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ORIGINAL ARTICLE

Gold nanoparticles synthesized by *Brassica oleracea* (Broccoli) acting as antimicrobial agents against human pathogenic bacteria and fungi

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Abstract Production of antimicrobial agents through the synthesis of gold nanoparticles using green technology has been extensively made consistent by various researchers; yet, this study uses the flower bud's aqueous extracts of *Brassica oleracea* (Broccoli) as a reducing agent for chloroauric acid (1 mM). After 30 min of incubation, synthesis of gold nanoparticles (AuNps) was observed by a change in extract color from pale yellow to purple color. Synthesis of AuNps was confirmed in UV–visible spectroscopy at the range of approximately 560 nm. The SEM analysis showed the average nanoparticles size of 12–22 nm. The antimicrobial activity of AuNps was analyzed by subjecting it to human pathogenic bacteria (Gram-positive *Staphylococcus aureus* and Gram-negative *Klebsiella pneumonia*) and fungi (*Aspergillus flavus, Aspergillus niger* and *Candida albicans*) using disc diffusion method. The broccoli-synthesized AuNps showed the efficient antibacterial and antifungal activity of above-mentioned microbes. It was confirmed that AuNps have the best antimicrobial agent compared to the standard antibiotics (Gentamicin and Fluconazole). When the concentrations of AuNps were increased (10, 25, and 50 μg/ml), the sensitivity zone also increased for all the tested microbes. The synthesized AuNps are capable of rendering high antimicrobial efficacy and, hence, have a great potential in the preparation of drugs used against major bacterial and fungal diseases in humans.

Keywords *Brassica oleracea* · Chloroauric acid · Gold nanoparticles · SEM · Human pathogens · Antimicrobial activity

Introduction

In this twenty-first century, most of the human pathogenic bacteria and fungi adversely change in their molecular level and are highly resistant in commonly used antibiotics. Therefore, researchers focus on preparation of new antimicrobial agents in different ways. In recent years, nanoscience and nanotechnology are the studies and applications of extremely small things and can be used across all the science fields, such as chemistry, biology, physics, materials science, and engineering. Nanoscale particles are not new in either nature or science. However, in the recent years, areas such as microscopy have given new tools to scientists to understand and take advantage of phenomena that occur naturally when matter is organized at the nanoscale. In addition, the fact that a majority of biological processes occur at the nanoscale gives scientists models and templates to imagine and construct new processes that can enhance their work in biomineralization (Robert and Schiffmant 1990; Rajesh et al. 2002), bioremediation (Francesco and Francesca 1997; Stephen and Macnaughton 1999; Watanabe 2001), bioleaching (Brierley and Brierley 2001; Harvey and Crundwell 1997), microbial corrosion (Peter Angell 1999), biomedical materials (Chad et al. 1996; David 2003) and many other fields. One of the major applications of nanotechnology is in biomedicine in that nanoparticles can be engineered as nanoplatforms for

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effective and targeted delivery of drugs and imaging labels by overcoming the barriers (Rajshri and Tarala 2007).

The extraction of biological systems such as microorganisms, (MubarakAli et al. 2013; Rajesh Kumar et al. 2012) plants and their parts is termed as green chemistry which approaches to develop the synthesis of nanoparticles. Gold nanoparticles are versatile materials for a broad range of applications with well-characterized electronic and physical properties because of well-developed synthetic procedures. These features have made gold nanoparticles as one of the most widely used nanomaterials for academic research and an integral component in point-of-care medical devices and industrial products worldwide.

Grapefruit extract-synthesized gold nanoparticles have high potential towards antibacterial and antifungal activity (Mohamed et al. 2009). The antibacterial efficacy of gold nanoparticles increases because of their larger total surface area per unit volume (Sun et al. 2005). As previously studied, silver nanoparticles synthesized from broccoli were found to be efficient in antibacterial activity when in combination with silver nanoparticles and antibiotics (Anita and Prakash 2013). From this fact, it is evident that preparation of antimicrobial agent of AuNps synthesized from broccoli aqueous extract. *Brassica oleracea* is a plant that has derivates from Europe and currently it is widely propagated all over the world. This family includes commonly available vegetables such as cabbage, sprouts, cauliflower. It belongs to the family Brassicaceae (Cruciferae) and, *Brassica oleracea* is a 6 species that includes Broccoli which is very nutritive, providing nutrients and health-promoting phytochemicals such as vitamins, carotenoids, fiber, soluble sugars, minerals, glucosinolates and phenolic compounds (Jahangir et al. 2009; Lanone and Boczkowski 2006). It is an edible green plant in cabbage family whose flower heads and stalk are succulent and readily edible. It has a rich fiber content surplus in Vitamin C and many studies have confirmed its counteraction against cancer. Broccoli helps in reducing cancer risks because the presence of isothiocyanate was observed to increase the death rate of cancer cells with the p53 mutation (Xiantao et al. 2011). 2-OH-E1 is a "good" estrogen metabolite that is produced by the cytochrome 1A1 enzymes of the liver, if the ratio is low, the risk for breast cancer is increased, and if high, the risk is reduced, and this compound is stimulated by broccoli.

