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In vitro Cytotoxicity and Antioxidation of a Whole Fruit Extract of *Liquidambar formosana* Exerted by Different Constituents

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JZ and GC designed the study. Author JZ performed fractionation, UPLC analysis, antioxidation study, literature search, and wrote the first draft of the manuscript. Authors ZL and GYK wrote the protocol and performed the cytotoxicity study. Authors ZL and GC revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The fruit of *Liquidambar formosana Hance* under the name of Lu Lu Tong (LLT) has been used as a traditional Chinese medicine in China for thousands of years. This study was undertaken to attempt to illustrate some of the pharmacological effects by screening for its cytotoxic and antioxidant activities with *in vitro* assays.
Methodology: LLT extract was initially prepared with 95% aqueous ethanol, and then fractionated based on solvent polarity into three fractions of petroleum ether (LLT-P), dichloromethane (LLT-C), and methanol (LLT-M). human colon adenocarcinoma Cells HT-29 cultured in Dulbecco's Modified

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Eagle's Medium were treated with LLT extracts in the concentration range of 0.39 µg/mL and 100 µg/mL and assayed by MTS. The antioxidant activities of each LLT fraction was reacted with a stable free radical of DPPH (1, 1-diphenyl-2-picrylhydrazyl) and ABTS•$^{+}$ (2, 20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt). Major constituents of three fractions were analyzed by UPLC-MS.

Results: Among the three fractions, LLT-M exhibited a strong antioxidant activity but the others had minimal or negligible effects. In contrast, the potent antioxidant fraction (LLT-M) showed essentially no cytotoxicity whereas the two fractions, LLT-P and LLT-C, were significantly cytotoxic.

Conclusion: Cytotoxicity and Antioxidant properties exhibited by LLT came from different constituents residing in different fractions of solvent affinity.

Keywords: Antioxidation; cytotoxicity; HT-29; fractionation; Liquidambar formosana.

1. INTRODUCTION

Four species are identified in the sweetgum genus of *Liquidambar* (*Hamamelidaceae*) with three residing in Asia and one in North America [1]. The mature fruit collected from the Chinese sweetgum tree *Liquidambar formosana Hance* is a Traditional Chinese Medicine (TCM) ingredient under the specific name of Lu Lu Tong (LLT). Despite the long history of use, its pharmacological mechanisms have not been fully and closely examined in modern scientific settings. In a multi-herb formulation of TCM, LLT has shown many pharmacological properties and was used to play the role of improving collateral circulation, amenorrhea [2], and arthralgia [3], promoting urinationtreating chronic renal diseases, removing numbness [4], reducing edema [5], relieving pain, activating collaterals, promoting blood circulation [6], and anti-inflammation and analgesics [7]. Oleanane triterpenoids in LLT exhibited a strong inhibition against the nuclear factor of activated T-cells and platelet aggregation induced by adenosine diphosphate [5,8]. LLT demonstrated the ability to reduce virus-induced cytopathic effects and virus yield in Madin-Darby canine kidney cells, and potently inhibited neuraminidase activity [9]. Among these triterpenes, lanostanes, cucurbitanes, and oleananes are probably the most interesting groups correlated to the immune responses and cancer or inflammation studies [10]. In addition to exerting cytotoxicity against cancer cells directly, antioxidation has become an approach for the prevention and therapy of diseases because oxidation was associated with the initiation and progression of cardiovascular, tumoral, and inflammatory conditions [11].

In recent natural product chemistry investigations, LLT was found to mainly contain triterpenoids such as betulinic acid, oleanolic acid, and lantanolic acid [6,12]. Other major constituents are tannins [13] and flavan glycosides [14]. In modern TCM, where each herbal ingredient is characterized by mostly one compound as qualitative index in China, betulinic acid is chosen to indicate LLT, [4] based primarily on chemical abundance, but not necessarily pharmacological activities responsible for reported bioactivities.

The North American species *L. styraciflua*, better known as sweetgum, was less used as an herbal medicine but more investigated in pharmacology. Sweetgum was found to possess anti-cancer properties, but little was known for the *L. formosana* species indigenous to China. TCM often uses the whole extract of all extractable constituents, while our objective is to narrow the whole extract to specific chemical constituents via fractionation. To assess the successful fractionation, fingerprinting analysis of each fraction is required. Subsequently, qualitative elucidation is needed to link chemical constituents to pharmacological activities. From this angle, this study was an expansion of traditional knowledge. In the meantime, antioxidation was screened for leaf extract [15], but not for the fruit. Based on the historical uses and exhibited pharmacological properties of limited scientific investigations, this study was undertaken to examine the dual pharmacological properties of antioxidation and cytotoxicity, and elucidate different constituents through phytochemical fractionation.

