

Effective Utilization of *Citrus unshiu* Plant Waste Extracts with Lipase Inhibitory Activities

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Abstract

During the course of *Citrus unshiu* fruit cultivation, large amounts of plant material including pruned leaves, thinned-out flowers and unripe fruit are obtained; these materials are generally considered unusable and discarded as plant wastes. We have previously attempted to establish an effective use of such plant wastes as beneficial natural resources and found that a methanolic extract of pruned *Citrus* leaves (CUL-ext) exhibited inhibitory activity against porcine pancreatic lipase. In this study, we sought to identify further the effective uses of *C. unshiu* plant wastes by determining the lipase inhibitory activity of methanolic extracts of thinning out flowers (CUF-ext) and unripe fruit (CUUF-ext). We accordingly found that the inhibitory activity of CUF-ext was superior to that of CUUF-ext and comparable to that previously observed using CUL-ext. Fractionation of CUF-ext and CUUF-ext, followed by chromatographic analyses, revealed that the pancreatic lipase inhibitory activities of these extracts could be attributed, at least in part, to the flavonoids hesperidin, nobiletin, narirutin and rutin. On the basis of present findings, we propose that, in addition to pruned *Citrus* leaves, the thinned-out flowers and unripe fruit of *C. unshiu* are natural resources which are suitable for preparing constituents with lipase inhibitory activity.

Keywords: *Citrus unshiu*, flower, leaf, lipase inhibitory activity, unripe fruit

1. Introduction

During cultivation of the fruit of *Citrus unshiu* ('Miyagawa wase' in Japanese, Figure 1-F), leaf pruning in early spring, thinning out of flowers in spring and thinning out of unripe fruit in summer are essential measures for optimizing the number of commercially available high-quality fruit, as shown in Figure 1. Pruning and thinning invariably generate large amounts of pruned leaves and thinned-out flowers and unripe fruit, which are typically considered worthless and are accordingly discarded as plant wastes. We have, however, previously attempted to identify a practical use of this waste material as a beneficial natural resource and have accordingly found that a methanolic extract of the pruned leaves of *C. unshiu* (CUL-ext) is characterized by inhibitory activity against porcine pancreatic lipase (Itoh et al., 2019).

Pancreatic lipase plays a key role in lipid absorption via the hydrolysis of total dietary fats (Seyedan et al., 2015) and in this regard, two pancreatic lipase inhibitors, orlistat (Xenical[®] and/or Alli[®]) (Ballinger & Peikin, 2002) and cetilistat (Oblean[®]) (Gras, 2013), have been approved for the treatment of obesity syndrome. With a view toward identifying novel pancreatic lipase inhibitors derived from natural resources, several studies have screened extracts obtained from plants such as chokeberry fruit (Sosnowska et al., 2015), *Nelumbo nucifera* leaves (Liu et al., 2013), *Coffea arabica* seeds (Patui et al., 2014) and *Panax japonicus* rhizomes (Han et al., 2005), which have been reported to have lipase inhibitory activities.

In this study, building on our previous findings regarding the lipase inhibitory activity of an extract of *C. unshiu*

leaves, we sought to further investigate the potential utility of *Citrus* plant wastes by comparing the porcine pancreatic lipase inhibitory activity of pruned leaves with that of other parts of the *C. unshiu* plant, namely, the thinned-out flowers and unripe fruit. In addition, we also performed analyses to identify the active components associated with the porcine pancreatic lipase inhibitory activities of the prepared extracts.

2. Materials and Methods

2.1 Plant Materials

The flowers (including flower buds), unripe fruit and leaves of *C. unshiu* were collected from commercially grown trees at the Experimental Farm of Kindai University, Wakayama Prefecture, Japan, in May 2015, July 2014, and March 2015, respectively (Figure 1-D, E, B and C). The plant materials were collected from 3000 trees of ages between 30 and 45 years, the typical lifespan of which is 50 to 70 years (Figure 1-A). The trees were propagated by grafting, and at the time of collection, had reached a height of 2.5 m and canopy width of 3.6 m. Data relating to the cultivation environment have been reported in our previous study (Itoh et al., 2019).

