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EVALUATION OF ANTI-OXIDANT AND ANTIBACTERIAL ACTIVITY OF POMEGRANATE (*PUNICA GRANATUM* L.) SEED EXTRACT

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ABSTRACT

In the present study Aqueous, Methanol, Acetone and Ethyl acetate extract of seeds of *Punica granatum* were prepared and evaluated for its antioxidant and antibacterial properties against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter aerogenes, Pseudomonas aeruginosa*. The results showed the highest extraction efficiencies were for Methanol and Acetone solvents in extraction of seed. The methanol extract has shown highest antibacterial activity compared to other extracts. Among the selected bacterial cultures, the highest antibacterial activity was recorded against *Staphylococcus aureus, Escherichia coli*. Antioxidative study was evaluated by DPPH assay. DPPH assay showed potential antioxidative activity by the various extracts. It can be concluded Pomegranate seed, which possesses high levels sources of the natural antioxidants with potent antibacterial properties.

KEYWORDS: Pomegranate seed, Antioxidant, antibacterial activity, bacterial pathogens.

INTRODUCTION

Pomegranate (*Punica granatum* L.), one of the fruit plants often grows in the garden as ornamental tree and the fruit is edible as well. It is an important fruit which originated in the Middle East, eastward to Asia (China and India). It has been extensively used as a traditional medicine in many countries for the treatment of different types (Arun and Singh, 2012). Pomegranates are popularly consumed in its in natural form, like juices, foods such as jams, jellies and extracts, (Goula and Adamapoulos, 2012) which are used as plant ingredients in natural medicine and dietary supplements (Mohagheghi *et al.*, 2011). According to Garau *et*

al. (2007), recent decades researchers have given special attention to the use of industry-derived residues, especially the food industry, both for the reduction of the environmental damage caused by them and consequent reduction of costs to the treatment process, and for their potential use in the development of high value-added products. Recent studies have revealed characterization and extraction techniques as well as the biological potential of agro-industrial residues such as antioxidant biomolecules obtained through simple extraction from chestnut and hazelnut shells (Nazzaro *et al.*, 2012), the drying temperature influence on the physico-chemical characteristics of dietary fibers and antioxidant capacity of orange byproducts (Garau *et al.,* 2007), tannin extraction from nuts residues (Capparucci *et al.,* 2011) and antioxidant activity of extracts of pomegranate peels compared with pomegranate juice extracts (Li *et al.,* 2006).

Many studies have shown the antioxidants contained in fruits and vegetables, including ascorbic acid, carotenoids, flavonoids, hydrolysable tannins, play an important role in the prevention of creation several diseases (Huxley and Neil 2003; Knekt *et al.*, 2002 ; Lampe 1999). Recently, natural antioxidants have become very popular for medical and food applications (Lapornik et al. 2005; Landbo and Meyer 2001; Spigno and De Faveri 2007). Extraction is the first step in the commercial isolation of these antioxidant compounds from fruits. Because of the presence of large amounts of certain pharmaceutical, nutraceutical and antioxidant components in different parts of fruits such as Pomegranate, type of solvent to obtain these components is highly demanded in the food industry. In addition, this plant is reported to have excellent antibacterial (Dahham *et al.,* 2010; Elfalleh *et al.,* 2011), antifungal (Abdollahzadeh *et al.,* 2011), and antioxidant activities due to an excellent source of phytochemical compounds such as polyphenolic compounds include falvonoids, anthocyanins, condensed and hydrolysable tannin (Al-Zoreky *et al.,* 2009). Considering that the seeds of pomegranate which is inedible part of fruit, can be used in pharmaceutical and other commercial products for developing the new value-added products from fruit wastes. Thus, the aims of the present study were to evaluate antioxidant and antibacterial activities of the extract of pomegranate seeds.

MATERIALS AND METHODS

Materials

Pomegranate fruits: The pomegranate fruits were purchased and collected from a well known market in Mumbai City. The mean weight of each fruit was 250 g. The Pomegranate

seeds (PS) were directly isolated from fruits by pressing them with soft cotton and then washed to remove any adhering Pomegranate flesh and finally oven-dried at 25°C for 24 h. dried Pomegranate seeds after removing their oil by cold press. Then they were stored at −18°C for subsequent tests.

Bacterial cultures

The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacterial cultures were rejuvenated in Mueller-Hinton broth (Hi-media laboratories, Mumbai, India) at 37^0C for 18h and then stocked at 40C in Mueller-Hinton Agar. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards (NCCLS, 2002) guideline. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 370C for 3h until the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 105 CFU/mL with the help of SPC and Nephlo-turbidometer.

Table 1: Bacterial cultures used in study (IMTECH, Chandigarh, India).

