

Original Article

ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT IN RIPE AND UNRIPE POMEGRANATE (*PUNICA GRANATUM* L.) FRUIT JUICE

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ABSTRACT

Objective: *P. granatum* L. is famous for antioxidant activity and utilized as a nourishing foodstuff. This work aimed to recognize the antioxidant activity and TPC in extracts of ripe and unripe fruit juice.

Methods: Competency of the solvents (water, methanol, acetone, chloroform, ethanol) were evaluated by analyzing extracts of ripe and unripe *P. granatum* L. fruit juice for TPC and DPPH* scavenging assay. FTC method measured the level of peroxides.

Results: In TPC extraction, the water solvent showed greater potential in both ripe (10.5±2.1) and unripe fruit juice (4.1±0.3) amongst all other solvents. Ethanol and water solvent showed the highest value of DPPH* scavenging activity (96%±6.81 and 72%±3.50 respectively) in ripe and unripe fruit juice. According to absorbance of DPPH radicals, the water solvent showed the highest antioxidant potential in ripe fruit juice (86%±6.78) like chloroform solvent in unripe fruit juice (14%±0.03). Unripe fruit juice showed lowest level of absorbance of DPPH radicals and highest antioxidant potential amid all solvents. In FTC method, unripe fruit juice showed the highest antioxidant activity and low amount of peroxides for consecutively seven days.

Conclusion: Ripe fruit juice showed the highest TPC and unripe fruit juice showed the maximum value of antioxidant potential. *P. granatum* L. provides an excellent supply of antioxidant activity and used in pharmaceutical and food industry.

Keywords: Antioxidant ability, DPPH scavenging assay, TPC, *P. granatum* L., Peroxides

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INTRODUCTION

P. granatum L. belongs to Lythraceae family as a deciduous shrub. It is a local fruit of Iran and now it is grown all over the world as fruit and ornamental plant. It is a good supplier of vitamin K, E, C, A as well as folate, minerals, carbohydrates, fat and fiber [1]. Fruit juice is sweet but sometimes has sour taste due to the presence of ellagitannins acid

[2]. Fruit have countless biological activities owing to the presence of active components like flavonoids, ascorbic acid, cynogenic glucosidase, papine and chymopepine etc. In latest research, *P. granatum* C. *papaya* L. extracts has been reported as a strong anti-dengue tonic that is supportive in recuperating the white blood cell hastily after illness [3]. In Pakistan, Balochistan considers the orchards of *P. granatum* L. and also grow in Punjab and KPK at a small scale.

