GREEN SYNTHESIS OF *PIPER LONGUM* SEED ASSISTED SILVER NANOPARTICLES AND EVALUATION OF ITS ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL

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Abstract:

Green synthesis of nanoparticles finds immense applicability in biomedical field. In the present study antimicrobial and antioxidant activities of silver nanoparticles synthesized from aqueous extractof *Piper longum* seeds were evaluated. The antibacterial and antifungal potential of synthesized nanoparticles is evaluated using agar well diffusion assay. In vitro antioxidant activities were studied using diphenyl- picryl- hydrazyl (DPPH) assay and total antioxidant assay. The synthesized nanoparticles showed promising antimicrobial activities against studied test bacteria and fungi. The nanoparticles also possess high antioxidant potential. Thus the silver nano particles from *piper longum* seeds can be used as potent antimicrobial and antioxidant agents that that can be used to reduce oxidative stress related diseases and control the bacterial and fungal pathogens.

Keywords: Antimicrobial, Antifungal, free radicals, inhibition, antioxidant.

Introduction:

In recent era, Nanotechnology acts as a Multidisciplinary field providing targeted applications in the areas of diagnostics, antimicrobial agents, drug delivery, cell labeling and cancer therapy (Nel *et al.,* 2006). Due to less toxic nature and potential antimicrobial activity, silver nanoparticles are considered as one of the important nanoparticles. Silver nanoparticles reported considerable antioxidant bactericidal, antifungal, antioxidant and anti inflammatory effects (El - Chaghaby & Ahmad, 2011; Veerasamy *et al.*, 2011).

Synthesis of nanoparticles using biomaterials, like plant products and microorganisms have been documented (Rajjou *et al.*, 2012; Chen & Arora, 2015; Thakkar *et al.*, 2009) and found effective due to their cost efficient, eco-friendly and simplicity in preparation (Mittal *et al.*, 2014).

Piper longum is commonly known spice plant in different Indian regions including subcontinent and referred as pippali or long pepper (Yadav *et al.,* 2019). The main chemical constituents found in *Piper longum* are piperine which is an alkaloid with pungent characteristics. It is a pain reliever, rejuvenator and recovers intestinal discomfort. The seeds of *Piper longum* are bitter in taste and known for its stomachic, abortifacient, aphrodisiac and digestive properties. In the present study an aqueous extract of *Piper longum* seeds was used for synthesis of silver nanoparticles and characterized using UV - visible spectroscopy, Fourier transform infrared spectroscopy (FTIR) and checked for their antioxidant and their antimicrobial potential.

Material and Methods:

1) Sampling and Preparation of extract

Dried seeds of *Piper longum* were collected from local market of Nanded, Maharashtra, India during March 2018. The seeds were ground to get fine powder 1 gm of dried powder was added to 100 ml distilled water in a 250 ml capacity glass beaker. The extract was prepared on a magnetic heating stirrer at 80°C for 15 min. The contents were filtered using Whatman filter paper no. 1 and stored at 4°C for synthesis of silver nanoparticles and evaluation of bioactivities.

2) Synthesis of Silver nanoparticles.

Silver nanoparticles of *Piper longum* seed extract were prepared as per the method described by Reddy et al. (2014). Briefly 0.1% silver nitrate solution and *Piper longum* seed extract (1%) were mixed vigorously in 1:1 proportion and incubated at room temperature in dark for 2 hrs. Change in color of silver nitrate solution from colorless to brown yellow was considered as an indication for nanoparticle synthesis. This was further confirmed by UV-visible spectroscopy. The synthesized nanoparticles were repeatedly centrifuged at 11,500 rpm for 20 min to remove any impurities. The extract was washed with distilled water and further dispersed in deionized water for Characterization.

3) Characterization of *Piper longum* nanoparticles.

The spectral analysis of synthesized silver nanoparticles was carried out by using UV - visible spectrophotometer (Shimadzu) and in the range of 300-700nm at varied time intervals. The FITR spectrometric analysis was recorded on FITR spectrophotometer (Perkin Elmer, Inc USA).

