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ORIGINAL PAPER

Evaluation of Antioxidant Capacity and Phenol Content in Jackfruit (*Artocarpus heterophyllus* Lam.) Fruit Pulp

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Abstract The antioxidant capacity of jackfruit (*Artocarpus heterophyllus* Lam. Fam. Moracae) fruit pulp (JFP) obtained from Western Ghats India was determined by evaluating the scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing power assays and *N, N*-dimethyl-*p*-phenylendiamine (DMPD) radical cation decolorization assay. JFP was analyzed for total phenolic content (TPC) and total flavonoids content (TFC). The ethanol and water are the best solvents for the extracting phenols and flavonoids from the JFP. The antioxidant activities of JFP extracts were correlated with the total phenolic and flavonoids content. The results indicated that the jackfruit pulp is one natural source of antioxidant compounds.

Keywords Antioxidant activity · *Artocarpus heterophyllus* · DMPD radical · DPPH radical · Total flavonoids · Total phenolic content

Abbreviations

DMPD	*N, N* dimethyl-*p*-phenylendiamine
DPPH	1,1-diphenyl-2-picrylhydrazyl
FRAP	ferric reducing antioxidant power
GAE	gallic acid equivalent
IC_{50}	inhibitory concentration showing 50% inhibition
JFP	jackfruit pulp
TFC	total flavonoids content
TPC	total phenolic content
TPTZ	2,4,6-tripyridyl-s-triazine
RSA	radical scavenging activity

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Introduction

Antioxidants regarded as compounds are able to delay, retard or prevent oxidation process [1]. The natural antioxidants in fruits and vegetables gained increasing interest among food scientists, nutrition specialists and consumers, as they reduce the risk of chronic diseases and promote human health as reported earlier from antioxidants of mango [2] and guava [3]. The jackfruit (*Artocarpus heterophyllus* Lam. Family-Moraceae) tree is a wild plant throughout the tropics and subtropics as it bears the largest known edible fruit (up to 35 kg) [4, 5]. The jackfruit has several uses. Young fruits and seeds are used as vegetables. The pulp of ripe fruit is eaten fresh and used in fruit salads as it posses high nutrient value, every 100 g of ripe fruit pulp contains carbohydrate 18.9 g, protein 1.9 g, fat 0.1 g, moisture 77%, fiber 1.1 g, total mineral matter 0.8 g, calcium 20 mg, phosphorus 30 mg, iron 500 mg, vitamin A 540 I.U., thiamin 30 mg, having caloric value 84 calories [6]. The jackfruit also contains useful antioxidant compounds [7]. The carotenoids composition of jackfruit was successfully determined and 14 of the 18 carotenoids were reported for the first time. Differences among batches might be due to the genetic and/or agricultural factors [8]. Ripe fruit are canned in syrup. The pulp is processed, dehydrated and sold as dried pulp, juice, biscuits, chutney, jam, jelly, toffee, and pastes. The plant is commonly referred to as 'poor man's food' as it is cheap and plentiful during the season [9].

Despite numerous advantages, the popularity of jackfruit as a commercial crop is very poor due to wide variation in fruit quality and the widespread belief that excessive consumption of jackfruit flakes leads to certain digestive ailments [6].

The purpose of this study was to investigate the antioxidant potentials of jackfruit pulp (JFP). The antioxidant properties of JFP extracts were tested for their total antioxidants capacity with 3-different methods.

Materials and Methods

Plant Material

The fully ripened fruits of *A. heterophyllus* were collected from Radhanagari locality Western Ghats, India. The fruits were cleaned and separated into pulp and seeds. The pulp was yellow in color having sweet taste. The JFP was stored in a freezer (−20 °C) until further analysis.

Chemicals

All solvents and chemicals were of analytical grade and obtained from local suppliers. Ascorbic acid, aluminum trichloride, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ferric chloride, Folin-Ciocalteu reagent, potassium phosphate (monobasic and dibasic), sodium carbonate, 2,4,6-tripyridyl-s-triazine (TPTZ) and trichloroacetic acid were purchased from Sigma Chemical Co., USA. *N, N*-dimethyl-*p*-phenylendiamine (DMPD) was purchased from Fluka (Schweiz, Switzerland). Glacial acetic acid (HPLC grade) and methanol (HPLC grade) were purchased from Merck Trolox (6-hydroxy 2,5,7,8 tetramethyl chroman 2-carboxylic acid) was purchased from Aldrich Chemical Co., USA.