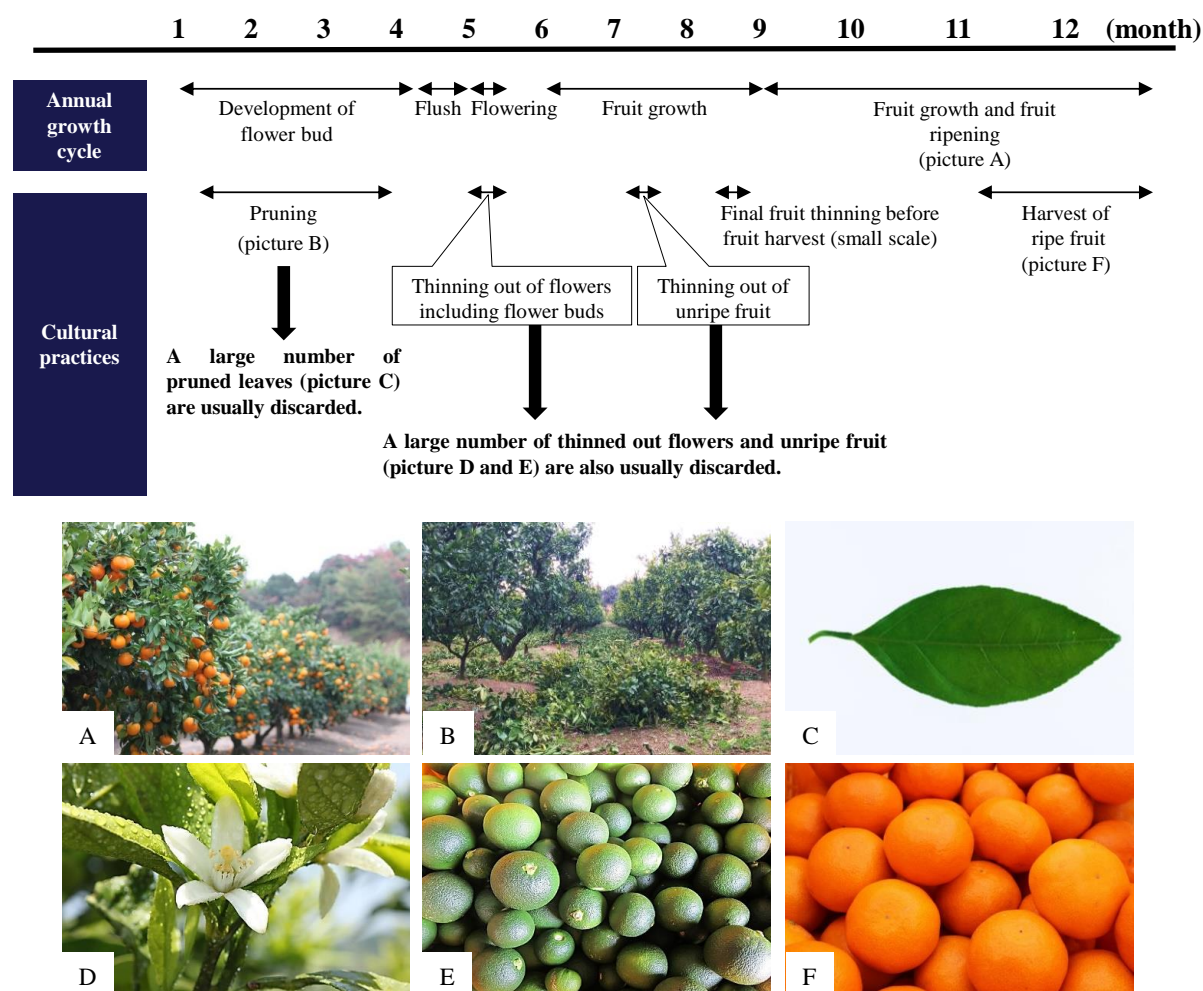


Figure 1. The cultural practices and the discarded agricultural resources of *Citrus unshiu* 'Miyagawa wase' in the Experimental farm, Kindai University

The picture of A, *Citrus unshiu* trees with fruit in November; B, pruning in late winter; C, pruned leaf; D, flowers; E, unripe fruit; F, ripe fruit.

