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Phytochemical and Antibacterial Potential of Punica granatum L. Leaves, Flower and Stem Bark Extracts against Human Pathogenic Bacteria

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Abstract : *The phytochemical and antibacterial activities' study of the Punica granatum leaves, flower and stem bark extracts were carried out. The phytochemical content was carried out according to the standard operated procedures. The antibacterial activities were carried out by standard agar well diffusion method. The methanol extract showed the highest presence of phytochemical, followed by ethanol, water and acetone extract. While, leaves showed the highest presence of phytochemical as compared to flower and stem bark. The methanol leaves' extract showed maximum zone of inhibition 21±01mm and 20±01 mm against Enterococcus fecalis and Staphylococcus aureus respectively. The aqueous leaves extract showed 19±0.5 mm zone of inhibition against Enterococcus fecalis. Ethanol leaves' extract observed 20±01 mm zone of inhibition against Escherichia coli. Generally, acetone leaves were found least zone of inhibition 09±0.4 mm against Pseudomonas aeruginosa. Maximum zone of inhibition 20±0.1 was noted against Escherichia coli by methanol flower extract and lowest zone of inhibition 07±0.4 mm was found by acetone flower extract against Pseudomonas aeruginosa. While stem bark acetone extract showed a low zone of inhibition 02±00 mm against Enterococcus fecalis and maximum zone of inhibition 15±0.2 mm by methanol extract against Salmonella typhi in this regard. These findings propose that pomegranate parts' extract has a considerable capacity to prepare a new broad spectrum of antibiotic plant based formulations.*

Keywords: *Pomegranate, Aerial Parts, Aqueous Extract, Organic Extract, Diseases, Bioactive Compounds, Antimicrobial Activity.*

INTRODUCTION

Punica granatum L. (pomegranate) is extensively grown in numerous subtropical and tropical regions. This is the oldest edible fruits of the world (Salaheddin & Kader, 1984). Pomegranate is broadly produced in India, USA, Japan, China, Argentina, France, Russia, Egypt, Spain and Iran (Patil & Karade, 1996). In the markets, pomegranate has been used for the preparation of therapeutic formula, cosmetics, juice and tinctures (Kim et al., 1991).

Due to the exhaustive utilization of antibiotics, microbes have developed a resistance against the antibiotics (Sydney et al., 1980). Furthermore, antibiotics connected with side effects make a difficulty in infectious

ailment treatment (Cunha, 2001). The utilization of herbal plants for antimicrobial agent has many advantages such as being renewable in nature, acceptance due to long history of use, cheap, better patient tolerance and less side effects (Vermani & Garg, 2002).

Herbal plants would be the main precursor to discover new drugs. So it is necessary to carry out research on herbal plants to better understand their efficacy, safety and properties. So, the aim of the current research project was to find out the phyto active compounds and antibiotic potential of aqueous and organic extracts of pomegranate parts.

Materials and Methods

Plant Parts Collection: Punica granatum L. flower, leaves and stem bark were collected from residential colony of Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Peshawar, Khyber Pakhtunkhwa-Pakistan. Flower, leaves and stem bark were washed with clean laboratory drinking H_2O, then rinsed with distilled H_2O and dried in Environmental Research Section Hall (PCSIR). The dried materials were powdered using grinding mill and stored in sterile plastic bags at 4°C until use.

Tested Bacteria: The bacteria (Table 2, 3 & 4) used in this study were procured from Environmental Research Section of PCSIR Peshawar- Pakistan. All stock cultures were subcultured and maintained in nutrient broth at 4°C.

Preparation of extracts

Flower, leaves and stem bark of pomegranate fifty-gram powder each were taken and extracted with methanol, ethanol, acetone and water (50 g/200ml of the solvent) for 24 h. The extracted processes were repeated three times. At the end, glass wool was used to filter the extracted material. The rotary evaporator at 40˚C under reduced pressure was used to evaporate the solvent and finally dry the extracted material. The dried extract was stored at 4°C till further analysis.

Preparation of McFarland Nephelometer standard: Mueller-Hinton broth (MHB) was mixed with 9.95 ml 1% H_2SO_4 and 1% Barium chloride (0.05 ml) in distilled H_2O in order to calculate the microbe's density (Baron et al., 1994). The vials were tight closed and whenever required utilized for bacterial suspension comparison.

Inoculum preparation and standardization: From the pure growth of each tested bacteria 4 to 5 colonies were shifted to MHB. For 18-24 hours at 35- 37°C the broth was incubated. The culture turbidity was matched to McFarland Nephelometer standards (0.5) to obtain 150 x 10^6 CFU/ml. Within 15-20 minutes, the inoculum suspension (standardized) was inoculated (Sabahat & Perween, 2005).

