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POMEGRANATE PEEL EXTRACTS ACTIVITY AS AN ANTIMICROBIAL AND ANTIOXIDANT AGENT AGAINST BACTERIAL DISEASES

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ABSTRACT

Aim and Objectives: In the present study, the *Punica granatum* (pomegranate) fruit rind was taken and subjected to assessment for its important therapeutic applications like antioxidant and antimicrobial and also screened for its pharmacognostic and phytochemical features. **Materials and Methods:** Pharmacognostical and phytochemical parameters determined in the present work can serve as major criteria for the identity, quality, and purity of a crude drug and extracts. Standard methods were used to screen *Punica granatum* fruit rinds' pharmacognostical, phytochemical, antimicrobial, and antioxidant potentials. **Results:** Results of antimicrobial activity revealed that the extracts showed good antimicrobial activity against all pathogens tested. The best activity was noted against *E.coli* (26mm) followed by *Klebsiella* (23mm) at 1600□g/disc concentration. Based on the phytochemical indicated that flavonoids, terpenoids, phenolic compounds and tannins might be responsible for the biological activity and *In Vitro* antioxidant assay results that the IC50 values based on the DPPH (47.93±100µg/ml) and Superoxide radical (91.28±100µg/ml) is concluded which exhibit antioxidant and free radical scavenging activity. It also chelates iron and possesses reducing power. **Conculsion:** *In vitro* assay systems confirm *Punica granatum* fruit rind as natural antioxidants but it is doubtful that specific components are responsible for antioxidant activity. This knowledge will be crucial in the future in drug discovery to develop innovative natural medicines to treat various diseases.

KEYWORDS: Pomegranate fruit peel, bacterial diseases, antioxidant, crude drugs.

INTRODUCTION

In the traditional system of medicines the drugs are primarily dispensed as aqueous or ethanol extract. The medicinal plants should be authentic and free from harmful materials like pesticides, heavy metals microbial, and radioactive contamination.[25] The extract of medicinal plants should be single solvent extraction once or repeatedly or aqueous extract or as described in the ancient texts. The extract should be then checked for biological activity in experimental animal models. The bioactive extract should be standardized based on active compounds.[10]

Pomegranate belongs to the family Punicaceae and its botanical name is *Punica granatum*.[44] In Ayurvedic medicine, the pomegranate is considered "a pharmacy unto itself" The root and bark are believed to have anti-helminthic and vermifuge properties.[18] The peels are a powerful astringent and cure for diarrhea and oral aphthae. The pomegranate plant contains important pharmacologic components. Flavonoids and tannins are more abundant in the rinds of the fruits. The tree also

has potent physiological effects and a long medical history.[23]

Pomegranate is traditionally used for various healing purposes.[38] They are used for curing dysentery and chronic diarrhea, expelling tapeworms and other worms from the body, strengthening and giving tone to the stomach, curing inflammation of the eye, elimination of kidney stones, gastric and asthmatic fever, inflammation of the urinary tract and hemorrhages.[27]

The antioxidant activity of pomegranate juices was evaluated by four different methods and compared to those of red wine and green tea infusion. Commercial pomegranate juices showed an antioxidant activity three times higher than those of red wine and green tea.[14] The activity was higher in commercial juices extracted from whole pomegranates than in experimental juices obtained from the arils only. HPLC-DAD and HPLC-MS analyses of the juices revealed that commercial juices contained the pomegranate tannin punicalagin while only traces of this compound were detected in the experimental juice obtained from arils in the laboratory.[29] This shows that pomegranate industrial processing extracts some of the hydrolyzable tannins present in the fruit rind. This could account for the higher antioxidant activity of commercial juices compared to the experimental ones. In addition, anthocyanins, ellagic acid derivatives, and hydrolyzable tannins were detected and quantified in the pomegranate juices.[15]

