

**INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH
TECHNOLOGY****A COMPARATIVE ANALYSIS OF ANTIMICROBIAL ACTIVITY OF SILVER
NANOPARTICLES FROM POMEGRANATE SEED, PEEL, AND LEAVES****M.Haniff Nisha*, R.Tamileaswari, Sr.S.Jesurani**

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ABSTRACT

The green synthesis of silver nanoparticles using plant extract has more advantages because they are eco-friendly, easily availability and cost efficient. The bio synthesis of silver nanoparticles using pomegranate seed, peel and leaf its act as a reducing agent as well as capping agent from 1mM AgNO₃ has been investigated. The formation of silver nanoparticles was characterized by UV-vis spectrum. The morphology was characterized by scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX) and X-ray diffraction (XRD), with the active functional groups present in the synthesized silver nanoparticles being confirmed by Fourier Transform Infra-red (FTIR) spectroscopy. Moreover, their antibacterial activity was evaluated against Pseudomonas, Bacillus cereus, staphylococcus albus and proteus pathogens. And anti-fungal activity was evaluated against Candida Tropical and Aspergillus. The applications of silver nanoparticles have the medical application like wound dressings, surgical instruments.

KEYWORDS: Green Synthesis. Pomegranate seed, peel and leaf. Antibacterial , Antifungal.

INTRODUCTION

Nanoparticles serve as the fundamental building blocks for various nanotechnology applications. Nanotechnology, and alongside nanostructured materials, play an ever increasing role in science, research and development as well as also in every day's life, as more and more products based on nanostructured materials are introduced to the market. Nanotechnology deals with materials with dimensions of nanometers, i.e. nanostructured materials. Nanoparticles are seen as solution to many technology and environmental challenges. By green synthesis methods of NPs reduced hazardous to the global efforts. Implantation of these sustainable processes should adopt the fundamental principles of green chemistry [1].

Silver nanoparticles can be synthesized by several physical, chemical and biological methods. However for the past few years, various rapid chemical methods have been replaced by green synthesis because of avoiding toxicity of the process and increased quality. Silver nanoparticles are one of the promising products in the nanotechnology industry. The development of consistent processes for the synthesis of silver nanomaterial's is an important aspect of current nanotechnology research. One of such promising process is green synthesis [2].

Silver nanoparticles have tremendous applications in the field of high sensitivity bimolecular detection and diagnostics, antimicrobials and therapeutics, catalysis and micro-electronics. It has some potential application like diagnostic biomedical optical imaging, biological implants (like heart valves) and medical application like wound dressings, contraceptive devices, surgical instruments and bone prostheses [3].

Pomegranate fruit is one of the most popular, nutritionally rich fruit with unique flavor, taste, and health promoting characteristics. Botanically, it is a small size fruit-bearing deciduous tree belonging within the *Lythraceae* family, of genus: *Punica*. The fruit is thought to originate in the Sub-Himalayan range of North India. Scientific name: *Punica*

granatum. Pomegranate tree grows to about five and eight meters tall. It is cultivated at a commercial scale in vast regions across Indian sub-continent, Iran, Caucuses, and Mediterranean regions for its fruits. Completely grown-up tree bears numerous spherical, bright red, purple, or orange-yellow colored fruits depending on the cultivar types. Each fruit measures about 6-10 cm in diameter and weighs about 200 gm. Health benefits of pomegranate, the fruit is suggested by nutritionists in the diet for weight reduction and cholesterol controlling programs. Regular inclusion of fruits in the diets boosts immunity, improves circulation, and offers protection from cancers [4]. Pomegranate seeds are an excellent source of dietary fiber which is entirely contained in the edible seeds. Compared to the pulp, the inedible pomegranate peel contains as much as three times the total amount of polyphenols. The higher phenolic content of the peel yields extracts for use in dietary supplements and food preservatives [5]. Pomegranate leaf has a wide range of potential health benefits. It has been shown to aid in digestion and to help treat certain infections and illnesses. More recently, it has been studied as an appetite suppressant and weight loss aid. One potential benefit of using pomegranate leaf is that it helps aid indigestion. The tea can be taken for upset stomach and related symptoms, or it may be taken as a supplemental capsule [6].

