行政院國家科學委員會專題研究計畫 期末報告

不同精油萃取物對大鼠子宮平滑肌收縮之影響效應（GM07）

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 101-2629-B-038-002-
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計 畫 主 持 人：夏詩閔
共 同 主 持 人：謝宗明

報 告 附 件 ：出席國際會議研究心得報告及發表論文

公 開 資 訊 ：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 102 年 11 月 14 日

中 文 摘 要 ： 現代婦女有一些常見的婦科疾病，而在女性月經期間所引發的眾多不適症狀中，仍然以生理痛的現象為婦科疾病中最常發生的現象。生理痛（dysmenorrhea）又叫經痛或月經困擾症，是指女性在月經的期間，會感覺到強烈的下腹部陣痛，且發生率極高，約佔女性七成以上。主要為前列腺素過度分泌刺激子宮收縮所造成。目前治療生理痛的方式很多，其中利用藥物來降低因子宮平滑肌過度收縮所造成之疼痛較為普遍。而目前一般用來治療經痛的藥物如：即非固醇類抗發炎藥（nonsteroidal anti-inflammatory drugs, NSAID）、前列腺素合成抑制劑（prostaglandin-synthesis inhibitors, PGSI）、口服避孕藥（oral contraceptive pill, OCP）、鈣離子管道阻斷劑（calcium channel blockers）、止痛劑（analgesics）、綜合維他命（vitamins）等。精油為自然界存在的化學物質，已被廣泛研究發現具有抗氧化、抗癌等生理活性功能，然而對於是否抑制子宮過度收縮而用來改善經痛的現象，目前尚未被充分研究，由實驗結果可以發現檜木精油、快樂鼠尾草精油以及薏苡油溶性萃取物具有抑制子宮過度收縮的現象，並可以進一步用來改善女性經痛問題。

中文關鍵詞： 經痛 經油 薏苡

英 文 摘 要 ：

英文關鍵詞：

行政院國家科學委員會補助專題研究計畫 □期中進度報告 ■期末報告

不同精油萃取物對大鼠子宮平滑肌收縮之影響效應 (GM07)

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計畫主持人：夏詩閔
共同主持人：謝宗明
計畫參與人員：

本計畫除繳交成果報告外，另含下列出國報告，共 一 份：
□移地研究心得報告
■出席國際學術會議心得報告
□國際合作研究計畫國外研究報告

處理方式：除列管計畫及下列情形者外，得立即公開查詢
■涉及專利或其他智慧財產權，□一年■二年後可公開查詢

中　華　民　國 102 年 9 月 1 日

中文摘要

現代婦女有一些常見的婦科疾病，而在女性月經期間所引發的眾多不適症狀中，仍然以生理痛的現象為婦科疾病中最常發生的現象。生理痛（dysmenorrhea）又叫經痛或月經困擾症，是指女性在月經的期間，會感覺到強烈的下腹部陣痛，且發生率極高，約佔女性七成以上。主要為前列腺素過度分泌刺激子宮收縮所造成。目前治療生理痛的方式很多，其中利用藥物來降低因子宮平滑肌過度收縮所造成之疼痛較為普遍。而目前一般用來治療經痛的藥物如：即非固醇類抗發炎藥（nonsteroidal anti-inflammatory drugs, NSAID）、前列腺素合成抑制劑（prostaglandin-synthesis inhibitors, PGSI）、口服避孕藥（oral contraceptive pill, OCP）、鈣離子管道阻斷劑（calcium channel blockers）、止痛劑（analgesics）、綜合維他命（vitamins）等。精油為自然界存在的化學物質，已被廣泛研究發現具有抗氧化、抗癌等生理活性功能，然而對於是否抑制子宮過度收縮而用來改善經痛的現象，目前尚未被充分研究，由實驗結果可以發現檜木精油、快樂鼠尾草精油以及薏苡油溶性萃取物具有抑制子宮過度收縮的現象，並可以進一步用來改善女性經痛問題。

關鍵字：經痛、生理痛、快樂鼠尾草精油

Abstract

Dysmenorrhea is directly related to elevated PGF2α (prostaglandin F2α) levels. It is treated with NSAIDs (nonsteroid antiinflammatory drugs), PGSI (prostaglandin-synthesis inhibitors), OCP (oral contraceptive pill), calcium channel blocker, analgesics and vitamins in Western medicine. Since NSAIDs produce many side effects, Chinese medicinal therapy is considered as a feasible alternative medicine. Many special physiological components (ex: flavonoid and Flavones) in Chinese medicine have been isolated and identified. Essential oils have a lot of physiological functions like anti-oxidatio and anti-cancer effects. However, the relationship between smooth muscle contraction and essential oils remains veiled. Therefore, we investigated this relationship in the rat uterus by measuring uterine contraction activity and recording the intrauterine pressure. We studied the *in vivo* and *in vitro* effects of the essential oils on uterine smooth muscle contraction. In our preliminary experimental results, the PGF2α-induced uterine contractions reduced significantly after treatment of quercetin or naringenin. Our data suggested that hinokitol could inhibit the PGF2α-induced uterine contractions. The objective of this study is to evaluate the effects of essential oils on uterine contractions *in vivo* and in vitro. It is helpful to understand the effects and the action mechanisms of essential oils on uterine contractions.

Keyword: Dysmenorrhea, hinokitol, adlay

前言

在眾多女性相關疾病中，經痛是臨床上常見困擾女性的問題(Jones et al., 2004)。根據美國在 1999 年的統計數據中發現約有高達 90%青春期少女有原發性痛經的困擾(Coco et al., 1999)。而從全美國的調查資料中顯示大約有 900 萬到 5500 萬女性有經痛的問題（Bullock et al., 1996）。而 1999 年在澳洲相關的研究統計數據顯示痛經盛行率約為 80%左右（Hillen et al., 1999）。另外 2000 年在西班牙的研究調查資料中發現經痛的盛行率更高達約 85% (Banikarim et al., 2000)。而在 2004 年針對國內約 15-18 歲的女學生調查研究中發現，約有 73%的人有過經痛的症狀。(邱，2004)。由以上的結果顯示經痛確實是一般女性常見的症狀，而經痛不只帶給女性很大的困擾，也會造成國家社會經濟生產力的降低，如影響女性的課業或工作皆會受到嚴重的影響。根據美國的研究統計每年因為經痛造成女性工作時數的損失更高達 14 億小時，而總損失美元約達 20 億（Coco et al., 1999）。經痛會造成女性生理上許多不適的現象產生如生理期疼痛、腸胃不適、頭痛等問題也會造成女性情緒上不穩定的一些問題，有些嚴重的甚至會造成心理上的一些問題障礙如：憂鬱、神經質、焦慮等症狀（周，1994）。故對於女性來說經痛確實是個嚴重困擾女性要的問題。

材料與方法

一、實驗動物

由樂斯科實驗動物公司購得 Sprague-Dawley（SD）種系，250-300 克之雌性大白鼠飼養於每日 14 小時（0600-2000）人工照明及空調設備（22± 2℃）之動物室，飲水及飼料不限。

二、實驗材料

精油由肯園芳療公司提供其他藥品採購至 Sigma 公司。

三、子宮平滑肌組織製備

將雌鼠犧牲並由腹腔迅速且小心的分別取出子宮，立刻置入 37℃的 Kreb’s solution（118.3 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 2.5 mM CaCl2, 25 mM NaCHO3, 0.026 mM CaEDTA and 11.1 mM glucose），持續以 95% 的 O2 及 5% CO2 充氣。將附著於子宮上的脂肪以及結締組織清除，沿著縱

行方向剪開子宮，小心的去除黏膜層，製備成 1 cm 長子宮平滑肌(縱肌)懸掛於組織槽中，內含 5 % Krebs 溶液，溫度保持於 37℃，連續以 95% O2 及 5% CO2 通氣。子宮縱肌肌條一端固定於槽底之固定鉤，另一端連於外側等長傳導器(external isometric force transducer)。子宮平滑肌縱肌肌條之運動則以記錄儀記錄(PowerLab recorder, ML785, Castle Hill, NSW, Australia)，經過約 30-60 分鐘的平衡，將催產素(Sigma, St. Louis, MO, USA)、前列腺素 F2α (Sigma, St. Louis, MO, USA)及測試藥劑以不同濃度加入組織槽中，觀察其對子宮縱肌肌條之作用。另外以催產素受體拮抗劑(atosiban, Ferring, Limhamn, Sweden)、蕈毒鹼受體拮抗劑(muscarinic receptor blocker, atropine, Sigma, St. Louis, MO, USA)、M3 receptor 拮抗劑(4-DAMP, Research Biochemicals International Company, Natick, MA., USA)、鈉離子通道阻斷劑(tetrodotoxin, TTX, Sigma, St. Louis, MO, USA)及 L 型鈣離子通道阻斷劑(nifidipine, Sigma, St. Louis, MO, USA)等不同藥劑來觀察其對子宮縱肌肌條之作用，並紀錄其收縮振幅 (mean contractile amplitude)及收縮頻率(contractile frequency)。

