



Antioxidant, α -amylase and α -glucosidase inhibitory activities of *Cedrela sinensis* (A. Juss) leaf with ethanol extract concentration

YingjinZhu WU¹, Young-Sil HAN¹, Myung-Hyun KIM^{1*} 

Abstract

Cedrela sinensis, a member of *Meliaceae* family, is a traditional Chinese woody vegetable widely used as food and in health since ancient times. In order to study antioxidant and anti-diabetic effects of different concentrations of ethanol extracts of *Cedrela sinensis* leaf, *Cedrela sinensis* leaf was extracted using five solvents based on different ethanol concentration (25 EE, 50 EE, 75 EE and 95 EE) and distilled water (DW). The antioxidant activity was analyzed using DPPH, ABTS⁺ radical scavenging ability, ORAC and reducing power assay. The contents of total phenolic and flavonoid compounds were also analyzed and assessed. The results showed 75 EE having higher polyphenol content (122.10 mg GAE/g) and flavonoid content (23.23 mg QE/g), showed better antioxidant and inhibitory effects against α -amylase and α -glucosidase. According to the test results, 75 EE had significant antioxidant activity and inhibitory ability to diabetes-related enzymes, indicating that it has potential as a functional food or nutritional food for the prevention and treatment of oxidation and diabetes.

Keywords: *Cedrela sinensis* leaf powder; antioxidant activities; α -amylase inhibitory activity; α -glucosidase inhibitory activity.

Practical Application: *Cedrela sinensis* represent a good alternative for as ingredients in functional foods.

1 Introduction

Cedrela sinensis (A. Juss) Roem. was a tall, woody plant of *Meliaceae* family that is native to Eastern and Southeastern Asia. It has been cultivated for more than 2,000 years in China and is commonly known as Chinese mahogany cedar or Chinese *Toona* (Yang et al., 2011). Traditional Chinese medicine makes extensive use of the numerous tissues and components of *Cedrela sinensis*. As the leaves of the *Cedrela sinensis* are crispy, juicy, aromatic and have a unique taste and high consumption value, fresh young leaves and shoots have long been used in Korea as part of a nutritious diet (Mu et al., 2007; Kakumu et al., 2014). *Cedrela sinensis* contains therapeutic properties in almost every part of the plant, including the seeds, root bark, petioles, and leaves (Lee et al., 2006). The leaves and stems of *Cedrela sinensis* have been used to treat itch, enteritis, and dysentery (Dong et al., 2013). *Cedrela sinensis* leaf extracts have a variety of effects, including anti-cancer (Chang et al., 2002b; Chang et al., 2006; Chen et al., 2009; Wang et al., 2010), anti-angiogenesis (Hseu et al., 2011), anti-inflammation (Bak et al., 2009), anti-diabetes (Hsu et al., 2003; Yang et al., 2003), and antioxidant effects (Cho et al., 2003), as well as inhibiting leydig cell steroidogenesis and improving the dynamic activity of human sperm quality (Poon et al., 2005). The bark has been used as an astringent and depurative, the powdered roots as a corrective, and the fruits were as an astringent and to treat eye infections (Dong et al., 2013).

Previous phytochemical investigations carried out on this plant have resulted in the isolation of flavonoids, phenolics, alkaloids, terpenes, anthraquinones, and limonoids (Lee et al., 2010; Dong et al., 2013). Gallic acid is a major phenolic compound in *Cedrela sinensis* leaf that has a wide spectrum of biological

and pharmacological effects (Huang et al., 2012). Several animal models and human investigations have shown that Gallic acid is extremely safe, even at large doses. Gallic acid's pharmacological safety and efficacy make it a promising treatment or preventative option for a wide range of human ailments (Huang et al., 2012).

Solvent extraction is a method of extracting functional substances from plants via a solvent which is absorbed by osmotic pressure and capillary phenomenon. Plant tissue is damaged by solvent concentration, and insoluble substances are leached and dissolved (Kim & Hong, 2012). In general, an extraction solvent that is easily dissolved and concentrated is used, and organic solvents used for extraction include ethanol, methanol, and hexane. The extraction content of polyphenols in plants varies depending on the solvent, and the polyphenol content of extracts with 70% ethanol as a solvent was twice as high as that of water extracts (Kim et al., 2006). Previous studies have used distilled water or ethanol extractive methods to extract *Cedrela sinensis* leaf. To our knowledge, *Cedrela sinensis* leaves exhibit good antioxidant activity, but a few reports have tested with different concentrations of ethanol. Therefore, this study was performed to analyze the antioxidant, α -glucosidase and α -amylase inhibitory activities of water or different concentrations of ethanol from the *Cedrela sinensis* leaf, for possible development of nutritional foods and functional materials.

2 Materials and methods

2.1 Sample preparation and extraction

Cedrela sinensis cultivated in Muan, South Korea was used as samples in this experiment. *Cedrela sinensis* leaf was ground and

Received 27 August, 2022

Accepted 01 October, 2022

¹Department of Food Nutrition, Sookmyung Women's University, Seoul, Korea

*Corresponding author: kimmh@sookmyung.ac.kr

stored, *Cedrela sinensis* leaf powder (CLP) was stored in -40 °C freezer to be used as the sample. Refer to Maulana et al. (2019) method and improve after, the freeze-dried material (10 g) was extracted with distilled water (WE), 25% ethanol (25 EE), 50% ethanol (50 EE), 75% ethanol (75 EE) and 95 ethanol (95 EE) of 200 mL for three times for 20 min at 25 °C using ultrasonic extraction. The extracts were filtered and evaporated under vacuum (NVC-2100, EYELA, Tokyo, Japan) and freeze-dried for 72 h at -40 °C.

