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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *PUNICA GRANATANUM* L. (*POMEGRANATE)* PEEL EXTRACT

Pooja G. Singh[2], Madhu S. B.[2], Raghu N.[1], Murugesan Karthikeyan[3], Ashok Gnanasekaran[3], Chandrashekrappa G. K.[4], Raviraja S.[5] and Kanthesh M. Basalingappa[1]*

[1]Division of Molecular Biology, Faculty of Life Sciences, Jagadguru Sri Shivarathreeswara (JSS) University, Mysuru-570015.
[2]Division of Biochemistry, Faculty of Life Sciences, Jagadguru Sri Shivarathreeswara (JSS) University, Mysuru-570015.
[3]Senior Lecturer, Department of Microbiology, Faculty of Medicine, Quest International University Perak, Malaysia.
[4]Chairman, Faculty of Life Sciences, Jagadguru Sri Shivarathreeswara (JSS) University, Mysuru-570015.
[5]Founder Chairman, Royal Research Foundation ®, Mysuru.

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***Corresponding Author**
Dr. Kanthesh M. Basalingappa
Division of Molecular Biology, Faculty of Life Sciences, Jagadguru Sri Shivarathreeswara (JSS) University, Mysuru-570015.

ABSTRACT

Pomegranate is a functional food of great interest, due to its multiple beneficial effects on human health. This fruit is rich in anthocyanin and ellagitannin, which exert a protective role towards degenerative diseases. Whole fruits or peels of pomegranate were subjected to several extraction methods. The obtained extracts were analysed for TPC, TFC, TAC and tannins contents. This was further evaluated for antioxidant capacity and tested for antimicrobial activity on microbes by using polar and nonpolar solvents extracts. Agar disc diffusion assay was conducted and methanol showed highest antimicrobial activity.

KEYWORDS: *Punica granatum*, antioxidant, antimicrobial, DPPH, reducing power assay, Polyphenols, tannins.

INTRODUCTION

Punica granatum Linn, commonly known as pomegranate, is the predominant member of two species comprising the Punicaceae family. The plant is known to have both medicinal and nutritional benefits. Pomegranate fruits comprise of four parts: the not edible exocarp and

mesocarp (the peel), and also the edible endocarp which contains the seeds, forming the arils. All of these contain bioactive molecules. There is an increasing epidemiological and pharmacological evidence that plants contains biologically active components (e.g. free radical scavengers) offering health benefits and protection against degenerative diseases.[1,2,3,4] There is increasing interest in applying fruit processing wastes as functional food ingredients since they are rich source of dietary fibers, and most of the beneficial bioactive compounds are remained in those byproducts.[5,6] In fact, phenolic compounds from plants exhibit various physiological properties, such as anti-allergenic, anti-inflammatory, anti-microbial, anti-oxidant effects.[7,8, 9,10,11]

MATERIALS AND METHOD

1kgs of fresh and healthy *Pomegranate kes*ar was collected from super market JP Nagar, Mysuru, Karnataka. The fresh Pomegranates were than washed, peels were separated from the fruits and then the peels were completely dried in a hot air oven at 35◦ C for 4 to 5 days. After complete drying, peels were then powdered. This powder was then weighed stored in air tight bottles and used for the compound extraction. About 61g of shade dried powdered Pomegranate peel was taken and divided into 5 groups of each 10g of peel powder. Each group was then subjected to polar, non-polar, acidified water and aqueous extract. The peel powder was soaked in 90ml of the respective solvents like methanol, ethanol, diethyl ether, acidified water and aqueous extract. The soaked plant extracts were then incubated at room temperature for two days. The obtained extract was further used to test the anti-oxidant and anti-microbial nature of the pomegranate peel extract against phytochemical tests, reducing power assay, and DPPH assay and test microorganism. These obtained plant extracts were then made into three different concentrations to get the comparative results on the antibacterial strength of the plant extract against *E.coli.* These concentrations were then used to make discs of various concentrations like 400mg/ml, 800mg/ml, 1000mg/ml. Likewise antioxidant process was carried out by Reducing Power assay and DPPH assay which 0.5g of extract was treated with 10ml of respective solvents to carry out phytochemical analysis tests.

