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# PHYTOFABRICATION OF SILVER NANOPARTICLES USING VARIOUS PARTS OF PUNICA GRANATUM AND THEIR ANTIMICROBIAL ACTVITIES

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## Abstract
In the present work, we described the green synthesis of silver nanoparticles (SNPs) using various parts of *Punica granatum* and their antimicrobial activities. The Bio-reduction of silver ions have been performed by the various parts of extract of *Punica granatum*. The antimicrobial activity of green synthesised SNPs is compared with $AgNO_3$ and the extract. *Punica granatum* extract showed good antibacterial activity against E.coli. The phytochemical investigation of *Punica Granatum* aqueous extract showed that it is rich in Xanthoproteins, Tannins, Terpenoids, Flavonoids, Phenols, Lacking steroids, Glycosides Coumarins, Fatty acids and Steroids. The biosynthesized SNPs were characterized by UV– visible spectrometry. Finally the phytochemical screening indicates that the plant parts are good source of bioactive principle for pharmaceutical industry.

**Keywords:** SNPs; Green synthesis, phytochemicals, antimicrobial activities.

## Introduction
Every plant kingdom contains their own phytochemicals. Phytochemicals come in a variety of forms and different vegetables have higher concentrations of a particular phytochemical than other. Some of the main phytochemicals include Carotenoids, Flavonoids, Isoflavonoids, Ligans, Omega 3, Omega 6, Fatty acids, and Plant sterols. Phytochemicals helps to protect the cells by blocking carcinogens that try to enter the cell walls and fight the malignant changes within cells that have already been penetrated by carcinogens, appear to boost enzyme activity to increase the benefits of various productive enzymes consumed within the diet. Combines with numerous vitamins boost antioxidant activity to scavenge free radicals before then can cause damage within the body. In this work, the phytochemical analyses have been performed on extracts of green synthesized silver nano particles of *Punica granatum*. The phytochemical screening indicates that the plant part is a good source of bioactive principle for pharmaceutical industry[i].

## Experimental

### Materials and Methods

### Preparation of the extract

The various parts of the medicinal plant *Punica granatum* were collected in Tirunelveli. The flower, leaf, stem, rind and fruit of Punica granatum were collected and were washed. The cleaned herbal parts were dried with water adsorbent paper (filter paper). Then, it was cut into small pieces, dispersed in 100 ml water sterile and boiled for one hour. Then the herbal extracts were collected in conical flasks by standard filtration method.

### Preliminary Phytochemical Analysis

Exactly 10g of various part of *Punica granatum* was boiled with 100ml of distilled water and the extract were tested for steroids, carbohydrates, reducing sugars, Alkaloids, Phenolic compounds, saponins, Xanthoproteins. Tannis and Flavonoids.

### Synthesis of silver nanoparticles

The $10^{-3}$ m silver nitrate solution was prepared and stored in brown bottles. 10ml of herbal extracts were taken in BOD bottles separately and to this 90ml of $AgNO_3$ solution was added. The same protocol was followed for all the five herbal extracts. The BOD bottles were incubated at room temperature. The color change of the extracts from pale yellow to dark brown was checked periodically and presented in photographs. The formation of brown color indicated that the silver nanoparticles were synthesized from the herbal extracts.

**Figure 1. Color change of *Punica granatum* extract with $AgNO_3$**

## Results and Discussions

### UV-Vis Spectra Analysis

The reduction of pure $Ag^+$ ions was monitored by measuring UV-Vis spectrum by diluting small aliquots of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrometer at the range of 300- 700nm and observed.

