Fourier-Transform Infrared Spectroscopy, Antioxidant, Phytochemical and Antibacterial Screening of N-Hexane Extracts of *Punica granatum*, A Medicinal Plant

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ABSTRACT.
Pomegranate is a fruit bearing plant belonging to *Lythraceae*; in botanic language it is called *Punica granatum.* It is been considered as a traditional medicine for the cure of many diseases. The purpose of this study was to investigate the antimicrobial activity and phytochemicals present qualitatively present in different parts of Pomegranate plant and to investigate antioxidant activity. Cold maceration process was used on seed, flower, stem and leaves of Pomegranate plant in N-hexane solvent. Antibacterial effect of pomegranate plant and their antibacterial effect on antibiotic drugs were evaluated by n-hexane extract against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Salmonella typhi* and *Klebsiella pneumoniae*. The antibacterial effects were determined by using disc and well diffusion method. The 25 mg extract of Pomegranate showed more zone of inhibition against as *Staphylococcus aureus* and *Salmonella typhi* and in 50 mg extract of AN-03 shows best zone of inhibition against *S. aureus*. Comparatively 25 mg/1 ml DMSO and 50 mg/1 ml DMSO showed equal and none results. Qualitative phytochemical screening showed the presence of phytochemicals present in n-hexane extracts. DDPH radical scavenging activity assay revealed the distinguished antioxidant activity of Pomegranate plant in n-hexane. Experimental results of *Punica granatum* extract possessed good antimicrobial

activity. *Punica granatum* plant contains secondary metabolites phenol, flavonoids, alkaloids, sterols, terpenes. The results of FT-IR analysis of the present study spectrum revealed the functional constituents such as alkanes, alkynes, amines, alcohol, alkenes, aldehyde and ketone, carboxylic acid, ketone etc. were present in the crude powder of *Punica granatum.* It has commercial interest in both research institutes and pharmaceuticals industries, also for the treatment of different diseases. Peel and leave extracts are considered as the most promising candidate for use as natural products based antioxidants for the health of human being.

Keywords: Pomegranate; Antioxidants; Antibacterial; Pathogens; FTIR

INTRODUCTION

To treat health diseases, plants have been used for thousands of years. In ancient time several herbs and spices were used in food, not only as a flavoring agent and food preservative but also as a folk medicine (Shan et al., 2007). Pomegranate is a ball shaped fruit comprising of a tough outer skin with sweet red jelly like flesh with many seeds, it is a fruit bearing plant that can grow up to 5 to 8 m in height (Jurenka, 2008). The plant has been used to treat conditions in the case of pathogens and is been considered as a folklore medicine (Mathabe et al., 2006). The fruit is found to be rich in phenolics acids, flavanols, flavones, flavonones, anthocyanidins and anthocyanin (Jurenka, 2008). In comparison with edible parts of this plant the non-edible parts i.e. leave, stem and roots, consists of apigenin, punicalin, punicalagins and luteolin (Gil et al., 2000). The appearance of pigments on the plant is because of anthocyanidins and ellagitannins present (Cui and Su, 2009).

These plants can be used in blood tonic, to heal aphthae, diarrhea and ulcers, it has majorly been in the Middle East and India, it can also be used as a remedy against diabetes (Jain et al., 2014). Peels and fruit have been proven as a rich source of polyphenolic compounds, also been known to exhibit antimicrobial activity against a number of bacteria whether they are Gram positive or Gram negative, alongside antifungal, anti-protozoal, antioxidant and anti-inflammatory properties. The use of this plant dates back to Biblical times (Longtin, 2003). The increase in antibiotic-resistant property of pathogens has led to the development of new tonic mediators that are active against these microbes. Now days, there has been extensive attention in the practice of several plant ingredients as a substitute medicine to treat most of the enteric infections and various compounds. Of plant products have been precisely used against resistant pathogens (Choi et al., 2009). Therefore the uses of indigenous medicinal plants as an alternative to antibiotics are being extensively evaluated these days and are considered to play a significant role (Ballal and Ramamurthy, 2005). However, to date, very few studies have been conducted on the antimicrobial activity of *P. granatum* peels. Since the prehistoric times, the *P. granatum* has been observed as medicinal food with several useful effects in numerous diseases (Vidal et al., 2003).

Fourier Transform-Infrared (FT-IR) has played a vital role in pharmaceutical analysis in recent years. FTIR spectroscopy is a physico-chemical analytical technique that does not determine concentrations of individual metabolites but provides a snapshot of the metabolic composition of a plant material at a given time. The FTIR method measures predominantly the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic “fingerprint” of the sample (Ahamed et al., 2020; Pednekar and Raman, 2013). The FT-IR analysis has proven to be a valuable tool for characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of flower extract (Sasidharan et al., 2011; Muhammad et al., 2020). It is a rapid, non-destructive technique with minimum sample preparation necessary (Baker et al., 2014). It allows the qualitative determination of organic compounds as the appearance of the bands in the infrared spectrum at a specific frequency, which is further influenced by the surrounding functional groups (Nivetha and Prasanna, 2016). Therefore, the present study was aimed to assess the FTIR, antibacterial activity, phytochemical and antioxidant potential of the n-hexane extracts of *P. granatum* plant.

