

Antioxidant Activity and Total Phenolic Contents of *Carcia papaya* L. (*Pawpaw*) fruit

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Abstract

Carica papaya L. is well-known for its antioxidant components and consider as a Nutraceutical. The aim of this research to determine the TPC and antioxidant activities in extracts of ripe and unripe *C. papaya* L. fruit. Competency of the solvents (water, methanol, acetone, chloroform, ethanol) was evaluated by analyzing extracts of ripe and unripe *C. papaya* L. fruit for TPC and DPPH scavenging assay. FTC method measured the level of peroxides. In TPC extraction, the water solvent showed greater potential in both ripe (10.5 ± 2.1) and unripe fruit (4.1 ± 0.3) amongst all other solvents. Ethanol and water solvent showed the highest value of DPPH* scavenging activity ($96\% \pm 6.81\%$ and $72\% \pm 3.50\%$) respectively in ripe and unripe fruit. According to an absorbance of DPPH radicals, the water solvent showed the highest antioxidant potential in ripe fruit ($86\% \pm 6.78\%$) like chloroform solvent in unripe fruit ($14\% \pm 0.03\%$). Unripe fruit showed the lowest level of absorbance of DPPH radicals and the highest antioxidant potential amid all solvents. In the FTC method, unripe fruit showed the highest antioxidant activity and low amount of peroxides for consecutively seven days. Ripe fruit showed the highest TPC and unripe fruit showed the maximum value of antioxidant potential. *C. papaya* L. is a good source of antioxidant activity and is used in the pharmaceutical and food industry.

Keywords: *C. papaya* L.; Total phenolic content; Antioxidant activity; DPPH scavenging assay; Peroxides

Abbreviations: TPC: Total Phenolic Content; DPPH: 2-2 Diphenyl-1-Picrylhydrazyl Radical; FTC: Ferric Thiocyanate

Received: February 09, 2021; **Accepted:** February 13, 2021; **Published:** February 28, 2021

Introduction

Carica papaya L. belongs to the *Caricaceae* family. Several centuries ago, it originated in Mexico and now it is cultivated all over the world. It is a good supplier of vitamin K, E, C, A as well as folate and fiber [1]. Fruit have countless biological activities owing to the presence of active components like flavonoids, ascorbic acid, cynogenic glucosidase, papine and chymopepine etc. In the latest research, *C. papaya* L. extracts have been reported as a strong anti-dengue tonic that is supportive in recuperating the white blood cell hastily after illness [2]. In Pakistan, Malir area of Karachi and the coastal area of Sindh province consider the orchards of papaya [3].

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 role of reactive oxygen species and free radicals is eminent in human beings after damaging the biomolecules (lipids, DNA, proteins, etc.). Oxidative stress has been reported as an

elementary mechanism for the development of manifold health disorders (neurodegenerative, autoimmune), infections and illnesses (Parkinson's disease, Alzheimer's disease and Ulcers) [5]. The present study was designed to determine the effect of solvents (water, acetone, ethanol, methanol, chloroform) on the extraction of total phenolic contents and antioxidant potential of ripe and unripe *C. papaya* L. fruit. These investigations will help secure maximum benefits associated with bioactive compounds present in *C. papaya* L.

Material and Methods

Fruit collection

C. papaya L. (genotype name Golden) was collected from the Local market of Bahawalpur, Punjab.

Preparation of *C. papaya* L. extract

Samples washed five times with tap water. The edible portion (unripe and ripe) peeled out, cut into pieces and then ground. After this, took 10 g papaya (ripe, unripe) from the paste and

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Citation: Amjad, Anwer, Shabbir, Khan, Raza (2021) Antioxidant Activity and Total Phenolic Contents of *Carcia papaya* L. (*Pawpaw*) fruit. Eur Exp Biol Vol.11 No. 3:130

dissolved it in different solvents (methanol, ethanol, acetone, chloroform, water). After this shake, put into orbital shaker at 200 rpm for 2 hours and centrifuge the mixture in centrifuge machine at 1000 rpm for 15 minutes. The mixture was filtered through a filter paper.