四、子宮平滑肌細胞之分離與培養

將雌鼠犧牲並由腹腔迅速且小心的以無菌鑷子分別取出子宮，上述取得之子宮利用顯小心撕下肌肉束以取得平滑肌縱肌。接著以 0.2% 蛋白質溶解酵素(protease)在 37℃下震盪 20 分鐘，再利用 0.2% trypsin inhibitor 及 0.2% collagenase 混和反應 60 分鐘，經離心沉澱後以分離子宮平滑肌細胞，緊接著細胞加入 DMEM-F12 培養液(含 10% FBS 和 1% antibiotic) 並培養於二氧化碳培養箱中(37℃,5% CO2)。

結果與討論

目前此國科會計畫之成果已發表於 Food Chemistry

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Application of the solvent extraction technique to investigation of the anti-inflammatory activity of adlay bran

Din-Wen Huang [a,1], Chi-Hao Wu [b,1], Chun-Kuang Shih [b], Chia-Yu Liu [a], Ping-Hsiao Shih [c], Tzong-Ming Shieh [d], Ching-I Lin [e], Wenchang Chiang [a], Shih-Min Hsia [b,*]

[a] Institute of Food Science and Technology, College of Bioresources and Agriculture, National Taiwan University, Taipei, Taiwan
[b] School of Nutrition and Health Science, Taipei Medical University, Taipei, Taiwan
[c] Department of Rehabilitation, Taipei Medical University-Wan Fang Hospital, Taipei, Taiwan
[d] Department of Dental Hygiene, College of Health Care, China Medical University, Taichung, Taiwan
[e] Department of Nutrition and Health Science, Kainan University, Taoyuan, Taiwan

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ABSTRACT

The current study utilised a bioassay-directed chemical analysis scheme to screen the anti-inflammatory activity of fractions and compounds from adlay bran (AB). Liquid–liquid extraction couple with liquid chromatography–mass spectrometry (LC–MS) was applied to the isolation, analysis and identification of active components in AB samples. Ethanol extracts of AB (ABE) and ethyl acetate extracts AB (ABEa) were obtained and further partitioned with different solvents. The results showed that among all 16 kinds of fractions from ABE and ABEa, ABEa-Ea-B (80% Ea/*n*-hexane sub-fraction from ABE-Ea) had the most potent inhibitory effects on NO production, iNOS and COX-2 expressions, and proinflammatory IL-6 and TNF-α secretion in lipopolysaccharide-activated RAW264.7 cells system. Mechanistic data from luciferase reporter-gene assay revealed that the anti-inflammatory action of ABEa-Ea-B may be associated with inhibition of NF-kB transcriptional activity. Notably, tangeretin, nobiletin, and *p*-hydroxybenzoic acid were found to be the main active compounds for the anti-inflammatory properties in ABEa-Ea-B.

1. Introduction

Inflammation is an important process generated by the body in response to infection, injury, and irritation. However, chronic low-grade inflammation plays a critical role in several diseases, including asthma, atherosclerosis, rheumatoid arthritis, cancer, Alzheimer's disease (Huang, Huang, & Deng, 2012), obesity, and its metabolic sequelae (Chawla, Nguyen, & Goh, 2011). Among the inflammatory mediators, inducible nitric oxidesynthase (iNOS) and cyclooxygenase (COX-2) are well known to cause the excess production of NO and prostaglandin E_2 (PGE_2), respectively, resulting in elevated oxidative stress. Moreover, activated macrophages, stimulated by exposure to proinflammatory cytokines, interferon-γ, and lipopolysaccharide (LPS), are major sources of tumor necrosis factor (TNF)-α, interleukin (IL)-6, and other inflammatory mediators (Lee et al., 2012).

Adlay (or Job's tears), an annual crop, has been consumed as both a herbal medicine and a food supplement in traditional Chinese medicine for a long time (Hsia, Chiang, Kuo, & Wang, 2006). Air-dried adlay seeds can be separated into four parts: the hull, testa, bran, and polished adlay. Although the antioxidative, anti-inflammatory and antitumor effects as well as their modulation of endocrine (Huang, Chung, Kuo, Lin, & Chiang, 2009; Huang, Kuo, Lin, Lin, & Chiang, 2009) and antimetabolic syndrome (Huang, Chiang, Yao, & Chiang, 2004) and by different parts of adlay seeds has been proven, adlay bran (AB) and polished adlay are the edible parts. Therefore, the active components of the ethanol extract of AB (ABE) were further investigated, and phenolic compounds, lactams, and other compounds were identified (Chen, Chung, Chiang, & Lin, 2011; Chung, Hsia, et al., 2011; Chung, Hsu, et al., 2011).

Our previous studies indicated that the ethyl acetate extract of ABE (ABE-Ea) expressed higher bioactivity than other fractions extracted using different solvents (Chen, Lo, & Chiang, 2012; Chung, Hsia, et al., 2011; Chung, Hsu, et al., 2011). However, sticky solid contents were found during the preparation process. Those sticky contents lowered the yield of ABE-Ea. Thus, in the present study, AB was extracted with ethanol or ethyl acetate. This study also examined the anti-inflammatory effects of the ABE-Ea and ABEa-Ea using lipopolysaccharide (LPS)-mediated RAW 264.7 macrophage cells, and analysed the variation of components of

* Corresponding author. Address: School of Nutrition and Health Science, Taipei Medical University, 250, Wu-Hsing Street, Taipei 110, Taiwan. Tel.: +886 2 7361661 6558 (S.-M. Hsia).
E-mail address: bryanhsia@tmu.edu.tw (S.-M. Hsia).
[1] These authors contribute equally to this work.

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ABE-Ea and ABEa-Ea by high-performance liquid chromatography (HPLC) tandem mass spectroscopy (MS).

2. Materials and methods

2.1. Materials and chemicals

The analytical standards used in this research were purchased from Sigma Chemical (St. Louis, MO) except for formononetin, luteolin, and nobiletin (ChromaDex, Irvine, CA). Chromatographic- and analytical-grade solvents used during the purification procedures and HPLC–MS [i.e., ethanol, hexane, ethyl acetate, butanol, acetonitrile, and amino acetate] were obtained from Merck (Darmstadt, Germany). Anti-iNOS and anti-COX-2 antibodies were purchased from Upstate (Lake Placid, NY, USA) and BD Biosciences (San Jose, CA, USA), respectively. Anti-β-actin was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Compatible horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Pierce (Rockford, IL), and goat anti-rabbit immunoglobulin G and goat anti-mouse antibodies were from Chemicon International (Temecula, CA).

2.2. Preparation and fractionation of the different extracts of AB and standards

Adlay was purchased from a local farmer who plated Taichung Shuenyu No. 4 (TCS4) of *Coix lachryma-jobi* L. var. *ma-yuen* Stapf in Taichung, Taiwan, in March 2010 and harvested it in July of the same year. After harvest, the air-dried adlay seeds were separated into four different parts, including adlay hull, adlay testa, adlay bran and polished adlay. The preparation of AB extract was from the previously described method, with minor modifications (Hsia et al., 2006). AB (33 or 10.3 kg, dry weight basis) was respectively extracted with six volumes (w/v) of 95% ethanol (200 L) or ethyl acetate (60 L) at room temperature. After each 24-h extraction, the ethanol or ethyl acetate and AB were separated by filtration. The pooled ethanol or ethyl acetate filtrates were vacuum-evaporated, and the 3.6 kg of ABE (10.9% of the initial dry weight) and 2.5 kg ABEa (24.3% of the initial dry weight) were stored at −20 °C.

