

# Biosynthesis of Silver nano Particles from Aqueous Extract of Apple Peel and Its Antimicrobial Activity Against *Fusobacterium nucleatum*

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## Abstract

**Aim & objective of the study:** - The main objective of this study is to evaluate the antimicrobial potential of biologically synthesized silver nanoparticles, against anaerobic bacteria *Fusobacterium nucleatum*. These bacteria are found in oral cavities as a part of the normal human flora and many times responsible for the oral disease like periodontitis. In this experiment silver nanoparticles were used, which are synthesized by using peel of apple.

**Result:**-Production of Silver nanoparticles was confirmed by UV-Visible and IR spectroscopy. Particle size has determined by Zeta Potential. Antimicrobial activity has done by well diffusion method. Silver nanoparticles made by using aqueous extract of fresh apple peel shown maximum zone of inhibition of  $18.25 \pm 0.500$  mm at 100mg/ml against *Fusobacterium nucleatum* and aqueous extract of apple peel shown maximum zone of inhibition of  $20.25 \pm 0.500$  mm at 200mg/ml

**Conclusion:**-The result suggested that the biogenic synthesized silver nanoparticle shown effective antimicrobial activity against *F.nucleatum*. Thus these particles can be utilized to produce biomedical product to control microbial population of infection or diseases causing by *Fusobacterium nucleatum*. In this study these antimicrobial effective silver nanoparticle were synthesized by using apple peel, therefore this technic can be replace chemical and physical method of nanoparticle.

## Introduction: -

Today there are lots of applications and technologies are comes into the picture with lots of future aspects, Nanotechnology is one of the most popular and widely utilized by the research to reveal and analysis the potential of nanoparticle to produce more product based on nanoparticles. Because of its property of having diameter of Nano scale, is become more popular now a day. Initially nanoparticles were made by using chemical and physical methods which can able to cause harmful effect[1], however now a day nanoparticle is synthesis by using less harmful biological material like microorganism ( fungal, algae etc. ), and plant material therefore this technic is called as biogenic synthesis or green synthesis of nanoparticles. Green synthesis of nanoparticle is more popular among research because of its environmental friendly, less time taking and cost effective properties which having less harmful effects on human being[3,4].

Silver is known as having antimicrobial properties from ancient times. Because of it antimicrobial properties silver is being use in many tradition and day to day life[2]. This properties of silver, made it more suitable for making nanoparticles to develop one kind of antimicrobial agent with less harmful effects. Biosynthesis of silver nanoparticle provides a wide range of environment friendly methodology.

Apple is known as very rich nutrient fruit with having many beneficial components like phenolic compound flavonoid compound and many more, therefore apple is always consumed by large group of people and many times selected by researcher as raw material for research. Many researchers have already prof that apple peel contains more beneficial properties than apple flash. Apple skin has flavonoid like quercetin glycosides and cyanidin glycosides which is not found in apple pulp. Apple is belong to the Kingdom:-

Plantae ,family:-Rosaceae,order:-Rosales,Genus:-Malus and species:-M.Pumila and its Binomial name is *Malus Pumila* and found in all over the world.

Human mouth is known as a great source of aerobic and mostly anaerobic microorganisms because mouth provides suitable condition for their growth by serving them good temperature, and food for proliferation. *Fusobacterium nucleatum* is anaerobic, gram-negative bacterium commonly found in oral cavity and play an important role in periodontal disease[6]. Compared to the healthy individual, patients with periodontitis and gingivitis having increased growth of F.Nucleatum.it is constantly associated bacteria with periodontitis and play an important role in biofilm formation [7,8]. As F. nucleatum are strongly associated with periodontitis, their suppression may be important in controlling the disease [11].

## **Method and material-**

### **Collection of Plant material**

Fresh apple peels were obtained by peeling fresh apples and some were collected from local juice shop where they use apple pulp for fruit custard and peels were no more useful for them. The apple peels were washed twice and thrice well by using distilled water, extra apple pulp was removing by the help of knife before washing. Apple peels were use in fresh condition for aqueous extract preparation.

