RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2014.40.121

ScienceAsia 40 (2014): **121–124**

The phytochemical properties of a new citrus hybrid (*Citrus hystrix* × *Citrus microcarpa*)

Abdul-Halim Yahya, Gun-Hean Chong*, Chin-Ping Tan

Department of Food Technology, Faculty of Food Science and Technology, Putra Malaysia University, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

*Corresponding author, e-mail: gunhean@upm.edu.my

Received 10 Apr 2013
Accepted 19 Jan 2014

ABSTRACT: The Merdeka lime is a new citrus hybrid with strong resistance against pests and disease. This work aims to provide information about the total phenolic content (TPC), total flavonoid (TF) content and antioxidant potential of the leaves, peel and pulp of this new hybrid along with those of its parent plants (*Citrus hystrix* and *C. microcarpa*). The TPC and TF contents were determined based on a colorimetric method, while antioxidant levels were determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the ferric-reducing antioxidant power (FRAP) techniques. The results revealed that Merdeka lime contained the highest TPC (4600 ± 140 μg gallic acid equivalents per gram of dry weight) and DPPH value (4260 ± 30 μg Trolox equivalents per gram of dry weight) in the leaves and the highest TF (16 400 ± 300 μg quercetin equivalents per gram dry weight) and FRAP value (13 430 ± 60 μg ascorbic acid equivalent per gram of dry weight) in the peel. The antioxidant activities of Merdeka lime were similar to the parent plants.

KEYWORDS: Merdeka lime, makrut lime, antioxidant, phenolic compounds, flavonoid compounds

INTRODUCTION

An antioxidant is a substance that is able to prevent an excessive amount of free radicals in biological systems[1]. It also plays an important role in food preservation by reducing lipid peroxidation preventing quality deterioration[2,3]. Natural antioxidants have therefore become a new focus in nutritional studies as the consumers believe that natural products are healthier and safer[1,4]. Numerous studies have proven that the phytochemical compounds produced in the plant are one of the best sources of natural antioxidant, such as those found in the citrus[5–7].

A new citrus hybrid (*C. hystrix* × *C. microcarpa*), known as the Merdeka lime in Malaysia, was introduced in 2010. The Merdeka lime has larger fruits (Fig. 1) and leaves than its parent plants, and it has been cultivated without the use of pesticides. The increased resistance to pests indicates that this hybrid might produce novel secondary metabolites or have undergone changes in metabolite composition. There is an urgent need for information about this hybrid to enable more detailed studies. The aim of this study was therefore to determine the total phenolic content (TPC), total flavonoid (TF) content and the antioxidant activity of this new citrus hybrid (*C. hystrix* × *C. microcarpa*) relative to its parent cultivars.

MATERIALS AND METHODS

Plant materials

Three lime varieties, namely *C. hystrix*, *C. microcarpa*, and the Merdeka lime (*C. hystrix* × *C. microcarpa*), were used in this study. The leaves of all three varieties and the fruit of the Merdeka lime were harvested from a Laverson Biotech farm in Batang Kali, Hulu Selangor, Malaysia. *C. hystrix* and *C. microcarpa* fruits were obtained from local markets in Serdang, Selangor, Malaysia. All leaf samples were dried in the open air in the laboratory until the moisture content reached less than 10% (wet basis). All fruit samples were manually cleaned and separated into peel and pulp samples, with the seeds removed. The peel and pulp were dried with a vacuum freeze-dryer (Labconco FreeZone, Kansas City, MO, USA) at −40 °C, 10 Pa until the moisture content was less than 10% (wet basis). All dried samples were ground into powder and stored at −10 °C.

Chemicals and reagents

Folin-Ciocalteu's reagent, gallic acid, Na_2CO_3, $NaNO_2$, $AlCl_3$, NaOH, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Chemical Co. (St. Louis,

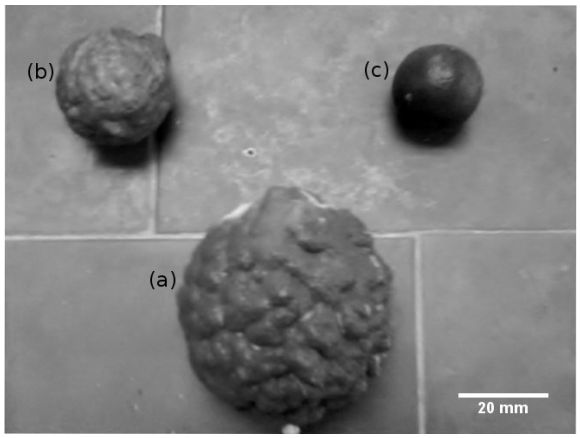

Fig. 1 (a) Merdeka lime crossbred from (b) *Citrus hystrix* and (c) *C. microcarpa.*

MO, USA); 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Merck (Darmstadt, Germany).