2.2 Analysis of phenolic acid in UPLC

A 0.1 g of the CLP was extracted with 6 mL of 2.6 M NaOH followed by sonication (Power sonic 410, Hwashin Technology Co., Korea) for 15 min. In order to cause the decomposition of plant cell wall components containing phenolic acids, the reaction was carried out at 200 rpm, 20 h, 25 °C in a shaking incubator. Extracts were centrifuged using a centrifuge at 25 °C, 3000 rpm, for 20 min. After putting 2 mL of the supernatant in a 15 mL tube, 0.5 mL of 35% HCl was added and refrigerated for 30 min. The extract was filtered through a syringe filter (PTFE, 13 mm, 0.2 µm; Advantec, Tokyo, Japan) prior to UPLC analysis. The identification of phenolic compounds was performed using UPLC (ultra performance liquid chromatography, Ultimate 3000. Dionex, Idstein, Germany), coupled with a quaternary solvent manager and a PDA detector. The column (XTerra MS C₁₈ Column, 5 µm, 3.9 mm*150 mm, Waters, MA, USA) was used at 30 °C. Mobile phase was a mixture of A: water + 0.1% formic acid, and B: methanol + 0.1% formic acid. The gradient conditions were as follows: solvent B, 12.5 min, 15%; 17.5 min, 25%; 20 min, 33%; 21 min, 50%; 22.5 min, 70%; 25 min, 15%. The flow rate was 0.8 mL/min, and the injection volume was 1.0 µL. Simultaneous monitoring was performed at 220 nm (gallic acid) and 330 nm (caffeic acid, coumaric acid, ferulic acid, sinapic acid).

2.3 Determination of Total Polyphenol Content (TPC)

The total polyphenol content was determined as Folin-Ciocalteu method (Lee et al., 2022). To measure TPC, 150 µL of sample solution, 2,400 µL of distilled water and 50 µL of 2 N Folin-Ciocalteu reagent were mixed and then incubated for 3 min. After the incubation, 300 µL of 5% Na₂CO₃ was mixed with reaction mixture and incubated for 2 h in the dark. After the incubation, the absorbance was measured at 725 nm using a UV/VIS spectrophotometer (T60UV, PG Instruments, Wibtotf, England). The results of the TPC were calculated as mg gallic acid equivalents (GAE)/g dry weight.

2.4 Determination of Total Flavonoid Content (TFC)

The total flavonoid content was determined as Davis method (Chang et al., 2002a). To measure TFC, 100 µL of sample solution, 1000 µL of 90% diethylene glycol and 100 µL of 4% NaOH were mixed and then incubated for 1 h in water bath at 37 °C. The absorbance was measured at 420 nm using a UV/VIS spectrophotometer. The TFC values were calculated as mg quercetin equivalents (QE) per gram of dry weight.

2.5 Determination of Oxygen Radical Absorbance Capacity (ORAC)

The oxygen radical absorbing capacity value was followed Ou et al. (2001). 25 µL of sample solution and 150 µL of fluorescein were added to a 96-well plate, and incubated at 37 °C for 30 min. After the incubation, 25 µL of AAPH was added and the fluorescence reduction rate was measured every minute for 120 min in a fluorescent microplate reader (SpectraMax i3x Multi-Mode Microplate Reader, Molecular Devices, CA, USA). The results were expressed as AUC (area under curve) values and as a standard, the trolox (6.25-100 µM) was employed. The ORAC value of the dry flour samples was given as µM TE/g (Equation 1).

$$AUC = 1 + f1/f0 + f2/f0 + f3/f0 + \dots + f80/f \quad (1)$$

2.6 Determination of DPPH radical scavenging activity

The radical scavenging activity of DPPH in CLP extracts was determined as Shafay et al. (2022). The sample solution and DPPH solution were stirred at a ratio of 3:1, left in a dark place blocked from light for 30 min, and then absorbance was measured at 517 nm using a UV/VIS spectrophotometer (Equation 2).

$$DPPH \text{ free radical scavenging activity (\%)} = \left[1 - \left(A_{\text{sample}} - A_{\text{sample blank}} \right) / A_{\text{control}} \right] \times 100 \quad (2)$$

2.7 Determination of ABTS radical scavenging activity

ABTS radical scavenging activity assay was performed as previously described Re et al. (1999). Prior to the assay, 900 µL of ABTS⁺ solution was mixed with 100 µL extracts to measure the absorbance at 734 nm using a UV/VIS spectrophotometer (Equation 3).

$$ABTS \text{ radical scavenging activity (\%)} = \left[1 - \left(A_{\text{sample}} - A_{\text{sample blank}} \right) / A_{\text{control}} \right] \times 100 \quad (3)$$

2.8 Determination of reducing power

The reducing power was measured according to the method of Silva et al. (2022). 1 mL of the sample solution in distilled water, and add 1 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide (K₃Fe(CN)₆) to a water bath (WBT-10, Chung Biotech, Incheon, Korea) for 20 min. Add 1 mL of 10% trichloroacetic acid (TCA: CCl₃COOH, w/v), centrifuge at 3,000 rpm for 10 min (COMBI-514R, Hanil Science Industry, Gimpo, Korea), and take 1 mL supernatant. After mixing with 5 mL of distilled water, 0.2 mL of 0.1% ferric chloride was added, and absorbance was measured at 700 nm.

