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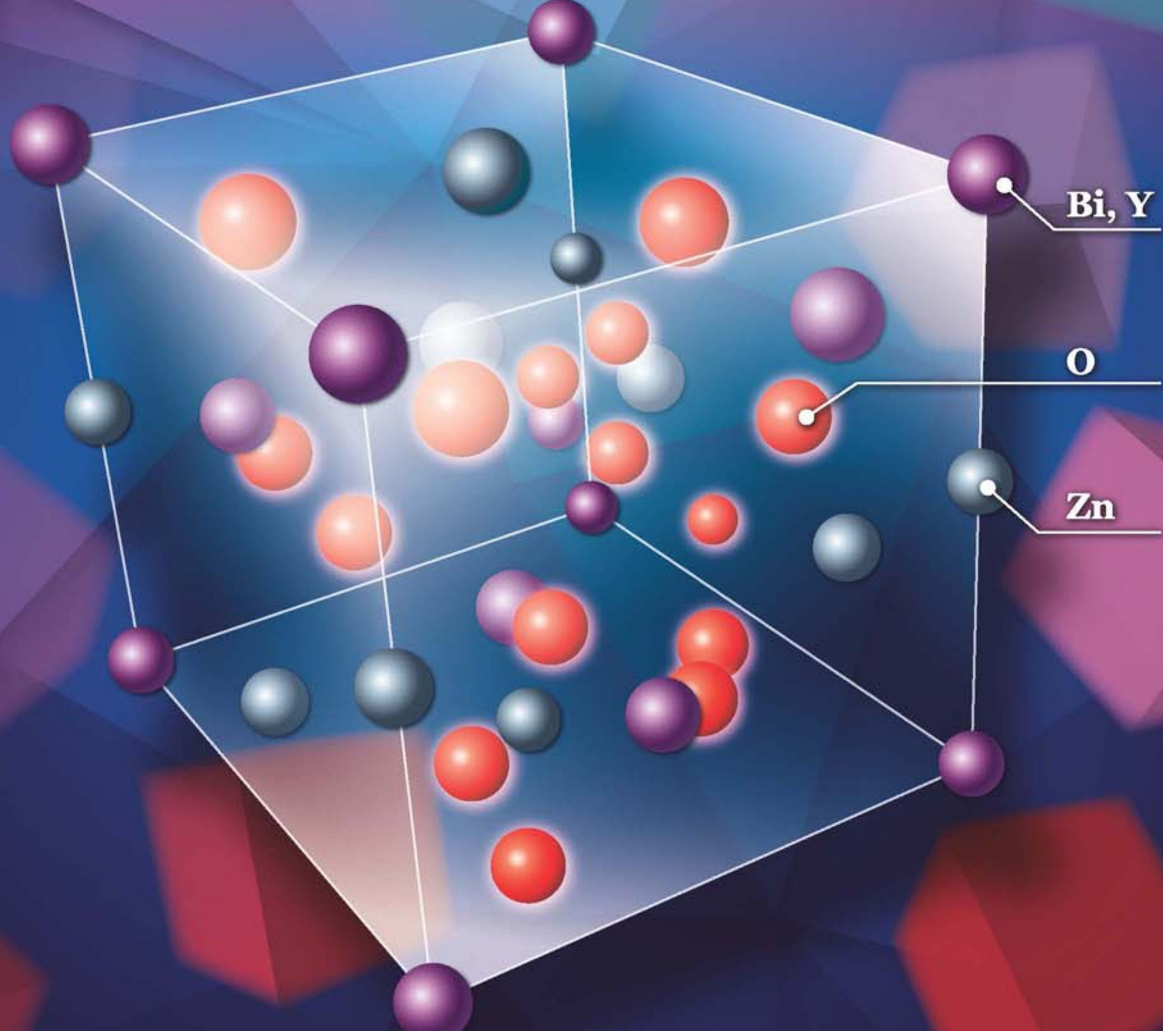

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Intracellular biosynthesis of Au and Ag nanoparticles using ethanolic extract of *Brassica oleracea* L. and studies on their physicochemical and biological properties

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ABSTRACT

In this present study, we reported broccoli (*Brassica oleracea* L.) as a potential candidate for the synthesis of gold and silver nanoparticles (NPs) in green chemistry method. The synthesized metal nanoparticles are evaluated their antimicrobial efficacy against different human pathogenic organisms. The physico-chemical properties of gold nanoparticles were analyzed using different analytical techniques such as a UV–Vis spectrophotometer, Field Emission Scanning Electron Microscopy, energy dispersive X-ray spectroscopy, X-ray diffraction and a Fourier Transform Infrared spectrophotometer. In addition, gold and silver NP antimicrobial efficacy was checked by disc diffusion assay. UV–Vis color intensity of the nanoparticles was shown at 540 and 450 nm for gold and silver nanoparticles respectively. Higher magnification of the Field Emission Scanning Electron Microscopy image shows the variable morphology of the gold nanoparticles such as spherical, rod and triangular shapes and silver nanoparticles were seen in spherical shapes. The average spherical size of the particles was observed in 24–38 nm for gold and 30–45 nm for silver NPs. X-ray diffraction pattern confirmed the presence of gold nanoparticles and silver nanoparticles which were crystalline in nature. Additionally, the functional metabolites were identified by the Fourier Transform Infrared spectroscopy. IR spectra revealed phenols, alcohols, aldehydes (sugar moieties), vitamins and proteins are present in the broccoli extract which are accountable to synthesize the nanoparticles. The synthesized gold and silver NPs inhibited the growth of the tested bacterial and fungal pathogens at the concentration of 50 μg/mL respectively. In addition, broccoli mediated gold and silver nanoparticles have shown potent antimicrobial activity against human pathogens.