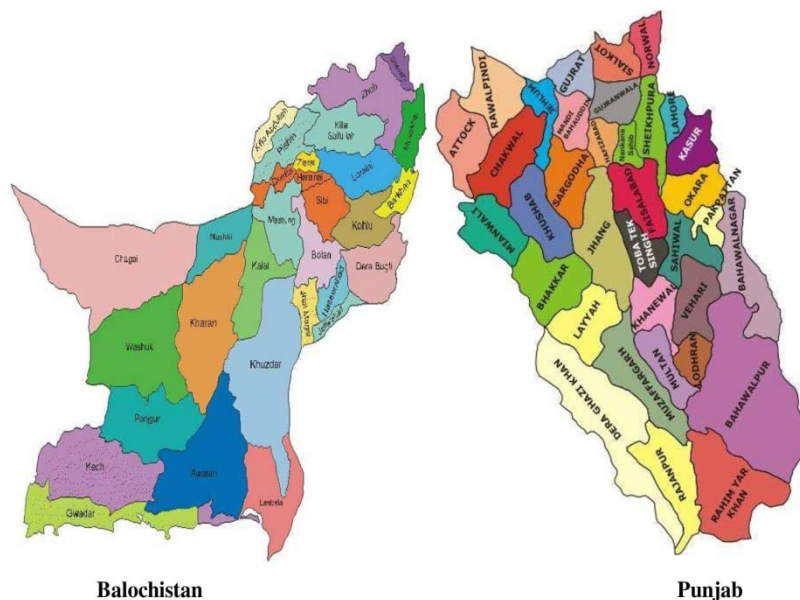


Fig. 1: Orchards of pomegranate in Punjab and Balochistan

Reactive oxygen species and free radicals are constantly formed in the individual body during ordinary liver functions, cellular metabolism and mitochondrial respiratory system [4]. The function of reactive oxygen species and free radicals is eminent in human being after damaging the biomolecules (lipids, DNA, proteins etc.). Oxidative stress has been reported as an elementary mechanism for the development of manifold health disorders, infections and illnesses like hemorrhage, respiratory problems, ulcers and dysentery etc. [5]. The current experiment was designed to determine the consequence of solvents (water, ethanol, methanol, chloroform and acetone) after extraction of TPC and antioxidant potential of ripe and unripe *P. granatum* L. fruit juice. These investigations will be helpful in securing maximum benefits associated with bioactive compounds present in *P. granatum* L.

MATERIALS AND METHODS

Fruit collection

P. granatum L. (genotype name Golden) was collected from Local market of Bahawalpur, Punjab.

Preparation of *P. granatum* L. fruit juice extract

Samples washed several time with tap water. The edible portion (unripe and ripe) peeled out, cut into pieces and then grinded. After this, took 10g papaya (ripe, unripe) from paste and dissolved in different solvents (methanol, ethanol, acetone, chloroform, water). After this shake, put into orbital shaker at 200rpm for 2 h and centrifuge the mixture in centrifuge machine at 1000rpm for 15 min. The filter paper (Whatman No. 4) was used for filtration of mixture.

Quantification assay

Estimation of total phenolic content (TPC)

Estimation of TPC evaluated by Folin-Ciocalteu reagent assay [6]. The reaction mixture was prepared by mixing 2 mg of papaya extract and 100 μ L of freshly prepared 0.5 ml Folin-Ciocalteu reagent. The primed mixtures were permitted to locate in dark for 15 min followed by the addition of 2.5 ml sodium carbonate (6%) and the resultant mixture was incubated in dark for 30 min. The absorbance was recorded at 725 nm by UV visible spectrophotometer (CECIL, Milton Technical Centre, Cambridge UK). The results were calculated by standard ascorbic acid [7].

Determination of antioxidant potential

DPPH radical scavenging assay

The capability of the extracts to scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals were calculated by reported method [8]. 3.9 ml of methanolic solution of DPPH radical (25 mg/ml) added

in the diluted papaya extract (0.2 ml). The mixture left in the dark for 30 min after shaking. The mixture's absorbance was recorded at 500 nm in opposition to absolute methanol without DPPH•. The outcomes were recorded in percentage which was used to evaluate the radical scavenging potential of *C. papaya* fruit extracts.

FTC method (Ferric thiocyanate)

Hydro peroxides produced when linoleic acid and reaction mixture was combined [9]. 2 ml of sample (methanol extract)+2.5% linolenic acid in 99.8% ethanol took 2.05 ml+4 ml of 0.05 mol. After this, 1.95 ml of water added in the flask and placed in a rotary incubator (150r/min at 40 °C) in a dark place. To calculate the antioxidant value, 0.1 ml of above sample mixture added in the test tube with added 9.7 ml of 75% ethanol+0.1 ml of 30% ammonium thiocyanate and 0.1 ml of 2×10^2 mol/l FeCl_2 in 3.5% HCl. FeCl_2 added in the reaction mixture and absorbance was recorded at 500 nm. Measurements were recorded after every 24h until the absorbance of the control and blank soln. reached its maximum [10].