Phytochemicals constituents and active ingredients

The active ingredient that has significant pharmacological action in pomegranate is designated by Indian scientist as "Phyllemblin". The fruit is rich in quercetin, phyllaemblic compounds, gallic acid, tannins, flavonoids, pectin, and vitamin C, vitamin K and also contains various polyphenolic compounds.[12,13,14]

QUANTITATIVE ANALYSIS

Determination of total phenolics

The total phenolic content was estimated using Folin-Ciocalteu colorimetric method. Test sample (100 µl) was reacted with 250µl of Folin-Ciocalteu reagent (previously diluted 10 fold with distilled water) and allowed to stand at 22°C for 5 min. Their action was neutralized with saturated sodium carbonate 1500µl and allowed to stand for 1.5 hr in the dark at 22°C. The absorbance of the resulting blue colour was measured at 765 nm (Hitachi U-3900 UV/visible spectrophotometer). Total phenolics were quantified by calibration curve obtained from measuring the absorbance of known concentrations of gallic acid standard (20 to 100µg). The total phenolic contents were expressed as gallic acid equivalence (GAE) in µg.

Determination of total flavonoids

The total flavonoid content was estimated using aluminium-trichloride by colorimetric method. Catechin was used as standard (1 – 5µg). Extract (10-50µl)were made up to 1ml using methanol, mixed with 4ml of distilled water and add 0.3ml of 5% $NaNO_2$ solution, 0.3ml of 10% aluminium-trichloride solution was added after 5 min of incubation and the mixture was allowed to stand for 6 min. Then, 2ml of 1mol/L NaOH solution was added, and the final volume of the mixture was brought to 20ml with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510nm. The total flavonoids content was calculated from a calibration curve observed from measuring the absorbance of known concentrations of Catechin as standard (10 to 50µg). The total flavonoid content was expressed as catechin equivalence (CE) in µg.

QUALITATIVE ANALYSIS

1. Test for tannins: About 0.1g of pomegranate peel extract was weighed and diluted using respective solvents. Diluted extract (0.5ml) was taken in test tube followed by addition of 10ml of distilled water and filtered using whatman no 1 filter paper. Ferric chloride reagent (3 drops) was added to the filtrate. The appearance of a blue – black or green precipitation confirmed the presence of gallic tannins or catechol tannins.[15]

Test for saponins: About 0.1g of pomegranate peel extract was weighed and diluted using respective solvents. Different solvent extracts (1ml) were taken in test tubes and 5ml of distilled water is added, then formation of stable foam indicated the presence of saponins.\

	Acidified water	Ethanol	Diethyl ether	Methanol
TANNINS	-	+	-	+
SAPONINS	-	+	+	+

***+ indicates presence - indicates absence of compounds

ANTIOXIDANT ACTIVITY

Evaluation of free radical scavenging activity.

DPPH Radical Scavenging Activity

The free radical scavenging property of pomegranate peel extract of different solvents such as acidified water, diethyl ether, ethanoland methanol were determined by DPPH method. The DPPH radical solution was prepared in methanol.[16] The reaction mixture contained 5µl of test samples and 95 µl of DPPH (50µM) in methanol. The reaction for scavenging DPPH radical was carried out by using 96 well plates at 37°C in dark for 30 min and the absorbance was recorded at 517 nm[17] (Thermo Scientific Varioskan Flash Microtiter plate reader). Percent radical scavenging activity was determined by comparing with a solvent added as control. The IC_{50} values were determined which denote the concentration of extracts required to scavenge 50% DPPH radicals.[18] 15 ascorbic acid (1–10µg) was used as standard. Percent scavenging effect was determined by the following equation.

$$\text{Inhibition (\%)} = \frac{[(\text{Absorbance of control} - \text{Absorbance of test sample})]}{\text{Absorbance of control X100}}$$

REDUCING POWER ESTIMATION

The reducing power assay of pomegranate peel extract was estimated by ferric chloride ($FeCl_3$). Test sample (0.1g) was mixed with equal volume of phosphate buffer (0.2M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). It was incubated at 50°C for 20min. Trichloro-acetic acid (TCA) (10%, 2.5 mL) was added to the mixture, which was then centrifuged at 3000rpm for 5min. After centrifugation, the supernatant (1ml) was taken in test tube and mixed with equal volume distilled water and $FeCl_3$ (0.5ml, 0.1%). Ascorbic acid was used as standard and phosphate buffer was used as blank solution. Absorbance was measured at 700nm (Beckman-coulter, DU730 life sciences UV/ visible spectrophotometer). Increased absorbance of the reaction mixture indicates stronger reducing power.