Introduction

In recent years, in order to enhance the growth of industrial and commercial sector, the nanotechnology has produced numerous value added products for daily life purposes. Nanotechnology is a field that incorporates life sciences, and has become enriched with activities in nanomaterial synthesis and energy production. The environmental friendly route synthesis of nanoparticles has

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abundant pharmaceutical applications such as treating and diagnosis of acute and chronic diseases (Shankar et al., 2004). Generally, physical and chemical methods have good exposure for the synthesis of nanomaterials in large quantities as well for specific size and shape (Noguez, 2007). However, these synthetic methods have an adverse effect on living things as a result of using strong chemicals as reductants and stabilizing agents. Accordingly, the development of novel environmentally benign methods for the biosynthesis of nanoparticles is widely explored for medical, pharmaceutical, electronic devices, solar energy and other commercial value added products. Nanoparticles have widespread application in different arenas due to their novel physicochemical properties (Kumar et al., 2011). According to that gold and silver nanoparticles have been used as cytotoxic drugs, antioxidant, antiinflammatory and antimicrobial agents (John et al., 2008). These advanced materials/metals such as gold, silver, copper, zinc, platinum and palladium nanoparticles have been used in diverse applications such as electronics, super capacitors, biolabelling and catalysis for chemical reaction (Park et al., 2011). The biosynthesis of nanoparticles using plant extracts is well designed, easy, single step reaction, to synthesize desired size and shape of the nanoparticles. However, the biosynthesis method does not use any reductants and stabilizing agents in the bioreduction steps (Klaus et al., 1999; Carlson et al., 2008). Recently, many bacteria biological substances such as, fungi, mushroom and plants are a greater effort to nanoparticles synthesis in green chemistry methods were reported, also the biological mediated nanoparticles can control various acute and chronic diseases (Becker, 1999). Broccoli (*Brassica oleracea* L.) vegetables belong to *Brassica* family. It contains a high amount of vitamins, antioxidants and anticarcinogenic compounds. Also, broccoli contain glucosinolates, a diverse class of sulfur- and nitrogen-containing metabolites (Nowack, 2010). Due to these compounds, broccoli has potential chemo protective properties and controlling various viral and bacterial diseases. Therefore, to our knowledge, this is the first scientific report on the use of broccoli extract to synthesize gold and silver nanoparticles in a non-hazardous way and monitor their biocide activity against various human pathogenic microorganisms.

1. Materials and methods

1.1. *Preparation of ethanolic extract of broccoli*

The healthy and cruciferous vegetable broccoli (family: *Brassicaceae*) was purchased from the commercial market at Kuantan, Malaysia. The plant materials were washed thoroughly using mild warm water to remove contamination from insects and fungal dust. The broccoli was cut into small pieces and dried at room temperature at shadow condition. The completely dried broccoli was powdered by using a mechanical grinder. A 1-g of broccoli fine powder was added with 200 mL of 90% ethanol and keep into a shaker at 37°C at 12 hr. The extract was filtered using a 0.22 μm membrane filter and the extract was stored at 4°C for further experimental analysis.

1.2. *Biosynthesis of gold and silver nanoparticles*

For the synthesis of desired size and shapes of AuNPs, AgNPs the various amount of broccoli extract used and also we optimized different physico-chemical parameters such as time (1, 2, 3, 6, 12 and 24 hr), metal ion concentration (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} mmol). The mixture solution was continuously monitored the product growth kinetics by UV–Vis spectrophotometer in the ranges of 300–700 nm. However, a color change occurred in the mixture solution, which indicates that reduction is occurring. After complete reduction, the synthesize medium was centrifuged at 12,000 rpm for 20 min. The pellet was collected and dried in an oven at 45°C. The biosynthesis reaction was attained without using any catalytic chemicals and polymer as a stabilizing and capping agent (Narayanan and Sakthivel, 2008).

1.3. *Structural characterization of gold and silver nanoparticles*

The UV–Vis spectra of gold and silver nanoparticles were recorded using a T80 UV–VIS spectrometer, PG instruments Ltd., Beijing, China with a resolution path of 10 nm. The field emission scanning electron microscope JEOL, JSM 7800F USA, and attached energy dispersive X-ray detector ware used and analysis the structure and composition of biosynthesized nanoparticles. The powder X-ray diffraction data of gold and silver nanoparticles were measured by XRD-Rigaku mini flux II, Japan with a built in nickel monochromator. The operating X-ray tube radiation used Cu Kα (λ = 1.5415 A°, 30 kV, 15 mA). The ICDD-40784 powder diffraction database was used and evaluate the nature of nanoparticles. IR spectra were obtained from FT-IR Perkin Elmer spectrum 100 CT, USA. The powder Au and Ag nanoparticle samples were prepared in thin pellets using potassium bromide (KBr). The sample was read at a 400–4000 cm^{-1} wavelength range with a resolution of 4 cm^{-1}.