The ABE or ABEa was then suspended in water and repeatedly partitioned with hexane until the hexane layer was not coloured. The hexane was collected and removed under a vacuum. The hexane residue was further partitioned with 70% ethanol until the 70% ethanol extract was colourless. The 70% ethanol was gathered and removed under a vacuum to acquire residue 1. The remaining hexane layer was the hexane fraction of ABE or ABEa (ABE-Hex or ABEa-Hex). The water phase as mentioned above was further partitioned into ethyl acetate until the ethyl acetate extract was not coloured. The ethyl acetate layer was gathered, and the ethyl acetate was removed under a vacuum to obtain residue 2. Residue 1 was combined with residue 2 to produce the Ea fraction (ABE-Ea or ABEa-Ea). The residue was dissolved in water and partitioned into butanol. Butanol was removed under a vacuum, leaving behind a residue (ABE-Bu or ABEa-Bu). Finally, the water fraction was lyophilised, and residues were recovered (ABE-Wa or ABEa-Wa).

The anti-inflammatory activities of the various fractions were assessed using the LPS/NO/RAW 264.7 system described below, and the anti-inflammatory activity of the EA fraction was better than those of other fractions. Thus, the Ea fraction was sub-fractionated using silica gel chromatography and a hexane/Ea/95% ethanol gradient. Four sub-fractions were manually collected and concentrated under a vacuum at 60 °C [30% Ea/Hex (A), 80% Ea/Hex (B), 100% Ea (C), 95% EtOH (D)]. The anti-inflammatory activities of the various sub-fractions were assessed. Fig. 1 shows the flowchart for the extraction of the anti-inflammatory sub-fractions from AB.

2.3. Determination of total phenolic contents

A Folin–Ciocalteu assay was used to determine the total phenolic contents of the various sub-fractions. In brief, 100 μL of a sample (2 mg/ml) was added to 2 ml deionised water and 1 ml Folin–Ciocalteu phenol reagent. Subsequently, 5 ml of sodium bicarbonate (20%, w/v) was added to the mixture as mentioned above, followed by incubation at ambient temperature in the dark for 20 min. The absorbance at 735 nm was measured with a spectrophotometer (Molecular Devices, San Francisco, CA, USA). The total phenolic contents of samples were compared with the calibration standard of gallic acid (0–800 μg/ml). The results were expressed as milligrammes of gallic acid equivalents (GAE) per gram of sample.

2.4. HPLC–MS analysis

The Agilent 1100 series HPLC coupled to a mass spectrometer (MS) (Palo Alto, CA, USA) was used to identify the phenolic compounds by their retention times (by comparing them to those of reference standards) and by the mass of the selected ions. The phenolic compounds in our in-house library were used as reference standards. In the HPLC system, an Alltech Alltima C18 column was used. The mobile phase consisted of solvents A (10 mM ammonia acetate containing 0.5% formic acid) and B (methanol containing 0.5% formic acid). The flow rate was 0.5 ml/min, and the column temperature was 25 °C. The gradient system was 10–90% B (0–45 min), 90–10% B (45–50 min), and 10% B (50–60 min). Data acquisition was via selected ion monitoring (SIM). Ions representing positive (only for tangeritin and nobiletin) or negative species of compounds were selected, and peak areas were measured. Calibration curves of authentic standards were linear over the concentration range of 0.02–40 μg/ml with correlation coefficients of $\geqslant 0.99$.

2.5. Cell culture and stimulation

The RAW 264.7 macrophage cell line was purchased from the Bioresource Collection and Research Center (Food Industry Research and Development Institute, Hsinchu, Taiwan) and cultured under a 5% CO_2 atmosphere at 37 °C in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated foetal bovine serum, 1% penicillin–streptomycin, and 1% glutamine. To investigate the anti-inflammatory activities of samples, cells were seeded in 96-well plates or 10 cm Petri dishes in serum-free medium overnight and then treated with only the carrier vehicle (0.1% DMSO), a test sample, and/or LPS.

2.6. Methyl thiazole tetrazolium (MTT) assay of cell viability

RAW 264.7 cells were seeded into 96-well plates (10^5 cells/well) and allowed to grow overnight. Then, the culture medium was replaced, and test samples in DMSO were added with or without LPS (1 μg/ml) and incubated for an additional 24 h. The concentration of DMSO after dilution in the culture medium was <0.1%. The filtered MTT solution in serum-free DMEM was added to each well (0.5 mg MTT/ml), and cells were incubated at 37 °C for 2 h. Any unreacted dye was then removed. The insoluble MTT formazan crystals were dissolved in DMSO at room temperature for 15 min, and the absorbance (at 570 nm) of each sample was measured. The viability of a test sample was calculated using the following equation: % viability = $(\text{absorbance}_{\text{test sample}})/(\text{absorbance}_{\text{control}}) \times 100$.

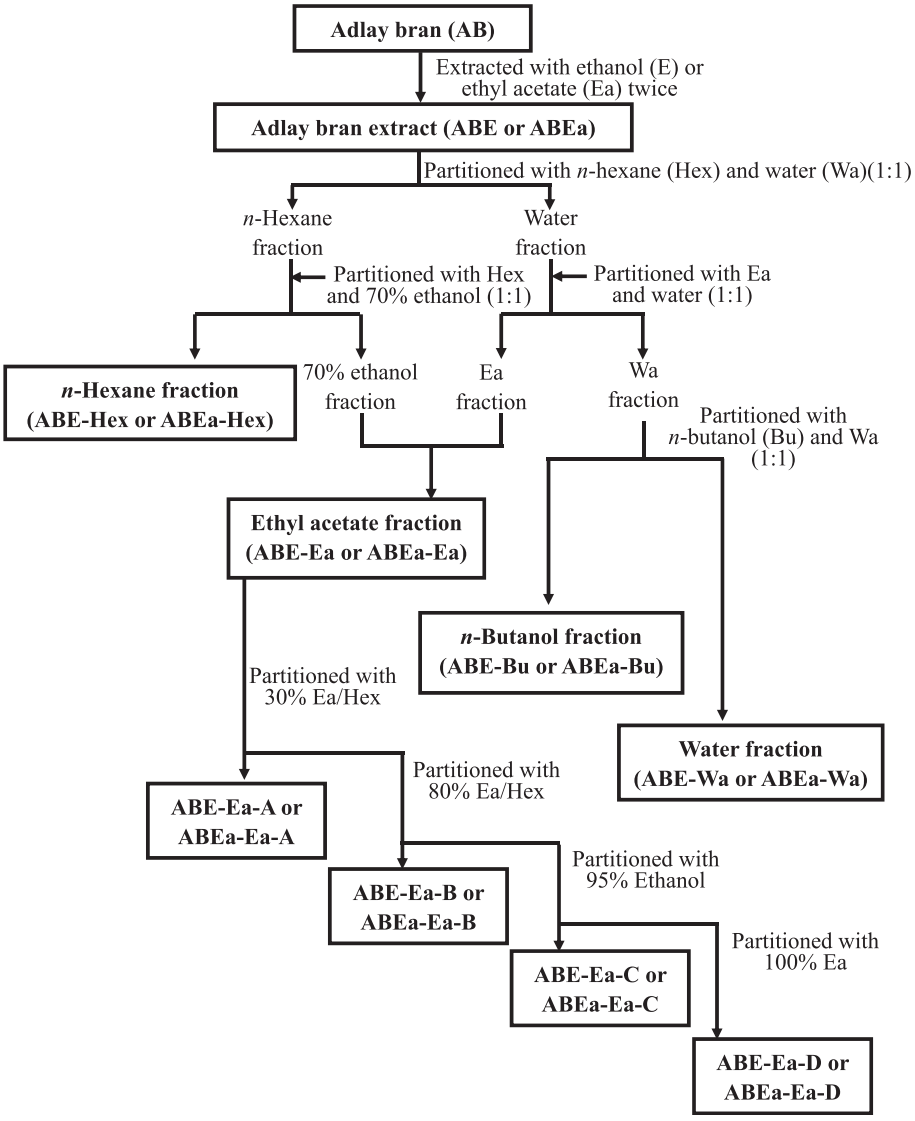

Fig. 1. Scheme of the separation methods used to prepare anti-inflammatory fractions of adlay bran (AB).

2.7. NO, TNF-α, and IL-6 formation

RAW264.7 macrophages were treated in a similar way to that described for cell viability. In brief, cells were treated with medium alone, with LPS, or with a test sample for 24 h. Thereafter, each supernatant (100 μl) was mixed with the same volume of Griess reagent [1% sulfanilamide in 5% phosphoric acid and 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride in water] and incubated for 15 min in the dark. The total amount of nitrite present was calculated based on the absorbance of a sample at 570 nm. The amount of NO synthesised in response to LPS stimulation was calculated using the following equation: % NO synthesis = $[(\text{absorbance}_{\text{sample-treated}} - \text{absorbance}_{\text{control}})/(\text{absorbance}_{\text{LPS-treated}} - \text{absorbance}_{\text{control}})] \times 100$.