The samples were identified by personnel at the Experimental Farm, Kindai University, air-dried at 50 °C for 72 h in an automatic air-drying apparatus (Vianove Inc., Tokyo, Japan) and powdered using a blender. Voucher specimens of flowers, unripe fruit and leaves of *C. unshiu* (CUF201505, CUUF201407 and CUL201503, respectively) have been deposited at the Experimental Farm, Kindai University.

2.2 Extraction

Samples of powdered flower, unripe fruit and leaf were extracted with twenty times the amount of methanol (MeOH) for 72 h at room temperature, as described previously by Itoh et al. (2016, 2019). The suspensions of each extract were evaporated under reduced pressure to give the MeOH extracts, the yields of which were as follows: flower extract (CUF-ext), 14%; unripe fruit extract (CUUF-ext), 29%; and leaf extract (CUL-ext), 15%.

2.3 Reagents

4-Methylumbelliferyl oleate, lipase (type II, from porcine pancreas, Lot #: SLBN3801V) and tangeretin standard were purchased from Sigma-Aldrich (St. Louis, MO, USA), and Orlistat was purchased from Tokyo Chemical Industry (Tokyo, Japan). Hesperidin and narirutin standards were purchased from Extrasynthese (Lyon, France). Other chemical and biochemical reagents were of reagent grade and purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) and/or Nacalai Tesque, Inc. (Kyoto, Japan) unless otherwise stated.

2.4 In vitro Pancreatic Lipase Inhibition Assay

This assay was performed as described previously by Itoh et al. (2016, 2019). Briefly, the test sample was dissolved with dimethyl sulfoxide (DMSO) and diluted with 13 mM Tris-HCl buffer containing 150 mM NaCl, 1.3 mM CaCl₂ (pH 8.0) to a final DMSO concentration of 2.5% v/v. 4-Methylumbelliferyl oleate (4-MU) was used as a substrate. The substrate and enzyme were both diluted in the above mentioned buffer immediately before use. An aliquot of 25 µL of the test solution and 50 µL of 0.1 mM 4-MU solution were mixed in back colored microtiter plates, 25 µL of 0.2 mg/mL enzyme solution was then added to each well to start the reaction. After incubation for 30 min at 37 °C, 100 µL of 0.1 M citrate buffer (pH 4.2) was added to stop the reaction. The fluorescence associated with the enzymatically released 4-methylumbelliferone product was monitored at an excitation wavelength of 355 nm and emission of 460 nm using a multi-label counter (PerkinElmer 2030 ARVO X4, PerkinElmer Life and Analytical Sciences). Orlistat, a known inhibitor of pancreatic lipase, was used as a reference compound.

2.5 Fractionation of CUF-ext

A suspension of powdered CUF-ext (10 g) in water (100 mL) was extracted with hexane (200 mL × 3), followed by ethyl acetate (200 mL × 3). Evaporation of the solvent gave a hexane-soluble fraction (1.0 g), an ethyl acetate-soluble fraction (0.6 g), a water-soluble fraction (7.0 g) and an ethyl acetate water-insoluble intermediate fraction (0.8 g), the latter of which was obtained as an intermediate layer during ethyl acetate extraction. For each fraction, we determined percentage pancreatic lipase inhibition.

2.6 Thin Layer Chromatographic Analysis

Thin layer chromatography (TLC) was carried in accordance with the method reported by Tosa et al. (1988), with minor modifications, as described by Itoh et al. (2016, 2019). The *R_f*-value obtained for hesperidin, nobiletin, narirutin, rutin and tangeretin were 0.53, 0.93, 0.51, 0.38 and 0.93, respectively.

