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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF POMEGRANATE (*PUNICA GRATANUM)* EXTRACT AGAINST URINARY TRACT INFECTIONS (UTI) PATHOGENS

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ABSTRACT

Urinary tract infections (UTIs), the second most common type of infection in the body are one the most serious health problem affecting millions of people each year. The urinary tract infection (UTI) involves infection in the kidneys, urinary bladder or urethra. These are the organs that urine passes through when eliminated from the body. Women are especially prone to UTIs, even though they generally have anatomically and physiologically normal urinary tract. Reasons for this are not yet well understood. These infections can be very serious when they do occur in men; however, UTIs in men are not as common as in women. A urinary tract infection (UTI) also known as Fearnes syndrome is a microbial infection that affects any part of the urinary tract. Normally, urine is sterile. It is usually free of microbes but does contain fluids, salts and waste products. The main cause agent in at least 90% of uncomplicated infections is Escherichia coli, which live in the bowel (colon) and around the anus. An infection occurs when bacteria get into the bladder or kidney and begin to grow. The infection often starts at the opening of the urethra where the urine leaves the body and moves upward into the urinary tract. The Pomegranate was once named as the “most medicinal fruit in the world”. In the present paper, health benefits of this fruit have been showed and proved. Antioxidant and antimicrobial activity were present in different parts of *Punica granatum*. Decreasing order of extract against UTI pathogens were PE > FE> WFE > BRE> LE > RE > SE respectively. The results of this study could provide efficacious and cost effective treatment of the UTI without any harmful effects on human body.

Keywords: Antioxidant, Antimicrobial, FRAP, *Punica granatum*, UTI.

INTRODUCTION

Urinary tract infections (UTIs), the second most common type of infection in the body are one of the most serious health problem affecting millions of people each year. The urinary tract infection (UTI) involves infection in the kidneys, ureters, urinary bladder or urethra. These are the organs through which urine passes when eliminated from the

body[1]. Women are especially prone to UTIs, even though they generally have anatomically and physiologically normal urinary tract. Reasons for this are not yet well understood. These infections can be very serious when they do occur in men, however, UTIs in men are not as common as in women [2, 3]. A urinary tract infection (UTI) also known as Fearnes syndrome is a microbial infection that affects any part of the urinary tract. Normally, urine is sterile. It is usually free of microbes but does contain fluids, salts and waste products [4, 5]. The main causal agent in at least 90% of uncomplicated infections is Escherichia coli, which lives in the bowel (colon) and around the anus. An infection occurs when bacteria get into the bladder or kidney and begin to grow. The infection often starts at the opening of the urethra where the urine leaves the body and moves upward into the urinary tract [6].Abnormalities of the urinary tract that hinder the flow of urine set the stage for an infection. The association between enteropathogenic Escherichia coli (EPEC) and diarrhoea has been reported in many countries. Virologists, bacteriologists, mycologists and parasitologists are just starting to understand the microbial world. Host - microbe interaction is as diverse as the organisms involved [7].

Plants are a valuable source of natural products for maintaining human health and the use of plant compounds for pharmaceutical purposes has increased [8].According to the WHO (World Health Organization), medical plants would be the best source to obtain a variety of drugs. The Pomegranate was once named the “most medicinal fruit in the world” and this paper will show and prove a number of health benefits of this fruit. The pomegranate tree, ***Punica granatum***, specially its fruit, possesses a vast ethnomedical history and represents a photochemical reservoir of heuristic medicinal value. The tree/fruit can be divided into several anatomical compartments: (1) Bark (2) flower (3) Fruit (4) Root (5) Branch (6) leaf and (7) Seed each of which has interesting pharmacologic activity. Juice and Peel, for example, possess potent antioxidant properties while Juice, Peel, and Oil are all weakly estrogenic and heuristically of interest for the treatment of menopausal symptoms and sequellae [9].

MATERIAL AND METHODS

Plant Material

The samples of the pomegranate *Punica granatum* (Peel, flower, Fruit, Root, Branch, leaf, Seed) were collected from Jogiwala nursery, Dehradun (Uttarakhand) India (Fig 1 and 2).