Extraction of phytochemicals

The leaves, peel and pulp (5 g) of *C. hystrix*, *C. microcarpa* and the Merdeka lime were extracted with 80% methanol (v/v) overnight at 25 °C on an orbital shaker (Hotech Instrument Corp., 903, Taiwan). The mixtures were then centrifuged for 5 min at 2380*g* (Beckman Coulter, Avanti J-25.15, USA). The supernatant was filtered through a Büchner funnel and Whatman no. 1 filter and collected. The crude extracts were obtained by evaporating the extracts to dryness under reduced pressure using a vacuum rotary evaporator (Stuart, RE300, UK) at a temperature below 40 °C. The extracts were re-suspended in 50 ml of 80% methanol (v/v) and used to measure phytochemical contents and to determine total antioxidant activity. The extraction was done in triplicate.

Determination of total phenolic content (TPC)

The total phenolic content of the lime extracts was measured by using the Folin-Ciocalteu colorimetric method as described by Almey et al[4]. Briefly, 100 µl of extract sample was transferred into a test tube and mixed with 0.75 ml Folin-Ciocalteu reagent (diluted 10-fold with distilled water). The mixture was allowed to stand for 5 min at 25 °C. Then 0.75 ml of 6% (w/v) Na_2CO_3 was added and mixed gently. The mixture was allowed to stand for 60 min, after which its absorbance was read at 725 nm (Thermo Electron Corporation, Genesys 20, California, USA). The results were expressed as µg gallic acid equivalents (GAE) per gram of dry weight, calculated using a standard calibration curve prepared with gallic acid (0–100 µg/ml). The analysis was done in triplicate.

Determination of total flavonoid content

The total flavonoid content of the samples was measured using the colorimetric method adapted from Jia et al[8], with modifications as described by Zhang et al[9]. Briefly, 0.25 ml of extract sample was added into a test tube containing 0.75 ml of distilled water. Then, 0.15 ml of $NaNO_2$ (5%) was added to the test tube, and the solution was mixed gently. The mixture was allowed to stand for 5 min, and then 0.3 ml of $AlCl_3$ (10%) was added; after an additional 5 min, 1 ml of NaOH (1 M) was added. The solution was mixed well, and the absorbance was read at 510 nm. Quercetin concentrations ranging of 0.04–0.20 mg/ml were used to prepare the standard calibration curve. The results were expressed as µg quercetin equivalents per gram of dry weight. The analysis was done in triplicate.

DPPH free radical-scavenging activity

The DPPH free radical scavenging activity method was adapted from Ismail et al[10]. Briefly, a 0.2 mM DPPH methanolic solution was freshly prepared immediately prior to the analysis. The analytical samples were prepared at 0.5 mg/ml, and 50 µl of each analytical sample was mixed with 195 µl of the 0.2 mM DPPH methanolic solution in a 96-well microplate. The plate was gently swirled and incubated for 60 min in the dark. All the extract samples were read at 515 nm in a 96-well ELISA microplate reader (BioTek, BIOTEL ELx800, Winoosk, USA). Trolox was used as the standard, and a calibration curve in the range of 20–100 µg/ml was prepared. The analysis was done in triplicate. The DPPH scavenging activity was determined by $((A_\mathrm{e} - A_\mathrm{n})/A_\mathrm{s})$, where A_e, A_n, and A_s are the absorbance of the extract, absorbance of the negative control, and standard absorbance of the negative control, respectively.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to procedure described by Zhang et al[9]. FRAP reagent was freshly prepared and it consisted of 10 ml ferric chloride (20 mM), 10 ml of 10 mM Fe(III)-2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 100 ml of 0.25 M sodium acetate buffer (pH 3.6). The FRAP reagent was warmed at 37 °C. Then 0.5 ml analytical sample was added to 1.5 ml of FRAP reagent and gently mixed. The absorbance was read at 593 nm after 10 min incubation at 37 °C. Calibration curve of ascorbic acid was used as standard at range of

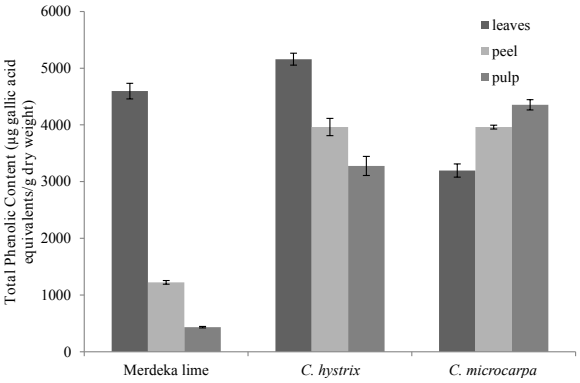

Fig. 2 Total phenolic content in Merdeka lime, *C. hystrix* and *C. microcarpa*.