Punica granatum extracts inhibited the growth of *Staphylococcus aureus* and stopped enterotoxin production. Growth of many gram-positive and gram-positive pathogens like *Escherichia coli, Salmonella* sp., *Shigella* sp. *Staphylococcus aureus* is inhibited by methanol fraction or rind.[5] Boiled water extracts of *Punica granatum* fruit pericarp showed antibacterial activity against multiple antibiotic- resistant *Salmonella typhi*. Decoction of *Punica granatum* peels showed the best bactericidal effect.[1] Studies showed the antibacterial activity of methanolic extracts on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans.* Petroleum ether extract, chloroform extract, and methanol extracts of *Punica granatum* fruit rind showed very good antibacterial activity against *Escherichia coli.*[32,37] The *Punica granatum* combined extract can be used as an alternative combination with the ability to inhibit antibiotic-resistant bacteria in single and biofilm forms.[33]

The global threats of synthetic drugs are a growing concern, with these substances posing a significant risk to public health, safety and security. Pomegranate therapeutic applications are gaining global attention due to their potential to address various health concerns. In the present study, the pomegranate fruit rind was taken and subjected to assessment for its important therapeutic applications like antioxidant, and antimicrobial and also screened for its pharmacognostic and phytochemical features.

Materials and Methods Clinical sample collection

A part of body fluid or any other material, which represents the actual infection of an individual, is called a clinical sample or specimen. In this study, stool, urine, and sputum happened to be the clinical sample or specimen. 5ml to 15ml of Stool, urine, and sputum samples were collected at hospitals in a wide- mouthed container (Hi-media) from clinically diagnosed patients. Various types of processing were done to find out the correct etiological agent of the disease. Various types of non-clinical samples like soil, water, and sewage samples were collected and processed.

Macroscopy & Microscopy of clinical sample

Fresh stool, urine, and sputum samples were looked for their physical nature like color, odor, consistency, and other such morphological features. In addition to nature, color, odor, and consistency, samples were also looked for the presence of mucous and blood. These findings would be of great help in identifying the nature of the infection, which would facilitate finding the type of infection. Microscopic examination of stool, urine, and sputum samples was done to get an idea about the nature of infection, inflammatory response, and type of microbial infection and its association.

Culture of the sample to look for bacterial etiology

The sample is inoculated on, a selective cum differential medium after enrichment which helps to isolate and identify the bacterial etiology of stool, urine, and sputum samples. An Enrichment medium is used to increase the number of bacterial isolates by making use of added ingredients. Particular groups of microorganisms were selected by adding selective ingredients to the medium, which allows the growth of only specific microorganisms. Generally, bile salts and deoxycholate are used to inhibit gram-positive microorganisms. Differentiation was done by adding an indicator to the medium. The indicator will differentiate the microbial group based on its physiological characteristics.

Enrichment, differential, and selective media were used to isolate bacterial pathogens from given samples. Bacterial pathogens were identified by making use of the staining tests, and biochemical tests in addition to its growth characteristics on nutrient agar, and microscopic analysis.[3,39]

Medicinal plants and their antimicrobial activity Plant material ---collection and process

Based on ancient and recent literature, traditional use, and Siddha and Ayurveda system of medicine fruit rind of pomegranate were chosen to look for their anti-bacterial activity. Good quality pomegranate fruit was purchased from the local markets of Srirangam, Tiruchirapalli, in the month of March 2023 and plant leaves were authenticated by Prof. John Britto,

Department of Botany, St. Joseph's College, Thiruchirapalli, Tamilnadu, India. Rind was collected from the fruit, shade dried, and powdered by making use of a mechanical blender (Smith mixie). The powder was stored in an airtight container and was used for extraction.

The organoleptic characters, physicochemical parameters, and fluorescence analysis of the samples were evaluated by the methods given in the Ayurvedic Pharmacopoeia of India.

Preparation of Extracts

The powdered plant material (150 g) was extracted with using water and alcohol by the cold maceration method. Both the extracts were filtered with a muslin cloth and the filterate was concentrated in vacuum evaporator. Vacuum evaporator was maintained under the temperaute at 40□C, gas pressure 1-30 mbar and 10-50litre/minutes of flow rates. Dried extracts were used for further studies.[24]

Antimicrobial activity test Test microorganisms

Escherichia coli, *Salmonella sp.* and *Pseudomonas sp. Klebsiella sp. Enterobacter sp. Staphylococcus sp. and Bacillus sp* isolated from the clinical and nonclinical samples were used as test organisms.

