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RESEARCH ARTICLE

GREEN SYNTHESIS AND CHARACTERIZATION OF BIOCOMPATIBLE SILVER NANOPARTICLES USING *BRASSICA OLERACEA* L. LEAF EXTRACT

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ABSTRACT

In recent years, the role of metal nanoparticles in medicine and biology has rapidly increased, due to ease of synthesis, surface functionalization and biocompatible unique properties. In this paper, extracellular biosynthesis of Silver nanoparticles (AgNaPs) using methanol extract of Cauliflower leaves (*Brassica oleracea* L.) has been attempted and achieved rapid formation of AgNaPs in a short duration. The resulting AgNaPs were analyzed using UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy, X-ray Diffraction, Scanning Electron Microscopy, High Resolution Transmission Electron Microscopy. The UV-visible spectrum showed a peak at 430nm corresponding to the Plasmon absorbance of the AgNaPs. FTIR spectra suggested the presence of biomolecules on the surface of the AgNaPs and XRD confirms the crystalline nature of the AgNaPs. The synthesized AgNaPs showed predominantly spherical structure and their sizes ranging from 20-70nm and 10-21nm under SEM and HRTEM observation respectively. The synthesized AgNaPs showed better antioxidant and efficiently inhibited various pathogenic organisms and reduced viability of the MDA-MB-231 and HeLa cells in a dose-dependent manner. This paper reported that the green chemistry approach for the synthesis of AgNaPs using methanol extract of cauliflower leaves would be a better alternative to the existing methods. The process may be helpful for the synthesis of plant based AgNaPs pharmaceutically useful drugs.

INTRODUCTION

Nanotechnology is an emerging area of nanoscience due to the remarkable difference in structural and physical properties to those of atoms, molecules and bulk materials of the same element. The synthesis of metallic nanoparticles have attracted extensive attention in the field of physical, biological and pharmaceutical studies due to its unique and considerably changed physical and chemical properties. The most effectively studied nanoparticles today are made from silver (Ag), gold (Au), platinum (Pt) and palladium (Pd) (Sulaiman, *et al.,* 2013). Among the above, silver nanoparticles play a significant role in the field of chemistry (catalysis), physics (optical, electrical and photothermal properties) and healthcare (therapeutics, diagnosis and immunoassay) (Cognet, *et al.*, 2003; O'Neal, *et al.*, 2004; Huang, *et al.*, 2007; Lee, *et al.*, 2011). Several researches have revealed that silver nanoparticles have significant anti-microbial and anti-cancer activity (Jain, *et al.*, 2009; Kumar, *et al.*, 2012). However silver nanoparticles have potential applications in many other areas such as biological deduction controlled drug delivery, low-threshold laser, consumer products, house hold equipments, optical filters and also sensors, among others (Cheng, *et al.*, 2004; Farkas, *et al.*, 2011).

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Conventionally nanoparticles can be prepared and stabilized by physical and chemical methods; the chemical approach include chemical reduction of silver ions in aqueous solution, thermal decomposition in organic solvent (Esumi, *et al.*,1990), radiochemical reduction techniques (Henglein, 1993), photo reduction in reverse micelles and microwave assisted reduction (Pileni, 2000). Some of these methods are easy and provide control over crystallite size by restoring the reaction environment. Still there is a problem of stability and achieving the desired nanosize. Most of these methods are expensive and uses hazardous chemicals such as pyridine, ethylene glycol and sodium borohydride which posse's potential environmental and biological risk (Christensen, *et al.*, 2011). As an alternative to the conventional methods several biological methods have been developed for the synthesis of nanoparticles due to the quest for environmentally sustainable synthesis process. Biological approaches for preparation of nanoparticles can be are based on microorganisms (Moazeni, *et al.*, 2012), enzymes (Ahmad, *et al.*, 2002) and plant extracts (Awwad, *et al.*, 2013). In microbial method, the silver nanoparticles are accumulated intra-cellularly and take more time for recovery. Further, the microbial process needs to be maintained under aseptic condition. On the other hand using plant extracts silver nanoparticles is accumulated extra-cellularly and therefore subsequent extraction is relatively easy. Also, they eliminate the maintenance cost of microbial culture and can be scaled up during large scale operation (Ramteke, *et al.*, 2013).

Researches are being carried out especially on the plant extract which secrete functional molecule for reaction are compatible with green chemistry principles. Recent researches reported the synthesis of silver nanoparticles using *Crossandra infundibuliformis* (Kaviya, *et al.,* 2012), *Trachyspermum ammi* and *Papaver someniferum* (Vijayaraghavan, *et al.,* 2012), *Acalypha Indica* (Krishnaraj, *et al.,* 2012), *Coriandrum sativum* (Sathyavathi, *et al.,* 2010), Lemongrass plant extract (Rai, *et al.,* 2009) and Banana peel extract (Ashok, *et al.,* 2010).

Cauliflower (*Brassica oleracea* L.) is a tropical crop of India and China in the family Brassicacea. Several research articles demonstrated that Brassica vegetables, especially broccoli and cauliflower are a rich source of phenolic compounds which is the major antioxidant of this plant (Guo, *et al*., 2001). Recently this research group has reported higher yield of phenolics and antioxidants from methanol extract of waste cauliflower leaves (Chowdhury, *et al.,* 2013). These polyphenolic compounds can neutralize the free radicals due to their redox properties. They have anti-inflammatory, anti-cancerous and hepatoprotective and anti-bacterial activities (Heim, *et al.,* 2002) and they can also reduce the diseases caused by oxidative stress (Wettasinghe and Shahidi, 1990). Brassica vegetables reduce the chances of cardio vascular diseases and other degenerative diseases (Kris-Etherton, *et al.,* 2002). Herein, we report the simple, rapid, cost effective and eco-friendly method for the synthesis of biocompatible silver nanoparticles with the help of cauliflower leaves methanol extract. The growth of nanoparticles was observed by UV-visible spectrophotometer. The crystalline nature of the nanoparticles was characterized by XRD (X-Ray diffraction) and the involvement of biomolecules was determined by FTIR. The elemental composition of silver nanoparticles was characterized by EDX. The morphological characterization was performed by SEM and TEM analysis. Further the efficacy of silver nanoparticles against pathogenic bacteria and fungi has been investigated in detail. In addition, we are submitting the preliminary results of cytotoxicity of biosynthesized silver nanoparticles for Human breast cancer cell lines (MDA-MB-231 and HeLa).

