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Synthesis of *Punica Granatum* Fruit Peel Extract Based Silver Nanoparticles and Evaluation of it's Antimicrobial Activity

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ABSTRACT

The main objective of the present work was to prepare pomegranate peel extract based nano particles by chemical complexation method. Ethanolic extracts of pomegranate peel were prepared by using Soxhlet apparatus and evaluated for phyto-chemical constituents. Qualitative analysis showed that pomegranate peel extract showed positive results for alkaloids, anthraquinones, saponins and terpenoids. The percentage moisture content and pH of the extract was found to be 72% and 3.6 respectively. A zeta potential and particle size of prepared nanoparticles was found in the range of -24.6 to– 34.5 mV and 118.6 nm to 231.7 nm, respectively. These range confirms that obtained particles were in nano range, i.e. <500 nm size. SEM results indicated the formation of nanoparticles and were relatively spherical in shape. Energy dispersive spectrometry (EDS) analysis confirms the presence of AgNPs. Prepared peel extracts based nanoparticle showed promising antimicrobial and antifungal properties.

Keywords: Pomegranate peel extract, Qualitative analysis, Nano particles

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INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm[1]. The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen[1].

Fruit peels are valuable source for maintaining human health. The use of peel extracts for antimicrobial properties can be of great significance in therapeutic treatments. Fruits by-products such as seeds, peels, stems, barks and leaves usually been discarded and currently the cause of a serious disposal problem in food and agricultural industries. Therefore, extensive researches on utilizing these wastes are being carried out worldwide. The peel was found to contain much higher beneficial compounds[2].

Currently there are several methods for the production of nanoparticles like chemical and physical methods. Greener way of synthesis nanoparticles provides an advantageous over chemical and physical method as it is eco friendly, cost effective and there is no need to use high temperature, pressure and toxic chemicals for large scale synthesis. Silver nanoparticles are used in a wide range of applications such as cosmetics, medical devices, pharmaceuticals, food ware, clothing, water purification and antimicrobial properties[3].

Punica granatum, commonly known as pomegranate belongs to family *Punicaceae*. Pomegranate is an important crop known by its taste and nutritional and medicinal properties. Several studies have reported the antimicrobial and insecticidal activities of extracts from different tree parts, such as bark, leaves, fruit and fruit peel[4].

Pomegranate peel is a rich source of tannins, flavonoids, polyphenols and some anthocyanins as delphinidins, cyanidins, etc. Antioxidant and antibacterial properties of pomegranate peel in *in-vitro* model systems have been reported. All the compounds of pomegranate peels are reported to have therapeutic properties. Extracts of peels of pomegranate show antibacterial property against bacterial strains of *E. coli*, *P. aeruginosa* and *S. aureus*[5].

The aim of the present study was to synthesize *Punica granatum* fruit peel extract based nano particle and to evaluate its antimicrobial activity.

MATERIAL AND METHODOLOGY

Material used

Pomegranate peels were collected from local market, Bellur. Chemicals like Silver nitrate and Sodium borohydride were obtained from S.D Fine chemicals, Mumbai, India. Milli Q water was used throughout the experiment.

Method used

Nanoparticles Preparation:

Fruit of pomegranate were collected, peels were separated and washed thoroughly with tap water. The washed peels were cut in to small pieces [1-5 cm] and air dried in sunlight for 20 days. The dried fruit peels were grinded properly using a mortar and pestle and later using a grinder, to obtain the powdered form and then passed through sieve no. 40 to get uniform powder and stored at room temperature. Powdered sample (100gm) was extracted with 800ml ethanol by Soxhlet extraction method for 6 hours. The mixture filtered through a Whatman filter paper (No. 2) for removal of peel particles. The extracts were filtered and evaporated to dryness under reduced pressure at 60°C by a rotary evaporator (Buchi, Singapore). The extracts were placed in dark bottles and stored in refrigerator at 4°C[6].

To a 50ml of freshly prepared 0.001M Silver nitrate solution, 5ml of 0.002M Sodium borohydride solution was added with continuous stirring and kept it aside for 15 minutes in a clean 250 ml beaker till a clear and slightly dark solution is obtained. Further, this

clear solution is heated and maintained in water bath at 45ºC for 30 min (solution A). Pomegranate peel extract in different concentration (200mg, and 500mg) was dissolved in 4ml of Milli Q water separately in test tube and heated slightly to get yellow brownish colored solution. This solution was added drop wise into solution A with continuous stirring using glass rod for 30-45 min till clear tea brown solution is obtained. The obtained solution is cooled to room temperature and 0.5ml of 0.5 mcg/ml PVP solution was added as a stabilizer and filtered to get clear tea brown colored solution of silver nanoparticles. This solution was stored in a dark place in a well closed container until further use. Then prepared nanoparticles were evaluated for FTIR, particle size, zeta potential, surface morphology by SEM, X-ray diffraction and energy dispersive spectrometry[7].

