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Contributed Paper

Antioxidative Properties and HPLC Profile of Chloroform Fraction from Ethanolic Extract of the Peel of *Citrus hystrix*

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ABSTRACT

Citrus fruits are known to possess high antioxidant activity due to active compounds present in it such as vitamins, carotenoids, flavonoids, and phytochemicals. The present study aimed to evaluate the antioxidant activity and total phenolic content of chloroform fraction from ethanol extract of peel of *Citrus hystrix*. The peel of *C. hystrix* was extracted with 77% ethanol and the crude extract was partitioned between hexane and formic acid (1:1). The formic acid layer was further fractionated with chloroform and n-butanol. The chloroform fraction showed the highest antioxidant activity; therefore it was subjected to further purification on column chromatography using hexane, ethyl acetate, and methanol in varying proportions. The eluates from the column were monitored using thin layer chromatography and aliquots with similar TLC profile were combined to give sub-fractions I, II, III, and IV. All the sub-fractions showed free radical scavenging activity. Qualitative HPLC analysis of all the sub-fractions showed presence of ascorbic acid, catechin, hesperetin, and apigenin; however, further purification and identification studies are needed to warrant their presence in the peel of *C. hystrix*.

Keywords: peel of *Citrus hystrix*, free radical scavenging activity, flavonoids, total phenolic, HPLC

1. INTRODUCTION

Citrus fruits are edible fruits of the plants under the family of Rutaceae or orange family and belong to the genus *Citrus*. They are considered to be an important fruit crop and are consumed freshly, processed into juice or added to dishes and beverages such as lemon and lime. Citrus fruits are widely consumed because they are rich in vitamin C, dietary fibre, beta-carotene, and folic acid [1]. The extracts of citrus fruits have been found to have antioxidant, anti-inflammatory, anti-tumour, anti-fungal, and blood clot inhibition activities [2]. Citrus fruits also have low ratio of sodium to potassium and low in fat and

dietary energy, which make citrus fruits as nutrient-dense, energy-dilute foods with low glycemic index [1].

Citrus fruits have been found to have effects in preventing cancer, coronary heart disease, stroke, diabetes, cataracts, arthritis, macular degeneration, Alzheimer's disease and inflammatory bowel disease [3]. The protective effects of citrus fruits are related to the biologically active elements such as carotenoids, flavonoids, phytochemicals such as limonoids as well as the high antioxidant activity among all the fruit classes [1]. Ascorbic acid is the major component responsible for the high antioxidant activity; however, other compounds such as carotenoids, polyphenols like flavonoids, glutathione and various enzyme systems also contribute to the high antioxidant activity index [4].

Citrus peel constitutes almost half of the fruit mass and it is a rich source of bioactive compounds. Citrus peel contains high amount of phytochemicals such as flavonoids, carotenoids and pectin which can contribute to health [4,5]. One of the flavanone glycosides in the citrus peels is hesperidin, which help to improve vascular integrity and is able to decrease capillary permeability; as well as anti-inflammatory and immunomodulatory effects [6].

Citrus hystrix known as Kaffir lime is a small evergreen tree and found commonly in Indonesia, and Kaffir lime tree is hardy and can reach up from 1.8 m to 7.5 m in height, the pyllodated leaves that are aromatic are dark glossy green colored that can reach 2.5 cm to 15.0 cm in length [7]. Therefore, the leaves and the rinds are often used as spice as well for flavouring purposes. The juice is not consumed directly but it is usually used in the preparation of food and beverages. Some studies had shown that common fruit and leaf possess potent antioxidant capacity, which include bitter melon [8] and *Vites negundo* (commonly known as the five-leaved chaste tree) [9]; as well as the edible portion of *C. hystrix* fruit extracts that had been shown to exert promising antioxidant activity [2,6]. However, there is still limited information on the antioxidant potential of *C. hystrix* peel extract. Therefore, the aims of this study were to evaluate the antioxidant activities and total phenolic content; and to identify qualitatively the presence of potent phenolic compounds in chloroform fraction of ethanolic extract of *C. hystrix* peel.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All the chemicals and reagents were of analytical or HPLC grade. Ascorbic acid, formic acid, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-s-triazine (TPTZ), sodium carbonate anhydrous, chloroform, iron (III) chloride anhydrous, and potassium persulfate were purchased from Fisher Scientific (Leicestershire, UK). Folin-Ciocalteu's phenol reagent, 2,2'-azino-di[3-ethylbenzthiazoline sulfonate] (ABTS), glacial acetic acid, hydrochloric acid, 1-butanol, diphenylboric acid-β-ethylamino ester (NP), polyethylene glycol (PEG), and methanol (HPLC grade) were from Merck (Darmstadt, Germany). Butylated hydroxyanisole (BHA), 2,2-diphenyl-1-picryhydrazyl (DPPH), sodium acetate buffer (0.3 M), α-tocopherol, catechin hydrate, and hesperetin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hexane, ethyl acetate, methanol were from Kofa Chemical Works (Kuala Lumpur, Malaysia). Water used was of Millipore quality.

