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In-vitro antioxidant activity of freeze dried extracts fruit peel waste (*Punica granatum* and *Citrus sinensis*) and its effect in α-linoleic acid model system

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Abstract
Every year huge quantities of fruit waste are generated by fruit Industries. These wastes are can be recover as natural antitoxins which would be beneficial to the society and individual. Antioxidants from fruit wastes have attracted the attention of researchers as they are cheaper in cost, easy to derive, and do not have any side effects. Thus the current investigation was focused on to extract natural antioxidant and evaluates their properties through various *in vitro* assays. The pomegranate peel exhibited higher total phenolic content (TPC) of 160.80 mg/g GAE while the orange peel (OPE) showed lower. Total flavonoid contents OPE showed the highest (2.955 mg/g quercetin equivalent), while the PPE showed 0.98mg/g quercetin equivalent. The DPPH radical scavenging activity exhibited a dose dependent activity. The PPE extract showed the radical scavenging activity of 94.25 at the concentration of 35µg/mL whereas the same at the same concentration BHA showed inhibition of 95.59% which was almost equal to BHA. The highest chelating activity was 28.42% for PPE recorded at a concentration of 1000 µg/mL, while at the same concentration the chelating activity of OPE was half of that of PPE. Lipid peroxidation inhibition activity of PPE and OPE was observed for 7 days under controlled temperature condition. The aqueous ethanolic extract of PPE was the strongest inhibitor compare to OPE. The results of present investigation reveals that the pomegranate peel extracts could be used as a natural antioxidant to food and food products to prevent the oxidative degradation.

Keywords: Fruit waste, pomegranate peel, total flavonoid and ethanolic extract

Introduction
Antioxidant is defined as any substance that when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1995) [10]. Pomegranate (*Punica granatum* L.) is known as one of the healthiest fruits due to its high phenolic content and antioxidant activity. Antioxidant potential of pomegranate *in vivo* and *in vitro* has been proved. In addition to its antioxidant activity, it has antimicrobial, antibacterial, antiviral, antifungal and antimutagenic properties as well as beneficial effects on the oral and cardiovascular diseases. The addition of synthetic antioxidants, such as propyl gallate (PG), butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and tertiary Butyl hydro quinine (TBHQ) has been widely used industrially to control lipid oxidation in foods and food products. However, the use of these synthetic antioxidants has been health hazardous and potential health risks and toxicity (Kalt & Kappus, 1993) [14]. In recent years, the research of natural antioxidants as alternative sources to synthesis antioxidants has emerged and the utilization of the diverse secondary metabolites of the plant was highlighted. These substances are able to reduce free radicals like superoxide, peroxyl, alkoxylate and hydroxyl (Benhammou, 2008) [3]. The objectives of this present investigation are to determine antioxidant activity and its effect in α-linoleic acid model system.

Material and Methods
Preparation of Extracts
The fruit peel waste (pomegranate and orange) were collected from juice vendors in Mangalore city India. The pulp was separated from the peel and washed to remove the unnecessary materials.

The washed peels were cut into small pieces and dried in hot air oven at 60 ºC for 12 h. The dried peels were ground in the kitchen grinder to make the fine powder to pass through 1 mm sieve. The extraction was carried out according to the methods described by Mansour and Khalil (2000) [15] with little modification. 25 g of peel powder was mixed with 100 mL of 70% ethanol. The mixture of peel powder and ethanol subjected to shaking in a rotary shaker (Orbitek Schigenics Biotech Pvt. Ltd. Chennai) at ambient temperature for 12 h at the speed of 190 rpm. The filtrates of the mixture were placed under a hood to remove the ethanol from the filtrates of each material using a rotary vacuum evaporator (Superfit Rotavap PBU-6D, Mumbai) at 40 °C. The residual aqueous extracts was frozen overnight and then freeze-dried (Thermo Fisher Scientific MODULYOD-230, USA). The extract was obtained from both the peels powder were weighed to calculate the yield. The both the extracts were stored at -20 ºC in a sample container for further analysis.

Analysis of *in-vitro* antioxidant activity

The concentration of total phenolics in the extracts was determined by following method of Singh *et al.* 2002 [22] and results were expressed as gallic acid equivalent (GAE)/g. Total flavonoid content (TFC) was Determined by the method suggested by Christ and Muller (1960) [6] using $AlCl_3$ colorimetric method with a suitable modification and expressed as mg quercetin (QE)/g extract. The Diphenyl-1-picrylhydrazyl DPPH free radical scavenging capacity of PPE and OPE was determined by using DPPH assay according to the method described by Abdulwahab *et al.* (2011) [1]. 2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid ABTS radical scavenging activity was assayed according to the method of (Re *et al*. 1999) [20]. Lipid peroxidation inhibition (LPI) assay of the fruit peel extracts was determined by the methods of Osawa and Namiki (1985) [8]. The degree of oxidation of linoleic acid was measured using the ferric thiocyanate method of Mitsuda *et al.* (1996) [17] during 7 days of storage at 40 ºC and results were compared with synthetic antioxidant BHA.

