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Original article

Synthesis and characterization of silver nanomaterial from aqueous extract of *Commelina forskaolii* and its potential antimicrobial activity against Gram negative pathogens



R. Vijay^a, V.M. Drisya^a, Daniel Raja Femil Selta^b, M.A. Rathi^{b,*}, VK. Gopalakrishnan^{c,*}, Dalal Hussien M. Alkhalifah^d, Wael N. Hozzein^e

^a Department of Biochemistry, Sree Narayana Guru College, Coimbatore – 641 105, TamilNadu, India

^b Department of Biochemistry and Cancer Research Center, FASCM, Karpagam Academy of Higher Education, Coimbatore 641 021, Tamil Nadu, India

^c School of Medicine, Bule Hora University Institute of Health, Bule Hora University, Bule Hora, Ethiopia

^d Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, B.O. Box 84428, Riyadh 11671, Saudi Arabia

^e Botany and Microbiology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt

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ABSTRACT

Aim: Green synthesis of silver nanoparticles from medicinal plants have been progressively acquiring attractiveness to the researchers due to its sustainable nature, nontoxic and economically beneficial. The present study was to synthesize silver nanoparticles (AgNPs) from aqueous extract of *Commelina forskaolii* Vahl and exhibit its potential antimicrobial and cytotoxic activity.

Material and Methods: The whole plant of *Commelina forskaolii* was used to synthesize AgNPs. The synthesized AgNPs was then characterized by UV – visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The AgNPs are widely tested for antibacterial, antifungal and cytotoxic property.

Results: The phytochemical screening of the aqueous extract showed the presence of secondary metabolites such as alkaloids, flavonoids, tannins, phenols, saponins, steroids, glycosides and proteins. The UV – vis absorption spectrum exhibited key peaks at 425 nm. FTIR spectrum revealed that the biochemical compounds are responsible for the reduction and capping material of AgNPs. SEM analysis showed, the average size of synthesized AgNPs ranged from 18 to 27 nm. TEM micrographs revealed that the particle size was to be 30–40 nm. The AgNPs exhibited potential antimicrobial activity against bacterial species (*Enterococcus faecalis*, *Pseudomonas aeruginosa*) showed MIC at about 62.5 µg/ml and 125 µg/ml respectively and fungal species (*Candida albicans* and *Aspergillus niger*) 250 µg/ml and 31.2 µg/ml respectively. The synthesized AgNPs showed potential cytotoxic activity against human breast cancer cell line (MCF-7) with the IC50 value of 50.2 µg/ml. The present investigation concludes the effectiveness of confirmed AgNPs might be used in pharmacological field for the treatment of bacterial, fungal and breast cancer.

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* Corresponding authors.

E-mail addresses: rathi.muthaiyan@kahedu.edu.in (M.A. Rathi), vkgopalakrishnan@gmail.com (VK. Gopalakrishnan).

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1. Introduction

Nanotechnology is one of the fast growing field and it has begun as an outstanding field with surplus of applications in plenteous areas including medicine, pharmacology, sensing devices, micro-electronics and drug delivery etc. (Saini et al., 2010). The physio-chemical properties of nanomaterials such as its nano size, high thermal conductivity they are widely used in the medical field for diagnosis and drug delivery approaches (Zhang et al., 2008). Physical and chemical approaches of nanoparticles are quite cost expensive and harmful to the environment because of the use of hazardous chemicals (Gurunathan et al., 2015). Green synthesis