These biogenic gold nanoparticles are cost-efficient, simpler to synthesize, and focus towards a greener approach through application as antimicrobial agent. In this study, it was found that broccoli-synthesized gold nanoparticles act as a very effective antimicrobial agent against the human pathogenic bacteria and fungi.

Materials and methods

Preparation of flower extract from broccoli

The healthy plant samples were collected and authenticated (No. AM 001) by Dr. S. John Britto, Director, Rapinat Herbarium and Centre for Molecular Systematic, St. Josephs College Tiruchirappalli, Tamilnadu. The plant specimen was confirmed by comparison with reference herbarium specimen and the herbarium sheet was deposited in the herbarium. The fresh extract of broccoli prepared by 25 g of broccoli was finely chopped and thoroughly washed with distilled water. Sterile distilled water of 100 ml was added and incubated for 10–15 min in water both at 100 °C. After incubation, the suspension was filtered using Whatman No. 1 filter paper (pore size 25 µm). The filtrate used as an aqueous extract of broccoli.

Synthesis of gold nanoparticles

Broccoli extract of 10 ml was added into 90 ml of aqueous solution of 1 mM Chloroauric acid ($HAuCl_4$) and incubated at room temperature in dark condition. Broccoli aqueous extract without chloroauric acid solution was used as control.

Characterization of synthesized gold particles

UV–visible spectral analysis

It is one of the most widely used standard techniques for characterization of gold nanoparticles. The bioreduction of gold ions was characterized by UV–visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu-Japan). Absorption measurements for both control (broccoli aqueous extract only) and synthesized nanoparticles were performed at a range of 400–750 nm.

FTIR spectroscopy analysis

For Fourier Transform Infrared Spectroscopy measurements, the biotransformed products present in the synthesized gold nanoparticles were analyzed. FTIR spectrum of samples was recorded on Shimazdu IR Prestige-21 instrument, and the measurement was performed in the range of 500–4000 cm^{-1}.

SEM and EDX analysis

For SEM observation, the sample was prepared using a drop of colloidal solution of synthesized gold and alloy on a carbon-coated copper grid and by allowing the drop to

dry completely in vacuum desiccators. The SEM image of the sample was obtained using Carl Zeiss, SIGMA, UK. Energy-dispersive X-ray (EDX) analysis was done using LEO 1430 VP, Carl Zeiss AG, Oberkochen, Germany to confirm the presence of gold in the particles, as well as to detect any other components, if present, performed on a SEM instrument.

XRD analysis

Broccoli-synthesized gold nanoparticles of XRD patterns were recorded by a SEIFERT X-ray diffractometer with Cu K α radiation. The samples were scanned in the 2θ range.

Antibacterial activity

The antibacterial assay was done with Gram-negative *Klebsiella pneumonia* (MTCC 530) and Gram-positive *Staphylococcus aureus* (MTCC 96) by standard disc diffusion method (Bauer et al. 1996). Sterile paper discs of 4 mm diameter were placed on the swabbed MHA plates and the nanoparticles were loaded over the discs at different concentrations (10, 25, and 50 µg/ml). Another disc with 30 µl of plant extract was loaded in each of the plates. A standard antibiotic disc (Gentamicin 10 mcg) was also placed. The plates were incubated at 37 °C for 24 h. Triplicate plates were maintained for each organism. After incubation, the zone of inhibition (mm) was measured.

Antifungal activity

The antifungal assay was done with *Aspergillus flavus, Aspergillus niger*, and *Candida albicans* by standard disc diffusion method (Bauer et al. 1996). The gold nanoparticles were loaded over the disc as mentioned above. A standard antibiotic disc (Fluconazole) was used. The plates were incubated at 25 °C for 48 h. Experiment was carried out in triplicate and the mean diameter (mm) of the inhibition zone was recorded.

