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Phytochemical analysis of different solvent extracts and antibacterial activity of silver nanoparticles from Punica granatum peel extract

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Abstract

The present study investigated the phytochemistry of silver nanoparticles derived from *Punica granatum* peel extract, as well as their potential to inhibit the growth of bacteria. Phytochemical analysis of pomegranate fruit peel extracts in methanol, ethanol, diethyl ether, and water showed the presence of glycosides, alkaloids, tannins, saponins, glycosides, flavonoids, quinones, and flavonoids; steroids, terpenoids, and phenols were exclusionary. The in-vitro agar well diffusion method was utilized in order to evaluate the effectiveness of three distinct doses of methanolic PGP extract of silver nanoparticles against pathogenic bacterial strains. These strains included *Escherichia coli* and *Protease.* All of the formulations showed good effect, and a conventional drug was used as a positive control.

Keywords: *Punica granatum*, phytochemical analysis, silver nanoparticles and anti-bacterial, gentamycin.

1. Introduction

Eighty percent of the global population uses some form of complementary or alternative medicine, therefore there has been a huge uptick in research into the potential medicinal benefits of plants in the last several decades. Phyto plants have emerged as viable substitutes for synthetic chemical antimicrobials and antibiotics, which pose significant risks such as resistance to these drugs, the development of new, rare infections, and the occurrence of harmful side effects [1]. Clinicians should caution patients about side effects such as urticaria, changes in taste, increased calculus formation, tooth and mucous membrane staining, and, in rare cases, desquamation of the oral mucosa and swelling of the parotid glands before prescribing chlorhexidine mouthwash as an antimicrobial. Of the 250,000–500,000 species of plants and animals on Earth, hardly 1% have had their medicinal potential investigated. The effects of native Iranian plant extracts have been documented in manuscripts [2-3]. A member of the Lythraceae family, the pomegranate tree or shrub (*Punica granatum* L.) is a popular fruiting plant grown in many parts of the world, including South Africa. Due to its various health benefits, all components of the plant, including fruit juice, are highly sought for. The fruit's peel is the most valuable yet wasted

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part, making up about 49% to 55% of the fruit's weight [4]. Its antibacterial, free radical scavenging, and anti-cancer properties are unparalleled by any other component of the pomegranate plant. The chemical makeup of pomegranate peel, especially the phenolics, is intimately linked to these biological activities since it includes numerous bioactive compounds, including ellagitannins, flavonoids, and phenolic acids, among many others. Even though there are a lot of different kinds of pomegranates available and more are being grown all the time, the "Wonderful" kind is still the most popular in South Africa due to its high yields and exceptional qualities. The pomegranate, or PGP, is a natural fruit of the area between Iran and northern India. However, it is currently grown extensively in certain regions of Africa, Mexico, Arizona, and Southwest America. Even ancient Greek and Egyptian writings make reference to the pomegranate's pharmacological benefits. Some of the many possible effects of pomegranate have been discovered in recent investigations [4-6]. These include: anthelmintic, stimulant, vermifuge, antifungal, antiviral, immunological modulation, styptic, astringent, stomachic, laxative, diuretic, and bacteriocidal. The cultivars 'Acco' and 'Herskawitz,' which together account for 9% of the market, are the second most prevalent. Several studies have shown that the beneficial compounds in pomegranate skin might differ across cultivars [7]. In addition, it helps lessen the negative effects of cardiovascular illness, vaginitis, denture stomatitis, erectile dysfunction, obesity, Alzheimer's disease, and baby brain ischemia are among the many medical diseases that it helps alleviate [8]. The pomegranate's seed oil, peels, and juice retain its numerous cancer-fighting and therapeutic characteristics. The plant also contains estriol, testosterone, 17-alphaestradiol, stigmasterol, campesterol, gamma-tocopherol, estriol, punicie acid, and coumesterol. Because of its many beneficial medicinal properties, the pomegranate deserves investigation from various branches of medicine [10].

Silver Nanoparticles serve as antitumor agents by decreasing progressive development of tumor cells. This might be because of their inhibitory actions in several signaling cascades liable for the development and pathogenesis of cancer. Taken together, this information recommends that Silver Nanoparticles can actuate cytotoxicity on cancer cells and hindering tumor progression without lethality to normal cells [9]. In this present investigation we have used fruit shell extract of PGP for the first time to synthesize silver nanoparticles and its application on anticancer activity. The synthesized nanoparticles were characterized using various spectroscopic and microscopic techniques.