4) Determination of total Phenolic content.

Folin- ciocalteu reagent method (Clarke *et al.,* 2013) was used to determine the phenolic content of synthesized nanoparticles. Briefly 200 µl of sample was mixed with 900 µl of Folin - ciocalteu reagent and 900 µl of 6% sodium carbonate. Tje mixture was incubated at room

temperature for 30 min. DMSO and Gallic acid were as negative and positive controls respectively. The phenolic content was expressed as gallic acid equivalents.

5) Determination if total flavonoid content.

The colorimetric method described by Bibi *et al.* (2012) was used to determine total flavonoid content of synthesized nanoparticles. The reaction mixture containing 100 µl samples, 500 µl aluminum chloride (10%), 500 µl potassium acetate (1M) & 1000 µl distilled water was incubator at room temperature for 30 min & the optical density was recorded at 415 nm. Quercetin (0 - 10 µg) & DMSO were used as positive and negative controls respectively. The total flavonoid content was expressed as quercetin equivalents.

6) Evaluation of antioxidant potential.

Antioxidant potential of synthesized nanoparticles was evaluated by using DPPH free radical scavenging assay (Clarke *et al.,* 2013) and total antioxidant assay (Banarjee and Narendrhirakann, 2011). Ascorbic acid was used as reference control.

7) Evaluation of Antibacterial potential

The bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Escherichia coli* were used to evaluate antibacterial potential of synthesized nanoparticles using agar well diffusion method. Active cultures of bacterial strains were spread inoculated on the surface of solidified nutrient agar plates. Wells of 5mm diameter were made on the agar surface using alcohol sterilized cork borer. 20 µl of sample were loaded in the wells and allowed to diffuse for 30 min in refrigerator. The plates were then incubated at 37°C for 24hrs. The diameter zones of inhibitions were measured.

8) Evaluation of Antimicrobial activity

Antifungal activity of synthesized nanoparticles was studied against *Candida albicans*, *Ustilago mydius, Aspergillus flavus* and *Aspergillus niger*.The active test culture or spore suspension of test fungi were inoculated on the surface of potato dextrose agar and wells of 5mm diameter were made as described above. The wells were loaded with sample and after diffusion the plates were incubated at 28 ± 2ºC for 24 to 48 hours. The zones of inhibitions around the wells were measured.

Results and Discussion:

In the present study, silver nanoparticles of *Piper longum* seed extract were prepared and tested primarily for their total phenolic and flavonoid content, antioxidant and Antimicrobial activities. Green synthesis of nanoparticles finds immense applicability in the field of modern nano technology due to eco-friendliness, non toxic nature and ease for large scale production (Kalishwaralal *et al.,* 2008). *Piper longum* is medicinally important plant known for treating

gonorrhoea, tuberculosis, respiratory tract infections, arthritis, gut associated and menstrual pain (Mehta *et al.,* 1998). Silver plays an important role in development of antimicrobial agents in treating the threat posed by antibiotic resistant microbes (Vyon *et al.,* 2009; Panaek *et al.,* 2006). Identification of synthesized silver nanoparticles from piper longum seeds was done by observingcolor change from colorlessto brown yellow after addition of piper longum seed aqueous extract in silver nitrate solution (Nogino *et al.,* 2007).

UV spectrophotometric analysis of synthesized nanoparticles showed maximum absorption at a wavelength of 430 nm. similar absorption maxima were observed in time dependent studies carried out using *Piper longum* fruit extract (Reddy *et al.,* 2014) and Dilleviia fruit extract (Susmita *et al.,* 2013)

FTIR spectra of aqueous extract of *Piper longum* revealed the presence of different functional groups. The presence of phenolic -OH group at 3240.17 cm-1 and corresponding-CH stretching of phenol group at920.5 cm-1. The presence of phenolic group in *Piper longum* seed extract might have contributed for capping and stabilization of silver nanoparticles. The presence of phenolic compounds were also confirmed by counting the phenolic and flavonoid content in aqueous extract and found in nanoparticles. The total phenolic and flavonoid contents of synthesized nanoparticles were recorded to be 18±0.3µg of Gallic acid equivalent / mg and 4.58 ±0.62 µg of quercetin equivalents / mg of nanoparticles.