2.2 Sample Extraction and Fractionation

Citrus hystrix fruits were bought from a local market in Cheras, Kuala Lumpur, Malaysia. The peel of the fruits was removed from the flesh and blended using a blender

(SHARP EM-11, Malaysia) and dried in oven (SHEL LAB 1350FX, Canada) at 40°C until constant weight was achieved. The dried peels were milled using milling machine (IKA®WERKE Model MF10 basic, Germany) at 3873 rpm. The fine powder was vacuum-packed using vacuum packager (Model DZQ 400/500, China) and wrapped with aluminum foil to prevent light exposure and was stored in dark at room temperature.

A total of 120 g of powder was extracted with 77% ethanol (1:10 (w/v) using water bath (Memmert, Germany) with shaking at 31°C for 120 min. These extraction conditions were based on the optimized conditions by response surface methodology as reported by Chan *et al.* [10]. The sample was first filtered and the residues went through the second extraction under the same condition. All the filtrates of both extractions were combined and centrifuged with centrifuge (Model UNIVERSAL 320R, Hettich Zentrifuge, Germany) at 3,000 rpm for 10 min. The supernatants were collected and then concentrated with rotary evaporator (Rotavapour R-200, BUCHI, Switzerland) at 45°C with rotation speed of 30 to 40 rpm. The crude 77% ethanol extract was partitioned between hexane and formic acid (98%) at a ratio of 1:1. The formic acid layer was further fractionated with chloroform and n-butanol. All the fractions (hexane, chloroform, n-butanol, and formic acid) were concentrated with rotary evaporator.

The chloroform fraction was fractionated using gravity column chromatography technique with silica gel as stationary phase and solvents mixture of 100% hexane, 5% methanol in ethyl acetate in order of increasing polarity till 100% methanol; and all the aliquots eluting from the column were monitored using thin layer chromatography (TLC) [11,12]. Aliquots with similar TLC profiles were pooled together and concentrated with rotary evaporator until dryness at 45°C to yield four sub-fractions of increasing polarity (SF I – SF IV). The crude ethanol extract, the chloroform fraction and sub-fractions were tested for antioxidant activity, as well as qualitative identification of potent antioxidative compounds present in the sub-fractions was determined using high-performance liquid chromatography equipped with diode-array detector (HPLC-DAD).

2.3 DPPH Radical Scavenging Activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used to measure the free radical scavenging activity of crude extract, fractions and sub-fractions based on the method by Sharma and Bhat [13] with slight modification. DPPH reagent was prepared by dissolving 7.8 mg of DPPH powder in small volume of ethanol. The solution was topped up to 100 mL with ethanol. Ethanolic DPPH solution (500 µL) was added to 2.0 mL of sample solution in test tubes wrapped with aluminium foil. Distilled water was used in replace of sample solution as control. The mixture was shaken vigorously using vortex (VORTEX V-1, BPECO, Germany). The test tubes were covered with aluminium foil and were left to stand still at room temperature for 30 minutes. The decrease in absorbance was read at 517 nm using a spectrophotometer (PRIM, Secomam, France) against ethanol as blank. The capability to scavenge DPPH radical was calculated using equation below:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100\%$$

Calibration curve was constructed by plotting the percentage of scavenged DPPH (y) against natural logarithm (Ln) of sample concentration (x) to form a calibration curve. From the linear regression equation, amount of sample required to scavenge DPPH radical by 50% (IC_{50}, expressed as µg/mL of extract) was determined.