MATERIALS AND METHODS

Collection and identification of plant materials

The plant required for the work were collected from Nishat nursery near Askari X. and were identified by Dr. Zahoor Ahmad Sajid, Assistant Professor, Department of Botany, University of Punjab, Lahore Pakistan. The plant specimen was deposited in the specially maintained garden, The University of Lahore.

Extract preparation

After the collection of the plant parts they were cleaned properly. The cleaning process involved the following steps; cleaning, washing, peeling or stripping the leaves of the stems. The need for drying was to remove the water content completely from the plants, so they are easier to work with and our basic requirement also included dry samples for better dipping. The plants were dried as soon as they were collected to avoid any spoilage. Drying could be done either by using a natural process or artificially. The natural process for drying was adapted and the plant parts were kept in the shady area for at least a week. The samples were then brought to the laboratory and crushed into fine powder by using an electrical grinder. After grinding for extraction preparation all the parts of the Pomegranate plant were dipped in n-hexane depending on the amount required and kept for a week, but the amount of samples was kept the same. All the samples were taken in grams and solvent in litres. After keeping the samples in the solvents for a week, samples were further filtered using a Whatman filter paper into a new container. The filtrates were evaporated under reduced pressure by vacuum rotary evaporator at 35°C to obtain crude extracts and stored at 4°C for further use. For the preparation of concentrations, extracts were weighed using a weighing balance and the weight for all the extract was kept the same. 25 mg of the extracts was added in 1 ml DMSO and mixed properly; no clumps of extract were seen, to have a clear solution of DMSO.

Bacteria culture

Total six bacteria were used in this study. Two Gram positive *Staphylococcus aureus* (ATCC 29213) and *Bacillus cereus* (ATCC 11778), and four Gram negative *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Salmonella enterica* (ATCC 14028), and *Klebsiella pneumoniae* (ATCC 10031) were obtained from microbiology department of University of Lahore.

Preparation of Mcfarland standards

In a conical flask add 100 mL of distilled water to dissolve 1.175 g of $BaCl_2$, now with the help of a glass pipette take 0.05 mL, mix it with 1% H_2SO_4, present in another test tube. This will create a turbid solution in the tube which we used further for turbidity analysis. This is a procedure for preparing 0.5 McFarland standards.

Procedure for preparing the inoculums

For proceeding further to apply the following techniques we had to prepare the strains for further analysis. Six different strains of bacteria were purified on Mueller Hinton agar and kept overnight to check any sort of contamination. With a sterile loop, loopful culture was taken and swabbed on the surface of the medium and transferred to the test tubes containing 5 ml of normal saline, a loopful culture was taken and added to each test tube, shaken well and then checked turbidity by matching it with the 0.5 McFarland Standard.

Antibacterial bioassays

For the antibacterial analysis two techniques such as disc and well diffusion methods were applied.

Disc diffusion method

20 ml of Muller Hinton agar was poured in petri plates and left to solidify. 100 µl of bacterial suspension was swabbed on the entire surface of the media in each petri plates and labelled accordingly. Filter paper discs were

then placed on swabbed surface of the media by using a sterilized forceps, then 10 µl and 20 µl of the extract was poured on each of the disc. The plates were then incubated for 24 hours at 37°C and then zone of inhibition around each disc was measured. Ciprofloxacin was used as a positive control while DMSO used as a negative control (Jebashree et al., 2011).

Well diffusion method

20 ml of Muller Hinton agar was poured in petri plates and left to solidify. 100 µl of bacterial suspension was swabbed on the entire surface of the media in each petri plates and labelled accordingly. For creating wells, a sterile cork borer of 6mm size was used, then with the help of 100 µl pipette, 10 µl and 20 µl of the extract was poured on each of the disc. The plates were then incubated for 24 hours at 37°C and then zone of inhibition around each disc was measured. Ciprofloxacin was used as a positive control while DMSO used as a negative control (Mikayel et al., 2017).

Phytochemical analysis

There are following tests which were used for the qualitative analysis of *Punica granatum* extracts (Nagy, 2014).

Detection of carbohydrates (Molisch test): A sensitive chemical test for carbohydrates, it works by the dehydration of the carbohydrate by any acid to produce an aldehyde, which condenses with two molecules of phenols (alpha-naphthol). α-naphthol is called the molisch reagent. To a solution few drops of H_2SO_4 is added and a violet ring forms which ensures the presence of carbohydrates.

Detection of proteins and amino acids (Ninhydrin test): Ninhydrin itself is a chemical which is been used to detect the ammonia or different amines group. All amino acids that have a free amino group will give positive result purple color, while not free amino group-proline- will give a yellow color. To the extract, ninhydrin reagent is added and boiled for few minutes, blue-violet color is added which shows the presence of amino acids.

Detection of alkaloids (Wagner's reagent): It is a mixture of iodine and potassium iodide used for the detection of alkaloids, mixture is called Wagner's solution. It gives a reddish brown precipitate which confirms the presence of alkaloids.

Detection of flavonoids (Alkaline reagent test): Extract sample are treated with a few drops of NaOH solution. Intense yellow color is formed which becomes colorless upon the addition of dilute acid is added, this indicates the presence of flavonoids.

Detection of Tannins (Braymer's test): The water extract of the crude dry powder of the plant is treated with 10% alcoholic $FeCl_3$, formation of black-blue or green color indicates tannins.

