Available Online at

http://www.ijcpa.in

October-December 2018

DOI: http://dx.doi.org/10.21276/ijcpa

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

Research Article | Volume-6 | Issue-1 | Article ID: 0002

COMBINATORIAL EFFECT OF SILVER NANOPARTICLES AND PLANT EXTRACT AGAINST SELECTED RESPIRATORY PATHOGENS

***Vaidehi V. Chandorkar, Anmol Varghese, Ashok V. Gomashe**

Department of Microbiology, Shivaji Science College, Congress Nagar, Nagpur, India.

***Corresponding Author:** Email: vaidehic21@rediffmail.com*

Received: 13 March 2018 / Revised: 10 May 2018 / Accepted: 25 July 2018 / Available online 31 December 2018

ABSTRACT

Respiratory infections are quite common these days due to immense amount of pollution in the environment. It ranges from common cold, to life-threatening entities like bacterial pneumonia, pulmonary embolism. The treatments available used to have an impact on the infection causing organisms initially but the current scenario is not so, as the pathogens have the tendency to develop resistance towards the drug. Pertaining to this issue there is a need to undertake multipronged approach for preventing and treating such infections. Silver Nanoparticles (AgNPs) have a lot of applications in the field of biotechnology, biomedicine, biosensors, catalyst and therapeutic areas. Synergistic activity of silver Nanoparticles and natural extracts have shown the enhanced activity, and it was observed even resistant organisms were sensitive to the combination. The present findings corroborate that silver Nanoparticles possess compounds with antimicrobial properties which supports its medicinal use.

Keywords *– Respiratory infection, Silver nanoparticles, Natural extracts, Antibiotics, Synergistic activity*

1. INTRODUCTION

Nanoparticle is a core particle which performs as a whole unit in terms of transport and property[1]. The biological synthesis of nanoparticle is a challenging concept which is very well known as green synthesis. The biological synthesis of nano material can solve the environmental challenges like solar energy conservation, agricultural production, catalysis[2] .Green synthesis of nanoparticle are cost effective, easily available, large scale production and act as reducing and capping agent[3] in comparison to the chemical method which is a very costly as well as it emits hazardous by-product which can have some deleterious effect on the environment [4].

Nanomaterials especially silver Nanoparticles have a lot of applications in the field of biotechnology, biomedicine, biosensors, catalyst, and therapeutic areas. Different methods are available for the synthesis of silver Nanoparticles like physical, chemical but recently development of biological method for the synthesis of Nanoparticles is simple, quite fast, eco-friendly, and free from any solvent or toxic chemicals involvement in the process. Biological method for synthesizing of silver Nanoparticles could have application in the field of medicine especially as anti-carcinogenic effect, drug carrier and diagnosis purposes. Different gold and silver Nanoparticles (nonmaterial) have been synthesized by the biological method using (fungi, bacteria, algae). By using

biological method, controlled silver Nanoparticles has been produced which increases the interest in this field of nanomedicine (development of drug) research. Different fungi have been reported for the synthesis of silver Nanoparticles10-12.

These fungi reduce the metal (silver and gold) extracellularly due to the presence of reductase enzyme present in the cell wall which generate stable silver and gold Nanoparticles in the deionized water. Silver has been used from the ancient times in the form of silver nitrate and silver sulfadiazine in order to treat various infections like wounds, burns, ophthalmic problems and also used as a disinfectant. Development of resistance by the bacterial pathogens to the antibiotics has become a major problem in the worldwide in the recent times. Biosynthesized Silver Nanoparticles have been used to counter these drug resistant microbes.

The importance of natural products for medicine and health has been enormous. The natural extracts Honey, *Ocimum tenuiflorum, Mentha arvensis, Piper nigrum, Zingiber officinale* has been reported to have an inhibitory effect to various species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives that cause respiratory tract disorder. Pathogens found to be sensitive to anti-infective properties of these natural extracts are manifold.

Respiratory infections can affect any part of the respiratory system. An infection of the lungs which is usually caused by bacteria, particularly *Streptococcus pneumoniae* in Western countries. Worldwide, tuberculosis is an important cause of pneumonia. Other pathogens such as viruses and fungi can cause pneumonia for example severe acute respiratory syndrome and pneumocystis pneumonia.

