

Biosynthesis and Characterization of Silver Nanoparticles using Wheatgrass Extract and Assessment of their Anticandidal Activity

SANGEETA KAMRADGI, SUHASINI ANGANELLIKAR, SHANKARAVVA BABANAGARE AND M V GUNAGAMBHIRE

Department of Botany, Gulbarga University, Kalaburagi, Karnataka 585106, India

Kamradgi *et al.*: Synthesis of Silver Nanoparticles from Wheatgrass Extract

Wheatgrass extract was used for eco-friendly extracellular synthesis of silver nanoparticles. Stable silver nanoparticles were formed by treating the wheatgrass extract with aqueous silver nitrate solution as reducing agents. Ultraviolet–visible spectroscopy was used to confirm the presence of silver nanoparticles. The X-ray diffraction analysis exhibited characteristic peaks signifying the crystalline nature of nanoparticles. The particle size and the involvement of various biomolecules in the synthesis of silver nanoparticles were determined using Atomic force microscopy and Fourier transform infrared spectroscopy, respectively. The particle structure was studied by scanning electron microscopy and the elemental silver present in reaction mixture was confirmed by Energy dispersive x-ray. The nanoparticles were tested for their anticandidal activity against *Candida albicans* MTCC3017, *Candida albicans* MTCC1637, *Candida albicans* MTCC183, *Candida tropicalis* MTCC230 and *Candida glabrata* MTCC3814 and determined their minimum inhibitory concentration.

Key words: Silver nanoparticles, wheatgrass, anticandidal activity, *Candida* species

Wheat (*Triticum aestivum* L.) is one of the most important cereals in the world belonging to Gramineae family; it has been an integral part of Indian culture for thousands of years and has been known to have outstanding healing properties^[1]. It is one of the major staple food for nearly 35 % of the world's population, grown in 102 countries, covers 220.69 million hectares of land, accounting about 32 % of the total cultivated land of the world^[2]. Wheatgrass has a great therapeutic potential due to the presence of many biomolecules including chlorophyll, vitamin A, C, E and B complex, bio flavonoids, minerals (Calcium and Magnesium), iron and 17 amino acids including 8 essential ones^[3]. Wheatgrass juice provides more energy by fulfilling nutritional deficiencies and removes wastes that clog cells, blood, tissues and organs^[4] and it could be an effective alternative of blood transfusion in terminally ill cancer patients. Recently, the Indian researchers examined the reduction in blood transfusion requirement up to 40 % with no adverse effect in children with thalassemia, who consumed 100 ml of wheatgrass juice per day^[5]. The Indian traditional folk and Ayurvedic medicine, reveals increase in hemoglobin levels in

thalassaemic and myelodysplastic patients who use aqueous extract of 7-8 d old germinated wheat seeds^[6-9].

Nanotechnology is referred as the term for fabrication, characterization, manipulation and application of structure by controlling shape and size at the nanoscale. It is emerging with its applications in science and technology for the purpose of manufacturing new materials. Nanoparticles could reach a biological target of interest by having a small size and offers a great possibility for biomedical applications, not only to deliver pharmaceuticals, but also to be used as novel diagnostic and therapeutic approaches^[10]. In recent years, many environment friendly methods have been employed for the synthesis of nanoparticles. The biological methods of silver nanoparticle synthesis using biological entities like bacteria^[11], yeast^[12], fungi^[13] and plants^[14] were reported. Over the past few

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*Address for correspondence
E-mail: gmvidyasagar@gmail.com

decades, the use of plants in medicine and industry has been significantly growing in the world, although the potential of higher plants and crops as source for this purpose is still largely unexplored^[15].

