



## Identification of phenolic compounds from a unique citrus species, finger lime (*Citrus australasica*) and their inhibition of LPS-induced NO-releasing in BV-2 cell line

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### ABSTRACT

In this study, a unique citrus species (*Citrus australasica*) was selected, and its fruit characteristics, phenolic compounds and ability to inhibit inflammation were preliminarily studied. Finger lime fruits showed distinctive features in shape, size, weight, colour, total soluble solids, water-soluble pectin, sugar and acids contents. Combining UPLC-HRMS and UPLC-DAD analysis, 31 phenolics, 1 secoiridoid derivative and 1 neolignan glycoside were preliminarily identified and quantified. The phenolics composition of finger limes showed cultivar and tissue specificity. Antioxidant evaluation showed that extracts from finger lime cultivar of 'XiangBin' exhibited better antioxidant capacities than cultivar of 'LiSiKe', especially in peel. LPS-induced NO-releasing model was performed in the mouse microglia BV-2 cell line. Results illustrated that finger limes inhibited the NO-releasing and the inflammation-related cytokines including IL-1 $\beta$ , IL-6 and TNF $\alpha$  elevation. QRT-PCR revealed that finger lime extracts alleviated LPS-induced upregulation of iNOS, IL-6, JAK2, TNF $\alpha$ , TLR2, TLR4, IL-1 $\beta$ , NF- $\kappa$ B and LPS-induced downregulation of  $I\kappa B\alpha$ . This study may expand our knowledge on the physiochemical characteristics and bioactive properties of citrus fruits.

### 1. Introduction

Citrus, characterized by charming aroma and distinctive tastes, is a popular fruit widely distributed and consumed around the world. Taxonomically, citrus fruits are classified within the angiosperm subfamily *Aurantioidae* of the Rutaceae family. While, commercially, citrus fruits are divided into mandarins (*Citrus reticulata*), oranges (*Citrus sinensis*), pomeloes (*Citrus maxima*), lemons (*Citrus limon*), limes (*Citrus aurantiifolia*) and citrons (*Citrus medica*). Different types of citrus fruits vary in shape, size, colour, aroma, maturation stage, as well as shelf life, consuming pattern and production method. These differences, which may vary due to complex genetic background, are firmly related to their regional distribution (Wu et al., 2018).

Citrus fruits are generally rich in bioactive components. Phenolics had been elucidated as the essential bioactive components in citrus with a broad spectrum of activity, exhibiting a wide range of promising pharmaceutical properties for human health (Wang et al., 2017a). Flavonoids, including flavones, flavanols, flavanones and coumarins, are the predominant soluble phenolic compounds in citrus, and the composition varied among different types and different parts of citrus

fruits (Wang et al., 2017b). Plenty of studies reported that the extracts and juices of citrus fruits had antioxidant capacities (Wang et al., 2017b), anti-tumor effects (Liu et al., 2017b), anti-inflammatory actions (Cheng et al., 2017), weight control (Raasmaja et al., 2013) and blood glucose regulation activities (Liu et al., 2017a).

Finger lime (*Citrus australasica*) is an Australia native citrus species with unique phenotype. This semi-wild citrus species was known for its finger-like shape and caviar-like juice sacs. The finger lime is attracting more and more attention, and the demand for this fascinating fruit is increasing and booming. However, compared with a large number of reports on other citrus species, the studies on physicochemical characteristics of finger lime are few. To the best of our knowledge, no phenolic identification and antioxidant evaluation had been carried out on finger limes. Thus, the present study was carried out to shed light on the physiochemical characteristics of this distinctive citrus species.

Inflammation is a stress response that occurs when tissues and cells of an organism are exposed to pathogens, harmful stimuli, or physical damage, and are closely related to the development of many diseases, such as cancer (Grivnenikov et al., 2010), atherosclerosis (Kriszbacher et al., 2005), Alzheimer's disease (Cassery and Topol, 2004). The

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principal pathways of inflammation include *Nuclear Factor kappa-light-chain-enhancer of activated B cells/Inhibitor of nuclear factor kappa-B kinase (NF- $\kappa$ B/I $\kappa$ B)* pathway (Baldwin, 1996), *Janus kinase-2/Signal Transducer and Activator of Transcription protein-3 (JAK2/STAT3)* pathway (Satriotomo et al., 2006), *Toll-like receptors (TLR)* pathway (Cario and Podolsky, 2000), and so on. Ingestion of substances that inhibit inflammation in daily diets has been a research trend in the field of natural products and food research. Citrus as one of the most popular fruits in the world had been paid close attention to anti-inflammatory research (Cui et al., 2010; Ho and Kuo, 2014; Lee et al., 2016; Shu et al., 2014). However, there are no reports of finger lime on the protection of microglia from inflammatory substances. This study found that finger lime extracts within the safe dose range inhibited the release of nitric oxide (NO) induced by lipopolysaccharide (LPS), decreased the concentrations of inflammatory factors Interleukin 1 beta (IL-1 $\beta$ ), Interleukin 6 (IL-6) and Tumor necrosis factor alpha (TNF $\alpha$ ), and the effects were related to the regulation of *JAK2/STAT3*, *NF- $\kappa$ B/I $\kappa$ B* and *TLR* pathways. Thus, Our research elucidated the diversity of anti-inflammatory mechanisms exerted by natural products.

## 2. Materials and methods

### 2.1. Materials

Finger lime fruits at commercial maturity were used in this study. Finger lime ripening criteria include the colour of the shady side of the fruit changes from green to yellow or red, the fruit is full and plump, and easy to pick off the branches. 'XiangBin' ('XB') and 'LiSiKe' ('LSK') were harvested from Hainan Province, China, in March 2018. Uniform and mechanical injury-free fruits were selected and separated into two parts, peel and flesh. Different parts of the fruit materials were frozen immediately in liquid nitrogen and were stored at -80 °C.

Mouse microglia BV-2 cell was purchased from Institute of Cells, Chinese Academy of Science (Shanghai, China).

### 2.2. Chemicals and reagents

All chemical standards were of high-performance liquid chromatography (HPLC) grade. Fructose, glucose, sorbitol, sucrose, malic acid, citric acid, quinic acid, gallic acid, Trolox, d-galacturonic acid sodium salt, fluorescein sodium, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2'-azobis(2-methylpropionamide) dihydrochloride (AAPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), Folin-Ciocalteu reagent, methanol, acetonitrile, narirutin, diosmin, poncirin, naringin, neohesperidin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cell counting kit-8 was purchased from Dojindo (Shanghai China). RPMI 1640 medium, trypsin-EDTA were purchased from Gibco (Waltham, MA, USA). Mouse Elisa TNF $\alpha$  kit, Mouse Elisa IL-6 kit, Mouse Elisa IL-1 $\beta$  kit were purchased from R&D Systems (Carlsbad, CA, USA). All other reagents were of analytical grade purchased from Sinopharm Chemical Reagents Co., Ltd. (Shanghai, China). Double-distilled water (ddH<sub>2</sub>O) was used in all experiments. Samples injected in UPLC systems were filtered through a 0.22 μM membrane.

