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# Bioactive compounds and antioxidant activity of selected Indian pummelo (*Citrus grandis* L. Osbeck) germplasm

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ABSTRACT

India is an important diversity center for different types of citrus species including pummelo (*Citrus grandis* L. Osbeck). The major objective of the present study was to determine variation in polyphenolic compounds, antioxidant activity, sugars and organic acid in 16 pummelo (red, pink and white flesh) genotypes. Significant differences ($p < 0.05$) in the fruit quality, polyphenolic content and antioxidant activity were observed among red, pink and white fleshed genotypes. Naringin was the predominant flavonoid compound identified in all genotypes, however, pummelo selection 8 (PS8) and pummelo selection 13 (PS13) were found to be particularly high in naringin. Other phenolics quantified in the juice included, caffeic, epicatechin, benzoic acid, neoeriocitrin, hesperidin, and narirutin. Hierarchical clustering revealed four definite clusters based upon their composition.Thin peeled and red fleshed genotypes, pummelo selection 4 (PS4) and pummelo selection 12 (PS12) with the high concentration of lycopene and phenolics seem to be promising genotypes for breeding elite cultivars.

## 1. Introduction

Pummelo (*Citrus grandis* L. Osbeck) is an ancient citrus species and an established source of dietary polyphenolic flavonoids (Zhang et al., 2011). The fruit holds tropical origin and is known to have a wide climatic adaptability and can grow well in warm areas of the world where other sweet citrus fruits cannot be grown (Paudyal, 1999). North-eastern Himalayan region and foothills of the central and western Himalayan tracts in India have rich citrus diversity and these regions are considered to be one of the important centers of origin for pummelo. The species is also a progenitor of the grapefruit (*Citrus paradisi*) and the tangelo among other modern citrus hybrids (Hazarika, 2012; Singh and Singh, 2003).

Pummelo has a unique and complex phytochemical composition encompassing the presence of dietary fiber, vitamin C, vitamin b-complex, lycopene and flavonoids such as naringin, naringenin, hesperidin, neohesperidin, ellagic acid, caffeic acid and epicatechin (Xu et al., 2008; Zhang et al., 2011). The phytochemicals are directly or indirectly responsible for a wide range of health-promoting effects including reducing risk of chronic and degenerative diseases (Moraga et al., 2012; Xu et al., 2008). Pummelo fruit extracts have been shown to demonstrate anti-hyperlipidemic properties, help reduce blood cholesterol and triglycerides levels in alloxan-induced diabetic rats (Oyedepo, 2012). Fruit extracts bind to primary and secondary bile acid and help reduce the risk factor in developing colorectal cancer by inhibiting the activity of enzymes pancreatic lipase and cholesterol esterase (Mäkynen et al., 2013). In addition, the fruit extracts have been shown to reduce reactive oxygen species in $H_2O_2$-treated HepG2 cells (Lim et al., 2006). However, despite the impressive phytochemical composition, pummelo is still an underutilized species in comparison to other citrus species because of its large sized fruit, thicker peels, and bitter juice.

In India, North-East region has diverse indigenous germplasm with high quality red, pink and white to yellow fleshed cultivars due to favorable climatic conditions (Roy et al., 2014; Singh and Singh, 2003). This diversity of fruits reverberates in its overall biochemical composition including phenolic compounds which directly affect quality and organoleptic traits. The germplasm adapted to difficult ecological conditions attract considerable attention, due to their high polyphenolic profile which may be used as a genetic resource for breeding elite cultivars.

At the same point, developing elite cultivars enriched in health-

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promoting polyphenols must realize potential limitations of consumer's acceptance as these compounds are also known to impart bitterness and astringency making the juice less desirable. A balance between sugars, acids and phenolic profile in the juice can overcome this bottleneck, thus improving quality and consumer acceptability. These reasons underpin the importance of information on chemical composition and phytochemical concentration in different germplasm. Documenting the information can help in developing quality and processing standards for regulatory authorities, authentication of variety, origin, and traceability (Du and Chen, 2010; Huang and Ho, 2010).

Phenolic composition of many citrus species from different parts of the world has been reported (Goulas and Manganaris, 2012; Jayaprakasha et al., 2008; Mäkynen et al., 2013; Ramful et al., 2011; Xi et al., 2014; Xi et al., 2015). Previous studies with respect to pummelo have been reported in cultivars from China, United States, Malaysia, and Thailand (Cheong et al., 2012; Jain et al., 2017; Mäkynen et al., 2013; Xi et al., 2014). However, to the best our knowledge, there is no information on the concentration of different metabolites in local pummelo genotypes grown under Indian conditions. Characterization of different metabolites including polyphenolics will assist in the retrieval of the indigenous resource for breeding elite cultivars and promotion of pummelo fruits as a functional food. Keeping this in mind, the aim of the present investigation was to characterize bioactive compounds, sugars and organic acids in 16 genotypes of pummelo (red, pink and white flesh) grown in India.

## 2. Materials and methods

### 2.1. Chemicals

Naringin, hesperidin, neoeriocitrin, narirutin, ferulic acid, epicatechin, caffeic acid, hydroxybenzoic acid, ellagic acid and salicylic acid were obtained from Sigma (Sigma-Aldrich Co., Bangalore, India). 2, 2-diphenyl-1-picrylhydrazyl radicals (DPPH), 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ), dimethyl sulfoxide (DMSO), acetic acid and acetonitrile were obtained from Merck (Darmstadt, Germany). All of the other reagents were analytical grade and were purchased from Sigma.