2.7 High-performance Liquid Chromatography-Photodiode Array Determination of Flavonoid Contents

The flavonoid contents of CUF-ext and CUUF-ext were simultaneously determined by high-performance liquid chromatography (HPLC)-photodiode array (PDA) in accordance with the method described by Zhao et al. (2015), with minor modifications. The analysis was performed using an LC-20A HPLC system (Shimadzu, Kyoto, Japan) equipped with a dual pump consisting of an LC-20AT pump and UV/VIS detector (SPD-20A). The samples were analyzed using a TSKgel ODS-120T reverse-phase column (4.6 i.d. × 250 mm, 5 µm; Tosoh, Tokyo, Japan) in conjunction with gradient elution with solvent A: water and solvent B: acetonitrile at a constant flow rate of 1.0 mL/min. Elution was carried out using the following linear gradient conditions: initial conditions were set at 15% B, followed by a linear gradient to 55% B for 15 min, then 55% to 60% B for 10 min and subsequently to 85% B, at which it was held for 5 min. The column temperature was set at 40 °C, and the eluted compounds were detected by monitoring UV absorption at wavelengths of 280 and 330 nm. At a wavelength of 280 nm, narirutin and hesperidin were eluted at retention times of 10.8 and 11.5 min, respectively, whereas at a wavelength of 330 nm, rutin, nobiletin and tangeretin were eluted at retention times of 9.5, 21.1, and 23.2 min, respectively. The concentrations of flavonoids were evaluated based on the absolute calibration curve method. Linear calibration curves were constructed from the peak areas analyzed at 280 nm in the range from 0.02 to 200 µg/mL for narirutin and 0.04 to 400 µg/mL for hesperidin, the determination coefficients of which were 0.9963 and 0.9956, respectively. Similarly, curves were prepared for rutin, nobiletin and tangeretin at 330 nm in the range from 0.01 to 100 µg/mL for rutin and 0.001 to 1.0 µg/mL for nobiletin and tangeretin, with respective determination coefficients of 0.9969, 0.9990 and 0.9991. Dried extracts were dissolved in MeOH under sonication, and appropriate amounts of each sample solution were subjected to HPLC analysis in triplicate. The reported values

represent the means \pm standard deviation.

2.8. Statistical Analysis

The experimental data were evaluated for statistical significance with using Bonferroni/Dunn's multiple-range test with GraphPad Prism for Windows ver. 5 (GraphPad Software Inc., 2007; Armonk, NY, USA).

3. Results and Discussion

3.1 A Comparison of the Porcine Pancreatic Lipase Inhibitory Activities of CUL-ext with those of CUF-ext and CUUF-ext

In a preliminary study using 4-methylumbelliferyl oleate as a substrate, we found that CUF-ext and CUUF-ext inhibited porcine pancreatic lipase activity with half maximal inhibitory concentration (IC_{50}) values of 43 and 105 $\mu\text{g}/\text{mL}$, respectively (Table 1), which compares with the IC_{50} value of 44 $\mu\text{g}/\text{mL}$ previously obtained for a methanol extract of pruned *Citrus* leaves (CUL-ext) (Itoh et al., 2019) (Figure 1-B, C). Accordingly, the inhibitory activity of CUF-ext was superior to that of CUUF-ext and comparable to that of CUL-ext. Although a large number of studies have examined the constituents of *Citrus* plants, among which Kim et al. (2016) have reported that mixed extracts of *C. unshiu* peel and *Diospyros kaki* fruit exhibited a lipase inhibitory effect with an IC_{50} value of 507 $\mu\text{g}/\text{mL}$, the present study is, to the best of our knowledge, the first to report on the lipase inhibitory activity of *C. unshiu* flowers.