Antibacterial Activity[8, 9]:

The petri plate were washed thoroughly and sterilized in autoclave at 121ºC for 20 min. 30 ml of sterile nutrient agar medium was poured into sterile petri dishes and allowed to solidify. The petri plates were incubated at 37ºC for 24 hour to check for sterility. The medium was seeded with the organism by spread plate method using sterile cotton swabs. Sterilized pepper disc were placed on media and compound samples of various concentration were placed on the paper disc. 0.1 ml of Ampicillin at a concentration of 100 µg/ml was taken as standard reference. A control having only DMSO was maintained in each plate. The petri plates were kept in refrigerator at 4ºC for 15 minutes, allowing diffusion to take place. The petri plates were incubated at 37ºC for 24 hour and zone of inhibition were observed and measured using a scale. Antibacterial activity of all the compounds was carried out against all four microorganisms (*B. subtilis*, *S. Aureus*, *E. Coli* and *K. Pneumoniae*). The zone of inhibition is as shown in table (1) the media was used for both sub culturing and also for estimating antibacterial activity.

Antifungal Activity[10, 11]:

The petri plate were washed thoroughly and sterilized in autoclave at 121º C for 20 min. 30 ml of sterile nutrient potato dextrose agar medium was poured into sterile petri dishes and allow to solidify. The petri plates were incubated at 37 ºC for 24 hour to check for sterility. The medium was seeded with the organism by spread plate method using sterile cotton swabs. Sterilized disc were made on media and compound samples of various concentration were placed on paper disc. 0.1 ml of Griseofulvin at concentrations of 100 µg/ml was taken as standard reference. A control having only DMSO was maintained in each plate. The petri plates were kept in refrigerator at 4ºC for 15 minutes, allowing diffusion to take place. The petri plates were incubated at 37ºC for 24 hour and zone of inhibition were observed and measured using a scale. Antifungal activity of all the compounds was carried out against all two microorganism species (*A. Flavus* and *C. Albicans*). The zone of inhibition is as shown in table (2) the media was used for both sub culturing and also for estimating antifungal activity.

RESULTS AND DISCUSSION

Pomegranate peel extracts were subjected to various tests to confirm the presence of photochemical constituents. The UV-spectroscopic analysis showed that pomegranate peel extract, a colored solution showed maximum absorbance at 461.0 nm (figure 1), wave length. Hence same wave length will be used for further studies. Results of qualitative analysis showed that pomegranate peel extract showed positive results for alkaloids, anthraquinones, saponins and terpenoids. The results were tabulated in table 1. Table 2 showed the equivalent weight, percentage moisture content and pH was found to be 345.14 mg/ml, 72% and 3.6, respectively. The obtained peel extract was acidic in nature.

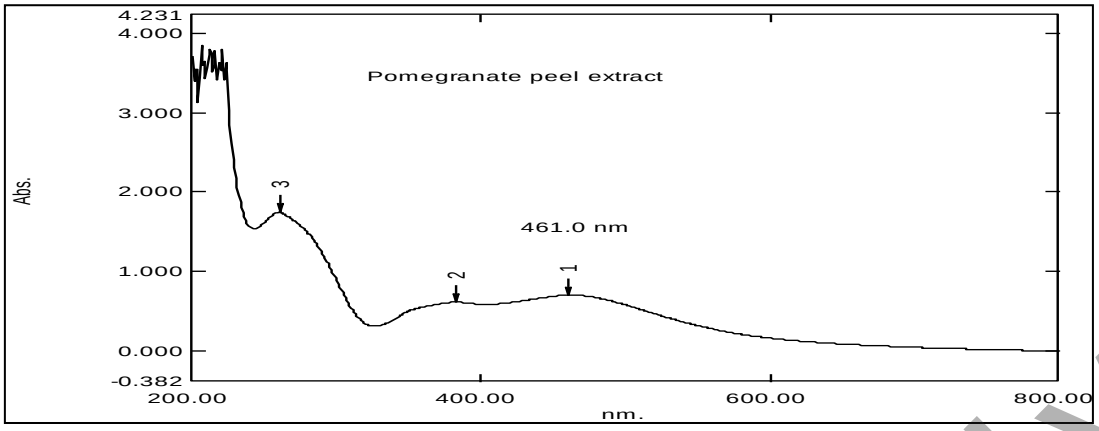

Fig 1: UV-Spectrum of pomegranate peel extract

Table 1: Phyto-chemical Analysis of pomegranate peel extract by Soxhlet Apparatus