2. MATERIALS AND METHODS

2.1 Standards and Reagents

The human colon adenocarcinoma (HT-29) cell line was obtained from the American Type Culture Collection (Maryland, USA). Dulbecco's

Modified Eagle's Medium (DMEM), 10% fetal bovine serum (FBS), N-2-Hydroxyethylpiperazine-N´-2-ethanesulfonic Acid (HEPES), penicillin, streptomycin, sodium pyruvate, L-glutamine, and non-essential amino acids were purchased from Invitrogen Corporation (Carlsbad, CA, USA). Curcumin (used as positive control for the cytotoxicity assay) with purity of 96.4% was purchased from Chromadex Inc. (Irvine, CA, USA). The agents of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT) and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt (ABTS) were purchased from Sigma-Aldrich China (Shanghai, China). Dichloromethane (CH_2Cl_2), petroleum ether, methanol, ethanol, formic acid were purchased from China National Medicine Group Shanghai Corporation (Shanghai, China). All chemicals and solvents were of analytical grade. Acetonitrile (Merck & Co. USA) and distilled water (Watson Group Shanghai Co., China) were of HPLC grade.

2.2 Preparation of *L. formosana* Extracts

Dried fruits of *L. formosana* (LLT) purchased from Kangqiao Herbal Material Company (Shanghai, China) were ground to particle sizes of approximately 5 mm, 500g of which was then extracted twice (1:10 w/v) with 5 L of 95% aqueous ethanol for 4 hours at 70°C. The solvent in the combined liquid extract (approximately 10 L) was then evaporated using a rotary evaporator (EYELA, Shanghai Ailang Co., Shanghai, China) under reduced pressure at 55°C to derive the ethanolic extract (30 g). This extract was then fractionated by silica gel (900 g, Particle size 100mesh, Qingdao Marine Chemical Factory, China) column chromatography. After loading the sample, the extract was sequentially eluted with petroleum ether, dichloromethane, and methanol. Solvent in each fractioned liquid extract was then evaporated to derive the fraction samples named as LLT-P, LLT-C, and LLT-M.

2.3 UPLC-MS Analysis

An ultra-performance liquid chromatography coupled with electrospray ionization mass spectrometry (UPLC-ESI-MS; Acquity-Quattro Premier, Waters Co.) was used to analyze LLTextracts. To perform the analyses, 10 mg each of LLT-P, LLT-C, and LLT-M samples was accurately weighed in 10 mL acetonitrile. The sample was extracted under sonication for 10 min and the solution was filtered through a 0.2 μm syringe filter prior to UPLC analysis. After a pretreatment procedure, the sample was separated on an Acquity UPLC BEH C18 column (20 ×100 mm, 1.7 μm) with a mobile phase consisting of A (acetonitrile with 0.1% formic acid, v/v) and B (distilled water with 0.1% formic acid, v/v). The gradient elution was designed as follows: from 0 to 15 min, a linear change for A from 5% to 25% (v/v); from 15 to 50 min, a linear change for A from 25% to 85% (v/v); and then hold A at 85% until 55 min, when the run was complete. Injection volume was kept at 5 μL and a flow rate was set at 0.20 mL/min. The column temperature was maintained at 30ºC. MS detection was performed by mass scan. The electrospray ionization source was applied and operated in both positive and negative ion modes (ESI±). The cone voltage was set at 40V and a full scan was obtained over the m/z range from 50 to 1000 Daltons to obtain total ion chromatography (TIC) for each sample.

2.4 Antioxidant Scavenging Activity

The antioxidant activities of each LLT sample was reacted with a stable free radical of DPPH (1, 1-diphenyl-2-picrylhydrazyl) and the DPPH radical scavenging activity was assayed using the modified method of Yosra [16]. Each extract sample was dissolved in ethanol to form its stock solution ranging from 1 to 10 mg/mL, 10 μL of which was transferred into 3 mL ethanol solution containing 0.1 mM of DPPH to allow reaction in an incubator at 30ºC. The scavenging activity of the DPPH radical was determined by UV spectrophotometer (757CR, Shanghai Precision Instrument Co., China) for the absorbance at 520 nm every 30 sec. for 15 min. Each LLT extract sample was reacted with the stable $ABTS^{\bullet+}$ (2, 20-azino-bis (3- ethylbenzothiazoline-6-sulfonic acid) diamonium salt) radical cation and then assayed according to the modified method of Wang [15]. $ABTS^{\bullet+}$ was produced by reacting ABTS with potassium persulfate ($K_2S_2O_8$). A stock solution of ABTS (7 mM) was prepared in phosphate-buffered saline (PBS, 50 mL). $ABTS^{\bullet+}$ was produced by reacting stock (50 mL) with $K_2S_2O_8$ water solution (50 mL). The mixture was left to stand in the dark at room temperature for about 15 h before use. For the evaluation of antioxidant activity, the $ABTS^{\bullet+}$ solution was diluted in PBS and equilibrated to 30°C to obtain an absorbance using UV spectrophotometer (757CRT, Shanghai Precision Instrument Co., China) at 730 nm. The mixture LLT sample solution (10 μL) with the $ABTS^{\bullet+}$ solution (3 mL)

were then assayed for absorbance at ambient temperature after 6 min. The antioxidant activity was expressed as percentage of scavenging activity on DPPH or $ABTS^{\bullet+}$ radical of the samples:

$$\text{Scavenging activity (\%)} = [(Ac_{(0)} - A_{A(t)}) / Ac_{(0)}] \times 100$$