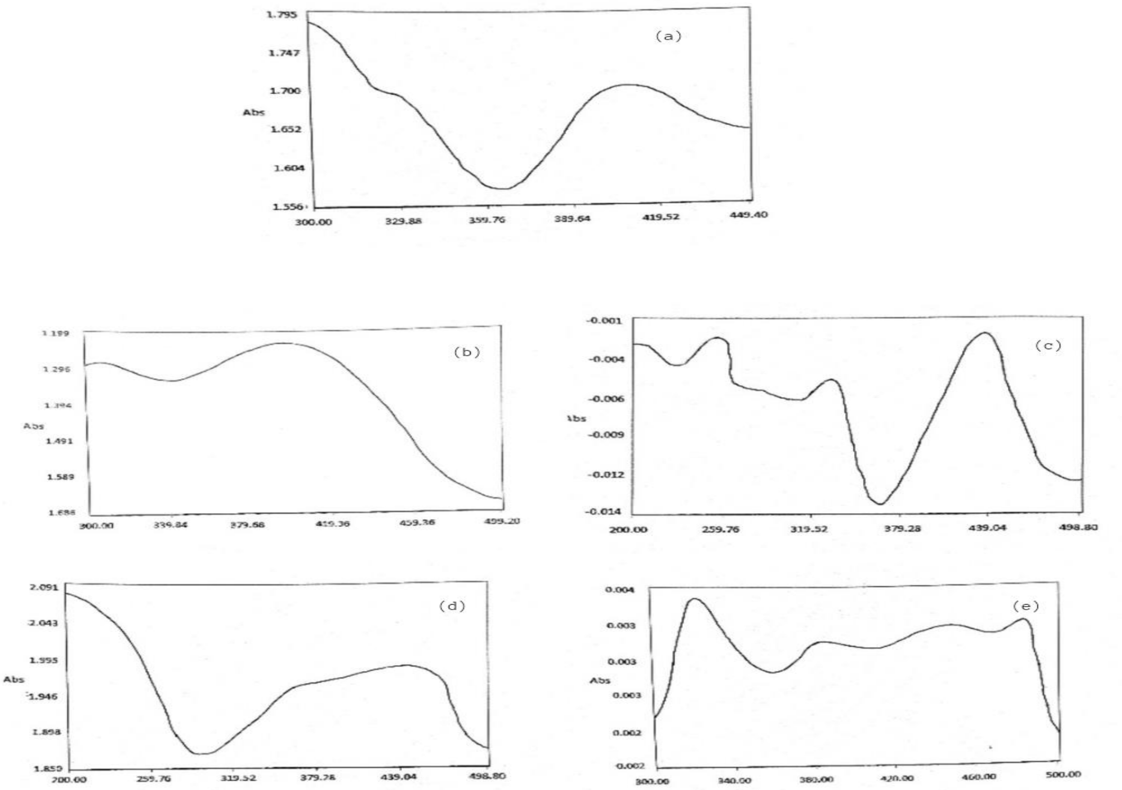


**Figure 2. UV-VIS spectrum of silver nanoparticles synthesized by the extract (a) fruit, (b) flower, (c) leaf, (d) rind and (d) stem of *Punica granatum***

**Antibacterial Activity**
**Disc diffusion Method**

The antibacterial[ii] assays were also performed by standard disc diffusion method. Nutrient broth agar (1 g beef extract, 1g peptone, 0.5g sodium chloride dissolved in 100 ml of double distilled water) was used to cultivate bacteria. The media was autoclaved and cooled. The media was poured in the petri dishes and kept for 30 minutes for solidification. After 30 minutes the fresh overnight cultures of inoculum (100 ml) of four different cultures were spread on to solidified nutrient agar plates. Sterile paper disc made of whatmann filter paper, 5mmm diameter (dipped in 50 mg per liter silver nanoparticle) along with standard antibiotic containing discs were incubated at $370^{o}C$ (310 K) for 24 hours. After 24 hour of incubation[iii] the zone of inhibition was investigated. The number of bacterial colonies grown on agar plates as a function of different concentration of silver nanoparticles when gradually declined when the concentration of nanoparticle increased.

Results clearly demonstrate that newly synthesized silver nanoparticles[iv] are promising antimicrobial agent against the pathogens employed. The mechanism of the bactericidal effect of silver colloid particles against bacterial is not very well known (Ales panacek et al., 2008), silver nanoparticles may attach to the surface of the cell membrane and disturb its power function such as permeability and respiration. It is reasonable to state that the binding of the particles to the bacteria depends on the surface area available for interaction will give more bacterial resistance to antimicrobial agents poses a serious problem in the treatment of infectious diseases as well as in epidemiological practice. Increasingly, new bacterial strains have emerged with dangerous levels of resistance, including both of Grams positive and Gram negative bacteria. Dealing with bacterial resistance will require precautions that lead to prevention of the emergence and spreading of multi resistant bacterial strains, and development of new antimicrobial substances (Ales panacek et al.. 2008), our results demonstrate the ability of the *Punica granatum* on synthesizing silver nanoparticles and their antimicrobial activity represent a significant advancement in the nanomaterial with realistic implications. The green chemistry approach addressed in the present work on the synthesis of silver nanoparticles is simple, cost effective and the resultant nanoparticles are highly stable and reproducible.

**Phytochemical result**

The phytochemical investigation of *Punica granatum* aqueous extract showed that it is rich in Xanthoproteins, Tannins, Terpenoids, Leucoantho Cyanins, Flavonoids, Phenols, lacking steroids, Glycosides, Coumarins, Fatty acids and Steroids. The fruit extract of Punica granatum contains carbohydrates, Phenolic compounds, Tannins and Falvonoids. The flower, leaf and extract of Punica granatum contains reducing sugars, carbohydrates, Phenolic compounds, saponins, Xanthoproteins, Tannins and Flavonoids. The stem extract of Punica granatum contains steroids, reducing sugars, carbohydrates, Xanthoproteins and Tannins.