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of silver nanoparticle production holds less cost effectiveness, eco-friendly and bulk scale synthesis without the utilization of high pressure, energy, temperature and hazardous chemicals (Singh et al., 2010). The synthesis of nanoparticles from plant based materials, bacteria, fungi and enzymes possesses numerous benefits in pharmaceutical and biomedical applications whereas toxic chemical are not involved for the synthesis procedure. Due to the unique properties of nanoparticles synthesized from biological origin the young researchers showed their interest to do research on nanoparticle worldwide (Verma et al., 2010). Numerous metals were used to synthesize nanoparticle such as silver, copper, gold, iron, platinum, silica and nickel (Ittiyavirah and Paul, 2016). Among these metals silver has the capability to cleave bacterial cell walls and inhibit the bacterial cell growth and interrupts the cell metabolism and cause cell death. It is a naturally occurring metal which is identified to be one of the antimicrobial constituents (Mohanta et al., 2017a,b; Logeswari et al., 2015). Nanoparticles can be synthesized from all the above mentioned metals. Among all these, silver nanoparticles possess distinctive properties such as chemical stability, good conductivity and showed potent antibacterial, antiviral, antifungal activity (Ahmad et al., 2003; Klaus-Joerger et al., 2001). In ancient days onwards medicinal plants were serving as a companion to humankind to treat against various disorders. Most commonly medicinal plants and microorganisms are used to synthesis nanoparticles (Mulfinger et al., 2007). The eco synthesis of nanoparticles from medicinal plants is due to the presence of secondary metabolites as reducing agents (Palaniselvam et al., 2016). The medicinal plant selected for the present investigation was *Commelina forskoalii*, belong to the family *Commelina*. It is an herbaceous plant commonly known as rat's ear also known as rat's ear, native to Africa, Arabia, and India. Since this plant is perennial, fast growing, and small herbs, we utilized the whole plant for synthesis of nanoparticles. *Commelina diffusa*, belongs to this family as used by the traditional people for wounds, joint pains and burns. In Africa and Southern part of Asia it has been used to treat urinary tract infection, respiratory tract infections, diarrhea, and hemorrhoids (David, 1998). Sultana et al., (2018) reported that this plant also used to treat fever, influenza, rheumatoid arthritis. The other species of *Commelina* such as *Commelina benghalensis*, used for skin softening, firing inflammation, smoothing emptying of bowel and also in treatment of leprosy. Since no any scientific investigation has been reported in *Commelina forskoalii*, and the above mentioned medicinal plants belong to this family exhibited potential activity in treating various illness prompt us to perform the current investigation to synthesis AgNPs from *Commelina forskoalii*, and carry out its potent antimicrobial and cytotoxic activity.

2. Materials and methods

2.1. Chemicals and reagents

Silver nitrate (AgNO₃), MTT, Fetal Bovine Serum (FBS) were purchased from Sigma – Aldrich. Ferric chloride, hydrochloric acid, DMSO, Nutrient Agar, Nutrient broth were purchased from Himedia, Pvt.ltd. All other chemicals used for the study were purchased of analytical grade.

2.2. Preparation of extract

The whole plant of *Commelina forskoalii* was collected in the month of June 2018 from Western Ghates, Coimbatore, Tamil Nadu and authenticated by Botanical Survey of India, Coimbatore. The plant materials were washed in sterile water to remove any dirt

or other unwanted objects, shade dried at room temperature and powdered using mixer. 300 g of plant powder were weighed and mixed with 1.5 L of water and boiled at 70 °C for 20 min. After cooling the extracts were filtered with Whatman filterpaper No 1 and dried by using rotatory evaporator and deposited at 20 °C for further use.

2.3. Phytochemical screening

The dried aqueous extract was diluted with water and screened qualitatively for the analysis of phytochemicals such as alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, steroids and glycosides by the method of (Evans, 2002).

2.4. Biosynthesis of silver nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) from aqueous plant extract were executed according to Geethalakshmi and Sarada (2012) with slight modifications. Aqueous plant extract was mixed with freshly prepared AgNO₃ solution in the ratio 1:1 and incubated the mixture at room temperature. The appearance of brownish color was noticed after 24 hrs of incubation at room temperature. After that the mixture were centrifuged at 18,000 rpm for 20 min. The deposited pellet was washed with double distilled water and dried at room temperature.

2.5. Characterization

The synthesized AgNPs were characterized using various spectroscopic and microscopic techniques. The UV – visible spectrum of AgNPs were characterized by UV – visible spectrophotometer (Shimadzu 1700 Pharm spec) at the wavelengths ranges from 200 nm to 600 nm. Fourier Transform Infrared (FTIR) analysis of AgNPs was performed using Fourier Transform Infrared Spectroscopy (Thermo Fisher Scientific). SEM determine the surface morphology of nanoparticles Size, shape. TEM analysis was used to determine the qualitative measurement of synthesized nanoparticles such as, particle size, distribution and morphology (Pyrz and Buttrey, 2008). Energy Dispersive Microanalysis techniques (EDX) was carried to confirm the presence of silver and other metal elements in the synthesized nanoparticles.