2.4 Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing power was determined according to the method of Benzie and Strain [14] with modification. FRAP reagent was prepared by mixing the acetate buffer, $FeCl_3 \cdot 6H_2O$ solution and TPTZ (2,4,6-tripyridyl-s-triazine) solution together. Acetate buffer was prepared by mixing 3.1 g of sodium acetate with 16 mL of glacial acetic acid and 1000 mL of distilled water. The $FeCl_3 \cdot 6H_2O$ solution was prepared by dissolving 3.24 g of ferric chloride in 1,000 mL of distilled water, whereas TPTZ solution was prepared by dissolving 0.031 g of TPTZ powder in 10 mL of 40 mM HCl. The freshly prepared FRAP solution was incubated in water bath at 37°C. Three milliliters of FRAP solution were added to 100 µL sample solution in test tube wrapped with aluminium foil. The mixture was shaken vigorously using vortex and was left to stand for 4 minutes. The increase in absorbance was measured at 593 nm against reagent as blank. The ferric reducing ability of sample was expressed as Trolox equivalent antioxidant capacity (µg TE/mL of extract).

2.5 Total Phenolic Content (TPC) Assay

The total phenolic content analysis was performed using the Folin-Ciocalteu method according to Zhao and Hall [15] with slight modification. Folin-Ciocalteu's reagent was prediluted 10-fold and the 7.5% sodium carbonate solution was prepared by dissolving 7.5 g of sodium carbonate in 100 mL of distilled water. Four milliliters of diluted Folin-Ciocalteu's reagent were added to 1.0 mL of sample in test tubes. The mixture was shaken vigorously using vortex and was left for 3 minutes. Five milliliters of 7.5% sodium carbonate solution was added into the test tube and the mixture was shaken vigorously using vortex. Next, the mixture in test tube was left to stand still for 30 minutes. Sample solution was replaced with distilled water as blank. The increase in absorbance was measured at 765 nm. Total phenolic content was expressed as mg of gallic acid equivalents (mg GAE/g of extract).

2.6 Thin Layer Chromatography (TLC) Analysis of Chloroform Sub-fractions

Sample solution was spotted on silica gel aluminium plate (Silica gel 60 F_{254}, Merck, Darmstadt, Germany). The mobile phase was a mixture of ethyl acetate and formic acid (10:0.1) for the TLC analysis. The TLC was sprayed with 1% methanolic NP (diphenylboric acid-β-ethylamino ester) followed by 5% methanolic PEG (polyethylene glycol). Instant florescence was produced in UV light at 365 nm. Flavonoids were appeared as orange-yellow bands, whereas phenolic acids formed blue fluorescent zones. The TLC plate was sprayed with 0.04% methanolic DPPH reagent to allow the free radical scavenging compounds to become visualized with positive results showed yellow spots on purple background. A separate TLC plate was sprayed with 0.5% FBB (Fast Blue B salt) reagent to allow the phenolic compounds to become visualized with positive results showed blue spots on white background [16].

2.7 High Performance Liquid Chromatography

HPLC analysis was performed using

Agilent 1,200 series (Agilent Technologies, CA, USA) equipped with G1311A quaternary pump with G1322A degasser, G1315D diode array detector (DAD). The chromatographic separations were performed on a Chromolith, RP-18e column (5 μm, 100 × 4.6 mm) (Merck, Darmstadt, Germany). The mobile phase comprising of HPLC grade methanol and deionized water (90:10, v/v) with a flow rate of 0.5 mL/min and a column temperature of 25°C. The mobile phase was filtered under vacuum through 0.45 μm pores nylon filter membrane (Agilent Technologies, CA, USA). Sample solution (1 mg/mL) was prepared for each sub-fraction by dissolving the dried sub-fraction in HPLC grade methanol. Similarly, standard solutions were also prepared by using HPLC grade methanol. The sample solutions and standard solutions were filtered using 0.45 μm syringe filter (Jet Biofil, Guangzhao, China) before being injected into HPLC system. UV detection was carried out at 280 nm. A volume of 20 μL of sample solution was injected each time. The peaks in the chromatogram were identified by the comparison of retention time and UV spectra of standard compounds [17].