| S.No | Test | Fruit | Flower | Leaf | Rind | Stem |
|---|---|---|---|---|---|---|
| **1** | Steroids | - | - | - | - | + |
| **2** | Reducing sugar | - | + | + | + | + |
| **3** | Carbohydrate | + | + | + | + | + |
| **4** | Alkaloids | - | - | - | - | - |
| **5** | Phenolic compound | + | + | + | - | - |
| **6** | Saponins | - | + | + | + | - |
| **7** | Xanthoproteins | - | + | + | + | + |
| **8** | Tannins | + | + | + | + | + |
| **9** | Flavonoids | + | + | + | - | - |

**Table 1: Phytochemical result**

**Silver nanoparticles synthesis**

The five parts of *Punica granatum* were used to produce SNPs, and the reduction of Ag ions into silver particles during exposure to the plant - extract is followed by color change from yellow to brown depends on the medicinal plant extract. It is yellow known that SNPs exhibit brown colour in aqueous solution due to excitation of surface Plasmon vibration in SNPs. As the plant extract was mixed in the aqueous solution of the silver nitrate, it started to change the colour from yellow to brown due to reduction of silver ion which may be the indication of formation of SNPs. Almost all the herbal mediated SN solution after incubation time, were showed the colour change from yellow to brown color.

| S.No | Plant extract | Color observed | pH | 0 hr | Day-1 | Day-2 | Day-3 |
|---|---|---|---|---|---|---|---|
| **1** | Fruit | Very dark brown | 6.5 | ++ | +++ | ++++ | +++++ |
| **2** | Flower | Dark brown | 4.8 | + | ++ | +++ | +++ |
| **3** | Leaf | Light brown | 5.5 | + | ++ | +++ | ++++ |
| **4** | Rind | Light brown | 5.0 | ++ | ++ | ++ | +++ |
| **5** | Stem | Dark brown | 4.6 | + | ++ | +++ | ++++ |

**Table 2: Indication of color change of the extracts after the addition of $AgNO_3$ (i.e) AgNP.**

**UV-Vis Spectra Analysis**

The reduction of pure $Ag^+$ ions was monitored by measuring the uv-vis spectrum by diluting a small aliquot of the sample into distilled water. The uv-vis spectrums for the herbal synthesized

Silver Nano Particles were in the range of 400-500. 'The various parts of Punica granatum were used to synthesis the SNPs. The uv-vis spectroscopy of colloidal solution of SNPs synthesized from rind extract of Punica granatum have the characteristic absorption peak at 419. Like wise to other parts.

| S.No | Extract | Peak Value ( λ max) |
|---|---|---|
| **1** | Fruit | 413 |
| **2** | Flower | 406 |
| **3** | Leaf | 401 |
| **4** | Rind | 439 |
| **5** | Stem | 476 |

**Table 3: Peak Value (λ max) of AgNP - plant extract**

**Antimicrobial Activity**

Medicinal plant medicated synthesized nanoparticles were efficient in inhibiting the bactericidal activity. Nanoparticle produced by various parts of *Punica granatum* extract showed good antibacterial activity against E-Coli and Pseudomonas aeruginosa[v]. It is confirmed that SNPs of various parts of Punica granatum capable of reducing antimicrobial efficacy and hence a great potential in the preparation of drug used bactericidal diseases.

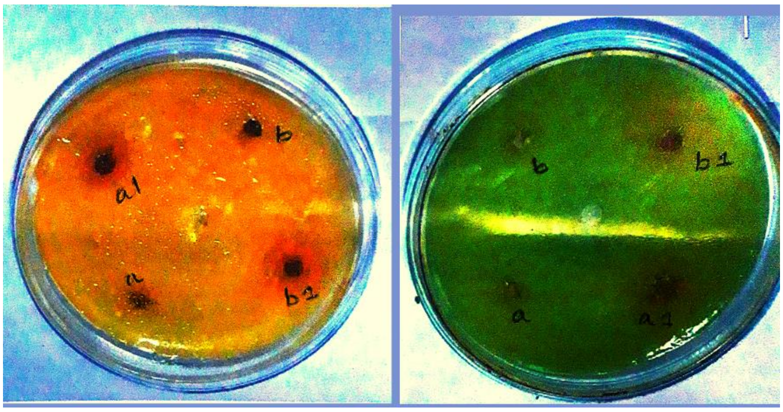


**Figure 3. Antibacterial activity of lea3f and flower extract of Punica granatum with micro organism E-Coli ( yellow) and Pseudomonas aeruginosa ( green) respectively ( a- leaf extracts, $a_1$- leaf extract AgNP, b- flower extract, $b_1$- flower extract AgNP)**

**Conclusion**

In the present work, the phytochemical analyses have been performed on *Punica granatum.* The phytochemical screening indicates that the plant part is a good source of bioactive principle for pharmaceutical industry. The Bio-reduction of silver ions have been performed by various parts of extract of *Punica granatum*. The syntheses of SNPs were confirmed by the change of colour of plant extracts. These environmentally begin SNPs were further confirmed by using UV-Vis spectroscopy. Finally the antimicrobial activity of green synthesized SNPs is compared with $AgNO_3$ and the extract.

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