Sl. No	Phyto-chemicals	Status*
1	Alkaloids	+
2	Amino acid	_
3	Tannin	-
4	Anthraquinones	+
5	Saponins	+
6	Protein	-
7	Terpenoids	+
8	Cardiac glycosides	_

+ = Present, - = Absent

Table 2: Quantitative analysis of pomegranate peel extract

Sl. No	Parameters	Values
1	Equivalent weight	345.14 (mg/ml)
2	Ash content	30%
3	Moisture content	72%
4	pH	3.6

The FT-IR spectrum of pomegranate peel extract showed the distinct peak in the range of 3036, 2928, 1734, 1102 and 713 cm^{-1}. The absorption peaks located mainly at 3036 cm^{-1} are generally attributed to aromatic or aliphatic C–H stretching, 2928 cm^{-1} are generally assigned to the alkyl C–H stretching, whereas peaks at 1734, 1375 and 1332 cm^{-1} are due to C–O–O stretching bands, 1102 and 1050 $cm^{-1}$are due to C–C stretching vibrations, 713 and 624 cm^{-1} are due to acetylenic C–H bending vibrations in the region of 40–4000 cm^{-1}. All the spectrum of pomegranate peel extracts was present in the pomegranate peel extract nanoparticles. Hence there was no any shift of functional groups are seen in pomegranate peel extract nanoparticles (figure 2 and 3).

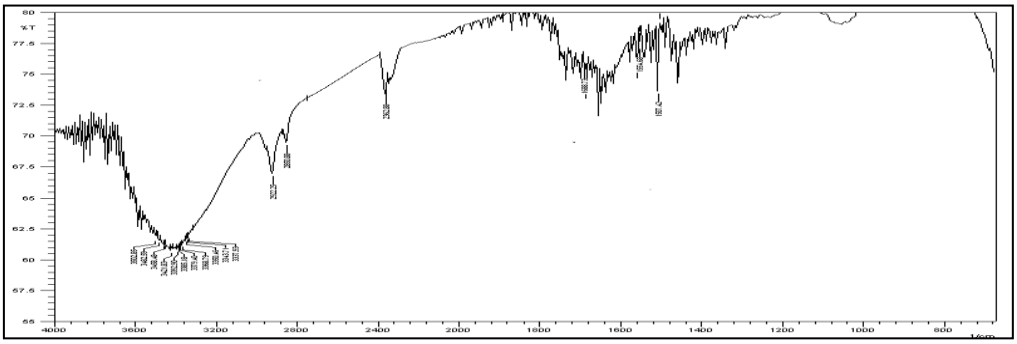

Fig 2: FTIR spectra for pomegranate peel extract

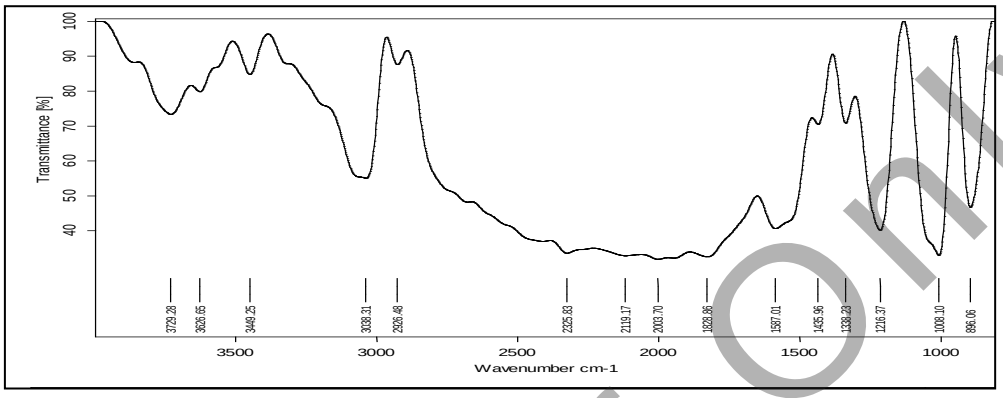

Fig 3: FTIR spectra for pomegranate peel extract based silver nanoparticles

The pomegranates peel extracts silver nanoparticles were subjected to zeta potential analysis. The values of zeta potential for pomegranate peel extract silver nanoparticles was found to be -34.5 mV to -24.6 mV for pomegranate P50 and Pomegranate P20, respectively (figure 4a, and 4b). Hence all batches of nanoparticles having higher surface charge which indicates there is least chance of aggregation.

