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Phytochemical Evaluation of Pummelo Fruits (*Citrus grandis*) in India for Enhancing Marketing Opportunities

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Pummelo *(Citrus maxima*) is a nutrient-rich citrus fruit that helps to preserve human health and provide nutritional security. The objective of the study was to investigate the effect of different sites on the phytochemicals content in eight accessions collected across India. Different varieties of pummelo were collected and analysed for their physico-chemical, biochemical and phytochemical properties, mainly total soluble solids (TSS), acidity, total phenols, limonoids, hesperidin and naringin. Results revealed that individual phytochemicals, flavonoids showed unique characteristics. Considerable differences were observed regarding the naringin (51.42-129.66 mg/l) and hesperidin (0.44-3.35 mg/l) contents among the different accessions. Naringin the predominant flavonone glycoside along with hesperidin was recorded highest in Pummelo from Salem (Tamil Nadu) as compared to the other accessions.

Key Words: *Citrus grandis*, Pummelo, Citrus fruits, Phytochemicals, Flavonoids

Introduction

Citrus fruits are one of the most traded horticultural crops in the world. They are considered as an important component of human diet because of their nutritional values. Pummelo (*Citrus grandis* Osbek.) native to South East Asia is the largest citrus fruit and many cultivars are grown all over the world. The health benefits of citrus fruits have mainly been attributed to the presence of bioactive compounds, such as phenolics (e.g. flavonoids, hydroxycinnamic acids) and ascorbic acid. Flavonoids have a wide range of biological effects including prevention and control of coronary heart disease and they have anti-inflammatory and antimicrobial activities (Silberberg *et al.*, 2006). Phenolic compounds naturally exist as secondary metabolites in fruits, and their accumulation is determined by genotypes as well as regional cultivation conditions. For a particular variety of fruit, different regional cultivation characteristics have been reported to regulate the flavonoid metabolism gene expressions and enzyme activities, and therefore play a very important role in affecting the accumulation of phenolic compounds. Therefore, understanding the impact of the cultivation regions on the pattern and content of phenolic compounds in pummelo is useful for the conservation of pummelo of better quality and nutritional value. Limonoids are a group of chemically related triterpene derivatives present in citrus fruit. The most prominent of this group are limonin and nomilin (Girard and Mazza, 1998). Hence the present study on the nutritional value of pummelo is very important as it will help in popularizing this crop from a nutraceutical point of view. In recent years, the pummelo has received much attention because of its nutritional and antioxidant properties, especially flavonoids in Asia *i.e*. Thailand, China, Taiwan and Japan (Wattanasiritham *et al*., 2005; Nagata *et al*., 2006; Wang *et al*., 2007; Xu *et al.*, 2008). Such studies have not been conducted on pummelo fruits in India and very meagre information is available regarding its physico-chemical, biochemical and phytochemical properties. In the present work, a comparative study among the different accessions grown under diverse climate conditions was carried out, which will enhance the marketing opportunities of pummelo diversity in India and in turn the conservation. Hence, this study is aiming to answer the phytochemical status of pummelo fruit grown in different climatic conditions and its place of origin.

Geographically, Chettalli (Karnataka) and Salem (Tamil Nadu) regions belong to the South India, whereas the Nagaland and Manipur regions are located in the North-East, pummelo cultivation area, respectively. Whereas, Andaman and Nicobar Islands belong to the South East region of India. The climate of Karnataka and Tamil Nadu regions possess a sub-tropical monsoon climate with average annual precipitation approximately 1200 mm (Table 1). The Andaman and Nicobar Island region possesses similar climate characteristics. It should

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Table1. Cultivation region, climate and regional characteristics of pummelo collection from different sites of India

Cultivation region	Climate	Regional characteristics
Karnataka	Sub- tropical monsoon	12.9702° N, 77.5603° E; average annual rainfall of 1248 mm
Tamil Nadu	Sub- tropical monsoon	13.0900° N, 80.2700° E; average annual rainfall of 1145 mm
Nagaland	Sub- tropical humid monsoon	25.6700° N, 94.1200° E; average annual rainfall of 1800–2500 mm
Manipur	Sub- tropical humid monsoon	24.8170° N, 93.9500° E; average annual rainfall of 1467 mm
Andaman and Nicobar Islands	Sub- tropical monsoon	11.6683° N, 92.7378° E; average annual rainfall of 3180 mm

be noted that the Nagaland and Manipur regions are located in the sub-tropical humid monsoon climate. This indicates that the microclimate of the regions might significantly alter the accumulation of flavonoids.

