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Antioxidant and anti-inflammatory activities of loquat (*Eriobotrya japonica*) tea

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ABSTRACT

Background: Fresh loquat leaves contain several kinds of flavonoids and have been reported to have preventive effects against some human diseases such as diabetes, coughs and ulcers,. Recently, fresh loquat leaves in Japan were processed to a beverage, called loquat tea, after the fresh leaves are roasted at 350°C for 30 minutes. However, the scientific evidence supporting the functions of these processed leaves is still minimal.

Objective: The aim of this study is to investigate the antioxidant and anti-inflammatory activities of roasted loquat tea extract (LTE) *in vitro* and in culture cells.

Methods: Bioactive fractions of LTE were separated by column chromatograph. Antioxidant activities were determined by DPPH and ROS assay. Pro-inflammatory mediators cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) were determined by Western blot and ELISA assay, respectively. Chemical quantification and characterization were analyzed by HPLC, FR-IR, and NMR. Phenolic content was measured by Folin-Ciocalteu assay.

Results: The results showed that loquat tea extract (LTE) possessed stronger DPPH scavenging activity than fresh. Cellular data revealed that LTE inhibited the production of reactive oxygen species (ROS), and further suppressed the production of COX-2 and PGE_2 in lipopolysaccharide (LPS)-activated RAW 264.7 cells. Chemical quantification and characterization data indicated that LTE contained new bioactive phenolic components that were produced from the roasting processes of fresh loquat leaves.

Conclusions: Loquat tea made from roasted loquat leaves contained new bioactive phenolic compounds that contribute to its antioxidant and anti-inflammatory activities.

Keywords: Loquat tea, Antioxidant activity, Anti-inflammatory activity, Chemical characterization

INTRODUCTION:

Loquat (*Eriobotrya japonica*) belongs to the *Rosaceae* family. All parts of loquat, such as fruits, leaves, and peels have been reported to have health benefits. In particular, the leaves have a higher flavonoid content than the peel or fruits, with stronger radical scavenging activity [1] and have been reported to have preventive effects against skin diseases, diabetes, chronic bronchitis, coughs, phlegm, ulcers, allergies, and cancer [2, 3]. Recently, fresh loquat leaves were processed into a beverage, called loquat tea, after the fresh leaves are roasted at 350°C for 30 minutes. However, the scientific evidence supporting the functions is still minimal.

Dietary antioxidants can scavenge reactive oxygen species (ROS), which are implicated in a wide range of human diseases such as atherosclerosis and certain cancers [4]. On the other hand, inflammation is the first physiological defense system and is present in two forms: short term inflammation and long term inflammation. Long term inflammation occurs in many kinds of inflammatory diseases and stimulated macrophages to produce excess amounts of inflammatory mediators, such as prostaglandins (PGE_2) [5]. COX-2 is one of the most pivotal enzymes, and is induced by proinflammatory stimuli and growth factors (LPS), and it is responsible for the production of PGE_2 at the inflammatory sites [6, 7]. Inhibition of the overproduction of PGE_2 in macrophages by inhibiting COX-2 expression may have therapeutic potential in inflammatory diseases.

The aim of the present study was to investigate the antioxidant and anti-inflammatory activities of roasted loquat tea. Therefore, fresh and different fractions of roasted loquat tea were extracted by boiling water according to folk customs. The antioxidant activities from the different fractions of loquat tea extracts (LTE) were compared with fresh loquat leaves by 1-diphenyl-2-picrylhydrazyl assay (DPPH) assay *in vitro* and by dichlorofluorescein-diacetate (DCFH-DA) assay in mouse RAW264.7 cells. Since RAW264.7 cells can be used to mimic a state of oxidative stress and inflammation [5, 8], the inhibitory effects on the production of cyclooxygenase-2 (COX-2) and prostagladin E_2 (PGE_2) were further examined in lipopolysaccharide (LPS)-activated RAW 264.7 cells. Finally, the HPLC profiles of LTE and its fractions were compared with the extract of fresh loquat leaves to clarify the bioactive compounds contributing to the antioxidant and anti-inflammatory activities in LTE.

METHODS:

Fresh and Loquat tea extraction

Loquat leaves were washed and dried, then roasted at 350°C for 30 minutes in a ceramic vessel. Then, for both the fresh and roasted samples, the leaves were boiled at 100ºC for 15 minutes, and supernatants were collected after centrifugation at 12000 rpm for 5 minutes. The supernatant fluid of roasted leaves (M fraction) was then separated by MCI gel column, and A, B, C, and D

fractions were obtained by eluting with water, 30% EtOH, 50% EtOH, and 100% acetone, respectively. According to the antioxidant activity-based purification, the C fraction was further separated with ODS gel column, and C1-C9 fractions were finally obtained by elution with 10 to 90 % MeOH (Figure 1A). All of extracts and fractions were evaporated and stored at -20 °C until use.

