科技部補助專題研究計畫成果報告
期末報告

不同萃取方式荔枝花精油之抗氧化、抗發炎、抗菌與抗癌及其機轉之研究

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中 文 摘 要 ： 從多種方法萃取的荔枝花精油以抗氧化試驗評估其抗氧化活性。結果顯示正己烷與低溫超臨界流體萃取的荔枝花精油，其抗氧化力比其他方式的結果較佳。水蒸餾萃取的荔枝花精油是所有之中抗氧化力最差的，尤其其多酚含量很低導致DPPH自由基清除力不佳。此外，氣相層析顯示，水蒸餾萃取的荔枝花精油含有豐富的揮發性成分，因此協助它彌補了在抗氧化反應中因多酚含量低所造成的缺點。

中 文 關 鍵 詞 ： 荔枝花精油，水蒸餾萃取，超臨界流體萃取，DPPH自由基清除，銅離子誘發低密度脂蛋白氧化，β-胡蘿蔔素漂白試驗，人類紅血球細胞溶血試驗

英 文 摘 要 ： Antioxidant activities of litchi flower essential oils extracted from different methods were assessed by varied assays. All the results showed the hexane solvent and low-temperature supercritical fluid extracts illustrated superior antioxidant power among the extracts. The essential oil from hydrodistillation revealed the weakest antioxidant activities compared to all the others, especially in the DDPH radical scavenging assay due to its low total phenol content. On the other hand, the gas chromatograph (GC) indicated the hydrodistillation essential oil contained the abundant volatile fraction which helped to reduce the deficiency of phenolic content in the antioxidant reaction.

英 文 關 鍵 詞 ： Litchi flower essential oil, hydrodistillation, supercritical fluid extraction, DPPH radical-scavenging, Cu2+-induced LDL oxidation, β-Carotene bleaching, human erythrocyte hemolysis

Antioxidant activities of litchi flower essential oils from hydrodistillation, hexane and supercritical fluid methods

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Keywords: Litchi flower essential oil, hydrodistillation, supercritical fluid extraction, DPPH radical-scavenging, Cu^{2+}-induced LDL oxidation, β-Carotene bleaching, human erythrocyte hemolysis

ABSTRACT

Antioxidant activities of litchi flower essential oils extracted from different methods were assessed by varied assays. All the results showed the hexane solvent and low-temperature supercritical fluid extracts illustrated superior antioxidant power among the extracts. The essential oil from hydrodistillation revealed the weakest antioxidant activities compared to all the others, especially in the DDPH radical scavenging assay due to its low total phenol content. On the other hand, the gas chromatograph (GC) indicated the hydrodistillation essential oil contained the abundant volatile fraction which helped to reduce the deficiency of phenolic content in the antioxidant reaction.

INTRODUCION

Plant flowers have been used as foods, drinks and medicinal herbs for a long time in human history. The extraction of flowers by various methods and solvents are continuously developed and studied worldwide for their applications in our life. Litchi (*Litchi chinenesis* Sonn.) is a tropical and subtropical fruit originating from South-east Asia (Rivera-Lopez, Ordorica-Falomir, & Wesche-Ebeling, 1999). It is one of the favorite and important economic crops in Taiwan, which blooms in late March and yields in late June. Litchi has been employed in traditional Chinese medicine to promote human health for a long time and its flower is also dried to make tea.

In a series of works, we had investigated the antioxidant activities of litchi flower (LF) by different extractive solvents and identified its major bio-active components (Liu, Lin, Wang, Chen & Yang, 2009; Chen, Lin, Liu, Lu & Yang, 2011; Yang, Chang, Chen, Liu, Hsu, & Lin, 2012). The anti-inflammatory effect of LF was examined by the inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), as well as the productions of nitric oxide (NO), prostaglandin E2 (PGE2) in lipopolysaccharide-induced (LPS-induced) RAW264.7 cells; the pathways of suppression of inflammatory mediators through inactivation of nuclear factor κB (NF-κB), extracellular signal-regulated kinase (ERK), and Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) was also explored therein (Yang, Chang , Lin, Chen, Hsu & Lin, 2014). The protective abilities against heavy metal induced hepatocytotoxicity and activation of hepatic stellate cells (HSCs) by LF extracts showed litchi flower posses excellent potential to be health food (Hwang, Lin, Liu, Hu, Shyu & Yang, 2013). Nevertheless, the antioxidant activities of LF essential oil (EO) are still unexplored. As well known, essential oil has been used daily since the ancient time for preservatives, cosmetics and medicines *etc.* (Tongnuanchan and

