Bioactive Phytochemical Contents on Fruit Peel of Several Citrus Species

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Abstract. Citrus fruits contain important sources of bioactive compounds, including antioxidants such as flavonoids and phenolic compounds that are beneficial for human health. These bioactive compounds also exist in non-edible fruit parts, like the peel. The research was conducted to evaluate these bioactive phytochemicals on the peels of different citrus species. The experiment was carried out from March to August 2018 at the citrus germplasm collection of the Indonesian Instrument Standard Assessment Institute for Citrus and Subtropical Fruits and The Central Laboratory of The Indonesian Instrument Standard Assessment Institute for Legumes and Tubers Crops. The steps of research methods included the preparation of extract samples, the determination of total flavonoids and phenol content, and the DPPH radical scavenging assay. The results showed that the bioactive content of the citrus peels of several citrus species was diverse among the studied citrus species. Tangerine citrus cv. Kintamani was observed to have a higher phenolic content. For flavonoid content, the peel of lime cv. Borneo had the highest and Mandarin cv. Satsuma showed the least. Higher DPPH radical-scavenging activity values were observed on Mandarin cv. Pachuan, tangerine cv. Kintamani, and pumello cv. Thn. The correlation between phenolic content and DPPH radical-scavenging activity was higher than that between phenolic-flavonoid and flavonoid-DPPH radical-scavenging activity, indicating the effect of active molecule structure on redox potential.

1 Introduction

Citrus is one of the most popular world fruit crops and is well known for its fragrance and thirst-quenching ability. The plant belongs to the family of Rutaceae and has a complex number of natural species, including important groups such as oranges, mandarin, lemons, grapefruits, pomelos, and limes [1, 2]. Since ancient times, citrus fruits have been recognized as a rich source of vital vitamins, minerals, fibres, bioactive phytochemicals, and other health-promoting substances [3]. The positive effects of citrus species on the prevention

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of life-threatening diseases have often been reported [4-7]. The phenolic profile and antioxidant qualities of citrus fruits, fruit extracts, and flavonoids also demonstrated a broad spectrum of biological characteristics [8-11].

Most citrus fruits are consumed around the world as fresh produce, juice, or other kinds of flesh derivatives. Citrus waste components, which include peel flavedo, albedo, and seed, make up around 44–60% of the weight of fresh fruit. These parts are thrown away [12]. As a result of the recent considerable rise in citrus production worldwide, a significant amount of peel is generated annually. There is an immediate need to investigate the potential applications of underutilized agricultural wastes for cosmetics, pharmaceuticals, and food preservation as the current focus of global attention is on developing valuable chemicals from these wastes [13]. Many studies have suggested that citrus peel is a possible source of many bioactive, including tannins, phenolics, flavonoids, and particularly limonoids, which are unique in other plants [12, 14-17]. Important biological effects of these bioactives include anti-inflammatory, antibacterial, antioxidant, and anti-cancer properties [18,19].

The mesocarp, or albedo, is the soft, white centre layer, and the epicarp, or avedo, is the coloured peripheral surface that makes up citrus peels. Some publications have documented the existence of polyphenols, minerals, vitamins, dietary fibres, essential oils, and carotenoids obtained from these peel parts [20, 21]. Prenyloxycoumarins that have been identified from citrus flesh and peel extracts, including auraptene, imperatorin, heraclenin, bergamottin, and oxypeucedanin, have also been found to be present in citrus fruits [22-24]. The preference of consumers and researchers for natural foods and food ingredients that are thought to be pure and healthful has changed as a result of these discoveries, as have their opinions about synthetic alternatives [12].

Since the concentrations of the bioactive compounds in citrus peel varied depending on the species, variety, quality, and degree of maturity, the method for identification and quantification of these phytochemicals was important to investigate. The paper elucidated the bioactive phytochemical containment on the peel of different citrus species, i.e. mandarin, tangerine, lime, sweet orange, and pumello that can act as potential nutraceutical resources. This citrus residue rich in bioactive compounds can be used to produce new nutraceuticals or enrich existing ones in an economical, environmentally friendly, and efficient method.