2.6. Antimicrobial activity

The pure culture of bacteria such as *Enterococcus faecalis*, *Pseudomonas aeruginosa* and fungi such as *Candida albicans* and *Aspergillus niger* were used to assess the antimicrobial activity of the AgNPs. The Minimum Inhibitory Concentration (MIC) of AgNPs was evaluated by broth dilution (resazurin) method Balachandran et al., (2015). Test was carried out in a 96 well plates under aseptic conditions separately for both bacterial and fungal species. A volume of 100 µl of sample was pipetted into the first well of the plate. To all other wells 50 µl of nutrient broth was added and serially diluted it and added 10 µl of resazurin indicator solution. The experiment was performed in different concentrations (7.8, 15.6, 25, 31.2, 50, 62.5, 100, 125 µg/ml) of AgNPs.

2.7. Cytotoxic activity

The human breast cancer cell lines MCF – 7, were procured from National Center for Cell Science, Pune and cultured in MEM (Minimal Essential Media) supplemented with 10 % Fetal Bovine Serum, 1 % penicillin - streptomycin at 37 °C atmosphere in 5 % CO₂. MTT (3-(4, 5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed to evaluate the *in vitro* cytotoxic

activity of AgNPs on MCF-7 by the method of Mosmann (1983). Cells were seeded on 96 well plates (5000 cells/well) cultured for a day and then treated with different concentration of extract for 48 h at 37 °C in 5 % CO₂. At the end of the incubation, medium was removed and MTT (5 mg/ml) was added and the cells were further incubated for 4 h after which the media was removed. DMSO was added in each well to solubilize the formazan crystals. The absorbance was read at a wave length 595 nm using a microtitre ELISA plate reader. Experiments for extract were carried out in triplicate including untreated cell control and blank cell – free control. Cell viability was expressed as percentage over the control.

2.8. Statistical analysis

All the assays were carried out in triplicates (n = 3) and the results represented as Mean ± S.D.

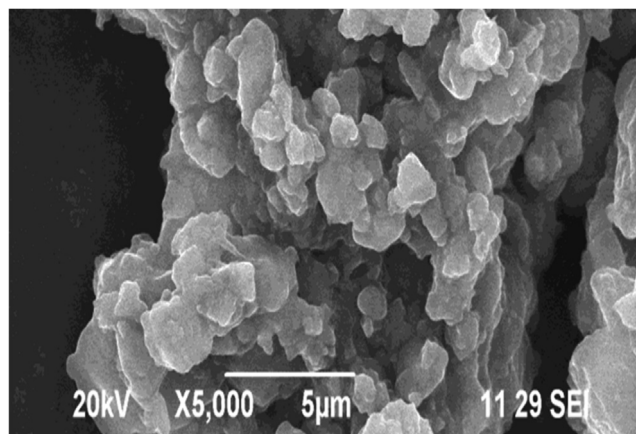


Fig. 3. Scanning Electron Microscopy images of synthesized AgNPs.

Table 1
Phytochemical analysis of aqueous extract of *Commelina forskoolii*.

S. No	Contents	Aqueous extract
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Phenols	+
5	Saponins	+
6	Steroids	+
7	Glycosides	+
8	Proteins	+

+: the components are present.

3. Results and discussion

3.1. Phytochemical screening

Results of qualitative phytochemical screening showed the presence of alkaloids, flavonoids, phenols, tannins, saponins, steroids, glycosides and proteins in aqueous extract of *Commelina forskoolii* (Table. 1). The presence of secondary metabolites such as alkaloids and flavonoids of plant origin are known for their numerous pharmacological significances. So far phytochemical screening was not assessed from this plant species. Similarly, Zhang and Dai, 2009 reported that one of the species of *Commelina* ie. *Commelina*

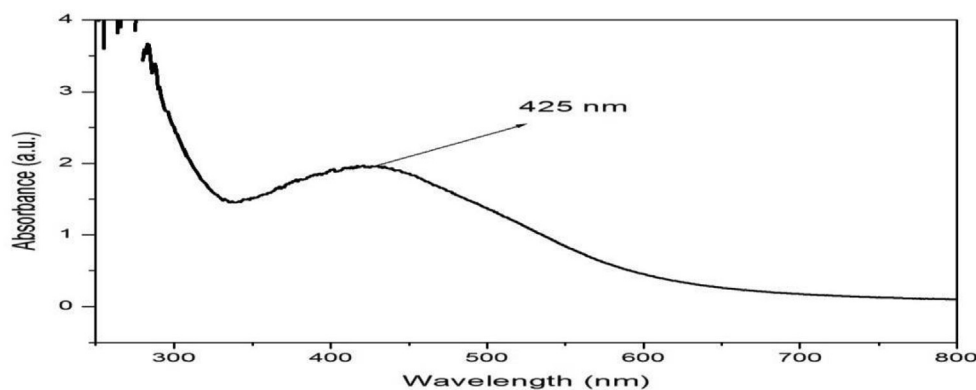


Fig. 1. UV- vis spectrum of AgNPs.