Results

Synthesis of gold nanoparticles

In our preliminary studies, the flower extract incubated without Chloroauric acid (control) did not show any color change, whereas the flower extract with 1 mM Chloroauric acid incubated in dark environmental conditions showed gradual change from transparent white to purple color. The appearance of the purple color was observed after 30 min of incubation, and this is an indication of formation of colloidal AuNps by the broccoli flower extract.

Characterization of gold nanoparticles

The UV spectral signatures divulging the formation of gold nanoparticles were monitored in the range of 400–750 nm using a UV–visible spectrophotometer. Short-term incubation was carried out within few minutes of introducing gold ions into the flask containing the range 500–625 nm. The UV–Visible spectra (Fig. 1) recorded at different (5, 10, 20 and 30 min) intervals showed increased absorbance with increasing time of incubation at around 560 nm.

The FTIR spectrum of synthesized gold nanoparticles is shown in Fig. 2. A peak at 3439.74 cm^{-1} assigned as –NH stretching of amide (II) band. The peak at 1627.63 cm^{-1} was O–H stretching vibration of alcohols and phenols. The band located at 1384.64 and 1077.05 cm^{-1} was because of C–N stretching vibration of aromatic and aliphatic amines, respectively. C–Br–stretching vibration was shown in 534.185 cm^{-1}; this was because of bio-extract present in the sample.

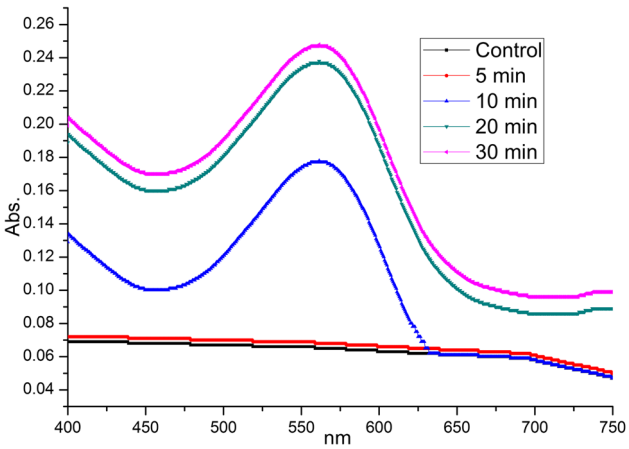

Fig. 1 UV–Vis spectrum showing the absorbance peak for gold nanoparticles for different time intervals up to 30-min incubation. Control—broccoli aqueous extract; (5, 10, 20 and 30 min) Broccoli aqueous extract and 1 mM $HAuCl_4$

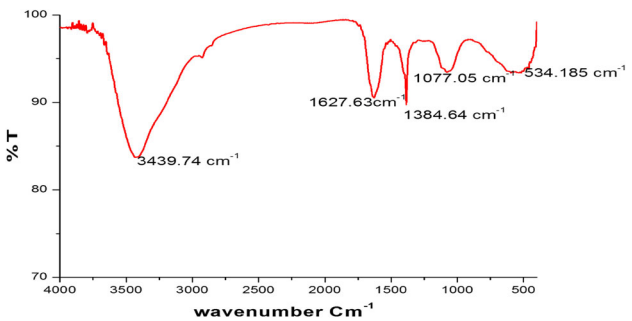

Fig. 2 FTIR spectrum for gold nanoparticles mediated green synthesis by broccoli flower extract