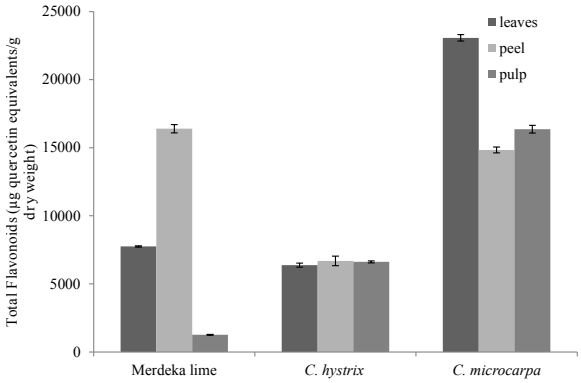

Fig. 3 Total flavonoid content in Merdeka lime, *C. hystrix* and *C. microcarpa*.

4–30 µg/ml and results were expressed as µg ascorbic acid per gram of dry weight. The analysis was done in triplicate.

Statistical analysis

The results are expressed as the means of the replicates ± standard deviations. Significant differences at the 95% confidence level were calculated based on Duncan's multiple range tests using IBM SPSS Statistic 19.0 (IBM SPSS Inc., USA).

RESULTS AND DISCUSSION

The result of univariate ANOVA showed that the parts of hybrid (peel, pulp, and leaves) and of parent plants presented significantly different TPC ($p < 0.05$). As shown in Fig. 2, in Merdeka lime the highest TPC was found in the leaves (4595.8 µg GAE per gram of dry weight) and the amount was comparable to that of *C. hystrix* (5158.3 µg GAE per gram of dry weight). The TPC in Merdeka lime was also comparable to white grapefruit (4201 µg GAE per gram of dry weight) and Jaffa sweetie grapefruits (4065 µg GAE per gram of dry weight)[11].

The TF contents were found significantly different in the hybrid and the parents plant ($p < 0.05$). In the Merdeka lime (Fig. 3), the peel contained the highest TF (16 395 µg quercetin equivalents per gram of dry weight) and the pulp had the lowest content of TF (1257 µg quercetin equivalents per gram of dry weight). The pulp of Mauritian *Citrus* was also found had the lowest content of TF[12].

The hybrid and the parent plants reacted differently in DPPH analysis ($p < 0.05$), except the leaves of Merdeka lime compared to the leaves of *C. hystrix*. In Merdeka lime (Fig. 4), the leaves presented the highest radical scavenging activity (4259.5 µg Trolox equivalents per gram of dry weight) followed by the

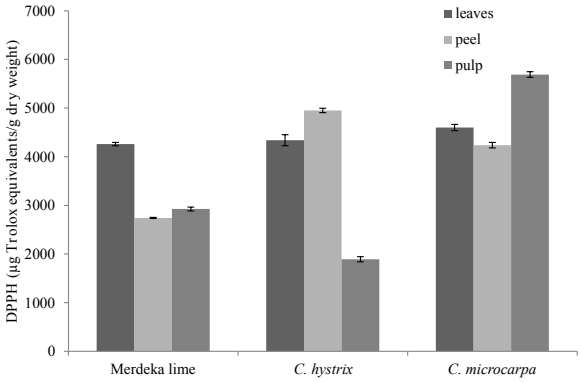

Fig. 4 DPPH radical scavenging activity of Merdeka lime, *C. hystrix* and *C. microcarpa*.

pulp (2925.0 µg Trolox equivalents per gram of dry weight) and the peel (2741.1 µg Trolox equivalents per gram of dry weight).

The result of FRAP showed that there was no significant difference ($p > 0.05$) in the antioxidant activities between the parts of Merdeka lime and part of the parent plants except the pulp of hybrid. In Merdeka lime (Fig. 5), the peel showed the highest antioxidant activity (13 427.0 µg ascorbic acid equivalent per gram of dry weight) and the pulp presented the lowest one (2510.0 µg ascorbic acid equivalent per gram of dry weight). Merdeka lime (leaves and peel) however had a good antioxidant activity as compared with some jujube cultivars, such as hamidazao (3724.9 µg ascorbic acid equivalent per gram of dry weight) and dongzao (9823.1 µg ascorbic acid equivalent per gram of dry weight)[9].

