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Bioactive compounds and total antioxidant activity of pummelo (*Citrus grandis* L.) ecotypes of Assam

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ABSTRACT
Biologically active compounds are the nutritional constituents such as ascorbic acid, flavonoids, limonoids etc. that have potential health promoting properties and have been widely used as medicine. In the present study ripe fruits from twenty four pummelo plants were analyzed to determine the important bioactive compounds and its antioxidant potential. Naringin and limonin content in the juice were estimated using high performance liquid chromatography method. The study revealed that there was significant variation among the pummelo accessions in terms of ascorbic acid content. The ascorbic acid content of pummelo fruits ranged from 37.70 to 84.49 mg/100ml juice. HPLC analysis of pummleo juice showed that naringin and limonin content varied from 6.07 to 47.71 mg/100ml and 2.37 to 7.01mg/100ml, respectively. Total flavoinoid content of the pummelo juice varied from 11.47 to 57.80 mg QE/100ml. Total antioxidant activity of pummelo juice showed significant variation among different genotypes and ranged from 0.78%/µl to 1.41%/ µl juice.

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INTRODUCTION

Pummelo or shaddock (Citrus grandis (L.) Osbeck, synonyms Citrus maxima (J. Burm.) has been regarded as an ancient species of the genus Citrus [Scora, 23]. It is an underutilized citrus fruit of Northeast region and India as a whole and mostly grown as backyard crop in homestead garden. It is mono embryonic in nature and highly cross pollinated. Citrus fruits are recognized as an important component of the human diet, providing a variety of constituents important to human nutrition including vitamin C, folic acid, potassium, flavonoids, coumarins, pectin and dietary fibres. Citrus flavonoids have a broad spectrum of biological activities including antibacterial, antioxidant, antidiabetic, anticancer, cardiovascular, analgesic, antiinflammatory, antianxiety [Sidana et al., 24]. Limonoids, first reported in 1864, are a group of chemically related triterpene derivatives found in the citrus family and 39 liminoid aglycones and 21 glucosides have been isolated so far, limonin and nomilin are the most prevalent citrus limonoids [Ohta et al., 15]. Therefore, the present investigation was carried out to determine the variation in bioactive compounds and its antioxidant potential.

MATERIALS AND METHODS

The experiment was conducted in Assam Agricultural University, Assam during 2015-2016. Four healthy pummelo trees between 10 to 20 years of age were selected in each district comprising of twenty four (24) numbers of trees in six districts representing six agro-climatic zones of Assam. . Each tree was given a number for future identification. The accession number consisted of letter 'AP' for Assam Pummelo, 01 to 06 for districts and T1 to T4 for plant number. Two to three branches with flowers were tagged during flowering (Feb.-March). The fruits were harvested at optimum maturity stage i.e. 200 days after fruit set. Five fruits from each plant were collected, washed, peeled and juice vesicles were separated from the segments for extraction of juice using power operated juicer. Extracted juice was evaluated for various bioactive compounds.

Estimation of bioactive compounds

Ascorbic acid content was determined by using 2,6-dichlorophenolindophenol dye method [Freed, 5]. The total antioxidant activity of fruit juice samples were tested on the basis of the radical scavenging effect on the DPPH free radical [Rekha *et al.,* 19] and the results were expressed in per cent total antioxidant activity per micro litre. The amount of sample necessary to decrease the absorbance of DPPH by 50 per cent i.e. IC_{50} was derived from the per cent scavenging activity versus concentration. Total flavonoid content of pummelo juice was determined based on aluminum chloride colorimetric assay as described by Liu *et al*. [12].

Naringin content in pummelo juice was determined by reverse phase HPLC method described by Rouseff [21]. The HPLC system consisted of Waters HPLC 2489 UV/Vis detector with two hydraulic pumps, Waters C_{18} column with particle size 5µm, internal diameter of 4.6 mm and column length of 250 mm and a computerized recorder. Mobile phase consisted of aetonitrile: deionised water (25:75) with a flow rate of 1 ml/minute and injection volume of 20 µl. The detector wavelength was 280 nm. Standard HPLC grade naringin with purity ≥ 95% was obtained from Sigma-Aldrich (USA), HPLC grade deionised water, acetonitrile and nylon filter were procured from Himedia.