So sufficient literature is not reported on the green synthesis of AgNO_3 from seed, peel & leaf extract of pomegranate. In this work, we have explored an inventive contribution for synthesis of silver nanoparticles using seed, peel & leaf of pomegranate. The procedures for development of stable silver nanoparticles, simple and viable synthesized nanoparticles were characterized by various methods, such as UV-visible spectroscopy. The interaction between nanoparticles with functional groups was confirmed by using Fourier transform infrared spectroscopy analysis (FTIR). The morphological characterizations are performed using scanning electron microscope (SEM) with Energy dispersive analysis (EDAX), X-ray diffractometer (XRD) and also further the antibacterial activity & antifungal activity of these biologically synthesized silver nanoparticles was evaluated against different pathogenic microorganisms. [7].

MATERIAL AND METHODS

PREPARATION OF POMEGRANATE SEED, PEEL AND LEAF EXTRACTS

Collection of pomegranate fruit and leaves was collected nearer shops and houses. And separated to the pomegranate seed and peel from the pomegranate fruit (Fig: 1). The seed, peel, and leaves weighing 25 g were thoroughly washed in de-ionized water. The peels and leaves were cut into small pieces and then take it seed. The cut pieces of peel and leaves were ground well in 100 ml of de-ionized water using motor and pestle. Here after the seed of pomegranate were ground well in 100 ml of de-ionized water using motor and pestle. Both extract was filtered twice by using Whatmanno-1 filter paper, this filtrate was collected for further study (Fig: 2).



Fig: 1 pomegranate seed, peel and leaves images.



Fig: 2 Pomegranate Seed, Peel and Leaf extract

SYNTHESIS OF SILVER NANOPARTICLES

90 ml of 1mM of silver nitrate solution was prepared in Erlenmeyer flask. 10 ml of freshly prepared pomegranate seed peel and leaf extract was added separately to the silver nitrate solution. The bio reduction of AgNO₃ ions occurred within minutes (fig: 3). The seed extracts before its pink colored solution which turned into the brown color slowly indicate the formation of silver nanoparticles. Peel extracts its yellow color solution which turned into the tea brown color slowly indicate the formation of silver nanoparticles. Leaf extracts its green color solution which turned into the tea brown color slowly indicate the formation of silver nanoparticles.

Mechanism of reaction could be expressed as follows [8],

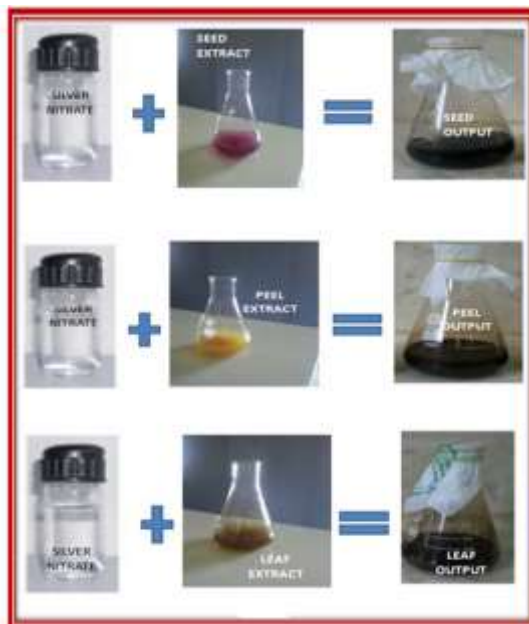
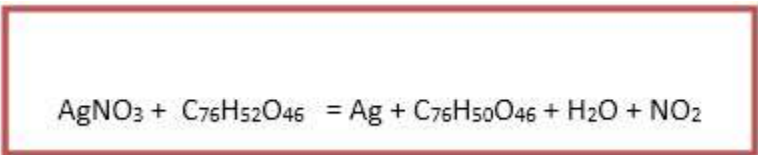


Fig: 3 Synthesis of silver nanoparticles using pomegranate seed, peel and leaf extracts.