ANTIBACTERIAL ASSAY

The anti-microbial nature of the peel extract was tested by disc diffusion method. Each petri plate which was swabbed with the test microorganism was placed with whatman No.1 filter paper discs of different concentrations. Now these plates were placed in the incubator for 48hrs. The zone of inhibition (in mm diameter) was measured at regular intervals of 0 hr., 12 hr., 24 hr and 48 hr. Control was simultaneously taken and the results were tabulated. The comparative potent of the anti-microbial nature of the different solvent extracts of the peel were then evaluated by comparing the ZOI exhibited.

RESULTS

Phytochemical constituents and active ingredients.

PHYTOCHEMICAL ANALYSIS

The physical properties of pH and moisture content of pomegranate peel were found to be slightly acidic. The Phytochemicals analysis of pomegranate peel extract is summarized in Table 1. Pomegranate peel contains lot of nutritive and antinutritive compounds.

Table 1: Total weight of extracts of peel in different solvents.

Sample	Solvent	Extracts in Grams
P.Granatum	Acidified water	0.362
	Diethyl ether	0.093
	Ethanol	0.2512
	Methanol	0.4416

Saponins, tannins (Table 2), flavonoids and phenolic content were found to be higher in methanolic extract than other solvent extraction. Smaller amounts of biologically active compound were extracted by acidified water, diethyl ether and ethanol.

Table 2: Qualitative analysis of Tannins and Saponins.

	ACIDIFIED WATER	ETHANOL	DIETHYL ETHER	METHANOL
TANINS	-	+	_	+
SAPONINS	-	+	+	+

+ indicates presence - indicates absence

The amount of total phenol was observed as 120µg (Figure 1) of peel as gallic acid equivalents and the amount of total flavonoids content was observed as 100µg (Figure 2) as catechin equivalents of pomegranate peel.

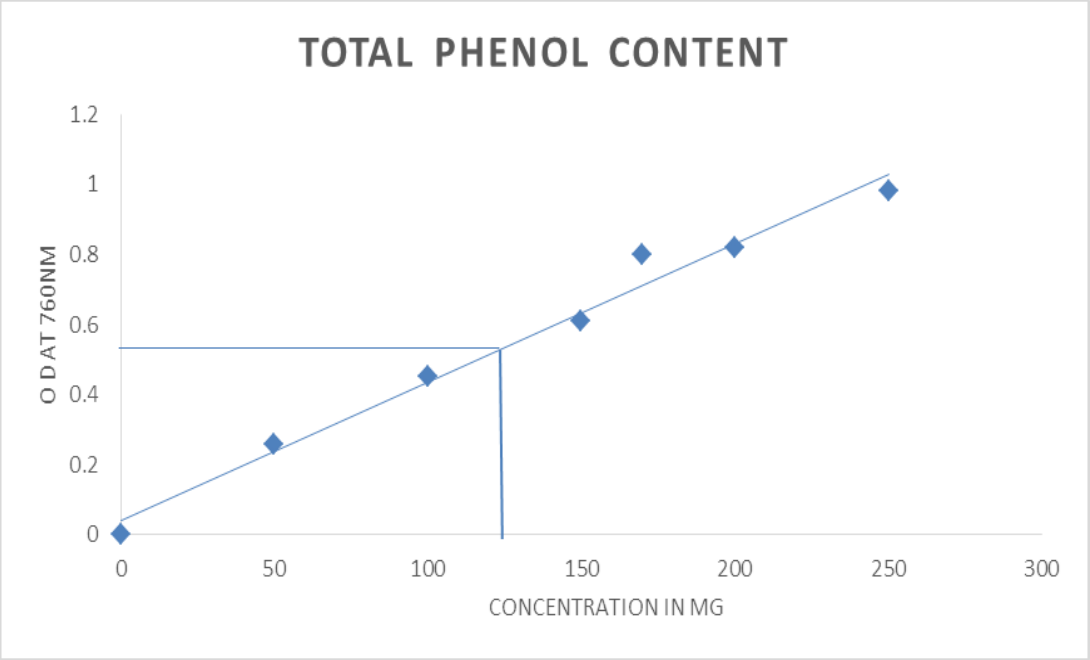

Figure 1: Total phenol content estimated from the standard graph of gallic acid and results are expressed as gallic acid equivalents (GAE).