In spite of this, a study is documented that aims to investigate the antibacterial characteristics of PGP peel extracts against four distinct pathogenic microorganisms. Additional objectives of this research include analyzing the peel extracts' phytochemical composition and determining which of the most promising extracts include biologically active components. To investigate the phytochemical properties (varieties of extracts) and antimicrobial investigation, we employed a suite of analytical tools.

2 Experimental

2.1 Preparation of Plant Extract

The plant extracts ready to start, fresh pomegranates (500 g) were bought from a market in the city of Tirupattur so that fresh extraction could be made. The peels of pomegranates were separated and dried for 7 days at 33 °C in an oven. An electric grinder was used to turn the dried peels into a powder, which was then put in plastic bags and kept for the next step. 200 mL of methanol (99.9%) was mixed with 100 grams of powder in an electric mixer for 30 minutes. For 30 days, this solution was filtered three times a day. It was always a new methanol dissolvent that was used. The methanol was then taken away in a rotating evaporator to make a dry powder [10]. The samples were then sent to the institute of biological science to be tested for phytochemicals and antibacterial activity.

2.2 Preparation of Extracts for Phytochemical Constituents

Before being chopped into bits and allowed to dry in the shade at room temperature, the selected medicinal plants were rinsed with distilled water after being cleaned with clean water. We crushed and sieved the dry samples before putting them in plastic bags to make sure they weren't contaminated. Using organic solvents instead of water is the optimal method for extracting bioactive chemicals, according to various research [11]. In this study, the powder was extracted from the peel of PGP using 80% methanol alcohol. Following that, there was a mixture of ethanol, diethyl ether, and water. This solvent triumphed due to its expanded extraction capabilities and higher yields of secondary bioactive chemicals. Two thousand milliliters of plant extract and one hundred grams of powdered material made up each reagent vial. Then, we filled each bottle with 1000 mL of methanol. Using an orbital shaking machine, the bottles were shaken after being left at room temperature for 24 hours. The leftover liquid was then filtered using a Whatman No. 1 strainer. Following filtration, the procedure was carried out twice using new solvent. Under reduced pressure and 40 °C, the methanol filtrate was concentrated using a rotary evaporator.

Then, to remove any residual organic solvents, it was moved to beakers & dried in a water bath set at 40 °C. A final step was to keep the samples at 2-8 °C until they could be analyzed further [12].

2.3 Qualitative Phytochemical analysis

The plant extract was tested for detecting various Phytochemicals present in the Chloroform and Ethanolic extract of *Punica granatum* using the standard procedure prescribed.

1) Test for carbohydrates

a) Molish's test

Plant extract was treated with few drops of Alpha naphthol and concentrated sulphuric acid added to it, the mixture in a slanting position. Observance of violet color ring indicates the presence of carbohydrates.

b) Benedict's test

0.5 mL of extract was treated with 0.5 mL of Benedict's reagent and kept for water bath for 2 minutes. Formation of precipitates indicates the presence of carbohydrates.

2) Test for tannins

a) Ferric chloride test

A small quantity of extract was mixed with water and heated in a water bath. The mixture was filtered and 5% of ferric chloride was added to the filtrate. A dark green color was formed. It indicates the presence of tannins.

b) Lead acetate test

3 ml of 10% lead acetate was added to the sample solution. Formation of bulky white precipitate indicates the presence of tannins.

3) Test for saponins

2 ml of distilled water was added with the sample solution and shakes well. Formation of foams indicates the presence of saponins.

4) Test for alkaloids

a) Mayer's test

The sample solution is treated with 2 drops of Mayer's reagent. Formation of white creamy precipitate indicates the presence of alkaloids.

b) Wagner's test

Few drops of Wagner's reagent were added with the sample. Formation of reddish-brown precipitate indicates the presence of alkaloids.

5) Test for Flavonoids

The sample solution is treated with 1 mL of 2N sodium hydroxide. Formation of yellow color indicates the presence of flavonoids.

6) Test for glycosides

The sample solution is treated with 3 ml of chloroform and 10% ammonia. Formation of pink color indicates the presence of glycosides.

7) Test for quinones

1 ml of concentrated sulphuric acid is added to the sample in slanting position. Formation of red color indicates the presence of quinones.

8) Test for Phenols

The sample solution is treated with few drops of 10% ferric chloride. Formation of blue or green color indicates the presence of phenols.

9) Test for terpenoids

The sample solution is added with 2 ml of chloroform and treated with concentrated Sulphuric acid. Formation of red brown color indicates the presence of terpenoids.