Preparation of samples and quantification of naringin

1 ml of refrigerated pummelo juice was extracted with 4 ml of HPLC grade methanol by shaking for about 1 minute using a vortex mixer and then centrifuged at 2500xg for 10 minutes. The extract was passed through 0.22 µm nylon filter prior to injection to HPLC. 20 µl aliquots of filtrate was injected to HPLC system, and naringin was identified in the samples by comparing the retention time with that of standard naringin (10-50 ppm) prepared from 1000 ppm stock solution in water and quantified by comparing the peak areas. The naringin content in pummleo juice was expressed in mg per 100 ml. All samples were analyzed in triplicate.

Limonin content in pummelo juice was determined by the method described by Abbasi [1]. The HPLC system consisted of Waters HPLC 2489 UV/Vis detector with two hydraulic pumps, Waters C_{18} column with particle size 5µm, internal diameter of 4.6 mm and column length of 250 mm and a computerized recorder. Mobile phase consisted of aetonitrile : deionised water (32:68) with a flow rate of 0.9 ml/minute and injection volume of 20 µl. The detector wavelength was 207 nm. Standard HPLC grade limonin (5 mg) with purity ≥ 90% was purchased from Sigma-Aldrich (USA), Sep-pak C_{18} cartridge (USA), HPLC grade deionised water, acetonitrile and nylon filter (0.45 µm) were procured from Himedia.

Preparation of samples and quantification of limonin

Prior to analysis, the pummelo juice samples were allowed to reach ambient temperature. The Millipore C_{18} Sep-pak cartridge (USA) was conditioned (rinsed) with 2 ml of acetonitrile and 5 ml of deionsed water. 2.5 ml of pummelo juice sample was gently passed through the cartridge followed by 2.5 ml of deionised water. 2.5 ml of acetonitrile was passed through the cartridge and permeates was collected in small glass vials. Permeates were filtered through nylon filter (0.45 µm) and collected in eppendorf tubes. 20 µl aliquots of filtrate was injected to HPLC and limonin content in juice was identified in the samples by comparing the retention time with that of standard limonin (5-50 ppm) prepared from 1000 ppm stock solution in acetonitrile and ethyl alcohol and quantified by comparing the peak areas. The limonin content in pummleo juice samples was expressed in mg per 100 ml. The data were statistically analyzed using Completely Randomized Design [Panse and Sukhatme, 16].

RESULTS AND DISCUSSION

Ascorbic acid content of the pummelo fruits (Table 1) varied significantly among the accessions. The highest ascorbic acid content of 84.49 mg/100ml was recorded in accession number AP02T3 followed by 76.16 mg/100ml in AP02T2 and the lowest was recorded in AP04T1 (35.70 mg/100ml). The mean value for ascorbic acid content was recorded to be 57.80 mg/100ml. The observation was fairly consistent with the earlier report of Chaiwong and Theppakorn [2] and Roy *et al.* [22] who recorded an average ascorbic acid content of 52.25 mg/100ml and 48.89 mg/100ml in pummelo fruits of Thailand and West Bengal, respectively. Yoo *et al.* [29] recorded high ascorbic acid content 90.4 mg/100g FW in *Citrus junos* fruits in Korea. The variation in ascorbic acid content among accessions might be due to genetic factor, position and maturity stage of the harvested fruits, soil nutrient status and environmental variation. Harris [7] reported that the fruits exposed to maximum sunlight contain higher amount of ascorbic acid than those inside the canopy or under shaded condition. In general, lower the light intensity during growth, lower is the ascorbic acid content of the fruits. Vitamin C content of citrus fruits increases with potassium soil fertilization and decreases with high nitrogen fertilization [Nagy, 14].

