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Biosynthesis and characterization of silver nanoparticles using ginger spent and their antibacterial activity

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ABSTRACT

Ginger spent is the byproduct of spice industries that remove the essential oils of ginger (*Zingiber officinale*) for the food industry and medicinal purposes. Ginger is a well known spice used often for seasoning in Indian cuisine. The de-oiled ginger has no specific use mostly goes to waste. Hence, we utilized this industrial waste product in the efficient synthesis of silver nanoparticles with the aid of UV irradiation from a solution of 1mM silver nitrate and spent extract in the ratio 9:1. Immediate colour change from pale yellow to dark brown was noted indicating the rapid synthesis of silver nanoparticles. These nanoparticles were centrifuged, dried and well characterized. UV Vis Spectroscopy, XRD analysis, Zeta potential and SEM analysis were carried out. It was commendable that the size of the nanoparticles fell well within the upper limit of 100nm. Agar well diffusion method was used to screen the antimicrobial activity of the well characterized silver nanoparticles. They were tested against seven pathogenic strains of three Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*) three Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus faecalis*) and a fungus (*Candida albicans*). It was seen that the zone of inhibition (ZOI) in the well plate method increased on increasing the concentration of silver nanoparticles. Further studies could lead to the application of these silver nanoparticles in the field of nanomedicine.

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INTRODUCTION

The field of nanoscience shows the good scope and is growing rapidly with several applications. Current research is aimed at developing new ways to efficiently scale up the synthesis of nanoparticles (NPs). There is huge demand for metallic nanoparticles produced from noble metals like gold, platinum and silver since they are inert and hence find several applications in therapeutics and biomedical devices. These silver nanoparticles are in demand as they exhibit surface Plasmon resonance (SPR) in the visible light region (Bhakya *et al.*, 2016). *Zingiber officinale* L. commonly known as ginger is a member of the *Zingiberaceae* family and is a flowering monocot. The edible portion is mainly the rhizome or root of the plant. Ginger is a good antibacterial agent and finds application in the ayurvedic systems of medicine since time immemorial. Most Asian countries like India use it to add spice to their daily cuisine and also as a handy remedy for treating cold, cough and indigestion problems (Sharma & Bhagatwala, 2019).

The essential oils present in ginger, namely, gingerol and zingiberine are extracted by distillation from the underground rhizome of ginger. Ginger oil has a unique smell that is spicy, strong and hot. The concentrated oil has a huge number of applications that include aromatherapy, use as a skin and hair care product and of course the universal use in cuisine worldwide to yield a distinct pungency to food and food products. The essential oil of ginger is immensely beneficial to human health as it is a proven antioxidant that scavenges free radicals. Its use is also reported in the treatment of arthritis and other acute or chronic inflammatory conditions to give pain relief. Hence we know that ginger oil is well established as an anti-oxidant, anti-inflammatory as well as anti-nociceptive agent (Jeena *et al.*, 2013). Various methods are available to synthesize nanoparticles which can be broadly classified into Chemical, Physical and Biological means. Of these, biological process is the most viable option as chemical methods involve the use of highly expensive as well as toxic substances that pollute the environment, while

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physical processes require sophisticated and costly equipment. Hence, bio-inspired green synthesis is growing rapidly into an important branch in nanoscience. Nanoparticles may be formed from scratch by precipitation in a colloidal solution (bottom-up) or by breaking down large substances to nanosize (top-down). The bottom-up approach is applied to chemical and biological methods, while physical methods rely largely on the top-down approach to break down larger particles by various techniques like grinding, laser ablation or milling (Ahmed *et al.*, 2016). Several studies are available that indicate the pharmacological importance and physiological activities of ginger and its essential oils. Ginger oil is of importance in the pharmaceutical industry and in high demand. Hence, several spice processing industries extract the oil of ginger to supply food and medical fields. This leaves the de-oiled ginger (spent) as an unnecessary byproduct often used as an adulterant in low grade food products. The present study is aimed at the efficient use of aqueous extracts of this industrial waste (ginger spent) to aid the green synthesis of silver nanoparticles. These biosynthesized silver nanoparticles have been well characterized for antimicrobial activity indicating their application in nanomedicine.