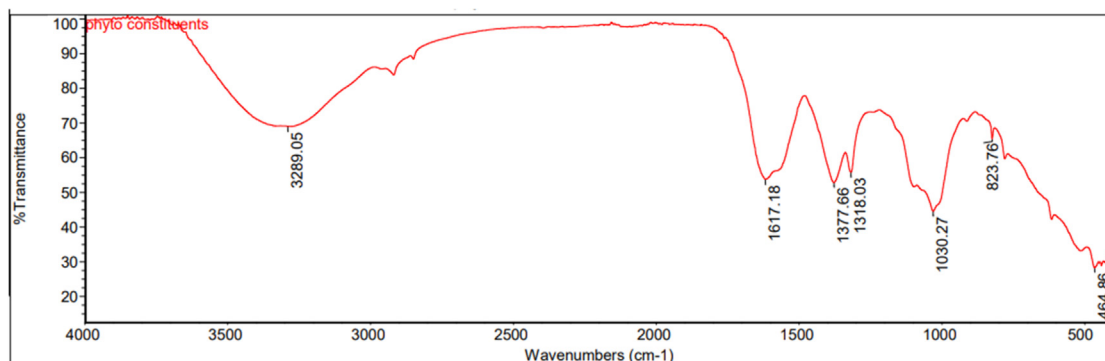


Fig. 2. FTIR spectra of AgNPs.

communis showed the presence of phytochemicals such as flavonoids, glycosides, phenolic acid, alkaloids, pyrimidine, sterols and polysaccharides. Bioactive compounds such as orientin, vitexin, rutin, apigenin, vanillic acid, caffeic acid, quercetin and isorhamnetin were present in *Commelina communis* (Xia Zhang et al., 2018). Ezeabara et al. (2019) reported that *Commelina diffusa* and *Commelina benghalensis* possessed alkaloids, ash, fat, flavonoids and carbohydrates.

3.2. UV- analysis

The maximum absorption was found with a broad peak at 425 nm which indicates the presence of AgNPs in the sample solution owing to the surface plasmon resonance effects (Fig. 1). According to the literature survey it was mentioned that UV – vis

spectroscopy reveals the maximum UV–visible absorption in the range of (400 nm–500 nm) for the initial characterization of synthesized nano particles and exhibit a surface plasmon resonance effects (Amendola et al., 2010). The AgNPs strongly interact with specific wave lengths of light is one of its unique optical property. Depending upon the size and morphology of synthesized nanoparticles varies from yellow – green to brown (Raza et al., 2016). This recommends that the phytochemicals present in the *Commelina forskoolii* acts as a reducing agent.

3.3. FTIR analysis

The FTIR spectra of AgNPs is shown in Fig. 2. The band values obtained at 3289.50, 1617.18, 1377.66, 1030.27, and 464.86 cm^{-1} , which revealed the presence of hydroxyl, amino