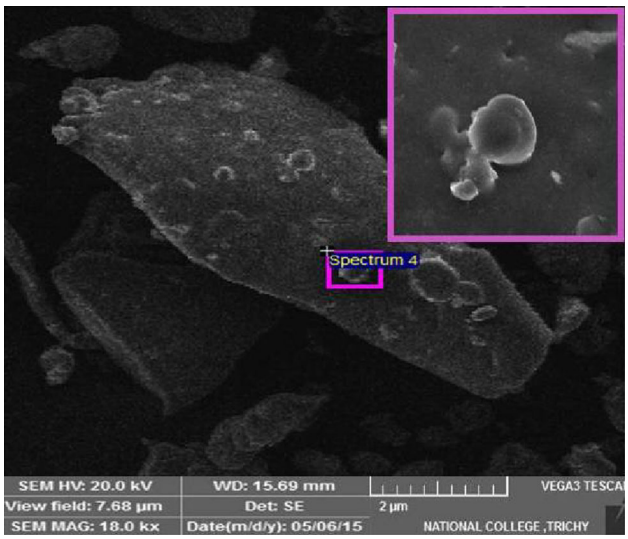

Fig. 3 SEM image of broccoli flower extract synthesized gold nanoparticles

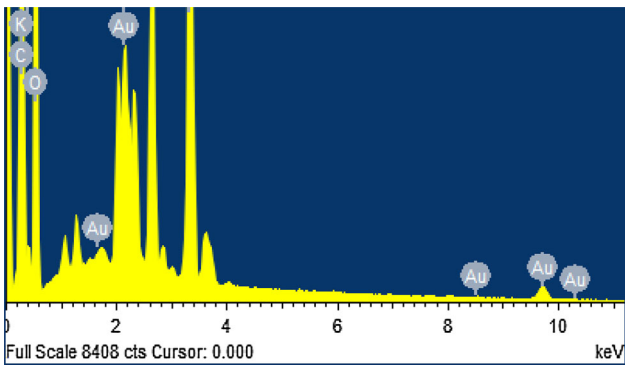

Fig. 4 EDX spectrum of broccoli-synthesized gold nanoparticles

Morphological size and structure of gold nanoparticles obtained from Scanning Electron Microscopy (SEM) is shown in Fig. 3. The synthesized AuNps were spherical in shape and showed a large distribution of size in the range of 12–22 nm. Elemental composition of synthesized gold nanoparticles was analyzed using EDX in SEM. The EDX spectrum (Fig. 4) provided further evidence for the presence of Au nanoparticles.

The integration of the gold nanoparticles with the plant extract of Broccoli was further studied by XRD analysis (Fig. 5). The XRD patterns correlate with the structural nature of the nano particles. The planes 111 and 220 illustrate the cubic and hexagonal structures of gold nanoparticles. Four intense peaks showed four distinct diffractions at 38.2°, 44.4°, 64.5° and 77.3°, at the spectrum of 2θ value which are indexed as (111), (200), (220) and (311) of the cubic face-centered gold. The obtained data was matched with the Joint Committee on Powder Diffraction Standards (JCPDS) card No. 893722. The

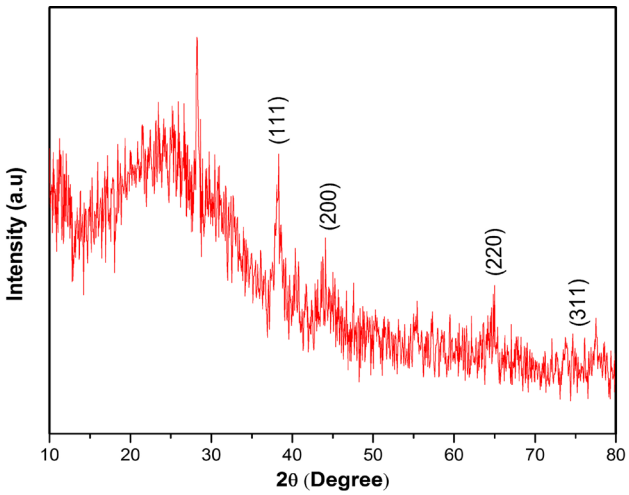

Fig. 5 XRD pattern of broccoli flower extract synthesized gold nanoparticles

crystalline size of the nanoparticles ranged from 22 to 13 nm which also correlates with the SEM analysis.

Antibacterial activity of gold nanoparticles

After 24 h of incubation, the zone of inhibition was measured in mm (Fig. 6) and the ranges were found to be 10, 14, and 20 mm, respectively, in width for the Gram-positive *Staphylococcus aureus* at the concentrations of 10, 25, and 50 µg/ml broccoli-synthesized AuNps. The mean value for zone inhibition was found to be 12, 18, and 22 mm, respectively, in width at the concentrations of 10, 25, and 50 µg/ml of AuNps in Gram-negative *Klebsiella pneumonia*.

Antifungal activity of gold nanoparticles

After 48 h incubation, the zone of inhibition was measured in all plates (width in mm). For three different fungal species, the formation zone of inhibition (Fig. 7) was observed for the different concentration (10, 25, and 50 µg/ml) of synthesized AuNps. For *Aspergillus flavus*, mean value of zone of inhibition was 5, 7, and 9 mm and for *Aspergillus niger*, whereas for *Candida albicans*, the mean value for zone of inhibition was 5, 8, and 9 mm and 5, 7, and 12 mm. There was no zone formed in the standard antibiotic fluconazole and broccoli flower aqueous extract for all the three types of fungal species.

