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Evaluation of Free Radical Scavenging Behaviour and Antioxidant Activity in Various Citrus Fruits

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ABSTRACT

The present study aims to evaluate the in vitro free radical scavenging behavour and antioxidant potential from the fruits of four different Indian medicinal plants (Punica granatum, Citrus limetta, Prunus armeniaca and Syzygium cumini). The free radical scavenging behavior and antioxidant potential of the aqueous extract of fruits of four different Indian medicinal plants were evaluated by employing different in vitro assays such as reducing power assay, Superoxide anion scavenging assay, and DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay. The total phenolic and flavanoid contents were assessed by Folin-Ciocalteau and aluminium chloride reagents. From this study, the results indicated that the aqueous extract of Punica granatum showed highest total phenolic content i.e. 46mg/ml and Prunus armeniaca was found to be rich in reducing power at the dose of 16.5mg/ml. Further Syzygium cumini showed fine DPPH radical scavenging activity by 85% inhibition and Citrus limetta showed good scavenging activity for Superoxide anion radical by inhibiting 60%. The results obtained in the present study indicate that the fruits of Punica granatum followed by Syzygium cumini are a potential source of natural antioxidants which may be due to its copious phenolic and flavanoid contents.

Key words: Free radical scavenging behaviour; Antioxidant Activity, DPPH antioxidant assay; Phenolic Content; reducing power assay

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INTRODUCTION

A free radical is any chemical species capable of independent existence and possessing one or more unpaired electrons, an unpaired electron being one that is alone in an orbital. The simplest radical is the hydrogen atom [1]. These are continuously produced by the body's normal use of oxygen [2]. When cells use oxygen to generate energy free radicals are produced by the mitochondria. These by-products are generally reactive oxygen species (ROS) that result from the cellular redox process. The free radicals have a special affinity for lipids, proteins, carbohydrates and nucleic acids [3]. The reason free radicals are often considered dangerous is because when they steal electrons, they cause damage to the other atoms. For example, if many free radicals are created inside the skin by repeated exposure to the sun, they may steal electrons from the nearby atoms that make up proteins used to build healthy skin. If many of these are damaged and the body isn't able to create new healthy skin like it used to, wrinkles may develop.

Antioxidants often come into play in these kinds of scenarios because they are able to give up electrons to the free radicals. In turn, this can help prevent some of the cells in the body from getting damaged. Free radicals are highly reactive and unstable compounds produced in the body during normal metabolic functions or introduced from the external environment such as pollution and cigarette smoke. Antioxidant works to maintain the oxidant at optimum level and to reduce free radical, stopping it from forming before it can disrupt living cells in our body. However, excessive oxidants or free radicals can cause cell damage and lead to chronic diseases. Researchers believe that on a large scale, this may lead to slowed aging and/or the prevention of certain diseases. For example, some studies have shown that vitamin A applied directly to the skin may help reduce wrinkles. Many experts believe this is due to the symbiotic relationship that antioxidants and free radicals have and the resulting protection of the body's atoms. Natural antioxidants occur in all parts of plants. These antioxidants include carotenoids, vitamins, phenols, flavnaoids, dietary glutathione and endogenous metabolites. Plant derived antioxidants have been shown to function as singlet and triplet oxygen quenchers, free redical scavangers, peroxide decomposers, enzyme inhibiters, and synergists [4].

Fruits and vegetables contain many different antioxidant components [5-7]. The consumption of fruits and vegetables has been associated with low incidences and mortality rates of cancer [8] and heart diseases [9]. Eating fruits and vegetables also reduces blood pressure, boosts immune system, detoxifies contaminants and pollutants, and reduces inflammation [10].

Present work is focused on exploring antioxidant potential of citrus fruits which have been valued as part of a nutritious and tasty diet. Citrus and citrus products are a rich source of vitamins, minerals and dietary fibre (non-starch polysaccharides) that are essential for normal growth and development and overall nutritional well-being.

MATERIALS AND METHODS

Sample Collection

Four different types of fruits were purchased from the local market of Jaipur, India. The fruits included *Punica granatum* L, *Citrus limetta*, *Prunus armeniaca* and *Syzygium cumini*.

Chemicals and Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) and 6-hydroxy-2,5,7,8–tetramethyl chromane-2-carboxylic acid (Trolox) were purchased from Sigma Chemicals Co. Methanol, Ethanol, Sodium acetate tri hydrate, ferric chloride hexahydrate ($FeCl_3$. $6H_2O$), Folin-Ciocalteu Phenolic reagent, Sodium carbonate were from Merck. All the chemicals used were of analytical grade.