### 2.3. General analysis (fruit weight, edible rate, colour, total soluble solids (TSS), water-soluble pectin)

Citrus colour index (CCI) and colour index of red grapes (CIRG)

were used for fruit colour measurement according to previous reports (Gao et al., 2015; Zhou et al., 2010). MiniScan XE plus Colourimeter (HunterLab, VA, USA) was used to adopt L\*, a\* and b\* values. The CCI was calculated as CCI = [1000 × a\*/(L\* × b\*)]. The CIRG was calculated as CIRG = (180 - H)/(L\* + C), while C = (a\*<sup>2</sup> + b\*<sup>2</sup>)<sup>0.5</sup> and H = arctan(b\*/a\*). For each fruit, four equatorially distributed area were measured while the mean value was calculated for 15 fruits for each cultivar. The edible rate was calculated as the weight percentage of flesh to the whole fruit and the mean value was calculated for 15 fruits for each cultivar. TSS contents were measured of 15 fruits per cultivar with a portable digital refractometer (Atago PR-101c, Tokyo, Japan) at 25 °C and were expressed as °Brix. The content of water-soluble pectin was measured according to the previous report (Dietz and Rouse, 1953) with slight modification. Samples of 1 g powder of 5 finger lime fruits mixed into a group were extracted in 25 mL 95% ethanol at 100 °C water bath for 30 min. The mixture was centrifuged for 10 min at 10000 r/min after cooling down to 25 °C, and then the precipitants were collected. The extraction steps were repeated for 3 times. After adding 20 mL ddH<sub>2</sub>O, the precipitant-water mixture was put into the 50 °C water bath for 30 min. After centrifugation, the supernatant was collected and diluted with ddH<sub>2</sub>O to 100 mL. The absorbance at 530 nm was measured using a microplate reader (Synergy H1, BioTek, VT, USA). Galacturonic acid was used as a standard.

### 2.4. Quantification of individual sugars and organic acids

Quantification of individual sugars and organic acids were measured according to a previous report (Lin et al., 2015). Samples of 0.1 g powder of 5 finger lime fruits mixed into a group were grounded in liquid nitrogen and extracted in 1.4 mL of methanol at 70 °C for 30 min. The mixture was centrifuged at 10000 r/min for 5 min. The supernatants were collected and 1.5 mL ddH<sub>2</sub>O (4 °C), 750 μL methenyl trichloride (-20 °C) were added. After full blending, the mixture was centrifuged at 4000 r/min for 10 min. The supernatants were collected and stored at -40 °C until analysis. Aliquots of 100 μL supernatants were dried in vacuum at 30 °C. The residue was dissolved in 60 μL of 20 mg/mL pyridine methoxyamine hydrochloride and incubated for 90 min at 37 °C. The sample was then treated with 40 μL Bis (trimethylsilyl) trifluoroacetamide (1% trimethylchlorosilane) for 30 min at 37 °C. 10 μL ribitol (0.2 mg/mL) was added into each sample as an internal standard.

The sample was analyzed on a gas chromatography (GC) system (Agilent 7890GC) equipped with a fused-silica capillary column (60 m × 0.25 mm, 0.25 μm, HB-5MS stationary phase) and flame ionization detector (FID) detector. The program for analysis was run at an initial column temperature at 100 °C for 1 min, increased to 184 °C with a ramp of 3 °C/min, next increased to 190 °C with a ramp of 0.5 °C/min, increased to 250 °C with a ramp of 10 °C/min and then held for 1 min, followed by increase to 280 °C with a ramp of 5 °C/min and then held for 3 min. The injector temperature was maintained at 250 °C and the helium carrier gas had a flow rate of 1.0 mL/min 1 μL of sample was injected with a split ratio of 10:1.

### 2.5. Phenolics extraction and detection

One-gram fruits powder from groups mixed with 5 finger limes were extracted with 90% methanol (containing 1% formic acid) at 25 °C in a material-to-solvent ration of 1:5 (m/v) for three times. The supernatants were collected by centrifuging at 3000 r/min for 5 min. The

methanol in the supernatants was removed by evaporating under reduced pressure at 37 °C. The sugars were removed and the phenolic fractions were enriched by solid-phase extraction with a Sep-Pak C18 cartridge (12 cc, 2 g sorbent, Water Corp., MA, USA).

### 2.6. DPPH free radical scavenging activity

The DPPH radical scavenging activity of finger lime components extracted from 1 g powder of 5 finger limes-mixed powder were measured according to our previous publication (Wang et al., 2017b). Briefly, 2 µL diluted finger lime extracts was added to 198 µL DPPH methanol solution (60 µM). The reaction was allowed to stand for 2 h at 25 °C and the absorbance at 515 nm was measured. Trolox was used as standards.

### 2.7. Ferric reducing antioxidant power (FRAP)

The FRAP assay was measured to test finger lime components extracted from 1 g powder of 5 finger limes-mixed powder according to our previous report (Wang et al., 2017b). In brief, 100 mL acetate buffer (300 mM, pH 3.6), 10 mL TPTZ solution (10 mM) and 10 mL ferric chloride (2 mM) was mixed up as FRAP reagent. The combination of 10 µL appropriately diluted finger lime extracts and 90 µL of FRAP reagent was reacted for 5 min. A microplate reader was used to test the 593 nm absorbance of the reactions and Trolox was used as standards.

### 2.8. Oxygen radical absorbance capacity (ORAC)

The ORAC assay was carried out to determine the finger lime components extracted from 1 g powder of 5 finger limes-mixed powder according to a previous report (Wang et al., 2017b). A mixture of 40 nM sodium fluorescein and appropriately diluted finger lime extracts were incubated for 10 min at 37 °C. AAPH of 150 mM was added into the mixture then the fluorescence detection was performed immediately. 485 nm was used as excitation wavelength and 535 nm was selected as emission wavelength, the detection lasted for 2 h with an interval of 2 min. Areas under the sodium fluorescein decay curve (AUC) was calculated as  $AUC = (f_0 + f_1 + f_2 + \dots + f_n)/f_0$ , while  $f_0$  is the initial reading data. The AUC difference between blank and samples was calculated as the ORAC value. The phosphate buffer solution was used as a blank control.

### 2.9. ABTS radical scavenging activity

The ABTS assay was measured to test finger lime components extracted from 1 g powder of 5 finger limes-mixed powder according to our previous report (Lv et al., 2015), with some modifications. ABTS<sup>+</sup> was produced by the reaction of 7 mM ABTS solution with 2.45 mM potassium persulfate. The mixture was incubated in the dark at 25 °C for 12 h. The ABTS<sup>+</sup> solution was diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance of 0.70 ± 0.02 at 730 nm. After the mixing of 10 µL of extracts (or antioxidant standard) with 390 µL ABTS<sup>+</sup> solution and incubated for 10 min. The absorbance at 730 nm was investigated using a microplate reader and Trolox was used as standards.

### 2.10. Determination and quantification of individual phenolics

Ultra-performance liquid chromatography-diode array detector (UPLC-DAD) and ultra-performance liquid chromatography-high

resolution mass spectrometry (UPLC-HRMS) were used for identification and quantification of individual phenolic compounds extracted from 1 g powder of 5 finger limes-mixed group. Phenolics were detected at 280 nm. Among all the phenolics, narirutin, naringin, poncirin, diosmin and neohesperidin were quantified with their respective standards while other phenolics were quantified as equivalents of neohesperidin at 280 nm.