The perusal of data pertaining to naringin content in pummelo juice is presented in Table 1 and Fig. 1. The data were obtained by HPLC analysis of juice with retention time of 6 minutes [Sun *et al.,* 25]. The results revealed that there was significant variation in naringin content among different accessions. The highest amount of naringin content in juice was recorded in accession number AP06T2 (47.71

mg/100ml) followed by AP01T3 (44.38mg/100ml) and AP05T1 (41.54 mg/100ml) and the lowest was recorded in accession number AP04T3 (6.07 mg/100ml) which was at par with AP03T2 (6.88 mg/100ml) and AP05T2 (7.52 mg/100ml), respectively. The mean naringin content in pummelo juice was recorded to be 19.66 mg/100ml. The variation in naringin content among accessions might be attributed to genetic differences among the genotypes. The present study is in conformity with the reports of Zhang *et al.* [30] who recorded naringin content ranging from 2.81 to 15.5 mg/100ml in three pummelo cultivars of China. Naringin content of six Thai pummelo cultivars ranged from 2.34 to 41.29 µg/mg dry weight [Makynen *et al.,*13]. Pichaiyongvongdee and Haruenkit [18] recorded naringin content ranging from 24.26 to 38.65 mg/100ml of juice in six pummelo cultivars. Kumar *et al.* [10] stated that the content of flavanones such as naringin can be used for identifying pummelo genotypes. Chaiwong and Theppakorn [2] recorded the highest naringin content of 71.90 mg/100g in juice of pink pummelo cultivar 'Thong Dee' in Thailand.

The data presented in Table 1 revealed that total flavonoid content of juice varied significantly among the accessions. The highest total flavonoid content of juice was recorded in accession number AP05T1 (57.80 mg QE/100ml) followed by AP06T2 (53.91 mg QE/100ml) and AP05T4 (51.01 mg QE/100ml). The lowest total flavonoid content of juice was recorded in accession number AP04T4 (11.47 mg QE/100ml) with an average value of 28.72 mg QE/100ml. The result of present study is fairly consistent with the reports of Toh *et al.* [26] and Petchlert *et al.* [17] who recorded total flavonoid content of 13.06 mgQE/100g and 84.0 mg HE/100ml of pummelo juice. Xi *et al.* [28] and Zhang *et al.* [31] in a study with local pummelo and mandarin accessions of China concluded that the variation patterns of flavonoid components and contents were largely same for different genotypes and were genetically controlled.

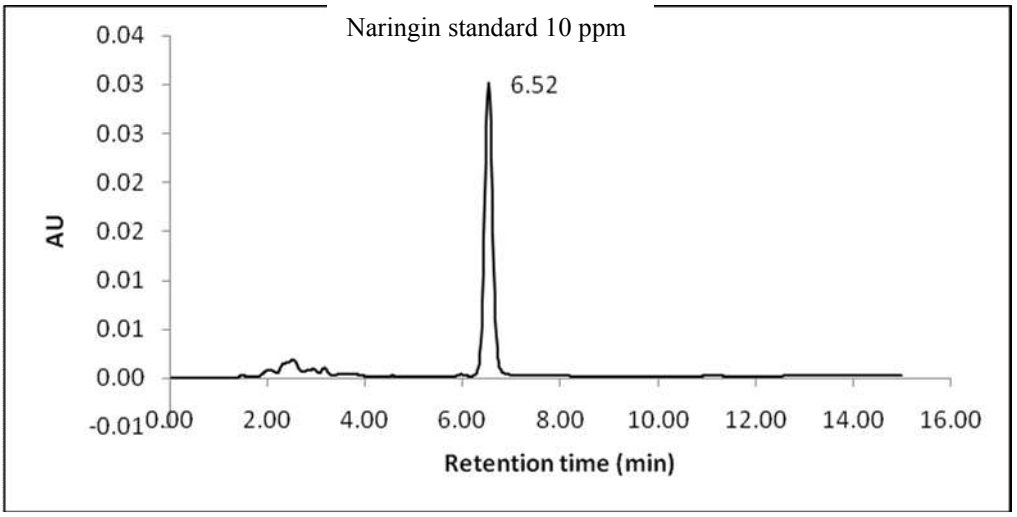
Significant variation was observed among the selected accessions for total antioxidant activity (Table 1). The highest total antioxidant activity of juice was recorded in accession number AP02T3 (1.41%/µl) followed by accession number AP03T4 (1.34%/µl) which was at par with AP03T2 (1.33%/µl), and AP06T2 (1.31%/µl), respectively. The lowest total antioxidant activity was recorded in accession number AP01T1 (0.78%/µl) with a mean total antioxidant activity of 1.15%/µl in the selected accessions.

