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Evaluation of the Antioxidant and Antibacterial Properties of *Punica granatum* Peel Extract to Treat Against Food Borne Bacteria

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Abstract: The widespread use of medicinal plants has a significant impact on the health of millions of people living in India. Approximately 80% of people living in the third world still rely nearly exclusively on traditional medicines to maintain overall health and treat a wide range of illnesses. *Punica granatum* L. (pomegranate) is one of the Mediterranean medicinal plants and has various curative properties. The aim of the study was to evaluate the antioxidant and antibacterial activity of the *Punica granatum* peel extract. The present study gives information regarding different assay techniques that were used to study the antioxidant activity of the peel extract. *In vitro* studies on the effect of *Punica granatum* peel extract on scavenging 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonate) radical cation (ABTS•⁺), hydroxyl radical (OH•) and superoxide anion radical (O₂•⁻) scavenging and confirmed the free radical antioxidant activity. The antibacterial activity of *Punica granatum* peel extract on the microorganisms Gram negative bacteria *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* and Gram positive bacteria *Enterococcus faecalis* produced inhibition zones which confirmed the antibacterial activity. The results of the present study showed significant antioxidant and antibacterial potential.

Keywords: *Punica granatum* peel, Antioxidant, Antibacterial, Radical scavenging, DPPH

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Introduction

The human life and their survival are nothing without plant and plant products, we humans are highly depending upon the nature. Plants are being used as a valuable source from the past, present and in the future. Humans learned to recognise and categorise plant materials suitable

for use in meeting the necessities of life over time and with the development of societies (Chakwuma *et al.*, 2015). The plant and the plant extracts have been used for various purposes. They play vital roles in treating many diseases and its uses in various pharmacological

properties. Plant diversity in terms of chemical and biological diversity represents a potentially limitless renewable resource for the development of new pharmaceuticals. Many biologically active chemical compounds are found in each medicinal plant, all of which work together in a synergistic manner. Each plant has an impact on the human body both directly and indirectly. The chemical and biological compounds produced have an impact on pharmacological action. Plants have produced many biological compounds where humans have developed a number of methods to depict those compounds and their structural studies which is called phytochemical analysis of the plants. The phytochemicals are the secondary metabolites with a variety of health benefits as well as colour, aroma, and flavour in plants. If scientifically confirmed, phytochemicals have the potential to be used as drugs, and the content and known pharmacological activity of these substances in medicinal plants is the scientific basis for their use in medicine. In this world, human can not live without food. Food is a very much essential need for every living being. But in this modern world human beings are facing a lots of food scarcity issues. However, it is believed that 12.5 per cent of the earth's people receive considerably less food than they need. as many as 50 per cent might be receiving a marginal level of food. The reason for this widespread hunger problem includes unequal distribution of food and money as well as cultural, religious and superstitions beliefs. Predictions for the future food supply range from very pessimistic, with famines expected (Ashraf *et al.*, 2018).

The use of microorganisms in food products is not a new idea. The action of yeast in the fermentations producing wine and beer and the leavening of doughs has been known for at least 4000 or 5000 years. Microorganisms are used in the fermentation of various foods and are consumed as a part of these foods. These are the evidence of cheese production penicillium antibiotic and many dairy productions. Therefore, the concept of using microorganisms as part of the food supply should not be completely

objectionable. Bacteria, yeasts, or Molds cannot create foods, but they can grow on cellulosic compounds that would otherwise be wasted. Algae can utilize solar energy to produce food. Every kilogram of food produced between 5 to 10 kg of waste materials are left in the field. These materials are regarded as wastes because using them would not be financially advantageous given their economic value. Oestrogens, tumorigenesis, carcinogens, cyanogen's, sea food toxins, nutritional inhibitors, and allergens are examples of naturally occurring toxins. These compounds are often present in little amounts in foods, and some of them undergo changes during processing that lessen their efficacy. People frequently consume raw foods, excessive amounts of a single type of food, or mistake similar-looking, edible plants for toxic ones, which results in problems brought on by natural toxicants. According to reports, even polyunsaturated fats speed up the ageing process in experimental animals and raise the risk of tumours and gallstones. They also increase the body's need for vitamin E. Many compounds that are not typically thought of as poisonous may pose a risk to some individuals. For instance, some people who ingest milk have significant digestive irritation due to an enzyme deficiency that causes a lactose intolerance (Banwart, 1989). Microbiological dangers are by far the most prevalent kind of food hazard according to data gathered by the Centres for Disease Control (CDC). The CDC figures are the most comprehensive data currently available although they are not exact because not all cases of foodborne illness are reported. Bacterial aetiologies account for more than 60 per cent of foodborne outbreaks each year. Less than 30 per cent of cases are caused by chemicals from different sources. Mycotoxins, a toxin generated by moulds are still regarded as naturally occurring toxins even though they have garnered great attention in the past ten to fifteen years in addition to food borne outbreaks. Food spoilage is the major threats have been increasing more in the present world. Diseases causing by these types of spoiled foods have been affecting many