CHARACTERIZATIONS OF SILVER NANOPARTICLES

The pomegranate seed and peel extract was centrifuged (1 or 2 times) to separate the nanoparticles & to remove the unwanted garbage. It was dried in hot air oven for 15 minutes and silver nanoparticles were obtained in a powder form at temperature 100°C. Synthesized nanoparticles were confirmed by UV-visible spectroscopy, and it was carried out in Perkin-Elmer spectrophotometer operating in a wavelength from 200-1100 nm. The morphology of the nanoparticles was studied in Philips Scanning Electron Microscope (SEM - Bruker Company). The Presence of elemental silver in the solution mixture was identified by Energy Dispersive X-ray Spectrophotometer (EDAX) and which was operated at the accelerating voltage of 129 KV. The crystalline nature of the nanoparticles was evident from XRD measurements. Perkin Elmer of Fourier Transform Infrared (FTIR) spectroscopic data was used to indicate the bonding of Silver nanoparticles with functional group of seed and peel extract through bridging linkage. The antibacterial and antifungal activities were done by using disc diffusion methods. The pomegranate seed, peel and leaves possess anti-inflammatory effects in laboratory animals at the doses investigated [9].

RESULTS AND DISCUSSION

UV-VISIBLE SPECTRA ANALYSIS

At the very beginning of the biosynthesis, reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the pomegranate fruit extract, peel extract and leaf extract was seen by the UV-Vis spectroscopy. After mixing the pomegranate fruit seed extract in the aqueous solution of the silver ion complex, solution slightly started to change color from light pink to dark brown color. After mixing the pomegranate peel extract in the aqueous solution of the silver ion complex, solution slightly started to change color from light yellow to dark tea brown. After mixing the pomegranate leaf extract in the aqueous solution of the silver ion complex, solution slightly started to change color from Light brown to dark tea brown. Colloidal solutions of Ag nanoparticles showed a very intense color, which is absent in the bulk material as well as formation of individual atoms. It was an indication of silver nanoparticles formation as the color change observed is due to excitation of surface Plasmon vibrations in the silver nanoparticles. The surface Plasmon resonance band observed around 435-475 nm (Fig: 4).

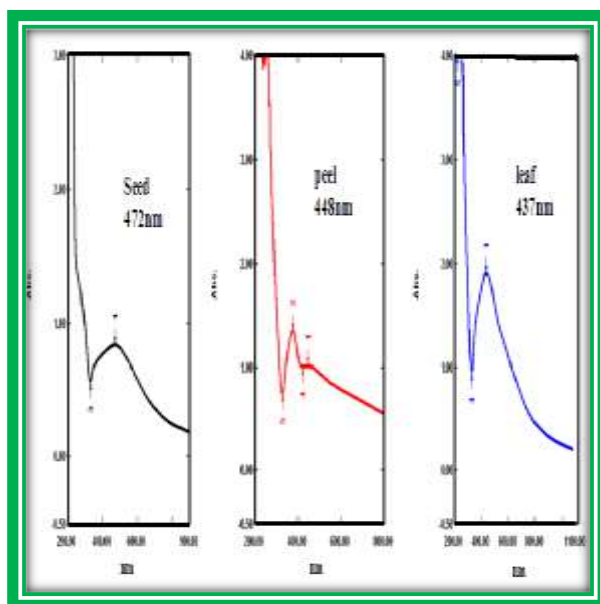


Figure: 4 UV-Vis absorption spectrum of AgNPs synthesized by treating 1mM of 90 ml AgNO₃ solution with Pome seed, peel & leaf extract.