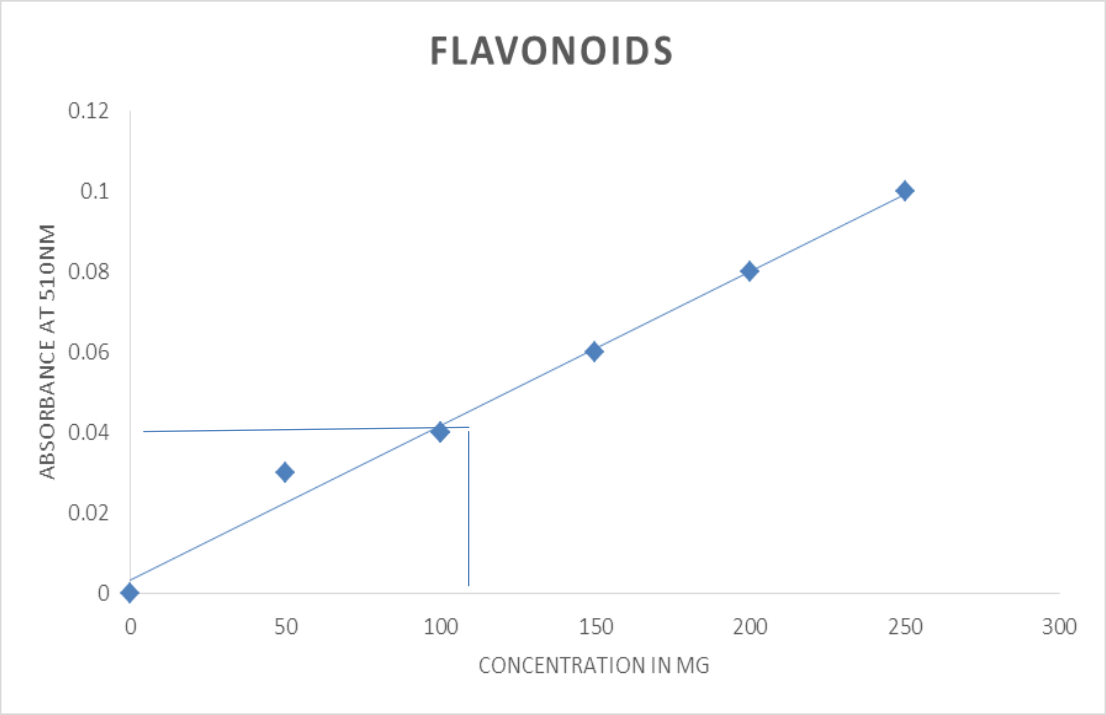

Figure 2: Total phenol content estimated from the standard graph of catechin and results are expressed as catechin equivalents

Estimation of DPPH free radical scavenging activity and Reducing Power Assay

Our study clearly demonstrated that the *P. Granatum peel* extracts have good antioxidant properties when assessed by DPPH and Reducing Power Assay. The percentage of DPPH

decolorization is attributed to hydrogen donating ability of test compounds. Variable DPPH activity was recorded for the pomegranate peel extract. The extracts of *P. Granatum peel* extracts had shown moderate activity. Methanolic extract exhibited higher antioxidant activity Fig. 3; IC_{50}24 µg) when compared to other extracts while Ethanol (Fig. 4) and Diethyl ether (Fig. 5) has the lowest DPPH radical scavenging activity. Reference standard ascorbic acid showed 50% inhibition at 42µg.

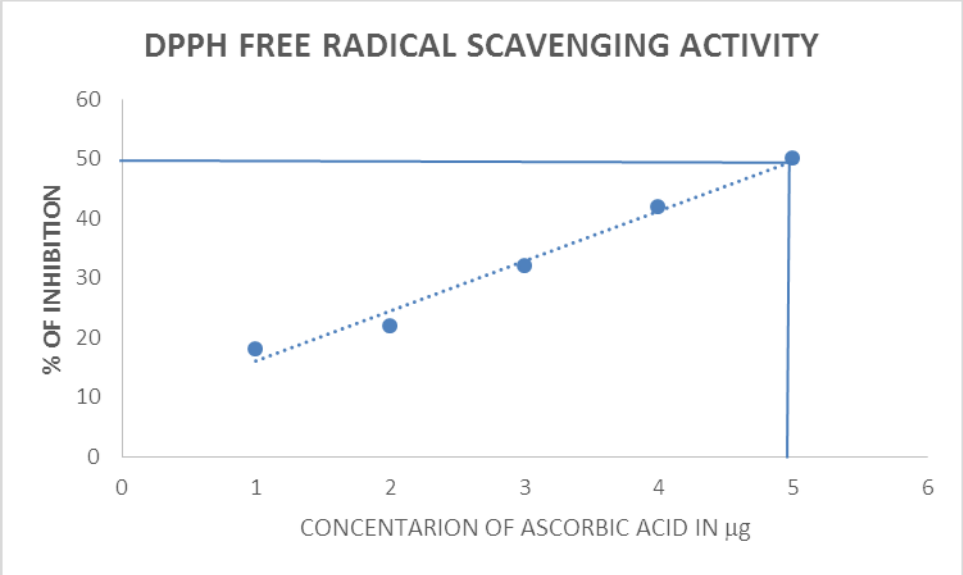

Figure 3: DPPH scavenging capacity estimated from the standard graph of ascorbic acid and results are expressed as IC_{50} values