Klebsiella infections is spread through exposure to the bacteria via respiratory tract, which causes pneumonia, or the blood to cause an infection in the blood stream. Upper respiratory tract infections (URI) can by *Chlamydia pneumonia E. coli, Mycoplasma pneumoniae, Streptococcus aureus, Streptococcus pyogenes, S. pneumoniae, Bordetella pertussis, and Hemophilus influenza* [5].

Antibiotics are generally prescribed for the treatment of any infection. As organisms become resistant to Antibiotics, Antibiotic susceptibility testing becomes mandatory.

2. MATERIALS AND METHODS

2.1 Preparation of silver Nanoparticles

Fungus was grown in 250ml Erlenmeyer flask. Flask containing 100ml of MGYP broth which consists of malt extract 0.3%, glucose 1%, yeast extract 0.3%, peptone 0.5%. At 29°C for 72 hours in static position. After this, biomass was washed with distilled water. This was repeated 2-3times to remove any traces of medium contents. Biomass was taken into flasks containing 100ml distilled water and incubate at same position for 48hours.Suspension was filtered with help of Whatman filter paper. Obtained filtrate was challenged with 1mM $AgNO_3$ at 29°C for reduction

2.2 Extracellular synthesis of silver Nanoparticles

Biomass of fungus was separated by filtration. Fungal filtrate was treated with equal volume of 1mM $AgNO_3$ solution. After 24 hours of incubation color changed from pale yellow to brown with Clear indication of formation of silver Nanoparticles in reaction mixture.

2.3 Preparation of Natural extract

Leaves of Tulsi and Pudina were collected, washed with tap water, and was blotted with filter paper. Spread over newspaper for air drying under sheet. Leaves were powdered using mixer grinder. 50gm (powdered form) of each plant was taken in 250ml flask. Add 100ml ethanol (95%). Kept at room temperature for 48 hours and rapidly stirred every 8 hours.

Honey, Black pepper, and Ginger juice was added directly.

2.4 Antimicrobial activity

Clinical pathogenic strains were used to determine the antibacterial activity of the silver nanoparticle. 1ml of suspension of approximately 10-5CFU/ml density of micro-organisms to be tested was distributed uniformly on agar plates. Wells were punctured on MHA agar plates on which lawn of organisms were made. Each well was filled with:

- Natural extracts (Honey, Tulsi, Pudina, Black pepper, Ginger)
- silver Nanoparticles
- Mixture of natural extracts and silver Nanoparticles.
- Antibiotics discs were placed on the agar

Plates were incubated at 37°C for 24hours. Results were observed.

3. RESULTS AND DISCUSSION

3.1 Silver nanoparticle

Silver Nanoparticle synthesized from Fungi; *Aspergillus* was confirmed by FTIR.

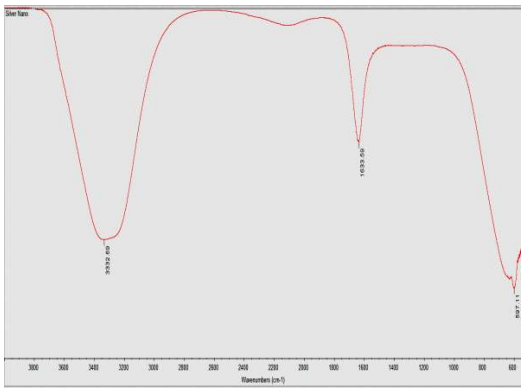

Fig 1: FTIR of Silver Nanoparticles

FTIR gives the information about functional groups present in the synthesized silver Nanoparticles for understanding their transformation from simple inorganic AgNO3 to elemental silver by the action of the different phytochemicals which would act simultaneously as reducing, stabilizing, and capping agent. FTIR spectrum clearly illustrates the bio fabrication of silver Nanoparticles. Fig : 1 represents the FTIR spectrum of silver Nanoparticles synthesized from the organism *Aspergillus niger* which showed peaks at 3332.69 cm-1, 1633.59cm-1, 597.11cm-1 shows the presence of functional group N-H, C=C and Halogen group [6].