MATERIALS AND METHODS

Materials

Silver nitrate was procured from Himedia Laboratories Pvt. Ltd. Mumbai, India. MTT, Rutin, DPPH [2, 2-diphenyl-1-picrylhydrazyl] and TPTZ [2, 4, 6-tripyridyl-s-triazine] were purchased from Sigma–Aldrich, MO, USA. All other chemicals and solvents used in this study were of analytical grade and obtained from Merck, Mumbai, India. Cauliflower leaves were obtained from a local vegetable market in Kolkata, India. The bacterial cultures such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella Sp*., *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Salmonella typhii* and fungal cultures such as *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* were obtained from Department of Microbiology, IPGMER SSKM Hospital, Kolkata, India. Human breast cancer cell lines (MDA-MB-231, HeLa cell) were received from the National Center for Cell Sciences, Pune, India. The glass wares (BOROSIL) supplied by Saradha chemicals, Kolkata were used. These were washed with dilute nitric acid and thoroughly rinsed with distilled water and dried in hot air oven before use.

Preparation of *Brassica oleracea* L. methanol extract

The cauliflower leaves were washed thoroughly with tap water followed by rinsing with double distilled water and were shade dried for 7days. The fine powder (≈60 mesh size) was obtained from dried leaves by using kitchen blender (Bajaj Electronics Ltd, India). The leaves powder was sterilized at 121˚C for 15 min. About 10g of cauliflower leaves powder was weighed and macerated with 50mL of 99.9% methanol in a 250mL conical flask and kept at room temperature for 24h. After 24h, the methanol extract of cauliflower leaves was filtered with Whatman No: 4filter paper. The filtered extract was centrifuged at 10000 rpm for 10 min at 4 ˚C. The supernatant was collected in a brown bottle and was stored in a refrigerator at 4°C until further use.

Green synthesis of silver nanoparticles

For synthesis of silver nanoparticles, 1mM solution of silver nitrate was prepared using de-ionized water. 5mL of (5%, v/v concentration) of the methanolic extract of *B. oleracea* L. was mixed with 25mL of 1mM silver nitrate solution and kept in shaker at 37°C for 4h. The colour change was observed. The bio reduction of silver ions in the solution was monitored by sampling the aqueous component at different time intervals (0min and 4h) in UV-visible spectrophotometry (Varian Cary 50 UV-Spec). A control reaction mixture was also maintained without cauliflower leaves extract. The UV-visible spectra were scanned at different wavelengths (320-720 nm) a scanning speed of 400 nm/minutes.

Characterisation of silver nanoparticles

Silver nitrate (aqueous), reacted with methanol extract of Cauliflower leaves solution, was centrifuged at 9000rpm for 10 minutes at 4˚C and the resultant supernatant solution was maintained at -80˚C for 24 hrs and then freeze dried in a lyophilizer (Christ Gefriertrocknungsanlagen GmbH Model 1–4) for 48 hrs. The lyophilized sliver nanoparticles were further used for various analyses such as Fourier Transform Infra Red Spectroscopy (FTIR), powder X-ray Diffraction (XRD), Scanning Electron Microscopy-Energy Dispersive X-ray Analysis (SEM-EDX), High Resolution Transmission Electron Microscopy (HRTEM) and Selected-area Electron Diffraction (SAED) patterns and Thermo Gravimetric Analysis (TG-DTA).

FTIR analysis

The Fourier Transform Infra Red Spectroscopy (FTIR) measurements were carried out using Perkin-Elmer, Spectrum 100, FTIR spectrophotometer and using the spectral range 4000-400cm-1 with resolution of 2cm and 5scans/sample. A small (≈1mg) amount of finely powered lyophilized silver nanoparticles was mixed with IR grade Potassium bromide (KBr) to obtain a round disc (with help of hydraulic press) suitable for FTIR measurement.

X-Ray diffraction analysis

The crystalline and the lattice characteristics of the synthesized silver nanoparticles were measured by powder X-ray diffraction analysis. The XRD measurement was carried out on thoroughly dried thin films of the purified lyophilized silver nanoparticles powder on a glass slab of a Schimadzu XRD 6000 instrument operated at a voltage of 20 keV and a current of 30mA with CuKα 1 radiation (λ=0.1542) in a Two-theta (degree) configuration.

Scanning Electron Microscopy- Energy Dispersive X-ray (SEM-EDX) Analysis

SEM observations were carried out on a JEOL JSM- 6700 scanning electron microscope. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5min. The elemental compositions of silver nanoparticles were obtained by using Energy Dispersive X-ray analysis (JEOL JSM- 6700) at variable pressure scanning electron microscope equipped with INCA Oxford EDX facility, at an acceleration voltage of 10 keV.

HRTEM analysis

Morphological details of biosynthesized silver nanoparticles were revealed under a transmission electron microscope (TEM-JEOL model 2100) operated at an acceleration voltage of 200 kV. To prepare HRTEM, silver nanoparticles were re-dispersed with 1mL distilled water. Few drops of dispersed silver nanoparticles was placed over the carbon coated copper grid and air-dried at 60˚C for 5min.

Thermo gravimetric analysis

Thermal stability of the synthesized silver nanoparticles was measured by thermo gravimetric- differential thermal analyses (TG-DTA) method. A TG-DTA was carried out at a heating rate of 10˚C/min using a Perkin Elmer (Singapore), Pyris Diamond TG/DTA model and Platinum crucible used with alpha alumina powder as reference standard.

Biological activities of silver nanoparticles

DPPH scavenging assay of silver nanoparticles

The antioxidant activity of the biosynthesized silver nanoparticles was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH*, according to the method suggested by Ramadan *et al.* with slight modifications. Stabilized AgNaPs solution (0.1mL) was added to 3mL of methanolic solution of DPPH (0.1µM). The mixture was shaken vigorously and allowed to stand for 30 minutes in the dark, and the absorbance was measured at 517nm against a blank. The capability to scavenge the free radical DPPH in percentage of sample (%DPPHSC) was calculated using the formula;

$$\%DPPHSC = (A0 - A1) \times 100/A0 \quad (1)$$

where *A*0= absorbance of the control; *A1* = absorbance of the sample.