Preparation of disc

Known quantities of extracts (10mg) or fractions were dissolved of DMSO: Methanol of 1:1 ratio. This, in turn, was diluted with an equal volume of phosphate-buffered saline (PBS pH 7). It was then filter sterilized by making use of a sartorious syringe filter of pore size 0.22 µm. Sterile discs of 6 mm diameter (Hi-Media) were loaded with various concentrations of extracts (200, 400, 800 & 1600 µg/disc) and fractions and were dried. Dried discs were stored in sterile containers till use. Solvent-loaded discs were also prepared and used as a negative control. Oxytetracycline-loaded Hi-Media discs were used as positive control.

Preparation of inoculum

Clinical isolates and referral strains were inoculated in nutrient broth and incubated at 37 ^{0}C for 4 hours in a shaker (Orbitech, Sciences, India) and were used for anti-bacterial activity test and to look for the Minimum Inhibitory Concentration (MIC) of various extracts and fractions.

Determination of antibacterial activity

Disc diffusion method was followed[7] to determine the anti-bacterial activity of various extracts and fractions. Petri plates containing 20 ml of Mueller Hinton agar were seeded with 4-hours-old fresh culture of clinical isolates and referral strains. By making use of template drawn extracts and fractions loaded discs were dispensed on the solidified Mueller Hinton agar with test organisms. Oxytetracycline antibiotic disc obtained from M/s Hi-Media Laboratories Ltd, Mumbai was used as a positive control, and solvent- loaded discs were used as a negative control. This was incubated at 37°C for 24 hours in an incubator (Rands SBC). The test was performed in triplicates. The zone of inhibition was measured by making use of Antibiotic zone scale (Hi - media).

Determination of Minimum Inhibitory Concentration (MIC)

The agar dilution method was used to find out the Minimal Inhibitory Concentration (**NCCLS, 1993**). The stock concentration of various plant extracts was prepared by making use of DMSO:Methanol, in the ratio of 1:1 which in turn was diluted with an equal volume of phosphate-buffered saline, pH 7. Mueller Hinton agar was prepared, sterilized, and kept ready in molten condition. 20ml of the molten media were taken and mixed with known concentrations of different extracts/fractions and were added in different tubes. This mixture was swirled carefully for complete mixing of extract and media and poured onto the plate. After getting solidified it was inoculated with the test organism and standard organism. The plates were incubated at 37°C for 24 hours. MIC was recorded based on the growth of the organisms.

Phytochemical analysis of secondary metabolites

The extracts were subjected for qualitative screening secondary metabolites like alkaloids, flavonoids, glycosides, steroids and other compounds by standard methods. To know the phytoconstituents, particularly for their nutritive value and medicinal value of *Punica granatum* fruit rind aqueous and acetone extracts were subjected to the analysis of macromolecules and secondary metabolites by using standard methods.[19]

Quantitative phytochemical analysis

The *Punica granatum* fruit rind aqueous and acetone extracts were subjected to qualitative screening of secondary metabolites like tannins,[36] flavonoids,[11] and phenols[28] by using standard methods.

***In-Vitro* antioxidant assay**

DPPH assay: (2, 2-diphenyl-1-picrylhydrazyl)

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as

(DPPH) + (H-A) →DPPH-H + (A) (Purple) (Yellow)

Antioxidants react with DPPH, a stable free radical which was reduced to DPPH-H and as consequence the absorbance were decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen-donating ability.

Antioxidant activity by DPPH staining

An aliquot (3□L) of each sample and standard (Quercetin and Ascorbic acid) was carefully loaded onto a 10cm X 10cm Silica gel plate (silica gel 60 F254; Merck) and allowed to dry for 3 minutes. Drops of each sample were loaded in an order of decreasing

concentration along the row. After 5 minutes, the TLC plate was sprayed with 0.2% DPPH in methanol. Discoloration of DPPH indicates the scavenging potential of the compound tested.[41]

DPPH assay by TLC

This preliminary test was performed with a rapid TLC screening method using the 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) as a spray reagent.[12,16] Analytical TLC silica gel plate (10cm X10cm) was developed using chloroform:methanol: water (61:32:7) after application of 5μL of each test compound solution (1mg/mL), dried and sprayed with DPPH solution (0.2%, MeOH). After 5 minutes, the active compounds appeared as yellow spots against a purple background. The purple stable free radical 2, 2- diphenyl-1-picrylhydrazyl was reduced to yellow diphenylpicryl hydrazine. Quercetin was used as a positive control.