1.4. *Phytochemical characterization of ethanolic broccoli extract*

To evaluate the presence of different phytochemicals such as phenolics, alkaloids, flavonoids, saponins, and ascorbic acid in the broccoli aqueous extract by standard method prescribed by Harborne, (1998) and Lewis and Ausubel, (2006).

1.5. *Antibacterial assay for biosynthesized Au and Ag nanoparticles*

The antibacterial activity of gold and silver nanoparticles was determined by using the agar disk diffusion method with minor modifications. Authentic microbial strains were cultured on nutrient agar medium (recommended by Himedia, India) for 24 hr at 37°C. A 1 mL authentic bacterial culture was taken and poured into a marked agar plate. The bacterial cultures were equally spread into the agar plate using a glass L rod. Besides, the prepared different concentrations of biosynthesized gold and silver nanoparticle antibacterial discs were placed into the bacterial cultured agar plate. After 24 hr incubation, the zone of inhibition was measured. The results were compared with the amphicillin which is a positive control. Experiments were carried out in triplicates to avoid the statistical errors.

1.6. ***In vitro*** *antifungal assay of metallic Ag and Au nanoparticles*

The gold and silver NPs effectively inhibited the growth of the fungal strains at the optimum concentrations compared with a standard drug. Potato dextrose agar plates were prepared according to the manufacture guidelines (NCCLS, 1998). The sterilized PDA plate was swabbed by selected fungal cultures on the surface of the plates. After that, the prepared gold and silver nanoparticle disks (50 μg/mL) were placed in the PDA plate and kept for incubation for 3 days. After 3 days zone of inhibition was calculated and tabulated.

2. Results

2.1. Conformation of gold and silver NPs from UV–Vis spectra

The color reaction has proven the reduction of ionic $HAuCl_4$ and $AgNO_3$ into metallic Au and Ag nanoparticles. On this note, our studies showed that the gold and silver nanoparticles exhibit stable purple and yellow colors respectively. The UV–Vis spectra provide strong evidence of nanoparticle formation and thus a gradual increase in the absorbance values indicates the productivity growth in the synthesis medium. The metal nanoparticles such as gold and silver have free electrons which stimulate the surface plasmon resonance absorption band. Fig. 1 shows the UV–Visible spectra recorded at different time intervals of gold and silver nanoparticles. Despite the fact that, there are clear UV–Vis peaks at 550 and 435 nm for gold and silver respectively, it is observed that the SPR (surface plasmon resonance) bands increase as a function of time.

2.2. Morphological studies on biosynthesized Au and Ag nanoparticles using FESEM

Fig. 2 represents the FESEM micrograph of gold and silver nanoparticles. The FESEM images clearly depict gold nanoparticles having a uniform size with different shapes such as spherical, rod and triangular. Electron microscopic studies on AuNPs have shown higher spherical shape nanoparticles, nevertheless, very few triangular and rod shaped nanoparticles were also identified. Hence, most of the Ag nanoparticles were spherical in size for which the average size of the nanoparticles was found to be 30–45 nm. Also, the average size of the gold nanoparticles was distributed, ranging from 24–38 nm. Although, the energy dispersive X-ray spectroscopy (EDX) spectra of Au and Ag nanoparticles revealed a strong signal of metallic gold at 1.6–2.6 keV and silver at 2.6–3.6 keV, and the presence of oxygen, nitrogen and carbon in the EDX spectrum indicates that organic moieties exist in the extracellular broccoli extract. Thus, these organic substances are partially involved in the reduction of gold and silver metallic nanoparticles.

2.3. Crystalline nature of metallic Au and Ag nanoparticles studied by X-ray diffraction (XRD)

X-ray diffraction patterns of gold and silver nanoparticle results are shown in Fig. 3. All patterns specify the occurrence of four diffraction peaks observed for gold nanoparticles at 2θ = 38.24, 44.28, 64.58, and 77.56 and silver nanoparticles at 2θ = 38.3, 46.3, 57.45, and 77.31, which is indexed to the planes of (111), (200), (220) and (311) reflections of the fcc (face centered cubic) structure of crystalline gold and silver respectively. Additionally, XRD planes confirmed the crystalline nature of the particles, and the XRD pattern showed the number of Bragg's reflections that may be indexed on the basis of the fcc structure of gold and silver nanoparticles.