DPPH assay

The radical scavenging activity of LTE and its fractions were measured by the DPPH (1-diphenyl-2-picrylhydrazyl) method [9]. Briefly, ten microliters of each extraction fraction (1mg/ml) was mixed with 190 μl of 100 μM DPPH in 96-well plates and a final concentration of 50 μg/ml. The plate was covered with aluminum foil and left for 30 minutes at room temperature, with the samples being mixed every 10 minutes. The absorbance was then measured at 492 nm with a microplate reader (Thermo scientific Multiscan FC, version 1.00.79). 6-Hydroxy -2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox), which has high antioxidant capacity, was used as a standard. The percentage activity of DPPH scavenging was calculated with the formula (A_0-A_1/ A_0) ×100 where A_0 was the absorbance of the control, and A_1 was the absorbance of LTE and its fractions [10].

Cell culture

Murine macrophage-like RAW 264.7 cells were purchased from RIKEN Bioresource Center Cell Bank of Japan (RCB0535), and cultured at 37ºC in a 5% CO_2 atmosphere in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS, 1% of penicillin and streptomycin, and 2% glutamin. Fetal bovine serum (FBS) was purchased from Equitech-Bio (Kerrville, TX, USA), and LPS (Escherichia coli Serotype 055:B5) was purchased from Sigma (St. Louis, MO, USA).

Cell viability assay

The cell survival rate was measured by a MTT assay [11]. Briefly, RAW 264.7 cells (2×10^4 cells/100 μl) were seeded into each well of 96-well plates. After an incubation period of 24 hours, the cells were treated with different concentrations of LTE or its fractions for 12 hours. Then, 10 μl of MTT solution (5 mg/ml) was added to each well. After incubating the cells for another 4 hours, the resulting MTT-formazan product was dissolved by adding 100 μl of 0.04 N HCl-isopropanol solution. The amount of formazan was determined by measuring the absorbance at 595 nm in a microplate reader (Thermo scientific Multiscan FC, version 1.00.79). The results were expressed as the optical density ratio of the treatment to control.

Measurement of ROS production

Intracellular ROS were determined using the oxidation-sensitive dichlorofluorescein-diacetate (DCFH-DA) fluorescent dye. RAW 264.7 cells were seeded into 96-well plates at a starting density of 2×10^4 cell/well. After pre-incubation for 24 hours, the culturing medium was replaced with a fresh one. The cells were treated with or without LTE and its fractions for 30 minutes before exposure to LPS (1μg/ml) for 12 hours, and DCFH-DA with a final concentration of 20 μM was then added for an additional 2 hours. Fluorescence was measured at 485 nm excitation and 530 nm emission using a fluorescent Multilable Counter (Perkin-Elmer). The relative

amount of intracellular ROS production was expressed as the fluorescence ratio of the treatment to control.

Measurement of PGE_2 production

PGE_2 in the culture medium was measured with a PGE_2 enzyme immunoassay kit (Cayman Co., St. Luris, MO, USA) according to manufacturer's manual [12]. In brief, RAW 264.7 cells (5×10^5 cells) were seeded into each well of 6-well plates. After an incubation period of 24 hours, the cells were starved by being cultured without serum for another 2.5 hours to eliminate the influence of FBS. The cells were then treated with or without LTE and its fractions for 30 minutes before exposure to LPS (40 ng/ml) for 12 hours. The amount of PGE_2 released into the medium was determined by measuring absorbance at 405 nm with a microplate reader.

Western blot analysis

Western blotting was performed as described previously [13]. RAW 264.7 (1×10^6) cells were pre-cultured in 6-cm dish for 21 hours and then starved by being cultured serum-free another 2.5 hours to eliminate the influence of FBS. The cells were treated with LTE and its fractions for 30 minutes and then exposed to LPS (40 ng/ml) for 12 hours. Equal amounts of lysated protein were separated on SDS-polyacrylamide gel and transferred onto the PVDF membrane. Afterwards, the membrane was blotted at room temperature for 2 hours in blocking buffer and incubated with specific primary antibody overnight at 4 ºC, following a three-time wash with TBS-Tween solution. The membrane was further incubated for 1 hour with HRP-conjugated secondary antibodies and washed three times again. Band intensities bound with antibodies were detected by ECL system in a luminivision PRO machine (TAITEC Co., Japan). Antibodies against COX-2 and α-tubulin were from Santa Cruz Biotechnology (CA, USA).

Chemical quantification and characterization

Ten microliters of each extract were analyzed using a HPLC unit and a 250 × 4.6 mm i.d., Crest Pak C_{18} T-5 column. The solvent system was a mixture of 0.05 μM H_3PO_4 in CH_3CN (A) and 0.05 μM H_3PO_4 in water (B), with a flow rate of 0.8 ml/min, and the gradient was as follows: 39 minutes - 4% A ; 96% B and 6 minutes -75% A ; 25% B. Spectroscopic data from all peaks were accumulated in the range of 200-700 nm, and chromatograms were recorded at 280 nm. C2 fraction separated from roasted loquat leaf extract was detected using a JASCO FT-IR/IRT-3000 ATR-30-Z (Tokyo, Japan) equipped with an ATR attachment. The FT-IR frequencies were detected between 400 and 4000 cm^{-1}. Moreover, the MALDI-TOF-MS was obtained in a 2,5-dihydroxybenzoic acid (DHB) matrix in positive ion mode on a Bruker Autoflex Speed/TOF/TOF(Bruker Daltonics, USA/CA) and the ^{1}H-NMR spectra were determined using a JEOL JNM-ECA600 (Tokyo, Japan). DMSO-d_6 was used as the solvent, and chemical shifts were expressed ppm with reference to tetramethylsilane.