Candida is the most common opportunistic human fungal pathogen causing diseases ranging from superficial mucosal to life-threatening systemic infections^[16-18] currently ranked by the Center for Disease Control as the third most commonly isolated bloodstream pathogen in hospitalized patients with a mortality rate of up to 50 %^[19-21]. The availability of antifungal agents for the treatment of fungal infections is significantly lower with limitations related to their spectrum of activity and mode of action. Therefore, new and safer antifungal drugs are very much in need to combat the *Candida* infections. Silver ions have long been known to exert a strong inhibitory and bactericidal effect as well as possess a broad spectrum of antimicrobial activities^[22]. The present studies have been conducted to understand the efficacy of silver nanoparticles (AgNPs) produced by aqueous wheatgrass extract against *Candida* species.

MATERIALS AND METHODS

Collection and preparation of plant material:

Fresh leaves of 15 d old wheatgrass were collected from the local Rajapur area near horticulture centre, Kalaburagi, Karnataka, India. The fresh wheatgrass was washed in tap water for several times to remove the dust particles and shade dried for 4-5 d. The dried wheatgrass material was ground and sieved to form a powder. 10g of powder was boiled in 90 ml of double distilled water and filtered. The filtrate thus obtained was used as plant extract for the synthesis of AgNPs.

Synthesis of AgNPs:

100 ml of 1mM of silver nitrate (AgNO_3) solution was mixed with equal volumes of freshly prepared dry powdered aqueous wheatgrass extract (1:1) in 250 ml of Erlenmeyer flask and incubated for 24 h^[23].

Characterization of silver nanoparticles:

After 24 h of incubation, the solution was subjected to UV-visible spectrophotometer (BL Elico 200 Double beam Biospectrophotometer) and recorded the spectral peaks. The X-ray diffraction (XRD) spectroscopy (Rigaku-Ultima-IV) reveals the phase variety and grain size of silver nanoparticles. The Atomic force microscopy (AFM) was employed to study the size, shape, absorption, structure, dispersion and aggregation of nanomaterials. The chemical nature of

silver nanoparticles was studied by Fourier transform infrared spectroscopy (FTIR) (NICOLET 6700). The purified and dried AgNPs mixed with Potassium bromide (KBr) to obtain a pellet and the spectrum was evaluated at 4000-400 cm^{-1} using FTIR spectroscopy. The morphology of silver nanoparticles was studied using Scanning electron microscopy (Quanta 200 FEG SEM) and confirmed the presence of elemental silver in the solution by Energy dispersive X-ray (EDX) spectroscopy in conjunction with Scanning electron microscopy (SEM).

Anti-candidal activity of silver nanoparticles:

The silver nanoparticles synthesized by wheatgrass aqueous extract were tested for anti-candidal activity against five different clinical isolates of *C. albicans* MTCC3017, *C. albicans* MTCC1637, *C. albicans* MTCC183, *C. tropicalis* MTCC230 and *C. glabrata* MTCC3814 (Microbial type culture collection and gene bank, Chandigarh, India) using agar well diffusion method^[24]. A 20 ml of YPDA (Yeast extract-1 g, peptone-2 g, dextrose-2 g, agar-1.5/100 ml of distilled water) medium was poured into sterilized petri plates and freshly grown 24 h old *Candida* cultures were seeded on YPDA medium. The synthesized AgNPs solution was centrifuged at 15 000 rpm for 15 mins, obtained residue was collected and dried. About 10 mg/ml of dried AgNPs was dissolved in 5 % of dimethyl sulphoxide (DMSO) was used as a test sample. The 5 mm diameter of 5 wells were prepared with the help of sterilized cork borer and wells were loaded with newly synthesized AgNPs (50 μl and 100 μl), fluconazole (10 mg/ml of 5 % DMSO) as positive control, DMSO and AgNO_3 as a negative control. The plates were incubated at 28° for 24 h and examined for the zone of inhibition. The mean value for each organism was recorded and expressed in millimeter (mm)^[23].