10) Test for steroids

Few drops of concentrated sulphuric acid are added to the sample solution in a slanting position. Occurrence of brown ring indicates the presence of steroids.

2.4 Synthesis of Silver Nanoparticles from *Punica granatum*

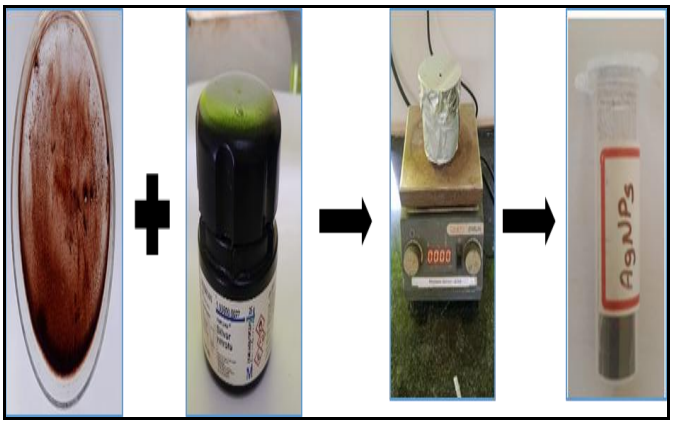

Figure 1: Synthesis of silver nanoparticles from *Punica granatum* peel

10 mM solution of Silver Nitrate was prepared in an Erlenmeyer flask. Then 400 mL of plant extract was added separately to 100 mL of 10 mM Silver Nitrate solution and

made up to 1000 mL with double distilled water. Then the mixture was stirred for 2 hours at 45 °C using hot plate with magnetic stirrer. Reduction of Silver ions (Ag^+) to Silver Nanoparticles (AgO) was confirmed by the color change of solution from colorless to brown [13]. Figure 1 shows brown colour of the prepared sample were silver nanoparticles and finally it was denoted as Ag NPs.

2.5 Anti-bacterial activity of Silver Nanoparticles

Silver Nanoparticles from *Punica granatum* extracts prepared were examined for their antibacterial capabilities. Two distinct bacterial strains were used to assess silver nanoparticles from PGP antibacterial capabilities. To prepare this nutrition agar medium, 2.5 grams of nutrient agar were mixed with 100 milliliters of distilled water and autoclaved at 37 °C for 24 hours. Apply the bacteria to the petri dish by means of the inoculation wire loop. The discs were made using grade 1 filter paper, which is the maximum level of purity. On infected agar plates, the discs were washed with 10, 20, and 30% w/v *Punica granatum* extracts [14]. The next step was to incubate the plates at 37 °C for a whole day. The antibacterial activity was determined by comparing the zone of inhibition to that of streptomycin, a commercial antibiotic, which served as a positive control. We tested every single extract and every single dose that could be administered.

3. Results and Discussion

3.1 Phytochemical screening of PGP Extract

Important therapeutic properties are provided by secondary metabolites for the benefit of human health. Some of these chemicals, in particular, show promise as cancer preventives and suppressors. For the treatment or prevention of a wide range of diseases, people turn to dietary supplements and pharmaceuticals containing compounds from the carbohydrate, alkaloids, quinones, and steroid families. After being washed with running tap water, the chosen fruits of the peel were carefully rinsed in distilled water. They were then let too dry at room temperature for approximately one month in the open air.

Table: 1 Phytochemical Analysis PGP Extracts

Phytochemical Constituents	Extraction of *Punica granatum* Peel			
	Methanol	Aqueous	Ethanol	Diethyl ether
Carbohydrates	+	+	+	+
Alkaloids	+	+	+	-
Flavonoids	+	+	+	+
Steroids	+	-	-	-
Terpenoids	+	+	+	+
Tannins	+	+	+	+
Quinones	+	+	-	-
Saponins	+	_	+	+
Glycosides	+	+	+	-
Phenols	+	+	+	-

Indicates: + Present and – Absent

The dried fruit was ground into a powder and placed in a sterile container for future use. The four solvents ethanol, methanol, water, and diethyl ether were used in 100 mL increments to extract the PGP powder from storage. Using air drying and an evaporator set at 40 °C, the solvents were removed to get crude extract after the extraction step. Following the standard protocol, the phytochemical

screening of the peel extract was conducted. Phytochemical analysis of plant sources in aqueous, methanolic, and ethanolic extracts showed the presence of a wide range of phytochemicals, including carbs, tannins, saponins, alkaloids, flavonoids, quinones, and glycosides; however, steroids, terpenoids, and phenols were not detected [15]. Table 1 lists the phytochemical components of the *Punica granatum* peel extracts that are suggested.