Materials and Methods

In this study, samples of eight indigenous accessions of pummelo (*C. grandis*) were collected from orchards in distinct sites. The most popular accessions grown in each province were selected and five fully ripened fruits were randomly picked. The accessions were from Salem, Tamil Nadu; Chettalli, Karnataka; Jonapotha, Nagaland; Tamenglang, Manipur; Port Blair, Andaman and Nicobar Islands and Devanahalli, Bengaluru, Karnataka and these accessions were analysed for their physico-chemical, biochemical and phytochemical properties at the laboratory of the National Research Centre (NRC) for Citrus, Nagpur (Table 2). Since GI has been given to Devanahalli pummelo, it is referred for the comparison. The fruits were analysed after seven days of harvesting. The fruits were washed and cleaned with distilled water to remove surface residues. The pulp and peel were separated and after that juice was squeezed manually from the fruits.

The total soluble solids (TSS) of pummelo juice was determined by using digital refractometer at temperature 25˚C and expressed as ºBrix. The titratable acidity was determined by the method given by Association of Analytical Chemists (AOAC, 1990) using Phenolphthalein (1%) as an indicator by titrating against N/10 NaOH. The acidity was expressed in gram of citric acid per 100 ml of juice. Ascorbic acid was estimated as per the method given by Association of Analytical Chemists (AOAC, 1990). Limonin (ppm) was estimated from juice by the method given by Wilson and Cruthchfield (1968). Total phenols content (mg/100ml) in the pummelo juice and peel was determined by the Folin-Ciocalteu method (Singleton *et al.*, 1999). Naringin (mg/l) and hesperidin (mg/l) were estimated from juice by the method given by Hendricson *et al*. (1958).

Results and Discussion

Results indicate that there were clear differences between the accessions for all the chemical constituents evaluated (Table 3). The TSS ranged from 6.2 to 12.5˚Brix. The TSS was found lower as compared to the Devanahalli pummelo in all the accessions except in red-fleshed pummelo from Tamenglang, Manipur. The juice acidity was in the range of 0.80-2.52%. Acidity was found maximum (2.52 %) in juice from white fleshed pummelo from Jonapotha, Nagaland and minimum in red-fleshed pummelo from Port Blair, Andaman and Nicobar Islands (0.80%) (Table 3). The limonin content ranged between 12.73 and 18.89 ppm. Ohta and Hasegawa (1995) reported that the total limonoid content in pummelo juice was 7-71 ppm. Limonin content was found maximum (18.89 ppm) in red-fleshed pummelo from Chettalli, Karnataka. However, in most of the fruits the limonin content was around 14 ppm. Ohta and Hasegawa (1995) reported the average limonin content in the pummelo juice of sixteen cultivars as 18 ppm. Wattanasiritham *et al.* (2005) found that the limonin concentration in the juice of eight pummelo cultivars averaged 21.07 ppm. The results

Table 2. Details of the pummelo accessions collected from different sites of India

Accession/ Collectors No.	Plant type	Colour of fruit flesh	Collection site	Sample size (No.)
Pummelo 1	Seedling	Red fleshed	Salem, Tamil Nadu	5
Pummelo 2	Seedling	White fleshed	Chettalli, Karnataka	5
Pummelo 3	Seedling	Red fleshed	Chettalli, Karnataka	5
Pummelo 4	Seedling	White fleshed	Jonapotha, Nagaland	5
Pummelo 5	Seedling	Red fleshed	Tamenglang, Manipur	5
Pummelo 6	Seedling	Red fleshed	Port Blair, Andaman and Nicobar Islands	5
Pummelo 7	Seedling	Red fleshed	Devanahalli, Karnataka	5
Pummelo 8	Seedling	Red fleshed	Devanahalli, Karnataka	5

Table 3. Comparative physico-chemical and phytochemical analysis of fruit juice of various accessions of pummelo

Pummelo types	Juice (%)	TSS (°B)	Acidity (%)	TSS Acidity ratio	pH	Limonin (ppm)	Total phenol (mg/100ml)
Pummelo, Salem, Tamil Nadu	19.54	7.5	1.36	5.36	2.94	14.72	7.86
White-fleshed pummelo, Chettalli, Karnataka	20.15	7.2	1.44	5.00	4.25	14.88	7.88
Red-fleshed pummelo, Chettalli, Karnataka	17.66	6.2	1.60	3.87	3.58	18.89	7.25
Red-fleshed, Devanahalli 1 pummelo, Bengaluru, Karnataka	20.32	10.9	1.37	7.95	4.44	12.73	5.10
Red-fleshed, Devanahalli 2 pummelo, Bengaluru, Karnataka	21.45	10.2	2.12	4.63	4.19	14.03	5.28
White-fleshed pummelo, Jonapotha, Nagaland	21.65	9.8	2.52	3.79	2.58	14.80	7.5
Red-fleshed pummelo, Tamenglang, Manipur	19.20	12.5	1.44	8.68	3.04	15.31	10.16
Red-fleshed pummelo, Port Blair, Andaman and Nicobar Islands	18.80	3.9	0.80	4.75	3.82	15.08	5.59