Ferric Reducing Antioxidant Power (FRAP) activity

The FRAP assay is based on the ability of AgNaPs to reduce Fe3+ in 2, 4, 6-tripyridyl -s-triazine (TPTZ) solution to Fe2+ and create blue colored complex Fe2+-TPTZ. The FRAP assay was used to estimate the antioxidant potential of the silver nanoparticles, according to Benzie and Strain method. The FRAP reagent was prepared using 300mM acetate buffer (3.1g Sodium acetate, and 16mL Acetic acid) at pH 3.6, 10mM TPTZ (2,4,6-tripyridyl -s-triazine) solution in 40mM hydrochloric acid solution, and 20mM FeCl3.6H2O solution in distilled water. The acetate buffer (25mL) and TPTZ (2.5mL) were mixed together with FeCl3.6H2O (2.5mL). The temperature of the solution was adjusted to 37˚C before it was used. Aqueous silver nanoparticles (40µL) were allowed to react with the FRAP solution (3mL) for 30min under dark conditions. The absorbance was measured at 593nm.The standard curve was linear between 200 and 1000 µM $FeSO_4$. Results were expressed in µM Fe (II)/g dry mass and compared with ascorbic acid as a standard.

H_2O_2 scavenging activity

H_2O_2 scavenging ability of synthesized AgNaPs was carried out by Nabavi *et al*. method with slight modifications[31]. H_2O_2 solution (40 mM) was prepared in phosphate buffer (pH 7.4). The *Brassica oleracea L.* methanolic extract mediated biosynthesized AgNaPs (1mL) in phosphate buffer (3.4 mL) was added to H_2O_2 solution (0.6 mL; 40 mM). Blank solution contained the phosphate buffer without H_2O_2. The absorbance of the silver nanoparticles was measured at 230 nm. Rutin was used as positive control. The percentage of H_2O_2 scavenging of silver nanoparticles was calculated using the following formula

$$\%\ H_2O_2sc = (A0 - A1) \times 100/A0 \quad (2)$$

where *A*0 = absorbance of the control; *A*1 = absorbance of the sample

Antimicrobial studies of silver nanoparticles

Biosynthesized silver nanoparticles antibacterial and antifungal activities were analyzed by well diffusion and disc diffusion methods respectively. Wells (3mm diameter) were prepared in the medium using sterile gel puncture. Overnight bacterial cultures were spread on the petri plate containing Nutrient agar medium using L-rod. Then 10µL of antibacterial solution and the 10µL of silver nanoparticles were added to the well. Wells with antibacterial solution alone were served as positive controls. The petri plates were incubated at 37˚C for 24h. For antifungal activities using stokes disc diffusion sensitivity testing technique; an inoculum containing fungal cells was applied onto Potato dextrose agar (PDA) plates. The samples (AgNaPs) and the control (antifungal solutions) of 10µL were loaded onto different filter paper disc (3mm diameter) prepared from Whatman No 1: filter paper. The discs were then placed on the PDA medium containing fungal cultures and incubated for 48h at 37°C. Each experiment was carried out in triplicates and diameter of the zone of inhibition was measured.

Cytotoxicity assay

The cytotoxicity effect of silver nanoparticles on cancer cells were examined by MTT assay (Ostad, *et al.*, 2010). Monocultures of the MDA-MB-231 and HeLa cell lines were incubated with increasing concentrations of sterilized silver nanoparticles for 24, 48, 72h and the cell viability was measured by MTT dye conversion assay. At least three independent experiments were conducted and six replicate wells were employed per concentration per plate in each independent experiment. Cells (1×105cells/mL in 100µL/well) were seeded in 1mL of minimal essential medium with 10% FBS/well in a 24- well plate (Gibco Company, Canada). After 24h of growth, the medium was replaced with the serum free medium that contained varied concentrations of silver nanoparticles (7.8-1000µg/mL). The medium was removed after 24, 48 and 72h of treatment and cells were washed with phosphate-buffered saline (PBS, 0.01M, pH-7.4) . This was followed by addition of 200µL (5mg/mL) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide cells (MTT) prepared in serum free medium solution to each well and incubated for 4h at 37°C in a 5% CO2. After 4h of incubation the medium was removed and 100µL of MTT fixative solution (isopropanol with 0.04 M HCl) was added. Viable cells were determined by the absorbance at 570 nm using UV-visible spectrophotometer. Wells containing cells not exposed with silver nanoparticles were taken as blanks. The cell viability was calculated with the following equation:

$$\text{Viability (\%)} = At/Ac \times 100 \quad (3)$$

where At, Ac are mean absorbance of silver nanoparticles for treated and control cells respectively, (n = 5; where n is the no. of independent experiments).

Statistical analysis

Values are expressed as mean ± standard deviation of at least three replicates of each experiment. Data within the groups are analyzed using one-way analysis of variance (ANOVA). Values were considered statistically significant when $p < 0.05$. Statistical analyses were carried out using SPSS statistics version 20 software.

RESULTS AND DISCUSSION

Green synthesis of silver nanoparticles

The green chemistry approach of biosynthesis of silver nanoparticles is a biocompatible eco-friendly method to produce environmentally sustainable nanoparticles. Although the exact mechanism of synthesis of silver nanoparticles using methanol extract of *Brassica oleracea L.* is not clear, results of current experimental studies suggest that presence of polyphenolic compounds and flavonoids may be responsible for reduction of silver ions (Ag^{3+}) to silver nanoparticles (Ag^0). The formation and stability of the silver nanoparticles was confirmed by monitoring colour change from original colorless solution into reddish brown colour. Fig.1 shows the colour intensity of the methanol extract of *Brassica oleracea* L. incubated with silver nitrate solution at the beginning and after 4h of reaction.

Fig. 1. Cauliflower leaves, 5% v/v *Brassica oleracea* L extract, 1mM silver nitrate solution and silver nanoparticles with reddish brown colour

UV-visible spectrophotometry analysis

UV–visible absorption spectrophotometry is one of the important techniques to ascertain the formation of metal nanoparticles in aqueous solution. For biosynthesized silver nanoparticles maximum absorption range (λmax) was observed at 430nm (Fig. 2). The reddish brown colour observed is characteristic for unique surface plasmon resonance (SPR) of silver nanoparticles. The optical absorption spectra of metal nanoparticles are dominated by surface plasmon resonances (SPR), which shift according to the particle size and shape. Fig. 3 shows the kinetics of the formation of silver nanoparticles using 5% v/v methanol extract of *Brassica oleracea* L. in 1mM silver nitrate solution.