Preparation of Extract

Fruits of the plant were cut into small pieces and then macerate in to pestal and mortar and finally material was extracted by successive soaking for three days using aqueous solvent. The aqueous extracts were filtered using muslin cloths and cottons and were ready for further investigation.

Antioxidant Activity Determination

Total Phenolic Content (Folin-Ciocalteu Method)

The total phenolic content was determined in all the four extracts using the Folin-Ciocalteu reagent according to the method of Sinkard *et al.* (1977) [11] using quercetin as a standard. The total phenolic content was expressed as Quercetin equivalents in milligrams per ml of extract.

DPPH Radical Scavenging Activity Assay

Free radical scavenging activity of the plant extracts was determined by the method of Yamaguchi *et al*. (1998) [12]. Briefly, 1.5 ml of DPPH solution (0.1 mm, in 95% ethanol) was incubated with fruit extract. The reaction mixture was shaken well and incubated for 20 min at room temperature and the absorbance of resulting solution was read at 517 nm against a blank. Quercetin was used as standard references. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$Scavenging\ effect(\%) = 1 - (sample_{OD}/control_{OD}) \times 100 \quad (1)$$

Total Flavanoid

The amount of total flavonoid content was determined by Aluminum chloride method [13]. The reaction mixture (3.0 ml) comprised of 1.0 ml of extract, 0.5 ml of aluminum chloride (1.2%) and 0.5 ml of potassium acetate (120 mM) which was incubated at room temperature for 30 min and absorbance was read at 415 nm.

Reducing Power

The reducing power was determined by the method of Athukorala *et al*. (2006) [14]. 1.0 ml extract was mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) and incubated at 50°C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (600 mM) was added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was collected and mixed with 2.5 ml of distilled water and 0.5 ml of FeCl3 (6 mM) and absorbance was read at 700 nm.

Superoxide Anion Radical Scavenging Assay

The assay for superoxide anion radical scavenging activity was measured as described by Robak and Gryglewski (1998) [15]. 1 ml of sample was taken and mixed with 0.5 ml of phosphate buffer (50 mM, pH 7.6), 0.3 ml riboflavin (50 mM), 0.25 ml PMS (20 mM), and 0.1 ml NBT (0.5 mM). After 20 min of incubation, the absorbance was measured at 560 nm. Ascorbic acid was used as standard. The scavenging ability of the fruit extract was determined by the equation (1).

RESULTS

Total Phenolic Content

The content of phenolic compounds (Quercetin equivalent/ 100 gm of fruits) was determined from a standard plot prepared taking quercetin as the standard. The highest activity was observed for *Punica granatum* (46 mg /ml) followed by *Syzygium cumini* (41mg/ml), *Prunus armeniaca* (34.5mg/ml) and *Citrus limetta* (20.5mg/ml).

DPPH Radical Scavenging Activity Assay

As shown in the figure 2, the antioxidant activity of the fruits tested, was found to vary over 170-fold from that at the lowest value. *Syzygium cumini* shows highest percent of DPPH inhibition of 82% and lowest is 28% shown by *Prunus armeniaca* fruit pulp.

Total Flavanoid Content

Figure 3 presents mean total flavonoid content of different fruits. Flavonoid compounds are considered to be the largest group of naturally occurring phenols [16]. Thus, it is not surprising to see that total flavonoid content accounts for almost half of the phenolic compounds in samples. Total flavonoid content of *Punica granatum (38mg/ml)* was found to be highest among all other fruits.