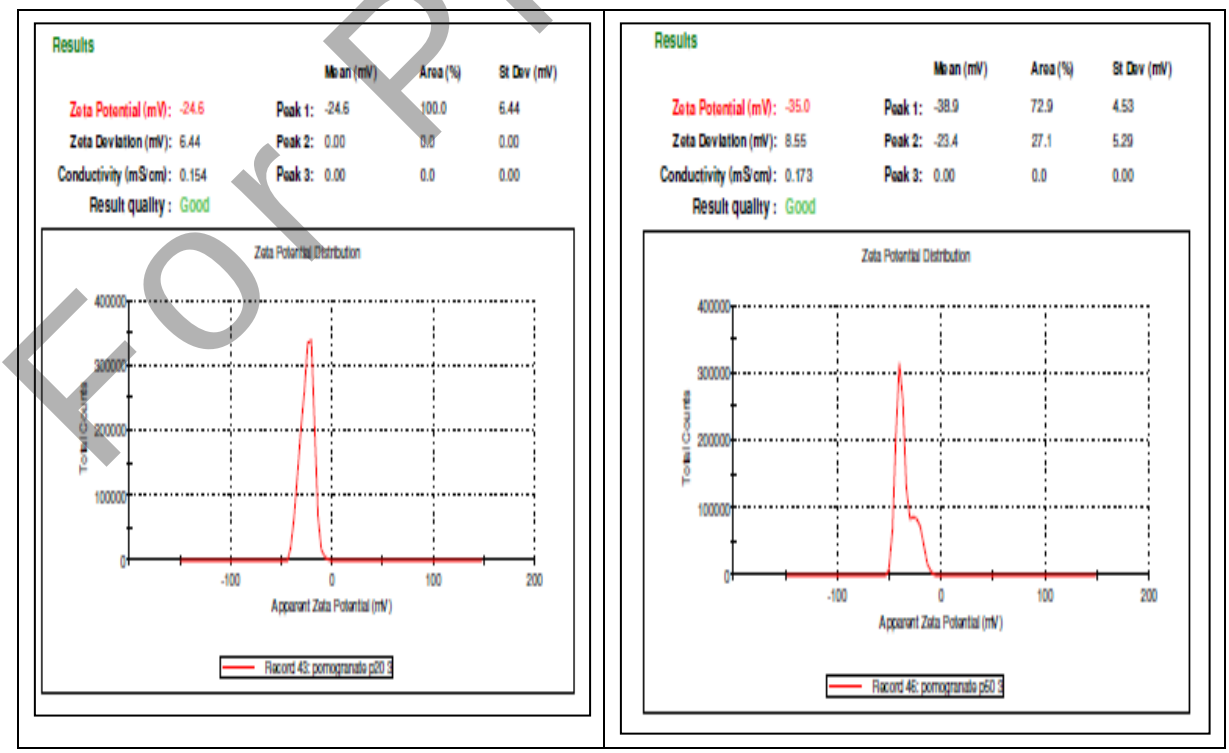

Fig 4a and 4b: Zeta potential of pomegranate extracts nanoparticles (P50, P20)

The particle sizes of prepared nanoparticles were determined by using Malvern particle size analyzer. The values of particle size for pomegranate peel extract silver nanoparticles were found in the range of 231.7 nm to 118.6 nm for pomegranate P50 and pomegranate P20, respectively (figure 5a and 5b). These range confirms that obtained particles were in nano range, i.e. <500 nm size.