MATERIALS AND METHODS

Materials

Raw material (Ginger spent or De-oiled *Zingiber officinale*) (Figure 1.) was procured from the world's largest manufacturer of natural spice powders and value added spice extracts, Synthite Industries Private Ltd., Kochi. Chemical reagents of high quality analytical grade were purchased and directly used without further purification. The silver nitrate used for the experiments was bought from Sigma-Aldrich, India. The culture medium used for the study, namely Muller Hilton Agar and Broth were brought from Hi-Media, Mumbai. All the seven strains of microorganisms used for screening of antimicrobial activity were donated by Gandhigram Rural Institute, Gandhigram. All steps mentioned below were carried out only with double distilled water.

Preparation of Extracts

The spent ginger sample was sun dried to remove moisture, repacked and stored in a refrigerator until further use. To prepare the aqueous extract, 10 gm of powder was weighed into a 500ml beaker and 100ml of double distilled water was added to it. The mixture was stirred thoroughly and heated to a temperature of 60 °C for 15 minutes. The extract was then obtained by filtering the resulting solution through Whatman No.1 filter paper. The aqueous extract (Figure 2.) so obtained was used immediately in the synthesis of silver nanoparticles (Pattabiraman *et al.*, 2016).

Silver Nanoparticles Biosynthesis

100ml of 1mM silver nitrate solution was first prepared in a standard flask. To 90ml of silver nitrate solution taken in an Erlenmeyer flask, 10ml of the prepared aqueous extract was



Figure 1: De-oiled *Zingiber officinale*



Figure 2: De-oiled *Zingiber officinale* extract

added and mixed evenly. The mixture was closed and placed in sunlight. After 30 minutes it was observed that the solution changed colour from pale yellow to dark brown. This colour change indicates the formation of silver nanoparticles.

Characterization of Biosynthesized Silver Nanoparticles

The formation of silver nanoparticles was confirmed by studying the optical properties of the solution using a UV visible spectroscope (UV 1800V of Shimadzu, Japan). A Flow cytometer was used to determine the stability of the silver nanoparticles by measuring the Zeta potential of the solution. A particle size analyzer was used to study the size and surface morphology of the newly synthesized silver nanoparticles. The functional groups involved in stabilizing the silver nanoparticles were identified using FTIR spectroscopy (Thermo Scientific Nicolet 380) by KBr pellet method. EDAX analysis was used to check the purity of silver present in the silver nanoparticles. XRD analysis (by using a Diffractometer by Shimadzu, Japan) was carried out at 30 mA, 40 kV using Cu ka radiations for a 2θ angle to study the crystalline structure of the silver nanoparticles.

Antimicrobial Activity

Agar well diffusion method (Valgas *et al.*, 2007) was followed to screen the biosynthesized silver nanoparticles for antimicrobial efficiency against seven human pathogens (*Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis*). The microorganisms were subcultured in Muller Hilton Agar (MHA) and incubated overnight at 37°C. The culture incubated for 24 hours was spread plated on MHA in Petri plates. Six wells were bored on the gel with a cork borer of diameter 6mm in a circle with one well in the centre to load antibiotic, Streptomycin (20 µg/ml) which functions as control. The other five wells were loaded with the prepared extract, pure silver nitrate and three concentrations of prepared silver nanoparticles (100 µg/ml, 150 µg/ml and 200 µg/ml) clockwise from top. The plates were again incubated overnight at room temperature. The zone of inhibition (ZOI) formed around the wells were measured in mm and results were tabulated to compare the antimicrobial activity of the silver nanoparticles with that of pure extract, silver nitrate and antibiotic.

RESULTS

Initially, a pale yellow solution resulted when an aqueous extract of ginger spent was added to a colourless solution of silver nitrate. When exposed to sunlight, the colour of the solution gradually changed to dark brown as seen in the picture below (Figure 3). It was noted that this colour change was a slow process when kept overnight in a dark room but immediately visible when exposed to sunlight. This confirms the photo catalytic effect of UV irradiation and its supportive role in the formation of silver nanoparticles.

Characterization of Silver Nanoparticles

Optical properties

Metallic silver shows a characteristic peak at 420nm due to Surface Plasmon Resonance (SPR) caused by vibration of surface electrons. This property is used to confirm the formation of silver nanoparticles using a UV visible spectroscope. The UV spectra revealed three peaks as shown in the graph below (Figure 4) of which peak 1 at 420nm corresponds to that of silver nanoparticles.