Table 1. Inhibitory activities of MeOH extracts of the flowers, unripe fruit and leaves on pancreatic lipase

Samples	IC_{50} values ^a ($\mu\text{g}/\text{mL}$ or μM)
Flower extract (CUF-ext)	43 $\mu\text{g}/\text{mL}$
Unripe fruit extract (CUUF-ext)	105 $\mu\text{g}/\text{mL}$
Leaf extract (CUL-ext)	44 $\mu\text{g}/\text{mL}$ ^b
Orlistat	0.1 μM (= 0.05 $\mu\text{g}/\text{mL}$)

Orlistat was used as a reference compound. a: IC_{50} values represent the concentrations required to inhibit 50% of pancreatic lipase activity. b: The IC_{50} value for CUL-ext was reported in our previous paper (Itoh et al., 2019).

3.2 Identification of the Constituents of CUF-ext and CUUF-ext Contributing to Porcine Pancreatic Lipase Inhibitory Activity

In order to identify the active constituents of CUF-ext, which exhibited the most potent activity among the three assessed extracts, we fractionated the extract via solvent extraction to give a hexane-soluble, ethyl acetate-soluble, water-soluble fractions and ethyl acetate water-insoluble intermediate fraction, among which, the hexane-soluble and ethyl acetate-soluble fractions showed potent inhibitory activity against pancreatic lipase (Table 2). In contrast, the water-soluble fraction and ethyl acetate water-insoluble intermediate fraction showed virtually no activity at the assessed concentrations (6.3, 25 and 100 $\mu\text{g}/\text{mL}$) (Table 2). As a reference compound, we used the established pancreatic lipase inhibitor orlistat, the IC_{50} value of which was determined to be 0.1 μM (corresponding to 0.0495 $\mu\text{g}/\text{mL}$), which is broadly consistent with the IC_{50} value of 0.05 $\mu\text{g}/\text{mL}$ reported by Ado et al. (2013), as shown in Tables 1 and 2.

Table 2. Inhibitory activities of fractions obtained from CUF-ext against pancreatic lipase

Samples	Concentration ($\mu\text{g/mL}$ or μM)	Percentage inhibition (%)	IC ₅₀ values ^a ($\mu\text{g/mL}$ or μM)
Control ^b		0	
CUF-ext	25 $\mu\text{g/mL}$	39	43 $\mu\text{g/mL}$
	100 $\mu\text{g/mL}$	68	
	400 $\mu\text{g/mL}$	83	
Hexane- soluble fraction	6.3 $\mu\text{g/mL}$	40	12 $\mu\text{g/mL}$
	25 $\mu\text{g/mL}$	61	
	100 $\mu\text{g/mL}$	89	
Ethyl acetate- soluble fraction	6.3 $\mu\text{g/mL}$	20	39 $\mu\text{g/mL}$
	25 $\mu\text{g/mL}$	37	
	100 $\mu\text{g/mL}$	69	
Water-soluble fraction	6.3 $\mu\text{g/mL}$	5	N.E. ^c
	25 $\mu\text{g/mL}$	5	
	100 $\mu\text{g/mL}$	12	
Ethyl acetate-water-insoluble intermediate fraction	6.3 $\mu\text{g/mL}$	5	N.E.
	25 $\mu\text{g/mL}$	6	
	100 $\mu\text{g/mL}$	7	
Orlistat	0.008 μM	7	0.1 μM
	0.04 μM	36	
	0.2 μM	70	

Orlistat was used as a reference compound. a: IC₅₀ values represent the concentrations required to inhibit 50% of pancreatic lipase activity. b: A 2.5% DMSO/buffer solution was used as a control. c: N.E. indicates no effect.