Antibacterial Activity

For antibacterial activity, Mueller-Hinton agar (MHA) was used and the experiments were carried out in PCSIR-Peshawar (Environmental Research Section). The media plates were inoculated with standardized inoculum (0.1 ml) of each experiment microbes. The sterile glass spreader was used to spread the inoculum uniformly over the plate. The inoculated plates were kept to dry for 20 minutes at 37°C in the incubator. On the surface of MHA, a uniform wells was drilled with the help of 8 mm diameter standard cork borer and in the well 100 µl (500 mg/ml) of each sample extract was poured. The experimented Petri dishes were kept for 24 hours at 35°C in an incubator and calculated inhibition zone in millimeter (Kivanc & Kunduhoglu, 1997). Dimethyl sulfoxide 10% (100 µl) was used as negative control, while 100 µl Streptomycin (25 mg/ml) used as positive control.

Statistical analysis: Mean and standard deviation of inhibition zone were calculated using SPSS Program.

Results and Discussion

The phytochemical analysis of pomegranate leaves, flower and stem bark is shown in table 1. Methanol extract shows the high content of phytochemical ingredient as compared to the rest of extracts. Moreover, leaves' extract showed the highest bioactive compounds as compared to flower and stem bark.

The antibiotic potential of leaves, flower and stem bark of pomegranate aqueous and organic extracts are shown in tables 2, 3 & 4. Methanol extract showed the highest antibacterial activities as compared to the other extracts, similarly leaves were found the maximum zone of inhibition as compared to flower and stem bark. The study of Kadi et al., 2011 were found that ethanol and water bark extracts have 21.30-23.75 mm and 15.85-22.85 mm zone of inhibition against all the tested microbes respectively.

The antimicrobial potential of decoctate and crude extracts (macerate) of pomegranate bark were due to tannins, which was found to be twenty-eight percent of bark ingredients (Lansky & Newman, 2007). In addition, the antibacterial potential observed were because of phytochemical such as saponins and phenolic compounds. The ethanol extract was more efficient than water extract because it permitted to isolate fine minimum polar substances like terpenic derives (Kadi et al., 2011). Flavonoid also forms and reacts with cell wall and soluble protein of microbes, while the saponins also exhibited antimicrobial activity which was leaded to its capability to originate leakage of certain enzyme and protein from cell (Reguieg & Hammadi, 2017).

Phenolics compounds were the main antimicrobial and among them gallic acid was marked as the prime antibiotic (Shoko et al. 1999). The bactericidal potential of polyphenolic substances should be made clearer by substrate and metal ion deprivation, interaction with enzyme and adsorption to cell membranes (Scalbert 1991). The current study also agrees with these findings as all extracts showed sufficient quantity of phytochemicals.

The antibiotic potential of herbs extracts is because of numerous compounds e.g. tannins and flavonoids, hydroxyl groups and other free phenolic compounds, which are categories as high potential of antibiotics. Tannins were found abundant in Punica granatum (Gil et al., 2000). The antibiotic potential of tannins was because of its capability to reduce enzyme production, inactivate bacterial adhesion and inhibit membrane synthesis of microbes by formation complex with polysaccharides. Furthermore, tannins also bind with substrate like vitamins, elements and sugars, finally microbes face shortage of these substances and death of microbes were taken place (Cowan, 1999). Usually, botanical products originate alteration in microbes' cell, such as cell wall collapse, cytoplasmic membrane disturbance, cytoplasm granulation, inhibition or inactivation of enzymatic functions outside the cell or within the cell (Brull & Coote 1999; Caccioni et al., 2000; Cox et al., 2002). Antifungal potential of methanolic extract of pomegranate flowers were due to tannins like ellagic acid, gallic acid and Pomegranatate, and flavonoids such as Punica flavones. The pomegranate's leaf methanol extracted tannins (Ellagic acid, Gallic acid, Corilagin and Brevifolin) and flavonoid (Luteoline and Apigenin). The extract of stem has abundance of tannins' substances like piperidine, Punicatonic, Punicalin, Punicalagin and Ellagitannin (Endo et al., 2010; Arun & Singh 2012; Jurenka, 2008). Furthermore, level of substances extracted and isolated from herbs depends on polarity of solvent and compounds, such as a polar solvent like methanol. Therefore, polar substances like flavonoids, tannins and phenols are highly effective isolated by polar solvent (Mahsa et al., 2012). As a result, maximum antimicrobial activities of methanol extracts are because of solvent polarity and abundant phytochemical content.

Conclusion

The pomegranate leaves, stem bark and flower were found abundant in phytochemical content. Methanol extract showed maximum bioactive content in leaves (1st), flower (2nd) and stem bark (3rd). Wide-ranging antimicrobial potential of pomegranate plant parts' extracts were due the phytochemical compounds such as saponins, phenols and tannins were plentiful in the study plant. Moreover, the value addition of stem bark, flower and leaves which are the byproduct could give healthiness advantages to man and may be utilized in pharmaceutical and food preservation purposes. These studies suggest the approach for further research and attention to discover the active ingredients accountable for the pharmacological activities along with animal and human trials.