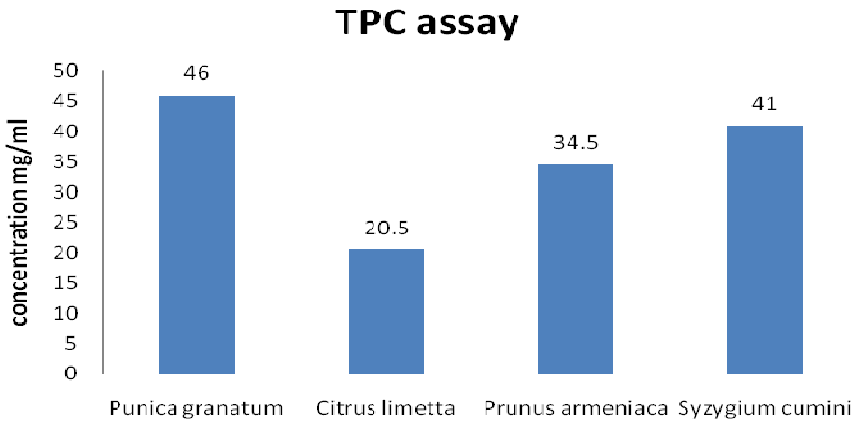

Fig. 1Total Phenolic Content in citrus fruits

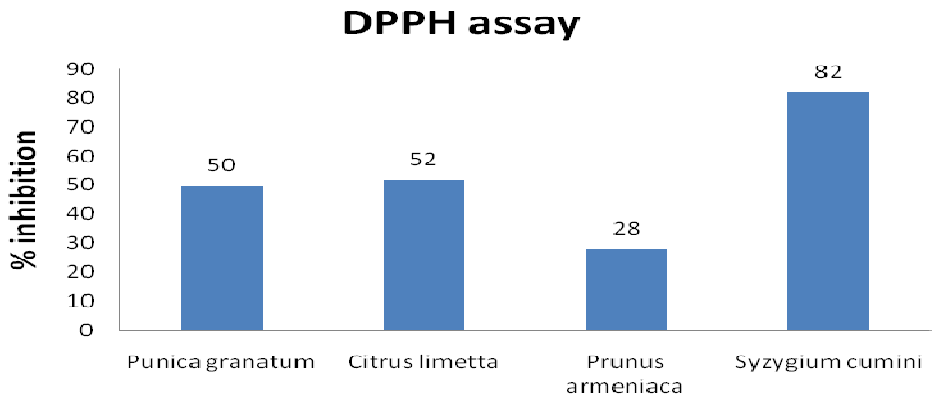

Fig. 2 DPPH Assay in citrus fruit

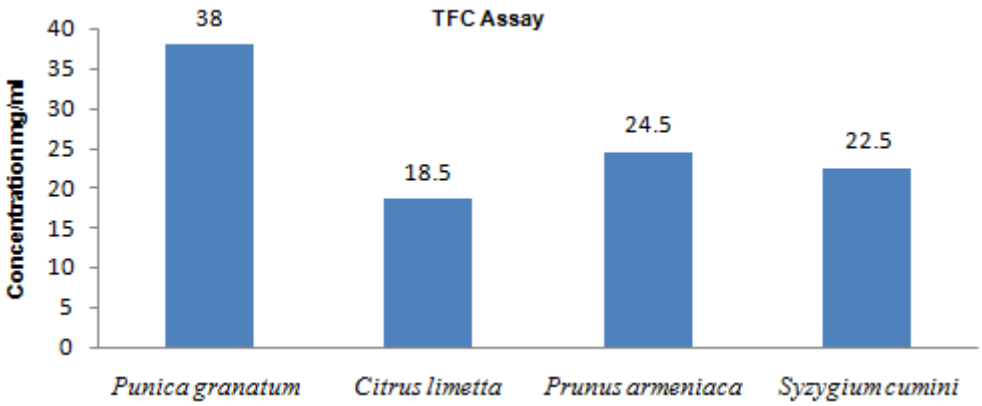

Fig. 3 Total Flavanoid Content in citrus fruit

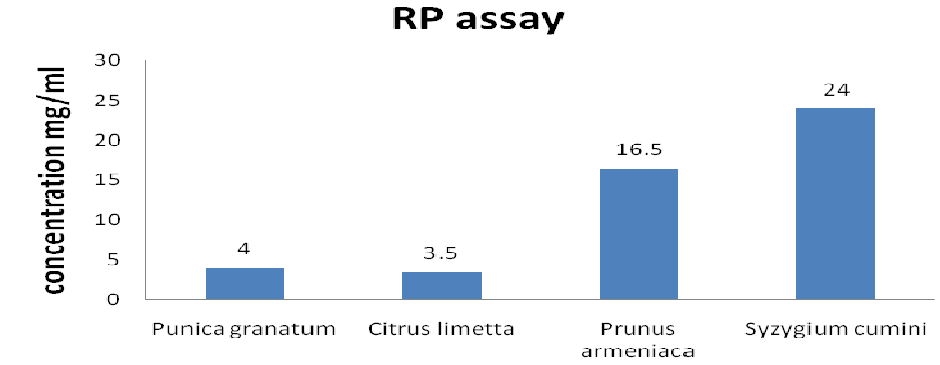

Fig. 4 RP Assay in citrus fruit

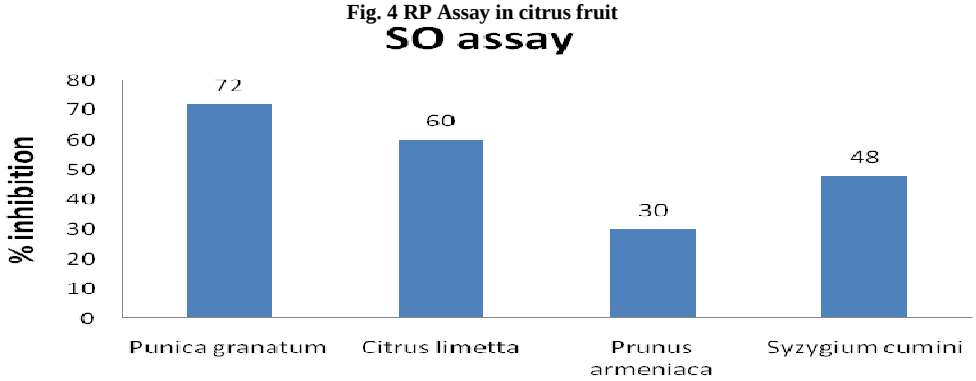

Fig. 5 Super Oxide Anion Radical Scavenging Assay in citrus fruit

DISCUSSION

Reducing Power

The reducing ability of extract of the fruits in descending order was *Syzygium cumini* > *Prunus armeniaca* / *Punica granatum* > *Citrus limetta*. In this assay, the presence of antioxidants in the extracts reduced Fe /ferricyanide complex to the ferrous form. Reducing power of *Syzygium cumini* (24mg/ml) was found to be maximum as compared to other citrus fruits.

Superoxide Anion Radical Scavenging Assay

The ability of the phytochemicals from the ethanol extract to scavenge the superoxide anion was carried out using the pyrogallol autooxidation method. Among the various citrus fruit pulp *Punica granatum* showed highest Superoxide activity (72%) among all the fruits followed by *Citrus limetta* (60%), *Syzygium cumini* (48%) and *Prunus armeniaca* (30%).

Total Phenolic Content

The plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, so it was reasonable to determine their total amount in the fruit extracts. Some authors have earlier reported a direct correlation between antioxidant activity and total phenolic content [17-18]. The antioxidant activity of phenolic constituents may be related to their redox properties, which allow them to act as reducing agents or hydrogen-atom donors, their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals [19].

DPPH Radical Scavenging Activity Assay

The bleaching of DPPH absorption by a test compound is representative of its capacity to scavenge free radicals generated independently of any enzymatic or transition metal-based system. This is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. Antioxidants react with DPPH which is a stable free radical and convert it to 1,1-diphenyl-2-(2,4,6-trinitrophenyl) hydrazine. The degree of decolourization indicates the scavenging potentials of the antioxidant compounds. The decrease in absorbance of DPPH radical is caused by antioxidant through the reaction between antioxidant molecule and radical results in the scavenging of the radical by hydrogen donation [20].