Discussion

In favour of living being concerned with their welfare, green-synthesized nanotechnology crossroads chemical synthesis and biological technologies to develop

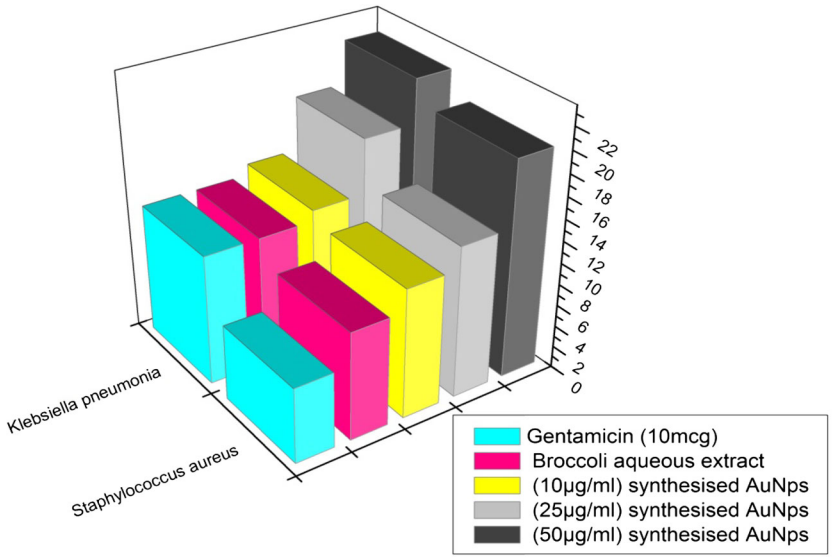

Fig. 6 Antibacterial activity of synthesized gold nanoparticles

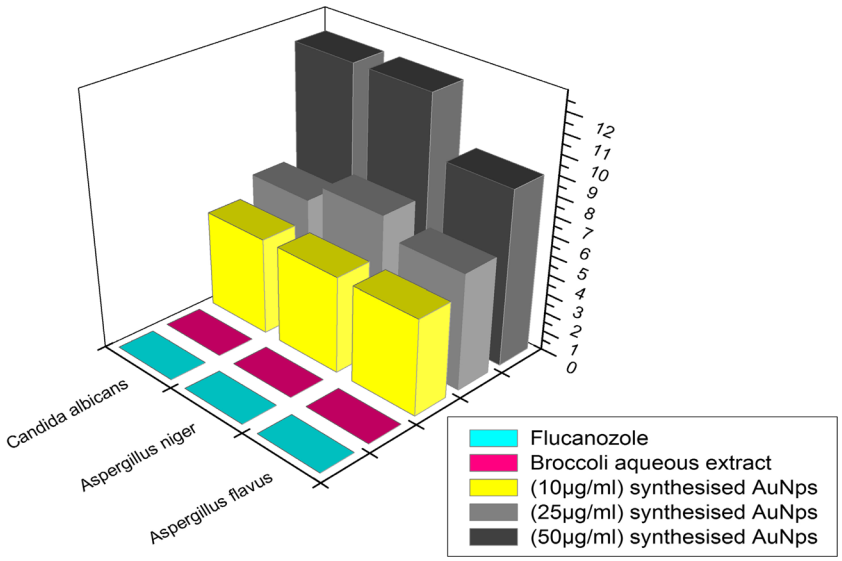

Fig. 7 Antifungal activity of synthesized gold nanoparticles

environmentally safe materials. After the addition of 1 mM of chloroauric acid, the broccoli aqueous extract formed the AuNps within 30 min of incubation. This might be because of the excitation of surface plasmon vibrations in the colloidal solution (Mulvaney 1996). The evidence of the primary confirmation of UV–visible spectra of gold nanoparticles revealed a distinct absorption peak at 542 nm corresponding to surface plasmon resonance with broadening peak-disclosed poly-dispersed gold nanoparticles (Kalishwaralal et al. 2010; Kalishwaralal et al. 2009; Agnihotri et al. 2009; He et al. 2007).