Where $A_{C(0)}$ is the absorbance of the control (blank) at t=0 and $A_{A(t)}$ is the absorbance in the presence of the sample at t=15 min(DPPH) or t=6min($ABTS^{\bullet+}$). Ethanol was used as a blank control. All tests were in duplicate. The DPPH or ABTS radical scavenging activity of BHT was measured for comparison.

2.5 Cytotoxicity Screening Assay

The human colon adenocarcinoma (HT-29) cell line was maintained at 37ºC in a humidified atmosphere with 5% CO_2. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), N-2-Hydroxyethylpiperazine-N´-2-ethanesulfonic Acid (HEPES), penicillin-streptomycin, sodium pyruvate, L-glutamine, and non-essential amino acids. *In vitro* cell viability assays were conducted using the MTS (3-(4, 5-Dimethylthiazol-2-yl) -5- (3-carboxymethoxyphenyl) -2- (4-sulfophenyl) - 2H-tetrazolium) assay. HT-29 cells were added to 96-well plates at 1×10^4 cells/well, respectively, and allowed to adhere for 16 hours. For the activity screening assay, a single dose method was used. In this case, the stock solutions of LLT extracts were made in DMSO at 10 mg/mL then diluted to a final solution of 100 µg/mL that contained 1% DMSO with culture medium supplemented with 10% FBS. The stock solution of curcumin (positive control) was prepared at 1.5 mg/mL in DMSO then diluted to 15.0 µg/mL with culture medium and DMSO in the final solution was kept at 1% v/v. For the determination of IC_{50} (half maximal inhibitory concentration), a range of multiple doses were used. In this case, the cells were treated with LLT extracts in the range of 0.39 µg/mL to 100 µg/mL or with curcumin (positive control) in the range of 0.12 to 30.0 µg/mL in triplicate wells and incubated at 37°C for 72 hrs. DMSO in the final solutions was all kept at 1% v/v. On day three, a 20 µL aliquot of MTS solution premixed with phenazine methosulfate was added directly to each well and the plate was incubated at 37°C for another 2 hrs. Absorbance was measured at 490 nm using a Bio-Rad Microplate Absorbance Reader (iMark, Bio-Rad Co., Hercules, CA, USA). Percent viability was calculated as cell viability relative to vehicle-treated control. The IC_{50} values were the average of at least two independent experiments.

2.6 Statistical Analysis

All data were analyzed using the paired Student's T-test (SAS, Cary, NC) and the means were compared. Significance of tests was set at $P < 0.05$ and data were expressed as mean ± SE (standard error) unless otherwise specified.

3. RESULTS AND DISCUSSION

3.1 Chromatographic Fingerprints Generated By UPLC-MS

Three samples were obtained from the fractionation and named LLT-P (8.2 g), LLT-C (10.7 g), and LLT-M (6.8 g), respectively. Eluting solvents of petroleum ether, dichloromethane, and methanol were used in a gradually increased polarity fashion. This sequential fractionation procedure resulted in the UPLC chromatographic fingerprints that showed variations in their peak occurrences and distribution.

Preliminary identification of major constituents in each of the three fractions was made based on UV and MS characteristics. Chromatographic fingerprints were developed for each fraction at wavelengths of 200-500 nm (Fig. 1). The number of identifiable peaks exhibited among the three fractions was more than thirty. Although same peaks appeared among the three fractions, differences in composition were obvious. Nine peaks were shown and numbered in the LLT-M fraction. The characteristic peaks were primarily detected in the first 10 min of the chromatogram. In a C18 reversed-phase column, the most polar constituents are predicted to elute first. This means LLT-M fraction contained more polar components. Peaks 2 and 3 showed absorptions at 203 to 205 nm and 254 to 262 nm, both of which highest peaks are characteristic of phenols. Mass fragments of m/z 181, m/z 137, and m/z 83 exhibited from these peaks are characteristic of phenols according to reference [17]. Those compounds with oxhydryl, carboxyl, carbonyl or methoxyl are subject to a logical loss by $[M-18]^+$, $[M-43]^+$, $[M-28]^+$, or $[M-30]^+$during ionization. Based on the predicted polarity as well as UV and MS spectra, these peaks are identified to be phenolic compounds (Fig. 2). There were no other obvious peaks after ten min of retention time, thus the LLT-M is characterized as the phenols-rich fraction.