X-RAY DIFFRACTION (XRD)

The X-ray diffraction (XRD) pattern clearly shows that synthesized Ag nanoparticles formed are crystalline in nature compared with the standard powder diffraction pattern (PDF# card No: 87-0720). The broadening of the Bragg's peaks indicates the formation of silver. The major planes correspond to (004), (110), (200), (220), (311), were found to be matched which confirmed the presence of Cubic Silver (Fig: 5). The average crystallite sizes of the silver nanoparticles using seed can be estimated from ~10 – 30 nm using peel the range was ~15-35 nm and using leaf, silver nanoparticles estimated from ~10 – 15 nm, from the X-ray peak broadening using Scherer's formula.

$$D = K \lambda / (\beta \cos \theta)$$

Where,

K is a constant taken as 0.9,

θ is the diffraction angle;

λ is the wavelength of the X-ray radiation.

β is the full width at half maximum (FWHM) of each phase and θ is the diffraction angle;

D = Average particle size of crystallite.

Compare among those three the best results obtained from pomegranate peel.

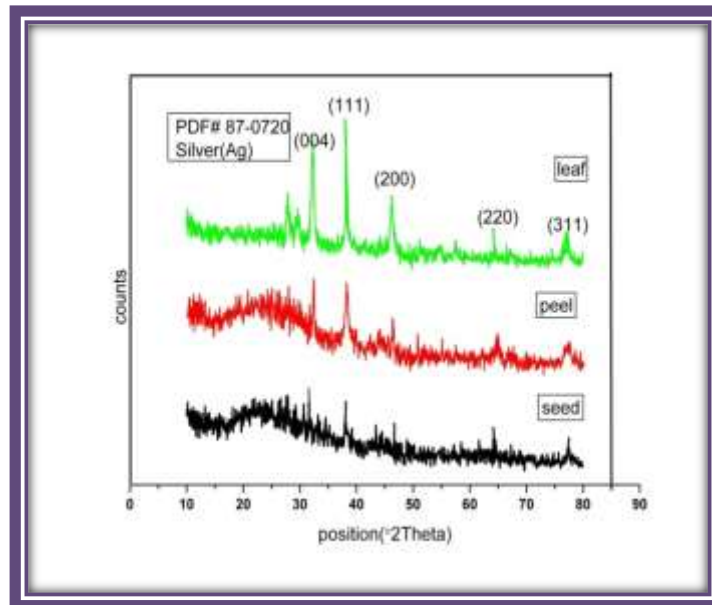
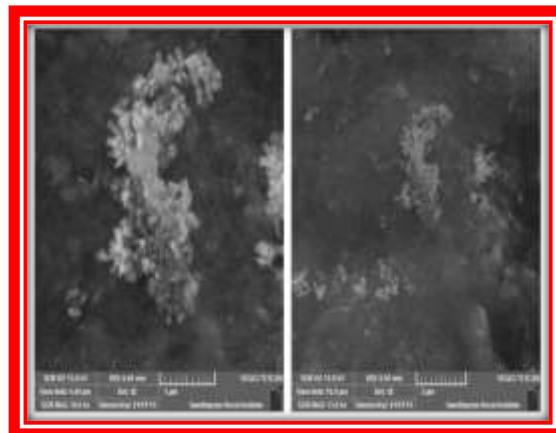


Figure: 5 XRD Spectrums of Silver Nanoparticles Synthesized By Pome Seed, Peel, Leaves Extracts.

SCANNING ELECTRON MICROSCOPE (SEM)

SEM technique was employed to visualize the size and shape of Ag nanoparticles. This scanning electron micrograph was taken using a Philips Scanning electron microscope. Dried powder of the silver was placed on carbon-coated copper grid. The SEM characterizations of the synthesized Ag nanoparticles are shown in Fig. 6 Image of SEM showed relatively nanoflowers, nanosquares and nanorods shapes nanoparticles formed with diameter range 10-30nm. The Nanoparticles diameter of SEM value compared with XRD value approximately equal.