Individual phenolic compounds were determined with a UPLC-DAD system (Waters Corp., MA, USA) coupled with a BEH C18 analytical column (ACQUITY UPLC, 2.1 × 150 mm, Waters Corp., MA, USA). A volume of 2 µL sample was injected under an operating temperature of 25 °C. The detection wavelength was 280 nm and the mobile phase of UPLC consisted of ddH<sub>2</sub>O (containing 0.1% formic acid, A) and acetonitrile (B) at a flow rate of 0.3 mL/min. The gradient program was as following: 0–5 min, 5% of B; 5–10 min, 5–8% of B; 10–20 min, 8–10% of B; 20–100 min, 10–30% of B; 100–110 min, 30–100% of B; 110–119 min, 100 to 20% of B.

Further UPLC-HRMS analyses were performed according to our previous publication (Lv et al., 2015). An AB Triple TOF 5600<sup>plus</sup> system (AB SCIEX, MA, USA) equipped with an electron Spray Ionization (ESI) source in negative mode was used for mass analysis. The operation conditions were as follows: nebulizer 45 psi, dry gas flow rate 5 L/min, sheath gas 11 L/min, 350 °C. Chromatographic separations were done on the same BEH C18 analytical column using a UPLC system. PeakView 1.2.0.3 (AB SCIEX, MA, USA) was used for data processing.

### 2.11. Cell culture and NO release analysis

Cell culture and NO release analysis were according to our previous report (Cao et al., 2018). Mouse microglia BV-2 cells were cultured at 37 °C and 5% CO<sub>2</sub> humidified incubator with RPMI 1640 complete medium (10% fetal bovine serum). Cells in the logarithmic growth phase were seeded in 96-well plates at a density of  $5 \times 10^4$  cells/well and cultured for 24 h before treatment. Different concentrations of finger lime extracts were dissolved in the dimethyl sulfoxide (DMSO) and then added into the fresh medium. After 24 h culture, the 0.1 µg/mL LPS was incubated with the pretreated reagents and cells for 12 h and the NO content was determined by NO detection kit according to the manufacturer's instruction. DMSO was used as a solvent control.

### 2.12. Cell viability assay

Cell viability assay was performed according to the manufacturer's instructions. Briefly, cells with a density of  $5 \times 10^4$  cells/well were plated in a 96-well plate and incubated overnight. The medium was moved, and fresh medium with indicated reagents was added. After 72 h incubation, the medium was dislodged and the cck-8 reagent diluted with FBS-free medium was incubated with the cells for 1 h, the absorbance of 450 nm and 620 nm was detected by a microplate reader. DMSO was used as a solvent and as a negative control.

### 2.13. Elisa assay

Elisa assay was according to our previous report (Cao et al., 2018) and performed following the manufacturer's instructions. BV-2 cells were plated onto the 96 well plates with a density of  $5 \times 10^4$  cells per well. DMSO was used as the solvent control while 0.1 µg/mL LPS was used as positive control.

**Table 1**  
Primers used in this study.

Genes	Sequences
<i>GAPDH</i>	F: TCA ACG GCA CAG TCA AGG R: ACT CCA CGA CAT ACT CAG C
<i>IL-1β</i>	F: AGT AAG TTC CTC TCT GCA AGA GAC T R: CAC TAG GTT TGC CGA GTA GAT CTC
<i>IL-6</i>	F: GAG ACT TCC ATC CAG TTG CCT R: CAG GTC TGT TGG GAG TGG TA
<i>TNFα</i>	F: CGG GCA GGT CTA CTT TGG AG R: ACC CTG AGC CAT AAT CCC CT
<i>iNOS</i>	F: CGG CAA ACA TGA CTT CAG GC R: GCA CAT CAA AGC GGC CAT AG
<i>TLR1</i>	F: TCT CTG AAG GCT TTG TCG ATA CA R: GAC AGA GCC TGT AAG CAT ATT CG
<i>TLR2</i>	F: TCT AAA GTC GAT CCC CGA CAT R: TAC CCA GCT CGC TCA CTA CGT
<i>TLR4</i>	F: CAA GAA CAT AGA TCT GAG CTT CAA CCC R: GCT GTC CAA TAG GGA AGC TTT CTA GAG
<i>TLR6</i>	F: AAC AGG ATA CGG AGC CTT GA R: CCA GGA AAG TCA GCT TCG TC
<i>JAK2</i>	F: AAG ATG CTT TCT GGG TTG G R: ACA TTG TCT AAG AGG GAG CAG
<i>STAT3</i>	F: ACC TCC AGG ACG ACT TTG AT R: TGT CTT CTG CAC GTA CTC CA
<i>IκBα</i>	F: TAC CCC TCT ACA TCT TGC CTG T R: GTG TCA TAG CTC TCC TCA TCC TC



Fig. 1. Finger lime fruits used in the present study.

**Table 2**  
Single fruit weight, edible rate and appearance colour of finger limes.

	Weight (g)	Edible rate (%)	L*	a*	b*	Colour (CCI)	Colour (CIRG)
LSK	10.76 ± 3.24 <sup>a</sup>	72.41 ± 1.01 <sup>a</sup>	23.91 ± 3.76 <sup>a</sup>	7.83 ± 3.32 <sup>a</sup>	11.00 ± 4.06 <sup>a</sup>	36.74 ± 13.15 <sup>a</sup>	5.17 ± 1.3 <sup>a</sup>
XB	9.94 ± 2.75 <sup>a</sup>	72.33 ± 2.58 <sup>a</sup>	30.43 ± 1.93 <sup>b</sup>	17.02 ± 3.58 <sup>b</sup>	18.15 ± 1.43 <sup>b</sup>	36.88 ± 12.69 <sup>a</sup>	3.27 ± 0.09 <sup>b</sup>

Data were represented as mean ± SD (n = 15). Values within each column followed by different superscript letters were significantly different at  $p < 0.05$  according to Tukey's tests.

#### 2.14. Quantitative Real-Time PCR assay

Quantitative Real-Time PCR assay was according to our previous report (Cao et al., 2018) with some modifications. BV-2 cells were plated onto the 6 well plates with a density of  $1 \times 10^6$  cells per well. The sequences of the primers for qRT-PCR are listed in Table 1. A house keeping gene *GAPDH* was used as control. The relative level of gene expression was calculated following the comparative ( $2^{-\Delta\Delta CT}$ ) method.

#### 2.15. Statistics

Experiments were repeated for 15 times for determination of basic fruit index and were repeated for 3 times for phenolic identification and cell assays. The results were expressed as the means ± standard deviation. SPSS 19.0 software (IBM, Armonk, NY, USA) was used for statistical analyses. Origin Pro 2016 (OriginLab Corp., MA, USA) was used for plotting. Significant differences in differences were calculated using Tukey's test.

### 3. Results and discussion

#### 3.1. Single fruit weight, edible rate and appearance colour of finger limes

Both cultivars of 'XB' and 'LSK' had finger-like shape with fruit weight around 10 g (Fig. 1), which were distinctive and much smaller than most citrus varieties (Wang et al., 2017b). The 'XB' cultivar had a red peel, while the 'LSK' had a dark red peel. The CCI value, which was applicable in evaluating the peel colour of most citrus fruits, did not show differences between the two cultivars. However, there was significant higher CIRG value, which was used to evaluate the red fruits, of 'LSK' than that of 'XB' (Table 2). Thus, finger lime fruits showed some unique characteristics in size and colour, which might be due to their distant genetic backgrounds and relatively isolated cultivation areas (Wu et al., 2018).