Minimum inhibitory concentration (MIC) of synthesized AgNPs:

The MIC of synthesized silver nanoparticles was determined using the plate count method against five *Candida* species. 10 ml of 1 % YPG (Yeast extract-1 g, peptone-1 g, glucose-1 g/100 ml of distilled water) broth was inoculated with 10⁵ cfu/ml of each *Candida* cell and cultured in shaking incubator at 28° for 24 h. The two fold diluted (2, 4, 8, 16, 32, 64, 128 and 256 μl /ml) AgNPs were mixed with 100 μl of 10⁵ cfu/ml cultured medium, made the final volume 1ml by adding 1 % YPG broth and incubated at 28° for 24 h. After incubation the medium was spread on the petriplates

containing 1 % YPG agar medium. The plates were incubated at 28° for 24 h and the numbers of colonies grown on the agar were counted^[25].

RESULTS AND DISCUSSION

The synthesis of silver nanoparticles was observed by visual observation of color change in plant extract (right Erlenmeyer flask) from pale yellowish to cherry red within 1 h after the addition of AgNO_3 (fig. 1). After 24 h of incubation at 35°, the changes in color to colloidal brown assured the production of silver nanoparticles in sample mixture (left Erlenmeyer flask). Kowshik *et al.*, reported that the reduction of silver nitrate into silver nanoparticles is clearly visible when the sample solution changes its color from colorless to brown with increase color intensity and also reported the brown color of the sample solution was due to the excitation of AgNPs in the surface plasmon vibration^[12]. That was initial sign of synthesis of silver nanoparticles can be detected by naked eyes has been reported from early reports^[26]. The UV-Visible spectra of silver nanoparticles gave a Plasmon resonance band and absorption peak at 400 nm (fig. 2). The early reports on silver nanoparticles showed the absorption peaks in visible range of 434 nm^[27], 410 nm^[28], 420 nm^[29], 436-446 nm^[30] and 423 nm^[15]. Fig. 2 showed a sharp single peak at 400 nm of visible spectra and confirms the reduction of Ag ions into Ag nanoparticles in wheatgrass aqueous extracts. The intensity of absorption peak and color intensity increase with the duration of incubation^[31]. The size of silver nanoparticle range 5-10 nm absorbs UV-visible light near 420 nm^[32]. According to Rong *et al.* the smaller nanoparticles possess less single surface Plasmon resonance that results in blue shift. In the present study, the absorption peak recorded from UV-visible spectra at 400 nm reveals the size (<20nm)^[33] of synthesized AgNPs and supports the report of Mahmut *et al.*^[15]. The XRD peaks at 28.0, 32.0, 38.0, 42.0, 48.0, 57.0, 65.0, 68.0 and 86.0 confirm the crystalline nature of nanoparticles and incorporated with organic compounds on the surface of silver atoms. The distinct broader diffraction peaks attributes smaller particle size. The diffraction peaks observed in 2θ range of 20-80° consequently, confirm that the nanoparticles have face center cubic (FCC) structure (fig. 3). AFM determines particle size between 20 to 85 nm (fig. 4). The possible biomolecules present in WG extract responsible for the reduction of Ag^+ ions to AgNPs were recorded and mentioned their respective functional groups (fig. 5 & Table 1). FTIR pattern of synthesized silver nanoparticles revealed the

presence of various organic compounds with different functionalities. A broader peak formed at 3382.45 cm^{-1} showed the presence of $-\text{OH}$ stretching vibration band. The O-H stretching carboxylic acid was observed at 2924.50 cm^{-1} . The peaks at 2854.95 cm^{-1} , 1630.46



Fig. 1: Wheatgrass (WG) aqueous extract treated with AgNO_3
Note: The right flask containing wheatgrass aqueous extract without AgNO_3 and left flask showing color changes with 1M AgNO_3 after 24 h of reaction.

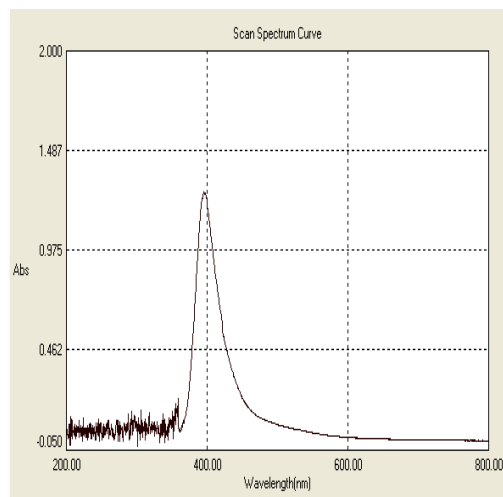


Fig. 2: UV-vis spectra of AgNPs
Note: UV-vis spectra of AgNPs obtained by the reduction of AgNO_3 into AgNPs.

