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Biogenic Synthesis of AgNP's from Fungal Endophytes of *V. vinefera:* An Approach towards Empowerment of Society

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Abstract
Worldwide fungi are considered as efficient nano factories on account of their reception towards higher bioaccumulation ability, comparatively economic, effortless synthesis method, simple downstream processing and biomass handling. Nowadays, exploration and implication of endophytic fungi in nano biotechnology has receiving extensive attention due to their promising potential for production of bioactive compounds as well as toleration and metal bioaccumulation capability. Present research has been focused to determine the potential of fungal endophytes for silver nanoparticles synthesis (AgNPs) and their possible applications in biomedical field. Total twenty-three isolates of fungal endophytes (FE1 to FE23) were isolated from *V. vinefera*. Biogenic synthesis of AgNPs was carried out by 0.1mM, 0.5mM and 1mM $AgNO_3$ solution with cell free supernatant of endophytic fungi (1:5 v/v). These AgNPs were further characterized by UV-Vis spectrophometer within the range 200-800 nm. The potent AgNPs sample was subjected to antioxidant activity using DPPH assay followed by antimicrobial activity against pathogenic bacteria *E.coli, S.aureus* and *K.aerogenes.* Findings of the study showed that 1mM $AgNO_3$ solution was more effective.Most of the fungal derived AgNPs exhibited maximum absorbance at 418 nm, 425nm, 430 nm, 437nm, 439nm etc. indicating outstanding potential for synthesis of AgNPs.Nanoparticles derived from FE20 showed highest antioxidant potential(81.65%).The inhibition potential was found in order, ,*K.aerogenes>S.aureus>E.coli.* Therefore, it can be concluded that the AgNPs biosynthesized by the endophytic fungi has significant therapeutic potential which may offer immense scope for their application in the field of biomedicine for empowerment of the society.

Keywords
Fungal endophytes, Antimicrobial, AgNPs, Antioxidant, Biomedical

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INTRODUCTION
Biomedical application of metallic nanoparticles has extensively increased the demands for their synthesis because of their large surface area to volume ratio and potential to exhibit different biomedical activities [1, 2]. Silver nanoparticles (AgNPs) are advantageous over

other metals because they can be effective against the broad range of antibiotic-resistant microorganisms and lower toxicity to mammalian cells [3]. Moreover, silver is known to be a health additive and traditionally used in ayurvedic medicine since the ancient times. It exhibits unique properties which are helpful in molecular diagnostics therapies as well as in the devices useful for several biomedical procedures. Therefore, Silver nanoparticles has gained more significance as therapeutic agent in biomedical field. Due to the hazardous effects of chemically or physically synthesized silver nanoparticles, there is emergence of nano biotechnology for development of ecofriendly biosynthesis of nanoparticles [4]. These days, problem of antibiotic resistant microbes has resulted in an urgent necessity for development of novel antimicrobial agents. Although, it is clinically proved that AgNPs have substantial potential for the development of novel biomedicines, physically or chemically synthesized AgNPs have limited applications over the AgNPs than the biosynthesized nanoparticles derived from different organisms. Worldwide fungal endophytes are greatly explored in terms of their nanoparticle synthesizing properties as compare to other organisms and considered as efficient nano factories on account of their reception towards higher bioaccumulation ability, comparatively economic, effortless synthesis method, simple downstream processing and biomass handling. Nowadays, exploration and implication of endophytic fungi in nano biotechnology has receiving extensive attention due to their promising potential for production of bioactive compounds as well as toleration and metal bioaccumulation capability they have emerged up as novel antimicrobial agents to overcome the outbreak of the infectious diseases caused by different antibiotic resistant pathogenic [5].Also these are relatively unexplored potent source for biosynthesis of silver nanoparticles [6]. Therefore, present research has been focused to determine the potential of fungal endophytes for silver nanoparticles synthesis (AgNPs) and their possible applications in biomedical field [7].