2 Materials and methods

The research was conducted at the citrus germplasm collection of the Indonesian Instrument Standard Assessment Institute for Citrus and Subtropical Fruits (formerly The Indonesian Citrus and Subtropical Fruits Research Institute (ICSFRI)) and The Central Laboratory of Indonesian Instrument Standard Assessment Institute for Legumes and Tubers Crops (formerly The Indonesian Legumes and Tubers Crops Research Institute (ILETRI)) from March to August 2018. The citrus species used were eight cultivars, comprised of 4 mandarin cultivars (Tankan Bimanu, Genensa Aceh, Satsuma, and Pachuan), one tangerine (Kintamani), one lime (Borneo), one pumello (Thn) and one sweet orange (Manis Taji). All the trees were cultivated at the Punten germplasm research station located at 900 masl.

2.1 Preparation of extract samples

After removing the edible part, the fruit peels were sliced into thin pieces (0.5–1.5 cm). The peels were blended into a powder after being dried for 72 hours at 350 degrees in an oven. Samples that had been dried and milled were extracted using methanol percolation at room temperature. The supernatant was extracted to quantify the antioxidant, flavonoid, and phenolic activity.

2.2 Determination of total flavonoid content

Flavonoid content was determined using the colourimetric aluminium chloride method based on Ebrahimzadeh et al. [25] with slight modification. In brief, a 0.5 ml solution of each plant extract in methanol was combined separately with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water and kept at room temperature for 30 min. A Perkin Elmer UV/Visible spectrophotometer with a double beam was used to measure the absorbance of the reaction mixture at 415 nm (USA). Quercetin was used as the measure of total flavonoid content based on a calibration curve. Quercetin solutions were made in methanol at concentrations ranging from 12.5 to 100 mg ml/l to create the calibration curve.

2.3 Determination of total phenol content

The Folin-Ciocalteau method [25] was used to determine the total phenolic component contents. Aqueous Na_2CO_3 (4 ml, 1 M) was added after the extract samples (0.5 ml of various dilutions) had been combined with the Folin-Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for five minutes. Following a 15-minute standing period, the mixture's phenol content was measured using colourimetry at 765 nm. Gallic acid solutions of 0, 50, 100, 150, 200, and 250 mg/ml in methanol: water (50:50, v/v) were used to create the standard curve. The common reference compound gallic acid equivalent (mg/g of dry mass) is used to express total phenol levels.

2.4 DPPH radical scavenging assay

The ability of the citrus peel extracts to scavenge the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to measure their antioxidant activity. The protocol for the DPPH assay was followed by Chatha et al. [26]. The samples (ranging in concentration from 0.2 to 500 mg/ml) were combined with 1 ml of a 90 mM DPPH solution and then filled to a final capacity of 4 ml with 95% methanol. After an hour at room temperature, the absorbance of the resultant solutions and the blank were measured. Positive control, butylated hydroxytoluene (BHT), was employed. A spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan) was used to investigate the disappearance of DPPH at 515 nm. Scavenging of free radical DPPH in per cent (%) was calculated in the following way.

$$\text{Scavenging\%} = 100 \text{ x } \left(\frac{\text{A blank} - \text{A sample}}{\text{A blank}}\right) \quad (1)$$

A blank is the absorbance of the control compounds, and A sample is the absorbance of the test compounds. IC50 (mg/ml) values representing the concentration of extracts that caused 50% neutralization of DPPH radicals were determined by plotting the inhibition percentage instead of concentration. The means ± SD are used to express the results of the experiment. Three duplicates of each measurement were made. An analysis of variance ($p < 0.05$) was used to assess the data, and the LSD test was used to separate the means.

3 Results and discussion

One of the most consumed fruits worldwide is the citrus fruit. More than 140 countries cultivate citrus. Citrus fruits that are grown, traded, and consumed most frequently are oranges, lemons, tangerines, and grapefruits [27]. The total production of citrus in the world was 143 755 600 tons [27], and 44-60% of the fruits were non-edible parts that were wasted

[12]. The citrus peel contains several compounds that show antioxidant properties: vitamin, flavonoid, lignin, carotenoids, saponin, plant sterols, and terpenoids [28].