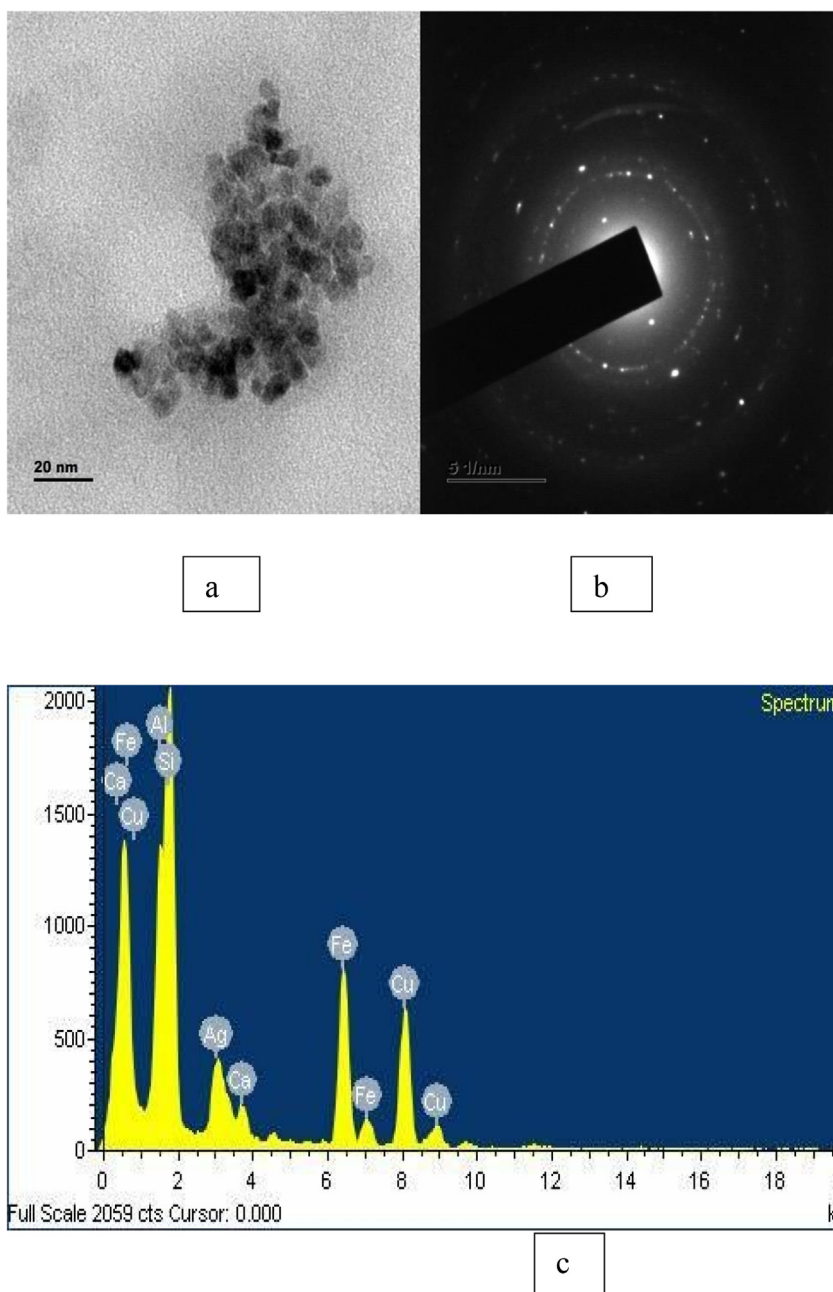


Fig. 4. (a) Transmission Electron Microscopy (TEM) image of synthesized AgNPs (b) Selected area of electron diffraction pattern of the synthesized AgNPs showing the rings. (c) EDX analysis EDX analysis displayed the chemical composition of synthesized AgNPs.

Table 2
Antimicrobial activity of *Commelina forskaolii* ethanol extract against bacteria and fungal species.

S. No	Microorganisms	MIC (µg/ml)
Bacterial species		
1	<i>Enterococcus fecalis</i>	62.5
2	<i>Pseudomonas aeruginosa</i>	125
Fungal Species		
1	<i>Candida albicans</i>	250
2	<i>Aspergillus niger</i>	31.2

and disulfide groups. The functional groups are responsible for the surface coating and effective stabilization of synthesized nanoparticles (Akintelu et al., 2020). The phytochemicals present in the plant extract is responsible for the the reduction and capping process during the synthesis of AgNPs.

3.4. SEM analysis

The morphology and size of the silver nanoparticles was determined using Scanning Electron Microscopy. Results of SEM analysis inferred, the average size of synthesized AgNPs ranged from 18 to 27 nm and showed the spherical and smooth surface morphology (Fig. 3). It is generally used to provide images about the size, shape, morphology and association of the surface topography of nanoparticles (Egerton, 2008). Similarly, Johnson and Prabu (2015) reported that *Commelina benghalensis* exhibited the spherical and uniform Ag nanoparticles in SEM analysis. These focused beam of electrons crash with sample surface to create secondary electrons. The evidence on the resulting electrons is exploited to restructure the sample structure morphology (Chandraker et al., 2021).