Benjakul, 2014; Shen, Tao, Li, Zhang, Luo & Xia, 2012). There are a large number of literatures concerning the essential oil effects including their antioxidant activity, anti-inflammation, antibacterial and anti-carcinogenesis (Vardar-Ünlü et al., 2003; Bhalla, Gupta & Jaitak, 2013; Frassinetti, Caltavuturo, Cini, Della Croce & Maserti, 2011; Mnayer et al., 2014).

In the present work, we extracted litchi flower essential oil (LFEO) by hydrodistillation (HD), hexane solvent extraction (SE) and carbon dioxide supercritical fluid extraction (CO_2 SFE) methods. The total phenol was compared for different extractive methods. The 2,2-diphenyl-1-picrylhydrazyl hydrate (DDPH) radical-scavenging activity, Cu^{2+}-induced LDL oxidation, β-carotene bleaching, and inhibition of erythrocyte hemolysis assays were used to evaluated the antioxidant power of these three extracts. In addition, the compositions of essential oils were determined by the gas chromatograph-mass spectrometer (GC-MS).

MATERIALS AND METHODS

Reagents and chemicals

Methanol (MeOH), n-hexane, acetic acid (CH_3COOH), were purchased from Merck (Darmstadt, Germany). Deionized water (dd H_2O) was prepared using an Ultrapure^{TM} water purification system (Lotun Co., Ltd. Taipei, Taiwan). Chemicals used for determination of contents of total phenols, including (+)-catechin, Folin–Ciocalteu's phenol reagent, gallic acid and sodium carbonate (Na_2CO_3) were obtained from Sigma Co. (St. Louis, MO, U.S.A.). Chemicals employed for antioxidant capacity assays such as β-carotene, tween 40, linoleic acid, chloroform ($CHCl_3$), sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium chloride (NaCl), ethylenediaminetetraacetic acid (EDTA), Copper (II) sulfate pentahydrate

($CuSO_4 \cdot 5H_2O$), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), phosphate-buffered saline (PBS). 2, 2-diphenyl-1-picryl-hydrazyl hydrate (DPPH), 2–2'-azino-bis-(3-amidinopropane) dihydrochloride (AAPH), α-tocopherol and n-alkanes (C7-C30) were purchased from Sigma Co. (St. Louis, Mo., U.S.A.).

Sample preparation

Fresh LF gathered from Taichung city, Taiwan was lyophilized (at 50 °C for 48 h) in a freeze-drying system (Vastech Scientific Co., Ltd., Taipei, Taiwan) before the EO extraction. There were three different methods used to extract the LFEO. A hundred and eighty grams of the dried LF was subjected to the 4-h hydrodistillation in 1200 mL using the Clevenger-type apparatus. The obtained EO was added with sodium chloride to separate the water phase. After centrifugation, the supernatant was kept at 4 °C under nitrogen until required. For solvent extraction, 20 g LF was extracted in 400 mL hexane for 24 h, the filtrate was dried by rotary evaporator and kept at 4 °C under nitrogen. The LF samples were also extracted at 300, 400 and 500 bar and 40, 60 and 80 °C by CO_2 SFE extractor (Applied Separations, Spe-ed SFE-NP, Allentown, PA, USA), respectively, each time 20 g dried LF in an extractor vessel. The samples were then dissolved in MeOH and filtrated prior to the assays.

