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Phenolic and Flavonoid Contents in the Seeds Extract of Most Commonly Consumed Fruits in India and Their Antioxidant Properties

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ABSTRACT

Background: Fruits residue may act as a source of environmental pollution by releasing nutrients or toxic chemicals in the soil as well as providing a platform for the reproduction of various disease-causing vectors. Thus, recycling fruit waste in pharmaceutical or agricultural sectors may be one of the options for their management. Because of these aspects, the present study was conducted to assess the total phenolic and flavonoid contents in methanol extract from the seed of most commonly consumed fruits in India and their antioxidant properties using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.

Method: The fruits were procured from the fruit shop corner and the seeds were separated manually. The total phenolic and flavonoid content in the methanol extract of seeds of test plants was quantified using standard methods. The DPPH scavenging potential of the extract was further evaluated.

Result: The results of the present study showed that the extract of the seed of the test plant has significant amounts of total phenolics, varying from a minimum of 153.72 µg GAE/g to maximum of 344.20 µg GAE/g and flavonoids compounds, varied from a minimum of 118.7 µg OE/g to a maximum of 168.3 µgQE/g. The results further showed that the seed extract possesses DPPH inhibition potential (78.86%-87.39) and their IC_{50} ranged between 102.60 µg/ml-45.69 µg/ml. The DPPH activity also showed a strong positive relationship with the concentration of extracts and with total phenolic and flavonoid content. The present study suggests that the seed extract of the tested plant has a significant amount of total phenolics and flavonoids compound and possesses higher antioxidant activity. The individual phenolic compound should be identified for its commercial utilization and promotion.

Key words: Antioxidants, Environmental pollutants, Fruit residue-seeds, Human promotion, ROS.

INTRODUCTION

Phytoantioxidants present in several medicinal plants are responsible for inhibiting the harmful effects of oxidative stress. Antioxidant substances, such as phenolic compounds, flavonoids, tocopherol and ascorbic acid, appear in many fruits and vegetables and seeds (Lourenco *et al*., 2019; Phuyal *et al*., 2020). Antioxidants are widely used in dietary supplement diet of natural foods with antioxidant compounds that can protect the human body from oxidative stress and has been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. In developing countries where the use of herbal medicines is widely used for their basic health needs. Medicinal plants have been used worldwide since ancient times for the treatment of various diseases, including asthma, abdominal disorders, skin diseases, respiratory and urinary complications and liver and cardiovascular disease. the medicinal plant has a maximum amount of antioxidant properties (Bruck *et al*., 2020). Antioxidants with different mechanisms of action are used to prevent or treat various diseases that are associated with oxidative stress and possess therapeutic effects in many cases (Hrelia *et al*., 2020; Shashank 2020; Vaiserman *et al*., 2020). Since the most important aspect of the treatment of viral diseases is the suppression of viral replication followed by cell survival, the search for drugs that have antiviral properties among antioxidants is promising. Interest in recent years in natural antioxidants from plants is increasing due to their free radicals (Fedoreyev *et al*., 2018, Rana and Dahiya 2019). A variety of species of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables, tea, *etc*. and free radical scavenging potential in several plant-based extracts have been screened for investigating their antioxidant and radical scavenging activities (Sahoo *et al*., 2013; Jan *et al*., 2020). Natural antioxidants are a stable part of nutrition as they occur in almost all edible plant products (Sonia *et al*., 2016; Xu *et al*., 2017). Plant and plant-based products are the natural sources of different