3.2 Antimicrobial Activity

Antimicrobial activity of the synthesized AgNPs from fungi *Aspergilus niger* along with five natural extracts testing was done against five clinically important Respiratory pathogens *Streptococcus, Escherichia coli, staphylococcus aureus and Klebsiella sp.* which showed promising antibacterial activity against all the pathogens. When only Natural extracts were examined, *Streptococcus sp.* didn't show any activity against Honey *while Klebsiella* sp. didn't show any activity against Honey as well as Ginger, whereas *E.coli* was found to be resistant to only Ginger, for rest of the natural extracts e.g. Black pepper, Tulsi, Pudina, organisms were found to be sensitive. Silver Nanoparticles did not show distinguishable results. On the contrary Antibiotics had shown very promising results except for *S. aureus* which was resistant to Erythromycin. Hence, Effort in the current research was to examine the effectiveness of joint action of Silver Nanoparticles and all-natural extracts. Similar activity as that in individual natural extract was observed. Distinctive activity was observed in Honey + AgNP and Ginger + AgNP combination. In this,

Organisms resistant to earlier tested extracts, Antibiotics and silver Nanoparticles were found sensitive to the combination, this demonstrates efficacious synergistic action as depicted in Fig 2.

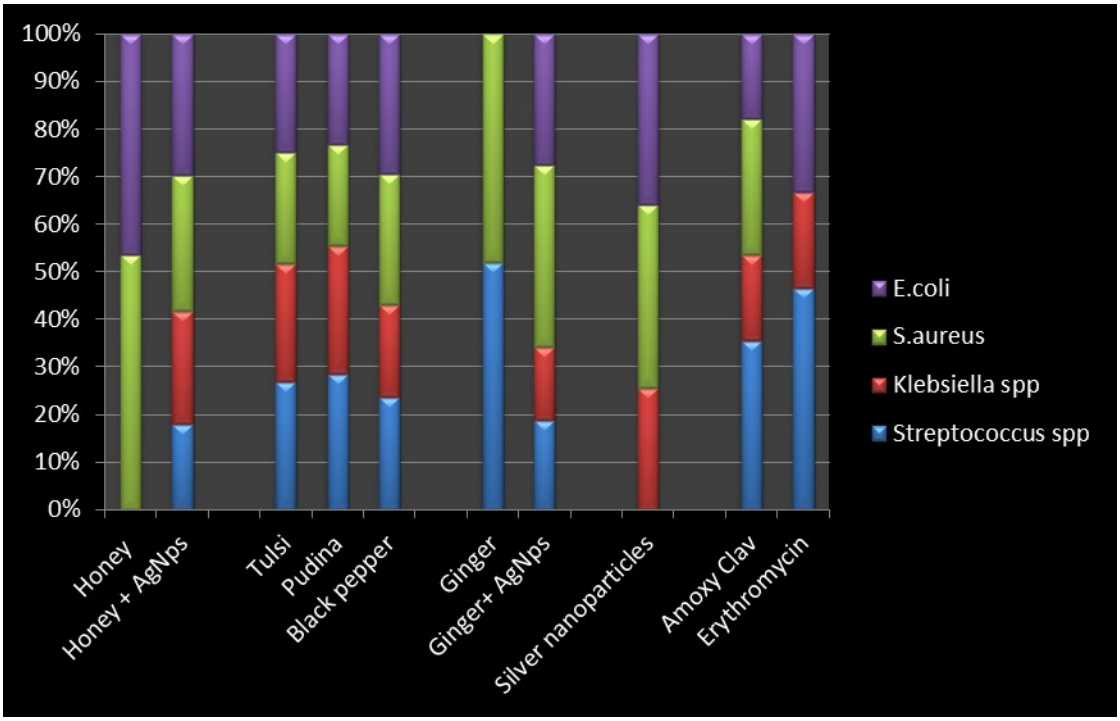

Fig 2: Sensitivity pattern of selected Respiratory pathogens to Natural extracts, Silver Nanoparticles, Extract+ AgNP and Antibiotics.