2.8 Statistical Analysis

Statistical analyses were conducted using SPSS 17.0 and the results were expressed as means ± standard deviations. All antioxidant activities were performed in triplicate and one-way analysis of variance (ANOVA) with Tukey's post-hoc comparison tests were carried out to assess for any significant differences among the means. Significant levels were based on the confidence level of 95% ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Extraction and Fractionation

Formic acid was used to dissolve the crude ethanol extract because its high polarity compared to hexane, chloroform, and *n*-butanol. The formic acid fraction was first partitioned with hexane, the least polar solvent to extract the non-polar compounds from the crude extract. As shown in Table 1, the yield of hexane fraction (Fraction I) was 1.05 g (1.51%). The formic acid-extract fraction was then partitioned with chloroform, which is more polar than hexane to extract compounds in middle range of polarity. The yield of the chloroform fraction (Fraction II) was 4.25 g (6.10%). The formic acid fraction was then further partitioned with *n*-butanol. Although *n*-butanol (polarity index = 4.0) and chloroform (polarity index = 4.1) had the similar polarity index, *n*-butanol extract was chosen for extraction to extract because it has the functional OH group, which could attract other compounds by forming hydrogen bond. The yield of *n*-butanol fraction (Fraction III) was 16.62 g (23.84%) and formic acid residue fraction was 9.40 g (13.48%). The chloroform fraction was fractionated using

Table 1. Percentage of yield of fractions from liquid-liquid partitioning chromatography.

Fraction	Weight of fraction (g)	Weight of crude extract (g)	Percentage of yield (%)
I	1.05	69.72	1.51
II	4.25	69.72	6.10
III	16.62	69.72	23.84
IV	9.40	69.72	13.48

Fraction I = Hexane fraction; Fraction II = Chloroform fraction; Fraction III = *n*-butanol fraction; Fraction IV = Formic acid fraction.

gravity column chromatography with hexane, ethyl acetate, and methanol in the order of increasing polarity. Aliquots with similar TLC profiles were combined to yield four sub-fractions of increasing polarity (SF I – SF IV).

3.2 Antioxidant Activities and Total Phenolic Content of Fractions and Sub-fractions

The free radical scavenging activities of *C. hystrix* peel fractions and its related sub-fractions are shown in Table 2. Based on the result, the scavenging activity of DPPH assay for chloroform fraction was significantly high among the crude extract and the fractions. The inhibitory concentration (IC_{50}) of sample solution at which the DPPH radicals were scavenged by 50% was determined, and the IC_{50} is inversely proportional to scavenging activities. The IC_{50} values increased as follows: Fraction II (chloroform fraction) < crude extract < Fraction IV (formic acid fraction) < Fraction III (*n*-butanol fraction) < Fraction I (hexane fraction). The scavenging activities of all the sub-fractions were significantly much lower than the chloroform fraction and standards. The IC_{50} values increased as follows: Chloroform < crude extract < SF IV < SF III < SF II < SF I.

The reducing power of Fraction II (chloroform fraction) showed the highest TEAC values (μg TE/mL) (Table 2). The TEAC values of crude extract and Fraction I, III, and IV were zero at sample concentrations of 10 μg/mL and 50 μg/mL. This could possibly due to the low amount of antioxidant compounds in the sample solutions. The TEAC values (in the decreasing order) was as follows: Fraction II (chloroform) > crude extract > Fraction III (*n*-butanol) > Fraction I (hexane) > Fraction IV (formic acid). This indicated that Fraction II (chloroform fraction) showed the highest reducing power. There was not much difference in TEAC values among SF I, SF II, SF III, and SF IV (Table 2). The TEAC of all

Table 2. Total phenolic content, DPPH radical scavenging activity, and reducing power of peel of *Citrus hystrix* extract, its liquid-liquid partitioning fractions, and sub-fractions of chloroform fraction.