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phytochemicals such as phenols, flavonoids, alkaloids, glycosides, lignins and tannins, flavonoids, phenols. Phenols and flavonoids are the most common phytoconstituents of different fruits, vegetables and medicinal and aromatic plants, which are responsible for antioxidant activities. Natural antioxidants such as phenols and flavonoid compounds from plant origin are gaining popularity in the coming years (Phuyal *et al*., 2020). The distribution ofphenolics and flavonoids in nature as antioxidants has therapeutic importance as the ability to trap free radicals (Phaniendra *et al*., 2015). During excessive oxidative stress conditions, the endogenous antioxidant is not enough to deal with the increased levels of ROS (Kumar *et al*., 2019). The accumulation of excessive ROS damages cellular macromolecules such as DNA, protein, lipid and plays a role in the development of many chronic diseases (Kumar *et al*., 2021). Further, oxidative stress may be associated with nearly two hundred diseases, such as cardiovascular, cancer, atherosclerosis, hypertension, ischemia, diabetes mellitus neurodegenerative disease (Alzheimer and Parkinson), rheumatoid, arthritis and aging (Liguori *et al*., 2018). It has been remarked that intake of vegetables and fruits is inversely associated with the risk of many chronic diseases and the use of antioxidant phytochemicals in vegetables and fruits is considered to overcome oxidative stress. Antioxidant phytochemicals can be found in many foods and medicinal plants and play an important role in the prevention and treatment of chronic diseases caused by oxidative stress (Zhang *et al*., 2015). Therefore, the present study was conducted to assess the phenolic and flavonoid contents in methanol extracts from seeds of the most commonly consumed fruits in the Indo-Gangetic plain and their antioxidant properties in pomegranate, watermelon, papaya and citrus fruits. These fruits have the higher antioxidant capacity and reduce the risk of many diseases and are consumed by local people in the Bareilly Rohilkhand region. The largest production of these fruits occurs in tropical and subtropical regions in India. It contains numerous antioxidants, such as vitamins, phenolics, flavonoids, minerals and pantothenic acids. These bioactive compounds provide defense against diseases, such as colon cancer and enhance cardiovascular functions. The seeds of these fruits might be a good source of dietary nutrients and phytochemicals but these seeds are wasted during the processing and consumption of the fruits. These fruit residue waste, which usually polluted our habitat, could be utilized. The fruits waste of several fruit has already been utilized as a new medicine as well as invigorant and cosmetic in recent years in several countries (Zhang *et al*., 2015; Liguori *et al*., 2018; Kumar *et al*., 2019). These present findings can contribute to increasing of medicinal plants or could be used as an antioxidant in food and medicinal preparation. Thus, the study aimed to determine the antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC) of the different plants including their different seeds. Two methods namely DPPH radical scavenging activity were used to determine the antioxidant activity to evaluate the relationship with the TPC and TFC for these purposes, methanolic extract (80%) was prepared and TPC was determined by the method folin-ciocalteureagent (FCR) while TFC by aluminum trichloride ($AlCl_3$) method. Fruits residue may act as a source of environmental pollution by releasing nutrients or toxic chemicals in the soil as well as providing a platform for the reproduction of various disease-causing vectors. Thus, utilization of fruit waste in pharmaceutical of agriculture sectors may be one of the options for their management. Therefore, the present study was conducted to assess the phenolic and flavonoid contents in methanol extracts from seeds of the most commonly consumed fruits in the Indo-gangetic plain and their antioxidant properties, especially in pomegranate, watermelon, papaya and citrus.These plants were selected for the present study based on their frequent consumption by the local people of the indo-gangetic plains, especially in the Bareilly district of Uttar Pradesh.

MATERIALS AND METHODS

DPPH (1,1-Diphenyl-2-picrylhydrazyl radical), FolinCiocaltue reagent (FCR) or folin's phenol reagent, gallic acid, Quercetin, methanol, ethanol, sodium. Carbonate, aluminum chloride, were obtained from Sigma-Aldrich Pvt.Ltd, India and Merck, Pvt. Ltd., India. All other chemicals used in the present study were of analytical grade. All the experimental work was carried out in the the Departmental laboratory of Bareilly College Bareilly. 2 kg of fresh fruits of *C. papaya, C. lanatus, P. granatum* and *C. sinensis* were collected in plastic bags from the juice corner shops in different areas of Bareilly and brought back to the laboratory. The seeds were separated from the fruits and air-dried till constant weight was achieved. The dried seeds were stored at room temperature till further analysis. Fresh seeds of all samples were peeled off by using a blade. Each type of seed was washed separately with water to remove dust. Then seeds were crushed in a motor and pestle by using 80% (w/v) methanol solution. Then the crushed seed sample was transferred into centrifugation tubes and covered with aluminum foil. The seed extract was placed in the refrigerator at 4°C for 72 hours. The dried extracts were sealed in sterilized tubes and stored in the refrigerator at 4°C for further analysis (Tiwari *et al*., 2011). The published protocol was used for the estimation of total phenolics contents in the methanol extracts of different samples involving Folin-Ciocalteaureagent (FCR) and gallic acid as standard (Wolfe *et al*., 2003). The amount of phenolic content is expressed as milligrams of GAE (gallic acid equivalent) per g of fresh weight (fw). Total flavonoid contents in the extract of each sample were estimated using aluminum chloride reagent and quercetin as standard according to the previously published method (Ordonez *et al*., 2006). The amount of flavonoid content is expressed as milligrams of quercetin equivalent per g of fresh weight (Total flavonoids in mg QE/ g fw). 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was done according to the published method (Liyana and Shahidi 2005). The antioxidant activity of seed extract was