2.9 α-Amylase inhibitory activity assay

The α-amylase inhibitory activity was measured according to the method of Bhandari et al. (2008). Samples and standard were diluted in distilled water, but reagents were diluted in 0.5 M Tris-HCl buffer (pH 6.9). 200 µL of sample and 200 µL of 0.5 M

α -amylase solution (1 U/mL) were mixed, and 300 μ L of starch azure solution was added to the mixture. And then the prepared solution was incubated at 37 °C for 10 min, and 100 μ L of 50% acetic acid was added. The reacted mixture was centrifuged 10 min at 3000 rpm and 4 °C. And as a standard, the acarbose (50-500 mg/mL) was employed. The absorbance was measured at 595 nm using a UV/VIS spectrophotometer (Equation 4).

$$\alpha - \text{Amylase inhibitory (\%)} = \left[\frac{1 - (A_{595\text{sample}} - A_{595\text{sample blank}})}{(A_{595\text{control}} - A_{595\text{control blank}})} \right] \times 100 \quad (4)$$

2.10 α - Glucosidase inhibitory activity assay

The α -glucosidase inhibitory activity was measured according to the method of Xu et al. (2018). Samples and standard were diluted in distilled water, but reagents were diluted in 0.05 M phosphate buffer (pH 6.8). 200 μ L of samples which was mixed with 10 μ L of 1 U/mL α -glucosidase solution. Then the mixture was incubated at 37 °C for 5 min. 200 μ L of 1 mM PNPG solution was added to the mixture and incubated at 37 °C for 20 min. Finally, the process was stopped by adding 500 μ L of 4% NaOH solution, and then 590 μ L of 0.05 M phosphate buffer was added to the mixture. And as a standard, the acarbose (50-500 mg/mL) was employed. The absorbance was measured at 405 nm using a UV/VIS spectrophotometer (Equation 5).

$$\alpha - \text{Glucosidase inhibitory (\%)} = \left[\frac{1 - (A_{595\text{sample}} - A_{595\text{sample blank}})}{(A_{595\text{control}} - A_{595\text{control blank}})} \right] \times 100 \quad (5)$$

2.11 Statistical analysis

All experiments in this study were performed in triplicate replicates and results were presented as the mean \pm SD of three independent experiments. SPSS program (Statistical Analysis Program, version 25, IBM Co., Amonk, NY, USA) was used, one-way ANOVA was used to verify the significance of the experiment, and Duncan's multiple range test was performed for post hoc testing. $p < 0.05$ was used as the threshold for statistical significance. Each sample was examined three times.

3 Results and discussion

3.1 Analysis of phenolic acid in CLP

The phenolic acid of CLP (*Cedrela sinensis* leaf powder) with five extracts is shown in Table 1 and Figure 1. The retention time of the CLP extracts were 2.68, 10.44, 15.7, 17.73, and 18.68 min

Table 1. Phenolic compounds detected in *Cedrela sinensis* leaf powder.

No	Compound	Retention time (min)	<i>Cedrela sinensis</i> leaf powder (mg/g)
1	Gallic acid	2.68	7.66 \pm 0.40 ^a
2	Caffeic acid	10.44	0.09 \pm 0.02 ^c
3	Coumaric acid	15.7	0.29 \pm 0.08 ^b
4	Trans-ferulic acid	17.73	0.12 \pm 0.04 ^b
5	Sinapic acid	18.68	ND

All values were expressed as mean \pm SD (n = 3). Values with different letter within a row differ significantly by Duncan's multiple range test ($p < 0.05$). ND: Not detect.

for gallic acid, caffeic acid, coumaric acid, trans-ferulic acid, and sinapic acid, respectively. The phenolic acid analysis demonstrated that gallic acid was 7.66 mg/g, caffeic acid 0.09 mg/g, coumaric acid 0.29 mg/g, and trans-ferulic acid 0.12 mg/g. The gallic acid content of CLP was the highest. Cheng et al. (2009) found that the CLP extracts showed high antioxidant capacity. According to a study by Chen et al. (2012) and Hseu et al. (2008), gallic acid and CLP extracts both have potent antioxidant properties in vitro that are effective against a variety of oxidative systems, and gallic acid and CLP extracts' capacity for reductive reactions, ability to chelate metals, and effectiveness at scavenging free radicals contribute to their numerous antioxidant qualities.

3.2 Total Phenolic Content (TPC) and Total Flavonoid Contents (TFC) in CLP

The TPC of CLP for the five extracts wear shown in Table 2. TPC in the WE, 25 EE, 50 EE, 75 EE, and 95 EE were 35.85, 76.50, 105.81, 122.10, and 36.08 mg GAE/g, respectively. The results showed the TPC of the five extracts increased from WE to 75 EE ($p < 0.05$). However, the results for the 95 EE dropped significantly. As the results showed, 75 EE of CLP had the highest TPC. Several studies have reported that the highest TPC and TFC were both observed in 75% ethanol extract, followed by 50% ethanol extract. This is similar to the results of our study (Sun et al., 2015; Zhang et al., 2015). In our study, TPC varied from 35.85 to 122.10 mg GAE/g. These results showed a large range of TPC, while other studies in China found the CLP extract reached 262.09 mg GAE/g, which indicates better antioxidant activity (Jiang et al., 2009). According to a study, the TPC of bacaba in powder was 290.93 mg GAE 100g (Santos et al., 2022). The major antioxidant components of these common foods are the phenolic compounds. Diet rich in fruits, vegetables, cereals, and olive oil can prevent cardiovascular diseases and certain forms of cancer (Bendary et al., 2013). According to a study by Shin & Lee (2011), regardless of the extraction method, the extraction efficiency of phenolic substances was best when extracted with 80% ethanol. These results are similar to this study, which improves the solvent affinity of various compounds in the sample.

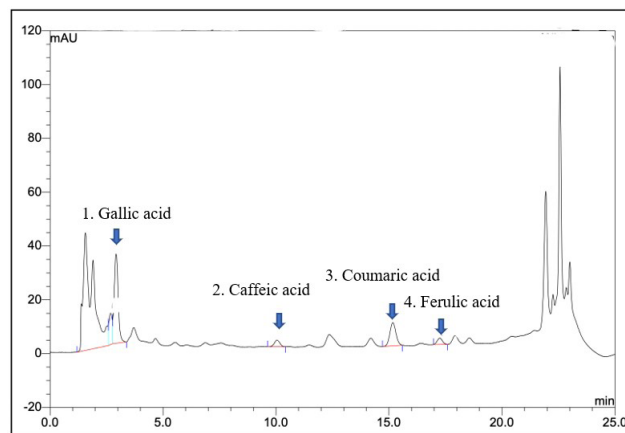


Figure 1. UPLC-PDA chromatogram of extracts from freeze-dried *Cedrela sinensis* leaf powder. Peaks: 1. Gallic acid; 2. Caffeic acid; 3. Coumaric acid; 4. Ferulic acid.