MATERIALS AND METHODS

Collection and Sterilization of Plant Material:

Young and healthy plant parts of various black cultivars of *V.vinifera* were randomly collected from vineyards of *Nashik* valley, Maharashtra India during year 2017. These plant parts were washed under running tap water. Sterilization was carried out by treating the plant parts in the solution of commercial bleach followed by washing with D/W. Then they were further treated with 70 % ethanol for few seconds followed by washing with sterile D/W for 3-4 times. Thereafter it was treated with 0.1% $HgCl_2$ solution followed by 3-4 times washing with sterile D/W. The chemicals were procured from HiMedia, Mumbai

Isolation of Fungal Endophytes:

The sterilized plant material was aseptically inoculated on the growth media and incubated at 25±2ºC.Observation of microbial colonies were recorded every after weekly interval. All the fungal colonies which were observed within 15 days were discarded and only the colonies observed during 15 to 45 days of inoculation were considered as true endophytes which were further transferred to Potato dextrose agar medium and incubated at room temperature for isolation and purification of endophytic fungi.

Myco-synthesis of AgNPs :

The fungal biomass of endophytic isolates was aseptically cultivated in 250 ml flask containing 100 ml of potato dextrose broth and further incubated at 28±1ºC for 7 days under the continuous shaking condition. After incubation, the fungal biomass was harvested by centrifugation (4000 rpm, 20 minutes). The pellets of fungal biomass were washed 3-4 times with sterile D/W to remove any attached media components as well as to prevent the contamination .Then approximately 10 gm of the mycelial biomass were suspended in 100 ml sterilized D/W in 250 ml flask and incubated at 28±0.5ºC, for further 3 days on rotary shaker. After wise, the mycelial biomass was separated from by filteration and the cell filtrate was used for the further biosynthesis of silver nanoparticles [8,9]. The reduction reaction for biosynthesis of silver nanoparticle was carried with different concentration of freshly prepared silver nitrate i.e. 0.1mM,0.5mM and 1mM by addition of cell free supernatant of endophytic fungi to the AgNO3 solution (1:5 v/v). The formation of silver nanoparticles was monitored visually by observing the colour change from yellowish to brown. The chemicals were procured from HiMedia,Mumbai

Characterization of Silver Nanoparticles:

Characterization of silver nanoparticles was carried out by UV-Vis spectrophotometer (Shimadzu, Japan, UV-1800 240V). The bioreduction of Ag+ ions in each sample was observed by the dilution with 2 ml of double distilled water. This AgNP synthesized mixture was scanned, in the range of 200 to 800 nm wavelengths. The spectra were recorded at the intervals of 1 min to 10 min and the distilled water was used as a baseline. [10]

Determination of Antioxidant Potential:

The antioxidant potential of potent sample of AgNPs was estimated by the free radical scavenging ability with reference to the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH)procured from HiMedia, Mumbai. Briefly, 5mg AgNPs were dissolved in 1ml double D/W and 100µL suspension solution was added to 100 µL methanolic solution of DPPH (0.1mM) final volume was made up to 2 mL with methanol. Whole mixture was shaken vigorously and incubated (30 min) in dark at room temperature. Absorbance was measured at 517 nm. Ascorbic acid was used as a positive control (standard) and methanol as a blank [10,11]. Radical-Scavenging Activity toward DPPH was estimated from the equation as:

% Inhibition = [A control –A sample / A control] x 100

Evaluation of Antibacterial Activity:

Antibacterial activity of mycosynthesized silver nanoparticles was studied as per agar well diffusion method [12]. The antibacterial activity was evaluated against the pathogenic bacteria viz. *Escherichia coli*(NCIM-2931*), Staphylococcus aureus* (NCIM-2127) and *Klebsiella aerogenes*(NCIM-2239).For this, the test organisms were inoculated on nutrient agar by spread plate method then the wells were made with sterile cork borer and loaded with 15µl of AgNPs under aseptic conditions and kept in refrigerator (30 min) for prediffusion and then incubated at 37°C for 24 hrs. After wise, the plates were observed for the zone of inhibition.

RESULTS AND DISCUSSION

Isolation and Purification of Endophytes:

Endophytes resides within the plant tissues and grows within different plant parts. Therefore, different plant parts of Black cultivars of *V.vinifera* were processed for isolation of endophytes. All the fungal colonies which were observed during 15 to 45days of inoculation were selected as true endophytes which were further transferred to Potato dextrose agar (PDA) medium and incubated at room temperature for isolation and purification of endophytic fungi. The purified isolates were labelled from FE1-FE23 and their morphology was observed under microscope. (Fig.1). Thereafter, they were transferred to Potato dextrose broth and incubated at room temperature on orbital shaker (110 rpm) for the further study.