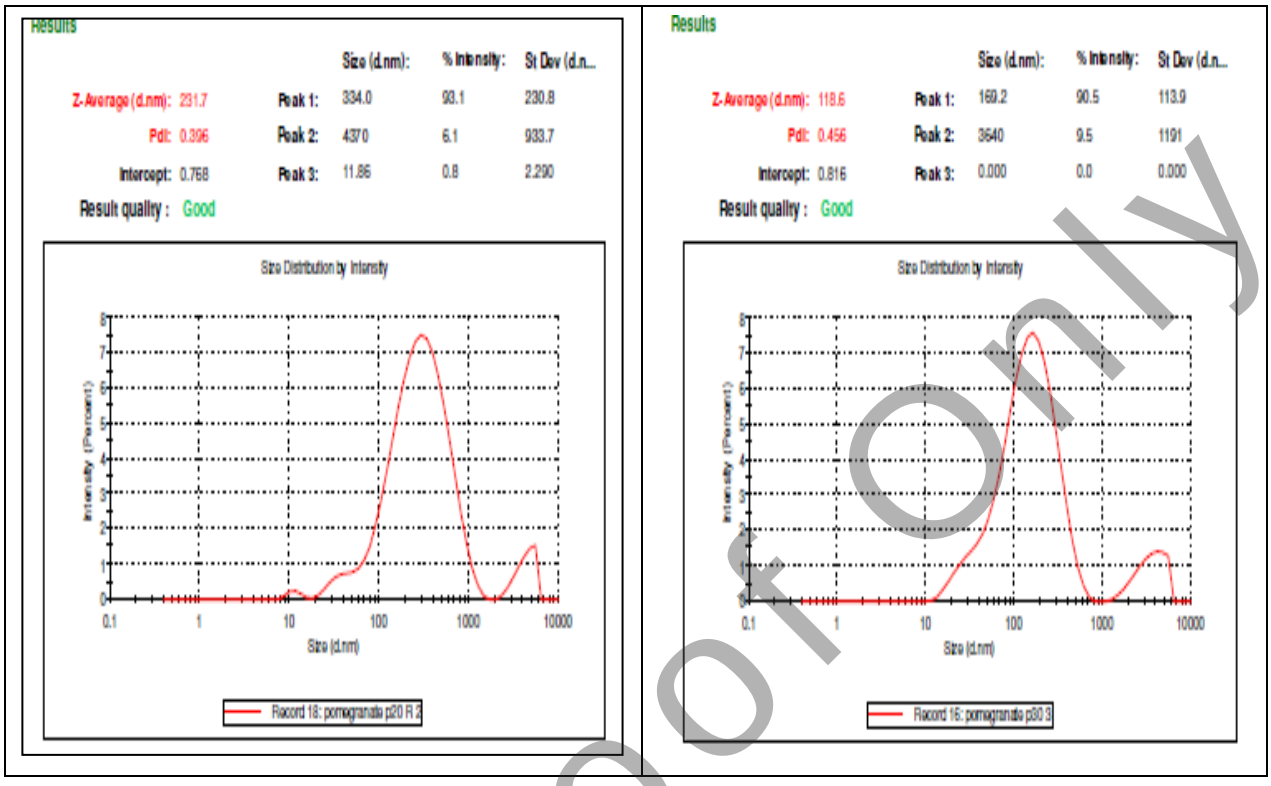

Fig 5a and 5b: Particle size distribution of pomegranate extracts silver nanoparticles (P50 and P20)

To confirm the crystalline nature of pomegranate peel extract, X-ray diffraction (XRD) patterns were obtained (figure 6). The peaks assigned to the diffraction pattern clearly showed peaks corresponding to 2θ = 11.36°, 16.26°, 19.04°, 27.48°, 49.26°, 59.56° and 64.07°. The surface morphology of prepared nanoparticles was determined by using SEM (Hitachi).