Quantification assay

Estimation of Total Phenolic Content (TPC): TPC was determined by Folin-Ciocalteu reagent assay [4]. 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 prepared mixtures were allowed to stand in dark for 15 minutes followed by the addition of 2.5 ml sodium carbonate (6%) and the resultant mixture was incubated in dark for 30 minutes. The absorbance was recorded at 725 nm using a UV visible spectrophotometer (CECIL, Milton Technical Centre, Cambridge UK). Ascorbic acid was used as standard and results were calculated [6].

Determination of antioxidant potential

DPPH radical scavenging assay: The capacity of the extracts to scavenge 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical was determined according to a reported method [7]. Diluted papaya extract (0.2 ml) was added to 3.9 ml of methanolic solution of DPPH radical (25 mg/ml). The mixture was shaken vigorously and left in the dark for 30 minutes. The absorbance of the mixture was recorded at 500 nm against absolute methanol without DPPH. The results were calculated as the percentage remaining of DPPH, which were used to compare the radical scavenging potential of *C. papaya* fruit extracts.

FTC method (Ferric Thiocyanate): Hydro-peroxide produced by linoleic acid added to the reaction mixture, which had oxidized by air during the experimental period was indirectly measured [8]. 2 ml of sample (methanol extract) +2.5% linolenic acid in 99.8% ethanol took (2.05+4) ml of 0.05 mol. After this, 1.95 ml water was added to the flask and placed in a rotary incubator (150 r/min at 40°C) in a dark place. To measure antioxidant value 0.1 ml of the above sample mixture was added to the test tube then added 9.7 ml of 75% ethanol+0.1 ml of 30% ammonium thiocyanate and 0.1 ml of (2 \times 10²) mol/L ferric chloride in 3.5% HCl. After three minutes, ferrous chloride was added to the reaction mixture and absorbance was measured at 500 nm. Measurement was taken after every 24 hr until the absorbance of the control reached its maximum value this mixture was also prepared without linoleic acid and negative control [9].

Sample preparation: Took 0.5 ml of a 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 an oven at 40°C. Took 0.1 ml from the above solution, then added 9.7 ml of 75% ethanol, 0.1 ml ammonium thiocyanate and 0.1 ml of FeCl₂ in HCl solution.

Control: 1 ml buffer, 0.5 ml water placed into an oven at 40°C. After this, took 0.1 ml of the above solution then added 9.7 ml of 75% ethanol 0.1 ml ammonium thiocyanate and 0.1 ml of FeCl₂ in HCl solution.

Blank: Add 0.5 ml of methanol, 0.5 ml of water, 1 ml buffer and then repeated the similar process of the above [10].

Statistical analysis

One way ANOVA was performed by using Statistics 8.1. Duncan test at 5% probability level was done with SPSS 16.0 version to compare the mean values.

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 [11]. The phenolic contents in ripe fruit were more than the unripe fruit [12]. The water has a high ability to solubilize a larger fraction of the phenolic components present in papaya [13]. Antioxidant potential and TPC had a direct relationship with each other.

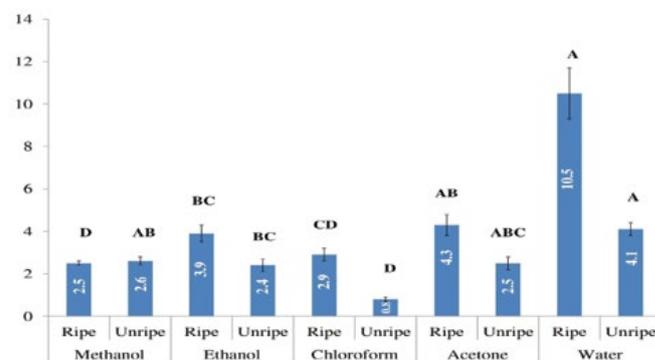


Figure 1 Comparison of total phenolic content (μ g/g) in different solvents.