Detection of steroids and triterpenoids (Salkowski test): The test which is not specific for cholesterol but may be used for other sterols. It yields a bluish red to purple color when cholesterol is treated with chloroform and concentrated sulfuric acid. To the mixture of extract sample and chloroform equal volume of H_2SO_4 is added. Different shades of red color in the chloroform layer indicates the presence of sterols and if the color is brownish-red it indicates triterpenoids.

Detection of diterpenes (Copper acetate test): Extracts selected for this test are first dissolved in water for few minutes and are the treated with fewer amount of copper acetate solution, appearance of emerald green indicates the presence of diterpenes.

Detection of saponins (Froth's test): The foam formed on the water surface indicates the presence of saponin. 10 mL distilled water is the reagent used in the said test. Froth produced is the indication of presence of saponins and if the froth is larger than 2 cm and is stable above the liquid layer than the test is said to be positive for saponins.

Detection of glycoside (Keller-Kiliani's test): A portion of dry extract is treated with 1mL of $FeCl_3$ reagent i.e.1 volume of 5% $FeCl_3$ and 99 volume of glacial acetic acid, to the solution little amount of H_2SO_4 was added which resulted in greenish blue color immediately, this indicated the presence of deoxy sugar of glycosides.

Detection of coumarins (Opened loop and closed loop response test): In the test tube, 2 drops of 1% sodium hydroxide solution was added and heated for 3 minutes to obtain a clear solution. 4 drops of 2% hydrochloric acid is added to the solution. If the solution becomes cloudy it indicates the presence of coumarins and lactones.

Antioxidant activity

DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) is a free radical, which is stable at 25°C. It gives a colorless ethanol solution when reduces due an antioxidant compound. For antioxidant analysis the standard DPPH assay was applied (Nithianantham et al., 2011). Numbers of different components are required for carrying out this assay, and along with all the components maintaining the concentrations of all is a must. To each of extract from 1-5 mg concentration equal amount of DPPH i.e., (0.004%) was added, for obtaining a clear solution the mixture was then vortexed and then was incubated for 30 minutes in relatively darker area, since DPPH is light sensitive. Their wavelengths were measured using spectrophotometer at 517 nm. Blank used was 80% volume/volume, DPPH value was measured using the following formula:

$$\text{DPPH scavenging effect (\%)} = \{Ao\text{-}A \div Ao\} \times 100$$

FTIR analysis

FTIR analysis was performed to detect the characteristic peaks and their functional groups using Perkin Elmer Spectrophotometer system at the range of 400 to 4000/cm. Peak values for FTIR was recorded (Suja and Yuvaranjani, 2018).

RESULTS

In the present study, the FTIR, phytochemical analysis, antioxidant and antibacterial activity of different extracts of pomegranate (*Punica granatum*) were evaluated. Antibacterial screening was done by disc and well diffusion method. Two concentrations such as 25 mg/ml and 50 mg/ml of flower, leave, stem and peel of n-hexane extracts of *Punica granatum* were used to observe their inhibitory effect against Gram positive and Gram negative bacterial isolates. Extracts were prepared in Dimethyl Sulfoxide (DMSO) solvent and also used as negative control. Antibacterial activity was also observed by using different doses such as 10 µl and 20 µl on each disc and well as well as different concentrations. Different codes such as PG01 (*Punica granatum* flower), PG02 (*Punica granatum* leaves), PG03 (*Punica granatum* stem) and PG04 (*Punica granatum* peel) were used. Results showed that zones were increased as concentrations and doses of plant extracts were increased. Zones of antibacterial activity were given in Tables 1-6.

Bacterial strains (10µl)	**Extracts (25 mg/1 mL DMSO with 10 µl extract)**					
	Positive control (mm)	**Negative control (mm)**	**PG 01 (mm)**	**PG 02 (mm)**	**PG 03 (mm)**	**PG 04 (mm)**

S. aureus	29	0	0	0	19	9
S. typhi	23	0	0	17	15	18
B. cereus	20	0	0	13	0	10
E. coli	27	0	0	12	10	8
P. aeruginosa	0	0	17	12	14	18
K. pneumoniae	24	0	18	10	0	19

Table 1. Antibacterial screening of *Punica granatum* crude extracts by Disc Diffusion Method (25 mg/1 mL DMSO with 10 µl extract).

Bacterial strains (10 µl)	**Extract (25 mg/1 ml DMSO with 20 µl)**					
	Positive control (mm)	**Negative control (mm)**	**PG 01 (mm)**	**PG 02 (mm)**	**PG 03 (mm)**	**PG 04 (mm)**
S. aureus	29	0	15	10	13	14
S. typhi	23	0	20	15	10	13
B. cereus	20	0	11	21	15	10
E. coli	27	0	11	18	15	14
P. aeruginosa	0	0	20	13	9	13
K. pneumoniae	24	0	9	11	9	10

Table 2. Antibacterial screening of *Punica granatum* crude extracts by Disc Diffusion Method (25 mg/1 ml DMSO with 20 µl).

Bacterial strains (10 µl)	**Extracts (50 mg/1 ml DMSO with 10 µl)**					
	Positive control (mm)	**Negative control (mm)**	**PG 01 (mm)**	**PG 02 (mm)**	**PG 03 (mm)**	**PG 04 (mm)**
S. aureus	29	0	14	20	16	19
S. typhi	23	0	14	11	12	16
B. cereus	20	0	14	16	15	14
E. coli	27	0	13	15	15	17
P. aeruginosa	0	0	15	11	13	14
K. pneumoniae	24	0	15	16	18	15

Table 3. Antibacterial screening of *Punica granatum* crude extracts by Disc Diffusion Method (50 mg/1 ml DMSO, 10 µl extract).