***Fig 1 Punica granatum* whole plant**

***Fig 2 Punica granatum* seeds**

Preparation of Plant Extract

We took different parts of *Punica granatum* like Peel, flower, Fruit, Root, Branch, leaf, Seed and washed them properly. Then weight before dried and after dried different parts of *Punica granatum*. BE (100g), FE (20.33g), WFE (54.05g), RE (32.73g), BRE (13.33g), LE (32.05g) and SE (79.25g) were chopped into small pieces and sterilized. Roots were crushed and dissolve in 500 ml of ethanol (99.9%) and the pericarp of ripened fruit was dried under shade and stored into fine powder using electric blender. 50g of dried powder sample was taken and extracted by soxhlet (BE, FE, RE) apparatus using ethanol (99.9%) and aqueous separately. The solvents were removed under reduced pressure in a rotary evaporator until they become completely dry. The extract was stored at 4 ℃ for further use.

Microorganism

The pure culture of *E.coli, Pseudomonas, Staphylococcus, Proteus mirabilis, Candida*, were collected from microbiology department of Dolphin Institute by the help of Deepika Bhatnagar. Culture was maintained at 4 ^{0}C until use. All these cultures were activated at 37 ^{0}C for 24 hours on Nutrient Agar media (NAM) prior to screening. The Muller Hinton Agar (MHA) and the nutrient broth were used for antimicrobial assay. The plates inoculated with *E.coli, Pseudomonas, Staphylococcus, Proteus mirabilis, Candida* were

then incubated at 37 ^{0}C for 24 hours. Antimicrobial activity was evaluated by measuring the diameter of hole and zone of growth inhibition around the hole [10].The assay was repeated on three plates and mean diameter was recorded as given below;

Area of growth zone inhibition= $\pi (r_2^2-r_1^2)$

Where r_2= radius from centre to periphery of growth zone

r_1= radius from centre to periphery of hole, $\pi = 3.14$

Determination of antibacterial activity

The disc diffusion method was used to screen the antibacterial activity. In-vitro antibacterial assay was screened by using Mueller Hinton Agar (MHA). The MHA plates were prepared by pouring 15ml of molten media into sterile petri dishes. The Plates were allowed to solidify for 10 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. Sterile paper disc (5mm) were soaked with 10 ml of extract residue diluted into corresponding extraction solvents, so that each disc was impregnated with 2.5mg of residue and dried at 37°C overnight. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37 °C for 24 hrs. Antibiotic discs containing Penicillin and Tetracycline (5-30µg) were used as controls. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone formed around the discs [11].

Minimal Inhibitory Concentration (MIC) determination by hole diffusion method

Further 10 fold decreasing dilutions of all the three fractions were done. So the curve should be in the range of 5-1000 µg/ml. Each dilution was tested by hole diffusion method as described earlier and MIC determined as the lowest concentration of extract that can show a visible inhibition zone (IZ) [12].

Statistical Evaluation

Statistical analysis of data was done by employing student t-test as described by [13]. P values less than 0.05 were considered significant.

Antioxidant Activity

Measurement of plasma "total antioxidant power" (FRAP) The method of Benzie and Strain, 1996 was used for measuring the ferric reducing ability, the FRAP assay, which estimate the "total antioxidant power", with minor modification [14]. Ferric to ferrous ion

reduction at low pH results in the formation of a colored ferrous-tripyridyl triazine complex. The assay was carried out in a total volume of 1.0 ml containing a suitable aliquot of plasma in 0.1 ml and 900 µl of freshly prepared FRAP reagent, prepared by mixing 10.0 ml of 22.78 mm sodium acetate buffer, pH 3.6, 1.0 ml of 20 mm ferric chloride and 1.0 ml of 10 mm 2, 4, 6-tripyridyl-s-triazine solution prepared in 40 mm HCl. Before starting the reaction, both FRAP reagent and plasma samples were pre incubated for 5 min at 30 ^{0}C. Incubation was done for 5 min at 30 ^{0}C and absorbance was recorded at 593 nm against a reagent blank in a Beckman DU 640 spectrophotometer. Ferrous sulphate was used as a standard for calculating the "total antioxidant power".

Antimicrobial activity

The reference antibiotic (RA) penicillin dissolved in BE, FE, WFE, RE, BRE, LE and SE was dissolved in ethanol 99.9% w/v. Whereas , antibiotic (RA) penicillin, BE, FE, WFE, RE, BRE, LE and SE were used at a concentration of 10 and 20 mg/ml and further dilution was done in distilled water with pH 7.4 each [12].