2.4. Functional characterization of AuNPs and AgNPs using infrared spectra

The Fourier Transform Infrared (FT-IR) spectra of gold and silver nanoparticles are depicted in Fig. 4. The strong bonds revealed at 3445.3 cm^{-1} (O–H stretching) can be assigned to alcohols and phenols; 2848.8 cm^{-1} H–C=O stretch, can be assigned to the presence of aldehyde group; the medium band 1739.3 cm^{-1} is assigned to C=O stretching of the aliphatic amines; and 1635.7 and 1524.7 cm^{-1} N–H bends, can be assigned to amide derivatives such as amino acid moieties and 1017.7 cm^{-1} C–O stretch indicates carboxylic acid. These functional metabolites are involved in the synthesis of gold and silver nanoparticles from broccoli bio-extract. Fig. 5

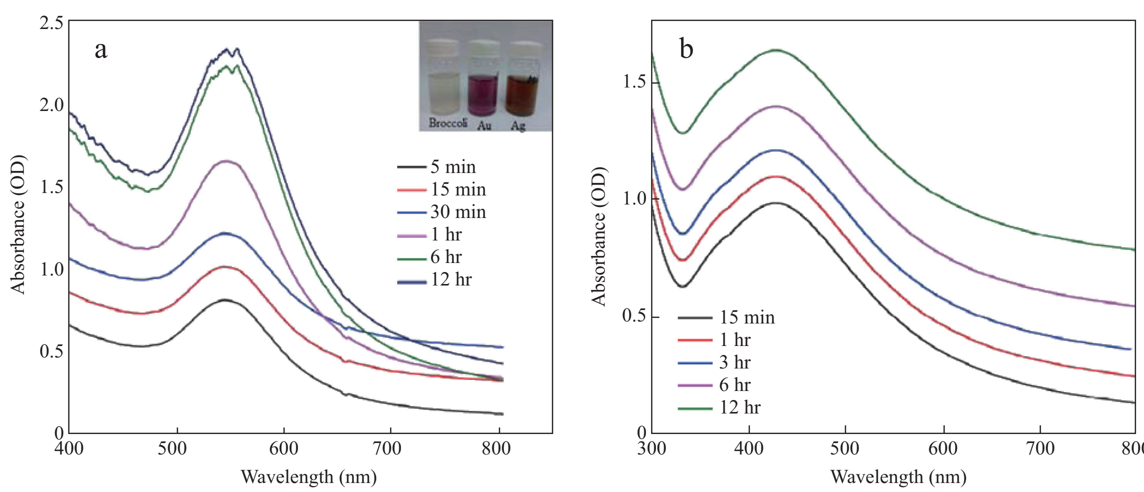

Fig. 1 – UV–Visible spectrum of synthesized gold and silver nanoparticles with function of time intervals. (a) Gold nanoparticles, (b) silver nanoparticles. Insect figure shows the color formation of gold and silver nanoparticles.

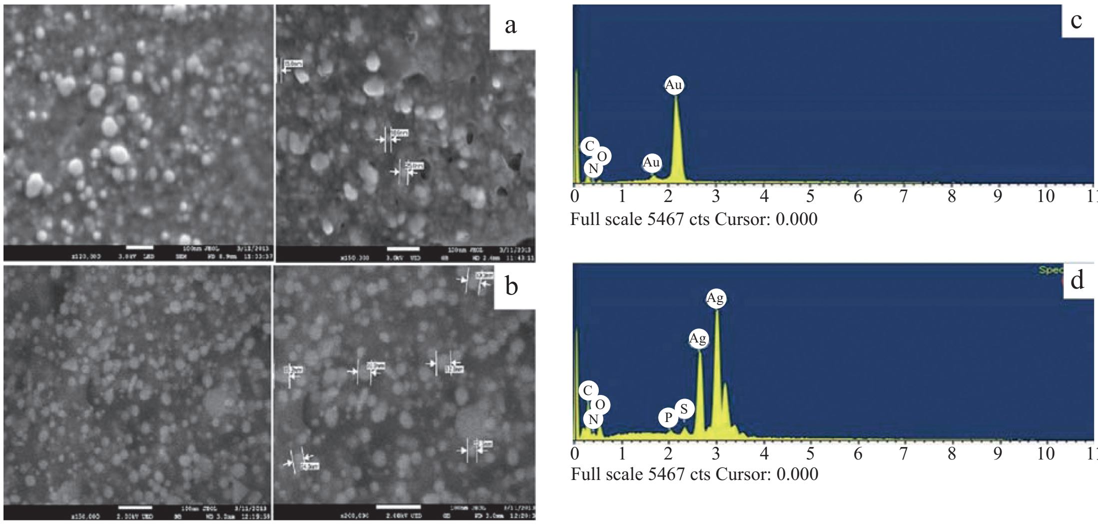

Fig. 2 – (a) and (b) FESEM images of the different morphologies of gold and silver nanoparticles at various magnifications, (c) EDX of AuNP spectrum, (d) EDX of AgNP spectrum.

shows the proposed mechanism of broccoli crude compounds involved in the production of gold and silver nanoparticles (Chen and Andreasson, 2001).

2.5. *Identification of phytochemical from ethanolic broccoli extract*

Table 1 illustrates the presence of various phytochemical constituents in the broccoli ethanolic extract. The screening of the bioactive compounds revealed that the alkaloids, ascorbic acid, phenolic compounds, flavonoids and saponins are present in the broccoli extract.