The DPPH activity testing showed that both *Piper longum* extract and synthesized nanoparticles were effective in scavenging of free radicals with 43.16 % and 48.29 % respectively. The nanoparticles also showed higher total antioxidant (52.61 ± 0.6 ascorbic acid equivalents) activity than found in extract. Ascorbic acid equivalents (40.18 ± 0.32)

The antimicrobial activities of *Piper longum* extract and synthesized nanoparticles were studied against 4 bacterial 4 fungal pathogens. Both the extract and nanoparticles showed antibacterial and antifungal potential. However the activities were higher in nanoparticles as compared to aqueous extract. The *Piper longum* nanoparticles also showed higher inhibition zones against fungi as compared to bacteria. Similar studies were reported by Reddy *et al.* (2014) and phull *et al.* (2016). The metallic nanoparticles act by weakening DNA replication and inactivating the proteins (thus acts as antimicrobial substance. (Feng *et al.,* 2000) .Antimicrobial activities of plant based silver nanoparticles have been studied and reported extensively (Vivek *et al.,* 2011; Reddy *et al.,* 2014). The results in the present study clearly indicated the antimicrobial potential of synthesized nanoparticles against bacterial and fungal pathogens.

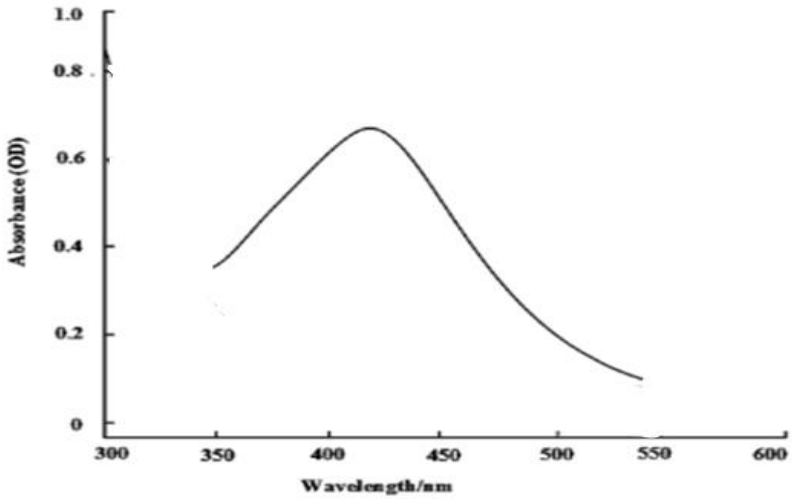

Figure 1: UV –Visible spectrum of synthesized silver nanoparticles

Table 1: Antibacterial activity of synthesized nanoparticles

Sr. No.	Name of the Bacteria	Gram Nature	Zone of inhibition (mm)
1	*Staphylococcus aureus*	+ve	10
2	*Bacillus subtilis*	+ve	11
3.	*Escherichia coli*	-ve	13
4.	*Salmonella typhi*	-ve	15

Table 2: Antifungal activity of synthesized nanoparticles

Sr. No.	Name of the Fungus	Zone of inhibition (mm)
1	*Candida albicans*	15
2	*Ustilago maydis*	10
3	*Aspergillus flavus*	17
4	*Aspergillus niger*	11

Conclusion:

In the present study, the silver nanoparticles with antioxidant and Antimicrobial potential were synthesized using *piper longum* seed extract using cost efficient approach. The promising antioxidant and Antimicrobial ability of synthesized nanoparticles will be beneficial to treat oxidative stress related disorder and diseases due to fungal and bacterial pathogens.

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