The variation in total antioxidant activity might be due to variation in genotypes selected and also due to different climatic conditions. The present study is in conformity with the reports of Xi *et al.* [28] who recorded DPPH inhibition per cent ranging from 0.58%/µl to 1.01%/µl of juice from twenty eight pummelo varieties. Kumari and Handique [11] recorded DPPH inhibition of 0.96%/µl of juice. Gardner *et al.* [6] in a study reported that ascorbic acid accounts for 60 to 100% of the antioxidant potential in citrus fruits whereas phenolic compounds are major contributor to antioxidant potential in non citrus fruits. Yoo *et al.* [29] recorded high positive correlation coefficient of r = 1.00 and r = 0.99 between vitamin C and total antioxidant activity in *Citrus junos* fruits. The IC_{50} value of selected pummelo genotypes is presented in Table 1. The IC_{50} is the amount of fruit extract required to scavenge the DPPH free radical to 50 per cent. The study revealed that there was significant variation in IC_{50} value among the selected pummelo accessions. The IC_{50} value of accession number AP01T1 (64.10 µl) was recorded highest among the accessions showing low antioxidant potential, followed by AP04T2 (59.52 µl) and AP06T4 (52.63 µl). The lowest IC_{50} value was recorded in accession number AP02T3 (35.46 µl) indicating high antioxidant potential over all other accessions. The mean IC_{50} value of 44.50 µl was recorded for selected pummelo genotypes. Similar results were reported by Devi *et al.* [4] and Petchlert *et al.* [17] who recorded IC_{50} value of 59 µl/ml and 50 µl/ml methanol extract, respectively in pummelo juice. Islam *et al.* [9] in a study with apple, jujube and hog plum, recorded an IC_{50} value of 10.74, 33.88 and 38.08 µl/ml extract in methanol, respectively. The results of HPLC analysis of juice for limonin content (Table 4.1 and Fig.1) revealed significant variations among pummelo accessions. The highest limonin content was recorded in accession number AP06T2 (7.09 mg/100ml) followed by AP02T3 (6.77 mg/100ml) and AP06T4 (6.49 mg/100ml), respectively. The lowest limonin content was recorded in accession number AP04T2 (2.37 mg/100ml) followed by accession number AP01T3 (2.77 mg/100ml) with an average content of 4.73 mg/100ml in juice of selected pummelo accessions. The findings on limonin differed with the findings of Wu *et al.* [27] who recorded higher limonin content in six pummelo cultivars of Taiwan, ranging from 43.3 mg/100ml to 125.18 mg/100ml of juice. They reported that the higher limonin content might be due to damage of fruits during harvesting and frost injury. Pichaiyongvongdee and Haruenkit [18] recorded limonin content ranging from 10 ppm to 29.62 ppm in seven pummelo cultivars. Slightly higher limonin content in the present study compared to the latter researcher might be attributed to development of bitterness during storage under refrigerated condition before quantitative analysis. Hasegawa [8] reported that limonin bitterness in juice develops within few hours at room temperature or overnight under refrigerator. The variation in limonin content among the accessions might be due to differences in genetic makeup and soil nutrient status. Variety is an important factor in determining limonin content of citrus fruits [Chandler *et al*., 3]. The soil nutrient status *i.e.* low nitrogen and potassium content in soil might increase limonin content of citrus juice [Rodrigo *et al.,* 20].