Further purification by Preparative HPLC analysis (Zhao et al. 2015) of the most active hexane-soluble fraction obtained from CUF-ext gave four fractions (fr. 1 to 4), the percentage pancreatic lipase inhibitory activities of which at concentrations of 10 and 100 $\mu\text{g/mL}$ were as follows: fr. 1, 0% and 19%; fr. 2, 21% and 80%; fr. 3, 15% and 72%; and fr. 4, 43% and 92%. As indicated, the inhibitory activity of fr. 4 was established to be strongest. However, we were unable to identify any components in this fraction owing to small chromatogram peaks. After successive purification of fr. 2 and fr. 3, we identified nobiletin and tangeretin as major flavonoids in the hexane-soluble fraction by comparing ¹H- and ¹³C-NMR spectral data with those of the respective standard samples (data not shown). Moreover, TLC analysis of the ethyl acetate-soluble fraction enabled us to identify hesperidin, narirutin and rutin as major flavonoids in the fraction, the inhibitory activities of which are shown in Table 3. As reported in our previous paper (Itoh et al., 2019), hesperidin, nobiletin and rutin show activity and narirutin exhibits potent activity (IC₅₀ = 189 μM). In contrast, tangeretin showed virtually no activity.

Table 3. Inhibitory activities of hesperidin, nobiletin, narirutin, rutin and tangeretin against pancreatic lipase activity

Samples	IC ₅₀ values ^a (μM)
Hesperidin	334 μM
Nobiletin	108 μM ^b
Narirutin	189 μM
Rutin	258 μM ^b
Tangeretin	N.E. ^{b, c}
Orlistat	0.1 μM

Orlistat was used as a reference compound. a: IC₅₀ values represent the concentrations required to inhibit 50% of pancreatic lipase activity. b: Values reported in our previous paper (Itoh et al., 2019). c: N.E. indicates no effect.

The pancreatic lipase inhibitory activities of hesperidin and rutin have previously been reported by Zeng et al. (2018), who found that hesperidin might be the most potent lipase inhibitor in the peel derived from *Citrus reticulata*, whereas Kawaguchi et al. (1997) have reported isolation of hesperidin from *C. unshiu* peel and identified this compound as a potent pancreatic lipase inhibitory constituent with an IC₅₀ value of 32 $\mu\text{g/mL}$ (52 μM). In contrast, we obtained an IC₅₀ value of 334 μM for hesperidin; however, given that Kawaguchi et al. did not use a reference compound such as orlistat, we are currently unable to ascertain the reason for the discrepancy

between these values. Nevertheless, we assume that differences in the activity of hesperidin could be attributable to certain differences in the respective experimental conditions, such as the substrate or evaluation method employed. With respect to compound rutin, Habtemariam (2013) have reported that the antihyperlipidemic effect of *Cassia auriculata* could be attributed to the direct lipase inhibitory effects of luteolin, quercetin and rutin.

To determine the contents of the flavonoids identified in CUF-ext, we examined the efficacy of a simultaneous HPLC analysis method based on a gradient mobile phase and PDA detector (Zhao et al., 2015), using which, we determined contents of 58.8 ± 1.4 , 0.03 ± 0.001 , 1.2 ± 0.04 , 8.8 ± 0.18 and 0.02 ± 0.002 mg/g extract for hesperidin, nobiletin, narirutin, rutin and tangeretin, respectively, in CUF-ext (Table 4). To the best of our knowledge, this is the first report of the identification of nobiletin, narirutin and rutin from the flowers of *C. unshiu*.

Table 4. Flavonoid contents in CUF-ext and CUUF-ext

Samples	Flavonoid contents (mg/g extract) \pm S.D. ^a				
	Hesperidin	Nobiletin	Narirutin	Rutin	Tangeretin
CUF-ext	58.8 ± 1.4	0.03 ± 0.001	1.2 ± 0.04	8.8 ± 0.18	0.02 ± 0.002
CUUF-ext	142.3 ± 3.7	0.36 ± 0.014	95.4 ± 3.1	3.9 ± 0.26	0.16 ± 0.01

a: Flavonoid contents were determined based on a linear absolute calibration curve method using gradient HPLC-PDA. b: Dried extracts were dissolved in MeOH under sonication, and appropriate amounts of sample solution were used for HPLC analysis in triplicate. Values represent the means \pm standard deviation.