In nature, flavonoids are the largest group of phenolic compounds. Flavonoid compounds belong to the class of phenolic or polyphenol compounds (Perez-Vizcaino & Fraga, 2018). The TFC of CLP for the five extracts were shown in Table 2. TFC in the WE, 25 EE, 50 EE, 75 EE, and 95 EE were 1.07, 11.98, 19.23, 23.23, and 4.73 mg QE/g, respectively. The results showed the TFC of five extracts increased from WE to 75 EE ($p < 0.05$). In one study, the extract of CLP was 324.61 mg QE/g, while in another study it was 108.57 mg RE/g (rutin equivalents). Pietta (2000) said that quercetin has more active hydroxyl groups than rutin, the glycosylated form of quercetin. These results indicated that the TFC in the extract was related to ethanol concentration. According to the study, 75% ethanol contains more flavonoids than other solvents.

3.3 Oxygen Radical Absorbance Capacity (ORAC) in CLP

The ORAC of CLP for the five extracts were shown in Table 2. WE, 25 EE, 50 EE, 75 EE, and 95 EE were 106.39, 170.93, 218.25, 223.65, and 135.18 $\mu\text{M TE/mg}$, respectively. The results showed that the 75 EE of CLP had the highest ORAC. Su et al. (2020) showed that the extract of CLP was 384 $\mu\text{M TE/mg}$. The ORAC assay is a reliable technique that combines the inhibition percentage of several varieties of reactive oxygen species of biologically relevant sources over time (Prior & Cao, 1999). Therefore, ORAC is largely utilized to assess the total antioxidant capacity, and it could also account for the ORAC values being much higher than the DPPH/ABTS⁺ values (Chai et al., 2020).

3.4 DPPH and ABTS radical scavenging activity in CLP

Several studies have shown that certain fruit and vegetable extracts have antioxidant properties (Ediriweera et al., 2017; Kevers et al., 2007). It shows that the DPPH scavenging abilities of all four broccoli extracts increased in a concentration-dependent manner, similar to in our study (Figure 2) (Kim et al., 2021). The results are expressed as IC_{50} , and the lower IC_{50} value indicates stronger antioxidant activity. The 75 EE has higher DPPH radical scavenging activity than other extracts ($> 80\%$ at 25 $\mu\text{g/mL}$). Also shown in Table 3, the IC_{50} values obtained from the DPPH radical scavenging activity of WE to 95 EE were 60.57, 30.08, 26.42, 17.64, and 67.26 $\mu\text{g/mL}$, respectively. Sun et al. (2015) also reported that 75% ethanol extract especially exhibited the strongest DPPH radical scavenging activity (IC_{50} 633 $\mu\text{g/mL}$).

All five extracts showed increasing ABTS radical scavenging activity in a concentration-dependent manner (Figure 2). The 75 EE demonstrated the strongest ABTS radical scavenging activity compared with the other extracts. The IC_{50} values obtained from ABTS radical scavenging activity of WE, 25 EE, 50 EE, 75 EE, and 95 EE were 233.69, 101.05, 78.90, 69.91, and 270.79 $\mu\text{g/mL}$, respectively. The IC_{50} values of different propolis extracts showed that 75% extract had the lowest IC_{50} value, indicating the highest ABTS radical scavenging activity (Sun et al., 2015). In our study, the antioxidant ability of L-ascorbic acid and trolox on DPPH and ABTS⁺ scavenging was better than the ethanol extract of CLP.

3.5 Reducing power of CLP

The yellow color of the sample solution changed to various green and blue colors depending on the reducing capacity

Table 2. Total polyphenol and flavonoid content and oxygen radical absorbance capacity (ORAC) of *Cedrela sinensis* leaf powder ethanol extract with different concentration.

Solvents	Total polyphenol contents (mg GAE/g)	Total flavonoid contents (mg QE/g)	ORAC ($\mu\text{M TE/mg}$)
Water extract	35.85 \pm 0.48 ^d	1.07 \pm 0.71 ^e	106.39 \pm 7.51 ^d
25% ethanol extract	76.50 \pm 0.57 ^c	11.98 \pm 1.77 ^c	170.93 \pm 2.52 ^b
50% ethanol extract	105.81 \pm 1.37 ^b	19.23 \pm 0.71 ^b	218.25 \pm 7.09 ^a
75% ethanol extract	122.10 \pm 1.27 ^a	23.23 \pm 0.71 ^a	223.65 \pm 4.33 ^a
95% ethanol extract	36.08 \pm 2.10 ^d	4.73 \pm 0.24 ^d	135.18 \pm 12.01 ^c

All values were expressed as mean \pm SD (n = 3). Means followed by different letters (a-e) within the same column are significantly different ($p < 0.05$). GAE: gallic acid equivalent; QE: quercetin equivalent; TE: trolox equivalent.

Table 3. DPPH and ABTS radical scavenging activity of *Cedrela sinensis* leaf powder ethanol extract with different concentration.

Solvents	DPPH radical scavenging activity IC_{50} ($\mu\text{g/mL}$)	ABTS radical scavenging activity IC_{50} ($\mu\text{g/mL}$)
Water extract	60.57 \pm 3.60 ^b	233.69 \pm 6.64 ^b
25% ethanol extract	30.08 \pm 0.37 ^c	101.05 \pm 4.73 ^c
50% ethanol extract	26.42 \pm 1.78 ^c	78.90 \pm 1.38 ^d
75% ethanol extract	17.64 \pm 0.21 ^d	69.91 \pm 3.31 ^d
95% ethanol extract	67.26 \pm 4.56 ^a	270.79 \pm 10.68 ^a
L-ascorbic acid	1.80 \pm 0.20 ^e	28.61 \pm 5.86 ^e
Trolox	2.16 \pm 0.12 ^e	29.93 \pm 1.81 ^e

All values were expressed as mean \pm SD (n = 3). Means followed by different letters (a-e) within the same column are significantly different ($p < 0.05$).