Bacterial Pathogens	MTCC Number
Staphylococcus aureus	96
Escherichia coli	739
Pseudomonas aeruginosa	424
Klebsiella pneumoniae	109
Enterobacter aerogenes	111

Preparation seed extracts

The seeds powders were mixed with solvent at a ratio of 1:10 (w/v). The solvents, were Water, Methanol, Acetone, and Ethyl acetate. After 24 h of shaking of seeds at room temperature in solvent liquid was separated from the solid using vacuum filtration through a Whatman No.1 filter paper. The residues were re-extracted by the same solvent. All extracts were concentrated under vacuum at 60°C and the concentrates were dried, desiccated and stored at −18 °C for subsequent test.

METHODS

Evaluation of antioxidant properties of Pomegranate seed extracts

Several methods have been developed to measure the antioxidant capacity of pure compounds and plant extracts. One of the most frequently used techniques for measuring antiradical capacity is depletion of the free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) by

addition of scavenger compounds. It determines the ability of crude extracts for trapping this unpaired-electron to disappearance of radical color. This will lead to the formation of less reactive species derived from the antioxidant (Brand-Williams *et al.,* 1995). There are many assays which have been used to assess the total antioxidant content of foods, e.g. the ferric-reducing ability of plasma (FRAP). The FRAP assay directly measures antioxidants with a reduction potential below the reduction potential of the Fe3+/Fe2+ couple (Halvorsen *et al.,* 2002). Therefore, the antioxidant properties of extracts were determined using two methods, DPPH. These methods seem to be rapid and accurate methods for assessing the antioxidant activity of Pomegranate seed extracts.

Antioxidant activity

DPPH Assay

The percentage of antioxidant activity of the aqueous peel was assessed by DPPH free radical assay. Thesamples were reacted with the stable DPPH radical in a methanol solution. The reaction mixture consisted of 3.7mL of absolute methanol in all test tubes along with blank. The blank tube was added with 100µL of absolute methanol and 100µL of respective samples to all other tubes marked as tests27. Finally 200µL of DPPH reagent were added to all the test tubes including blank. The test tubes were incubated in dark condition for 30minutes to the reaction to take place. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read (absorbance) at 517nm. The scavenging activity percentage was determined according;

% Antioxidant activity={(absorbance at blank)-(absorbance at test)/absorbance at blank)} X100

Preparation of disc for antibacterial activities: The aqueous, ethanol, methanol and acetone extracts were prepared in their respective solvents and the sterile blotting paper disc (10 mm) were soaked in the diluted extract in such concentration that the amount of solution absorbed by each disc was 1mg, 2mg, 3mg, 4mg, 5mg of each extracts of Pomegranate seed. The prepared disc were dried in controlled temperature to remove excess of solvent and used in study.

Antibacterial activity using disc diffusion method: The modified paper disc diffusion method was employed to determine the antibacterial activity of aqueous, ethanol, methanol and acetone extracts. Turbidity of inoculums was matched with McFarland turbidity standard (NCCLS, 2002). Inoculums were spread over the Nutrient agar plate using a sterile cotton

swab in order to get a uniform microbial growth. Then the prepared antibacterial disc were placed over the lawn and pressed slightly along with positive and negative controls. Ampicillin 10 mcg/disc (Hi-Media, Mumbai) were used as positive control while disc soaked in various organic solvents and dried were placed on lawns as negative control. The plates were incubated for 18h at 37^0C. The antibacterial activity was evaluated and diameters of inhibition zones were measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. The antibacterial activity was classified as strong (>20mm), moderate (16-19mm) and mild (12-15mm) and less than 12mm was taken as inactive.

RESULTS AND DISCUSSION

The antibacterial activity has been attributed to the presence of some active constituents in the extracts. Most plants are able to produce a diverse range of bioactive molecules which become a rich source of different types of medicines. An interesting feature of plants in the present is focused on phytochemical compounds as potential sources of functional substances such as antioxidant and antimicrobial substances.

Table 2: Antibacterial activity of Pomegranate seed extracts against bacterial pathogens (Zone of inhibition of growth in mm, average of 3 readings).

Medicinal Plants	Solvent extract	*S. aureus*	*E. coli*	*K. pneumonia*	*E. aerogenes*	*P.aeruginosa*
Pomegranate seed extracts	Aqueous	14	13	12	-	-
	Ethanol	18	17	14	17	-
	Methanol	23	20	17	19	14
	Acetone	25	23	21	20	17
Negative control	Water	-	-	-	-	-
	Ethanol	-	-	-	-	-
	Methanol	-	-	-	-	-
	Acetone	-	-	-	-	-
Positive control	Ampicillin (10mcg/disc)	24	11	16	30	19

According to antibacterial profile (Table 2), maximum inhibitory effect of the aqueous extract observed only on *Staphylococcus aureus* and moderate antibacterial against *Escherichia coli, Enterobacter aerogenes,* but mild inhibitory effect on *Pseudomonas aeruginosa,*. Methanol and ethanol extract showed strong antibacterial effect against *Staphylococcus aureus* and moderate antibacterial against *Escherichia coli, Enterobacter*

aerogenes, but mild effect on *Pseudomonas aeruginosa.* Acetone extract showed maximum inhibitory effect on *Staphylococcus aureus, Pseudomonas aeruginosa,* but moderate inhibitory effect on *Escherichia coli, Enterobacter aerogenes.* Several researchers have reported on the medicinal properties of plants derived compounds. These classes of compounds are known to show curative activity against several bacterial and it is not surprising that these plants extracts are used traditionally by herbalist to cure bacteria related ill-health.