Hole diffusion method

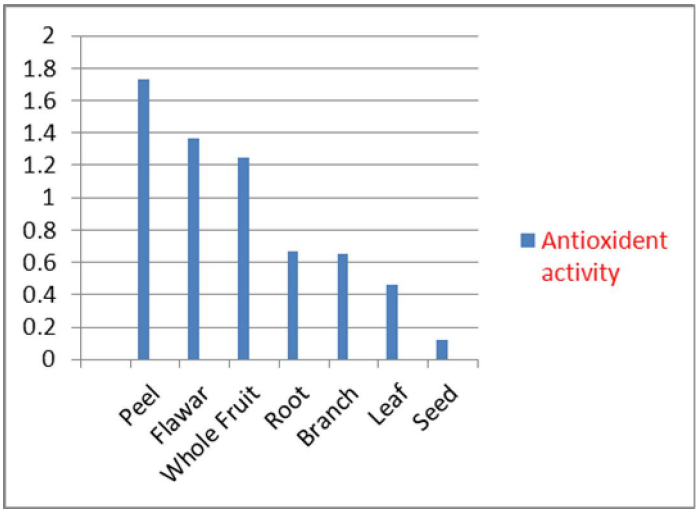
Screening test for antimicrobial activity were carried out by the hole diffusion method by using a cell suspension from the pure culture of about 1.0×10^4 colony forming unit (CFU/ml) obtained by McFarland turbidity standard number 0.5. The concentration of suspension was standardized by taking the OD to 0.1 at 600 nm (Beckman DV 640 Spectrophotometer) [15]. Holes of about 3 mm diameter were made on the MHA plate and tilted with the 100 µl (20 mg/ml, 10 mg/ml) of antibiotic (RA) penicillin, BE, FE, WFE, RE, BRE, LE and SE.

RESULTS AND DISCUSSION

Table 1 showed Antioxidant activity (nmol/mg extract) of PE, FE, WFE, RE, BRE, LE and SE isolated from the *Punica granatum.* All values are mean ± Standard deviation of triplicate values, Significant for Standard Error (SE). As seen in Fig.2, the antioxidant impact of PE, FE, WFE, RE, BRE, LE and SE at different concentration range 20 µg/ml extract respectively is depicted.

Table 1 Showing antioxidant activity in nmol/mg

Test Sample	Peel (20 µg /ml)	Flower (20 ug/ml)	Whole Fruit (20 µg/ml)	Root (20 µg/ml)	Branch (20 µg/ml)	Leaf (20 µg/ml)	Seed (20 µg/ml)
Antioxidant activity (nmol/mg extract)	1.73±0.13	1.37±0.12	1.25±0.16	0.6673±0.025	0.6531±0.023	0.4628±0.013	0.1214±0.014