people and causing many illnesses and leads to severe health problems. This food spoilage is caused by certain microorganisms like bacteria and fungi. The food spoilage is caused by Gram positive and Gram-negative bacteria like *Bacillus cereus*, *Staphylococcus aureus*, *Escherichiacoli*, *Pseudomonas aeruginosa* and *Salmonella typhi* etc. which will cause many illnesses to peoples who consumed the food. To avoid the spoilage of food it is necessary to preserve the food from certain food pathogens. Usage of chemical preservatives to prevent food deterioration and food poisoning has undesirable drawbacks, including risks to human health such as presence of chemical residues in food and feed chains. Because of such concerns, the necessity to find a potentially effective, safer, and natural alternative preservatives have been increased (Ashraf *et al.*, 2018). Usage of plant extract as a natural preservative does not show any toxic effects on food. These plant extracts have been used to control food poisoning diseases and preserve foodstuff. The phytochemicals found in medicinal plants are what give them their therapeutic qualities. Phytochemicals found in plants have positive health effects and are actively used in the treatment of diseases but are not necessary for the body to function on a regular basis. The most crucial resource for treating crippling disorders are these phytochemicals. Plants are coloured and have organoleptic characteristics due to phytochemicals, which can also enhance immunomodulatory reactions. Additionally, it guards against several ailments. It is recognised that several phytochemicals, including phenols, tannins, flavonoids, saponins, carbohydrates, alkaloids, phytosterols, and others, have physiological and therapeutic effects. It is generally known that plants have a variety of naturally occurring antioxidants that guard and maintain their physical and metabolic integrity as well as their ability to pass on their heredity through their seeds. Reactive oxygen species (ROS) and other oxidants are thought to be key players in the emergence of a wide range of illnesses and diseases, according to a large body

of research. The disease protection offered by medicinal plants may be influenced by their antioxidant content. Antioxidants are common in dietary supplements, nutraceuticals, and functional food additives. They help preserve food by preventing oxidation processes and promote health (Lansky and Newman, 2007). Before attacking targets in biological cells, free radicals are frequently stabilized or inactivated by antioxidants. Due to their diversity and high level of activity, as well as their extensive range in redressing imbalance, naturally occurring antioxidants have recently attracted a lot of attention from those interested in using them in culinary, cosmetic, and pharmaceutical goods. A traditional lifestyle that lowers the incidence of diabetes may include traditional plant treatments with antioxidant activity. Researchers are interested in natural antioxidants because of their capacity to scavenge free radicals. Vitamins C and E, two antioxidants, are essential for ROS defence. But rather than vitamins C, E, or β -carotene, chemicals like phenolic acids and flavonoids are responsible for the majority of plant antioxidant action. In this study, pomegranate peel extract (*Punica granatum* L.) was used as the sample material. The pomegranate peel is regarded as an agricultural waste that can be utilised in soils but can also be used as a source of potential chemicals such as antioxidants, flavonoids, phenols, tannins, and also has antibacterial and antifungal activity. Pomegranate peel has more antioxidant activity and overall phenolic content than the pulp. The pomegranate peel is reported to have several health benefits due to the presence of various tannins, flavonoids, alkaloids, and organic acids. Various bioactive compounds such as galloyldilacton, gallic acid, granatin B showed anti-inflammatory activity and tannins possessing antioxidant activities are punicalin, punicalagin, pedunculagin, gallic acid and casuarinin. In addition, various flavonoids, including catechin, epicatechin, epigallocatechin-3-gallate, flavan-3-ol, kaempferol, kaempferol-3-O-glucoside, luteolin, luteolin 7-O-glucoside, Naringin, pelargonidin, prodelphinidin,

quercetin, and rutin, have been discovered in pomegranate peel extracts. These peel extracts have antimutagenic qualities as well as advantageous cardiovascular disease preventive benefits. Outbreaks of foodborne disease can cause serious illnesses in people. Rapid and precise pathogen identification is required to quickly select the most effective antibiotic therapy to reduce the burden of such outbreaks. The purpose of the present study was to evaluate the antioxidant and antibacterial activity of the peel extract of *Punica granatum* L. and evaluated treatment against food borne illness (Badami and Channabasavaraj, 2007).

Materials and Methods

Sample Collection:

The fresh peel of *Punica granatum* were collected from the gardens and wild of Chennai, India. The collected peel was washed thoroughly first with tap water and then with distilled water and kept for shade drying. The peels were shade dried for two days and then powdered (Tanaka *et al.*, 1990).

Preparation of Peel Extract:

The dried peels were powdered using a grinder and extracted with ethanol using Soxhlet apparatus. It was carried out to obtain extracts for the phytochemical screening. Around 20 g of the powder was packed in a thimble of filter paper prepared manually. The thimble was then inserted into the Soxhlet apparatus and extraction was done by using 200 ml Ethanol as a solvent. The temperature was maintained at 60-70 °C and extraction was continued for 4-5 h. Then the ethanolic extract was collected and powder from the thimble was discarded. Then the extract was used for the phytochemical screening (Matsukawa *et al.*, 1993).

Qualitative Phytochemical Screening:

Semi-quantitative phytochemical screening of the extract was performed. The extract was analysed for the presence of alkaloids, carbohydrates, flavonoids, phenolic compounds, saponins,

phytosterols, protein, cardiac glycosides, tannins, terpenoids and the results were tabulated (Amakura *et al.*, 2000)

Phytochemical Analysis:

Phytochemical screening was done for analysing secondary metabolites that are responsible for curing ailments (Gil *et al.*, 2000).

Test for Alkaloids:

Picric acid test: 3-4 drops of 2% picric acid solution was added to 1ml of the plant extract. Formation of orange colour showed the presence of alkaloids.

Test for Carbohydrates:

Molisch's test: 2 ml of ethanolic extract, add 1ml of Alpha-naphthol solution, add concentrated H₂SO₄ acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of carbohydrates.