HPLC-PDA and TLC analyses of CUUF-ext revealed that hesperidin, nobiletin, narirutin, rutin and tangeretin are the major flavonoids of this extract (data not shown), among which hesperidin, nobiletin, narirutin and rutin were found to have potent inhibitory activity. Taking into account the flavonoid contents in CUUF-ext (Table 4), we speculate that hesperidin, nobiletin, narirutin and rutin contribute to the pancreatic lipase inhibitory activity of CUUF-ext. However, Kawaguchi et al. (1997) have reported that narirutin does not inhibit the lipase derived from porcine pancreas, whereas these authors found that the concentrations of plasma triglycerides in rats fed a diet containing hesperidin were significantly lower than that in those fed a control diet (Kawaguchi et al., 1997). Accordingly, on the basis of the determined flavonoid contents and findings of previous studies, we speculate that hesperidin, nobiletin and narirutin are the principal factors contributing to the lipase inhibition of CUUF-ext, and to the best of our knowledge, this is the first report indicating the lipase inhibitory activity of narirutin.

Collectively, our observations would thus tend to indicate that the pancreatic lipase inhibitory activities of CUF-ext and CUUF-ext are attributable, at least in part, to the activities of hesperidin, nobiletin, narirutin and rutin. Accordingly, in addition to the pruned leaves of *C. unshiu*, the thinned-out flowers and unripe fruit of *C. unshiu* could also serve as suitable natural resources for the preparation of constituents with lipase inhibitory activity. However, we should not exclude the possibility that the potent inhibitory activities of the flower and unripe fruit extracts could also be attributable to other constituents, and further studies are thus necessary to identify additional active constituents.

4. Conclusion

We determined that both the CUF-ext and CUUF-ext obtained from *Citrus unshiu* exhibited pancreatic lipase inhibitory activity, with the inhibitory activity of the former being superior to that of the latter and comparable to that obtained for CUL-ext. Fractionation of CUF-ext and CUUF-ext, followed by chromatographic analyses, revealed that the lipase inhibitory activities of these extracts are partially attributable to hesperidin, nobiletin, narirutin and rutin. We believe this to be the first report of the lipase inhibitory activity of *C. unshiu* flowers, as well as being the first study in which nobiletin, narirutin and rutin have been identified in *C. unshiu* flowers and the first to establish the lipase inhibitory activity of narirutin.

Accordingly, we established that, in addition to the previously reported utility of pruned *Citrus* leaves, the thinned-out flowers and unripe fruit of *C. unshiu* that are generally discarded during *Citrus* fruit cultivation may represent suitable resources for preparing constituents for the treatment of obesity. This study supports the effective utilization of *C. unshiu* plant wastes. However, further investigations are required to examine the safety of administration, to elucidate the mechanisms involved and to identify other potentially active constituents.