TNF-α and IL-6 were determined using a commercial mouse enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, San Diego, CA, USA) according to the instructions. Concentrations of TNF-α and IL-6 were calculated based on the absorbance of a sample at 450 nm.

2.8. Western blotting

RAW264.7 macrophages were treated in a manner similar to that described for the NO and cell viability assays. Cells were washed twice with ice-cold phosphate-buffered saline (PBS), lysed in 50 mM Tris–HCl (pH 8.0) containing 1 mM NaF, 150 mM NaCl, 1 mM EGTA, 1 mM phenylmethanesulfonyl fluoride, 1% (w/v) NP-40, and 10 μg/ml leupeptin, and then centrifuged at $10^4 g$ for 30 min at 4 °C. Amounts of cytosolic proteins recovered in the supernatant were measured using the Bradford assay, with bovine serum albumin (BSA) as the standard. To detect iNOS, COX-2, and β-actin, total cytosolic extracts (40 μg protein) were first separated over 8% sodium dodecylsulfate (SDS)–polyacrylamide minigels and then transferred to Immobilon polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA) using a transfer buffer composed of 25 mM Tris–HCl (pH 8.9), 192 mM glycine, and 20% MeOH. Membranes were each incubated in PBS containing 5% BSA for 1 h at room temperature and then overnight at 4 °C in the presence of an appropriate primary antibody (1:1000 dilution of the stock solution). After hybridisation with a primary antibody, membranes were washed with 0.1% (w/v) Tween 20 in PBS three times, incubated with an HRP-labelled secondary antibody overnight at 4 °C, and then washed with the same buffer three more times. To detect the targeted proteins, enhanced chemiluminescence Western blotting reagents (Amersham Pharmacia Biotech) were used.

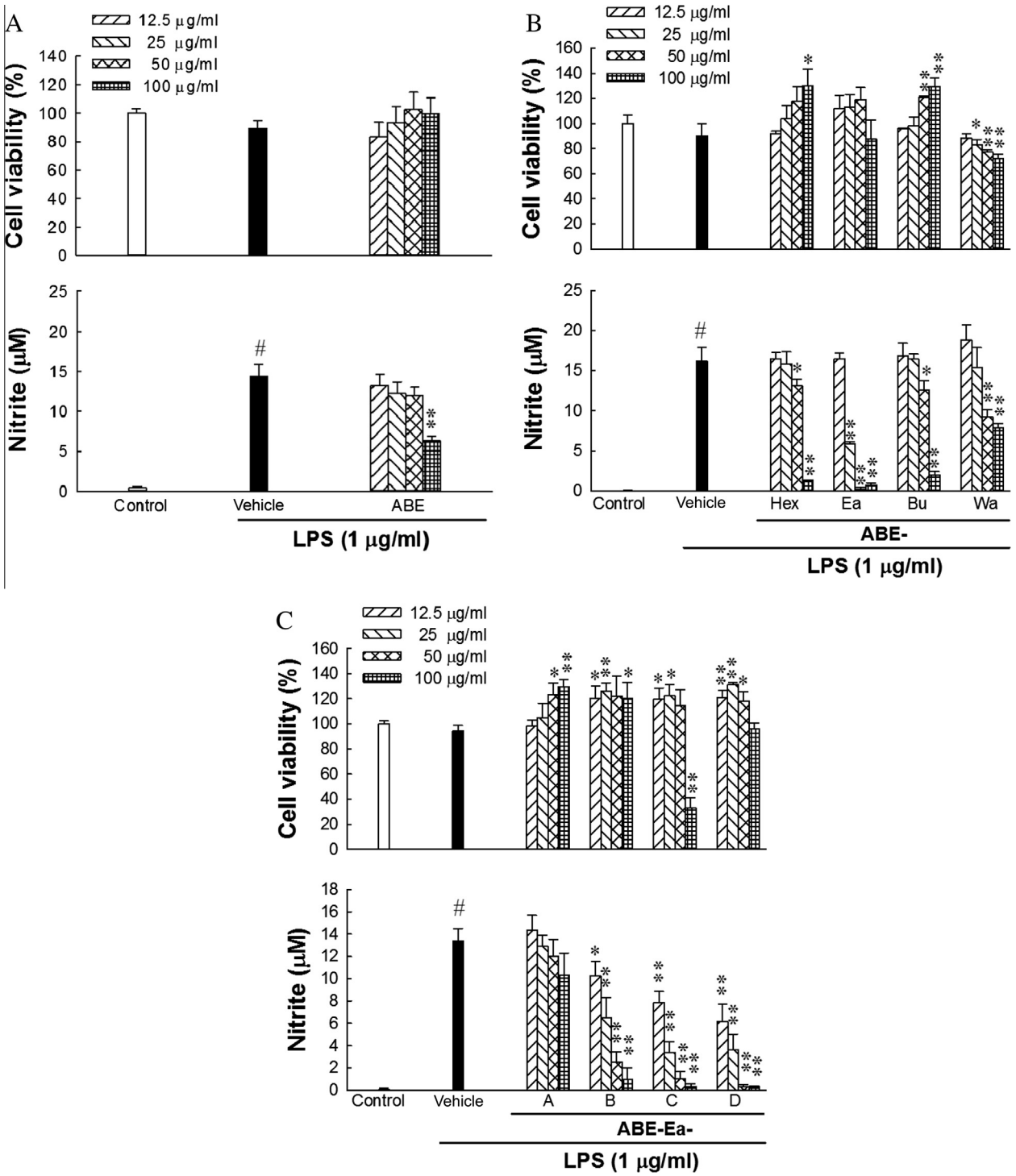

Fig. 2. Effects of the ethanol extract of adlay bran (ABE) (A), fractions partitioned from the ABE (B) and the ethyl acetate sub-fraction prepared from the ABE (ABE-Ea) (C) on cell viability (top) and lipopolysaccharide (LPS)-induced nitric oxide (NO) formation (bottom) in RAW 264.7 cells. Values represent the mean ± SD of three independent experiments. $^{\#}p < 0.05$ as compared with control; $^{*}p < 0.05$ and $^{**}p < 0.01$ as compared with LPS.

2.9. Transfection and NFκB-luciferase assay

To measure NFκB transcriptional activity, RAW 264.7 cells (1×10^6/well) were cultured in 6-well plates and transfected with a NFκB-dependent luciferase reporter gene construct (Stratagene, Jalla, CA) using Lipofectamine and Plus reagent following Invitrogen's protocols. For transient expression, the cells were harvested 48 h after transfection. The resistant clones were pooled and confirmed by Western blot. To assay the effect of individual AB extracts and their isolated compounds on the activation of NFκB in response to LPS, the transfected cells were treated with 10–75 μg/ml AB extracts or isolated compounds in the absence or presence of LPS (1 μg/ml) for 1 h. Luciferase assay was performed with a dual luciferase assay kit and the manual of the manufacturer (Promega). NFκB mediated luciferase activity was normalised by the amounts of proteins in total cell lysate of the stable cell line, which was measured by Bradford (Bio-Rad Laboratories).

Table 1
Total phenolic contents of ethyl acetate sub-fractions partitioned from the ethanol extract (ABE-Ea) and ethyl acetate extract (ABEa-Ea) of adlay bran.

Sub-fraction	mg GAE/g dry sample[a]
ABE-Ea	
(A) 30% Ea/Hex	10.2 ± 1.1
(B) 80% Ea/Hex	29.7 ± 1.4
(C) 100% Ea	77.5 ± 2.8
(D) 95% Ethanol	32.0 ± 0.8
ABEa-Ea	
(A) 30% Ea/Hex	1.40 ± 2.7
(B) 80% Ea/Hex	28.4 ± 0.5
(C) 100% Ea	40.5 ± 4.1
(D) 95% Ethanol	12.9 ± 0.7

*Each data point represents the mean ± SD of three independent experiments.
[a] GAE, gallic acid equivalent.

2.10. Statistical analysis

Data are presented as the mean ± standard deviation (SD). Differences between specific means were analysed by a one-way analysis of variance (ANOVA) using the SPSS system, vers. 11.0 (SPSS, Chicago, IL, USA). Group means were compared using a one-way ANOVA and Duncan's multiple-range test. For comparison of two groups, Student's *t*-test was used. The difference between two means was considered statistically significant when $p < 0.05$ and highly significant when $p < 0.01$.

3. Results and discussion

Numerous reports proved that chronic inflammation can stimulate several diseases, e.g., cancers, atherosclerosis, obesity, and diabetes (Kim, Son, Chang, & Kang, 2004).