Sample	TPC (GAE, mg/g)	FRAP (TEAC, μg/mL)	DPPH radical scavenging IC_{50}† (μg/mL)
Crude extract	34.66 ± 1.34^{c}	37.03 ± 2.38^{b}	100.05
Hexane fraction	14.83 ± 0.16^{c}	13.30 ± 2.99^{d}	> 500
n-Butanol fraction	48.08 ± 2.33^{b}	27.62 ± 1.37^{c}	464.77
Formic acid fraction	15.75 ± 0.78^{c}	11.36 ± 0.55^{d}	> 500
Chloroform fraction	69.67 ± 5.26^{a}	43.13 ± 1.27^{a}	397.75
Sub-fraction I	1.26 ± 0.06^{f}	20.64 ± 1.61^{e}	> 500
Sub-fraction II	1.37 ± 0.05^{f}	20.67 ± 0.85^{e}	> 500
Sub-fraction III	4.00 ± 0.35^{e}	20.56 ± 0.73^{e}	> 500
Sub-fraction IV	7.69 ± 0.30^{d}	21.79 ± 0.84^{e}	> 500
BHA	NA	864.28 ± 98.45^{a}	10.53

Each value was expressed as mean ± standard deviation (n = 3). NA: not applicable. *Different superscripts within the column (TPC and FRAP) denote significantly different ($p < 0.05$).

†Defined as effective concentration that was able to scavenge 50% of the total DPPH radicals; IC_{50} was calculated by interpolation of linear regression analysis (based on concentration-dependent result (100–500 μg/mL) – data not shown).

the sub-fractions were lower than chloroform fraction (Fraction II) and the standards. The TEAC value of the chloroform fraction was higher than the crude extract. From the results of the assays, the antioxidant activities of all the sub-fractions were lower than the chloroform fraction. This may due to many factors including inability to elute the entire active compound by the fractionation process into the four sub-fractions and degradation in the column during the partial purification process. Degradation of phenolic compounds due to decomposition and polymerization was has been reported by Chirinos *et al.* [18].

Fraction II showed significantly higher TPC compared to other fractions and the TPC values follow the decreasing order as follows: Fraction II > Fraction III > Crude extract > Fraction IV > Fraction I (Table 2). Amongst all the sub-fractions, SF IV showed significantly higher TPC, but significantly lower when compared to Fraction II that follow the decreasing order of Fraction II > crude extract SF IV > SF III > SF II > SF I.

Fraction II the highest antioxidant activity and this was significant for all the assays (DPPH and FRAP) followed by fraction III. Fraction I and Fraction IV showed significantly lower antioxidant activity compared to crude extract, Fraction II and Fraction III. Based on this observation, it can be assumed that most of the antioxidant compounds in the peel of *Citrus hystrix* were in the middle range of polarity as chloroform had the polarity index of 4.1. This shows that chloroform has a better retention capability for the antioxidant compounds in the crude 77% ethanol extract [19]. The crude 77% ethanol extract was lower in antioxidant activity than Fraction II because the crude extract contained all the compounds including antioxidant compounds as well as non antioxidant compounds.

The fractionation and concentration of phenolics throughout the partitioning procedure was responsible for high free radical scavenging activity of the chloroform fraction compared to the crude extract. Although *n*-butanol and chloroform had similar polarity, polarity index of 4.0 and 4.1, respectively, Fraction II showed higher antioxidant activity than Fraction III. This may be due to the partitioned of the sample with chloroform before being partitioned with *n*-butanol. As a result, most of the antioxidant compounds were retained in chloroform. Fraction I and Fraction IV were the lowest in antioxidant activity. This was due to the characteristics of hexane being non polar (polarity index of 0.0) and of formic acid being too polar. However, most of antioxidant compounds in the sample were neither in the low polarity range nor in the high polarity range.

SF IV and SF III showed the highest antioxidant activity among the sub-fractions from the results of the *in vitro* assays. Generally, it was quite obvious that antioxidant activities of SF III and SF IV were significantly higher than SF I and SF II in all assays. This was because the compounds in SF IV and SF III were eluted out mostly with ethyl acetate and methanol whereas compounds in SF II and SF I were eluted out with high proportion of hexane. This showed that the antioxidant compounds in the sample are substantially polar and small amount of antioxidant compounds were in the low polarity range. There was no significant difference between SF I and SF II from DPPH assay and FRAP assay. However, the total phenolic content of SF II was significantly higher than SF I.