Bacterial strains (10 µl)	**Extracts (50 mg/1 ml DMSO with 20 µl)**					
	Positive control (mm)	**Negative control (mm)**	**PG 01 (mm)**	**PG 02 (mm)**	**PG 03 (mm)**	**PG 04 (mm)**
S. aureus	29	0	16	9	15	13
S. typhi	23	0	20	16	20	19
B. cereus	20	0	16	15	17	18
E. coli	27	0	20	19	16	11
P. aeruginosa	0	0	23	20	18	17
K. pneumoniae	24	0	21	16	10	15

Table 4. Antibacterial screening of *Punica granatum* crude extracts by Disc Diffusion Method (50 mg/1 ml DMSO, 20 µl extract).

Bacterial strains (10µl)	**Extracts (25 mg/ 1 ml DMSO 10 µl extract)**					
	Positive control (mm)	**Negative control (mm)**	**PG 01 (mm)**	**PG 02 (mm)**	**PG 03 (mm)**	**PG 04 (mm)**
S .aureus	29	0	9	14	13	12
S. typhi	23	0	15	13	19	14
B. cereus	20	0	14	13	16	14
E. coli	27	0	16	15	14	1
P. aeruginosa	0	0	9	10	0	11
K. pneumoniae	24	0	10	15	10	9

Table 5. Antibacterial screening of *Punica granatum* crude extracts by well Diffusion Method (25 mg/1 ml DMSO 10 µl extract).

The preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their qualitative estimation and also in locating the source of pharmacologically active chemical compound. The qualitative analyses of bioactive compounds for the four extracts have been analysed in this study and there is wide range of phytochemical compounds present in the extracts as shown in Table 7. The hexane being highly nonpolar in nature was able to extract compound characterized like carbohydrates, phenols, steroids and tannins, saponin, coumarins.

Bacterial strains (10µl)	**Extracts (50 mg/ 1 ml DMSO 20 µl extract)**					
	Positive control (mm)	**Negative control (mm)**	**PG 01 (mm)**	**PG 02 (mm)**	**PG 03 (mm)**	**PG 04 (mm)**

S. aureus	29	0	7	12	15	11
S. typhi	23	0	20	15	14	10
B. cereus	20	0	11	12	15	11
E. coli	27	0	16	15	15	9
P. aeruginosa	0	0	11	10	12	19
K. pneumoniae	24	0	11	18	9	10

Table 6. Antibacterial screening of *Punica granatum* crude extracts by well Diffusion Method (50 mg/1 ml DMSO 20 µl extract).

S. No.	**Phytochemical Tests**	**PG 01**	**PG 02**	**PG 03**	**PG 04**
1.	Molisch test	+	+	+	+
2.	Detection of alkaloids	+	+	+	+
3.	Flavonolids	_	+	+	+
4.	Tannis	_	_	_	+
5.	Steroids	+	_	_	_
6.	Triterpenoids	_	+	+	+
7.	Saponins	+	+	_	_
8.	Cardiac glycosides	+	+	+	+
9.	Coumarins	_	+	+	+
10.	Protein/amino acid	+	_	_	_

Table 7. Phytochemical analysis of *Punica granatum* crude extracts.

The DPPH scavenging activity has been widely used to detect antiradical activity of different samples, due to its sensitivity to lower concentrations of active principles from natural sources. The stable radical, DPPH, has a maximum A at 517 nm and could readily undergo scavenging by antioxidants. Higher free radical scavenging activities of samples is indicated by lower A at 517 nm. Flower, leave and stem of *Punica granatum* showed good antioxidant potential in comparison with ascorbic acid which was used as positive control. Flower and leaves showed highest antioxidant activity while Peel did not show antioxidant activity. DPPH free radical scavenging activity of extracts increased with increasing concentration showing a dose dependent effect (Table 8).

Extracts	**Conc. (µg/ml)**	**Absorbance (reading 1)**	**Absorbance (reading 2)**	**Absorbance (reading 3)**	**Mean**	**% SCV**	**IC50 (µg/ml)**
PG01	50	0.2249	0.2234	0.2288	0.226	45.483	135.689
	100	0.2146	0.2142	0.2145	0.214	48.205	
	150	0.2038	0.2071	0.2056	0.206	50.362	
	200	0.1979	0.1974	0.1977	0.198	52.254	
	250	0.1771	0.1762	0.1711	0.175	57.778	
PG02	50	0.2286	0.2265	0.2234	0.226	45.370	

	100	0.2176	0.2132	0.2111	0.214	48.317	135.665
	150	0.2061	0.2041	0.2033	0.205	50.604	
	200	0.1992	0.1973	0.1936	0.197	52.488	
	250	0.1788	0.1788	0.1743	0.177	57.174	
PG03	50	0.2316	0.2394	0.2348	0.235	43.172	130.866
	100	0.2196	0.2146	0.2136	0.216	47.842	
	150	0.2081	0.2048	0.2098	0.208	49.863	
	200	0.1932	0.1922	0.193	0.193	53.430	
	250	0.1449	0.1432	0.144	0.144	65.209	
PG04	50	0.206	0.205	0.2016	0.204	50.676	25.141
	100	0.1756	0.1722	0.1746	0.174	57.939	
	150	0.1541	0.1521	0.1561	0.154	62.778	
	200	0.1382	0.1362	0.1381	0.138	66.787	
	250	0.1275	0.127	0.1274	0.127	69.251	
Ascorbic acid	50	0.262	0.269	0.261	0.264	36.232	116.159
	100	0.256	0.254	0.256	0.2553	38.325	
	150	0.141	0.145	0.144	0.1433	65.378	
	200	0.111	0.109	0.113	0.1110	73.188	
	250	0.101	0.106	0.108	0.1050	74.638	

Table 8. Antioxidant activity of *Punica granatum* crude extracts by DPPH method.