Test for Flavonoids: Added 2 ml of ethanolic extract, 2 ml of 2% NaOH solution and few drops of dil. HCl or few drops of 10% ammonium hydroxide solution. Formation of yellow fluorescence confirmed the presence of flavonoids.

Test for Phenols: 2 ml of ethanolic extract and few drops of 5% ferric chloride solution was mixed. Formation of dark green bluish black colour confirmed the presence of phenols

Test for Saponins:

Foam test: 1 ml of ethanolic extract, 2 ml of water is added and shaken vigorously. Presence of saponins indicated by the formation of foams.

Test for Phytosterols:

Salkowski's test: 1 ml of extract, and few drops of conc.H₂SO₄ was shaken well and allowed to stand. Formation of red colour in lower layers revealed the presence of phytosterols.

Test for Protein:

Millon's test: 2 ml of extract was mixed with few drops of Millon's reagent and formation of white

precipitate indicated the presence of proteins.

Test for Cardiac Glycosides:

Bromine water test: To 2 ml of extract, added few ml of bromine water. Formation of yellow colour precipitate was observed for cardiac glycosides.

Test for Tannins:

Braymer's test: To 2 ml of extract, added 3 ml of distilled water and 3 drops of 10% ferric chloride solution. Formation of Blue green colour was appeared indicating the presence of tannins.

Test for Terpenoids:

Salkowski's test: To 1 ml of extract, few drops of conc. H₂SO₄ was added. Golden yellow layer was formed at the bottom which indicated the presence of terpenoids.

Antioxidant Assays:

DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging assay:

DPPH radical scavenging assay is a simple method for measuring the antioxidant activity (Cook and Samman, 1996). Different concentrations of the extracts (10, 20, 30, 40, 50 µg/ml) were added and the sample was added in a range of 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml and 0.5 ml. Then it was made up to 3 ml using ethanol. Then, 1 ml of freshly prepared ethanolic solution of DPPH was added. Twenty minutes later, the absorbance was measured at 517 nm. 2 ml of ascorbic acid was used as standard control. Lower Absorbance of the reaction mixture indicated higher free radical scavenging activity. IC₅₀ values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals. IC₅₀ value is calculated from % inhibition which was calculated from the following formula:

$$\% \text{ of DPPH Inhibition} = ((A_0 - A_s) / A_0) \times 100$$

Where A₀ is the absorption of control and A_s is the absorption of the tested extract solution.

ABTS (2, 2'- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay:

The antioxidant activity of the plant extracts in

the research against ABTS was evaluated by the method described by Bernal-Mercado *et al.* (2018). The ABTS stock solution was prepared by dissolving 0.037 g of potassium persulfate in 20 ml of distilled water, which is labelled as solution A. And 0.027 g of ABTS was weighed and dissolved in 25 ml of distilled water, which is labelled as solution B. Combine 20 ml each of solution A and B, mix well, store in a dark room for 16 h. The concentration of ABTS is 7 mM and 2.5 mM of potassium persulfate. Different concentrations of the extract (10, 20, 30, 40, 50 µg/ml) were added to 3.5 ml of ABTS solution. The absorbance was read at 734 nm using UV-VIS spectrophotometer and the % inhibition was calculated using the following formula:

$$\% \text{ of ABTS Inhibition} = ((A_0 - A_s) / A_0) \times 100$$

Where A₀ is the absorption of control and A_s is the absorption of the tested extract solution.

Superoxide Anion Scavenging Assay:

Superoxide anion scavenging assay Scavenging of the superoxide (O₂ •-) anion radical was determined by a modified method (Vázquez-Armenta *et al.*, 2017). Superoxide radical anion was developed in PMS-NADH system by oxidation of NADH. Different concentrations of the extract (10, 20, 30, 40, 50 µg/ml) were added to the reaction mixture containing 1 ml of NBT, 1 ml of NADH and 1 ml of PMS. The reaction mixture was incubated at 30 °C for 15 min and the absorbance was measured at 560 nm using UV-VIS spectrophotometer. The percentage inhibition of Superoxide anion generation was calculated using the following formula:

$$\% \text{ of superoxide anion scavenging inhibition} = (A_0 - A_1) \times 100$$

Where A₀ was the absorbance of the control, and A₁ was the absorbance of ethanol extract and standards.

Antibacterial Activity:

The antibacterial activity of the test sample was carried out by Agar well-diffusion method. In this method, the microorganisms used were Gram

negative bacteria *S. typhi*, *E. Coli* and *P. aeruginosa* and Gram positive bacteria *E. faecalis*.

Agar Well Diffusion Method:

Preparation of inoculums:

Stock cultures were maintained at 4°C on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated for 24 h at 37°C. The assay was performed by agar well diffusion method. The Muller Hinton agar medium was weighed as 3.8 g and dissolved in 100 ml of distilled water and add 1 g of agar. Then the medium is kept for sterilization. After sterilization the media was poured into sterile Petri plates and were allowed to solidify for 1hr. After the medium was solidified, the inoculums was spread on the solid plates with sterile swab moistened with the bacterial suspension. Wells were made using cork borer. Samples and Positive control Streptomycin (1mg/ml - 20 µl) was loaded in respective wells. These plates were incubated for 24 h at 37°C. Then the microbial growth was determined by measuring the diameter of zone of inhibition and expressed in millimetres as its antibacterial activity (Fratianni *et al.*, 2011).