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References

- Ado, M. A., Abas, F., Mohammed, A. S., & Ghazali, H. M. (2013). Anti- and pro-lipase activity of selected medicinal, herbal and aquatic plants, and structure elucidation of an anti-lipase compound. *Molecules*, *18*, 14651-14669. <https://doi.org/10.3390/molecules181214651>
- Ballinger, A., & Peikin, S. R. (2002). Orlistat: its current status as an anti-obesity drug. *European Journal of Pharmacology*, *440*, 109-117. [https://doi.org/10.1016/S0014-2999\(02\)01422-X](https://doi.org/10.1016/S0014-2999(02)01422-X)
- Gras, J. (2013). Cetilistat for the treatment of obesity. *Drugs Today*, *49*(12), 755-759. <https://doi.org/10.1358/dot.2013.49.12.2099318>
- Han, L. K., Zheng, Y. N., Yoshikawa, M., Okuda, H., & Kimura, Y. (2005). Anti-obesity effects of chikusetsusaponins isolated from *Panax japonicus* rhizomes. *BMC Complementary and Alternative Medicine*, *5*, 9. <https://doi.org/10.1186/1472-6882-5-9>
- Habtemariam, S. (2013). Antihyperlipidemic components of *Cassia auriculata* aerial parts: identification through *in vitro* studies. *Phytotherapy Research*, *27*, 152-155. <https://doi.org/10.1002/ptr.4711>
- Itoh, K., Murata, K., Nakagaki, Y., Shimizu, A., Takata, Y., Shimizu, K., ... Matsuda, H. (2016). A pancreatic lipase inhibitory activity by Mango (*Mangifera indica*) leaf methanolic extract. *Journal of Plant Studies*, *5*(2), 72-78. <https://doi.org/10.5539/jps.v5n2p72>
- Itoh, K., Matsukawa, T., Murata, K., Nishitani, R., Yamagami, M., Tomohiro, N., ... Matsuda, H. (2019). Pancreatic lipase inhibitory activity of *Citrus unshiu* leaf extract. *Natural Product Communications*, *14*(9), 1-5. <https://doi.org/10.1177/1934578X19873439>
- Kawaguchi, K., Mizuno, T., Aida, K., & Uchino, K. (1997). Hesperidin as an inhibitor of lipases from porcine pancreas and *Pseudomonas*. *Bioscience, Biotechnology, and Biochemistry*, *61*(1), 102-104. <https://doi.org/10.1271/bbb.61.102>
- Kim, G. N., Shin, M. R., Shin, S. H., Lee, A. R., Lee, J. Y., Seo, B. I., ... Roh, S. S. (2016). Study of antiobesity effect through inhibition of pancreatic lipase activity of *Diospyros kaki* fruit and *Citrus unshiu* peel. *Biomed. Research International*, *2016*, 1-7. <https://doi.org/10.1155/2016/1723042>
- Liu, S., Li, D., Huang, B., Chen, Y., Lu, X., & Wang, Y. (2013). Inhibition of pancreatic lipase, α -glucosidase, α -amylase, and hypolipidemic effects of the total flavonoids from *Nelumbo nucifera* leaves. *Journal of Ethnopharmacology*, *149*, 263-269. <https://doi.org/10.1016/j.jep.2013.06.034>
- Patui, S., Clincon, L., Peresson, C., Zancani, M., Conte, L., Terra, L. D., ... Braidot, E. (2014). Lipase activity and antioxidant capacity in coffee (*Coffea arabica* L.) seeds during germination. *Plant Science*, *219*-220, 19-25. <https://doi.org/10.1016/j.plantsci.2013.12.014>
- Seyedan, A., Alshawsh, M. A., Alshagga, M. A., Koosha, S., & Mohamed, Z. (2015). Medicinal plants and their inhibitory activities against pancreatic lipase: a review. *Evidence-Based Complementary and Alternative Medicine*, *2015*, 1-13. <https://doi.org/10.1155/2015/973143>
- Sosnowska, D., Podśędek, A., Redzyna, M., & Żyżelewicz, D. (2015). Effects of fruit extracts on pancreatic lipase activity in lipid emulsions. *Plant Foods for Human Nutrition*, *70*, 344-350. <https://doi.org/10.1007/s11130-015-0501-x>
- Tosa, S., Ishihara, S., Toyota, M., & Yoshida, S., Nakazawa, H., & Tomimatsu, T. (1988). Studies of flavonoids in *Citrus*. Analysis of flavanone glycosides in the peel of *Citrus* by high performance liquid chromatography. *Shoyakugaku Zasshi*, *42*(1), 41-47.
- Zeng, S. L., Li, S. Z., Lai, C. J. S., Wei, M. Y., Chen, B. Z., Li, P., ... Liu, E. H. (2018). Evaluation of anti-lipase activity and bioactive flavonoids in the *Citri Reticulatae* Pericarpium from different harvest time. *Phytomedicine*, *43*, 103-109. <https://doi.org/10.1016/j.phymed.2018.04.008>
- Zhao, B. T., Kim, E. J., Son, K. H., Son, J. K., Min, B. S., & Woo M. H. (2015). Quality evaluation and pattern recognition analyses of marker compounds from five medicinal drugs of Rutaceae family by HPLC/PDA. *Archives of Pharmacological Research*, *38*, 1512-1520. <https://doi.org/10.1007/s12272-015-0583-x>

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