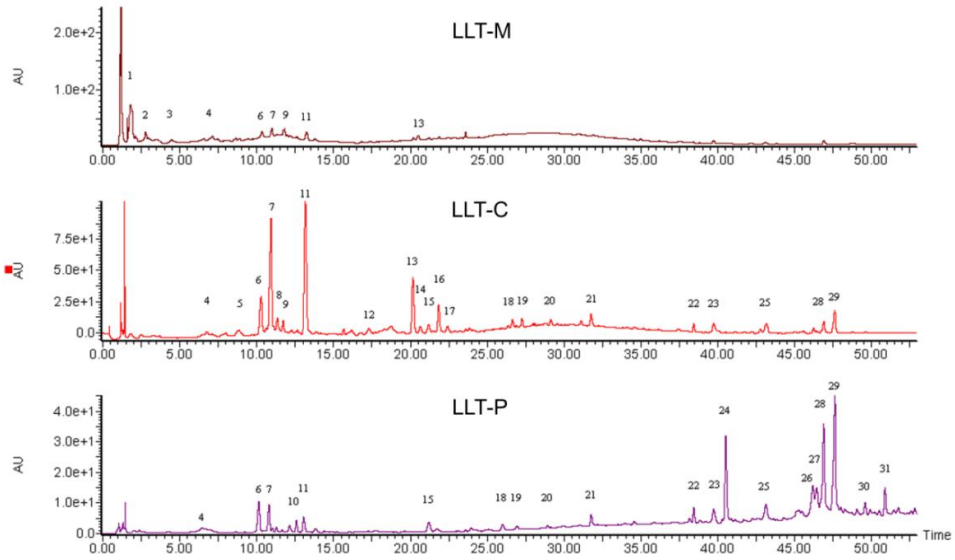

Fig. 1. Chromatographic fingerprints of three fraction extracts prepared from LLT at wavelengths of maximal absorption in the range of 200-500 nm. Upper: LLT-M; middle: LLT-C; and lower: LLT-P

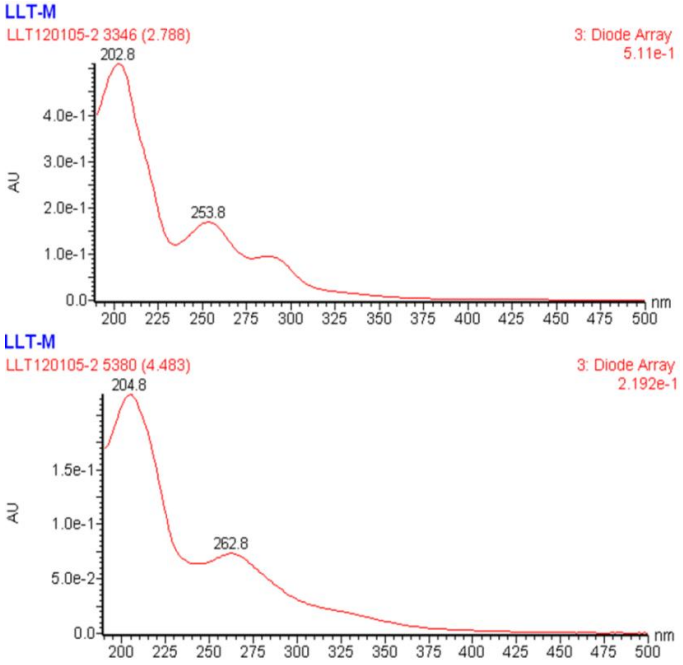

Fig. 2. UV spectrum of peak 2(upper) and peak 3(down) selected from the LLT-M fraction. UV absorption spectrum over the range of 200 and 500 nm of two representative peaks from one of the three fraction extracts prepared from the LLT

Twenty-two peaks were identified and numbered in the LLT-C fraction. Peak 7 showed two major absorption peaks at 216 nm and 309 nm; and Peak 11 showed two major absorption peaks at 245 nm and 342 nm. Dual absorption peaks between 216 nm and 245 nm and between 309 nm and 342 nm are characteristic of flavonoids (Fig. 3). UV spectra and MS fragments showed that the characteristics of those compounds matched flavone or isoflavone, flavonol, and chalcone [18]. Usually flavonoids produce mass fragments of $[A_1+H]^+$ m/z 121 (peak 6, peak 21),

B_1^+ m/z 102 (peak 13, peak 16, peak 21), and $[A_1\text{-CO}]^+$ m/z 92 (peak 6). Flavonols with one hydroxyl on the B ring produce a mass fragment of B_2^+ m/z 135 (peak 6) or B_2^+ m/z 165 (peak 7) when two hydroxyl groups are on the B ring. Those isoflavones with methoxyl group on the ring produce two mass fragments of m/z 181 (peak 7) and m/z 191 (peak 7, peak 11). Most of the peaks eluted between retention time of 10 and 35 min are flavonoids according totheir UV and MS spectra Similar as peak 7and peak 11. The LLT-C fraction displayed most abundant and numerous peaks during this period, thus is considered a flavonoids-rich fraction.