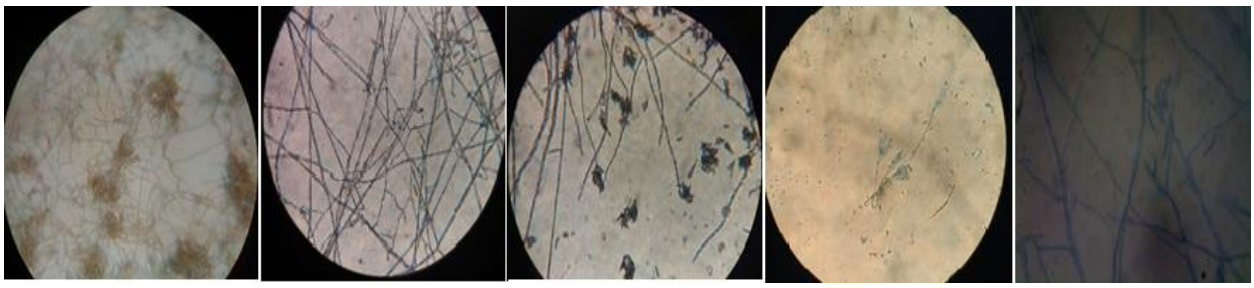
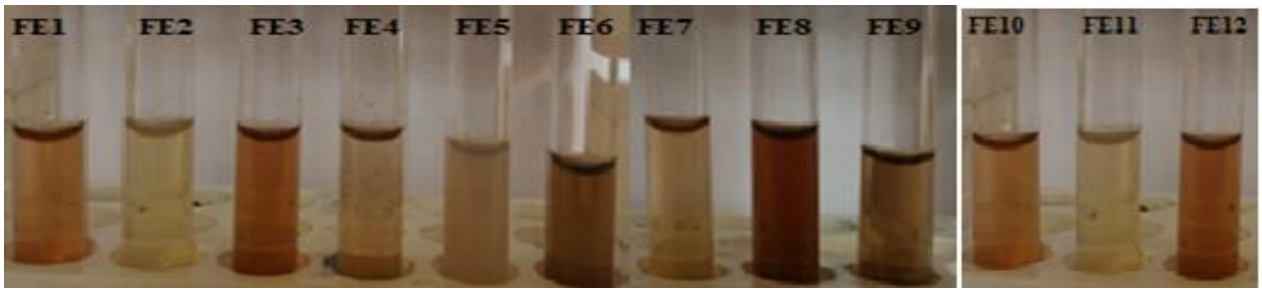