#### 3.2. Acid, sugar and pectin contents

The flesh of the 'LSK' and 'XB' had 3 to 4 times higher TSS values (36.54 °Brix and 37.92 °Brix, respectively) in comparison to most citrus varieties varying from 8.98 °Brix to 12.44 °Brix.

The soluble components in the flesh were evaluated. The content for acids and sugars were determined by gas chromatography (GC). As shown in Tables 3 and 3 acids (malic acid, citric acid and quinic acid) and 4 sugars (fructose, glucose, sorbitol and sucrose) were detected both in finger lime peel and flesh. Citric acid was the primary acid in finger lime fruits, the concentration of citric acid was 71.50 mg/g of fresh weight (FW) in 'LSK' flesh and 73.49 mg/g FW in 'XB' flesh, respectively. The citric acid contents in these finger limes were much higher than in other citrus fruits where were reported with a range from

**Table 3**

Taste quality indexes of finger limes.

	TSS (°Brix)	Malic acid (mg/g FW)	Citric acid (mg/g FW)	Quinic acid (mg/g FW)	Fructose (mg/g FW)	Glucose (mg/g FW)	Sorbitol (mg/g FW)	Sucrose (mg/g FW)	Water soluble pectin
LSK peel	–	1.35 ± 0.05 <sup>a</sup>	8.26 ± 0.22 <sup>a</sup>	5.48 ± 0.22 <sup>d</sup>	0.89 ± 0.19 <sup>a</sup>	0.71 ± 0.13 <sup>a</sup>	0.96 ± 0.04 <sup>b</sup>	2.27 ± 0.10 <sup>b</sup>	14.91 ± 1.10 <sup>b</sup>
LSK flesh	36.54 ± 3.03 <sup>a</sup>	4.80 ± 0.34 <sup>d</sup>	71.50 ± 6.47 <sup>c</sup>	1.13 ± 0.10 <sup>b</sup>	4.16 ± 0.28 <sup>c</sup>	3.15 ± 0.18 <sup>c</sup>	1.66 ± 0.12 <sup>c</sup>	2.77 ± 0.20 <sup>c</sup>	8.28 ± 0.43 <sup>a</sup>
XB peel	–	2.23 ± 0.17 <sup>b</sup>	20.75 ± 1.57 <sup>b</sup>	4.39 ± 0.34 <sup>c</sup>	2.56 ± 0.22 <sup>b</sup>	2.08 ± 0.16 <sup>b</sup>	0.59 ± 0.04 <sup>a</sup>	0.68 ± 0.06 <sup>a</sup>	18.72 ± 1.60 <sup>c</sup>
XB flesh	37.92 ± 2.50 <sup>a</sup>	4.08 ± 0.27 <sup>c</sup>	73.49 ± 4.10 <sup>c</sup>	0.58 ± 0.05 <sup>a</sup>	4.39 ± 0.31 <sup>c</sup>	3.34 ± 0.26 <sup>c</sup>	1.02 ± 0.08 <sup>b</sup>	2.25 ± 0.14 <sup>b</sup>	11.40 ± 2.08 <sup>b</sup>

Results were represented as mean ± SD (n = 3). –, indicated the value was unavailable; Water-soluble pectin was calculated as mg galacturonic acid equivalents (GTAE)/g FW. Values within each column followed by different superscript letters were significantly different at *p* < 0.05 according to Tukey's tests.

**Table 4**

Antioxidant capacities of finger limes.

	DPPH	ORAC	FRAP	ABTS
LSK peel	1.25 ± 0.05 <sup>b</sup>	14.32 ± 0.84 <sup>ab</sup>	1.13 ± 0.14 <sup>a</sup>	2.83 ± 0.80 <sup>b</sup>
LSK flesh	0.92 ± 0.13 <sup>a</sup>	14.74 ± 1.46 <sup>ab</sup>	1.08 ± 0.11 <sup>a</sup>	1.57 ± 0.53 <sup>ab</sup>
XB peel	1.60 ± 0.05 <sup>c</sup>	16.36 ± 0.49 <sup>b</sup>	1.46 ± 0.01 <sup>b</sup>	3.60 ± 0.16 <sup>c</sup>
XB flesh	0.84 ± 0.10 <sup>a</sup>	13.68 ± 0.62 <sup>a</sup>	0.92 ± 0.09 <sup>a</sup>	1.24 ± 0.27 <sup>a</sup>

Results were the mean ± SD (n = 3). Antioxidant capacities (DPPH, ORAC, FRAP and ABTS) were calculated as mg Trolox equivalent antioxidant capacities (TEAC)/g FW. Values within each column followed by different superscript letters were significantly different at *p* < 0.05 according to Tukey's tests.

1.1 to 67.8 mg/g FW (Asencio et al., 2018; Lin et al., 2015). The peels also contained a certain amount of these three organic acids, with low malic acid and citric acid content and high quinic acid. Also, the acid contents showed tissue and cultivar specificities. 'XB' had higher malic acid and citric acid content in the peel than 'LSK', and lower malic acid and quinic acid content in the flesh than 'LSK'.

Fructose, glucose and sucrose were the three dominant sugar components in the flesh of finger limes (Table 3), which was similar to most of the citrus fruits. The fructose was the dominant component, the glucose content was a little lower than that of fructose, following by the sucrose and sorbitol. The sorbitol and sucrose contents in 'LSK' were higher than that in 'XB' for both flesh and peel. For the fructose and glucose, the difference between the content in peel and flesh in 'LSK' were greater than in 'XB'; while for sorbitol and sucrose, the results showed an opposite situation. The content difference in the two parts of 'LSK' was smaller than in 'XB'. Together, comparing to previous reports (Canan et al., 2016; Tang et al., 2017), finger lime fruit showed a higher content in acid and a lower content in sugar, which corresponded to their sour taste.

Water-soluble pectin content was an important indicator of fruit quality, which could affect the tastes and health value of the fruit. Finger lime fruits showed higher water soluble pectin in peel (14.91 mg (GTAE)/g FW of 'LSK' and 18.72 mg (GTAE)/g FW of 'XB') than in flesh (8.28 mg (GTAE)/g FW of 'LSK' and 11.40 mg (GTAE)/g FW of 'XB'), this result was in consistent with our previous studies (Tang et al., 2017).

### 3.3. Antioxidant activity of finger limes

Antioxidant capacities of finger lime fruits were estimated by the DPPH, FRAP, ORAC and ABTS assays (Table 4). Based on the mechanisms of these four antioxidant tests, the DPPH, FRAP and ABTS tests were mainly electron transfer type antioxidant methods (Kamiloglu et al., 2015; Wang et al., 2017b), while ORAC was a hydrogen supply ability type antioxidant method (Wang et al., 2017b). For the electron transfer type antioxidant capacity, the 'XB' peel extracts showed the highest value than the other samples tested. The DPPH values of 'LSK' and 'XB' had tissue specificity, in which the peel extracts (1.25 mg TEAC/g FW for 'LSK' and 1.60 mg TEAC/g FW for 'XB') showed significant higher value than the flesh extracts (0.92 mg TEAC/g FW for 'LSK' and 0.84 mg TEAC/g FW for 'XB'). For FRAP and ABTS testes, only the 'XB' extracts showed significantly different values between different parts of fruits, though the values of 'LSK' peel were also slightly higher than those of 'LSK' flesh. Results of the ORAC assay showed that the 'XB' peel extracts value was higher than 'XB' flesh extracts value. The ORAC values of two different parts of 'LSK' fruits were similar.