TSS= Total soluble solids

from other studies indicated that the limonin content in our pummelo is in the same range as in other cases. Total phenols were maximum (16.57 mg/100ml) in peel of white-fleshed pummelo from Jonapotha, Nagaland and were maximum (10.16) in juice from Red-fleshed pummelo, Tamenglang, Manipur. The phenol content in other accessions were either lower or equal to the phenol content in Devanahalli pummelo juice and peel. The peel showed the higher amount of phenol, as compared to the juice in accordance with previous studies and expressed as mg catechin equivalent per 100 ml. Total phenols have reportedly been linked with the antioxidant capacity of fruits. Increasing the total phenol content also increased the antioxidant efficacy in fruits (Proteggente *et al*., 2003). Total phenols were reported to be the major antioxidant of citrus fruits. Tsai *et al.* (2007) showed that pink pummelo juice had higher total polyphenol content and antioxidant ability than white pummelo juice due to pigments. Fig. 1 and 2 shows considerable differences regarding the naringin and hespiridin contents among the different cultivars; naringin content ranged between 51.42 and 129.66 mg/l. The naringin content in the pummelo from Manipur and Nagaland was also higher as compared to the Devanahalli pummelo. The hespiridin content ranged between 0.44 and 3.35 mg/l. Pummelo from Salem, Tamil Nadu had the highest naringin and hesperidin contents (129.66 mg/l and 3.35mg/l), followed by pummelo from Andaman and Nicobar Islands (Figs. 1 & 2). This indicates that there was a variation in naringin content in the local accessions of pummelo in India. Factors such as environment, production area, cultivation and cultural practices might cause the divergence. Xu *et al.* (2008) also reported that naringin was the major flavanone followed by hesperidin in cultivars. The antioxidant power of flavanones (hesperidin, naringin, neohespiridins) was also reported by Majo *et al.* (2005). It has been proposed that the expressions of genes and activities of enzymes related to flavonoid biosynthesis and metabolism can be regulated and altered in different cultivation regions under different temperatures, precipitations, and sunshine exposures, which eventually impact the accumulation of the flavonoids in fruits

The observations in the present study indicate higher phenolic contents which indicate potent antioxidant ability. Similar findings were reported by other workers who found high correlation of the total phenolic and antioxidant activity of citrus extracts (Gorinstein *et al.*, 2004). It has been found that composition and level of metabolites in fruits varied greatly between cultivars and was independent of species and geographical locations similar to the report of Wahyuni *et al*. (2011).

The content of flavanones in each accession of pummelo juice could be used to identify pummelo accessions. Strategy to conserve fruit diversity while exerting a positive influence on rural livelihoods would be the development of local value-added products that would capitalize on the many nutritional and medicinal properties of pummelo. The unique and significant traits as summarized in Table 4 can help to create added value and increase profit margins for all actors in the pummelo value chain from farmer to retailer through product differentiation, targeting specific consumer segments and explore fresh sales and novel products based on unique traits for local and distant markets.

The expression of unique nutritional and health traits as found in this study due to distinct climate and cultivation practices in different sites suggest the