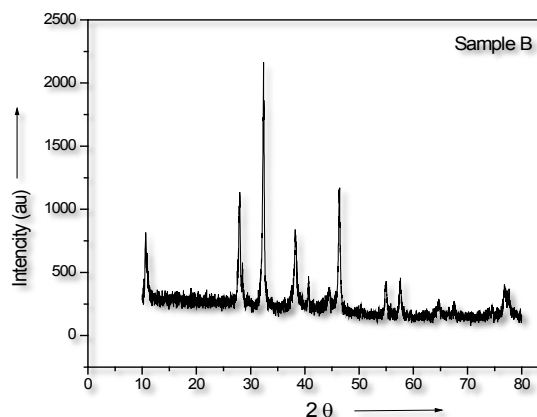


Fig. 3: XRD pattern of synthesized AgNPs.

cm^{-1} and 1384.16 cm^{-1} showed the presence of ZnO particles, primary amides and monosubstituted alkynes respectively. The band at 1052.05 cm^{-1} assigned Si-O-Si symmetric stretching vibrations of polypeptides and proteins. An alkaline stretching and bending molecular vibrations used to obtain the information about surface topography and composition. The morphological view of synthesized silver nanoparticles by SEM analysis reveals the spherical shape of silver nanoparticles with particle size below 100 nm (fig. 6). The presence of Ag metals in reaction mixture analyzed by EDX confirms the synthesis of silver nanoparticles (fig. 7). EDX in conjugation with SEM determined the elemental composition of the silver nanoparticles. Nowadays

biosynthesis of silver nanoparticles has gained more popular and advanced among the biologists, chemists and biotechnologist.

The anticandidal activity of silver nanoparticles carried out against five different strains of *Candida* using agar well diffusion method showed highest 15 mm zone of inhibition at $50 \mu\text{l}$ concentration against *C. albicans* MTCC1637 and found equal zone of inhibition by standard fluconazole indicates the similar efficacy of synthesized AgNPs whereas AgNO_3 solution has shown least inhibitory activity against all tested *Candida* spp. compared with AgNPs. The Lowest 9 and 10 mm zone of inhibition was recorded against *C. tropicalis* MTCC230 and *C. glabrata* MTCC3814 respectively (Table 2). The low activity does not mean that the silver particles do not have any activity; probably it will show the activity with high concentration of AgNPs. Very little zone of inhibition (8 mm) was observed in standard fluconazole against *C. albicans* MTCC183 indicating the development of resistance against drug. However, the AgNPs showed better inhibition (13 mm) in *C. albicans* MTCC183 as compared to fluconazole. According to Jiaxin Gao *et al.* the *C. albicans* gains azole resistance by altering sphingolipid composition via genetic alternation of the drug target Erg11. The reason for easily acquired resistance to multiple antifungal drugs by *Candida* spp. remains unidentified. However, a wide genome genetic screening has been conducted in detail for fluconazole resistance^[34]. The percent resistance acquired by *Candida* spp. against fluconazole was also reported by Berkow EL and