Twenty peaks were detected in the LLT-P fraction. Although some peaks eluted before 35 min, major peaks appeared after 35 min. Most non-polar compounds including triterpenoids typically elute during this period. Unless α, β-unsaturated ketones were linked in the side chain, UV absorption only occurs at 205 nm or shorter wavelengths for triterpenoids. The highest absorption for peaks 28 and 29 was at 193 nm (Fig. 4). Six oleanane or ursane types of triterpenoids were reportedly isolated and identified from *L. styraciflua* chloroform ($CHCl_3$) extract and *L. formosana* $CHCl_3$ extract [19,20]. Oleanolic acid and ursolic acid with $[M-1]^-$ m/z 454, betulinic acid with $[M-1]^-$ m/z 453, Betulonic acid have characteristic mass fragments of $[M-1]^-$ m/z 454, m/z 439, m/z 234,m/z 189, and other oleanane triterpenoids have characteristic mass fragments of $[M-1]^-$ m/z 471, m/z 469,m/z 453, m/z 409, m/z 301, m/z 234, m/z 189, and m/z 149 matching the data reported in the reference [21]. Oleanolic acid and ursolic acid generate of fracture fragment m/z 189 according to the inverse Dells - Alder rule. Those compounds (peak 22- peak 31) peaked similarly as peak 28 or peak 29 in this retention time period have shown spectral data characteristic of those found for triterpenoids [13]. Triterpenoids appeared to be most abundant in this fraction, thus the LLT-P is characterized as a triterpenoids-rich fraction.

Column chromatography was successful in separating phenols, flavonoids, and triterpenoids into the LLT-M, LLT-C, and LLT-P fractions, which were tested for bioactivities.

3.2 Effects on Antioxidant Activity

The scavenging abilities of various fractions against DPPH radical were concentration-dependent. Compared with the positive control BHT, the phenols-rich LLT-M fraction was found to be a more active DPPH radical scavenger than the other two fractions. The flavonoids-rich LLT-C fraction showed a similar scavenging capacity to that of BHT. In contrast, the triterpenoids-rich LLT-P fraction showed minimal scavenging activity to less than 20%, whereas the phenols-rich and flavonoids-rich fractions generated 35% and 58% scavenging activity, respectively (Fig. 5). This result is in agreement with the findings that the extracts richer in total phenolic compounds and total flavonoids are more potent DPPH radical scavengers [16,22]. The low radical scavenging activity displayed by the LLT-P fraction may have been the result of lacking abundant phenolic compounds and/or flavonoids.

Similar to the DPPH assay, the phenols-rich LLT-M fraction exhibited a stronger antioxidant activity than the positive control BHT and the flavonoids-rich LLT-C fraction. Once again, there was no difference in ABTS radical scavenging activity between the flavonoids-rich LLT-C fraction and the positive control BHT. The triterpenoids-rich LLT-P fraction showed weaker ABTS radical scavenging capacity than BHT or LLT-C (Fig. 6).

The antioxidant properties normalized and expressed by the inhibitory rate on 50 percentage concentration are summarized in Table 1. According to the two methods used for measuring antioxidant potency by the DPPH and ABTS radical assays, antioxidation capability was in the order of LLT-M > LLT-C > LLT-P. Good linear correlations were reported between various antioxidant activities and contents of total phenols [23] or flavonoids [24]. These findings coincide with the results of this study.

Table 1. Antioxidant properties of IC_{50} by DPPH and ABTS radicals of the LLT fraction extracts

Sample	DPPH IC_{50} Value [a] (μg/mL)	ABTS IC_{50} Value [b] (μg/mL)
BHT [c]	54.34±0.86	32.08±0.48
LLT-P	89.08±0.83	35.81±0.17
LLT-C	70.34±0.03	31.28±0.27
LLT-M	25.00±0.03	10.71±0.02

[a] *The inhibitory concentration at which DPPH radicals were scavenged by 50%. Results are expressed as the mean ±SD (n = 2) in each group.*

[b] *The inhibitory concentration at which ABTS radicals were scavenged by 50%. Results are expressed as the mean ±SD (n = 2) in each group.*