Measurement of phenolic contents

The concentration of the total phenolic substances was measured according to the previous method with some modification [9]. Briefly, 10 μl of LTE or its fractions was mixed with 200 μl of 2% Na_2CO_3 in 96-well plates. After 3 minutes, 10 μl of 50% diluted Folin-Ciocalteu reagent

was added to each well. The mixture was allowed to stand for 30 minutes at room temperature with mixing every 10 minutes, and then the absorbance was measured at 595 nm with a microplate reader (Thermo scientific Multiscan FC, version 1.00.79). The gallic acid was used as standard, and the total phenolic content was expressed as a gallic acid equivalent (GAE) in milligrams per gram of LTE or its fractions.

Statistical Analysis

All data were statistically analyzed by a student's *t*-test. Differences were considered significant for $p < 0.05$ and $p < 0.01$.

RESULTS:

In vitro antioxidant activities of fresh and LTE

Antioxidant activities of fresh leaves and fractions of LTE were examined using a DPPH assay. As shown in Figure 1B**,** DPPH scavenging activities of fresh (Fresh) and LTE (M) at the concentration of 50 μg/ml were 18.34 and 44.81, respectively. Thus, LTE possessed higher levels of antioxidant activity than fresh loquat leaves. Furthermore, the DPPH value of fraction A, B, C, or D fractionated from LTE (M) were 44.24, 54.00, 69.31, and 25.24 % at the concentration of 50μg/ml, respectively. According to antioxidant activity-guided purification, C fraction was further separated into C1~C9 fractions by ODS gel column. Their DPPH scavenging activities at the concentration of 50 μg/ml were 69.02, 75.00, 68.52, 67.61, 65.28, 38.54, 21.44, 27.25, and 49.67%, respectively (Figure 1C), suggesting that C1~C5 fractions contain higher antioxidant activity than C6~C9. Thus, we chose C2 fraction with highest antioxidant activity as a sample to further studies.

Antioxidant activities of LTE in culture cells

To investigate whether the LTE also showed antioxidant activity in cellular level, we measured the change of ROS level in LPS-activated RAW 264.7 cells with or without treatment of LTE, using DCFH-DA fluorescent dye. As shown in Figure 2A**,** LPS induced ROS production (lane 2), and C fraction showed the highest inhibitory effect on LPS-induced ROS among M, A, B, C, and D fractions at a concentration of 25 μg/ml. We further investigated the inhibitory effects of C and its C2 fractions on LPS-induced ROS, and found a dose-dependent inhibition of the ROS at the concentration of 0-50 μg/ml (Figure 2B). These data revealed that LTE and its fractions also had antioxidant activity at the cellular level.

Innhibition of LTE on PGE_2 production in LPS-stimulated RAW 264.7 cells

Since the antioxidant activity of phytochemicals has been considered to link to anti-inflammation [8], we next investigated the anti-inflammatory activities of LTE in mouse macrophage-like cell RAW 264.7, which is a cell model to investigate inflammation mechanisms. As shown in Figure 3A, LPS-induced PGE_2 productions were significantly attenuated by treatment with LTE (M, A, B, C, and D). Moreover, C and C2 fraction strongly inhibited PGE_2 production at the concentration range of 100-200 μg/ml (Figure 3B). In addition, there is no significant difference in the cell viability between the treatments and controls (Figure 3A and 3B). Thus, the inhibitory effects by LTE and its fractions were not caused by their cytotoxicity.