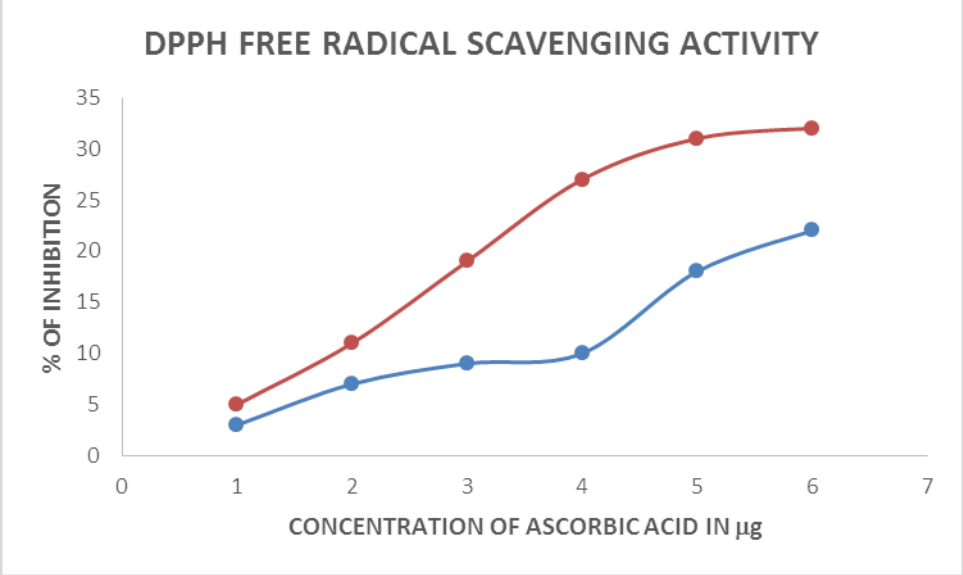

Figure 4: DPPH scavenging capacity estimated from the standard graph of ascorbic acid and results are expressed as IC_{50} values for ethanol and diethyl ether extract.

REDUCING POWER ASSAY

The IC_{50} for methanol extract of methanolic extract is 40µg in Reducing Power assay (Fig. 6). The methanol extracts of pomegranate peel had good activity, whereas Ethanol and diethyl ether showed moderate activity. Reference standard ascorbic acid showed 50% inhibition. Lower IC_{50} value implies higher antioxidant power. The higher reducing power indicates the presence of reductones which are able to break free radical chain by donating hydrogen atoms and thus, converting them to a more stable non-reactive species.

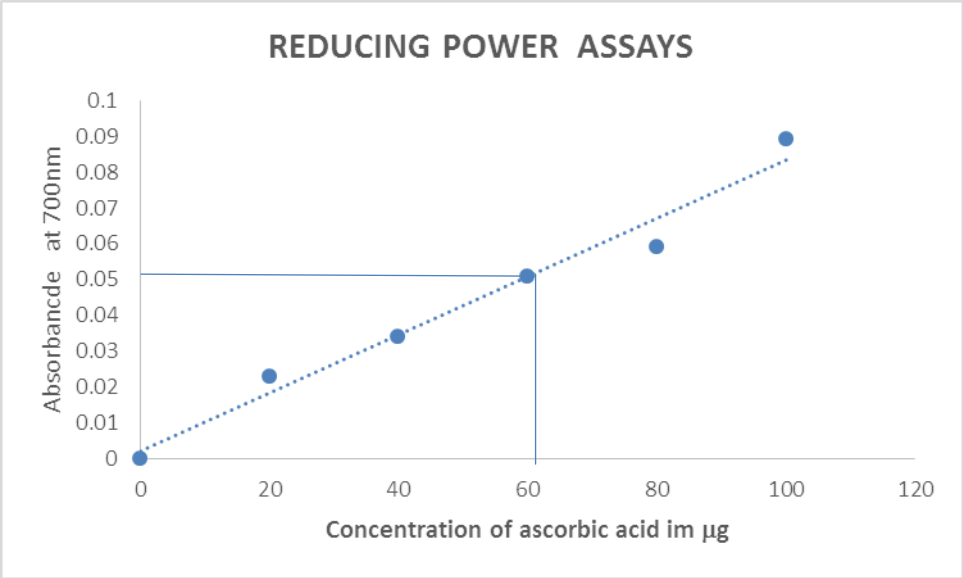

Figure 6: Reducing power capacity estimated from the standard graph of ascorbic acid And results are expressed as IC_{50} values for methanolic extract.