DPPH Radical Scavenging Activity (Spectrophotometer)

The free radical scavenging capacity of the extracts of *Punica granatum* aqueous and acetone extract was determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% methanol. Extracts of *Punica granatum* were mixed with 95% methanol to prepare the stock solution (10mg/100mL). The concentration of thr extract solution was 10mg/100mL or 100□g/mL. From stock solution 2mL, 4mL, 6mL, 8mL, and 10mL of the solution were taken in five test tubes and serially diluted, this was made up to a final volume of each test tube to 10mL whose concentration was then 20□g/mL, 40□g/mL, 60□g/mL, 80□g/mL and 100□g/mL respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing extracts and after 10 minutes, the absorbance was taken at 517nm using a spectrophotometer(Systronics UV-Visible Spectrophotometer 119, INDIA). Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (10mg/100mL or 100□g/mL) of extracts. A control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank.[8]

Reducing power assay

Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form Potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form a ferric ferrous complex that has an absorption maximum at 700nm. 1mL of plant extract solution (final concentration 100-500mg/L) was mixed with 2.5mL phosphate buffer (0.2M, pH 6.6) and 2.5mL potassium ferricyanide [K_3Fe (CN_6)] (10g/L), then the mixture was incubated at 50^0C for 20 minutes. To this 2.5mL of trichloroacetic acid (100g/L) was added, and centrifuged at 3000rpm for 10 minutes.

Finally, 2.5mL of the supernatant solution was mixed with 2.5mL of distilled water and 0.5mL $Fecl_3$ (1g/L), and the absorbance was measured at 700nm in UV-Visible Spectrophotometer (Systronics UV-Visible Spectrophotometer 119, India). Ascorbic acid was used as standard and phosphate buffer as a blank solution. The absorbance of the final reaction mixture of two parallel experiments was expressed as mean ± standard deviation. Increased absorbance of the reaction mixture indicates stronger reducing power.

$$\% \text{ increase in Reducing Power} = \frac{\text{A test}}{\text{A Blank}} - 1 \times 100$$

A_{test} is the absorbance of the test solution; A_{blank} is the absorbance of the blank. The antioxidant activity of the root extract was expressed as IC_{50} and compared with the standard.

Superoxide radical scavenging activity (PMS- NADH System)

Superoxide anions were generated using the PMS/NADH system. The superoxide anions are subsequently made to reduce nitroblue tetrazolium, which yields a chromogenic product, which is measured at 560nm. The phenazine methosulfate- nicotinamide adenine dinucleotide (PMS-NADH) system was used for the generation of superoxide anion. It was assayed by the reduction of nitroblue tetrazolium (NBT).

About 1mL of nitroblue tetrazolium (156µM), and 1mL NADH (468µM) in 100mM phosphate buffer of pH 7.8, and 0.1mL of sample solution of different concentrations were mixed. The reaction started by adding 100µl PMS (60µM). The reaction mixture was incubated at 25°C for 5 minutes and absorbance of the mixture was measured at 560nm against blank samples. The percentage inhibition was determined by comparing the results of control and test samples.[33]

Assessment of % inhibition and IC[50]

Radical scavenging activity of extracts and standards were expressed in terms of % inhibition. It is calculated by using the formula $[(A_{Control}-A_{Sample}) /A_{Control}] \times 100$. Where $A_{Control}$ is the absorbance of the control, and A_{Sample} is the absorbance in the presence of the sample of aqueous and alcoholic extracts. The IC_{50} value is defined as the concentration (in µg/mL) of extracts that produced a 50% antioxidant effect. IC_{50} = Concentration of extract / % inhibition X 50.