2.6. *Biosynthesized AuNPs and AgNPs for antimicrobial studies*

The bactericidal effect of gold and silver nanoparticles was determined by using the agar disk diffusion method. Recently the biosynthesized gold and silver nanoparticles are focused as novel antibiotics sources for treating various chronic microbial diseases. Different concentrations of nanoparticles disk (50 μg/mL) were prepared and placed in the experimental plates. In addition, a clear zone of inhibition was formed around the disk of control and test samples. The results of the synthesized gold and silver nanoparticles ZOI were calculated against *Salmonella typhi* at 9 and 11 mm respectively. The moderate activity was observed in *Escherichia coli* and *Staphylococcus aureus* and measured the inhibition rate at 8 and 9 mm of gold and silver nanoparticles and vice versa. Simultaneously the lowest activity of gold and silver nanoparticles showed *Bacillus subtilis* at 6 and 7 mm zone of inhibition rate respectively (Fig. 6). Likewise the control ampicillin exhibits moderate activity against the test organisms showed 8, 7 and 8 mm for *E. coli*, *B. subtilis* and *S. aureus* respectively. Thus, this is may be due to that cell wall of *B. subtilis* as a G^+ bacterium encompasses a thick peptidoglycan layer. Also, commercial antibiotics have also shown lots of drawbacks such as highly resistant, poor soluble, and less activity in target site and thus more quantity of the drug is needed to control the diseases. Table 2 shows the antifungal activity of AuNPs and AgNPs against two pathogenic fungal strains. Among these, the two fungus strains such as *Aspergillus* sp. and *Pneumocystis* sp. were used in the disc diffusion assay. In this study, the Au metallic nanoparticles show the higher zone of inhibition against *Aspergillus* sp. (6 mm) and silver nanoparticles exhibits *Pneumocystis* sp. (7 mm) respectively. Similarly the positive control fluconazole shows

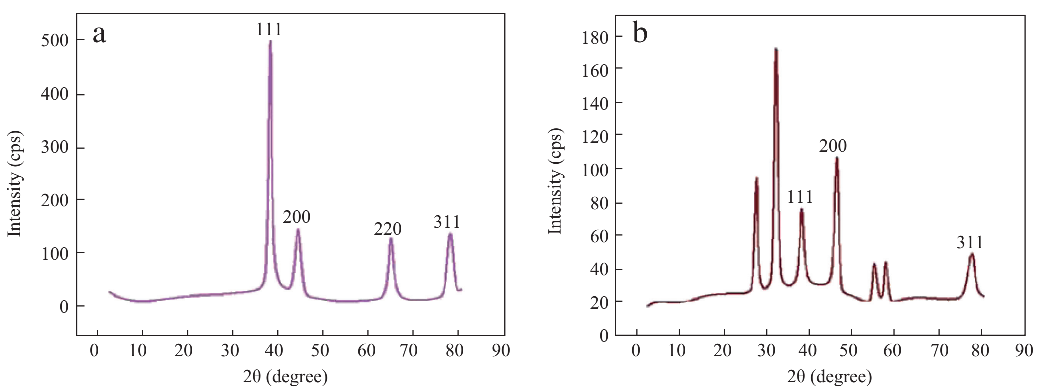

Fig. 3 – XRD pattern of crystalline (a) AuNPs and (b) AgNPs obtained by broccoli extract.

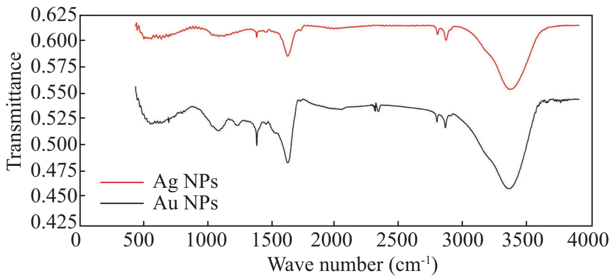

Fig. 4 – FT-IR spectra of gold and silver nanoparticles synthesized by broccoli extract.

Table 1 – Preliminary identification of phytochemicals from ethanolic extract of broccoli.

Series No.	Phytochemicals	Results obtained
1	Alkaloids	+
2	Flavonoids	+
3	Phenolic acids	+
4	Terpenoids	–
5	Saponins	+
6	Essential oils	–
7	Vitamin C	+

+: indicates presence; –: indicates absence.

equal activity (6 mm) against both fungal pathogens compared with metallic gold and silver nanoparticles.

Accordingly, nano-drug was developed as unique potential antimicrobial agents with minimal drug are enough to act on particular active sites of the pathogens. Therefore, these smaller sizes of particles can easily pass through the membrane channel and act as specific sites to control the cell division of the bacteria. Hence, our results clearly demonstrated that the broccoli extract mediated gold and silver nanoparticles act as powerful antimicrobial agents.

3. Discussion

Physico-chemical factors such as temperature, extract concentration and pH are controlling the nucleation, growth of nanoparticles and metal affinity with oxygen radicals. These typically controlled factors are producing smaller size nanoparticles as well. Gold and silver ions are reduced by the plant ingredients as a novel strategy for the synthesis of nanomaterials. However, the plant pigment from edible *Spinacia oleracea* extract reacts with tetrachloroaurate and silver nitrate solution to produce nanosized metals. Nevertheless, the silver nitrate mixture was turned from pale yellow to brown and gold solution was changed in ruby red color. Hence, this gradual changes in color indicates the formation of gold and silver nanoparticles (Bunghez et al., 2011).