In previous studies, several beneficial components were purified and identified from adlay seeds, such as phytosterols,

Table 2
Contents of phenolic compounds of the ethyl acetate sub-fractions partitioned from ABE-Ea and ABEa-Ea.

Classification	Compound	Phenolic contents of sub-fractions of ABE-Ea (μg/g)			
		A	B	C	D
Phenolic acids					
Hydroxybenzoic acids	*p*-Hydroxybenzoic acid	16.6	295	1603	2770
	Protocatechuic acid	ND	ND	Trace	88.5
	Syringic acid	ND	ND	ND	5.06
	Vanillic acid	ND	Trace	Trace	275
Hydroxycinnamic acids	Caffeic acid	ND	Trace	Trace	100
	p-Coumaric acid	ND	ND	678	750
	Ferulic acid	ND	Trace	Trace	412
Phenolic aldehydes	*p*-Hydroxybenzaldehyde	251	46.5	173	41.7
	Syringaldehyde	ND	ND	940	102
	Vanillin	622	638	582	87.5
Flavonoids					
Chalcones	Isoliquiritigenin	191	259	97.7	2.67
Flavonols	Quercetin	6.46	Trace	23.2	433
Flavones	Apigenin	2.25	34.0	26.3	1.35
	Luteolin	Trace	Trace	Trace	29.7
	Nobiletin	Trace	55.9	337	2.05
	Tangeretin	Trace	409	8.15	0.82
Flavanones	Eriodictyol	Trace	2.82	4.86	298
	Naringenin	283	530	508	3.31
Isoflavones	Formononetin	74.3	30.9	4.14	0.80
Others	*p*-Hydroxyacetophenone	11.6	4.66	5.08	3.64
Total		1457	2306	4990	5404
		Phenolic contents of sub-fractions of ABEa-Ea (μg/g)			
Phenolic acids					
Hydroxybenzoic acids	*p*-Hydroxybenzoic acid	ND	991	169	155
	Protocatechuic acid	ND	ND	ND	47.8
	Syringic acid	ND	ND	1.07	35.6
	Vanillic acid	ND	136	2415	Trace
Hydroxycinnamic acids	Caffeic acid	ND	ND	0.90	Trace
	p-Coumaric acid	ND	386	574	Trace
	Ferulic acid	ND	11.8	1457	15.2
Phenolic aldehydes	*p*-Hydroxybenzaldehyde	5.24	86.7	3.70	1.77
	Syringaldehyde	ND	92.2	Trace	292
	Vanillin	ND	719	267	Trace
Flavonoids					
Chalcones	Isoliquiritigenin	0.28	250	Trace	Trace
Flavonols	Quercetin	ND	Trace	Trace	61.1
Flavones	Apigenin	ND	2.13	Trace	ND
	Luteolin	ND	0.59	ND	ND
	Nobiletin	Trace	1423	110	9.97
	Tangeretin	Trace	1579	2.10	2.09
Flavanones	Eriodictyol	ND	ND	Trace	7.55
	Naringenin	ND	237	2.53	ND
Isoflavones	Formononetin	ND	171	ND	ND
Others	*p*-Hydroxyacetophenone	ND	18.1	1.71	ND
Total		5.52	6104	5002	628

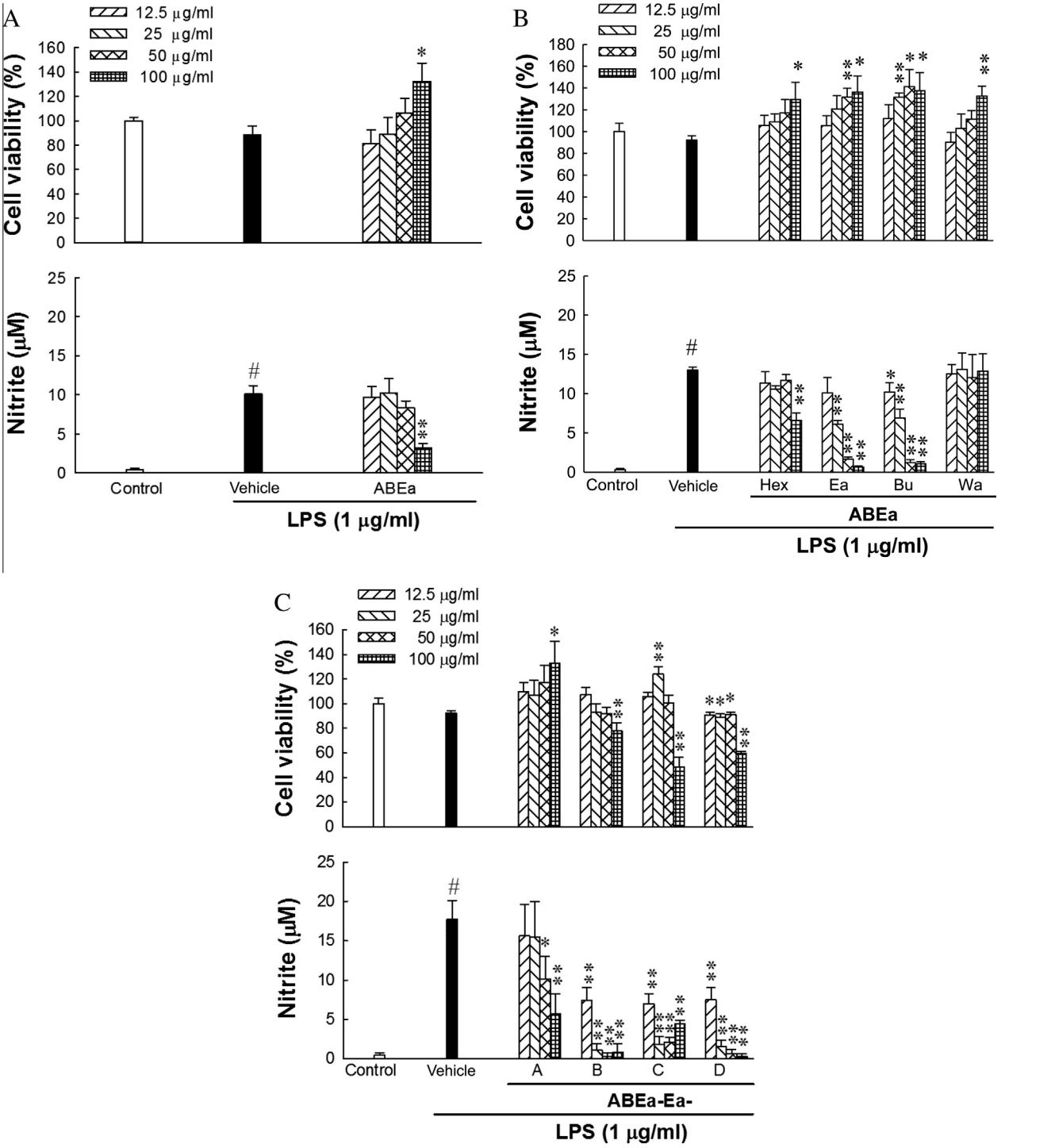

Fig. 3. Effects of the ethyl acetate extract of adlay bran (ABEa) (A), fractions partitioned from the ABEa (B) and the ethyl acetate sub-fraction prepared from the ABEa (ABEa-Ea) (C) on cell viability (top) and lipopolysaccharide (LPS)-induced nitric oxide (NO) formation (bottom) in RAW 264.7 cells. Values represent the mean ± SD of three independent experiments. $^{\#}p < 0.05$ as compared with control; $^{*}p < 0.05$ and $^{**}p < 0.01$ as compared with LPS.

lactams, and phenolic compounds, which had different activities and antitumor effects, modulated immunology, and were gastroprotective (Chen et al., 2012; Chung, Hsia, et al., 2011; Chung, Hsu, et al., 2011; Lee, Lin, Cheng, Chiang, & Kuo, 2008). Thus, in the present study, LPS-mediated inflammation of RAW 264.7 cells, a murine macrophage cell line, was used to investigate the anti-inflammatory activity of AB extracted with different solvents, ethanol or ethyl acetate.