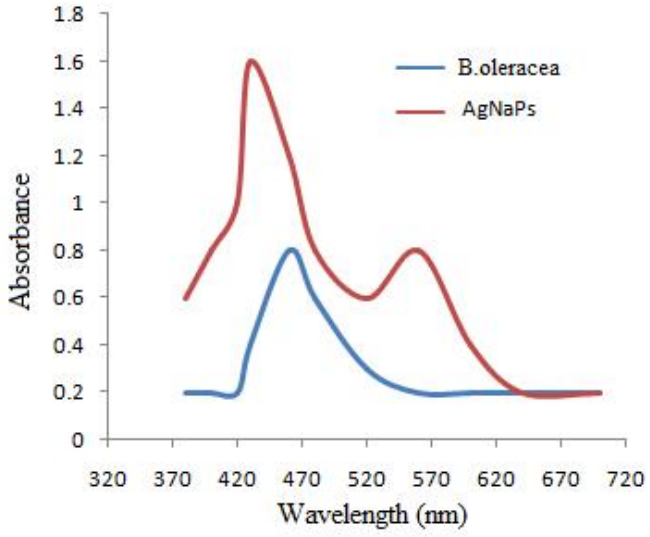


Fig. 2. UV-visible absorption spectra of AgNaPs synthesized by reacting 1mM silver nitrate aqueous solution with 5%v/v methanol extract of *Brassica oleracea* L. leaves

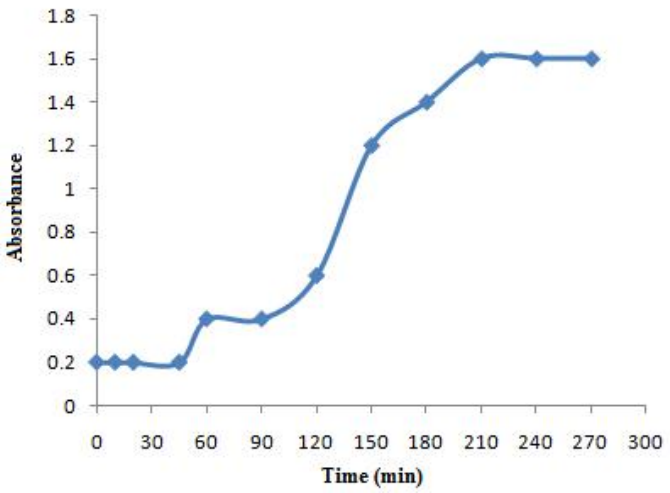
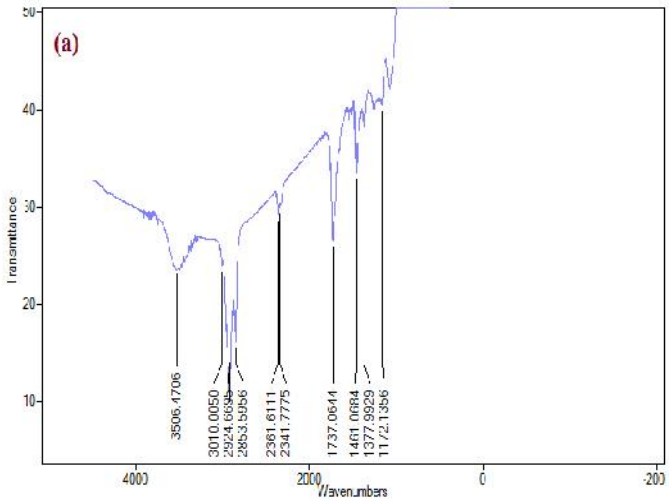


Fig. 3. Kinetics of the formation of silver nanoparticles with 5% v/v methanol extract of *Brassica oleracea* L. leaves extract in 1mM silver nitrate solution

FTIR analysis of silver nanoparticles

The FTIR spectra of methanol extract of *Brassica oleracea* L. and biosynthesized silver nanoparticles are shown in Fig.4 (a b). The FTIR spectra of lyophilized silver nanoparticles were observed to posses prominent peaks (*V*max) at 3459-, indicating the presence of O-H stretching of carboxyl groups and N-H stretching of secondary amides; 2353- C-H stretching bonds; 1575- represent the bonds with C-N stretching, N-H deformation, COO anions and C=C aromatic conjugates; 1412, 1343- C=O groups from aromatic rings having conjugation; 1023,936- bending vibrations of the C-OH alcoholic group and C-O single bond vibrations of ether linkage; 678-assigned to C-H out of plane bending vibrations substituted ethylene systems –CH=CH(cis). Based on the previous literature, the functional groups C-O, C-OH, C=O, N-H and COO from amino acids and proteins has strong affinity to find metals to produce highly stable nanoparticles (Wu and Chen, 2010). The plant extract and the nanoparticles have nearly similar peaks as our result suggests. An FTIR result thus indicates the presence of some biomolecules which may be responsible for the reduction and stabilization of silver nanoparticles.



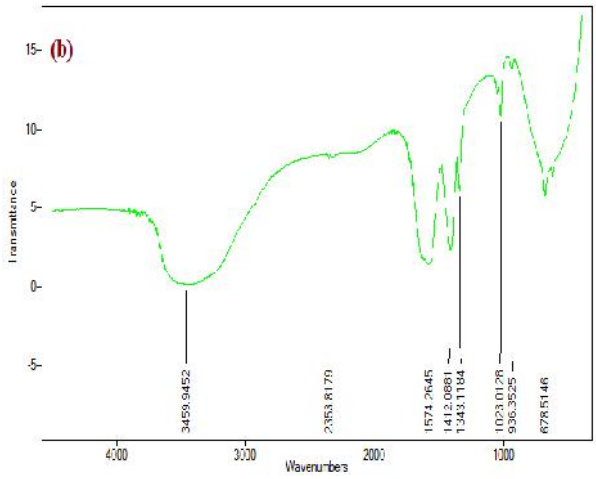


Fig. 4. FTIR spectra of (a) methanol extract of *Brassica oleracea* L. (b) biosynthesized silver nanoparticles

X-Ray diffraction analysis

The X-ray diffraction pattern (XRD) of synthesized silver nanoparticles is shown in Fig 5. The characteristic Bragg's reflections with 2θ values of 38.11, 45.25, 65.08 and 78.5 which correspond to the (111), (200), (220) and (311) sets of lattice planes which are indexed to the face-centered cubic (fcc) structures for silver. The XRD pattern thus clearly shows that the silver nanoparticles are formed by the reduction of silver ions (Ag^{3+}) by biomolecules present in the methanol extract of *Brassica oleracea* L. leaves. The result indicates that the biosynthesized silver nanoparticles were crystalline in nature. The size of the silver nanoparticles was calculated by Debye-Scherrer's equation.

$$D = \frac{K^{a}}{{}_{s}\cos^{-}} \qquad (4)$$

Where, D: crystallite size, K: size-dependent Debye-Scherrer's constant (0.94 for spherical particles), λ: incident X-radiation wavelength (1.548 ˚A) and *βs*: full peak width at half maxima. Average size of the synthesized silver nanoparticles is calculated to be 20nm (Equation 4).