Fourier Transform Infrared Spectroscopy

The chemical groups responsible for the reduction and stabilization of the green synthesized silver nanoparticles were identified by FTIR analysis. The spectrum showed several peaks as shown in the graph below (Figure 5). The five major peaks are at 1028.95 cm⁻¹, 1379.97 cm⁻¹, 1601.77 cm⁻¹, 2360.71 cm⁻¹ and 3421.48 cm⁻¹. There are also five minor peaks at 533.28 cm⁻¹, 773.4 cm⁻¹, 1227.61 cm⁻¹, 1310.54 cm⁻¹ and 2924.85 cm⁻¹. The largest narrow peak at 2360.71 cm⁻¹ is of the same absorbance as that of CO₂. The next biggest

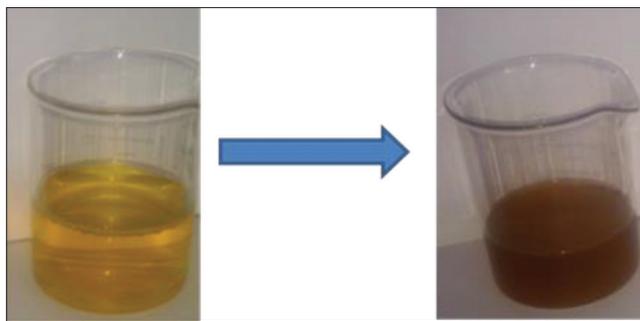


Figure 3: Formation of Silver Nanoparticles using Ginger spent extract

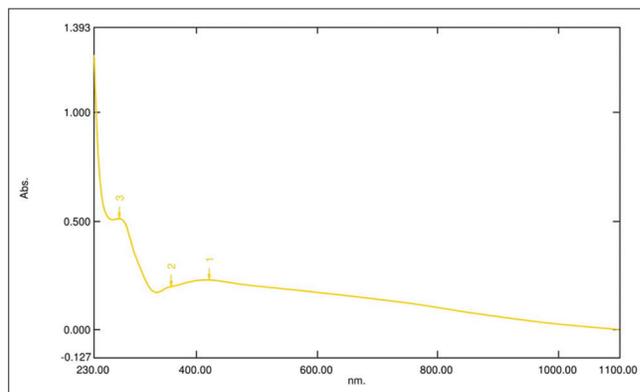


Figure 4: UV spectra- peak 1 at 420 nm indicates presence of silver metal

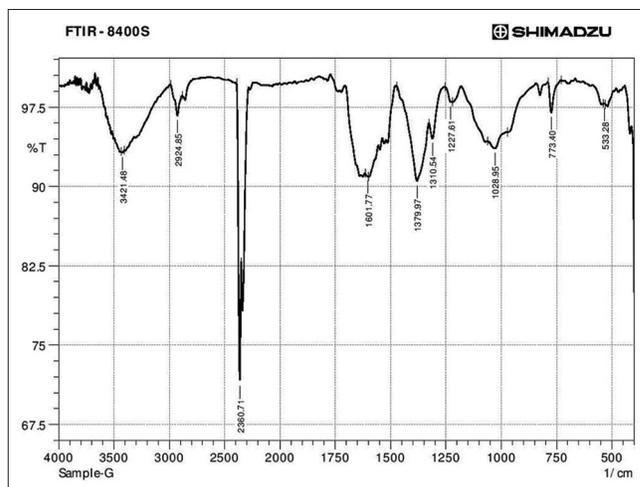


Figure 5: FTIR spectra of silver nanoparticles indicates presence of stabilizing groups

peak is at 1379.97 which corresponds to that of aliphatic nitro compounds. The next two peaks at 3421.48 cm⁻¹ and 1601.7 cm⁻¹ can be assigned to that of primary amines while the overlapping peaks at 1028.95 cm⁻¹ indicate the presence of aliphatic amines and the one at 1310.54 cm⁻¹ belongs to aromatic amines. The smaller peak at 533.28 cm⁻¹ shows bromoalkanes, 773.40 cm⁻¹ reveals aromatic benzene ring, 1227.61 cm⁻¹ is for aromatic ethers and 2924.85 cm⁻¹ shows the presence of ammonium ions (N-H bond) (Sanati & Andersson, 1993).