Fig. 1. Microscopic Images of Fungal Isolates of Endophytes of V.Vinefera



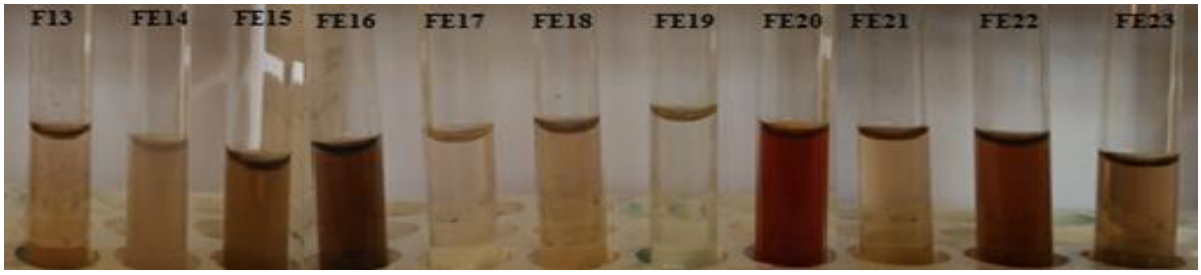


Fig.2. Reaction Mixtures Indicating Synthesis of AgNPs

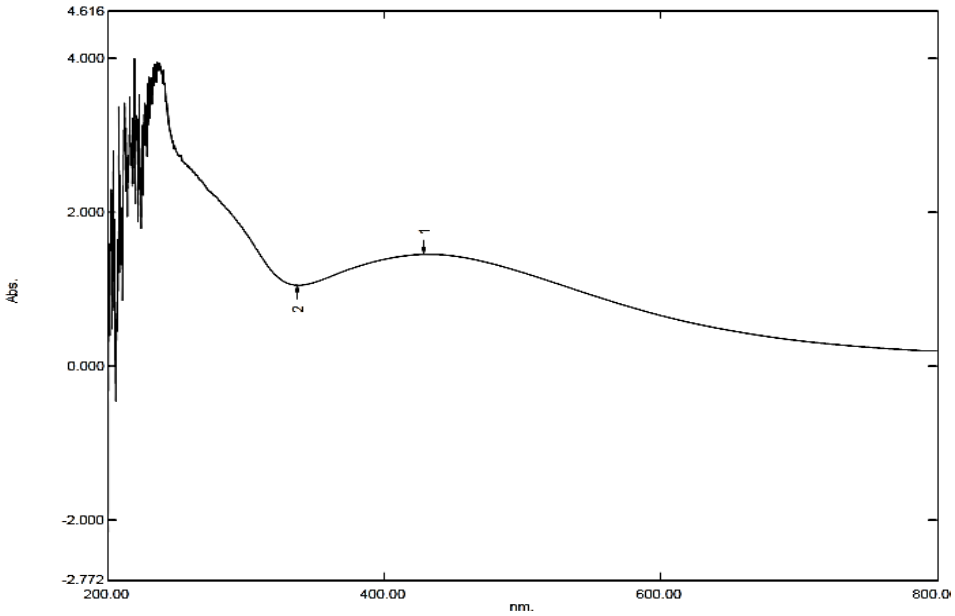
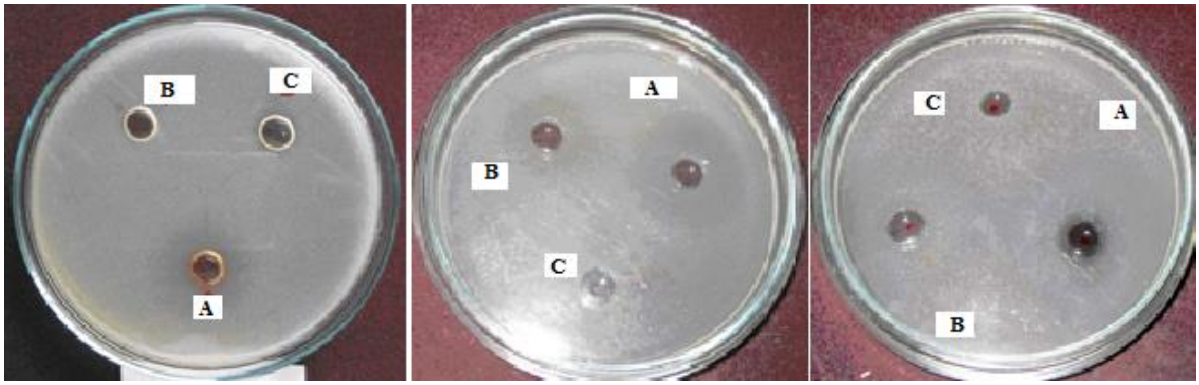


Fig.3.UV-Vis spectra of potent sample

S. Sample C.Control

Fig.4. DPPH Assay exhibiting Antioxidant Activity of Sample



E.coli ***S.aureus*** ***K.aerogenes***

Fig.5. Antimicrobial Activity of Potent AgNPs Against Test Organisms
[A. AgNPs by 1mM $AgNO_3$ B. AgNPs by 0.5mM $AgNO_3$ C. AgNPs by 0.1mM $AgNO_3$]

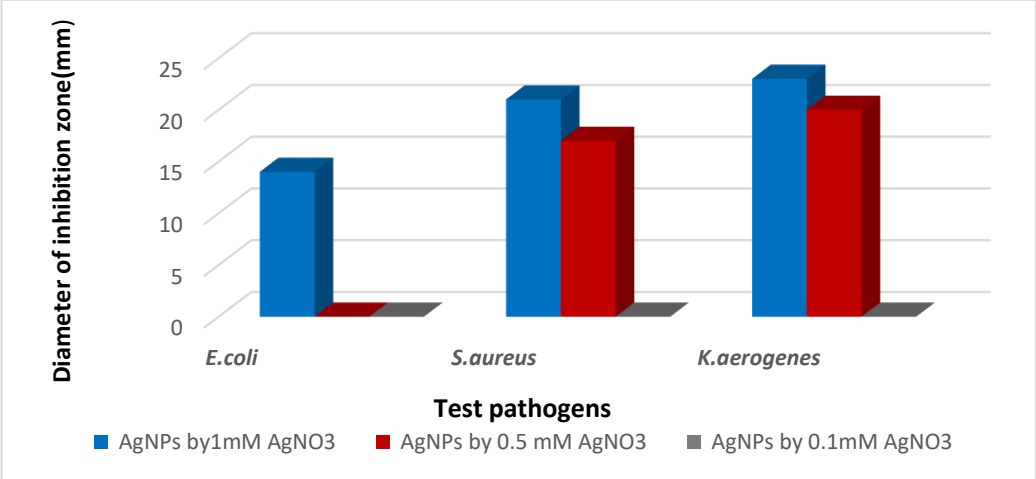


Fig.6.Zone of Inhibition by Potent AgNPs with Different Concentration of $AgNO_3$