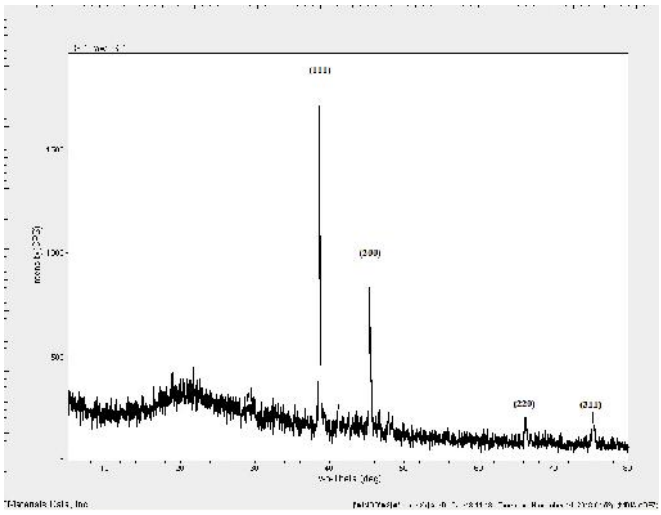


Fig. 5. XRD pattern of lyophilized silver nanoparticles

Scanning Electron Microscopy- Energy Dispersive X-ray (SEM-EDX)

Figure 6 (a, b, c) show the SEM images of the biosynthesized silver nanoparticles. The overall morphological shapes of the silver nanoparticles are spherical at higher magnifications. The average sizes of the silver nanoparticles were 40-80nm. Energy Dispersive X-ray (EDX) analysis of silver nanoparticles provided in Fig. 7 observed strong signals for elemental silver. The silver nanocrystallites display an optical absorption band peaking at 2.15 keV. EDX analysis suggested that reduction of silver ions into elemental silver.

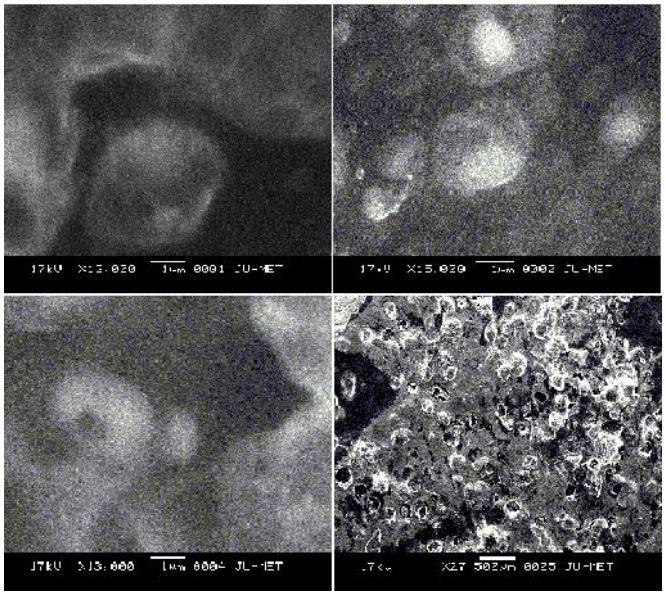
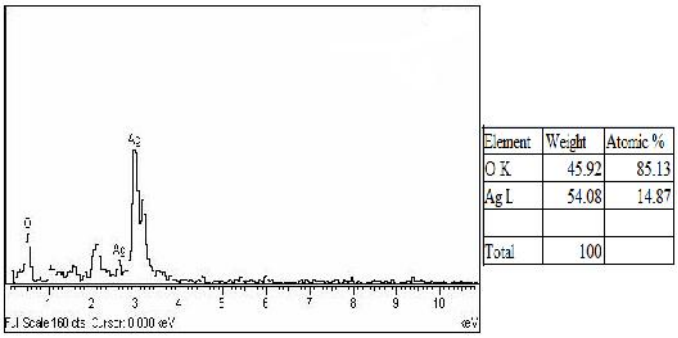


Fig. 6. SEM images of 5% v/v *Brassica oleracea* L. extract mediated synthesis of silver nanoparticles



Element	Weight	Atomic %
O K	45.92	85.13
Ag L	54.08	14.87
Total	100	

Fig. 7. EDX pattern of silver nanoparticles synthesized with 5%v/v *Brassica oleracea* L. extract in aqueous 1mM silver nitrate solution

High resolution Transmission electron microscope (HRTEM)

Transmission electron microscopy experiment proved the formation of silver nanoparticles, shown in Fig 8 (a, b, c and d). Most of the silver nanoparticles were spherical, triangular and hexagonal in nature. The obtained nanoparticles were quite uniform in size and up to 10-25nm. Furthermore, the selected area electron diffraction (SAED) pattern (Fig.9) proved that the silver nanoparticles are single crystalline in nature. The spots are indexed according to face-centered cubic (fcc) structure of silver nanoparticles.