X-Ray Diffraction Studies

XRD data indicates the crystalline shape and size of the silver nanoparticles. The size can be calculated using the following equation by Holzwarth and Gibson (2011):

$$\tau = K\lambda/\beta \cos \theta$$

where:

- τ is the calculated crystal size measured in nanometers.
- K is the shape factor – a dimensionless value close to unity. It is often taken as 0.9 but may vary slightly depending on the crystal shape.
- λ is the wavelength of X-ray.
- β often referred to as 2θ is the full width half maximum (FWHM)
- and θ is Bragg's angle

With the use of XRD tabulated data in Table 1 and this equation, the average crystal size was calculated as 22.7nm.

The major peaks are seen at 32.35, 38.27 and 46.26 as shown in the graph (Figure 6) below. The d-spacing values were compared to standard values in JCPDF software to arrive at the crystal structure. It was seen that the values matched PDF#411402 which corresponds to hexagonal crystal structure corresponding to 112(1.386) and 201(1.2400) planes.

Particle Size Analyzer (SPS)

A Flow cell cytometer (Shimadzu SALD-2300) was used to determine the exact grain size of the newly synthesized nanoparticles. It was seen that their size varied from 85nm to 120 nm with most particles averaging a grain size of 96nm (Figure 7). The increase in grain size, compared to crystal size measured using XRD data, could be attributed to functional groups around the particles forming a free floating layer in solution.

Zeta Potential

The same instrument was used in calculating the zeta potential of the nanoparticles. The silver nanoparticles showed good stability in solution with a negative value of -31mV as shown in Figure 8 (Clogston & Patri, 2011).

SEM Analysis

The nanoparticles were studied using scanning electron microscope to confirm the size and shape of the nanoparticles. It can be seen that the particles varied in size with most falling within 100nm as indicated with red lines in the figure below (Figure 9). The particles showed crystalline shapes.

Antimicrobial Activity of Silver Nanoparticles

Silver nanoparticles are highly effective in controlling the growth of bacteria and fungi and these hybrid nanoparticles

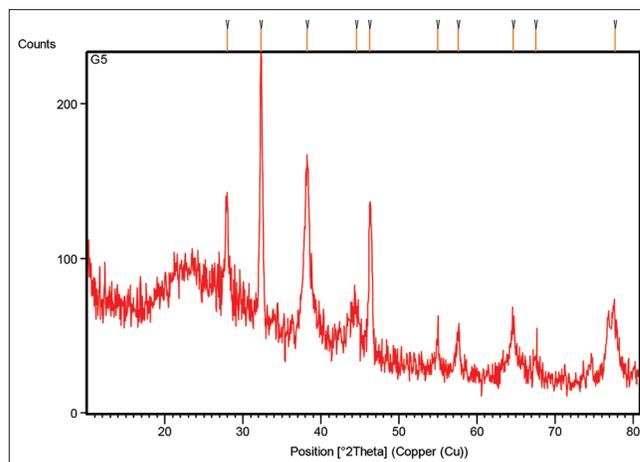


Figure 6: XRD of silver nanoparticles revealing hexagonal structure

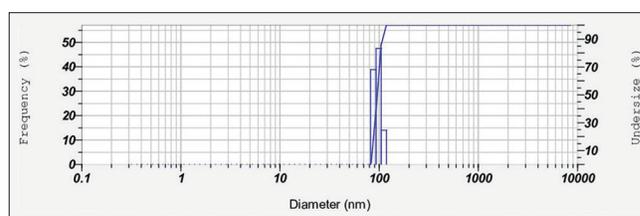


Figure 7: Particle Size Analysis of silver nanoparticles synthesized using Ginger spent