Fourier Transform Infrared Spectroscopy (FTIR) analysis:

Fourier Transform Infrared Spectroscopy was used to the unknown components; determine the quality or consistency of a sample and the number of components in a mixture. Based on the peak values in the IR radiation area, the FTIR spectrum was utilised to determine the functional groups of the active components included in the extract. Based on the ratio of its peak, the functional groups of the constituents were separated after the extract was run through the FTIR. The existence of alcohol, phenol, alkanes, aldehyde, aromatic compound, secondary alcohol, aromatic amines, and halogen compound was confirmed by the FTIR results of the study. The sensitivity and accuracy of FT-IR detectors, along

with a wide variety of software algorithms, have dramatically increased the practical use of infrared for quantitative analysis. Quantitative methods can be easily developed and calibrated and can be incorporated into simple procedures for routine analysis. The infrared spectra of combustion gases were analysed. There are various factors that affect the ability of a FTIR to detect certain species of gases. In each volume of gas, there are fewer absorbing molecules than in a more condensed phase sample (i.e. liquids, solids), so a greater sample thickness is required to record the infrared spectrum. Long path gas cells provide this ability, and typically range anywhere from 10 cm to several hundred meters in length. Low pressures also affect the ability of the FTIR to detect absorbing molecules, since at lower pressures there are fewer molecules present, and the chances for interaction are low. Therefore, analysis must be performed near atmospheric pressure. Attempts were made in the present experiment to try to analyse pure gas components at approximately 20 torr, with concentrations as low as 50 ppm. In these trials the signal intensity was so low that the peaks produced by the samples were indistinguishable from the noise produced in the spectrum (Katalinice *et al.*, 2006).

Gas Chromatography–Mass Spectrometry (GC-MS) analysis:

Gas chromatography-mass spectrometry (GC-MS) is a tool that is used to separate, quantify, and analyze organic compounds that are naturally volatile. Helium, an inert gas was utilized as the carrier gas, with a flow rate of 1.5 ml/min, a split ratio of 10:1, a sample volume of 1 µl, and the fused capillary silica column HP-5 (30 m x 0.25 mm x 0.25 µm). Injector: 260°C; detector: 240 °C, 10 min⁻¹. The length of the GC as a whole is 35 min. At 70 eV, the MS was recorded. The settings for the MS scan included a mass range of m/z 40-1000, a detector voltage of 1.0 kV, a scan interval of 0.5 s, a scan speed of 2000 amu s⁻¹. Using the NIST08, WILEY8, and FAME Library databases, chemicals were identified. Each unknown compounds' mass

Table 1: Phytochemical Analysis

S. No.	Phytochemicals	Inference
1.	Alkaloids	-
2.	Carbohydrates	+
3.	Flavanoids	+
4.	Phenolic compound	+
5.	Saponins	+
6.	Phytosterols	+
7.	Protein	+
8.	Cardiac glycosides	+
9.	Tannins	+
10.	Terpenoids	+

(+) Presence of the compound; (-) Absence of the compound

spectra were compared to known compounds kept in the software database Libraries. The components of the test materials names, molecular weights, and structures were determined (Stratil *et al.*, 2006).

Results and Discussion

Phytochemical Analysis:

Qualitative estimation of the phytochemicals conducted on peel extract of *Punica granatum* revealed the presence of carbohydrates, flavonoids, phenolic compound, saponins, phytosterols, protein, cardiac glycosides, tannins and terpenoids (Table 1). Based on record, the Phytochemical screening of extracts show the presence of certain important components such as phenols, glycosides, flavonoids, terpenoids, carbohydrates, proteins and amino acids. Phytochemical constituents afford imperative pharmaceutical properties for human health. These compounds can be used as drugs or as dietary supplements to heal or to prevent various diseases (Hyland *et al.*, 1983).

Antioxidant Activity:

DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging assay:

In the present study, DPPH is a free radical molecule which has affinity to accept electron and

becomes stable. At maximum absorbance at 517 nm, the DPPH radical can be quantified. The acceptance of electrons caused by the antioxidants is indicated by a decrease in absorbance. Based on the creation of the non-radical form DPPH-H by the reaction, this approach involves reducing an ethanolic DPPH solution in the presence of a hydrogen-donating antioxidant. The extract's IC₅₀ for antioxidant activity is 65.7 µg/ml. The results indicate that extracts have a high potential for scavenging free radicals (Fig. 1), which may be due to their capacity for hydrogen donation. Evaluation of free radical scavenging activity revealed that the extract showed highest radical scavenging potential with IC₅₀ value of 55.4 µg/ml. It is comparable to standard antioxidant ascorbic acid as recorded. An assay on DPPH activity of extracts of pomegranate peel was done.