### 3.4. Identification of phenolic components in finger limes

The phenolic composition of finger lime fruits was identified and quantified by combining UPLC-HRMS and UPLC-DAD. A total of 33 compounds were identified, including 31 phenolics (25 flavonoids, 3 coumarin derivatives, 2 resin phenol derivatives and 1 phenol derivative), 1 neolignan glycoside and 1 secoiridoid derivate (Table 5, Fig. 2). The phenolic profiles showed tissue specificities. Neohesperidin (18) and α-glucosyl hesperidin (9) were the main phenolics in the peel. Neohesperidin (18) accounted for 17.22% of total phenolics in 'LSK' peel and 14.87% of total phenolics in 'XB' peel. α-glucosyl hesperidin (9) (accounted for 10.47% of total phenolics in 'LSK' peel and 13.17% of total phenolics in 'XB' peel). Quercetin 3-rutinoside-7-glucoside (5) (accounting for 6.93% of total phenolics in 'LSK' flesh and for 8.90% of total phenolics in 'XB' flesh) and chrysoeriol 7-O-rutinoside (15) (accounting for 5.10% of total phenolics in 'LSK' flesh and for 4.97% of total phenolics in 'XB' flesh) were the first and second high content phenolics in the flesh. The (7S,8S)-4,7,9,9'-tetrahydroxy-3,3'-dimethoxy-8-4'-oxyneolignan-9'-O-D-glucopyranoside (29), lyoniiresinol 9'-O-glucoside (30) and poncirin (31) were detected only in the peel.

Of all the compounds, seven of them were firstly identified from

**Table 5**  
Determination (UPLC-HRMS) and quantification (UPLC-DAD) of phenolics and other compounds from finger lime fruits.

Peak No.	TR (min)	$\lambda_{\text{max}}$	[M-H] (m/z)	Fragment Ions (m/z)	Formula	Tentative Compounds
1	17.583	325.8	325.0929	187.0404, 163.0406, 145.0307 463.0907, 301.0356	$\text{C}_{15}\text{H}_{18}\text{O}_8$ $\text{C}_{27}\text{H}_{30}\text{O}_{17}$	$\alpha$ -Comaroyl glucose Quercetin 3,4'-di-O- $\beta$ -Hexoside
2	19.459	315.1	625.1412	223.0622, 205.0520, 190.0290	$\text{C}_{17}\text{H}_{22}\text{O}_{10}$	1-O-sinapoyl- $\beta$ -D-glucose
3	21.458	328.2	385.1147	503.1226, 473.1110, 383.0790, 353.0683	$\text{C}_{29}\text{H}_{30}\text{O}_{15}$	Vicenin-2
4	24.483	318.6	593.1514	609.1502, 463.0903, 301.0368	$\text{C}_{29}\text{H}_{30}\text{O}_{15}$	Quercetin 3-rutinoside-7'-glucoside
5	27.167	325.8	771.1989	639.1567	$\text{C}_{29}\text{H}_{32}\text{O}_{17}$	Isorhamnetin 3,7-O-diglucoside
6	29.115	341.1	609.1464	476.0996, 314.0447, 313.0366	$\text{C}_{27}\text{H}_{30}\text{O}_{16}$	Kaempferol 3-O-sophoroside
7	30.365	342.2	609.1467	463.0900, 300.0279, 271.0248	$\text{C}_{27}\text{H}_{30}\text{O}_{16}$	Rutin
8	32.041	350.3	771.2353	609.0291, 271.0261, 178.9933, 151.0043	$\text{C}_{34}\text{H}_{44}\text{O}_{20}$	$\alpha$ -Glucosyl hesperidin
9	34.301	284.1, 341.1	507.1146	492.0947, 344.0554, 329.0314, 315.0146	$\text{C}_{23}\text{H}_{24}\text{O}_{13}$	Syringin 3-O-glucoside
10	37.183	352.7	579.1722	271.0628, 151.0049	$\text{C}_{27}\text{H}_{32}\text{O}_{14}$	Narinutin
11	38.132	342.2	537.2557	375.2044, 359.2043	$\text{C}_{24}\text{H}_{42}\text{O}_{13}$	Limocitrol 3-O-glucoside
12	40.538	353.9	579.1719	459.1184, 271.0623	$\text{C}_{27}\text{H}_{32}\text{O}_{14}$	Naringin
13	41.996	280.5, 342.2	477.1042	315.0525, 317.0250, 243.0306	$\text{C}_{29}\text{H}_{22}\text{O}_{12}$	Isorhamnetin 3-O-glucoside
14	43.495	342.2	607.1677	299.0574, 284.0339	$\text{C}_{28}\text{H}_{32}\text{O}_{15}$	Chrysotin 7-O-rutinoside
15	45.273	274.6, 342.2	607.1676	299.0582, 284.0341, 255.0319	$\text{C}_{28}\text{H}_{32}\text{O}_{15}$	Diosmin
16	46.510	284.1, 342.2	607.1678	341.0678, 299.0571, 284.0339	$\text{C}_{28}\text{H}_{32}\text{O}_{15}$	Neohesperidin
17	48.001	286.5, 325.8	609.1825	489.1453, 301.0735, 286.0501	$\text{C}_{28}\text{H}_{34}\text{O}_{15}$	Isorhamnetin 3-O-(6'-acetyl)-galactoside
18	48.734	284.1, 337.7	519.1146	689.1535, 463.0906, 301.0368	$\text{C}_{29}\text{H}_{22}\text{O}_{13}$	Quercetin 3-O-sinapoyl-phloroside
19	50.403	353.9	831.2009	473.1534, 431.1374, 285.0780	$\text{C}_{34}\text{H}_{44}\text{O}_{21}$	Luteolin 7-O-rutinoside-4'-O-glucoside
20	51.660	342.2	755.2419	351.1566, 285.1055, 151.1251	$\text{C}_{29}\text{H}_{34}\text{O}_{14}$	Methylnaringenin 7-O-neohesperidose
21	52.803	342.2	593.1816	427.1635, 409.1674, 247.1142	$\text{C}_{26}\text{H}_{36}\text{O}_{14}$	8a-(O- $\beta$ -gentiotiosilyoxy)-7, 8-9,9-dimethylpyranoside
22	54.850	342.2	571.2039	327.0833, 309.0759, 285.0763	$\text{C}_{29}\text{H}_{34}\text{O}_{14}$	Hydrocumamic acid
23	56.429	342.4	593.1857	593.2569, 319.1322, 301.1213	$\text{C}_{34}\text{H}_{44}\text{O}_{14}$	Didymin
24	57.813	342.2	675.2686	475.1261, 431.1157, 269.0458	$\text{C}_{27}\text{H}_{30}\text{O}_{14}$	Kaempferol-3-arabin-7-rhamnosid-permethylether
25	63.416	277.0, 342.2	577.1565	373.1878, 331.1771, 125.0733	$\text{C}_{24}\text{H}_{36}\text{O}_{12}$	Apigenin 7-O-neohesperidose
26	64.876	342.2	517.229	327.0893, 285.0784	$\text{C}_{29}\text{H}_{34}\text{O}_{14}$	3-O-methyl-15-pentylscorcinol-1-O-[ $\beta$ -D-glucopyranoside-6]- $\beta$ -D-glucopyranoside
27	66.062	342.2	539.2145	493.2138, 161.0255	$\text{C}_{24}\text{H}_{36}\text{O}_{12}$	Methylnaringenin 7-O-rutinoside
28	68.575	284.1, 342.2	593.1882	7(8S)-4,7,9,9-Tetrahydroxy-3',3'-dimethoxy-4'-oxynolignan-9-O-D-glucopyranoside	$\text{C}_{29}\text{H}_{34}\text{O}_{14}$	
29	71.137	342.2	539.2145	Lyoniressinol 9-O-glucoside	$\text{C}_{29}\text{H}_{34}\text{O}_{14}$	
30	80.460	342.2	581.2247	Poncirin	$\text{C}_{29}\text{H}_{36}\text{O}_{13}$	
31	81.379	342.2	593.1856	Lyoniressinol 2a-O- $\beta$ -glucoside	$\text{C}_{28}\text{H}_{38}\text{O}_{13}$	
32	82.972	325.8	581.2250	Lonicera japonin B	$\text{C}_{34}\text{H}_{44}\text{O}_{17}$	
33	88.295	328.2	723.2535	679.2699, 635.2797, 161.0253		
				'LSK' peel (ug/g FW)	'LSK' flesh (ug/g FW)	'XB' flesh (ug/g FW)
				References		'XB' flesh (ug/g FW)
1		Coumarin derivatives	(Anttonen and Karjalainen, 2006; Sommella et al., 2017)	8.40 ± 0.16 ab	5.40 ± 0.14 a	12.11 ± 2.96 b
2		Flavanols	12.39 ± 0.69 b	4.15 ± 0.04 a	14.49 ± 1.68 b	4.53 ± 0.21 a
3		Coumarin derivatives	6.11 ± 0.66 ab	4.00 ± 0.03 a	8.23 ± 1.62 b	4.07 ± 0.06 a
4		Flavanones	17.89 ± 1.44 a	4.67 ± 0.24 b	26.76 ± 7.63 a	5.12 ± 0.19 b
5		Flavanols	16.48 ± 0.91 b	10.17 ± 0.62 a	9.52 ± 2.66 a	13.26 ± 1.58 ab
6		Flavanols	9.22 ± 1.85 a	4.64 ± 0.59 a	8.21 ± 3.22 a	5.57 ± 0.13 a
7		Flavanols	7.28 ± 1.26 ab	6.26 ± 0.10 a	8.03 ± 0.25 b	5.83 ± 0.30 a
8		Flavanones	9.48 ± 1.31 bc	8.19 ± 1.57 ab	11.28 ± 1.13 c	6.01 ± 0.32 a
9		Flavanones	42.80 ± 3.26 b	5.19 ± 0.01 a	77.17 ± 4.16 c	4.25 ± 0.38 a
10		Flavanones	5.07 ± 0.89 b	4.64 ± 0.04 ab	4.90 ± 0.29 ab	3.83 ± 0.04 a
11		Flavanones	5.13 ± 0.30 a	5.27 ± 0.02 a	11.54 ± 3.57 b	4.30 ± 0.09 a
12		Flavanones	12.37 ± 0.35 b	4.07 ± 0.19 a	32.10 ± 3.24 c	5.95 ± 0.49 a
13		Flavanones	21.42 ± 3.42 b	5.41 ± 0.33 a	39.50 ± 8.08 c	6.64 ± 0.49 a