Table 1. Phytochemical contents of citrus species

Citrus cultivar		**Phenolic content**[*)****)]	**Flavonoid content**[**)))****)]	**DPPH radical-scavenging activity**[***)))****)]
		(mg GE/g)	(mg QE/g)	(µmol TE/g)
Tankan Bimanu	Mandarin	10.56 ± 0.93 b	6.17 ± 0.01 b	13.49 ± 0.93 b
Genensa Aceh	Mandarin	10.08 ± 0.17 b	3 ± 0.06 a	13.265 ± 0.17 b
Satsuma	Mandarin	8.94 ± 0.11 a	2.98 ± 0.03 a	8.73 ± 0.11 a
Pacuan	Mandarin	13.61 ± 0.53 d	7.75 ± 0.098 bc	15.0805 ± 0.52 c
Kintamani	Tangerine	14.76 ± 0.57 d	8.48 ± 0.11 c	15.1 ± 0.57 c
Borneo	Lime	8.42 ± 0.08 a	11.48 ± 0.31 d	10.265 ± 0.08 ab
Thn	Pumello	12.01 ± 0.24 cd	6.33 ± 0.72 b	15.145 ± 0.24 c
Manis Taji	Sweet Orange	11.99 ± 0.09 c	4.81 ± 0.057 ab	8.73 ± 0.09a

Remarks: [*)] mg gallic acid equivalent/g of extract powder
[**)] mg quercetin equivalent/g of extract powder
[***)] µmol Trolox equivalent/g of extract powder
[****)] Values in int the same column followed by different letters differ significantly based on LSD (α≤5%)

3.1 Total phenolic content

Using the Folin-Ciocalteu technique, the total phenol compounds were calculated and presented as gallic acid equivalents. The most significant total phenol in the peel was detected in Tangerine citrus (Kintamani), followed by Mandarin (Pacuan), Pumello (Thn), and Sweet Orange (Manis Taji), respectively (Table 1). The lowest peel phenolic content was detected on lime cv. Borneo and Mandarin cv. Satsuma.

Based on the citrus group, tangerine tends to have higher peel phenolic content, as also reported by Ghasemi et al. [20], though it was in the lower value range in this experiment. Bitter orange peels have 0.39 mg/g phenolic acid, whereas satsuma mandarin has 0.21 mg/g and yuzu has 0.33 mg/g [29]. Chen et al. [30] showed Mandarin has a higher phenolic content than Sweet Orange and Pumello. The different peel phenolic content and other biochemical compounds among the citrus species were also reported by Sir Elkhatim et al. [31], which also indicated the different capabilities of each species in producing indigenous biochemical compounds. Its variations could be related to cultivar factors as well as to other elements including growing region, climate, and fruit ripeness [30]. The findings of beneficial compounds within the citrus peel also widened the opportunity for the utilization of non-edible plant parts as the source of various useful substances for human health [12].

Phenolic compounds, which have anti-ageing, anti-inflammatory, antioxidant, and anti-proliferative properties, were essential for defence responses. Certain chronic diseases will become less prevalent as a result of phenolic compounds. Phenolic in plants were distributed throughout the entire metabolic process. Although Kintamani has the highest phenolic concentration, further investigation was required to identify the specific type of phenolic.

3.2 Flavonoid content

The total flavonoid contents of the experiment citrus species were presented in Table 1 and reported as mg quercetin equivalent/g peel extract—the peel of Lime cv. Borneo was observed to have higher flavonoid content (11.48 ± 0.31 mg QE/g peel extract), followed by Tangerine cv. Kintamani (8.48 ± 0.11 mg QE/g peel extract), and Mandarin cv. Pacuan and Tankan Bimanu with the values of 7.75 ± 0.098 and 6.17 ± 0.01 mg QE/g, respectively. Mandarin citrus has 220.96-420.35 µg/mL flavonoids content in the juice, it varies among the varieties [30, 32]

The flavonoid contents on the peel within the mandarin citrus species were also varied indicating no consistent trend within the mandarin citrus group. The lowest flavonoid content was found in Mandarin cv. Satsuma. Based on the citrus group, lime citrus tends to have higher flavonoid content followed by tangerine. These findings were not in line with the reports of Ghasemi et al. [20], Chen et al. [30], and Sowmya et al. [33] that found orange citrus to be the species that have high flavonoid in the peel. They also reported that the method of extraction also contributed to the different flavonoid quantification. Citrus fruits are identified with more than 250 flavonoids, categorized into flavonones, flavones, flavonols, and anthocyanins [34], and when it comes to antioxidant activity, citrus peel leads pulp and seed [28]. Further study is needed regarding the types of flavonoids contained in citrus which may require different methods.