Mycosynthesis of AgNPs:

Mycosynthesis of nanoparticles by endophytic fungi of *V.vinefera* was visually evaluated by observing change in colour of the reaction mixure.It was found that most of the isolates showed change in colour from yellow to brown. The colour change was due to the reduction of silver ions to silver nanoparticles i.e. Ag^+ to Ag^0.and due to surface plasmon resonance excited by electromagnetic waves in the visible region [13]. It was found that 1mM concentration of $AgNO_3$ was found to be more effective as compared to the 0.1mM and 0.5 mM concentation as do not showed any change in colour except the isolate FE20.It was found that among the 23 isolates of fungal endophytes ,73.91% isolates showed brown coloration indicating formation of AgNPs whereas,26.09 % isolates were unable to show change in colour or showed only brown tinge indicating the inability of isolates for biogenic synthesis of AgNPs.(Fig 2).The biosynthesis of AgNPs assessed by UV-Vis spectroscopy showed that most of the isolates showing brown coloration of reaction mixture exhibited maximum absorbance at 418 nm, 425nm, 430 nm, 437nm,439nm etc. indicating the outstanding potential for biogenic synthesis of AgNPs. It was found that isolate FE20 which showed remarkable brown coloration has shown maximum absorbance at 420nm showing its strong potential for nanoparticle synthesis (Fig.3.). Hereafter, nanoparticles biosynthesized by isolate FE20 were considered as potent sample for further antioxidant as well as antimicrobial study.

Antioxidant Potential of Potent Sample:

The DPPH radical scavenging activity of potent sample was carried out using ascorbic acid as reference compound. It was observed that the reaction mixture showed colour change from purple to yellow (Fig. 4).It showed noteworthy antioxidant potential with 81.65 % radical scavenging activity.

Antimicrobial Activity of AgNPs:

The antibacterial activity of potent sample of silver nanoparticles was evaluated against the pathogenic bacteria viz. *E.coli , S.aureus and K.aerogenes* .It was found that the AgNPs biosynthesized by fungal endophytes showed remarkable antimicrobial activity against these pathogenic bacteria .However, the maximum inhibitory activity was observed against *K.aerogenes followed by S.aureus* whereas comparatively less inhibitory activity was shown by *E.coli* (Fig 5)**.** The comparative inhibitory action of different concentrations of AgNPs on the selected pathogenic bacteria showed that silver nanoparticles synthesized by using 1mM $AgNO_3$ have successfully shown antimicrobial potential against almost all the test organisms. The order for inhibitory activity against the organisms was observed as, *K.aerogenes> S.aureus> E.coli*. It was revealed that 0.5 mM $AgNO_3$ has found to be comparatively less effective to exhibit antibacterial activity. Whereas, 0.1 mM $AgNO_3$ was unable to show any inhibitory action against all the pathogenic bacteria (Fig.6).It is reported that the Silver nanoparticles shows inhibitory effect over microorganisms by various mechanisms. They enter inside the microbial cells and disturb functions of cell membranes such as permeability and respiration. Besides this they inhibit enzyme functioning of the microbial cells by interacting with sulphur containing proteins and phosphorus-containing compounds such as DNA. Thus, silver nanoparticles interrupt the respiratory chain and cell division leading to cell death [14, 15]. Such complex action mechanisms of silver decrease the probability of development of microbial resistance against them.