The present study revealed that the pummelo accessions of Assam are rich source of different biologically active compounds viz. ascorbic acid, total flavonoid, naringin and limonin. As pummelos are highly cross-pollinated, significant differences have been observed among the genotypes for these compounds. Present investigation also revealed that pummelo fruit juices have high antioxidant potential which is useful against various ailments.

Table 1. Bioactive compounds in pummelo (*Citrus grandis*) fruits of Assam

Plant No.	Accession No.	Ascorbic acid (mg/100ml)	TFC (mgQE/100ml)	Naringin (mg/100ml)	TAA (%/µl)	IC_{50} (µl juice)	Limonin (mg/100ml)
1	AP01T1	41.65	22.97	8.81	0.78	64.10	4.12
2	AP01T2	47.62	37.14	32.21	1.15	43.48	6.42
3	AP01T3	42.84	49.02	44.38	1.13	44.25	2.77
4	AP01T4	45.22	26.72	21.71	0.97	51.55	5.46
5	AP02T1	64.26	20.32	12.32	1.17	42.74	4.50
6	AP02T2	76.16	13.82	11.53	1.28	39.06	4.18
7	AP02T3	84.49	37.49	33.26	1.41	35.46	6.77
8	AP02T4	66.16	25.21	9.76	1.24	40.32	5.22
9	AP03T1	72.49	19.92	8.98	1.29	38.76	4.81
10	AP03T2	65.68	13.57	6.88	1.33	37.59	4.71
11	AP03T3	61.88	17.12	12.49	1.09	45.87	4.20
12	AP03T4	66.64	40.72	34.17	1.34	37.31	3.71
13	AP04T1	35.70	34.72	14.20	0.95	52.63	4.06
14	AP04T2	52.12	16.33	10.63	0.84	59.52	2.37
15	AP04T3	58.31	12.04	6.07	1.01	49.50	3.20
16	AP04T4	67.35	11.47	7.80	1.21	41.32	3.24
17	AP05T1	53.55	57.80	41.54	1.23	40.65	3.90
18	AP05T2	51.17	26.40	7.52	1.16	43.10	4.17
19	AP05T3	53.51	32.68	26.32	1.08	46.30	5.06
20	AP05T4	47.60	51.01	40.10	1.20	41.67	5.35
21	AP06T1	59.50	27.74	11.64	1.25	40.00	5.82
22	AP06T2	67.41	53.91	47.71	1.31	38.17	7.09
23	AP06T3	62.24	20.25	10.91	1.19	42.02	5.99
24	AP06T4	43.33	20.77	10.93	0.95	52.63	6.49
Mean		57.80	28.72	19.66	1.15	44.50	4.73
SEd (±)		2.73	0.69	0.75	0.03	1.56	0.11
$CD_{(0.05)}$		5.04	1.37	1.48	0.06	3.09	0.22



