoparticles the cell viabilities decreased further and reached 31.08%, 42.84% and 88.17 % respectively at 60 µg/ml **(Figure 8c)**. Lower cell viability was seen in all the cell lines for Au and Ag/Au nanoparticles at almost all concentrations checked, but the Ag nanoparticles were found non cytotoxic for all the three cell lines checked. The results indicated highest cytotoxicity of Au nanoparticles on all the cell lines. The results thus obtained showed that though highest toxicity was seen for Au nanoparticles in all cell lines but individually Au nanoparticles were highest effective on MCF-7 cell lines and Ag/Au nanoparticles were on HepG2 cell lines; while Ag nanoparticles had no considerable cytotoxicity. Cell Morphology at IC_{50} concentrations of different nanoparticles are represented in **Figure 9**.

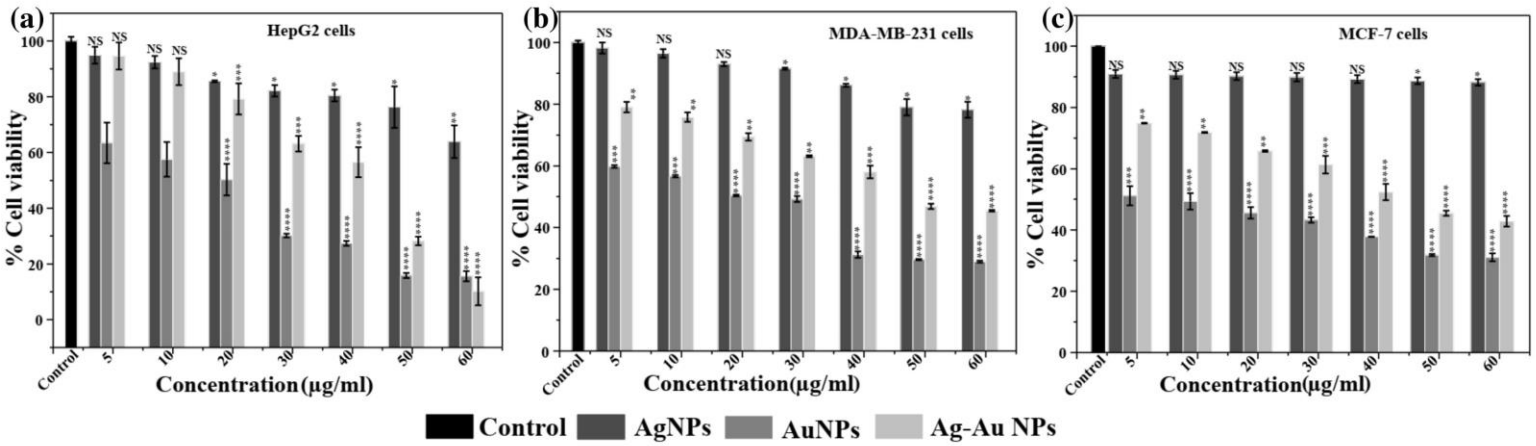

Figure 8: Effect of Ag, Au and Ag/Au bimetallic nanoparticles on human cancer cell lines. Graph represents the percentage of cell viability of different cell lines after 24hrs of exposure to different concentrations of nanoparticles; **a)** HepG2 **b)** MDA-MB-231 **c)** MCF-7.

The values plotted are average of mean of triplicates ± SD; * signifies the p value compared to control. Where NS= $p > 0.05$; *= $p< 0.05$; **= $p< 0.01$; ***= $p< 0.001$ and ****= $p< 0.0001$.

Table 2: IC_{50} ± SD values for different types of nanoparticles tested on the different human cancer cell lines.

IC_{50} (µg/ml) ± SD			
Nanoparticles	**HepG2**	**MDA-MB-231**	**MCF-7**
Ag	92.19 ± 2.19	128.28 ± 1.8	821.01 ± 2.77
Au	19.02 ± 4.46	21.46 ± 1.4	9.2 ± 0.93
Ag-Au	37.22 ± 2.63	49.94 ± 0.22	45.61 ± 0.15

These results suggested that the silver nanoparticles were least affecting all the cancer cell lines and therefore had higher values of IC_{50} in each case, but usually, Ag nanoparticles have self-toxicity affects [39]. However

it has been earlier reported that the biosynthesized Ag nanoparticles using *Moringa oleifera* are different, these nanoparticles have a reduced toxicity effect which is attributed by the plant extract [40]. At the same instance, Au nanoparticles biosynthesized using *Moringa oleifera* have proved and reported to have a cytotoxic effect on cancer cells [41]. But the phytosynthesized Ag/Au bimetallic nanoparticles by *Moringa oleifera* extract that have shown cytotoxic effects in the cancer cell lines in our study have not been reported yet to the best of our knowledge.

Figure 9: Morphological analysis of HepG2, MDA-MB-231 and MCF-7 cell lines control and exposed to different nanoparticles.