3.3 DPPH radical scavenging activity

The DPPH radical scavenging assay is frequently utilized to evaluate antioxidants' capacity to scavenge free radicals. It is widely known that the DPPH radical scavenging or antioxidant activity of plant extract or a similar compound increases with the concentration of phenolic compounds or the degree of hydroxylation of phenolic components [35]. The values of DPPH radical-scavenging activity on the peel of the evaluated citrus species were presented in Table 1 and reported as Trolox equivalent.

Among the tested citrus species, the DPPH radical-scavenger values were diverse and ranged from 8.73 to 15.145 µmol TE/g (Table 1). Higher DPPH radical-scavenging activity values were observed on Mandarin cv. Pachuan, tangerine cv. Kintamani and pumello cv. Thn. The lowest values were observed on Mandarin cv. Satsuma and sweet orange cv. Manis Taji. The variation of DPPH radical-scavenging activity on the peels within the mandarin citrus group also indicated no consistent tendency of DPPH radical-scavenging activity in the mandarin citrus group. The variation of DPPH radical scavenging activity on the peel of the citrus species was also reported by Muthiah et al. [36] when using three citrus species, i.e. *C. aurantium*, *C. limetta* and *C. limon*. Erba et al. [37] reported the peel of orange, mandarin, lime and lemon has the highest DPPH scavenging activity but different result of DPPH radical scavenging activity was also reported by Singh & Immanuel [38].

3.4 Correlation among the bioactive components

The correlations among the evaluated bioactive components of citrus peel was presented in Table 2. The correlation of phenolic–flavonoid and flavonoid–DPPH radical was considered low. A higher relation was detected in the phenolic content with DPPH radical-scavenging activity. Similar results were also published by Ghasemi et al. [20] that the extract fraction exhibited a significant phenolic content along with significant radical scavenging activity.

Table 2. Correlation of the peel bioactive components on studied citrus species

Bioactive components	Correlation (r)

Phenolic – Flavonoid	0.156
Phenolic – DPPH radical scavenging activity	0.641
Flavonoid – DPPH radical-scavenging activity	0.237

According to Oboh & Ademosun [39], Plants contain phenolics in both bound and free forms. Bound phenolics, mostly as β-glycosides, endure stomach and small intestine digestion and proceed to make it to the colon, where they are released and serve their biological function of digestion. In general, the amount of free phenolic was greater than the amount of bound phenolic. Citrus peels had greater amounts of free and bound phenolic content than cucumber, onion, and red pepper peels [31]. Phenolics can reduce α-tocopherol radicals, chelate metal catalysts, scavenge free radicals, activate antioxidant enzymes, and inhibit oxidases [6]. Due to the redox characteristics of their hydroxyl groups, they have potent antioxidant behaviour [40].

Citrus peel flavonoid amount and radical scavenging activity were also found to have an insufficient correlation [21]. The ability to donate protons and exhibit radical scavenging activity is restricted to flavonoids with specific molecular structures, especially those having hydroxyl positions. Additionally, the bioactive ingredients of citrus peels are complex combinations of several compounds with various functions [17].

4 Conclusion

The bioactive content on the citrus peel of several citrus species was diverse among the studied citrus species. Total phenol determined by the Folin-Ciocalteu method revealed tangerine cv. Kintamani has higher phenolic content with 14.76 ± 0.57 mg GE/g. While the lowest was detected on lime cv. Borneo (8.42 ± 0.08 mg GE/g) and Mandarin cv. Satsuma (8.94 ± 0.11 mg GE/g). For flavonoid content, the peel of lime cv. Borneo had the highest with 11.48 ± 0.31 mg QE/g and Mandarin cv. Satsuma showed the least with 2.98 ± 0.03 mg QE/g. Higher DPPH radical-scavenging activity values were observed on Mandarin cv. Pachuan (15.0805 ± 0.52 µmol TE/g), tangerine cv. Kintamani (15.1 ± 0.57 µmol TE/g) and pumello cv. Thn (15.145 ± 0.24 µmol TE/g). While the lowest values were observed on Mandarin cv. Satsuma (8.73 ± 0.11 µmol TE/g) and sweet orange cv. Manis Taji (8.73 ± 0.09 µmol TE/g). The correlation between phenolic content with DPPH radical-scavenging activity was higher than phenolic–flavonoid and flavonoid–DPPH radical scavenging activity, indicating the active molecule structure on redox potential.

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