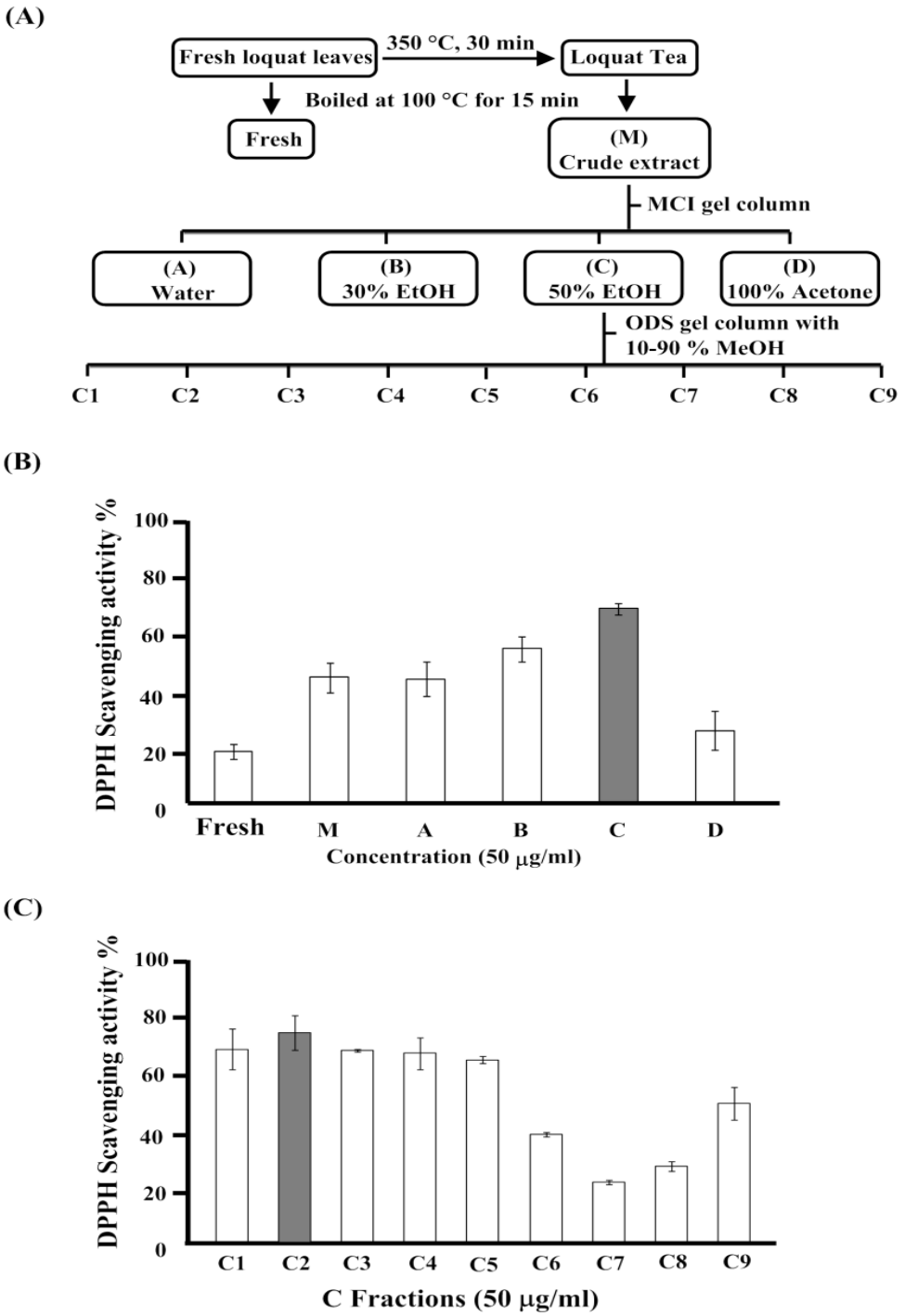

Fig. 1. **(A)** LTE fractionation. Fresh loquat leaves were roasted at 350°C for 30 minutes. Both fresh and roasted leaves were boiled at 100 ºC for 15 minutes. The aqueous layer of roasted leaves was chromatographed by MCI gel column with water, 30% MeOH, 50% EtOH and acetone. The fraction which contained high antioxidant activity was further chromatographed by ODS gel column with 10-90% MeOH. DPPH scavenging activities of fresh loquat leaves and LTE **(B)**, and C1-9 fractions and **(C)**. The values were expressed as the percentage of the control value. M, crude extract; A, water elution fraction; B, 30% EtOH fraction; C, 50% EtOH fraction; D, 100% acetone fraction. C1~C9, fractions 1-9 obtained from C fraction eluted by 10~90% MeOH, respectively. The data represent the mean ± SD of three separated experiments.