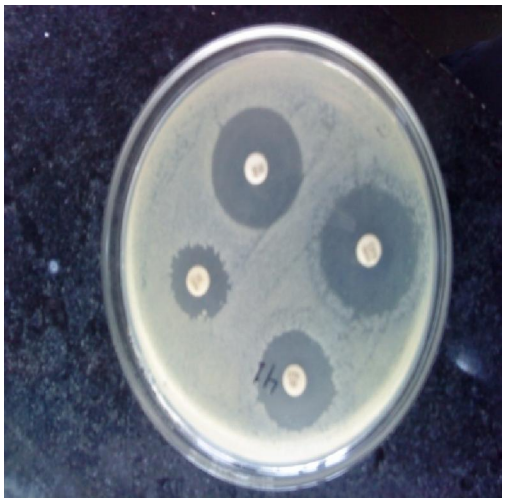


Fig 3 showing antioxidant activity ***Fig 4 showing inhibition zone***

Fig. 4 showing Inhibition zone area (mm^2) for the PE, FE, WFE, RE, BRE, LE, SE isolated from the *Punica granatum* plant and Reference antibiotic (RA) against *UTI pathogens*. Inhibition zone area is mean (mm^2) ± Standard deviation of triplicate values, Inhibition zone area is Calculated from the difference of radial of inhibition zone and hole (3 mm), Reference antibiotics (penicillin), significantly different from BE, FE, WFE, RE, BRE, LE and SE. The results obtained from BE was more efficient scavengers of proxy radicals. It was observed that the antioxidant power (Scavengers of proxy radicals) of 20 µg/ml BE, and 20µg/ml of FE 20 µg/ml WFE, 20 µg/ml RE, 20 µg/ml BRE, and 20 µg/ml SE. There is a decrement of Purified BE, 1.73±0.13, provide a high antioxidant activity as compared to FE 1.37±0.12 FRE 1.25±0.16, RE 0.6673± 0.025, BRE 0.6531±0.023, LE 0.4628±0.013, SE 0.1214±0.014 as shown in Table 1.

TABLE 2: SHOWING INHIBITION ZONE AREA IN PERCENTAGE

Test Sample	*E. coli*	*Pseudomonas*	*Staph*	*Proteus mirabilis*	*Candida*
Peel	2.77±.003 (71.67%)	379.9±.004 (51.35%)	379.9±.003 (15%)	379.9±.023 (20%)	1.791±.042 (16%)
Flower	1.913±.004 (10.31%)	313.93±.003 (11.39%)	0.8873±.065 (1.60%)	313.93±.003 (34.54%)	1.732±.042 (2.297%)
Whole fruit	1.612±.053 (7.34%)	313.93±.072 (39.93%)	313.93±.052 (1.219%)	0.5733±.053 (3.837%)	1.801±.053 (8.724%)
Branch	1.9471±.072 (39.16%)	0.722±.053 (73.27%)	313.93±.031 (8.54%)	0.4399±.072 (5.499%)	0.779±.072 (3.119%)
Root	1.21±.004 NA	0.552±.062 (14.75%)	1.02±.039 (32.53%)	1.13±.062 (3.073%)	1.46±.052 (14.55%)
Leaf	(43.26%)	0.433±.053 (24.32)	1.264±.065 (15.06%)	0.7255±.072 (34.97%)	1.4761±.092 (13.42%)
Seed	NA	0.133±.072 (0.9826%)	0.7255±.059 (10.57%)	0.4399 (3.289%)	0.5225 (20.23%)
RA	452±.032 (87.32%)	379.9±.003 (81.96%)	313.93±.003 (62.24%)	379.9±.003 (67.34%)	313.93±.003 (57.35%)

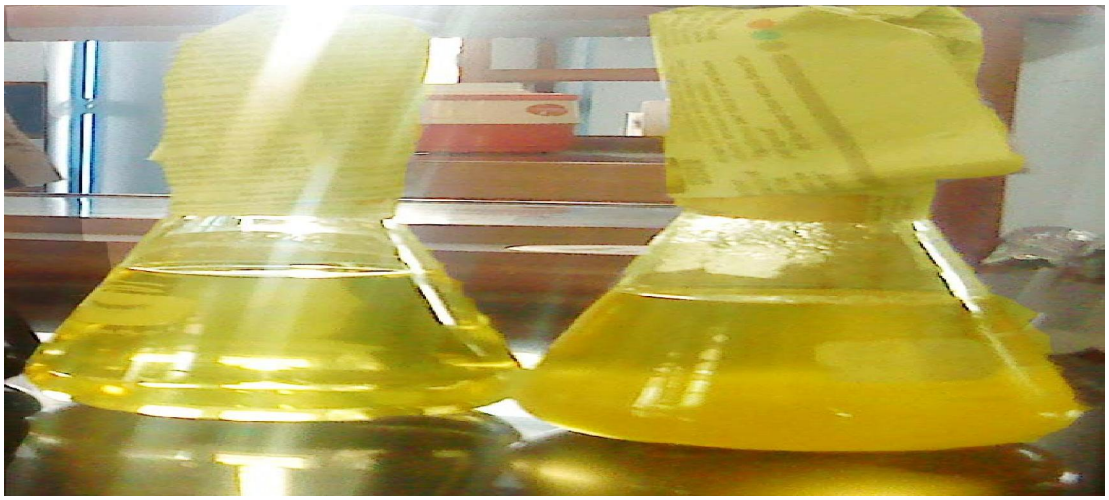


Fig. 5 showed the antioxidant activity as in the form of visible bacterial zone formation