[c] *Used as Positive control. Tests were in two replications*

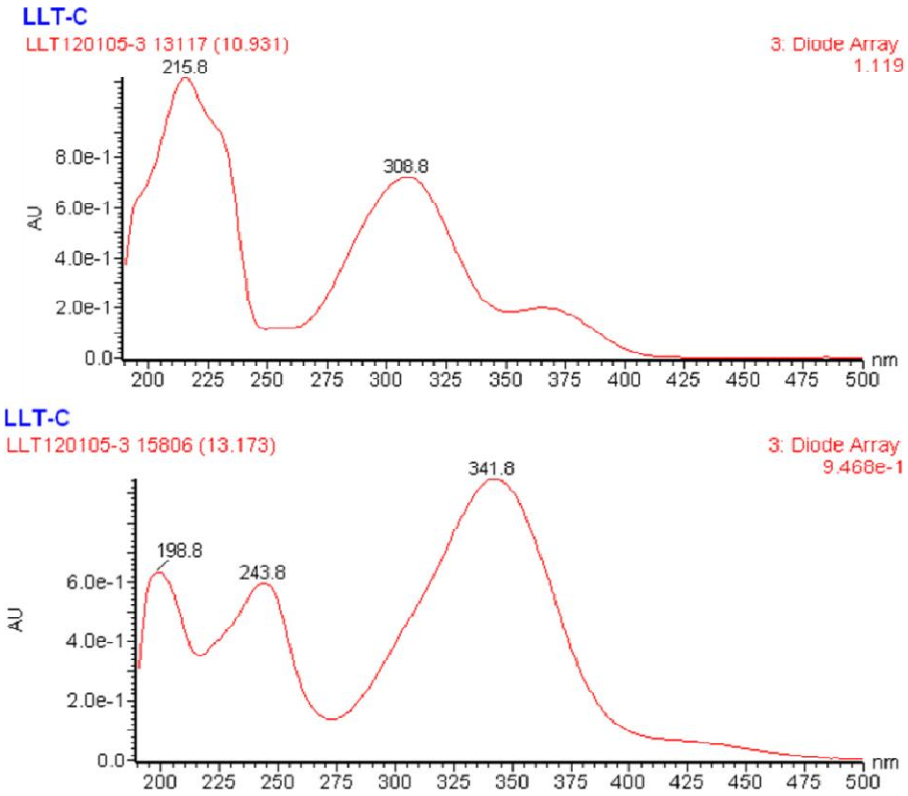

Fig. 3. UV spectrum of peak 7(upper) and peak 11(down) selected from the LLT-C fraction. UV absorption spectrum over the range of 200 and 500 nm of two representative peaks from one of the three fraction extracts prepared from the LLT

3.3 Effects on Cell Viability

At 100 µg/mL, the triterpenoids-rich LLT-P and flavonoids-rich LLT-C fractions inhibited the growth of HT-29 cells by 92.9% and 78.0%, respectively, whereas the phenols-rich LLT-M fraction had negligible cytotoxicity (Table 2). Curcumin (positive control) at 15.0 µg/mL inhibited 50.2%, similar to the report by Zhang [25]. The triterpenoids-rich fraction and flavonoids-rich fraction decreased the viability of HT-29 in a concentration-dependent manner between 12.5 µg/mL and 100 µg/mL with IC_{50} values of 50.9 µg/mL and 73.0 µg/mL, respectively (Fig. 7). The curcumin control displayed an IC_{50} of 11.90µg/mL between 0.12 and 30.0 µg/mL, in agreement with a previous finding of IC_{50} at 36.89 µM (13.58µg/mL) [25]. Further isolation of major triterpenoids and structural elucidation will enable the identification of responsible compounds. The IC_{50} value of 50.9 µg/mL was promising because this fraction is still a mixture of triterpenoids. As it becomes purer, it is generally predicted bioactivity would improve. Some triterpenoids isolated from the sweetgum tree were potently cytotoxic against 39 human cancer cell lines [21]. Triterpenoids of uvaol and oleanolic acid from multiple botanical sources were found to affect cell viability, proliferation, and cycle; entice apoptosis; and increase reactive oxygen species level and oxidative DNA damage to human MCF-7 breast cancer cell line [16]. The multiple bioactivities displayed by the triterpenoids oleanolic acid and its derivatives prompted wide-spread interests in pre-clinical and clinical studies for their anti-tumor, antioxidant, anti-inflammatory, and anti-angiogenic properties [26]. It will be interesting to know if triterpenes in the current study are some of these reported or new. Meanwhile, non-cancerous cells are needed to show that a cytotoxic compound targets cancer cells. For example, Hong [27] found that isolates from Genkwa Flos were almost 400 times more potent against cancer cells than non-cancerous cells. There were no reports on LLT's cytotoxicity against non-cancerous cells. When isolates from our LLT extracts are completed in future studies, it will be necessary to compare them with non-cancerous cells.