determined by the radical scavenging capacity of the methanolic extract of seed using DPPH. DPPH solution (0.004% w/v) was prepared in methanol (Blois *et al*.,1998). Methanol extract of seeds was mixed with methanol to prepare the stock solution (100 µg/10 ml). The concentration of this seeds methanol extract solution was 100 µg/10 ml from stock solution, 100 µg/ml, 80 µg/ml, 60 µg/ml, 20 µg/ml and solution (0.004%w/v) was added in each of these test tubes containing seeds methanolic extract and after 30 min, the absorbance was taken at 517nm using a UV-visible spectrophotometer.

The statistical analysis was performed by using Graph-pad Prism 8 software USA. All experiments were carried out in triplicate and the results are expressed as the mean ±standard deviation (SD).

RESULTS AND DISCUSSION

Annually, approximately one-third of the world's food production is wasted or lost. According to the food and agriculture organization, 40% of food was lost in developed countries. During fruit processing in the industry, an enormous amount of solid waste is produced in developing countries like India. These fruit residues might be a rich source of potential antioxidants. In India, approximately 80% of people rely on traditional plant-based drugs for their primary healthcare needs (Hashempour *et al*., 2010; Yadav *et al*., 2018). The fruit residues such as peel and seeds are may contain several natural compounds such as carotenoids, polyphenols and flavonoids. These fruits waste may be used as an alternative remedy to cure various harmful human diseases in the human. Therefore, the present study was performed to explore the occurrence of natural Phyto antioxidants in the fruit waste (seed) from most common and large-scale consumed fruits in India. In the present study, the total phenolic and flavonoid content in seed extract of most commonly consumed tropical fruits were analyzed and the results are presented in Fig 1 and Fig 2. The total phenolic contents in the tested seeds of tropical fruits ranged from a minimum of 154.72 µg GAE/ gm to a maximum of 341.72 µg GAE/ gm. The total phenolic content was found maximum in *C. reticulata* (341.72 µg GAE/ gm) followed by *C. lanatus* (327.07µg GAE/ gm), *P. granatum* (308.19 µg GAE/ gm), *C. sinensis* (253.28 µg GAE/ gm) and the lowest in *C. papaya* (154.72 µg GAE/ gm). The results obtained show a trend toward higher TPC to lower TPC as *C. reticulate*>*C. lanatus*>*P. granatum*>*C. sinensis*>*C. papaya* (Fig 1). A large amount of fruit residue including seeds and peels are wasted from these fruits. It may contain numerous antioxidants, such as vitamin B, vitamin C, flavonoids, phenol, minerals and pantothenic acids. These waste products can therefore be converted into high-value products and thereby reducing problems associated with environmental pollution (Vesna *et al*., 2019; Ali *et al*., 2020). It is also well established that natural antioxidants from plant products are safer than their synthetic counterparts (Phuyal *et al*., 2020). Earlier reports suggested that pomegranate seeds and peel may possess extraordinary phytochemicals that have medicinal value (Vesna *et al*., 2019, Ávila *et al*., 2020). Gallic acid is the major phenolic acid in pomegranate and it has antimutagenic, antiallergic, anticarcinogenic and anti-inflammatory activities (Maissa *et al*., 2021). The total flavonoid content was determined using the aluminum chloride spectrophotometric method, reported as quercetin equivalent standard (QE) by reference to the standard curve (Y= 0.0838X+0 and R^2= 0.99). The TFC of respective fruits seed extract is given in Fig 2. The total flavonoid content in the tested seeds of tropical fruits ranged from a minimum of 119.13 µg QE/gm to a maximum of 167.4 µg QE/gm. The total flavonoid content was found in *C. lanatus* (167.4 µg QE/gm) followed by *C. reticulata* (162.23 µg QE/gm), *P. granatum* (143.76 µg QE/gm), *C. sinensis* (117.46 µg QE/gm) and lowest in papaya (119.13 µg QE/gm) (Fig 2). Polyphenolic compounds are vital antioxidants and it contains a minimum of two hydroxyl groups attached to an aromatic ring. The antioxidant properties of fruits and vegetables are largely contributed by the polyphenolic compounds(Abinaya *et al*., 2020). Due to their electron-