The exact mechanism behind the antimicrobial activity of Nanoparticles is not clearly known but some of the hypotheses may be: Attachment to the bacterial cell wall and changing the permeability of the cell membrane, Production of Reactive oxygen species and damage the cell membrane, Bind to DNA and leads to problem in DNA replication.

Similar work is carried out by Mano priya[7] *et al*., (2011) who observed the antimicrobial activity of synthesized Ag Nanoparticles against six different bacteria such as *E. coli, S. pyrogens, S. aureus, B. Subtilis, S. typhi and Citrobacter* sp. As it showed a clear inhibition zone.

Kumbalwar M.M [8] *et.al* evaluated the antibacterial activity of M. Arvensis L, which indicated that *M. arvensis* L. have the potential to generate novel metabolites. The plant extract demonstrated anti-bacterial activity.

Dr.Zaki[9] *et.al* studied that ginger (alcoholic and aqueous) extracts have bactericidal activity, and it found that the stock solution of the extracts inhibited the growth of all the bacterial isolates, but when the dilutions made, the efficacy reduced. The widest inhibition zone of crude alcoholic extract obtained from *Streptococcus spp.* was (44mm), then (23, 11, 6 mm) from *E. coli* at 10-1, 10-2, and 10-3 conc. respectively, no inhibition zones at 10-4. *Enterobacter spp*. showed highest inhibition zone (23 mm) from crude aqueous extract, then *Streptococcus sp.* (14, and 8 mm) at 10-1, and at 10-2 (8mm), no inhibition zones at 10-3, 10-4.

4. CONCLUSION

Biological synthesis of silver Nanoparticles by the help of *Aspergillus niger* is a very cost effective, safe, non-toxic, eco-friendly route of synthesis which can be manufactured at a large scale. The AgNPs + natural extracts possess great antimicrobial activity against respiratory pathogen.

The present findings corroborate that silver Nanoparticles may possess compounds with antimicrobial properties which may support its medicinal use. The results indicate significant capacity and future scope for the use against a wide range of microbial populations. The work can be extended to reveal specific secondary metabolites that attributes to their antimicrobial activity.

REFERENCES

1. Nour, A.E., Kholoud, M.M., Eftaiha, A., Reda A.A.W and Ammar, A.A. Synthesis and applications of silver Nanoparticles. Arab. J. Chem., 2010, 3: 135– 140.
2. Kumar L, Chakraborthy G, Singh V, Mazumder A, *Hibiscus rosa-sinensis*: A Review on Divine Herb, Journal of Advances in Pharmacy and Healthcare Research, 2012. 2(4):6.
3. T. Sriram and V. Pandidurai, Synthesis of Silver Nanoparticles from Leaf Extract of *Psidium guajava* and its Antibacterial Activity Against Pathogens, Int.J.*Curr.Microbiol.App.Sci.* 2014, (3): 146-152
4. Kaler A, Jain S, Banerjee U. Green and rapid synthesis of anticancerous silver Nanoparticles by *Saccharomyces boulardii* and insight into mechanism of nanoparticle synthesis. BioMed Res Int, 2013:1–8
5. Bisno AL. Acute pharyngitis. N Engl J Med, 2001; 344(3):205–11.
6. Kong J, Yu S. Fourier transform infrared spectroscopic analysis of protein secondary structures, Acta Biochim Biophys Sin (Shanghai). 2007;39(8):549-59
7. Mano Priya, M., Karunai Selvia B.A. John Paul. J.A. Green synthesis of silver Nanoparticles from the leaf extracts of *Euphorbia hirta* and *Nerium indicum*. Digest Journal of Nanomaterials and Biostructures, 2011, 6(2): 869 877.
8. Kumbalwar M M., Ingle A B. Shende M.H. Antimicrobial Activity of Mentha Arvensis L (Pudina) Against on Gram Negative Bacteria Indian Journal of Applied Research, 2014, 4(4):488-489.
9. Zaki NH, Al-Oqaili RM, Tahreer H. Antibacterial effect of ginger and black pepper extracts (alone and in combination) with sesame oil on some pathogenic bacteria. World Journal of Pharmacy and Pharmaceutical Sciences (WJPPS). 2015;4(4):774-84.