Measurement of the total phenolic contents (TPCs)

The contents of total phenols were determined through the method (Julkunen-Titto, 1985). An aliquot (50 μL) of LF sample (1 mg/mL) was mixed with 1 mL of dd H_2O and 0.5 mL of Folin-Ciocalteu's phenol reagent. Subsequently, the mixture was added 2.5 mL of 10% Na_2CO_3 solution and placed in the dark at ambient

temperature for 20 min. The absorbance against blank was read at 735 nm (Multiskan Spectrum microplate spectrophotometer, Thermo Co., Vantaa, Finland). Gallic acid was used to establish a standard curve (0.1–1 mg/mL; Y = 0.8747X - 0.0519; r = 0.9984; Y is the value of the absorbance; X is the value of the solution concentration). The results were expressed as mg gallic acid equivalent (GAE)/g sample.

DPPH radical-scavenging activity

DPPH radical-scavenging activity was estimated according to the method of Shimada, Fujikawa, Yahara, and Nakamura (1992). Each LF sample or standard solution (200 μL) was mixed with 50 μL of 1 mM DPPH, all dissolved in MeOH. The mixture was shaken followed by incubating at ambient temperature for 30 min in the dark. The absorbance against blank was measured at 517 nm. The EC_{50} value, the extract concentration that could scavenge 50% of the DPPH radicals, was obtained from the plot of scavenging activity *vs.* the concentration of extract. The scavenging activity was estimated based on the percentage of DPPH radical scavenged compared to the blank sample. (+)-Catechin standards were used for comparison.

***β*-Carotene bleaching assay**

The assay was modified from that given by Elzaawely et al. (2007). First, 2 mg of *β*-carotene in 10 mL of chloroform was mixed with 20 mg of linoleic acid and 200 mg of Tween 40 and then chloroform was removed under nitrogen. Subsequently, 100 mL of dd H_2O was added with vigorous shacking to prepare *β*-carotene linoleate emulsion. An aliquot of each sample (30 μL, 1 mg/mL) was mixed with 250 μL of the emulsion, and then the absorbance was determined at 470 nm at 45 °C for 2 h. *β*-Carotene bleaching inhibition was evaluated from inhibition activity value:

inhibition (%) = $[(A_{A(120)}-A_{C(120)})/(A_{C(0)}-A_{C(120)})] \times 100$, where $A_{A(120)}$ is the absorbance of the antioxidant at t = 120 min, and $A_{C(120)}$ and $A_{C(0)}$ are the absorbance of the control at time 0 and 120 min, respectively. The α-tocopherol was used as standard for the positive reference.

Inhibition of Cu^{2+}-induced LDL oxidation

The LDL (d = 1.019–1.063 g/mL) was prepared as the method described in the report of Chen et al. (2011). The plasma of fasting healthy volunteers was used to separate LDL through sequential density ultracentrifugation in a Beckman Ultracentrifuge (model: LE-80K; Beckman Instruments Inc., Palo Alto, CA) at 4 °C. The isolated LDL was dialyzed using 10 mM sodium phosphate buffer (PBS, pH 7.4) overnight at 4 °C in the dark to remove the excess of buffer salt. The cholesterol content of the isolated LDL was calculated with the CHOD-PAP enzymatic test kit (Merck, Darmstadt, Germany), and then adjusted with 5 mM PBS to a final cholesterol concentration of 50 μg/mL. Each LF sample was dissolved in DMSO and then diluted with PBS to a final concentration of 1 mg/mL (5% DMSO). The LDL (100 μL) in each well of 96-well flat-bottom plates was mixed with 10 μL of each sample solution and 130 μL of 5 mM PBS, and then added with 10 μL of 125 μM $CuSO_4$ (in 5 mM PBS) at 37 °C. The formation of conjugated diene was recorded at 234 nm at every 5 min interval with the Multiskan Spectrum microplate spectrophotometer to establish the kinetics of LDL oxidation. The lag time was determined from the time-absorbance plot. (+)-Catechin was used for comparison.