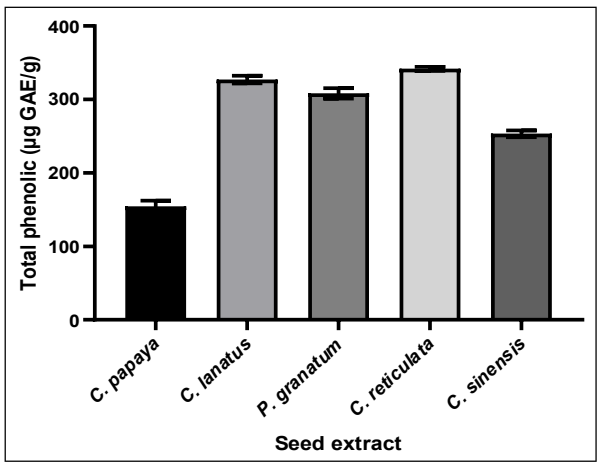

Fig 1: Showing variations in the total phenolics content in seed extracts from tropical fruit.

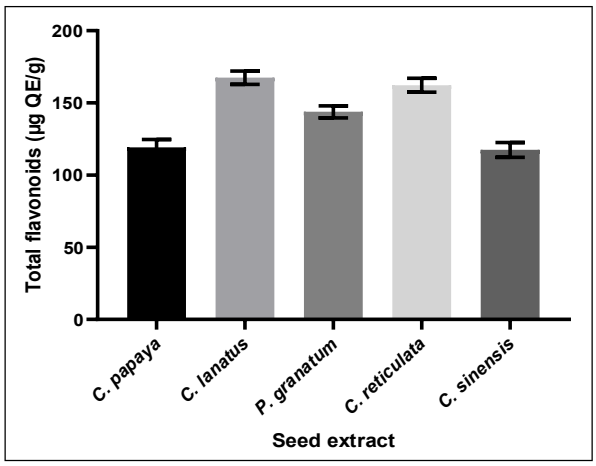

Fig 2: Showing variation in the total flavonoid content in seed extracts from tropical fruits

donating properties, these phenolics are capable of scavenging reactive oxygen species (ROS) (Fischer *et al*., 2018, Alam *et al*., 2020). The quantitative analysis of phenolic acid and flavonoids by using a spectrophotometer is also well known (Sankhalkar *et al*., 2016). In the present study, the total phenolic contents in the tested seeds of tropical fruits ranged from a minimum of 154.72 µg GAE/g to a maximum of 341.72 µg GAE/ g. Interestingly, the present total phenolic contents were found higher than other Citrus fruits reported earlier (Hashempour *et al*.,2010). Then the total flavonoid content was also determined and reported as quercetin equivalent standard (QE) by reference to the standard curve (Y= 0.0838X+0 and R^2= 0.99). The total flavonoid content in the tested seeds of tropical fruits was ranged from a minimum of 119.13µg QE/ g fw to a maximum of 167.4 µg QE/g. Several studies have shown that high phenolic and flavonoid content is associated with greater antioxidant activity or vice versa (Zheng *et al*., 2001). However, significant variation was observed in the total phenolic and total flavonoid content in different seeds of plant species but it was higher than those reported earlier (Ghasemi *et al*., 2009; Gattuso *et al*., 2007; Bag and Chattopadhyay 2015).