The hole diffusion method results are presented in table 2, showed the antioxidant activity as in the form of visible bacterial zone formation. There is no bacterial inhibition zone formation without the application of either purified extract or its purified fractions. It was found that on application of 20 µg/ml BE on inoculated MHA media plate, the inhibition area correspond to 1.73±0.13 mm^2 in BE, whereas an application of FE, WFE,

RE, BRE, LE and SE it was 1.37±0.12, 1.25±0.16, 0.6673±0.025, 0.6531±0.023, 4628±0.013 0.1214±0.0140 mm2 respectively. It is clear from the data that inhibition zone area, obtained from BE, was increased by (71.67%) than FE (10.31%) and WFE (7.34%) BRE (39.16%) applied plates, whereas (87.32%) increment was observed when compared with (RA) applied plates. On further dilution of the BE, FE, LE, SE, RE, WFE and BRE fractions the same pattern was obtained. The inhibition zone area was increased by 89.90%, 112.04%, 95.56% and 96.38%, than BE compared to reference antibiotics (*penicillin*) applied plates as shown in Table 2. Thus the BE, FE, WFE, RE, BRE, LE and SE exhibited both antibacterial and antimicrobial effect, since the BE extract fraction and RA were tested at different concentrations, the real extend of their inhibitory activities against the test microorganism *E.coli , Pseudomonas, Staphylococcus, Proteus mirabilis, Candida,*could be well established only by comparing the minimal inhibitory concentration (MIC) values obtained.

Table 3 and 4 below indicates the respective MIC values from for 5-1000 µg/ml for BE, FE, WFE, RE, BRE, LE and SE. The MICs of the BE and its other functions on test pathogen was either greater or equal to that of RA. The results thus obtained, confirmed that in UTI the organism isolated from urine sample was sensitive to extracts and its purified fractions obtained from the *Punica granatum* plant. Minimal inhibitory concentration (MIC)5-1000 µg/ml, Peel extract (PE), Flower extract(FE), Whole fruit extract(WFE), Leaf extract(LE) and Seed extract(SE), Branch extract (BRE), Root extract (RE), Inhibition zone area (- Absent, + Present, ++ Abundant, +++ Very abundant, NA- Not available) The bacterial growth curve is in term of optical density (OD) at 600 nm for different time interval (0-90 h) and concentrations of PE, FE, WFE, RE, BRE, LE, and SE (50 µg/ml). It is observed that without the application of plant extract either PE, FE, WFE, RE, BRE, RE ,BRE, LE, and SE, the increase in O.D was observed, whereas on application of BE and its purified fractions the maximal bacterial growth inhibition in term of decrement in O.D was noted. The maximal bacterial growth inhibition was found in Seed extract PE followed by PE, FE, WFE, RE, BRE, LE and SE in all concentrations (50µg/ml) as shown in Table no 4, there is a decrement of PE, FE, WFE, RE, BRE, LE, SE in concentrations (50µg/ml) of respectively. Intervals in absence of extract and presence of BE 50 µg/ml, FE 50 µg/ml, RE 50 µg/ml, LE 50

µg/ml, SE 50 µg/ml, WFE 50 µg/ml, Values are mean (OD) ± Standard deviation for bacterial growth for triplicate assay.

TABLE 3: INDICATES THE RESPECTIVE MIC VALUES FROM FOR 5-1000 µG/ML IN +VE AND –VE VALUES

Test Sample	5 µg/ml	10 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml	1000 µg/ml	Without Extract
Bark	-	-	+	++	++	++	+++	+++	---
Flower	-	-	+	+	++	++	++	+++	---
Whole Fruit	-	-	+	+	++	++	++	+++	---
Branch	-	-	-	+	+	+	++	+++	---
Root	-	-	-	+	+	+	+	++	---
Leaf	-	-	-	+	+	+	+	++	---
Seed	-	-	-	-	+	+	+	++	---
RA	+	+	++	++	++	++	+++	+++	---