Wave number cm^{-1}	bonds	Functional groups
3900-3700	O-H stretch, free hydroxyl	alcohols, phenols
3449	O-H stretch, H-bonded	alcohols, phenols
2372	C≡N stretch	nitriles
2345	C=C stretch	alkynes
1648	N-H bend	1° amines
1400	C-C stretch	Aromatics
1053	C-O stretch	alcohols, carboxylic acids, esters, ethers
675	=C-H bend	alkynes

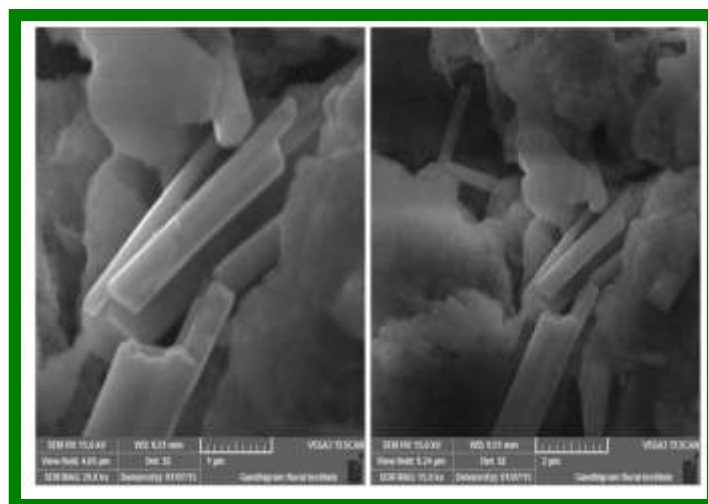
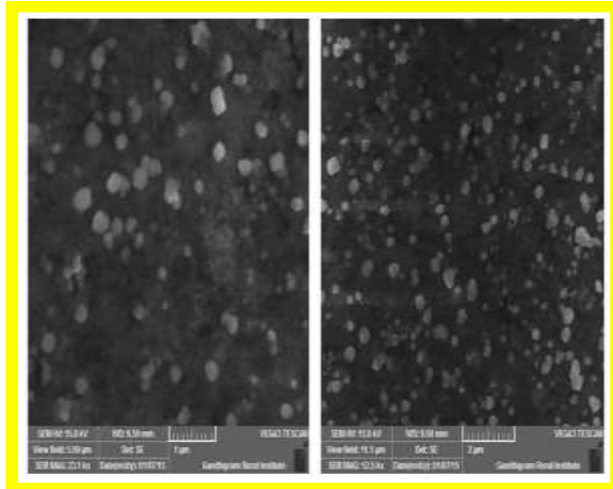


Figure: 6 SEM Image of synthesized silver nanoparticles.

ENERGY DISPERSIVE X-RAY ANALYSIS (EDAX)

EDAX exposed strong signal in the silver region and confirmed the formation of silver nanoparticles (fig: 7). Metallic silver nanocrystals generally show typical absorption peak approximately at 3KeV due to surface Plasmon resonance. And also a strong signal for Ag.

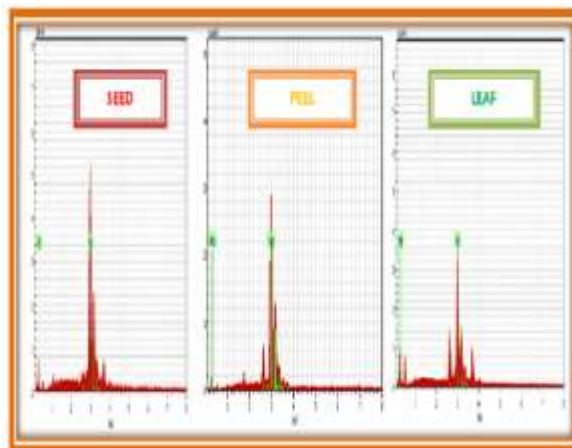


Figure: 7 XRD pattern of the green synthesis of AgNPs.