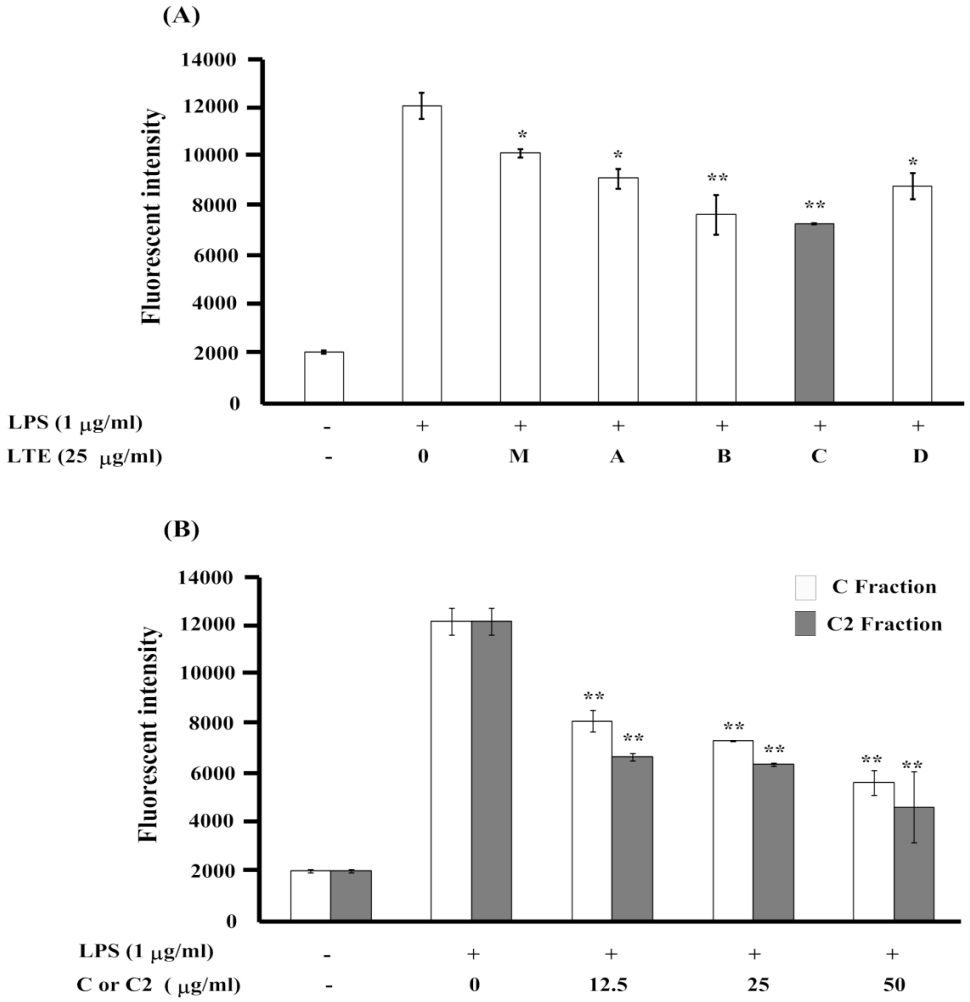

Fig. 2. Iinhibition of LTE **(A)**, C and C2 fractions (**B**) on ROS production in LPS-activated RAW 264.7 cells. The cells were seeded into 96-well plate (2×10^4 cells/well) and pre-cultured for 24 hours. The cells were treated with or without LTE fractions at different concentrations for 30 minutes before exposure to LPS (1 μg/ml) for 12 hours. DCFH-DA was then added to the medium with a final concentration of 20 μM for an additional 2 hours. The fluorescence intensity was then measured at an excitation (485 nm) and emission (530 nm) wavelength using a fluorescent Mutilabel Counter (Perkin-Elmer), and was expressed as the percentage of control in the absence of LPS. Data are the mean ± SD of three separated experiments. Asterisk shows significant inhibition to LPS only ($P< 0.05$) and ($P< 0.01$).

Inhibition of LTE on COX-2 expression in LPS-stimulated RAW 264.7 cells

PGE_2 is usually synthesized at inflammatory site by the enzyme, cyclooxygenase-2 (COX-2). Thus, we further investigated the effect of LTE and its fractions on the LPS-induced COX-2 expression. As shown in Figure 4A, LPS-induced COX-2 production was markedly inhibited by LTE at the concentration (200 μg/ml), and C and C2 fractions showed a dose-dependent inhibition on LPS-induced COX-2 expression (Figure 4B and 4C). As a control, α-tubulin expression was not changed. These results suggested that LTE, especially C fraction, inhibited PGE_2 production by suppressing COX-2 expression.

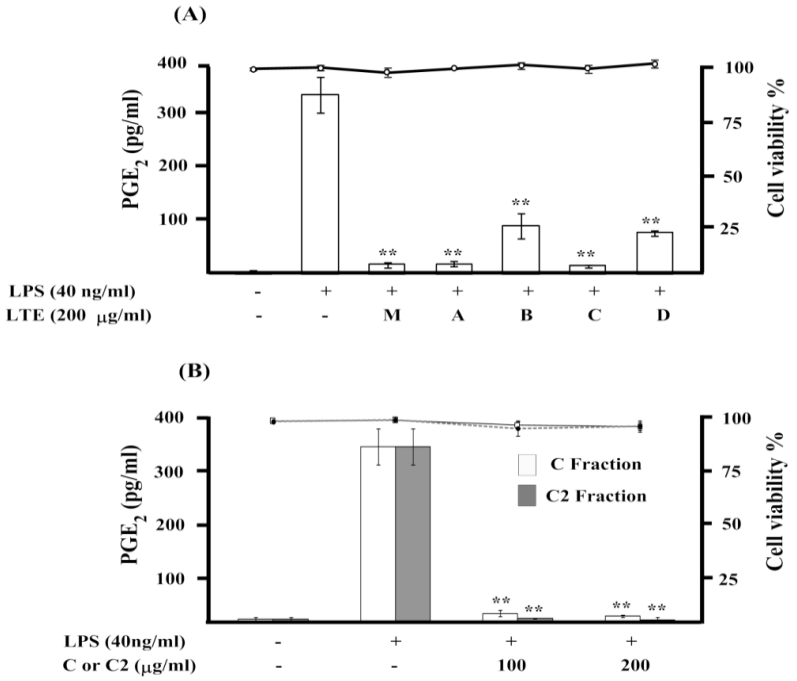

Fig. 3. Inhibition of PGE_2 production and influence on cell viability by LTE (**A**), C fraction and C2 fraction (**B**) in LPS-induced RAW 264.7 cells. RAW 264.7 (5×10^5 cells) were pretreated with the indicated concentrations of LTE, C and C2 fractions for 30 minutes and then incubated with LPS (40 ng/ml) for 12 hours. The level of PGE_2 production in culture media was determined using enzyme immunoassays (ELISA) kit, and expressed as pg/ml (left vertical axis). Asterisk shows significant inhibition to LPS only ($P< 0.01$). The cell viability was simultaneously estimated by MTT assay, and expressed as viability percentage to control cell (right vertical axis). The data represent the mean ± SD of three separated experiments.