### **Extraction method:**

#### **Aqueous Extraction of fresh apple peel:-**

#### **By Boiling Method:-**

For obtain this type of extract washed fresh apple peel were used. The 20 gm. of apple peel were soaked in 200 ml of distilled water and boil for 30 minute. The extract was then filtered using firstly by muslin cloth then filter paper .Then evaporate excesses water to thick paste by using water bath at 60°C thick paste of extract was obtained .Extract was transferred to glass vials and keep at low temperature.

#### **Biosynthesis of nanoparticles: -**

Preparation of Silver nitrate solution:- Silver nitrate solution was prepared. To prepare 1mM AgNO<sub>3</sub> solution 16.9 mg AgNO<sub>3</sub> is dissolve in 100 ml deionized water.

**Silver nanoparticle biosynthesis by using aqueous extract of apple peel:-** For synthesis of this type of silver nanoparticles, a known concentration of fresh apple peel extract was interacted with 1mM AgNO<sub>3</sub> solution at a the ratio of 9:1 of AgNO<sub>3</sub> and extract to make up 100ml volume in 250 ml flasks then flask was incubate for a desired time (commonly 24hr) at 28°C.

The formation of AgNPs(silver nanoparticles) was observed by the development of yellowish-dark brown color. The incubation was done for 24 h. the observations were made after define time interval and observe a change in color from light brown to dark brownish.

## **Characterization of silver nanoparticles**

### **UV-Vis Spectroscopy**

Silver Nanoparticle synthesis by adding apple peel extract to the silver nitrate solution is conforming by observing change in color of reaction mixture. Synthesized silver nanoparticles were characterized by UV-Spectroscopy. UV-Vis spectrometer records the intensity of absorbance and show a band in specific wavelength, which is one of the characteristic of nanoparticle. Added plant extract help to reduce Ag<sup>+</sup> to Ag<sup>0</sup> [1].In term of optical density the higher OD indicate the high conversion of Ag<sup>+</sup> to Ag<sup>0</sup>. Silver nanoparticles show specific optical properties in particular wavelength of light, by observing it spectrum band it can be easily characterized.

### **IR spectroscopy:-**

The Peel extracts and AgNPs samples were analyzed by FTIR spectra. The different functional groups present in the samples were identified by FTIR spectroscopy. The various modes of vibration were identified and assigned. FTIR measurements were made to identify the presence of biomolecules, which are responsible for the reduction of silver ions to AgNPs and stabilization of AgNPs in colloidal solution [5].

**Zeta Potential:-** Silver nano particle size was determined by the help of Zeta potential technics. Zeta potential gives the net surface charge to the nanoparticles, this technic also measure the stability of nano particles. With the help of Zeta Potential we can understand the state of particle surface charge and it is also help to determine the stability of the nanoparticle [12]. Sizes and shapes of silver nanoparticles are affected by a number of factors which includes pH, precursor concentration, reductant concentration, time of incubation, temperature as well as preparation method of nano particles. [13].

### **Experiment for testing antimicrobial activities of Prepared Silver Nanoparticles**

**Test Microorganisms:-** ATCC *F.nucleatum* culture was used in this experiment. This Microorganism was sub cultured on the brain heard infusion broth and incubates anaerobically at 37°C for 42 to 72 hours or more.

**MIC determination:-**To obtain antimicrobial effectiveness of silver nano particle, MIC of silver nanoparticle was determined with the help of broth dilution method. MIC is define as lowest concentration of any compound/extract/drug which can able to completely inhibits the visible growth of microorganism in 24 h[14,15,16] . The microbial activity of AgNPs was recorded by the determination of minimum inhibitory concentration (MIC). The selected bacterial suspensions were prepared and seeded on the Brain heard infusion broth having 0.03 mg/ml to 20 mg/ml of AgNPs in ten different test tube. All the tube was incubated at 37°C for 24 hours, and the MIC concentration was recorded.