FTIR measurement was performed to find the possible biomolecules responsible for capping and efficient stabilization agent for synthesized gold nanoparticles. FTIR analysis was performed both with the plant extract and synthesized gold nanoparticles. The purpose of this analysis is to identify whether interactions have taken place between gold and the plant extract. The bioactive molecules in the plant intermingle with the synthesized gold nanoparticles and act as a capping material thereby stabilizing it. The FTIR spectrum of the gold nanoparticles (Fig. 2) constitutes two characteristic peaks 3439 and 1637 cm^{-1} of ν(OH) and ν(NH2) indicating the presence of amino groups and the N–H bend in the primary amines. The plant extract also consists of the similar groups but with a slight variation in the peak at 3439 and 1627 cm^{-1}. The presence of peak at 1077 cm^{-1} relates with the ν(C–O–C) of glycosidic linkage belonging to the plant. This confirms the integration of the plant compounds with the synthesized nanoparticles.

This purple color formation clearly indicated that the size of the nanoparticles was less than 100 nm in SEM

image. The particle size increases with the color change, which was found to be changed from pink to blue because of the surface plasmon of AuNps (Husseiny et al. 2007). The EDX spectrum (Fig. 4) provided further evidence for the presence of Au nanoparticles.

The integration of the gold nanoparticles with the plant extract of Broccoli was further studied by XRD analysis (Fig. 5). The XRD patterns correlate with the structural nature of the nanoparticles. The planes 111 and 220 illustrate the cubic and hexagonal structures of gold nanoparticles. Four intense peaks showed four distinct diffractions at 38.2°, 44.4°, 64.5° and 77.3°, at the spectrum of 2θ value which are indexed as (111), (200), (220) and (311) of the cubic face-centered gold. The obtained data were matched with the Joint Committee on Powder Diffraction Standards (JCPDS) card No. 893722. The crystalline size of the nanoparticles ranged from 22 nm to 13 nm which also coincides with the SEM analysis.

The antibacterial activity of the synthesized dispersed gold nanoparticles solution was used in different concentrations of 10, 25, and 50 µg/ml against the human pathogenic Gram-positive *Staphylococcus aureus* and Gram-negative *Klebsiella pneumonia* bacteria by standard disc diffusion method. Gold nanoparticles exhibited effective zone of inhibition against both Gram-positive and Gram-negative bacteria when compared to the standard antibiotic Gentamicin (10 mcg) and broccoli flower extract (control). From this result, Gram-negative bacteria zone of inhibition was higher than the Gram-positive bacteria because the Gram-negative bacteria cell wall peptidoglycan layer is very thin. The AuNps bind to the bacterial cell membrane and break through bacteria cell wall and interact with protein- and phosphorous-containing compounds, such as DNA. After interaction, AuNps may attack the respiratory mechanisms, cell division, and finally leads to death (Rai et al. 2009). Several studies reported that gold ions react with SH groups of proteins and play a vital role in bacterial inactivation (Guzman et al. 2012). Uncoupled respiratory electron transport from oxidative phosphorylation which inhibits respiratory chain enzymes and interaction with nucleic acids probably results in the impairment of DNA replication (Feng et al. 2000).

Antifungal activity of the synthesized AuNps was studied on 3 different human pathogenic fungal species, namely *Aspergillus flavus, Aspergillus niger*, and *Candida albicans.* When gold nanoparticle concentration increases, the zone of inhibition also increases in all the three fungal species (Fig. 5). The broccoli-synthesized AuNps were highly efficient and have the high level of antifungal activity compared to the previous report of green synthesis gold nanoparticles from different sources (Geethalakshmi and Sarada 2013; Ahmad et al. 2013). The antimicrobial ability of AuNPs might be referred to their small size (12–22 nm), which is 275× smaller than a bacterium. This makes them easier to adhere with the cell wall of the microorganisms causing destruction and leads to the death of the cell. Metal nanoparticles are the damaging agents to bacteria and fungi (Chwalibog et al. 2010).

Furthermore, studies will be performed based on molecular level changes that occur in human pathogenic bacteria and fungi. Moreover, AuNps can target against tumor proliferation, which will be future perceptions drawn from this study.

Conclusion

To summarize, it could be stated that we succeeded in biological reduction of gold nanoparticles by broccoli (*Brassica oleracea*). The gold nanoparticles were characterized under UV–visible spectroscopy, SEM, FTIR and XRD. The synthesized gold nanoparticles were tested for human pathogenic bacteria (Gram-positive and Gram-negative) and fungi. From this study, it was found that gold nanoparticles synthesized from broccoli had higher levels of antimicrobial agent activity in both bacteria and fungi. In future, the use of broccoli-synthesized AuNps direct or coated drugs could minimize the treatment durations and side effects of drugs in chemical preparation. Our work could assist profoundly in the field of biomedicine in rendering therapies and treatments with gold nanoparticles.

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