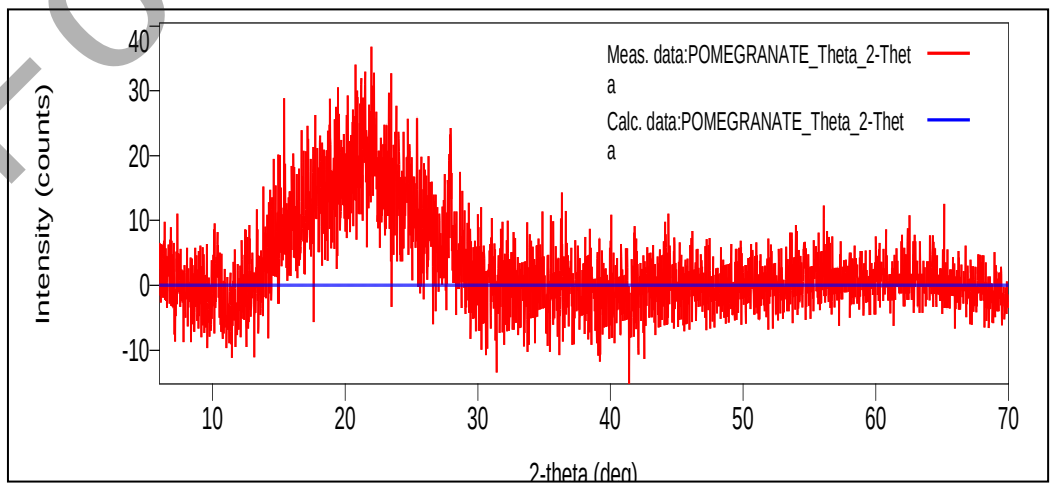

Fig 6: XRD pattern of Pomegranate peel extract

SEM results clearly showed the formation of nanoparticles and were relatively spherical in shape and also showed there was only a small degree of agglomeration. The SEM results were shown in figure 7. Energy dispersive spectrometer (EDS) analysis was performed for the detection of elemental silver. The EDS microanalysis confirms the presence of AgNPs which is known to provide information on the chemical analysis of the elements or the composition at specific locations. The spectrum analysis reveals signal in the silver region and then confirms the formation of AgNPs. Metallic silver nanocrystals generally showed a typical optical absorption peak at approximately 2 keV due to the surface plasmon resonance. The EDS results were shown in figure 8.