RESULTS

Test organisms required for the antimicrobial activity screening were isolated from various samples. Isolated organisms were identified by making use of colony morphology on nutrient agar, colony morphology on selective cum differential agar, microscopic methods and Biochemical methods. Tables 1, 2, 3, and 4 revealed the identification features of various test organisms.

Based on these tests the organisms were identified as *E.coli, Salmonella, Staphylococcus aureus, Klebsiella, Bacillus, Pseudomonas and Enterobacter.* These organisms are involved in various human, animal and

plant infections control of these organisms through herbal way will be more helpful to human society because most current antibiotics are toxic to human cells and tissues some of the organisms develop resistance against various current antibiotics used.

The plant's pharmacognostial nature of plant provides proper authenticity for the quality raw material. *Punica granatum* fruit rind was collected from Srirangam market and powdered using a mechanical blender. The resulting power showed a light brown colour, tamarind odour, bitter taste, and smooth texture, and fracture (Table 5).

Physiochemical data of the plant powder also provide the specific nature of the *Punica granatum* plant powder. Results of physiochemical parameters revealed that the plant powder did not have any foreign matter. Ash content of the powder material is directly proportional to the quantity of foreign matter present in the given sample. Total ash value, acid insoluble ash value, and water soluble ash value were within the limit of ayurvedic pharmacopoeia of India. *P. granatum* fruit rind exhibited 5.1% total ash, 1.7% water-soluble ash and 2.7% acid-soluble ash. Powder showed a higher percentage of water-soluble extractives (42.4%) followed by alcohol soluble extractives (24.8%). Higher extractive values indicated that the plant showed higher polar compounds like alkaloids, flavonoids, and phenolic compounds etc., and 0.24µm size particles were used for assaying biological activity (Table -6)

Fluorescence analysis also indicated the quality of the raw material used. Chroomatophoeres are produced when the powder is treated with different chemicals. *P. granatum* plant powder showed characteristic green, yellow, yellowish green, and brown black colour (Table 7). The table 8 showed the microbial loads available in the plant powder which was examined before subjecting the powder to extraction. There were no fungal and other intestinal pathogenic organisms like *Salmonella, Shigella and E. coli*. Total aerobic microbial load is within the limits of Ayurvedic and international pharmacopoeial standards.

A disc diffusion test was followed to assess antimicrobial activity against test organisms. The zone of inhibition was produced by the aqueous extract of *P.granatum* ranges from 10mm to 26mm. The best activity was noted against *E.coli* (26mm) followed by *Klebsiella* (23mm) at 1600□g/disc concentration (Table 9).

Acetone extract inhibited all pathogens and other organisms in a dose-dependent manner. The best activity was noted against *Staphylococcus* and *Enterobacter* (16mm each) followed by *E.coli* and *Klebsiella* (15mm each) at 400□g/disc concentration. There was no zone of inhibition at 100□g/disc concentration. Acetone extract showed the best results when compared to aqueous extract (Table 10).

MIC value of aqueous and acetone extracts revealed that acetone extract yielded the best result. MIC value for acetone extract ranges from 75□g/ml to 175□g/ml concentration with best activity against *E. coli* (75□g/ml). MIC value of aqueous extract ranges from 150-250□g/ml concentration. *Bacillus* was best inhibited by aqueous extract at 150□g/ml concentration (Table 11).

Punica granatum extracts inhibited the growth of *Staphylococcus aureus* and stopped enterotoxin production at 0.05% (v/v) concentrations[10]. Growth of many gram-positive and gram-positive pathogens like *Escherichia coli, Salmonella* sp., *Shigella* sp. *Staphylococcus aureus* is inhibited by methanol fraction or rind. Boiled water extracts of *Punica granatum* fruit pericarp showed antibacterial activity against multiple antibiotic- resistant *Salmonella typhi*. Decoction of *Punica granatum* peels showed the best bactericidal effect. Studies showed the antibacterial activity of methanolic extracts on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans.* Petroleum ether extract, chloroform extract and methanol extracts of *Punica granatum* fruit rind showed very good antibacterial activity against *Escherichia coli.*[32,37]