The DPPH· scavenging activity has been commonly used to detect antioxidant activity of different samples sources, due to its sensitivity to lower concentrations of active standards from natural sources. The steady radical, DPPH, has a maximum absorbance at 517nm and could swiftly undergo scavenging by antioxidants. Complex free radical scavenging activities of samples are indicated by lower absorbance at 517nm. It was found that the highest concentration of aqueous extract at around 500µL had the highest percentage of antioxidant activity

Table 3: Antioxidant activity of Pomegranate seed extracts

Sr.No	Concentration (uL)	Solvent extract			
		Aqueous	Ethanol	Methanol	Acetone
1.	100	30.8	31.2	32.8	33.2
2.	200	56.2	58.1	60.2	61.4
3.	300	78.8	80.1	82.4	84.7
4.	400	86.2	82.3	85.3	88.5
5.	500	90.3	91.2	93.4	94.8

CONCLUSION

Our results suggest that the various extract of pomegranate is a potential source of antibacterial and antioxidant agents and could be used as a natural antioxidant and preservative in food and nonfood systems. Over viewing the reducing capacity, the use of these aqueous seed extract of pomegranate might contribute a certain level of health fortification against oxidative damages. With the established antioxidant activity of this extract, the specific isolation of the active components in the aqueous extract of pomegranate and characterization should be further examined.

REFERENCES

1. Abdollahzadeh SH, Mashouf RY, Mortazavi H, Moghaddam MH, Roozbahani N, Vahedi M. Antibacterial and antifungal activities of Punica granatum peel extracts against oral pathogens. J Dent (Tehran), 2011; 8(1): 1-6.

2. Al-Zoreky NS. Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels. Int J Food Microbiol, 2009; 134(3): 244-8.
3. Arun N and Singh DP. Punica granatum: A review on pharmacological and therapeutic properties. Inter. J Pharm Sci Res, 2012; 3(5): 1240-45.
4. Dahham SS, Ali MN, Tabassum H, Khan M. Studies on antibacterial and antifungal activity of pomegranate (Punicagranatum L.). American-Eurasian J Agric & Environ Sci, 2010; 9(3): 273-81.
5. Elfalleh W, Tlili N, Nasri N, Yahia Y, Hannachi H, Chaira N, et al. Antioxidant capacities of phenolic compounds and tocopherols from tunisianpome granate (Punica granatum) fruits. J Food Sci, 2011; 76(5): 707-13.
6. Goula, M. A.; Adamapoulos, G. K., 2012, A method for pomegranate seed applicatios in food industries: seed oil encapsulation. Food and Bioproducts Processing, 90: 639-652.
7. Huxley RR, Neil H. The relationship between dietary flavonol intake and coronary heart disease mortality: a meta-analysis of prospective cohort studies. Eur J Clin Nutr, 2003; 57: 904–908.
8. Knekt P, Kumpulainen J, Jarvinen R, Rissanen H, Heliovaara M, Rreunanen A, et al. Flavonoid intake and the risk of chronic diseases. Am J Clin Nutr, 2002; 76: 560–568.
9. Lampe JW. Health effects of vegetables and fruits: assessing mechanism of action in human experimental studies. Am J Clin Nutr, 1999; 70: 475s–490s.
10. Landbo AK, Meyer AS. Enzyme-assisted extraction of antioxidative phenols from black currant juice press residues (Ribes nigrum) J Agric Food Chem, 2001; 49(7): 3169–3177.
11. Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol, 2007; 109(2): 177–206.
12. 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.
13. Mohagheghi, M.; Rezaei, K.; Labbafi, M.; Mousavi, S. M. E., 2011, Pomegranate seed oil as a functional ingredient in beverages. European Journal of Lipid Science and Technology, 113: 730–736.
14. Nazzaro M., Mottola V., La Cara F., Del Monaco G., Aquino R. P. and Volpe M.G., 2012, Extraction and characterization of biomolecules from agricultural wastes. Chemical Engineering Transactions, 27: 331-336.

15. Spigno G, De Faveri DM. Antioxidants from grape stalks and marc: Influence of extraction procedure on yield, purity and antioxidant power of the extracts. J Food Eng, 2007; 3(78): 793–801.