Antibacterial estimation

Punica granatum. is a plant of great medicinal importance and has been extensively used as antipyretic, analgesic and immunomodulatory agent. Anti-bacterial activity of the extracts was initially screened as reported earlier by the standard agar disc diffusion method.[19] Streptomycin inhibited the growth of test bacterial samples. In this study the *in vitro* antibacterial activity of extracts of peel from *Punic* were assayed alone, and also in comparison with commonly used the antibiotic, Streptomycin.

The methanolic solvent extract of the peel showed antibacterial activity against the clinical isolates of bacteria like *Escherichia coli* and the results were recorded at different time intervals of 12h, 24 h and 48h and the results for *E. coli* is presented in Fig. 7. Maximal antibacterial activity was obtained for *E. coli* (4.32 mm; Table 3).

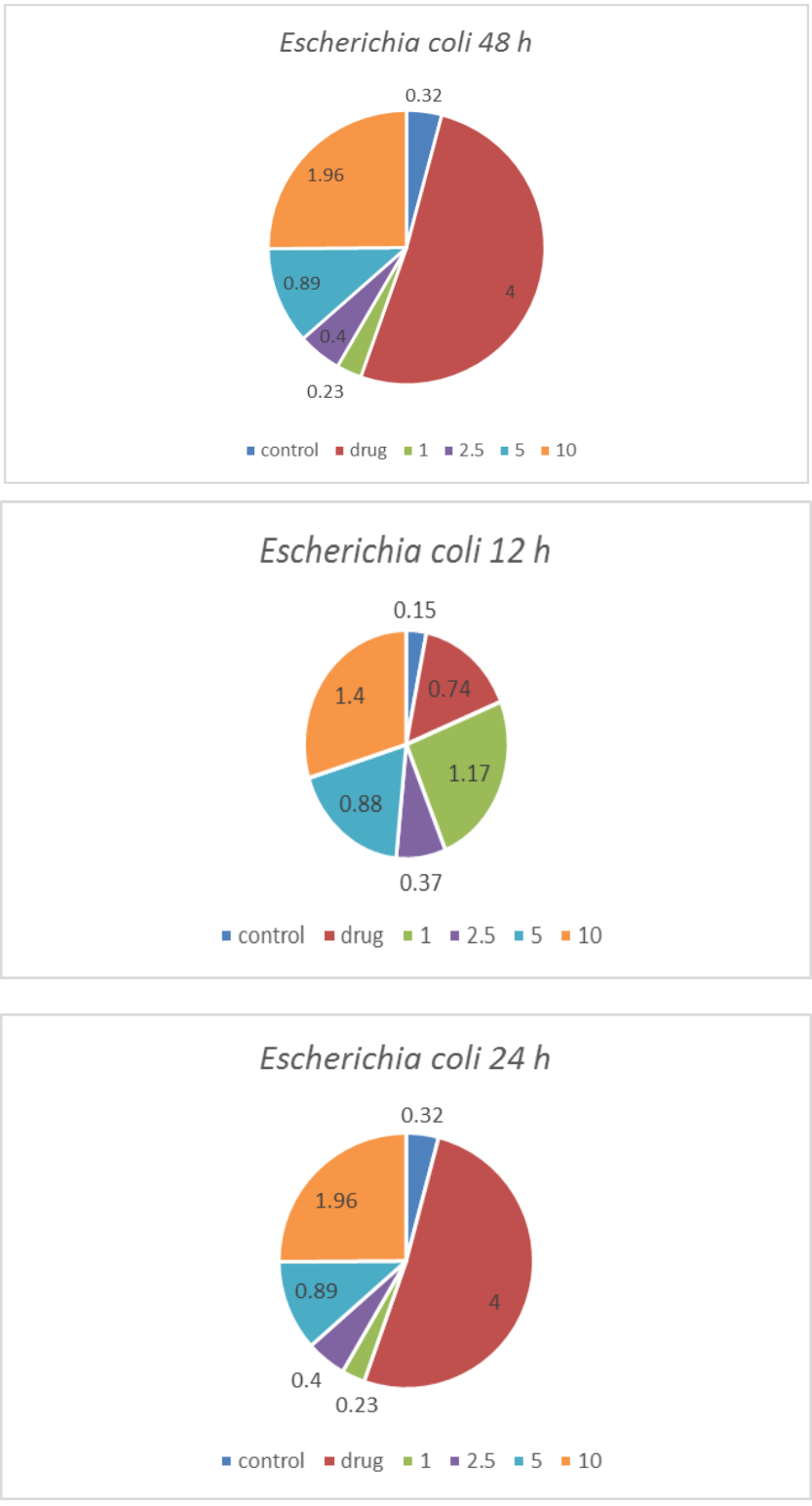

Figure 7: Results of methanolic seed extract inhibiting *Escherichia coli* at different time intervals.

Table 3: Antibacterial activity of methanolic extract of pomegranate peel exhibited towards test microbe *Escherichia coli.*

SL NO.	SOLVENT USED	TEST MICRO ORGANISM	CONCENTRATION	ZONE OF INHIBITION T0	T12	T24	T48
1	METHANOL	*Escherichia. coli*	CONTROL	0.0	0.15mm	0.32mm	0.37mm
			DRUG	0.0	0.74mm	4.0mm	4.32mm
			1	0.0	1.17mm	0.23mm	0.26mm
			2.5	0.0	0.37mm	0.40mm	0.47mm
			5	0.0	0.88mm	0.89mm	1.36mm
			10	0.0	1.4mm	1.96mm	2.55mm

DISCUSSION

Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development.[20] The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries for health programs.[21] Besides the investigation of single compounds, a growing interest persists on application of standardized extracts. Studies have been devoted to assess the capacity of natural products and consequently attenuating disease conditions involving inhibition of ROS.

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased.[22] In general bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents. In the present study, in DPPH method, a freshly prepared DPPH solution is used that exhibits a deep purple colour with absorption maximum at 517nm.[23] The purple colour generally fades or disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench the DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH molecule) and convert them to a colorless (i.e., 2, 2- diphenyl-1-hydrazine, or a substituted analogous hydrazine)[24] resulting in a decrease in absorbance at 517nm. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant of the extract. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.[25,26] The reducing ability is generally associated with the presence of reductones, which breaks the free radical chain by donating a hydrogen atom. The extract had reductive ability which increased with increasing concentrations of the extract.[27]

Screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective compounds. Plant-derived antimicrobial compounds might inhibit bacteria through different mechanisms than conventionally used antibiotics and could therefore be of clinical value in the treatment of infections caused by resistant microbes. Methanol extracts were most effective against Gram (−) bacteria *viz., E. coli*. Differential sensitivity of bacterial species (gram-negative) toward phenolics and phenolic acids were reported earlier.[28]

CONCLUSION

The present study deals with pomegranate peel for antibacterial and antioxidant properties. The bioactive compounds were extracted through different solvents. The Phytochemicals in pomegranate peel were flavonoids, phenols, tannins and saponins. The polyphenols and ascorbic acids lead to the medicinal and antioxidant activity. This therapeutic nature of peel also supports the treatment of diseases and discovery of new drugs.

REFERENCE

1. Ahmad, I., Beg, A.Z., 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethno pharmacology, 7*4: 113–133.
2. Arun N, Singh D.P., 2012. *Punica granatum*: A review on pharmacological and therapeutic properties. *International journal of pharmaceutical sciences and research,* 3: 1240-5.
3. Sastravaha, G., Yotnuengnit, P., Booncong, P., Sangtherapitikul, P., 2003. Adjunctive periodontal treatment with *Centella asiatica* and *Punica granatum* extracts. A preliminary study. *Journal of the Interntional Academy of Periodontology*, 5: 106-15.
4. Dai, J., Russell, J. M., 2010.Plantphenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15: 7313–7352.
5. Naz, S., Siddiqi, R., Ahmad, S., Rasool, S., Sayeed, S., 2007. Antibacterial activity directed isolation of compounds from *Punica granatum*. *Journal of Food Sciences,* 72: 341–345.
6. Kim, N.D., Mehta, R., Yu, W., Lansky, E., 2002. Chemo preventive and adjuvant therapeutic potential of pomegranate (*Punica granatum*) for human breast cancer. *Breast Cancer Research and Treatment*, 71: 203–217.