Total Flavanoid Content

Calorimetrically analysis of polyphenolic and total flavonoid contents indicated that the ethanolic extract of orange peel had highly amounts of TPC and TFC and this in agreement with Ma *et al.*2008 [21] who studied the physical and chemical characteristics of citrus peel and traced high amount of TP.

Reducing Power

Regarding the reducing power, it is found that the amount of phenolic compounds was high in Pomegranate and there was a tight relationship between the amount of total phenolic content and the reducing power. These results

were previously recorded [21] indicating that the reducing power of bioactive compounds is associated with antioxidant activity. Thus, it is necessary to determine the reducing power of phenolic constituents to elucidate the relationship between their antioxidant effects and the reducing power [22].

Superoxide anion radical scavenging assay

Dioxygen form superoxide anions O_2^- by a single electron transfer during the pyrogallol autooxidation in basic solutions. The superoxide anions are scavenged by antioxidants and consequently, decrease the rate of pyrogallol autooxidation or even inhibit it.

CONCLUSION

In the present study, antioxidant property and free radical scavenging behaviour of citrus fruits has been examined. Results showed that *Punica granatum* had the highest Phenolic content, Total flavanoid content, and Superoxide activity followed by *Syzygium cumini*. There were significant differences in the contents of all activity of studied citrus fruits. These investigations clearly show the potential value of Pomengrate (*Punica granatum*) and Jamun (*Syzygium cumini*) fruits as a significant source of phenolic compounds. These fruits can be considered as a good source of natural antioxidants and may show potential future use in food and nutraceutical supplement formulations. Therefore, daily consumption of the juices of citrus fruits with food may reduce malnutrition and the risk of cardiovascular and cancer diseases as well.