(continued on next page)

Table 5 (continued)

Peak No.	Type	References	'LSK' peel (µg/g FW)	'LSK' flesh (µg/g FW)	'XB' peel (µg/g FW)	'XB' flesh (µg/g FW)
14	Flavanols	Martucci et al. (2018)	6.09 ± 0.78 a	4.75 ± 0.09 a	20.30 ± 10.48 b	4.46 ± 0.58 a
15	Flavanones	Wang et al. (2017a)	15.44 ± 7.93 ab	7.48 ± 0.18 a	27.81 ± 11.87 b	7.41 ± 0.23 a
16	Flavanones	Wang et al. (2017a)	19.75 ± 4.03 b	5.07 ± 0.02 a	32.45 ± 3.18 c	4.71 ± 0.24 a
17	Flavanones	Wang et al. (2017a)	27.52 ± 6.97 b	4.46 ± 0.13 a	44.58 ± 14.45 b	4.17 ± 0.10 a
18	Flavanones	Wang et al. (2017a)	70.38 ± 10.72 b	6.12 ± 0.22 a	87.19 ± 28.97 b	6.62 ± 0.49 a
19	Flavanols	Simrigiotis et al. (2016)	6.56 ± 0.12 b	4.38 ± 0.15 a	7.90 ± 0.28 c	4.21 ± 0.09 a
20	Flavanols	Schmidt et al. (2010)	5.49 ± 0.59 ab	5.34 ± 0.24 ab	6.04 ± 0.05 b	4.77 ± 0.19 a
21	Flavanones	Wang et al. (2017a)	7.45 ± 0.95 b	4.13 ± 0.02 a	8.38 ± 0.15 b	4.19 ± 0.11 a
22	Flavanones	Wang et al. (2017a)	7.42 ± 2.35 ab	4.15 ± 0.18 a	9.61 ± 1.47 b	4.25 ± 0.05 a
23	Coumarin derivatives	Lacroix et al. (2011)	6.22 ± 1.41 a	3.97 ± 0.06 a	6.46 ± 2.26 a	4.07 ± 0.08 a
24	Flavanones	Wang et al. (2017b)	5.72 ± 0.86 b	4.20 ± 0.15 a	6.76 ± 0.40 b	3.87 ± 0.08 a
25	Flavanols	Seligmann and Wagner (1973)	7.24 ± 1.07 b	4.56 ± 0.06 a	10.16 ± 0.46 c	4.40 ± 0.04 a
26	Flavones	Lou et al. (2015)	6.09 ± 1.61 b	3.81 ± 0.04 a	6.80 ± 0.27 b	3.80 ± 0.04 a
27	Phenol derivatives	Wubshet et al. (2016)	5.34 ± 0.15 b	3.79 ± 0.02 a	5.99 ± 0.25 c	3.77 ± 0.03 a
28	Flavanones	Wang et al. (2016b)	17.52 ± 0.63 b	4.65 ± 0.18 a	19.25 ± 1.76 b	4.41 ± 0.10 a
29	Neolignan glycosides	Gan et al. (2008)	4.02 ± 0.13 b	n.d. a	4.59 ± 0.33 c	n.d. a
30	Resin phenol derivatives	Marchal et al. (2015)	4.83 ± 0.30 b	n.d. a	5.40 ± 0.20 c	n.d. a
31	Flavanones	Wang et al. (2017b)	4.16 ± 0.05 b	n.d. a	4.61 ± 0.11 c	n.d. a
32	Resin phenol derivatives	Jiao et al. (2018)	11.49 ± 0.29 b	3.83 ± 0.01 a	12.43 ± 0.85 b	3.94 ± 0.02 a
33	Secoiridoid derivatives	Kashiwada et al. (2013)	6.65 ± 0.43 b	3.75 ± 0.03 a	7.95 ± 0.83 c	3.75 ± 0.03 a

n.d., not detected. Results were the mean ± SD (n = 3).