FOURIER TRANSFORMS INFRARED SPECTROSCOPY (FTIR) ANALYSIS

FTIR is used for determination of find out the functional group associated with the synthesized Silver nanoparticles from seed, peel & leaves absorption peak (Fig: 8).

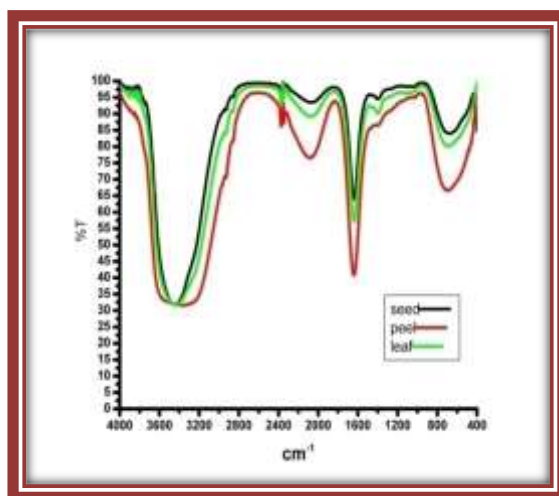


Figure: 8 FTIR-Analysis of the green synthesis of AgNPs.

The spectrum showed in the figure indicated the major peak at 3449 cm^{-1} and other peaks were obtained at 1648 cm^{-1} respectively (Table: 1).

ANTI-BACTERIAL ACTIVITY

Test bacterial assay

The effect of various fruit extracts (pomegranate seed, peel and leaves) on the several bacteria strains were assayed by Disc diffusion method. The minimum concentration of extracts to inhibit the micro-organisms.

Test microorganism

The four bacterial strains were used in the present study, BACILLUS CEREUS, STAPHYLOCOCCUS ALBUS, PSEUDOMONAS, PROTEUS. First two bacteria is denoted as gram positive and another bacteria is denoted as gram negative.

SAMPLE CODE	PSEUDOMONAS	BACILLUS CERUEUS	STAPH.ALBUS	PROTEUS
PEEL	16	17	13	R
LEAF	15	15	15	R
SEED	13	14	13	R
CONTROL	R	R	R	R
STANDARD DISC (AMIKACIN)	16	19	16	16

Table: 2 Antibacterial activity of Ag nanoparticles against PSEUDOMONAS, BACILLUS CERUEUS, STAPH.ALBUS, PROTEUS.

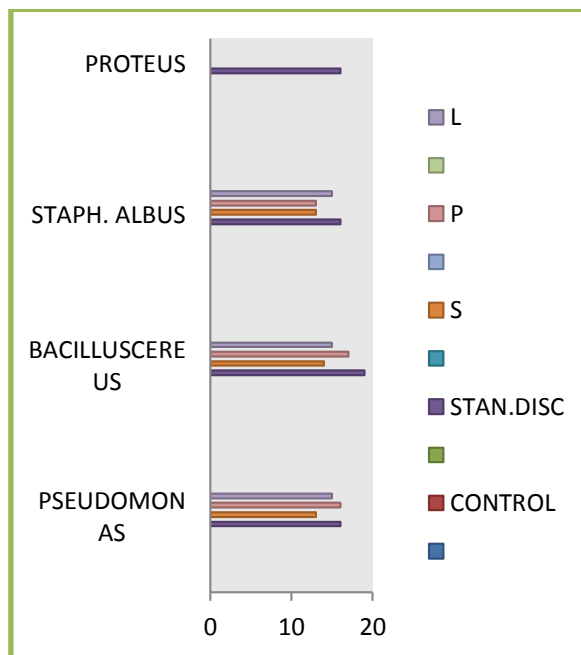
The Antibacterial activity of biogenic Silver nanoparticles was evaluated by using standard Zone of Inhibition (ZOI) microbiology assay. The nanoparticles showed inhibition zone against almost all the studied bacteria (Table: 2& Fig: 9).