3.1. Effects of ABE-Ea and its sub-fractions on NO formation

Under pathological conditions, excess NO is secreted by inflammatory cells and synergistically acts with other inflammatory mediators (Korhonen, Lahti, Kankaanranta, & Moilanen, 2005). Therefore, the inhibitory effect of NO formation was initially used to screen the anti-inflammatory activity of samples. The ABE inhibited the formation of NO in a dose-dependent manner, with an IC_{50} of 84.7 μg/ml

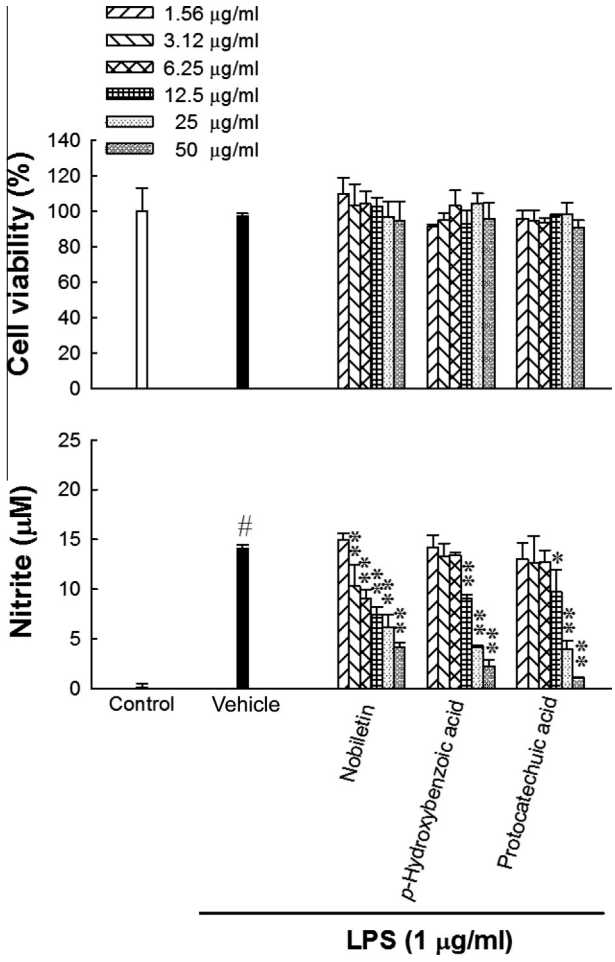

Fig. 4. Effects of nobiletin, *p*-hydroxybenzoic acid, and protocatechuic acid on cell viability (top) and lipopolysaccharide (LPS)-induced nitric oxide (NO) formation (bottom) in RAW 264.7 cells. Values represent the mean ± SD of three independent experiments. $^{\#}p < 0.05$ as compared with control; $^{*}p < 0.05$ and $^{**}p < 0.01$ as compared with LPS.

(Fig. 2A). The inhibitory activity of ABE-Ea on NO formation was better than those of the other fractions (IC_{50} = 27.7 μg/ml) and did not show cell toxicity at the tested concentrations (Fig. 2B). ABE-Ea was further separated into four sub-fractions. Fig. 2C showed that ABE-Ea-B, ABE-Ea-C, and ABE-Ea-D exhibited better inhibitory effects (with respective IC_{50} values of 24.3, 15.7, and <12.5 μg/ml) against NO formation than ABE-Ea-A (with an IC_{50} of >100 μg/ml). To confirm the anti-inflammatory activity of the tested subfractions, cell viability was simultaneously determined using a colorimetric MTT assay. Only ABE-Ea-C showed cytotoxic effect at the higher concentration, it reduced 67% of cell viability at 100 μg/ml. Of note, the inhibitory effects of the ABE-Ea sub-fractions were consistent with results of the total phenolic contents (Table 1) and demonstrate that more phenolic contents express higher inhibitory activities, except for the ABE-Ea-D sub-fraction. This may have resulted from the ABE-Ea-D sub-fraction having higher contents of quercetin, luteolin, and eriodictyol (433, 29.7, and 298 μg/g, respectively; Table 2). The anti-inflammatory effects of quercetin, luteolin, and eriodictyol are well known (Huang, Chung, et al., 2009; Huang, Kuo, et al., 2009; Kim, Cheon, Kim, Kim, & Kim, 1999).

3.2. Effects of ABEa and its fractions and sub-fractions on NO formation

The ABEa inhibited the formation of NO in a dose-dependent manner, with an IC_{50} of 81.8 μg/ml (Fig. 3A). Fig. 3B shows the

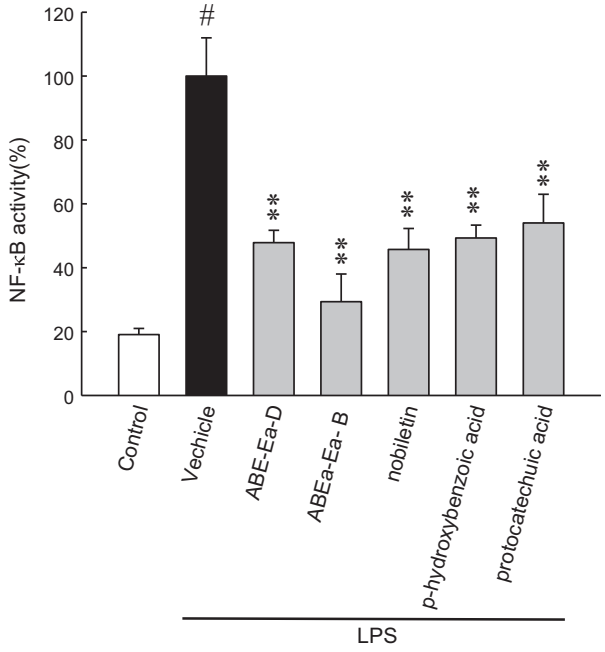

Fig. 5. Effects of individual AB fractions and their isolated compounds on LPS-induced transcriptional activity of NF-κB in RAW 264.7 cells. The cells were transfected with 1 μg of NF-κB-Luc reporter gene and treated with indicated extracts or compounds (50 μg/ml) in the presence or absence of LPS (1 μg/ml) for 18 h. Values represent the mean ± SD of three independent experiments. $^{\#}p < 0.05$ as compared with control; $^{*}p < 0.05$ and $^{**}p < 0.01$ as compared with LPS.

effect of different ABEa fractions on cell viability and NO formation. Results demonstrated that both the ABEa-Ea (with an IC_{50} of 28.2 μg/ml) and ABEa-Bu (with an IC_{50} of 29.2 μg/ml) decreased NO secretion. However, the yield of the ABEa-Ea was 1.16% higher than that of the ABEa-Bu (0.02%), and the ABEa-Ea was further separated into four sub-fractions by column chromatography. The inhibitory effects on NO formation of the ABEa-Ea-B, ABEa-Ea-C and ABEa-Ea-D sub-fractions were better than that of the ABEa-Ea-A sub-fraction (Fig. 3C, bottom). Fig. 3C (top) shows the cell viability, and the results indicate that the high concentration (100 μg/ml) of ABEa-Ea-B, ABEa-Ea-C, and ABEa-Ea-D significantly expressed cytotoxicity toward RAW 264.7 cells.

Fig. 4 shows the effects of the components of AB on cell viability and NO formation. The data show that nobiletin, *p*-hydroxybenzoic acid, and protocatechuic acid all inhibited the formation of NO in dose-dependent manners without cytotoxicity.

3.3. AB extracts suppresses NFκB activation

Because the transcription factor NFκB induces expression of many immediate pro-inflammatory genes, activation of NFκB was also analysed. Transcriptional activity of NFκB was evaluated to assess whether AB extracts and their active compounds inhibited NFκB binding activity in LPS-stimulated RAW 264.7 cells. As shown in Fig. 5, treatment with AB extracts (ABE-Ea-D or ABEa-Ea-B) and their active compounds (nobiletin, *p*-hydroxybenzoic acid or protocatechuic acid) significantly inhibited LPS-induced NFκB transcriptional activity in RAW 264.7 cells. The results of this study indicate that nobiletin, *p*-hydroxybenzoic acid, and protocatechuic acid in AB are bioactive compounds with potential anti-inflammatory activity.