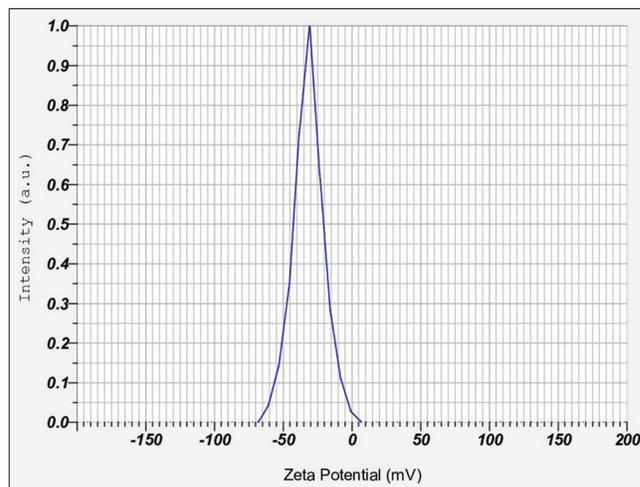


Figure 8: Zeta potential of silver nanoparticles synthesized using Ginger spent

Table 1: X-Ray Diffraction Studies

Position [°2θ]	Height [cts]	FWHM Left [°2θ]	d-spacing [Å]	Rel. Int. [%]
27.9948	67.93	0.2952	3.18730	39.28
32.3459	172.94	0.2460	2.76780	100.00
38.2731	112.25	0.3936	2.35171	64.91
44.5249	28.47	0.5904	2.03494	16.46
46.2613	95.95	0.2952	1.96253	55.48
54.9780	25.77	0.2952	1.67022	14.90
57.6254	27.04	0.3936	1.59963	15.63
64.6199	36.90	0.4920	1.44235	21.34
67.5399	12.01	0.4920	1.38693	6.94
77.6495	41.88	1.1808	1.22968	24.21