Assay for inhibition of human erythrocyte hemolysis

The assay reported by Barreira and others (2008) was used. Erythrocytes were

separated from the plasma of a fasting healthy volunteer, which was centrifuged at 1500 g for 10 min at 4 °C. The separated erythrocytes were further washed with 10 mL of 10 mM PBS and centrifuged at 1500 g for 5 min, 3 times. After that, 0.1 mL of 20% suspension of erythrocytes was mixed with 0.2 mL of 200 mM AAPH solution (in PBS) and 0.1 mL LF sample which was dissolved in DMSO and diluted with dd H_2O to 5% DMSO, the mixture was incubated at 37 °C for 3 h in a water bath shaker (Firstek Scientific Co., Ltd, Taipei, Taiwan), at 30 rpm. The reaction mixture was diluted with 2 mL of PBS and centrifuged at 3000 g for 10 min, at 25 °C. The absorbance of its supernatant was then measured at 540 nm. The hemolysis inhibition (percent) was calculated according to the equation: hemolysis inhibition (percent) = $[(A_{AAPH} - A_E)/A_{AAPH}] \times 100$ (A_{AAPH} is the absorbance of the sample without LFEO, and A_E is the absorbance of the sample with LFEO). The EC_{50} is the value which the EO concentration could inhibit 50% of the erythrocyte hemolysis, (+)-Catechin was used as positive control.

Identification of LFEO compositions by GC-MS

The analyses of the LFEO and extracts volatile compounds were carried out on a Hewlett-Packard GC-MS system (GC 5890 series II; MSD 5972, Hewlett Packard, Wilmington, DE, U.S.A.). A CD-5MS Poly-(diphenyl/dimethylsiloxane) column (30 m × 0.25 mm, 0.25 μm thickness, CNW, Düsseldorf, Germany) was directly coupled to the mass spectrometer. The flow rate of carrier gas (helium) was 1 mL/min. The temperature gradient was set as following : Initially, 2 min isothermal at 50 °C, then 2 °C /min to 90 °C , 1 °C /min to 160 °C , 3 °C /min to 230 °C and finally 10 min isothermal. The injection port temperature and the detector temperature was 200 °C

and 250 °C , respectively. Ionization of the sample components was performed in the EI mode (70 eV). The linear retention indices for all the compounds were determined by co-injection of the sample with a solution containing the homologous series of C7-C30 n-alkanes (Van Den Dool, & Kratz, 1963). The individual components were identified by their retention indices corresponding to the compounds from literature data (Babushok, Linstrom, & Zenkevich, 2011), and also by comparing their mass spectra with those stored in the NIST02/Wiley275 mass spectral databases (Hewlett-Packard, Vienna, Austria).

Statistical analysis

Determination of antioxidant contents and all antioxidant capacity assays in LFEO were performed in triplicate, and the mean values were calculated. The data were subjected to analysis of variance (ANOVA) and Duncan's multiple-range tests were used to assess differences between means. A significant difference was presumed at a level of $p < 0.05$.

RESULTS AND DISCUSSION

Extraction yield and volatile compositions of LFEO

The extraction yield of dried LF ranged from 4.71% to 0.39% (Table 1). The total phenolic contents were richer for the SE and SFE, and the lowest for HD extraction, respectively. From Table 1, we could see that the SFE at 40 °C, 400 and 500 bar and hexane SE extracted more total phenols than the others although their extraction yields were not prominent compared to the other methods.

Table 2 shows 20 compounds of the LF extracts identified by the GC-MS from the libraries and RI values which contained mainly sesquiterpenes with minor oxygenated

sesquiterpenes and monoterpenes. The α-zingiberene, α-curcumene and β-caryophyllene were the primary components (totally 67.7~77.5%) in LFEO followed by the secondary abundant components α-humulene and β-sesquiphellandrene (8.6~9.0%) in the LFEO. In addition, we could notice that the LFEO from hydrodistillation had great amount of terpene contents than the other two extractive methods.

Antioxidant activities

As it is well known, the TPC is closely related to the antioxidant power of extracts and this trend could be observed from Table 3, 4 and 5. In Table 3, the EC_{50} values of DDPH free radical scavenging effect for the lowest three (0.63, 0.69 and 0.77 mg extract/mL) and the highest one (29.75 mg extract/mL) were SE, SFE(40 ◦C, 400 and 500 bar) and HD, respectively, which corresponded to the TPCs of these extracts. The inhibition of AAPH-induced erythrocyte hemolysis also shows the same consistency between the EC_{50} values (0.272, 0.287, 0.305 and 0.462 mg extract/mL) and the TPCs (14.06, 13.75, 10.64 and 1.28 mg GAE/ g extract). The three highest *β*-carotene bleaching inhibitory activity and inhibition of Cu^{2+}-induced LDL oxidation in Table 4 and 5 were also the SE and SFE (40 ◦C, 400 and 500 bar) extracts, respectively. The HD essential oil was still the lowest one as compared to the others. The largest difference of DDPH EC_{50} values was about 40 folds among SE, SFE and HD essential oils. On the contrast, the inhibition of AAPH-induced hemolysis erythrocyte, *β*-carotene bleaching and Cu^{2+}-induced LDL oxidation for HD essential oil was around 1.5~4.0 times weaker than those strong antioxidant extracts.