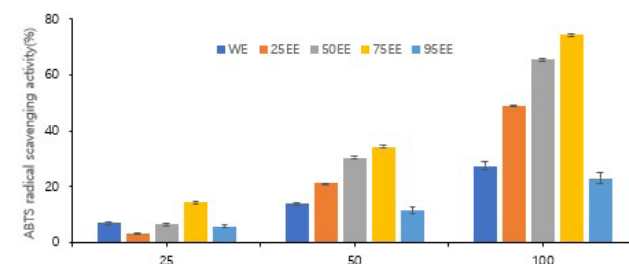
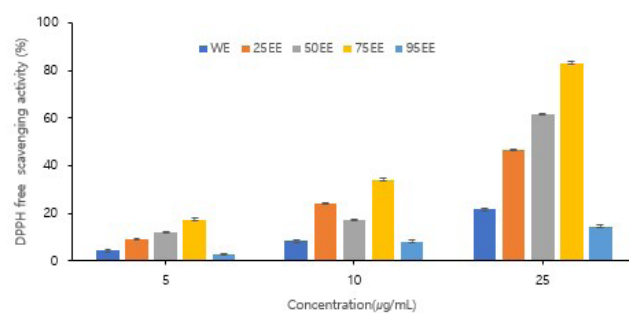


Figure 2. DPPH and ABTS⁺ scavenging activity of *Cedrela sinensis* leaf powder ethanol extract with different concentration, water extract (WE), 25% ethanol extract (25 EE), 50% ethanol extract (50 EE), 75% ethanol extract (75 EE), and 95% ethanol extract (95 EE).

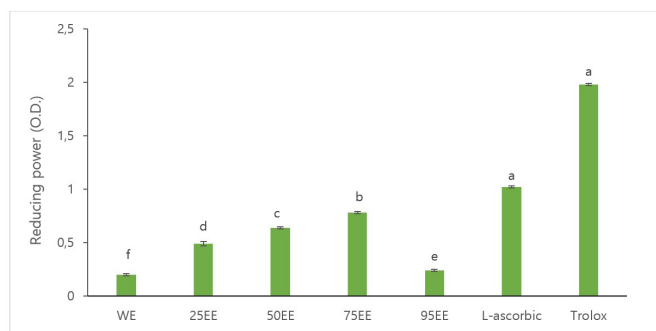


Figure 3. Reducing power of *Cedrela sinensis* leaf powder ethanol extract with different concentration, water extract (WE), 25% ethanol extract (25 EE), 50% ethanol extract (50 EE), 75% ethanol extract (75 EE), and 95% ethanol extract (95 EE).

Table 4. α -Amylase and α -glucosidase inhibitory activity of *Cedrela sinensis* leaf powder ethanol extract with different concentration.

Solvents	α -Amylase inhibitory activity IC_{50} (μ g/mL)	α -Glucosidase inhibitory activity IC_{50} (μ g/mL)
Water extract	> 10,000	1849.14 \pm 62.41 ^a
25% ethanol extract	4969.70 \pm 386.93 ^a	44.65 \pm 1.86 ^c
50% ethanol extract	183.54 \pm 12.14 ^b	1.92 \pm 0.01 ^c
75% ethanol extract	158.34 \pm 1.87 ^b	1.86 \pm 0.01 ^c
95% ethanol extract	> 10,000	> 10,000
Acarbose	214.37 \pm 7.53 ^b	332.36 \pm 43.10 ^b

Means followed by different letters (a-c) within the same column are significantly different ($p < 0.05$). All values were expressed as mean \pm SD ($n = 3$).

(Chen et al., 2012). The reducing power of CLP with the five extracts are shown in Figure 3, and the WE, 25 EE, 50 EE, 75 EE, and 95 EE were 0.10, 0.49, 0.64, 0.78, and 0.24, respectively, at 500 μ g/mL. The reducing power value increased from WE to 75 EE, but 95 EE of CLP decreased significantly. The use of L-ascorbic acid and Trolox as reference standards demonstrated that the concentrations of extracts had a dependent effect. 75 EE of CLP is very close to the value of L-ascorbic acid and Trolox compared to other concentrations. The greater reducing power of the *Cedrela sinensis* leaf extracts and gallic acid correlates well with their marked antioxidant abilities, indicating the possible contribution of reducing power to this activity (Hseu et al., 2008).

3.6 α -Amylase and α -glucosidase inhibitory activity in CLP

α -Amylase and α -glucosidase inhibitory effects increased with increasing ethanol concentrations from 25% to 75%, but not by the 95 EE ($p < 0.05$). As seen in Table 4, the IC_{50} values of 75 EE exhibited the highest inhibitory effect against α -amylase and α -glucosidase among the five extracts, with IC_{50} values of 158.34 and 1.86 μ g/mL, respectively. Compared with acarbose with IC_{50} values at 214.37, 332.36, with 75 EE exhibited strong inhibitory activity against α -amylase and α -glucosidase, which is comparable with positive control of acarbose. These results confirm that CLP have α -glucosidase and α -amylase inhibitory properties. Srinuanchai et al. (2021) also reported that the 75% ethanol extract with *Gymnema inodorum* (Lour.) Decne leaf had a similarly high increase of α -amylase and α -glucosidase inhibitory activity.