Conclusion

In gist, *Moringa oleifera* a plant considered a medicinal hub was successfully used in the facile synthesis of Ag, Au, and Ag/Au bimetallic nanoparticles. It was confirmed the biomolecules of *Moringa oleifera* to be responsible for reducing and capping agents of the nanoparticles.

Additional, the nanoparticles were put forward to show their cytotoxic effect of different human cancer cell lines, which were HepG2, MDA-MB-231, and MCF-7. The nanoparticles were effective in reducing the survival of the cancer cells in their presence in different concentrations for different cell lines. Thus, this report adds on another feature to the medicinal hub plant *Moringa oleifera* to successfully formulate bimetallic nanoparticles which could be used effectively for their cytotoxic effect.

Declaration of interest

None. The authors declare no conflict of interest.

Funding

The authors are grateful to the Science and Engineering Research Board (SERB) for their financial support (Project No: EEQ/2016/000696).

Acknowledgment

Authors also thank Material Research Centre, MNIT, Jaipur, India for FT-IR and DLS analysis. Authors do thank Department of Physics, Central University of Rajasthan, Ajmer, India for XRD analysis. Authors even thank University of Hyderabad, Hyderabad, India for TEM and EDAX analysis.

Data availability

All data generated or analysed during this study are included in this published article.

References

1. Mandal, R., et al., *Evolving trends in bio/chemical sensor fabrication incorporating bimetallic nanoparticles.* Biosens Bioelectron, 2018. **117**: p. 546-561.
2. Wysocka, I. and E. Kowalska, *Morphology, Photocatalytic and Antimicrobial Properties of TiO2 Modified with Mono- and Bimetallic Copper, Platinum and Silver Nanoparticles.* 2019. **9**(8).
3. Bao, Z. and C.Q. Lan, *Advances in biosynthesis of noble metal nanoparticles mediated by photosynthetic organisms-A review.* Colloids Surf B Biointerfaces, 2019. **184**: p. 110519.
4. Sharma, M., et al., *Biofabrication and characterization of flavonoid-loaded Ag, Au, Au-Ag bimetallic nanoparticles using seed extract of the plant Madhuca longifolia for the enhancement in wound healing bio-efficacy.* Prog Biomater, 2019. **8**(1): p. 51-63.
5. Kumari, Y., et al., *Gold nanoparticles: New routes across old boundaries.* Adv Colloid Interface Sci, 2019. **274**: p. 102037.
6. Emam, H.E., M.M. El-Zawahry, and H.B. Ahmed, *One-pot fabrication of AgNPs, AuNPs and Ag-Au nano-alloy using cellulosic solid support for catalytic reduction application.* Carbohydr Polym, 2017. **166**: p. 1-13.
7. Elegbede, J.A. and A. Lateef, *Silver-gold alloy nanoparticles biofabricated by fungal xylanases exhibited potent biomedical and catalytic activities.* 2019. **35**(5): p. e2829.
8. Shmarakov, I., et al., *Antitumor Activity of Alloy and Core-Shell-Type Bimetallic AgAu Nanoparticles.* Nanoscale Res Lett, 2017. **12**(1): p. 333.
9. Murugavelu, M. and B. Karthikeyan, *Synthesis, characterization of Ag-Au core-shell bimetal nanoparticles and its application for electrocatalytic oxidation/sensing of l-methionine.* Mater Sci Eng C Mater Biol Appl, 2017. **70**(Pt 1): p. 656-664.
10. Katifelis, H., et al., *Ag/Au bimetallic nanoparticles induce apoptosis in human cancer cell lines via P53, CASPASE-3 and BAX/BCL-2 pathways.* Artif Cells Nanomed Biotechnol, 2018. **46**(sup3): p. S389-s398.
11. Ahmad, N., et al., *Biosynthesized composites of Au-Ag nanoparticles using Trapa peel extract induced ROS-mediated p53 independent apoptosis in cancer cells.* Drug Chem Toxicol, 2019. **42**(1): p. 43-53.
12. Kumari, M.M., J. Jacob, and D. Philip, *Green synthesis and applications of Au–Ag bimetallic nanoparticles.* Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2015. **137**: p. 185-192.
13. Gopinath, K., et al., *Green synthesis of silver, gold and silver/gold bimetallic nanoparticles using the Gloriosa superba leaf extract and their antibacterial and antibiofilm activities.* Microb Pathog, 2016. **101**: p. 1-11.
14. Subbiah, K.S. and S.R. Beedu, *Biogenic synthesis of biopolymer-based Ag-Au bimetallic nanoparticle constructs and their anti-proliferative assessment.* IET Nanobiotechnol, 2018. **12**(8): p. 1047-1055.
15. Botha, T.L., E.E. Elemike, and S. Horn, *Cytotoxicity of Ag, Au and Ag-Au bimetallic nanoparticles prepared using golden rod (Solidago canadensis) plant extract.* 2019. **9**(1): p. 4169.
16. Vergara-Jimenez, M., M.M. Almatrafi, and M.L. Fernandez, *Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease.* 2017. **6**(4).
17. Dhakad, A.K., et al., *Biological, nutritional, and therapeutic significance of Moringa oleifera Lam.* Phytother Res, 2019. **33**(11): p. 2870-2903.
18. Tuorkey, M.J., *Effects of Moringa oleifera aqueous leaf extract in alloxan induced diabetic mice.* Interv Med Appl Sci, 2016. **8**(3): p. 109-117.
19. Kou, X., et al., *Nutraceutical or Pharmacological Potential of Moringa oleifera Lam.* 2018. **10**(3).
20. Tejavath, K.K., *Studies on some biologically important proteins from Moringa oleifera seeds.*
21. Rehana, D., et al., *Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts.* Biomed Pharmacother, 2017. **89**: p. 1067-1077.
22. Ezhilarasi, A.A., et al., *Green synthesis of NiO nanoparticles using Moringa oleifera extract and their biomedical applications: Cytotoxicity effect of nanoparticles against HT-29 cancer cells.* Journal of Photochemistry and Photobiology B: Biology, 2016. **164**: p. 352-360.
23. Tiloke, C., et al., *Moringa oleifera Gold Nanoparticles Modulate Oncogenes, Tumor Suppressor Genes, and Caspase-9 Splice Variants in A549 Cells.* Journal of cellular biochemistry, 2016. **117**(10): p. 2302-2314.
24. Nayak, D., et al., *Biologically synthesised silver nanoparticles from three diverse family of plant extracts and their anticancer activity against epidermoid A431 carcinoma.* J Colloid Interface Sci, 2015. **457**: p. 329-38.