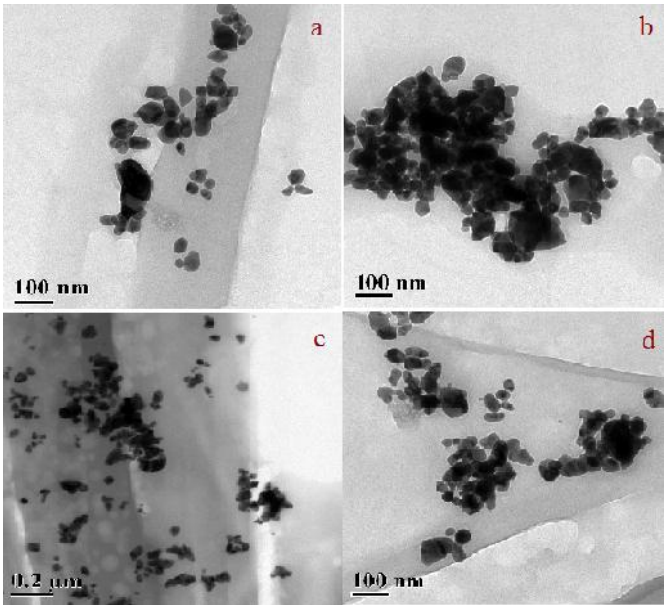


Fig. 8. HRTEM images of silver nanoparticles

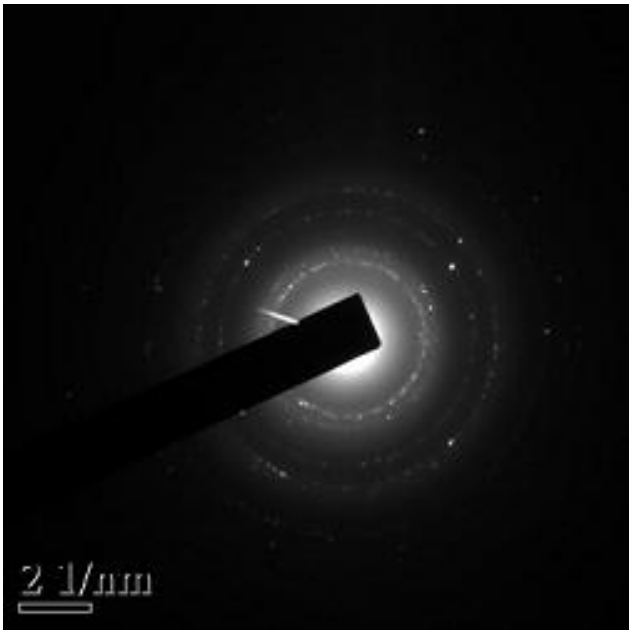


Fig. 9. SAED pattern of synthesized silver nanoparticles

Thermo gravimetric analysis

The TG-DTA (Thermo gravimetric-Differential thermal) plot of the silver nanoparticles prepared using methanol extract of Brassica oleracea L. is given in Fig.10. The powdered silver nanoparticles were subjected to heating from 50 to 800˚C. The thermogram showed a multiple weight loss in the temperature ranges from 50-70˚C, 220-360˚C, 360-5400˚C and 540-770˚C. The weight loss of the nanoparticles powder due to desorption of bioorganic compounds in the biosynthesized silver nanoparticles was 51%. This indicates the presence of high amount of bioorganic compounds in the biosynthesized silver nanoparticles.

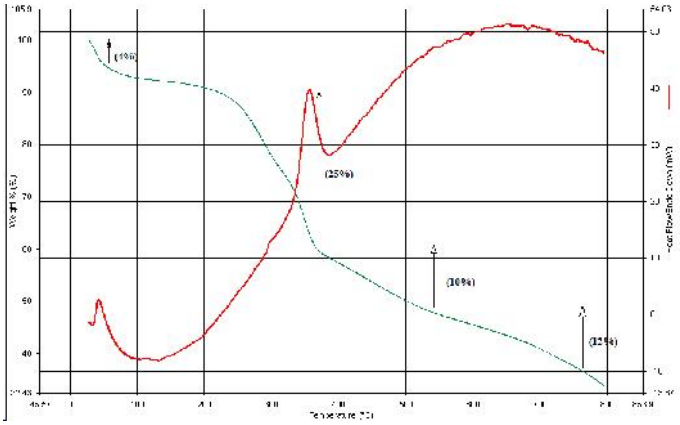


Fig. 10. TG-DTA thermogram of silver nanoparticles

Determination of antioxidant activities of silver nanoparticles

The methanol extract of *Brassica oleracea* L. leaves mediated synthesized silver nanoparticles was subjected to screening for their antioxidant activity. DPPH is a purple-colored stable radical of organic nitrogen with a maximum absorbance at 517 nm and it is widely used to study radical scavenging activities of extracts, pure compounds and nanoparticles. When the odd electron becomes paired off in the presence of a free radical scavenger to form hydrazine, the absorption reduces and the DPPH solution is decolorized from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the nanoparticles. Free radical scavenging capacity of the *Brassica oleracea L.* leaves mediated synthesized silver nanoparticles, measured by DPPH assay, is 78.76 ± 1.39%. The results are in agreement with the content of flavonoids found in the surface of the silver nanoparticles. The ferric reducing ability of biosynthesized silver nanoparticles was of the range of 802.03 ± 5.3µm of FeSO4/mg. The ferric reducing potential of the biosynthesized silver nanoparticles was estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The FRAP values for the silver nanoparticles were significantly lower than those of L-ascorbic acid. Scavenging ability of hydrogen peroxide by silver nanoparticles solution may be attributed to the flavonoids present in the surface of the silver nanoparticles, which can donate electrons to H2O2, to form water. The ability of the silver nanoparticles to effectively scavenge hydrogen peroxide, are compared with that of standard Rutin. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. Scavenging capacities of the silver nanoparticles, measured by H2O2 assay, are 81.13 ± 2.30%.

Determination of antimicrobial activities of silver nanoparticles

The anti-microbial activity of AgNaPs, were compared with the standard antimicrobial drugs (control). The zone of inhibition for standard drugs and silver nanoparticles over bacterial and fungal species were shown in Table 1. Ampicillin, penicillin, cepifime and fluconazole were used for anti-microbial studies against *S. aureus*, *E. coli*, *Klebsiella Sp.*, *P. aeruginosa*, *B. subtilis* and *S. typhii* and fungal cultures such as *A. niger*, *A. flavus* and *C. albicans.* The silver nanoparticles were showed considerable inhibitory action against all bacterial and fungal species (*A. niger*), except *A. flavus*, *C. albicans.*

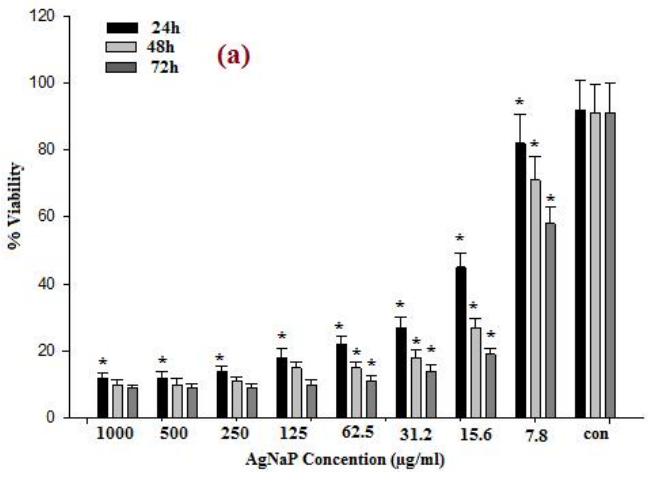
Table 1. Antimicrobial activity of the silver nanoparticles synthesized from methanol extract of *Brassica oleracea* L.