• **Sample preparation:** Took 0.5 ml of sample (ripe, unripe, vitamin E) then added 0.5 ml linoleic acid, 0.5 ml water and 1 ml phosphate buffer. After this, covered and placed into oven at 40 °C. Took 0.1 ml from the above soln. then added 9.7 ml of 75% ethanol, 0.1 ml ammonium thiocyanate and 0.1 ml of FeCl_2 in HCl solution.

• **Control:** 1 ml buffer, 0.5 ml water placed into oven at 40 °C. After this, took 0.1 ml of above soln then added 9.7 ml of 75% ethanol 0.1 ml ammonium thiocyanate and 0.1 ml of FeCl_2 in HCl soln.

• **Blank:** Add 0.5 ml of methanol, 0.5 ml of water, 1 ml buffer and a then similar process of the above [11].

RESULTS AND DISCUSSION

Determination of total phenolic contents (TPC)

Water showed greater potential among all other solvents. All these observations proposed that phenolic compounds are highly polar in polar solvents. The range of polarity of solvents in ripe and unripe papaya fruit is Methanol<Chloroform<Ethanol<Acetone<Water. The polarity of methanol solvent in unripe papaya fruit is greater than ripe papaya fruit. Phenolic constituents are also called secondary metabolites, which are familiar due to free radical scavenging potential [12]. The TPC in ripe fruit was highest than the unripe fruit [13]. Phenolic contents with tannins, anthocyanin and flavonoids consider the main antioxidant phytochemical because of free radical scavenging [14]. The water has a high ability to soluble a larger fraction of the phenolic components present in papaya [15]. Antioxidant potential and TPC had a direct relationship with each other [16].

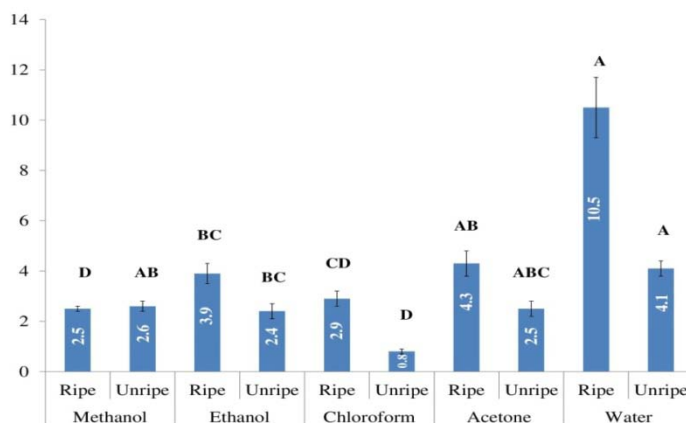


Fig. 2: Comparison of total phenolic content (µg/g) in different solvents

Estimation of DPPH scavenging activity

The DPPH scavenging activity highest in ripe fruit as compared to unripe fruit in all solvents. Ethanol solvent showed the highest DPPH

radicals scavenging activity in ripe fruit and water solvent showed the best result in unripe fruit juice as compared to all other solvents. In ripe fruit extraction, all solvents showed the significance difference unlike unripe fruit extraction. The order of solvent

potential in ripe fruit juice was Ethanol>Chloroform> Acetone>Methanol>Water and in unripe fruit was Water>Ethanol>Methanol>Acetone>Chloroform. Water solvent showed the highest capacity as antioxidant in ripe fruit juice like chloroform solvent in unripe fruit juice, because antioxidant

activity decreases the absorbance of DPPH radicals. The colour of end product in the reaction was changed due to the donation of hydrogen [17]. That's why, the DPPH radicals showed stability at room temperature [18]. Odd electron in DPPH* given a strong absorption [19].