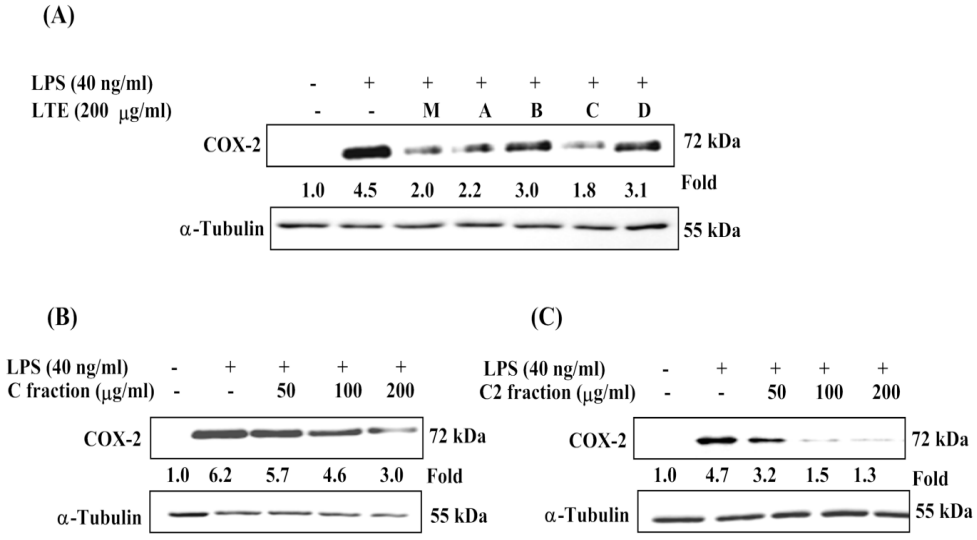

Fig. 4. Inhibition of LTE (**A**), C fraction (**B**) and C2 fraction (**C**) on COX-2 expression in LPS-activated RAW 264.7 cells. The cells were treated by different fractions of Loquat tea with indicated concentrations for 30 minutes, and stimulated with 40 ng/ml LPS for 12 hours. COX-2 and α-tubulin were detected by Western Blotting and analysis with their antibodies, respectively. The values show the densitometry fold of COX-2 protein normalized to α-tubulin. The data represent the mean of three separated experiments.

Chemical quantification and characterization of LTE

To analyze the bioactive components in LTE, we first compared the HPLC profiling of LTE to fresh loquat leaves with known compounds as standards. As shown in Figure 5A and Table 1, larger amounts of 5-caffeonylquinic acid and 3-caffeonylquinic acid, and smaller amounts of (-)-epicatechin and procyanidin B2 were detected in fresh loquat leaves, but smaller amounts of everything were in LTE crude (b) and none were detected in the C and C2 fraction (c and d) of LTE. Some new peaks were detected (c and d). To characterize these chemicals, C2 fraction was further investigated by FT-IR spectra. The strong and broad band of O-H stretching was observed at 3252 cm^{-1}. The existence of one or more aromatic rings in a structure is normally readily determined from the C–H, C=C and C–C ring. Medium strong and finger peaks at 1593, 1543, and 1515 assigned to C=C stretching modes. The generated C-C aromatic stretch was observed at 1387.53, with strong absorptions of FT-IR. The peaks at 7193 and 6733 cm^{-1} could be assigned as mono substitute benzene. These data suggest that some new phenolic compounds might be produced during the roasting process of fresh loquat leaves. Moreover, the C2 fraction showed primary three peaks at m/z 170 - m/z 330 on MALDI-TOF MS spectra, and the signals for aromatic or olefinic protons at 6.256-8.308 ppm as well as for the methylene or alicyclic protons at 0.823-2.995 ppm were detected in the ^{1}H-NMR spectra. Thus, we gather from these data that these compounds in LTE might be several kinds of phenolic compounds.

Since phenolic compounds generally have antioxidant capacity, we thus quantified the phenolic contents of fresh and its fractions of LTE, using gallic acid as reference standard. As shown in Figure 5B, phenolic contents of LTE fractions (M) are higher than fresh loquat leaves. Moreover, the fraction C and C2 contained higher phenolic amount among these fractions.