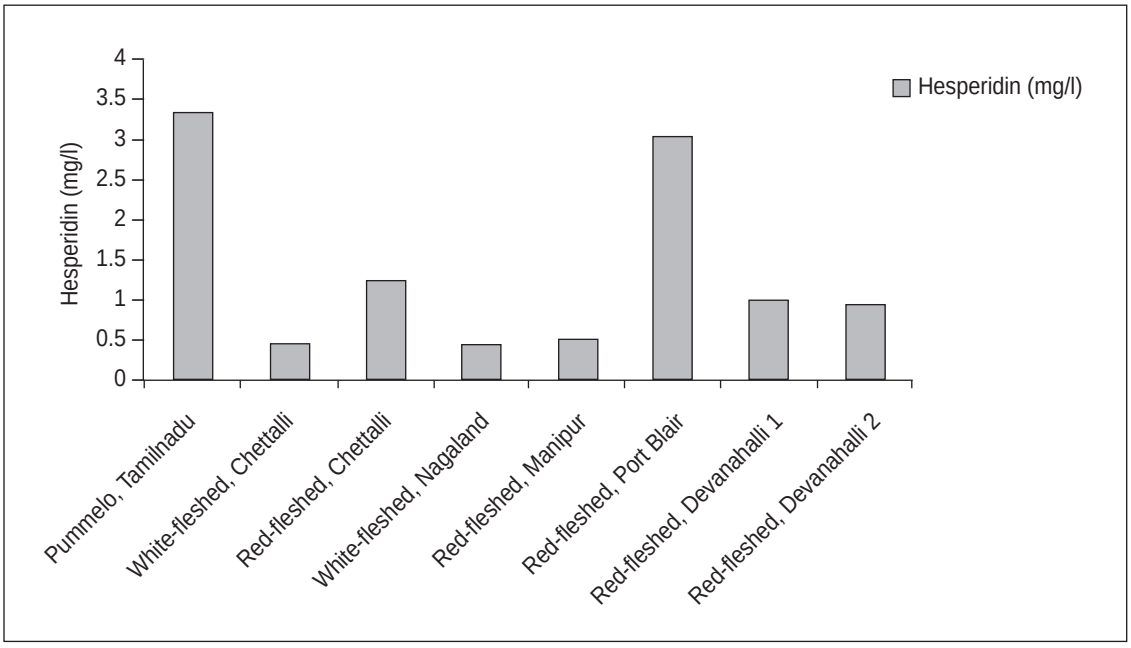

Fig. 1. Hesperidin content of juice from collected accessions of pummelo

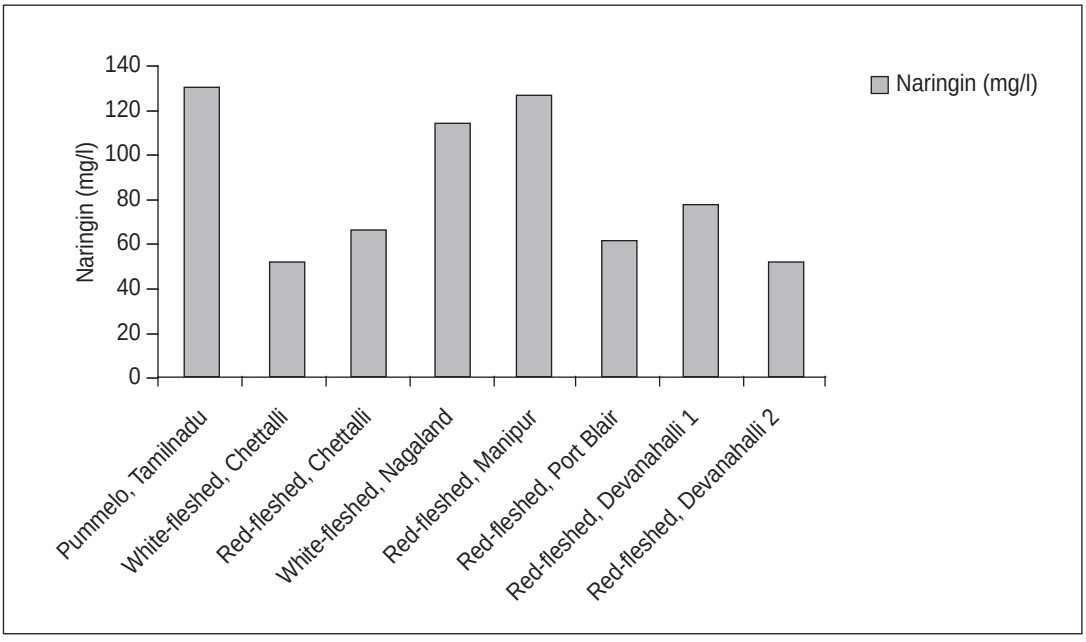

Fig. 2. Naringin content of juice from collected accessions of pummelo

Table 4. Unique traits and potential markets of the collected accessions of pummelo

Various pummelo types	Unique and significant nutritional traits	Potential consumer and market interest
Pummelo, Tamil Nadu	High hesperidin and naringin	Health-aware consumers
Red-fleshed, Devanahalli 2	High juice content	Juice industry
Red-fleshed, Chettalli	High limonin	Health-aware consumers
Red-fleshed, Manipur	High phenols, naringin and TSS	Health-aware consumers
White-fleshed, Nagaland	High naringin, high juice content	Juice industry and health-aware consumers
Red-fleshed, Port Blair	High hesperidin	Health-aware consumers

potential of a marketing strategy for specific accessions based on area of origin (geographic indication). Innovative marketing approaches based on unique traits that combines traditional knowledge with global or local economic trends, are key when pursuing market development strategies based on unique crop diversity and can help to ensure the on-farm conservation of pummelo diversity in India.

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