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay:

In the present study, the total antioxidant capacity (TAC) of natural products is estimated using the stable free radical 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt radical cation (ABTS). The powerful ABTS radical anion scavenging assay of peel of *Punica granatum* uses an approach that generates a blue/green ABTS chromophore by

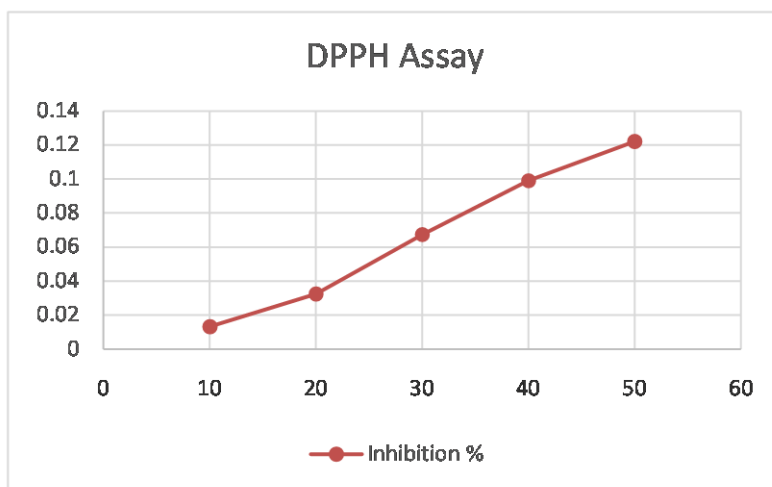


Fig.1: DPPH assay.

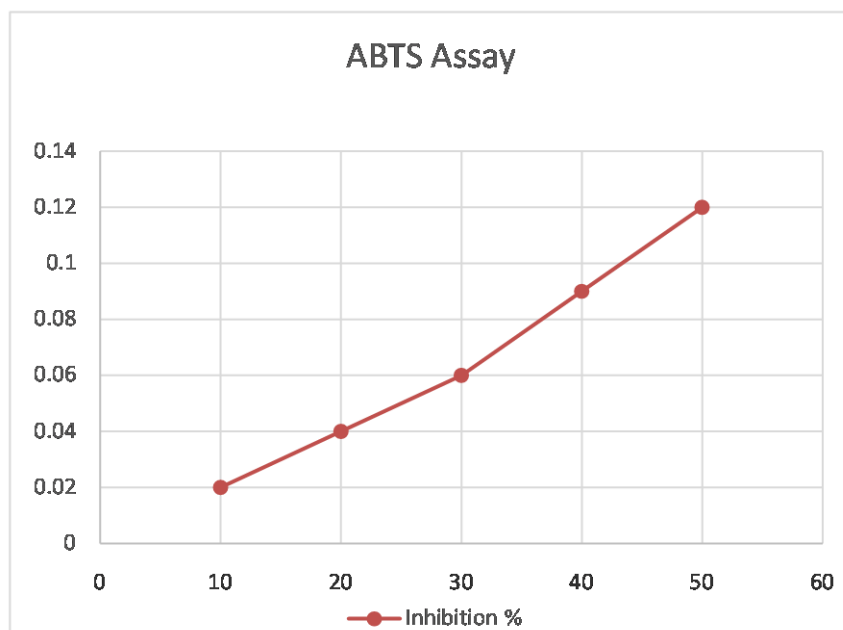


Fig. 2: ABTS assay.

the interaction of ABTS with potassium per sulphate. Its reduction is produced spectrophotometrically at 745 nm in the presence of antioxidants that donate hydrogen. The extract's antioxidant activity (Fig. 2) has an IC_{50} of 0.19 $\mu\text{g/ml}$. Pomegranate peel displayed an IC_{50} value of 116.40 $\mu\text{g/ml}$ in the ABTS assay. 5.96 $\mu\text{g/ml}$ of standard quercetin's synthetic radicals were determined to be the IC_{50} value for free radical

scavenging assays and shown in Figure 2. In ABTS assay, *Punica granatum* L (1) exhibited IC_{50} value of 116.40 $\mu\text{g/ml}$ and *Melia dubia*leaves² showed IC_{50} values of 55.20 $\mu\text{g/ml}$. IC_{50} Free radical scavenging assays using synthetic radicals of standard quercetin was found to be 5.96 $\mu\text{g/ml}$.

Superoxide Anion Scavenging Assay:

In the present study, Superoxide radicals were

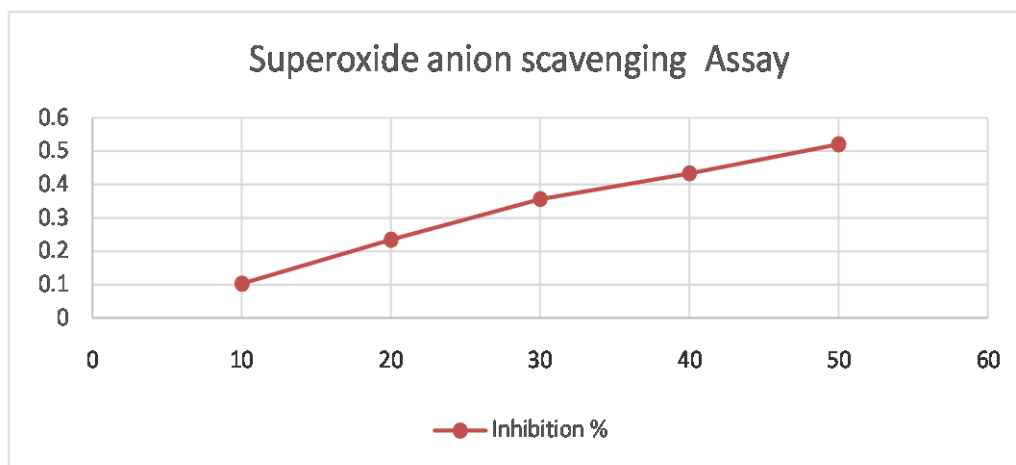


Fig. 3: Superoxide Anion assay.