Extractions

JFP extracts were obtained by homogenizing the JFP in four different solvents (acetone, methanol, ethanol, and water) keeping JFP to solvent ratio of 1:10. Extraction was carried out on an orbital shaker for 24 h at room temperatures. The homogenates were centrifuged at 15,000 rpm at 4 °C for 10 min and the supernatants were recovered and stored at −20 °C.

Analysis of Total Phenolic Content (TPC)

The TPC of JFP in four different solvents was determined spectrophotometrically using the Folin-Ciocalteu assay. An aliquot of 125 µl extract was mixed with 1.8 ml of Folin-Ciocalteu reagent which was previously diluted 10-fold with distilled water. The solution was allowed to stand at 25 °C for 5 min before adding 1.2 ml of 15% sodium carbonate solution in distilled water. After 90 min at room temperature, absorbance was measured at 765 nm using a spectrophotometer. This was compared to standard curve of gallic acid concentrations and expressed as mg of gallic acid equivalents per g (mg GAE g^{-1}) of dry powder [10].

Analysis of Total Flavonoids Content (TFC)

Total flavonoid contents of all four extracts were also measured by colorimetric method [11]. JFP extracts (0.5 ml) were mixed with methanol (1.5 ml), to which 10% aluminum chloride (0.1 ml), 1 M potassium acetate (0.1 ml) and distilled water (2.8 ml) were added. The solution was vortexed, allowed to stand for 30 min at room temperature. The absorbance of reaction mixture was measured at 415 nm using a UV–vis spectroscopic analysis (Hitachi U-2800; Hitachi, Tokyo, Japan). The total flavonoids content was quantified according to the standard curve prepared for rutin and the concentration of flavonoids was reported as mg of rutin equivalents per g (mg RE g^{-1}) of sample.

DPPH Radical Scavenging Activity

Antioxidant activity of the JFP extracts was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay [12] with some modifications. Briefly, the stock reagent solution was prepared by dissolving 24 mg of DPPH in 100 ml methanol and stored at −20 °C until use. The working solution was obtained by mixing 10 ml of stock solution with 45 ml methanol to obtain an absorbance value of 1.1±0.02 at 517 nm, using a spectrophotometer. The different concentrations of JFP extracts were allowed to react with 3 ml of DPPH solution. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. The mixture was measured spectrophotometrically at 517 nm. A control sample with no added extract was also analyzed and the results were expressed as radical scavenging activity (% RSA).

$$\%\mathrm{RSA} = \left(\mathrm{A}_{\mathrm{control}} - \mathrm{A}_{\mathrm{sample}}\right) \times 100/\mathrm{A}_{\mathrm{control}}$$

Where, A = absorbance at 517 nm.

A standard curve was then prepared by plotting the percentage (%) of free radical scavenging activity of ascorbic acid versus its concentration. The final results were expressed as mg ascorbic acid equivalent antioxidant capacity in 1 g of sample (mg AEAC g^{-1}).

FRAP (Ferric Reducing/Antioxidant Power) Assay

The FRAP assay was performed as previously described by Benzie and Strain [13] with some modifications. Briefly the working FRAP reagent produced by mixing 300 mM acetate buffer (pH 3.6) 10 mM 2,4,6-tripyridyl-s-triazine

(TPTZ) in 40 mM HCL and 20 mM $FeCl_3 \cdot 6H_2O$ in 10:1:1 ratio prior to use and heated to 37°C in water bath for 10 min. JFP extracts of various concentrations (1–5 mg/ml) were allowed to react with 2.7 ml of the FRAP reagent. The final volume of the reaction mixture was made up to 3 ml with distilled water. The reaction mixture was kept in dark for 30 min. The readings of the colored product (ferrous tripyridyltriazine complex) were then taken at 593 nm. The results were expressed as mM Trolox equivalent g^{-1} dry weight of sample.