Discussions

From the results of these antioxidant assays, the hexane and SFE essential oils showed superior antioxidant capacities than HD essential oil which agreed with their phenolic contents. Because nonpolar solvents were used in the extractive process and the LFEO was mainly composed of low-polarity hydrocarbons, their TPCs were lower and resulted in lower antioxidant capacities compared with those extracted by polar solvents (Liu et al., 2009). For the DDPH assay, the antioxidant process was the capture of radical which the phenolic compounds could provide this requirement by hydrogen donating and the terpenes in EO were hard to participate this event, therefore the EC_{50} value of HD LFEO was about 40 folds smaller than the SE and SFE's revealing the key role of TPC in the assay. On the other hand, the antioxidant mechanism in the LDL, β-carotene and erythrocyte hemolysis assays were mainly the inhibiting of the lipid peroxidation, where the terpenes could also play an antioxidant role except the polyphenol. The HD essential oil contained higher level of terpenes than the others and gave rise to the antioxidant activities within 4 times smaller than those of SE and SFE extracts by compensating the deficiency of TPC as compared to the DDPH 40-fold difference.

Conclusion

We had extracted the essential oil of litchi flower which was taken as waste before fruiting by different methods and estimated their antioxidant capacities. In general, the TPC and antioxidant capacities of LFEO were lower as compared to our previous results. Nevertheless, the LFEO could have the potential to be exploited as flavor in the day life. Besides, anti-inflammation and anti-cancer of LFEO are still unexplored and it will be carried on in the near future.

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Table 1. Extraction yields and total phenols contents of litchi flower essential oils

Extractive method	Temperature	Pressure	Extraction yield (%)[a]	Total Phenols (mg GAE[b] /g extract)
Supercritical fluid		300 bar	1.59 ± 0.04	9.95 ± 0.18^{D}
	40 ℃	400 bar	1.90 ± 0.02	10.64 ± 0.11^{C}
		500 bar	2.38 ± 0.04	13.75 ± 0.37^{B}
		300 bar	2.75 ± 0.03	6.17 ± 0.17^{I}
	60 ℃	400 bar	3.23 ± 0.16	7.14 ± 0.27^{F}
		500 bar	4.08 ± 0.04	6.29 ± 0.24^{H}
		300 bar	3.74 ± 0.02	6.68 ± 0.25^{G}
	80 ℃	400 bar	3.91 ± 0.08	7.96 ± 0.20^{E}
		500 bar	4.71 ± 0.05	5.51 ± 0.15^{J}
n-Hexane			2.86 ± 0.49	14.06 ± 0.50^{A}
Hydrodistillation			0.39 ± 0.02	1.28 ± 0.09^{K}

Values (mean ± SD, n = 3) in the same column followed by a different letter are significantly different ($p < 0.05$)

[a]Extraction yield(%) = (extract weight / sample weight) × 100%

[b]GAE, Gallic acid equivalent

Table 2. Chemical compositions of the volatile fraction of litchi flower essential oils