REFERENCES

[1] B Halliwell, Antioxidants and Human Disease: A General Introduction, *Nutrition Reviews*, **1997**, 55(1), S44–S49.
[2] AK Tiwari, Antioxidants: New Generation Therapeutic Base for Treatment of Polygenic Disorders, *Current Science*, **2004**, 86(8), 1092-1102.
[3] S Velavan, Free radicals in health and diseases, *Pharmacology Online*, **2011**, 1, 1062-1077
[4] RA Larson, The antioxidants of higher plants, *Phytochemistry*, **1988**, 27(4), 969-978
[5] G Cao, E Sofic and RL Prior, Antioxidant Capacity of Tea and Common Vegetables, *Journal of Agriculture and Food Chemistry*, **1996**, 44, 3426-3441.
[6] YS Velioglu, G Mazza, L Gao and BD Oomah, Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables and Grain Products, *Journal of Agriculture and Food Chemistry*, **1998**, 46, 4113-4117.
[7] H Wang, G Cao and RL Prior, Total Antioxidant Capacity of Fruits, *Journal of Agriculture and Food Chemistry*, **1996**, 44, 701-705.
[8] BM Ames, MK Shigena and TM Hagen, Oxidants, Antioxidants and the Degenerative Diseases of Aging, *Proceedings of National Academy of Sciences*, USA, **1993**, 90, 7915-7922.
[9] AJ Verlangieri, JC Kapeghian, S el-Dean and M Bush, Fruit and Vegetable Consumption and Cardiovascular Mortality, *Med Hypothesis*, **1985**, 16(1), 7-15.
[10] A Ascherio, EB Rimm, EL Giovannucci, GA Colditz, B Rosner, WC Willett, F Sacks and MJ Stampfer, A Prospective Study of Nutritional Factors and Hypertension Among US Men, *Circulation*, **1992**, 86(5), 1475-1484.
[11] K Slinkard, VL Singleton, Total Phenol Analysis: Automation and Comparison with Manual Methods, *American Journal of Enology and Viticulture*, **1977**, 28(1), 49-55.
[12] T Yamaguchi, H Takamura, T Matobavand J Terao, HPLC Method for Evaluation of the Free Radical Scavenging Activity of Foods by using 1,1,-Diphenyl-2-Picrylhydrazyl, *Bioscience, Biotechnology and Biochemistry*, 1998, 62(6), 1201–1204.
[13] C Chang, M Yang, H Wen and J Chem, Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods, *Journal of Food Drug Analysis*, **2002**, 10(3) 178-182.
[14] Y Athukorala, Y Jeon and K Kim, Antiproliferative and Antioxidant Properties of an Enzymatic Hydrolysate from Brown Alga, Ecklonia Cava, *Food Chemical Toxicology*, **2006**, 44 (7), 1065-1074.
[15] J Robak and J Gryglewski, Flavonoids are Scavengers of Superoxide Anions, *Biochemical Pharmacology*, **1998**, 37 (5), 837-841.
[16] S Vijayakumar, G Presnnakumar and NR Vijayalakshmi, Antioxidant Activity of Banana Flavonoids, *Fitoterapia*, **2008**, 79(4), 279-282.
[17] YS Velioglu, G Mazza, L Gao and BD Oomah, Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables and Grain Products, *Jouranl of Agriculture and Food Chemistry*, **1998**, 46(10), 4113-4117.
[18] L Barros, M Joao Ferreira, B Queiros, IC Ferreira and P Baptista, Total Phenol, Ascorbic Acid, β-carotene and Lycopene in Portuguese Wild Edible Mushroom and their Antioxidant Activities, *Food Chemistry*, **2007**, 103(2), 413-419.
[19] EA Decker, Phenolics: Prooxidants or antioxidants, *Nutrtion Reviews*, **1997**, 55(11), 396–398
[20] CJ Hong and H Chi-Tang, *Journal of Agriculture Food Chemistry* **1997**, 45, 2357.
[21] YQ Ma, JC Chen, DH Liu and XQ Ye, Effect of Ultrasonic Treatment on the Total Phenolic and Antioxidant Activity of Extracts from Citrus Peel, *Journal of Studies in Natural Products Chemistry Elsevier Food Sci*ences, **2008**, 73, 115-120.
[22] B Shan, Cai M Sun and H Corke, *Journal of .Free Radicals in Biology and Medicine*, **2005**, 26, 1231.