Table1 HPLC data of fresh loquat leaves and roasted loquat tea

Fractions	Chemical compound	Retension time (min)	Peak area (μV.S)	Amount (mg/kg)
Fresh leaves	5-caffeonylquinic acid	21.07	1673131	6.59
	3-caffeonylquinic acid	28.08	4225609	16.64
	Procyanidin B2	31.27	295307	1.16
	Epicatechin	36.26	1174750	4.62
LTE (M)	5-caffeonylquinic acid	21.07	27036	0.26
	3-caffeonylquinic acid	28.08	106130	1.03
	Procyanidin B2	n.d	-	-
	Epicatechin	n.d	-	-
LTE (C or C2)	5-caffeonylquinic acid	n.d	-	-
	3-caffeonylquinic acid	n.d	-	-
	Procyanidin B2	n.d	-	-
	Epicatechin	n.d	-	-

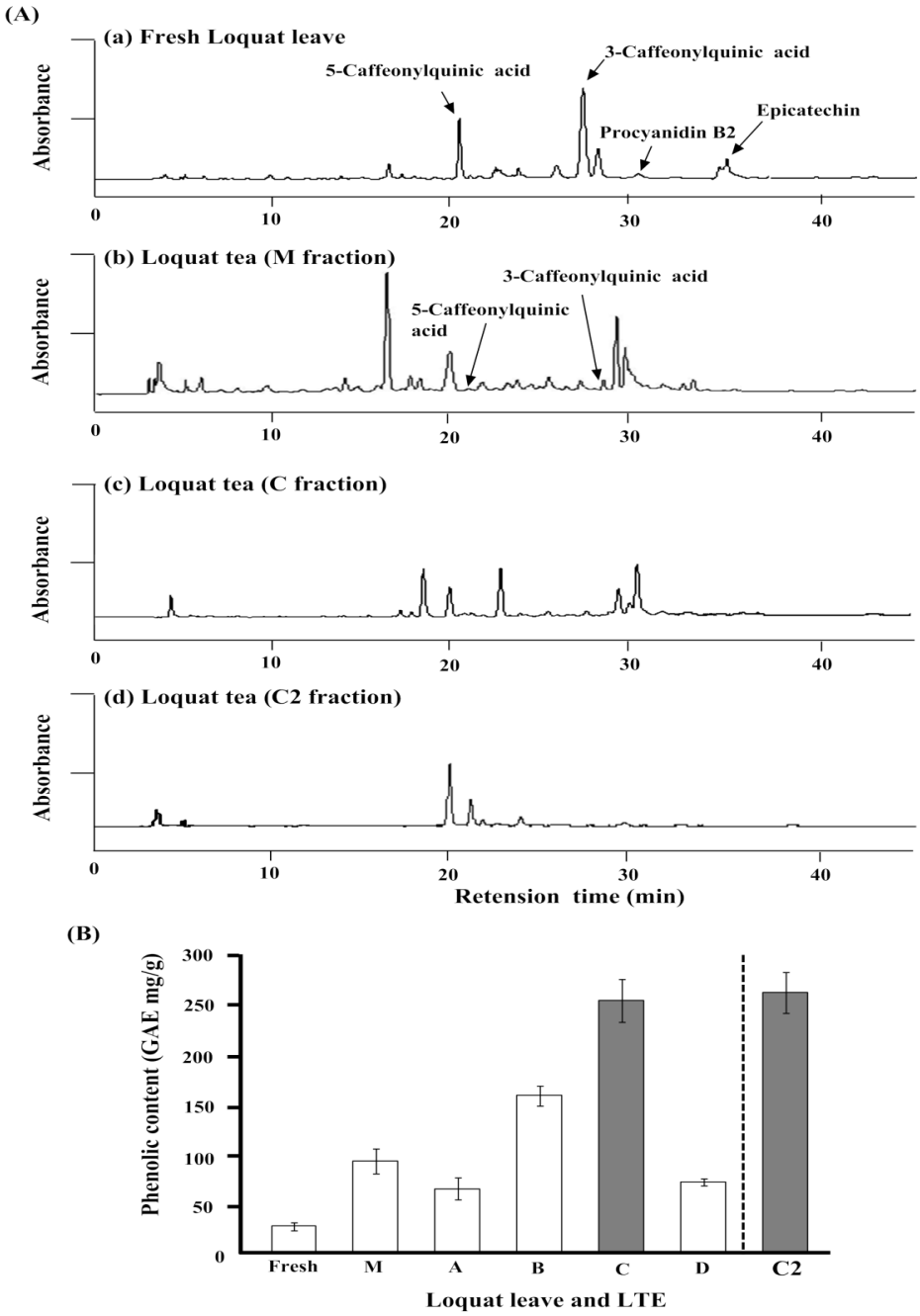

Fig. 5. HPLC profiles (**A**) of fresh Loquat leave (**a**), LTE M (**b**), C fraction (**c**) and C2 fraction (**d**). (-)-Epicathechin, 3-caffeonylquinic acid, 5-caffeonylquinic acid and procyanidin B2 were used as standards. The CresPak C_{18}T-5 column (4.6 mm i.d. × 250 mm) was set in 40 ºC. Ten microliters of standards or LTE solution was injected to the column after filtered with a millipore filter (0.45 μM) and flowed with 0.05 M of H_3PO_4 in CH_3CN from 4% to 30% for 39 minutes and then changed 30% to 75% for 6 minutes under a flow rate of 0.8 ml/min. Spectroscopic data from all peaks were accumulated in the range of 200-700 nm, and chromatograms were recorded at 280 nm. Phenolic contents (**B**) of fresh loquat leaf, LTE, LTE and C2 fractions. Phenolic contents were determined by Folin-Ciocalteu method. The amounts were presented as gallic acid equivalents (GAE mg/ml). M, crude extract; A, water elution fraction; B, 30% EtOH fraction; C, 50% EtOH fraction; D, 100% acetone fraction, C2 fraction purified from C fraction, respectively. The data represent the mean ± SD of three separated experiments.