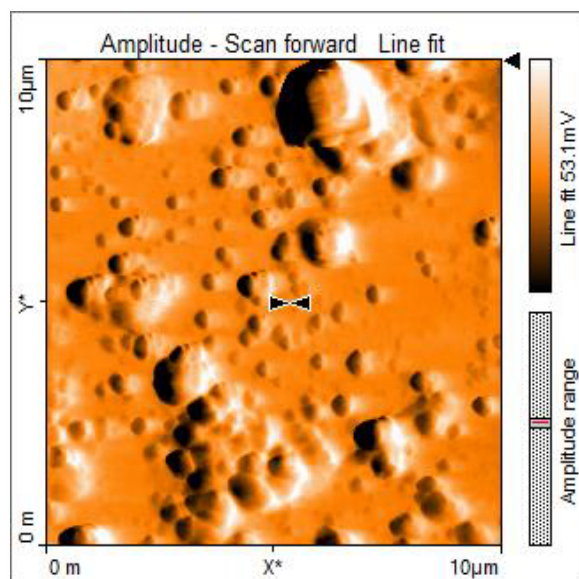


Fig. 4: AFM images of AgNPs

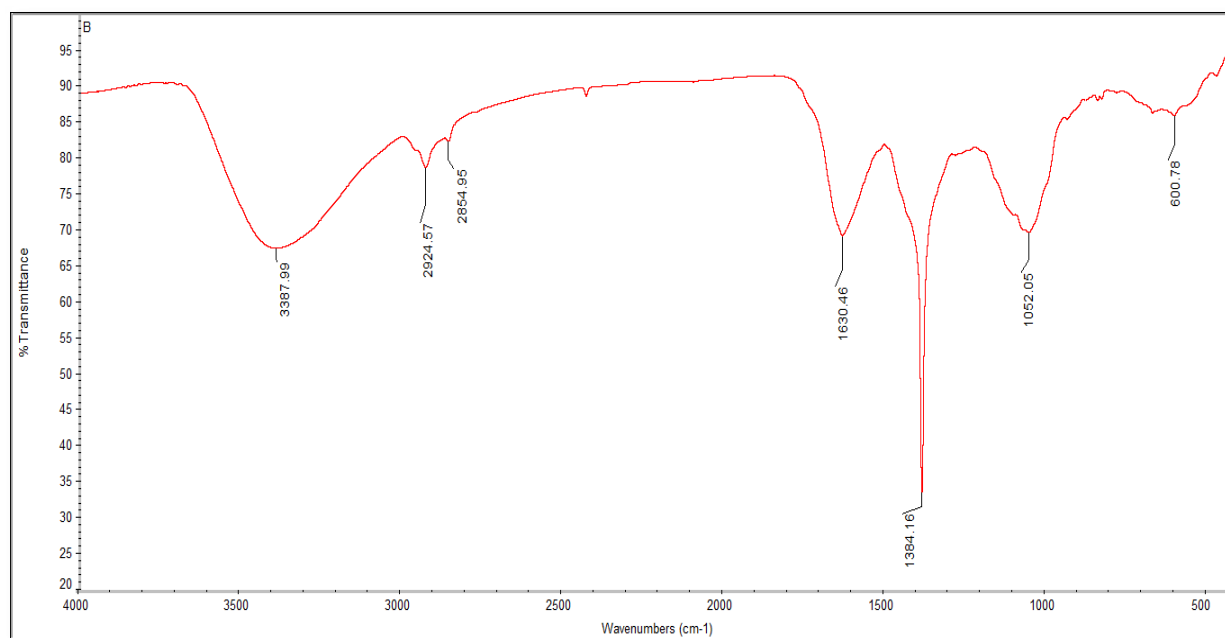
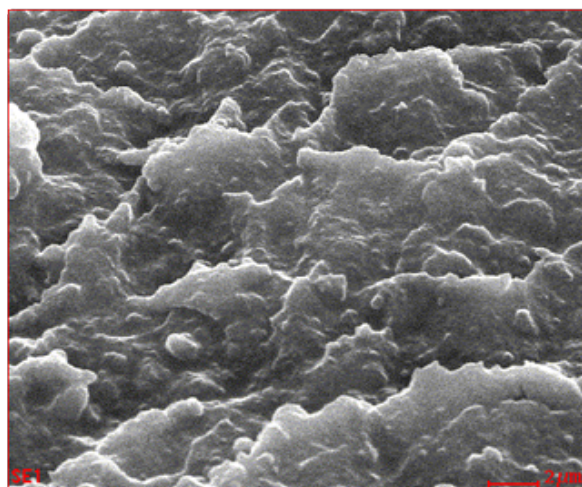