**Screening of antimicrobial activities by well diffusion method:-** Biosynthesized Silver nanoparticles were tested for its antimicrobial activity by well diffusion method against *Fusobacterium nucleatum*. The pure bacteria cultures were anaerobically sub cultured on Brain heart infusion broth. Sub cultured bacterial were used to test antimicrobial properties of silver nano particle. Brain heard infusion agar media was used to determine the antimicrobial potential of nano particle. After incubation at 37±C for 42 to 72 hours, the diameter of zone of inhibition for different concentration of silver nanoparticle synthesized by aqueous extract and aqueous extract of apple peel were determined.

### **Result:-**

**UV- Spectroscopy:-** Reduction of Silver ion into Silver nanoparticles by using two type of peel extract of apple was evidenced by change of color of reaction mixture from yellow to reddish brown[17,18] . The result of UV-Vis spectroscopy of prepare reaction mixture confirmed the formation of Ag nanoparticles. The surface Plasmon resonances (SPR) of produced silver nanoparticles was observed at around 400 nm, which prof that aqueous extract of apple peel is able to reduce silver ion into silver nanoparticle

### **IR Spectroscopy:-**

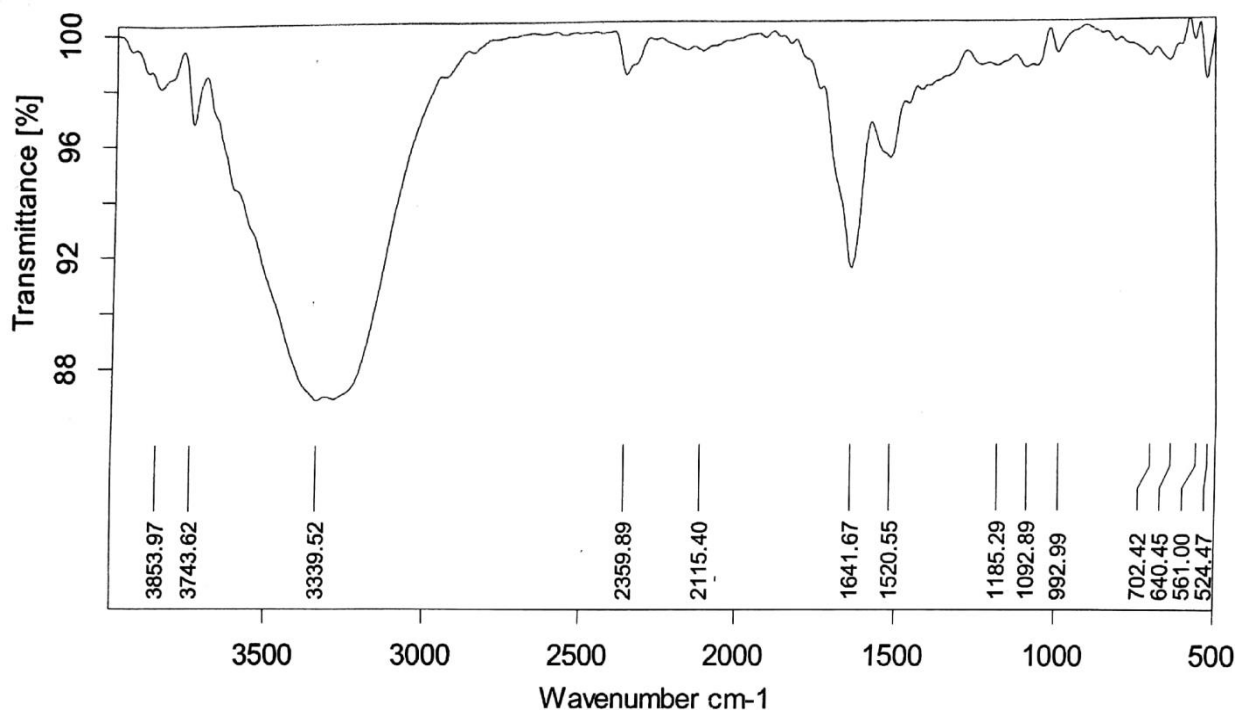
- The presence of functional group or biomolecules in the reaction mixture was determined with the help of IR spectroscopy. These Biomolecule are responsible for reducing and capping of silver nanoparticle, and also helps to produce more stabilized silver nanoparticle[18,19]. Silver nano particle made by aqueous extract of apple peel shows( Image no.1) the Infrared absorption peaks of silver nano particle made by aqueous extract shows a very weak O-H stretch at 3853 & 3740, very broad O-H stretch hydrogen bond shifted to lower frequency at 3339 ,at 1641 peak less intense C=C broad stretch observed, the peak at 1526 correspondence to C=C aromatic ring stretch ,methylene C-