Quercetin was the most abundant flavonoid of the ABE-Ea-D; nobiletin and tangeretin were more abundant than the other flavonoids in the ABEa-Ea-B (Table 2). Quercetin can downregulate messenger RNA expressions of TNF-α and IL-6 through decreasing the

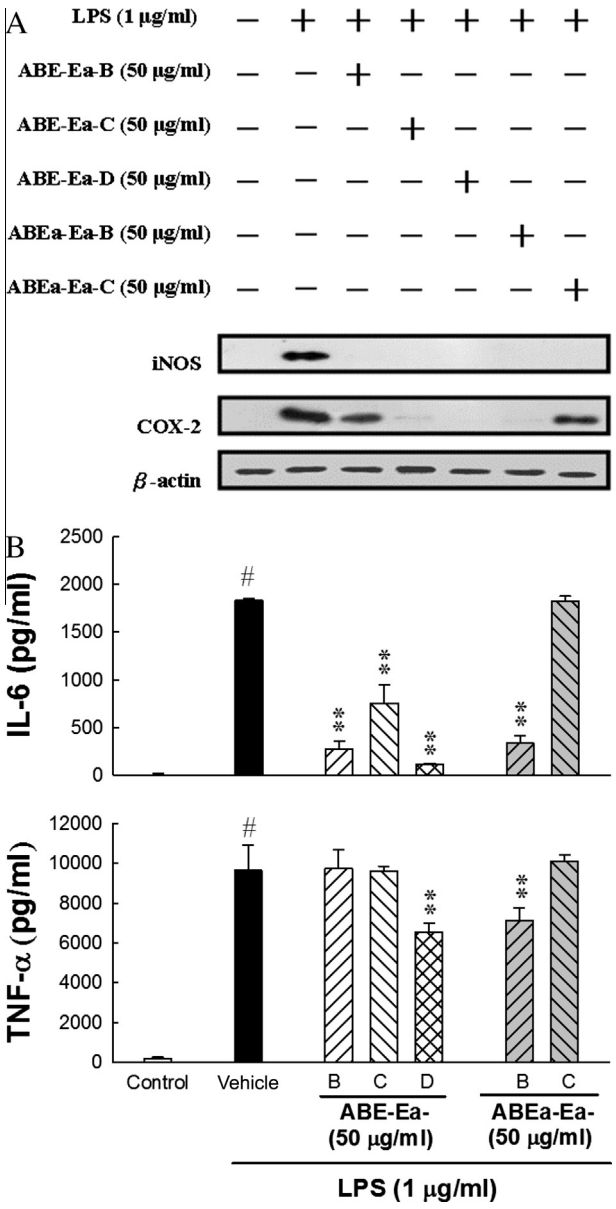

Fig. 6. Ethyl acetate sub-fractions of the ethanol extract of adlay bran (ABE-Ea)-B, ABE-Ea-C, ABE-Ea-D, and of the ethyl acetate extract of AB (ABEa-Ea)-B, and ABEa-Ea-C lowered the expression of lipopolysaccharide (LPS)-induced inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 protein (A), and secretion of the proinflammatory cytokines interleukin (IL)-6 (top) and tumor necrosis factor (TNF)-α (bottom) in RAW 264.7 cells. Values represent the mean ± SD of three independent experiments. $^{\#}p < 0.05$ as compared with control; $^{*}p < 0.05$ and $^{**}p < 0.01$ as compared with LPS.

phosphorylation of extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) (Cho et al., 2003). On the other hand, nobiletin cannot downregulate the phosphorylation of ERK and p38 MAPK, but does downregulate transcription of NFκB (Murakami et al., 2000). Therefore, at least partially, variations in different anti-inflammatory effects between sub-fractions are related to their contents of phenolic compounds.

3.4. Effects of sub-fractions on the expressions of iNOS and COX-2

In inflammatory situations, NO is principally produced by the enzyme, iNOS, and COX-2 secretes PGE_2 to induce macrophage assembly In the present study, protein expressions of iNOS and COX-2 were both downregulated by the ABE-Ea-B, ABE-Ea-C, ABE-Ea-D, ABEa-Ea-B, and ABEa-Ea-C (Fig. 6A), consistent with the inhibitory effects on NO formation (Fig. 2C bottom, Fig. 3C bottom).

3.5. Effects of sub-fractions on the secretion of TNF-α and IL-6

TNF-α and IL-6 are both classic proinflammatory mediators The ABE-Ea-B, ABE-Ea-C, and ABEa-Ea-B inhibited the secretion of IL-6; however, the ABEa-Ea-D was ineffective (Fig. 6B, top). Moreover, only the ABE-Ea-D and ABEa-B lowered TNF-α secretion (Fig. 6B, bottom).

4. Conclusions

Expressions of iNOS and COX-2 protein, and the secretion of NO, TNF-α, and IL-6 were all inhibited by the ABE-Ea-D and ABEa-Ea-B. The most abundant phenolic components of the ABE-Ea-D were *p*-hydroxybenzoic acid (2770 μg/g), *p*-coumaric acid (750 μg/g), and quercetin (433 μg/g sample), and the most abundant phenolic components of the ABEa-Ea-B were tangeretin (1579 μg/g), nobiletin (1423 μg/g), and *p*-hydroxybenzoic acid (991 μg/g).

This is the first investigation to compare variations in extracts of AB using different solvents. The results suggest that regardless of the solvent used, both the ABE and ABEa exhibited anti-inflammatory properties, and the yield of the ABEa-Ea was higher than that of the ABE-Ea. Therefore, Ea may be a better solvent to extract the anti-inflammatory components of AB.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2013.08.071.

References

Chawla, A., Nguyen, K. D., & Goh, Y. P. S. (2011). Macrophage-mediated inflammation in metabolic disease. *Natural Review Immunology, 11*(11), 738–749. http://dx.doi.org/10.1038/nri3071.

Chen, H. J., Chung, C. P., Chiang, W., & Lin, Y. L. (2011). Anti-inflammatory effects and chemical study of a flavonoid-enriched fraction from adlay bran. *Food Chemistry, 126*, 1741–1748. http://dx.doi.org/10.1016/j.foodchem.2010.12.074.

Chen, H. J., Lo, Y. C., & Chiang, W. (2012). Inhibitory effects of adlay bran (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) on chemical mediator release and cytokine production in rat basophilic leukemia cells. *Journal of Ethnopharmacology, 141*, 119–127. http://dx.doi.org/10.1016/j.jep.2012.02.009.

Cho, S. Y., Park, S. J., Kwon, M. J., Jeong, T. S., Bok, S. H., Choi, W. Y., et al. (2003). Quercetin suppresses proinflammatory cytokines production through MAP kinases and NF-kappa B pathway in lipopolysaccharide-stimulated macrophage. *Molecular and Cellular Biochemistry, 243*(1–2), 153–160.

Chung, C. P., Hsia, S. M., Lee, M. Y., Chen, H. J., Cheng, F., Chan, L. C., et al. (2011a). Gastroprotective activities of adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) on the growth of the stomach cancer AGS cell line and indomethacin-induced gastric ulcers. *Journal of Agricultural and Food Chemistry, 59*(11), 6025–6033. http://dx.doi.org/10.1021/jf2009556.

Chung, C. P., Hsu, C. Y., Lin, J. H., Kuo, Y. H., Chiang, W., & Lin, Y. L. (2011b). Antiproliferative lactams and spiroenone from adlay bran in human breast cancer lines. *Journal of Agricultural and Food Chemistry, 59*(4), 1185–1194. http://dx.doi.org/10.1021/jf104088x.

Hsia, S. M., Chiang, W., Kuo, Y. H., & Wang, P. S. (2006). Downregulation of progesterone biosynthesis in rat granulosa cells by adlay (*Coix lachryma-jobi* L.

var. *ma-yuen* Stapf) bran extracts. *International Journal of Impotence Research, 18*(3), 264–274.

Huang, B. W., Chiang, M. T., Yao, H. T., & Chiang, W. (2004). The effect of high-fat and high-fructose diets on glucose tolerance and plasma lipid and leptin levels in rats. *Diabetes, Obesity and Metabolism, 6*(2), 120–126.

Huang, D. W., Chung, C. P., Kuo, Y. H., Lin, Y. L., & Chiang, W. (2009a). Identification of compounds in adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) seed hull extracts that inhibit lipopolysaccharide-induced inflammation in RAW 264.7 macrophages. *Journal of Agricultural and Food Chemistry, 57*(22), 10651–10657. http://dx.doi.org/10.1021/jf9028514.

Huang, G. J., Huang, S. S., & Deng, J. S. (2012). Anti-Inflammatory activities of inotilone from *Phellinus linteus* through the inhibition of MMP-9, NF-κB, and MAPK activation in vitro and in vivo. *PLoS ONE, 7*(5), 1–12. http://dx.doi.org/10.1371/journal.pone.0035922.

Huang, D. W., Kuo, Y. H., Lin, F. Y., Lin, Y. L., & Chiang, W. (2009b). Effect of adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) testa and its phenolic components on Cu^{2+}-treated low-density lipoprotein (LDL) oxidation and lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 macrophages. *Journal of Agricultural and Food Chemistry, 57*(6), 2259–2266. http://dx.doi.org/10.1021/jf803255p.