4 Conclusion

This study evaluated the total phenolic and total flavonoid content, antioxidant activity, and enzyme inhibition activity of CLP extracts with distilled water and ethanol of different concentrations (WE, 25 EE, 50 EE, 75 EE, and 95 EE). According to our experimental results, 75 EE may be an excellent solvent for extracting the chemical constituents of CLP. Additionally, 75 EE acts as an antioxidant and had excellent phenolic and flavonoid content. The 75EE also had better potential to inhibit α -amylase and α -glucosidase. Moreover, analysis of phenolic compounds in CLP by LC-ESI-MS indicated the presence of gallic acid, caffeic acid, coumaric acid, and ferulic acid. Based on these results, we speculate that the CLP extracts and gallic acid have powerful antioxidant activity against various oxidative systems in vitro. This study guides the development of natural antioxidants and supports the use of *Cedrela sinensis* leaf as a nutritious food.

References

- Bak, M. J., Jeong, J. H., Kang, H. S., Jin, K. S., Ok, S., & Jeong, W. S. (2009). *Cedrela sinensis* leaves suppress oxidative stress and expressions of iNOS and COX-2 via MAPK signaling pathways in RAW 264.7 cells. *Preventive Nutrition and Food Science*, 14(4), 269-276. <http://dx.doi.org/10.3746/jfn.2009.14.4.269>.
- Bendary, E., Francis, R. R., Ali, H. M. G., Sarwat, M. I., & Hady, S. (2013). Antioxidant and structure-activity relationships (SARs) of some phenolic and anilines compounds. *Annals of Agricultural Science*, 58(2), 173-181. <http://dx.doi.org/10.1016/j.aos.2013.07.002>.
- Bhandari, M. R., Jong-Anurakkun, N., Hong, G., & Kawabata, J. (2008). α -Glucosidase and α -amylase inhibitory activities of nepalese medicinal herb pakhanbhed (*Bergenia ciliata*, Haw.). *Food Chemistry*, 106(1), 247-252. <http://dx.doi.org/10.1016/j.foodchem.2007.05.077>.
- Chai, Z., Tian, L., Yu, H., Zhang, L., Zeng, Q., Wu, H., Yan, Z., Li, D., Hutabarat, R. P., & Huang, W. (2020). Comparison on chemical compositions and antioxidant capacities of the green, oolong, and red tea from blueberry leaves. *Food Science & Nutrition*, 8(3), 1688-1699. <http://dx.doi.org/10.1002/fsn3.1455>. PMID:32180976.
- Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002a). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Yao Wu Shi Pin Fen Xi*, 10(3), 3. <http://dx.doi.org/10.38212/2224-6614.2748>.
- Chang, H. C., Hung, W. C., Huang, M. S., & Hsu, H. K. (2002b). Extract from the leaves of *Toona sinensis* roemor exerts potent antiproliferative effect on human lung cancer cells. *The American Journal of Chinese Medicine*, 30(2-3), 307-314. <http://dx.doi.org/10.1142/S0192415X02000223>. PMID:12230019.
- Chang, H. L., Hsu, H. K., Su, J. H., Wang, P. H., Chung, Y. F., Chia, Y. C., Tsai, L. Y., Wu, Y. C., & Yuan, S. S. (2006). The fractionated *Toona sinensis* leaf extract induces apoptosis of human ovarian cancer cells and inhibits tumor growth in a murine xenograft model. *Gynecologic Oncology*, 102(2), 309-314. <http://dx.doi.org/10.1016/j.ygyno.2005.12.023>. PMID:16466781.
- Chen, C. M., Lin, C. Y., Lin, L. C., & Wan, T. C. (2012). Antioxidation activity and total phenolic contents of various *Toona sinensis* extracts. *African Journal of Biotechnology*, 11(73), 13831-13837. <http://dx.doi.org/10.5897/AJB12.2086>.
- Chen, H. M., Wu, Y. C., Chia, Y. C., Chang, F. R., Hsu, H. K., Hsieh, Y. C., Chen, C. C., & Yuan, S. S. (2009). Gallic acid, a major component of *Toona sinensis* leaf extracts, contains a ROS-mediated anti-cancer