Organisms		Diameter zone of inhibition (mm)			
		Antibiotics			
		Ampicillin	Penicillin	Cefipime	Fluconazole
S. aureus	Control	12.68±0.45	14.65±0.51	16.44±0.43	-
	AgNaPs	13.32±0.54	13.32±0.54	13.32±0.54	-
E. coli	Control	14.21±0.49	14.6±0.48	16.02±0.52	-
	AgNaPs	13.16±0.58	13.16±0.58	13.16±0.58	-
Klebsiella Sp.,	Control	13.76±0.57	16.26±0.57	17.02±0.61	-
	AgNaPs	12.28±0.61	12.28±0.61	12.28±0.61	-
P. aeruginosa	Control	11.98±0.38	13.45±0.42	15.28±0.52	-
	AgNaPs	12.62±0.51	12.62±0.51	12.62±0.51	-
B. subtilis	Control	12.08±0.38	15.16±0.44	15.66±0.54	-
	AgNaPs	12.67±0.55	12.67±0.55	12.67±0.55	-
S. typhii	Control	14.21±0.40	16.10±0.51	17.85±0.54	-
	AgNaPs	13.34±0.55	13.34±0.55	13.34±0.55	-
A. niger	Control	-	-	-	18.52±0.42
	AgNaPs	-	-	-	14.34±0.46
A. flavus	Control	-	-	-	16.21±0.32
	AgNaPs	-	-	-	6.63±0.32
C. albicans	Control	-	-	-	14.56±0.43
	AgNaPs	-	-	-	4.58±0.46

Values are listed as mean± SD of three independent experiments.

Determination of cytotoxicity activities

The *in vitro* cytotoxicity effects of silver nanoparticles were screened against cancer cell lines, and viability of tumor cells was confirmed by MTT assay. The silver nanoparticles were able to reduce viability of the cells in a dose-dependent manner, as shown in Fig 11(a) and (b). The viability was found to be decreasing with increasing exposure time in both the cell lines. The number of HeLa cells were slightly higher as compared to MDA-MB-231 cells after 24h exposure, but then again get lowered after 48h and 72h exposure time. After 24h treatment, the silver nanoparticles at concentration 500µg/mL decreased the viability of HeLa cells to be 60% of the initial level and longer exposures resulted in additional toxicity to the cells. The result revealed that *B. oleracea* L. leaves mediated synthesized silver nanoparticles possess great selectivity to cancer cell and can display potential application in cancer chemoprevention and chemotherapy. In summary, it has been demonstrated that cauliflower leaves extract could be used to synthesize silver nanoparticles, which could be a better alternative to chemical synthesis, which is simple, biocompatible and environmental friendly.



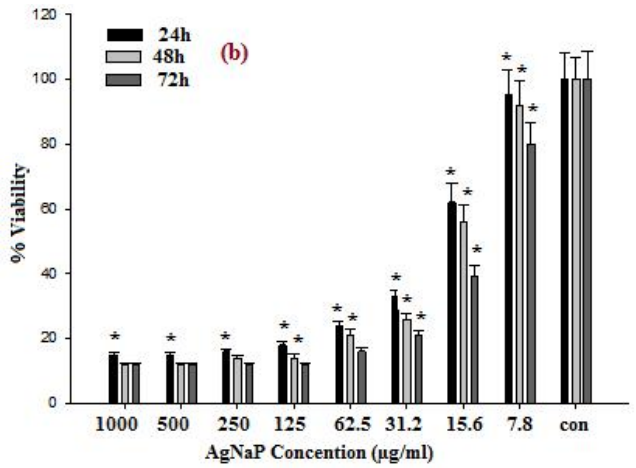


Fig. 11. Cytotoxicity of AgNaPs after 24, 48 and 72h exposures determined by MTT assay. (a) in MD-MBA-231 cells (b) in HeLa cells. Data were expressed as percent of control mean±SD of three independent experiments.*denotes a statistically significant ($p < 0.05$) difference from the unexposed control

Further, the biosynthesized silver nanoparticles showed good antimicrobial, antioxidant and possessed considerable cytotoxicity effect against human breast cancer cell lines (MDA-MB-231 and HeLa). From the data, one can affirm that waste cauliflower leaves can play an important role in the bioreduction and stabilization of silver ions to silver nanoparticles. Further studies are, however, required to investigate the clear mechanisms involved in the antimicrobial, antioxidant and anticancer activities of silver nanoparticles, and that could lead to the development of novel drugs for human welfare in future.

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REFERENCES

Ahmad, A., Mukherjee, P., Mandal, D., Senapati, S., Khan, M.I., Kumar, R and Sastry, M. 2002. Enzyme mediated extracellular synthesis of CdS nanoparticles by the fungus, *Fusarium oxysporum*. *Journal of the American Chemical Society.*, 124:12108-12109.

Ashok, B., Bhagyashree, J., Ameeta, R and Smita, Z. 2010. Banana peel extract mediated novel route for the synthesis of silver nanoparticles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects.*, 368:58-63.

Awwad, A.M., Salem, N.M and Abdeen, A.O. 2013.Biosynthesis of silver nanoparticles using Loquat leaf extract and its antibacterial activity. *Advanced Materials Letter.*, 4:338-342.

Benzie, I.F.F and Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" the FRAP assay. *Analytical Biochemistry.*, 239:70–76.

Cheng, D., Yang, J and Zhao, Y. 2004. Antibacterial materials of silver nanoparticles application in medical appliances and appliances for daily use. *Chinese Medical Equipment Journal.*, 4: 26–32.

Chowdhury, A., Selvaraj, K., Bhattacharjee, C and Chowdhury, R. 2013.Optimization of the solvent extraction of phenolics and antioxidants from waste Cauliflower leaves (*Brassica oleracea* L.) using Response Surface Methodology (RSM). *International Journal of Biological Sciences and Technology.*, 5:4-12.

Christensen, L., Vivekanandhan, S., Mishra, M and Mohanty, A.K. 2011. Biosynthesis of silver nanoparticles using *Murraya koenigii* (curry leaf): An investigation on the effect of broth concentration in reduction mechanism and particle size. *Advanced Materials Letter.*, 2:429-434.