TABLE 4: SHOWING MAXIMUM GROWTH INHIBITION ZONES

Sample (50µg/ml)	**Time (h)**	***E. coli***	***Pseudomonas***	***Staph***	***Proteus mirabilis***	***Candida***	**Control**
Bark	0	0.377	0.513	0.108	0.180	0.186	0.012
	6	0.406	0.541	0.131	0.208	0.447	0.014
	12	0.559	0.551	0.118	0.230	0.783	0.235
	18	0.590	0.577	0.084	0.331	0.908	0.395
	24	o.592	0.532	0.119	0.380	0.940	0.489
	36	0.593	0.599	0.222	0.486	0.868	0.533
	48	0.475	0.637	0.318	0.521	1-039	0.677
	60	0.399	0.718	0.551	0.606	1.078	0.775
	72	0.676	0.760	0.721	0.599	1.037	0.987
	90	0.809	0.999	0.720	0.521	1.162	0.995
Fold		**1.992**	**1.846**	**5.496**	**2.504**	**2.599**	**71.071**
Flower	0	0.076	0.095	0.117	0.123	0.078	0.011
	6	0.466	0.086	0.093	0.130	0.336	0.121
	12	0.532	0.085	0.109	0.201	0.572	0.198
	18	0.559	0.087	0.094	0.382	0.876	0.287
	24	0.574	0.089	0.030	0.539	0.898	0.365
	36	0.579	0.110	0.156	0.648	0.848	0.444
	48	0.609	0.596	0.392	0.672	0.775	0.543
	60	0.501	0.130	0.562	0.700	0.911	0.765
	72	0.657	0.935	0.736	0.817	0.816	0.876
	90	0.737	0.834	0.730	0.814	0.894	1.543
Fold		**1.581**	**9.697**	**7.849**	**6.261**	**2.660**	**12.752**
Fruit	0	0.243	0.399	0.018	0.018	0.267	0.121
	6	0.303	0.412	0.198	0.212	0.143	0.022
	12	0.318	0.470	0.020	0.222	0.380	0.443
	18	0.347	0.459	0.024	0. 309	0.636	0.532
	24	0.330	0.327	0.003	0. 356	0.707	0.654
	36	0.342	0.480	0.123	0.460	0.784	0.699
	48	0.225	0.596	0.412	0.427	0.791	0.746
	60	0.338	0.679	0.578	0.452	0.903	0.801
	72	0.365	0.794	0.786	0.470	0.914	0.9221
	90	0.331	0.999	1.476	0.469	0.912	0.782
Fold		**1.877**	**2.424**	**6.962**	**2.368**	**6.377**	**35.545**
Branch	0	0.047	0.439	0.065	0.358	0.224	0.067
	6	0.041	0.348	0.066	0.395	0.245	0.013
	12	0.046	0.441	0.070	0.440	0.626	0.237
	18	0.050	0.437	0.061	0.510	0.690	0.339
	24	0.051	0.330	0.186	0.537	0.687	0.412
	36	0.036	0.445	0.337	0.583	0.685	0.532
	48	0.036	0.633	0.411	0.721	0.761	0.654

	60	0.057	0.511	0.612	0.590	0.893	0.776
	72	0.061	0.513	0.781	0.700	0.719	0.832
	90	0.120	0.599	0.761	0.651	0.718	0.989
Fold		**2.926**	**0.5718**	**11.530**	**1.648**	**2.930**	**76.076**
Leaf	0	0.350	0.207	0.132	0.199	0.224	0.089
	6	0.616	0.204	0.232	0.221	0.442	0.197
	12	0.559	0.219	0.321	0.224	0.765	0.289
	18	0.590	0.215	0.398	0.382	0.824	0.339
	24	0.588	0.232	0.456	0.539	0.750	0.453
	36	0.593	0.291	0.599	0.645	0.865	0.543
	48	0..475	0.466	0.654	0.684	0.891	0.599
	60	0.399	0.548	0.765	0.646	0.912	0.692
	72	0.676	0.691	0.887	0.664	0.923	0.771
	90	0.809	0.851	0.876	0.569	0.912	0.906
Fold		**1.567**	**4.171**	**3.775**	**2.574**	**2.063**	**4.598**
Root	0	0.360	0.126	0.205	0.037	0.173	0.011
	6	0.167	0.133	0.225	0.189	0.200	0.055
	12	0.152	0.148	0.299	0.233	0.582	0.254
	18	0.195	0.168	0.321	0.535	0.937	0.369
	24	0.193	0.279	0.228	0.519	0.886	0.476
	36	0.193	0.242	0.202	0.663	0.929	0.554
	48	0.171	0.479	0.392	0.495	1.027	0.676
	60	0.250	0.616	0.462	0.561	1.092	0.769
	72	0.240	0.532	0.631	0.570	1.101	0.897
	90	0.272	0.854	0.630	0.999	1.189	0.531
Fold		**1.628**	**6.421**	**2.800**	**5.285**	**5.945**	**9.654**
Seed	0	0.018	0.013	0.033	0.025	0.173	0.176
	6	0.079	0.321	0.123	0.036	0.200	0.014
	12	0.085	0.122	0.222	0.271	0.582	0.432
	18	0.101	0.144	0.342	0.856	0.637	0.546
	24	0.103	0.614	0.432	0.911	0.664	0.789
	36	0.084	0.630	0.566	0.983	0.675	0.897
	48	0.539	0.150	0.643	0.783	0.654	0.963
	60	0.556	0.677	0.786	0.843	0.765	1.323
	72	0.664	0.646	0.432	0.788	0.865	1.333
	90	0.751	1.323	0.312	0.760	0.855	0.633
Fold		**9.506**	**4.121**	**2.536**	**21.111**	**4.275**	**45.214**