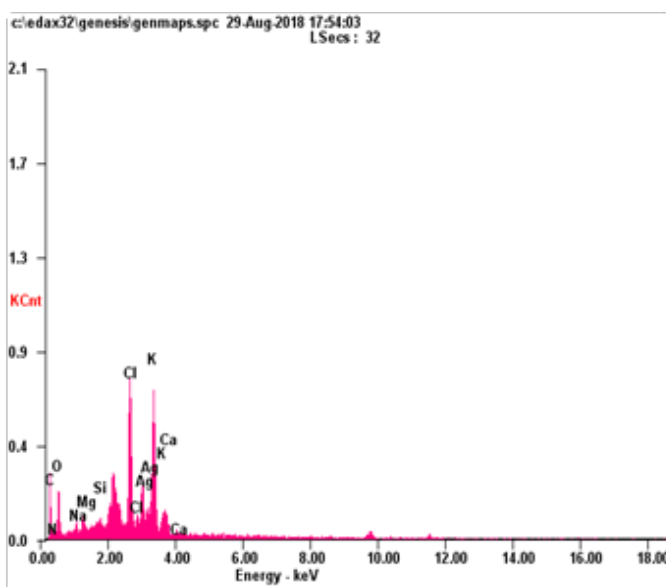
Fig. 5: FTIR pattern of AgNPs.

TABLE 1: FTIR ABSORPTION PEAKS AND THEIR FUNCTIONAL GROUPS

Absorption peaks (cm ⁻¹)	Functional groups
600.78	A terminal alkaline C-H bending vibration
1052.05	Si-O-Si stretching vibration of proteins
1384.16	Monosubstituted alkynes
1630.46	Amide bond-1 of polypeptide
2854.95	Diol on ZnO nanoparticles
2924.57	O-H stretching carboxylic acid
3387.99	-OH stretching vibration band

**Fig. 6: SEM micrographs of AgNPs**

Note: Morphological view of AgNPs by scanning electron microscopy (SEM) of reduced silver nanoparticles.

**Fig. 7: EDX elemental analysis of AgNPs****TABLE 2: ANTICANDIDAL ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES**

ZOI in mm (mg/ml)						
<i>Candida</i> spp.	Fluconazole	AgNPs		AgNO ₃	Plant extract	DMSO
		50μl	100μl			
<i>C. albicans</i> MTCC3017	15	13	14	06	-	-
<i>C. albicans</i> MTCC1637	15	15	14	03	-	-
<i>C. albicans</i> MTCC183	08	13	13	03	-	-
<i>C. tropicalis</i> MTCC230	12	09	12	08	-	-
<i>C. glabrata</i> MTCC3814	17	10	14	03	-	-

Lockhart^[35]. The wheatgrass AgNPs exhibited good anti-fungal activity against *Candida* spp (fig. 8).

MIC of AgNPs performed by using two fold diluted concentration of AgNPs against five clinical isolates of *Candida* spp. to determine the minimum concentration that completely inhibit the visible growth of *C. albicans* MTCC3017, *C. albicans* MTCC1637, *C. albicans* MTCC183, *C. tropicalis* MTCC230 and *C. glabrata* MTCC3814. The MIC of AgNPs against

tested organisms was found at 128 $\mu\text{g/ml}$ against all test organisms except *C. tropicalis* MTCC230 (Table 3). A few *Candida* colonies were observed at 2, 4, 8, 16, 32 and 64 $\mu\text{g/ml}$ concentration and there is no visible growth of *Candida* strains at 128 $\mu\text{g/ml}$. These results could be very much useful in designing the new anticandidal drug. The multiple applications of AgNPs in health care system have attracted the current researcher towards the synthesis, characterization and pharmacological properties of nanoparticles.