DMPD (*N, N*-dimethyl-*p*-phenylendiamine) Assay

The principle of this assay is based on the reduction of the purple radical cation $DMPD^+$ (*N, N*-dimethyl-*p*-phenylendiamine). A 100 mM DMPD solution was prepared by dissolving 209 mg DMPD in 10 ml distilled water. One ml of this solution was added to 100 ml 0.1 M acetate buffer (pH 5.25). Adding 0.2 ml of a 0.05 M ferric chloride solution resulted in the purple radical cation $DMPD^+$, which was measured at 505 nm and equilibrated to an absorbance of 0.900±0.100. The DMPD radical cation was stable up to 12 h. One µl of $DMPD^+$ solution and 50 µl antioxidant solutions were mixed continuously for 10 min at 25°C. After mixing, the absorbance of this solution was taken at 505 nm [14]. The antioxidative potential of the four substances was evaluated as shown for the DPPH assay

Statistical Analysis

In this study, three analyses of each sample were made and each experiment was carried out in triplicate (n=3). The mean value and standard deviation were calculated from the data obtained. Values representing the concentration of investigated extracts that cause 50% of inhibition (IC_{50}) were determined by linear regression analysis of obtained RSA (Microsoft Excel programme for Windows XP).

Results and Discussion

Total Phenolic Content (TPC)

The amount of TPC determined in different solvent extracts of JFP is shown in Table 1. Results revealed that ethanol was the best solvent for extracting phenolic compounds followed by water, methanol, and acetone. JFP contained lower amounts of total phenolics (0.46 mg GAE g^{-1}) as compared to *A. odoratissimus* flesh (4.39 mg GAE g^{-1}) [15]. The seeds of jackfruit showed higher amounts of total phenolic content (27.7 mg GAE g^{-1}) than the edible portions [16].There are great variations in the TPC of the different fruits or same fruits reported by different authors, due to the complex nature of these groups of compounds and the methods of extraction and analysis [17]. It was shown that the phenolic content of plants are influenced by a number of intrinsic (genus, species, cultivar) and extrinsic (agronomic, environmental, handling and storage) factors [18].

Table 1 Total phenolic and flavonoids contents for the studied extracts of JFP

JFP extract	Total phenolics (mg GAE/g)	Total flavonoids (mg RE/g)
Water	0.25±0.017	1.20±0.020
Methanol	0.21±0.012	0.24±0.012
Ethanol	0.46±0.014	0.23±0.016
Acetone	0.18±0.012	0.19±0.021

Data shown as mean ± SD, n=3

Total Flavonoids Content (TFC)

Flavonoids are a group of polyphenolic compounds naturally present in most edible fruit and vegetable plants. They constitute most of the yellow, red and blue colors in the fruit [19]. Flavonoids from the fruit and vegetables are currently widely studied as components that have the potential to provide multiple health benefits. Epidemiological and clinical studies have provided evidence of a potential role for flavonoids in lowering the risk of coronary heart disease prevention, cardiovascular diseases, free radical scavenging capacity, neurodegenerative diseases, osteoporosis, and lung cancer [20, 21]. The amount of TFC in different solvent extracts of JFP is shown in Table 1. The results revealed that extractability of flavonoids was also affected by the solvent used. The water was the best solvent for extracting flavonoid compounds (1.20 mg of RE g^{-1}). The methanol, ethanol and acetone were not good solvents for extraction of flavonoids from JFP. Because the solubility of flavonoids changed during ripening process, the majority of flavonoids from under ripe pulp were methanol soluble while the flavonoids from over ripe pulp were water soluble [22].