citrus fruit: isorhamnetin 3-O-(6'-acetyl)-galactoside (19), quercetin 3-O-sinapoyl-sophoroside (20), 3-O-methyl-5-pentylresorcinol-1-O-[ $\beta$ -D-glucopyranosyl-(1 → 6)]- $\beta$ -D-glucopyranoside (27), (7S,8S)-4,7,9,9'-tetrahydroxy-3,3'-dimethoxy-8-4'-oxyneolignan-9'-O-D-glucopyranoside (29), lyoniresinol 9'-O-glucoside (30), lyoniresinol 2a-O- $\beta$ -glucoside (32) and Lonicerjaponin B (33). Isorhamnetin 3-O-(6'-acetyl)-galactoside (19) was previously reported in pear fruit (Simrigiotis et al., 2016). Quercetin 3-O-sinapoyl-sophoroside (20) was naturally occurring flavonoid glycosides found in kale (Schmidt et al., 2010). 3-O-methyl-5-pentylresorcinol-1-O-[ $\beta$ -D-glucopyranosyl-(1 → 6)]- $\beta$ -D-glucopyranoside (27) was found in *Eugenia catharinae* (Wubshet et al., 2015). (7S, 8S)-4,7,9,9'-tetrahydroxy-3,3'-dimethoxy-8-4'-oxyneolignan-9'-O-D-glucopyranoside (29) was a glycoside from *Iodes cirrhosa* (Gan et al., 2008). Lyoniresinol 9'-O-glucoside (30) and lyoniresinol 2a-O- $\beta$ -glucoside (32) were two resin phenol derivatives. Lyoniresinol 9'-O-glucoside (30) was an oak lignin contributed to wine taste (Marchal et al., 2015) and lyoniresinol 2a-O- $\beta$ -glucoside (32) were found in *Tinospora sagittata* and *Tinospora sinensis* (Jiao et al., 2018). Lonicerjaponin B (33) was previously found in the bud of *Lonicera japonica* (Kashiwada et al., 2013).

Different types of citrus fruit showed unique and characteristic phenolics profiles. Pomelo fruits had flavanones as their main phenolics (Wang et al., 2017b). Besides flavanones, polymethoxyflavonoids also accounted for a large proportion of phenolics in mandarin and orange fruits, especially in their peels (Wang et al., 2017a, 2017b). The most two common polymethoxyflavonoids in mandarin were nobiletin and tangeretin, while sinensetin and isosinensetin were more common in oranges (Wang et al., 2017b). Citron fruit had rutin as high proportion of all the phenolics (Venturini et al., 2014). Lemon fruits had hesperidin and eriocitrin as main phenolics in flesh and high hesperidin, eriocitrin and neoeriocitrin content in peel (Caristi et al., 2003). The most abundant extracts in lime were apigenin, rutin, quercetin kaempferol and nobiletin (Loizzo et al., 2012). Finger lime showed a unique phenolic content comparing to other citrus fruits.  $\alpha$ -glucosyl hesperidin was identified as one of the dominant phenolic substances for the first time. Polymethoxyflavonoids were not detected in finger lime fruits, which were different from other lime fruits (Loizzo et al., 2012).

### 3.5. Finger lime extracts inhibited LPS-induced NO release in BV-2 cells

Lipopolysaccharides (LPS), also known as lipoglycans, is an endotoxin which is used as a positive drug to induce an inflammatory reaction. It was reported that LPS treatment could sensitize cell inflammatory pathways, activate and accumulate inflammatory molecules, promote NO production and release and further exacerbates inflammatory responses (Shi et al., 2014). In this study, finger lime extracts were used to determine the inhibition effects of NO release induced by LPS in mouse microglia BV-2 cell line. To avoid interference caused by the cytotoxicity of finger lime extracts, CCK-8 assay was performed to select safe, noncytotoxic doses of the 4 extracts from 2 varieties. Results indicated that the cellular activities under finger lime extract treatments were all higher than 85% at doses below 800 µg/mL (Fig. 3A). Therefore, the doses between 200 and 800 µg/mL were used for further experiments. As shown in Fig. 3B and 0.1 µg/mL LPS treatment induced the NO concentration elevation to more than 24 times of the control group, while all the treatments significantly prevented NO release in BV-2 cells (Fig. 3B). Inflammatory factors were detected by Elisa assay, LPS significantly elevated cellular release of IL-1 $\beta$ , IL-6 and TNF $\alpha$  for about 310, 840 and 300 times. While finger lime extracts, within the treatment dose range, inhibited the inflammatory factors releasing in a dose-dependent manner (Fig. 3C).

Essential gene expression for 3 inflammation-related pathways including the JAK2/STAT3 pathway, NF- $\kappa$ B/I $\kappa$ B $\alpha$  pathway, and TLR pathway were investigated using qRT-PCR. As shown in Fig. 3D, finger lime extracts restrained LPS-induced upregulation of iNOS, IL-6, JAK2, TNF $\alpha$ , IL-1 $\beta$ , NF- $\kappa$ B, TLR2, TLR4 and LPS-induced downregulation of