Compound Seed exhibited potential antibacterial Maximum ZOI was found to be 14 mm for *Bacillus Cereus*. Whereas, the other three bacterial strains of *Pseudomonas*, *Staph.Albus* , *proteus* showed ZOI of 13,13,0 mm.

Compound Peel exhibited potential antibacterial Maximum ZOI was found to be 17mm for *Bacillus Cereus* Whereas, the other three bacterial strains of *Pseudomonas*, *Staph.Albus*. *proteus* showed ZOI of 16,13,0 mm.

Compound Leaves exhibited potential antibacterial Maximum ZOI was found to be 15mm,15mm,15mm for *Bacillus Cereus* ,*Pseudomonas* ,*Staph.Albus* Whereas, the other bacterial strains of *proteus* showed ZOI of 0 mm.

Compare those three, the best results obtained from the pomegranate peel that is 17 mm for BACILLUS CERUES pathogens (Fig: 9) & (Graph: 1).



Graph: 1 Graphical Representation for Antibacterial Activity.

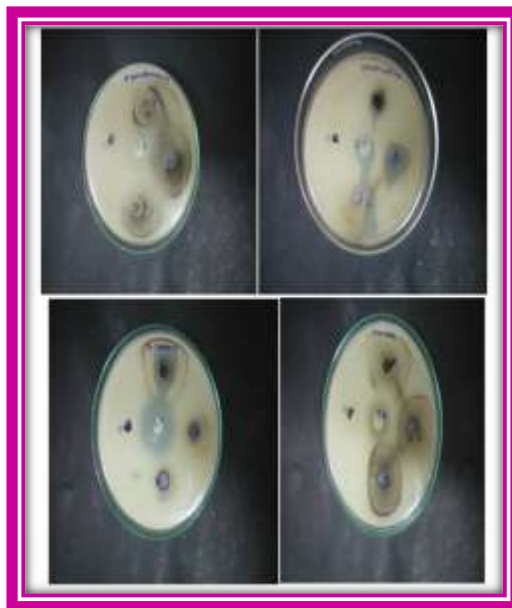


Figure: 9 Antibacterial activities of silver nanoparticles synthesized from pome seed, peel and leaf extract against microorganism.

ANTI-FUNGAL ACTIVITY

Test Fungal assay

The activity of fruit extract (pomegranate seed, peel and leaves) on various fungal strains was assayed by agar plug methods.

Test microorganism

The two fungal strains were used in the present study,

1. CANDIDA TROPHICA,
2. ASPERGILLUS

Antifungal activity

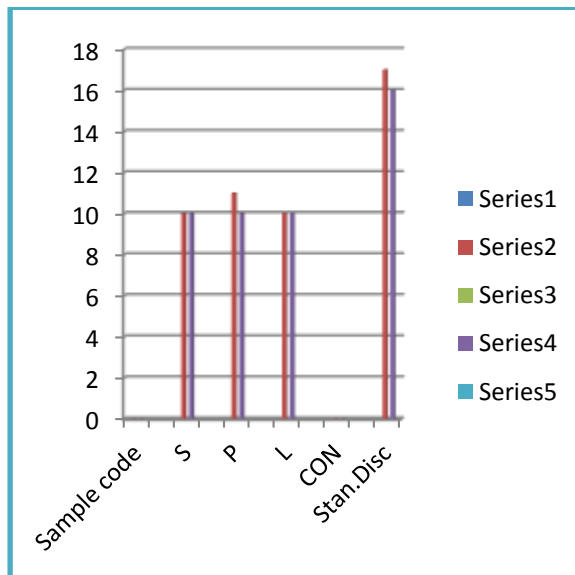
Agar plates were prepared, sterilized and solidified, after solidification fungal cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (10mg/ml) and placed in the agar plate and kept for incubation for 24 hrs. After 24 hrs days zone of inhibition was measured.