Punica granatum is commonly used in Korea as a traditional medicine for the treatment of pathogenic bacteria. In this study, we investigated the *in vitro* and *in vivo* antimicrobial activity of *Punica granatum* peel EtOH extract (PGPE) against 16 strains of *Salmonella*. The minimal inhibitory concentrations of PGPE were in the range of 62.5-1000 □g ml^{-1}. In addition, the *in vivo* antibacterial activity of the PGPE extract was examined in an *S. typhimurium* infection mouse model. Mice were initially infected with *S. typhimurium* and then with PGPE. The extract was found to have significant effects on mortality and the number of viable *S. typhimurium* recovered from faces. Although clinical signs and histological damage were rarely observed in the treated mice, the untreated controls showed signs of lethargy and histological damage in the liver and spleen. Taken together, the results of this study indicate that PGPE has the potential to provide an effective treatment for salmonellosis.[2,21]

In the present study, the preliminary phytochemical screening of the aqueous extract revealed the presence of terpenoids, flavonoids, saponins, phenolic compound, tannins, lignin, protein and total carbohydrates whereas acetone extract showed only flavonoids, phenolic compound and tannins. This test is required to elicit the primary nature of the phytochemicals available in the extract. This study also indicated that flavonoids, terpenoids, phenolic compounds and tannins might be responsible for the biological activity (Table 12).

In order to quantify important phenolic compounds available in the extracts, standard spectrophotometric

methods were used and the results indicated that acetone extract yielded 24.40% flavonoid, 20% tannins and 32.4%, phenolic acid (Table 13) and aqueous extracts showed lower concentrations of these phytochemicals (8% flavonoid, 12.2% tannin and 18% phenols).

DPPH test provides a simplified version to detect the antioxidant properties of various molecules present in the extracts. DPPH solution is decolourized when the odd electron becomes paired off in the presence of a free radical scavenger. The colour becomes light yellow from deep violet. Dot assay, TLC assay and spectrophotometric assay indicated the DPPH scavenging nature of the extracts.

The corresponding increase in absorbance is noted in extracts as well as standard when the concentrations of extracts and standard were increased. The percentage of DPPH radical scavenging activity of aqueous (at 100 µg/ml) and acetone extracts (at 40µg/ml) and standard (at 50 µg/ml) were 47.93, 58.46 and 52.67 respectively (Table 14). Figure 1 indicates IC_{50} exhibited by different extracts of *P.granatum* fruit rind, Acetone extract showed good IC_{50} value when compared to others Aqueous and acetone extracts of *P.granatum* fruit rind showed potent antioxidant activity by reducing power ability. Aqueous extract yielded better antioxidant power (72.8) (at 100 µg) than acetone extract (68.7) at 40 µg/ml concentrations respectively. Ascorbic acid is produced at 96.26 reducing power at 50 µg/ml concentrations. The results of the reducing power assay were significantly different among the various concentrations tested (Table 15).

Aqueous extract of *Punica granatum* fruit rind showed significant free radical scavenging activity against superoxide ions. The percentage of scavenging was found to be (72.57) which is slightly higher than acetone extract (45.64). Ascorbic acid was used as a reference standard, which exhibited 50.70±1.24% superoxide radical scavenging power at 50µg/ml concentration (Table 16).

DISCUSSION

Biological and chemical research in Lifescience evidenced that free radical and reactive oxygen species can be involved in a high number of diseases.[22] Numerous physiological and biochemical processes in the human body may produce oxygen- centered free radicals and other reactive oxygen species and byproducts. Overproduction of such free radicals causes oxidative damage to biomolecules leading to many chronic diseases.[17] Plants are an important source of free radical scavenging molecules. Intake of natural antioxidants has been associated with reduced risk of cancer; cardiovascular diseases, diabetes and other diseases associated with ageing.