CONCLUSION

In present study, total twenty three endophytic fungal isolates i.e. FE1-FE23 were successfully isolated from different parts of black cultivar of *V.vinefera*.During the biogenic synthesis of AgNPs, It was revealed that 1mM concentration of $AgNO_3$ was more effective .It was observed that among the 23 isolates of fungal endophytes ,73.91% isolates showed brown coloration indicating formation of AgNPs whereas,26.09 % isolates were unable to show any change in colour indicating the inability for biogenic synthesis of AgNPs. Characterization of AgNPs by UV-Vis spectroscopy showed that the isolates showing brown coloration exhibited maximum absorbance at 418 nm, 425nm, 430 nm, 437nm,439nm etc. indicating their outstanding potential for biogenic synthesis of AgNPs. Among all the samples of AgNPs, sample derived from FE20 was found to be potent sample. Therefore, only it was considered for further antioxidant as well as antimicrobial study. The DPPH radical scavenging activity of this sample showed noteworthy activity antioxidant potential of 81 .65 %. During the antibacterial study, inhibition potential against pathogenic test organisms was found in the order, *K.aerogenes> S.aureus> E.coli*. Thus, it can be concluded that the AgNPs biosynthesized by using fungal endophytes of *V.vinefera* has noteworthy therapeutic potential. Therefore, the AgNPs derived from such endophytic fungus may offer immense scope for their application in the field of biomedicine for empowerment of the society.

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REFERENCES:

1. Bhattacharya R, Mukherjee P., Biological properties of "naked" metal nanoparticles. Adv. Drug Deliv. Rev. 60:1289-1306, (2008).
2. Hirst S., Karakoti A., Tyler R., Sriranganathan N, Seal S, Reilly CM., Anti-inflammatory properties of cerium oxide nanoparticles. Small ,5:2848-2856, (2009)
3. Sharma S, Kumar S, Bulchandani B., Taneja S, Banyal S., Green Synthesis of Silver Nanoparticles and their antimicrobial activity against Gram positive and Gram-Negative Bacteria. Int. J. Biotechnol. Bioeng. Res. 7: 711-714, (2013)
4. Kalishwaralal K, Deepak V, Ramkumarpandian S, Nellaiah H, Sangiliyandi G., Extracellular biosynthesis of silver nanoparticles by the culture supernatant of Bacillus licheniformis. Mater. Lett. 62:4411-4413, (2008)

5. Kim J., Kuk E, Yu K., Antimicrobial effects of silver nanoparticles. Nanomedicine 3:95-101, (2007)
6. Petrini O.,Fungal endophytes of tree leaves. In. Andrews J., Hirano S., editors. . Microbial ecology of leaves. New York.:179-197, (1991)
7. Singh S, Shukla H and Shukla S.,Biosynthesis of silver nanoparticles by endophytic fungi: Its mechanism, characterization techniques and antimicrobial potential.African Journal of Biotechnology , 16(14): 683-698, (2017)
8. HemanthN, Gaurav K., Karthik L., Bhaskara R.,Extracellular biosynthesis of silver nanoparticles using the filamentous fungus *Penicillium sp*. Arch. Appl. Sci. Res. 2:161-167,(2010)
9. Rahi D. and Parmar A., Mycosynthesis of silver nanoparticles by an endophytic Penicillium species of Aloe vera root, evaluation of their antibacterial and antibiotic enhancing activity. International Journal of Nanomaterials and Biostructures 4(3): 46-51, (2014)
10. Kharat S., Mendhulkar V., Synthesis, Characterization and studies on Antioxidant activity of Silver Nanoparticles using Elephantopus scaber leaf extract. Materials Science and Engineering C ,62:719–724, (2016)
11. Sannigrahi S, Mazuder U, Pal D, Parid S,and Jain S.,Antioxidant Potential of Crude Extract and Different Fractions of Enhydra fluctuans Lour. Iranian Journal of Pharmaceutical Research ,9(1):75-82, (2010)
12. Midolo, P. Turnidge J., Lambert J.., Bell J., Validation of a modified Kirby-Bauer disk diffusion method for Metronidazole susceptibility testing of Helicobacter pylori. Diagn. Microbiol. Infect. Dis.,21: 135-140, (1995)
13. Netala V., Bobbu P., Ghosh S.,Tartte V.,Endophytic fungal assisted synthesis of silver nanoparticles, characterization, and antimicrobial activity.Asian Journal of Pharmaceutical and Clinical Research, 8(3):113-116 , (2015)
14. Sondi I, Sondi B., Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J. Colloid Interface Sci. 275:177-182. (2004)
15. Song H, Ko K, Oh L, Lee B.,Fabrication of Silver Nanoparticles and their Antimicrobial Mechanisms. Eur. Cell. Mat. 11(Suppl 1):58, (2006)