3.3 Qualitative TLC and HPLC Analyses of Sub-fractions

The four sub-fractions were analyzed by TLC and the results of R_f values, color, and bioautographic sprays (indicating DPPH radical scavenging and phenolic compounds) were

shown in Table 3. The retention factor (R_f) values are used to compare polarity among spots on TLC profile. Spots with large R_f values were considered non-polar compounds, whereas the spots with small R_f values were considered more polar compounds and strongly adsorb to the silica gel. Therefore, these compounds appeared on the lower part of the plate [20].

The HPLC profiles of SF I, SF II, SF III, and SF IV were quite similar as shown in Figure 1. The peaks were eluted before 10 minutes except one peak which was detected around 12 minutes in all sub-fractions. This indicated that all the sub-fractions contained the similar type of antioxidant compounds, but in different concentrations since the area and the height of the peak corresponded to the concentration of a particular compound [21]. The peaks were identified qualitatively by comparing the retention times and UV spectra of the peak of standard compounds to the peaks in the sample chromatographs.

Table 3. Thin layer chromatographic analysis of sub-fractions.

Sub-fraction	Dry weight (g)	Colour of spot	R_f	DPPH spray	FBB Spray
SF I	6.46	Blue	0.23	+	+
SF II	6.41	Green	0.35	+	+
		Blue	0.65	+	+
SF III	4.48	Yellow	0.00	+	+
		Light blue	0.20	+	+
		Blue	0.18	+	+
		Green	0.30	+	+
		Yellow	0.37	+	+
		Orange	0.43	+	+
		Red	0.46	+	+
		Blue	0.70	+	+
SF IV	1.62	Yellow	0.00	–	+
		Light blue	0.03	–	+
		Blue	0.20	–	+
		Green	0.33	–	+
		Yellow	0.41	–	+
		Orange	0.45	–	+
		Red	0.51	–	+
		Blue	0.73	+	+

TLC plates were visualized under UV light at 365 nm.
SF: Sub-fraction.
+: Presence of free radical scavenging compound/phenolic compound
–: Absence of free radical scavenging compound/phenolic compound

Four possible antioxidant compounds were identified to be present in the sub-fractions, which include ascorbic acid, hesperetin, and apigenin. The sub-fractions showed the presence of similar compounds such as catechin in SF I and SF IV; ascorbic acid in SF I, SF II, and SF III; and hesperetin in SF II and SF III; however, with different concentration as observed based on their peak areas and peak heights. The antioxidant