Rajasekharreddy et al. (2010) evaluated non-edible plants *Tridax procumbens*, *Jatropha curcas* and *Calotropis gigantea* as suitable candidates for the nanoparticles synthesis in green chemistry method. The bragg diffraction peak at 2θ = 38.18°, 44.37°, 64.48° and 77.63° indexed to (111), (200), (220) and (311) orientations respectively, confirmed the crystalline nature of Ag nanoparticles. Likewise, there are different lattice planes observed in XRD studies, including (111), (200), (220) and (311); 2θ = 38.16°, 44.34°, 64.50° and 77.50° respectively. While the corresponding data of Au and Ag lattice planes are closely related to the standard metals, also the XRD peak distances for Au and Ag nanoparticles are relatively close to a small discrepancy (Dwivedi and Gopal, 2010; Philip and Unni, 2011). The FT-IR spectrum of gold nanoparticles reveals prominent absorption bands at 1017, 1229, 1511 and 1634 cm^{-1}. These organic peaks are indirectly explains secondary metabolites are potential source for the synthesis of metal nanoparticles. On the other hand, the IR spectrum of tulsi leaves synthesized silver nanoparticles were shown at 1027, 1318 and 1610 cm^{-1}. The C–H stretching and O–H functional groups are adapted from flavonoids, terpenoids and proteins. Therefore, the results confirmed that these biomolecules are responsible for efficient reductant and stabilizer compound (Morones et al., 2005; Mott et al., 2010).

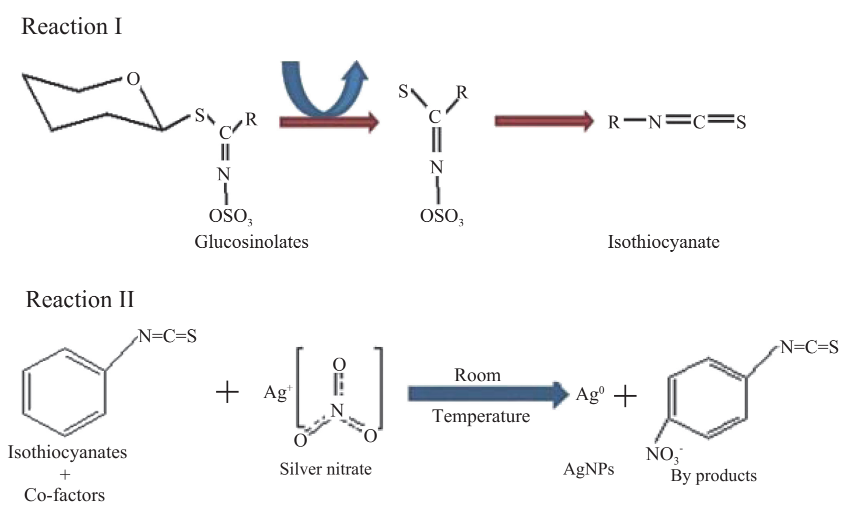

Fig. 5 – Reaction I (adapted from Chen and Andreasson (2001)) and reaction II explains the possible mechanism of metallic nanoparticles production using broccoli crude extract.

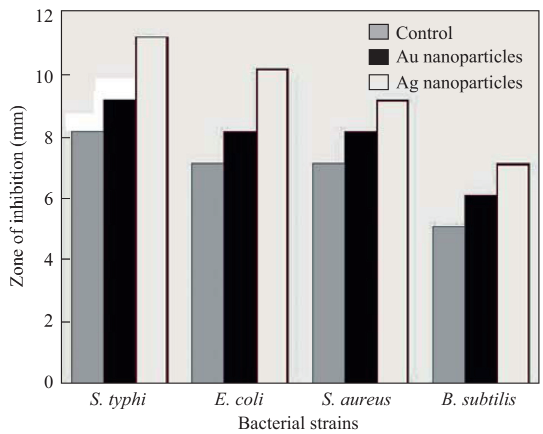

Fig. 6 – Antibacterial efficacy of broccoli mediated gold and silver nanoparticles (50 μg/mL respectively) against different pathogenic bacteria.

In the same manner, susceptibility of microorganisms has been studied using gold and silver nanoparticles at different concentrations against diverse pathogenic organisms. Similarly, MubarakAli et al. (2011) reported that silver nanoparticles have also greater antimicrobial action compared to the gold nanoparticles. Such bio-based silver nanoparticles play a vital role in pharmacological industries because of their extensive physicochemical properties. Additionally, the smaller size of nanoparticles has a larger surface area and high electric conductivity. These effects, hence improve the nanoparticle binding capacity with very high microbial entities compared to other synthetic drugs (Zhou et al., 2012). The green syntheses of AgNPs using *Podophyllum hexandrum* leaf extract were analyzed against human cervical carcinoma cells by various apoptosis assays. The *P. hexandrum* synthesized AgNPs can comparatively inhibit the over expression of HeLa cell activity and similarly induction caspase mechanism (Jeyaraj et al., 2013b). Likewise *P. hexandrum* plant extract biosynthesized crystalline gold nanoparticles have potential anticancereous properties against human cervical carcinoma cells (HeLa). Synthesized AuNPs have drastic induction of cell cycle arrest and DNA damage in HeLa cells. Moreover, it was proved that AuNP treated cells are undergoing apoptosis through the activation of caspase and mitochondrial dysfunction (Jeyaraj et al., 2014).