Results and Discussion

Total Phenolic Compounds (TPC) and total flavonoid content (TFC)

Phenolic compounds are secondary metabolites, which are produced in the shikimic acid of plants and pentose phosphate through phenyl propanoid metabolization Randhir *et al.* (2004) [19]. They contain benzene ring with one or more hydroxyl substituent's and range from simple phenolic molecules to highly polymerized compounds Velderrain-Rodríguez *et al.* 2014 [24]. Mainly they constitutes is one of the major groups of compounds acting as primary antioxidants. The content of phenolic compounds is expressed as mg of Gallic acid equivalent (GAE) /g extract. The Folin-Ciocalteu method is used for the determination of total phenolic compounds.

Table 1: Total Phenolic Compounds and total flavonoid content

Extracts	TPC (GAE Equ./g)	TFC(QE Equ./g)
PPE	160.80	1.75
OPE	36.71	3.25

The pomegranate peel exhibited higher total phenolic content (TPC) of 160.80 mg/g GAE while the orange peel showed lower TPC of 36.71 mg/g GAE (Table 1.) The results of PPE were higher compare to our pervious study which was extracted by absolute ethanol without fridge drying (Pal *et al.* 2017) [13]. This higher value of TPC may be due to the extraction methods. The sample extracted by aqueous ethanol was may be able to extract the compounds which is only soluble in aqueous ethanol. The flavonoids are a class of secondary plant phenolics as they are having significant amounts of antioxidant activity and chelating properties. In the human diet, they are most concentrated in fruits, vegetables, wines, teas and cocoa (Heim *et al.* 2002) [12]. The flavonoids constitute the largest group of plant phenolics, accounting for over half of the eight thousand naturally occurring phenolic compounds (Harborne *et al*. 1999) [11]. The OPE showed the highest flavonoid content compared to PPE. The orange peel extract showed 2.955 mg/g quercetin equivalent (Table 1.) while the PPE showed 0.98mg/g quercetin equivalent. The result of TFC of OPE was in agreement with the results of Singh and Immanue (2014) [23] which was lower than pervious study. The citrus fruit contain three types of flavonoids compounds *viz.*, flavanones, flavones and flavonols. The main flavonoids found in citrus species are hesperidine, narirutine, naringin and eriocitrin (Schieber *et al.* 2001) [21].

DPPH free radical scavenging capacity

The stable radical DPPH has been used widely for the determination of primary antioxidant activity; it is the free radical scavenging activities antioxidants compounds. The assay is based on the reduction of DPPH radicals in methanol or ethanol which causes an absorbance fall at 515 nm. In this study, the antioxidant activity was expressed as % of inhibition. In the existing investigation the results of DPPH radical scavenging activity of PPE and OPE are presented in figure 1. The DPPH radical scavenging activity exhibited a dose dependent activity radical scavenging activity increased with increase in the concentration of extract. The DPPH radical scavenging activity of both the extracts showed at different concentration range 5 µg/mL to 35 µg/mL in PPE while OPE showed at the concentration range of 100 µg/mL to 700 µg/mL. The PPE extract showed the radical scavenging activity of 94.25 at the concentration of 35µg/mL whereas the at the same concentration BHA showed inhibition of 95.59% which is about equivalent to PPE. The OPE showed the radical scavenging activity 89.67% at a higher concentration of 700 µg/mL. The higher antioxidant activity of PPE may be due to PPE contain higher amount phenloic contents. The present study also support with the results of Chidambara *et al*. 2002 [5] they reported pomegranate peels showed 83 and 81% DPPH antioxidant activity at the concentration 50 ppm.