				Area % (relative amount[c])		
N	T_R[a]	Compound	RI[b]	HD	SE	SFE[e]
1	21.043	limonene	1029.9	3.7	0.3(0.02)	ND
2	24.319	cis-Linalool oxide	1072.8	0.1	ND[d]	ND
3	25.549	trans-Linalool oxide	1089.0	0.2	ND	ND
4	26.737	linalool	1103.6	0.1	ND	ND
5	27.109	nonanal	1107.5	0.1	ND	ND
6	54.556	cycloisosativene	1362.8	0.6	0.4(0.13)	0.3(0.10)
7	55.539	α-copaene	1371	1.0	0.8(0.16)	0.6(0.15)
8	60.997	β-caryophyllene	1416.7	16.3	13.1(0.16)	13.3(0.21)
9	62.523	trans-α-bergamotene	1429.4	0.8	0.7(0.18)	0.6(0.20)
10	65.070	α-humulene	1453.1	4.4	3.7(0.17)	3.7(0.22)
11	67.508	α-amorphene	1470.8	0.5	0.4(0.15)	0.3(0.16)
12	68.116	germacrene D	1475.9	1.0	0.7(0.13)	1.7(0.44)
13	68.974	α-curcumene	1483.0	22.6	41.2(0.37)	30.5(0.35)
14	70.845	α-zingiberene	1498.6	36.7	13.4(0.07)	33.7(0.24)
15	71.784	cis-α-bisabolene	1513.9	0.7	0.4(0.13)	0.4(0.14)
16	72.790	δ-cadinene	1521.7	0.6	0.3(0.11)	0.4(0.20)
17	73.702	β-sesquiphellandrene	1528.9	4.2	5.3(0.25)	5.1(0.31)
18	83.235	trans-α-bisabolene epoxide	1604.2	0.1	0.4(1.59)	ND
19	92.570	6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl-	1686.5	0.1	0.5(1.45)	0.5(1.95)
20	113.325	palmitic acid, ethyl ester	1995.3	0.03	1.2(9.39)	0.6(6.35)

[a] Retention time (in minutes). [b] RI: Linear retention index to n-alkanes on CD-5MS column. [c] Relative amount of component to hydrodistillation extract.

[d] ND: Not detected. [e] SFE extract at 40 °C and 500 bar

Table 3. DPPH free radical scavenging effect and AAPH-induced erythrocyte hemolysis inhibition of litchi flower essential oils

Temperature	Pressure	EC_{50}[a] of DPPH radical scavenging activity (mg extract/mL)	EC_{50} of AAPH-induced erythrocyte hemolysis (mg extract/mL)
40 ℃	300 bar	1.44 ± 0.02^{C}	0.367 ± 0.016
	400 bar	0.77 ± 0.03^{I}	0.305 ± 0.021
	500 bar	0.69 ± 0.02^{J}	0.287 ± 0.002
60 ℃	300 bar	1.00 ± 0.04^{D}	0.316 ± 0.007
	400 bar	1.45 ± 0.01^{B}	0.375 ± 0.031
	500 bar	0.92 ± 0.01^{E}	0.352 ± 0.005
80 ℃	300 bar	0.85 ± 0.01^{G}	0.383 ± 0.009
	400 bar	0.88 ± 0.01^{F}	0.395 ± 0.022
	500 bar	0.82 ± 0.01^{H}	0.496 ± 0.030
		0.63 ± 0.01^{K}	0.272 ± 0.011
		29.75 ± 1.16^{A}	0.462 ± 0.011
		0.01 ± 0.00^{L}	0.028 ± 0.002

Values (mean ± SD, n = 3) in the same column followed by a different letter are significantly different ($p < 0.05$)

[a]EC_{50} means the effective concentration of extract that can decreases DPPH concentration or inhibits AAPH-induced erythrocyte hemolysis by 50%

Table 4. Inhibition of β-carotene bleaching activity value of litchi flower essential oils

Extractive method	Temperature	Pressure	Inhibitory activity value (%)
Supercritical fluid	40 ℃	300 bar	62.11 ± 0.79^{G}
		400 bar	80.42 ± 3.54^{B}
		500 bar	69.51 ± 3.29^{D}
	60 ℃	300 bar	67.37 ± 1.80^{E}
		400 bar	65.07 ± 2.06^{F}
		500 bar	62.20 ± 0.66^{I}
	80 ℃	300 bar	61.32 ± 2.09^{H}
		400 bar	59.44 ± 1.02^{J}
		500 bar	54.89 ± 0.48^{L}
n-Hexane			75.96 ± 2.59^{C}
Hydrodistillation			58.25 ± 1.26^{K}
α-Tocopherol[a]			89.36 ± 1.08^{A}
Control			31.16 ± 4.04^{M}

Values (mean ± SD, n = 3) in the same column followed by a different letter are significantly different ($p < 0.05$)

[a]The concentrations of LFEO and α-tocopherol are 1 mg/mL and 0.1 mg/mL, respectively.