3.5. TEM and EDX analysis

To confirm the structure morphology TEM analysis was performed. TEM micrographs of AgNPs revealed that the particle size

was observed to be 30–40 nm (Fig. 0.4 a). It is the powerful technique for providing exhaustive confirmation about the size and shape of nanoparticles (Dudkiewicz et al, 2011). The TEM characterization result in nm range confirms the synthesis of nanoparticle from the extract. EDX micro analysis is achieved by determining the energy and intensity distribution of X – ray signals produced by electron beam on a specimen. The peaks inferred in EDX analysis is represented in Fig. 4b. The spectra represent the presence of Ag in the synthesized AgNPs, addition to other metal elements like copper (Cu), iron (Fe), and calcium (Ca). The other elements present served as capping organic agents bound to the surface of the silver nanoparticles (Dada et al., 2017). Selected area of electron diffraction (SAED) pattern was recorded from the spherical AgNPs and it evidently shows the ring like electron diffraction patterns (Fig. 4c). Tai and Yang, 2011, reported that the diffraction rings of the AgNPs have been indexed as (1 1 1), (2 0 0) and (2 2 0) consistent with the face centered cubic (fcc) structure of Ag, typical of the polycrystalline AgNPs structure (Mehmood et al., 2014).

3.6. Antimicrobial activity

The antimicrobial activity of AgNPs were tested against two bacteria (*Enterococcus fecalis*, *Pseudomonas aeruginosa*) and two fungi such as *Candida albicans* and *Aspergillus niger*. Inhibition of *Enterococcus fecalis*, *Pseudomonas aeruginosa* in treatment of AgNPs was found to be 62.5 µg/ml and 125 µg/ml respectively. Inhibition of *Candida albicans* and *Aspergillus niger* in treatment of AgNPs was found to be 250 µg/ml and 31.2 µg/ml respectively (Table 2). The lipo-solubility property of active compounds present in AgNPs could be responsible for the degradation of the bacterial cell membrane and enter in to the bacterial cell (Elemike et al., 2017). Most AgNPs obtained from medicinal plants have been credited with high antimicrobial properties, especially against drug-resistant human pathogenic and food spoilage microbes (Abdel-Aziz et al., 2014; Reddy et al., 2014). The results from this study correlate with other reports that most plant extract AgNPs exhibit enhanced broad-spectrum antibacterial activities (Mohanta et al., 2017a,b).

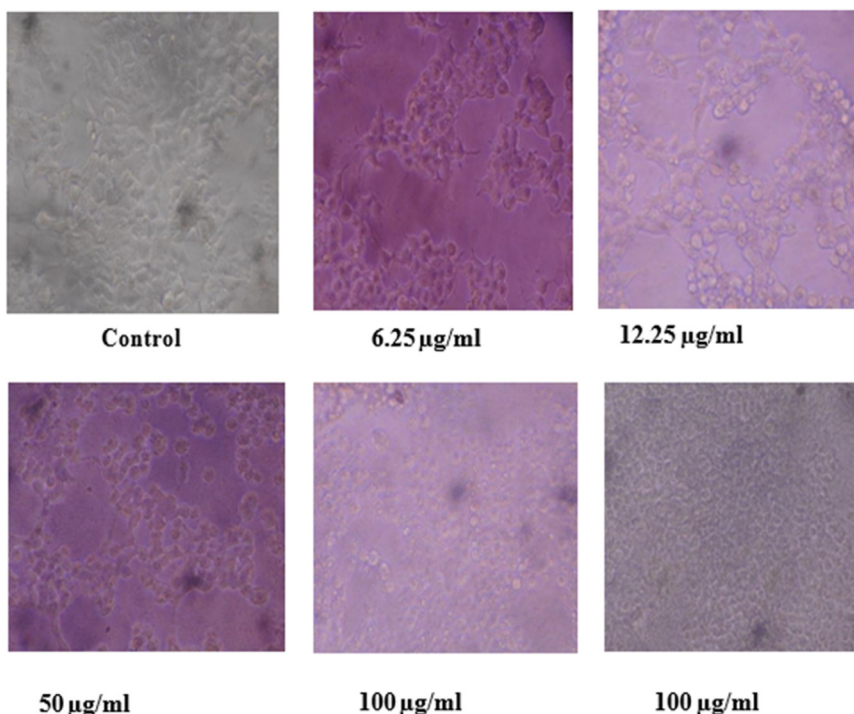


Fig. 5. Morphological changes in MCF – 7 cell lines treated with AgNPs at different concentration.