- activity in human prostate cancer cells. *Cancer Letters*, 286(2), 161-171. <http://dx.doi.org/10.1016/j.canlet.2009.05.040>. PMID:19589639.
- Cheng, K. W., Yang, R. Y., Tsou, S. C., Lo, C. S., Ho, C. T., Lee, T. C., & Wang, M. (2009). Analysis of antioxidant activity and antioxidant constituents of Chinese toon. *Journal of Functional Foods*, 1(3), 253-259. <http://dx.doi.org/10.1016/j.jff.2009.01.013>.
- Cho, E. J., Yokozawa, T., Rhyu, D. Y., Kim, H. Y., Shibahara, N., & Park, J. C. (2003). The inhibitory effects of 12 medicinal plants and their component compounds on lipid peroxidation. *The American Journal of Chinese Medicine*, 31(6), 907-917. <http://dx.doi.org/10.1142/S0192415X03001648>. PMID:14992543.
- Dong, X. J., Zhu, Y. F., Bao, G. H., Hu, F. L., & Qin, G. W. (2013). New limonoids and a dihydrobenzofuran norlignan from the roots of *Toona sinensis*. *Molecules*, 18(3), 2840-2850. <http://dx.doi.org/10.3390/molecules18032840>. PMID:23455673.
- Ediriweera, M. K., Tennekoon, K. H., Samarakoon, S. R., Thabrew, I., & Silva, E. D. (2017). Induction of apoptosis in MCF-7 breast cancer cells by Sri Lankan endemic mango (*Mangifera zeylanica*) fruit peel through oxidative stress and analysis of its phytochemical constituents. *Journal of Food Biochemistry*, 41(1), e12294. <http://dx.doi.org/10.1111/jfbc.12294>.
- Hseu, Y. C., Chang, W. H., Chen, C. S., Liao, J. W., Huang, C. J., Lu, F. J., Chia, Y. C., Hsu, H. K., Wu, J. J., & Yang, H. L. (2008). Antioxidant activities of *Toona Sinensis* leaves extracts using different antioxidant models. *Food and Chemical Toxicology*, 46(1), 105-114. <http://dx.doi.org/10.1016/j.fct.2007.07.003>. PMID:17703862.
- Hseu, Y. C., Chen, S. C., Lin, W. H., Hung, D. Z., Lin, M. K., Kuo, Y. H., Wang, M. T., Cho, H. J., Wang, L., & Yang, H. L. (2011). *Toona sinensis* (leaf extracts) inhibit vascular endothelial growth factor (VEGF)-induced angiogenesis in vascular endothelial cells. *Journal of Ethnopharmacology*, 134(1), 111-121. <http://dx.doi.org/10.1016/j.jep.2010.11.058>. PMID:21130856.
- Hsu, H. K., Yang, Y. C., Hwang, J. H., & Hong, S. J. (2003). Effects of *Toona sinensis* leaf extract on lipolysis in differentiated 3T3-L1 adipocytes. *The Kaohsiung Journal of Medical Sciences*, 19(8), 385-389. [http://dx.doi.org/10.1016/S1607-551X\(09\)70481-4](http://dx.doi.org/10.1016/S1607-551X(09)70481-4). PMID:12962425.
- Huang, P.-J., Hseu, Y.-C., Lee, M.-S., Kumar, K. J. S., Wu, C.-R., Hsu, L.-S., Liao, J.-W., Cheng, I.-S., Kuo, Y.-T., Huang, S.-Y., & Yang, H.-L. (2012). In vitro and in vivo activity of gallic acid and *Toona sinensis* leaf extracts against HL-60 human promyelocytic leukemia. *Food and Chemical Toxicology*, 50(10), 3489-3497. <http://dx.doi.org/10.1016/j.fct.2012.06.046>. PMID:22771367.
- Jiang, S.-H., Wang, C.-L., Chen, Z.-Q., Chen, M.-H., Wang, Y.-R., Liu, C.-J., Zhou, Q.-L., & Li, Z.-J. (2009). Antioxidant properties of the extract and subfractions from old leaves of *Toona sinensis* roem (meliaceae). *Journal of Food Biochemistry*, 33(3), 425-441. <http://dx.doi.org/10.1111/j.1745-4514.2009.00226.x>.
- Kakumu, A., Ninomiya, M., Efdi, M., Adfa, M., Hayashi, M., Tanaka, K., & Koketsu, M. (2014). Phytochemical analysis and antileukemic activity of polyphenolic constituents of *Toona sinensis*. *Bioorganic & Medicinal Chemistry Letters*, 24(17), 4286-4290. <http://dx.doi.org/10.1016/j.bmcl.2014.07.022>. PMID:25074815.
- Kevers, C., Falkowski, M., Tabart, J., Defraigne, J. O., Dommès, J., & Pincemail, J. (2007). Evolution of antioxidant capacity during storage of selected fruits and vegetables. *Journal of Agricultural and Food Chemistry*, 55(21), 8596-8603. <http://dx.doi.org/10.1021/jf071736j>. PMID:17880151.
- Kim, D. I., & Hong, J. H. (2012). Optimization of ethanol extraction conditions for functional components from *Lespedeza cuneata* using response surface methodology. *Korean Journal of Food and Cookery Science*, 28(3), 275-283. <http://dx.doi.org/10.9724/kfcs.2012.28.3.275>.
- Kim, H. Y., Ediriweera, M. K., Boo, K. H., Kim, C. S., & Cho, S. K. (2021). Effects of cooking and processing methods on phenolic contents and antioxidant and anti-proliferative activities of broccoli florets. *Antioxidants*, 10(5), 641. <http://dx.doi.org/10.3390/antiox10050641>. PMID:33922092.
- Kim, S. J., Kweon, D. H., & Lee, J. H. (2006). Investigation of antioxidative activity and stability of ethanol extracts of licorice root (*Glycyrrhiza glabra*). *Korean Journal of Food Science Technology*, 38(4), 584-588.
- Lee, I. S., Kim, H. J., Youn, U. J., Chen, Q. C., Kim, J. P., Ha, D. T., Ngoc, T. M., Min, B.-S., Lee, S.-M., Jung, H.-J., Na, M.-K., & Bae, K.-H. (2010). Dihydrobenzofuran norlignans from the leaves of *Cedrela sinensis* A. Juss. *Helvetica Chimica Acta*, 93(2), 272-276. <http://dx.doi.org/10.1002/hlca.200900180>.
- Lee, I. S., Wei, C. H., Thoung, P. T., Song, K. S., Seong, Y. H., & Bae, K. H. (2006). Antioxidant constituents from the leaves of *Cedrela sinensis* A. Juss. *Korean Journal of Medicinal Crop Science*, 14(5), 267-272.
- Lee, S., Cho, J.-H., Park, K. D., Kim, Y.-D., & Yim, S.-H. (2022). Assessment of validation and antioxidant activities of novel 12 Korean strawberry cultivars. *Food Science and Technology*, 42, e76121. <http://dx.doi.org/10.1590/fst.76121>.
- Maulana, T. I., Falah, S., & Andrianto, D. (2019). Total phenolic content, total flavonoid content, and antioxidant activity of water and ethanol extract from Surian (*Toona sinensis*) leaves. *IOP Conference Series: Earth and Environmental Science*, 299, 012021. <http://dx.doi.org/10.1088/1755-1315/299/1/012021>.
- Mu, R., Wang, X., Liu, S., Yuan, X., Wang, S., & Fan, Z. (2007). Rapid determination of volatile compounds in *Toona sinensis* (A. Juss.) Roem. by MAE-HS-SPME followed by GC-MS. *Chromatographia*, 65(7-8), 463-467. <http://dx.doi.org/10.1365/s10337-007-0183-0>.
- Ou, B., Hamsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry*, 49(10), 4619-4626.
- Perez-Vizcaino, F., & Fraga, C. G. (2018). Research trends in flavonoids and health. *Archives of Biochemistry and Biophysics*, 646, 107-112. <http://dx.doi.org/10.1016/j.abb.2018.03.022>. PMID:29580946.
- Pietta, P. G. (2000). Flavonoids as antioxidants. *Journal of Natural Products*, 63(7), 1035-1042. <http://dx.doi.org/10.1021/np9904509>. PMID:10924197.
- Poon, S. L., Leu, S.-F., Hsu, H.-K., Liu, M.-Y., & Huang, B.-M. (2005). Regulatory mechanism of *Toona sinensis* on mouse leydig cell steroidogenesis. *Life Sciences*, 76(13), 1473-1487. <http://dx.doi.org/10.1016/j.lfs.2004.08.026>. PMID:15680312.
- Prior, R. L., & Cao, G. (1999). In vivo total antioxidant capacity: comparison of different analytical methods. *Free Radical Biology & Medicine*, 27(11-12), 1173-1181. [http://dx.doi.org/10.1016/S0891-5849\(99\)00203-8](http://dx.doi.org/10.1016/S0891-5849(99)00203-8). PMID:10641708.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, 26(9-10), 1231-1237. [http://dx.doi.org/10.1016/S0891-5849\(98\)00315-3](http://dx.doi.org/10.1016/S0891-5849(98)00315-3). PMID:10381194.
- Santos, O. V., Viana, A. A., Soares, S. D., Vieira, E. L. S., Martins, M. G., Nascimento, F. C. A., & Teixeira-Costa, B. E. (2022). Industrial potential of Bacaba (*Oenocarpus bacaba*) in powder: antioxidant activity, spectroscopic and morphological behavior. *Food Science and Technology*, 42, e62820. <http://dx.doi.org/10.1590/fst.62820>.
- Shafay, S. E., El-Sheekh, M., Bases, E., & El-Shenody, R. (2022). Antioxidant, antidiabetic, anti-inflammatory and anticancer potential