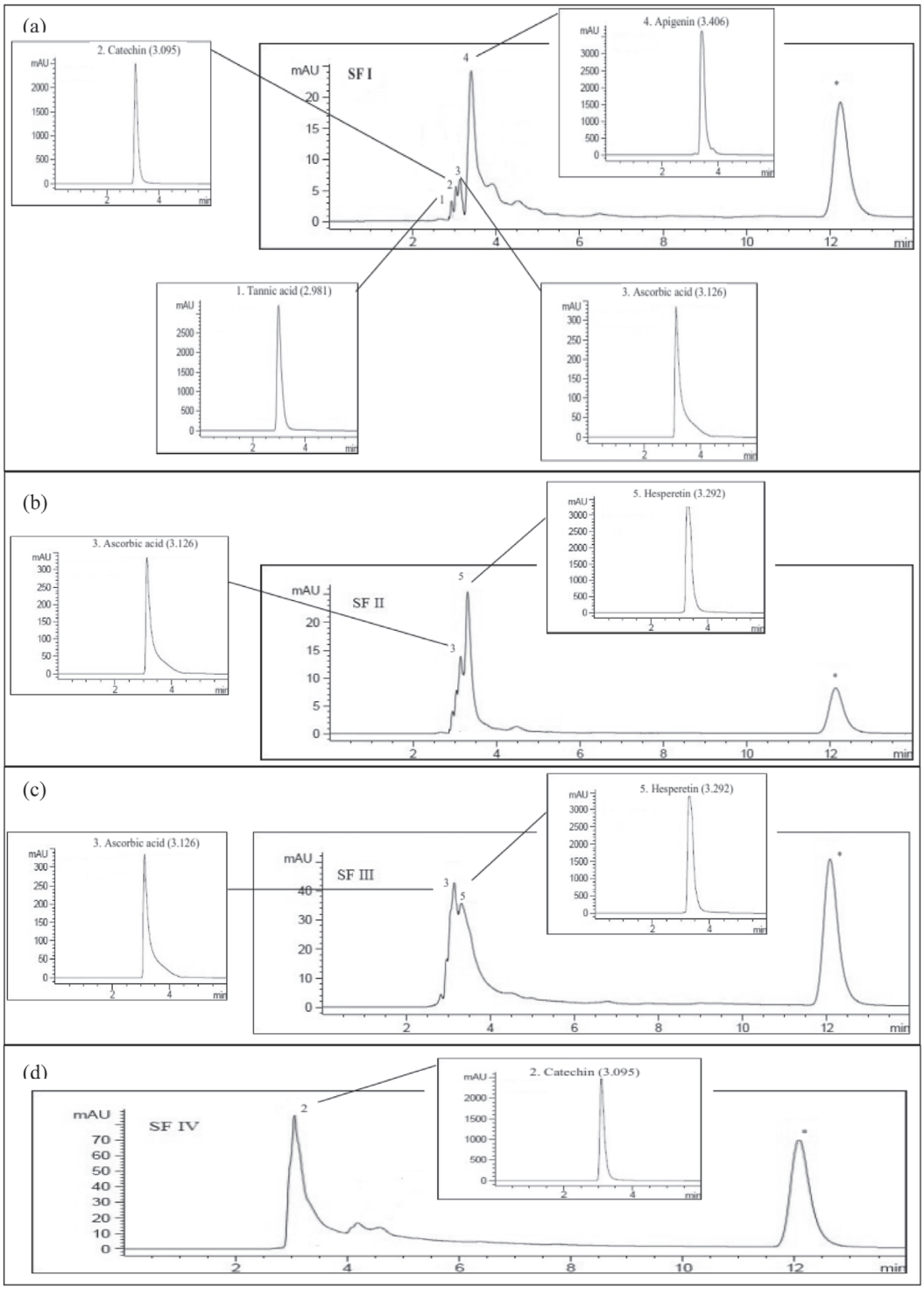

Figure 1. HPLC chromatograms of sub-fractions of peel of *Citrus hystrix* at 280 nm. (a) SF I; (b) SF II; (c) SF III; and (d) SF IV. Inserts showed the HPLC chromatograms of standard compounds with respective retention time. * Indicates unknown.

activities of all of the sub-fractions were considered low especially SF I and SF II. This was due to small amounts of catechin, ascorbic acid, hesperetin, and apigenin present in the sub-fractions based on their peak heights and peak areas. SF III and SF IV had the higher antioxidant activities compared to SF I and SF II. This was possibly due to the presence of higher amounts of ascorbic acid and hesperetin in SF III; catechin in SF IV, based on their ratio of peak height to peak area. In SF II, the ratio of peak height to peak area of ascorbic acid and hesperetin were smaller. By comparing SF III and SF II, the ratio of peak height to peak area of ascorbic acid in SF III was about 5.2 times of that in SF II, whereas the ratio of peak height to peak area of hesperetin in SF III was about 3 times of that present in SF II. By comparing ratio of peak height to peak area of catechin in SF IV and SF I, the amount of catechin in SF IV was about 9.2 times of that in SF I. The smaller amounts of antioxidant compounds present in SF I and SF II made them have lower antioxidant activities than SF III and SF IV.

As mentioned, catechin, ascorbic acid, hesperetin, and apigenin are among the compounds that could be responsible for the antioxidant activities of sub-fractions. These compounds have been reported to dem onstrate antioxidative and free radical scavenging activities including DPPH radical scavenging, ABTS radical scavenging, superoxide anion radical scavenging hydrogen peroxide scavenging, Fe^{3+} reducing power and ferrous ions chelating activities [22-24].

4. CONCLUSION

Chloroform fractions from the peels of *Citrus hystrix* showed promising antioxidant activity to a certain extent with the presence of potent phenolic compounds. This suggests that the peel of *C. hystrix* could be utilized as a source of natural antioxidant instead of being discarded. However, extensive purification and identification work of the antioxidant compounds in the peel of *C. hystrix* need to be further studied.

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AUTHORS DISCLOSURE STATEMENT

No competing financial interests exist.

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