3.2 Antibacterial Activity of AgNPs

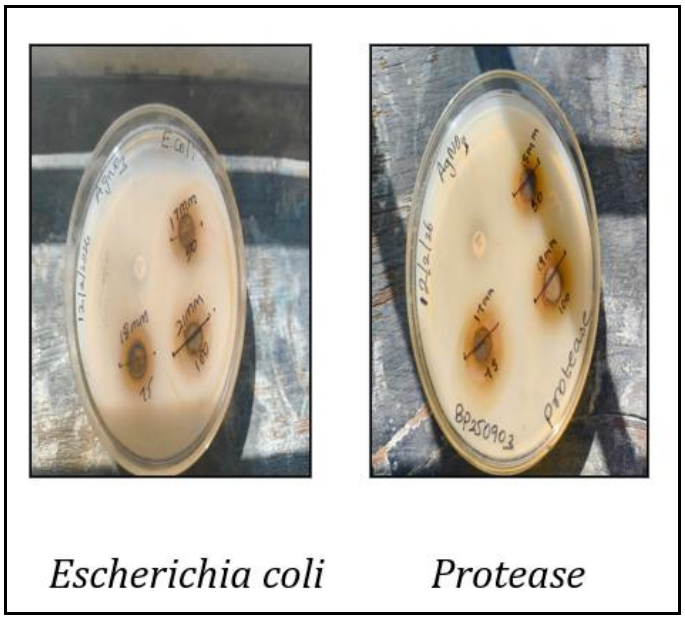

Figure 2: Antibacterial Activity of Silver NPs from PGP Extract

Using the conventional zone of inhibition assay, we assessed the antibacterial activity of the PGP methanolic extract of silver nanoparticles that was produced. Utilizing a sterile cotton scrub, a pathogenic organism culture was swabbed from a new culture of 1.5 x 10^8 CFU/mL of the bacterial species on a Muller Hinton Agar plate. A 1 mg/1 mL stock solution of PGP extract was produced in sterile distilled water. The sterile disk was filled with varying volumes of PGP extract, ranging from 50 to 100 µL. The discs containing the drugs were then placed on top of the swab culture plate. The milli meter was used to measure the zones of inhibition after incubating the plate for 24 hours at 37 °C [16]. Because of this, it hinders the system's normal functioning, which in turn causes the bacteria to die because it disrupts their respiration mechanism. In order to analyze the surface morphological alterations of the test pathogens, all of the photos were captured (Figure 2).

Table 2: Anti-bacterial activity of Silver Nanoparticles

S.No.	Organisms	Zone of inhibition (mm in dm)			
		PC(Gen)	50 µg/mL	75 µg/mL	100µg/mL
1.	*Protease*	20 (mm)	15	17	19
2.	*Escherichia coli*	20 (mm)	17	18	21

Traditional medicinal plants in Tirupattur District had high informant consensus factor values; antibacterial tests were done to confirm their therapeutic effects. The results showed that almost all of the therapeutic plant extracts tested had antibacterial effects against at least one of the Gram-positive and Gram-negative bacteria. From Table 2 showed that the concentrations of 100 and 150 mg/mL, the crude PGP extract exhibited a much larger mean zone of inhibition against *Escherichia coli* than the positive control [17-18]. Likewise, against *Protease*, the Peel extract showed the largest mean zone of inhibition at a dosage of 150 mg/mL. No previous research has ever looked at the

effects of PGP extract like this one. This precludes any meaningful comparisons between the results of this study and those of related studies carried out in the same or similar locations around the world.

4. Conclusions

Based on the results of this investigation, Peel extracts of Ag NPs showed promise in antibacterial activity testing and preliminary phytochemical analyses of different extractions. Selected extracts from the peel of the Pomegranate tree were discovered to be extremely sensitive to two different bacterial strains, one each of Gram positive and Gram negative. Alkaloids, polyphenols, saponins, terpenoids, steroids, flavonoids, and tannins were also identified through phytochemical study. According to the study's findings, *Punica granatum* peel extracts of silver nanoparticles had greater mean zones of inhibition against a number of harmful bacteria, including *Protease,* and *Escherichia coli.* Two bacterial strains are comparison to the conventionally prescribed medications. Consequently, in order to create new antibiotics, it is crucial to undertake more in-depth research to extract the bioactive secondary components from PGP peel of silver NPs and identify them.

Conflict of Interest: Nil

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