DISCUSSION:

Loquat tea is made from loquat leaves roasted at 350°C for 30 minutes, and usually used as beverage according to the folk customs. Although fresh loquat leaves have been reported to have biological activities such as antioxidant and anti-inflammatory activities [14-16], there is no report on the biological activities of roasted loquat leaves (loquat tea). In the present study, we used antioxidant activity-guided fractionation to investigate the biological activities of loquat tea. Our data showed that loquat tea had stronger antioxidant activities than fresh loquat leaves. Loquat tea revealed stronger antioxidant activities not only *in vitro,* but also in mouse macrophage-like cell RAW264.7, which is a cell model to investigate antioxidant and anti-inflammation mechanisms. COX-2 is only induced during inflammation by pro-inflammatory stimuli including bacterial LPS, growth factor and cytokines. COX-2 is the rate-limiting enzyme in the conversion of arachidonic acid to PGE_2 which is a pro-inflammatory mediator present in the inflammatory site [17,18]. Persistence inflammation and continuous production of COX-2 has been linked to development of cancer and autoimmune disorders [19,20]. In this study, we found that loquat tea could attenuate the production of PGE_2 and COX-2 induced by LPS. Therefore, our data indicate that loquat tea has antioxidant and anti-inflammatory properties.

We were then interested in the bioactive compounds contributing to the antioxidant and anti-inflammatory activities in loquat tea extract (LTE). Thus, we compared the HPLC profiles of LTE fractions with the extract of fresh loquat leaves because some bioactive flavonoids such as (-)-epicatechin, 3-caffeonylquinic acid, 5-caffeonylquinic acid, and procyanidin B2 have been clarified in fresh loquat leaves, and have been suggested to contribute the biological activities [21-23].

We confirmed these compounds in fresh loquat leaves using their standard samples (Figure 5A-a). These results are in agreement with the previous report [1, 24]. However, their compounds were much lower in LTE (Figure 5A-b), and finally disappeared in the C and C2 fractions. In place of them, some new compounds were detected in the C fractions (Figure 5A-d). Although we could not determine the chemical structure at this moment, the data from FT-IR spectra showed the existence of aromatic rings and broad band of O-H stretching. Moreover, the C2 fraction showed primary three peaks at m/z 170 - m/z 330 on the MALDI-TOF MS spectra, and the signals for aromatic or olefinic protons at 6.256-8.308 ppm as well as for the methylene or alicyclic protons at 0.823-2.995 ppm were detected in the ^{1}H-NMR spectra. Thus, we gather that the bioactive compounds in LTE might be, at least partly, several kinds of phenolic compounds, which are produced from the release and/or degradation of bound phenolic compounds in fresh loquat leaves during roasting process.

Some similar findings have also been reported that (-)-epicatechin and procyanidin significantly decreased after roasting cocoa beans and coca ingredients due to epimerization [25, 26]. Since phenolic compounds have been reported to have antioxidant activities, we next measured the phenolic amount of the C and C2 fractions, comparing with LTE using gallic acid as standard. As shown in Figure 5B, phenol content in C and C2 fraction of LTE were 258 ± 22mg/g and 267 ± 21 mg/g. In fresh loquat leaves and LTE (M), the total phenolic contents were 26 ± 4.2 mg/g and 77.4 ± 2.2 mg/g, respectively. These data indicated that phenolic contents were increased when loquat leaves were roasted at 350°C for 30 min. It has been reported that high temperature treatment changed phenolic content of samples caused by the release of bound

phenolic compound, release of phenolic acid derivatives, and thermal degradation of the phenolic compounds [27].

CONCLUSION:

Loquat tea, made from roasted loquat leaves, revealed stronger antioxidant activity than its fresh leaves by scavenging DPPH and suppressing cellular ROS, and also showed anti-inflammatory activity by suppressing the production of pro-inflammatory mediators such as COX-2 and PGE_2. The bioactive components are speculated to be phenolic compounds that were produced from fresh loquat leaves during the roasting processes.

Abbreviations:

LTE, loquat tea extract; COX-2, cyclooxygenase-2; DCFH-DA, dichlorofluorescein-diacetate; DMEM, Dulbecco's Modified Eagle Medium; DPPH, 1-diphenyl-2-picrylhydrazyl; HPLC, High performance liquid chromatography; LPS, lipopolysaccharide; PGE_2, prostaglandin E_2; ROS, reactive oxygen species; Trolox, 6-Hydroxy -2.5.7.8-tetramethyl chroman-2-carboxylic acid.

Competing interest:

The authors have no financial interests or other conflicts of interest.

Authors' Contribution:

Ms. Phyu Phyu Khine Zar is the primary investigator in this study. Dr. Kozue Sakao participated in chemical characterization. Dr. Fumio Hashimoto and Dr. Koji Wada participated in the extraction and purification. Ms. Akiko Morishita helped the culture cell experiments. Dr. Makato Fujii helped the preparation of roasted loquat leaves. Dr. De-Xing Hou designed this study and wrote the manuscript as corresponding author.

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