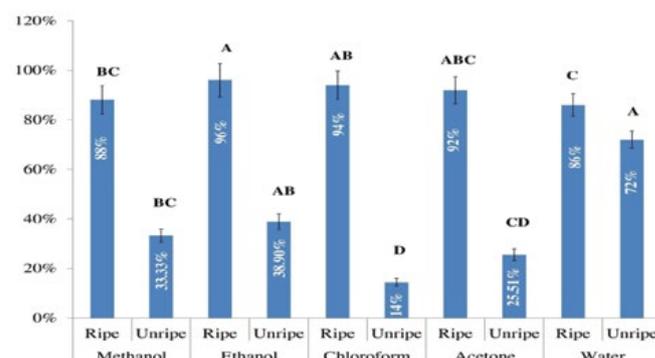


Figure 2 Comparison of radical DPPH scavenging potential (%) among different solvents.

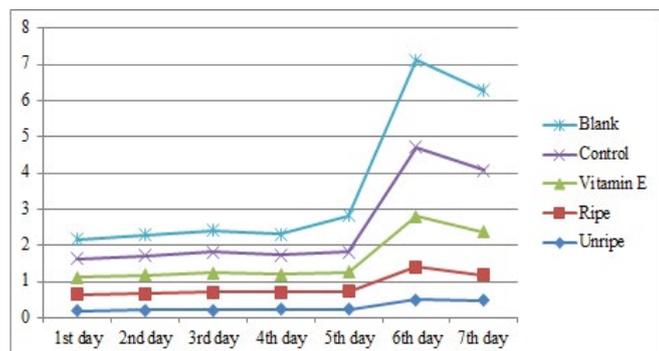


Figure 3 Amount of peroxides in consecutive seven days.

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 as compared to all other solvents. In ripe fruit extraction, all solvents showed a significant difference unlike unripe fruit extraction. The order of solvent potential in ripe fruit was Ethanol>Chloroform>Acetone>Methanol>Water and in unripe fruit was Water>Ethanol>Methanol>Acetone>Chloroform. Water solvent showed the highest capacity as an antioxidant in ripe fruit like chloroform solvent in unripe fruit because antioxidant activity decreases the absorbance of DPPH radicals. The colour of the end product in the reaction was changed due to the donation of hydrogen [14]. The DPPH radicals showed stability at room temperature [15]. Odd electron in DPPH given a strong absorption.

FTC method (Ferric Thiocyanate)

FTC method used to determine the level of peroxides at the initial stage of lipid oxidation. All extracts showed a significant

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 terms of antioxidant activity, the unripe fruit showed the highest antioxidant capacity consecutively seven days in all extracts. Absorbance rate reached at the maximum level from (1-6) day and eventually start to drop from 7th day. A large number of peroxides decreased the antioxidant capacity [16].

Conclusion

C. papaya L. fruit is the principal source of phenolic compounds. It is also called a nutraceutical plant due to the presence of vitamins, enzymes and bioactive compounds. Ripe fruits contained the highest total phenolic contents and unripe fruits have antioxidant potential. According to an absorbance of DPPH* scavenging assay, chloroform solvent showed maximum antioxidant potential in unripe fruit like water solvent in ripe fruit. Unripe fruit showed the lowest amount of peroxides and the highest potential as antioxidant activity. Unripe fruit showed maximum strength like an antioxidant potential in both DPPH scavenging assay and FTC method. It can be used for the treatment of many diseases like wound healing, corns, blood pressure, constipation, warts and cancer, etc. It's dressing safe and cost-effective.

Acknowledgement

My sincere gratitude goes towards Siddra Shabbir, Wajeaha Khan and Suleman Raza for their continuous advice, encouragement and support.

Author contributions

Conceived and designed the experiment: M Amjad, Z Anwer. Performed the Experiment: M Amjad, Z Anwer, Analyzed the data: M Amjad, Z Anwer. Contributed reagents, materials and analysis tools: M Amjad, S Shabbir, W Khan, S Raza. Wrote the paper: M Amjad, Z Anwer. All authors revised the manuscript.

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