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. By using FT-IR, we can confirm the functional constituents present in the plant extract, and identify the medicinal materials from the adulterants and even evaluate the qualities of medicinal materials. The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The powder of *Punica granatum* was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The results of *Punica granatum* FTIR analysis confirmed the presence of Amines, Alkanes, carboxylic acids, Aldehydes, Ketones, aromatic amines, Alcohols and Alkenes compounds which shows different peaks (Figures 1-4) (Table 9).

S. No	**Wavelength cm^{-1} (Test samples)**	**Transmittance**	**Wavelength cm^{-1} (Reference article)**	**Functional group assignment**	**Phyto-compounds identified**
PG01	3388.2	53.223	3570-3200	O-H stretch, Hydroxy group, H-bonded	Poly Hydroxy compound
	3276.3	46.008	3570-3200	O-H stretch, Hydroxy group, H-bonded	Poly Hydroxy compound
	2946.5	67.140	3500-2400	O-H stretch, Acidic	Carboxylic acids
	2970.7	67.374	3500-2400	O-H stretch, Acidic	Carboxylic acids
	2901.7	67.146	3500-2400	O-H stretch, Acidic	Carboxylic acids
	1718.3	77.882	1700-1725	C=O stretch	Carbonyl

				Carbonyl group	compound
	1617.7	78.493	1650-1600	C=O stretching vibration, Ketone group	Ketone compound
	1280.3	54.059	1340-1250	CN stretch	Aromatic primary amine
	1075.3	29.762	1100-1000	Phosphate ion	Phosphate compound
	1041.8	31.844	1100-1000	Phosphate ion	Phosphate compound
	1015.7	19.523	1100-1000	Phosphate ion	Phosphate compound
	930.0	59.201	995-850	P-O-C stretch	Aromatic phosphates
	877.8	45.042	995-850	P-O-C stretch	Aromatic phosphates
PG02	3270.7	75.935	3570-3200	O-H stretch, H bonded	Hydroxy compound
	2924.1	58.240	3000-2840	C-H stretching	alkane
	2853.3	65.987	3000-2840	C-H stretching	alkane
	1686.6	59.940	2000-1650	C-H bending	aromatic compound
	1617.7	69.737	1620-1610	C=C stretching	α,β-unsaturated ketone
	1507.7	79.620	1550-1500	N-O stretching	nitro compound
	1237.5	54.892	1400-1000	C-F stretching	fluoro compound
	1190.9	54.302	1210-1163	C-O stretching	ester
	1034.3	44.686	1250-1020	C-N stretching	amine
	922.5	63.398	995-850	P-O-C stretch	Aromatic phosphates
	881.5	62.800	995-850	P-O-C stretch	Aromatic phosphates
	754.8	54.615	800-700	C-Cl stretch	Aliphatic Chloro compound
PG03	3267.0	68.959	3300-2500	O-H stretching	carboxylic acid
	2920.4	62.520	2935–2915	Asymmetric stretching of –CH (CH2) vibration	Lipids, protein
	2849.5	69578	2865-2845	Symmetric stretching of –CH (CH2) vibration	Lipids, protein
	1707.1	56.151	1725-1705	C=O stretching	aliphatic ketone or or cyclohexanone or cyclopentenone
	1617.7	62.804	1650-1580	N-H bending	amine
	1507.7	79.809	1550-1500	N-O stretching	nitro compound
	1450.1	66.988	1510-1450	C=C-C, Aromatic ring stretch	Aromatic compound
	1315.8	56.292	1390-1310	O-H bending	phenol
	1196.5	49.891	1400-1000	C-F stretching	fluoro compound
	1026.9	41.508	1250-1020	C-N stretching	amine
	877.8	63.399	995-850	P-O-C stretch	Aromatic phosphates

PG04	3365.8	76.388	3400-3300	N-H stretching	aliphatic primary amine
	2920.4	62.255	2935–2915	Asymmetric stretching of –CH (CH2) vibration,	Lipids, protein
	2849.5	70.940	2865-2845	Symmetric stretching of –CH (CH2) vibration,	Lipids, protein
	1742.5	79.614	1750-1735	C=O stretching	δ-lactone
	1638.2	83.712	1620-1610	C=C stretching	α,β-unsaturated ketone
	1459.3	77.805	1510-1450	C=C_C, Aromatic ring stretch	Aromatic compound
	1377.3	80.344	1390-1310	O-H bending	phenol
	1239.3	81.989	1275-1200	C-O stretching	alkyl aryl ether
	1169.2	77.468	1400-1000	C-F stretching	fluoro compound
	1047.4	74.609	1050-1040	CO-O-CO stretching	anhydride
	719.4	66.445	800-700	C-Cl stretch	Aliphatic Chloro compound

Table 9. Fourier-transform infrared spectroscopy interpretation of *Punica granatum* plant extracts.