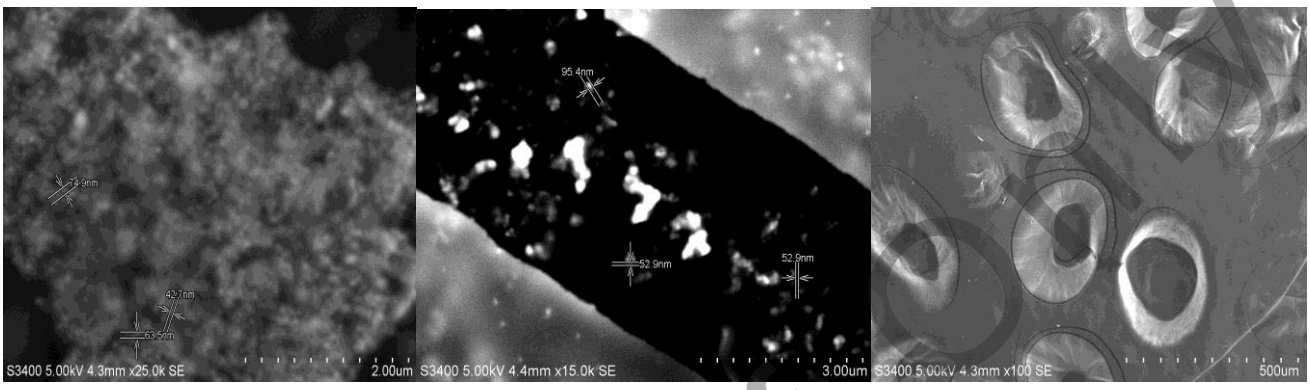

Fig 7: SEM images of pomegranate peel AgNPs

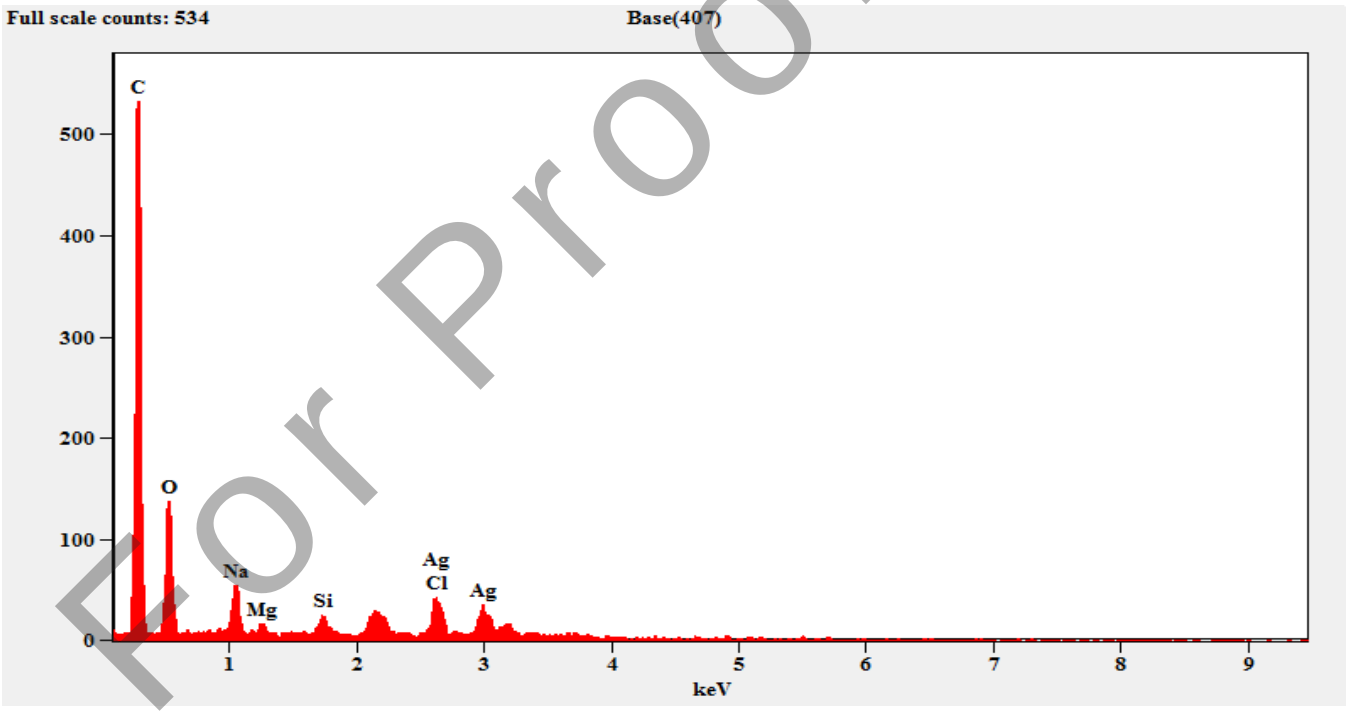

Fig 8: EDS pattern of spherical pomegranate peel extract AgNPs prepared.

Antibacterial activity results:

The antibacterial activity of orange and pomegranate peel extract were carried out by disc diffusion method by using Ampicillin as a standard. Different concentration of samples and standard were prepared. Results revealed that pomegranate peel extracts showed promising antibacterial activity against gram +ve and gram –ve bacteria (table 3).