Kim, H. K., Cheon, B. S., Kim, Y. H., Kim, S. Y., & Kim, H. P. (1999). Effects of naturally occurring flavonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure–activity relationships. *Biochemical Pharmacology, 58*, 759–765.

Kim, H. P., Son, K. H., Chang, H. W., & Kang, S. S. (2004). Anti-inflammatory plant flavonoids and cellular action mechanisms. *Journal of Pharmacological Sciences, 96*(3), 229–245.

Korhonen, R., Lahti, A., Kankaanranta, H., & Moilanen, E. (2005). Nitric oxide production and signaling in inflammation. *Current Drug Targets–Inflammation and Allergy, 4*(4), 471–479.

Lee, J. Y., Lee, M. S., Choi, J. W., Shin, T. S., Woo, H. C., & Kim, H. R. (2012). Dichloromethane fraction of *Laminaria japonica* ethanolic extract inhibits lipopolysaccharide-Induced nitric oxide synthase and cyclooxygenase-2 expression in RAW 264.7 cells via NF-κB pathway. *Inflammation, 35*(5), 1650–1658. http://dx.doi.org/10.1007/s10753-012-9481-2.

Lee, M. Y., Lin, H. Y., Cheng, F., Chiang, W., & Kuo, Y. H. (2008). Isolation and characterization of new lactam compounds that inhibit lung and colon cancer cells from adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) bran. *Food and Chemical Toxicology, 46*(6), 1933–1939. http://dx.doi.org/10.1016/j.fct.2008.01.033.

Murakami, A., Nakamura, Y., Torikai, K., Tanaka, T., Koshiba, T., Koshimizu, K., et al. (2000). Inhibitory effect of citrus nobiletin on phorbol ester-induced skin inflammation oxidative stress, and tumor promotion in mice. *Cancer Research, 60*(18), 5059–5066.

國科會補助專題研究計畫出席國際學術會議心得報告

日期：102 年 7 月 25 日

計畫編號	NSC 101-2629-B-038-002		
計畫名稱	不同精油萃取物對大鼠子宮平滑肌收縮之影響效應 (GM07)		
出國人員姓名	夏詩閔	服務機構及職稱	台北醫學大學保健營養學系助理教授
會議時間	102 年 6 月 8 日至 102 年 6 月 13 日	會議地點	日本東京
會議名稱	(中文)2013 亞太地區臨床營養大會 (英文) The 8th Asia Pacific Conference on Clinical Nutrition (APCCN 2013)		
發表題目	(中文)薏苡種皮萃取物合併抗癌藥物 doxorubicin 對人類子宮內膜癌細胞之影響效應 (英文) Combination effects of adlay testa extracts and doxorubicin on the growth inhibition of human uterine sarcoma cancer cells		

一、參加會議經過

此會議乃一國際性之臨床營養會議，每兩年在亞洲著名城市舉辦一次，是營養學界非常重要的國際會議之一，至今已是第八屆舉辦約有二十的歷史。此次與會的營養學界學者專家有400多人，大部分以亞洲地區國家為主如日本、韓國及台灣等，本次共有壁報發表約三百篇，我國也有包括臺北醫學大學、台灣大學、中國醫藥大學、實踐大學等師生參加、發表壁報及研討會口頭報告共十幾篇。於大會期間每天均有許多不同領域與臨床營養相關主題之演講與研討會，此外也有不同領域的壁報展覽。

二、與會心得

本人研究論文「薏苡種皮萃取物與化療藥物 doxorubicin 並用對於子宮內膜癌細胞之影響效應 Combination effects of adlay testa extracts and doxorubicin on the growth inhibition of human uterine sarcoma cancer cells 」獲 2013 年亞太地區臨床營養大會接受並發表。此次經費感謝由國科會專題研究計畫補助。本次開會於日本東京舉辦，與會成員多為亞太地區營養與食品相關領域之專家學者參與。本次年會保健營養系共有 3 位教師一起參加。除參觀食品營養展覽會場，蒐集最新市場動態以外，筆者同時也參加乳酸菌保健機能性以及非熱加工技術對食品保存的重要管制及液態食品包裝新技術等相關專題討論。同時，筆者一行人亦參觀與會廠商所陳列之分析儀器功能展示，值得注意的是，日本已經開發出能快速分析皮膚高度醣化終產物含量之非侵入性儀器，售價約折合台幣九十萬元左右，由於其具有非侵入、亦檢測之優點，筆者未來將考慮引進採用，設計人體試驗進行分析，受益良多。

三、發表論文全文或摘要

Combination effects of adlay testa extracts and doxorubicin on the growth inhibition of human uterine sarcoma cancer cells

Shih-Min Hsia[1], Tzong-Ming Shieh[2], Kai-Lee Wang[3], Chi-Ho Wu[1]

[1]School of Nutrition and Health Sciences, Taipei Medical University, Taipei 11031, Taiwan; [2]Department of Dental Hygiene, College of Health Care, China Medical University, Taichung , Taiwan; [3]Department of Physiology, School of Medicine, National Yang-Ming University, Taipei 11221, Taiwan.

Abstract:

Background/Aims

Cancer has been the top ten causes of death in Taiwan from 1982. Chemotherapy is one of the cancer treatments. However, chemotherapy will cause many side effects and multiple drug resistance (MDR). These are the main poor effects of chemotherapy. MDR can be the result of a variety of mechanisms that are not completely understood, but many studies have shown P-glycoprotein expression was higher in tumor cells, and it induced MDR. On the other hand, there are evidences that P-gp positive cells are resistant to apoptosis. In addition, it's also closely related between the poor effects of chemotherapy and tumor metastasis. Previous studies revealed that adlay seed extracts could have anti-cancer activity. Thus, this study will investigate that combination of adlay testa ethanolic extracts (ATE) and doxorubicin has on the growth inhibition of human uterine sarcoma cells.

Methods

The human uterine sarcoma cell line MES-SA and MES-SA/Dx5 cells were used fo ATE anti- human study in vitro. Cell viability is detected by MTT assay after variant ATE 24 h~72 h treatment. Cell cycle and apoptosis was analyzed by flow cytometry. Messenger RNA and protein expression were detected by RT-PCR and western blotting, respectively.

Results

Results demonstrated that (1) ATE-Hex had the best effects of inhibition on MES-SA and MES-SA/Dx5 cells. Co-treatment of ATE-Hex and sub-toxic doxorubicin could synergistically or additively inhibit cancer cells proliferation. (2) ATE-Hex reduced the rhodamine efflux in MES-SA/Dx5 cells, indicated that ATE-Hex could reduce P-gp expression. ATE-Hex also could inhibit migration of MES-SA and MES-SA/Dx5 cancer cells. (3) Combination of ATE-Hex and doxorubicin induced apoptosis by increasing sub G1 phase and PARP being cleaved. (4) Analysis of anti-cancer activity, phytosterols had better inhibition on cancer cells growth than fatty acids, especially campesterol and β-sitosterol.

Conclusion

These present findings showed that ATE could inhibit on the growth of human uterine sarcoma cancer cells. Furthermore, the combination of ATE and doxorubicin could decrease drug resistance and increase synergistic effect.

Keyword：adlay testa, multi-drug resistance, apoptosis, migration, phytosterol

四、建議

本次發筆者發表的題目為薏苡種皮萃取物與化療藥物doxorubicin並用對於子宮內膜癌細胞之影響效應。本研究獲致之成果將有助於瞭解薏苡種皮萃取物降低化療藥物的使用劑量，可以做為輔助治療癌症製劑的潛力。

五、攜回資料名稱及內容

大會手冊、手提袋、文具等

六、其他

國科會補助計畫衍生研發成果推廣資料表

日期:2013/11/14

國科會補助計畫	計畫名稱: 不同精油萃取物對大鼠子宮平滑肌收縮之影響效應 (GM07)
	計畫主持人: 夏詩閔
	計畫編號: 101-2629-B-038-002- 學門領域: 性別主流科技計畫

無研發成果推廣資料

101 年度專題研究計畫研究成果彙整表

計畫主持人：夏詩閔	計畫編號：101-2629-B-038-002-
計畫名稱：不同精油萃取物對大鼠子宮平滑肌收縮之影響效應（GM07）	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	1	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	1	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

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。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

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：

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 100 字為限)

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）

本研究發現不同精油萃取物具有抑制大鼠子宮平滑肌過度收縮之效果, 其中以快樂鼠尾草精油, 肉桂精油及薏苡油溶性萃取物具有較佳之抑制子宮收縮之效果