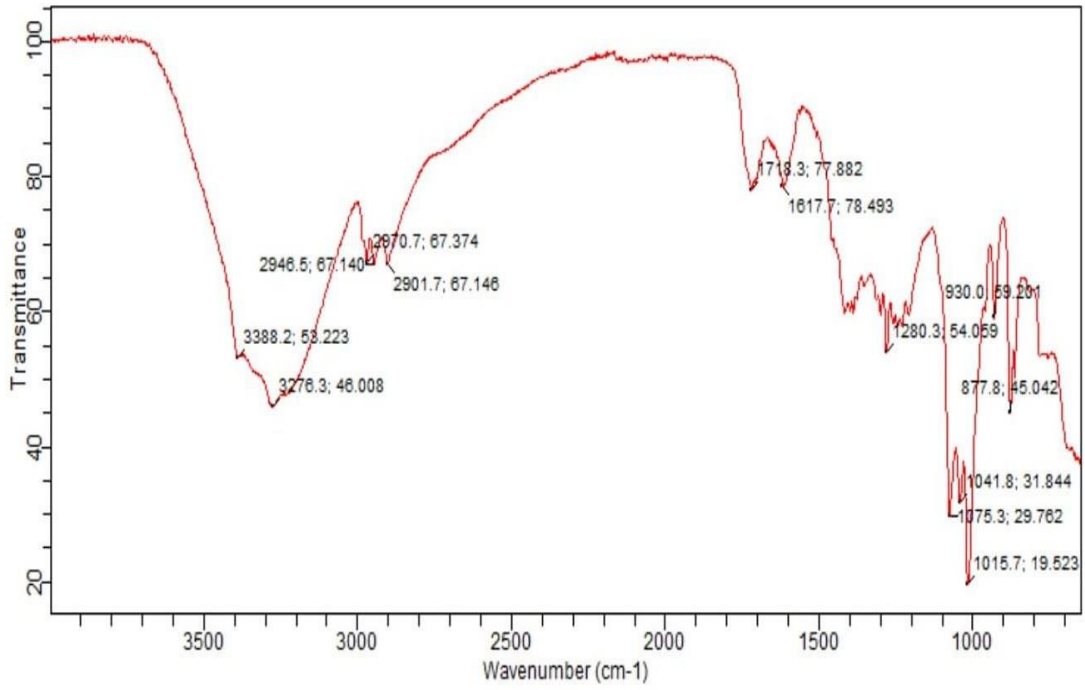

Figure 1. Fourier transform-infrared spectrum of flower extract of *Punica granatum*.

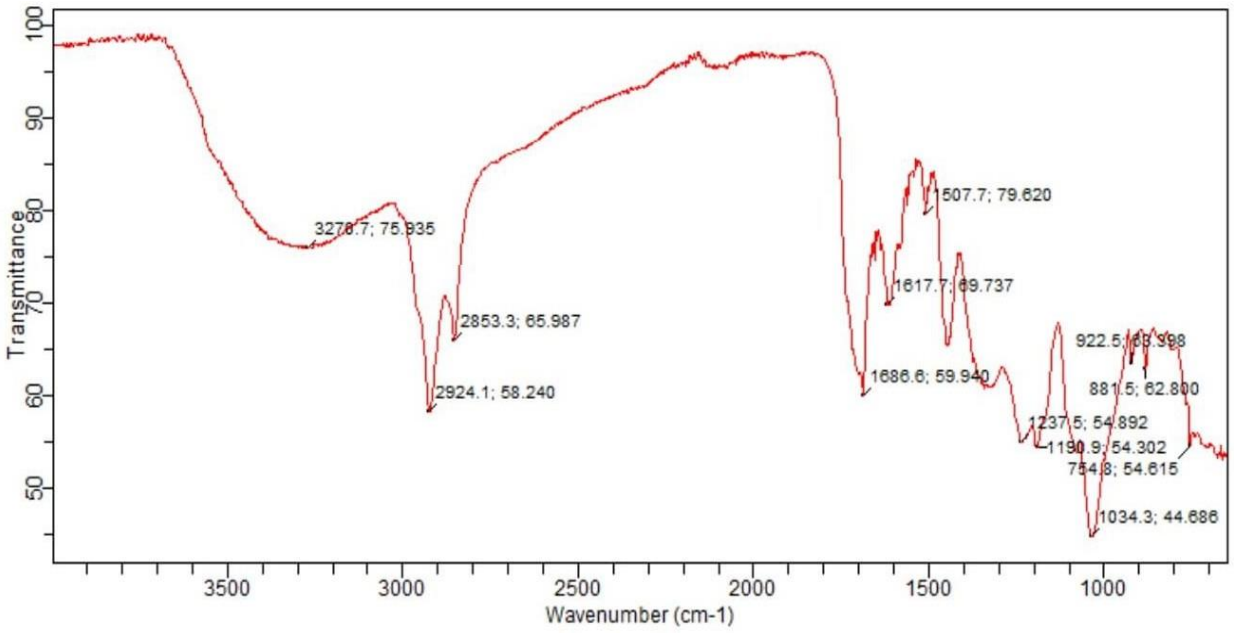

Figure 2. Fourier transform-infrared spectrum of leaves extract of *Punica granatum*.