Table 2: Antibacterial Activity

Microorganisms	Zone of Inhibition in (diameter) mm				
	1	2	3	4	5
<i>Salmonella typhi</i>	-	-	-	12	20
<i>Escherichia coli</i>	24	26	28	31	19
<i>Pseudomonas aeruginosa</i>	23	24	26	28	26
<i>Enterococcus faecalis</i>	25	27	29	31	18

produced in the PMS-NADH system by the oxidation of NADH and measured through the reduction of NBT. As a precursor to the most reactive oxygen species (ROS), it is well recognised to be extremely toxic to cellular components and to contribute to tissue damage and a number of illnesses. *Punica granatum's* ethanolic extract demonstrated a significant level of superoxide anion radical scavenging activity. The extract's IC₅₀ for antioxidant activity (Fig. 3) is 66.6 μg/ml. In the ethanolic extract, superoxide radical scavenging with an IC₅₀ of 100 μg/ml was seen. During typical physiological function, superoxide radicals are produced, primarily in a mitochondria. It is evident that superoxide anion is a very weak oxidant, undergoes oxidation to give hydroxyl radical and singlet oxygen, which are harmful, leading to oxidative stress (Fig. 3). Superoxide radicals are generated during the normal physiological process, mainly in mitochondria. It is evident that superoxide anion is a very weak oxidant, undergoes oxidation to

give hydroxyl radical and singlet oxygen, which are harmful, leading to oxidative stress (Ramya *et al.*, 2022).

Anti Bacterial Activity:

Agar Well Difussion Method:

In the present study, Test samples (20 μl, 40 μl, 60 μl, 80 μl) were loaded in respective wells. Standard drug Streptomycin (20 μg) was used as a positive reference standard to determine the sensitivity of microbial species tested (Table 2). After incubation period at 37°C for 24 h, the diameter of the clear zone around the well for Gram negative bacteria *S. typhi*, *E. coli* and *P. aeruginosa* was measured as 20 mm, 19 mm and 26 mm, respectively. For Gram positive bacteria, *Enterococcus faecalis* it was measured as 31 mm. Diameter of inhibition zone (DIZ) against *S. aureus* and *E. coli* was found to be comparatively less than the other bacterial strains. The antimicrobial properties of a number of tropical plants from Puerto Rico have been evaluated

Table 3: FTIR analysis

BOND TYPE	FREQUENCY RANGE	FUNCTIONAL GROUP
-OH	3307.41	Hydroxyl group
-CH ₃	2920.85	Methyl group
-CH ₃	2851.36	Methyl group
C=O	1710.66	Carbonyl compound
C=O	1602.43	Carbonyl compound
-COOH	1512.97	Phenol ring
C ₆ H ₆	1444.1	Aromatic ring
C ₆ H ₆	11324.81	Aromatic ring
C ₆ H ₆	1180.94	aromatic ring
C-O-C	1028.15	alkene group
C-O-C	921.16	alkene group
C-O-C	871.47	alkene group
C-H	756.9	methine group
C-S	635.54	sulphur compound
C-Br	589.5	halogen compound
C-Br	577.96	halogen compound
C-Br	565.8	halogen compound

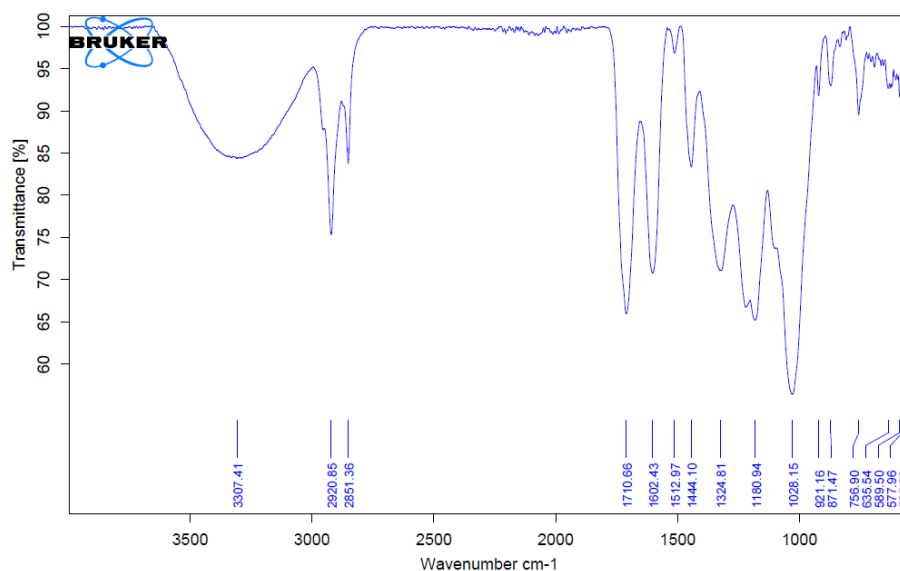


Fig. 4: FTIR Analysis of peaks in graph.

using the disc diffusion method against *E. coli* and *S. aureus*. They demonstrated that pomegranate extract produced inhibition zone sizes of 11 and 20 mm, for *E. coli* and *S. aureus*, respectively (Sudan and Rupasinghe, 2015).