Radical Scavenging Activity on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Radical

The DPPH· method is used worldwide in the quantification of free radical scavenging activity, *in vitro* and is foreign to biological system [23]. One of the mechanisms to investigate antioxidant activity is to study the scavenging effect on proton radicals. In the present study, the investigation of total antioxidant capacity was measured as the cumulative capacity of the compounds present in the sample to scavenge stable organic free radicals with a deep violet color, which gave the absorbance maxima within 515–

528 nm range, using the DPPH reaction. The presence of antioxidant in the sample leads to disappearance of DPPH radical chromogens, which can be detected spectrophotometrically at 517 nm. This method is sensitive to light, oxygen, pH, and type of solvent used [24]. The radical-scavenging effects of all extracts of JFP are represented in Fig. 1. All the assessed extracts were able to reduce the stable, purple colored DPPH· radical reaching 50% of reduction. The minimum and maximum IC_{50} value was 0.4 mg/ml and 0.7 mg/ml for methanolic extract. It has been proved that phenolic and flavonoids compounds present in the plants are mainly responsible for antioxidant activity [25]. From the above results, the methanolic and water extracts of JFP proved their efficiency as an antioxidant.

FRAP (Ferric Reducing/Antioxidant Power) Assay

The FRAP assay is one of the most simple, rapid, inexpensive tests and very useful for routine analysis. The FRAP assay is developed for direct test of total antioxidant power of a sample. The antioxidant activities of JFP extracts using FRAP assay are shown in Fig. 2, and different concentrations (1–5 mg/ml) were prepared. The ferric reducing power of JFP extracts increased with increasing in concentration (1–5 mg/ml). The JFP extract (5 mg/ml) showed higher ability to reduce Fe^{3+} to Fe^{2+}, 1.7 mM TEAC g^{-1} for methanolic and 1.4 mM TEAC g^{-1} for water extract. It revealed that a methanol soluble factor was most likely responsible for reducing potential of the extracts. The radical scavenging capacity of fruit pulp decreased with increasing ripening status [22]. The phenolic phytochemicals exhibited redox properties which played a crucial role in determining the antioxidant properties [26]. Therefore the reducing ability of extracts was strongly correlated with the phenolic and flavonoid content.