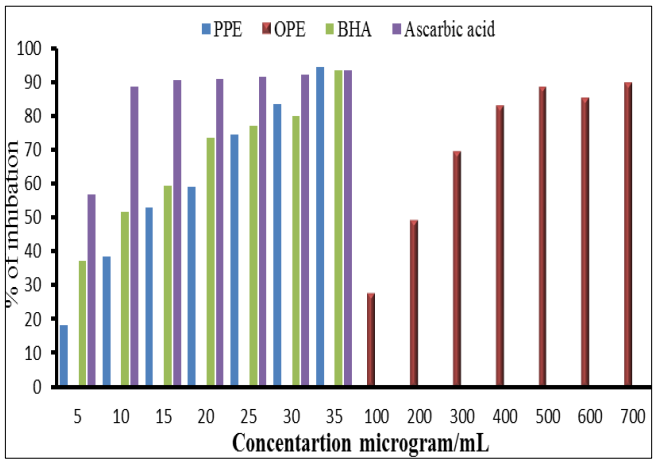

Fig 1: DPPH radical scavenging activity of PPE and OPE

Metal chelating activity

The metals such as iron and copper are known to be able to generate reactive oxygen substance in biological systems; hence they can accelerate oxidation in the system. Though iron is an essential mineral in the blood of the meat it is one of the most active pro-oxidants promoting lipid oxidation, also called as Fenton reagent (Chemizmu and Fentona, 2009) [4]. The metal chelation may provide important antioxidative effect by retarding metal-catalyzed oxidation reactive oxygen species (singlet oxygen) as observed by Gulcin *et al.* (2010) [9]. The chelating activity slightly increased with increase in concentration of the extracts. The highest chelating activity was 28.42% for PPE recorded at a concentration of 1000 µg/mL, while at the same concentration the chelating activity of OPE was half of that of PPE. The results of present investigation are also supported with the finding of Orak *et al.* (2012) [18] where they found that the metal chelating activity of pomegranate peel was 37.22% and it was remarkably higher than the pomegranate seed. The results of previous study were higher than the present study as reported by Orak *et al.* (2012) [18]. However, EDTA showed an excellent chelating ability of 79.23% at the concentration of 100µg/mL which might be because of EDTA is a potent metal chelating activity agent.

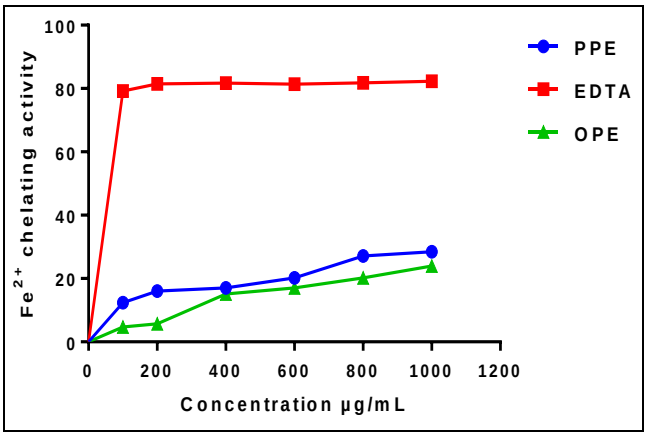

Fig 2: Metal chelating activity of PPE and OPE
A-linoleic acid model system

Linoleic acid, an unsaturated fatty acid, served as an efficient model compound for lipid oxidation and antioxidant studies in the emulsion system. The formation of peroxides in the linoleic acid model system results in increased absorption at 500 nm (Yen and Chen, 1995) [25]. These hydroperoxides are highly unstable and react readily with ferrous ions to produce ferric ions. The resulting ferric ions are detected using thiocyanate ion as chromogen, with an optimum wavelength at 500 nm (Mihaljevic *et al.* 1996) [16]. The presence of an antioxidant in the solution it inhibits auto-oxidation of linoleic acid, resulting ultimately in low production of the ferric thiocyanate complex. Low absorbance is the means a high peroxidation inhibitory activity of a sampled for antioxidant activity. Lipid peroxidation inhibition activity of PPE and OPE was observed for 7 days under controlled temperature (Figure 3.) The aqueous ethonlic extract of PPE was the strongest inhibitor compare to OPE. The PPE extracts showed almost similar behaviour activity compare to BHA. Similar results also reported by Ahmed *et al.* 2015 [2] they reported that aqueous extract of *Adiantum caudatum* Leaves was the strongest inhibitor of hydroperoxide after two days of storage.

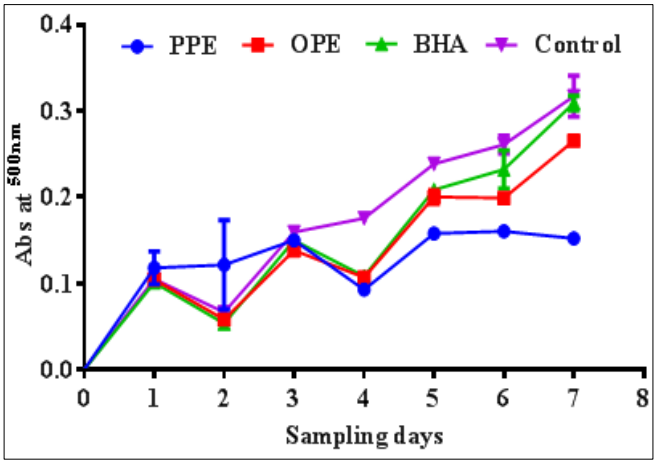

Fig 3: Lipid peroxidation inhibition of PPE and OPE

Conclusion

Based on the results of present instigation it can be conclude that the fridge-dried extracts of pomegranate and orange peel is the good source natural antioxidants. Pomegranate peel showed the highest activity in comparison to orange peels. In α-linoleic acid model system it showed strong peroxidation inhibition activity. The current investigation suggested that pomegranate peel can be utilized as a natural antioxidant to food and food products to prevent oxidative degradation.

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