CONCLUSION

Punica gratanum is one of the most popular herbal remedies in India. It is an important ayurvedic medicine that is provided in different part of country. It is protective against Prostate Cancer, Lung Cancer, Colon Cancer, Skin Cancer, Oesophageal Cancer,

Diabetes, Osteoarthritis, Hypercholesterolemia, Atherosclerosis, Obstructive Pulmonary Disease, Alzheimer's Disease, Tuberculosis, Macular Degeneration and Vision Loss, Erectile Dysfunction, Chronic inflammation (arthritis and cystic fibrosis), Menopausal Symptoms, Anti-histaminic [16,17]. Pomegranate helps to stabilize crease sperm quality. It has anti-malarial properties which protects the neonatal brain against hypoxic-ischemic injury and is classified within the cosmetic industry as a cosmetically (combining a feature of both cosmetic and pharmaceutical[3].It was observed that *Punica gratanum* has antimicrobial and anti amylo proliferative activity as purified extract and its in-vitro. We have made an attempt to provide basic approach for the evaluation of its traditional preparation in order to verify the therapeutic effect of the BE, FE, WFE, RE, BRE, LE and SE in the form of isolated purified extracts of *Punica granatum* against the Urinary tract infections (UTI) The main causative agent of UTI to the best of our knowledge are microbes. There are reports showing the efficacy of *Punica gratanum* products [18].The bark was more efficient scavengers of peroxyl radicals. It was observed that the antioxidant power of PE, FE, WFE, RE, BRE, LE and SE and there is a decrement of extract PE, FE, WFE, RE, BRE, LE and SE respectively. Thus BE showing high antioxidant activity as compared to FE, WFE, RE, BRE, LE and SE. On the other hand, the MICs of the extracts and its fractions observed against the sensitive strain of *E.coli*, *Pseudomonas, Staphylococcus, Proteus mirabilis, Candida* ranged from 5-1000 µg/ml, the values were equally effective to R.A. It was further observed that the antimicrobial activity was increased gradually as on application with BE followed by FE, WFE, RE, BRE, LE and SE. This antimicrobial activity may be due to alkaloids, phenols, polyphenols, tannins, triterpenes and steroids which were found in *Punica granatum* extract and its purified fractions. This phytochemical growth was known to cause antibacterial activities. It was observed that the bacterial growth in term of Optical Density (at 600 nm) was increased by fold without application of extract whereas on addition of extract BE, FE, WFE, RE, BRE, LE and SE (50 µg/ml), there is a decrement of fold, respectively. Further purification and characterization of the active principal compounds from PE and its purified fractions will provide a better antioxidant, antimicrobial and anti lympho proliferative mechanism [19]. In conclusion, the extract and its purified fraction obtained from the *punica granatum* were found to be active enough

for the antimicrobial and anti lympho proliferative activity against the Urinary tract infection pathogens (UTI). Thus the result of this study could provide efficacious and cost effective treatment of the UTI without any harmful effects on human body.

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