H stretch peak is observed at 1418 , An absorption band appeared at 1185 due to the stretching vibration of O-C-O, while an absorption band at 1092 is due to the presence of very weak alkene out of plane deformation. In the reaction mixture the presence of hydroxyl, alkene, carbonyl functional groups are indicated By Infra-Red absorption spectrum.

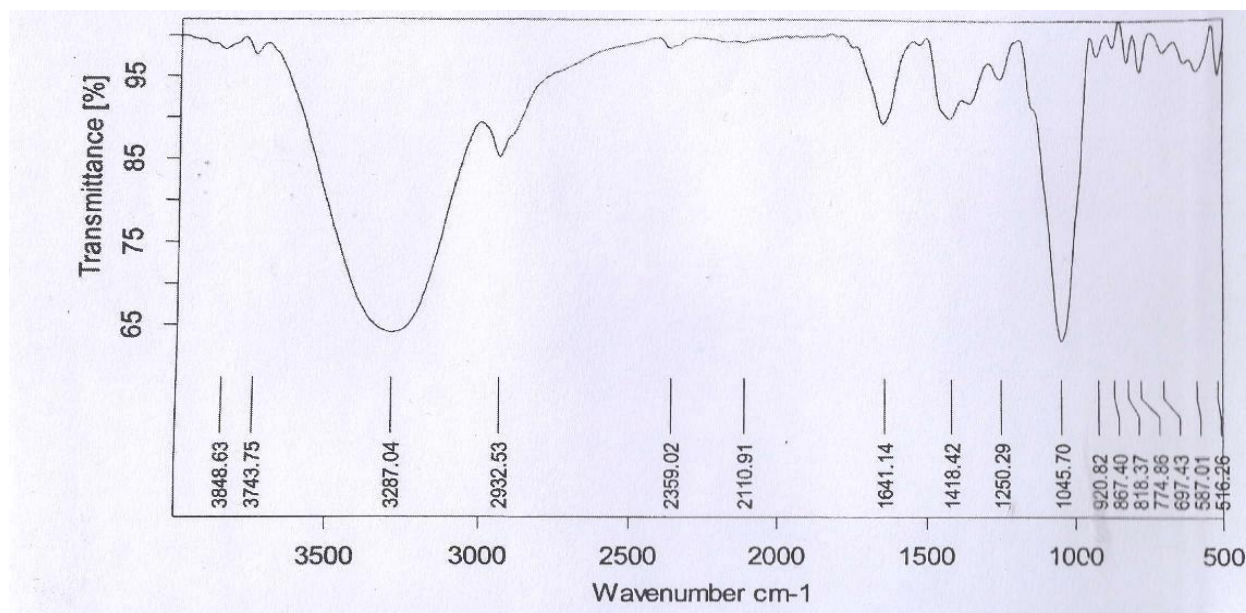
- Infrared absorption peaks of aqueous extract of fresh apple peel at  $\text{vcm}^{-1}$  shows very weak O-H stretch at 3854 & 3743, hydrogen bonded very broad O-H stretch was observed at 3287 nm, hybridize C-H stretch occurs at 2932, due to less intense C=C broad stretch peak observed at 1641 nm, at 1418 nm peak occur due to methylene C-H stretch, peak at 1250 nm was correspondence to C=O weak stretch, strong sharp alkene of plane deformation was observed at 1045nm, as well as alkene out of plane bending was appears at 920-867, aromatic substitution ring bending occurs at 774 nm. Major functional groups indicated by Infra Red absorption spectrum are hydroxyl, alkene, substituted aromatic ring, and carbonyl.