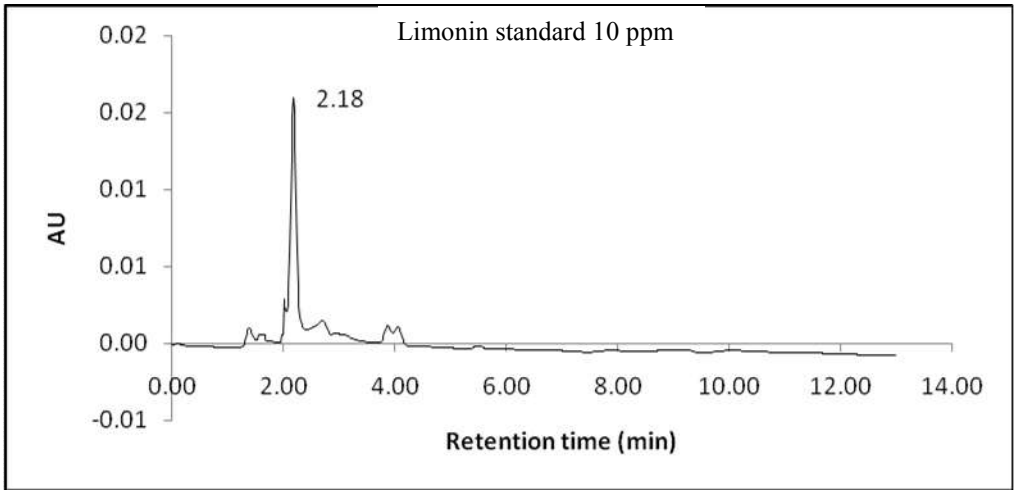


Fig.1. HPLC chromatogram of naringin and limonin standards at 280 nm and 207 nm

REFERENCES

1. Abbasi, S., P. Zandi and E. Mirbagheri. 2005. Quantification of limonin in Iranian orange juice concentrates using high performance liquid chromatography and spectrophotometric methods. *Eur. Food Res. Technol*. **221**: 202-207.
2. Chaiwong, S and T. Theppakorn. 2010. Bioactive compounds and antioxidants capacity of pink pummelo (*Citrus grandis* (L.) Osbeck) cv. "Thong Dee" in Thailand. *J. ISSAAS*. **16** (2): 10-16.
3. Chandler, B. V., K. J. Nicol and C. V. Biedermann. 1976. Factors controlling the accumulation of limonin and soluble constituents within orange fruits. *J. Sci. Food Agri.* **27** (9): 866-876.
4. Devi, R., T. Mudoi, D. C. Deka and D. Devi. 2012. In vitro and in vivo antioxidant activity of fresh juice of *Citrus grandis* of the northeastern region of India. *J. Herbs Med. Plants.* **18** (4): 349-359.
5. Freed, M. 1966. Method of vitamin assay. Inter-science publication Inc. New York.
6. Gardner, P. T., T. A. C. White, D.B. McPhail and G.G. Duthie. 2000. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chem.* **68**: 471-474.
7. Harris, R. S. 1975. Effects of agricultural practices on the composition of foods. In: Nutritional Evaluation of Food Processing (R.S. Harris and E. Karmas (Eds.), pp. 33-57AVI pub., Westport, CT.
8. Hasegawa, S. 1999. Limonin bitterness in citrus juices. In: Flavor chemistry, Three years of progress (R. Teranishi, E. L. Wick, and I. Hornstein, Eds.). p. 89, Plenum publishers, New York.
9. Islam, S., O. Rahman, M. Hossain and A. Khaleque. 2015. Antioxidant activity of some seasonal fruits of Bangladesh. *Biores. Comm.* **1** (1): 28-31.
10. Kumar, D., H. Lamers, I. P. Singh, M. S. Ladaniya and B. Sthapit. 2015. Phytochemical evaluation of pummelo fruits (*Citrus grandis*) in India for enhancing marketing opportunities. *Indian J. Plant Genet. Resour*. **28** (1): 50-54.
11. Kumari, S and A. K. Handique. 2013. Total phenolic content and in vitro antioxidant activity of indigenous citrus fruits of northeast India. *Int. J. Sci. Engg. Res*. **4** (6): 2125-2128
12. Liu, H., N. Qiu, H. Ding and R. Yao. 2008. Polyphenol content and antioxidant capacity of Chinese herbals suitable for medical or food uses. *Food Res. Int*. **41**: 363-370.
13. Makynen, K., S. Jitsaardkul, P. Tachasamran, N. Sakai, S. Puranachoti, N. Nirojsinlapachai, V. Chattapat, N. Caengprasath, S. Ngamukote and S. Adisakwattana. 2013. Cultivar variation of antioxidant and antihyperlipidemic properties of pomelo pulp [*Citrus grandis* (L.) Osbeck] in Thailand. *Food Chem*. **139**: 735-743.
14. Nagy, S. 1980. Vitamin C contents of citrus fruit and their products: A Review. *J. Agric. Food Chem.* **28**: 8-18.
15. Ohta, H., M. Berhow, R. D. Bennett and S. Hasegawa. 1992. Limonoids in seeds of *Citrus hanaju*. *Phytochem*. **31**: 3905-3907.
16. Panse, V. S and P. V. Sukhatme. 1985. Statistical methods for agricultural workers, 4th Edn. ICAR, New Delhi.
17. Petchlert, C., R. Kaewnoi, A. Siriboot and O. Suriyapan. 2013. Antioxidant capacity of commercial citrus juices from supermarket in Thailand. Pure and Applied Chemistry International Conference, pp. 1-4.
18. Pichaiyongvongdee, S and R. Haruenkit. 2009. Comaparative studies of limonin and naringin distribution in different parts of pummelo [*Citrus grandis* (L.) Osbeck] cultivars grown in Thailand. *Kasetart J. (Nat. Sci.)*. **43**: 28-36.
19. Rekha, C., G. Poornima, M. Manasa, V. Abhipsa, J. Pavithradevi, H. T. Vijaykumar and T. R. Prashithkekuda. 2012. Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. *Chem. Sci. Trans*. **1** (2) : 303-310.
20. Rodrigo, M. I., D. Mallent and A. Casas. 1985. Relationship between the acid and limonin content of Washinton navel orange juice. *J. Sci. Food Agri.* **38** (11)**:** 1125-1129.
21. Rouseff, R. L. 1988. Liquid chromatographic determination of naringin as a dectector of grapefruit juices in orange juices. *J. Assoc. Off. Anal. Chem*. **71**:789-802.
22. Roy, D., S. Kundu, B. Ghosh, P. Dutta, and P. Pal. 2014. Performance of pummelo germplasm in new alluvial zone of West Bengal. *J. Crop. Weed.* **10** (2)**:** 179-182.