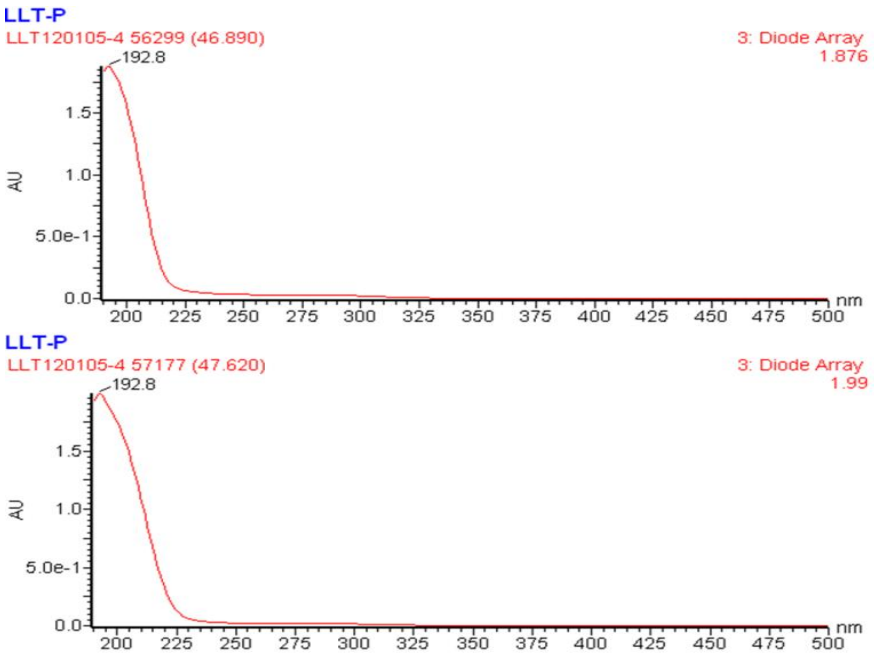

Fig. 4. UV spectrum of peak 28(upper) and peak 29(down) selected from the LLT-P fraction. UV absorption spectrum over the range of 200 and 500 nm of two representative peaks from one of the three fraction extracts prepared from the LLT

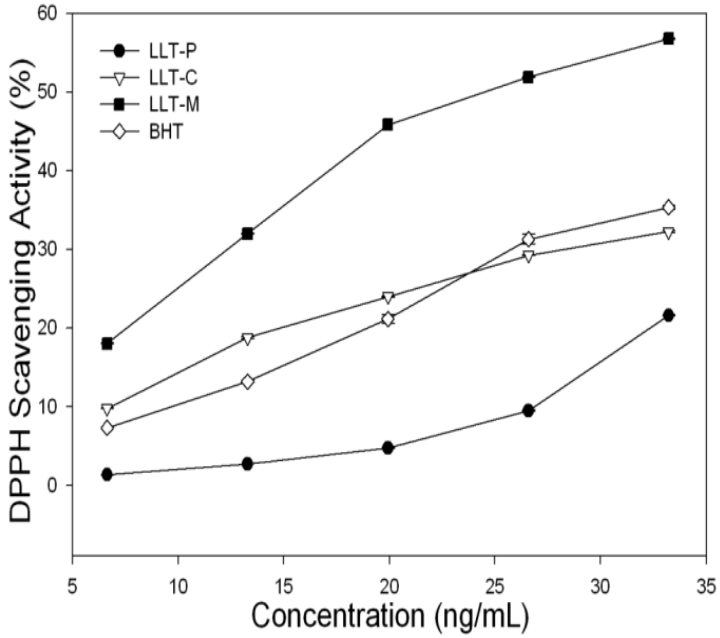

Fig. 5. Different DPPH free radical scavenging activity exerted by the fraction extracts of obtained from the fruit of *Liquidambar formosana*

LLT-M is the methanol fraction; LLT-C is the dichloromethane fraction; LLT-P is the petroleum ether fraction; and BHT is the positive control. Each data point is the mean ± SD of two replications. P < 0.05. The vertical bars across each data point represent one standard deviation.

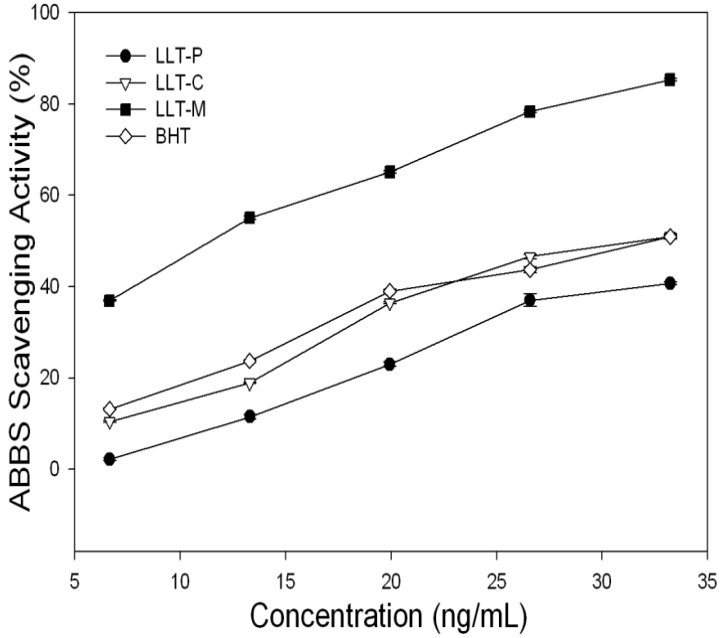

Fig. 6. Different ABTS free radical scavenging activity exerted by the fraction extracts of obtained from the fruit of *Liquidambar formosana*