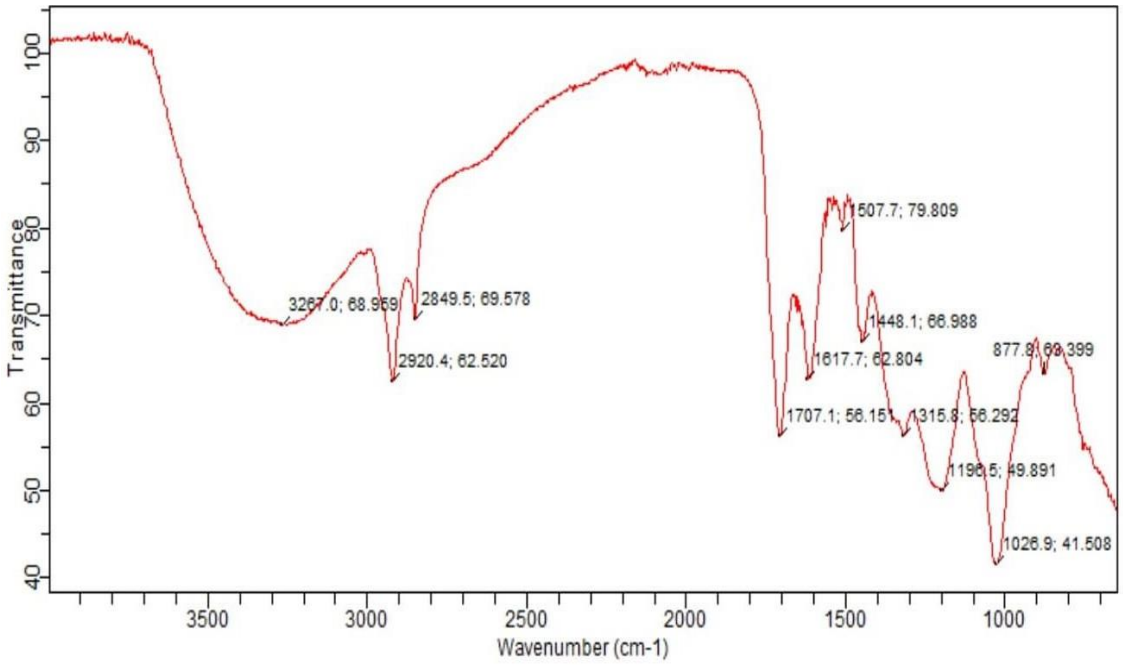

Figure 3. Fourier transform-infrared spectrum of stem extract of *Punica granatum*.

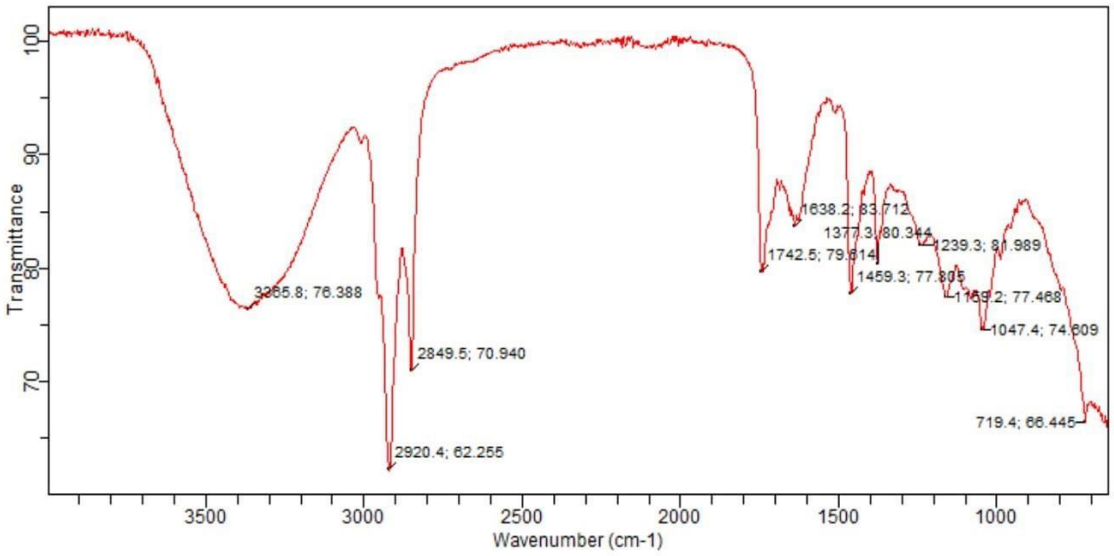

Figure 4. Fourier transform-infrared spectrum of peel extract of *Punica granatum.*