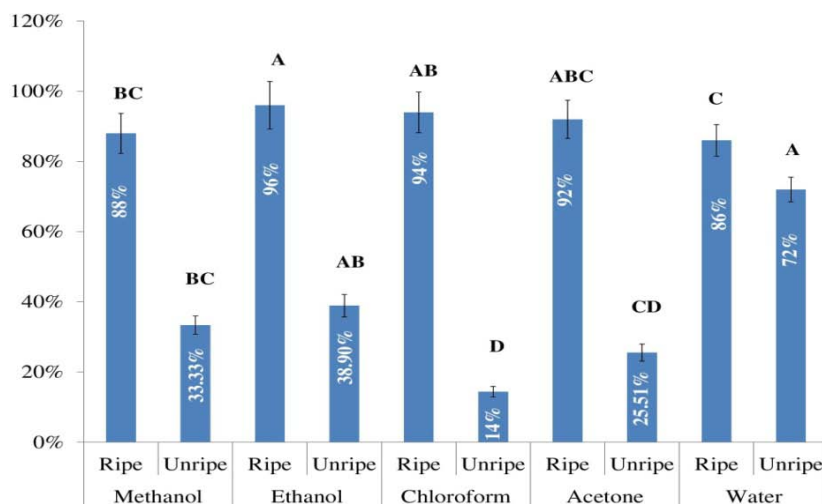


Fig. 3: Comparison of radical DPPH scavenging potential (%) among different solvents

FTC method (ferric thiocyanate)

The level of peroxides recognize by FTC method at the beginning stage of lipid oxidation. All extracts showed the significance result for consecutively seven days. The highest concentration of peroxides showed by blank and ripe fruit extract showed the lowest peroxides

potential. The lowest amount of peroxides showed the highest antioxidant capacity. In term of antioxidant activity, the unripe fruit showed highest antioxidant capacity consecutively seven days in all extracts. Absorbance rate reached at the maximum level from 1st to 6th day and eventually start to drop from 7th day. The large amount of peroxide decreased the antioxidant capacity [20].

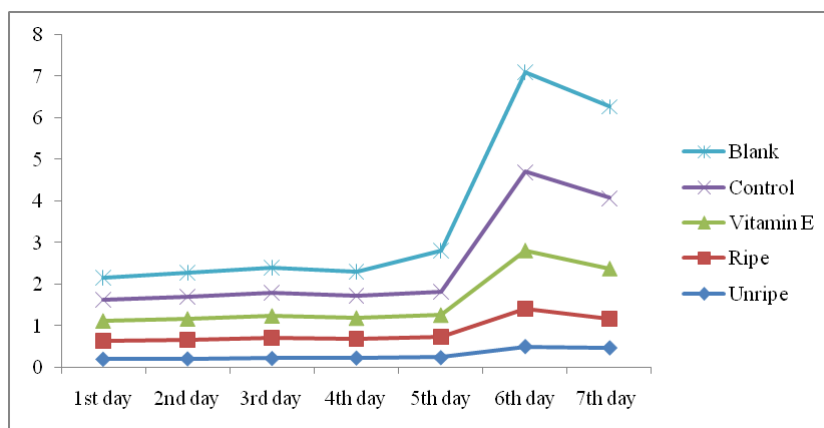


Fig. 4: Amount of peroxides in consecutive seven days

CONCLUSION

P. granatum L. fruit juice is the principal source of phenolic compounds. It also called nutraceutical fruit due to presence of vitamins, enzymes, carbohydrates and bioactive compounds. Ripe fruits juice contained highest total phenolic contents and unripe fruits juice have highest antioxidant potential. According to absorbance of DPPH* scavenging assay, chloroform solvent showed maximum antioxidant potential in unripe fruit juice like water solvent in ripe fruit juice. Unripe fruit juice showed the lowest amount of peroxides and the highest potential as antioxidant activity. Unripe fruit juice showed maximum strength like antioxidant potential in both DPPH* scavenging assay and FTC method. It can be used for cure of much disease like blood pressure, constipation, warts and cancer etc.

ABBREVIATION

TPC, Total Phenolic Content; DPPH*, 2-2 diphenyl-1-picrylhydrazyl radical; FTC, Ferric thiocyanate.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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