The preliminary antimicrobial activity of *P.granatum* studied here can be seen as a potential source of useful drugs. In the present study, the preliminary phytochemical screening of the various chemicals revealed the presence of alkaloids, steroids, flavonoids, saponins, reducing sugar, quinone and coumarins. The plant studied here can be a source of high pharmacological importance and a potential source of new drugs. Further studies on such bioactive compounds screening and their antimicrobial activity will unravel the potentiality of these traditional medicines.[3]

The antibacterial activity of *P.granatum* L. was studied using the disk diffusion method, micro broth dilution, and microtiter plate methods. Given the disc diffusion test (MIC and MBC), the extracts had inhibitory effects on the individual forms of bacteria. However, the ethanolic extract had greater effectiveness than the methanolic extract.[33]

An Antioxidant is one of the most essential ingredients of today's menu/therapy because the antioxidative system protects the animal against reactive oxygen species (H_2O_2, superoxide, OH, singlet oxygen & nitrogen species) induced oxidative damage. Various synthetic antioxidants Butylated Hydroxy Toluene (BHT) are in use, but they are suspected to be carcinogenic.[4,40] Natural antioxidants, therefore, have gained importance. Aqueous & alcoholic extracts of *Punica granatum* fruit rind have been studied for their antioxidant properties using different in vitro antioxidant methods.

Flavonoids, phenolic acids, tannins, and steroids are found in the extracts of *Punica granatum* fruit rind. *Punica granatum* fruit rind extracts showed a good antioxidant effect, which could be due to the available phytoconstituents. In this respect, polyphenolic compounds commonly found in plants have been reported to have multiple biological effects like anticancer.[42] and antiproliferative.[42] antimicrobial.[43] wound healing.[30,31] and antibacterial activities[13] including antioxidant activity.[15]

The potent antioxidant activity of *Punica granatum* fruit rind extracts was analysed by making use of 6 different methods. However, the efficiency of each extract is different against various free radicals depending on the specific assay methodology, which reflects the complexity of the mechanisms and diversity of the chemical nature of the plant material.

Numerous scientists have reported the potent antioxidant capacity of pomegranate fruit juice and its components using multiple assay systems.[35] However, this is the first kind of this kind of work on fruit rind antioxidant assay. The effect of antioxidants on DPPH is thought to be due to their hydrogen-donating activity.[6]

The aqueous & alcoholic extracts had a significant radical scavenging effect on DPPH radical. As DPPH is considered a lipophilic radical, it readily accepts

electrons from the antioxidant compound and converts its colour from violet to yellow which is detected at 516nm. Hydrogen donors in the extract may be responsible for the DPPH radical scavenging power of the extracts which the increased with the increased concentration of the extract.

Antioxidants present in the sample reduce Fe^{3+} to Fe^{2+} by donating electrons. The amount of Fe^{2+} can be assessed by measuring OD at 700 nm. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging.[20]

Standard methods were adopted to assess the antioxidant activity and phytochemical nature of the plant materials. The extent of radical scavenging was determined by the calculated IC50 value. The results revealed that hexane, aqueous, ethyl acetate and ethanol extracts showed good antioxidant activity when compared to Ascorbic acid standard. The ethyl acetate soluble fractions have shown the maximum activity among all.[4]

The results of the present study suggested that the extracts of *Punica granatum* rind are a more potent scavenger with IC_{50} value (Figure 1). Superoxide anions are highly toxic to cellular components. The reported has been updated flavonoids are effective antioxidants mainly because they scavenge superoxide anions.[20]

CONCLUSION

P.granatum fruit rind has been used as a medicine for treating various diseases including diarrhea and dysentery. Standard methods were used to screen *P.granatum* fruit rinds' pharmacognostical, phytochemical, antimicrobial and antioxidant potentials. Pharmacognostical and phytochemical parameters determined in the present work can serve as major the criteria for identity, quality and purity of a crude drug and extracts. Results of antimicrobial activity revealed that the extracts showed good antimicrobial activity against all pathogens tested. Based on the phytochemical and *In Vitro* antioxidant assay results it is concluded that the extracts contain higher quantities of phenolic compounds, which exhibit antioxidant and free radical scavenging activity. It also chelates iron and possesses reducing power. *In vitro* assay systems confirm *P.granatum* fruit rind as natural antioxidants but it is doubtful that specific components are responsible for antioxidant activity. Further *In vivo* assessment is also needed to confirm the antioxidant nature of *Punica granatum* fruit rind. This knowledge will be crucial in the future in drug discovery to develop innovative natural medicines to treat various diseases.

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Conflict of interests

The authors declare there are no competing interests.

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Ethics approval and consent to participate Not applicable.

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