CONCLUSIONS

The antioxidant properties of the Merdeka lime (leaves, peel, and pulp) were investigated in this

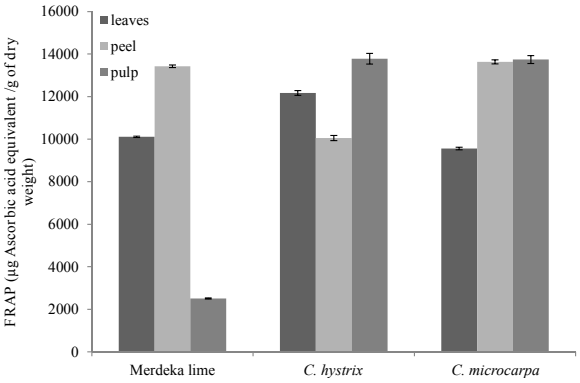

Fig. 5 Ferric-reducing antioxidant activity (FRAP) of Merdeka lime, *C. hystrix* and *C. microcarpa*.

study. Of the tissues analysed, the leaves contained the highest TPC, and the peel contained the highest TF. The Merdeka lime also presented antioxidant activity comparable to that of other fruits. As a new hybrid, however, the Merdeka lime should be studied further.

Acknowledgements: This study received financial support from RUGS grants (Vot no. 9 300 360). The authors wish to acknowledge Mr Devandran from LeVarson Biotech Sdn. Bhd. for supplying the leaves and the fruits of Merdeka lime and other samples.

REFERENCES

1. Conde-Hernández LA, Guerrero-Beltrán JA (2014) Total phenolics and antioxidant activity of *Piper auritum* and *Porophyllum ruderale*. *Food Chem* **142**, 455–60.
2. Moure A, Cruz JM, Franco D, Domínguez JM, Sineiro J, Domínguez H, Núñez MJ, Parajó JC (2001) Natural antioxidants from residual sources. *Food Chem* **72**, 145–71.
3. Delgado Adámez J, Gamero Samino E, Valdés Sánchez E, González-Gómez D (2012) In vitro estimation of the antibacterial activity and antioxidant capacity of aqueous extracts from grape-seeds (*Vitis vinifera* L.). *Food Contr* **24**, 136–41.
4. Almey AAA, Khan CAJ, Zahir IS, Suleiman KM, Aisyah MR, Rahim KK (2010) Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plants' leaves. *Int Food Res J* **17**, 1077–84.
5. Mäkynen K, Jitsaardkul S, Tachasamran P, Sakai N, Puranachoti S, Nirojsinlapachai N, Chattapat V, Caengprasath N, Ngamukote S, Adisakwattana S (2013) Cultivar variations in antioxidant and antihyperlipidemic properties of pomelo pulp (*Citrus grandis* [L.] Osbeck) in Thailand. *Food Chem* **139**, 735–43.
6. Park HY, Choi HD, Eom H, Choi I (2013) Enzymatic modification enhances the protective activity of citrus flavonoids against alcohol-induced liver disease. *Food Chem* **139**, 231–40.
7. Pająk P, Socha R, Gałkowska D, Rożnowski J, Fortuna T (2014) Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food Chem* **143**, 300–6.
8. Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* **64**, 555–9.
9. Zhang H, Jiang L, Ye S, Ye Y, Ren F (2010) Systematic Evaluation of Antioxidant capacities of the ethanolic extract of different tissues of jujube (*Ziziphus Jujuba* Mill.) from China. *Food Chem Toxicol* **48**, 1461–5.
10. Ismail HI, Chan KW, Mariod AA, Ismail M (2010) Phenolic content and antioxidant activity of cantaloupe (*Cucumis melo*) methanolic extracts. *Food Chem* **119**, 643–7.
11. Gorinstein S, Cvikrová M, Machackova I, Haruenkit R, Park YS, Jung ST, Yamamoto K, Martinez Ayala AL et al (2004) Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits. *Food Chem* **84**, 503–10.
12. Ramful D, Tarnus E, Rondeau P, Robert Da Silva C, Bahorun T, Bourdon E (2010) *Citrus* fruit extracts reduce advanced glycation end products (AGEs)- and H_2O_2-induced oxidative stress in human adipocytes. *J Agr Food Chem* **58**, 11119–29.