7. Ibrahim, M.I., 2010. Efficiency of Pomegranate Peel Extract as Antimicrobial, Antioxidant and Protective Agents. *World Journal of Agricultural Sciences*, 6(4): 338-344.
8. Kulkarni, A., Aradhya, S., 2005. Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chemistry*, 93: 319-324.
9. Braga, L.C., Leite, A.A., Xavier, K.G., 2005. Synergic interaction between pomegranate extract and antibiotics against *Staphylococcus aureus*. *Canadian Journal of Microbiology*, 51: 541-547.
10. Elfalleh, W., Hannachi, H., Tlili, N., Yahia, Y., Nasri, N., Ferchichi, A., 2012. Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. *Journal of Medicinal Plants Research*, 6: 4724– 4730.
11. Prashanth, D., Asha, M., Amit, A., 2001. Antibacterial activity of *Punica granatum*. *Fitoterapia*, 72: 171–173.
12. Carballo, S., G., Rodriguez, C.G., Krueger, M., Dreher., Reed, J.D., 2009. Pomegranate (*Punica granatum*) supplements: Authenticity, antioxidant and polyphenol composition. *Journal of Functional Foods*, 35: 1-6.
13. Chidambara, M.K., Reddy, V.K., Veigas, J.M., Murthy, U.D., 2004. Study on wound healing activity of *Punica granatum* peel. *Journal of Medicinal Food*, 7: 256-259.
14. Jasuja, N.D., Saxena, R., Chandra, S., Sharma, R., 2012 Pharmacological Characterization and Beneficial Uses of Punica granatum. *Asian Journal of Plant Sciences, 11(6): 251-267.*
15. Bhattacharya, A., Chatterjee, A., Ghosal, S., Bhattacharya, S.K., 1999. Antioxidant activity of active tannoid principles of *Emblica officinalis*. *Indian Journal of Experimental Biology*, 37: 676–680.
16. Rackova, L., Oblozinsky, M., Kostalova, D., Kettmann, V., Bezakova, L., 2007. Free radical scavenging activity and lipoxygenase inhibition of *Mahonia aquifolium* extract and isoquinoline alkaloids. *Journal of Inflammation*, 4: 15-21.
17. Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., Cheng, S., 2006. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry*, 96: 254–260
18. Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. *LWT –Food Science and technology*, 28: 25-30.

19. Nabavi, S.M., Ebralimzadeh, M.A., Nabavi, S.F., Jafari, M., 2008. Free radical scavenging activity and antioxidant capacity of *Euryngium Caucasicum Trautv* and *Froripia sub pinnata. Pharmacology online*, 3: 19-22.
20. Orech, F.O., Akenga, T., Ochora, J., Friis, H., Aagaard, H.j., 2005 Potential Toxicity of some traditional leafy vegetables consumed in Nyangoma division, Western Kenya. *African Journal Food Nutrition Sciences*, 5(1): 1-13.
21. Puupponen-Pimiä, R., Nohynek, L., Meier, M., Kähkönen, M., Heinonen, A., Hopia., Oksman-Caldentey, K. M., 2001. Antimicrobial properties of phenolics compounds from berries. *Journal of Applied Microbiology*, 90: 494–507.
22. Leval, X. D., F. Julemont, J., Delarge, B., Pirotte., and Dogne, J. M., 2002. New trends in dual 5-LOX/COX inhibition. *Current Medicinal Chemistry*, 9: 941–962.
23. Nadheesha, M.K.F., Bamunuarachchi, A., Edirisinghe, B. M., Weerasinghe, K., 2007. Studies on Antioxidant Activity of Indian Gooseberry Fruit and Seed. *Journal of Science of the University of Kelaniya Sri Lanka*, 3: 83-92.
24. Nair, R., Sumitra, V., Chanda, C., 2007. Antibacterial activities of some medicinal plants of western regions of India. *Turkish Journl of Biology*, 3(1): 231-236.
25. Sharma, S.K., Perianayagam, B.J., Joseph, A., Christina, A.J.M., Anti inflammatory activity of ethanol in aqueous extracts. Hamdard Medicines, 46: 75-78.
26. Perianayagam, J.B., Narayana, S., Ganasekar, G., Pandurengan, A., Rajagopal, K., Vijayakumar, S.G., 2005. Evolution of ant diarrheal potential of *Emblica officinalis*. *Pharmaceutical Biology*, 43(4): 373-377.
27. Khan, J.A., Hanee, S., 2011. Antibacterial properties of *punica granatum* peels. *International journal of applied biology and pharmaceutical technology, 2(3).*
28. Panda, S., Kar, A., 2003. Fruit extract of ameliorates hyperthyroidism and hepatic lipid peroxidation in mice. *Pharmazie*, 58: 753–761.