Cognet, L., Tardin, C., Boyer, D., Choquet, D., Tamarat, P and Lounis, B. 2003. Single metallic nanoparticle imaging for protein detection in cells. *Proceedings of the National Academy of Science U.S.A.*, 100:11350–11355.

Esumi, K., Tano, T., Torigoe, K and Meguro, K. 1990. Preparation and characteristic of biometalic Pd-Cu colloids by Thermal decomposition of their acetate compounds in organic solvents. *Journal of Materials chemistry.*, 2:564-567.

Farkas, J., Peter, H., Christian, P., Gallego Urrea, J. A., Hassellov, M., Tuoriniemi, J., Gustafsson, S., Olsson, E., Hylland, K and Thomas, K.V. 2011.Characterization of the effluent from a nanosilver producing washing machine. *Environment International journal.*, 37:1057-1062.

Guo, J.T., Lee, H.L., Chiang, S.H., Lin, F.I and Chang, C.Y. 2001. Antioxidant properties of the extracts from different parts of broccoli in Taiwan. *Journal of Food and Drug Analysis.*, 9:96-101.

Heim, K.E., Taigliaferro, A.R and Bobilya, D.J. 2002. Flavonoids antioxidants: Chemistry, metabolism and structure- activity relationships. J*ournal of Nutritional Biochemistry.*, 13:572-584.

Henglein, A. 1993. Physicochemical properties of small metal particles in solutions: Microelectrode reactions, chemisorptions, composite metal particles, and the atom-to-metal transition. *Journal of Physical Chemistry B.*, 97:5457-5471.

Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X., Wang, H., Wang, Y., Shao, W and He N. 2007.Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnology.*, 18:1–11.

Jain, D., Daima, H.K., Kachhwaha, S and Kothari, S.L. 2009. Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. *Digest Journal of Nanomaterials Biostructures.*, 4:723-727.

Kaviya, S., Santhanalakshmi, J and Viswanathan, B. 2012. Biosynthesis of silver nano-flakes by *Crossandra infundibuliformis* leaf extract. *Materials Letter.*, 67:64-66.

Kris-Etherton, P.M., Hecker, K.D, Bonanome, A, Coval, S.M., Binkoski, A.E., Hilpert, K.F., Grie, A.E and Etherto, T.D. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine.*, 113:71S-88S.

Krishnaraj, C., Jagan, E.G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P.T and Mohan, N. 2010. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antimicrobial activity against water borne pathogens. *Colloids Surfaces B: Biointerfaces Journal.*, 76:50–56.

Krishnaraj, C., Jagan, E.G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P.T and Mohan, N. 2010. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. *Colliods and Surfaces B: Biointerfaces.*, 76:50-56.

Lee, J., Kim, Y.H., Zhou, H., Hwang, S., Koh, K., Han, W.D and Lee J. 2011.Green synthesis of phytochemical-stabilized Au nanoparticles under ambient conditions and their biocompatibility and antioxidative activity. *Journal of Materials Chemistry.*, 21: 13316–13326.

Moazeni, M., Rashidi, N., Shahverdi, A.R., Noorbakhsh, F and Rezaie, S. 2012. Extracellular production of silver nanoparticles by using three common species of dermatophytes: Trichophyton rubrum, Trichophyton mentagrophytes and Microsporum canis. *Iranian Biomedical Journal.*, 16:52–58.

Nabavi, S.M., Ebrahimzadeh, M.A., Nabavi, S.F., Hamidinia, A and Bekhradnia, A.R. 2008. Determination of antioxidant activity, phenol and flavonoid content of *Parrotia persica* mey. *Pharmacologyonline.*, 2:560–567.

O'Neal, D.P., Hirsch, L.R., Halas, N.J., Payne, J.D and West J.L. 2004. Photo-thermal tumor ablation in mice using near infrared-absorbing nanoparticles. *Cancer Letter.*, 209:171–176.

Ostad, S.N., Dehnad, S., Nazari, Z.E., Fini, S.T., Mokhtari, N., Shakibaie, M and Shahverdi, AR. 2010. Cytotoxic activities of silver nanoparticles and silver ions in parent and Tamoxifen-resistant T47D human breast cancer cells and their combination effects with Tamoxifen against resistant cells. *Avicenna Journal of Medical Biotechnology.*, 2: 187-196.

Pileni, M.P. 2000. Fabrication and physical properties of self-organised silver nanocrystals. *Pure and Applied Chemistry.*, 72:53-65.

Rai, M., Yadava, A and Gadea, A. 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances.*, 27:76-83.

Ramadan, M.F., Kroh, L.W and Morsel, J.T. 2003. Radical scavenging activity of black Cumin (*Nigella sativa* L), Coriander (*Coriandrum sativum* L), and Niger (*Guizotia abyssinica* Cass.) crude seed oils and oil fractions. *Journal of Agricultural and Food Chemistry.*, 51:6961–6969.

Ramteke, C., Chakrabarti, T., Sarangi, B.K and Pandey, R.A. 2013. Synthesis of silver nanoparticles from the aqueous extract of leaves of *Ocimum sanctum* for enhanced antibacterial activity. *Journal of Chemistry.*, 1-7.

Sathyavathi, R., Krishna, M.B., Rao, S.V., Aritha, R and Rao, D.N. 2010. Biosynthesis of silver nanoparticles using *Coriandrum sativum* leaf extract and their application in nonlinear optics. *Advanced Science Letters.*, 3:1-6.

Sulaiman, G.M., Mohammed W.H., Marzoog T.R., Amir Al-Amiery A.A., Amir H. Kadhum A and Mohamad, A.B. 2013. Green synthesis, antimicrobial and cytotoxic effects of silver nanoparticles using *Eucalyptus chapmaniana* leaves extract. *Asian Pacific Journal Tropical Biomedicine.*, 3:58-63.

Vijayaraghavan, K., Nalini, S.P.K., Prakash, U.N and Madhankumar, D. 2012. One step green synthesis of silver nano/microparticles using extracts of *Trachyspermum ammi* and *Papaver somniferum*. *Colliods and Surfaces B: Biointerfaces.*, 94:114-117.

Wettasinghe, M and Shahidi F. 1999. Antioxidant and free radical scavenging properties of ethanolic extracts of defatted borage (*Borago officinalis* L.) seeds. *Food Chemistry.*, 67:399-414.

Wu, C.C and Chen, D.H. 2010.Facile green synthesis of gold nanoparticles with gum arabic as a stabilizing agent and reducing agent. *Gold Bulletin.*, 43:234-240.

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