23. Scora, R. W. 1975. On the history and origin of Citrus. Bull Torrey Bot. Club. 102:369-375
24. Sidana, J., V. Saini, S. Dahiya, P. Nain, and S. Bala. 2013. A Review on Citrus : The Boon of Nature. *Int. J. Pharm. Sci. Rev. Res*. **18**: 20-27.
25. Sun, Y., J. Wang, S. Gu, Z. Liu, Y. Zhang and X. Zhang. 2010. Simultaneous Determination of flavonoids in different parts of *Citrus reticulata* 'Chachi' fruit by High performance liquid chromatography-Photodiode Array Detection. *Molecules,* pp. 5378-5388.
26. Toh, J. J., H. E. Khoo and A. Azrina. 2013. Comparison of antioxidant properties of pummelo [*Citrus grandis* (L.) Osbeck] varieties. *Int. Food Res. J*. **20** (4):1661-1668.
27. Wu, S. J., C. C. Ng, W. S. Tzeng, K. C. Ho and Y. T. Shyu. 2011. Functional antioxidant and tyrosinase inhibitory properties of extracts of Taiwanese pummelo (*Citrus grandis* Osbeck). *African J. Biotechnol*. **10** (39): 7668-7674.
28. Xi, W., B. Fang, Q. Zhao, B. Jiao and Z. Zhou. 2014. Flavonoid composition and antioxidant activities of Chinese local pummelo (*Citrus grandis* Osbeck) varieties. *Food Chem*. **161**: 230-238
29. Yoo, K. M., K. W. Lee, J. B. Park, H. J. Lee and I. K. Hwang. 2004. Variation in major antioxidants and total antioxidant activity of Yuzu (*Citrus junos* Tanaka) during maturation and between cultivars. *J. Agric. Food Chem*. **52** (19): 5907-5913.
30. Zhang, M., C. Duan, Y. Zang, Z. Huang and G. Liu. 2011. The flavonoid composition of flavedo and juice from the pummelo cultivar (*Citrus grandis* L. Osbeck) and the grapefruit cultivar (*Citrus paradidsi*) from China. *Food Chem*. **129**: 1530-1536.
31. Zhang, M., H. Nan, Y. Wang, X. Jiang and Z. Li. 2014. Comparison of flavonoid compounds in the flavedo and juice of two pummelo cultivars (*Citrus grandis* L. Osbeck) from different cultivation regions of China. *Molecules* **19**: 17314-17328.

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