Fourier Transform Infrared Spectroscopy (FTIR):

Fourier Transform Infrared Spectroscopy (FTIR) was also used as it was known to be a substantial analytical method as it can detect a few functional groups in the compounds. It was discovered that there were significant impacts to the chemical bond in a liquid once it interacted with the

Table 4: GC-MS Analysis

S. No	NAME OF THE COMPOUND	Molecular weight	PEAKS%	molecular formula
1	Dicholoacetamide	164	11.755	C ₂ H ₃ Cl ₂ NO
2	Furfural	134	15.93	C ₅ H ₄ O ₂
3	hydroxymethylfurfural	129	17.225	C ₆ H ₆ O ₃
4	2,6-Di- ter-butylphenol	114	18.329	C ₁₄ H ₂₂ O
5	3,4 -bis(trimethylsilyloxymethyl)pyridin	268	19.251	C ₁₄ H ₂₈ N ₂ O ₄ S ₁₂
6	Ethyl Oleate	156	20.132	C ₂₀ H ₃₈ O ₂
7	n- Nitrosoazacyclononane	242	20.32	C ₉ H ₁₇ NO
8	1-Methylethyl acetate	140	20.98	C ₅ H ₁₀ O ₂
9	Cis-Dimethyl Morphine	234	21.859	C ₁₉ H ₂₃ NO ₃
10	Cis-Oleic Acid	294	22.849	C ₁₈ H ₃₄ O ₂
11	Norolean-12-ene	158	23.996	C ₃₀ H ₄₈
12	alpha- Tocophenol -beta-D-mannoside	208	24.21	C ₃₅ H ₅₃ NO ₆
13	Ethyl palmitate	154	25.355	C ₂₂ H ₄₄ O ₂
14	Lupenone	168	26.942	C ₃₀ H ₅₀ O
15	gamma- Sitosterol	128	28.52	C ₂₉ H ₅₀ O

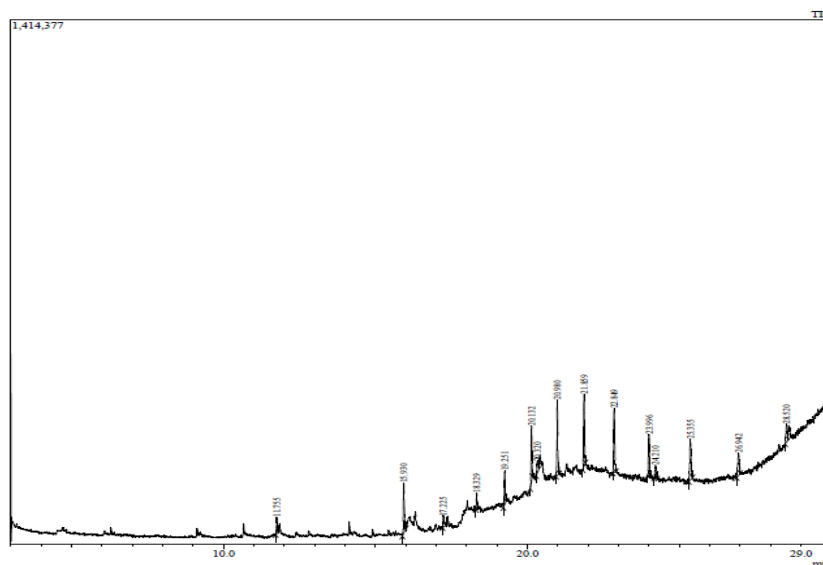


Fig. 5: GC-MS Analysis.

infrared light. The chemical bond will elongate, contract, and absorb the radiation when other molecules were present at a particular wavelength. Due to concerns of sample contamination, the gas cell was evacuated of the previously collected combustion products. The needle valve at the exit of the gas cell was opened and the pressure was reduced down to

approximately 5 torr. Then, the inlet valve is opened to enable a purge of UHP N₂, which was allowed to flow for 15 min to purge the gas cell. Then, the procedure for sample collection was repeated for the next location in the flame. The time scale involved during the sample flow through the probe, sample lines, and gas cell is long compared with the amount of time it takes

for radicals to react with the surfaces or other species. Therefore, with this method, only stable species can be identified. It was discovered that there were significant impacts to the chemical bond in a liquid once it interacted with the infrared light. The chemical bond will elongate, contract, and absorb the radiation when other molecules were present at a particular wavelength. Therefore, the main functional groups in the compound were recorded are hydroxyl compound, methyl group, phenol ring, aromatic ring, halogens (Taylor and Richardson, 1980). Based on record FTIR analysis (Table 3, Fig. 4) of extract of *Punica granatum* peel proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxylic acids, Esters, Nitro Compounds, Alkanes, H-bonded H-X group.

GC-MS Analysis:

GC – MS investigation of ethanolic extract of peel of *Punica granatum* exhibited compounds revealed that Dicholoacetamide, Furfural, Hydroxymethylfurfural ,2,6-Di- ter-butylphenol, 3,4 -bis(trimethylsilyloxymethyl)pyridine, Ethyl Oleate, n- Nitrosoazacyclononane, 1-Methylethyl acetate, Cis-Dimethyl Morphine, Cis-Oleic Acid, Norolean-12-ene, alpha- Tocophenol -beta-D-mannoside, Ethyl palmitate, Lupenone, gamma-Sitosterol (Table 4, Fig. 5). GC-MS chromatogram of the ethanolic extract of *P. granatum* peel showed 26 peaks indicating the presence of twenty six phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST08, WILEY8 and FAME libraries the twenty-six phytoconstituents were characterized and identified. The major phytochemical constituent's mass spectra are Glycerin, Hydroxymethylfurfurole, Guanosine and Pyrogallol. The *V. vinifer* seed extract showed sixteen peaks in GC-MS chromatogram indicating the presence of sixteen phytochemical constituents (Meléndez and Capriles, 2006).