Table 5. Inhibition of Cu^{2+}-induced oxidation of LDL of litchi flower essential oils

Extractive method	Temperature	Pressure	Cu^{2+}-induced LDL oxidation Δt_{lag}(min)
Supercritical fluid	40 ℃	300 bar	91.01 ± 7.92^{E}
		400 bar	160.36 ± 2.77^{A}
		500 bar	154.82 ± 11.70^{B}
	60 ℃	300 bar	138.30 ± 4.78^{C}
		400 bar	63.35 ± 3.91^{G}
		500 bar	69.33 ± 4.54^{F}
	80 ℃	300 bar	58.57 ± 4.04^{I}
		400 bar	56.32 ± 2.84^{K}
		500 bar	56.48 ± 5.69^{J}
n-Hexane			122.59 ± 29.62^{D}
Hydro distillation			43.02 ± 6.96^{L}
(+)-Catechin[a]			61.95 ± 3.01^{H}

Values (mean ± SD, n = 3) in the same columm followed by a different letter are significantly different ($p < 0.05$)

[a]The concentrations of LFEO and (+)-catechin are 1 mg/mL and 0.01 mg/mL, respectively.

參加 2016 CIGR-AgEng 會議報告

計畫編號：MOST 104-2320-B-040-019-

計畫名稱：不同萃取方式荔枝花精油之抗氧化、抗發炎、抗菌與抗癌及其機轉之研究

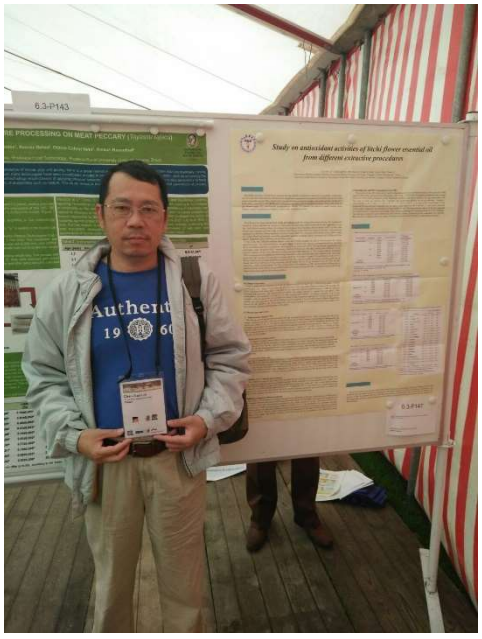
報告者： 中山醫學大學 醫學應用化學系 林昭田

時間與地點：2016.06.26～2016.06.30，丹麥 Aarhus

去年 12 月收到 CIGR 的徵求投稿的 e-mail，在了解之後，知道他是屬於歐盟的一個相關學術組織 Ag-Eng，主要是與農業工程及其他與農業有關的研究課題。此次會議中共分 11 個 sessions，其中第 6 個 session Post-harvest technologies 的 Food processing, technologies and influence on nutrition, losses and flavour 與我們正在研究的各種萃取方式的荔枝花精油的抗氧化活性有相關性，吸引我們的興趣。加諸往年我們較著重於參加較專業性高的單一主題會議，因此我們覺得應該拓展我的視野，了解其他相關領域的進展與知識，多元的學習進而充實教學內涵；所以就嘗試投稿，也很幸運的被接受，並承科技部惠允同意計畫撥款補助。

CIGR-AgEng 是屬歐盟的組織，每年由各會員國輪流主辦，這次是由丹麥的 Aarhus 大學於 6 月 26-30 日間負責主辦。Aarhus 是丹麥第二城，人口約 32 萬，而 Aarhus 大學校齡約 90 年是丹麥第二大大學，世界百大，有產生一位諾貝爾化學獎得主及程式語言 C++發明人。