Similarly Jeyaraj et al. (2013a) reported the biogenic silver nanoparticles act against human breast cancer (MCF-7) cells. The silver nanoparticles synthesized using *Sesbania grandiflora* leaf extract as a reducing agent. Biologically synthesized AgNPs showed a cytotoxic effect against MCF-7 cell lines which was confirmed by MTT, AO-EB and COMET assays. The AgNPs was an immediate induction of cellular damage in terms of loss of cell membrane integrity, oxidative stress and apoptosis in MCF-7 cancer cells. The *Tribulus terrestris* L. dried fruit body extract was mixed with silver nitrate in order to synthesize silver nanoparticles. The active phytochemicals involved in quick reduction of silver ion (Ag^+) to metallic silver nanoparticles (Ag^0) produced spherical shaped silver nanoparticles with size range between 16–28 nm. The smaller sizes of silver nanoparticles effectively act against different gram positive and gram negative bacteria such as *Streptococcus pyogens*, *Pseudomonas aeruginosa*, *E. coli*, *B. subtilis* and *S. aureus* (Gopinath et al., 2012).

Table 2 – Antifungal activity of metallic gold and silver nanoparticles from broccoli extract.

Pathogenic fungus	Antifungal activity (zone of inhibition in mm)		
	Control (fluconazole 50 μg/mL)	AuNPs (50 μg/mL)	AgNPs (50 μg/mL)
Aspergillus sp.	6	6	6
Pneumocystis sp.	6	5	7

Despite that, the active interaction of the microorganism and nanoparticles is due to the higher surface area of the nanomaterials, as well as the binding capacity of the nanoparticles which depends on the shapes and functional active site on the surface of the bacterial cell. Nevertheless, these important factors of nanoparticles have potentially enhanced the antimicrobial activity (Lim et al., 2013). On concordent, the gold and silver NPs inhibit the growth of the *Aspergillus* sp. and *Pneumocystis* sp. at 50 μg/mL concentrations which is comparable to that of common antifungal fluconazole. The differences in the metallic nanoparticle concentrations probably show different inhibition rates between the various types of fungal cells. Also, due to the composition of cell wall structure, evolutionarily types of fungus strains are unable to control spore formation and resistivity to grow better toxification medium (Panáček et al., 2009). Although, our research focused on the green chemistry developed of gold and silver nanoparticles, it could also serve as a useful antibiotic against pathogenic bacteria and fungus. Therefore, these findings revealed that broccoli synthesized gold and silver nanoparticles with controlled size and shapes. The broccoli reduced nanoparticles have been controlled bacterial and fungal infections for human and also alternative for drug resistance problem against multidrug resistance (MDR) microorganism.

4. Conclusions

This work highlights the biosynthesis of gold and silver nanoparticles using an intracellular extract of broccoli, which was successfully evaluated in the study. BCE (broccoli extract) is a quick reduction of gold and silver ions and formation of metallic gold and silver NPs with controlled size and shapes such as spherical, triangular and rod. Moreover, the FT-IR peak ranges exhibit the presence of phenols, alkanes, amines and alcohol. Therefore, the functional bioactive metabolites are mainly responsible for the production of the gold and silver nanoparticles in the broccoli extract. In addition, the broccoli reduced gold and silver NPs as a novel and alternative for commercial antibiotics, it could be effectively control the growth of human pathogenic bacteria. However, the biosynthesized gold and silver nanoparticles have potent activity against gram positive and gram negative bacteria. Also the biosynthesized gold and silver NPs effectively control the growth of the tested fungus at the concentration of 50 μg/mL. Further studies we are putting more attention on cytotoxicity of broccoli synthesized gold and silver nanoparticles against different cancer cells and their therapeutic efficacy.

Acknowledgments

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REFERENCES

Becker, R.O., 1999. Silver ions in the treatment of local infections. Metal-Based Drugs 6 (4–5), 311–314.

Bunghez, I.R., Fierascu, R.C., Dumitriu, I., Ghiurea, M., Ion, R.M., 2011. Biosynthesis of silver and gold nanoparticles via pigments extracted from *Spinacia oleracea*. Environ. Eng. Manag. J. 10 (2), 231–235.

Carlson, C., Hussain, S.M., Schrand, A.M., Braydich-Stolle, L.K., Hess, K.L., Jones, R.L., et al., 2008. Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. J. Phys. Chem. B 112 (43), 13608–13619.

Chen, S., Andreasson, E., 2001. Update on glucosinolate metabolism and transport. Plant Physiol. Biochemistry 39 (9), 743–758.

Dwivedi, A.D., Gopal, K., 2010. Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. Colloids Surf. A 369 (1–3), 27–33.

Gopinath, V., MubarakAli, D., Priyadarshini, S., Priyadharsshini, N.M., Thajuddin, N., Velusamy, P., 2012. Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: a novel biological approach. Colloids Surf. B 96, 69–74.