The Anti-fungal activity of biogenic Silver nanoparticles was evaluated by using standard Zone of Inhibition (ZOI) microbiology assay. The nanoparticles showed inhibition zone against two fungi that is CANDIDA TROPHICAL and ASPERGILLUS (Table: 3 and Fig: 10)

Table: 3 Antifungal activity of Ag nanoparticles against CANDIDA TROPHICAL AND ASPERGILLUS.

SAMPLE CODE	CANDIDA TROPHICAL	ASPERGILLUS
P	11	10
L	10	10
S	10	10
CON	R	R
SD	17	16

Compound Seed exhibited potential antifungal Maximum ZOI was found to be 11 mm for *Candida Tropical*. Whereas, the other fungal strains of *Aspergillus* showed ZOI of 10 mm.



Graph: 2 Graphical Representations for Antifungal Activity.

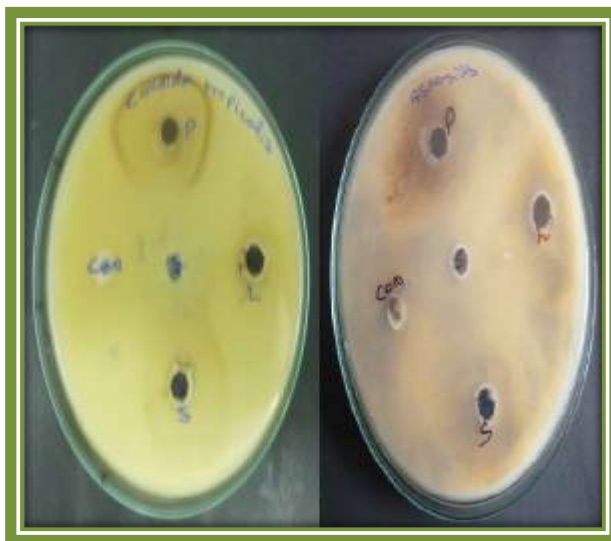


Figure: 10 The nanoparticles showed inhibition zone against fungus (CANDIDA TROPICALIS AND ASPERGILLUS)

Compound Peel exhibited potential of antifungal Maximum ZOI was found to be 10 mm for *Candida Tropicalis*. Whereas, the other fungal strains of *Aspergillus* showed ZOI of 10 mm.

Compound leaf exhibited potential antifungal Maximum ZOI was found to be 10 mm for *Candida Tropicalis*. Whereas, the other fungal strains of *Aspergillus* showed ZOI of 10 mm.

Among these three results, the best results obtained from pomegranate peel for *Candida Tropicalis* fungal (Graph: 2).

CONCLUSION

In the present work we found that pomegranate seed, peel and leaf can also a good source for synthesis of silver nanoparticles and also environmental friendly process etc. Analytical techniques such as UV-Vis spectroscopy, FT-IR, SEM, XRD, Anti-bacterial and Anti-fungal activity applied to characterize the synthesized nanoparticles. The phase formation of Ag was confirmed by an XRD pattern. Compare those three the maximum size of results showed the synthesis of Ag nanoparticles with average size of 18 nm measured by SEM and confirmed the presence of Ag with EDAX for peel. The anti-bacterial activity showed the maximum zone of inhibition obtained from peel for

BACILLUS CEREUS bacteria. The size was 17mm. The anti-fungal activity showed the maximum zone of inhibition obtained from peel for CANDIDA TROPICAL fungi. The size was 11mm. finally I have concluded from this, pomegranate seed, peel, and leaf compare among those three the best results obtained from pomegranate peel.

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