### Zeta Potential:-

The size of silver nanoparticle and its charge were determined with the help of Zeta Potential reveals the average particle size of aqueous SNPs was, these particles carries negative charge on it.



**Image:1 IR spectrum of Silver Nanoparticle**

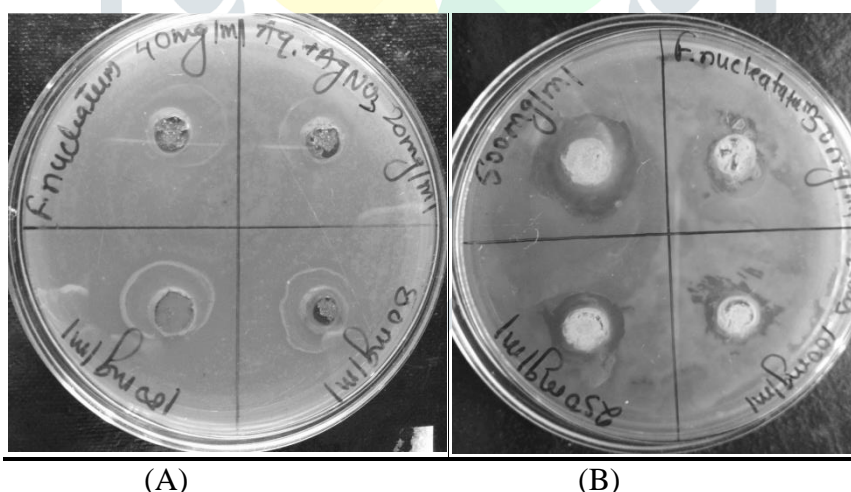


**Image:2 IR spectrum of Aqueous Extract**

### **Antimicrobial Test Results**

#### **Well Diffusion Method:-**

Antimicrobial activity of silver nanoparticles and aqueous extract was determined by well diffusion method. Synthesized nanoparticles were tested against Gram-negative bacteria *Fusobacterium nucleatum* and zone of inhibition was recorded. Different concentration of silver nanoparticles (20mg/ml, 40 mg/ml, 80 mg/ml, 100 mg/ml) and extract (50mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml) were tested. 100 mg/ml SNPs prepared by aqueous extract shows  $18.25 \pm 0.500$  mm zone of inhibition however peel extract shows maximum Zone of Inhibition  $20.25 \pm 0.500$  at the 200 mg/ml concentration.



**(A) Image shows ZOI of SNP against F.nucleatum**

**(B) Image shows ZOI of aqueous extract against F.nucleatum**

### **Conclusion:-**

This study demonstrated that silver nano particle obtained from aqueous extract of apple peel as well as aqueous extract shows a good antimicrobial activity against *F. nucleatum* which is association with oral microbial disease mainly with periodontal pathogens. As result shows that the reduction of *F. nucleatum* population was increases with the increasing concentration of Silver nano particle and aqueous extract it can be conclude that extract and Silver nano particle synthesis with the help of apple peel can be used to control

the microbial population of *F. nucleatum* for maintaining oral hygiene and also to avoid serious oral disease like periodontitis. Besides that Nanoparticles, have immense applications in the field of early diagnosis and management of diseases, also including in caused of multidrug-resistant pathogens. The present study also concludes that the peel of apple can use as reducing agent to synthesize Silver nanoparticles. As green synthesized nanoparticles are ecofriendly it must be promote to utilized more than chemical method of SNPs synthesis; besides that it is also a cost effective procedure.

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