Conclusion

Phytochemical analysis of ethanolic peel extract

of *Punica granatum* revealed that the presence of significant secondary metabolites, contributes to antibacterial and antioxidant activities. It contains high antimicrobial activity, with more resources and time, further investigation of chemical constituents of *Punica granatum* with food borne pathogens and its effectiveness can be revealed. Therefore, more of the research and studies are required.

References

- Amakura Y, Umino Y, Tsuji S and Tonogai Y. (2000) Influence of jam processing on the radical scavenging activity and phenolic content in berries. J Agricult Food Chem. 48(12): 6292-6297.
- Ashraf A Mostafa, Abdulaziz A Al-Askar, Khalid S Almaary, Turki M Dawoud, Essam N Sholkamy and Marwah M Bakri (2018) Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases, Saudi J Biol Sci. 25(2): 361-366.
- Badami S and Channabasavaraj KP. (2007) In vitro antioxidant activity of thirteen medicinal plants of India's Western Ghats. Pharmaceut Biol45(5): 392-396.
- Banwart G.J. (1989) Control of microorganisms by retarding growth. In: Basic Food Microbiol., (ed.) Banwart G.J., Springer; New York, NY, pp. 545-650.
- Bernal-Mercado AT, Acevedo-Hernandez C, Silva-Espinoza AB, Cruz-Valenzuela MR, Gonzalez-Aguilar, AG, Nazzaro F, Siddiqui MW, Ayala-Zavala JF, Fratianni F and Vazquez-Armenta FJ. (2018) Antioxidant and antimicrobial capacity of phenolic compounds of mango (*Mangifera indica* L.) seed depending upon the extraction process. J Med Plants By-product 7(2): 209-219.
- Chakwuma EC, Mike OS and Roseline TF. (2015) Traditional medicine and the future of medicinal plants in Nigeria. J Med Plants Stud. 3: 23-29.
- Cook NC and Samman S. (1996) Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutritional Biochem. 7(2): 66-76.
- Devi DR and Battu GR. (2019) Qualitative phytochemical screening and FTIR spectroscopic analysis of *Grewia tilifolia* (Vahl) leaf extracts. Int J Curr Pharm Res. 11(4): 100-107.
- Fratianni F, Coppola R and Nazzaro F. (2011) Phenolic composition and antimicrobial and antiquorum sensing activity of an ethanolic extract of peels from the apple cultivar Annurca. J Med Food 14(9): pp.957-963.

- Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM and Kader AA. (2000) Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J Agricult Food Chem.* 48(10): 4581-4589.
- Hebbar DR and Nalini MS. (2020) GC-MS characterization of antioxidative compounds from the stem bark and flower extracts of *Schefflera* species, from Western Ghats. *Der Pharm Lett.* 12: 51-60.
- Hyland K, Voisin E, Banoun H and Auclair C. (1983) Superoxide dismutase assay using alkaline dimethylsulfoxide as superoxide anion-generating system. *Analytical Biochem.* 135(2): 280-287.
- Katalinic V, Milos M, Kulisic T and Jukic M. (2006) Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem.* 94(4): 550-557.
- Khan JA and Hane S. (2011) Antibacterial properties of *Punica granatum* peels. *Int J Appl Biol Pharmaceut Technol.* 2(3):23-27.
- Lansky EP and Newman RA. (2007) *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *J Ethnopharmacol.* 109(2): 177-206.
- Matsukawa Y, Marui N, Sakai T, Satomi Y, Yoshida M, Matsumoto K, Nishino H and Aoike A. (1993) Genistein arrests cell cycle progression at G2-M. *Cancer Res.* 53(6): 1328-1331.
- Meléndez PA and Capriles VA. (2006) Antibacterial properties of tropical plants from Puerto Rico. *Phytomed.* 13(4): 272-276.
- Ramya S, Vijayakumar S, Vidhya E, Bukhari NA, Hatamleh AA, Nilavukkarasi M and Pham TH. (2022) TiO₂ nanoparticles derived from egg shell waste: eco synthesis, characterization, biological and photocatalytic applications. *Environ Res.* 214: 113829.
- Stratil P, Klejdus B and Kubáň V. (2006) Determination of total content of phenolic compounds and their antioxidant activity in vegetables evaluation of spectrophotometric methods. *J Agricult Food Chem.* 54(3): 607-616.
- Sudan S and Rupasinghe HV. (2015) Antiproliferative activity of long chain acylated esters of quercetin-3-O-glucoside in hepatocellular carcinoma HepG2 cells. *Exp Biol Med.* 240(11): 1452-1464.
- Tanaka K, Miura N, Satokata I, Miyamoto I, Yoshida MC, Satoh Y, Kondo S, Yasui A, Okayama H and Okada Y. (1990) Analysis of a human DNA excision repair gene involved in group A xeroderma pigmentosum and containing a zinc-finger domain. *Nature* 348(6296): 73-76.
- Taylor MJ and Richardson T. (1980) Antioxidant activity of cysteine and protein sulfhydryls in a linoleate emulsion oxidized by haemoglobin. *J Food Sci.* 45(5): 1223-1227.
- Vázquez-Armenta FJ, Silva-Espinoza BA, Cruz-Valenzuela MR, González-Aguilar GA, Nazzaro F, Fratianni F and Ayala-Zavala JF. (2017) Antibacterial and antioxidant properties of grape stem extract applied as disinfectant in fresh leafy vegetables. *J Food Sci Technol.* 54: 3192-3200.