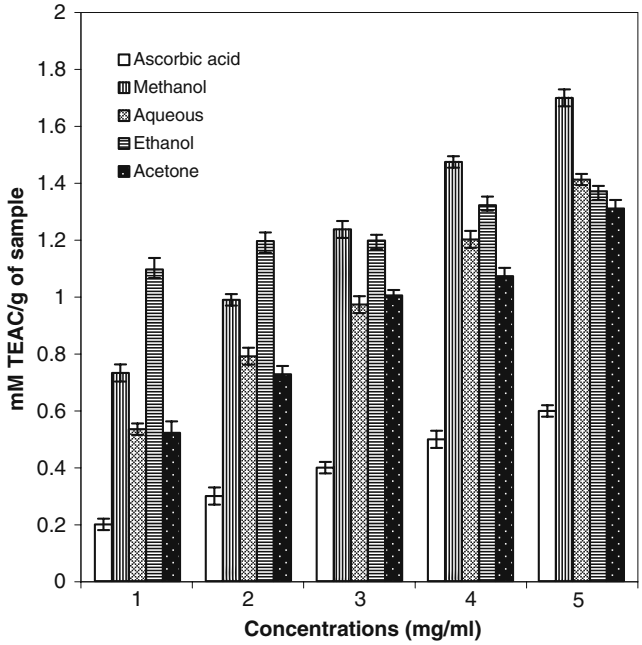

Fig. 2 FRAP assay of different JFP extracts, data expressed in mean ± SD, n=3

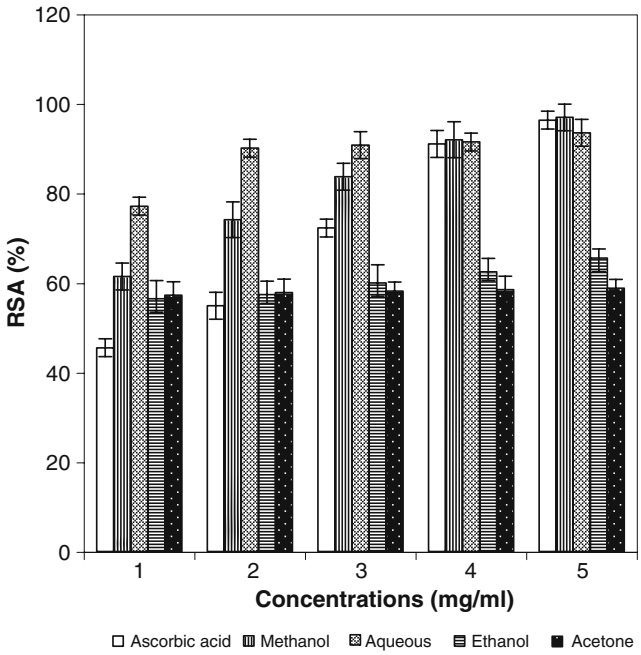

Fig. 1 DPPH radical scavenging activity of different JFP extracts, data expressed in mean ± SD, n=3

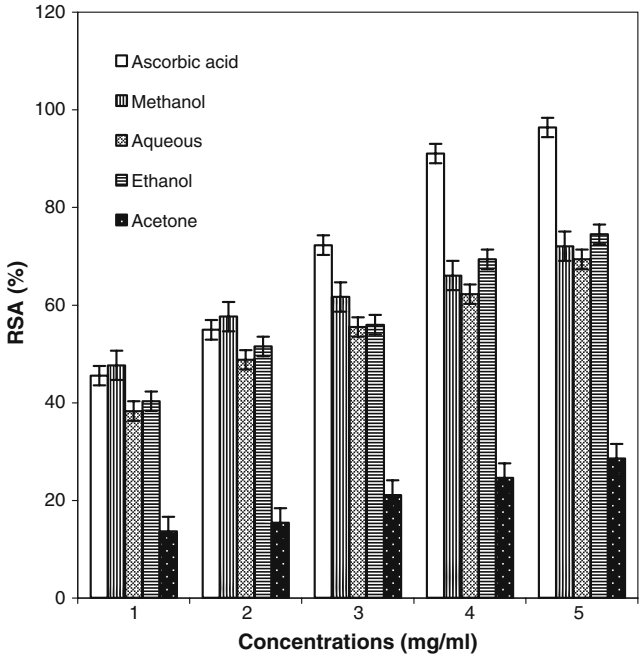

Fig. 3 DMPD radical scavenging activity of different JFP extracts, data expressed in mean ± SD, n=3

DMPD (*N*, *N*-dimethyl-*p*-phenylendiamine) Assay

The DMPD assay has some advantages as high stability of the end point, quick reaction time, cost effective and less cumbersome. In the presence of an oxidant solution (ferric chloride) at acidic pH, DMPD is converted to stable and colored DMPD radical cation (DMPD·+, absorption maxima 505 nm). The antioxidant compounds, which were present in sample able to transfer a hydrogen atom to DMPD·+ and caused discoloration, which was proportional to their concentration [27]. Data in Fig. 3 showed the antioxidant activity (% RSA) of the different JFP extracts. Results (Fig. 3) clearly indicated that all the extracts exhibited antioxidant activity and it was dose dependent methanol > ethanol > water > acetone. The concentrations of JFP extracts that cause 50% inhibition (IC_{50}) were as follows: 3.43 mg/ml for methanolic extract, 3.6 mg/ml for ethanolic extract and 3.9 mg/ml for water extract. It was observed that all JFP extracts exhibited lower free radical scavenging activity than the standard ascorbic acid.

Conclusion

In the present study, the extracts from jackfruit pulp were found to possess strong antioxidant activity. The antioxidant mechanisms of JFP extracts might be attributed to their free radical scavenging activity. In addition, phenolic and flavonoid compounds appeared to be responsible for the antioxidant activity of JFP extracts. On the basis of the results presented here, jackfruit pulp possesses good antioxidant properties. Evaluation of the total antioxidant capacity of fruits, vegetables and other plant products could not be performed accurately by any single method due to the complex nature of phytochemicals. In this study, we have reported their antioxidant capacity against DPPH radical, ferric reducing power and DMPD radical. The jackfruit is a rich source of phytochemicals including phenolic compounds and offers opportunities for development of value-added products from jackfruit, neutraceutical and food applications to enhance health benefits.

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