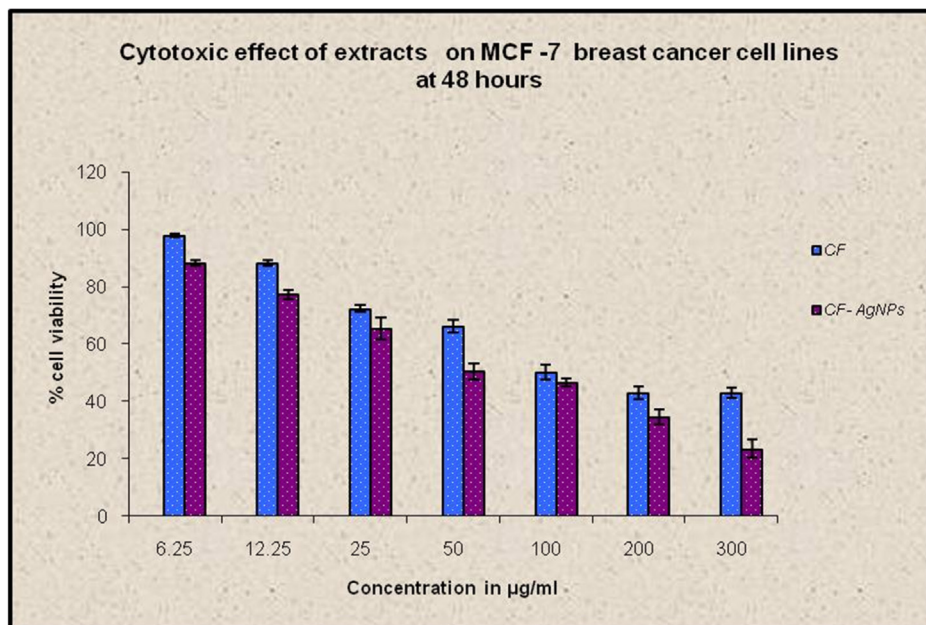


Fig. 6. Cytotoxic effect of plant extracts on breast cancer cell lines at 48 h. The AgNPs and plant extract showed the IC₅₀ value of 50.2 µg/ml and 100.5 µg/ml respectively.

3.7. Cytotoxic activity

The cytotoxic potency of plant extract and AgNPs were evaluated against MCF – 7 (human breast cancer cell lines) by MTT at different concentration. The phase contrast photographs of MCF – 7 (Figs. 5 and 6) at different concentration displayed the abnormal cellular morphologies with detachment of cells and shrinking with a significant reduction in cell viability. Cell growth was inhibited and eventually the cell death occurred and aggregated to form round dead cells at the highest concentration. The plant extract and AgNPs showed the IC₅₀ value of 50.2 µg/ml and 100.5 µg/ml respectively. The present investigations show significant cytotoxic potential of CF-AgNPs against MCF – 7 cell lines. Such potential activity could be due to the effect of biosynthesized nanoparticles and the bioactive components attached to their surface (Valsalam et al., 2019). The result of the present study demonstrates that the cytotoxic activity of AgNPs mediate a dose and time dependent manner.

4. Conclusion

The current study presents the environmentally friendly and cost effective procedure for the synthesis of AgNPs by exploiting renewable natural resources. The AgNPs were synthesized from *Commelina forskoalii* at room temperature and were characterized using UV – vis spectroscopy, FTIR, SEM, TEM and EDX analysis. The UV-Vis spectra confirmed the surface plasmon resonance of green-synthesized silver nanoparticles. The FTIR spectra revealed that the biomolecules were responsible for reducing and capping of AgNPs. Gram negative bacteria are the most common pathogen present in critically ill patients. Bacterial and fungal infections cause crucial diseases in worldwide. AgNPs exhibited good antimicrobial against pathogens and also possess potent cytotoxic activity against MCF – 7 (breast cancer cell lines). Further *in vivo* studies should be carried out for successful application in pharmaceutical and nanotechnology based industries in emerging of new drugs to fight against bacterial, fungal pathogens and to suppress the proliferation of cancer cells.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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