TABLE 3: MINIMUM INHIBITORY CONCENTRATION (MIC) OF AgNPs

<i>Candida</i> spp.	MIC in $\mu\text{g/ml}$		
	Fluconazole	AgNPs	Plant extract
<i>C. albicans</i> MTCC3017	16	128	>256
<i>C. albicans</i> MTCC1637	32	128	>256
<i>C. albicans</i> MTCC183	64	128	>256
<i>C. tropicalis</i> MTCC230	32	>256	>256
<i>C. glabrata</i> MTCC3814	16	128	>256

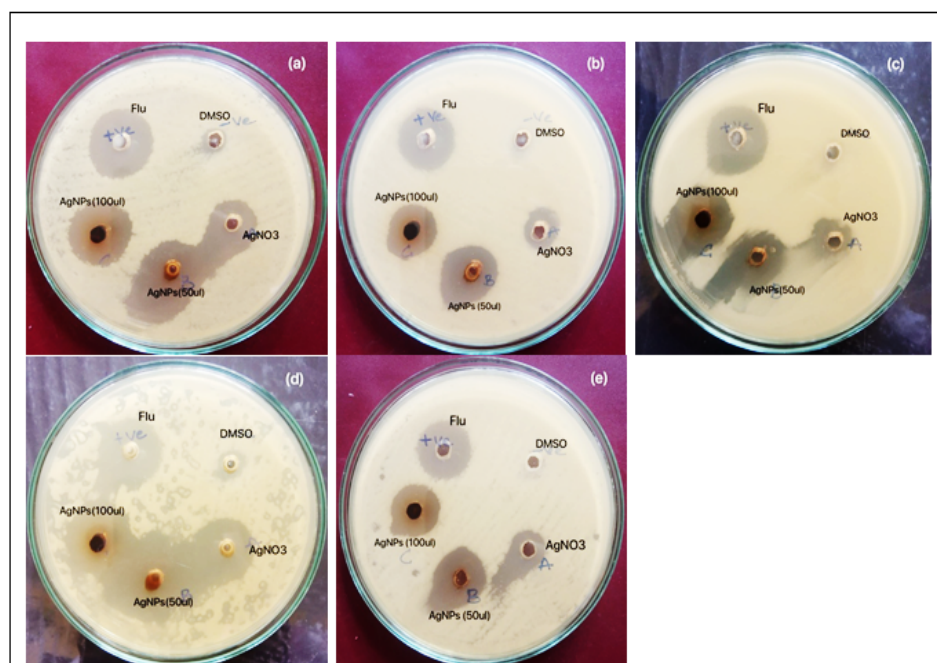
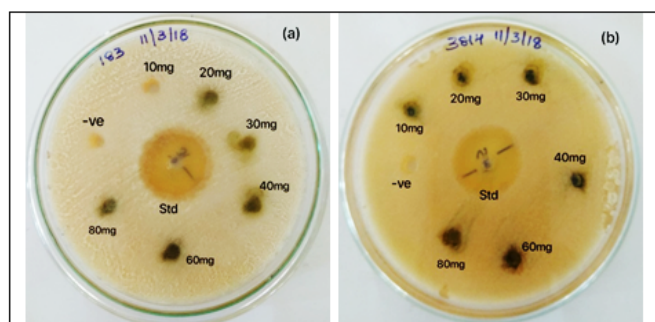


Fig. 8: Anticandidal activity of biosynthesized silver nanoparticles

Note: Anticandidal activity of wheat grass extract (10mg, 20mg, 30mg, 40mg, 60mg and 80mg/ml) against (a) *C. albicans* MTCC183, (b) *C. glabrata* MTCC3814; Anticandidal activity: Flu (Fluconazole), DMSO (dimethyl sulfoxide), AgNO_3 (Silver nitrate), AgNPs (Biosynthesized silver nanoparticles 50 μl & 100 μl). (a) *C. albicans* MTCC3017, (b) *C. albicans* MTCC1637, (c) *C. albicans* MTCC183, (d) *C. tropicalis* MTCC230, (e) *C. glabrata* MTCC3814

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Conflict of interests:

The authors declared no conflicts of interest.

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