25. Hu, D., et al., *Biosynthesis of Silver nanoparticles using Bauhinia acuminate flower extract and their effect to promote osteogenesis of MSCs and improve meniscus injury healing.* J Photochem Photobiol B, 2019. **197**: p. 111536.
26. Moustafa, N.E. and A.A. Alomari, *Green synthesis and bactericidal activities of isotropic and anisotropic spherical gold nanoparticles produced using Peganum harmala L leaf and seed extracts.* Biotechnol Appl Biochem, 2019.
27. Xia, B., F. He, and L. Li, *Preparation of bimetallic nanoparticles using a facile green synthesis method and their application.* Langmuir, 2013. **29**(15): p. 4901-7.
28. Sanchez-Ramirez, J., et al., *Synthesis and optical properties of Au-Ag alloy nanoclusters with controlled composition.* Journal of Nanomaterials, 2008. **2008**.
29. Banerjee, P., et al., *Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis.* Bioresources and Bioprocessing, 2014. **1**(1): p. 3.
30. Dubey, S.P., M. Lahtinen, and M. Sillanpää, *Green synthesis and characterizations of silver and gold nanoparticles using leaf extract of Rosa rugosa.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2010. **364**(1-3): p. 34-41.
31. Boote, B.W., H. Byun, and J.-H. Kim, *One-pot synthesis of various Ag–Au bimetallic nanoparticles with tunable absorption properties at room temperature.* Gold Bulletin, 2013. **46**(3): p. 185-193.
32. Vasanth, K., et al., *Anticancer activity of Moringa oleifera mediated silver nanoparticles on human cervical carcinoma cells by apoptosis induction.* Colloids Surf B Biointerfaces, 2014. **117**: p. 354-9.
33. Bindhu, M.R., V. Sathe, and M. Umadevi, *Synthesis, characterization and SERS activity of biosynthesized silver nanoparticles.* Spectrochim Acta A Mol Biomol Spectrosc, 2013. **115**: p. 409-15.
34. Shankar, S.S., et al., *Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth.* J Colloid Interface Sci, 2004. **275**(2): p. 496-502.
35. Awwad, A.M., N.M. Salem, and A.O. Abdeen, *Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity.* International journal of Industrial chemistry, 2013. **4**(1): p. 29.
36. Ibrahim, H.M., *Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms.* Journal of Radiation Research and Applied Sciences, 2015. **8**(3): p. 265-275.
37. Li, J., et al., *Biosynthesis of Au, Ag and Au-Ag bimetallic nanoparticles using protein extracts of Deinococcus radiodurans and evaluation of their cytotoxicity.* (1178-2013 (Electronic)).
38. Philip, D., *Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract.* (1386-1425 (Print)).
39. Marin, S., et al., *Applications and toxicity of silver nanoparticles: a recent review.* Curr Top Med Chem, 2015. **15**(16): p. 1596-604.
40. El-Khadragy, M., et al., *Clinical Efficacy Associated with Enhanced Antioxidant Enzyme Activities of Silver Nanoparticles Biosynthesized Using Moringa oleifera Leaf Extract, Against Cutaneous Leishmaniasis in a Murine Model of Leishmania major.* Int J Environ Res Public Health, 2018. **15**(5).
41. Tiloke, C., et al., *Moringa oleifera and their phytonanoparticles: Potential antiproliferative agents against cancer.* Biomed Pharmacother, 2018. **108**: p. 457-466.