DISCUSSION

Plant product drugs and herbal remedies have been employed since prehistoric times to treat human and animal diseases and several countries still rely on plants and herbs as the main sources of drugs. It is well known that the medicinal compounds comprise hundreds of components and produce their curative effects through mutual effects with many ingredients, so the limited numbers of specific components cannot reflect the real qualities of the herbal medicines. Therefore, an effective and inexpensive analysis method to entirely monitor the whole constituents of the medicinal materials and their corresponding extract products is required. The antimicrobial effects of pomegranate were previously studied. Indeed, it is reported that the bark leaves, flowers and fruits of pomegranate are widely used as phytotherapeutic agents in Brazil (Mathabe et al., 2005). Alcohol extracts of pomegranate fruits showed antibacterial activity when tested against *S. aureus*, *E. coli* and *Shigella dysentriae* (Ahmad and Beg, 2001; Yaqoob et al., 2020). Methanolic extracts of *Punica granatum* fruit rind to be active against all microorganisms tested in their study. These results are in accordance to results obtained in the present study for bacteria wherein antibacterial activity was observed for all the bacterial cultures tested (Prashanth et al., 2001). Methanol, ethanol, acetone and water extracts obtained from pomegranate were active and effective against the tested microorganisms (*S. aureus*, *E. coli*, *Salmonella typhi*, *Vibrio cholera*, *S. dysenteriae*, *S. sonnei*, *S. flexneri* and *S. boydii*), showing an inhibition Zones of 12-31 mm (Mathabe et al., 2005; Ali et al., 2020). In this study, two Gram positive bacterial isolates such as *Staphylococcus aureus* and *Bacillus cereus* and four Gram negative bacterial isolates such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enterica* and *Klebsiella pneumoniae* were used to assess the *Punica granatum* antibacterial potential. Two concentrations 25 mg and 50 mg of *Punica granatum* were used and showed different zones of inhibition.

Santos et al., (2014) also examined the inhibitory effects of pomegranate peel extract on *S. aureus* isolates from cases of bovine mastites, which has also been checked by us in preliminary studies (Moreira et al., 2014; Khalil et al., 2020b). However, for the conditions evaluated by the present study, antimicrobial activity for Gram-negative bacteria was also observed. These bacteria are more resistant to antimicrobials based on natural extracts (Carvalho et al., 2013) because they have phospholipid external layer that is impermeable for solute lipophilic. Additionally, the porins create a barrier against hydrophilic solutes, restricting the penetration of antimicrobial compounds. On the other hand, the Gram positive bacteria have only peptidoglycan on the cell wall (Rabelo et al., 2014). Antibacterial activity of pomegranate extracts for Gram positive and negative bacteria's, but they used the pericarp for making the extract. In this study, the use of the peel was prioritized as residue in order to promote the economic sustainability of the activity (Moorthy et al., 2013; Danish et al., 2020). Phenolic compounds, but they use the fruit to do the extract (Karaaslan et al., 2014). The extract confectioned from the pomegranate peel, and just as

the present study, they found a high antioxidant activity, but without correlation to phenolic content. The pomegranate has complex composition; perhaps other alkaloids may have been responsible for the antioxidant activity (Silva et al., 2013). Noda et al., (2002) and Duman et al., (2009) associated the antioxidant activity with anthocyanins. In this study leaves and flowers extracts showed good antioxidant potential. DPPH free radical scavenging activity of extracts increased with increasing concentration showing a dose dependent effect.

In this study flower extract showed peaks at 3388.2, 3276.3, 2946.5, 2970.7, 2901.7, 1718.3, 1617.7, 1280.3, 1075.3, 1041.8, 1015.7, 930.0, 877.8, leave extract showed peak at 3270.7, 2924.1, 2853.3, 1686.6, 1617.7, 1507.7, 1237.5, 1190.9, 1034.3, 922.5, 881.5, 754.8, stem extract showed peak at 3267.0, 2920.4, 2849.5, 1707.1, 1617.7, 1507.7, 1450.1, 1315.8, 1196.5, 1026.9, 877.8, peel extract showed peak at 3365.8, 2920.4, 2849.5, 1742.5, 1638.2, 1459.3, 1377.3, 1239.3, 1169.2, 1047.4, 719.4. The results of *Punica granatum* flower FTIR analysis confirmed the presence of amines, alkanes, carboxylic acids, aldehydes, ketones, aromatic amines, alcohols and alkenes compounds which shows major peaks at 3384.74, 2940.11, 1699.41 cm-1, 1619.49cm-1, 1446.01cm-1, 1368.73cm-1, 1191.32cm-1, 1077.57, 1058.06 cm-1 and 923.75 cm-1 respectively (Suja and Yuvaranjani, 2018). FTIR analysis of dry methanolic extract of *Punica granatum* peel proved the presence of alkenes, aliphatic fluoro compounds, alcohols, ethers, carboxlic acids, esters, nitro compounds, alkanes, H-bonded H-X group, hydrogen bonded alcohols and phenols which shows major peaks at 671.23, 688.59, 707.88, 754.17, 802.39, 875.68, 921.97, 1016.49, 1145.72, 1226.73, 1317.38, 1338.60, 2860.43, 2929.87, 3082.25, 3176.76, 3219.19, 3246.20, 3265. 49 and 3334.92 (Ghaidaa et al., 2016).

CONCLUSION

Now-a-days herbs are extensively used for the research purpose and it possesses more than one chemical entity so it has been widely used for the research investigations. The plant based compounds have the effective dosage response and minimal side effects when compared to the synthetic compounds Phytochemical screening of *Punica granatum* leaves reveals it as a valuable medicinal plant with numerous medicinal properties. Experimental results of *Punica granatum* extract possessed good antibacterial activity. *Punica granatum* plant contains secondary metabolites phenol, flavonoids, alkaloids, sterols, terpenes. It has commercial interest in both research institutes and pharmaceuticals industries for manufacturing of new drugs for the treatment of different diseases. Flower, leave and stem extracts are a promising candidate for use as natural products based antioxidants for the health of human beings. A typical research and developmental work needs to be carried out for its better therapeutic and commercial utilization.

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