The DPPH is a very stable free radical than in vitro generated free radicals such as the hydroxyl radical and superoxide anion. In the present study, the percentage of scavenging activity in the tested seed of tropical fruits ranged from a minimum of 77.31% to a maximum of 88.26% (Fig 3). The percentage of scavenging activity was found maximum in *C. reticulate* (88.26%) followed by *C. lanatus* (86.45), *P. granatum* (82.98) and *C. sinensis* (82.72) and the lower in *C. papaya* (77.31). The results obtained indicated that higher percentage of DPPH. The values of DPPH scavenging activities found in the present study were higher than those in wild edible mushrooms (Ferreira *et al*., 2007). The results obtained show a trend towards higher DPPH radical scavenging activity to lower DPPH radical scavenging activity as *C. reticulata*>*C. lanatus*>*P. granatum*>*C. sinensis*>*C. papaya* (Fig 3). A lower IC_{50} indicates a higher antioxidant activity of a compound in the blow table shows the IC_{50} values in the DPPH radical scavenging activity assay of the extracts. In the present study, the IC_{50} in the tested seeds of tropical fruits ranged from a minimum of 75.56µg/ ml to a maximum of 102.60µg/ml were observed. The IC_{50} was found minimum in BHT (45.69 µg/ml) as standard substances followed by *C. lanatus* (75.56 µg/ml), *C. reticulata* (83.20 µg/ml), *P. granatum* (89.20 µg/ml), *C. sinensis* (98.91 µg/ml) and the lowest in *C. papaya* (102.60 µg/ml) (Table 1). The IC_{50} value is the amount of antioxidants required to scavenge 50% DPPH free radicals (Bag and Chattopadhyay 2015, Hymery *et al*., 2021). In the present study, we observed the IC_{50} in the tested seeds of tropical fruits ranging from a minimum of 102.60 µg/ml to a maximum of 45.69 µg/ml, which is indicating suitability to develop a remedy for the prevention of human disease-related to excessive free radicals generation.

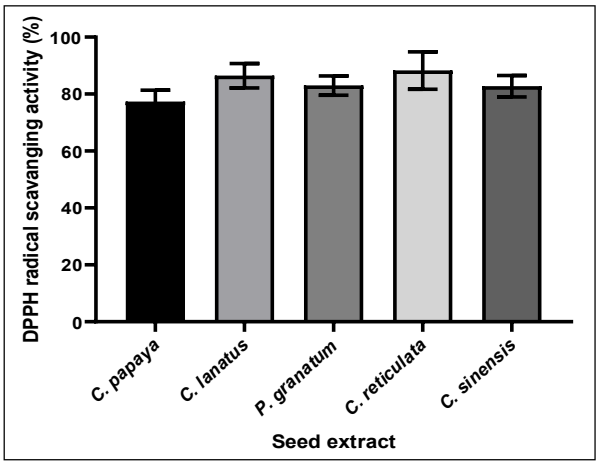

Fig 3: Showing variation in the DPPH Inhibition capacity (%) of seed extracts from tropical fruits.

Table 1: Inhibition concentration (IC_{50}) values of seed extracts from tropical fruits.

Seeds extracts	Y=m X+c	R^2	IC_{50} (µg/ml)
BHT (control)	Y=15.51X+17.74	0.71	45.69
C. papaya	Y=0.6060X-4.206	0.97	75.56
C. lanatus	Y=0.4616X +2.698	0.97	102.60
P. granatum	Y=0.5520X+0.726	0.87	89.26
C. reticulata	Y=0.574X+2.248	0.89	83.20
C. sinensis	Y=0.501X+0.444	0.94	98.91

CONCLUSION

In conclusion, the total phenolics and flavonoid contents in methanol extracts of seed of *C. papaya, C. lanatus, P. granatum, C. reticulata* and *C. sinensis* plants and their antioxidant properties were evaluated. The study revealed that tested seeds of *C. reticulata, C. lanatus* are found as a rich source of nutritional total phenolics and possess potent antioxidant activities as compared to *P. granatum, C. reticulate* and *C. sinensis*. The DPPH activity also showed a strong positive relationship with the DPPH inhibition potential of the extract. The present study suggests that test plants could be used for the health benefits of local people. The identification of individual phenolic and flavonoid compounds in the extracts of the test plant further suggested its commercial utilization.

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Conflict of interest: None.

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