### 2.2. Fruit materials

Sixteen pummelo (*C. grandis* Osbeck.) selections (PS) grown in the research field of the Horticulture division, Indian Agricultural Research Institute, New Delhi, India were selected and coded as PS1 to PS18. The selected experimental location has typical sub-tropical climatic conditions characterized by hot and dry summer followed by cold winter. May and June are the hottest months with the maximum temperature varying between 22.0–44.0 °C, and December and January being the coolest months, with the temperature ranging between 3.0–27.0 °C. This belongs to trans-Gangetic plains of agro-climatic zones of India. Sunshine hour varied from 1.2 h $day^{-1}$ in January to 10.8 h $day^{-1}$ in June. The management practices are adopted as per the recommendations. The budded plants of selected strains were transplanted at 5 m × 5 m distance. Whole experimental orchard was on drip irrigation system and water was given at a rate of 6 L $h^{-1}$ $tree^{-1}$ thrice a week for 4–6 h during summar season, and twice a week for 3–4 h during winter season. Irrigation water used had the electrical conductivity of 1.0 dS $m^{-1}$. As shown in Fig. 1, fruits were harvested at the physiological maturity as adjudged by TSS/TA ratio equal to 12. After harvest, pummelo fruits were weighed and after dividing into peel, pulp (segment epidermis and juice vesicle) and seed; peel thickness was measured using vernier caliper. The pulps were hand squeezed and filtered juice was stored at −20 °C until analysis. All the parameters were evaluated in 3 replications. Each replication of a genotype consisted of 6 randomly selected fruits from all the directions i.e. total 18 fruits of each genotype were used.

### 2.3. Titratable acidity and soluble solids content determination

The AOAC official method for the titratable acidity (TA) of fruit products was used (Association of Official Analytical Chemists (AOAC), 2000). Five mL of the juice was diluted to 10 mL with distilled water and titrated against 0.1N NaOH using phenolphthalein as an indicator to pink end point persisting for 30 s. The percentage acidity was reported in terms of citric acid.

For determination of total soluble solids (TSS), 1 drop of juice was placed on a digital hand refractometer (Atago Co. Ltd.) prism plate of range 0–50%, which was then covered. The reading on the prism scale was noted to one decimal place. The temperature of the sample at the time of measurement was also recorded. The °Brix of the juice was then calculated and temperature correction applied. All determinations were performed in triplicates. Maturity index is an indicator of fruit sweetness and was calculated as the ratio of soluble solids and titratable acidity.

### 2.4. Extraction of phenolic compounds

Extraction of polyphenolics was conducted as described by Ramful et al. (2011) and Xi et al. (2014) with some modification. One mL of juice sample was extracted using 10 mL of 80% methanol. The extract was sonicated for 30 min at 25 °C and then centrifuged at 10,000*g* for 10 min at 4 °C and the extract was decanted, the residue was extracted twice following the same procedure. The combined extracts adjusted to 30 mL and were filtered with 0.4 µm nylon filter and stored at −20 °C until used for the determination of total phenol, total flavonoids and for the antioxidant assays.

### 2.5. Total phenolic content (TPC)

TPC was estimated spectrophotometrically using Folin–Ciocalteu reagent (FCR) (Singleton et al., 1999). To 100 µL of extract, 2.9 mL of deionized water, 0.5 mL of Folin–Ciocalteu reagent and 2.0 mL of 20% $Na_2CO_3$ solution were added. The mixture was allowed to stand for 60 min and absorption was measured at 750 nm against a reagent blank in UV–vis spectrophotometer (Varian Cary 50). Results were expressed as gallic acid equivalent (mg GAE 100 $mL^{-1}$ FW (fresh weight)).

### 2.6. Total flavonoids content (TFC)

Total flavonoids content of pummelo juice was measured by a colorimetric assay developed by Zhishen et al. (1999). A known volume (0.3 mL) of sample extract in acidified ethanol (2.1 mL) was taken. At zero time, 0.3 mL of 5% w/v sodium nitrite ($NaNO_2$) was added; followed by addition of 0.3 mL of 10% w/v aluminium chloride ($AlCl_3$) and 2 mL NaOH (1 M) after 5 min and 6 min respectively. The solution was mixed well again, appearing pink to yellow color. The absorbance was read at 510 nm against a reagent blank using 80% aqueous methanol instead of the sample in UV–vis spectrophotometer (Varian Cary 50). The results expressed as quercetin equivalent (mg QE 100 $mL^{-1}$ FW).

### 2.7. Antioxidant activity

#### 2.7.1. Ferric reducing antioxidant power (FRAP)

FRAP assay for juice was determined according to the procedure described by Benzie and Strain (1996). FRAP reagent of 3 mL was mixed with 100 µL of sample extract in a test tube, vortexed and incubated at 37 °C in a water bath for 4 min. Reduction in the ferric–-tripyridyltriazine to the ferrous complex formed an intense blue color which was measured using UV–vis spectrophotometer (Varian Cary 50) at 593 nm at the end of 4 min. The results were expressed in terms of trolox equivalent (µmol TE $mL^{-1}$ FW).