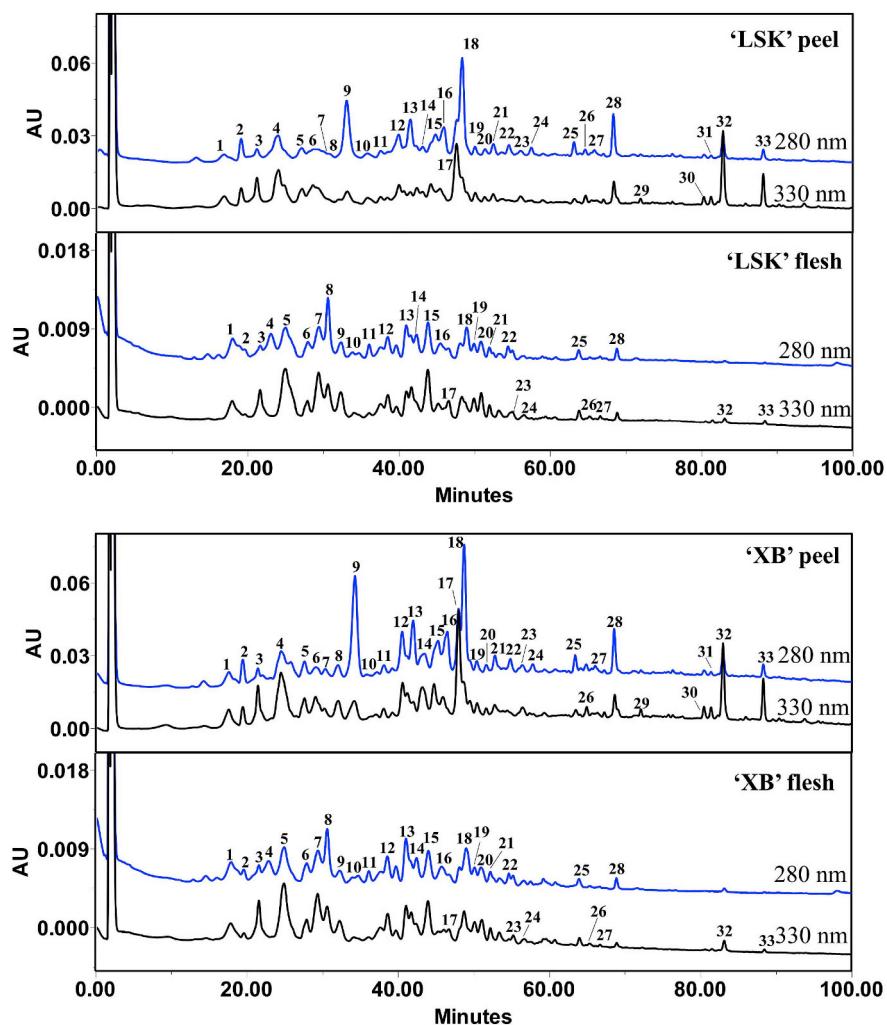
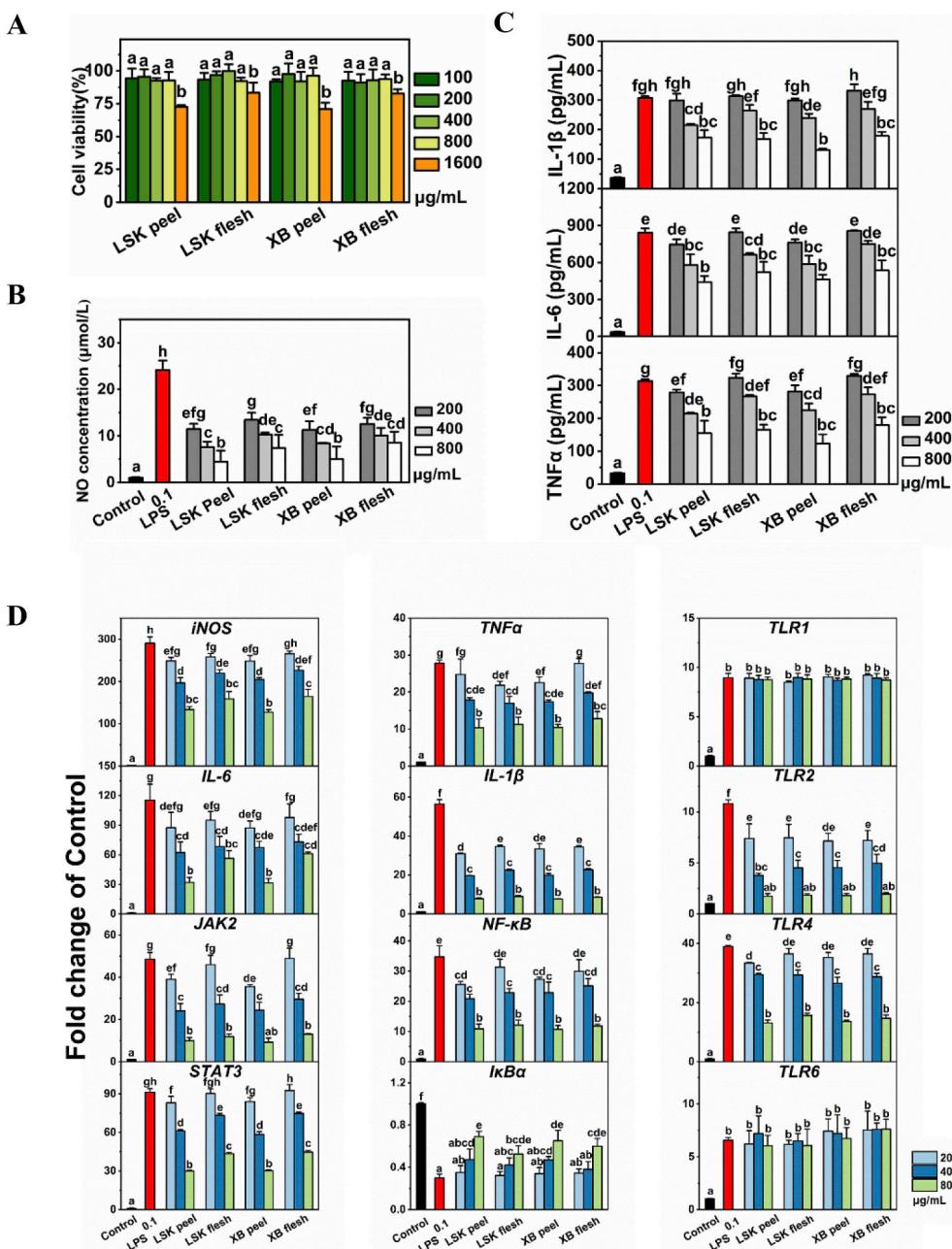


Fig. 2. UPLC spectrum of finger lime peel and flesh.

*IκBα*. While for *TLR1* and *TLR6*, finger limes did not affect LPS-induced gene upregulation. Among the 4 extracts, the peel fraction showed stronger regulation ability especially in *STAT3*, which may be relevant with the flavonoids contents differences. Previous reports have shown that citrus extractions and citrus phenolic compounds could inhibit inflammation responses in various models. Citrus extractions showed the suppressing effect of NO release induced by LPS in RAW264.7 cells and the inhibitory effect had a positive and significant correlation with contents of nobiletin and tangeretin (Choi et al., 2007). Naringenin inhibited LPS excessive production of NO by downregulating of iNOS and COX-2 expression and *IL-1β* and *TNFα* inhibition (Park et al., 2012). Tangeretin inhibited IL-1 $\beta$ , IL-6 and TNF $\alpha$  concentration in BV-2 medium and was concerned with *NF-κB/IκBα* pathway regulation (Shu et al., 2014). Naringenin inhibited TNF $\alpha$  and TLR2 expression in C57BL/6J mice adipocytes (Yoshida et al., 2013). Our results were consistent with previous reports and indicated that the extracts of citrus finger lime prevented the LPS-induced NO elevation by affecting the gene expression of the 3 inflammation pathways including *JAK2*, *STAT3*, *NF-κB*, *IκBα*, *TLR2*, *TLR4* regulation.

#### 4. Conclusion

The finger lime fruits showed unique features especially in shape, size, TSS, colour, taste quality indexes and phenolic composition comparing to other species of citrus. A total of 33 compounds were identified in the peel and flesh of two cultivars of 'XB' and 'LSK' finger lime fruits. Among these compounds, 7 of them (isorhamnetin 3-O-(6'-acetyl)-galactoside, quercetin 3-O-sinapoyl-sophoroside, 3-O-methyl-5-pentylresorcinol-O-[ $\beta$ -D-glucopyranosyl-(1 → 6)]- $\beta$ -D-glucopyranoside, (7S,8S)-4,7,9,9'-tetrahydroxy-3,3'-dimethoxy-8-4'-oxyneolignan-9'-O-D-glucopyranoside, lyoniressinol 9'-O-glucoside, lyoniressinol 2 $\alpha$ -O- $\beta$ -glucoside and Lonicerjaponin B) were first reported in citrus fruits. It was also found that all finger lime extracts could restrain the LPS-induced NO-releasing and reduce the expression of inflammation-related cytokines IL-1 $\beta$ , IL-6 and TNF $\alpha$ . Gene expression analyses explored the mechanism of finger lime suppressing LPS-induced NO elevation might relate to the regulation of *JAK2/STAT3*, *NF-κB/IκBα* and *TLR* pathway. Our experiments offered a preliminary basis of finger lime for further research on inflammation and neuronal cell protection and would



**Fig. 3.** Inhibition of NO release in BV-2 cells by finger lime extractions. (A) Cell viability detected by cck-8 assay; (B) NO release detection; (C) Elisa detection of IL-1 $\beta$ , IL-6 and TNF $\alpha$ . (D) Relative gene expression detected by qRT-PCR. Column followed by different superscript letters were significantly different at  $p < 0.05$  according to Tukey's tests.

expand our knowledge on the physicochemical characteristics of citrus fruits.

#### Conflicts of interest

The authors declare no conflict of interest.

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