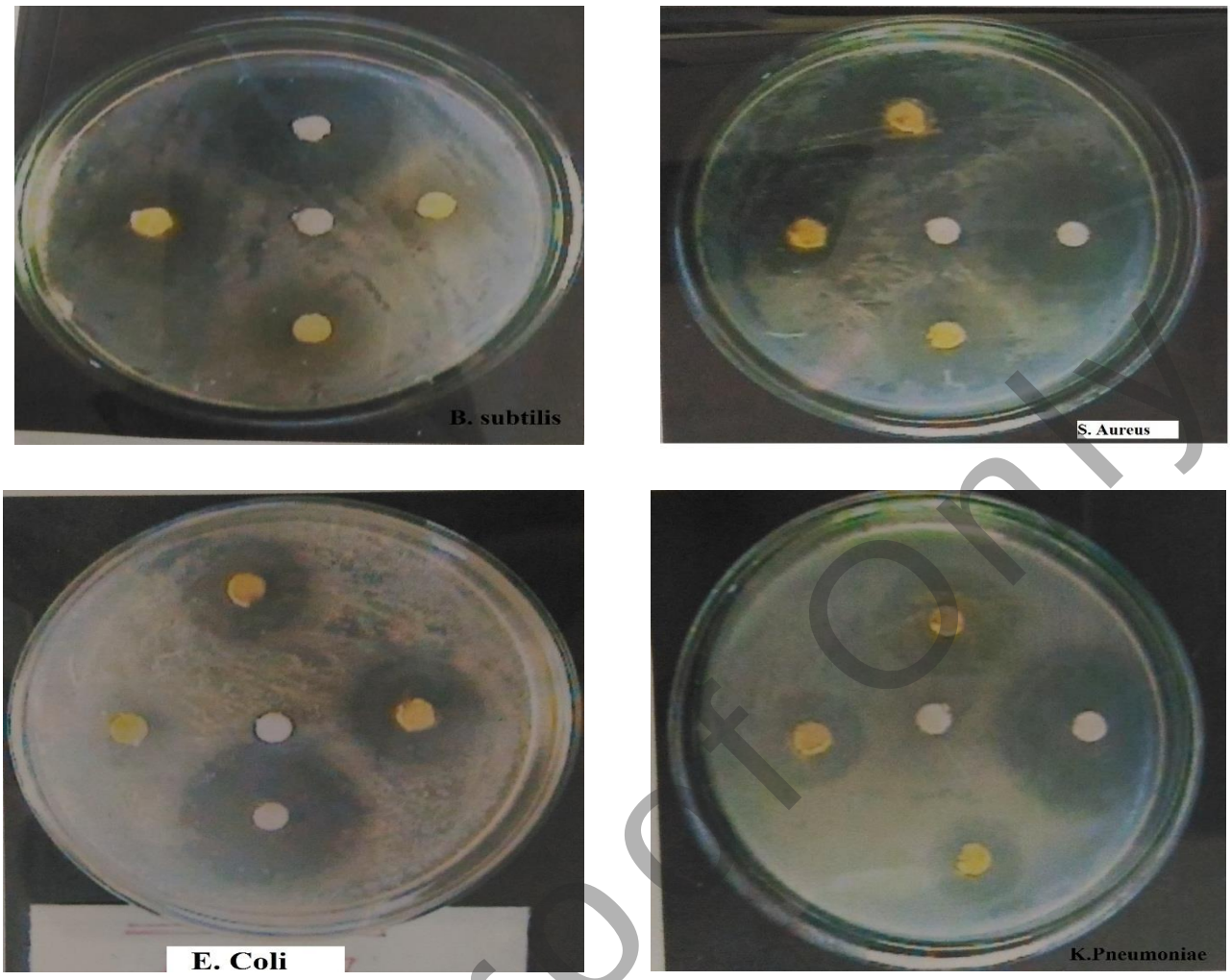

Fig 9: Antibacterial activity of Pomegranate peel extract on: (A) *Bacillus subtilus* (B) *Staphylococcus aureus* (C) *Escherichia coli* (D) *Klebsella pneumonia*

Table 3: Results of antibacterial activity (zone of inhibition)

Compound	Mean zone of inhibition (in mm)															
	B. subtilis				*S. Aureus*				*E. Coli*				*K. Pneumoniae*			
	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml
Pomegranate*	10	12	15	18	10	13	15	19	9	12	15	19	11	14	18	23
Standard *	18	20	22	24	19	21	23	25	19	20	21	22	18	20	22	24

*Zone of inhibition measured in mm

'-'no activity, 7-9 mm poor activity, 10-15 moderate activity, 15-20 good activity, 20-25 excellent activity

Antifungal activity results:

The pomegranate peel extracts based nanoparticle was tested for antifungal activity. Prepared nanoparticle showed prominent antifungal activity against both strains of fungi (table 4).

Table 4: Results of antifungal activity (zone of inhibition)

Compound code	Mean zone of inhibition (in mm)			
	A. Flavus		*C. Albicans*	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
Pomegranate	9	21	6	14
Standard 1	15	24	-	-
Standard 2	-	-	12	16

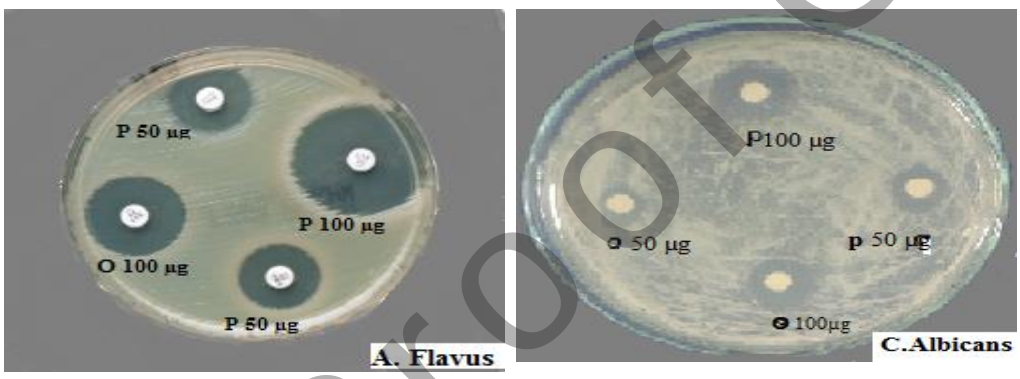

Fig 10: Antifungal activity of Pomegranate peel extract on: (A) A. flavus, (B) C. Albicans

CONCLUSION

Pomegranate peel extract based nano particles were prepared by chemical complexation method using silver nitrate. Particle size, surface morphology and elemental analysis confirm that prepared formulations were silver particle based nano particle. Pomegranate peel based nano particles showed promising antibacterial and antifungal activity. Further research will be extended for anti-fungal and anti-inflammatory activities in suitable animal model. Such herbal based nano particles might be safe, economically cheap and user friendly.

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