- of some seaweed extracts. *Food Science and Technology*, 42, e20521. <http://dx.doi.org/10.1590/fst.20521>.
- Shin, S. L., & Lee, C. H. (2011). Antioxidant activities of ostrich fern by different extraction methods and solvents. *Journal of Life Science*, 21(1), 56-61. <http://dx.doi.org/10.5352/JLS.2011.21.1.56>.
- Silva, E. S., Santos, H. B. Jr., Guedes, T. J. F. L., Sandes, R. D. D., Rajan, M., Leite, M. T. S. Na., & Narain, N. (2022). Comparative analysis of fresh and processed mango (*Mangifera indica* L, cv. "Maria") pulps: influence of processing on the volatiles, bioactive compounds and antioxidant activity. *Food Science and Technology*, 42, e54020. <http://dx.doi.org/10.1590/fst.54020>.
- Srinuanchai, W., Nooin, R., Pitchakarn, P., Karinchai, J., Suttisansanee, U., Chansrinoyom, C., Jarussophon, S., Temviriyankul, P., & Nuchuchua, O. (2021). Inhibitory effects of *Gymnema inodorum* (Lour.) Decne leaf extracts and its triterpene saponin on carbohydrate digestion and intestinal glucose absorption. *Journal of Ethnopharmacology*, 266, 113398. <http://dx.doi.org/10.1016/j.jep.2020.113398>. PMID:32971162.
- Su, S., Wang, L., Ni, J., Geng, Y., & Xu, X. (2020). Diversity of red, green and black cultivars of Chinese Toon [*Toona sinensis* (A. Juss.) Roem]: anthocyanins, flavonols and antioxidant activity. *Journal of Food Measurement and Characterization*, 14(6), 3206-3215. <http://dx.doi.org/10.1007/s11694-020-00560-8>.
- Sun, C., Wu, Z., Wang, Z., & Zhang, H. (2015). Effect of ethanol/water solvents on phenolic profiles and antioxidant properties of Beijing propolis extracts. *Evidence-Based Complementary and Alternative Medicine*, 2015, 595393. <http://dx.doi.org/10.1155/2015/595393>. PMID:26351514.
- Wang, C. Y., Lin, K. H., Yang, C. J., Tsai, J. R., Hung, J. Y., Wang, P. H., Hsu, H. K., & Huang, M. S. (2010). *Toona sinensis* extracts induced cell cycle arrest and apoptosis in the human lung large cell carcinoma. *The Kaohsiung Journal of Medical Sciences*, 26(2), 68-75. [http://dx.doi.org/10.1016/S1607-551X\(10\)70010-3](http://dx.doi.org/10.1016/S1607-551X(10)70010-3). PMID:20123594.
- Xu, Y., Niu, X., Liu, N., Gao, Y., Wang, L., Xu, G., Li, X., & Yang, Y. (2018). Characterization, antioxidant and hypoglycemic activities of degraded polysaccharides from blackcurrant (*Ribes nigrum* L.) fruits. *Food Chemistry*, 243, 26-35. <http://dx.doi.org/10.1016/j.foodchem.2017.09.107>. PMID:29146337.
- Yang, Y., Yang, J. X., Wang, R., & Li, W. Y. (2011). The extraction and determination of total flavonoids of *Toona sinensis* cultivated in Taihe county. *Zhongguo Shengwuzhipinxue Zazhi*, 28, 91-93.
- Yang, Y.-C., Hwang, J.-H., Hong, S.-J., & Hsu, H.-K. (2003). Enhancement of glucose uptake in 3T3-L1 adipocytes by *Toona sinensis* leaf extract. *The Kaohsiung Journal of Medical Sciences*, 19(7), 327-332. [http://dx.doi.org/10.1016/S1607-551X\(09\)70433-4](http://dx.doi.org/10.1016/S1607-551X(09)70433-4). PMID:12926517.
- Zhang, H., Wang, X., Wang, K., & Li, C. (2015). Antioxidant and tyrosinase inhibitory properties of aqueous ethanol extracts from monofloral bee pollen. *Journal of Apicultural Science*, 59(1), 119-128. <http://dx.doi.org/10.1515/jas-2015-0013>.