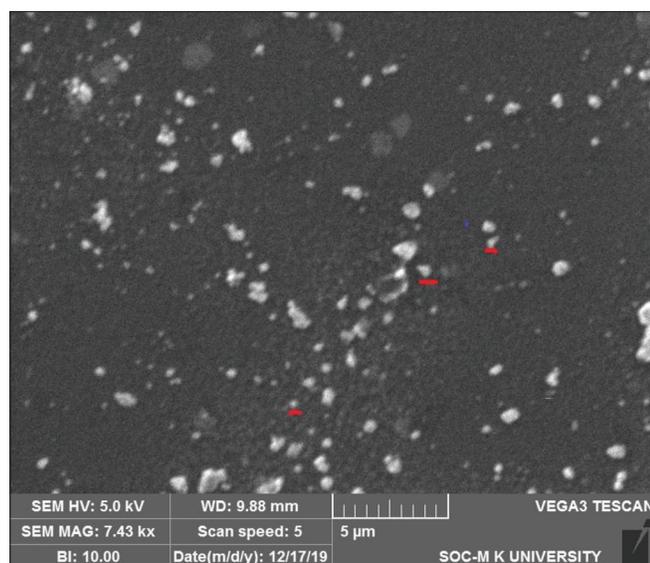


Figure 9: SEM image of silver nanoparticles synthesized using Ginger spent

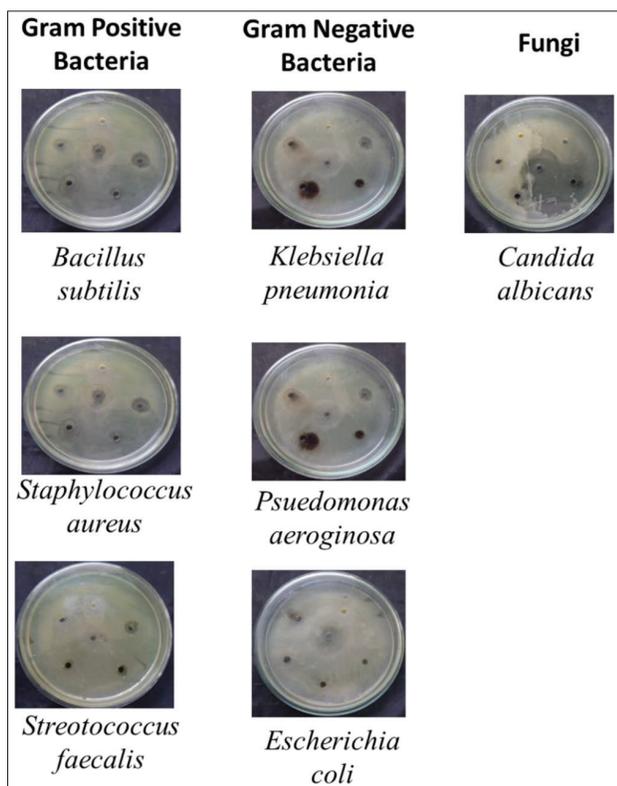


Figure 10: Antimicrobial activity by agar well plate method

proved to be no different. The particles were screened for antimicrobial activity against seven human pathogens of which one is a fungus (*Candida albicans*), three are Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and three Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus faecalis* and *Bacillus subtilis*) by agar well diffusion method as shown below (Figure 10 & Table 2). The ZOI was calculated for each microbe with respect to plant extract, silver nitrate, and three different concentrations of silver nanoparticles and compared to that of standard antibiotics to arrive at an accurate value. It was seen that the AgNPs showed average activity against the pathogens with a maximum zone of inhibition as 13.0 ± 1.0 mm seen against *Bacillus subtilis* and *Pseudomonas aeruginosa* equally. The activity naturally increased with increasing concentrations of silver nanoparticles. All the microbes were fairly susceptible to the AgNPs paving the way for its significant use in the reduction of microbial populations where necessary.

DISCUSSION

Dawadi *et al.* (2021) and Garibo *et al.* (2020) noted that polyphenols, alkaloids and other antioxidants present in plant extracts function as reducing as well as capping agents in the synthesis of silver nanoparticles from silver nitrate. Also, reaction temperature, PH and ratio of salt: plant extract all control the size of the green synthesized silver nanoparticles. Silver nanoparticles find applications as water purifiers, pesticides and novel drugs due to their efficient antimicrobial action. The toxicity of the silver nanoparticles needs to be studied before further use in large scale. Previously, Roy *et al.* (2019) showed that green synthesized silver nanoparticles show enhanced antimicrobial activity due to the combined effect of silver and plant antioxidants and can hence be used to treat multidrug resistant bacteria by easily disrupting their membranes due to this synergistic effect. In 2020, Mangindaana and coworkers were able to synthesize silver nanoparticles using spent coffee grounds, as reducing and capping agents, which are considered a waste product of the coffee industry but are actually rich in biofunctional molecules.

In the present study, FTIR analysis of the silver nanoparticles revealed the presence of phenols which could function as reducing agents. The silver nanoparticles showed better antimicrobial activity than silver nitrate alone or spent extract alone (Table 2) proving the synergistic effect. Ginger spent contains traces of residual polyphenols which are sufficient to

Table 2: Mean Zone Of Inhibition (in mm) of Silver nanoparticles synthesized using Ginger spent extract

S. No	Cultures	Ginger spent extract(10µl)	AgNO ₃ (10µl)	AgNPs(10µl)	AgNPs(20µl)	AgNPs(30µl)	Streptomycin(10µl)
1.	<i>Bacillus subtilis</i>	8±0.6	9±0.7	11±1.0	13±0.6	13±1.0	27±1.2
2.	<i>Staphylococcus aureus</i>	7±1.0	8±0.3	8±0.5	10±0.5	11±0.7	25±0.6
3.	<i>Streptococcus faecalis</i>	8±0.3	8±0.5	9±0.4	9±0.6	10±1.0	20±1.0
4.	<i>Klebsiella pneumoniae</i>	9±1.0	8±1.2	9±0.7	11±0.8	12±0.6	21±1.0
5.	<i>Pseudomonas aeruginosa</i>	8±0.7	10±1.0	12±0.4	12±0.9	13±1.3	20±0.7
6.	<i>E. coli</i>	7±0.5	8±0.4	8±0.5	10±0.6	10±0.7	22±0.6
7.	<i>Candida albicans</i>	6±0.4	8±0.6	9±0.3	9±1.0	11±0.5	25±0.8

reduce silver nitrate to efficiently synthesize silver nanoparticles and can be used in large scale processes.

CONCLUSION

We can conclude that there are several other active ingredients present in ginger beyond Zingiberine and Gingerol that have antimicrobial activity since even the ginger spent was effectively able to control the growth of several microorganisms. Rapid synthesis of silver nanoparticles using ginger spent as stabilizing agent and sunlight as catalyst was feasible and the silver nanoparticles so synthesized were well characterized. This was done using UV Visible Spectroscopy, FTIR, XRD studies, SPS and SEM analysis. It was seen that the silver nanoparticles were hexagonal with an average size of 96nm. The particles were stabilized mostly by nitro groups. They showed moderate stability with a negative zeta potential of -31.6mV. The AgNPs were fairly active against the screened bacteria and fungi. An increase in the concentration of the silver nanoparticles resulted in an increase in antimicrobial activity. Best antibacterial activity was seen against *Streptococcus faecalis* and was equal to that of Streptomycin. This effective use of hybrid silver nanoparticles can be applied in the medical field.

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