LLT-M is the methanol fraction; LLT-C is the dichloromethane fraction; LLT-P is the petroleum ether fraction; and BHT is the positive control. Each data point is the mean ± SD of two replications. P < 0.05. The vertical bars across each data point represent one standard deviation

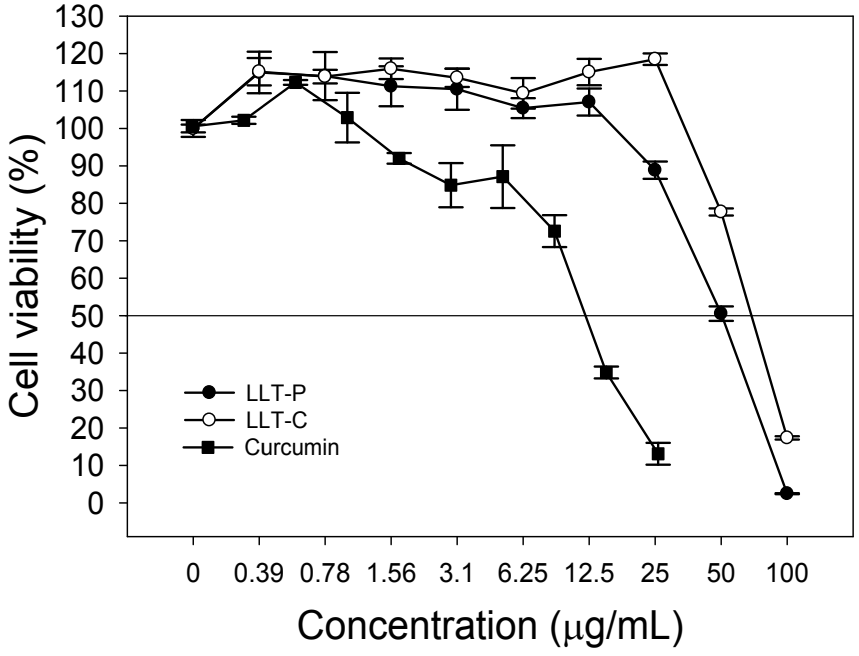

Fig. 7. Cell viability assay showing cytotoxicity of the two LLT fraction extracts against human colon adenocarcinoma cell line (HT-29) by MTS Assay

Absorbance was measured at 490 nm by a Bio-Rad Microplate Reader. Curcumin was used as a positive control. Each data point is the mean± SE of two independent experiments and vertical bars represent one standard error

Table 2. Inhibition of human colon adenocarcinoma (HT-29) cell growth by LLT fraction extracts[a]

Sample	% Inhibition
LLT-P	92.86±0.57
LLT-C	78.01±0.32
LLT-M	0.00
Curcumin	50.20±2.84

[a] *HT-29 human colon cells were added to 96-well plates at 1 × 10^4 cells/well, respectively. The cells were treated with LLT extracts at the dose of 100 µg/mL (LLT-P, LLT-C and LLT-M) and curcumin (positive control) at the dose of 15 µg/mL in triplicate wells.*

4. CONCLUSION

Fractionation of a whole fruit extract using column chromatography techniques was successful in obtaining phenols-rich, flavonoids-rich, and triterpenoids-rich fractions, as indicated from the UV and MS analyses. Corresponding to compositional variations among the three fractions, bioactivities separated. In this study, the antioxidant activity of the *Liquidambar formosana* fruit extract came primarily from the methanol fraction (LLT-M). In contrast, cytotoxicity against a human colon cancer cell line came from the petroleum ether fraction (LLT-P) rich in triterpenoids and the dichloromethane fraction (LLT-C) rich in flavonoids, but not from the methanol fraction (LLT-M) rich in phenols. These interesting findings illustrate the versatility of a single herbal extract in exerting multiple activities of antioxidation and cytotoxicity. If both properties contribute to a concerted effect, chemo-preventive efficacy could be augmented significantly compared to the use of any single component such as the antioxidant fraction or the cytotoxic fractions. Further studies could be designed to elucidate responsible cytotoxic compounds in both fractions and consider the possibility of merging the two into one.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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