Table 1*:* Phytochemical Compositions of Pomegranate Parts

Phytochemical	Extracts											
	Aqueous			Methanol			Ethanol			Acetone		
	L	F	B	L	F	B	L	F	B	L	F	B
Phenols	++	++	+	+++	+++	++	++	++	+	+	+	+
Tannins	+	+	+	+++	+++	++	++	++	+	+	+	+
Steroids	-	-	-	++	++	-	+	+	-	+	+	-
Proteins	+	+	+	++	++	+	+	+	+	+	+	+
Flavonoids	+	+	+	+++	+++	++	++	++	++	+	+	+
Saponins	++	++	-	++	++	-	-	-	-	-	-	-
Alkaloids	+	+	+	+++	+++	++	++	++	+	+	+	+
Terpenoids	-	-	-	+	+	-	+	+	-	+	+	-
Glycosides	+	+	-	+++	+++	+	++	++	+	+	+	+

L=Leaves, F= Flower, B= Stem Bark. - = absent, + = low, ++ = moderately present, +++ = highly present

Table 2*:* Antibacterial Activity of Pomegranate Leaves Extract

Tested Microorganism	Extracts/Zone of Inhibition (mm)				*S	DMSO
	Aqueous	Methanol	Ethanol	Acetone		
Salmonella typhi	17±0.2	19±01	18±1.4	16±0.1	22±0.5	00
Escherichia coli	15±0.6	17±0.1	20±01	14±0.5	25±01	00
Staphylococcus aureus	18±0.5	20±01	19±01	17±0.5	23±1.2	00
Bacillus subtilis	13±0.3	15±0.6	14±0.2	12±0.8	26±0.3	00
Bacillus cereus	11±0.8	13±0.4	12±0.9	10±0.5	21±0.4	00
Pseudomonas aeruginosa	10±0.1	12±0.7	11±0.1	09±0.4	20±0.5	00
Klebsilla pneumoniae	12±0.4	14±0.2	13±0.8	11±0.1	24±00	00
Proteus mirabilis	15±0.7	17±0.8	16±0.3	14±0.3	27±00	00
Enterococcus fecalis	19±0.5	21±01	16±01	18±0.7	25±00	00
Vibrio cholerae	14±0.2	16±0.5	15±0.4	13±0.2	24±00	00
Shigella dysenteriae	16±01	18±01	17±0.7	15±0.9	26±02	00

Each values are means of 3 replicates (n = 3) ± standard deviations, *S= Streptomycin= positive control, DMSO=negative control

Table 3*:* Antibacterial Activity of Pomegranate Flower Extract

Tested Microorganism	Extracts/Zone of Inhibition (mm)				*S	DMSO
	Aqueous	Methanol	Ethanol	Acetone		
Salmonella typhi	15±0.2	18±01	16±1.4	14±0.1	22±0.5	00
Escherichia coli	14±0.6	20±0.1	18±01	12±0.5	25±01	00
Staphylococcus aureus	17±0.5	19±01	17±01	15±0.5	23±1.2	00
Bacillus subtilis	12±0.3	14±0.6	12±0.2	10±0.8	26±0.3	00
Bacillus cereus	10±0.8	12±0.4	10±0.9	08±0.5	21±0.4	00
Pseudomonas aeruginosa	08±0.1	11±0.7	09±0.1	07±0.4	20±0.5	00
Klebsilla pneumoniae	11±0.4	13±0.2	11±0.8	09±0.1	24±00	00
Proteus mirabilis	14±0.7	16±0.8	15±0.3	10±0.3	27±00	00
Enterococcus fecalis	18±0.5	19±01	14±01	10±0.7	25±00	00
Vibrio cholerae	13±0.2	15±0.5	13±0.4	11±0.2	24±00	00
Shigella dysenteriae	15±01	17±01	15±0.7	13±0.9	26±02	00

Each values are means of 3 replicates (n = 3) ± standard deviations, *S= Streptomycin= positive control, DMSO=negative control

Table 4: Antibacterial Activity of Pomegranate Stem Bark Extract

Tested Microorganism	Extracts/Zone of Inhibition (mm)				*S	DMSO
	Aqueous	Methanol	Ethanol	Acetone		
Salmonella typhi	13±0.4	15±0.2	14±0.9	11±0.3	22±0.5	00
Escherichia coli	11±0.9	13±0.4	12±0.5	09±0.8	25±01	00
Staphylococcus aureus	09±0.5	11±0.7	10±0.2	07±0.2	23±1.2	00
Bacillus subtilis	07±0.6	09±0.9	08±0.6	06±0.1	26±0.3	00
Bacillus cereus	05±00	07±0.1	06±0.3	04±00	21±0.4	00
Pseudomonas aeruginosa	00	05±0.2	04±00	03±00	20±0.5	00
Klebsilla pneumoniae	00	06±0.6	05±00	04±00	24±00	00
Proteus mirabilis	03±00	08±0.8	07±00	05±00	27±00	00
Enterococcus fecalis	06±0.7	10±0.3	09±0.8	02±00	25±00	00
Vibrio cholerae	08±0.2	12±01	11±0.4	00	24±00	00
Shigella dysenteriae	09±0.8	14±0.5	10±0.7	00	26±02	00

Each values are means of 3 replicates (n = 3) ± standard deviations, *S= Streptomycin= positive control, DMSO=negative control.

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