這次會議共有土壤與水資源工程，精緻家畜飼養及環境技術，植物生產技術，能源問題與研究，資訊技術，管理系統，農產品加工技術等十幾個 senssions 的小組報告及相關海報展示，個人在此會議中是以海報方式投稿，主要是討論在水蒸餾、正己烷、二氧化碳超臨界萃取的樣品之抗氧化能力比較。期間看到許多其他農業領域的應用，例如：以紅外線顯示器監督養雞場的雞隻群聚效應與最佳飼養密度之研究，以原子力顯微鏡研究馬鈴薯的烹調過程中其組織成分之探討，農產品運輸程序最佳化，利用無人飛機與資訊系統於農業生產過程等等。



本人於 CIGR Poster 現場

我們也參加了許多的 oral report，有介紹如何提升農業加工工廠的熱機效率，介紹加拿大相關的食品安全與管制之發展及 UV 光與高壓對果汁營養品質的影響，與研磨稻米的品質之研究。最後也聆聽了同行同事劉教授對廢棄香蕉心的回收並對其萃取物之抗氧化能力的精彩演講。發人省思的是這次會議主詞人是一位由中國留歐在英國任教的人士所擔

任的，可見中國的開放留學政策在這 30 年來已經取得了顯著成果，這對台灣來說是一大警訊，不論是從政治外交或是經濟與科技合作來看，都會對台灣形成巨大的挑戰，台灣當局應當亟思對策，加強與歐美日之學術科技之交流，並且有計畫的協助台灣學生的國外深造，甚或協助他們畢業後在當地的就業，為台灣之將來布局。會後我們亦順道參觀了哥本哈根大學，對其校園之規劃不愧為北歐最高學府，世界排名十一、二之頂尖大學，希望台灣能夠有朝一日也能夠正常的發展出一個這樣的世界知名大學。

總而言之，這次的會議讓我們看到北歐國家的生活態度、建設及學術的精神與樣貌；同時也拓展了我們農業學門的視野，不再侷限於狹窄的單一研究領域。

科技部補助計畫衍生研發成果推廣資料表

日期:2016/10/20

科技部補助計畫	計畫名稱: 不同萃取方式荔枝花精油之抗氧化、抗發炎、抗菌與抗癌及其機轉之研究
	計畫主持人: 林昭田
	計畫編號: 104-2320-B-040-019- 學門領域: 食品科學

無研發成果推廣資料

104年度專題研究計畫成果彙整表

計畫主持人：林昭田	**計畫編號：**104-2320-B-040-019-
計畫名稱：不同萃取方式荔枝花精油之抗氧化、抗發炎、抗菌與抗癌及其機轉之研究	

成果項目					量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文			0	篇	
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
國外	學術性論文	期刊論文			0	篇	
		研討會論文			1		2016,June Demark Aarhus, CIGR conference Poster Title:Study on antioxidant activities of litchi flower essential oil from different extractive procedures
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		

		積體電路電路布局權	0		
		著作權	0		
		品種權	0		
		其他	0		
	技術移轉	件數	0	件	
		收入	0	千元	
參與計畫人力	本國籍	大專生	0	人次	
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）					

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）
   論文：□已發表　□未發表之文稿　■撰寫中　□無
   專利：□已獲得　□申請中　■無
   技轉：□已技轉　□洽談中　■無
   其他：（以200字為限）
   初步成果已於今年(2016)六月於丹麥,Aarhus之CIGR中以Poster方式展示
   研究成果正在整理撰寫,俟完成後將投稿國際期刊

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性，以500字為限）
   荔枝花經由目前文獻搜尋並未發現,而其為荔枝生產過程中的農業廢棄物
   今將其加工後可將其成品用於各種農產再製品或食品添加,或直接用於精油的使用
   可增加農民之額外收益並成為開發出新的產品之原物料

4. 主要發現
   本研究具有政策應用參考價值：□否　■是，建議提供機關農委會
   （勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
   本研究具影響公共利益之重大發現：■否　□是
   說明：（以150字為限）
   日常慣用之水蒸餾精油之抗氧化效果並沒有
   hexane,CO2SFE萃取的效果好
   而SFE萃取條件中以 40度C 400與500bar的萃取物抗氧化效果
   較其他溫度壓力條件加