Harborne, J.B., 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. Chapman and Hall, London.

Jeyaraj, M., Rajesh, M., Arun, R., MubarakAli, D., Sathishkumar, G., Sivanandhan, G., et al., 2013a. An investigation on the cytotoxicity and caspase-mediated apoptotic effect of biologically synthesized silver nanoparticles using *Podophyllum hexandrum* on human cervical carcinoma cells. Colloids Surf. B 102, 708–717.

Jeyaraj, M., Sathishkumar, G., Sivanandhan, G., MubarakAli, D., Rajesh, M., Arun, R., et al., 2013b. Biogenic silver nanoparticles for cancer treatment: an experimental report. Colloids Surf. B 106, 86–92.

Jeyaraj, M., Arun, R., Sathishkumar, G., MubarakAli, D., Rajesh, M., Sivanandhan, G., et al., 2014. An evidence on G2/M arrest, DNA damage and caspase mediated apoptotic effect of biosynthesized gold nanoparticles on human cervical carcinoma cells (HeLa). Mater. Res. Bull. 52, 15–24.

John, C.P., Zheng, L.Z., Faaizah, K., Tania, S., David, J.S., 2008. Birch nanomedicine and its potential in diabetes research and practice. Diabetes Metab. Res. Rev. 24 (8), 604–610.

Klaus, T., Joerger, R., Olsson, E., Granqvist, C.G., 1999. Silver-based crystalline nanoparticles, microbially fabricated. Proc. Natl. Acad. Sci. U. S. A. 96 (24), 13611–13614.

Kumar, P., Singh, K., Kumari, S., Mozumdar, R., 2011. A green approach for the synthesis of gold nanotriangles using aqueous leaf extract of *Callistemon viminalis*. Mater. Lett. 65 (4), 595–597.

Lewis, K., Ausubel, F.M., 2006. Prospects for plant-derived antibacterials. Nat. Biotechnol. 24 (12), 1504–1507.

Lim, E., Guerra, R., Lara, V., Guzmán, A., 2013. Gold nanoparticles as efficient antimicrobial agents for *Escherichia coli* and *Salmonella typhi*. Chem. Cent. J. 7, 11. http://dx.doi.org/10.1186/1752-153X-7-11.

Morones, J.R., Elechiguerra, L.J., Camacho, A., Holt, K., Kouri, J.B., Ramírez, J.T., et al., 2005. The bactericidal effect of silver nanoparticles. Nanotechnology 16 (10), 2346–2353.

Mott, D., Thuy, N.T.B., Aoki, Y., Maenosono, S., 2010. Aqueous synthesis and characterization of Ag and Ag–Au nanoparticles: addressing challenges in size, monodispersity and structure. Phil. Trans. R. Soc. A 368 (1927), 4275–4292.

MubarakAli, D., Thajuddin, N., Jeganathan, K., Gunasekaran, M., 2011. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids Surf. B 85 (2), 360–365.

Narayanan, K.B., Sakthivel, N., 2008. Coriander leaf mediated biosynthesis of gold nanoparticles. Mater. Lett. 62 (30), 4588–4590.

NCCLS and Antimicrobial Susceptibility Testing, 1998. Performance standards for antimicrobial susceptibility testing. Blood Safety and Clinical Technology Guidelines on Standard Operating Procedures for Microbiology. Eight Informational Supplement. M100-S8 18 (1). NCCLS, Pennsylvania, USA.

Noguez, C., 2007. Surface plasmons on metal nanoparticles: the influence of shape and physical environment. J. Phys. Chem. C 111 (10), 3806–3819.

Nowack, B., 2010. Nanosilver revisited downstream. Science 330 (6007), 1054–1055.

Panáček, A., Kolář, M., Večeřová, R., Prucek, R., Soukupová, J., Kryštof, V., et al., 2009. Antifungal activity of silver nanoparticles against *Candida* spp. Biomaterials 30 (31), 6333–6340.

Park, M.V.D.Z., Neigh, A.M., Vermeulen, J.P., Fonteyne, L.J.J., Verharen, H.W., Briedé, J.J., et al., 2011. The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. Biomaterials 32 (36), 9810–9817.

Philip, D., Unni, C., 2011. Extracellular biosynthesis of gold and silver nanoparticles using Krishna tulsi (*Ocimum sanctum*) leaf. Phys. E. 43 (7), 1318–1322.

Rajasekharreddy, P., Rani, P.U., Sreedhar, B., 2010. Qualitative assessment of silver and gold nanoparticle synthesis in various plants: a photobiological approach. J. Nanopart. Res. 12 (5), 1711–1721.

Shankar, S.S., Rai, A., Ahmad, A., Sastry, M., 2004. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. J. Colloid Interface Sci. 275 (2), 496–502.

Zhou, Y., Kong, Y., Kundu, S., Cirillo, J.D., Liang, H., 2012. Antibacterial activities of gold and silver nanoparticles against *Escherichia coli* and *bacillus* Calmette-Guérin. J. Nanobiotechnol. 10, 19. http://dx.doi.org/10.1186/1477-3155-10-19.

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